0071988

SAF-RC-048
100 Area and 300 Area Component of the RCBRA Water Sampling
FINAL DATA PACKAGE

COMPLETE COPY OF DATA PACKAGE TO:

Jackie Queen H4-22 KW 1/10/07
Initial/Date

COMMENTS:

SDG BC1722 SAF-RC-048

Rad only X Chem only Rad & Chem

X Complete Partial

Waste Site: **Cr 2 Pore Water (2nd event)**
Cr 4 Pore Water (2nd event)
Cr 6 Pore Water (2nd event)
Sr 4 Pore Water (2nd event)

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JAN 2007

BIOASSAY REPORT
CHRONIC SCREENING BIOASSAYS
Conducted November 30 through December 7, 2006

Prepared for

ELR CONSULTING, INC.
WASHINGTON HANFORD CLOSURE
RICHLAND, WASHINGTON

Prepared by

CH2M HILL
2300 NW Walnut Boulevard
Corvallis, Oregon 97330

December 19, 2006
Lab I.D Number B1722-01 through -04
SDG NUMBER BC1722

RC-048

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INTRODUCTION

CH2M HILL conducted a series of chronic screening bioassay tests using the water flea (*Ceriodaphnia dubia*) on pore water samples provided by ELR Consulting for Washington Closure Hanford, Richland, Washington. The test was conducted from November 30 through December 7, 2006.

METHODS AND MATERIALS

TEST METHODS

Ceriodaphnia dubia chronic three brood toxicity test method is adapted from *Standard Guide for Conducting Three-Brood, Renewal Toxicity Tests with Ceriodaphnia dubia*, ASTM, designation: E1295-01 (2002); and *Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms*, EPA Method 1002.0 "*Ceriodaphnia* Survival and Reproduction Test," EPA-821-R-02-013 (2002).

Additional guidance for test interpretation was provided by: *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program*, (EPA June 2000), EPA 833-R-00-003: and *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)*, (EPA July 2000), EPA 821-B-00-004.

TEST ORGANISMS

The *Ceriodaphnia dubia* were obtained from CH2M HILL's in-house cultures. The *Ceriodaphnia dubia* used in the chronic test were less than 24 hours old and within an eight hour age range at test initiation. All organisms tested were fed and maintained during culturing, acclimation, and testing as prescribed by the EPA. The test organisms appeared vigorous and in good condition prior to testing.

DILUTION WATER

The specific hardness and alkalinity of the dilution water used with each test is listed in Table 2 below.

TEST CONCENTRATIONS

The concentrations tested in the chronic tests were 100 percent porewater samples with dilution water alone for the control. For the *Ceriodaphnia* chronic test, 1 organism per chamber with 10 chambers per concentration for a total of 10 organisms per concentration was used.

SAMPLE COLLECTION

All samples were delivered to CH2M HILL's Corvallis Aquatic Toxicology Laboratory by Federal Express. The samples were stored in the dark at 4°C until test solutions were prepared and tested. Chain of custody for sample collection is provided in Appendix C.

The sample identification and collection, receipt, and testing dates are listed in Table 1 below.

Table 1
Sample Collection and Cross-Reference Data

Client I.D	SAMPLE DATE	RECEIPT DATE	LAB SDG I.D.	ANALYSIS DATE
J13HH2	11-25-2006	11-29-2006	B1722-01	11-30-2006
J13HH3	11-25-2006	11-29-2006	B1722-02	11-30-2006
J13LM9	11-25-2006	11-29-2006	B1722-03	11-30-2006
J13HH4	11-25-2006	11-29-2006	B1722-04	11-30-2006

SAMPLE PREPARATION

Temperature was adjusted on the sample used during the test prior to each daily renewal.

MONITORING OF BIOASSAYS

The sample was monitored on arrival for hardness, alkalinity, total residual chlorine, ammonia, and temperature.

For the chronic test, pre- and post-renewal solutions were monitored for dissolved oxygen and pH daily in the control and all concentrations tested. Conductivity was measured at test initiation. Temperature was monitored continuously throughout the testing period.

Ceriodaphnia dubia survival and neonate production were measured daily in the chronic tests. In accordance with ASTM E1295-01, survival data were determined as the number of 1st generation organisms surviving at day 7. In accordance with ASTM E1295-01 and EPA-821-R-02-013, the collection of reproduction data were complete when 60 percent of the control organisms have produced a third brood (day 6 or day 7). Reproduction data are defined as the total neonates produced in the first three broods by the 1st generation test organism.

DATA ANALYSIS

The effects measured during the *Ceriodaphnia dubia* chronic test included survival and reproduction over the exposure period. The statistical analyses performed were those outlined in *Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms,* EPA Method 1002.0 "*Ceriodaphnia* Survival and Reproduction Test," EPA-821-R-02-013 (2002), using CETIS version 1.1.2. Fisher Exact test was used to compare the survival data between the control and each sample treatment. Equal variance t Two-Sample test was used to compare the reproduction data between the control and each sample treatment When the assumptions of normality or homogeneity of variance necessary for Equal variance t Two-Sample test could not be met, Unequal variance t Two-Sample test or Wilcoxon Two-Sample Test was used to analyze the data.

RESULTS AND DISCUSSION

CHRONIC BIOASSAYS

Table 2 summarizes the survival and reproduction data, statistical results, and water chemistry data for the *Ceriodaphnia dubia* chronic tests.

The J13HH2, J13HH3, J13LM9, and J13HH4 samples indicated no statistically significant reduction in either survival or in reproduction when compared to the laboratory control. The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were 100 and greater than 100 percent for this sample, respectively.

The dissolved oxygen levels in the chronic tests remained above 4 mg/L throughout the test period and test temperatures remained at 25±1°C. The tests proceeded without any interruption that could have affected test results.

Table 2: *Ceriodaphnia dubia* Chronic Test Results for Washington Closure Hanford

F, significantly different from control by use of Fisher's Exact test; E, significantly different from lab control by use of Equal Variance Two-Sample t Test; ns indicates a non significant result; *, indicates significant at alpha (p) = 0.05; –, indicates no statistical test performed.

Lab ID:	Sample Number:	*Ceriodaphnia dubia* Survival Endpoint Percent Survival	Significantly different compared to Lab Control?	*Ceriodaphnia dubia* Reproduction Endpoint Young per female	Significantly different compared to Lab Control?	Total Residual Chlorine (mg/L)	Ammonia (mg/L as NH_3-N)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Tests initiated on Nov 30, 2006									
Laboratory Control		100	–	26.2	–	na	na	86	64
B1722-01	J13HH2	100	ns	26.1	ns	0.03	0.06	118	90
B1722-02	J13HH3	90	ns	26.1	ns	0.03	0.08	70	64
B1722-03	J13LM9	100	ns	26.5	ns	0.01	0.05	84	66
B1722-04	J13HH4	100	ns	30.1	ns	0.02	0.19	102	66

REFERENCE TOXICANT TESTS

The results of monthly reference toxicant test conducted with sodium chloride indicate that the test organisms were within their respective sensitivity range based on EPA guidelines. The IC_{25} values, and Control Chart Limits are listed in Table 3 below. The data sheets for the reference toxicant tests are provided in Appendix B.

Table 3
Chronic Reference Toxicant Tests (g/L)

Species (test)	IC_{25}	Control Chart limits
Ceriodaphnia dubia (survival)	1.64	1.34 to 1.81
Ceriodaphnia dubia (reproduction)	0.46	0.30 to 0.80

CERTIFICATION STATEMENT

I certify that this data package is in compliance with the Statement of Work, both technically and for completeness, for other than the conditions detailed above. Release of the data contained in this hard copy data package has been authorized by the Laboratory Manager or a designee, as verified by the following signature:

APPENDIX A
RAW DATA SHEETS

CH2M HILL *TOXICITY TEST ORGANISM AND WATER QUALITY DATA*

Client: ELR - Washington Closure Hanford — Test Initiation: Date Nov 30, 2006 — Test Termination: Date Dec 7, 2006

Contact: — Technician: Mucky, Pittinger, Winn

Test Species/ID: *Ceriodaphnia dubia* / Cd 2007 / /

Sample ID Number	Field ID	Collected Date	Collected Time	Total Residual Chlorine (mg/l) As Received	Total Residual Chlorine (mg/l) As Dechlor.	Ammonia NH_3-N mg/l	Hardness mg/l as $CaCO_3$	Alkalinity mg/l as $CaCO_3$
B1722-01	J13HH2	11/25/06	1915	0.03	-	0.06	116	90
B1722-02	J13HH3	11/25/06	2030	0.03	-	0.08	70	64
B1722-03	J13LM9	11/25/06	2230	0.01	-	0.05	84	66
B1722-04	J13HH4	11/25/06	2120	0.02	-	0.19	102	66

Test Species Information	ID# Cd 2007	ID#	ID#	ID#
	Chronic			
Organism Age at Initiation	< 24 hours within an 8 hour span			
Test Container Size	30 ml			
Test Volume	15 ml			
Feeding: Type	0.1 ml Algae &			
Amount	YCT daily			
Aeration: Began	none			
Amount	—			
Dilution Water ID#	2728, 2735			
Acclimation Period	<24 hrs			
Test Location	# 5			
Organism Source	In-House			
Size (mm)	-	-	-	-
Loading Rate	-	-	-	-

Dilution Water	ID#	Hardness mg/l as $CaCO_3$	Alkalinity mg/l as $CaCO_3$	Initial pH
Recon MH (FHM)	2728	86	64	7.9
1	2735	90	66	7.6

Comments: ☑ Indicates the following action was taken, (☐ Indicates action not taken):

☐ Sample(s) filtered through 60 µm mesh prior to use.

Water Quality Meters Used/ID#

Dissolved Oxygen #2 pH #3 Conductivity #2

CH2M HILL

Ceriodaphnia dubia
Survival and Reproduction
Test Data Summary

Client: ELR - Washington Closure Hanford

Test Start Date: Nov 30, 2006

Sample Description:

Sample ID#: see randomization chart

Ceriodaphnia Lot#: Cd 2007

Statistician: [illegible]

Percent or Concentration	Total Live Young Produced in First 3 Broods per Replicate										# Alive Adults	Total Live Young
	A	B	C	D	E	F	G	H	I	J		
XIII # 1	35	25	25	28	34	31	24	15	20	25	10	262
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
XIII # 2	36	30	34	31	28	31	28	25	28	30	10	301
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
XIII # 3	28	26	27	28	26	28	24	16	31	27	10	261
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
XIII # 4	31	24	25	35	27	33	22	25	20	23	10	265
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
XIII # 5	30	27	30	23	26	15	30	25	25	30	9	261
	AD?	AD?	AD?	AD?	AD?	AD? ✓	AD?	AD?	AD?	AD?		
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		

Test Organism Mortality (Adult dead) = AD? ✓

Test Organism identified as Male = AD? M

Test Organism Injured during test = AD? I

# of Alive Adults = Number of test organism alive at termination (for WDOE only, = Number of test organisms alive at Day 7)

Total Live Young = Total neonates produced in first 3 broods

Footnote: As per EPA-821-R-02-013 (13.10.9.1), *Ceriodaphnia dubia* test should be terminated when 60% of the surviving control organisms have produced their third brood, or at the end of eight days, whichever occurs first.

Also as per EPA-821-R-02-013 (13.10.9.1), "In this three-brood test, offspring from fourth or higher broods should not be counted and should not be included in the total number of neonates produced during the test."

Observations:

CH2M HILL

CERIODAPHNIA 7-DAY SURVIVAL AND REPRODUCTION DATA

Client: ELR - Washington Closure Hanford Test Initiation: Date: 11-30-06 Time: 1330

Sample Description: _____ Test Termination: Date: 12-7-06 Time: 1000

Initial Sample ID #: see randomization chart Ceriodaphnia Lot#: Cd 2007

Technician: Day 1 Bm Day 2 Bm Day 3 TP Day 4 Bm Day 5 TP Day 6 TP Day 7 DW

Time: Day 1 1430 Day 2 100 Day 3 1215 Day 4 1430 Day 5 1300 Day 6 1400 Day 7 1000

Percent	Day	Daily Number of Live Young for each Replicate										No. Live Adults	Daily Total Live Young
		A	B	C	D	E	F	G	H	I	J		
XIII # 1	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	5	4	2	5	7	4	5	3	0	0	10	35
	4	0	0	0	10	0	0	0	0	2	0	10	12
	5	0	10	9	13	0	10	8	0	5	10	10	65
	6	19	11	14	0	16	17	11	12	0	0	10	100
	7	11	0	0	15	11	0	15	0	13	15	10	80
XIII # 2	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	3	6	6	6	5	4	4	2	4	4	10	44
	4	13	11	0	12	9	0	0	9	0	0	10	54
	5	0	0	12	0	0	12	10	0	11	9	10	54
	6	20	13	16	13	14	15	14	14	13	0	10	132
	7	0	11	0	10	16	0	0	10	0	17	10	64
XIII # 3	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	7	6	4	5	4	5	2	2	5	0	10	40
	4	0	7	10	9	10	10	8	4	0	4	10	62
	5	0	0	0	0	0	0	0	0	9	11	10	20
	6	21	13	13	14	12	13	14	10	0	0	10	110
	7	0	12	11	0	13	8	18	11	17	12	10	102
XIII # 4	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	3	6	6	5	5	4	4	3	4	10	44
	4	0	9	9	0	11	0	8	0	0	0	10	37
	5	0	0	0	11	0	12	0	11	8	8	10	50
	6	16	12	10	18	11	16	10	10	0	0	10	103
	7	11	0	12	0	15	0	12	0	9	11	10	70
XIII # 5	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	5	6	0	4	5	6	6	2	4	5	10	43
	4	6	9	5	11	8	0	0	11	0	0	10	50
	5	4/0	0	10	8	0	9 AD	11	0	8	10	9	60
	6	15	12	0	0	13	(	13	12	13	0	9	78
	7	0	11	15	16	0	)	0	0	0	15	9	57
	1												
	2												
	3												
	4												
	5												
	6												
	7												

"AD" = Adult Dead, "AY" = Aborted young, "M" = male organism, " / " = split brood (carry-over brood / current day brood)
"Inj" = Adult Injured during test solution renewal, replicate removed from analysis. "AM" = Adult missing. "F" = Female

Footnote: As per EPA, *Ceriodaphnia dubia* test should be terminated when 60% of the surviving control organisms have produced their third brood, or at the end of eight days, whichever occurs first.

CH2M HILL

CERIODAPHNIA WATER QUALITY DATA

Client: ELR - Washington Closure Hanford
Initiated Date: 11-30-06 Time: 1330
Adults Isolated Date: 11-29-06 Time: 0235
Sample Description: see randomization chart
Initial Sample ID #: —
Neo's Collected Date: 11-29-06 Time: 1340

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Technician	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	DW
Time	1345	1440	1020	1215	1545	1355	1400	1000

Percent	Dissolved Oxygen (mg/l) Day 0	1	2	3	4	5	6	7
XIII # 1	7.9	8.1 / 7.8	7.9 / 8.2	8.0 / 8.1	8.0 / 7.8	8.0 / 8.2	8.3 / 7.9	7.9
XIII # 2	7.8	7.9 / 8.0	7.9 / 8.2	8.1 / 8.1	8.0 / 8.0	8.0 / 8.2	8.1 / 8.2	7.8
XIII # 3	7.9	7.9 / 8.0	7.8 / 8.2	8.1 / 8.2	7.9 / 8.0	7.9 / 8.2	8.0 / 8.2	7.8
XIII # 4	8.0	8.0 / 8.1	7.8 / 8.2	8.1 / 8.2	8.0 / 7.9	8.0 / 8.3	8.1 / 8.2	7.8
XIII # 5	8.1	8.1 / 8.1	8.0 / 8.2	8.1 / 8.2	8.1 / 8.0	8.1 / 8.3	8.2 / 8.2	7.9

Percent	pH Day 0	1	2	3	4	5	6	7
XIII # 1	7.7	7.9 / 7.7	7.8 / 7.7	8.1 / 7.7	8.0 / 7.7	7.7 / 7.7	8.2 / 7.9	7.8
XIII # 2	7.6	7.9 / 7.5	7.8 / 7.3	8.0 / 7.5	7.9 / 7.7	7.7 / 7.6	8.1 / 7.5	7.7
XIII # 3	7.6	8.0 / 7.6	7.8 / 7.4	8.1 / 7.6	8.0 / 7.7	7.7 / 7.7	8.1 / 7.6	7.8
XIII # 4	7.5	7.9 / 7.4	7.8 / 7.3	8.0 / 7.3	8.0 / 7.5	7.7 / 7.5	8.0 / 7.3	7.8
XIII # 5	7.4	7.9 / 7.4	7.9 / 7.2	8.0 / 7.3	7.9 / 7.6	7.7 / 7.6	7.9 / 7.3	7.7

Percent	Temperature (°C) / Conductivity (µmhos/cm) Day 0	1	2	3	4	5	6	7
XIII # 1	24.8 / 292	24.6	25.1	25.5	25.8	25.6	24.3	24.1
XIII # 2	24.8 / 214	24.1	25.2	25.9	25.2	24.8	24.2	24.5
XIII # 3	24.7 / 241	24.0	25.3	25.4	24.8	24.8	24.6	24.0
XIII # 4	24.6 / 172	24.8	24.6	25.6	25.6	25.3	24.6	24.1
XIII # 5	24.9 / 160	25.4	24.8	25.4	25.6	24.5	24.5	24.1

Comments:

Randomization chart for *Ceriodaphnia* tests initiated on Nov 30, 2006

Lab ID:	Field ID	Alternate ID	Random number	Random position	Board ID	Postion on Board	Sample Number
Lab Control	ReconMH only		0.853113719	1	XIII	#1	XIII #1
B 722 -01	J13HH2		0.552365596	3	XIII	~~#2~~ 3	XIII ~~#2~~ 3
B -02	J13HH3		0.180654974	5	XIII	~~#3~~ 5	XIII ~~#3~~ 5
B -03	J13LM9		0.251994801	4	XIII	#4	XIII #4
B -04	J13HH4		0.71031824	2	XIII	~~#5~~ 2	XIII ~~#5~~ 2
					XIII	#6	XIII #6

Test Board Slot Location Information

Board ID: XIII

	Slot #	Sample #	Slot #	Sample #	Slot #	Sample #	Slot #	Sample #	Slot #	Sample #	Slot #	Sample #
rep A	1	XIII # 1	11	XIII # 5	21	XIII # 4	31	XIII # 6	41	XIII # 2	51	XIII # 3
rep B	2	XIII # 3	12	XIII # 5	22	XIII # 2	32	XIII # 1	42	XIII # 4	52	XIII # 6
rep C	3	XIII # 4	13	XIII # 1	23	XIII # 3	33	XIII # 6	43	XIII # 2	53	XIII # 5
rep D	4	XIII # 3	14	XIII # 6	24	XIII # 5	34	XIII # 1	44	XIII # 2	54	XIII # 4
rep E	5	XIII # 2	15	XIII # 5	25	XIII # 4	35	XIII # 3	45	XIII # 6	55	XIII # 1
rep F	6	XIII # 5	16	XIII # 4	26	XIII # 3	36	XIII # 1	46	XIII # 6	56	XIII # 2
rep G	7	XIII # 5	17	XIII # 6	27	XIII # 4	37	XIII # 1	47	XIII # 3	57	XIII # 2
rep H	8	XIII # 6	18	XIII # 4	28	XIII # 3	38	XIII # 2	48	XIII # 1	58	XIII # 5
rep I	9	XIII # 1	19	XIII # 3	29	XIII # 2	39	XIII # 5	49	XIII # 6	59	XIII # 4
rep J	10	XIII # 6	20	XIII # 1	30	XIII # 2	40	XIII # 4	50	XIII # 3	60	XIII # 5

CETIS Test Summary

Report Date: 13 Dec-06 2:28 PM
Test Link: 14-9937-6925/B172201cdc

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Test No:	09-1592-2397	Test Type:	Reproduction-Survival (7d)	Duration:	6d 20h
Start Date:	30 Nov-06 01:30 PM	Protocol:	EPA/821/R-02-013 (2002)	Species:	Ceriodaphnia dubia
Ending Date:	07 Dec-06 10:00 AM	Dil Water:		Source:	In-House Culture
Setup Date:	30 Nov-06 01:30 PM	Brine:			

Sample No:	08-2790-7347	Code:	B1722-01	Client:	
Sample Date:	25 Nov-06	Material:	Unknown	Project:	
Receive Date:		Source:	ELR-Washington Closure Hanford		
Sample Age:	5d 13h	Station:			

Comments: J13HH2

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
19-0541-9266	7d Proportion Survived	100	> 100	N/A	N/A	Fisher Exact
01-3612-9509	Reproduction	100	> 100	N/A	15.29%	Equal Variance t Two-Sample

Test Acceptability

Analysis	Endpoint	Attribute	Statistic	TAC Range	Overlap	Decision
19-0541-9266	7d Proportion Survived	Control Response	1	0.8 - NL	Yes	Passes acceptability criteria
01-3612-9509	Reproduction	Control Response	26.2	15 - NL	Yes	Passes acceptability criteria
01-3612-9509	Reproduction	PMSD	0.15293	0.13 - 0.47	Yes	Passes acceptability criteria

7d Proportion Survived Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
100		10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%

Reproduction Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	26.2	15	35	1.9368	6.1246	23.38%
100		10	26.1	16	31	1.2601	3.9847	15.27%

7d Proportion Survived Detail

Conc-%	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	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
100		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000

Reproduction Detail

Conc-%	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	35	25	25	28	34	31	24	15	20	25
100		28	26	27	28	26	28	24	16	31	27

CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
7d Proportion Survived	Comparison	14-9937-6925	14-9937-6925	13 Dec-06 2:28 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Fisher Exact	C > T	Untransformed		100	>100	1	N/A	

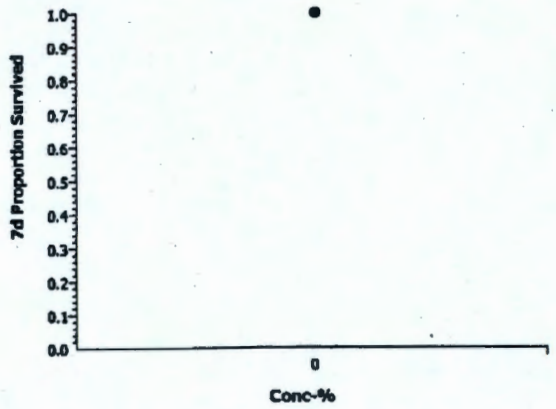
Group Comparisons

Control	vs	Conc-%	Statistic	P-Value	Decision(0.05)
Dilution Water		100	1.00000	1.00000	Non-Significant Effect

Data Summary

Conc-%	Control Type	Non-Responders	Responders	Total Observed
0	Dilution Water	10	0	10
100		10	0	10

Graphics



CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
Reproduction	Comparison	14-9937-6925	14-9937-6925	13 Dec-06 2:28 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Equal Variance t Two-Sample	C > T	Untransformed		100	>100	1	N/A	15.29%

Group Comparisons

Control	vs	Conc-%	Statistic	Critical	P-Value	MSD	Decision(0.05)
Dilution Water		100	0.04328	1.73406	0.4830	4.00673	Non-Significant Effect

ANOVA Table

Source	Sum of Squares	Mean Square	DF	F Statistic	P-Value	Decision(0.05)
Between	0.05	0.05	1	0.00	0.96596	Non-Significant Effect
Error	480.5	26.69444	18			
Total	480.550000	26.744445	19			

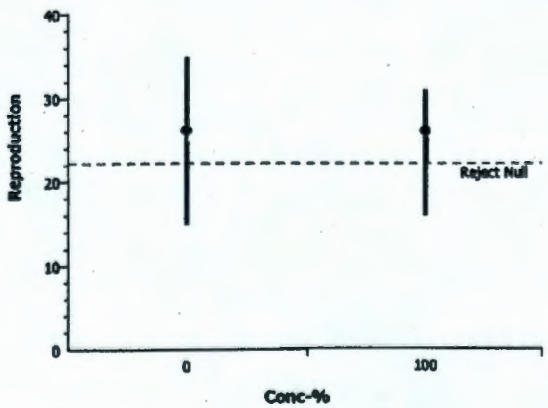
ANOVA Assumptions

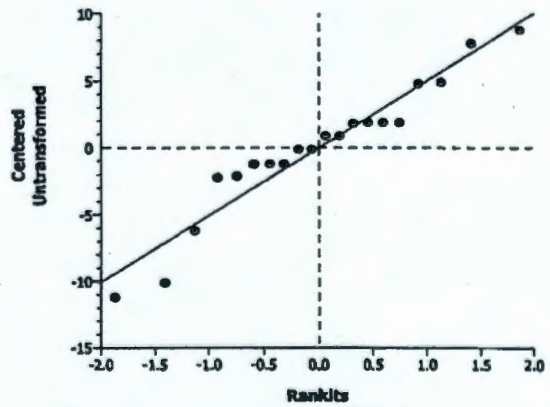
Attribute	Test	Statistic	Critical	P-Value	Decision(0.01)
Variances	Variance Ratio F	2.36249	6.54109	0.21635	Equal Variances
Distribution	Shapiro-Wilk W	0.93500		0.19265	Normal Distribution

Data Summary

			Original Data				Transformed Data			
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
0	Dilution Water	10	26.2	15	35	6.1246				
100		10	26.1	16	31	3.9847				

Graphics





CETIS Test Summary

Report Date: 13 Dec-06 2:29 PM
Test Link: 14-3274-3882/B172202cdc

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Test No:	07-2683-4663	**Test Type:**	Reproduction-Survival (7d)	**Duration:**	6d 20h
Start Date:	30 Nov-06 01:30 PM	**Protocol:**	EPA/821/R-02-013 (2002)	**Species:**	Ceriodaphnia dubia
Ending Date:	07 Dec-06 10:00 AM	**Dil Water:**		**Source:**	In-House Culture
Setup Date:	30 Nov-06 01:30 PM	**Brine:**			

Sample No:	08-2188-8480	**Code:**	B1722-02	**Client:**	
Sample Date:	25 Nov-06	**Material:**	Unknown	**Project:**	
Receive Date:		**Source:**	ELR-Washington Closure Hanford		
Sample Age:	5d 13h	**Station:**			

Comments: J13HH3

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
11-4027-5933	7d Proportion Survived	100	> 100	N/A	N/A	Fisher Exact
13-3335-1116	Reproduction	100	> 100	N/A	16.13%	Equal Variance t Two-Sample

Test Acceptability

Analysis	Endpoint	Attribute	Statistic	TAC Range	Overlap	Decision
11-4027-5933	7d Proportion Survived	Control Response	1	0.8 - NL	Yes	Passes acceptability criteria
13-3335-1116	Reproduction	Control Response	26.2	15 - NL	Yes	Passes acceptability criteria
13-3335-1116	Reproduction	PMSD	0.16129	0.13 - 0.47	Yes	Passes acceptability criteria

7d Proportion Survived Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
100		10	0.90000	0.00000	1.00000	0.10000	0.31623	35.14%

Reproduction Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	26.2	15	35	1.9368	6.1246	23.38%
100		10	26.1	15	30	1.4791	4.6774	17.92%

7d Proportion Survived Detail

Conc-%	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	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
100		1.00000	1.00000	1.00000	1.00000	1.00000	0.00000	1.00000	1.00000	1.00000	1.00000

Reproduction Detail

Conc-%	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	35	25	25	28	34	31	24	15	20	25
100		30	27	30	23	26	15	30	25	25	30

CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
7d Proportion Survived	Comparison	14-3274-3882	14-3274-3882	13 Dec-06 2:29 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Fisher Exact	C > T	Untransformed		100	>100	1	N/A	

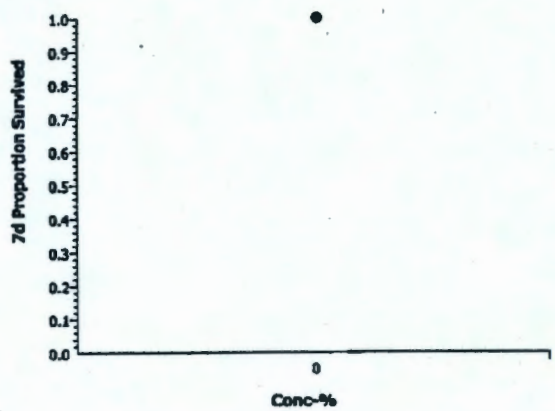
Group Comparisons

Control	vs	Conc-%	Statistic	P-Value	Decision(0.05)
Dilution Water		100	0.50000	0.50000	Non-Significant Effect

Data Summary

Conc-%	Control Type	Non-Responders	Responders	Total Observed
0	Dilution Water	10	0	10
100		9	1	10

Graphics



CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
Reproduction	Comparison	14-3274-3882	14-3274-3882	13 Dec-06 2:29 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Equal Variance t Two-Sample	C > T	Untransformed		100	>100	1	N/A	16.13%

Group Comparisons

Control	vs	Conc-%	Statistic	Critical	P-Value	MSD	Decision(0.05)
Dilution Water		100	0.04103	1.73406	0.4839	4.22588	Non-Significant Effect

ANOVA Table

Source	Sum of Squares	Mean Square	DF	F Statistic	P-Value	Decision(0.05)
Between	0.05	0.05	1	0.00	0.96772	Non-Significant Effect
Error	534.5	29.69444	18			
Total	534.550000	29.744445	19			

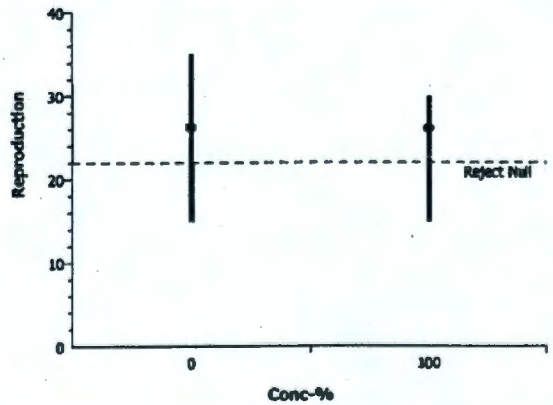
ANOVA Assumptions

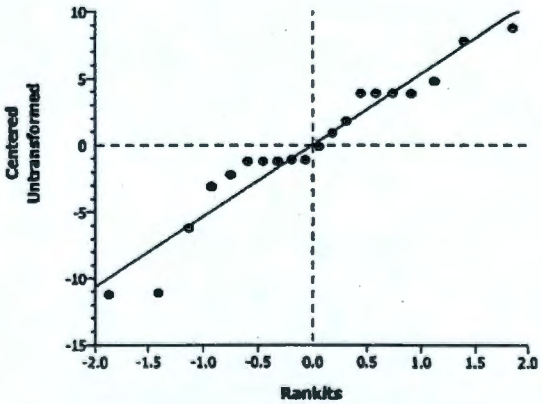
Attribute	Test	Statistic	Critical	P-Value	Decision(0.01)
Variances	Variance Ratio F	1.71458	6.54109	0.43416	Equal Variances
Distribution	Shapiro-Wilk W	0.93529		0.19510	Normal Distribution

Data Summary

			Original Data				Transformed Data			
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
0	Dilution Water	10	26.2	15	35	6.1246				
100		10	26.1	15	30	4.6774				

Graphics





CETIS Test Summary

Report Date: 13 Dec-06 2:31 PM
Test Link: 05-2672-4080/B172203cdc

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M HILL

Test No:	13-9465-4851	**Test Type:**	Reproduction-Survival (7d)	**Duration:**	6d 20h
Start Date:	30 Nov-06 01:30 PM	**Protocol:**	EPA/821/R-02-013 (2002)	**Species:**	Ceriodaphnia dubia
Ending Date:	07 Dec-06 10:00 AM	**Dil Water:**		**Source:**	In-House Culture
Setup Date:	30 Nov-06 01:30 PM	**Brine:**			

Sample No:	02-7210-3178	**Code:**	B1722-03	**Client:**	
Sample Date:	25 Nov-06	**Material:**	Unknown	**Project:**	
Receive Date:		**Source:**	ELR-Washington Closure Hanford		
Sample Age:	5d 13h	**Station:**			

Comments: J13LM9

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
10-7322-9551	7d Proportion Survived	100	> 100	N/A	N/A	Fisher Exact
10-0557-9783	Reproduction	100	> 100	N/A	16.48%	Equal Variance t Two-Sample

Test Acceptability

Analysis	Endpoint	Attribute	Statistic	TAC Range	Overlap	Decision
10-7322-9551	7d Proportion Survived	Control Response	1	0.8 - NL	Yes	Passes acceptability criteria
10-0557-9783	Reproduction	Control Response	26.2	15 - NL	Yes	Passes acceptability criteria
10-0557-9783	Reproduction	PMSD	0.16482	0.13 - 0.47	Yes	Passes acceptability criteria

7d Proportion Survived Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
100		10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%

Reproduction Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	26.2	15	35	1.9368	6.1246	23.38%
100		10	26.5	20	35	1.5652	4.9497	18.68%

7d Proportion Survived Detail

Conc-%	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	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
100		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000

Reproduction Detail

Conc-%	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	35	25	25	28	34	31	24	15	20	25
100		31	24	25	35	27	33	22	25	20	23

CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
7d Proportion Survived	Comparison	05-2672-4080	05-2672-4080	13 Dec-06 2:31 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Fisher Exact	C > T	Untransformed		100	>100	1	N/A	

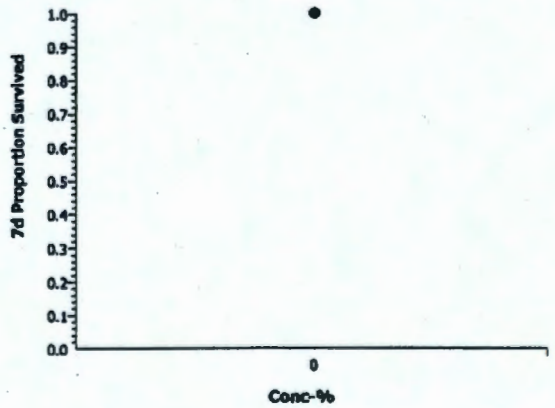
Group Comparisons

Control	vs	Conc-%	Statistic	P-Value	Decision(0.05)
Dilution Water		100	1.00000	1.00000	Non-Significant Effect

Data Summary

Conc-%	Control Type	Non-Responders	Responders	Total Observed
0	Dilution Water	10	0	10
100		10	0	10

Graphics



CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
Reproduction	Comparison	05-2672-4080	05-2672-4080	13 Dec-06 2:31 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Equal Variance t Two-Sample	C > T	Untransformed		100	>100	1	N/A	16.48%

Group Comparisons

Control	vs	Conc-%	Statistic	Critical	P-Value	MSD	Decision(0.05)
Dilution Water		100	-0.1205	1.73406	0.5473	4.31817	Non-Significant Effect

ANOVA Table

Source	Sum of Squares	Mean Square	DF	F Statistic	P-Value	Decision(0.05)
Between	0.45	0.45	1	0.01	0.90544	Non-Significant Effect
Error	558.1	31.00556	18			
Total	558.549976	31.455556	19			

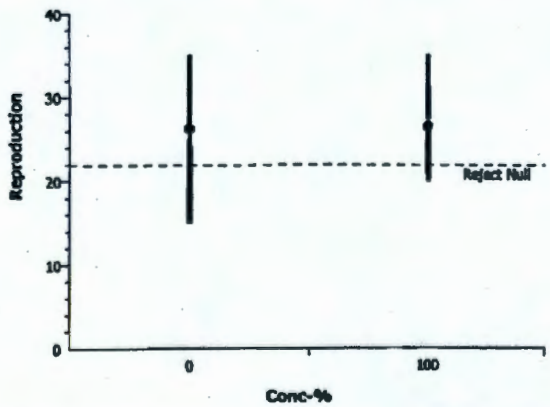
ANOVA Assumptions

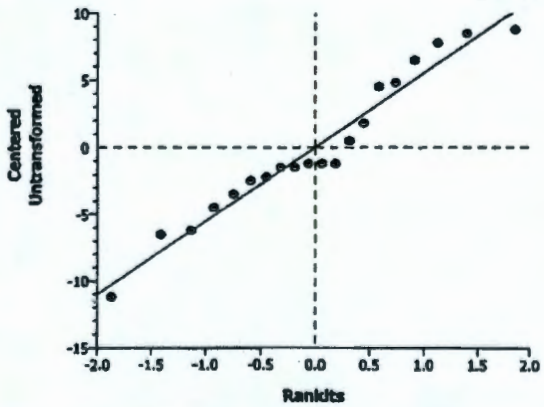
Attribute	Test	Statistic	Critical	P-Value	Decision(0.01)
Variances	Variance Ratio F	1.53107	6.54109	0.53577	Equal Variances
Distribution	Shapiro-Wilk W	0.95282		0.41185	Normal Distribution

Data Summary

			Original Data				Transformed Data			
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
0	Dilution Water	10	26.2	15	35	6.1246				
100		10	26.5	20	35	4.9497				

Graphics





CETIS Test Summary

Report Date: 13 Dec-06 2:32 PM
Test Link: 19-3880-5676/B172204cdc

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Test No:	07-3444-6883	**Test Type:**	Reproduction-Survival (7d)	**Duration:**	6d 20h
Start Date:	30 Nov-06 01:30 PM	**Protocol:**	EPA/821/R-02-013 (2002)	**Species:**	Ceriodaphnia dubia
Ending Date:	07 Dec-06 10:00 AM	**Dil Water:**		**Source:**	In-House Culture
Setup Date:	30 Nov-06 01:30 PM	**Brine:**			

Sample No:	13-8316-7056	**Code:**	B1722-04	**Client:**	
Sample Date:	25 Nov-06	**Material:**	Unknown	**Project:**	
Receive Date:		**Source:**	ELR-Washington Closure Hanford		
Sample Age:	5d 13h	**Station:**			

Comments: J13HH4

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
14-7752-4901	7d Proportion Survived	100	> 100	N/A	N/A	Fisher Exact
05-0731-7197	Reproduction	100	> 100	N/A	14.44%	Equal Variance t Two-Sample

Test Acceptability

Analysis	Endpoint	Attribute	Statistic	TAC Range	Overlap	Decision
14-7752-4901	7d Proportion Survived	Control Response	1	0.8 - NL	Yes	Passes acceptability criteria
05-0731-7197	Reproduction	Control Response	26.2	15 - NL	Yes	Passes acceptability criteria
05-0731-7197	Reproduction	PMSD	0.14442	0.13 - 0.47	Yes	Passes acceptability criteria

7d Proportion Survived Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
100		10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%

Reproduction Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	26.2	15	35	1.9368	6.1246	23.38%
100		10	30.1	25	36	1.005	3.1780	10.56%

7d Proportion Survived Detail

Conc-%	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	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
100		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000

Reproduction Detail

Conc-%	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	35	25	25	28	34	31	24	15	20	25
100		36	30	34	31	28	31	28	25	28	30

CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
7d Proportion Survived	Comparison	19-3880-5676	19-3880-5676	13 Dec-06 2:32 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Fisher Exact	C > T	Untransformed		100	>100	1	N/A	

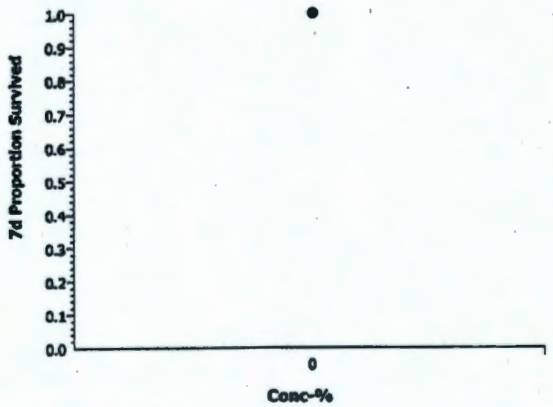
Group Comparisons

Control	vs	Conc-%	Statistic	P-Value	Decision(0.05)
Dilution Water		100	1.00000	1.00000	Non-Significant Effect

Data Summary

Conc-%	Control Type	Non-Responders	Responders	Total Observed
0	Dilution Water	10	0	10
100		10	0	10

Graphics



CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
Reproduction	Comparison	19-3880-5676	19-3880-5676	13 Dec-06 2:32 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Equal Variance t Two-Sample	C > T	Untransformed		100	>100	1	N/A	14.44%

Group Comparisons

Control	vs	Conc-%	Statistic	Critical	P-Value	MSD	Decision(0.05)
Dilution Water		100	-1.7874	1.73406	0.9546	3.78372	Non-Significant Effect

ANOVA Table

Source	Sum of Squares	Mean Square	DF	F Statistic	P-Value	Decision(0.05)
Between	76.05	76.05	1	3.19	0.09073	Non-Significant Effect
Error	428.5	23.80556	18			
Total	504.550003	99.855558	19			

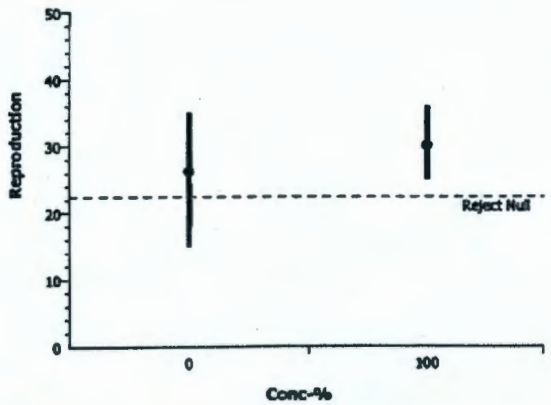
ANOVA Assumptions

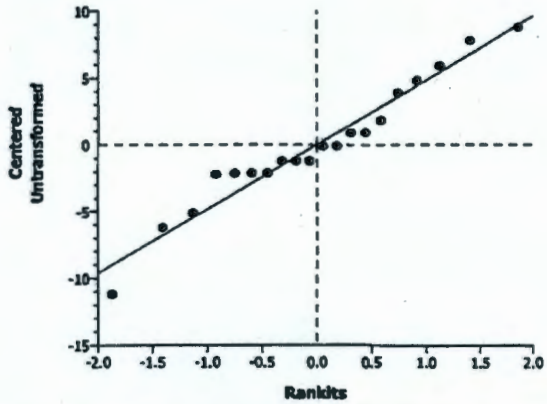
Attribute	Test	Statistic	Critical	P-Value	Decision(0.01)
Variances	Variance Ratio F	3.71397	6.54109	0.06382	Equal Variances
Distribution	Shapiro-Wilk W	0.96086		0.56119	Normal Distribution

Data Summary

			Original Data				Transformed Data			
Conc-%	Control Type	Count	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
0	Dilution Water	10	26.2	15	35	6.1246				
100		10	30.1	25	36	3.1780				

Graphics





APPENDIX B
REFERENCE TOXICANT DATA SHEETS

CH2M HILL

Ceriodaphnia dubia
Survival and Reproduction
Test Data Summary

Client: QA / QC Test Start Date: 12-12-2006

Sample Description: NaCl - 50 g/L Stock Sample ID#: 1B057-02

Ceriodaphnia Lot#: Cd 2011 Statistician: [illegible]

Percent or Concentration	Total Live Young Produced in First 3 Broods per Replicate										# Alive Adults	Total Live Young
	A	B	C	D	E	F	G	H	I	J		
Control	31	31	29	32	27	34	30	35	35	35	10	319
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
0.25 g/L	31	22	33	30	32	30	15	31	31	33	9	288
	AD?	AD?	AD?	AD?	AD?	AD?	AD? X	AD?	AD?	AD?		
0.50 g/L	13	25	29	18	28	25	21	25	23	22	10	229
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?		
1.0 g/L	18	0	17	6	8	16	0	16	7	7	8	95
	AD?	AD?	AD?	AD? X	AD?	AD?	AD? X	AD?	AD?	AD?		
1.5 g/L	2	9	4	3	8	11	3	7	10	0	9	57
	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD?	AD? X		
2.0 g/L	0	0	0	0	0	0	0	0	0	0	5	0
	AD? X	AD?	AD?	AD? X	AD?	AD?	AD? X	AD?	AD? X	AD? X		
4.0 g/L	0	0	0	0	0	0	0	0	0	0	0	0
	AD? ✓	AD? ✓	AD? ✓	AD? ✓	AD? ✓	AD? ✓	AD? ✓	AD? ✓	AD? ✓	AD? ✓		

Test Organism Mortality (Adult dead) = AD? ✓

Test Organism identified as Male = AD? M

Test Organism Injured during test = AD? I

# of Alive Adults = Number of test organism alive at termination (for WDOE only, = Number of test organisms alive at Day 7)

Total Live Young = Total neonates produced in first 3 broods

Footnote: As per EPA-821-R-02-013 (13.10.9.1), *Ceriodaphnia dubia* test should be terminated when 60% of the surviving control organisms have produced their third brood, or at the end of eight days, whichever occurs first.

Also as per EPA-821-R-02-013 (13.10.9.1), "In this three-brood test, offspring from fourth or higher broods should not be counted and should not be included in the total number of neonates produced during the test."

Endpoint	IC25	Cusum Chart Limits
Survival	1.64	1.34 to 1.21
Reproduction	0.46	0.30 to 0.80

Task Manager: [signature]

Project Manager: [signature]

QA Officer: [signature]

CH2M HILL

CERIODAPHNIA 7-DAY SURVIVAL AND REPRODUCTION DATA

Client: QA / QC — Test Initiation: Date: 12-12-06 Time: 0945

Sample Description: NaCl - 50 g/L — Test Termination: Date: 12-18-06 Time: 1030

Initial Sample ID #: 1BC57-02 — Ceriodaphnia Lot#: Cd 2011

Technician: Day 1 DW Day 2 DW Day 3 DW Day 4 MS Day 5 MS Day 6 MS Day 7 ____

Time: Day 1 0930 Day 2 0940 Day 3 0930 Day 4 1000 Day 5 0930 Day 6 1030 Day 7 ____

Concentration (g/L)	Day	Daily Number of Live Young for each Replicate										No. Live Adults	Daily Total Live Young
		A	B	C	D	E	F	G	H	I	J		
Control	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	5	4	5	3	6	5	4	5	5	10	46
	4	0	0	0	10	8	10	8	0	0	0	10	36
	5	11	12	10	0	0	0	0	13	12	13	10	71
	6	16	14	15	17	16	18	17	16	18	17	10	166
	7												
0.25 g/L	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	5	2	5	5	4	5	5	4	5	5	10	45
	4	0	10	11	11	11	10	10	11	0	0	10	74
	5	12	10	0	0	0	0	AD 0	0	10	11	9 ~~10~~ AM	43
	6	14	0	17	14	17	15		16	16	17	9	126
	7												
0.50 g/L	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	3	4	0	4	4	3	4	4	4	10	34
	4	0	0	0	6	10	8	9	11	0	0	10	44
	5	9	8	10	0	0	0	0	0	10	10	10	47
	6	0	14	15	12	14	13	9	16	9	8	10	164
	7												
1.0 g/L	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	2	0	2	0	2	3	0/AD	2	2	0	9	13
	4	0	0	0	6	0	7	|	7	0	0	9	20
	5	7	0	7	0	6	0	|	0	5	7	9	32
	6	9	0 AD	8	0 AD	0	6	|	7	0	0	8	30
	7												
1.5 g/L	1	0	0	0	0	0	0	0	0	0	0	10	0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	2	1	1	0	0	2	0	0	1	0/AD	9	5
	4	0	0	3	0	0	0	3	0	0	|	9	6
	5	0	3	0	3	2	3	0	2	3	|	9	16
	6	0	5	0	0	6	6	0	5	6	|	9	28
	7												
2.0 g/L	1	0	0	0	0	0	0	0	0	0	0	10	DW 0
	2	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	0	0	0	0	0/AD	0	0/AD	0	8	0
	4	0	0	0	0	0	0	|	0	|	0	8	0
	5	0 AD	0	0	0 AD	0	0	|	0	|	0	6	0
	6	|	0	0	|	0	0	|	0	|	AD	5	0
	7	|			|			|		|		
4.0 g/L	1	0/AD	0/AD	0/AD	0/AD	0/AD	0/AD	0/AD	0/AD	0/AD	0/AD	0	0
	2												
	3												

"AD" = Adult Dead, "AY" = Aborted young, "M" = male organism, " / " = split brood (carry-over brood / current day brood)
"Inj" = Adult Injured during test solution renewal, replicate removed from analysis. "AM" = Adult missing.

Footnote: As per EPA, *Ceriodaphnia dubia* test should be terminated when 60% of the surviving control organisms have produced their third brood, or at the end of eight days, whichever occurs first.

CH2M HILL

CERIODAPHNIA WATER QUALITY DATA

Client: QA/QC — Initiated Date: 12-12-06 Time: 0945 — Adults Isolated Date: 12-11-06 Time: 0825

Sample Description: NaCl (50 g/L stock) — Reagent Log #: 1B057-02 — Neo's Collected Date: 12-11-06 Time: 1350

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Technician	DW	DW	DW	DW	MS	MS	MS	
Time	0945	0930	0940	0930	1000	0930	1030	

Percent or Concentration	Dissolved Oxygen (mg/l) Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	8.2	7.9 / 8.1	7.8 / 8.0	8.0 / 7.9	7.5 / 7.9	7.6 / 8.0	7.7 / —	
0.25 g/L	8.1	7.9 / 8.0	7.7 / 8.0	8.0 / 7.9	7.8 / 7.9	7.7 / 8.0	7.8 / —	
0.50 g/L	8.1	8.0 / 8.0	7.7 / 8.0	7.9 / 7.9	7.8 / 7.9	7.7 / 8.0	7.9 / —	
1.0 g/L	8.1	8.0 / 8.0	7.8 / 8.0	7.9 / 7.9	7.8 / 7.9	7.8 / 8.0	7.9 / —	
1.5 g/L	8.1	8.0 / 8.0	7.8 / 8.1	7.9 / 7.9	7.8 / 7.8	7.8 / 7.9	7.9 / —	
2.0 g/L	8.2	8.0 / 8.0	7.9 / 8.1	7.9 / 7.9	7.8 / 7.9	7.8 / 7.9	7.9 / —	
4.0 g/L	8.1	8.0 / —	— / —					

Percent or Concentration	pH Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	7.9	7.9 / 8.0	7.8 / 7.7	8.0 / 8.0	8.0 / 7.9	8.0 / 7.7	8.2 / —	
0.25 g/L	7.8	8.0 / 7.9	7.8 / 7.7	8.0 / 8.0	8.0 / 7.8	7.9 / 7.7	8.3 / —	
0.50 g/L	7.8	8.0 / 7.9	7.8 / 7.8	8.0 / 7.9	7.7 / 7.8	7.9 / 7.7	8.1 / —	
1.0 g/L	7.8	8.0 / 7.9	7.8 / 7.8	8.0 / 7.8	7.9 / 7.8	7.8 / 7.7	8.0 / —	
1.5 g/L	7.8	8.0 / 7.8	7.9 / 7.8	8.0 / 7.8	8.0 / 7.8	7.9 / 7.7	8.0 / —	
2.0 g/L	7.8	8.0 / 7.8	7.9 / 7.8	8.0 / 7.8	8.0 / 7.8	7.9 / 7.7	8.0 / —	
4.0 g/L	7.8	8.1 / —	— / —					

Percent or Concentration	Temperature (C) / Conductivity (µmhos/cm) Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24.5 / 316	25.0 / 308	25.5 / 308	25.2 / 312	25.1 / 333	25.0 / 390	25.1 / —	
0.25 g/L	24.9 / 771	24.6 / 756	25.5 / 756	25.0 / 761	25.0 / 778	25.1 / 750	25.0 / —	
0.50 g/L	25.0 / 1216	24.7 / 1198	25.4 / 1221	25.0 / 1216	24.9 / 1232	24.6 / 1217	25.3 / —	
1.0 g/L	25.0 / 2140	24.7 / 2060	25.6 / 2090	24.6 / 2110	24.7 / 2150	24.9 / 2140	25.3 / —	
1.5 g/L	25.0 / 2550	24.5 / 3010	25.5 / 2960	24.5 / 2980	25.0 / 3000	25.1 / 2970	25.3 / —	
2.0 g/L	24.9 / 3850	24.2 / 3870	25.3 / 3650	24.6 / 3840	25.1 / 3850	25.0 / 3900	—	
4.0 g/L	25.0 / 7130	24.7 / —	— / —					

COMMENTS:

Note: All Day 0 data represents conditions at initiation. All other days: numerator represents pre-renewal conditions, denominator represents post-renewal conditions.

CETIS Test Summary

Report Date: 18 Dec-06 11:10 AM
Test Link: 11-9469-2123/rcdc2011

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Test No:	15-4933-0512	Test Type:	Reproduction-Survival (7d)	Duration:	6d 0h
Start Date:	12 Dec-06 09:45 AM	Protocol:	EPA/821/R-02-013 (2002)	Species:	Ceriodaphnia dubia
Ending Date:	18 Dec-06 10:30 AM	Dil Water:		Source:	In-House Culture
Setup Date:	12 Dec-06 09:45 AM	Brine:			

Sample No:	10-2151-1140	Code:	1B057-02	Client:	
Sample Date:	13 Nov-06	Material:	Sodium chloride	Project:	
Receive Date:		Source:	Reference Toxicant		
Sample Age:	29d 9h	Station:			

Point Estimate Summary

Analysis	Endpoint	% Effect	Conc-gm/L	95% LCL	95% UCL	Method
07-7105-9142	6d Proportion Survived	25	1.642857	0.875	2.125	Linear Interpolation
07-8855-5805	Reproduction	25	0.4565678	0.3294643	0.5621019	Linear Interpolation

Test Acceptability

Analysis	Endpoint	Attribute	Statistic	TAC Range	Overlap	Decision
07-8855-5805	Reproduction	Control Response	31.9	15 - NL	Yes	Passes acceptability criteria

6d Proportion Survived Summary

Conc-gm/L	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
0.25		10	0.90000	0.00000	1.00000	0.10000	0.31623	35.14%
0.5		10	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
1		10	0.80000	0.00000	1.00000	0.13333	0.42164	52.70%
1.5		10	0.90000	0.00000	1.00000	0.10000	0.31623	35.14%
2		10	0.50000	0.00000	1.00000	0.16667	0.52705	105.41
4		10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00%

Reproduction Summary

Conc-gm/L	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	10	31.9	27	35	0.8876	2.8067	8.80%
0.25		10	28.8	15	33	1.8245	5.7697	20.03%
0.5		10	22.9	13	29	1.5015	4.7481	20.73%
1		10	9.5	0	18	2.1615	6.8354	71.95%
1.5		10	5.7	0	11	1.1930	3.7727	66.19%
2		10	0	0	0	0	0	0.00%
4		10	0	0	0	0	0	0.00%

CETIS Test Summary

Report Date: 18-Dec-06 11:10 AM
Test Link: 11-9469-2123/rcdc2011

6d Proportion Survived Detail

Conc-gm/L	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	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
0.25		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	0.00000	1.00000	1.00000	1.00000
0.5		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
1		1.00000	1.00000	1.00000	0.00000	1.00000	1.00000	0.00000	1.00000	1.00000	1.00000
1.5		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	0.00000
2		0.00000	1.00000	1.00000	0.00000	1.00000	1.00000	0.00000	1.00000	0.00000	0.00000
4		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Reproduction Detail

Conc-gm/L	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	31	31	29	32	27	34	30	35	35	35
0.25		31	22	33	30	32	30	15	31	31	33
0.5		13	25	29	18	28	25	21	25	23	22
1		18	0	17	6	8	16	0	16	7	7
1.5		2	9	4	3	8	11	3	7	10	0
2		0	0	0	0	0	0	0	0	0	0
4		0	0	0	0	0	0	0	0	0	0

CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
6d Proportion Survived	Linear Interpolation	11-9469-2123	11-9469-2123	18 Dec-06 11:09 AM	CETISv1.1.2

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2895624	280	Yes	Two-Point Interpolation

Point Estimates

% Effect	Conc-gm/L	95% LCL	95% UCL
25	1.642857	0.875	2.125

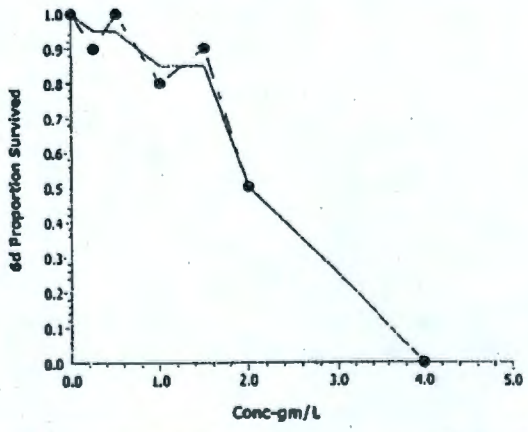
Data Summary

			Calculated Variate(A/B)						
Conc-gm/	Control Type	Count	Mean	Minimum	Maximum	SE	SD	A	B
0	Dilution Water	10	1.00000	1.00000	1.00000	0.00000	0.00000	10	10
0.25		10	0.90000	0.00000	1.00000	0.06455	0.31623	9	10
0.5		10	1.00000	1.00000	1.00000	0.00000	0.00000	10	10
1		10	0.80000	0.00000	1.00000	0.08607	0.42164	8	10
1.5		10	0.90000	0.00000	1.00000	0.06455	0.31623	9	10
2		10	0.50000	0.00000	1.00000	0.10758	0.52705	5	10
4		10	0.00000	0.00000	0.00000	0.00000	0.00000	0	10

Data Detail

Conc-gm/L	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	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
0.25		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	0.00000	1.00000	1.00000	1.00000
0.5		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
1		1.00000	1.00000	1.00000	0.00000	1.00000	1.00000	0.00000	1.00000	1.00000	1.00000
1.5		1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	0.00000
2		0.00000	1.00000	1.00000	0.00000	1.00000	1.00000	0.00000	1.00000	0.00000	0.00000
4		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Graphics



CETIS Analysis Detail

Ceriodaphnia 7-d Survival and Reproduction Test — CH2M Hill

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
Reproduction	Linear Interpolation	11-9469-2123	11-9469-2123	18 Dec-06 11:09 AM	CETISv1.1.2

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2895624	280	Yes	Two-Point Interpolation

Point Estimates

% Effect	Conc-gm/L	95% LCL	95% UCL
25	0.4565678	0.3294643	0.5621019

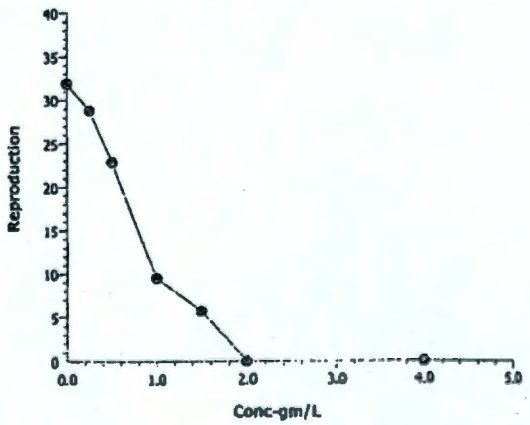
Data Summary

			Calculated Variate				
Conc-gm/	Control Type	Count	Mean	Minimum	Maximum	SE	SD
0	Dilution Water	10	31.9	27	35	0.57292	2.80674
0.25		10	28.8	15	33	1.17773	5.76965
0.5		10	22.9	13	29	0.96920	4.7481
1		10	9.5	0	18	1.39526	6.83537
1.5		10	5.7	0	11	0.77010	3.77271
2		10	0	0	0	0	0
4		10	0	0	0	0	0

Data Detail

Conc-gm/L	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	31	31	29	32	27	34	30	35	35	35
0.25		31	22	33	30	32	30	15	31	31	33
0.5		13	25	29	18	28	25	21	25	23	22
1		18	0	17	6	8	16	0	16	7	7
1.5		2	9	4	3	8	11	3	7	10	0
2		0	0	0	0	0	0	0	0	0	0
4		0	0	0	0	0	0	0	0	0	0

Graphics



APPENDIX C
CHAIN OF CUSTODY

Washington Closure Hanford	CHAIN OF CUSTODY/SAMPLE ANALYSIS REQUEST	RC-048-425	Page 1 of 1
Collector B. TILLER	Company Contact JOAN KESSNER Telephone No. 375-4688	Project Coordinator KESSNER, JH	Price Code 7N Data Turnaround 21 Days
Project Designation 100 Area and 300 Area Component of the RCBRA Water Sa	Sampling Location Cr 2 PORE WATER, 2 nd event	SAF No. RC-048	Air Quality ☐
Ice Chest No. GRP-03-018	Field Logbook No. EL-1597 / 1612 "3T 11-28-06 COA BESRAS6520	Method of Shipment ~~GROUND TRANSPORT~~ DAS 11/27/06 FED EX	
Shipped To CH2MHill - Corvallis	Offsite Property No. A070116	Bill of Lading/Air Bill No. N/A SEE OSPC	

POSSIBLE SAMPLE HAZARDS/REMARKS

POTENTIAL RADIOACTIVE

Special Handling and/or Storage

COOL 4C

Preservation	None	None									
Type of Container	Cubitainer	Cubitainer									
No. of Container(s)	1	0									
Volume	2gal	2gal									
SAMPLE ANALYSIS	Water Toxicity Ceriodaphnia dubia ASTM E1295	Water Frog Embryo Teratogenesis ASTM E1439									
Sample No. / Matrix * / Sample Date / Sample Time											
J13HH2 / WATER / 11-25-06 / 1915	X	X									

CHAIN OF POSSESSION	Sign/Print Names	SPECIAL INSTRUCTIONS
Relinquished By/Removed From: BRETT TILLER — Date/Time 0100 11-26-06	Received By/Stored In: EAS LOCKED STORAGE — Date/Time 0100 11-26-06	14.2°C on arrival B1722-01 COC seals intact.
Relinquished By/Removed From: EAS LOCKED STORAGE — Date/Time 1200 11-27-06	Received By/Stored In: DAVID SIGNATURE WEB — Date/Time 11/27/06 1200	
Relinquished By/Removed From: DAVID SIGNATURE WEB — Date/Time 11/27/06 1300	Received By/Stored In: FED EX — Date/Time	
Relinquished By/Removed From: Fed Ex — Date/Time	Received By/Stored In: CH2M HILL — Date/Time 11/29/06 1035	
Relinquished By/Removed From — Date/Time	Received By/Stored In — Date/Time	
Relinquished By/Removed From — Date/Time	Received By/Stored In — Date/Time	

Matrix *: S=Soil; SE=Sediment; SO=Solid; Sl=Sludge; W=Water; O=Oil; A=Air; DS=Drum Solids; DL=Drum Liquids; T=Tissue; Wi=Wipe; L=Liquid; Ve=Vegetation; X=Other

LABORATORY SECTION	Received By	Title	Date/Time
		Assoc. Sci - Chem	11/29/06 1035
FINAL SAMPLE DISPOSITION	Disposal Method	Disposed By	Date/Time

BHI-EE-011 (08/29/2005)

Washington Closure Hanford	CHAIN OF CUSTODY/SAMPLE ANALYSIS REQUEST	RC-048-426	Page 1 of 1

Collector B. TILLER	Company Contact JOAN KESSNER	Telephone No. 375-4688	Project Coordinator KESSNER, JH	Price Code 7N	Data Turnaround 21 Days
Project Designation 100 Area and 300 Area Component of the RCBRA Water Sa	Sampling Location Cr 4 PORE WATER, 2 nd event		SAF No. RC-048	Air Quality ☐	
Ice Chest No. GRP-03-018	Field Logbook No. EL-1592/1617 BT 11-25-06	COA BESRAS6520	Method of Shipment ~~GROUND TRANSPORT~~ ~~DB 11/27/06~~ FED EX		
Shipped To CH2MHill - Corvallis	Offsite Property No. A070116		Bill of Lading/Air Bill No. N/A SEE DSAC		

POSSIBLE SAMPLE HAZARDS/REMARKS

POTENTIAL RADIOACTIVE

Special Handling and/or Storage

COOL 4C

Preservation	None	None									
Type of Container	Cubitainer	Cubitainer									
No. of Container(s)	1	0									
Volume	2gal	2gal									
SAMPLE ANALYSIS	Water Toxicity Ceriodaphnia dubia ASTM E1295	Water Frog Embryo Teratogenesis ASTM E1439									
Sample No. / Matrix * / Sample Date / Sample Time											
J13HH3 / WATER / 11-25-06 / 2030	X	X									

CHAIN OF POSSESSION	Sign/Print Names	SPECIAL INSTRUCTIONS
Relinquished By/Removed From: BRETT TILLER; Date/Time: 11-26-06 0100	Received By/Stored In: EAS LOCKED STORAGE; Date/Time: 11-26-06 0100	14.2°C on arrival COC seals intact B1722.02
Relinquished By/Removed From: EAS LOCKED STORAGE; Date/Time: 11-27-06 1200	Received By/Stored In: David Stanton WCH; Date/Time: 11/27/06 1200	
Relinquished By/Removed From: David Stanton WCH; Date/Time: 11/27/06 1300	Received By/Stored In: FED EX; Date/Time:	
Relinquished By/Removed From: Fed Ex; Date/Time:	Received By/Stored In: [signature]; Date/Time: 11/29/06 1035	
Relinquished By/Removed From: Date/Time:	Received By/Stored In: Date/Time:	
Relinquished By/Removed From: Date/Time:	Received By/Stored In: Date/Time:	

Matrix *: S=Soil; SE=Sediment; SO=Solid; Sl=Sludge; W = Water; O=Oil; A=Air; DS=Drum Solids; DL=Drum Liquids; T=Tissue; Wi=Wipe; L=Liquid; Ve=Vegetation; X=Other

LABORATORY SECTION	Received By: [signature]	Title: Assoc. Sci - CH2MHill	Date/Time: 11/29/06 1035
FINAL SAMPLE DISPOSITION	Disposal Method	Disposed By	Date/Time

BHI-EE-011 (08/29/2005)

Washington Closure Hanford	CHAIN OF CUSTODY/SAMPLE ANALYSIS REQUEST	RC-048-435	Page 1 of 1
Collector B. TILLER	Company Contact JOAN KESSNER Telephone No. 375-4688	Project Coordinator KESSNER, JH	Price Code 7N Data Turnaround 21 Days
Project Designation 100 Area and 300 Area Component of the RCBRA Water Sa	Sampling Location Sr # 4 pore water, 2nd event	SAF No. RC-048	Air Quality ☐
Ice Chest No. GRP-03-018	Field Logbook No. BT 11-25-06 EL-~~1502~~ 1612 COA BESRAS6520	Method of Shipment ~~GROUND TRANSPORT~~ FED EX DAS 11/27/06	
Shipped To CH2MHill - Corvallis	Offsite Property No. A070116	Bill of Lading/Air Bill No. N/A SEE OSPC	

POSSIBLE SAMPLE HAZARDS/REMARKS

Special Handling and/or Storage
COOL 4C

Preservation	None	None										
Type of Container	P/G	P/G										
No. of Container(s)	1	0										
Volume	2gal	2gal										
SAMPLE ANALYSIS	Water Toxicity Ceriodaphnia dubia ASTM E1295	Water Frog Embryo Teratogenesis ASTM E1439										
Sample No. / Matrix * / Sample Date / Sample Time												
J13LM9 / WATER / 11-25-06 / 2230	X	X										

CHAIN OF POSSESSION	Sign/Print Names	SPECIAL INSTRUCTIONS	Matrix *
Relinquished By/Removed From: BRETT TILLER — Date/Time 0100 11-26-06	Received By/Stored In: EAS LOCKED STORAGE — Date/Time 0100 11-26-06	14.2°C on arrival COC seals intact B1722-03	S=Soil SE=Sediment SO=Solid Sl=Sludge W=Water O=Oil A=Air DS=Drum Solids DL=Drum Liquids T=Tissue Wi=Wipe L=Liquid Ve=Vegetation X=Other
Relinquished By/Removed From: EAS LOCKED STORAGE — Date/Time 1200 11-27-06	Received By/Stored In: David Stephen Wert — Date/Time 11/27/06 1200		
Relinquished By/Removed From: David Stephen Wert — Date/Time 11/27/06 1300	Received By/Stored In: FED EX — Date/Time		
Relinquished By/Removed From: Fed Ex — Date/Time	Received By/Stored In: [signature] — Date/Time 11/29/06 1035		
Relinquished By/Removed From — Date/Time	Received By/Stored In — Date/Time		
Relinquished By/Removed From — Date/Time	Received By/Stored In — Date/Time		

LABORATORY SECTION	Received By: [signature]	Title: ASSOC. SCI - CH2M HILL	Date/Time: 11/29/06 1035
FINAL SAMPLE DISPOSITION	Disposal Method	Disposed By	Date/Time

BHI-EE-011 (08/29/2005)

Washington Closure Hanford	CHAIN OF CUSTODY/SAMPLE ANALYSIS REQUEST	RC-048-427	Page 1 of 1

Collector B. TILLER	Company Contact JOAN KESSNER	Telephone No. 375-4688	Project Coordinator KESSNER, JH	Price Code 7N	Data Turnaround 21 Days
Project Designation 100 Area and 300 Area Component of the RCBRA Water Sa	Sampling Location Cr 6 PORE WATER, 2 nd event		SAF No. RC-048	Air Quality ☐	
Ice Chest No. S4WS-114	Field Logbook No. BT 11/25-06 EL-1591/0/2	COA BESRAS6520	Method of Shipment ~~GROUND TRANSPORT~~ DTS 11/27/06 FED EX		
Shipped To CH2MHill - Corvallis	Offsite Property No. A070116		Bill of Lading/Air Bill No. N/A SEE DSPC		

POSSIBLE SAMPLE HAZARDS/REMARKS

POTENTIAL RADIOACTIVE

Special Handling and/or Storage

COOL 4C

Preservation	None	None									
Type of Container	Cubitainer	Cubitainer									
No. of Container(s)	1	0									
Volume	2gal	2gal									
SAMPLE ANALYSIS	Water Toxicity Ceriodaphnia dubia ASTM E1295	Water Frog Embryo Teratogenesis ASTM E1439									
Sample No. / Matrix * / Sample Date / Sample Time											
J13HH4 / WATER / 11-25-06 / 2120	X	X									

CHAIN OF POSSESSION	Sign/Print Names	SPECIAL INSTRUCTIONS	Matrix *
Relinquished By/Removed From: BRETT TILLER, Date/Time 11-26-06 0100	Received By/Stored In: EAS LOCKED STORAGE, Date/Time 11-26-06 0100	COC seals intact B1722-04 Temp. on Arrival = 14.1°C	S=Soil SE=Sediment SO=Solid SL=Sludge W = Water O=Oil A=Air DS=Drum Solids DL=Drum Liquids T=Tissue WI=Wipe L=Liquid V=Vegetation X=Other
Relinquished By/Removed From: EAS LOCKED STORAGE, Date/Time 11-27-06 1200	Received By/Stored In: David Stockhowell, Date/Time 11/27/06 1200		
Relinquished By/Removed From: David Stockhowell, Date/Time 11/27/06 1300	Received By/Stored In: FED EX, Date/Time		
Relinquished By/Removed From: Fed Ex, Date/Time 11-29-06 1130	Received By/Stored In: Nalisha Johnson, Date/Time 11-29-06 1135		
Relinquished By/Removed From, Date/Time	Received By/Stored In, Date/Time		
Relinquished By/Removed From, Date/Time	Received By/Stored In, Date/Time		

LABORATORY SECTION	Received By: Nalisha Johnson	Title: Staff Scientist	Date/Time: 11-29-06 1135
FINAL SAMPLE DISPOSITION	Disposal Method	Disposed By	Date/Time

BHI-EE-011 (08/29/2005)

From: Origin ID: (509)376-7768
SHIPPING DEPT
FLUOR HANFORD
for the U.S. Dept. of Energy
2355 STEVENS DR BLDG 1162
RICHLAND, WA 99352

FedEx Express

Ship Date: 27NOV06
ActWgt: 79 LB
System#: 5851986/INET2500
Account#: S *********

REF: BESRAS 6520

Delivery Address Bar Code

SHIP TO: (541)752-4271 BILL THIRD PARTY

SAMPLE RECEIVING
CH2M HILL
APPLIED SCIENCES LAB
2300 NW WALNUT BLVD.
CORVALLIS, OR 97339

PRIORITY OVERNIGHT **TUE**

Deliver By: 28NOV06

TRK# 7915 9092 4902 FORM 0201

PDX AA

97339 -OR-US

86 CVOA

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View all labels

Place label in shipping label pouch and affix it to your shipment so that the barcode portion of the label can be read and scanned.

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B1722-01, -02, -03

From: Origin ID: (509)376-7768
SHIPPING DEPT
FLUOR HANFORD
for the U.S. Dept. of Energy
2355 STEVENS DR BLDG 1162
RICHLAND, WA 99352

FedEx Express

Ship Date: 27NOV06
ActWgt: 33 LB
System#: 5851986/INET2500
Account#: S *********

REF: BESRAS 6520

Delivery Address Bar Code

SHIP TO: (541)752-4271 BILL THIRD PARTY

SAMPLE RECEIVING
CH2M HILL
APPLIED SCIENCES LAB
2300 NW WALNUT BLVD.
CORVALLIS, OR 97339

PRIORITY OVERNIGHT **TUE**

Deliver By: 28NOV06

TRK# 7915 9092 4913 FORM 0201

PDX AA

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B1722-04

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AMPHIBIAN BIOASSAY REPORT

FROG EMBRYO TERATOGENESIS ASSAY – *XENOPUS* (FETAX)

Sampling Conducted November 25, 2006

Prepared for

ELR CONSULTING, INC.
WASHINGTON CLOSURE HANFORD
RICHLAND, WASHINGTON

Prepared by

FORT ENVIRONMENTAL LABORATORIES, INC.
515 S. Duncan Street
Stillwater, Oklahoma 74074

December 20, 2006
SDG 00133

BC1722
RC-048

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INTRODUCTION

As a component of an ecological hazard assessment battery, Frog Embryo Teratogenesis Assay – *Xenopus* (FETAX) was chosen as a biomonitoring test to evaluate potentially adverse effects of pore water on the amphibian, *Xenopus laevis*. Fort Environmental Laboratories (FEL) was contracted to conduct FETAX screening on pore water samples from Washington Closure Hanford (WCH), Richland, Washington. The methods used and the results and conclusions derived from the FETAX tests are presented in this report.

METHODS AND MATERIALS

TEST METHODS

The 96-hour whole embryo assay was performed in accordance with ASTM E1439-98 (Reapproved 2004), *Standard Guide for Conducting the Frog Embryo Teratogenesis Assay – Xenopus (FETAX)* (1) with minor modification. The aqueous field samples were tested in replicates of 4, using test vessels containing 25 embryos and 200 mL of each test sample. Embryos were cultured at 23±3°C. All dishes were renewed every 24-hours of the 4-day test by removing dead embryos and spent water, and adding back fresh sample. A summary of test conditions for FETAX is presented in Table 1. At the end of the 4 days of exposure (larvae reaching developmental stage 46 [2]), larvae were preserved in 3% formalin, and the numbers of live, malformed larvae determined using a dissecting microscope. FETAX solution water was used as the laboratory control for the bioassay. FETAX solution is purified water of uniform quality, which has been ionically balanced to provide satisfactory survival without affecting test results (1). Two concentrations (2,500 mg/L and 5.5 mg/L) of the chemical 6-aminonicotinamide (6-AN) were used as positive controls for embryo-lethality and malformation endpoints, respectively. The FETAX endpoints included: mortality, malformation, and growth.

TEST ORGANISMS

The *Xenopus laevis* (South African clawed frog) larvae used for the FETAX screens were obtained from in-house cultures originally purchased from *Xenopus* I (Dexter, MI.). Adult care and breeding were conducted in accordance with modified standard guidelines (1). To induce breeding, adult female and male breeding stock were injected with human chorionic gonadotropin (hCG). Essentially, 4 to 6 h prior to breeding, female and male specimens were injected with approximately 800 and 600 IU, respectively, of hCG dissolved in 0.9% sterile saline solution. Fertilized egg collection was performed as described in ASTM E1439-98 (1) and the *Atlas of Abnormalities: A Guide for the Performance of FETAX* (3).

SAMPLE COLLECTION

Four pore water samples were collected by WCH personnel on November 25, 2006, shipped via commercial carrier by CH2M Hill, and received at FEL on November 30, 2006. A copy of the sample chain of custody is provided in Appendix A. Samples were recorded in the laboratory check-in logbook and laboratory tracking numbers 00133-001 through 00133-004 were assigned.

Samples measured 4°C upon receipt and were stored at 4°C ± 1° throughout the holding period. The chronological timelines for sample collection, receipt, and testing are presented in Table 2.

SAMPLE PREPARATION

FETAX screens were conducted using 100% sample volumes without dilution. Sample temperatures were adjusted to 23°C ± 3°C prior to test setup and daily renewal.

SAMPLE MONITORING

Temperature, pH, and dissolved oxygen (DO) were measured daily on each sample throughout the FETAX screens. General water chemistry (hardness, conductivity, alkalinity, ammonia-nitrogen, and residual oxidants) was also performed on each sample (Appendix B).

DATA ANALYSIS

Mortality and malformation frequencies were determined for each sample tested. Head-to-tail lengths of the surviving larvae were measured as an index of growth (sample growth over control growth, expressed as a percentage) using a personal computer, digital camera, and SigmaScanPro® 5.0 image analysis software (SPSS®, Inc., Chicago, IL). Kruskal-Wallis (K-W) ANOVA or ANOVA with multiple comparison tests (Dunn's Method, Dunnett's Method, or Bonferroni t-test [$P < 0.05$]), as appropriate, were performed on the FETAX screens to determine if statistically significant differences existed between the FETAX solution control and each of the pore water samples at 100% concentrations. All statistical calculations were performed using SigmaStat® 2.03 statistical software (SPSS® Inc., Chicago, IL). The raw data (including organism scoring and malformation, growth measurements and statistics, and mortality and malformation statistics) from the FETAX screens are presented in Appendix C.

QUALITY ASSURANCE / QUALITY CONTROL

All data, documents, and final report generated for the WCH Project have been reviewed and meet the criteria set forth in the FEL Quality Management Program (QMP). Specifically, the goals of FEL are to ensure the accuracy and precision of all laboratory operations, including data generation, collection, and presentation.

RESULTS AND DISCUSSION

LABORATORY CONTROLS AND REFERENCE SAMPLES

FETAX screens were performed on pore water samples J13HH2, J13HH3, J13LM9, and J13HH4 over a 4-day period. The results of the FETAX screens conducted on the laboratory control (FETAX solution water) and reference samples are presented in Table 3. The laboratory control induced 2.0% mortality and 2.0% malformation. The results were within the acceptable limits (≤10.0%) for mortality and malformation, as outlined in ASTM E1439-98 (1). The 6-AN

positive reference samples produced acceptable results with 100.0% mortality induced by the 2,500 mg/L concentration and 52.0% malformation induced by the 5.5 mg/L concentration.

PORE WATER SAMPLES

The results of the FETAX screens conducted on the 4 pore water samples are presented in Table 4. The frequencies of mortality ranged from 0.0% to 1.0%, and were not significantly different than the laboratory control (KW-ANOVA, P = 0.202). The frequencies of malformation for the samples tested ranged from 5.0% to 7.1%, and were not significantly different from the laboratory control (ANOVA, P = 0.377). The percent growth (mean sample length [cm] divided by mean laboratory control length) of the test organisms exposed to each of the pore water samples were 101.8% (J13HH2), 99.7% (J13HH3), 98.6% (J13LM9), and 96.9% (J13HH4), which were not significantly different than the laboratory control (K-W ANOVA and Dunn's Method, P ≥ 0.050).

Based on the results of this study, no developmental toxicity was induced by the samples evaluated. If you have any questions concerning this report, please do not hesitate to contact me at 405-624-6771 or djfort@fortlabs.com, or Robert Rogers, Laboratory Director, at rrogers@frotlabs.com.

I certify that this data package is in compliance with the Statement of Work (SOW), both technically and for completeness, for other than the conditions detailed above. The Laboratory Director or designee, as verified by the following signature, has authorized release of the data contained in this data package.

Douglas J. Fort, Ph.D.
President

REFERENCES

1. Standard Guide for Conducting the Frog Embryo Teratogenesis Assay - *Xenopus* (FETAX). American Society for Testing and Materials (ASTM), E 1439-98, 1998 (Reapproved 2004).

2. Nieuwkoop, P.D. and Faber, J. Normal Tables of *Xenopus laevis* (Daudin). Garland Publishing, London, 1994.

3. Bantle, J.A., Dumont, J.N., Finch, R.A., and Linder, G., Fort, D.J., *Atlas of abnormalities: a guide for the performance of FETAX*, 2nd Ed., Oklahoma State Publications Department, 1998.

Table 1
Summary of Test Conditions for WCH FETAX Screens

Test Parameter	Test Condition
Test type:	Static renewal
Renewal frequency	Daily
Temperature:	23°C ± 3°C
Light quality:	Ambient laboratory
Light intensity:	50 to 100 foot-candles (ambient levels)
Photoperiod:	12 h light, 12 h. dark
Test chamber size:	500 mL chamber
Test water volume:	200 mL
Larval mortality counts:	Daily
Overlying water type:	Pore water
Age of organisms:	Small cell blastula (Stage 8-10)
Number of organisms per vessel:	25
Number of replicate test vessels / concentration:	4
Feeding:	None
Laboratory control water:	FETAX solution (reconstituted laboratory water)
Sample test concentrations:	100%
Dilution factor:	None
Test duration:	96 h
Overlying water quality:	Daily temperature, pH, and dissolved oxygen
Effects measured (endpoints):	Mortality, malformation, growth (length [cm])
Test validation:	90% survival and ≤ 10% malformation in control

Table 2
Chronological Timelines for WCH Sample Collection, Receipt, and Testing

WCH Field Sample No.	FEL Lab No.	Sample Date	FEL Receipt Date	Test Initiation Date	Test Termination Date
J13HH2	00133-001	11/25/06	11/30/06	12/5/06	12/9/06
J13HH3	00133-002	11/25/06	11/30/06	12/5/06	12/9/06
J13LM9	00133-003	11/25/06	11/30/06	12/5/06	12/9/06
J13HH4	00133-004	11/25/06	11/30/06	12/5/06	12/9/06

Table 3
Results of WCH FETAX Screens on
Laboratory Control and Reference Samples

Sample ID	Test Initiation Date	Mean Mortality (%)	Mean Malformations (%)
FETAX Solution Control	12/5/06	2.0	2.0
6-AN Reference (2,500 mg/L)	12/5/06	100.0	-
6-AN Reference (5.5 mg/L)	12/5/06	0.0	52.0

Table 4
Results of FETAX Screens on WCH Pore Water Samples

WCH Field Sample No.	Mean Mortality (%)	Mean Malformation (%)	Mean Growth[1] (%)	Mortality Significantly Different From Control[2]	Malformation Significantly Different From Control[3]	Growth Significantly Different From Control[4]
J13HH2	0.0	6.0	101.8	No	No	No
J13HH3	0.0	7.0	99.7	No	No	No
J13LM9	1.0	7.1	98.6	No	No	No
J13HH4	0.0	5.0	96.9	No	No	No

[1] Mean sample length (cm) divided by mean laboratory control length (cm), expressed as % growth.
[2] Results based on statistical analysis using K-W ANOVA (P = 0.202).
[3] Results based on statistical analysis using ANOVA (P = 0.377).
[4] Results based on statistical analysis using K-W ANOVA and Dunn's Method (P = 0.05).

Appendix A

Chain of Custody Form

CH2MHILL CHAIN OF CUSTODY RECORD FOR ALL BIO COMM. ANALYSES

Client: ELR - Washington Closure Hanford
NPDES#:
Address:
Composite Sample Information:
Samples/Hour: Volume/Sample:
Total Hours: Total Volume:
Initiated: Date: Time:
Ended: Date: Time:
Chilled During Collection: Yes ☐ No ☐
Contact Person: Rich Weiss - CH2m Hill
Phone: (509) 372-9631
CH2M HILL Project #:

Ship Samples to: CH2M HILL, Inc.
Attention: Aquatic Toxicology Laboratory
2300 NW Walnut Blvd.
Corvallis, OR 97330
Phone: (541) 752-4271 Ext. 3160
Message: (541) 758-0235 Ext. 3160
P. O.#
Check Chlorine (Y/N):
Temp. Upon Arrival (°C): 4°C @ FEL
Check Ammonia (Y/N): Dechlorinate (Y/N):

Analysis Required/Comments

Sample ID	Date	Time	Sample Type: Comp.	Sample Type: Grab	# of Containers	Lab ID#	Fathead Acute	Fathead Chronic	Ceriodaphnia Acute	Ceriodaphnia Chronic	Daphnia Acute	Trout Acute	SHM Acute	SHM Chronic	MB Acute	MB Chronic	MYS Acute	MYS Chronic	Algae	Haz Waste	FETAX			Concentrations and/or Comments
J13 HH2	11/25/06	1915				001															X			CH2MØ5-00133
J13 HH3		2030				002															X			
J13 LM9		2230				003															X			
J13 HH4	11/25/06	2120				004															X			

Sampled By & Title (Please sign and print name): Brett Mukey	Date/Time: 11-29-06 11:00	Relinquished By (Please sign and print name): Brett Mukey - CH2m Hill	Date/Time: 11-29-06 11:30
Received By (Please sign and print name): Robert Rogers ROBERT ROGERS	Date/Time: 11/30/06 - 1300	Relinquished By (Please sign and print name):	Date/Time:
Received By (Please sign and print name):	Date/Time:	Relinquished By (Please sign and print name):	Date/Time:
Received By (Please sign and print name):	Date/Time:	Shipped Via: UPS ___ Bus ___ Fed-Ex ___ Hand ___ Other ___	Shipping #:
Work Authorized By (Please sign and print name):	Remarks: Samples subdivided by CH2m on 11-29-06. Original sample by Brett Tiller on 11-25-06		

Rev. 10/97

Appendix B

General Water Chemistry

CH2M05-00133
Washington Closure Hanford
FORT ENVIRONMENTAL LABORATORIES

GENERAL WATER CHEMISTRY

Analysis Date	Tech Initials	FEL W.O.	Sample ID	Temp (C)	pH (su)	DO (mg/L)	Conduct. (µS/cm)	Hardness (mg/L)	Alkalinity (mg/L)	NH_3-N (mg/L)	Residual Oxidants (mg/L)
12/01/06	MLB/BB	-	FETAX Control	-	-	-	1707	114	50	<0.06	<0.01
		001	J13HH2 Pore Water	-	-	-	264	140	74	<0.06	0.02
		002	J13HH3 Pore Water	-	-	-	165	72	57	0.12	<0.01
		003	J13LM9 Pore Water	-	-	-	176	78	56	<0.06	0.01
		004	J13HH4 Pore Water	-	-	-	224	113	52	<0.06	0.01
12/05/06	MLB/BB	-	FETAX Control	23.0	8.0	8.9					
		001	J13HH2 Pore Water	23.0	7.9	9.9					
		002	J13HH3 Pore Water	23.0	7.6	9.9					
		003	J13LM9 Pore Water	23.0	7.5	9.8					
		004	J13HH4 Pore Water	23.0	7.6	9.6					
12/06/06	GP/MB	-	FETAX Control	23.5	7.9	8.2					
		001	J13HH2 Pore Water	23.5	8.0	7.9					
		002	J13HH3 Pore Water	23.5	7.6	7.7					
		003	J13LM9 Pore Water	23.5	7.5	7.5					
		004	J13HH4 Pore Water	23.5	7.6	7.4					
12/07/06	MLB	-	FETAX Control	23.9	7.9	8.2					
		001	J13HH2 Pore Water	25.3	7.9	8.1					
		002	J13HH3 Pore Water	25.3	7.7	7.9					
		003	J13LM9 Pore Water	25.5	7.6	7.6					
		004	J13HH4 Pore Water	25.3	7.7	7.7					
12/08/06	BB	-	FETAX Control	23.0	7.9	8.1					
		001	J13HH2 Pore Water	22.5	8.0	7.9					
		002	J13HH3 Pore Water	23.0	7.7	7.6					
		003	J13LM9 Pore Water	23.0	7.6	7.8					
		004	J13HH4 Pore Water	22.5	7.6	7.5					

Appendix C

Raw and Statistical Data

FETAX SCORE SHEET / MALFORMATION DATA
FORT ENVIRONMENTAL LABORATORIES

Investigator:	DF	Client/Project-WO No.:	CH2M05-00133	Test Start Date:	12/05/06
Test Material:	Controls	FEL Sample No.:	001-004	Test End Date:	12/09/06
Test Type:	FETAX Screen with X. laevis			Test Score Date:	12/11/06

Malformation:	Sample ID/Concentration *(unit)*															
	FETAX Ctl												5.5mg/L 6AN		2500mg/L 6AN	
Stunted																
Edema				1												
Blister																
Head/Facial			1	1									15	11		
Jaw																
Mouth			1	1									15	11		
Eye													5	7		
Brain																
Cardiac																
Gut			1	1									15	11		
Hemorrhage																
Tail/Myotome																
Notochord																
Fin																
Other:																
Other:																
Other:																
Other:																
Other:																
Total Malformed	0	0	1	1									15	11	-	-
Total Survived	25	25	24	24									25	25	0	0
Total Tested	25	25	25	25									25	25	25	25
% Mortality	0.0	0.0	4.0	4.0									0.0	0.0	100.0	100.0
% Malformed	0.0	0.0	4.2	4.2									60.0	44.0	-	-
Avg % Mortality	2.0												0.0		100.0	
SEM (Mortality)	1.2												0.00		0.00	
Avg % Malformed	2.0												52.0		-	
SEM (Malformed)	1.2												8.00		-	

MALFORMATION DATA

CH2M05-00133
FETAX Screen with X. laevis

SAMPLE ID	REPLICATE NUMBER	NUMBER MALFORMED	TYPE OF MALFORMATION/DELTS OBSERVED																		
			Stunted	Edema	Blister	Head/ Facial	Jaw	Mouth	Eye	Brain	Cardiac	Gut	Hemor-rhage	Tail/ Myotome	Noto-chord	Fin	Other (specify)	Other (specify)	Other (specify)	Other (specify)	Other (specify)
FETAX Ctl	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
	4	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Total Survived	96																				
Total Malformed		2	0	1	0	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0
% Malformed			0.00	1.02	0.00	2.04	0.00	2.04	0.00	0.00	0.00	2.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00	0.25	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM			0.00	0.25	0.00	0.29	0.00	0.29	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)			na	49.00	na	28.29	na	28.29	na	na	na	28.29	na	na	na	na	na	na	na	na	na
5.5mg/L 6AN	1	15	0	0	0	15	0	15	5	0	0	15	0	0	0	0	0	0	0	0	0
	2	11	0	0	0	11	0	11	7	0	0	11	0	0	0	0	0	0	0	0	0
Total Survived	50																				
Total Malformed		26	0	0	0	26	0	26	12	0	0	26	0	0	0	0	0	0	0	0	0
% Malformed			0.00	0.00	0.00	52.00	0.00	52.00	24.00	0.00	0.00	52.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00	0.00	0.00	8.00	0.00	8.00	2.00	0.00	0.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM			0.00	0.00	0.00	2.00	0.00	2.00	1.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)			na	na	na	5.44	na	5.44	5.89	na	na	5.44	na	na	na	na	na	na	na	na	na
2500mg/L 6AN	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total Survived	0																				
Total Malformed		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
% Malformed			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Var (S2)			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SEM			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CV (%)			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

SCORE SHEET / MALFORMATION DATA
FORT ENVIRONMENTAL LABORATORIES

Investigator:	DF	Client/Project-WO No.:	CH2M05-00133	Test Start Date:	12/05/06
Test Material:	Pore Water	FEL Sample No.:	001-004	Test End Date:	12/09/06
Test Type:	FETAX Screen with X. laevis			Test Score Date:	12/11/06

Malformation:	Sample No./Sample ID/Concentration *(unit)*															
	J13HH2				J13HH3				J13LM9				J13HH4			
Stunted																
Edema			1													
Blister																
Head/Facial		1	3	2	2	2	2	1	2	3	2		1	1	2	1
Jaw																
Mouth		1	3	2	2	2	2	1	1	3	2		1	1		1
Eye		1	1	2				1	1	2	1		1	1	2	1
Brain					1	1		1	1	1	1			1	1	1
Cardiac		1	3	2												
Gut		1	2	1	2	2	2	1	2	3	1		1	1	2	1
Hemorrhage		1				1	1		1				1	1		
Tail/Myotome					1			1		1	1				1	1
Notochord																
Fin																
Other:																
Other:																
Other:																
Other:																
Other:																

Total Malformed	0	1	3	2	2	2	2	1	2	3	2	0	1	1	2	1
Total Survived	25	25	25	25	25	25	25	25	25	25	25	24	25	25	25	25
Total Tested	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
% Mortality	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0
% Malformed	0.0	4.0	12.0	8.0	8.0	8.0	8.0	4.0	8.0	12.0	8.0	0.0	4.0	4.0	8.0	4.0
Avg % Mortality	0.0				0.0				1.0				0.0			
SEM (Mortality)	0.0				0.0				1.0				0.0			
Avg % Malformed	6.0				7.0				7.1				5.0			
SEM (Malformed)	2.6				1.0				2.5				1.0			

MALFORMATION DATA

CH2M05-00133
FETAX Screen with X. laevis

SAMPLE ID	REPLICATE NUMBER	NUMBER MALFORMED	TYPE OF MALFORMATION/DELTS OBSERVED																		
			Stunted	Edema	Blister	Head/ Facial	Jaw	Mouth	Eye	Brain	Cardiac	Gut	Hemor-rhage	Tail/ Myotome	Noto-chord	Fin	Other (specify)	Other (specify)	Other (specify)	Other (specify)	Other (specify)
J13HH2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	1	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0
	3	3	0	1	0	3	0	3	1	0	3	2	0	0	0	0	0	0	0	0	0
	4	2	0	0	0	2	0	2	2	0	2	1	0	0	0	0	0	0	0	0	0
Total Survived	100																				
Total Malformed		6	0	1	0	6	0	6	4	0	6	4	1	0	0	0	0	0	0	0	0
% Malformed			0.00	1.00	0.00	6.00	0.00	6.00	4.00	0.00	6.00	4.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00	0.25	0.00	1.67	0.00	1.67	0.67	0.00	1.67	0.67	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM			0.00	0.25	0.00	0.65	0.00	0.65	0.41	0.00	0.65	0.41	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)			na	50.00	na	21.52	na	21.52	20.41	na	21.52	20.41	50.00	na	na	na	na	na	na	na	na
J13HH3	1	2	0	0	0	2	0	2	0	1	0	2	0	1	0	0	0	0	0	0	0
	2	2	0	0	0	2	0	2	0	1	0	2	1	0	0	0	0	0	0	0	0
	3	2	0	0	0	2	0	2	0	0	0	2	1	0	0	0	0	0	0	0	0
	4	1	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0	0	0
Total Survived	100																				
Total Malformed		7	0	0	0	7	0	7	1	3	0	7	2	2	0	0	0	0	0	0	0
% Malformed			0.00	0.00	0.00	7.00	0.00	7.00	1.00	3.00	0.00	7.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00	0.00	0.00	0.25	0.00	0.25	0.25	0.25	0.00	0.25	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM			0.00	0.00	0.00	0.25	0.00	0.25	0.25	0.25	0.00	0.25	0.29	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)			na	na	na	7.14	na	7.14	50.00	16.67	na	7.14	28.87	28.87	na	na	na	na	na	na	na
J13LM9	1	2	0	0	0	2	0	1	1	1	0	2	1	0	0	0	0	0	0	0	0
	2	3	0	0	0	3	0	3	2	1	0	3	0	1	0	0	0	0	0	0	0
	3	2	0	0	0	2	0	2	1	1	0	1	0	1	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Survived	99																				
Total Malformed		7	0	0	0	7	0	6	4	3	0	6	1	2	0	0	0	0	0	0	0
% Malformed			0.00	0.00	0.00	7.07	0.00	6.06	4.04	3.03	0.00	6.06	1.01	2.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00	0.00	0.00	1.58	0.00	1.67	0.67	0.25	0.00	1.67	0.25	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM			0.00	0.00	0.00	0.63	0.00	0.65	0.41	0.25	0.00	0.65	0.25	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)			na	na	na	17.80	na	21.30	20.21	16.50	na	21.30	49.50	28.58	na	na	na	na	na	na	na
J13HH4	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0
	2	1	0	0	0	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0
	3	2	0	0	0	2	0	0	2	1	0	2	0	1	0	0	0	0	0	0	0
	4	1	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0	0	0
Total Survived	100																				
Total Malformed		5	0	0	0	5	0	3	5	3	0	5	2	2	0	0	0	0	0	0	0
% Malformed			0.00	0.00	0.00	5.00	0.00	3.00	5.00	3.00	0.00	5.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Var (S2)			0.00	0.00	0.00	0.25	0.00	0.25	0.25	0.25	0.00	0.25	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM			0.00	0.00	0.00	0.25	0.00	0.25	0.25	0.25	0.00	0.25	0.29	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)			na	na	na	10.00	na	16.67	10.00	16.67	na	10.00	28.87	28.87	na	na	na	na	na	na	na

FIGURE 1
CH2M05-00133 (001-004)
FETAX Screen with *X. laevis*
FETAX Solution Control

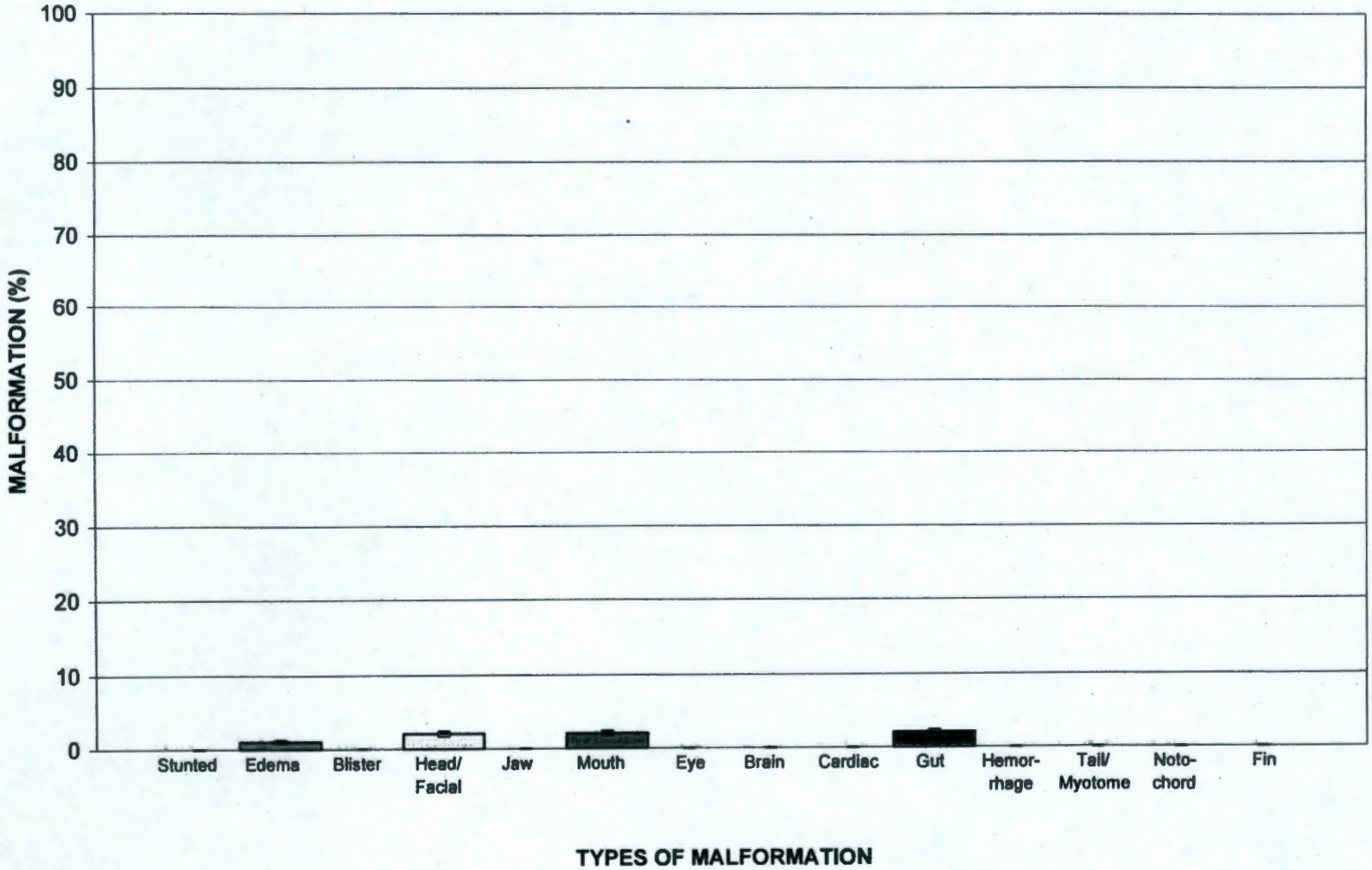


FIGURE 2
CH2M05-00133 (001)
FETAX Screen with *X. laevis*
J13HH2

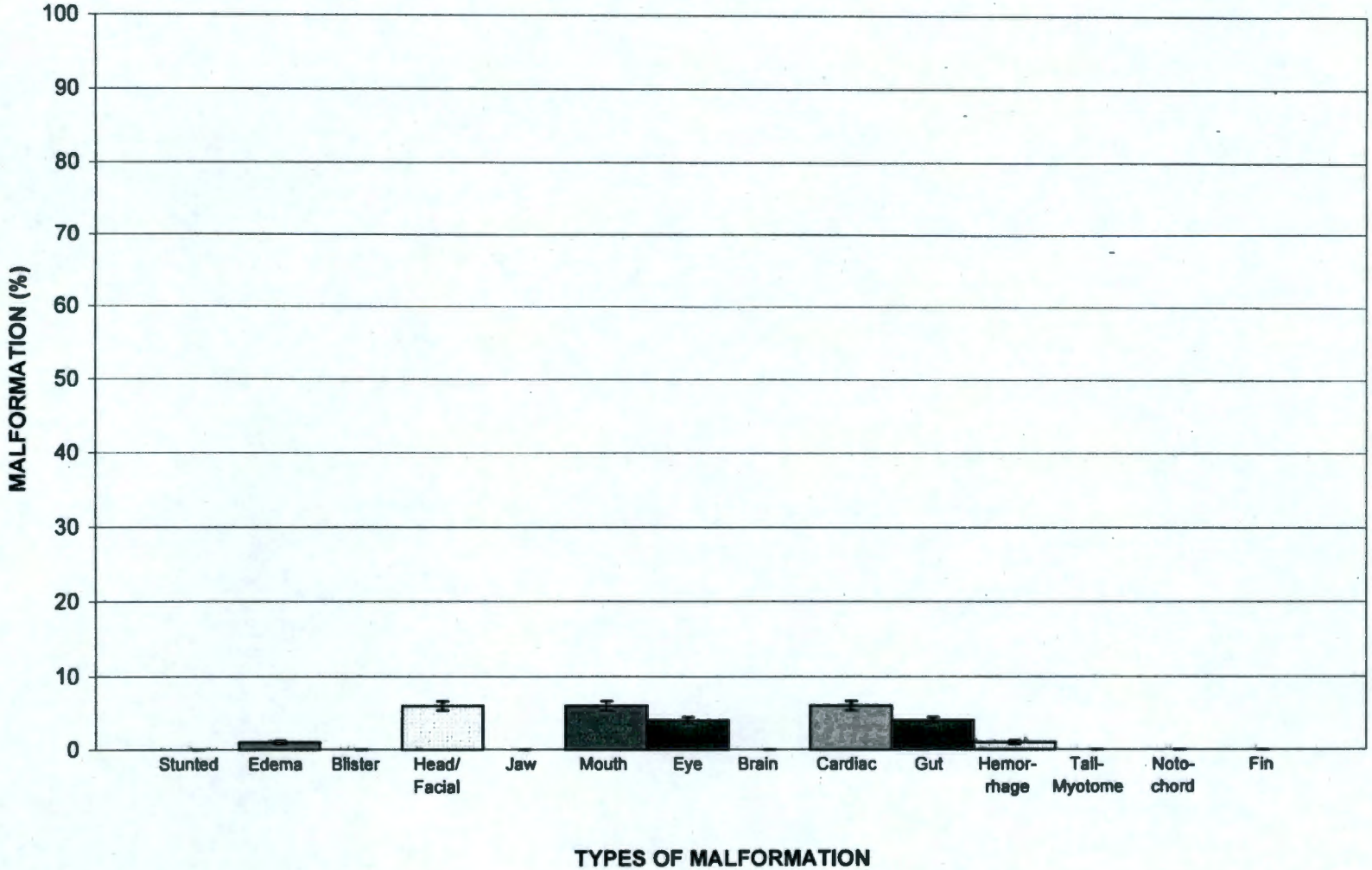


FIGURE 3
CH2M05-00133 (002)
FETAX Screen with *X. laevis*
J13HH3

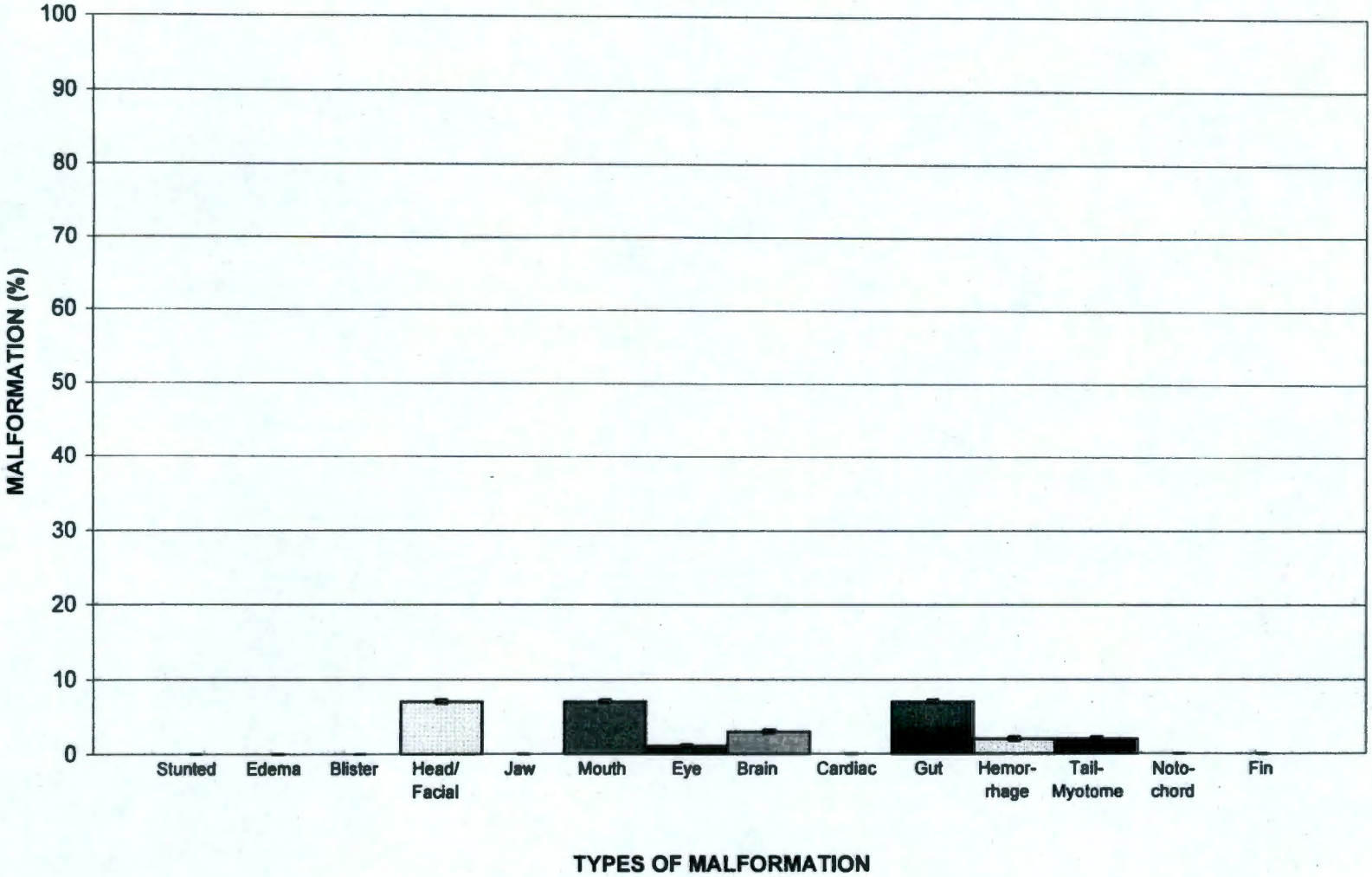


FIGURE 4
CH2M05-00133 (003)
FETAX Screen with *X. laevis*
J13LM9

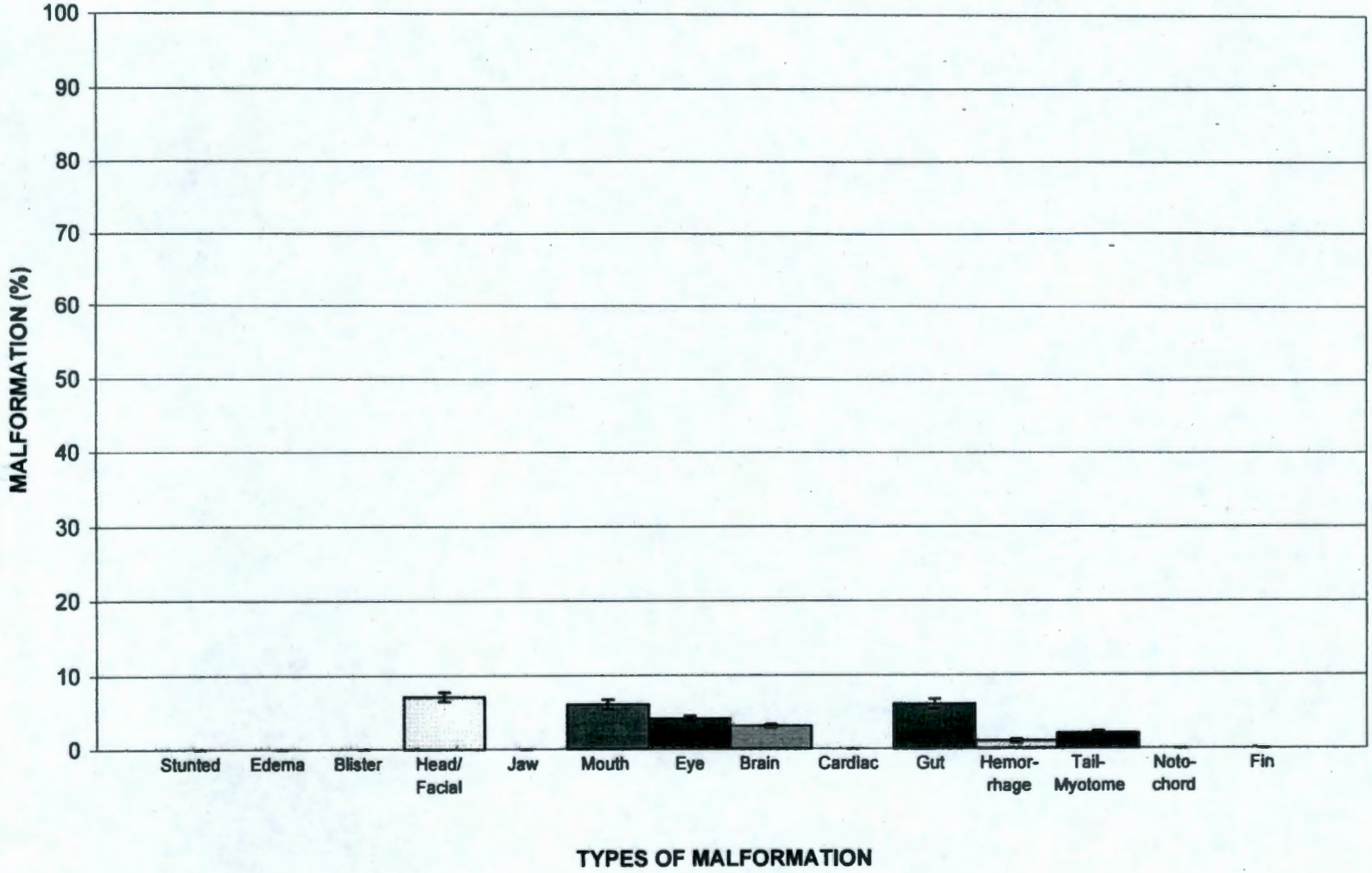


FIGURE 5
CH2M05-00133 (004)
FETAX Screen with *X. laevis*
J13HH4

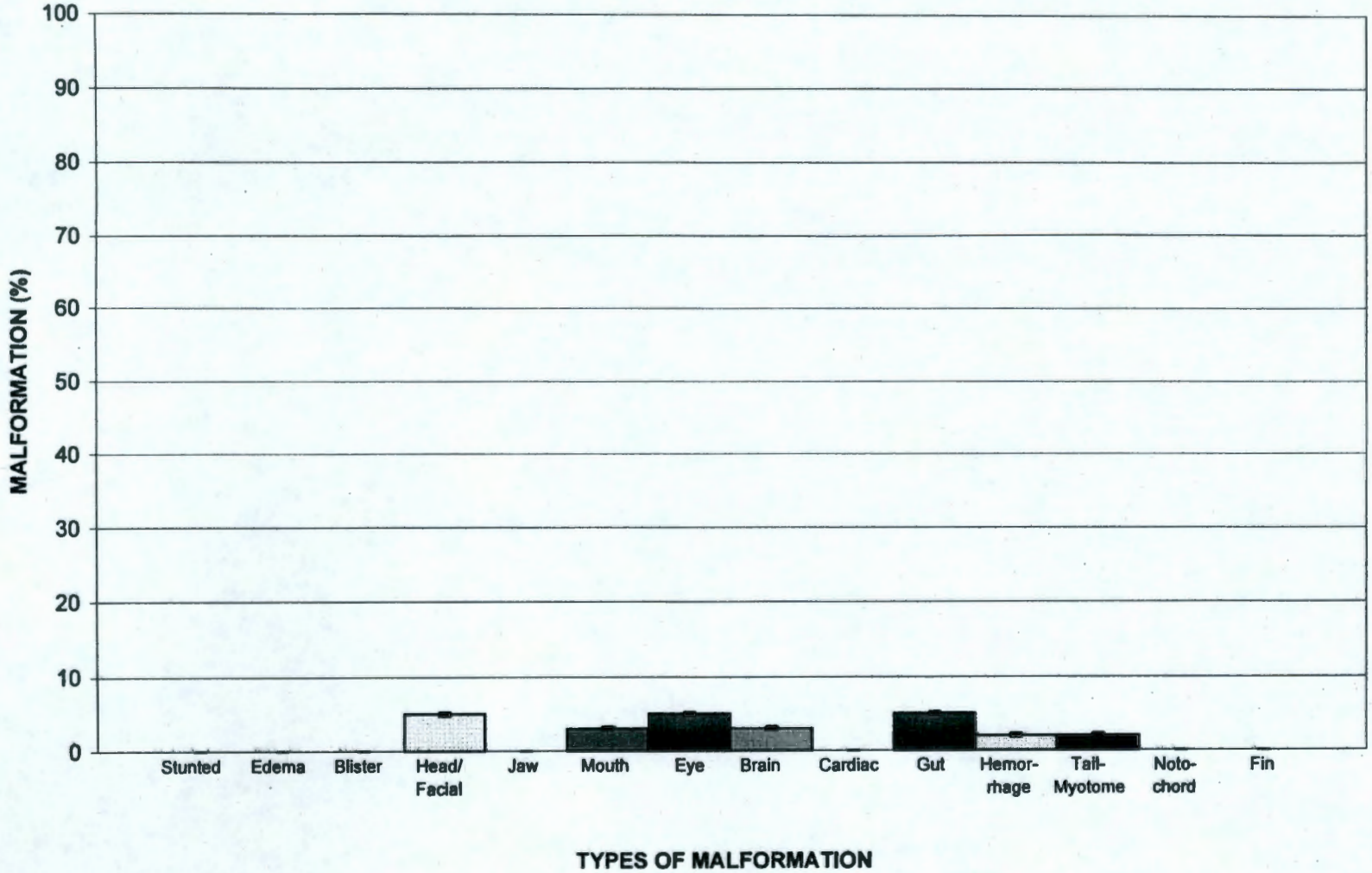


FIGURE 6
CH2M05-00133
FETAX Screen with *X. laevis*

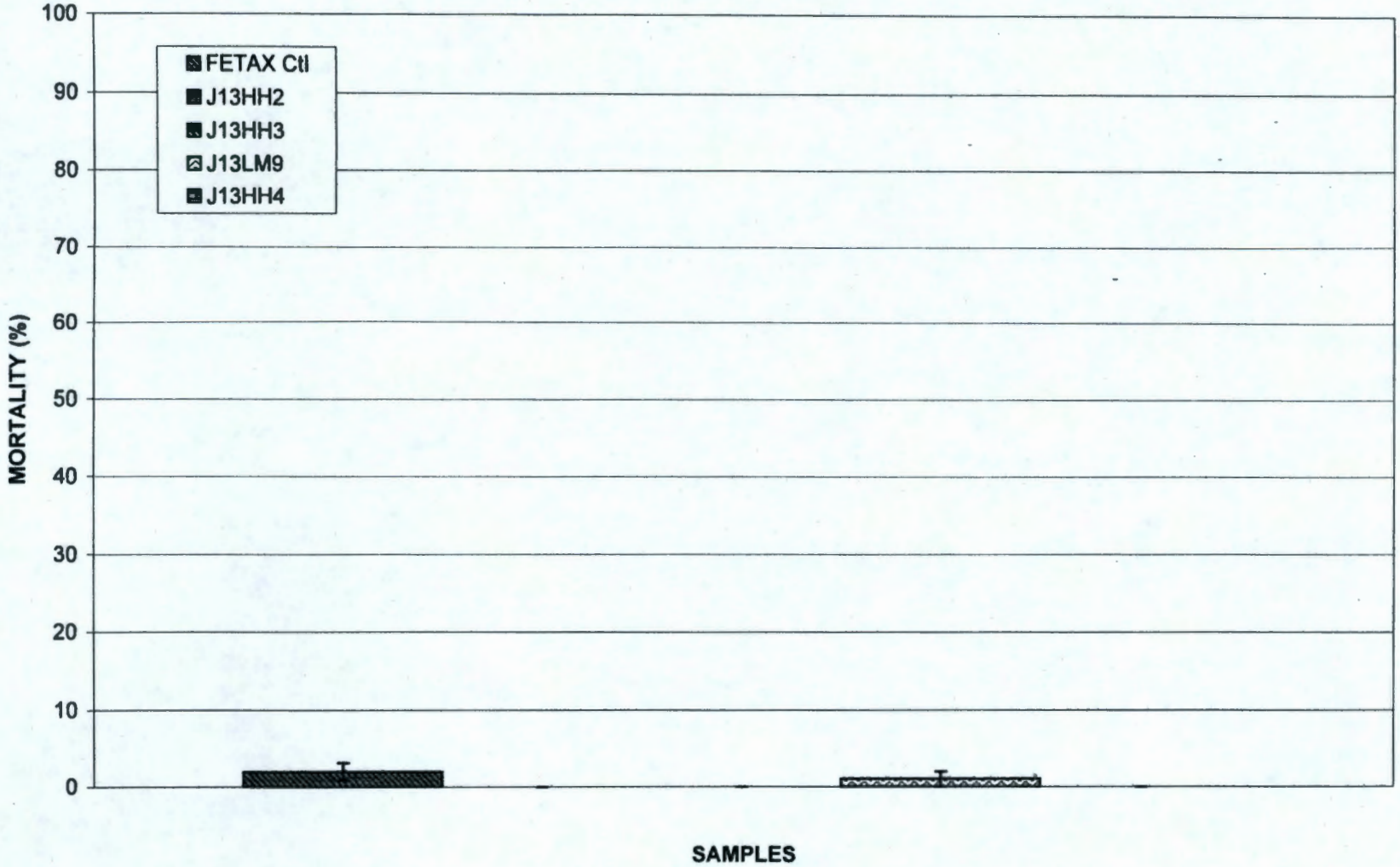
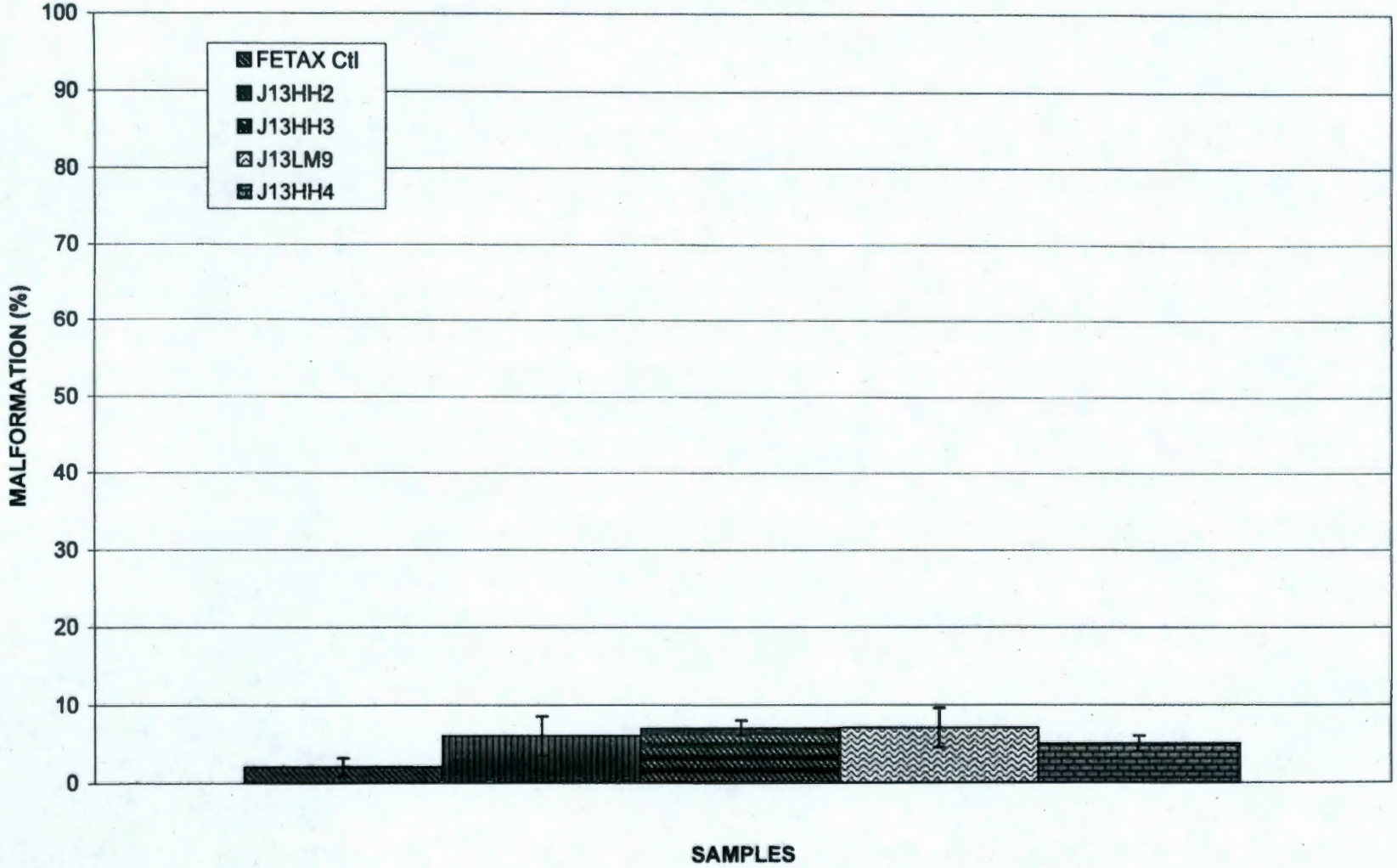


FIGURE 7
CH2M05-00133
FETAX Screen with *X. laevis*



FEL

CH2M05-00133_Pore Water Growth Data				
FX Ctl	J13HH2	J13HH3	J13LM9	J13HH4
1.089	1.001	0.993	0.874	1.021
1.029	1.026	1.027	0.850	0.975
0.976	0.926	1.048	1.016	0.979
0.967	1.096	0.905	0.968	1.034
1.068	0.999	1.030	1.002	1.098
1.054	1.035	0.993	1.063	0.995
0.881	0.990	1.063	1.006	0.975
1.059	1.007	0.981	1.027	1.030
0.972	0.997	0.898	1.065	0.996
1.023	0.988	0.941	1.112	1.025
1.048	0.973	0.939	0.960	1.042
0.984	1.026	0.997	1.035	0.983
1.013	1.007	0.987	0.966	0.841
1.044	1.018	1.037	0.908	1.034
0.966	0.891	1.059	0.942	1.043
1.013	0.930	0.996	0.940	0.994
1.032	1.048	1.061	1.023	1.070
0.849	0.981	0.886	0.939	0.975
1.116	1.013	1.010	1.048	1.079
1.054	0.953	1.000	1.041	1.041
1.082	1.071	1.022	0.990	1.022
1.060	1.024	0.880	1.093	0.931
1.086	0.949	1.008	1.026	1.104
0.872	1.023	0.932	0.945	0.739
0.972	0.956	0.853	1.041	0.803
1.124	0.981	1.096	1.123	0.974
0.996	0.957	1.054	1.046	0.955
1.067	1.094	1.031	1.024	0.925
0.955	0.996	1.021	0.991	1.029
1.037	1.177	0.945	1.014	0.963
1.031	1.046	0.962	1.021	1.023
1.073	1.034	0.910	1.081	0.953
1.030	1.031	1.064	0.879	0.987
1.042	1.050	0.983	1.093	1.009
1.073	1.035	1.041	0.957	1.053
1.024	1.089	1.029	0.812	0.865
1.058	0.966	1.068	0.850	1.042
1.064	1.053	1.044	0.925	0.982
0.962	0.998	1.089	0.923	0.760
0.889	1.033	1.065	1.054	0.919
1.064	1.091	1.000	0.981	1.071
1.036	0.872	0.899	0.939	0.881
0.923	1.005	0.967	0.925	1.020
1.024	1.001	0.869	1.032	0.910
1.059	0.906	1.011	1.065	0.985
1.054	1.064	0.954	0.673	0.926
1.152	0.949	0.990	1.023	0.981
0.853	1.104	0.985	1.046	1.000
1.189	1.067	1.024	1.061	0.851
1.114	1.045	1.017	0.971	0.925
1.009	1.035	0.945	0.939	0.917

FEL

CH2M05-00133_Pore Water Growth Data				
FX Ctl	J13HH2	J13HH3	J13LM9	J13HH4
0.818	1.066	0.952	0.975	1.000
0.969	0.975	0.975	1.054	0.999
1.069	1.038	0.860	0.798	0.913
1.013	1.077	0.670	0.832	1.035
1.005	1.053	0.807	0.907	0.983
0.934	1.013	0.937	1.021	1.067
0.972	0.996	1.081	1.042	1.006
0.844	1.057	1.023	0.998	1.013
0.980	1.038	1.064	0.935	0.940
0.930	1.090	0.989	0.893	1.045
0.922	1.001	1.006	1.007	0.836
0.890	1.137	1.098	0.507	0.974
0.856	1.021	1.032	0.991	0.937
0.986	1.014	0.979	0.978	1.011
0.859	1.067	0.815	1.030	1.065
1.039	1.032	0.983	0.917	0.964
1.013	1.086	0.972	0.893	1.027
0.983	1.066	1.081	1.038	0.941
0.919	1.100	0.936	0.991	0.791
1.055	0.773	0.991	1.087	1.051
0.980	0.907	1.061	0.841	1.022
0.957	0.957	0.907	1.019	1.111
0.923	0.907	0.944	1.057	1.063
1.029	0.986	1.067	0.900	0.881
0.950	1.020	0.923	1.033	0.985
0.951	0.991	1.063	0.924	0.836
0.989	0.921	1.218	1.024	0.868
0.953	1.026	0.905	1.067	1.039
0.984	1.017	0.985	1.003	0.869
0.969	1.026	1.036	1.007	0.929
1.031	1.073	0.986	0.804	0.883
0.963	0.923	1.055	1.099	0.744
1.009	1.001	0.993	1.022	0.951
0.964	0.900	0.921	0.975	0.902
0.706	1.061	1.068	1.036	0.789
1.043	0.964	1.031	1.073	0.950
0.980	1.058	1.053	1.027	0.949
1.000	0.986	1.068	1.011	0.969
1.043	0.966	1.011	1.043	0.972
0.975	1.132	0.932	0.984	1.007
0.946	1.045	1.020	1.086	1.063
1.267	1.100	1.127	1.017	0.924
1.027	0.944	0.961	0.899	1.063
0.976	1.110	0.975	0.955	0.952
0.841	1.081	0.792	0.963	0.836
0.942	1.093	1.076	0.971	0.899
0.964	1.048	1.133	1.030	0.940
	0.965	1.029	1.095	0.793
	0.902	1.070		0.936

Descriptive Statistics:

Data source: Mortality in CH2M05-00133 Pore Water

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
FX Ctl	4	0	0.02	0.0231	0.0115	0.0367
J13HH2	4	0	0	0	0	0
J13HH3	4	0	0	0	0	0
J13LM9	4	0	0.01	0.02	0.01	0.0318
J13HH4	4	0	0	0	0%	0%

Column	Range	Max	Min	Median	5%	95%
FX Ctl	0.04	0.04	0	0.02	0	0.04
J13HH2	0	0	0	0	0	0
J13HH3	0	0	0	0	0	0
J13LM9	0.04	0.04	0	0	0	0.04
J13HH4	0	0	0	0	0	0

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
FX Ctl	0	-6	0.307	0.203	0.08	0.0032
J13HH2	0	-6	0	<0.001	0	0
J13HH3	0	-6	0	<0.001	0	0
J13LM9	2	4	0.441	0.006	0.04	0.0016
J13HH4	0	-6	0	<0.001	0	0

One Way Analysis of Variance

Data source: Mortality in CH2M05-00133 Pore Water

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
asinsqrtFX Ctl	4	0	0.101	0	0.201
asinsqrtJ13HH2	4	0	0	0%	0%
asinsqrtJ13HH3	4	0	0	0%	0%
asinsqrtJ13LM9	4	0	0	0%	10%
asinsqrtJ13HH4	4	0	0	0	0

H = 5.961 with 4 degrees of freedom. (P = 0.202)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; **there is not a statistically significant difference (P = 0.202)**

Descriptive Statistics:

Data source: Malformation in CH2M05-00133 Pore Water

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
FX Ctl	4	0	0.021	0.0242	0.0121	0.0386
J13HH2	4	0	0.06	0.0516	0.0258	0.0822
J13HH3	4	0	0.07	0.02	0.01	0.0318
J13LM9	4	0	0.07	0.0503	0.0252	0.0801
J13HH4	4	0	0.05	0.02	1%	3%

Column	Range	Max	Min	Median	5%	95%
FX Ctl	0.042	0.042	0	0.021	0	0.042
J13HH2	0.12	0.12	0	0.06	0	0.12
J13HH3	0.04	0.08	0.04	0.08	0.04	0.08
J13LM9	0.12	0.12	0	0.08	0	0.12
J13HH4	0.04	0.08	0.04	0.04	0.04	0.08

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
FX Ctl	0	-6	0.307	0.203	0.084	0.00353
J13HH2	0	-1.2	0.151	0.71	0.24	0.0224
J13HH3	-2	4	0.441	0.006	0.28	0.0208
J13LM9	-1.129	2.227	0.329	0.138	28%	3%
J13HH4	2	4	0.441	0.006	0.2	0.0112

One Way Analysis of Variance

Data source: Malformation in CH2M05-00133 Pore Water

Normality Test: **Passed** **(P = 0.101)**

Equal Variance Test: **Passed** **(P = 0.334)**

Group Name	N	Missing	Mean	Std Dev	SEM
asinsqrtFX Ctl	4	0	0.103	0.119	0.0596
asinsqrtJ13HH2	4	0	0.21	0.154	0.0768
asinsqrtJ13HH3	4	0	0.265	0.0427	0.0213
asinsqrtJ13LM9	4	0	0.232	0.158	0.0789
asinsqrtJ13HH4	4	0	0.223	4%	2%

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.0602	0.0151	1.136	0.377
Residual	15	0.199	0.0133		
Total	19	0.259			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; **there is not a statistically significant difference (P = 0.377).**

Power of performed test with alpha = 0.050: 0.071

The power of the performed test (0.071) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Descriptive Statistics:

Data source: Growth in CH2M05-00133 Pore Water

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean	%Growth
FX Ctl	98	0	0.996	0.0818	0.00827	0.0164	-
J13HH2	100	0	1.014	0.0644	0.00644	0.0128	101.81
J13HH3	100	0	0.993	0.0795	0.00795	0.0158	99.70
J13LM9	99	0	0.982	0.0925	0.0093	0.0185	98.59
J13HH4	100	0	0.965	0.0827	1%	2%	96.89

Column	Range	Max	Min	Median	5%	95%
FX Ctl	0.561	1.267	0.706	1.003	0.851	1.115
J13HH2	0.404	1.177	0.773	1.021	0.904	1.102
J13HH3	0.547	1.218	0.67	0.999	0.856	1.092
J13LM9	0.616	1.123	0.507	1.006	0.821	1.093
J13HH4	0.372	1.111	0.739	0.977	0.792	1.07

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
FX Ctl	-0.273	1.864	0.0821	0.102	97.628	97.908
J13HH2	-0.559	1.189	0.0694	0.27	101.421	103.272
J13HH3	-0.851	2.42	0.0942	0.029	99.266	99.163
J13LM9	-1.954	6.861	0.114	0.003	97.195	96.261
J13HH4	-0.756	0.216	0.0971	0.021	96.456	93.715

One Way Analysis of Variance

Data source: Growth in CH2M05-00133 Pore Water

Normality Test: **Failed** **(P = <0.001)**

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
FX Ctl	98	0	1.003	0.957	1.048
J13HH2	100	0	1.021	0.978	1.058
J13HH3	100	0	0.999	95%	105%
J13LM9	99	0	1.006	94%	104%
J13HH4	100	0	0.977	0.924	1.026

H = 18.817 with 4 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group **(Dunn's Method)** :

Comparison	Diff of Ranks	Q	P<0.05
J13HH4 vs FX Ctl	50.554	2.476	No
J13HH2 vs FX Ctl	36.456	1.786	Do Not Test
J13LM9 vs FX Ctl	12.512	0.611	Do Not Test
J13HH3 vs FX Ctl	1.914	0.0938	Do Not Test

Note: The multiple comparisons on ranks do not include an adjustment for ties.