BIOASSAY REPORT
96-HOUR STATIC SCREENING
BIOASSAY CONDUCTED
March 28 through April 1, 2000

Prepared for

WASHINGTON DEPARTMENT OF ECOLOGY
PORT ORCHARD, WASHINGTON

Prepared by

CH2M HILL
2300 NW Walnut Boulevard
Corvallis, Oregon 97330

April, 2000
Lab I.D. C02532

CONTENTS

INTRODUCTION

CH2M HILL conducted one 96-hour Washington State Hazardous Waste Regulation bioassay using 100 mg/L of sample provided by the Washington Department of Ecology, Port Orchard, Washington. The test was conducted from March 28 through April 1, 2000 and the organism tested was the rainbow trout (*Oncorhynchus mykiss*).

METHODS AND MATERIALS

TEST METHODS

The test was performed according to: *Biological Testing Methods*, Washington State Department of Ecology, DOE 80-12, Revised August 1996.

TEST ORGANISMS

The rainbow trout used in the test were obtained from College of Southern Idaho, Twin Falls, Idaho, and were 41±4 mm in length, and 59 days old at test initiation. All test organisms were acclimated to dilution water for 11 days, appeared vigorous and in good condition prior to testing, and were not treated for any disease.

DILUTION WATER

The water used for acclimation and dilution water during the static testing was reconstituted moderately hard water with a hardness of 88 mg/l as $CaCO_3$, alkalinity of 66 mg/l as $CaCO_3$, and a pH of 8.0.

TEST CONDITIONS

The test concentration was 100 mg/L of sample with dilution water alone for the control. The bioassay was run in a 5-gallon glass aquaria, with a volume of 10 liters. The sample was run in triplicate with 10 organisms per replicate. The photoperiod was 16 hours light, 8 hours dark, and the temperature remained at 12±1°C throughout the test. Loading of test organisms was 0.70 g wet fish weight per liter. The test was initiated by adding organisms to test chambers within 30 minutes after adding test substances. Mortality was measured by lack of response to tactile stimulation and lack of respiratory movement.

SAMPLE COLLECTION AND DESCRIPTION

One sample was collected by Washington Department of Ecology personnel, shipped to CH2M HILL's Corvallis laboratory, and received on March 24, 2000. The sample was stored at 4°C in the dark until test initiation. Chain of custody for sample collection is provided in Appendix C.

MONITORING OF BIOASSAY

The static test was analyzed at initiation for total hardness, pH, conductivity, and dissolved oxygen, and every 24 hours thereafter for mortality, dissolved oxygen, and pH. Temperature was monitored continuously throughout the 96-hour test period. Conductivity was taken again at test termination.

RESULTS AND DISCUSSION

STATIC BIOASSAY

The raw data sheets are presented in Appendix A and the results of the test are summarized in Table 1. The sample failed the definition below and is considered a dangerous waste.

Table 1
Test Start Date: January 22, 2000

Sample	Concentration (mg/L)	Number Dead/ Number Tested
Control	0	0/30
10-8022	100	22/30

Dissolved oxygen concentrations remained at 60 percent saturation or greater throughout the test period. The test proceeded without interruption or incidence that could have affected test results.

DEFINITIONS

Extremely hazardous waste: 96 hr LC_{50} concentration less than or equal to 10 mg/L.

Dangerous waste: 96 hr LC_{50} concentration greater than or equal to 100 mg/L.

REFERENCE TOXICANT TEST

The 48-hour LC_{50} value and Control Chart limits for the reference toxicant (sodium lauryl sulfate) test conducted in March are listed in Table 2. The results indicate that the organisms were within their expected sensitivity range.

Table 2
Reference Toxicant Tests

Species	LC_{50} (mg/L)	Control Chart limits
Oncorhynchus mykiss	3.2	1.7 to 3.5

APPENDIX A
RAW DATA SHEETS

CH2M HILL TOXICITY TEST ORGANISM AND WATER QUALITY DATA

Client Washington Dept. of Ecology — Test Initiation: Date 3-28-00 Time 1330 — Test Termination: Date 4-1-00 Time 1200

Contact Pam Covey — Technician Winn, Stanaway, Janssen, Muckey

Test Species/ID Oncorhynchus mykiss / RBT 184 / / / /

Sample Information								Test Species Information	ID#	ID#	ID#	ID#
Sample ID Number	Field ID	Collected Date	Collected Time	~~Total Residual Chlorine (mg/l)~~ Concentration ~~As Received / As Dechlor.~~	Ammonia NH_3-N mg/l	Hardness mg/l as $CaCO_3$	Alkalinity mg/l as $CaCO_3$					
								(Age) or Size	59 days			
									ABL			
c02532-01	108022	3-21-00	—	100 mg/L	—	108	—	Test Container Size	5 gal.			
				/				Test Volume	10 L			
				/				Feeding: Type	None			
				/				Amount	—			
				/				Aeration: Began	None			
				/				Amount	—			
				/				Dilution Water ID#	1812			
				/				Acclimation Period	11 days			
				/				Test Location	#1			
				/				Condition of Survivors	Good			
				/				Size (mm)	41 ± 4 mm			
				/				Loading Rate	0.70 g/L			
				/				Comments				

Dilution Water Source	ID#	Hardness mg/l as $CaCO_3$	Alkalinity mg/l as $CaCO_3$
Recon MH (RBT)	1812	88	66

Water Quality Meters Used/ID#

Dissolved Oxygen #1 pH #1 Temperature _____

Conductivity #1 Refractometer _____ Other _____

CH2M HILL

48 or (96) HOUR FRESHWATER TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Client: Washington Dept. of Ecology CH2M ID #: CO2532-01
Sample Description: 108022
Test Species: Oncorhynchus mykiss ID#: RBT 184

Beginning, Date: 3-28-00 Time: 1330
Ending, Date: 4-1-00 Time: 1200
Technician: 0 hr MD 24 hr KJ 48 hr KT 72 hr KT 96 hr KS
Time: 0 hr 1330 24 hr 0930 48 hr 0700 72 hr 1000 96 hr 1200

Conc or Percent	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Temperature (°C)					Conductivity (µmohs/cm)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10	10	10	10.4	8.6	8.4	8.5	8.4	8.0	7.7	7.7	7.7	7.7	12	12	12	12	12	250				307
	B	10	10	10	10	10	10.4	8.6	8.7	8.5	8.4	8.0	7.7	7.7	7.7	7.6										
	C	10	10	10	10	10	10.4	8.6	8.6	8.4	8.2	8.0	7.7	7.7	7.6	7.6										
100	A	10	10	10	10	3	10.4	8.5	8.4	8.1	7.2	8.2	7.7	7.7	7.6	7.5	12	12	12	12	12	262				314
mg/L	B	10	10	10	10	3	10.4	8.5	8.4	8.1	7.2	8.2	7.7	7.6	7.5	7.5										
	C	10	10	9	7	2	10.4	8.6	8.4	7.9	7.2	8.0	7.7	7.6	7.6	7.5										

Summary of Test Results for **WDOE**						
LabID: **2532-01**			Start Date:	**03/28/2000**		
	Control		10 mg/L		100 mg/L	
Replicate	Number Dead	Proport. Dead	Number Dead	Proport. Dead	Number Dead	Proport. Dead
A	0	0.0000	n/a	n/a	7	0.7000
B	0	0.0000	n/a	n/a	7	0.7000
C	0	0.0000	n/a	n/a	8	0.8000
Mean		0.0000		n/a		0.7333
Variance		0.0000		n/a		0.0033

	F statistic for variance test	
	10 mg/L	100 mg/L
Calculated F statistic	n/a	Unequal Variance
Critical F degrees of freedom (Numerator, Denominator)	2 , 2	2 , 2
Cricital F (See Table 2 WDOE 80-12)	39	39
Equal Variance?	n/a	No

	t-Test (Modified t-Test if Unequal Variance)	
	10 mg/L	100 mg/L
Calculated t staistic	n/a	7.0343
Critical t degrees of freedom	n/a	2
Critical t value (See Table 3 WDOE 80-12)	n/a	-1.8860
Does Waste Designate ...	**... as an Extremely Hazardous Waste ?** **n/a**	**... as a Dangerous Waste ?** **Yes**

Reviewed by

APPENDIX B
REFERENCE TOXICANT DATA SHEETS

REFERENCE TOXICANT DATA SHEET

Client QA/QC | Reference Toxicant SLS | Test Begin Date 3-28-00 Time 1340
Test Organism Oncorhynchus mykiss | CID# 161 | Stock Solution 5 g/L | Test End Date 3-30-00 Time 1330
Source College of Southern Idaho | Solvent Distilled water
ID# RBT 184 | Age 59 5 days ABW | *Dilution Water Recon MH | ID# 1612
Size 41 ± 4 mm | Total Hardness as CaCO3 <56 | Total Alkalinity as CaCO3 66
Loading 0.70 g/L | Conductivity (µmhos/cm) / Salinity (ppt) 285 | Temperature 12
Volume = 10 L

		0 hr	24 hr	48 hr	72 hr	96 hr
Technician		MS	ES	MS		
Time		1340	0930	1330		

Toxicant Concen.	Test Chamber Number	Number Surviving					Dissolved Oxygen mg/l					pH					Temperature °C					Cond
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0
Control		10	10	10			10.2	8.6	8.2			8.0	7.7	7.6			12	12	12			280
1		10	10	10			10.2	8.6	8.3			8.0	7.7	7.6			12	12	12			270
2		10	10	10			10.2	8.4	8.2			8.1	7.7	7.6			12	12	12			274
3		10	10	8			10.2	8.4	8.2			8.1	7.7	7.7			12	12	12			283
4		10	8	0			10.2	8.6	8.9			8.1	7.7	7.8			12	12	12			289
10 8 ms Dec		10	0	—			10.2	9.0	—			8.1	7.9	—			12	12	—			300
Test Acceptability Limits:		Survival in Controls: > or = 90%					For RBT: >6.0 and <10.8 All Others (at 20°C): > 4.0 and < 9.1					pH: > 6.0 and < 9.0					Temperature ±1 °C					

*Dilution Water Code
Recon. - reconstituted water
VS - very soft
S - soft
MH - moderately hard
H - hard
VH - very hard

46 h LC50 3.2

Cusum Chart Limits 1.7 TO 3.5

Statistical Method Trimmed Spearman Karber

We verify this data is true and correct.

Task Manager Mike Stanovsky

Project Manager Doug Wilson

QA Officer Ginger Collins

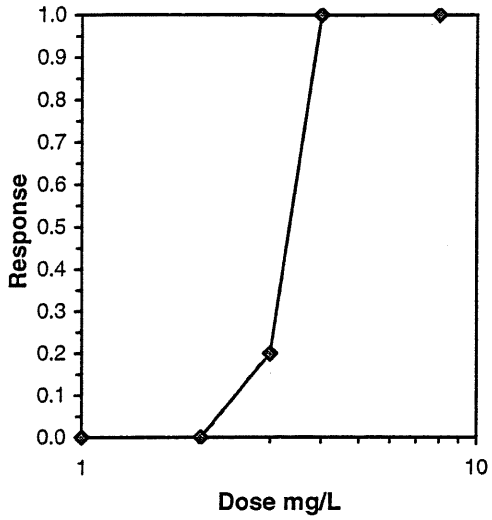
Acute Test-48 Hr Survival

Start Date:	03/28/2000 13:40	Test ID:	roma184	Sample ID:	REF-Ref Toxicant
End Date:	03/30/2000 13:30	Lab ID:	ORCHM-Corvallis Lab	Sample Type:	SLS-Sodium lauryl sulfate
Sample Date:		Protocol:	EPAA 93	Test Species:	OM
Comments:					

Conc-mg/L	1
LAB-Control	1.0000
1	1.0000
2	1.0000
3	0.8000
4	0.0000
8	0.0000

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	3.2321	2.9608	3.5283
5.0%	3.2728	2.9589	3.6200
10.0%	3.3071	2.9188	3.7471
20.0%	3.3417	3.1570	3.5373
Auto-0.0%	3.2321	2.9608	3.5283



Reviewed by: B~

APPENDIX C
CHAIN OF CUSTODY

WASHINGTON STATE DEPARTMENT OF ECOLOGY

REQUEST FOR LABORATORY SERVICES

PAGE 1 OF 1

SIC NO. _____

Contact: Doug Winn	Project: Treoil	Date: 3/23/00
Laboratory: CH2M Hill; Bioassay Lab	Client and Address: WA STATE DEPT. OF ECOLOGY MANCHESTER LABORATORY 7411 BEACH DRIVE EAST PORT ORCHARD WA 98366-8204	☐ Enforcement ☒ Return to Client ☐ Dispose ☒ Return Cooler
Address: 2300 NW Walnut Blvd Corvallis, OR 97339		

ITEM NO.	SAMPLE NO.	PROJECT NAME AND/OR DESCRIPTION	QUANTITY	UNIT PRICE	TOTAL COST
1	10-8022	Analyze one sample for 80-12 Dangerous Waste Designation Fish BIOASSAY. Ore Concentration: 100 ppm	1	450	450
		Deliverables to include a copy of this form, raw data, reports, tables & invoice.			
		Data due within 30 days of sample receipt			
		Sample collected 3/21/00 Please return remaining sample			
		TOTAL			450

Requested By (Your contact if any questions arise): Pam Carey — Phone No. (360) 871-8827

CHAIN OF CUSTODY*

Lab ID = C02532-01

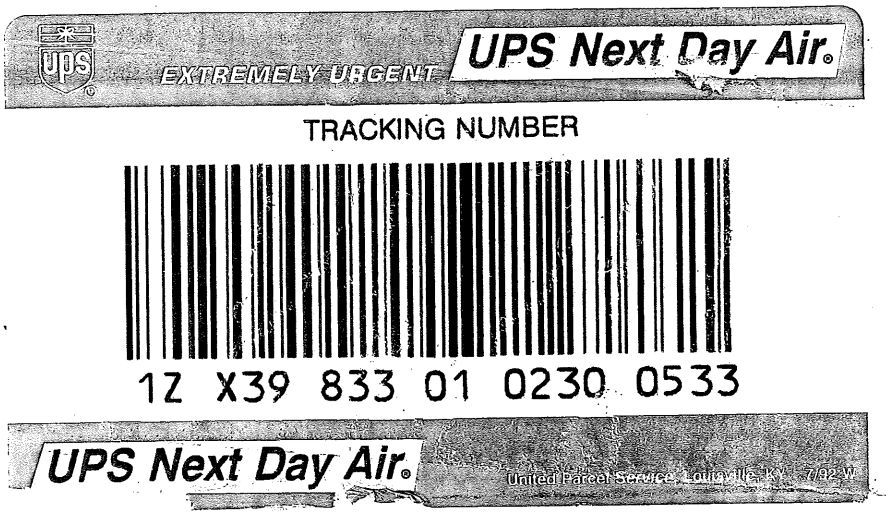
Relinquished By:	Received By:	Yr	Mo	Day	Hr	Min
Pam Carey	Doug Winn/LAB	01	01	21	09	15

*Signatures on this part of the form pertain to the custody of these samples and not to the cost of the analysis.

Invoice will be paid after sample analyses have passed a QA/QC review.

ECY 050-11 (Rev. 9/98)

DOUG WINN
CH2M HILL - BIOASSAY LAB
2300 NW WALNUT BOULEVARD
CORVALLIS OREGON 97339



MANCHESTER LABORATORY
WA STATE DEPARTMENT OF ECOLOGY
7411 BEACH DRIVE EAST
PORT ORCHARD WASHINGTON 98366

C02532-01