



ANALYTICAL SYSTEMS, INC.

2433 Impala Drive, Carlsbad, CA 92008 / (760) 931-8081 / (760) 931-1580 FAX

RECEIVED  
DEC 17 2002  
DEPT OF ECOLOGY

December 13, 2002

Linda Dawson  
Maul, Foster, and Alongi, Inc.  
17171 Bothell Way NE #264  
Seattle, Washington 98155

Re: Interim Report  
Whole Effluent Toxicity Tests, Unocal Edmonds Terminal

Dear Linda:

This Interim Report transmits the preliminary results of the Tier 1, freshwater, Whole Effluent Toxicity (WET) Tests conducted for the Unocal Edmonds Terminal evaluation. Tests were performed in accordance with *Whole Effluent Toxicity Testing Work Plan, Unocal Edmonds Terminal*, June 19, 2002, as amended. This preliminary report includes a brief description of the test methods, a summary of test acceptability, and a summary of test results.

### Product

Product collected from monitoring wells MW-133 and MW-112R was received on October 10, 2002. Four, 1-liter bottles were gently blended by pouring them into a large beaker, separating residual groundwater from the product, and storing the product in two, 1-liter bottles. The separated water was not used in the water-accommodated fraction preparations. A portion of the product was shipped to Columbia Analytical Services, Inc. (CAS) for chemical analyses.

### Preparation of Laboratory Water and Stock Solution

Synthetic (laboratory) fresh water was first prepared (hardness = 100 mg/L CaCO<sub>3</sub>; alkalinity = 80 mg/L CaCO<sub>3</sub>; pH = 8.20) to match conditions at Willow Creek (hardness = 112 mg/L CaCO<sub>3</sub>; alkalinity = 93 mg/L CaCO<sub>3</sub>; pH = 5.9-6.5) as closely as possible, while remaining within the acceptable criteria for the test species. A stock solution was then prepared by adding product to the laboratory water using the water-accommodated fraction (WAF) preparation methodology in Appendix A of the work plan.

To prepare the stock solution, 1,200 milliliters (mL) of product was placed over 12 liters of the laboratory water in a 20-liter container, for a loading rate of 1:10 product to water. The preparation was then placed on a magnetic stirrer and stirred for 24 hours at approximately 180 revolutions per minute (rpm). The product created a 5-10 millimeter layer on the water surface. As required by the WAF preparation procedure, the stirring was sufficient to cause movement within the water, without creating a vortex that would draw the product downward and potentially create suspended droplets in the test waters.

After the mixing period, the underlying water was siphoned into sample bottles for chemical analyses and two 4-L amber glass bottles for use in the bioassays. This stock solution was considered the 100% WAF solution. The WAF preparation was initiated on October 10 and completed on October 11. A portion of the 100% WAF stock solution was shipped to CAS for chemical analyses. The remainder of the stock solution was held in the dark, with no headspace, at 4°C, until needed for testing.

### Freshwater WET Tests

After receiving TPH results for the 100% WAF stock solution (100% WAF), the dilution series for the chronic toxicity tests with the fathead minnow (*Pimephales promelas*) and *Ceriodaphnia dubia* was determined in order to bracket the combined total petroleum hydrocarbon (TPH) concentration of 1.8 mg/L (i.e., the combined human-health-based concentration of TPH in the gasoline, diesel and heavy oil ranges from Table 720-1 of chapter 173-340 WAC). Tests were conducted with the following concentrations of WAF in fresh laboratory water: 30 percent of the WAF-generated stock solution (30% WAF), 15% WAF, 7.5% WAF, 3.75% WAF, 1.875% WAF, and 0% WAF (fresh water, only). Thirty percent WAF was the highest concentration tested. The concentration of petroleum in the 30% WAF preparation was estimated to be 3,831 µg/L, which was calculated as 30% of the TPH concentration of the 100% WAF (12,770 µg/L) (Table 1).

Dilutions were prepared at test initiation and Days 1 through 6, immediately prior to renewal. Each dilution was prepared by mixing the appropriate volume of stock solution with the corresponding volume of laboratory dilution water for each WAF concentration. Dilutions were then placed in each test chamber. At test initiation, samples were collected from each test (dilution) and shipped to CAS for analysis of TPH in the diesel range (DRO), heavy oil range (HO), and gasoline range (GRO). The results of these analyses are presented in Table 1.

#### *Pimephales promelas*

A 7-day, chronic WET test was conducted with *P. promelas* with each of the five dilutions of the 100% WAF and a laboratory control. Ten *P. promelas* were exposed in each of four replicates of each WAF dilution. Survival and growth were the test endpoints. Growth was defined by the total biomass at test termination divided by the number of individuals at test initiation. Test results are summarized in Table 2. Test data for all replicates are presented in Table 3. This test was validated by 100% survival and 0.38 mg/day mean growth per individual in the controls. Control survival and growth were calculated based on three replicates, as a pH probe tip broke in one of the *P. promelas* controls. That replicate was not included in the control results. Control survival in the four additional replicates included for the *P. promelas* reference-toxicant tests was 96.7%. The 100% survival within the three replicates of the control and the additional 96.7% survival in the four reference-toxicant control replicates provide acceptable survival in excess of the required 90%, validating the *P. promelas* test. Reference-toxicant test results for *P. promelas* were within the MEC control chart limits, as required by the SOP, demonstrating appropriate test organism sensitivity. Dissolved oxygen, temperature, and pH remained within acceptable limits throughout the 7-day test. Hardness ranged from 98 mg/L to 112 mg/L CaCO<sub>3</sub> and alkalinity ranged from 68 mg/L to 88 mg/L CaCO<sub>3</sub>. Alkalinity was lower than the target of 93 mg/L CaCO<sub>3</sub>; however, this is a value, relative to hardness, that is typical of this synthetic water preparation.

Mean percent survival in the *P. promelas* test ranged from 100% in the controls to 35% in the 30% WAF treatment. Mean growth in the *P. promelas* test ranged from 0.38 mg/individual/day in the controls to 0.12 mg/individual/day in the 30% WAF treatment.

#### *Ceriodaphnia dubia*

The 7-day chronic test was also conducted with *C. dubia*. One *C. dubia* was exposed in each of ten test replicates of each of the five dilutions of 100% WAF. The test endpoints were survival and

reproduction. Reproduction was defined as the number of offspring per individual. If an individual died prior to hatching any offspring, the number of offspring for that individual was "0". Test results are summarized in Table 4 and test results for each replicate are presented in Table 5. This test was validated by 90% survival and 24.9 offspring per individual in the controls. Reference-toxicant test results were within the MEC Control Chart limits, as required by the SOP, demonstrating appropriate test organism sensitivity. Dissolved oxygen and temperature remained within acceptable limits throughout the 7-day test; however, pH increased to >9.0 between renewals in the test solutions containing WAF. This did not appear to affect survival or reproduction, however, as control survival and reproduction exceeded acceptable levels. Water hardness in the controls ranged from 100 mg/L to 106 mg/L CaCO<sub>3</sub> and alkalinity in the controls ranged from 72 mg/L to 78 mg/L CaCO<sub>3</sub>.

Mean percent survival in the *C. dubia* test ranged from 70% to 100%. The reproduction endpoint ranged from 32.5 offspring/individual in the 3.75% WAF to 12.7 offspring/individual in the 30% WAF.

Please call me if there are any questions on this interim report.

Sincerely,

William Gardiner  
Senior Scientist

Attachments:

Table 1. Measured TPH Concentrations in WAF Preparations

Table 2. Summary of Chronic Toxicity Test Results for *Pimephales promelas*

Table 3. Chronic Toxicity Test Results for *Pimephales promelas*

Table 4. Summary of Chronic Toxicity Test Results for *Ceriodaphnia dubia*

Table 5. Chronic Toxicity Test Results for *Ceriodaphnia dubia*

**Table 1**  
**Measured TPH Concentrations in WAF Preparations**  
**Unocal Edmonds Terminal**  
**October 2002**

Percent WAF	Diesel Range Organics (µg/L)	Heavy Oil Range Organics (µg/L)	Gasoline Range Organics (µg/L)	Sum of Ranges (µg/L)
100%	8,900	3,200	670	12,770
30%	2,800	1,100	170	4,070
15%	1,100	<500 <sup>a</sup>	70	1,420
7.50%	560	<500	<50	835 <sup>b</sup>
3.75%	290	<500	<50	565 <sup>b</sup>
1.88%	280	<500	<50	555 <sup>b</sup>

<sup>a</sup> Analyze not detected at indicated reporting limit.

<sup>b</sup> TPH concentration is a sum of the DRO, HO, and GRO concentrations. One-half the detection limit was used for non-detected values.

**Table 2**  
**Summary of Chronic Toxicity Test Results for *Pimephales promelas***  
**Unocal Edmonds Terminal**  
**October 2002**

Sample	Concentration <sup>a</sup>	Mean % Survival	Mean Growth <sup>b</sup>	SD
0% WAF	0 µg/L	100.0	0.38	0.01
1.88% WAF	555 µg/L	92.5	0.36	0.06
3.75% WAF	565 µg/L	87.5	0.34	0.06
7.5% WAF	835 µg/L	92.5	0.35	0.02
15% WAF	1,420 µg/L	80.0	0.34	0.09
30% WAF	4,070 µg/L	35.0	0.12	0.02

<sup>a</sup> TPH concentration based on detected concentrations of DRO, HO, and GRO.

<sup>b</sup> Growth calculated as total biomass divided by number initiated.

**Table 3**  
**Chronic Toxicity Test Results for *Pimephales promelas***  
**Unocal Edmonds**  
**October 2002**

Sample	Concentration <sup>a</sup>	Rep	Number Initiated	Number Surviving	% Survival	Mean % Survival	Total Biomass	Growth <sup>b</sup>	Mean Growth	SD
0% WAF	0 µg/L	1	10	10	100.0		3.69	0.37		
0% WAF	0 µg/L	2	10	10	100.0		3.99	0.40		
0% WAF	0 µg/L	3	10	10	100.0		3.84	0.38		
0% WAF	0 µg/L	4	10	ND <sup>c</sup>	NC <sup>d</sup>	100.0	NC	NC	0.38	0.01
1.88% WAF	555 µg/L	1	10	9	90.0		3.58	0.36		
1.88% WAF	555 µg/L	2	10	10	100.0		4.07	0.41		
1.88% WAF	555 µg/L	3	10	8	80.0	92.5	2.80	0.28		
1.88% WAF	555 µg/L	4	10	10	100.0		3.84	0.38	0.36	0.06
3.75% WAF	565 µg/L	1	10	9	90.0		3.51	0.35		
3.75% WAF	565 µg/L	2	10	8	80.0		2.68	0.27		
3.75% WAF	565 µg/L	3	10	9	90.0	87.5	4.07	0.41		
3.75% WAF	565 µg/L	4	10	9	90.0		3.41	0.34	0.34	0.06
7.5% WAF	835 µg/L	1	10	10	100.0		3.67	0.37		
7.5% WAF	835 µg/L	2	10	7	70.0		3.52	0.35		
7.5% WAF	835 µg/L	3	10	10	100.0	92.5	3.65	0.37		
7.5% WAF	835 µg/L	4	10	10	100.0		3.20	0.32	0.35	0.02
15% WAF	1420 µg/L	1	10	8	80.0		4.71	0.47		
15% WAF	1420 µg/L	2	10	8	80.0		3.05	0.31		
15% WAF	1420 µg/L	3	10	8	80.0	80.0	2.62	0.26		
15% WAF	1420 µg/L	4	10	8	80.0		3.23	0.32	0.34	0.09
30% WAF	4070 µg/L	1	10	4	40.0		1.30	0.13		
30% WAF	4070 µg/L	2	10	4	40.0		1.43	0.14		
30% WAF	4070 µg/L	3	10	3	30.0	35.0	0.90	0.09		
30% WAF	4070 µg/L	4	10	3	30.0		1.04	0.10	0.12	0.02

<sup>a</sup> TPH concentration is a sum of the DRO, HO, and GRO concentrations. One-half the detection limit was used for non-detected values.

<sup>b</sup> Growth calculated as total biomass divided by number initiated.

<sup>c</sup> ND= No data - pH probe broken in test chamber; replicate not included in mean.

<sup>d</sup> NC= Not Calculable.

**Table 4**  
**Summary of Chronic Toxicity Test Results for *Ceriodaphnia dubia***  
**Unocal Edmonds Terminal**  
**October 2002**

Sample	Concentration <sup>a</sup>	Mean % Survival	Number of Offspring							Total Offspring	Mean Offspring/ Individual	SD
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
0% WAF	0 µg/L	90.0	0	0	2	27	51	94	50	225	22.5	13.7
1.88% WAF	555 µg/L	90.0	0	0	3	14	62	67	53	199	19.9	17.9
3.75% WAF	565 µg/L	100.0	0	0	1	48	85	126	65	325	32.5	10.5
7.5% WAF	835 µg/L	90.0	0	0	4	14	54	52	44	168	16.8	17.9
15% WAF	1,420 µg/L	70.0	0	0	4	21	39	42	47	153	15.3	19.4
30% WAF	4,070 µg/L	90.0	0	0	4	21	42	46	14	127	12.7	12.9

<sup>a</sup> TPH concentration is the sum of the DRO, HO, and GRO concentrations. One-half of the detection limit was used for non-detected values.

**Table 5**  
**Chronic Toxicity Test Results for *Ceriodaphnia dubia***  
**Unocal Edmonds Terminal**  
**October 2002**

Sample	Concentration <sup>a</sup>	Rep	Number Initiated	Number Surviving	% Survival	Mean % Survival	Number of Offspring							Total Offspring	Mean Offspring per Individual <sup>b</sup>	SD
							Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
0% WAF	0 µg/L	1	1	1	100.0		0	0	0	6	9	15	0	30		
0% WAF	0 µg/L	2	1	1	100.0		0	0	0	0	9	9	11	21		
0% WAF	0 µg/L	3	1	0	0.0		ND <sup>c</sup>	ND	ND	ND	ND	ND	ND	0		
0% WAF	0 µg/L	4	1	1	100.0		0	0	7	0	16	18	18	41		
0% WAF	0 µg/L	5	1	1	100.0		0	0	4	9	13	0	0	26		
0% WAF	0 µg/L	6	1	1	100.0		0	0	0	0	0	0	0	0		
0% WAF	0 µg/L	7	1	1	100.0		0	0	2	14	0	0	0	16		
0% WAF	0 µg/L	8	1	1	100.0		0	0	0	0	18	12	12	30		
0% WAF	0 µg/L	9	1	1	100.0		0	0	0	11	9	9	0	26		
0% WAF	0 µg/L	10	1	1	100.0	90.0	0	0	0	8	14	14	9	35	22.5	13.7
1.88% WA	555 µg/L	1	1	1	100.0		0	0	0	3	17	8	0	28		
1.88% WA	555 µg/L	2	1	0	0.0		ND	ND	ND	ND	ND	ND	ND	0		
1.88% WA	555 µg/L	3	1	1	100.0		0	0	3	9	11	11	16	39		
1.88% WA	555 µg/L	4	1	1	100.0		0	0	0	4	12	8	0	24		
1.88% WA	555 µg/L	5	1	1	100.0		0	0	0	1	10	18	13	42		
1.88% WA	555 µg/L	6	1	1	100.0		0	0	0	0	0	0	0	0		
1.88% WA	555 µg/L	7	1	1	100.0		0	0	0	0	11	12	14	37		
1.88% WA	555 µg/L	8	1	1	100.0		0	0	0	6	3	10	10	29		
1.88% WA	555 µg/L	9	1	1	100.0		0	0	0	0	0	0	0	0		
1.88% WA	555 µg/L	10	1	1	100.0	90.0	0	0	0	0	0	0	0	0	19.9	17.9
3.75% WA	565 µg/L	1	1	1	100.0		0	0	0	7	11	10	8	36		
3.75% WA	565 µg/L	2	1	1	100.0		0	0	0	8	0	17	12	37		
3.75% WA	565 µg/L	3	1	1	100.0		0	0	1	0	12	13	23	49		
3.75% WA	565 µg/L	4	1	1	100.0		0	0	0	9	14	18	0	41		
3.75% WA	565 µg/L	5	1	1	100.0		0	0	0	0	13	12	13	38		
3.75% WA	565 µg/L	6	1	1	100.0		0	0	0	3	9	11	0	23		
3.75% WA	565 µg/L	7	1	1	100.0		0	0	0	7	10	19	0	36		
3.75% WA	565 µg/L	8	1	1	100.0		0	0	0	4	7	10	9	30		
3.75% WA	565 µg/L	9	1	1	100.0		0	0	0	6	4	11	0	21		
3.75% WA	565 µg/L	10	1	1	100.0	100.0	0	0	0	4	5	5	0	14	32.5	10.5



**Table 5**  
**Chronic Toxicity Test Results for Ceriodaphnia dubia**  
**Unocal Edmonds Terminal**  
**October 2002**

Sample	Concentration <sup>a</sup>	Rep	Number Initiated	Number Surviving	% Survival	Mean % Survival	Number of Offspring							Total Offspring	Mean Offspring per Individual <sup>b</sup>	SD
							Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
7.5% WAF	835 µg/L	1	1	0	0.0		0	0	ND	ND	ND	ND	ND	0		
7.5% WAF	835 µg/L	2	1	1	100.0		0	0	0	0	0	0	0	0		
7.5% WAF	835 µg/L	3	1	1	100.0		0	0	3	0	8	16	36	0		
7.5% WAF	835 µg/L	4	1	1	100.0		0	0	1	0	10	13	34	0		
7.5% WAF	835 µg/L	5	1	1	100.0		0	0	0	5	13	0	27	0		
7.5% WAF	835 µg/L	6	1	1	100.0		0	0	0	0	0	0	0	0		
7.5% WAF	835 µg/L	7	1	1	100.0		0	0	0	9	14	12	35	0		
7.5% WAF	835 µg/L	8	1	1	100.0		0	0	0	0	8	13	36	0		
7.5% WAF	835 µg/L	9	1	1	100.0		0	0	0	0	0	0	0	0		
7.5% WAF	835 µg/L	10	1	1	100.0	90.0	0	0	0	0	0	0	0	0	16.8	17.9
15% WAF	1420 µg/L	1	1	1	100.0		0	0	0	7	8	6	24	3		
15% WAF	1420 µg/L	2	1	1	100.0		0	0	2	0	10	16	45	17		
15% WAF	1420 µg/L	3	1	1	100.0		0	0	2	1	9	6	29	11		
15% WAF	1420 µg/L	4	1	1	100.0		0	0	0	10	7	14	47	16		
15% WAF	1420 µg/L	5	1	1	100.0		0	0	0	3	5	0	8	0		
15% WAF	1420 µg/L	6	1	1	100.0		0	0	0	0	0	0	0	0		
15% WAF	1420 µg/L	7	1	0	0.0		0	0	ND	ND	ND	ND	0	ND		
15% WAF	1420 µg/L	8	1	0	0.0		0	0	ND	ND	ND	ND	0	ND		
15% WAF	1420 µg/L	9	1	0	0.0		0	ND	ND	ND	ND	ND	0	ND		
15% WAF	1420 µg/L	10	1	1	100.0	70.0	0	0	0	0	0	0	0	0	15.3	19.4
30% WAF	4070 µg/L	1	1	1	100.0		0	0	0	2	6	7	17	2		
30% WAF	4070 µg/L	2	1	1	100.0		0	0	0	0	6	12	22	4		
30% WAF	4070 µg/L	3	1	1	100.0		0	0	0	5	9	13	35	8		
30% WAF	4070 µg/L	4	1	1	100.0		0	0	0	8	10	5	23	0		
30% WAF	4070 µg/L	5	1	1	100.0		0	0	3	0	0	0	3	0		
30% WAF	4070 µg/L	6	1	0	0.0		0	ND	ND	ND	ND	ND	0	ND		
30% WAF	4070 µg/L	7	1	1	100.0		0	0	0	4	11	9	24	0		
30% WAF	4070 µg/L	8	1	1	100.0		0	0	1	2	0	0	3	0		
30% WAF	4070 µg/L	9	1	1	100.0		0	0	0	0	0	0	0	0		
30% WAF	4070 µg/L	10	1	1	100.0	90.0	0	0	0	0	0	0	0	0	12.7	12.9

<sup>a</sup> TPH concentration is a sum of the DRO, HO, and GRO concentrations. One-half the detection limit was used for non-detected values.

<sup>b</sup> Calculated as the number of offspring divided by the number initiated.

<sup>c</sup> ND= No data. Mortality in this replicate.