

ENVIRONMENTAL TOXICOLOGY SPECIALISTS

FINAL REPORT

**COMPARATIVE 7-DAY TOXICITY OF SACRAMENTO RIVER
WATER TO LARVAL FATHEAD MINNOWS AND LARVAL
CHINOOK SALMON**

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COMPARATIVE 7-DAY TOXICITY OF SACRAMENTO RIVER WATER TO LARVAL FATHEAD MINNOWS AND LARVAL CHINOOK SALMON

1.0 EXECUTIVE SUMMARY

A series of five 7-day chronic bioassays were conducted on larval fathead minnows and larval (sac-fry and swim-up) chinook salmon using samples of Sacramento River water collected at Freeport Marina during December, 1996 to March, 1997. Three of the five river samples tested produced significant mortality (31-83%) and four of the five produced significant growth effects on the larval fathead minnows. In contrast, none of the river samples produced significant mortality to the larval salmon under the conditions of these tests. Identification of any sublethal effects in the salmon caused by the river samples awaits histopathological evaluation of the fish preserved in these studies.

2.0 BACKGROUND

Since December of 1990, quarterly bioassays on larval fathead minnows have been conducted with samples of Sacramento River water collected at Garcia Bend (river mile 52) or Freeport Marina (river mile 46.5). The bioassays were conducted as part of Sacramento Regional Wastewater Treatment Plant's (SRWTP) NPDES permit requirements. The testing occurred from 1990-1996 during four periods corresponding to wet weather (December - February), agricultural run-off (April - June), tomato processing (July - September), and low river flow (October - December). The results of the bioassays have been summarized in a separate document¹. Briefly, the bioassays with Sacramento River water demonstrated toxicity to fathead minnows about 50% of the time (18 of 35 testing events). In these bioassays, the river water samples caused 20% to 97% mortality to the test species within 7 days. The toxicity in Sacramento River water samples most frequently occurs during the January to May period in which sensitive life stages of important species are present in the river system including salmon, striped bass, steelhead and rainbow trout.

This study was conducted to determine if Sacramento River water, which demonstrated pervasive toxicity to larval fathead minnows, also caused toxicity to sensitive life stages of chinook salmon, a species in decline. The study incorporated parallel bioassays with larval fathead minnows and chinook salmon in order to assess the relative sensitivity of the two species to any toxicants which may be present in samples of Sacramento River water during the study period.

3.0 METHODS AND MATERIALS

3.1 Test Dates

The test period incorporated the interval between 12/11/96 and 3/6/97. This period corresponds to the period in which toxicity had been previously identified in samples of Sacramento River during 1990-1996 (Appendix I).

3.2 Control Water and Test Samples

3.2.1 Control Water

The control water used in the fathead minnow and salmon bioassays was reverse-osmosis, double carbon-filtered well water amended with dry salts to achieve EPA moderately hard specifications, or Sierra Spring Water alone, as shown in Table 1.

3.2.2 Test Samples

Sacramento River water samples were subsurface grabs collected at Freeport Marina (River mile 46.5). Sample volumes were 5 gallons for Test 1 and 35 gallons for Tests 2-5. Samples were stored in 5 gallon polycarbonate carboys at 4 °C in the dark until used. Aliquots of a single sample were used for test change-out during the 7-day test.

3.3 Bioassay Procedures

Test procedures for the fathead minnow and salmon bioassays are summarized in Table 1 and detailed below:

Table 1 Summary of Test Protocol for Fathead Minnows and Chinook Salmon

<i>Parameter</i>	<i>Larval Fathead Minnow Bioassays</i>	<i>Chinook Salmon Bioassays</i>
<i>Source of Test Animals</i>	Aquatox, Inc. Yellow Springs, AK (Tests 1-5)	Mokelumne Hatchery Clements, CA (Test 1) Nimbus Hatchery Rancho Cordova, CA (Tests 2-5)
<i>Age/Life Stage of Test Animals</i>	Larval (<24 hours old)	Swim-up (Tests 1-3, 5) Sac-fry (Test 4) (1-2 days post yolk sac "button-up")
<i>Protocol Reference</i>	EPA. 1994 ²	EPA. 1993 ³ and EPA. 1996 ⁴
<i>Exposure Regimen and Duration</i>	24-hour renewal (Tests 1-5)	48-hour renewal (Test 1) 24-hour renewal (Tests 2-5)

<i>Parameter</i>	<i>Larval Fathead Minnow Bioassays</i>	<i>Chinook Salmon Bioassays</i>
<i>Test Volume</i>	500 mL	4,000 mL
<i>Test Endpoints</i>	Daily survival and growth after 7 days (Tests 1-5)	Daily survival and growth after 7 days (Test 2) Daily survival and preservation for histopathology (Tests 1, 3-5)
<i>Control Water</i>	EPA Moderately Hard (Tests 1, 3, 4) Sierra Spring Water (Tests 2,5)	Sierra Spring Water (Tests 1-5)
<i>Test Sample</i>	Grab sample of Sacramento River water at Freeport Marina	
<i>Exposure Duration</i>	7 days	
<i>Replicates</i>	4 per treatment (Control and River)	
<i>Number of Fish per Replicate</i>	10	
<i>Water Quality Parameters</i>	Initial: Dissolved oxygen (D.O.), pH, alkalinity and hardness Sample renewal: D.O. and pH Continuous temperature monitoring	

3.3.1 Fathead Minnow Bioassays

Fathead minnow chronic bioassays were conducted using methods published by EPA². Three or four replicates of 10 fish (<24 hours old) were exposed for 7 days to the Control and River samples. Control water for Tests 1, 3, and 4 was EPA moderately hard water. Control water for Tests 2 and 5 was Sierra Spring Water (used as received). Water quality parameters including temperature, dissolved oxygen (D.O.), pH, alkalinity, hardness and conductivity were measured daily in freshly prepared test solutions. D.O. and pH were measured at change-out in the 24-hour solutions. Test samples were renewed daily and fish were fed throughout the test period. Mortality was monitored daily and fish were dried and weighed at test termination for determination of growth. Statistical evaluation of the mortality and growth data was conducted using a computer program (ToxCalc™ 5.0).

3.3.2 Larval Salmon Bioassays

Salmon bioassays were conducted using methods published by EPA^{3,4} as guidelines. Four replicates of 10 fish (1-2 days post swim-up) were exposed for 7 days to the Control and River samples. Sources of the fish used in the five bioassays are shown in Table 1. Control water in all tests was Sierra Spring Water, which had water quality parameters that were very similar to the River water samples. Water quality parameters, including temperature, D.O., pH, alkalinity, hardness and conductivity, were measured daily in freshly prepared test solutions. D.O. and pH were measured at change-out in the 24-hour solutions. Test samples were renewed at 48-hour intervals in Test 1, and at 24-hour intervals in Tests 2-5. Fish were offered feed throughout the test period and mortality was monitored daily. Fish from Test 1 were

preserved in Bouin's fixative, and fish from Tests 3-5 were preserved in 10% neutral buffered formalin for future histopathological evaluation. Fish from Test 2 were dried and weighed for determination of growth. Statistical evaluation of the mortality and growth data was conducted using a computer program (ToxCalc™ 5.0).

4.0 RESULTS

4.1 Water Quality Parameters

A summary of water quality measurements taken during the fathead minnow and salmon bioassays are shown in Tables 2 and 3, respectively.

Briefly, all water quality parameters were within protocol requirements in the fathead minnow bioassays. In Test 1 of the salmon bioassays, which were renewed at 48-hour intervals, the D.O. values in some 24-hour test solutions were slightly less than the recommended level of 60% of saturation. In subsequent tests, samples were renewed at 24-hour intervals and D.O. values in these tests were all in excess of 60% saturation.

Table 2 Summary of Water Quality Parameters for Larval Fathead Minnow Bioassays

Test No.	Sample	Initial Solutions						Change-Out Solutions	
		Temp (°C)	D.O. (mg/L)	pH (units)	Alk. (mg/L)	Cond. (µmohs)	Hard (mg/L)	D.O. (mg/L)	pH (units)
1	C	24-24	8.0-8.4	7.61-8.01	100-120	330-330	80-100	6.9-7.9	7.35-7.70
	R	24-24	8.1-8.7	7.24-7.63	80-80	120-120	40-40	6.0-7.9	7.09-7.49
2	C	24-24	8.2-8.6	6.70-7.58	52-53	100-100	40-42	7.9-8.3	7.05-7.54
	R	24-24	8.3-8.6	7.13-7.39	48-48	110-110	48-49	7.7-8.3	7.01-7.55
3	C	24-25	7.6-8.2	7.45-7.74	68-77	320-330	84-92	7.6-8.4	7.47-7.81
	R	24-25	7.7-8.7	7.23-7.42	60-68	140-140	58-62	7.6-8.2	7.27-7.69
4	C	24-25	7.9-8.3	7.33-7.76	56-77	325-335	84-92	7.0-8.5	7.55-7.90
	R	24-25	7.7-8.6	7.23-7.58	50-54	125-125	48-50	7.0-8.4	7.32-7.71
5	C	24-25	7.7-8.8	6.85-7.08	54-64	110-115	28-52	8.0-8.8	7.34-7.90
	R	24-25	8.1-8.6	7.18-7.40	48-53	110-135	49-52	8.0-8.8	7.12-7.99

C = Control samples

R = River samples

Table 3 Summary of Water Quality Parameters for Chinook Salmon Bioassays

Test No.	Sample	Initial Solutions						Change-Out Solutions	
		Temp (°C)	D.O. (mg/L)	pH (units)	Alk. (mg/L)	Cond. (µmohs)	Hard. (mg/L)	D.O. (mg/L)	pH (units)
1	C	12-12	8.9-9.2	6.95-7.01	80-80	110-110	60-60	6.4-8.2	6.43-7.02
	R	12-12	9.8-10.2	7.04-7.14	80-80	100-100	60-60	5.1-7.4	6.48-7.25
2	C	12-13	10.3-10.8	7.07-7.50	53-57	80-85	38-45	7.6-8.7	6.47-6.91
	R	12-13	10.3-10.9	6.84-7.30	48-48	80-80	48-49	7.5-8.8	6.55-6.84
3	C	12-13	9.20-11.0	6.98-7.39	54-60	80-110	38-91	9.2-10.2	6.73-7.50
	R	12-13	10.8-11.2	6.79-7.40	60-60	110-125	40-58	9.0-10.1	6.52-7.44
4	C	13-14	10.2-11.2	6.76-7.11	52-62	80-100	40-46	8.2-9.7	6.50-6.98
	R	11-13	10.4-11.1	6.92-7.26	50-52	90-100	48-50	7.8-9.4	6.47-6.93
5	C	13-13	10.2-10.8	6.83-7.19	50-58	90-95	38-47	8.6-10.4	6.87-7.19
	R	13-13	10.0-10.5	6.99-7.23	48-56	90-100	48-52	8.3-9.9	6.67-7.15

C = Control samples R = River samples

4.2 Bioassay Results

The results of the bioassays for each test series are discussed below and are summarized in Table 4 and Figure 1.

Table 4 Mortality and Growth of Larval Fathead Minnows and Swim-Up Chinook Salmon Exposed to Sacramento River Water for Seven Days

Test No. (Test Date)	Species	Treatment	Cumulative % Mortality							Mean Weight of Fish ^a (mg)
			1	2	3	4	5	6	7	
1 (12/11/96)	FHM	Control	3	3	3	7	10	10	10	0.363 ± 0.025
		River	0	0	0	17	77	83	83*	0.047 ± 0.050*
	Salmon	Control	0	0	0	0	0	0	0	n/t ^b
		River	0	0	0	0	0	0	0	n/t
2 (1/22/97)	FHM	Control	0	0	0	0	0	0	0	0.355 ± 0.013
		River	0	0	5	20	25	35	48*	0.143 ± 0.032*
	Salmon	Control	0	0	0	0	0	0	0	348.5 ± 16.2
		River	0	0	0	0	0	0	0	332.6 ± 10.9
3 (2/20/97)	FHM	Control	0	0	0	0	0	0	3	0.332 ± 0.018
		River	0	3	3	10	17	21	31*	0.243 ± 0.043*
	Salmon	Control	0	0	0	0	0	0	0	n/t
		River	0	0	3	3	3	3	3	n/t

Table 4 (continued)

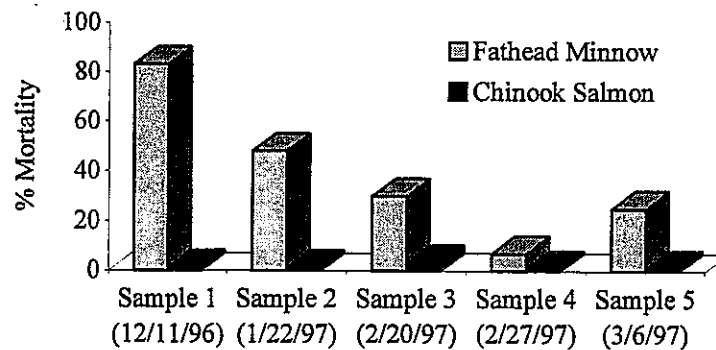
Test No. (Test Date)	Species	Treatment	Cumulative % Mortality							Mean Weight of Fish ^a (mg)
			1	2	3	4	5	6	7	
4 (2/27/97)	FHM	Control	0	0	0	0	0	0	0	0.359 ± 0.013
		River	0	0	0	0	0	0	7	0.306 ± 0.024*
	Salmon	Control	0	0	0	0	0	0	0	n/t ^b
		River	0	0	0	0	0	0	0	n/t
5 (3/6/97)	FHM	Control	3	3	3	3	3	3	3	0.325 ± 0.022
		River	0	0	3	8	13	25	25	0.254 ± 0.075
	Salmon	Control	0	0	0	0	0	0	0	n/t
		River	0	0	0	0	0	0	0	n/t

* Significantly different from control ($p < 0.05$)

a Mean ± SD of fish from each treatment replicate. There were 10 fish per replicate on day 0 of the study.

b n/t = weights not taken because salmon were fixed for histopathology evaluation immediately after termination.

Figure 1 Mortality of Larval Fathead Minnow and Swim-Up Chinook Salmon Exposed to Sacramento River Water for Seven Days



4.2.1 Test 1

Mortality was observed in the fathead minnows exposed to the river sample beginning on day 4 (17%). By day 7, the mortality was 83%, which was significantly greater than the control mortality (10%). Growth in the fathead minnows exposed to the river sample was also significantly reduced (0.047 ± 0.050 mg/fish) compared to the control (0.362 ± 0.025 mg/fish). No mortality was observed in salmon exposed to either the control or the river sample. The salmon in this test were not weighed because they were preserved in Bouin's fixative immediately after termination.

4.2.2 Test 2

Mortality was observed in the fathead minnows exposed to the river sample beginning on day 3 (5%). By day 7, the mortality was 48%, which was significantly greater than the control mortality (0%). Growth in the fathead minnows exposed to the river sample was also significantly reduced (0.143 ± 0.032 mg/fish) compared to the control (0.355 ± 0.013 mg/fish). No mortality was observed in salmon exposed to either the control or the river sample. Growth in the salmon exposed to the river sample (348.5 ± 16.2 mg/fish) was not significantly different than the controls (332.6 ± 10.9 mg/fish).

4.2.3 Test 3

Mortality was observed in the fathead minnows exposed to the river sample beginning on day 2 (3%). By day 7, the mortality was 31%, which was significantly greater than the control mortality (3%). Growth in the fathead minnows exposed to the river sample was also significantly reduced (0.243 ± 0.043 mg/fish) compared to the control (0.332 ± 0.018 mg/fish). Mortality was observed in salmon exposed to the river sample (3%), which was not significantly different from the control mortality (0%). The salmon in this test were not weighed because they were preserved in formalin immediately after termination.

4.2.4 Test 4

Mortality was observed in the fathead minnows exposed to the river sample on day 7 (7%), which was not significantly different from the control mortality (0%). However, growth in the fathead minnows exposed to the river sample was significantly reduced (0.306 ± 0.024 mg/fish) compared to the control (0.359 ± 0.013 mg/fish). No mortality was observed in salmon exposed to either the control or river samples. The salmon in this test were not weighed because they were preserved in formalin immediately after termination.

4.2.5 Test 5

Mortality was observed in the fathead minnows exposed to the river sample beginning on day 3 (3%). By day 7, the mortality was 25% which was not significantly greater than the control mortality (3%). Growth in the fathead minnows exposed to the river sample (0.254 ± 0.075 mg/fish) was not significantly different compared to the control (0.325 ± 0.022 mg/fish). No mortality was observed in salmon exposed to either the control or river samples. The salmon in this test were not weighed because they were preserved in formalin immediately after termination.

5.0 DISCUSSION

Five 7-day bioassays were conducted with larval fathead minnows and larval chinook salmon using five samples of Sacramento River water collected at Freeport Marina during the period from 12/11/96 to 3/6/97. The river samples produced significant mortality (31-83%) in fathead minnows in three of the five test events, and growth effects in four of the five test events. In contrast, the river samples did not produce significant mortality or growth effects to the salmon. These studies showed that the salmon did not respond to the toxicant(s) which affected the fathead minnows under the conditions of this study.

Overall, these results may indicate that the salmon were less sensitive to the toxicant(s) in the river water samples which affected the fathead minnows or, alternatively, that the study design, including the timing of sample collection, duration of exposure and the measured endpoints, may not have been appropriate to produce and detect toxic effects in the salmon. For example, although the 7-day exposure period used in these studies has been shown to be adequate to identify chronic effects of metal and organic toxicants in fathead minnows¹, this relatively short-term exposure may not have been of sufficient duration to produce detectable effects in salmon, which have a much longer life history than fathead minnows. Moreover, previous studies have identified toxicity to fathead minnows in virtually every month of the year, while this study incorporated only the four-month period from early December to early March. Potentially sensitive life-stages of salmon are in the river system during much of the year, so the exposure scenario to river-borne toxicants would potentially be much longer than the one used in the present study. It is also possible that the river water exposures produced sublethal effects in the salmon which were not detected by the endpoints used in this study. This possibility could be addressed by conducting a histopathological evaluation of the salmon that were preserved from this study.

This study should be viewed as a reconnaissance level effort. Additional studies, using more rigorous study designs, will be needed before it can be concluded that toxicants in the river water samples which affected fathead minnow survival do not cause adverse effects to salmon. In future studies, consideration should be given to conducting longer-term exposures under flow-through conditions. Study endpoints should include survival, growth, histopathology and genotoxicity measurements. These longer-term and more definitive studies would involve considerable effort and expense, but they are required to adequately address the role of toxics in the decline of the salmon populations.

Acknowledgments: We are grateful for the cooperation of the California Department of Fish and Game (CDFG) for providing the salmon used in this study. Special thanks to Mr. Michael Cochran at the Mokelumne River CDFG Hatchery (Clements, CA) and Mr. Bruce Barngrover at the Nimbus CDFG Hatchery (Rancho Cordova, CA). We appreciate the careful review and useful comments provided by Dr. Phyllis Fox (Fox Environmental).

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2. EPA. 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms. EPA 600/4-91/002.
3. EPA. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA 600/4-90/027F.
4. EPA. 1996. A 7-Day Survival and Growth Test for Rainbow Trout, *Oncorhynchus mykiss* and Brook Trout, *Salvelinus fontinalis*. Draft Protocol.

APPENDIX I

Toxicity of Sacramento River Water to Larval Fathead Minnows

Sample Date	Cumulative Number Dead Out of 30 Animals							7-Day Mortality (%)	Control Mortality (%)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
12/12/90	0	0	1	1	1	1	9	30	10
2/5/91	0	0	0	2	3	5	6	20	0
5/9/91	1	1	1	1	1	8	21	70	7
6/14/91	0	0	0	10	17	20	21	70	3
8/29/91	0	0	19	20	22	24	25	83	3
9/25/91	0	0	0	10	22	24	25	86	17
10/16/91	0	0	0	0	1	4	9	30	0
11/19/91	0	0	0	0	0	0	1	3	3
2/11/92	0	0	5	5	7	7	7	24	0
5/12/92	0	0	0	0	0	0	0	0	0
6/17/92	0	0	0	1	4	4	4	13	7
8/11/92	0	0	0	0	0	0	0	0	10
8/28/92	1	1	1	2	2	2	2	7	23
10/14/92	1	1	2	2	4	4	5	17	3
11/17/92	0	0	2	2	2	2	2	7	3
11/16/93	0	0	0	0	1	2	2	7	6
2/16/94	2	2	3	9	18	29	29	97	7
3/25/94	0	0	7	13	14	14	14	47	17
5/25/94	0	0	2	9	14	15	18	60	7
8/17/94	0	1	1	3	3	3	3	10	2
10/19/94	0	0	0	0	0	0	0	10	3
2/15/95	0	0	4	9	13	14	14	47	5
5/23/95	0	0	2	5	5	6	6	20	9
8/15/95	0	0	0	0	0	0	0	0	10
11/14/95	0	0	0	0	0	1	1	3	7
11/28/95	0	0	2	3	3	7	9	30	9
1/16/96	0	1	4	7	11	17	17	57	1
2/21/96	0	0	4	11	15	17	17	57	1
5/22/96	0	0	0	1	3	3	7	23	4
6/19/96	0	0	1	1	1	2	2	7	2
8/21/96	0	3	9	14	16	20	20	67	8
10/16/96	0	0	1	1	1	1	1	3	2
12/11/96	0	0	0	5	23	25	25	83	5
2/18/97	0	0	6	12	12	15	16	53	5

RAW DATA

RAW DATA

RAW DATA

TEST 1
12/11/96

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Sac. Amb. Salmon/FHM 96-01	Study Director:	J. L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Wiborg
Test Material:	Sacramento River Ambient (Freeport Marina 12/10/96)		
Test Species:	<i>O. tshawytscha/P. promelas</i>	Animal Lot No.:	Mokelumne 10/21/96
Initiation Date:	December 12, 1996	Termination Date:	December 19, 1996

Sample Description	OBSERVATIONS: 12/12 - 12/19						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	D. O.	pH
12/12 Control	12	9.2	6.95	80	110	60	6.8	7.02
12/12 Ambient	12	10.0	7.14	80	100	60	7.4	7.25
12/14 Control	12	9.0	7.01	80	110	60	6.1	6.43
12/14 Ambient	12	10.2	7.08	80	100	60	5.1	6.48
12/16 Control	12	8.9	6.98	80	110	60	See 72 hr	
12/16 Ambient	12	9.8	7.04	80	100	60	WQ Below	

UNIT/INSTRUMENTATION LEGEND

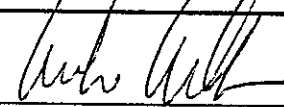
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit


ADDITIONAL COMMENTS: SALMON NOT BUTTERED UP @ TEST INITIATION 12/12/96 KJIM

Control- Sierra Spring water
 Ambient-Sacramento River at Freeport Marina 12/10/96
 FINAL WATER QUALITY (72 HR)

CONTROL	PH	DO	100% AMBIENT	PH	DO
A	6.75	8.2	A	6.64	6.6
B	6.75	8.1	B	6.66	6.6
C	6.71	7.0	C	6.58	5.8 = 56% SAT.
D	6.64	6.4 = 64% SAT.	D	6.51	5.9

mm 12/19/96

Technician:  Date: 12/12/96

Study Director:  Date: 1/5/97

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 12/11/96	Test ID: a2439701	Sample ID: Freeport Marina 12/10/96
End Date: 12/18/96	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 12/10/96	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 96-01 - FHM Survival and Growth Bioassay		

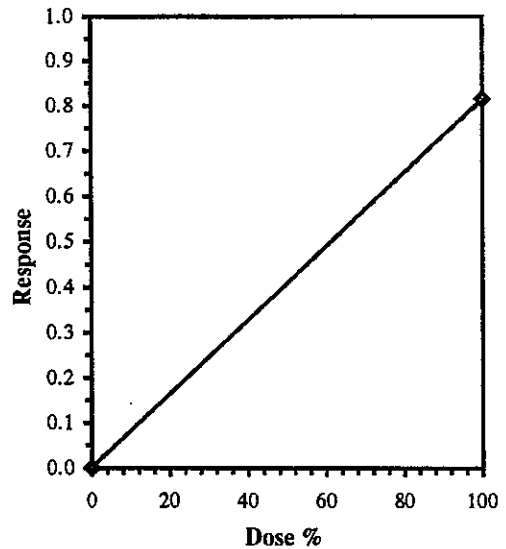
Conc-%	1	2	3
Control	0.9000	0.9000	0.9000
100	0.3000	0.2000	0.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	I-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Control	0.9000	1.0000	1.2490	1.2490	1.2490	0.000	3				0.9000	1.0000
*100	0.1667	0.1852	0.4007	0.1588	0.5796	54.251	3	6.760	2.920	0.0460	0.1667	0.1852

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.87961	0.713	-0.9439	2.5
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)				
Heteroscedastic t Test indicates significant differences				

Point	%	SD	Linear Interpolation (80 Resamples)		
			95% CL(Exp)	Skew	
IC05*	6.136	0.538	3.191	10.012	0.1161
IC10*	12.273	1.077	6.382	20.024	0.1161
IC15*	18.409	1.615	9.573	30.036	0.1161
IC20*	24.545	2.153	12.764	40.048	0.1161
IC25*	30.682	2.691	15.955	50.060	0.1161
IC40*	49.091	4.306	25.527	80.096	0.1161
IC50*	61.364	5.383	31.909	100.120	0.1161

* indicates IC estimate less than the lowest concentration


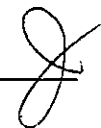


AQUA-Science
 Environmental Toxicology Consultants
FHM MORTALITY STUDY WEIGHT SHEET

Test Number:	CUWA 96-01	Study Director:	J. L. Miller
Protocol No.:	EPA 600/4-91/002	Technician(s):	Miller/Silverman/Wiborg
Test Material:	Sacramento River Ambient at Freeport Marina ; collected 12/10/96		
Test Species	<i>Pimephales promelas</i>	Animal Lot No.:	AQTX: 121196
Initiation Date:	December 11, 1996	Termination Date:	December 18, 1996

Conc. (%)	A Replicate			B Replicate			C Replicate			Dead	Average
	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	Dosed	Dry wt. (mg)
Ctrl.	9	3.6	0.36	9	3.9	0.39	9	3.4	0.34	2/29	
Amb.	3	1.0	0.10	2	0.4	0.04	∅	-	-	25/30	
DAY 1	10	1.1	0.11	10	0.7	0.07	∅	-	-	-	

Comments	Reference Weights 1 mg <u>1.0</u> 5 mg <u>5.0</u> 10 mg <u>10.1</u>
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Technician(s):  Date: 12/19/96
 Study Director:  Date: 1/5/97

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 12/11/96	Test ID: a2439701	Sample ID: Freeport Marina 12/10/96
End Date: 12/18/96	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 12/10/96	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 96-01 - FHM Survival and Growth Bioassay		

Conc-%	1	2	3
Control	0.3600	0.3900	0.3400
100	0.0100	0.0400	0.0000

Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	I-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Control	0.3633	1.0000	0.3633	0.3400	0.3900	6.926	3				0.3633	1.0000
*100	0.0167	0.0459	0.0167	0.0000	0.0400	124.900	3	18.385	2.132	0.0008	0.0167	0.0459

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89397	0.713	0.47656	-1.7212
F-Test indicates equal variances (p = 0.81)	1.46154	199.012		

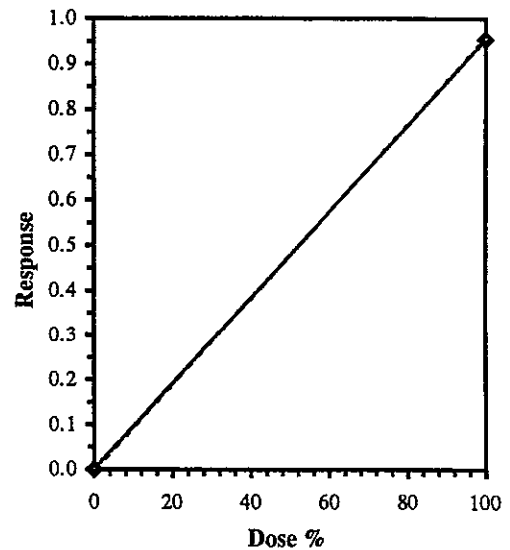
Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	5.240	0.130	4.456	6.135	0.0713
IC10*	10.481	0.260	8.912	12.271	0.0713
IC15*	15.721	0.391	13.368	18.406	0.0713
IC20*	20.962	0.521	17.823	24.542	0.0713
IC25*	26.202	0.651	22.279	30.677	0.0713
IC40*	41.923	1.042	35.647	49.084	0.0713
IC50*	52.404	1.302	44.558	61.355	0.0713

* indicates IC estimate less than the lowest concentration



AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 96-01	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 12/10/96)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 121196
Initiation Date:	December 11, 1997	Termination Date:	December 18, 1997

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 12/11-12/14						48 Hour Obsv.		
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH	
FHM									
Control-12/11	24	8.2	7.63	100	330	100	7.8	7.63	BSJ
Ambient 12/11	24	8.1	7.33	80	120	40	7.2	7.22	12/1
Control-12/12	24	8.2	7.82	100	330	80	7.6	7.64	WJM
Ambient-12/12	24	8.6	7.36	80	120	40	7.3	7.11	12/1
Control-12/13	24	8.2	7.68	100	330	100	6.9	7.70	WJM
Ambient-12/13	24	8.6	7.48	80	125	40	7.0	7.49	12/1
Control-12/14	24	8.0	8.01	100	330	100	7.7	7.49	BSJ
Ambient-12/14	24	8.4	7.63	80	120	40	6.0	7.14	12/1

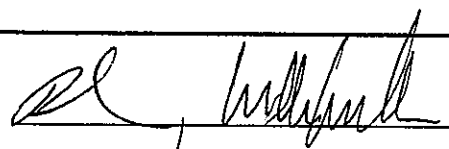
UNIT/INSTRUMENTATION LEGEND


*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = 2x carbon filtered R/O well water at EPAMH using EPA salts.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 12/11-14/97

Study Director:  Date: 1/5/97

AQUA-Science

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WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 96-01	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 12/10/96)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 121196
Initiation Date:	December 11, 1997	Termination Date:	December 18, 1997

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 12/15-12/17						48 Hour Obsv.		
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH	
FHM									
Control-12/15	24	8.2	7.74	100	330	80	6.9	7.35	BJ 12/1
Ambient 12/15	24	9.2/8.7	7.37	80	120	40	6.6	7.09	12/1
Control-12/16	24	8.4	7.68	120	320	100	7.9	7.44	BJ 12/1
Ambient-12/16	24	8.4	7.28	80	120	40	7.9	7.21	12/1
Control-12/17	24	8.4	7.61	100	330	100	7.4	7.35	BJ
Ambient-12/17	24	6.3	7.33	80	120	40	6.8	7.29	12/1


UNIT/INSTRUMENTATION LEGEND

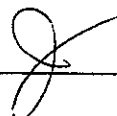
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = 2x carbon filtered R/O well water at EPAMH using EPA salts.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 12/15-17/96

Study Director:  Date: 1/5/97

TEST 2
1/22/97

Environmental Toxicology Consultants

FISH LENGTH AND WEIGHT REPORT

Test Number:	<u>CUWA 97-02</u>	Study Director:	<u>J. L. Miller</u>
Protocol No.:	<u>EPA 600/4-90/027</u>	Technician(s):	<u>Miller/Silverman</u>
Test Material:	<u>Sacramento River at Freeport Mariana 1/21/97</u>		

Test Species:	<u><i>Oncorhynchus tshawytscha</i></u>	Test Initiation Date:	<u>January 22, 1997</u>
Lot Number:	<u>American River strain 11/12/96</u>	Test Termination Date:	<u>January 29, 1997</u>

Replicate Number	Day 0-Weight (g)	Day 7-Weight (g)	Comments
Stock A	2.1499	3.3814	10 fish /Replicate
Stock B	2.3069	3.2415	↓
Stock C	2.0993	2.2307	
Stock D	2.0384	2.4638	
Mean ± S. D.	2.1485 ± 11.52	333 ± 11.27	
Control A	—	3.4954	10 fish /Replicate
Control B	—	3.2537	↓
Control C	—	3.6221	
Control D	—	3.5682	
Mean ± S. D.	—	348.48 ± 16.24	

100% Ambient A	—	3.3692	10 fish /Replicate
100% Ambient B	—	3.2076	↓
100% Ambient C	—	3.4550	
100% Ambient D	—	3.2703	
Mean ± S. D.	—	332.55 ± 10.90	

Technician: *John Hall* Date: 1/29/97

Study Director: _____ Date: 2/5/97

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 1/22/97	Test ID: a2439702	Sample ID: Freeport Marina 1/22/97
End Date: 1/29/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 1/21/97	Protocol: EPAF 91-EPA Freshwater	Test Species: Onchorhynchus tshawytscha
Comments: CUWA 97-02 Salmon Survival and Growth Bioassay		

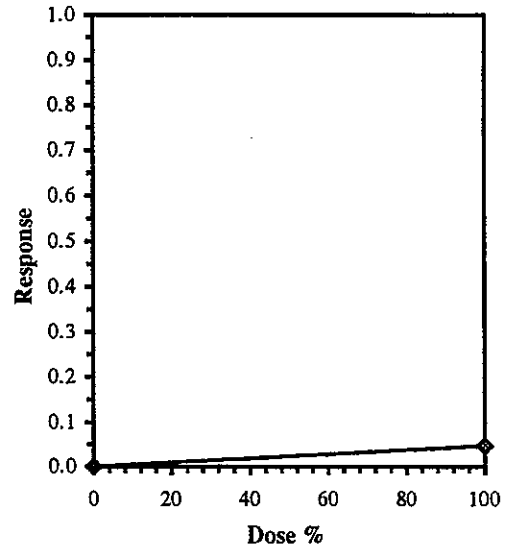
Conc-%	1	2	3	4
Control	349.54	325.37	362.21	356.82
100	336.92	320.76	345.50	327.03

Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	I-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Control	348.49	1.0000	348.49	325.37	362.21	4.666	4				348.49	1.0000
100	332.55	0.9543	332.55	320.76	345.50	3.277	4	1.628	1.943	186.15	332.55	0.9543

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93159	0.749	-0.791	-0.1385
F-Test indicates equal variances (p = 0.53)	2.22656	47.4683		

Hypothesis Test (1-tail, 0.05)
 Homoscedastic t Test indicates no significant differences

Linear Interpolation (80 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	> 100			
IC10	> 100			
IC15	> 100			
IC20	> 100			
IC25	> 100			
IC40	> 100			
IC50	> 100			



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WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-02	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 1/21/97)		
Test Species:	<i>Oncorhynchus tshawytscha</i>	Animal Lot No.:	Nimbus Hatchery 012297
Initiation Date:	January 22, 1997	Termination Date:	January 29, 1997

Sample @ 100%	OBSERVATIONS: DAY (0-3) 1/25 Date: 1/22-1/25-29 <small>OK MIM</small>						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^{^^}	Alkalinity ^{**}	Conductivity or Salinity [^]	Water Hardness [~]	DO	PH
Salmon								
Control-1/22	13	10.9	7.36	56	80	38	8.7	6.71
Ambient-1/22	12	12.3/10.9	6.84	48	80	48	8.8	6.66
Control-1/23	13	10.8	7.25	57	85	45	8.7	6.67
Ambient-1/23	12	10.8	6.95	48	80	48	8.8	6.57
Control-1/24	12	10.4	7.19	54	85	45	7.8	6.71
Ambient-1/24	12	10.4	7.00	48	80	49	7.7	6.64
Control-1/25	13	10.3	7.07	-	85	-	8.6	6.91
Ambient-1/25	13	10.3	6.93	-	80	-	8.6	6.84


UNIT/INSTRUMENTATION LEGEND

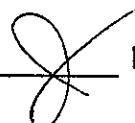
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = Sierra Spring™ water unammended.

All surface waters filtered through 35 µm screen daily..

Technician:  Date: 1/22/97 - 1/25/97

Study Director:  Date: 2/5/97

AQUA-Science

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WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	<u>CUWA 97-02</u>	Study Director:	<u>J.L. Miller</u>
Protocol No.:	<u>EPA 600/4-90/027</u>	Technician(s):	<u>Miller/Silverman/Ramos</u>
Test Material:	<u>Sacramento River Ambient (Freeport Marina 1/21/97)</u>		
Test Species:	<u>Oncorhynchus tshawytscha</u>	Animal Lot No.:	<u>Nimbus Hatchery 012297</u>
Initiation Date:	<u>January 22, 1997</u>	Termination Date:	<u>January 29, 1997</u>

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 1/26-1/28						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-1/26	13	10.8	7.33	-	80	-	7.6	6.88
Ambient-1/26	13	10.9	7.20	-	80	-	7.5	6.71
Control-1/27	13	11.2/10.6	7.49	53	80	38	7.8	6.89
Ambient-1/27	13	11.4/10.7	7.30	-	80	-	7.8	6.87
Control-1/28	13	11.0/10.8	7.50	-	80	-	7.6	6.47
Ambient-1/28	13	11.7/10.8	7.18	-	80	-	7.5	6.55

UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = Sierra Spring™ water unammended.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 1/26-28/97

Study Director:  Date: 2/5/97

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 1/22/97	Test ID: a2439702	Sample ID: Freeport Marina 1/22/97
End Date: 1/29/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 1/22/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-02 PHM Survival and Growth Bioassay		

Conc-%	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
100	0.5000	0.4000	0.7000	0.5000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4				1.0000	1.0000	
*100	0.5250	0.5250	0.8117	0.6847	0.9912	15.860	4	9.327	2.353	0.0097	0.5250	0.5250	

Auxiliary Tests

Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	Statistic	Critical	Skew	Kurt
	0.79138	0.749	1.17767	3.92758

Equality of variance cannot be confirmed

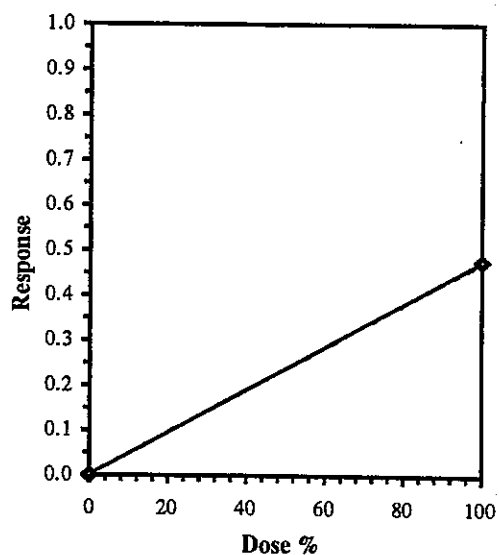
Hypothesis Test (1-tail, 0.05)

Heteroscedastic t Test indicates significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)	Skew	
IC05*	10.526	1.234	6.682	14.671	0.4923
IC10*	21.053	2.467	13.364	29.342	0.4923
IC15*	31.579	3.701	20.046	44.013	0.4923
IC20*	42.105	4.934	26.728	58.684	0.4923
IC25*	52.632	6.168	33.410	73.355	0.4923
IC40*	84.211				
IC50	> 100				

* indicates IC estimate less than the lowest concentration





AQUA-Science
Environmental Toxicology Consultants
FHM MORTALITY STUDY WEIGHT SHEET

Test Number:	CUWA 97-02	Study Director:	J. L. Miller
Protocol No.:	EPA 600/4-91/002	Technician(s):	Miller/Silverman
Test Material:	Sacramento River at Freeport Marina 1/21/97	Animal Lot No.:	AQTX:012297
Test Species:	<i>Pimephales promelas</i>	Termination Date:	January 29, 1997
Initiation Date:	January 22, 1997		

Sample ID	A Replicate			B Replicate			C Replicate			D Replicate			Dead Average	
	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	Dosed	Dry wt. (mg)
Control	10	3.7	0.37	10	3.6	0.36	10	3.4	0.34	10	3.5	0.35	6/40	
Ambient	5	1.7	0.17	4	1.1	0.11	7	1.7	0.17	5	1.2	0.12	19/40	
Day 0	10	1.0		10	1.2									

REF. wt. 1mg = 1.0mg
5mg = 5.0mg
10mg = 9.9mg

Technician(s):  Date: 1/30/97
Study Director:  Date: 2/5/97

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 1/22/97	Test ID: a2439702	Sample ID: Freeport Marina 1/22/97
End Date: 1/29/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 1/22/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-02 FHM Survival and Growth Bioassay		

Conc-%	1	2	3	4
Control	0.3700	0.3600	0.3400	0.3500
100	0.1700	0.1100	0.1700	0.1200

Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
Control	0.3550	1.0000	0.3550	0.3400	0.3700	3.637	4				0.3550	1.0000	
*100	0.1425	0.4014	0.1425	0.1100	0.1700	22.467	4	12.312	1.943	0.0006	0.1425	0.4014	

Auxiliary Tests

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93845	0.749	-0.0681	-1.4583
F-Test indicates equal variances (p = 0.17)	6.15	47.4683		

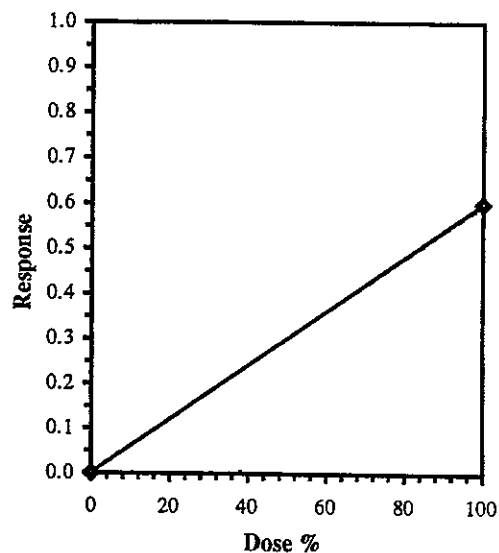
Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	8.353	0.636	6.174	11.093	0.5780
IC10*	16.706	1.272	12.348	22.185	0.5780
IC15*	25.059	1.907	18.522	33.278	0.5780
IC20*	33.412	2.543	24.696	44.370	0.5780
IC25*	41.765	3.179	30.869	55.463	0.5780
IC40*	66.824	5.086	49.391	88.741	0.5780
IC50*	83.529	6.358	61.739	110.926	0.5780

* indicates IC estimate less than the lowest concentration



AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-02	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 1/21/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 012297
Initiation Date:	January 22, 1997	Termination Date:	January 29, 1997

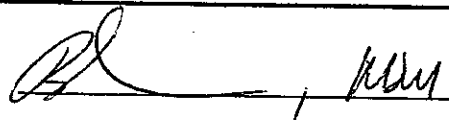

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 1/22-1/25						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-1/22	24	9.6/8.6	7.12	53	100	42	8.3	7.05
Ambient-1/22	24	10.7/8.6	7.26	48	110	48	8.3	7.28
Control-1/23	24	8.6	6.70	53	100	42	8.3	7.36
Ambient-1/23	24	8.6	7.13	48	110	48	8.1	7.40
Control-1/24	24	8.4	6.88	52	100	41	7.9	7.21
Ambient-1/24	24	8.4	7.15	48	110	49	8.0	7.01
Control-1/25	24	8.3	7.11	-	100	-	7.9	7.49
Ambient-1/25	24	8.3	7.22	-	110	-	7.9	7.43

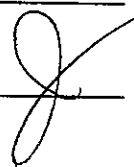
UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = Sierra Spring™ water unammended.
 All surface waters filtered through 35 µm screen daily.

Technician: ,  Date: 1/22/97 - 1/25/97

Study Director:  Date: 2/5/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-02	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 1/21/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 012297
Initiation Date:	January 22, 1997	Termination Date:	January 29, 1997


Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 1/26-1/28						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-1/26	24	8.2	7.58	52	100	40	7.9	7.43
Ambient-1/26	24	8.6	7.27	—	110	—	7.7	7.39
Control-1/27	24	8.8/8.5	7.56	52	100	40	7.9	7.47
Ambient-1/27	24	9.0/8.6	7.39	—	110	—	7.8	7.41
Control-1/28	24	8.3	7.58	—	100	—	8.0	7.54
Ambient-1/28	24	9.0/8.6	7.36	—	110	—	8.0	7.55

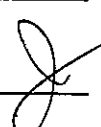
UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = Sierra Spring™ water unammended.
 All surface waters filtered through 35 µm screen daily.

Technician:  Date: 1/26-28/97

Study Director:  Date: 2/5/97

TEST 3
2/20/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number: <u>CUWA 97-03</u>	Study Director: <u>J.L. Miller</u>
Protocol No.: <u>EPA 600/4-90/027</u>	Technician(s): <u>Miller/Silverman</u>
Test Material: <u>Sacramento River Ambient (Freeport Marina 2/18/97)</u>	
Test Species: <u>O. tshawytscha</u>	Animal Lot No.: <u>Nimbus 21997</u>
Initiation Date: <u>February 19, 1997</u>	Termination Date: <u>February 26, 1997</u>

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 2/20-2/22						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-2/19	13	10.7	7.39	65	250 110	91	9.5	7.50
Ambient-2/19	13	10.7	7.02	60	110	58	9.5	7.35
Control-2/20	12	10.2	6.86/7.01	54	80	38	10.2	7.27
Ambient-2/20	12	10.3	7.08	60	110	58	10.1	7.12
Control-2/21	12	11.0	7.01	60	110 80	58	9.6	7.28
Ambient-2/21	12	11.2	7.08	60	110	40	9.7	7.44
Control-2/22	12	9.7	7.01	60	110	40	9.7	7.21
Ambient-2/22	12	9.3	7.40	60	125	58	9.7	7.31


UNIT/INSTRUMENTATION LEGEND


*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS: ① USED R/O EPAMH DAY 6 2/19 97
 ② #17 ✓ 13L S.S WITH 20DAPS 1.0N NaOH

S.S. Control= Sierra Spring Water unammended

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 2/23-22/97

Study Director:  Date: 3/12/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-05	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 2/18/97)		
Test Species:	<i>O. tshawytscha</i>	Animal Lot No.:	Nimbus 21997
Initiation Date:	February 19, 1997	Termination Date:	February 26, 1997

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 2/23-2/25						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-2/23	13	10.6	6.55/6.98	60	80	40	9.2	7.18
Ambient-2/23	13	10.7	7.12	60	110	58	9.0	7.20
Control-2/24	13	10.2	6.70/6.98	60	80	40	9.9	7.20
Ambient-2/24	13	10.2	6.96	60	110	58	9.8	7.17
Control-2/25	13	10.6	6.86/7.01	60	80	40	9.4	6.73
Ambient-2/25	12	10.8	6.79	60	110	58	9.0	6.52
Control-2/26								
Ambient-2/26								

UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

S.S. Control= Sierra Spring Water unammended

All surface waters filtered through 35 µm screen daily.

Technician:

Date: 2/23-26/96

Study Director:

Date: 3/12/97

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 2/20/97	Test ID: a2439703	Sample ID: Freeport Marina 2/18/97
End Date: 2/27/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 2/18/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-03 FHM Survival and Growth Bioassay		

Conc-%	1	2	3
Control	0.9000	1.0000	1.0000
100	0.9000	0.6000	0.5556

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	0.9667	1.0000	1.3577	1.2490	1.4120	6.930	3				0.9667	1.0000
*100	0.6852	0.7088	0.9921	0.8411	1.2490	22.548	3	2.610	2.132	0.0418	0.6897	0.7134

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88102	0.713	0.94398	0.26465
F-Test indicates equal variances (p = 0.30)	5.65182	199.012		

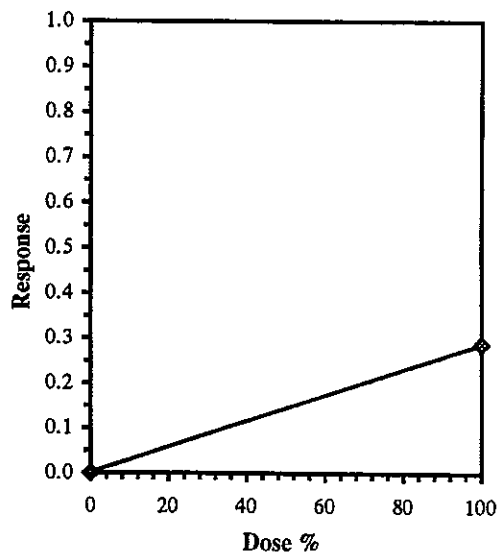
Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	17.448	6.813	0.000	87.656	1.7665
IC10*	34.896				
IC15*	52.344				
IC20*	69.793				
IC25*	87.241				
IC40	> 100				
IC50	> 100				

* indicates IC estimate less than the lowest concentration




AQUA-Science
Environmental Toxicology Consultants
FHM MORTALITY STUDY WEIGHT SHEET

Test Number:	A/S FHM Comparison 97-23	Study Director:	J. L. Miller
Protocol No.:	EPA 600/4-91/002	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River at Freeport Marina; collected 2/18/97		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	AQTX:030597
Initiation Date:	March 5, 1997	Termination Date:	March 12, 1997

Conc. (%)	A Replicate			B Replicate			C Replicate			D Replicate			Dead	
	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	Dosed	Average Dry wt. (mg)
Control plastic	9	3.12	0.312	10	3.44	0.344	10	3.41	0.341					
100% Amb. plastic	9	2.90	0.290	6	2.07	0.207	5	2.31	0.231					

Comments Samples warmed to test temperature overnight. Test Animals >24 hrs. old at test initiation. (UCDAQTXL)	Reference Weights 1 mg 1.09 10 mg 9.99 5 mg 5.01
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Technician(s):  Date: 2-28-97

Study Director: _____ Date: 3/12/97

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 2/20/97	Test ID: a2439703	Sample ID: Freeport Marina 2/18/97
End Date: 2/27/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 2/18/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-03 FHM Survival and Growth Bioassay		

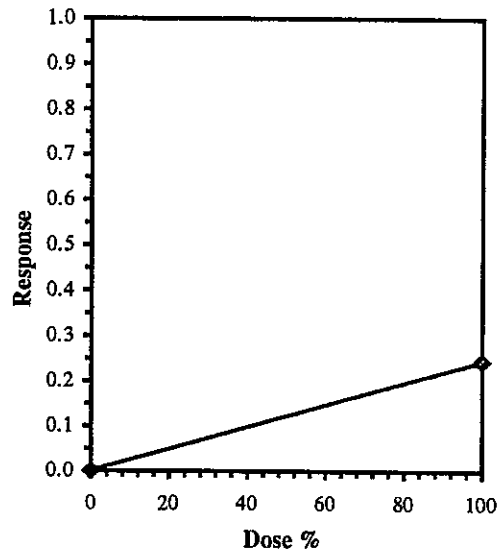
Conc-%	1	2	3
Control	0.3120	0.3440	0.3410
100	0.2900	0.2070	0.2567

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	0.3323	1.0000	0.3323	0.3120	0.3440	5.318	3				0.3323	1.0000
*100	0.2512	0.7559	0.2512	0.2070	0.2900	16.626	3	3.098	2.132	0.0015	0.2512	0.7559

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9538	0.713	-0.4345	0.24611
F-Test indicates equal variances (p = 0.30)	5.58532	199.012		
Hypothesis Test (1-tail, 0.05)				
Homoscedastic t Test indicates significant differences				

Point	Linear Interpolation (80 Resamples)			
	%	SD	95% CL(Exp)	Skew
IC05*	20.486	5.270	0.000	86.027
IC10*	40.973	10.540	0.000	172.053
IC15*	61.459			
IC20*	81.945			
IC25	> 100			
IC40	> 100			
IC50	> 100			

* indicates IC estimate less than the lowest concentration



AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	A/S FHM Comparison 97-03	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 2/18/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 21997
Initiation Date:	February 20, 1997	Termination Date:	February 27, 1997

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 2/20-2/23						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-2/20	24	8.2	7.74	68	325	90	8.1	7.60
Ambient-2/20	24	8.7	7.43	60	140	58	8.1	7.54
Control-2/21	24	7.9	7.59	68	330	92	8.1	7.59
Ambient-2/21	24	8.2	7.33	68	140	58	8.0	7.49
Control-2/22	25	7.6	7.46	66	320	86	8.2	7.81
Ambient-2/22	25	7.7	7.23	66	140	62	8.0	7.69
Control-2/23	24	8.0	7.58	66	330	88	7.6	7.76
Ambient-2/23	24	8.3	7.27	66	140	62	7.6	7.56

JLM
2/2
JL
2/2
B
2/1
B
2/1


UNIT/INSTRUMENTATION LEGEND

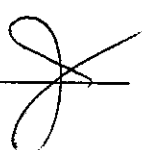
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = R/O water amended to EPAMH specification.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 2/20-23/97

Study Director:  Date: 3/1/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	A/S FHM Comparison 97-03	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 2/18/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 21997
Initiation Date:	February 20, 1997	Termination Date:	February 27, 1997

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 2/24-2/26						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM							8.1	
Control-2/24	24	8.0	7.72	68	330	91	7.6 7.47	2.1
Ambient-2/24	24	8.3	7.42	66	140	62	7.9	7.27 2/2
Control-2/25	24	8.0	7.67	77	330	84	8.4	7.76 6.1
Ambient-2/25	24	8.2	7.43	66	140	62	8.0	7.59 6.1
Control-2/26	24	8.0	7.45	77	330	84	8.4	7.66 6.1
Ambient-2/26	24	8.2	7.42	66	140	62	8.2	7.38 6.1
Control-2/26								


UNIT/INSTRUMENTATION LEGEND

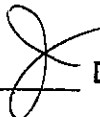
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = R/O water ammended to EPAMH specification.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 2/24-27/97

Study Director:  Date: 3/12/97

TEST 4
2/27/97

AQUA-Science
Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number: <u>CUWA 97-04</u>	Study Director: <u>J.L. Miller</u>
Protocol No.: <u>EPA 600/4-90/027</u>	Technician(s): <u>Miller/Silverman</u>
Test Material: <u>Sacramento River Ambient (Freeport Marina 2/27/97)</u>	
Test Species: <u>O. tshawytscha</u>	Animal Lot No.: <u>Nimbus 22797</u>
Initiation Date: <u>February 27, 1997</u>	Termination Date: <u>March 6, 1997</u>

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 2/27-3/2						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-2/27	13	11.0	6.76	57	100	44	9.7	6.94
Ambient-2/27	11	11.2	6.44	52	90	48	9.4	6.95
Control-2/28	14	10.6/10.5	6.80	52	90	42	9.0	6.83
Ambient-2/28	13	11.1	7.04	- [Ⓞ]	100	-	8.9	6.86
Control-3/1	13	11.2	6.96	62	90	42	8.2	6.68
Ambient-3/1	13	11.1	7.15	50	100	48	7.8	6.68
Control-3/2	13	10.2	7.10	-	85	-	8.8	6.50
Ambient-3/2	13	10.4	7.14	-	90	-	8.8	6.47

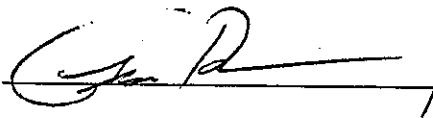
UNIT/INSTRUMENTATION LEGEND

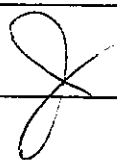
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS: Ⓞ Ambient ALK/HARD Done every other day

S.S. Control= Sierra Spring Water unammended

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 2-27-3-2

Study Director:  Date: 3/2/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-04	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 2/27/97)		
Test Species:	<i>O. tshawytscha</i>	Animal Lot No.:	Nimbus 22797
Initiation Date:	February 27, 1997	Termination Date:	March 6, 1997

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 3/3-3/5						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-3/3	13	10.6	7.11	53	80	46	9.6	6.90
Ambient-3/3	13	10.8	7.26	50	90	49	9.2	6.81
Control-3/4	13	10.9	6.90	—	100 ⁹⁵⁰	—	9.4	6.97
Ambient-3/4	13	10.9	6.85 ^{7.17}	—	100	—	9.0	6.93
Control-3/5	13	10.8	6.94	52	90	40	8.6	6.98
Ambient-3/5	13	10.8	6.92	52	100	50	8.2	6.93

6.3
6.3
6.3

UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS: ① Entry error 6.3-4.97

S.S. Control= Sierra Spring Water unammended
 All surface waters filtered through 35 µm screen daily.

Technician: Bl, Gel Date: 3/3-6/97
 Study Director: J Date: 3/2/97

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 2/27/92	Test ID: a2439704	Sample ID: Freeport Marina 2/27/97
End Date: 3/6/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 2/27/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-04 FHM Survival and Growth Bioassay		

Conc-%	1	2	3
Control	1.0000	1.0000	1.0000
100	0.9000	0.9000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	I-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	3				1.0000	1.0000
100	0.9333	0.9333	1.3034	1.2490	1.4120	7.219	3	2.000	2.920	0.0086	0.9333	0.9333

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.81394	0.713	1.36931	2.5

Equality of variance cannot be confirmed

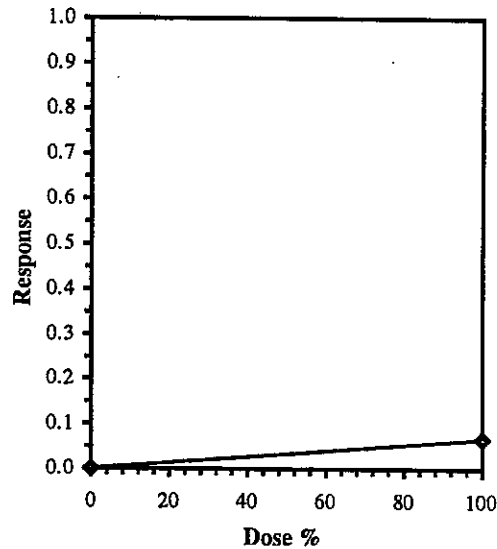
Hypothesis Test (1-tail, 0.05)

Heteroscedastic t Test indicates no significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	75.000			
IC10	> 100			
IC15	> 100			
IC20	> 100			
IC25	> 100			
IC40	> 100			
IC50	> 100			

* indicates IC estimate less than the lowest concentration



Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 2/27/92	Test ID: a2439704	Sample ID: Freeport Marina 2/27/97
End Date: 3/6/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 2/27/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-04 FHM Survival and Growth Bioassay		

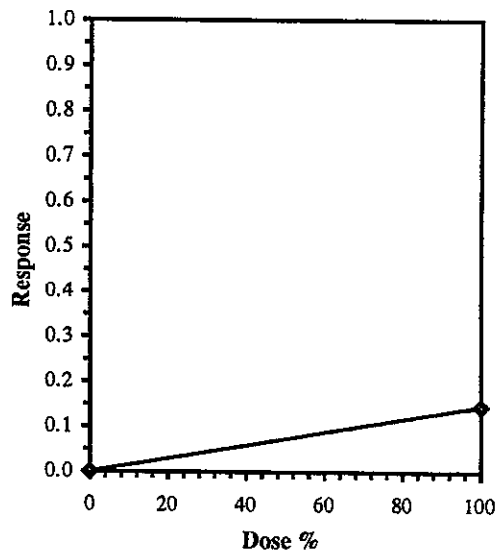
Conc-%	1	2	3
Control	0.3620	0.3440	0.3700
100	0.2900	0.2950	0.3340

Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
Control	0.3587	1.0000	0.3587	0.3440	0.3700	3.713	3				0.3587	1.0000
*100	0.3063	0.8541	0.3063	0.2900	0.3340	7.864	3	3.293	2.132	0.0005	0.3063	0.8541

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89735	0.713	0.77911	-0.6373
F-Test indicates equal variances (p = 0.47)	3.27256	199.012		
Hypothesis Test (1-tail, 0.05)				
Homoscedastic t Test indicates significant differences				

Linear Interpolation (80 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05*	34.268	11.116	0.000 140.021	2.7896
IC10*	68.535			
IC15	> 100			
IC20	> 100			
IC25	> 100			
IC40	> 100			
IC50	> 100			

* indicates IC estimate less than the lowest concentration



AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	FHM Comparison	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 2/27/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 22797
Initiation Date:	February 27, 1997	Termination Date:	March 6, 1997

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 2/27-3/2						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-2/27	24.7	8.0	7.76	77	330	84	8.4	7.64
Ambient-2/27	25.1	9.2/8.4	7.58	52	125	48	8.4	7.45
Control-2/28	24.8	7.9	7.57	— ^o	335	— ^o	8.5	7.60
Ambient-2/28	24.9	8.1	7.45	— ^o	125	— ^o	8.4	7.41
Control-3/1	24.7	8.0	7.60	68	325	84	7.0	7.55
Ambient-3/1	24.9	8.2	7.49	54	125	50	7.0	7.36
Control-3/2	24.8	8.3	7.75	—	330	—	8.1	7.90
Ambient-3/2	24.8	8.6	7.40	—	125	—	8.0	7.71

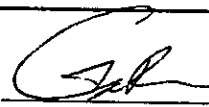
UNIT/INSTRUMENTATION LEGEND


*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS: ^o ALK/HARD MEASURED EVERY OTHER DAY FROM 2/28

Control = R/O well water Amended with EPA salts to EPAMH specifications.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 2/27 - 3/2/97

Study Director:  Date: 3/21/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	FHM Comparison	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 2/27/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 22797
Initiation Date:	February 27, 1997	Termination Date:	March 6, 1997

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 3/3-3/5						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-3/3	25	8.2	7.50	56	325	92	8.2	7.67
Ambient-3/3	25	8.1	7.33	50	125	49	8.0	7.46
Control-3/4	25	8.0	7.38	—	335	—	8.0	7.68
Ambient-3/4	24	8.2	7.27	—	125	—	8.0	7.53
Control-3/5	25	7.9	7.33	54	320	92	8.1	7.55
Ambient-3/5	24	7.7	7.23	52	125	50	8.0	7.32

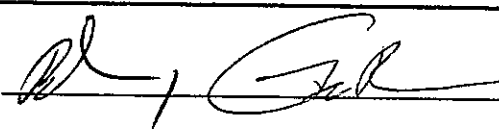
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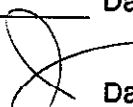
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = R/O well water Ammended with EPA salts to EPAMH specificatons.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 3/3-6/97

Study Director:  Date: 3/2/97

TEST 5
3/6/97

AQUA-Science

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MORTALITY AND BEHAVIOR OBSERVATIONS

Test Number:	CUWA 97-05	Study Director:	J. L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River at Freeport Marina 3/6/97		
Test Species:	<i>Oncorhynchus tshawytscha</i>	Animal Lot No.:	Nimbus 2/27/97
Initiation Date:	March 6, 1997	Termination Date:	March 13, 1997

Sample @ 100%	Vessel No.	CUMULATIVE MORTALITY							Comments
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Control	A	0	0	0	0	0	0	0	n=10 animals/rep
Plastic	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	Test Initiation Info. Time: 1700 Tech.: RL
	D	0	0	0	0	0	0	0	
Ambient	A	0	0	0	0	0	0	0	Test Termination Info. Time: 1420 Tech.: RL
Plastic	B	0	0	0	0	0	0	0	
	C	0	0	0	0	0	0	0	
	D	0	0	0	0	0	0	0	

Technician Initials: RL RL BS BS RL RL RL

Observation Date: 3-7-97 3-8-97 3/9 3/10 3/11 3/12 3/13

Study Director: J Date: 3/21/97

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WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-05	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 3/6/97)		
Test Species:	<i>O. tshawytscha</i>	Animal Lot No.:	Nimbus 22797
Initiation Date:	March 6, 1997	Termination Date:	March 13, 1997

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 3/6-3/9						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-3/6	13	10.8	6.83	40 ⁵⁰	90	38	10.4	6.92
Ambient-3/6	13	10.5	7.04	100 ⁴⁸	100	52	9.9	6.85
Control-3/7	13	11.2 / 10.5	6.97	54	90	46	8.7	7.03
Ambient-3/7	13	10.8 / 10.5	7.09	56	100	50	9.0	6.75
Control-3/8	13	11.4 / 10.3	7.11	58	95	47	9.0	6.96
Ambient-3/8	13	11.0 / 10.4	6.99	52	100	48	8.8	6.97
Control-3/9	13	10.2	7.19	54	90	46	9.0	7.03
Ambient-3/9	13	10.0	7.23	53	100	49	8.6	6.67


UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS: ① Entry error for 3-7-97

S.S. Control= Sierra Spring Water unammended

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 3/6 - 3/9/97

Study Director: _____ Date: 3/2/97

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WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-05	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman
Test Material:	Sacramento River Ambient (Freeport Marina 3/6/97)		
Test Species:	<i>O. tshawytscha</i>	Animal Lot No.:	Nimbus 22797
Initiation Date:	March 6, 1997	Termination Date:	March 13, 1997

Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 3/10-3/12						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
Salmon								
Control-3/10	13	10.2	7.00	54	90	46	8.6	6.87
Ambient-3/10	13	10.2	7.05	53	100	49	8.3	6.80
Control-3/11	13	10.2	6.98	54	90	46	9.0	6.91
Ambient-3/11	13	10.2	7.19	53	100	49	8.8	6.70
Control-3/12	13	10.2	6.97	54	90	46	8.7	7.19
Ambient-3/12	13	10.4	7.05	53	100	49	8.5	7.15


UNIT/INSTRUMENTATION LEGEND

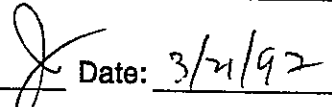
*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

S.S. Control= Sierra Spring Water unammended

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 3/10-13/97

Study Director:  Date: 3/21/97

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 3/6/97	Test ID: a2439705	Sample ID: Freeport Marina 3/6/97
End Date: 3/13/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 3/6/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-05 FHM Survival and Growth Bioassay		

Conc-%	1	2	3	4
Control	1.0000	1.0000	1.0000	0.9000
100	0.9000	0.6000	1.0000	0.5000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4				0.9750	1.0000
100	0.7500	0.7692	1.0831	0.7854	1.4120	27.345	4	1.876	1.943	0.0458	0.7500	0.7692

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96431	0.749	0.10472	-0.2765
F-Test indicates equal variances (p = 0.06)	13.2116	47.4683		

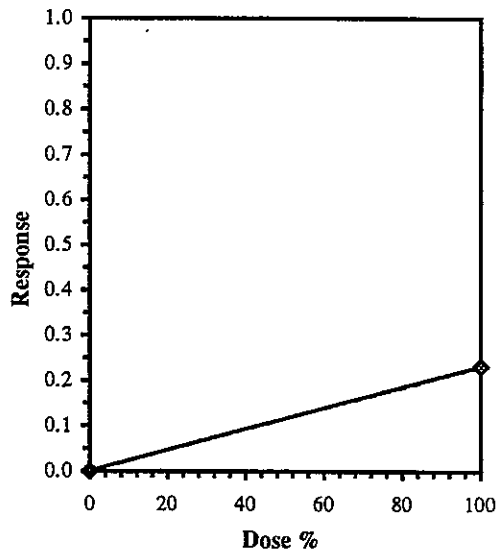
Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates no significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	21.667			
IC10*	43.333			
IC15*	65.000			
IC20*	86.667			
IC25	> 100			
IC40	> 100			
IC50	> 100			

* indicates IC estimate less than the lowest concentration



AQUA-Science
 Environmental Toxicology Consultants
FHM MORTALITY STUDY WEIGHT SHEET

Test Number:	CUWA 97-05	Study Director:	J. L. Miller
Protocol No.:	EPA 600/4-91/002	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River at Freeport Marina 3/6/97	Animal Lot No.:	AQTX:030697
Test Species:	<i>Pimephales promelas</i>	Termination Date:	March 13, 1997
Initiation Date:	March 6, 1997		

Sample ID	A Replicate			B Replicate			C Replicate			D Replicate			Dead Dosed	Average Dry wt. (mg)
	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)	No. fish	Total wt (mg)	Wt. per fish (mg)		
Control	10	3.30	0.330	10	3.52	0.352	10	3.00	0.300	9	3.16	0.316		
Ambient	9	3.39	0.339	6	2.26	0.226	10	2.86	0.286	5	1.65	0.165		
Day 0	10	0.69	0.069	10	0.79	0.079								

Technician(s):  Date: 3/14/97

Study Director: _____ Date: 3/14/97

Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 3/6/97	Test ID: a2439705	Sample ID: Freeport Marina 3/6/97
End Date: 3/13/97	Lab ID: CAAS-AQUA Science 94616	Sample Type: AMB1-Ambient water
Sample Date: 3/6/97	Protocol: EPAF 91-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: CUWA 97-05 FHM Survival and Growth Bioassay		

Conc-%	1	2	3	4
Control	0.3300	0.3520	0.3000	0.3160
100	0.3390	0.2260	0.2860	0.1650

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	0.3245	1.0000	0.3245	0.3000	0.3520	6.796	4				0.3245	1.0000
100	0.2540	0.7827	0.2540	0.1650	0.3390	29.597	4	1.800	1.943	0.0030	0.2540	0.7827

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97719	0.749	-0.105	0.92455
F-Test indicates equal variances (p = 0.07)	11.6203	47.4683		

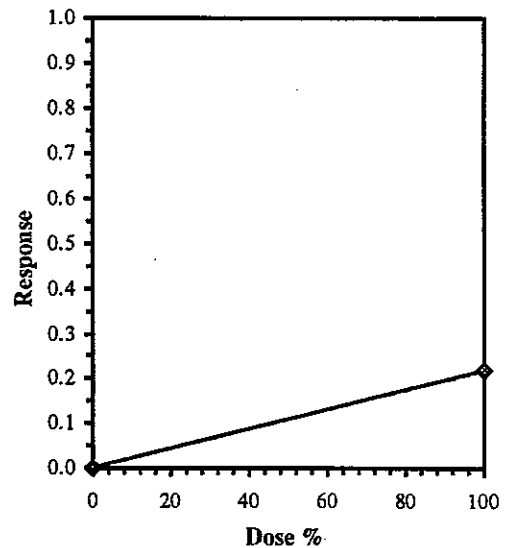
Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates no significant differences

Linear Interpolation (80 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	23.014			
IC10*	46.028			
IC15*	69.043			
IC20*	92.057			
IC25	> 100			
IC40	> 100			
IC50	> 100			

* indicates IC estimate less than the lowest concentration



AQUA-Science

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WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-05	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 1/21/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 030697
Initiation Date:	March 6, 1997	Termination Date:	March 13, 1997

Sample @ 100%	OBSERVATIONS: DAY (0-3) Date: 3/6-3/9						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-3/6	25	10.2 / 8.8	6.85	56	110	28	8.7	7.58
Ambient 3/6	25	8.3	7.26	48	100	52	8.6	7.62
Control-3/7	25	8.0	6.94	64	115	34	8.8	7.46
Ambient-3/7	25	8.1	7.40	48	130	52	8.8	7.49
Control-3/8	25	8.0	7.01	60	115	46	8.0	7.55
Ambient-3/8	25	8.2	7.32	48	130	50	8.0	7.59
Control-3/9	24	8.4	7.02	54	110	46	8.4	7.50
Ambient-3/9	24	8.6	7.28	53	125	49	8.3	7.12

M/M
 3/7
 3/8
 3/9
 3/10

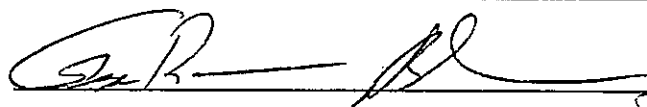
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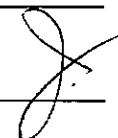
* = Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^ = Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 ** = Alkalinity (mg/L CaCO₃); HACH Test Kit ^^ = Beckman Model 11 pH Meter
 ~ = Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = Sierra Spring™ water unammended.

All surface waters filtered through 35 µm screen daily.

Technician:  Date: 3/6 - 9/97

Study Director:  Date: 2/21/97

AQUA-Science

Environmental Toxicology Consultants

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	CUWA 97-05	Study Director:	J.L. Miller
Protocol No.:	EPA 600/4-90/027	Technician(s):	Miller/Silverman/Ramos
Test Material:	Sacramento River Ambient (Freeport Marina 1/21/97)		
Test Species:	<i>Pimephales promelas</i>	Animal Lot No.:	Aquatox 030697
Initiation Date:	March 6, 1997	Termination Date:	March 13, 1997

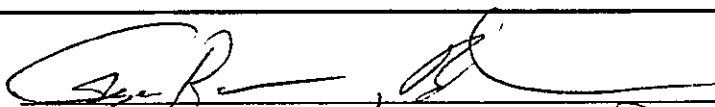
Sample @ 100%	OBSERVATIONS: DAY (4-6) Date: 3/10-3/12						48 Hour Obsv.	
	Temperature (°C)	Dissolved Oxygen *	pH ^^	Alkalinity **	Conductivity or Salinity ^	Water Hardness ~	DO	PH
FHM								
Control-3/10	24	9.5	7.08	54	110	46	8.5	7.34
Ambient 3/10	24	8.8/9.5	7.36	53	125	49	8.6	7.33
Control-3/11	25	7.7	7.00	54	110	46	8.4	7.45
Ambient-3/11	25	8.5	7.37	53	135	49	8.2	7.42
Control-3/12	24	8.2	6.86	54	110	49	8.2	7.90
Ambient-3/12	25	8.6	7.18	53	130	49	8.1	7.99

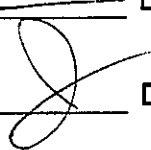
UNIT/INSTRUMENTATION LEGEND

*=Dissolved Oxygen (mg/L); YSI Model 51-8 oxygen meter ^=Conductivity/Salinity (µmhos); YSI Model 33 SCT meter
 **=Alkalinity (mg/L CaCO₃); HACH Test Kit ^^=Beckman Model 11 pH Meter
 ~=Water Hardness (mg/L CaCO₃); HACH Test Kit

ADDITIONAL COMMENTS:

Control = Sierra Spring™ water unammended.
 All surface waters filtered through 35 µm screen daily.

Technician:  Date: 3/10-12/97

Study Director:  Date: 3/24/97