

Flambeau Mining Company
Subsidiary of Kennecott Corporation
N4100 Highway 27
Ladysmith, WI 54848
(715) 532-6690
FAX (715) 532-6885

Rec'd 1-14-94

Kennecott

January 12, 1994

Mr. Tom Bauman
Department of Natural Resources
Bureau of Wastewater Management
101 S. Webster Street
P.O. Box 7921
Madison, WI 53707

RE: Flambeau Mining Company - WPDES Permit No. WI-0047376-1
Acute & Chronic Toxicity Test Battery Results

Dear Mr. Bauman:

Pursuant to Flambeau Mining Company's (Flambeau) WPDES Permit No. WI-0047376-1 Condition E. (7), Flambeau is submitting the results of acute and chronic toxicity test batteries performed on Outfall 001 discharge December 9 - 17, 1993 and acute test batteries performed December 21 - 25, 1993 and January 6 - 10, 1994. The test batteries were performed by Integrated Paper Services.

The initial test battery was performed during the period December 9 - 17, 1993. As previously reported to the Department in a letter dated December 17, 1993, two of three test species passed the acute bioassay. The Ceriodaphnia dubia at 50% effluent produced a 100% survival rate and at 100% effluent produced a 10% survival rate. Upon notification of the results Flambeau immediately began investigating the potential cause of the positive toxicity experienced by the C. dubia as discussed in a report of Flambeau's investigation dated January 11, 1994. The chronic toxicity test battery resulted in no chronic effect.

In accordance with Flambeau's WPDES permit, two additional acute test batteries were performed on Outfall 001 discharge. These test batteries were performed during the periods December 9 - 17, 1993 and January 6 - 10, 1994. The results of the two test batteries showed no evidence of toxicity according to Flambeau's WPDES Permit compliance criterion.

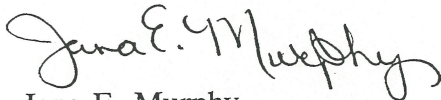
Mr. Tom Bauman

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January 12, 1994

If you should have any questions please contact me at 715-532-6690 Ext. 717.

Sincerely,
FLAMBEAU MINING COMPANY



Jana E. Murphy
Supervisor of Environmental Affairs

Enclosure

cc: Greg Fauquier, Flambeau
Larry Lynch, WDNR
Jim Hansen, WDNR
Janet Hopke, WDNR
Bernice Dukerschein, Rusk Co.
Al Christanson, City of Ladysmith
Jim Anderson, Rusk Co. Zoning
Tom Riegel, Town of Grant
Jim Hutchison, Foth & Van Dyke



Environmental and Analytical Services

1-4-94

January 5, 1994
Project 5000-601

Ms Jana E. Murphy
Flambeau Mining Company
N4100 Highway 27
Ladysmith, WI 54848

Dear Jana:

Enclosed please find the final report on the outfall 001 effluent acute and chronic test batteries (using the WDNR split samples) we performed on your behalf during December 9-17, 1993.

An acute toxicity positive result was obtained for Ceriodaphnia; all other results were toxicity negative (i.e., $\geq 50\%$ acute survival in 100% effluent and chronic IC_{25} 's \geq IWC) according to permit compliance criteria.

Supplemental tests conducted on these samples will be issued under separate cover (report in progress).

Please call if you have any questions or comments.

Best regards.

Sincerely,

David F. Sanders
Manager
Environmental Bioassessment

Enclosure

Copy w/ enclosure:

Mr. William M. West
Foth & Van Dyke
2737 S. Ridge Road
P.O. Box 19012
Green Bay, Wisconsin 54307-9012



Flambeau Mining Company
Ladysmith, Wisconsin
January 5, 1994

(IPS Project 5000-601)

Integrated Paper Services, Inc.

101 West Edison Avenue, Suite 250
P.O. Box 446
Appleton, WI 54912-0446
(414) 749-3040 FAX: (414) 749-3046

Effluent Bioassays For
Flambeau Mining Company
Ladysmith, Wisconsin

(IPS Project 5000-601)

INTRODUCTION

This report presents the results of a biological assessment of treatment plant effluent quality conducted by Integrated Paper Services, Inc. (IPS). This study was conducted on behalf of Flambeau Mining Company (Flambeau) during the period of December 9-17, 1993.

An acute/chronic bioassessment was implemented to

- 1) estimate the acute toxicity of plant effluent to the cladocerans, Ceriodaphnia dubia and Daphnia magna, and the fish, Pimephales promelas, using the definitive format of the U. S. Environmental Protection Agency (EPA) acute protocols (1), and
- 2) estimate the chronic toxicity of plant effluent diluted to an instream waste concentration (IWC) of 1% and additional concentrations of 10%, 30%, 60%, and 100% (v:v) to the cladoceran, C. dubia, and the fish, P. promelas, using a modified EPA format for chronic toxicity testing (2,3).

Acute toxicity in these tests is defined as greater than fifty percent mortality to C. dubia or D. magna at 48 hours exposure, or to P. promelas at 96 hours exposure. Chronic toxicity was determined using the EPA IC₂₅ endpoint. It is defined as a 25 percent reduction, from control, in C. dubia reproduction or in P. promelas growth (biomass = total replicate dry weight/number of larvae used to initiate the replicate) as measured using linear interpolation.

Positive test results are defined, by WPDES Permit, as acute toxicity in 100% effluent or chronic toxicity at < IWC.

METHODS

IPS methods, including test organism culture, quality assurance, sample handling, test procedures, and data analyses were in accordance with EPA and Wisconsin Department of Natural Resources (WDNR) procedures (1-4). These tests were undertaken as described in the QA/QC plan submitted pursuant to Flambeau's current WPDES Permit No. WI-00047376-1 and approved by the WDNR (changes from the original plan are in accordance with the December, 1992 modified permit).

SAMPLE COLLECTION AND PREPARATION

Effluent for the tests consisted of three composite samples [split samples from Wisconsin Department of Natural Resources (WDNR) composites]; control/dilution water was a Flambeau River grab sample. These samples were collected by Flambeau personnel (Table 1), iced and delivered to IPS within 38 hours of collection.

Table 1. Sample collection schedule for Project 5000-601.

Sample	Type	Collection Dates	Test Use Days
Effluent	12-hr Comp.	12/7	Chronic(1-2)/Acute(1-2)
Effluent	13.5-hr Comp.	12/8	Chronic(3-5)/Acute(3-4)
Effluent	14.5-hr Comp.	12/13	Chronic(6-7)
River	Grab	12/8	Chronic(1-7)/Acute(1-4)

Sample temperatures were measured and recorded upon receipt; total residual chlorine was measured on and a NH₃-N sample taken [and preserved (5) if necessary] from effluent samples upon receipt. Samples not used immediately for test initiation or solution renewal were placed in cold storage (4° C) until needed. All samples were filtered through a 63μ mesh sieve to remove interfering organisms prior to test use.

LABORATORY ANALYSIS

Laboratory procedures followed appropriate protocols and special requirements (1-3). Test conditions are summarized in Tables 2-6. Dissolved oxygen, pH, and conductivity were measured initially and after 24-hours exposure on all test solutions. Total hardness and total alkalinity were measured initially each day on control and highest effluent concentration renewal solutions. Test temperature was monitored hourly by thermocouple thermometry in all exposure areas.

Acute tests were concluded by counting the surviving organisms in each test chamber. In addition, the surviving secondary control fish were sacrificed (exposed to 70% ethanol) and total length and wet weight measurements were made to determine the loading rate.

C. dubia daily survival and reproduction data were tallied at the conclusion of the Ceriodaphnia chronic test. Upon conclusion of the P. promelas chronic test, the surviving fish in each chamber were counted, sacrificed (exposed to 70% ethanol), dried at 100° C

(minimum time 3 hours), and weighed by replicate to the nearest 0.01 mg. C. dubia reproduction and P. promelas biomass data were subjected to IC_p analysis (4).

Table 2. Summary of test conditions for the acute toxicity test with Ceriodaphnia dubia.

1. Test organism:	<u>Ceriodaphnia dubia</u> (Crustacea:Cladocera)
2. Test type:	Static renewal
3. Age of test organisms:	Less than 24 hours
4. Test chamber size:	1 oz
5. Test solution volume:	15 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	4
8. Test organisms/chamber:	5
9. Control/dilution water:	Primary - Flambeau River Secondary - Flow-through culture water
10. Effluent test concentrations:	6.25, 12.5, 25, 50 & 100% (v:v)
11. Temperature:	20 +/- 2° C
12. Feeding regime:	None
13. Aeration:	River and effluent initially to reduce D.O. supersat.
14. pH adjustment:	None
15. Test duration:	48 hours
16. Effects measured:	Mortality (Immobilization)

TEST VALIDATION AND RESULTS

PHYSICOCHEMICAL PARAMETERS

Effluent temperature upon receipt at the laboratory was $\leq 1.2^{\circ}$ C. Dechlorination of effluent samples was not necessary (total residual chlorine < 0.02 ppm). Physicochemical parameters measured

Table 3. Summary of test conditions for the acute toxicity test with Daphnia magna.

1. Test organism:	<u>Daphnia magna</u> (Crustacea:Cladocera)
2. Test type:	Static renewal
3. Age of test organisms:	Less than 24 hours
4. Test chamber size:	100 mL
5. Test solution volume:	50 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	2
8. Test organisms/chamber:	10
9. Control/dilution water:	Primary - Flambeau River Secondary - Flow-through culture water
10. Effluent test concentrations:	6.25, 12.5, 25, 50 & 100% (v:v)
11. Temperature:	20 +/- 2° C
12. Feeding regime:	None
13. Aeration:	River and effluent initially to reduce D.O. supersat.
14. pH adjustment:	None
15. Test duration:	48 hours
16. Effects measured:	Mortality (Immobilization)

during the bioassays satisfied requirements for aquatic life and the bioassays (1,2). Test exposure temperatures were within protocol specified ranges (Table 7). Chain-of-custody forms with sample collection information, chemical data sheets for the bioassays, and laboratory bench sheets are appended to this report.

Table 4. Summary of test conditions for the acute toxicity test with Pimephales promelas.

1. Test organism	<u>Pimephales promelas</u> (Osteichthyes: Cyprinidae)
2. Test type:	Static renewal
3. Age of test organisms:	Juvenile, 26 days
4. Test chamber size:	1 L
5. Test solution volume:	750 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	2
8. Test organisms/chamber:	10
9. Control/dilution water:	Primary - Flambeau River Secondary - Dechlorinated tap water
10. Effluent test concentrations:	6.25, 12.5, 25, 50 & 100% (v:v)
11. Temperature:	20 +/- 2° C
12. Feeding regime:	None
13. Aeration:	River (days 1-4) and effluent (days 1 & 2) initially to reduce D.O. supersat.
14. pH adjustment:	None
15. Test duration:	96 hours
16. Effects measured:	Mortality

Table 5. Summary of test conditions for the chronic toxicity test with Ceriodaphnia dubia.

1. Test organism:	<u>Ceriodaphnia dubia</u> (Crustacea:Cladocera)
2. Test type:	Static Renewal
3. Age of test organisms:	Less than 24 hours, all released within an 8-hour period, same generation from even-aged parents
4. Test chamber size:	1 oz (plastic cup)
5. Test solution volume:	15 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	10
8. Test organisms/chamber:	1
9. Control/dilution water:	Primary - Flambeau River Secondary - Moderately hard synthetic water
10. Effluent test concentrations:	1, 10, 30, 60 & 100% (v:v)
11. Temperature:	25 +/- 1° C
12. Feeding regime:	0.1 mL "YCT"/day 0.1 mL Algae/day
13. Aeration:	River and effluent initially to reduce DO supersat.
14. pH adjustment:	None
15. Test duration:	7 days
16. Effects measured:	Survival and reproduction
17. Endpoint:	IC ₂₅ at time of 3rd brood release by ≥ 60% of surviving test controls

Table 6. Summary of test conditions for the chronic toxicity test with Pimephales promelas.

1. Test organism:	<u>Pimephales promelas</u> (Osteichthyes: Cyprinidae)
2. Test type:	Static Renewal
3. Age of test organisms:	Larval, less than 24 hours
4. Test chamber size:	600 mL
5. Test solution volume:	250 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	4
8. Test organisms/chamber:	10
9. Control/dilution water:	Primary - Flambeau River Secondary - Moderately hard synthetic water
10. Effluent test concentrations:	1, 10, 30, 60 & 100% (v:v)
11. Temperature:	25 +/- 1° C
12. Feeding regime:	0.15 mL brine shrimp nauplii concentrate twice daily
13. Aeration:	River and effluent initially to reduce DO supersat.
14. pH adjustment:	None
15. Cleaning:	Siphoning
16. Test duration:	7 days
17. Effects measured:	Survival and growth (biomass)
18. Endpoints:	IC ₂₅ at 7 days

Table 7. A summary of temperature data (date is start of 1200 hr to 1200 hr recording period) for Project 5000-601.

	<u>Date</u>	<u>Mean</u>	<u>Max.</u>	<u>Min.</u>	
Acute Tests:	12/9	20.1	20.3	19.8	
	12/10	20.1	20.3	19.8	
	12/11	20.1	20.3	20.0	
	12/12	20.0	20.2	19.8	
Chronic Tests:					
	<u>Ceriodaphnia</u> -	12/10	25.1	25.3	25.0
		12/11	25.1	25.2	25.0
		12/12	25.1	25.3	24.9
		12/13	25.1	25.3	25.0
		12/14	25.2	25.3	25.0
		12/15	25.2	25.6	25.0
		12/16	25.1	25.6	25.0
	<u>Pimephales</u> -	12/10	25.2	25.3	25.1
		12/11	25.1	25.3	25.1
		12/12	25.2	25.3	25.1
		12/13	25.1	25.3	25.1
		12/14	25.2	25.4	25.0
		12/15	25.2	25.4	25.1
	12/16	25.1	25.3	25.0	

BIOLOGICAL PARAMETERS

Loading rate for the P. promelas acute test satisfied the ≤ 0.65 g/L range criterion for 20° C acute tests (Table 8). Primary and secondary controls for all acute tests met or exceeded the 90% survival criterion for an acceptable test (Tables 9-11).

C. dubia chronic test primary and secondary controls met or exceeded minimum performance criteria for an acceptable test, i.e., $\geq 80\%$ survival, ≥ 15 neonates/surviving adult, $\geq 60\%$ of surviving adults releasing a third brood within 7 days (Tables 12 and 13). P. promelas chronic test primary and secondary controls met or exceeded minimum performance criteria of $\geq 80\%$ survival and ≥ 0.25 mg average individual dry weight (Tables 14 and 15). Control performance and test endpoints are summarized in Table 16.

Table 8. Mean length and weight of juvenile fathead minnows used for the December 9-13, 1993 acute test.

Mean length = 13.30 mm
Mean weight = 15.45 mg
Loading factor = 0.21 g/L

Table 9. Ceriodaphnia dubia survival data for the December 9-11, 1993 acute toxicity test.

<u>Test Solution</u>	<u>Rep</u>	<u>Fatalities/Expos. Per. (HRS.)</u>		<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	
Secondary Control	A/B/C/D	0/0/0/0	0/0/0/0	100
Primary Control	A/B/C/D	0/0/0/0	0/0/0/0	100
6.25% Effluent	A/B/C/D	0/0/0/0	0/0/0/0	100
12.5%	A/B/C/D	0/0/0/0	0/0/0/0	100
25%	A/B/C/D	0/0/0/0	0/0/0/0	100
50%	A/B/C/D	0/0/0/0	0/0/0/0	100
100%	A/B/C/D	4/3/3/4	1/1/1/1	10

Table 10. Daphnia magna survival data for the December 9-11, 1993 acute toxicity test.

<u>Test Solution</u>	<u>Rep</u>	<u>Fatalities/Expos. Per. (HRS.)</u>		<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	
Secondary Control	A/B	0/0	0/0	100
Primary Control	A/B	0/0	0/0	100
6.25% Effluent	A/B	0/0	0/0	100
12.5%	A/B	0/0	0/0	100
25%	A/B	0/0	0/0	100
50%	A/B	0/0	0/0	100
100%	A/B	0/0	0/0	100

Table 11. Pimephales promelas survival data for the December 9-13, 1993 acute toxicity test.

<u>Test Solution</u>	<u>Rep</u>	Fatalities/Expos. Per. (HRS.)				<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	<u>72</u>	<u>96</u>	
Secondary Control	A/B	0/0	0/0	0/0	0/0	100
Primary Control	A/B	0/0	0/0	0/0	0/0	100
6.25% Effluent	A/B	0/0	0/0	0/0	0/0	100
12.5%	A/B	0/0	0/0	0/0	0/0	100
25%	A/B	0/0	0/0	0/0	0/0	100
50%	A/B	0/0	0/0	0/0	0/0	100
100%	A/B	0/0	0/0	0/0	0/0	100

Table 12. Ceriodaphnia dubia survival data (%) for the December 10-17, 1993 chronic test.

<u>Test Solution</u>	3 - brood <u>Endpoint (day 7)</u>
Secondary Control	100
Primary Control	100
1% Effluent	100
10%	100
30%	100
60%	90
100%	100

Table 13. Number of offspring produced per Ceriodaphnia dubia at the point of third brood release by $\geq 60\%$ of test control organisms (i.e., day 7) for the December 10-17, 1993 chronic toxicity test.

Test Solution	Neonates per Replicate										Mean
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	
Secondary Control	35	30	32	34	34	33	31	32	32	29	32.2
Primary Control	29	35	36	36	36	35	31	31	30	28	32.7
1% Eff.	28	35	32	31	30	34	33	32	37	30	32.2
10%	32	31	39	33	35	33	35	33	40	31	34.2
30%	34	32	35	22	36	34	32	31	31	34	32.1
60%	31	33	36	34	33	28	34	4	32	12	27.7
100%	30	21	36	26	32	29	33	28	30	30	29.5

Table 14. Pimephales promelas survival data (%) for the December 10-17, 1993 chronic toxicity test.

Test Solution	Replicate				Mean
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	
Secondary Control	90	100	100	89	95
Primary Control	60	100	78	100	84
1% Eff.	90	70	80	100	85
10%	70	70	30	90	65
30%	90	100	80	100	92
60%	100	90	90	100	95
100%	100	100	100	100	100

Table 15. Pimephales promelas biomass data (mean weight in parentheses) for the December 10-17, 1993 chronic toxicity test (all values are mg).

<u>Test Solution</u>	<u>Replicate</u>				<u>Mean</u>
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	
Secondary Control	0.43 (0.47)	0.60 (0.60)	0.59 (0.59)	0.54 (0.61)	0.54 (0.57)
Primary Control	0.36 (0.60)	0.62 (0.62)	0.37 (0.48)	0.63 (0.63)	0.50 (0.58)
1% Eff.	0.48	0.46	0.44	0.61	0.50
10%	0.50	0.37	0.24	0.44	0.39
30%	0.38	0.49	0.40	0.54	0.45
60%	0.47	0.44	0.46	0.41	0.44
100%	0.48	0.51	0.57	0.44	0.50

Table 16. Project 5000-601 control performance and statistical results summary.

CHRONIC TOXICITY (IWC = 1%)

Organism: <u>Ceriodaphnia dubia</u> ^a	Survival	Reprod. ^b	
Secondary Control	100%	32.2 (100)	
Primary Control	100%	32.7 (100)	
IC ₂₅ (% effluent)		Not Calculable	
Organism: <u>Pimephales promelas</u>	Survival	Mean	Mean
Secondary Control	95%	Biomass	Weight
Primary Control	84%	0.54 mg	0.57 mg
		0.50 mg	0.58 mg
IC ₂₅ (% effluent)		Not Calculable	

ACUTE TOXICITY

Organism: <u>Ceriodaphnia dubia</u>	Survival (@ 48 hours)
Secondary Control	100%
Primary Control	100%
100% Effluent	10%
Organism: <u>Daphnia magna</u>	Survival (@ 48 hours)
Secondary Control	100%
Primary Control	100%
100% Effluent	100%
Organism: <u>Pimephales promelas</u>	Survival (@ 96 hours)
Secondary Control	100%
Primary Control	100%
100% Effluent	100%

a = EPA/600/4-89/001 test endpoint, i.e., point at which $\geq 60\%$ of surviving test controls release third brood.

b = (number) is % of surviving control C. dubia producing three broods.

REFERENCES

1. Weber, C. I. (ed.). 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (fourth edition). EPA/600/4-90/027. U.S. Environmental Protection Agency, Environmental Monitoring and Support Lab., Cincinnati, OH. 293 p.
2. Weber, C. I., et al. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Second edition. EPA/600/4-89/001. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH. 258 p.
3. Quality Assurance/Quality Control Plan for whole effluent toxicity monitoring of Flambeau Mining Company's effluent in accordance with WPDES Permit requirements.
4. BOOTSTRP. Version 1.1 Program for IC₅₀ determination. USEPA-Duluth.
5. A.P.H.A. 1989. Standard Methods for the Examination of Water and Wastewater (17th edition). American Public Health Association, Washington, DC.

INTEGRATED PAPER SERVICES, INC.

Laboratory Analysis:

David Christel
Matt Harp
Tom Perzentka
Jeff Seidensticker
Todd Traeder

Laboratory Supervisor:

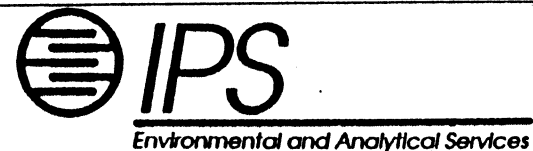
David J. Christel
David J. Christel
Aquatic Toxicologist
Environmental Bioassessment

Quality Assurance Review:

David F. Sanders
David F. Sanders
Manager
Environmental Bioassessment

Chain-of-Custody Record

Client Jana E Murphy 715 532 6690 EXT 717
Flambeau Mining Co
N4100 Hwy 27
Ladysmith, WI 54848



Return Samples To:

Integrated Paper Services, Inc.
 Aquatic Toxicology Lab
 101 W. Edison Ave., Suite 250
 Appleton, WI 54915

Sampled by:

[Signature]
 (signature)

Sample Identification	# Containers	Grab	Composite		Collection				Receipt				
		Date/Time	Start Date/Time	End Date/Time	Temp. (°C)	pH	NH ₃ -N	Cl ₂	Ice	Temp. (°C)	pH	DO	Cl ₂
① outfall (001) Disch	2	12/07/93 12:03	12/07/93 11:44 PM	2.6	7.62			y	0.2	7.0	10.4	20.01	2.4
② Flambeau River	3	12/08/93 2:00 PM		3.9	7.97			y	0.0	7.2	12.4	-	-
③													
④													
⑤													

Remarks:

Laboratory ID:

① AC1-2 CH1-2
 ② AC1-4 CH1-7

③
 ④
 ⑤

Custody Seal: ~~OK~~ Broken

Relinquished by: [Signature]
 (to carrier)

12/08/93 5:45 P
 Date/Time

Received by: Jana E. Murphy

12/8/93 4:00 PM
 Date/Time

Relinquished by: Jana E. Murphy

12/9/93 7:45 AM
 Date/Time

Received for lab: Tom Perretho

12/9/93 0745
 Date/Time

Carrier Identification (UPS, Fed-Ex, etc.)

~~Fed-Ex~~ Hand Delivered

* 12 hr composite

≤ 32 hr

Chain-of-Custody Record

Client Jana E Murphy 715 532680 EXT 217
Plambeau Mining Co ✓
N4100 Hwy 27
Lady Smith, WI 54848



Return Samples To:

Integrated Paper Services, Inc.
 Aquatic Toxicology Lab
 101 W. Edison Ave., Suite 250
 Appleton, WI 54915

Sampled by: [Signature] ✓
 (signature)

Sample Identification	Containers	Grab	Composite		Collection				Receipt					
		Date/Time	Start Date/Time	End Date/Time	Temp. (°C)	pH	NH ₃ -N	Cl ₂	Ice	Temp. (°C)	pH	DO	Cl ₂	NH ₃ -N
① outfall pool Disch ✓	2	/	12/08/93 ✓ 10:13 A	12/08/93 ✓ 11:45 P	6.2	7.30			Y	0.4	6.3	10.2	20.01	21.4 ✓
②														
③														
④														
⑤														

Remarks: _____

Laboratory ID: ③ _____
 ④ _____
 ⑤ _____

Custody Seal: OK / Broken

Relinquished by: [Signature] ✓ 12/09/93 4:00 P ✓
 (to carrier) Date/Time

Received by: _____ Date/Time

Relinquished by: _____ Date/Time

Received for lab: Judd Jaeder ✓ 12/10/93 10:10 ✓
 Date/Time

Carrier Identification (UPS, Fed-Ex, etc.) UPS ✓

* 13.5 hr composite

35h ✓

Chain-of-Custody Record

Client Jana E Murphy 215 532 6690 Ext 717

Flambeau Mining Co
N4100 Hwy 27 ✓
LadySmith, WI 54849



Environmental and Analytical Services

Sampled by: [Signature] ✓

(signature)

Return Samples To:

Integrated Paper Services, Inc.
 Aquatic Toxicology Lab
 101 W. Edison Ave., Suite 250
 Appleton, WI 54915

Sample Identification	# Containers	Grab	Composite		Collection				Receipt					
		Date/Time	Start Date/Time	End Date/Time	Temp. (°C)	pH	NH ₃ -N	Cl ₂	Ice	Temp. (°C)	pH	DO	Cl ₂	NH ₃ -N
① <u>outfall-ool Disch</u> ✓	1	↗	<u>12/13/93</u> ✓ <u>0914</u> <u>05c</u> ✓	<u>12/13/93</u> ✓ <u>0944</u> <u>05c</u> ✓	4.4	6.92			4	1.2	6.6	10.0	40.0	21.4
②														
③														
④														
⑤														

Remarks:

Laboratory ID:

① CH 6-7 ✓
 ② _____
 ③ _____
 ④ _____
 ⑤ _____

Custody Seal: OK Broken

Relinquished by: [Signature] 12-14-93 4:00p ✓
 (to carrier) Date/Time

Received by: _____ Date/Time

Relinquished by: _____ Date/Time

Received for lab: [Signature] 12-15-93 1330 ✓
 Date/Time

Carrier Identification (UPS) Fed-Ex, etc.) _____

* 14.5 hr composite

✓
 38hr

D

Four Day Bioassay Chemical Data - Initial

Project No. 5000-601 Start Date 12-9-93

Client Flambeau Mining

Test Organism P. promelas / D. magna / C. dubia

Test Solution	Parameter	Exposure Day				Comments
		1	2	3	4	
6.25% Eff. (2)	DO	8.6	7.7	8.6	8.5	✓
	pH	7.6	7.6	7.4	7.5	
	Cond.	119	119	121	123	
	Alk.					
	Hard.					
12.5% (3)	DO	8.6	7.7	8.6	8.5	✓
	pH	7.6	7.6	7.4	7.4	
	Cond.	127	125	136	139	
	Alk.					
	Hard.					
25% (4)	DO	8.6	7.8	8.6	8.6	✓
	pH	7.6	7.6	7.3	7.4	
	Cond.	142	137	165	171	
	Alk.					
	Hard.					
50% (5)	DO	8.6	7.8	8.6	8.6	✓
	pH	7.6	7.5	7.2	7.3	
	Cond.	169	165	222	231	
	Alk.					
	Hard.					
100% (6)	DO	8.7	7.9	8.6	8.7	E ↓ 1, 2 X Initially aerated d 1-2, to reduce DO supersaturation
	pH	7.5	7.4	7.2	7.2	
	Cond.	214	217	327	346	
	Alk.	68	24	24	16	
	Hard.	100	100	105	170	
	DO					
	pH					
	Cond.					
	Alk.					
	Hard.					
	DO					
	pH					
	Cond.					
	Alk.					
	Hard.					
	Initials	JS	JP	JP	JP	

Four Day Bioassay Chemical Data - Initial

Project No. 5000-601 ✓ Start Date 12-9-93 ✓

Client Flambeau Mining ✓

Test Organism P. promelas ✓ / D. magna ✓ / C. dubia ✓

Test Solution	Parameter	Exposure Day				Comments
		1	2	3	4	
Internal Control ✓ (dechlor H ₂ O) for P. promelas (e)	DO	7.6	7.5	7.7	7.8	✓
	pH	7.6	7.4	7.5	7.5	
	Cond.	195	175	182	188	
	Alk.	32	30	30	32	
	Hard.	90	90	85	85	
Internal Control ✓ (flow-thru H ₂ O) for daphnids (e)	DO	7.6	7.4	X	X	✓
	pH	7.4	7.4			
	Cond.	197	191			
	Alk.	30	28			
	Hard.	105	105			
River Control ✓ (d)	DO	8.6 ↓	7.7 ↓	8.6 ↓	8.5 ↓	R ↓ 1,2,3,4 ✓ X Initial aeration d 1-4 to reduce DO supersaturation
	pH	7.6	7.5	7.4	7.5	
	Cond.	112	111	107	107	
	Alk.	38	40	42	42	
	Hard.	60	55	55	55	
	DO					
	pH					
	Cond.					
	Alk.					
	Hard.					
	DO					
	pH					
	Cond.					
	Alk.					
	Hard.					
	Initials ✓	JS	JP	JP	JP	

JP

Two Day Bioassay Chemical Data - Final

Project No. 5000-601 Start Date 12-9-93

Client Flambeau Mining

Test Organism Ceriodaphnia dubia

Test Solution	Parameter	Exposure Day		Comments
		1	2	
Internal Control (flow-thru H ₂ O) ②	DO	7.7	8.0	✓
	pH	7.3	7.2	
	Cond.	199	197	
	Alk.			
	Hard.			
River Control ①	DO	7.8	8.6	✓
	pH	7.7	7.5	
	Cond.	109	110	
	Alk.			
	Hard.			
6.25% Effluent ②	DO	7.8	8.6	✓
	pH	7.7	7.5	
	Cond.	111	115	
	Alk.			
	Hard.			
12.5% ③	DO	7.7	8.1	✓
	pH	7.7	7.5	
	Cond.	119	121	
	Alk.			
	Hard.			
25% ④	DO	7.7	8.1	✓
	pH	7.7	7.5	
	Cond.	132	141	
	Alk.			
	Hard.			
50% ⑤	DO	7.7	8.2	✓
	pH	7.6	7.5	
	Cond.	162	166	
	Alk.			
	Hard.			
100% ⑥	DO	7.7	8.2	✓
	pH	7.6	7.4	
	Cond.	225	218	
	Alk.			
	Hard.			
Initials ✓		JP	JL	

Two Day Bioassay Chemical Data - Final

Project No. 5000-601 ✓ Start Date 12-9-93 ✓

Client Flambeau Mining ✓

Test Organism Daphnia magna ✓

Test Solution	Parameter	Exposure Day		Comments
		1	2	
Internal Control (flow-thru H ₂ O) ①	DO	7.0	7.6	✓
	pH	7.2	7.2	
	Cond.	214	199	
	Alk.			
	Hard.			
River Control ②	DO	7.0	7.8	✓
	pH	7.3	7.6	
	Cond.	110	111	
	Alk.			
	Hard.			
6.25% Effluent ③	DO	7.0	7.8	✓
	pH	7.3	7.6	
	Cond.	122	118	
	Alk.			
	Hard.			
12.5% ④	DO	7.1	7.8	✓
	pH	7.3	7.6	
	Cond.	130	131	
	Alk.			
	Hard.			
25% ⑤	DO	7.2	7.8	✓
	pH	7.2	7.6	
	Cond.	146	149	
	Alk.			
	Hard.			
50% ⑥	DO	7.3	7.9	✓
	pH	7.2	7.5	
	Cond.	175	175	
	Alk.			
	Hard.			
100% ⑦	DO	7.4	7.9	✓
	pH	7.2	7.4	
	Cond.	228	226	
	Alk.			
	Hard.			
Initials ✓		JP	JP	

JP

Four Day Bioassay Chemical Data - Final

Project No. 5000-601 ✓ Start Date 12-9-93 ✓

Client Flambew Mining ✓

Test Organism Pimephales promelas ✓

Test Solution	Parameter	Exposure Day				Comments
		1	2	3	4	
Dechlor Control ① ✓	DO	7.1	7.5	7.6	7.4	✓
	pH	7.1	7.0	7.0	7.1	
	Cond.	184	178	178	181	
	Alk. Hard.					
River Control ① ✓	DO	7.1	7.6	7.9	7.9	✓
	pH	7.2	7.2	7.2	7.2	
	Cond.	107	106	105	108	
	Alk. Hard.					
6.25% Effluent ② ✓	DO	7.1	7.6	7.9	7.9	✓
	pH	7.2	7.2	7.2	7.2	
	Cond.	112	110	117	118	
	Alk. Hard.					
12.5% ③ ✓	DO	7.1	7.6	7.9	7.9	✓
	pH	7.2	7.2	7.2	7.2	
	Cond.	122	117	121	134	
	Alk. Hard.					
25% ④ ✓	DO	7.2	7.6	8.0	7.9	✓
	pH	7.2	7.1	7.1	7.2	
	Cond.	137	129	158	160	
	Alk. Hard.					
50% ⑤ ✓	DO	7.2	7.6	8.0	8.0	✓
	pH	7.2	7.1	7.0	7.1	
	Cond.	162	155	224	219	
	Alk. Hard.					
100% ⑥ ✓	DO	7.2	7.7	8.0	8.0	✓
	pH	7.1	7.1	7.0	7.0	
	Cond.	211	206	318	319	
	Alk. Hard.					
Initials ✓		JF	JF	JF	SS	

48 HOUR ACUTE BIOASSAY SURVIVAL DATA SHEET

PROJECT: 5000-601 COMPANY: Flambeau Mining

TEST ORGANISM: Ceriodaphnia dubia AGE: <24 hrs.

START DATE/TIME: 12-9-93 1100 FINISH DATE/TIME: 12-11-93 1150

TEST BY: SS

TEST SOLUTION	REP.	FAT/SURV. PER EXPOSURE PERIOD (HRS)		TOTAL		PERCENT SURVIVAL	MEAN SURVIVAL	COMMENTS
		24	48	FAT.	SUR.			
Internal Control (flow-thru) (2)	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
River Control (1)	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
6125% Effluent (3)	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
125% (4)	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
25% (5)	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
50% (6)	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
100% (7)	A	4/1	1/0	5	0	0	10	
	B	3/2	1/1	4	1	20		
	C	3/2	1/1	4	1	20		
	D	4/1	1/0	5	0	0		
	A							
	B							
	C							
	D							

DETERMINED BY: JP JP DATA CHECK: SCM

48 HOUR ACUTE BIOASSAY SURVIVAL DATA SHEET

PROJECT: 5000-601 COMPANY: Flambeau Mining

EST ORGANISM: Daphnia magna AGE: <24 hrs.

START DATE/TIME: 12-9-93 1110 FINISH DATE/TIME: 12-11-93 1200

TEST BY: SS

TEST SOLUTION	REP.	FAT/SURV. PER EXPOSURE PERIOD (HRS)		TOTAL		PERCENT SURVIVAL	MEAN SURVIVAL	COMMENTS
		24	48	FAT.	SUR.			
Internal Control (flow-thru) ①	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
River Control ②	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
6.25% Effluent ③	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
12.5% Effluent ④	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
25% Effluent ⑤	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
50% Effluent ⑥	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
100% Effluent ⑦	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
	A							
	B							
	C							
	D							

DETERMINED BY: SP SP

DATA CHECK: DC/1/93

96 HOUR ACUTE BIOASSAY SURVIVAL DATA SHEET

PROJECT: 5000-601 ✓ COMPANY: Flambeau Mining ✓
 TEST ORGANISM: Pimephales promelas ✓ AGE: 26d ✓
 START DATE/TIME: 12-9-93 ✓, 11045 ✓ FINISH DATE/TIME: 12-13-93 ✓, 11205 ✓
 SET BY: SS ✓

TEST SOLUTION	REP.	FAT/SURV. PER EXPOSURE PERIOD (HRS)				TOTAL		PERCENT SURVIVAL	MEAN SURVIVAL	COMMENTS
		24	48	72	96	FAT.	SUR.			
Internal Control (dechlor) (C)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
River Control (1)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
6.25% Effluent (2)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
12.5% (3)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
25% (4)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
50% (5)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
100% (6)	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
	A									
	B									
	C									
	D									
DETERMINED BY: ✓		JP	JP	JP	SS					

DATA CHECK: OCB ✓

ACUTE BIOASSAY LENGTH, WEIGHT AND LOADING RATE DATA

Project: 5000-601 ✓ Company: Flintknox Mining ✓
 Test Organism: Pimephales promelas ✓ Age: 26 d ✓
 Analysis Date: 12.13.93 ✓ Completed By: SS ✓

Mean Length (mm):

	<u>Rep A</u>		<u>Rep B</u>		<u>Rep C</u>	<u>Rep D</u>
1.	<u>11</u>	<u>11</u>	<u>11</u>	<u>12</u>	_____	_____
2.	<u>12</u>	<u>12</u>	<u>12</u>	<u>11</u>	_____	_____
3.	<u>12</u>	<u>13</u>	<u>13</u>	<u>14</u>	_____	_____
4.	<u>13</u>	<u>17</u>	<u>13</u>	<u>16</u>	_____	_____
5.	<u>13</u>	<u>17</u>	<u>14</u>	<u>14</u>	_____	_____
Total	<u>131</u> ✓		<u>135</u> ✓		_____	_____

$$\text{Mean Length} = \frac{\text{Total of Reps A + B + C + D}}{\text{Total No. Organisms}}$$

$$= \frac{266 \checkmark}{20 \checkmark} = \underline{13.3 \text{ mm}} \checkmark$$

Mean Weight (mg):

	<u>Rep A</u>	<u>Rep B</u>	<u>Rep C</u>	<u>Rep D</u>
Tare+Fish	<u>1756.27</u>	<u>1763.65</u>	_____	_____
- Tare	<u>1605.82</u>	<u>1605.04</u>	_____	_____
= Fish	<u>150.45</u> ✓	<u>158.61</u> ✓	_____	_____

$$\text{Mean Weight} = \frac{\text{Total of Reps A + B + C + D}}{\text{Total No. Organisms}}$$

$$= \frac{309.06 \checkmark}{20 \checkmark} = \underline{15.45 \text{ mg}} \checkmark$$

Loading Rate (g/L):

$$= \frac{\text{Mean Weight (mg)} \times \text{Number of Organisms per Replicate}}{\text{Volume of Test Solution per Replicate (mL)}}$$

$$= \frac{15.45 \checkmark (10) / 75 \checkmark}{\text{_____}} = \underline{0.21 \checkmark} \text{ g/L}$$

Seven Day Bioassay Chemical Data - Final

Project No. 5000-601 ✓ Start Date 12-10-93 ✓

Client Flambeau Mining ✓

Test Organism Ceriodaphnia dubia ✓

Test Solution	Parameter	Exposure Day							Comments
		1	2	3	4	5	6	7	
MHSW CONTROL ② ✓	DO	7.8	7.9	8.1	7.5	7.8	7.5	8.0	✓
	pH	8.2	8.0	8.1	8.0	8.3	8.1	8.1	
	Cond.	285	275	293	295	286	266	298	
	Alk.								
	Hard.								
River Control ① ✓	DO	7.6	7.7	8.0	7.4	7.7	7.4	7.9	✓
	pH	8.1	8.0	8.0	7.9	8.2	8.0	8.0	
	Cond.	127	125	128	130	128	137	136	
	Alk.								
	Hard.								
1% Effluent ② ✓	DO	7.5	7.7	7.9	7.3	7.5	7.4	7.9	✓
	pH	8.1	7.9	7.9	7.9	8.2	8.0	8.0	
	Cond.	127	126	129	128	129	127	131	
	Alk.								
	Hard.								
10% ③ ✓	DO	7.5	7.6	7.9	7.4	7.6	7.4	7.9	✓
	pH	8.1	7.9	7.9	7.8	8.2	8.0	8.0	
	Cond.	138	138	153	152	155	141	148	
	Alk.								
	Hard.								
30% ④ ✓	DO	7.5	7.6	8.0	7.4	7.7	7.4	7.8	✓
	pH	8.0	7.9	7.8	7.8	8.1	7.9	8.0	
	Cond.	163	157	263	206	213	174	186	
	Alk.								
	Hard.								
60% ⑤ ✓	DO	7.5	7.6	8.0	7.4	7.7	7.4	7.9	✓
	pH	7.9	7.9	7.9	7.7	8.1	7.9	7.9	
	Cond.	201	198	274	278	287	214	235	
	Alk.								
	Hard.								
100% ⑥ ✓	DO	7.5	7.6	8.1	7.5	7.7	7.4	8.0	✓
	pH	7.8	7.8	7.8	7.6	7.9	7.9	7.9	
	Cond.	243	239	371	373	385	271	290	
	Alk.								
	Hard.								
Initials ✓		JP	SS	#	#	#	JS	JP	

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Ceriodaphnia Survival and Reproduction Data

PROJECT: 5000-601 ✓ COMPANY: Flambeau Mining ✓
 START DATE/TIME: 12-10-93 / 1515 FINISH DATE/TIME: 12-17-93 / 1405 ✓
 SET BY: # ✓ 13RD BROOD RELEASE: DAY 6 0 ✓ DAY 7 100 ✓
 TEST ENDPOINT: DAY 6 _____ DAY 7 ✓ ✓

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
MHSW CONTROL (C)	1	OK	G	E	7	10	18	E	35/35	3/3	0/0
	2				6	10	E	14	16/30	2/3	0/0
	3				6	11	E	15	17/32	2/3	0/0
	4				6	11	17	E	34/34	3/3	0/0
	5				6	9	E	19	15/34	2/3	0/0
	6				6	11	16	E	33/33	3/3	0/0
	7				5	10	E	16	15/34	2/3	0/0
	8				5	9		18	14/32	2/3	0/0
	9				5	8		19	13/32	2/3	0/0
	10				5	10		14	15/29	2/3	0/0
Determined by: ✓		JP	JS	#	#	#	JS	JS	20.7 / 32.2	30% 38rd d6 100% 38rd d7	0/10 / 0/10

Comments: _____

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
River Control (1)	1	OK	G	E	4	9	E	16	13/29	2/3	0/0
	2				5	11		19	16/35	2/3	0/0
	3				5	11		20	16/36	2/3	0/0
	4				5	13		18	18/36	2/3	0/0
	5				7	12		17	19/36	2/3	0/0
	6				6	11		18	17/35	2/3	0/0
	7				5	8		18	13/31	2/3	0/0
	8				4	8		19	12/31	2/3	0/0
	9				6	9		15	15/30	2/3	0/0
	10				5	8		15	13/28	2/3	0/0
Determined by: ✓		JP	JS	#	#	#	JS	JS	15.2 / 32.7	0% 38rd d6 100% 38rd d7	0/10 / 0/10

Comments: _____

Ceriodaphnia Survival and Reproduction Data

PROJECT: 5000-601 ✓ COMPANY: _____

START DATE/TIME: _____ / _____ FINISH DATE/TIME: _____ / _____

SET BY: _____ Z3RD BROOD RELEASE: DAY 6 _____ DAY 7 _____

TEST ENDPOINT: DAY 6 _____ DAY 7 ✓✓

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
1% effluent (2)	1	OK	G	E	4	8	16	E	28	3	
	2				6	11	E	18	35	3	
	3				5	10	↓	17	32	3	
	4				6	6	19	E	31	3	
	5				5	9	E	16	30	3	
	6				5	13	16	E	34	3	
	7				5	8	E	20	33	3	
	8				5	10	↓	17	32	3	
	9				6	12	↓	19	37	3	
	10	↓	↓	↓	5	6	↓	19	30	3	
Determined by: ✓	JP	JS	#	#	#	JS	SS	32.2		0/10	

Comments:

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
10% (3)	1	OK	G	E	5	13	14	E	32	3	
	2				4	10	E	17	31	3	
	3				6	14	↓	19	39	3	
	4				5	10	↓	18	33	3	
	5				6	11	↓	18	35	3	
	6				5	13	↓	15	33	3	
	7				6	13	↓	16	35	3	
	8				6	11	↓	16	33	3	
	9				6	12	42	20	40	3	
	10	↓	↓	↓	5	10	16	E	31	3	
Determined by: ✓	JP	JS	#	#	#	JS	SS	34.2		0/10	

Comments:

JS

D = Adult Dead YD = Young Dead G = Gravid E = Eyed AbY = Aborted Young

Seven Day Bioassay Chemical Data - Initial

Project No. 5000-601 ✓ Start Date 12-10-93 ✓

Client Flambeau Mining ✓

Test Organism P. promelas / C. dubia ✓

Test Solution	Parameter	Exposure Day							Comments
		1	2	3	4	5	6	7	
MHSW Control ② ✓	DO	8.0	7.9	7.9	8.2	7.9	8.0	7.6	✓
	pH	8.1	8.0	8.1	8.1	8.0	8.0	8.1	
	Cond.	272	273	270	278	265	258	261	
	Alk.	56	58	59	59	57	57	57	
	Hard.	85	85	80	85	105	85	80	
River Control ① ✓	DO	7.4 ↓	7.8 ↓	8.1 ↓	8.1 ↓	7.7 ↓	8.0 ↓	7.7 ↓	R ↓ 1-7 X Initial aeration d1-7 to reduce DO supersaturation
	pH	7.6	7.8	7.6	7.6	7.9	7.9	8.0	
	Cond.	119	121	121	114	116	118	115	
	Alk.	40	42	42	44	42	42	42	
	Hard.	55	55	55	55	55	60	60	
0.15% Effluent ② ✓	DO	7.4	7.8	8.1	8.1	7.7	8.0	8.0	✓
	pH	7.6	7.8	7.6	7.6	7.9	7.9	7.9	
	Cond.	113	117	121	116	118	118	118	
	Alk.								
	Hard.								
10% ③ ✓	DO	7.4	7.8	8.1	8.1	7.7	8.0	8.0	✓
	pH	7.7	7.8	7.6	7.6	7.8	7.9	7.9	
	Cond.	136	128	144	141	143	132	131	
	Alk.								
	Hard.								
30% ④ ✓	DO	7.4	7.8	8.0	8.1	8.0	8.0	8.0	✓
	pH	7.7	7.7	7.5	7.6	7.7	7.8	7.9	
	Cond.	140	152	196	191	198	166	159	
	Alk.								
	Hard.								
66% ⑤ ✓	DO	7.5	7.8	8.0	8.1	7.8	8.0	8.0	✓
	pH	7.7	7.7	7.4	7.6	7.6	7.8	7.8	
	Cond.	187	187	271	263	267	217	202	
	Alk.								
	Hard.								
100% ⑥ ✓	DO	7.4 ↓	8.0 ↓	7.9 ↓	8.1 ↓	7.7 ↓	8.0 ↓	8.0 ↓	E ↓ 1-7 X Initial aeration d1-7 to reduce DO supersaturation
	pH	7.7	7.6	7.3	7.5	7.6	7.7	7.8	
	Cond.	231	232	362	352	356	263	256	
	Alk.	24	24	16	16	16	22	32	
	Hard.	100	105	170	180	160	140	140	
Initials ✓		DC	JF	JF	JF	SS	MH		

12/10/93

Ceriodaphnia Survival and Reproduction Data

PROJECT: 5000-601[✓] COMPANY: _____

START DATE/TIME: _____ / _____ FINISH DATE/TIME: _____ / _____

SET BY: _____ 13RD BROOD RELEASE: DAY 6 _____ DAY 7 _____

TEST ENDPOINT: DAY 6 _____ DAY 7 ✓✓

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
30% effluent (4)	1	OK	G	E	5	12	17	E	34 [✓]	3 [✓]	1 [✓]
	2				5	10	17	E	32 [✓]	3 [✓]	
	3				5	15	15	E	35 [✓]	3 [✓]	
	4				5	4	E	13	22 [✓]	3 [✓]	
	5				7	12	↓	17	36 [✓]	3 [✓]	
	6				4	13	17	E	34 [✓]	3 [✓]	
	7				5	10	E	17	32 [✓]	3 [✓]	
	8				5	10	↓	16	31 [✓]	3 [✓]	
	9				5	9	↓	17	31 [✓]	3 [✓]	
	10	↓	↓	↓	7	12	↓	15	34 [✓]	3 [✓]	
Determined by: ✓ JP		JS	#	#	#	JS	JS	32.1 [✓]		0/10 [✓]	

Comments:

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
60% (5)	1	OK	G	E	5	10	16	E	31 [✓]	3 [✓]	
	2				6	10	17	E	33 [✓]	3 [✓]	
	3				5	12	E	19	36 [✓]	3 [✓]	
	4				6	10	18	E	34 [✓]	3 [✓]	
	5				6	10	E	17	33 [✓]	3 [✓]	
	6				3	9	↓	16	28 [✓]	3 [✓]	
	7				5	10	↓	19	34 [✓]	3 [✓]	
	8				ok	ok	↓	4	4 [✓]	1 [✓]	
	9				5	9	↓	18	32 [✓]	3 [✓]	
	10	↓	↓	↓	4	8	↓ A	AD	12 [✓]	2 [✓]	1 [✓]
Determined by: ✓ JP		JS	#	#	#	JS	JS	27.7 [✓]		1/10 [✓]	

Comments: A: possible injury ✓

JS

Ceriodaphnia Survival and Reproduction Data

PROJECT: 5000-601 ✓ COMPANY: _____

START DATE/TIME: _____ / _____ FINISH DATE/TIME: _____ / _____

SET BY: _____ 3RD BROOD RELEASE: DAY 6 _____ DAY 7 _____

TEST ENDPOINT: DAY 6 _____ DAY 7 ✓✓

Test Solution	Rep	Neonates / Exposure Day							Total Neonates Day 6 / 7	No. Broods Day 6 / 7	Mort. Day 6 / 7
		1	2	3	4	5	6	7			
100% Effluent (6)	1	OK	G	E	5	12	E	13	30 ✓	3 ✓	
	2				5	10		6	21 ✓	3 ✓	
	3				5	14		17	36 ✓	3 ✓	
	4				4	8		14	26 ✓	3 ✓	
	5				5	12		15	32 ✓	3 ✓	
	6				5	12		12	29 ✓	3 ✓	
	7				5	13		15	33 ✓	3 ✓	
	8				5	9		14	28 ✓	3 ✓	
	9				5	9		16	30 ✓	3 ✓	
	10		↓	↓	↓	5	9	↓	16	30 ✓	3 ✓
Determined by: ✓		JP	SS	#	#	#	JS	SS	29.5 ✓		0/10

Comments: ((32.7) * 25% = 24.5 ✓ (N) JS ✓

	1	2	3	4	5	6	7			
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Determined by: _____

Comments: _____

7 DAY CHRONIC LARVAL FISH BIOASSAY DATA SHEET

PROJECT: 5000-60 ✓ COMPANY: Flambeau Mining ✓

TEST ORGANISM: Pimephales promelas ✓ AGE: <24hr ✓

START DATE/TIME: 12-10-93 ✓ 1515 ✓ FINISH DATE/TIME: 12-17-93 ✓ 11520 ✓

SET BY: DJC ✓

*changed beaker

TEST SOLUTION	REP.	FATALITIES/SURVIVORS PER DAY							TOTAL		SUR. PROP.	COMMENTS
		1	2	3	4	5	6	7	FAT.	SUR.		
MHSW CONTROL (2)	A	0/10	0/10	1/9	0/9	0/9	0/9	0/9	1	9	0.9	A. set with nine ✓ $\bar{x} = 0.95$ ✓
	B	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
	C	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
	D	1/8 ^A	0/8	0/8	0/8	0/8	0/8	0/8	1	8	0.875	
River Control (1)	A	1/9	0/9	0/9	0/9	3/6*	0/6	0/6	4	6	0.6	A. one missing ✓ $\bar{x} = 0.84$ ✓
	B	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
	C	1/9	1/8	0/8	0/8	0/8	0/8	0/7	2	7	0.78	
	D	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
125% Effluent (2)	A	1/9	0/9	0/9	0/9	0/9	0/9	0/9	1	9	0.9	A. one missing ✓ $\bar{x} = 0.85$ ✓
	B	0/10	0/10	0/10	0/10	1/9	0/9	2/7	3	7	0.7	
	C	0/10	1/9	0/9	0/9	0/9	0/9	1/8	2	8	0.8	
	D	0/10	0/10	0/10	0/9 ^A	0/9	0/9	0/9	0	9	1.0	
10% (3)	A	3/7*	0/7	0/7	0/7	0/7	0/7	0/7	3	7	0.7	$\bar{x} = 0.65$ ✓
	B	0/10	0/10	0/10	0/10	0/10	1/9	2/7	3	7	0.7	
	C	0/10	0/10	0/10	0/10	6/4*	1/3	0/3	7	3	0.3	
	D	0/10	1/9	0/9	0/9	0/9	0/9	0/9	1	9	0.9	
30% (4)	A	0/10	0/10	0/10	0/10	0/10	1/9	0/9	1	9	0.9	$\bar{x} = 0.92$ ✓
	B	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
	C	0/10	0/10	0/10	0/10	1/9	0/9	1/8	2	8	0.8	
	D	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
60% (5)	A	0/11	0/11	0/11	0/11	0/11	0/11	0/11	0	11	1.0	$\bar{x} = 0.95$ ✓
	B	1/9	0/9	0/9	0/9	0/9	0/9	0/9	1	9	0.9	
	C	0/10	0/10	0/10	0/10	0/10	0/10	1/9	1	9	0.9	
	D	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
100% (6)	A	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	A. one missing ✓ $\bar{x} = 1.0$ ✓
	B	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
	C	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0	10	1.0	
	D	0/10	0/10	0/10	0/10	0/10	0/9 ^A	0/9	0	9	1.0	
	A											
	B											
	C											
	D											
DETERMINED BY: ✓	#	#	#	#	55	#	55					DATA CHECK ✓

Larval Fathead Minnow Weight Data

Project 60060 Client Frambeau Mining

Date 12-17-93 Initials SW/DC

Reference Wgts: Nominal 1.0 Actual 1.00008

Balance Calibration: Yes ✓ No 5.0 5.00004 / 5.00000

Test Solution	Rep	Pan ID	Tare + Sample (g)	Tare (g)	Sample (mg)	Biomass		Mean Wgt.		Comments
						# of Fish @ Start	Weight (mg)	# of Fish @ Term.	Weight (mg)	
MHSW	A		1.59005		1.58638	10 ✓		9 ✓		
	B		1.58763		1.58368	10 ✓		10 ✓		
	C		1.58885		1.58236	10 ✓		10 ✓		
	D		1.60133		1.59643	9 ✓		8 ✓		
River	A		1.58356		1.57917	10 ✓		6 ✓		
	B		1.58897		1.58275	10 ✓		10 ✓		
	C		1.59920		1.59585	9 ✓		7 ✓		
	D		1.59835		1.59203	10 ✓		10 ✓		
1% Euclett	A		1.57847		1.57363	10 ✓				
	B		1.57794		1.57331	10 ✓				
	C		1.58288		1.57850	10 ✓				
	D		1.58880		1.58335	9 ✓				
10% 3	A		1.58608		1.58103	10 ✓				
	B		1.58278		1.57909	10 ✓				
	C		1.58149		1.57906	10 ✓				
	D		1.58572		1.58129	10 ✓				
30% 4	A		1.59448		1.59069	10 ✓				
	B		1.59532		1.59045	10 ✓				
	C		1.59268		1.58868	10 ✓				
	D		1.58948		1.58407	10 ✓				
60% 5	A		1.59603		1.59141	11 ✓				
	B		1.59409		1.58970	10 ✓				
	C		1.59358		1.58899	10 ✓				
	D		1.59341		1.58934	10 ✓				
100% 6	A		1.59694		1.58609	10 ✓				
	B		1.58690		1.58184	10 ✓				
	C		1.58797		1.58226	10 ✓				
	D		1.58788		1.58326	9 ✓				

LARVAL FATHEAD MINNOW WEIGHT DATA

PROJECT: 5000-601

DATE: DECEMBER 11, 1993

BALANCE CALIBRATE: YES/YES

CLIENT: FLAMBEAU MINING
INITIALS: BEV/DC

REFERENCE WEIGHTS (TARE)

NOMINAL ACTUAL
1.00000 1.00008/1.00009
5.00000 5.00004/5.00003

TEST	SOLUTION REPLICATE	PAN ID	TARE+ SAMPLE(g)	TARE(g)	SAMPLE(g)	TARE(g)	BIOMASS		MEAN WEIGHT		COMMENTS	AVERAGE	
							# FISH @ START	WEIGHT (mg)	# FISH @ TERM.	WEIGHT (mg)		BIOMASS MEAN WT.	BIOMASS MEAN WT.
CONTROL (MHSW)	A	C1	1.59065	1.58638	4.27	10	0.43	9	0.47		0.54	0.57	
	B	C2	1.58963	1.58368	5.95	10	0.60	10	0.60				
	C	C3	1.58825	1.58236	5.89	10	0.59	10	0.59				
	D	C4	1.60133	1.59643	4.9	9	0.54	8	0.61				
RIVER	A	1A	1.58356	1.57997	3.59	10	0.36	6	0.60		0.50	0.58	
	B	1B	1.58897	1.58275	6.22	10	0.62	10	0.62				
	C	1C	1.59920	1.59585	3.35	9	0.37	7	0.48				
	D	1D	1.59835	1.59203	6.32	10	0.63	10	0.63				
1% EFFLUENT	A	2A	1.57847	1.57363	4.84	10	0.48				0.50		
	B	2B	1.57794	1.57331	4.63	10	0.46						
	C	2C	1.58288	1.57850	4.38	10	0.44						
	D	2D	1.58880	1.58335	5.45	9	0.61						
10% EFFLUENT	A	3A	1.58608	1.58103	5.05	10	0.51	0.50			0.39		
	B	3B	1.58278	1.57909	3.69	10	0.37						
	C	3C	1.58149	1.57906	2.43	10	0.24						
	D	3D	1.58572	1.58129	4.43	10	0.44						
30% EFFLUENT	A	4A	1.59448	1.59069	3.79	10	0.38				0.45		
	B	4B	1.59532	1.59045	4.87	10	0.49						
	C	4C	1.59268	1.58868	4	10	0.40						
	D	4D	1.58948	1.58407	5.41	10	0.54						
60% EFFLUENT	A	5A	1.59663	1.59141	5.22	11	0.47				0.44		
	B	5B	1.59409	1.58970	4.39	10	0.44						
	C	5C	1.59358	1.58899	4.59	10	0.46						
	D	5D	1.59341	1.58934	4.07	10	0.41						
100% EFFLUENT	A	6A	1.59094	1.58609	4.85	10	0.48	0.48			0.50		
	B	6B	1.58696	1.58184	5.12	10	0.51						
	C	6C	1.58797	1.58226	5.71	10	0.57						
	D	6D	1.58722	1.58326	3.96	9	0.44						

(N) 0.50 - 2576 = 0.38

REFERENCE TOXICANT DATA SUMMARY

**Integrated Paper Services, Incorporated
Aquatic Toxicology Laboratory**

Through November, 1993

Sodium Chloride Reference Toxicant LC50 Data

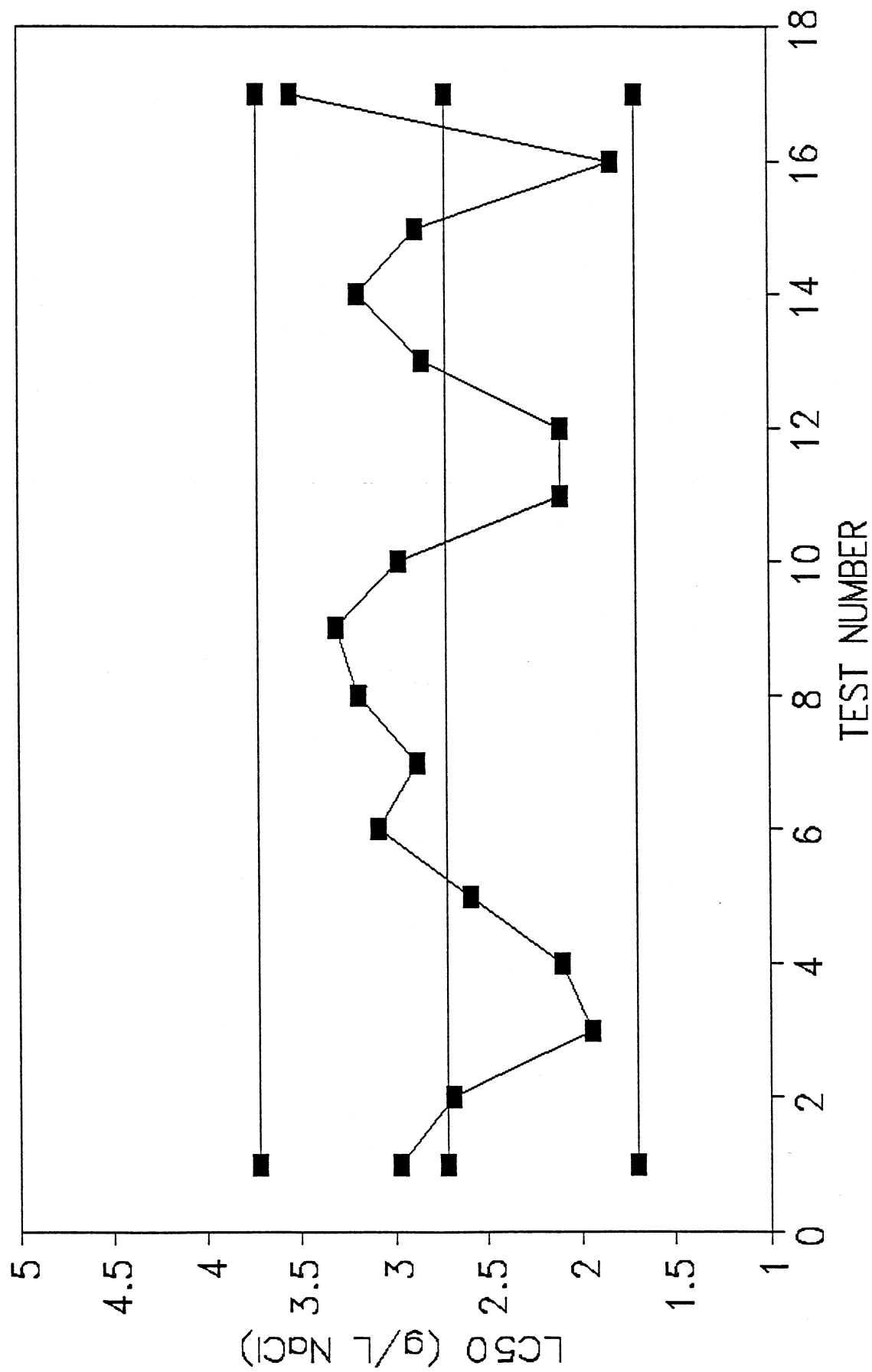
Test Duration: 48 hrs

Species: Ceriodaphnia dubia

Test No.	Test Date	LC50	
1	July 23, 92	2.97	
2	Aug. 27, 92	2.68	
3	Sept 17, 92	1.94	Mean = 2.72
4	Oct. 15, 92	2.10	-----
5	Nov. 03, 92	2.59	
6	Dec. 22, 92	3.08	
7	Jan. 29, 93	2.87	Mean + 2 Std = 3.73
8	Feb. 18, 93	3.19	-----
9	Mar. 11, 93	3.30	
10	Apr. 06, 93	2.97	
11	May 13, 93	2.10	Mean - 2 Std = 1.71
12	June 01, 93	2.10	-----
13	July 06, 93	2.84	
14	Aug. 10, 93	3.19	
15	Sept. 7, 93	2.87	
16	Oct. 12, 93	1.83	
17	Nov. 23, 93	3.54	

NaCl 48 Hour LC50 Data

Species: *Ceriodaphnia dubia*



Sodium Chloride Reference Toxicant LC50 Data

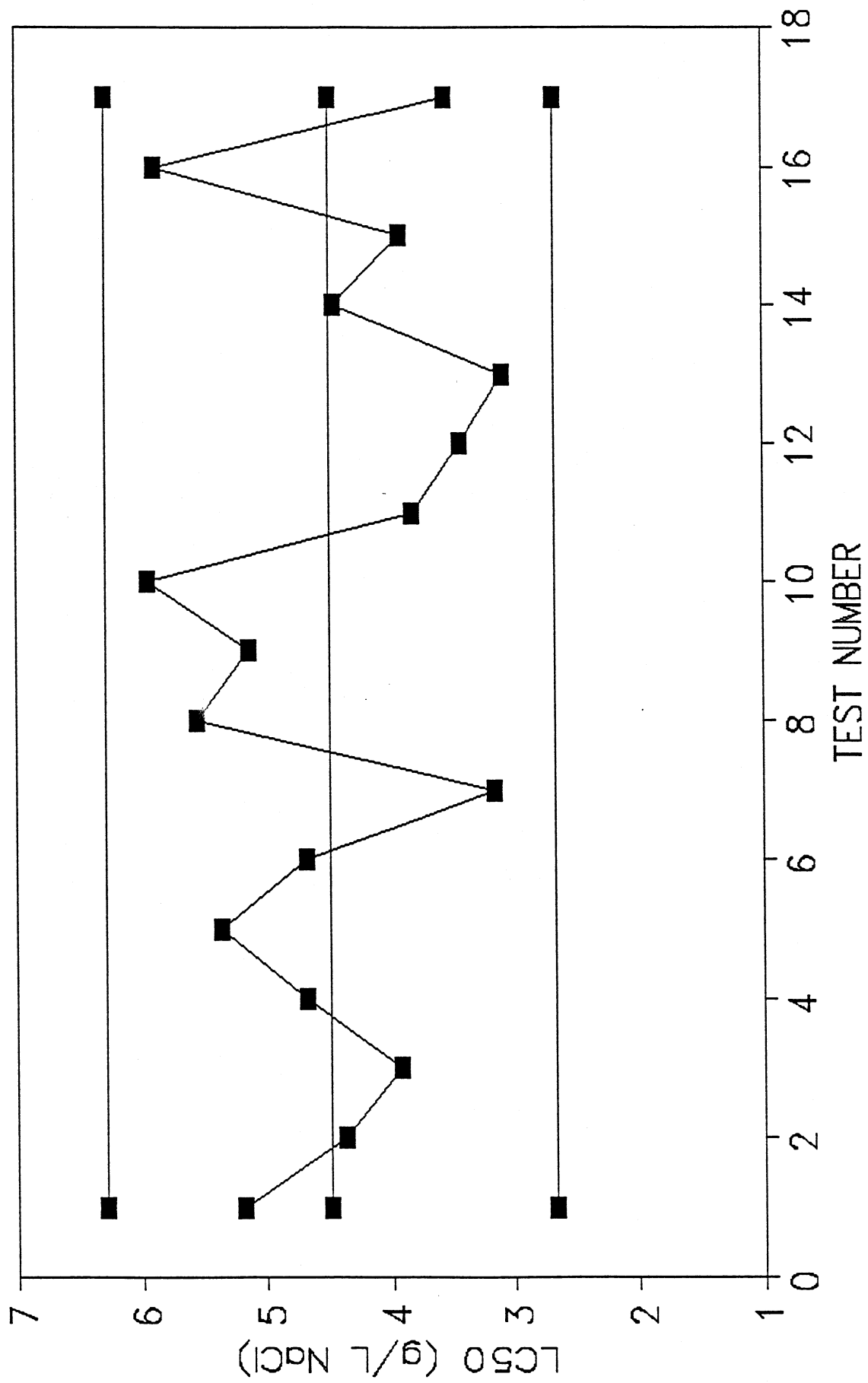
Test Duration: 48 hrs

Species: Daphnia magna

Test No.	Test Date	LC50	
1	July 23, 92	5.18	
2	Aug. 27, 92	4.35	
3	Sept 17, 92	3.92	Mean = 4.47
4	Oct. 15, 92	4.67	-----
5	Nov. 03, 92	5.36	
6	Dec. 22, 92	4.67	
7	Jan. 28, 93	3.15	Mean + 2 Std = 6.28
8	Feb. 19, 93	5.55	-----
9	Mar. 11, 93	5.14	
10	Apr. 06, 93	5.95	
11	May 13, 93	3.82	Mean - 2 Std = 2.67
12	June 01, 93	3.42	-----
13	July 06, 93	3.08	
14	Aug. 10, 93	4.44	
15	Sept. 7, 93	3.92	
16	Oct. 12, 93	5.89	
17	Nov. 23, 93	3.54	

NaCl 48 Hour LC50 Data

Species: *Daphnia magna*



Sodium Chloride Reference Toxicant LC50 Data

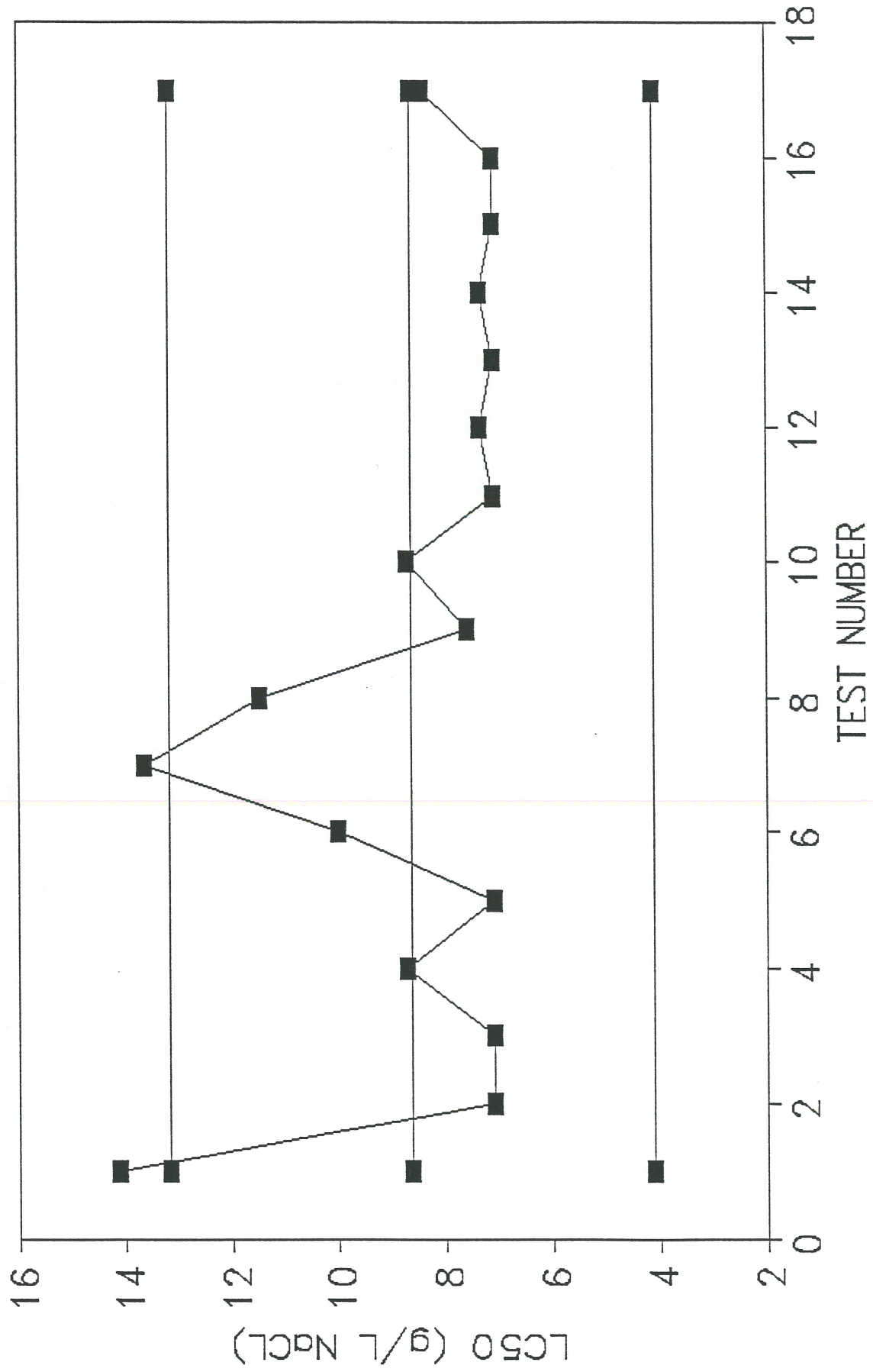
Species: Pimephales promelas

Test Duration: 96 Hours

Test No.	Test Date	LC50	
1	July 23, 92	14.14	
2	Aug. 27, 92	7.07	
3	Sept 17, 92	7.07	Mean = 8.64
4	Oct. 15, 92	8.71	-----
5	Nov. 03, 92	7.07	
6	Dec. 20, 92	10.00	
7	Jan. 26, 93	13.66	Mean + 2 Std = 13.17
8	Feb. 19, 93	11.49	-----
9	Mar. 11, 93	7.58	
10	Apr. 06, 93	8.71	
11	May 13, 93	7.07	Mean - 2 Std = 4.10
12	June 01, 93	7.32	-----
13	July 06, 93	7.07	
14	Aug. 10, 93	7.32	
15	Sept. 7, 93	7.07	
16	Oct. 21, 93	7.07	
17	Nov. 23, 93	8.41	

NaCl 96 Hour LC50 Data

Species: *Pimephales promelas*



Sodium Chloride Reference Toxicant IC25 Data

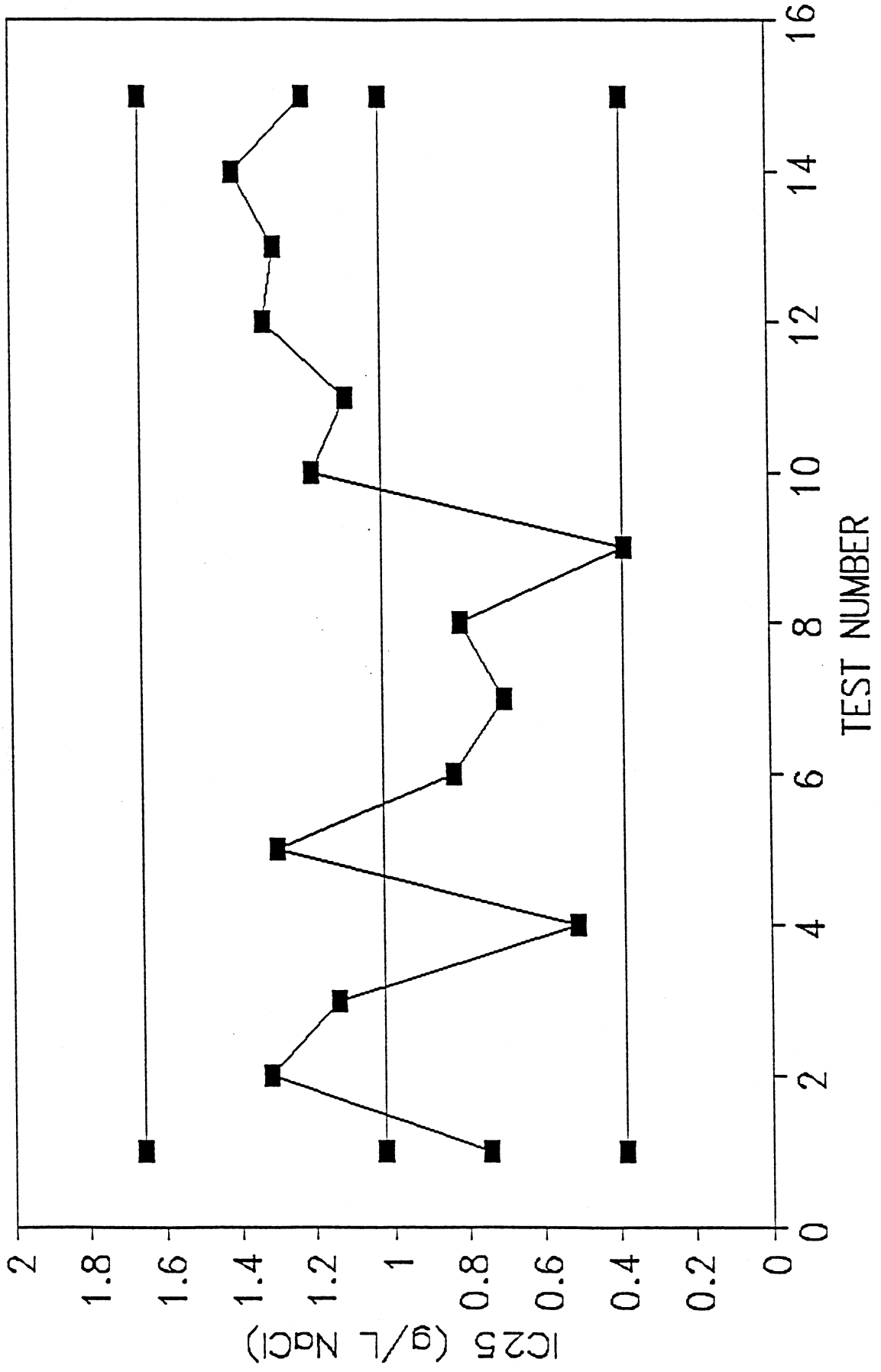
Test Duration: Seven Days

Species: Ceriodaphnia dubia

Test No.	Test Date	IC25	
1	Jan. 14, 91	0.74	
2	May 14, 91	1.32	
3	July 05, 91	1.14	Mean = 1.02
4	Nov. 07, 91	0.51	-----
5	Jan. 07, 92	1.30	
6	May 28, 92	0.83	
7	Sept 29, 92	0.70	Mean + 2 Std = 1.66
8	Nov. 19, 92	0.81	-----
9	Feb. 02, 93	0.38	
10	Jun. 01, 93	1.20	
11	July 06, 93	1.11	Mean - 2 Std = 0.38
12	Aug. 10, 93	1.33	-----
13	Sept. 7, 93	1.30	
14	Oct. 12, 93	1.41	
15	Nov. 23, 93	1.22	

NaCl IC25 Data

Species: *Ceriodaphnia dubia*



Sodium Chloride Reference Toxicant IC25 Data

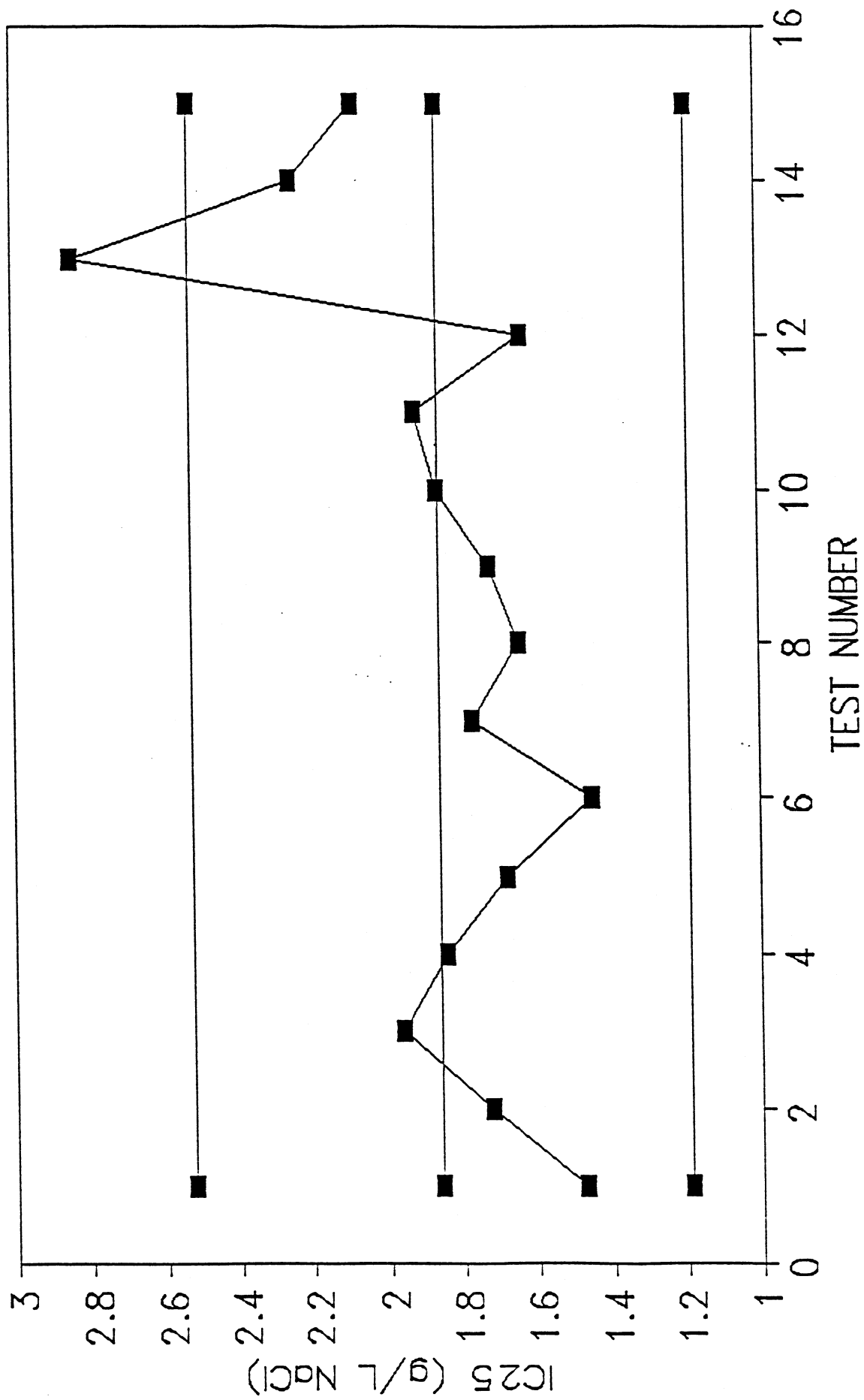
Test Duration: Seven Days

Species: Pimephales promelas

Test No.	Test Date	IC25	
1	Jan. 14, 91	1.47	
2	May 14, 91	1.72	
3	July 05, 91	1.96	Mean = 1.86
4	Nov. 07, 91	1.84	-----
5	Jan. 07, 92	1.68	
6	May 28, 92	1.45	
7	Sept 29, 92	1.77	Mean + 2 Std = 2.52
8	Nov. 19, 92	1.64	-----
9	Feb. 02, 93	1.72	
10	Jun. 01, 93	1.86	
11	July 06, 93	1.92	Mean - 2 Std = 1.19
12	Aug. 10, 93	1.63	-----
13	Sept. 7, 93	2.84	
14	Oct. 12, 93	2.25	
15	Nov. 23, 93	2.08	

NaCl IC25 Data

Species: *Pimephales promelas*



1-14-94



Flambeau Mining Company
Ladysmith, Wisconsin
January 11, 1994

(IPS Project 5000-611)

Integrated Paper Services, Inc.

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Effluent Bioassays For
Flambeau Mining Company
Ladysmith, Wisconsin

(IPS Project 5000-611)

INTRODUCTION

This report presents the results of a biological assessment of treatment plant effluent quality conducted by Integrated Paper Services, Inc. (IPS). This study was conducted on behalf of Flambeau Mining Company (Flambeau) during the period of January 6-10, 1994, and represents the second of two retests subsequent to a positive acute toxicity result in the December 9-12, 1993, Wisconsin Department of Natural Resources (WDNR) split-sample test batteries.

An acute test format bioassessment was implemented to estimate the acute toxicity of outfall 001 effluent to the cladocerans, Ceriodaphnia dubia and Daphnia magna, and the fish, Pimephales promelas, using the definitive format of the U. S. Environmental Protection Agency (EPA) acute test protocols (1).

Acute toxicity in these tests is defined as greater than fifty percent mortality to C. dubia or D. magna at 48 hours exposure, or to P. promelas at 96 hours exposure. A positive test result is defined, by WPDES Permit, as acute toxicity in 100% effluent.

METHODS

IPS methods, including test organism culture, quality assurance, sample handling, test procedures, and data analyses were in accordance with EPA and WDNR procedures (1,2). These tests were undertaken as described in the QA/QC plan submitted pursuant to Flambeau's current WPDES Permit No. WI-00047376-1 and approved by the WDNR (changes from the original plan are in accordance with the December, 1992 modified permit).

SAMPLE COLLECTION AND PREPARATION

Effluent for the tests consisted of two 16-hour composite samples; control/dilution water was a Flambeau River grab sample. These samples were collected by Flambeau personnel (Table 1), kept cool and delivered to IPS within 37 hours of collection.

Sample temperatures were measured and recorded upon receipt; total residual chlorine was measured on and a NH₃-N sample taken [and preserved (3) if necessary] from effluent samples upon receipt. Samples not used immediately for test initiation or solution

renewal were placed in cold storage (4° C) until needed. All samples were filtered through a 63 μ mesh sieve to remove interfering organisms prior to test use.

Table 1. Sample collection schedule for Project 5000-611.

Sample	Type	Collection Dates	Test Use Days
Effluent	16-hr Comp.	1/4	Acute(1-2)
Effluent	16-hr Comp.	1/5	Acute(3-4)
Flambeau River	Grab	1/5	Acute(1-4)

LABORATORY ANALYSIS

Bioassay test procedures followed acute bioassay protocols and special requirements (1,2); chemical parameter measurements followed the equipment manufacturer's instructions or A.P.H.A. methods (3). Test conditions are summarized in Tables 2-4.

Dissolved oxygen, pH, and conductivity were measured initially and after 24-hours exposure on all test solutions. Total hardness and total alkalinity were measured daily on the control and highest effluent concentration renewal solutions. Test temperature was monitored hourly by thermocouple thermometry in the exposure areas.

Acute tests were concluded by counting the surviving organisms in each test chamber. In addition, the surviving secondary control fish were sacrificed (exposed to 70% ethanol) and total length and wet weight measurements were made to determine the loading rate.

TEST VALIDATION AND RESULTS

PHYSICOCHEMICAL PARAMETERS

Effluent temperature upon receipt at the laboratory was $\leq 1.2^\circ$ C. Dechlorination of effluent samples was not necessary (total residual chlorine < 0.02 ppm). Physicochemical parameters measured as part of the bioassays satisfied requirements for aquatic life and the bioassays (1,2). Test temperatures were within protocol specified ranges (Table 5). Chain-of-custody forms with sample collection information, chemical data sheets, and laboratory bench sheets are appended to this report.

BIOLOGICAL PARAMETERS

Loading rate for the P. promelas acute test satisfied the ≤ 0.65 g/L range criterion for 20° C acute tests (Table 6). Primary and secondary controls for all acute tests met or exceeded the 90% survival criterion for an acceptable test (Tables 7-9).

Table 2. Summary of test conditions for the acute toxicity test with Ceriodaphnia dubia.

1. Test organism:	<u>Ceriodaphnia dubia</u> (Crustacea:Cladocera)
2. Test type:	Static renewal
3. Age of test organisms:	Less than 24 hours
4. Test chamber size:	30 mL
5. Test solution volume:	15 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	4
8. Test organisms/chamber:	5
9. Control/dilution water:	Primary - Flambeau River Secondary - Flow-through culture water
10. Effluent test concentrations:	6.25, 12.5, 25, 50 & 100% (v:v)
11. Temperature:	20 +/- 2° C
12. Feeding regime:	None
13. Aeration:	River and effluent initially to reduce DO supersaturation
14. pH adjustment:	None
15. Test duration:	48 hours
16. Effects measured:	Mortality (immobilization)

Table 3. Summary of test conditions for the acute toxicity test with Daphnia magna.

1. Test organism:	<u>Daphnia magna</u> (Crustacea:Cladocera)
2. Test type:	Static renewal
3. Age of test organisms:	Less than 24 hours
4. Test chamber size:	100 mL
5. Test solution volume:	50 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	2
8. Test organisms/chamber:	10
9. Control/dilution water:	Primary - Flambeau River Secondary - Flow-through culture water
10. Effluent test concentrations:	6.25, 12.5, 25, 50 & 100% (v:v)
11. Temperature:	20 +/- 2° C
12. Feeding regime:	None
13. Aeration:	River and effluent initially to reduce DO supersaturation
14. pH adjustment:	None
15. Test duration:	48 hours
16. Effects measured:	Mortality (immobilization)

Table 4. Summary of test conditions for the acute toxicity test with Pimephales promelas.

1. Test organism	<u>Pimephales promelas</u> (Osteichthyes: Cyprinidae)
2. Test type:	Static renewal
3. Age of test organisms:	Juvenile, 22 days
4. Test chamber size:	1 L
5. Test solution volume:	750 mL
6. Renewal of test solutions:	Daily
7. Replicate chambers/treatment:	2
8. Test organisms/chamber:	10
9. Control/dilution water:	Primary - Flambeau River Secondary - Dechlorinated tap water
10. Effluent test concentrations:	6.25, 12.5, 25, 50 & 100% (v:v)
11. Temperature:	20 +/- 2° C
12. Feeding regime:	None
13. Aeration:	River and effluent initially to reduce DO supersaturation
14. pH adjustment:	None
15. Test duration:	96 hours
16. Effects measured:	Mortality

Table 5. A summary of temperature data (date is start of 1200 hr to 1200 hr recording period) for Project 5000-611.

<u>Date</u>	<u>Mean</u>	<u>Max.</u>	<u>Min.</u>
1/6	20.3	20.5	20.1
1/7	20.1	20.3	19.7
1/8	20.2	20.3	19.8
1/9	20.2	20.3	20.1

Table 6. Mean length and weight of juvenile fathead minnows used for the January 6-10, 1994 acute test.

Mean length = 11.30 mm
Mean weight = 4.91 mg
Loading factor = 0.07 g/L

Table 7. Ceriodaphnia dubia survival data for the January 6-8, 1994 acute toxicity test.

<u>Test Solution</u>	<u>Rep</u>	<u>Fatalities/Expos. Per. (HRS.)</u>		<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	
Secondary Control	A/B/C/D	0/0/0/0	0/0/0/0	100
Primary Control	A/B/C/D	0/0/0/0	0/0/0/0	100
6.25% Effluent	A/B/C/D	0/0/0/0	0/0/0/0	100
12.5%	A/B/C/D	0/0/0/0	0/0/0/0	100
25%	A/B/C/D	0/0/0/0	0/0/0/0	100
50%	A/B/C/D	0/0/0/0	0/0/0/0	100
100%	A/B/C/D	0/0/0/0	0/0/0/0	100

Table 8. Daphnia magna survival data for the January 7-9, 1994 acute toxicity test.

<u>Test Solution</u>	<u>Rep</u>	<u>Fatalities/Expos. Per. (HRS.)</u>		<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	
Secondary Control	A/B	0/0	0/0	100
Primary Control	A/B	0/0	0/0	100
6.25% Effluent	A/B	0/0	0/0	100
12.5%	A/B	0/0	0/0	100
25%	A/B	0/0	0/0	100
50%	A/B	0/0	0/0	100
100%	A/B	0/0	0/0	100

Table 9. Pimephales promelas survival data for the January 6-10, 1994 acute toxicity test.

<u>Test Solution</u>	<u>Rep</u>	<u>Fatalities/Expos. Per. (HRS.)</u>				<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	<u>72</u>	<u>96</u>	
Secondary Control	A/B	0/0	0/0	0/0	0/0	100
Primary Control	A/B	0/1	0/0	0/0	0/0	95
6.25% Effluent	A/B	1/0	0/0	0/0	0/0	95
12.5%	A/B	0/0	0/0	0/0	0/0	100
25%	A/B	0/0	0/0	0/0	0/0	100
50%	A/B	0/0	0/0	0/0	0/0	100
100%	A/B	0/0	0/0	0/0	0/0	100

REFERENCES

1. Weber C. I. (ed.). 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (fourth edition). EPA/600/4-90/027. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory., Cincinnati, OH. 293 p.
2. Quality Assurance/Quality Control Plan for whole effluent toxicity monitoring of Flambeau Mining Company's effluent in accordance with WPDES Permit requirements.
3. A.P.H.A. 1989. Standard Methods for the Examination of Water and Wastewater (17th edition). American Public Health Association, Washington, DC.

INTEGRATED PAPER SERVICES, INC.

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Chain-of-Custody Record



Environmental and Analytical Services

Integrated Paper Services, Inc.
 Aquatic Toxicology Lab
 101 W. Edison Ave., Suite 250
 Appleton, WI 54915

Client Jana E Murphy 715-532-6690 GYF 717

Fleming Mining Co
N400 Hwy 27

Lady Smith, WI 54848

Sampled by: [Signature]
 (signature)

Return Samples To:

Sample Identification	# Containers	Grab		Composite		Collection			Receipt				
		Date/Time	Start Date/Time	End Date/Time	Temp. (°C)	pH	NH ₃ -N	Cl ₂	Ice	Temp. (°C)	pH	DO	Cl ₂
① Outfall - cool Disch	1	/	01-04-94 ✓ 8:00 A	01-04-94 ✓ 11:50 PM	3.2	8.01		Y	0.1	6.6	11.4	20.01	<1.4
② Flambeau River	2	01-05-94 ✓ 1:15 PM	/	/	3.4	8.07		Y	0.1	6.7	11.0	—	—
③													
④													
⑤													

Remarks: ✓ 16hr composite

Laboratory ID: ③
① AC 1-2 ✓
② AC 1-4 ✓

Custody Seal: ① / Broken

Relinquished by: [Signature] ✓ 01-05-94 5:30 PM ✓
 (to carrier) Date/Time

Received by: _____ Date/Time ✓

Relinquished by: _____ Date/Time

Received for lab: Tom Pennington ✓ 1-6-94 / 1030
 Date/Time

Carrier Identification (UPS, Fed-Ex, etc.) Fed-Ex

✓
 ≤ 35hr
 ✓
 NC

Chain-of-Custody Record

Client Jana E Murphy 715522-4490-54717
Flambeau Mining Co.
N 4100 Hwy 27
Lady Smith, WI 54848
 Sampled by: *Jana E Murphy*
 (signature)

IPS
 Environmental and Analytical Services
 Integrated Paper Services, Inc.
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 101 W. Edison Ave., Suite 250
 Appleton, WI 54915

Return Samples To:

Sample Identification	# Containers	Grab		Composite		Collection				Receipt				
		Date/Time	Start Date/Time	End Date/Time	Temp. (°C)	pH	NH ₃ -N	Cl ₂	Ice	Temp. (°C)	pH	DO	Cl ₂	NH ₃ -N
① outflow/disch	1		1-5-94 ✓ 8:00 A	1-5-94 ✓ 11:50 P	4.2	7.62			4	1.2	6.8	10.3	<0.01	<1.4
②														
③														
④														
⑤														

Remarks: ✓
 - 16 hr composite

Laboratory ID: ① AC 3-4 ✓
②
③
④
⑤

Custody Seal: OK / Broken

Relinquished by: *Jana E Murphy* ✓ Date/Time 1-6-94 4:00 P
 (to carrier)

Received by: _____ Date/Time _____

Relinquished by: _____ Date/Time _____

Received for lab: Tom Payne ✓ Date/Time 1-7-94/1230

Carrier Identification (UPS, Fed-Ex, etc.) UPS ✓

③37hr

Four Day Bioassay Chemical Data - Initial

Project No. 5000-611 ✓ Start Date 1-6-94 ✓

Client Flambeau Mining ✓

Test Organism P. promelas ✓ / C. dubia ✓

Test Solution	Parameter	Exposure Day				Comments
		1	2	3	4	
Control (Dechlor) ①	DO	8.0	7.8	7.9	8.0	✓
	pH	7.5	7.4	7.3	7.3	
	Cond.	191	186	196	195	
	Alk.	33	32	33	32	
	Hard.	105	115	115	120	
River ②	DO	8.2 ↓	8.6 ↓	8.5 ↓	8.9 ↓	R ↓ 1-4 ✓
	pH	7.4	7.3	7.3	7.3	X Initial aeration d 1-4 to reduce DO supersaturation
	Cond.	117	116	120	120	
	Alk.	48	44	44	44	
	Hard.	50	90	90	90	
Effluent 6.25% ③	DO	8.4	8.5	8.6	8.9	✓
	pH	7.4	7.4	7.4	7.3	
	Cond.	127	127	127	127	
	Alk.					
	Hard.					
12.5% ④	DO	8.5	8.5	8.7	8.9	✓
	pH	7.4	7.4	7.4	7.3	
	Cond.	137	137	135	136	
	Alk.					
	Hard.					
25% ⑤	DO	8.5	8.4	8.7	8.9	✓
	pH	7.4	7.4	7.4	7.4	
	Cond.	157	158	153	155	
	Alk.					
	Hard.					
50% ⑥	DO	8.6	8.3	8.8	8.9	✓
	pH	7.4	7.4	7.4	7.4	
	Cond.	196	199	187	189	
	Alk.					
	Hard.					
100% ⑦	DO	8.7 ↓	8.6 ↓	8.2 ↓	8.8 ↓	E ↓ 1-4 ✓
	pH	7.3	7.4	7.4	7.3	X Initial aeration d 1-4 to reduce DO supersaturation
	Cond.	268	280	250	251	
	Alk.	50	46	50	48	
	Hard.	125	140	140	145	
Initials ✓		JH	JH	JS	JS	

2 of 2 ✓

Two Day Bioassay Chemical Data - Initial

Project No. 5000-611 ✓

Start Date 1-6-94 ✓

Client Flambeau ✓

Test Organism C. dubia ✓

Test Solution	Parameter	Exposure Day		Comments
		1	2	
Control (Flow-thru) Daphnids only (C) ✓	DO	7.5	7.7	✓
	pH	7.4	7.3	
	Cond.	196	196	
	Alk.	27	28	
	Hard.	105	105	
	DO			
	pH			
	Cond.			
	Alk.			
	Hard.			
	DO			
	pH			
	Cond.			
	Alk.			
	Hard.			
	DO			
	pH			
	Cond.			
	Alk.			
	Hard.			
	Initials ✓	JH	JH	

Handwritten initials/signature

Two Day Bioassay Chemical Data - Initial

Project No. 5000-611 ✓ Start Date 1-7-94 ✓

Client Flambeau ✓

Test Organism Daphnia magna ✓

Test Solution	Parameter	Exposure Day		Comments
		1	2	
Control (F.T.) (1)	DO	7.7	8.0	✓
	pH	7.3	7.3	
	Cond.	196	209	
	Alk.	28	31	
	Hard.	105	120	
RIVER	DO	8.6 ↓	8.5 ↓	R ↓ 1, 2 ✓ X Initial aeration d 1-2 to reduce DO supersaturation
	pH	7.3	7.3	
	Cond.	116	120	
	Alk.			
	Hard.			
6.25% Effluent (2)	DO	8.5	8.5	✓
	pH	7.4	7.4	
	Cond.	127	136	
	Alk.			
	Hard.			
12.5% (3)	DO	8.5	8.5	✓
	pH	7.4	7.4	
	Cond.	137	140	
	Alk.			
	Hard.			
25% (4)	DO	8.4	8.5	✓
	pH	7.4	7.4	
	Cond.	158	156	
	Alk.			
	Hard.			
50% (5)	DO	8.3	8.5	✓
	pH	7.4	7.4	
	Cond.	199	197	
	Alk.			
	Hard.			
100% (6)	DO	8.6 ↓	8.5 ↓	E ↓ 1, 2 ✓ X Initial aeration d 1-2 to reduce DO supersaturation
	pH	7.4	7.4	
	Cond.	281	280	
	Alk.	46	50	
	Hard.	140	140	
	Initials ✓	FS	FS	

Two Day Bioassay Chemical Data - Final

Project No. 5000-611 ✓ Start Date 1-6-94 ✓

Client Flambeau ✓

Test Organism Ceriodophnia dubia ✓

Test Solution	Parameter	Exposure Day		Comments
		1	2	
Control (F.T.) ①	DO	7.9	7.9	✓ /
	pH	7.1	7.1	
	Cond.	207	199	
	Alk.			
	Hard.			
River ②	DO	8.0	7.9	✓ /
	pH	7.3	7.3	
	Cond.	127	114	
	Alk.			
	Hard.			
6.25% Effluent ③	DO	7.9	8.0	✓ /
	pH	7.4	7.4	
	Cond.	130	122	
	Alk.			
	Hard.			
12.5% ④	DO	7.9	8.0	✓ /
	pH	7.4	7.4	
	Cond.	136	131	
	Alk.			
	Hard.			
25% ⑤	DO	7.9	8.0	✓ /
	pH	7.4	7.4	
	Cond.	159	160	
	Alk.			
	Hard.			
50% ⑥	DO	7.9	8.0	✓ /
	pH	7.4	7.4	
	Cond.	197	199	
	Alk.			
	Hard.			
100% ⑦	DO	8.2	8.1	✓ /
	pH	7.6	7.6	
	Cond.	303	293	
	Alk.			
	Hard.			
Initials ✓		JP	SS	

✓
OC

Two Day Bioassay Chemical Data - Final

Project No. 5000-611 ✓

Start Date 1-7-94 ✓

Client Flambeau ✓

Test Organism Daphnia magna ✓

Test Solution	Parameter	Exposure Day		Comments
		1	2	
Control (F.T.) ①	DO	7.4	7.6	✓ ✓
	pH	7.2	7.3	
	Cond.	206	197	
	Alk.			
	Hard.			
River ②	DO	7.4	7.5	✓ ✓
	pH	7.4	7.4	
	Cond.	129	126	
	Alk.			
	Hard.			
6.25% Effluent ③	DO	7.3	7.5	✓ ✓
	pH	7.4	7.4	
	Cond.	135	130	
	Alk.			
	Hard.			
12.5% ④	DO	7.3	7.5	✓ ✓
	pH	7.4	7.4	
	Cond.	146	140	
	Alk.			
	Hard.			
25% ⑤	DO	7.3	7.4	✓ ✓
	pH	7.4	7.4	
	Cond.	165	155	
	Alk.			
	Hard.			
50% ⑥	DO	7.5	7.5	✓ ✓
	pH	7.4	7.4	
	Cond.	206	195	
	Alk.			
	Hard.			
100% ⑦	DO	7.7	7.6	✓ ✓
	pH	7.4	7.4	
	Cond.	275	261	
	Alk.			
	Hard.			
Initials ✓		JS	JS	

Four Day Bioassay Chemical Data - Final

Project No. 5600-611 Start Date 1-6-94

Client Flambeau

Test Organism Pimephales promelas

Test Solution	Parameter	Exposure Day				Comments
		1	2	3	4	
Control (Dechlor) ①	DO	7.2	7.4	7.7	7.5	✓ ✓
	pH	7.2	7.1	7.1	7.1	
	Cond.	192	186	184	189	
	Alk.					
	Hard.					
River ①	DO	7.3	7.5	7.8	7.6	✓ ✓
	pH	7.3	7.2	7.2	7.2	
	Cond.	118	118	117	114	
	Alk.					
	Hard.					
6.25% Effluent ②	DO	7.3	7.5	7.8	7.6	✓ ✓
	pH	7.3	7.2	7.2	7.2	
	Cond.	126	121	121	129	
	Alk.					
	Hard.					
12.5% ③	DO	7.4	7.7	7.8	7.5	✓ ✓
	pH	7.3	7.3	7.3	7.2	
	Cond.	136	131	130	129	
	Alk.					
	Hard.					
25% ④	DO	7.3	7.7	7.6	7.2	✓ ✓
	pH	7.3	7.3	7.2	7.2	
	Cond.	154	149	144	146	
	Alk.					
	Hard.					
50% ⑤	DO	7.5	7.7	7.0	6.6	✓ ✓
	pH	7.3	7.2	7.2	7.1	
	Cond.	187	186	175	177	
	Alk.					
	Hard.					
100% ⑥	DO	7.8	8.0	7.5	6.8	✓ ✓
	pH	7.4	7.2	7.2	7.1	
	Cond.	256	252	240	243	
	Alk.					
	Hard.					
Initials ✓		JH	JS	SS	JH	

48 HOUR ACUTE BIOASSAY SURVIVAL DATA SHEET

PROJECT: 5000-611 ✓ COMPANY: Flambeau ✓

TEST ORGANISM: Ceriodaphnia dubia ✓ AGE: 224 hrs ✓

START DATE/TIME: 1-6-94 ✓, 1345 ✓ FINISH DATE/TIME: 1-8-94 ✓, 1155 ✓

SET BY: JH ✓

TEST SOLUTION	REP.	FAT./SURV. PER EXPOSURE PERIOD (HRS)		TOTAL		PERCENT SURVIVAL	MEAN SURVIVAL	COMMENTS
		24	48	FAT.	SUR.			
Control (F.T.) ①	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
River ②	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
6.25% Effluent ③	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
12.5% ④	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
25% ⑤	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
50% ⑥	A	0/5	0/5	0	5	100	100	
	B	0/5	0/5	0	5	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
100% ⑦	A	0/5	0/5	0	5	100	100	
	B	0/4	0/4	0	4	100		
	C	0/5	0/5	0	5	100		
	D	0/5	0/5	0	5	100		
	A							
	B							
	C							
	D							
DETERMINED BY:	JH		JS					DATA CHECK <u>DC</u>

48 HOUR ACUTE BIOASSAY SURVIVAL DATA SHEET

PROJECT: 5000-611 ✓ COMPANY: Flambeau ✓

TEST ORGANISM: Daphnia magna ✓ AGE: 224 hrs ✓

START DATE/TIME: 1-7-94 ✓, 1500 ✓ FINISH DATE/TIME: 1-9-94 ✓, 1310 ✓

SET BY: # ✓

TEST SOLUTION	REP.	FAT./SURV. PER EXPOSURE PERIOD (HRS)		TOTAL		PERCENT SURVIVAL	MEAN SURVIVAL	COMMENTS
		24	48	FAT.	SUR.			
Control (F.T.) (1)	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
River (1)	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
6.25% Effluent (2)	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
12.5% (3)	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
25% (4)	A	0/9	0/9	0	9	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
50% (5)	A	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
100% (6)	A	0/9	0/9	0	9	100	100	
	B	0/10	0/10	0	10	100		
	C							
	D							
	A							
	B							
	C							
	D							
DETERMINED BY: ✓		JS	JS					DATA CHECK <u>DC/MS</u>

96 HOUR ACUTE BIOASSAY SURVIVAL DATA SHEET

PROJECT: 5000-611 ✓ COMPANY: Flambeau ✓
 TEST ORGANISM: Pimephales promelas ✓ AGE: 22 days ✓
 START DATE/TIME: 1-6-94 ✓, 1415 ✓ FINISH DATE/TIME: 1-10-94 ✓, 1445 ✓
 SET BY: JH ✓

TEST SOLUTION	REP.	FAT/SURV. PER EXPOSURE PERIOD (HRS)				TOTAL		PERCENT SURVIVAL	MEAN SURVIVAL	COMMENTS
		24	48	72	96	FAT.	SUR.			
Control (Dechlor) ①	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
River ②	A	0/10	0/10	0/10	0/10	0	10	100	95	
	B	1/9	0/9	0/9	0/9	1	9	90		
	C									
	D									
6.25% Effluent ③	A	1/9	0/9	0/9	0/9	1	9	90	95	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
12.5% ④	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
25% ⑤	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
50% ⑥	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
100% ⑦	A	0/10	0/10	0/10	0/10	0	10	100	100	
	B	0/10	0/10	0/10	0/10	0	10	100		
	C									
	D									
	A									
	B									
	C									
	D									
DETERMINED BY: ✓ JH										DATA CHECK ✓ JH

ACUTE BIOASSAY LENGTH, WEIGHT AND LOADING RATE DATA

Project: 5000-611 ✓ Company: Flambeau ✓
 Test Organism: Pimephales promelas ✓ Age: 22 days
 Analysis Date: 1-10-94 ✓ Completed By: JH ✓

Mean Length (mm):

	Rep A		Rep B		Rep C	Rep D
1.	<u>11</u>	<u>11</u>	<u>13</u>	<u>11</u>	_____	_____
2.	<u>12</u>	<u>11</u>	<u>10</u>	<u>11</u>	_____	_____
3.	<u>13</u>	<u>12</u>	<u>11</u>	<u>10</u>	_____	_____
4.	<u>14</u>	<u>10</u>	<u>11</u>	<u>11</u>	_____	_____
5.	<u>14</u>	<u>10</u>	<u>10</u>	<u>10</u>	_____	_____
Total	<u>118</u> ✓		<u>108</u> ✓		_____	_____

$$\text{Mean Length} = \frac{\text{Total of Reps A + B + C + D}}{\text{Total No. Organisms}}$$

$$= \frac{226}{20} = 11.30 \text{ mm}$$

Mean Weight (mg):

	Rep A	Rep B	Rep C	Rep D
Tare+Fish	<u>1.66328g</u> ✓	<u>1.62683g</u> ✓	_____	_____
- Tare	<u>1.60217g</u> ✓	<u>1.58971g</u> ✓	_____	_____
= Fish	<u>61.11mg</u> ✓	<u>37.12mg</u> ✓	_____	_____

$$\text{Mean Weight} = \frac{\text{Total of Reps A + B + C + D}}{\text{Total No. Organisms}}$$

$$= \frac{98.23}{20} = 4.91 \text{ mg}$$

Loading Rate (g/L):

$$= \frac{\text{Mean Weight (mg)} \times \text{Number of Organisms per Replicate}}{\text{Volume of Test Solution per Replicate (mL)}}$$

$$= \frac{4.91(10)}{750} = \frac{0.06}{0.07 \text{ M}} \text{ g/L}$$

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REFERENCE TOXICANT DATA SUMMARY

**Integrated Paper Services, Incorporated
Aquatic Toxicology Laboratory**

Through December, 1993

Sodium Chloride Reference Toxicant LC50 Data

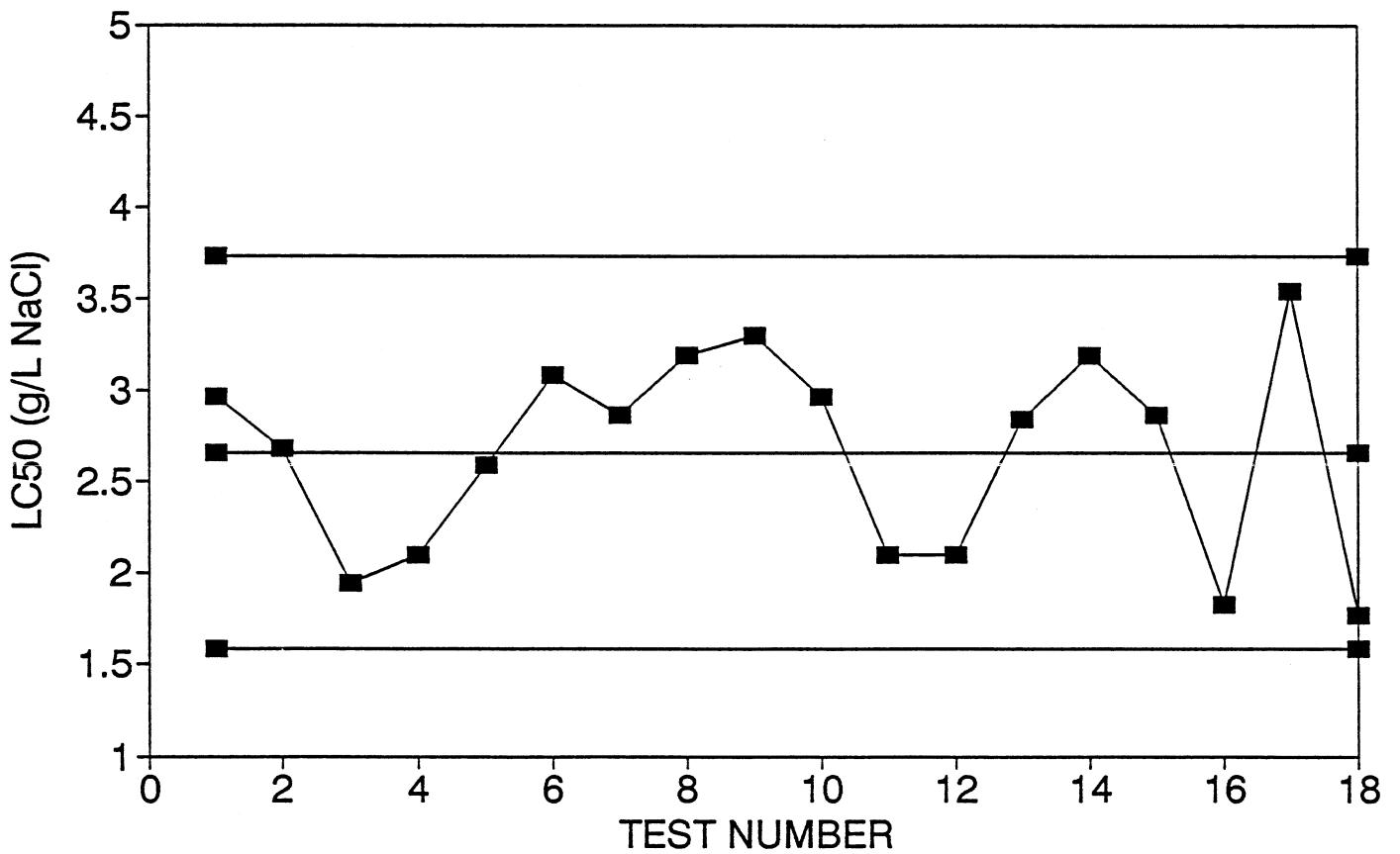
Test Duration: 48 hrs

Species: Ceriodaphnia dubia

Test No.	Test Date	LC50	
1	July 23, 92	2.97	
2	Aug. 27, 92	2.68	
3	Sept 17, 92	1.94	Mean = 2.66
4	Oct. 15, 92	2.10	-----
5	Nov. 03, 92	2.59	
6	Dec. 22, 92	3.08	
7	Jan. 29, 93	2.87	Mean + 2 Std = 3.74
8	Feb. 18, 93	3.19	-----
9	Mar. 11, 93	3.30	
10	Apr. 06, 93	2.97	
11	May 13, 93	2.10	Mean - 2 Std = 1.59
12	June 01, 93	2.10	-----
13	July 06, 93	2.84	
14	Aug. 10, 93	3.19	
15	Sept. 7, 93	2.87	
16	Oct. 12, 93	1.83	
17	Nov. 23, 93	3.54	
18	Dec. 28, 93	1.77	

NaCl 48 Hour LC50 Data

Species: *Ceriodaphnia dubia*



Sodium Chloride Reference Toxicant LC50 Data

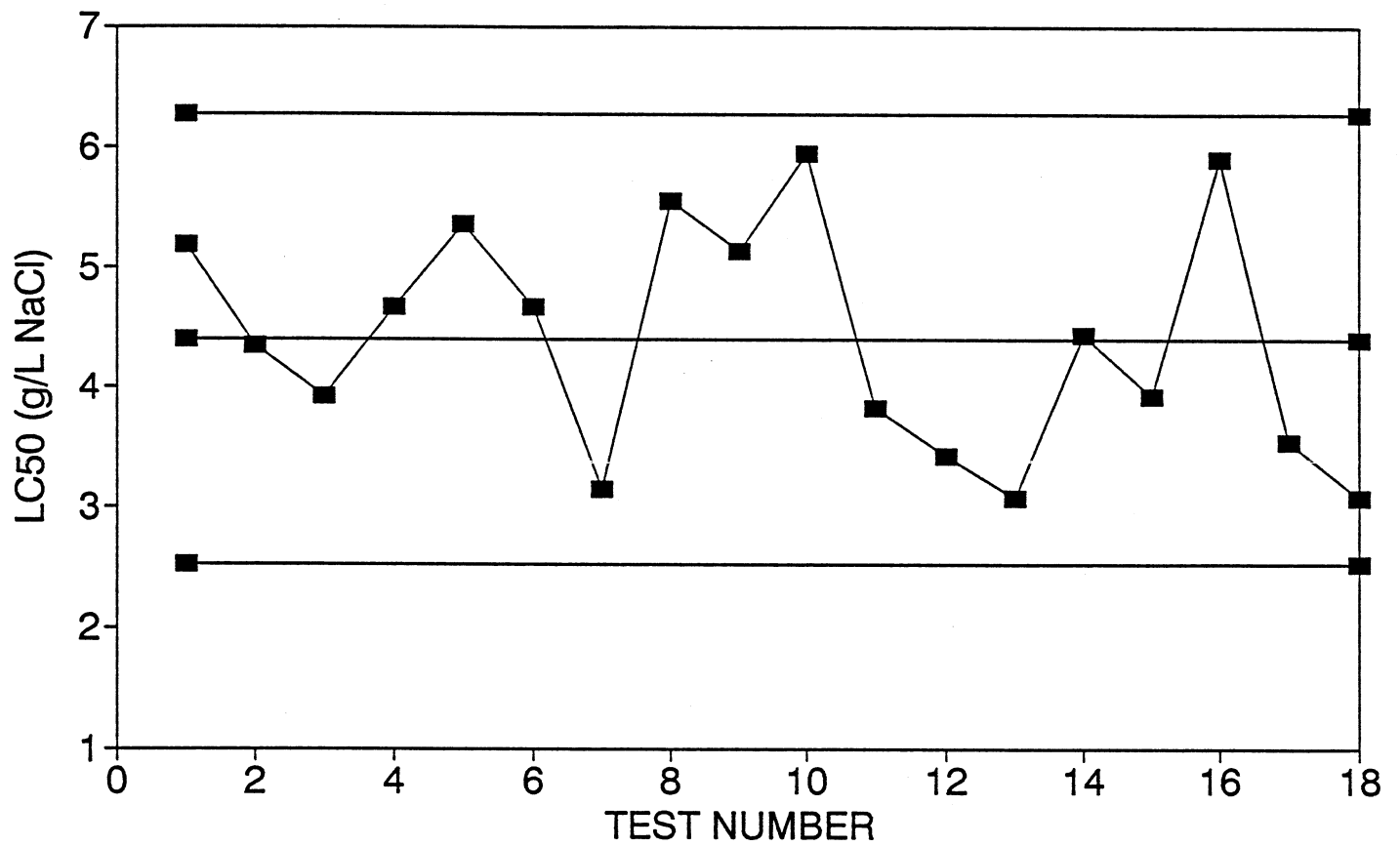
Test Duration: 48 hrs

Species: Daphnia magna

Test No.	Test Date	LC50	
1	July 23, 92	5.18	
2	Aug. 27, 92	4.35	
3	Sept 17, 92	3.92	Mean = 4.40
4	Oct. 15, 92	4.67	-----
5	Nov. 03, 92	5.36	
6	Dec. 22, 92	4.67	
7	Jan. 28, 93	3.15	Mean + 2 Std = 6.26
8	Feb. 19, 93	5.55	-----
9	Mar. 11, 93	5.14	
10	Apr. 06, 93	5.95	
11	May 13, 93	3.82	Mean - 2 Std = 2.53
12	June 01, 93	3.42	-----
13	July 06, 93	3.08	
14	Aug. 10, 93	4.44	
15	Sept. 7, 93	3.92	
16	Oct. 12, 93	5.89	
17	Nov. 23, 93	3.54	
18	Dec 28, 93	3.08	

NaCl 48 Hour LC50 Data

Species: *Daphnia magna*



Sodium Chloride Reference Toxicant LC50 Data

Species: Pimephales promelas

Test Duration: 96 Hours

Test No.	Test Date	LC50	
1	July 23, 92	14.14	
2	Aug. 27, 92	7.07	
3	Sept 17, 92	7.07	Mean = 8.66
4	Oct. 15, 92	8.71	-----
5	Nov. 03, 92	7.07	
6	Dec. 20, 92	10.00	
7	Jan. 26, 93	13.66	Mean + 2 Std = 13.07
8	Feb. 19, 93	11.49	-----
9	Mar. 11, 93	7.58	
10	Apr. 06, 93	8.71	
11	May 13, 93	7.07	Mean - 2 Std = 4.25
12	June 01, 93	7.32	-----
13	July 06, 93	7.07	
14	Aug. 10, 93	7.32	
15	Sept. 7, 93	7.07	
16	Oct. 21, 93	7.07	
17	Nov. 23, 93	8.41	
18	Dec. 28, 93	9.01	

NaCl 96 Hour LC50 Data

Species: *Pimephales promelas*

