

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

**Kennecott
 Minerals**

May 30, 1995

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
 April 1995 Acute Toxicity Test Battery Results

Dear Mr. Bauman:

Pursuant to the Flambeau Mining Company (Flambeau) WPDES Permit No. WI-0047376-1 Condition E. (7), Flambeau is submitting the toxicity test battery results performed on Outfall 001 discharge. The tests were performed by Integrated Paper Services and CH2M Hill during the period April 12 - 16, 1995. The results summarized in the table below show no evidence of toxicity within Flambeau's effluent in accordance with Flambeau's WPDES Permit compliance criterion. The IPS and CH2M Hill test reports are enclosed.

APRIL 1995 ACUTE BIOASSAY TEST RESULT SUMMARY

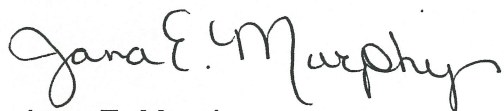
Test Solution	Mean % Survival					
	<i>C. dubia</i>		<i>D. magna</i>		<i>P. promelas</i>	
	IPS	Hill	IPS	Hill	IPS	Hill
Secondary Control	100	100	100	100	100	100
Primary Control	100	95	95	100	100	100
Effluent 6.25%	100	100	100	100	100	100
12.50%	100	100	95	100	95	100
25%	100	100	100	100	100	100
50%	100	100	100	100	100	100
100%	*	90	100	100	100	100

*Multiple testing was performed within 100% effluent with the following per cent survival: 100, 90, 54, 56, and 95.

Mr. Tom Bauman
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May 30, 1995

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

Sincerely,



Jana E. Murphy
Supervisor of Environmental Affairs

Enclosure

cc: Tom Myatt, Flambeau
Larry Lynch, WDNR
Jim Hansen, WDNR
Janet LaRose, WDNR
Ken Markart, WDNR
Bernice Dukerschein, Rusk Co.
Al Christianson, City of Ladysmith
Melvin Spencer, Rusk Co. Zoning
Tom Riegel, Town of Grant
Jim Hutchison, Foth & Van Dyke

BIOASSAY REPORT

ACUTE TOXICITY TESTS
Conducted April 12 through 16, 1995

Prepared for
Flambeau Mining Company
Ladysmith, Wisconsin

Prepared by

CH2M HILL
Bioassay Laboratory
15779 West Ryerson Road
New Berlin, Wisconsin 53151

Lab I.D. No. 655
May 1995

Summary

CH2M HILL conducted acute toxicity tests on effluent samples provided by Flambeau Mining Company, Ladysmith, Wisconsin. The bioassays were conducted from April 12 through 16, 1995, as part of NPDES compliance monitoring for the State of Wisconsin. *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows were used as the test organisms. The following is a summary of the test results:

Test Media	Acute Toxicity		
	<i>Ceriodaphnia dubia</i>	<i>Daphnia magna</i>	Fathead Minnow
Flambeau River Control	Pass	Pass	Pass
100 % Effluent	Pass	Pass	Pass
Laboratory Control	Pass	Pass	Pass

For NPDES compliance purposes, the tests results show that:

- The effluent samples were not acutely toxic to *Ceriodaphnia dubia*, *Daphnia magna*, or fathead minnows at 100 percent concentrations using the 50 percent lethality criteria.
- Flambeau River and laboratory control data were acceptable in all bioassays.

Introduction

This report presents the results of laboratory acute toxicity tests conducted by CH2M HILL on effluent samples provided by Flambeau Mining Company, Ladysmith, Wisconsin. The bioassays used *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows as the test organisms and were performed from April 12 through 16, 1995, as part of NPDES compliance biomonitoring for the State of Wisconsin.

Methods

All laboratory methods, including organism culture, sample handling, test procedures, and data analyses, were in accordance with the recommendations of the United States Environmental Protection Agency (EPA) [1, 3], the CH2M HILL Bioassay Laboratory's Standard Operating Procedures, and the Wisconsin Department of Natural Resources (DNR) biomonitoring requirements as specified in the Flambeau Mining Company NPDES permit.

Sample Collection and Handling

Photocopies of the chain-of-custody forms are included in Appendix B. Two composite effluent samples and one receiving water grab sample were used as follows:

Description	Sample No.	Date Collected	Date Tested
Flambeau River	655.01	4/11	4/12-4/16
Effluent	655.02	4/11	4/12-4/14
Effluent	655.03	4/12	4/14-4/16

All samples were collected by Flambeau Mining Company personnel and delivered on ice the same day or by overnight courier to the CH2M HILL Bioassay Laboratory. Upon arrival, samples were logged in and physicochemical characterizations were conducted. Samples not immediately prepared for testing were refrigerated (4°C) for later use.

Test Organisms

All test organisms were cultured at the CH2M HILL Milwaukee Laboratory.

Test Procedures

Bioassays

Bioassay test conditions are summarized in Tables 1 through 3.

Physicochemical Monitoring

Total alkalinity, hardness, and total ammonia were measured initially on each sample. Total residual chlorine was measured initially on each effluent sample. Total alkalinity and hardness were measured once in the laboratory control media.

Dissolved oxygen (DO), pH, and conductivity were measured initially and daily thereafter in all test treatment renewals. DO and pH were measured in one test chamber or composite of each 24-hour-old test solution.

Bioassay incubator temperature was electronically monitored hourly by a thermocouple and data logger, and a 24-hour summary of mean values was recorded.

Data Analysis

Pass/fail criteria were applied to acute toxicity data.

Toxicity was defined according to the DNR criteria:

- < 50 percent survival of test organisms at test termination (48 hours for *Ceriodaphnia dubia* and *Daphnia magna*, and 96 hours for fathead minnow).

Quality Assurance

Part of the quality assurance and quality control (QA/QC) program at the CH2M HILL Milwaukee Laboratory includes the performance of organisms concurrently tested in laboratory media. Tables 1 through 3 present the test acceptability criteria for laboratory control data. The results of the laboratory control tests are listed in Table 4.

In addition, other QA/QC procedures include performing monthly reference toxicant tests using reagent-grade sodium chloride. The results of reference toxicant tests conducted during the past 12 months on the appropriate test organisms are summarized in Appendix C.

Table 1
Summary of Test Conditions for the
***Ceriodaphnia* Acute Bioassay**
Conducted for the Flambeau Mining Company
Ladysmith, Wisconsin
April 12 through 14, 1995

1.	Test organism	<i>Ceriodaphnia dubia</i> (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	25 mL
6.	Renewal of test solutions	Daily.
7.	Replicate chambers per treatment	4
8.	Test organisms per chamber	5
9.	Primary control/dilution water	Receiving water: Flambeau River.
10.	Internal control water	Laboratory culture medium.
11.	Effluent concentrations	6.25, 12.5, 25, 50, and 100%
12.	Temperature	20 ± 1°C
13.	Feeding regime	None.
14.	Aeration	None.
15.	Test duration	48 hours.
16.	Sampling scheme	One composite effluent sample (see Chain-of-Custody record in Appendix B). Maximum holding time of 48 hours between completion of collection and initial test use. One receiving water grab sample collected within 48 hours of test initiation. Laboratory water used was collected daily.
17.	Effects measured	Survival.
18.	Test acceptability	90% or greater mean survival in the laboratory or receiving water control.

Table 2
Summary of Test Conditions for the
***Daphnia* Acute Bioassay**
Conducted for the Flambeau Mining Company
Ladysmith, Wisconsin
April 12 through 14, 1995

1.	Test organism	<i>Daphnia magna</i> (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	25 mL
6.	Renewal of test solutions	Daily.
7.	Replicate chambers per treatment	4
8.	Test organisms per chamber	5
9.	Primary control/dilution water	Receiving water: Flambeau River.
10.	Internal control water	Laboratory culture medium.
11.	Effluent concentrations	6.25, 12.5, 25, 50, and 100%
12.	Temperature	$20 \pm 1^{\circ}\text{C}$
13.	Feeding regime	None.
14.	Aeration	None.
15.	Test duration	48 hours.
16.	Sampling scheme	One composite effluent sample (see Chain-of-Custody record in Appendix B). Maximum holding time of 48 hours between completion of collection and initial test use. One receiving water grab sample collected within 48 hours of test initiation. Laboratory water used was collected daily.
17.	Effects measured	Survival.
18.	Test acceptability	90% or greater mean survival in the laboratory or receiving water control.

Table 3
Summary of Test Conditions for the
Fathead Minnow Acute Bioassay
Conducted for the Flambeau Mining Company
Ladysmith, Wisconsin
April 12 through 16, 1995

1.	Test organism	<i>Pimephales promelas</i> (Osteichthyes: Cyprinidae).
2.	Test type	Static renewal.
3.	Age of test organisms	33 days old.
4.	Test chamber size	500 mL
5.	Test solution volume	400 mL
6.	Renewal of test solutions	Daily.
7.	Number of replicate chambers per treatment	2
8.	Number of test organisms per chamber	10
9.	Primary control/dilution water	Receiving water: Flambeau River.
10.	Internal control water	Laboratory culture medium
11.	Effluent concentrations	6.25, 12.5, 25, 50, and 100%
12.	Temperature	$20 \pm 1^{\circ}\text{C}$
13.	Feeding regime	None
14.	Aeration	None, unless DO concentration falls below 40% saturation (then, continuous at rate not exceeding 100 bubbles/minute).
15.	Test duration	96 hours.
16.	Loading rate	Less than 0.65 g/L.
17.	Sampling scheme	Two separate composite effluent samples, each used for a 48-hour exposure (see Chain-of-Custody record in Appendix B). Maximum holding time of 48 hours between completion of collection and initial test use for each sample. . One grab sample of receiving water collected within 48 hours of test initiation. Laboratory water used was collected daily.
18.	Effects measured	Survival
19.	Test acceptability	90% or greater mean survival in the laboratory or receiving water control.

Results

Photocopies of the laboratory data are found in Appendix A. There were no excursions from the protocols and all test conditions were within the limits required by the DNR. The results of tests are summarized below.

Acute Bioassays

Table 4 presents the results of the acute bioassays. No acute toxicity was demonstrated to *Ceriodaphnia dubia*, *Daphnia magna*, or fathead minnows in the 100 percent effluent concentrations. Flambeau River and laboratory control water were acceptable in all tests.

Table 4
Summary of Results of Acute Bioassays
Conducted for the Flambeau Mining Company
Ladysmith, Wisconsin
April 12 through 16, 1995

Test Media	Mean Percent Survival		
	<i>Ceriodaphnia dubia</i>	<i>Daphnia magna</i>	Fathead Minnow
Flambeau River Control	95	100	100
6.25% Effluent	100	100	100
12.5% Effluent	100	100	100
25% Effluent	100	100	100
50% Effluent	100	100	100
100% Effluent	90	100	100
Laboratory Control	100	100	100

Physicochemical Data

All physicochemical parameters measured satisfied the bioassay requirements (see Appendix A).

Conclusions

Based on the results of the laboratory bioassays, the effluent samples collected by the Flambeau Mining Company, Ladysmith, Wisconsin on April 11 and 12, 1995, passed the Wisconsin DNR's whole effluent acute toxicity criteria for all species tested for NPDES compliance purposes. In summary, the test results show:

- The effluent samples were not acutely toxic to *Ceriodaphnia dubia*, *Daphnia magna*, or fathead minnows at 100 percent concentrations using the 50 percent lethality criteria.
- Both Flambeau River and laboratory control water met test acceptability criteria in all acute bioassays.

Reference

1. Weber, C. I. (ed.). 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (Fourth Edition). EPA/600/4-90/027F. U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. 293 p.
2. Denny, J. S. 1987. *Guidelines for the Fathead Minnows, Pimephales promelas, for Use in Toxicity Tests*. EPA/600/3-87/001. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. 42 p.

APPENDIX A
LABORATORY DATA SHEETS
AND STATISTICAL ANALYSES

CH2M HILL MILWAUKEE BIOASSAY LABORATORY

CLIENT: Flambeau Mining Co.

TEST DATE: 4-12-95

To the best of our knowledge, the laboratory data reported is true and accurate.

Report and Data:

Reviewed by:

Michael Coleman Date: 5-17-95

_____ Date: _____

Approved by:

James Stank Date: 5-17-95

48-HOUR ACUTE TEST INITIAL CHEMICAL DATA*
(1-7 TREATMENTS)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Ceriodaphnia dubia LAB MEDIA /No.: Culture media /655-ADL
 SAMPLE No.(s): 655.01, .02, 075D CONTROL/DILUENT: Flambeau River
 SAMPLE DESCRIPTION: Effluent
 TEST START DATE: 4-12-95 TIME: 1315 TEST END DATE: 4-16-95 TIME: 1130
 ANALYST(s): J. Trammel CODE: _____

INITIAL CHEMICAL MEASUREMENT

TREAT. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HR)		COMMENTS
			0	24	
1	Lab	DO	8.4	8.8	
		pH	8.2	8.3	
		COND	.20	.20	
2	Flambeau River	DO	8.3	8.9	
		pH	8.0	8.3	
		COND	.11	.11	
3	6.25%	DO	9.0	8.9	
		pH	8.0	8.2	
		COND	.11	.12	
4	12.5%	DO	9.0	8.8	
		pH	7.9	8.1	
		COND	.12	.12	
5	25%	DO	9.4	8.8	
		pH	7.8	8.0	
		COND	.13	.14	
6	50%	DO	9.4	8.8	
		pH	7.8	7.9	
		COND	.17	.17	
7	100%	DO	9.3	8.7	
		pH	7.7	7.9	
		COND	.26	.24	
DATE			4-12	4-13	
SAMPLE No.			.02	.02	
DETERMINED BY			JT	JT	

*DO as mg/L COND as mmho

Handwritten initials/signature

48-HOUR ACUTE TEST FINAL CHEMICAL DATA*

(1-7 TREATMENTS)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Ceriodaphnia dubia LAB MEDIA No.: Culture media/655.ADL
 SAMPLE No.(s): 655.01, 02 CONTROL/DILUENT: Flambeau River
 SAMPLE DESCRIPTION: Effluent
 TEST START DATE: 4-12-95 TIME: 1315 TEST END DATE: 4-14-95 TIME: 1130
 ANALYST(s): J. Trammel CODE: _____

FINAL MEASUREMENT

TREAT. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HRS)		COMMENTS
			24	48	
1	Lab	DO	8.8	8.5	
		pH	8.3	8.3	
2	Flambeau River	DO	8.8	8.5	
		pH	8.3	8.3	
3	6.25%	DO	8.9	8.6	
		pH	8.2	8.1	
4	12.5%	DO	8.9	8.6	
		pH	8.1	8.0	
5	25%	DO	8.9	8.6	
		pH	7.9	8.0	
6	50%	DO	8.9	8.6	
		pH	7.8	7.9	
7	100%	DO	8.9	8.5	
		pH	7.8	7.8	
DATE			4-13	4-14	
DETERMINED BY			JT	JT	

*DO as mg/L COND as mmho

JS

48-HOUR ACUTE BIOASSAY SURVIVAL DATA

(4 Reps. 1-7 Treatments)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co
 TEST ORGANISM: Ceriodaphnia dubia AGE: < 24H LOT No.: 620
 SAMPLE DESCRIPTION: EFFLUENT
 SAMPLE No.(s): 655.01, 02
 LAB MEDIA/No.: Culture media/655.APL CONTROL/DILUENT: Flambeau River
 TEST START DATE: 4-12-95 TIME: 1315 TEST END DATE: 4-14-95 TIME: 1130
 ANALYST(s): J. Trammel CODE: _____

TREAT. NO.	TEST SOLN	REP	FATALITIES PER EXPOSURE PERIOD (Hrs)		TOTAL FATAL.	MEAN SURV.	COMMENTS
			24	48			
1	Lab	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
2	Flambeau River	A	0	0	0	95%	
		B	0	0	0		
		C	0	0	0		
		D	1	0	1		
3	6.25%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
4	12.5%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
5	25%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
6	50%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
7	100%	A	0	0	0	90%	
		B	1	0	1		
		C	0	1	1		
		D	0	0	0		
DATE			4-13	4-14			
DETERMINED BY			JT	JT			

JS ✓

48-HOUR ACUTE TEST INITIAL CHEMICAL DATA*
(1-7 TREATMENTS)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Daphnia magna LAB MEDIA /No.: Culture media/655.ADL
 SAMPLE No.(s): 655.01.02 CONTROL/DILUENT: Flambeau River
 SAMPLE DESCRIPTION: Effluent
 TEST START DATE: 4-12-95 TIME: 1330 TEST END DATE: 4-16-95 TIME: 1145
 ANALYST(s): J. Trammel CODE: _____

INITIAL CHEMICAL MEASUREMENT

TREAT. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HR)		COMMENTS
			0	24	
1	Lab	DO	8.4	8.8	
		pH	8.2	8.3	
		COND	0.20	0.20	
2	Flambeau River	DO	8.3	8.9	
		pH	8.0	8.3	
		COND	0.11	0.11	
3	6.25%	DO	9.0	8.9	
		pH	8.0	8.2	
		COND	0.11	0.12	
4	12.5%	DO	9.0	8.8	
		pH	7.9	8.0	
		COND	0.12	0.12	
5	25%	DO	9.4	8.8	
		pH	7.8	8.0	
		COND	0.13	0.14	
6	50%	DO	9.4	8.8	
		pH	7.8	7.9	
		COND	0.17	0.17	
7	100%	DO	9.3	8.9	
		pH	7.7	7.9	
		COND	0.26	0.24	
DATE			4-12	4-13	
SAMPLE No.			655.02	02	
DETERMINED BY			JT/JS	JT/JS	

*DO as mg/L COND as mmho

JS

48-HOUR ACUTE TEST FINAL CHEMICAL DATA*

(1-7 TREATMENTS)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Daphnia magna LAB MEDIA /No.: Culture media/655.AVL
 SAMPLE No.(s): 655.01.02 CONTROL/DILUENT: Flambeau River
 SAMPLE DESCRIPTION: Effluent
 TEST START DATE: 4-12-95 TIME: 1330 TEST END DATE: 4-14-95 TIME: 1145
 ANALYST(s): J. Trammel CODE: _____

FINAL MEASUREMENT

TREAT. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HRS)		COMMENTS
			24	48	
1	Lab	DO	8.9	8.6	
		pH	8.2	8.0	
2	Flambeau River	DO	8.9	8.7	
		pH	8.4	7.9	
3	6.25%	DO	8.7	8.6	
		pH	8.0	7.9	
4	12.5%	DO	8.6	8.6	
		pH	8.0	7.9	
5	25%	DO	8.6	8.5	
		pH	7.8	7.9	
6	50%	DO	8.5	8.6	
		pH	7.9	7.9	
7	100%	DO	8.5	8.6	
		pH	7.8	8.1	
DATE			4-13	4-14	
DETERMINED BY			JT	JT	

*DO as mg/L COND as mmho

JT

48-HOUR ACUTE BIOASSAY SURVIVAL DATA

(4 Reps. 1-7 Treatments)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Daphnia magna AGE: <24 H LOT No.: 413-414
 SAMPLE DESCRIPTION: Effluent
 SAMPLE No.(s): 655.02, 01
 LAB MEDIA/No.: Culture media/655.APL CONTROL/DILUENT: Flambeau River
 TEST START DATE: 4-12-95 TIME: 1330 TEST END DATE: 4-14-95 TIME: 1145
 ANALYST(s): J. Trammel CODE: _____

TREAT. NO.	TEST SOLN	REP	FATALITIES PER EXPOSURE PERIOD (Hrs)		TOTAL FATAL	MEAN SURV.	COMMENTS
			24	48			
1	Lab	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
2	Flambeau River	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
3	6.25%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
4	12.5%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
5	25%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
6	50%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		
7	100%	A	0	0	0	100%	
		B	0	0	0		
		C	0	0	0		
		D	0	0	0		

DATE	4-13	4-14
DETERMINED BY	JT	JT

JT

96-HOUR ACUTE TEST INITIAL CHEMICAL DATA*

(1-7 TREATMENTS)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Fathead minnow LAB MEDIA /No.: Culture media/655.AFL
 SAMPLE No.(s): 655.01, .02, .03 CONTROL/DILUENT: Flambeau River
 SAMPLE DESCRIPTION: Effluent
 TEST START DATE: 4-12-95 TIME: 1300 TEST END DATE: 4-16-95 TIME: 1430
 ANALYST(s): J. Trueme CODE: _____

INITIAL CHEMICAL MEASUREMENT

TREAT. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HRS)				COMMENTS
			0	24	48	72	
1	Lab	DO	8.3	8.6	8.5	8.4	
		pH	8.2	8.0	8.0	8.2	
		COND	.20	.20	.20	.20	
2	Flambeau River	DO	8.3	8.9	8.4	8.4	
		pH	8.0	8.3	8.1	8.1	
		COND	0.11	.11	.12	.12	
3	6.25%	DO	9.0	8.9	8.8	8.4	
		pH	8.0	8.2	8.0	8.0	
		COND	.11	.12	.12	.12	
4	12.5%	DO	9.0	8.8	8.8	8.4	
		pH	7.9	8.1	7.9	7.9	
		COND	.12	.12	.12	.13	
5	25%	DO	9.4	8.8	8.7	8.4	
		pH	7.9	8.0	7.8	7.9	
		COND	.13	.14	.13	.14	
6	50%	DO	9.4	8.8	8.8	8.4	
		pH	7.8	7.9	7.7	7.8	
		COND	.17	.17	.17	.18	
7	100%	DO	9.3	8.9	8.8	8.2	
		pH	7.7	7.9	7.7	7.7	
		COND	.26	.24	.23	.25	
DATE			4-12	4-13	4-14	4-15	
SAMPLE No.			.02	.02	.03	.03	
DETERMINED BY			JT	JT	JT	JT	

*DO as mg/L COND as mmho

JT

96-HOUR ACUTE TEST FINAL CHEMICAL DATA*
(1-7 TREATMENTS)

PROJECT No.: ASE142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Fathead minnow LAB MEDIA /No.: Culture media/655A
 SAMPLE No.(s): 655.01, 02, 03 CONTROL/DILUENT: Flambeau River
 SAMPLE DESCRIPTION: Effluent
 TEST START DATE: 4-12-95 TIME: 1300 TEST END DATE: 4-16-95 TIME: 1430
 ANALYST(s): J. Trammel CODE: _____

FINAL MEASUREMENT

TREAT. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HRS)				COMMENTS
			24	48	72	96	
1	Lab	DO	8.8	8.5	8.8	8.6	
		pH	8.2	8.0	8.3	8.2	
2	Flambeau River	DO	8.4	8.4	8.7	8.6	
		pH	8.0	8.1	8.4	8.4	
3	6.25%	DO	8.2	8.8	8.9	8.7	
		pH	7.8	7.9	8.3	8.4	
4	12.5%	DO	8.1	8.8	8.8	8.8	
		pH	7.8	7.8	8.3	8.4	
5	25%	DO	8.1	8.7	8.8	8.8	
		pH	7.7	7.8	8.3	8.4	
6	50%	DO	8.1	8.6	8.8	8.8	
		pH	7.6	7.7	8.3	8.4	
7	100%	DO	8.1	8.4	8.8	8.9	
		pH	7.6	7.6	8.3	8.4	
DATE			4-13	4-14	4-15	4-16	
DETERMINED BY			JT	JT	JT	JT	

*DO as mg/L

96-HOUR ACUTE BIOASSAY SURVIVAL DATA

(1 - 7 Treatments)

PROJECT No.: ASE 142.32 CLIENT: Flambeau Mining Co.
 TEST ORGANISM: Fathead minnow AGE: 33 D LOT No.: 1695
 SAMPLE DESCRIPTION: Effluent
 SAMPLE No.(s): 655.01, 02, 03
 LAB MEDIA/No.: Culture media/655.AFL CONTROL/DILUENT: Flambeau River
 TEST START DATE: 4-12-95 TIME: 1300 TEST END DATE: 4-16-95 TIME: 1430
 ANALYST(s): J. Trammel CODE: _____

TREAT. NO.	TEST SOLN	REP	FATALITIES PER EXPOSURE PERIOD (Hrs)				TOTAL FATALITIES	MEAN SURVIVAL
			24	48	72	96		
1	Lab	A	0	0	0	0	0	100%
		B	0	0	0	0		
2	Flambeau River	A	0	0	0	0	0	100%
		B	0	0	0	0		
3	6.25%	A	0	0	0	0	0	100%
		B	0	0	0	0		
4	12.5%	A	0	0	0	0	0	100%
		B	0	0	0	0		
5	25%	A	0	0	0	0	0	100%
		B	0	0	0	0		
6	50%	A	0	0	0	0	0	100%
		B	0	0	0	0		
7	100%	A	0	0	0	0	0	100%
		B	0	0	0	0		

DATE	4-13	4-14	4-15	4-16
DETERMINED BY	JT	JT	JT	JT

COMMENTS:

SUPPLEMENTAL CHEMICAL DATA SUMMARY

PROJECT NO. ASE 142-32
 TEST DATE 4-12-95

CLIENT Flambeau Mining Co.
 SUMMARIZED BY J. Stark

LABORATORY CONTROL	CONTROL I.D.			
	655-A0L	655-AFL		
TOTAL ALKALINITY mg/L CaCO ₃	90/88 *	92		
HARDNESS mg/L CaCO ₃	105/105 *	105		

SAMPLE DESCRIPTION	SAMPLE NO.			
	655.01			
Flambeau River				
TOTAL ALKALINITY mg/L CaCO ₃	32			
HARDNESS mg/L CaCO ₃	60			
TOTAL RESIDUAL CHLORINE mg/L	NA			
TOTAL AMMONIA mg/L	0.10			

SAMPLE DESCRIPTION	SAMPLE NO.			
	655.02	655.03		
100% Effluent				
TOTAL ALKALINITY mg/L CaCO ₃	42	40		
HARDNESS mg/L CaCO ₃	110	105		
TOTAL RESIDUAL CHLORINE mg/L	0	0		
TOTAL AMMONIA mg/L	0.23	0.18		

SAMPLE DESCRIPTION	SAMPLE NO.			
TOTAL ALKALINITY mg/L CaCO ₃				
HARDNESS mg/L CaCO ₃				
TOTAL RESIDUAL CHLORINE mg/L				
TOTAL AMMONIA mg/L				

NA = Not Analyzed
 * = Duplicate for QA

TEST TEMPERATURE SUMMARY

PROJECT No.: ASE 142-32 CLIENT: Flambeau Mining Co.

ACUTE TEST TEMPERATURE

	DATE								
	4-12-95	4-13	4-14	4-15	4-16				
AVG	20.3	20.3	20.4	20.4	20.4				
MAX									
MIN									
SUM. BY:	JS	JS	JS	JS	JS				

CHRONIC TEST TEMPERATURE

	DATE							
AVG								
MAX								
MIN								
SUM. BY:								

COMMENTS:
 * Data logger malfunctioned, temperatures taken by thermometer

BIOASSAY SAMPLE RECEIPT CHARACTERIZATION

CLIENT Flambeau Mining Co

DATE RECVD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS
4-12-95	655.01 RIVER	3°	13.3	8.1	.09	JT

SIEVED DECHLORINATE _____ USE: IMMEDIATE STORE (4 C) _____
 ALIQUOTS HOMOGENIZED _____ CONTAINER TYPE (G/P) P ODOR _____
 APPEARANCE : CLEAR CLOUDY _____ SOLIDS _____ COLOR TAN
 ALIQUOT FOR : HARDNESS ALKALINITY
 COMMENTS:

DATE RECVD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS
4-12	655.02 EFFLUENT	3°	11.9	7.8	.20	JT

SIEVED DECHLORINATE _____ USE: IMMEDIATE STORE (4 C) _____
 ALIQUOTS HOMOGENIZED _____ CONTAINER TYPE (G/P) P ODOR _____
 APPEARANCE : CLEAR CLOUDY _____ SOLIDS _____ COLOR _____
 ALIQUOT FOR : HARDNESS ALKALINITY
 COMMENTS:

DATE RECVD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS
4-14	655.03 EFFLUENT	3°	12.5	7.7	.17	MC

SIEVED DECHLORINATE _____ USE: IMMEDIATE STORE (4 C) _____
 ALIQUOTS HOMOGENIZED _____ CONTAINER TYPE (G/P) P ODOR _____
 APPEARANCE : CLEAR CLOUDY _____ SOLIDS _____ COLOR _____
 ALIQUOT FOR : HARDNESS ALKALINITY
 COMMENTS:

DATE RECVD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS

SIEVED _____ DECHLORINATE _____ USE: IMMEDIATE _____ STORE (4 C) _____
 ALIQUOTS HOMOGENIZED _____ CONTAINER TYPE (G/P) _____ ODOR _____
 APPEARANCE : CLEAR _____ CLOUDY _____ SOLIDS _____ COLOR _____
 ALIQUOT FOR : HARDNESS _____ ALKALINITY _____
 COMMENTS:

APPENDIX B
CHAIN-OF-CUSTODY FORMS



CHAIN OF CUSTODY RECORD FOR NPDES COMPLIANCE BIOMONITORING

Client Name Flambeau Mining Co		Client Shipping Address N 4100 Hwy 27 Ladysmith, WI 54848		NPDES Number					
Sample Kit Tracking Information		Method of Shipment (Check One)		Prepared by/Date: MW / 4-4-95					
No. of Cooler _____ of _____		<input type="checkbox"/> Fed X <input type="checkbox"/> Pickup _____		Shipped by/Date:					
Total No. of Bottles _____		<input type="checkbox"/> UPS _____ <input type="checkbox"/> Other _____							
Composite Sample Information		Description of Sampling Site							
Flow Proportional <input checked="" type="checkbox"/> Time Interval <input type="checkbox"/>									
Samples/Hour _____ Volume/Sample _____									
Total Hours _____ Total Volume _____									
Initiated: Date 4/11/95 Time 8:40AM									
Ended: Date 4/11/95 Time 6:22PM									
Chilled During Collection Yes <input checked="" type="checkbox"/> No <input type="checkbox"/>									
Sample Description	Date	Time	Sample Type	Comp	Grab	No. of Containers	Volume	Sampled By (Signature)	
Flambeau River	4/11/95	2:32	X	X		1	5	<i>Joseph Schuel</i>	
05-R-001	4/11/95	8:40-8:22P	X	X		1		<i>J. Johnson Schuel</i>	
Relinquished By and Title (Signature)		Date 4-12-95		Time 4:22 PM		Relinquished By: (Signature)		Date 4/12/95	
<i>Johnson Schuel</i>						<i>Johnson Schuel</i>		Time 1105	
Received By: (Signature)		Date 4-12-95		Time 4:22 PM		Received By Lab: (Signature)		Date 4/12/95	
<i>James Parker</i>						<i>James Cooke</i>		Time 1105	
Sample Container		Plastic <input checked="" type="checkbox"/> Glass <input type="checkbox"/> New <input type="checkbox"/> Used <input type="checkbox"/>		Refrigerant Used For Shipping		Wet Ice <input checked="" type="checkbox"/> Blue Ice <input type="checkbox"/> Other <input type="checkbox"/>		Sample(s) Shipped Via	
								UPS <input type="checkbox"/> Fed X <input type="checkbox"/> Other <input checked="" type="checkbox"/>	
								Bioassays Required	
								Acute <input checked="" type="checkbox"/> Chronic <input type="checkbox"/> Other <input type="checkbox"/>	
								For Lab Use Sample ID No. 655.01 655.02	
								Condition of Seal Upon Receipt by Lab	
								Intact <input type="checkbox"/> Other (Describe) _____	



CHAIN OF CUSTODY RECORD FOR NPDES COMPLIANCE BIOMONITORING

Client Name E. Ambreau Mining Co		Client Shipping Address N 4100 Hwy 27 Ladysmith, WI 54848		NPDES Number			
Sample Kit Tracking Information		Method of Shipment (Check One) <input type="checkbox"/> Fed X <input type="checkbox"/> Pickup <input type="checkbox"/> Other		Ship Samples to: CH2M HILL Bioassay Laboratory 15779 W. Ryerson Road New Berlin, WI 53151 Phone: (414) 784-0448 Fax: (414) 784-0353			
No. of Cooler _____ of _____		Prepared by/Date: <i>MW / 4/4/95</i>					
Total No. of Bottles _____		<input type="checkbox"/> UPS <input type="checkbox"/> Other _____		Sample Container Plastic <input checked="" type="checkbox"/> Glass <input type="checkbox"/> New <input checked="" type="checkbox"/> Used <input type="checkbox"/> Refrigerant Used For Shipping Wet Ice <input checked="" type="checkbox"/> Blue Ice <input type="checkbox"/> Other <input type="checkbox"/> Sample(s) Shipped Via UPS <input checked="" type="checkbox"/> Fed X <input type="checkbox"/> Other <input type="checkbox"/> Air			
Composite Sample Information		Description of Sampling Site					
Flow Proportional <input checked="" type="checkbox"/> Time Interval <input type="checkbox"/>		Samples/Hour _____ Volume/Sample _____					
Total Hours _____ Total Volume _____		Initiated: Date <i>4-12-95</i> Time <i>9:11 AM</i>					
Ended: Date <i>4-12-95</i> Time <i>11:01 PM</i>		Chilled During Collection Yes <input checked="" type="checkbox"/> No <input type="checkbox"/>					
Sample Description	Date	Time	Sample Type	No. of Containers	Volume	Sampled by (Signature)	For Lab Use Sample ID No.
<i>outfall-001</i>	<i>4-13-95</i>	<i>7:45</i>	<i>1</i>	<i>1</i>	<i>2 1/2</i>	<i>Jack (Signature)</i>	<i>655-03</i>
Relinquished By and Title (Signature) <i>Jack (Signature)</i>		Date	Relinquished By: (Signature)		Condition of Seal Upon Receipt by Lab		
Received By: (Signature) <i>Michael Colura</i>		Date	Received By Lab: (Signature)		Intact <input checked="" type="checkbox"/> Other (Describe) _____		
Time		Time	Time		Date <i>4-13-95</i>		
Time		Time	Time		Date <i>4-14-95</i>		
Time		Time	Time		Time <i>1000</i>		

APPENDIX C
REFERENCE TOXICANT DATA

Table 1. Reference Toxicant Summary

Laboratory: CH2M HILL - Milwaukee, WI

Test Type: Acute

Organism: *Ceriodaphnia dubia*

Age < 24 Hours

of Organisms / Conc.: 20

Test Duration: 48 Hours

Toxicant: Sodium Chloride

Response: Mean % Survival

Calculation: LC50

Reporting Period: May 94 - April 95

Test No	Test Date	Test LC50 (g/L)	12 Month Control Limits			In or Out of Control	Action if Out of Control
			Mean LC50	Mean +2 S.D.	Mean -2 S.D.		
56	5/31/94	2.67	2.48	2.80	2.17	IN	
57	6/29/94	2.32	2.47	2.80	2.14	IN	
58	7/29/94	2.45	2.48	2.80	2.17	IN	
59	8/31/94	2.52	2.49	2.80	2.17	IN	
60	9/28/94	2.58	2.47	2.73	2.21	IN	
61	10/18/94	2.45	2.47	2.73	2.21	IN	
62	11/28/94	1.54	2.38	2.95	1.80	OUT	a
63	12/14/94	2.60	2.39	2.98	1.80	IN	
64	12/20/94	2.63	2.42	3.02	1.83	IN	
65	12/27/94	2.53	2.43	3.03	1.84	IN	
66	1/24/95	2.32	2.42	3.02	1.82	IN	
67	2/28/95	2.10	2.39	3.02	1.77	IN	
68	3/28/95	2.25	2.36	2.96	1.75	IN	
69	4/27/95	1.83	2.32	2.99	1.64	IN	

a = water batch had low hardness (<70 mg/L); Retest 12/14/94

Table 2. Reference Toxicant Summary

Laboratory: CH2M HILL - Milwaukee, WI

Test Type: Acute

Organism: *Daphnia magna*

Age: < 24 Hours

of Organisms / Conc.: 20

Test Duration: 48 Hours

Toxicant: Sodium Chloride

Response: Mean % Survival

Calculation: LC50

Reporting Period: May 94 - April 95

Test No	Test Date	Test LC50 (g/L)	12 Month Control Limits			In or Out of Control	
			Mean LC50	Mean +2 S.D.	Mean -2 S.D.	Control	Action if Out of Control
54	5/31/94	4.99	4.82	5.28	4.35	IN	
55	6/29/94	4.90	4.87	5.18	4.56	IN	
56	7/29/94	4.90	4.87	5.18	4.56	IN	
57	8/31/94	4.65	4.89	5.06	4.73	OUT	a
58	9/28/94	4.51	4.86	5.14	4.58	OUT	a
59	10/18/94	4.64	4.84	5.14	4.53	IN	
60	11/28/94	3.83	4.75	5.40	4.10	OUT	b
61	12/20/94	4.90	4.74	5.41	4.06	IN	
62	12/27/94	4.90	4.74	5.41	4.06	IN	
63	1/24/95	5.34	4.77	5.53	4.01	IN	
64	2/28/95	3.74	4.66	5.63	3.69	IN	c
65	3/28/95	4.78	4.64	5.60	3.69	IN	
66	4/25/95	3.77	4.54	5.61	3.47	IN	

a = The LC50s of tests 57 and 58 were 5% and 7% different, respectively, from the mean LC50. As the variance has been too low (tight) due to many of the previous tests with identical results, no action was taken. This is due to an artifact of the statistical analysis.

b = Lab water hardness decreased below 70 mg/L; Retest when cultures back on-line.

c = New concentrations used.

Table 3. Reference Toxicant Summary

Laboratory: CH2M HILL - Milwaukee, WI
Test Type: Acute
Organism: *Pimephales promelas*
Age: 10 ± 2 Days
of Organisms / Conc.: 20
Test Duration: 96 Hours
Toxicant: Sodium Chloride
Response: Mean % Survival
Calculation: LC50
Reporting Period: May 94 - April 95

Test No	Test Date	Test LC50 (g/L)	12 Month Control Limits			In or Out of Control	Action if Out of Control
			Mean LC50	Mean +2 S.D.	Mean -2 S.D.		
54	5/16/94	9.53	7.23	9.56	4.90	IN	
55	6/13/94	6.49	7.26	9.54	4.98	IN	
56	7/11/94	5.47	7.17	9.64	4.69	IN	
57	8/8/94	5.41	7.07	9.72	4.42	IN	
58	9/12/94	5.81	6.91	9.60	4.22	IN	
59	10/10/94	5.79	6.85	9.61	4.09	IN	
60	11/7/94	5.76	6.61	9.16	4.06	IN	
61	12/8/94	5.98	6.63	9.16	4.09	IN	
62	1/9/95	5.28	6.58	9.19	3.98	IN	
63	2/6/95	5.28	6.39	8.96	3.81	IN	
64	3/6/95	5.81	6.28	8.82	3.74	IN	
65	4/17/95	7.05	6.31	8.87	3.74	IN	



Environmental and Analytical Services

April 20, 1995
Project 5000-860

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 acute test battery we performed during April 12-16, 1995.

All results were acute toxicity negative (i.e., \geq 50% acute survival in 100% effluent).

Additional C. dubia test treatments of 100% effluent were included to evaluate differences related to exposure chamber type or other protocol acceptable procedural alternatives. Survival of pretest-unfed C. dubia in rinsed and unrinsed plastic cups was similar (i.e., 54% and 56%); survival of pretest-fed organisms in unrinsed plastic cups was 100%. Survival of pretest-unfed organisms in glass beakers and in unrinsed plastic cups with CO₂ entrapment was 90% and 95%, respectively.

WDNR recently stated that both pretest-fed and unfed daphnids are presently being used for acute testing in Wisconsin and that either is acceptable. The pretest availability of food in this case apparently made a substantial difference. We will initiate reference toxicant testing using pretest-fed daphnids shortly.

Please call if you have any questions or comments.

Best regards.

Sincerely,

David F. Sanders, Manager
Environmental Bioassessment

enclosures



Flambeau Mining Company
Ladysmith, Wisconsin
April 20, 1995

(IPS Project 5000-860)

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 5000-860)

INTRODUCTION

This report presents the results of a biological assessment of wastewater treatment plant effluent quality conducted by Integrated Paper Services, Inc. (IPS) on behalf of Flambeau Mining Company (Flambeau) during the period of April 12-16, 1995.

An aquatic toxicity 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 50% 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 followed EPA and Wisconsin Department of Natural Resources (WDNR) procedures (1,2). The tests were undertaken as described in the QA/QC plan submitted pursuant to Flambeau's WPDES Permit No. WI-0047376-1 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 (9.75-13.75 hour) composite samples; control/dilution water was a Flambeau River grab sample. These samples (Table 1) were kept cool and delivered to IPS within 41 hours of collection.

Table 1. Sample collection schedule for Project 5000-860.

Sample	Type	Collection Dates	Test Use Days
Effluent	9.75-hr Comp.	4/11	Acute(1-2)
Effluent	13.75-hr Comp.	4/12	Acute(3-4)
River	Grab	4/11	Acute(1-4)

Sample temperatures were measured and recorded upon receipt; total residual chlorine was measured on and a $\text{NH}_3\text{-N}$ sample taken 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

Bioassay test procedures followed acute test 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. Additional C. dubia test treatments of 100% effluent were included to evaluate potential differences related to exposure chamber type (i.e., rinsed and unrinsed plastic cups and glass beakers) or other protocol acceptable procedural alternatives (i.e., feeding of pooled organisms prior to test exposure and CO_2 entrapment). Bench sheets for these additional treatment are appended.

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 area. 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 $\leq 1^\circ\text{C}$. 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 tests (1,2). Mean test temperatures were within protocol specified ranges (Table 5). Chain-of-custody forms with sample collection information, 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 7-10).

Table 2. Ceriodaphnia dubia acute toxicity test conditions.

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) and additional 100% effluent treatments
11. Temperature:	20 +/- 1° 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. Daphnia magna acute toxicity test conditions.

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:	2 oz
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 +/- 1° 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. Pimephales promelas acute toxicity test conditions.

1. Test organism	<u>Pimephales promelas</u> (Osteichthyes: Cyprinidae)
2. Test type:	Static renewal
3. Age of test organisms:	Juvenile - 48 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 +/- 1° 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 (°C: date is start of 1200 hr to 1200 hr recording period) for Project 5000-860.

<u>Date</u>	<u>Mean</u>	<u>Max.</u>	<u>Min.</u>
4/12	20.0	20.3	19.6
4/13	20.0	20.5	19.8
4/14	19.8	20.3	19.6
4/15	20.1	20.5	19.6

Table 6. Fathead minnow acute test loading rate; April 12-16, 1995.

Mean length = 14 mm
Mean weight = 14.37 mg
Loading factor = 0.19 g/L

Table 7. Ceriodaphnia dubia acute test data; April 12-14, 1995.

<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	3/2/2/1	54

Table 8. Ceriodaphnia dubia acute test data for the additional 100% effluent treatments; April 12-14, 1995.

<u>Test Solution</u>	<u>Rep</u>	Fatalities/Expos. Per. (HRS.)		<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	
Unrinsed Cups w/fed <u>C. dubia</u>	A/B/C/D	0/0/0/0	0/0/0/0	100
Glass Beakers	A/B/C/D	0/0/0/0	0/0/1/1	90
Rinsed Cups ^a	A/B/C/D	0/0/0/0	3/2/2/1	54
Unrinsed Cups	A/B/C/D	0/0/0/0	3/2/1/2	56
Unrinsed Cups w/CO ₂	A/B/C/D	0/0/0/0	0/0/1/0	95

a From 0.5 dilution series treatments

Table 9. Daphnia magna acute test data; April 12-14, 1995.

<u>Test Solution</u>	<u>Rep</u>	Fatalities/Expos. Per. (HRS.)		<u>Mean % Survival</u>
		<u>24</u>	<u>48</u>	
Secondary Control	A/B	0/0	0/0	100
Primary Control	A/B	0/1	0/0	95
6.25% Effluent	A/B	0/0	0/0	100
12.5%	A/B	1/0	0/0	95
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 10. Pimephales promelas acute test data; April 12-16, 1995.

<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	0/0	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	0/1	0/0	0/0	95
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.

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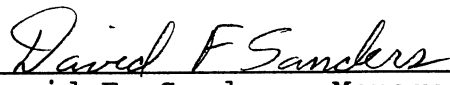
Laboratory Analysis:

David Christel
Tom Perzentka
Jason Taege
Todd Traeder

Laboratory Supervisor:

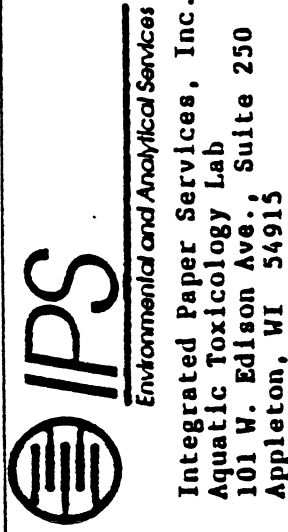
David J. Christel

Quality Assurance Review:



David F. Sanders, Manager
Environmental Bioassessment

Chain-of-Custody Record



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

Client Flambeau Mining Co
N 4100 Hwy 27
Lady Smith, WI 54848
James E. Murphy 715 532-6680 Ext 717
 Returned Samples To:
 Sampled by: [Signature] (signature)

Sample Identification	Containers	Grab Date/Time	Composite		Collection				Receipt					
			Start Date/Time	End Date/Time	Temp. (°C)	pH	NH ₃ -N	Cl ₂	Temp. (°C)	pH	DO	Cl ₂	NH ₃ -N	
① <u>Flambeau River</u>		<u>4/11/95</u>			<u>3.7</u>	<u>8.59</u>			<u>y</u>	<u>0.5</u>	<u>8.0</u>	<u>14.2</u>	<u>-</u>	<u>-</u>
② <u>outfall-ool</u>		<u>4/11/95</u>	<u>8:40 A</u>	<u>6:22 PM</u>	<u>8.5</u>	<u>7.75</u>			<u>y</u>	<u>0.5</u>	<u>7.8</u>	<u>12.0</u>	<u>0.01</u>	<u>< 1.4</u>
③														
④														
⑤														

Remarks: #9.75 hour composite

Laboratory IDs:
 ① A 1-4
 ② A 1-2
 ③ _____
 ④ _____
 ⑤ _____

Relinquished by: [Signature] 4-11-95 8:00 PM Date/Time
 (to carrier)

Received by: _____ Date/Time

Relinquished by: [Signature] 4-12-95 Date/Time
 Received for lab: Sam Carpenter 4-12-95/0825 Date/Time

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

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 Fambourne Mining Co
N4100 Hwy 37
Lady Smith, WI 54048
Jana E Murphy 715 5326690 EVT717

Return Samples To:

Sampled by: [Signature]
 (signature)

Sample Identification	Grab Date/Time	Composite		Collection			Receipt					
		Start Date/Time	End Date/Time	Temp. (°C)	pH	MI ₃ -N	Cl ₂	Ice	Temp. (°C)	pH	DO	Cl ₂
① outfall-001	4/12/95 9:11 AM	4/12/95 11:01 PM	5.7	7.57			Y	16	7.2	9.6	<0.01	<1.4
②												
③												
④												
⑤												

Remarks: * 13.75 hr composite.

Laboratory ID: ① A34
②
③
④
⑤

Custody Seal: OK Broken

Relinquished by: [Signature] Date/Time: 4-13-95 10:30
 (to carrier)

Received by: [Signature] Date/Time: 4-14-95 10:30

Relinquished by: _____ Date/Time: _____
 Received for lab: [Signature] Date/Time: _____

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

≤ 12hr

Four Day Bioassay Chemical Data - Initial

Project No: 5000-860

Start Date: 4-12-95

Client: Flambeau Mining

Organism: Ceriodaphnia dubia Daphnia magna Pimephales promelas

Test Solution	Param.	Exposure Day				Comments
		1	2	3	4	
Control Dechlor (fish) (C)	D.O.	8.4	8.4	8.0	8.2	/
	pH	7.9	7.9	8.0	7.9	
	Cond.	181	181	176	189	
	Alk.	39	42	42	41	
	Hard.	80.0	80.0	80.0	—	
Control River (1)	D.O.	8.5 ↓	8.0 ↓	8.1 ↓	7.8 ↓	R↓1-4
	pH	7.7	7.8	7.5	7.7	
	Cond.	79	82	77	87	
	Alk.	48	28	34	30	
	Hard.	37.5	35.0	35.0	37.5	
6.25% effluent (2)	D.O.	8.6	8.5	8.5	7.8	
	pH	7.7	7.8	7.5	7.7	
	Cond.	88	99	88	94	
	Alk.					
	Hard.					
12.5% effluent (3)	D.O.	8.6	8.5	8.5	7.9	/
	pH	7.7	7.8	7.5	7.7	
	Cond.	99	97	99	104	
	Alk.					
	Hard.					
25% effluent (4)	D.O.	8.5	8.6	8.5	7.9	/
	pH	7.7	7.7	7.6	7.7	
	Cond.	120	118	108	124	
	Alk.					
	Hard.					
50% effluent (5)	D.O.	8.5	8.5	8.5	7.9	/
	pH	7.7	7.8	7.6	7.7	
	Cond.	159	156	153	162	
	Alk.					
	Hard.					
100% effluent (6)	D.O.	8.7 ↓	8.6 ↓	8.7 ↓	8.2 ↓	E↓1-4
	pH	7.8	7.8	7.6	7.7	
	Cond.	236	228	225	243	
	Alk.	44	44	38	38	
	Hard.	112.5	107.5	105.0	102.5	
Determined By:		JH	JH	JH	JH	

R↓1-4

E↓1-4

R/S
DL

Two Day Bioassay Chemical Data - Initial

Project No: 5000-860

Start Date: 4-12-95

Client: Flambeau Mining

Organism: Ceriodaphnia dubia Daphnia magna

Test Solution	Param.	Exposure Day		Comments
		1	2	
Control Flow-thru (daphnids) (C)	D.O.	8.0	8.0	
	pH	7.8	7.9	
	Cond.	217	207	
	Alk.	36	37	
	Hard.	82.5	80.0	
(1)	D.O.			
	pH			
	Cond.			
	Alk.			
	Hard.			
(2)	D.O.			
	pH			
	Cond.			
	Alk.			
	Hard.			
(3)	D.O.			
	pH			
	Cond.			
	Alk.			
	Hard.			
(4)	D.O.			
	pH			
	Cond.			
	Alk.			
	Hard.			
(5)	D.O.			
	pH			
	Cond.			
	Alk.			
	Hard.			
(6)	D.O.			
	pH			
	Cond.			
	Alk.			
	Hard.			
Determined By:		<i>H</i>	<i>Jm</i>	

Two Day Bioassay Chemical Data - Final

Project No: 5000-860

Start Date: 4-12-95

Client: Flambeau Mining

Organism: Ceriodaphnia dubia

Test Solution	Param.	Exposure Day		Comments
		1	2	
Control Flow-thru (C)	D.O.	7.7	7.6	
	pH	7.8	7.7	
	Cond.	223	216	
Control River (1)	D.O.	7.7	7.6	
	pH	7.9	7.8	
	Cond.	89	84	
6.25% effluent (2)	D.O.	7.7	7.7	
	pH	7.8	7.7	
	Cond.	93	89	
12.5% effluent (3)	D.O.	7.6	7.7	✓
	pH	7.8	7.7	
	Cond.	102	99	
25% effluent (4)	D.O.	7.6	7.7	✓
	pH	7.8	7.7	
	Cond.	119	123	
50% effluent (5)	D.O.	7.7	7.7	✓
	pH	7.8	7.7	
	Cond.	158	160	
100% effluent (6)	D.O.	7.7	7.7	✓
	pH	7.8	7.8	
	Cond.	231	242	
(7)	D.O.			
	pH			
	Cond.			
Determined By:		DC	JH	

12
DC

Two Day Bioassay Chemical Data - Final

Project No: 5000-860

Start Date: 4-12-95

Client: Flambeau Mining

Organism: Daphnia magna

Test Solution	Param.	Exposure Day		Comments
		1	2	
Control Flow-thru (C)	D.O.	7.5	7.6	-
	pH	7.7	7.7	
	Cond.	218	215	
Control River (1)	D.O.	7.5	7.6	-
	pH	7.7	7.7	
	Cond.	81	8.2	
6.25% effluent (2)	D.O.	7.6	7.6	✓
	pH	7.7	7.7	
	Cond.	88	92	
12.5% effluent (3)	D.O.	7.6	7.6	-
	pH	7.7	7.7	
	Cond.	100	99	
25% effluent (4)	D.O.	7.6	7.7	-
	pH	7.7	7.7	
	Cond.	121	117	
50% effluent (5)	D.O.	7.6	7.7	✓
	pH	7.7	7.7	
	Cond.	158	160	
100% effluent (6)	D.O.	7.7	7.7	✓
	pH	7.8	7.8	
	Cond.	234	241	
(7)	D.O.			
	pH			
	Cond.			
Determined By:		DC	HT	

Four Day Bioassay Chemical Data - Final

Project No: 5000-860

Start Date: 4-12-95

Client: Flambeau Mining

Organism: Pimephales promelas

Test Solution	Param.	Exposure Day				Comments
		1	2	3	4	
Control Dechlor (C)	D.O.	7.5	7.2	7.7	7.4	
	pH	7.6	7.6	7.6	7.6	
	Cond.	187	188	170	185	
Control River (1)	D.O.	7.1	6.9	7.5	7.3	
	pH	7.4	7.4	7.4	7.4	
	Cond.	82	80	79	81	
6.25% effluent (2)	D.O.	7.1	7.0	7.6	7.2	
	pH	7.4	7.4	7.4	7.4	
	Cond.	92	89	79	89	
12.5% effluent (3)	D.O.	7.0	6.9	7.7	7.3	
	pH	7.4	7.4	7.4	7.4	
	Cond.	99	99	96	100	
25% effluent (4)	D.O.	7.2	7.1	7.7	7.4	
	pH	7.4	7.4	7.4	7.3	
	Cond.	112	118	117	117	
50% effluent (5)	D.O.	7.1	7.1	7.8	7.5	
	pH	7.4	7.4	7.3	7.3	
	Cond.	139	156	154	154	
100% effluent (6)	D.O.	7.4	7.2	7.5	7.5	
	pH	7.5	7.4	7.3	7.3	
	Cond.	222	227	225	228	
(7)	D.O.					
	pH					
	Cond.					
Determined By:		<i>Jen</i>	<i>Jen</i>	<i>Jen</i>	<i>J</i>	

48 Hour Ceriodaphnia dubia Survival Data

Project No: 5000-860

Client: Flambeau Mining

Start Date: 4-12-95

Finish Date: 4-14-95

Start Time: 1545

Finish Time: 1400

Set By: JH

Age/Source: 424 hrs Wed Synth

Test Solution	Rep.	Fatal. / Surv. Per Exposure Period (Hrs)		Comments	Total		% Surv.	Mean Surv.
		24	48		Fatal.	Surv.		
Control Flow-thru (C)	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	
Control River (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	
6.25% effluent (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	
12.5% 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	
25% effluent (4)	A	0/5	0/5		0	5	100	100
	B	0/5	0/5		0	5	100	
	C	0/4	0/4		0	4	100	
	D	0/5	0/5		0	5	100	
50% effluent (5)	A	0/5	0/5	D. 50% 100% bugs small & pale compared to river bugs.	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% effluent (6)	A	0/5	2/3 A.	A. one not located, bug surviving fairly immobilized or appears stressed. B. one missing C. one bug surviving appears near death - stressed D. bugs surviving not as robust compared with controls	3	1	25	54
	B	0/5	2/2 B.		2	2	50	
	C	0/5	2/3 C.		2	3	60	
	D	0/5	1/4 D.		1	4	80	
(7)	A							
	B							
	C							
	D							
Determined By:		DC	JH	Data Check: DC				

15

48 Hour Ceriodaphnia dubia Survival Data

Start Date: 4-12-95 Finish Date: 4-14-95

Start Time: 1545 Finish Time: 1400

Set By: JH Age/Source: 224 hrs Wed Synth

Effluent I.D. #: -320 1st - 100%

Test Solution	Rep.	Fatal. / Surv. Per Exposure Period (Hrs)		Comments	Total		% Surv.	Mean Surv.
		24	48		Fatal.	Surv.		
Flow-Thru Control	A							
	B							
	C							
	D							
Rinsed Cups	A							
	B							
	C							
	D							
Glass Beakers	A							
	B							
	C							
	D							
Un-rinsed Cups	A							
	B							
	C							
	D							
Un-rinsed Cups CO2	A							
	B							
	C							
	D							
(R) Un-rinsed Cups Feed Cerio	A	0/5	0/5	D ₁ -No visual difference vs. unfed Cerio. 100% EFF	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	
Determined By:		<u>OC</u>	<u>JH</u>		Data Check:		<u>OC</u>	

organisms fed pretest

Two Day Bioassay Chemical Data - Initial / Final

Test Solution	Param.	INITIAL / DAY		FINAL / DAY		Comments
		1	2	1	2	
Rinsed Cups	D.O.	8.7 ↓	8.7			EFF 100% EFF
	pH	7.8	7.6			
	Cond.	236	225			
Glass Beakers						
Un-rinsed Cups						
Un-rinsed Cups CO2						
Un-rinsed Cups Fed Cerio				7.7		D ₂ - final chems not taken OC
				7.9		
				265		
Determined By:		<u>JH</u>	<u>JH</u>	<u>OC</u>		

Day 1: Alk 44 Chlorine 68.01
Hard 172.5 Ammonia 21.4

48 Hour Ceriodaphnia dubia Survival Data

Start Date: 4-12-95 Finish Date: 4-14-95

Start Time: 1545 Finish Time: 1400

Set By: JH Age/Source: 224 hrs Wed Synth

Effluent I.D. #: -300 (100% eff)

Test Solution	Rep.	Fatal. / Surv. Per Exposure Period (Hrs)		Comments	Total		% Surv.	Mean Surv.
		24	48		Fatal.	Surv.		
Flow-Thru Control	A							
	B							
	C							
	D							
Rinsed Cups	A							
	B							
	C							
	D							
Glass Beakers (9)	A	0/5	0/5	D ₁ - bugs small & pale vs. river bugs.	0	5	100	90% 100% eff
	B	0/5	0/5		0	5	100	
	C	0/5	1/4	D ₂ - bugs small & pale	1	4	80	
	D	0/5	1/4		1	4	80	
Un-rinsed Cups (10)	A	0/4	3/1	d ₂ - surviving bugs transparent and small	3	1	25	56% 100% eff
	B	0/5	2/3		2	3	60	
	C	0/5	1/4		1	4	80	
	D	0/5	2/3		2	3	60	
Un-rinsed Cups CO ₂ (11)	A	0/5	0/5	D ₂ - bugs pale, not as robust as control	0	5	100	95% 100% eff
	B	0/5	0/5		0	5	100	
	C	0/5	1/4		1	4	80	
	D	0/5	0/5		0	5	100	
Un-rinsed Cups Fed Cerio	A	X	X					
	B	X	X					
	C	X	X					
	D	X	X					

Determined By: DC JH

Data Check: DC

Two Day Bioassay Chemical Data - Initial / Final

Test Solution	Param.	INITIAL / DAY		FINAL / DAY		Comments
		1	2	1	2	
Rinsed Cups	D.O.	8.7 ↓	8.7 ↓			E ↓ 1,2 ✓
	pH	7.8	7.6			
	Cond.	236	225			
Glass Beakers				7.7	7.7	-
				7.9	7.9	
				269	257	
Un-rinsed Cups				7.6	7.8	✓
				8.0	8.0	
				269	289	
Un-rinsed Cups CO ₂				7.6	7.8	✓
				7.4	7.4	
				220	240	
Un-rinsed Cups Fed Cerio		X	X	X	X	
		X	X	X	X	
		X	X	X	X	

Determined By: JH DC JH

Day 1: Alk 44 Chlorine 40.01
 Hard 112.5 Ammonia 4.4

48 Hour Daphnia magna Survival Data

Project No: 5000-860 Client: Flambeau Mining

Start Date: 4-12-95 Finish Date: 4-14-95

Start Time: 1530 Finish Time: 1500

Set By: JH Age: 224 hrs

Test Solution	Rep.	Fatal. / Surv. Per Exposure Period (Hrs)			Comments	Total		% Surv.	Mean Surv.
		24	48			Fatal.	Surv.		
Control Flow-thru (C)	A	0/10	0/10	D ₁ - bugs floating	0	10	100	100	
	B	0/9	0/9		0	9	100		
	C								
	D								
Control River (1)	A	0/10	0/10	D ₁ - bugs floating	0	10	100	95	
	B	1/9	0/9		1	9	90		
	C								
	D								
6.25% effluent (2)	A	0/7 A.	0/7	A. Several floaters - 3 dead. D ₁ - bugs floating	0	7	100	100	
	B	0/11	0/11		0	11	100		
	C								
	D								
12.5% effluent (3)	A	1/9	0/9		1	9	90	95	
	B	0/10	0/10		0	10	100		
	C								
	D								
25% effluent (4)	A	0/10	0/10		0	10	100	100	
	B	0/10	0/10		0	10	100		
	C								
	D								
50% effluent (5)	A	0/10	0/10		0	10	100	100	
	B	0/10	0/10		0	10	100		
	C								
	D								
100% effluent (6)	A	0/9 A.	0/9	D ₁ - bugs floating A. one floater dead	0	9	100	100	
	B	0/9 A.	0/9		0	9	100		
	C								
	D								
(7)	A								
	B								
	C								
	D								
Determined By:		DC	JH	Data Check: <u>DC</u>					

96 Hour Pimephales promelas Survival Data

Project No: 5000-860 Client: Flambeau Mining

Start Date: 4-12-95 Finish Date: 4-16-95

Start Time: 1520 Finish Time: 1410

Set By: JH Age: 48 days

Test Solution	Rep.	Fatal. / Surv. Per Exposure Period (Hrs)				Total		% Surv.	Mean Surv.
		24	48	72	96	Fatal.	Surv.		
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								
Control River (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% effluent (3)	A	0/10	0/10	0/10	0/10	0	10	100	95
	B	0/10	1/9	0/9	0/9	1	9	90	
	C								
	D								
25% effluent (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% effluent (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% effluent (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								
(7)	A								
	B								
	C								
	D								
Determined By:		<u>JH</u>	<u>JH</u>	<u>JH</u>	<u>JH</u>	Data Check: <u>De</u>			

ACUTE BIOASSAY LENGTH, WEIGHT AND LOADING RATE DATA

Project: 5000-860 Company: Flambeau Mining
 Test Organism: Pimephales promelas Age: 48 days
 Analysis Date: 4-16-95 Completed By: JF

Mean Length (mm):

	Rep A		Rep B	
1.	<u>14</u>	<u>6.20</u>	<u>12</u>	<u>6.12</u>
2.	<u>15</u>	<u>7.14</u>	<u>13</u>	<u>7.12</u>
3.	<u>15</u>	<u>8.14</u>	<u>13</u>	<u>8.13</u>
4.	<u>16</u>	<u>9.11</u>	<u>17</u>	<u>9.14</u>
5.	<u>16</u>	<u>10.13</u>	<u>18</u>	<u>10.14</u>
	Total <u>148</u>		Total <u>138</u>	

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

$$= \frac{14}{20} = \frac{286}{20}$$

Mean Weight (mg):

	Rep A	Rep B
Tare+Fish	<u>1713.00</u>	<u>1721.13</u>
- Tare	<u>1556.79</u>	<u>1589.97</u>
= Fish	<u>156.22</u>	<u>131.16</u>

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

$$= \frac{14.37}{20} = \frac{287.38}{20}$$

Loading Rate (g/L):

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

$$= \frac{14.37(10)}{750} = 0.19 \text{ g/L}$$

MS
Dr

REFERENCE TOXICANT DATA SUMMARY

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

Through March, 1995

Sodium Chloride Reference Toxicant LC50 Data

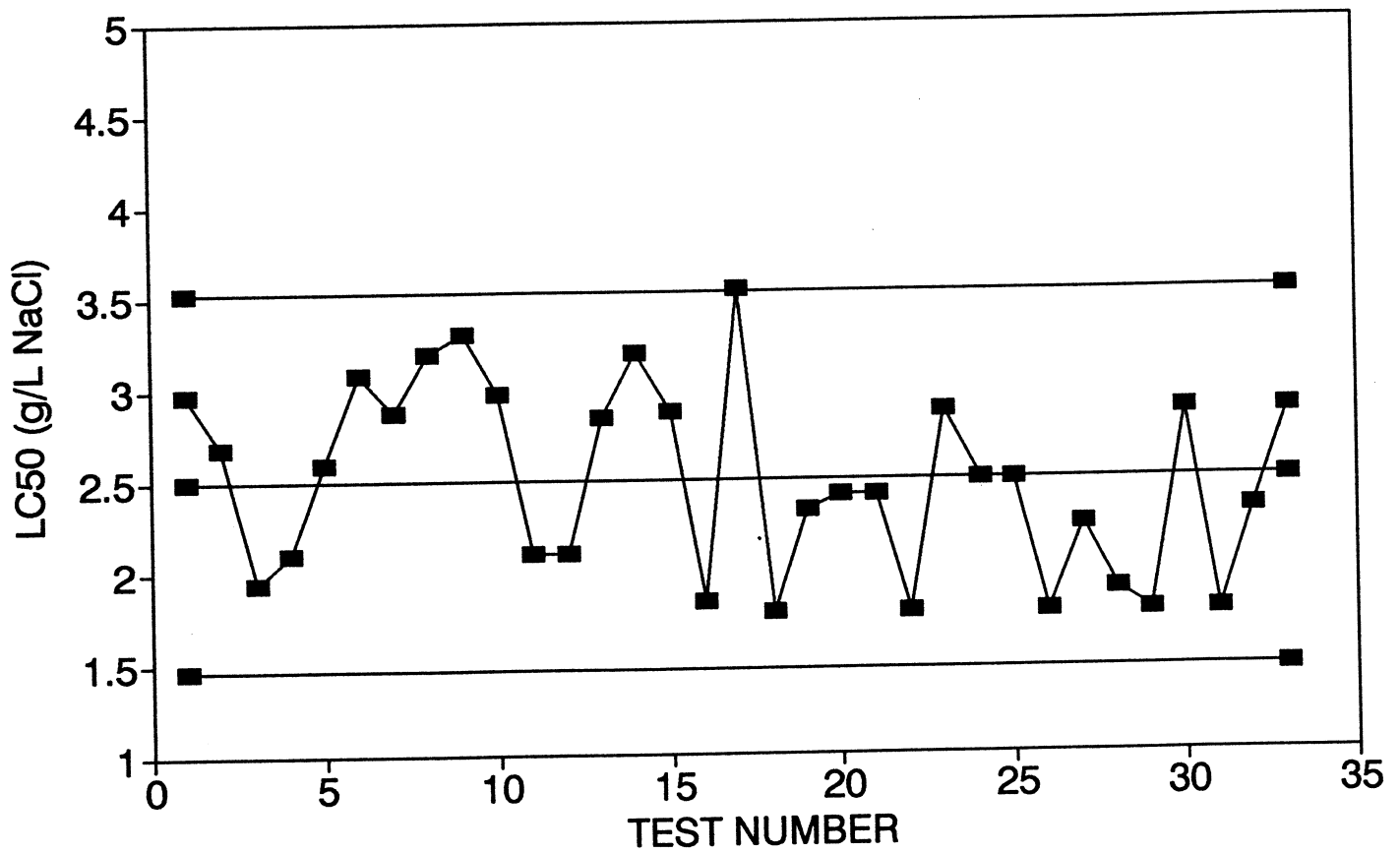
Test Duration: 48 hrs

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 = 2.49
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 = 3.52
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 = 1.46
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	
19	Jan. 18, 94	2.33	
20	Feb. 01, 94	2.41	
21	Mar. 07, 94	2.41	
22	Apr. 06, 94	1.77	
23	May 06, 94	2.87	
24	June 01, 94	2.50	
25	July 14, 94	2.50	
26	Aug. 02, 94	1.77	
27	Sep. 06, 94	2.25	
28	Oct. 04, 94	1.89	
29	Nov. 22, 94	1.77	
30	Dec. 13, 94	2.87	
31	Jan. 03, 95	1.77	
32	Feb. 14, 95	2.33	
33	Mar. 07, 95	2.87	

NaCl 48 Hour LC50 Data

Species: *Ceriodaphnia dubia*



Sodium Chloride Reference Toxicant LC50 Data

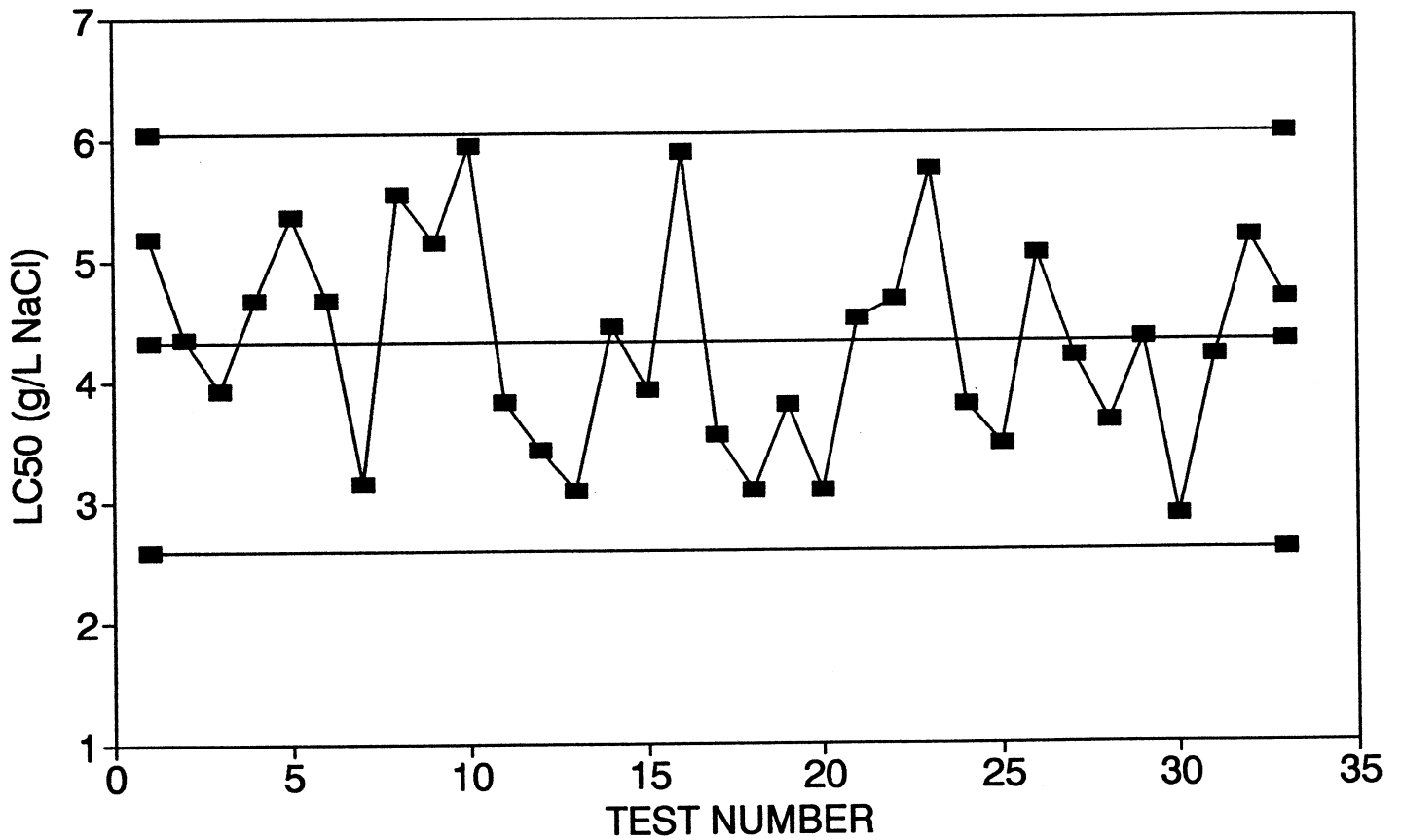
Test Duration: 48 hrs

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 = 4.31
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 = 6.05
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 = 2.58
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	
19	Jan. 20, 94	3.79	
20	Feb. 08, 94	3.08	
21	Mar. 07, 94	4.51	
22	Apr. 06, 94	4.67	
23	May 06, 94	5.74	
24	June 01, 94	3.80	
25	July 14, 94	3.47	
26	Aug. 02, 94	5.04	
27	Sep. 29, 94	4.20	
28	Oct. 04, 94	3.66	
29	Nov. 22, 94	4.35	
30	Dec. 13, 94	2.87	
31	Jan. 19, 95	4.20	
32	Feb. 14, 95	5.18	
33	Mar. 07, 95	4.67	

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 = 8.16
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 = 11.77
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 = 4.54
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	
19	Jan. 18, 94	10.00	
20	Feb. 01, 94	7.58	
21	Mar. 07, 94	7.58	
22	Apr. 28, 94	7.07	
23	May 06, 94	6.83	
24	June 01, 94	7.07	
25	July 05, 94	8.12	
26	Aug. 02, 94	6.60	
27	Sep. 06, 94	7.35	
28	Oct. 04, 94	8.71	
29	Nov. 22, 94	7.58	
30	Dec. 23, 94	7.07	
31	Jan. 03, 95	6.83	
32	Feb. 14, 95	7.32	
33	Mar 07, 95	7.64	

NaCl 96 Hour LC50 Data

Species: *Pimephales promelas*

