



Arkansas Analytical, Inc.

Toxicity Test Results

MAGCOBAR MINE SITE
NPDES PERMIT NUMBER: AR0049794
March 2004

Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test
Test 1000.0

Ceriodaphnia dubia, Survival and Reproduction Test
Test 1002.0

Prepared for: **Mr. Alan B. Brown**
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Introduction

This report contains test results for toxicity testing for the Magcobar Mine Site. The NPDES permit number is AR0049794. The facility is located one mile northeast of Magnet Cove in Sections 10, 11, 14, & 15, Township 3 South, Range 17 West in Hot Springs County, Arkansas. The facility discharges into Chamberlain Creek, thence to Cove Creek, thence to Quachita River in Segment 2F of the Ouachita River Basin.

The permit requires chronic biomonitoring testing once per month for both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for March of 2004.

Plant Operations

To be provided by permittee.

Source of Effluent and Dilution Water

Effluent samples were collected as follows:

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-17-04, 1100	3-18-04, 1100
Sample #2:	3-18-04, 1100	3-19-04, 1100
Sample #3:	3-22-04, 1000	3-23-04, 1000

The sample was a composite collected at the final discharge from the Magcobar mine site.

The following information was collected upon immediate receipt of the samples at the laboratory:

Sample Receiving Information:	Date, Time Sample(s) Received	Storage Temperature (°C)
Sample #1:	3-18-04, 1350	4
Sample #2:	3-19-04, 1525	4
Sample #3:	3-23-04, 1415	4

Chain of custody documentation is located in Appendix A.

The permit designates the receiving water to be used as dilution water for the toxicity tests. Synthetic dilution water was substituted either because zero flow conditions existed or due to an earlier characterization of the receiving water as being toxic.

Each sample was analyzed for pH, hardness, total alkalinity, and conductivity. Results are provided in Appendix B.

Dilution Series

Five dilutions in addition to a control (0% effluent) were used in the toxicity tests. The dilutions, which were made with synthetic water, were 32%, 42%, 56%, 75%, and 100%. The low-flow effluent concentration (**critical dilution**) was defined as **100% effluent**.

Test Methods

EPA Method 1000.0, Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test, was used in this bioassay. Larvae are exposed in a static renewal system for seven days and the results are based on the survival and growth (increase in weight) of the larvae. There were no deviations from the reference method. The test chambers were 500 ml plastic cups, and each chamber contained ten organisms in a test solution volume of 250 mls. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix C.

EPA Method 1002.0, Cladoceran, *Ceriodaphnia dubia*, Survival and Reproduction Test, was also used. Neonates are exposed in a static renewal system until at least 60% of the control organisms have produced a third brood. Results are based on the survival and reproduction of the organisms. One neonate was placed in each of ten replicate chambers using a randomizing template. Test chambers were 30 ml plastic cups filled with 15 ml of test solution. The test temperature was 25 degrees Centigrade. Raw data and statistics are provided in Appendix D.

Test Organisms

The organisms used in Test 1000.0 were < 48 hour old Fathead Minnows, *Pimephales promelas*, which were purchased from Aquatox; a copy of the organism history is provided in Appendix E.

The organisms used in Test 1002.0 were < 24 hour old *Ceriodaphnia dubia* neonates, (all born within the same eight hours), obtained from an in-house culture. An organism history is provided in Appendix E.

Quality Assurance

Test Acceptability

TEST ACCEPTANCE CRITERIA for *Ceriodaphnia dubia*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	80%	X	
Average of 15 or more young per surviving female	16.3	X	
At least 60% of surviving females should have produced 3 broods	60%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	36.9%	X	

TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	100%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	0%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.349	X	
The percent coefficient of variation between replicates must be 40% or less for growth	17.4%	X	

Reference Toxicant

The reference toxicant used was Potassium Chloride prepared in-house. The tests were performed using moderately hard synthetic as dilution water. The results of the reference toxicant were:

REFERENCE TOXICANT

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	125 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	250 ppm KCl	LOEC Growth:	1000 ppm KCl

Quality Assurance charts are provided in Appendix F.

Summary of Results Magcobar Mine Site

<i>Ceriodaphnia dubia</i>		<i>Pimephales promelas</i>	
NOEC / LOEC Survival	100% / NA	NOEC / LOEC survival	100% / NA
NOEC / LOEC Reproduction	100% / NA	NOEC / LOEC growth	100% / NA
Mean number of neonates (critical dilution)	15.6	%CV survival (critical dilution)	0%
%CV Reproduction (critical dilution)	32.7%	Mean dry weight (critical dilution) in milligrams	0.631
		%CV growth (critical dilution)	9.72%

Conclusion

Chronic static renewal larval survival and growth test using fathead minnow, *Pimephales promelas*, (Method 1000.0).

The permit issued to the Magcobar Mine Site, AR0049794, specifies that the **critical dilution is 100% effluent**. The effluent samples did not exhibit lethal effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

Chronic static renewal survival and reproduction test using *Ceriodaphnia dubia*, (Method 1002.0).

The permit issued to the Magcobar, AR0049794, specifies that the **critical dilution is 100% effluent**. The effluent samples did not exhibit lethal effects or sublethal effects at the critical dilution, and, as such, **passed** both portions of the test.

Biomonitoring Analysts:


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**SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL
*PIMEPHALES PROMELAS***

PERMITTEE: Magcobar Mine Site

NPDES #: AR0049794

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-17-04, 1100	3-18-04, 1100
Sample #2:	3-18-04, 1100	3-19-04, 1100
Sample #3:	3-22-04, 1000	3-23-04, 1000

Test initiated (date, time): 3-19-04, 1250 Test terminated (date, time): 3-26-04, 1630

Dilution water used: Soft Synthetic

DATA TABLE FOR FATHEAD MINNOW SURVIVAL

Effluent Conc %	Percent Survival in Replicate Chambers						Mean Percent Survival			
	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	100	100	100	100		100	100	100	0.00
32%	100	100	100	100	100		100	100	100	
42%	100	100	100	100	100		100	100	100	
56%	100	100	100	100	100		100	100	100	
75%	100	100	100	100	100		100	100	100	
100%	100	100	100	100	100		100	100	100	0.00

DATA TABLE FOR GROWTH OF FATHEAD MINNOWS

Effluent Conc %	Average Dry Weight in milligrams in replicate chambers						Mean Dry Weight	CV%
	A	B	C	D	E			
0%	0.382	0.427	0.358	0.292	0.284		0.349	17.4
32%	0.549	0.559	0.492	0.473	0.586		0.532	
42%	0.584	0.558	0.489	0.598	0.437		0.533	
56%	0.561	0.632	0.653	0.539	0.598		0.597	
75%	0.599	0.572	0.547	0.624	0.490		0.566	
100%	0.561	0.604	0.636	0.628	0.728		0.631	9.72

Coefficient of Variation = standard deviation / mean * 100

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL

Pimephales promelas

1. Dunnett's procedure or Steel's Many-One Rank Test as appropriate:
Is the mean survival at 7 days significantly different ($p=0.05$) than the control survival for:

a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X

2. Dunnett's Procedure
Is the mean dry weight (growth) at 7 days significantly different ($p=0.05$) than the control's dry weight (growth) for:

a) LOW FLOW OR CRITICAL DILUTION, (100%) YES _____ NO X

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C): 0

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C): 0

5. Enter percentage corresponding to each parameter below:

a) NOEC survival (parameter TOP6C)= 100 % effluent

b) NOEC growth (parameter TPP6C)= 100 % effluent

c) Coefficient of variation (parameter TQP6C)= 0 %

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING
Ceriodaphnia dubia SURVIVAL AND REPRODUCTION

Permittee: Magcobar Mine Site

NPDES #: AR0049794

Sample Collection:	Date, Time Started	Date, Time Ended
Sample #1:	3-17-04, 1100	3-18-04, 1100
Sample #2:	3-18-04, 1100	3-19-04, 1100
Sample #3:	3-22-04, 1000	3-23-04, 1000

Test initiated (date, time): 3-19-04, 1010 Test terminated (date, time): 3-25-04, 0920

Dilution water used: Soft Synthetic

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION
 NUMBER OF YOUNG PRODUCED PER FEMALE @ TEST TERMINATION
 PERCENT EFFLUENT

Replicate	0%	32%	42%	52%	75%	100%
A	19	11	16	21	14	15
B	15	X2	20	19	14	11
C	14	18	16	8	5	19
D	10	12	27	28	17	15
E	7	16	14	18	8	17
F	X0	16	22	14	7	14
G	22	17	24	15	0	8
H	X3	18	13	22	22	11
I	25	24	X0	10	17	21
J	18	X0	15	24	31	25
Mean	13.3	13.4	16.7	17.9	13.5	15.6
Mean/surviving female	16.3	16.5	18.6	17.9	13.5	15.6
CV%*	36.9					32.7

X= Dead Adult; M= Male (Not considered in statistics)

*Coefficient of Variation = standard deviation/ mean * 100; CV% calculation based on young per surviving female

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING *Ceriodaphnia dubia* SURVIVAL AND REPRODUCTION

Permittee: Magcobar Mine Site

NPDES #: AR0049794

PERCENT SURVIVAL

PERCENT EFFLUENT	0%	32%	42%	56%	75%	100%
Time of Reading: 24 HOURS	100	100	100	100	100	100
48 HOURS	90	90	100	100	100	100
Test termination	80	80	90	100	100	100

1. Fisher's Exact Test:

Is the mean survival at test termination significantly different (p=0.05) than the control survival for:

a) LOW FLOW OR CRITICAL DILUTION, (100%): YES _____ NO X

2. Dunnett's Procedure or Steel's Many One Rank Test:

Is the mean number of young produced per female significantly different (p=0.05) than the controls number of young per female for:

a) LOW FLOW OR CRITICAL DILUTION, (100%): YES _____ NO X

3. If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP3B): 0

4. If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP3B): 0

5. Enter percentage corresponding to each parameter below:

a) NOEC survival (parameter TOP3B)= 100 % effluent

b) NOEC reproduction (parameter TPP3B)= 100 % effluent

c) Coefficient of variation (parameter TQP3B)= 36.9 %



APPENDIX A

Chain of Custody Forms



APPENDIX B

Effluent and Dilution Water Data

CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Fathead Minnow

Lab # / Sample ID		K403681							Test Start (Date/Time)	3-19-04/1250
Client		Weston							Test End (Date/Time)	3-20-04/11030
		Day of Test								
		1	2	3	4	5	6	7	notes/remarks	
Control		3/19	3/20	3/21	3/22	3/23	3/24	3/25	SS 94	
D.O (mg/L)	INITIAL	7.9	7.8	7.8	7.8	7.7	7.7	7.7		
	FINAL	7.5	7.0	7.0	7.7	7.1	6.5	6.5		
pH(mg/L)	INITIAL	6.2	6.8	6.9	6.3	7.0	6.9	7.0		
	FINAL	7.6	6.5	6.8	6.7	7.5	7.3	7.8		
temp(C)	INITIAL	21.3	20.7	21.3	21.6	21.5	21.3	21.4		
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0		
ALKALINITY(mg/L)		30								
HARDNESS(mg/L)		42								
CONDUCTIVITY(umhos/cm)		1101								
CHLORINE(mg/L)		60.05								
CONC:		32%	32%	32%	32%	32%	32%	32%		
D.O (mg/L)	INITIAL	7.9	7.8	8.0	8.1	7.9	8.0	7.8		
	FINAL	7.4	6.7	7.2	7.9	7.2	6.6	6.4		
pH(mg/L)	INITIAL	7.0	7.1	7.2	6.8	7.0	7.0	7.0		
	FINAL	7.3	6.6	6.8	6.9	7.3	7.2	7.4		
temp(C)	INITIAL	21.3	20.7	21.5	21.6	21.5	21.4	21.4		
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:		42%	42%	42%	42%	42%	42%	42%		
D.O (mg/L)	INITIAL	7.9	7.8	8.0	8.1	8.0	8.0	7.9		
	FINAL	7.4	7.0	7.3	7.9	7.1	6.7	6.8		
pH(mg/L)	INITIAL	7.0	7.2	7.3	6.8	7.0	7.2	7.1		
	FINAL	7.2	6.7	6.9	6.9	7.3	7.2	7.4		
temp(C)	INITIAL	21.4	20.8	21.6	21.6	21.5	21.4	21.4		
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:		56%	56%	56%	56%	56%	56%	56%		
D.O (mg/L)	INITIAL	7.9	7.9	8.0	8.1	8.0	7.9	7.9		
	FINAL	7.6	6.4	7.4	7.9	7.1	6.7	7.2		
pH(mg/L)	INITIAL	6.8	7.2	7.5	6.8	7.0	7.2	7.1		
	FINAL	7.3	6.8	7.0	7.1	7.3	7.2	7.4		
temp(C)	INITIAL	21.4	20.8	21.7	21.6	21.5	21.5	21.4		
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:		75%	75%	75%	75%	75%	75%	75%		
D.O (mg/L)	INITIAL	7.9	8.1	8.3	8.1	8.1	7.9	7.9		
	FINAL	7.6	6.4	7.5	7.9	7.1	6.8	7.2		
pH(mg/L)	INITIAL	6.8	7.3	7.7	6.8	7.0	7.3	7.1		
	FINAL	7.2	6.8	7.0	7.1	7.3	7.2	7.4		
temp(C)	INITIAL	21.4	20.8	21.7	21.6	21.5	21.5	21.5		
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:		100%	100%	100%	100%	100%	100%	100%		
D.O (mg/L)	INITIAL	7.9	8.4	8.4	8.2	8.1	8.0	7.9		
	FINAL	7.6	6.5	7.6	7.9	6.9	6.9	6.7		
pH(mg/L)	INITIAL	6.8	7.5	7.7	6.8	7.0	7.4	7.1		
	FINAL	7.1	6.8	7.0	7.2	7.2	7.2	7.3		
temp(C)	INITIAL	21.4	20.8	21.8	21.6	21.5	21.5	21.5		
	FINAL	25.0	25.0	25.0	25.0	25.0	25.0	25.0		
CONC:		100%	A	B	A	B	C	C		
ALKALINITY(mg/L)		17		17	17	17	25			
HARDNESS(mg/L)		1130		980	1130	980	1490			
CONDUCTIVITY(umhos/cm)		2300		2370	2360	2370	2410			
CHLORINE(mg/L)		60.05		60.05	60.05	60.05	60.05			



APPENDIX C

Fathead Minnow Raw Data and Statistics

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB #/S:	1403681		TEST DATES (BEGIN/END):	3/19-26/04
CLIENT:	Weston		WEIGHING DATE/TIME:	3-29-04/1600
ANALYST/S:	mg, AD		DRYING TEMPERATURE (DEGREES C):	60°C
SAMPLE ID:			DRYING TIME (HOURS):	24 hrs.

	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVA (mg)		REMARKS
CONTROL	A61	0.95779	0.95397	0.00382	10	0.382	AVG DRY	
	B62	0.96017	0.95590	0.00427	10	0.427	WEIGHT (mg)	
	C63	0.96354	0.95996	0.00358	10	0.358		0.349
	D64	0.96677	0.96385	0.00292	10	0.292	CV	
	E65	0.96683	0.96399	0.00284	10	0.284		17.4%
32% CONC:	A66	0.96422	0.95873	0.00549	10	0.549	AVG DRY	
	B67	0.96110	0.95551	0.00559	10	0.559	WEIGHT (MG)	
	C68	0.96002	0.95510	0.00492	10	0.492		0.532
	D69	0.96016	0.95543	0.00473	10	0.473	CV	
	E70	0.96222	0.95636	0.00586	10	0.586		
42% CONC:	A71	0.96286	0.95702	0.00584	10	0.584	AVG DRY	
	B72	0.96397	0.95839	0.00558	10	0.558	WEIGHT (MG)	
	C73	0.95938	0.95449	0.00489	10	0.489		0.533
	D74	0.95967	0.95369	0.00598	10	0.598	CV	
	E75	0.96040	0.95603	0.00437	10	0.437		
56% CONC:	A76	0.96552	0.95991	0.00561	10	0.561	AVG DRY	
	B77	0.97050	0.96418	0.00632	10	0.632	WEIGHT (MG)	
	C78	0.96697	0.96044	0.00653	10	0.653		0.597
	D79	0.96687	0.96148	0.00539	10	0.539	CV	
	E80	0.96863	0.96265	0.00598	10	0.598		
75% CONC:	A81	0.96766	0.96167	0.00599	10	0.599	AVG DRY	
	B82	0.96299	0.95727	0.00572	10	0.572	WEIGHT (MG)	
	C83	0.96143	0.95596	0.00547	10	0.547		0.566
	D84	0.96164	0.95540	0.00624	10	0.624	CV	
	E85	0.96835	0.96345	0.00490	10	0.490		
100% CONC:	A86	0.96383	0.95822	0.00561	10	0.561	AVG DRY	
	B87	0.97207	0.96603	0.00604	10	0.604	WEIGHT (MG)	
	C88	0.97049	0.96413	0.00636	10	0.636		0.631
	D89	0.96815	0.96187	0.00628	10	0.628	CV	
	E90	0.96877	0.96149	0.00728	10	0.728		9.72%

CV = (STANDARD DEVIATION/MEAN)*100

AA# K403681 FATHEAD MINNOW SURVIVAL, 3-19-04
File: k403681s Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

D = 0.000

W = 0.000

Critical W (P = 0.05) (n = 30) = 0.927

Critical W (P = 0.01) (n = 30) = 0.900

Data FAIL normality test. Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normal data and should not be performed.

AA# K403681 FATHEAD MINNOW SURVIVAL, 3-19-04
File: k403681s Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: AA# K403681 FATHEAD MINNOW SURVIVAL, 3-19-04
FILE: k403681s
TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.4120
1	CONTROL	2	1.0000	1.4120
1	CONTROL	3	1.0000	1.4120
1	CONTROL	4	1.0000	1.4120
1	CONTROL	5	1.0000	1.4120
2	32 % EFFLUENT	1	1.0000	1.4120
2	32 % EFFLUENT	2	1.0000	1.4120
2	32 % EFFLUENT	3	1.0000	1.4120
2	32 % EFFLUENT	4	1.0000	1.4120
2	32 % EFFLUENT	5	1.0000	1.4120
3	42 % EFFLUENT	1	1.0000	1.4120
3	42 % EFFLUENT	2	1.0000	1.4120
3	42 % EFFLUENT	3	1.0000	1.4120
3	42 % EFFLUENT	4	1.0000	1.4120
3	42 % EFFLUENT	5	1.0000	1.4120
4	56 % EFFLUENT	1	1.0000	1.4120
4	56 % EFFLUENT	2	1.0000	1.4120
4	56 % EFFLUENT	3	1.0000	1.4120
4	56 % EFFLUENT	4	1.0000	1.4120
4	56 % EFFLUENT	5	1.0000	1.4120
5	75 % EFFLUENT	1	1.0000	1.4120
5	75 % EFFLUENT	2	1.0000	1.4120
5	75 % EFFLUENT	3	1.0000	1.4120
5	75 % EFFLUENT	4	1.0000	1.4120
5	75 % EFFLUENT	5	1.0000	1.4120
6	100 % EFFLUENT	1	1.0000	1.4120
6	100 % EFFLUENT	2	1.0000	1.4120
6	100 % EFFLUENT	3	1.0000	1.4120
6	100 % EFFLUENT	4	1.0000	1.4120
6	100 % EFFLUENT	5	1.0000	1.4120

AA# K403681 FATHEAD MINNOW SURVIVAL, 3-19-04

File: k403681s Transform: ARC SINE(SQUARE ROOT(Y))

STEEL'S MANY-ONE RANK TEST

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.412				
2	32 % EFFLUENT	1.412	27.50	16.00	5.00	
3	42 % EFLLUENT	1.412	27.50	16.00	5.00	
4	56 % EFFLUENT	1.412	27.50	16.00	5.00	
5	75 % EFFLUENT	1.412	27.50	16.00	5.00	
6	100 % EFFLUENT	1.412	27.50	16.00	5.00	

Critical values use $k = 5$, are 1 tailed, and $\alpha = 0.05$

AA # K403681, FATHEAD MINNOW GROWTH, 3-19-04
File: k403681g Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.074

W = 0.974

Critical W (P = 0.05) (n = 30) = 0.927

Critical W (P = 0.01) (n = 30) = 0.900

Data PASS normality test at P=0.01 level. Continue analysis.

AA # K403681, FATHEAD MINNOW GROWTH, 3-19-04
File: k403681g Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.40

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

TITLE: AA # K403681, FATHEAD MINNOW GROWTH, 3-19-04
FILE: k403681g
TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3820	0.3820
1	CONTROL	2	0.4270	0.4270
1	CONTROL	3	0.3580	0.3580
1	CONTROL	4	0.2920	0.2920
1	CONTROL	5	0.2840	0.2840
2	32 % EFFLUENT	1	0.5490	0.5490
2	32 % EFFLUENT	2	0.5590	0.5590
2	32 % EFFLUENT	3	0.4920	0.4920
2	32 % EFFLUENT	4	0.4730	0.4730
2	32 % EFFLUENT	5	0.4860	0.4860
3	42 % EFFLUENT	1	0.5840	0.5840
3	42 % EFFLUENT	2	0.5580	0.5580
3	42 % EFFLUENT	3	0.4890	0.4890
3	42 % EFFLUENT	4	0.5980	0.5980
3	42 % EFFLUENT	5	0.4370	0.4370
4	56 % EFFLUENT	1	0.5610	0.5610
4	56 % EFFLUENT	2	0.6320	0.6320
4	56 % EFFLUENT	3	0.6530	0.6530
4	56 % EFFLUENT	4	0.5390	0.5390
4	56 % EFFLUENT	5	0.5980	0.5980
5	75 % EFFLUENT	1	0.5990	0.5990
5	75 % EFFLUENT	2	0.5720	0.5720
5	75 % EFFLUENT	3	0.5470	0.5470
5	75 % EFFLUENT	4	0.6240	0.6240
5	75 % EFFLUENT	5	0.4900	0.4900
6	100 % EFFLUENT	1	0.5610	0.5610
6	100 % EFFLUENT	2	0.6040	0.6040
6	100 % EFFLUENT	3	0.6360	0.6360
6	100 % EFFLUENT	4	0.6280	0.6280
6	100 % EFFLUENT	5	0.7280	0.7280

AA # K403681, FATHEAD MINNOW GROWTH, 3-19-04
File: k403681g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.246	0.049	15.935
Within (Error)	24	0.074	0.003	
Total	29	0.321		

Critical F value = 2.62 (0.05,5,24)
Since $F > \text{Critical } F$ REJECT H_0 : All equal

AA # K403681, FATHEAD MINNOW GROWTH, 3-19-04
 File: k403681g Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.349	0.349		
2	32 % EFFLUENT	0.512	0.512	-4.640	
3	42 % EFFLUENT	0.533	0.533	-5.249	
4	56 % EFFLUENT	0.597	0.597	-7.051	
5	75 % EFFLUENT	0.566	0.566	-6.192	
6	100 % EFFLUENT	0.631	0.631	-8.041	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

AA # K403681, FATHEAD MINNOW GROWTH, 3-19-04
 File: k403681g Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	5			
2	32 % EFFLUENT	5	0.083	23.8	-0.163
3	42 % EFFLUENT	5	0.083	23.8	-0.185
4	56 % EFFLUENT	5	0.083	23.8	-0.248
5	75 % EFFLUENT	5	0.083	23.8	-0.218
6	100 % EFFLUENT	5	0.083	23.8	-0.283



APPENDIX D

Ceriodaphnia dubia Raw Data and Statistics

SURVIVAL AND REPRODUCTION TEST

Ceriodaphnia dubia
 Discharger: WESTON
 Location: WUO3L81

Analyst: MG, AD, MW
 Test Start-Date/Time: 3-19-04 / 10:10
 Test Stop-Date/Time: 3-25-04 / 09:20

Day	Replicate								No. of Young/Adults		Analyst		
	A	B	C	D	E	F	G	H	I	J		Young	Adults
1	0	0	0	0	0	0	0	0	0	0	0	0	MW
2	0	0	0	0	X	0	0	0	0	0	0	0	MW
3	4	0	4	3	0	-	0	3	X	4	22	8	2.4 AD
4	6	5	0	2	2	-	0	-	10	0	25	8	3.1 AD
5	0	5	5	0	0	-	10	-	11	7	38	8	4.8 AD
6	9	5	5	5	5	-	12	-	0	7	48	8	6.0 AD
7													
8													
Total	19	15	14	10	7	X0	22	X3	25	18	133	10.3	CV=36.9%

Conc 1		Conc 4												Total			
Day	%	Replicate												No. of Young	No. of Adults	Analyst	
		A	B	C	D	E	F	G	H	I	J	Young	Adults				
1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MW
2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MW
3		0	4	0	4	0	3	0	0	3	0	0	3	0	14	10	1.4 AD
4	50	3	6	4	1	5	0	5	5	4	1	5	4	11	5	4	AD
5		9	1	4	0	9	7	7	8	0	12	5	7	10	5	7	AD
6		9	8	0	13	4	4	3	9	3	1	5	4	10	5	4	AD
7																	
8																	
Total		21	19	8	28	18	14	15	22	10	24	179					

Day	Replicate								No. of Young/Adults		Analyst		
	A	B	C	D	E	F	G	H	I	J		Young	Adults
1	0	0	0	0	0	0	0	0	0	0	0	0	MW
2	0	0	0	0	0	0	0	0	0	0	0	0	MW
3	0	0	0	0	4	1	0	0	4	-	9	9	1.0 AD
4	1	2	5	8	5	6	6	9	6	-	48	9	5.3 AD
5	0	0	9	0	4	5	6	9	1	-	34	9	3.8 AD
6	10	X0	4	4	3	4	5	0	13	-	43	8	4.8 AD
7													
8													
Total	11	X2	18	12	16	16	17	18	24	X0	134		

Conc 2		Conc 5												Total			
Day	%	Replicate												No. of Young	No. of Adults	Analyst	
		A	B	C	D	E	F	G	H	I	J	Young	Adults				
1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MW
2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MW
3	32	0	0	0	0	4	1	0	0	4	0	5	2	1	10	2	1.1 AD
4		1	2	5	8	5	6	6	9	6	-	32	10	3	2	10	3.2 AD
5		0	0	9	0	4	5	6	9	1	-	48	10	4	8	10	4.8 AD
6		10	X0	4	4	3	4	5	0	13	-	34	10	3	4	10	3.4 AD
7																	
8																	
Total		14	14	5	17	8	7	0	22	17	31	135					

Day	Replicate								No. of Young/Adults		Analyst		
	A	B	C	D	E	F	G	H	I	J		Young	Adults
1	0	0	0	0	0	0	0	0	0	0	0	0	MW
2	0	0	0	0	0	0	0	0	0	0	0	0	MW
3	3	0	3	0	4	0	3	0	0	X0	13	9	1.3 AD
4	8	4	7	6	0	5	10	0	-	3	43	9	4.8 AD
5	0	6	0	12	0	11	13	-	8	-	62	9	6.9 AD
6	5	10	6	9	10	5	0	0	-	4	49	9	5.4 AD
7													
8													
Total	16	20	16	27	14	22	24	13	X0	15	167		

Conc 3		Conc 6												Total			
Day	%	Replicate												No. of Young	No. of Adults	Analyst	
		A	B	C	D	E	F	G	H	I	J	Young	Adults				
1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MW
2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MW
3	42	3	0	3	0	4	0	3	0	0	X0	20	10	2	20	10	2.0 AD
4		8	4	7	6	0	5	10	0	-	3	37	10	3	37	10	3.7 AD
5		0	6	0	12	0	11	13	-	8	-	58	10	5	58	10	5.8 AD
6		5	10	6	9	10	5	0	0	-	4	41	10	4	41	10	4.1 AD
7																	
8																	
Total		15	19	15	17	14	8	11	21	25	15	150	150	150	150	150	150

X=DEAD; Y=MALE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	8	2	10
32% effluent	8	2	10
TOTAL	16	4	20

CRITICAL FISHER'S VALUE (10,10,8) (p=0.05) IS 3. b VALUE IS 8.
 Since b is greater than 3 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
42% effluent	1	9	10
TOTAL	3	17	20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 1.
 NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
56% effluent	0	10	10

TOTAL 2 18 20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
75% effluent	0	10	10
TOTAL	2	18	20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	2	8	10
100% effluent	0	10	10
TOTAL	2	18	20

CRITICAL FISHER'S VALUE (10,10,2) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	2	

1	32% effluent	10	2
2	42% effluent	10	1
3	56% effluent	10	0
4	75% effluent	10	0
5	100% effluent	10	0

AA# K403681, CERIODAPHNIA REPRODUCTION, 3-19-04
File: k403681c Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

***** Shapiro - Wilk's Test is aborted *****

This test can not be performed because total number of replicates
is greater than 50.

Total number of replicates = 60

AA# K403681, CERIODAPHNIA REPRODUCTION, 3-19-04
File: k403681c Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.29

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

TITLE: AA# K403681, CERIODAPHNIA REPRODUCTION, 3-19-04
 FILE: k403681c
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	19.0000	19.0000
1	CONTROL	2	15.0000	15.0000
1	CONTROL	3	14.0000	14.0000
1	CONTROL	4	10.0000	10.0000
1	CONTROL	5	7.0000	7.0000
1	CONTROL	6	0.0000	0.0000
1	CONTROL	7	22.0000	22.0000
1	CONTROL	8	3.0000	3.0000
1	CONTROL	9	25.0000	25.0000
1	CONTROL	10	18.0000	18.0000
2	32 % EFFLUENT	1	11.0000	11.0000
2	32 % EFFLUENT	2	2.0000	2.0000
2	32 % EFFLUENT	3	18.0000	18.0000
2	32 % EFFLUENT	4	12.0000	12.0000
2	32 % EFFLUENT	5	16.0000	16.0000
2	32 % EFFLUENT	6	16.0000	16.0000
2	32 % EFFLUENT	7	17.0000	17.0000
2	32 % EFFLUENT	8	18.0000	18.0000
2	32 % EFFLUENT	9	24.0000	24.0000
2	32 % EFFLUENT	10	0.0000	0.0000
3	42 % EFFLUENT	1	16.0000	16.0000
3	42 % EFFLUENT	2	20.0000	20.0000
3	42 % EFFLUENT	3	16.0000	16.0000
3	42 % EFFLUENT	4	27.0000	27.0000
3	42 % EFFLUENT	5	14.0000	14.0000
3	42 % EFFLUENT	6	22.0000	22.0000
3	42 % EFFLUENT	7	24.0000	24.0000
3	42 % EFFLUENT	8	13.0000	13.0000
3	42 % EFFLUENT	9	0.0000	0.0000
3	42 % EFFLUENT	10	15.0000	15.0000
4	56 % EFFLUENT	1	21.0000	21.0000
4	56 % EFFLUENT	2	19.0000	19.0000
4	56 % EFFLUENT	3	8.0000	8.0000
4	56 % EFFLUENT	4	28.0000	28.0000
4	56 % EFFLUENT	5	18.0000	18.0000
4	56 % EFFLUENT	6	14.0000	14.0000
4	56 % EFFLUENT	7	15.0000	15.0000
4	56 % EFFLUENT	8	22.0000	22.0000
4	56 % EFFLUENT	9	10.0000	10.0000
4	56 % EFFLUENT	10	24.0000	24.0000
5	75 % EFFLUENT	1	14.0000	14.0000
5	75 % EFFLUENT	2	14.0000	14.0000
5	75 % EFFLUENT	3	5.0000	5.0000
5	75 % EFFLUENT	4	17.0000	17.0000
5	75 % EFFLUENT	5	8.0000	8.0000
5	75 % EFFLUENT	6	7.0000	7.0000
5	75 % EFFLUENT	7	0.0000	0.0000
5	75 % EFFLUENT	8	22.0000	22.0000
5	75 % EFFLUENT	9	17.0000	17.0000

5	75	%	EFFLUENT	10	31.0000	31.0000
6	100	%	EFFLUENT	1	15.0000	15.0000
6	100	%	EFFLUENT	2	11.0000	11.0000
6	100	%	EFFLUENT	3	19.0000	19.0000
6	100	%	EFFLUENT	4	15.0000	15.0000
6	100	%	EFFLUENT	5	17.0000	17.0000
6	100	%	EFFLUENT	6	14.0000	14.0000
6	100	%	EFFLUENT	7	8.0000	8.0000
6	100	%	EFFLUENT	8	11.0000	11.0000
6	100	%	EFFLUENT	9	21.0000	21.0000
6	100	%	EFFLUENT	10	25.0000	25.0000

AA# K403681, CERIODAPHNIA REPRODUCTION, 3-19-04
 File: k403681c Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	13.300				
2	32 % EFFLUENT	13.400	104.50	75.00	10.00	
3	42 % EFFLUENT	16.700	117.00	75.00	10.00	
4	56 % EFFLUENT	17.900	121.00	75.00	10.00	
5	75 % EFFLUENT	13.500	102.50	75.00	10.00	
6	100 % EFFLUENT	15.600	112.50	75.00	10.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05



APPENDIX E

Organism History

AQUATOX, INC.

100 Springwood Drive #15
Hot Springs, Arkansas 71913
(501) 767-9120

TEST ORGANISM HISTORY

DATE SHIPPED 3-18-04 Arkansas Analytical

SPECIES Pimephales promelas

QUANTITY SHIPPED 850+

AGE/LIFE STAGE 44hrs 3-18 1500xST

BROODSTOCK SOURCE Anderson Farms, Inc.

CULTURE WATER groundwater

ALKALINITY (Mg/l as CaCO₃) =180

HARDNESS (Mg/l as CaCO₃/Salinity (ppt)) =160

FEEDING Artemia

COMMENTS _____

PACKAGED BY WLL

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

ORGANISM HISTORY

DATE: 1/17/01

SPECIES: Ceriodaphnia dubia

AGE: Variable

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, Selenastrum

Water Chemistry Record:

	Mean	Range
TEMPERATURE:	<u>24 °C</u>	<u>21-24°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO ₃):	<u>112 mg/l</u>	<u>90-124 mg/l</u>
TOTAL ALKALINITY (as CaCO ₃):	<u>85 mg/l</u>	<u>50-85 mg/l</u>
pH:	<u>8.09</u>	<u>7.68-8.14</u>

Comments:



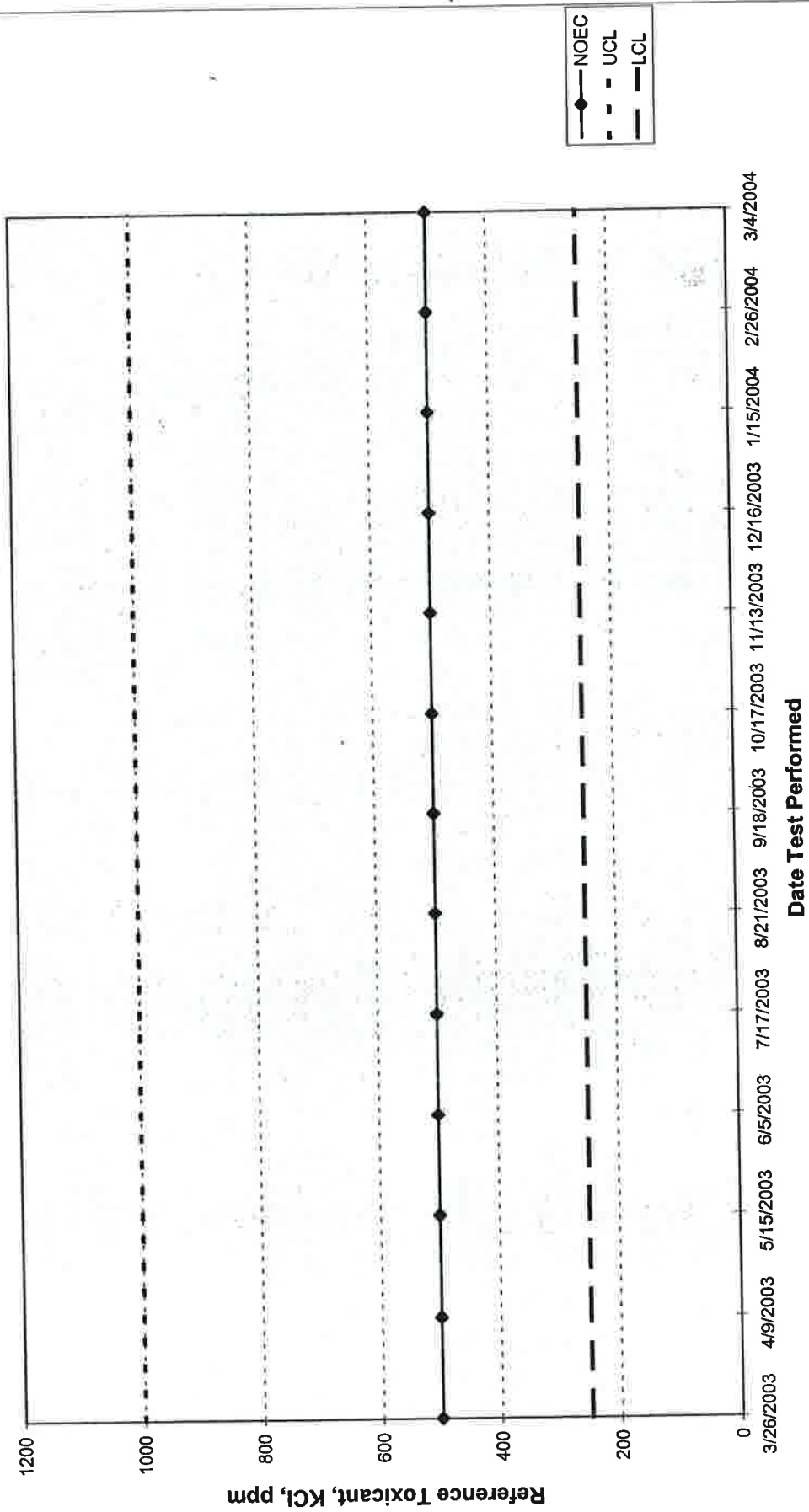
Facility Supervisor



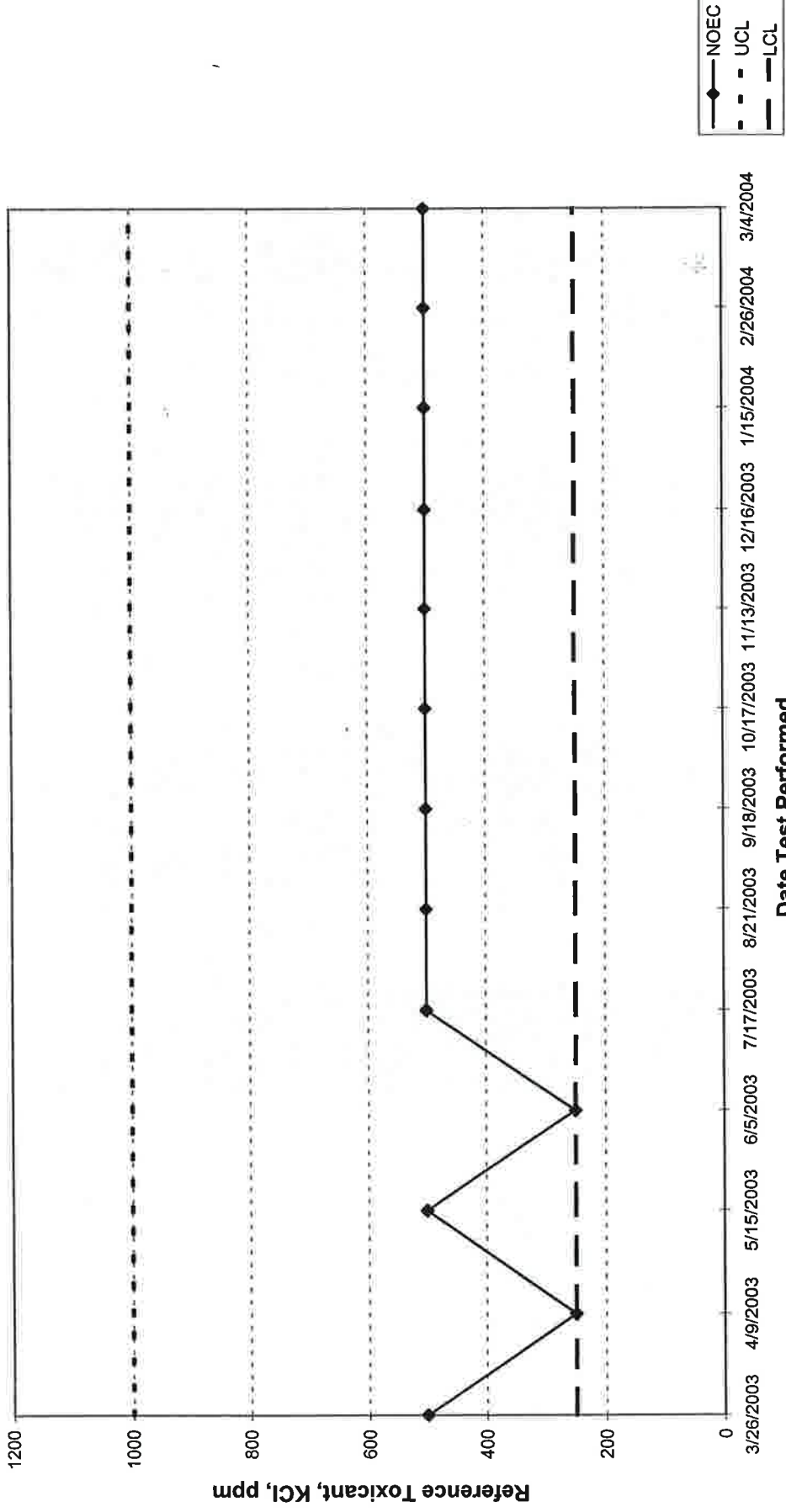
APPENDIX F

Quality Assurance Charts

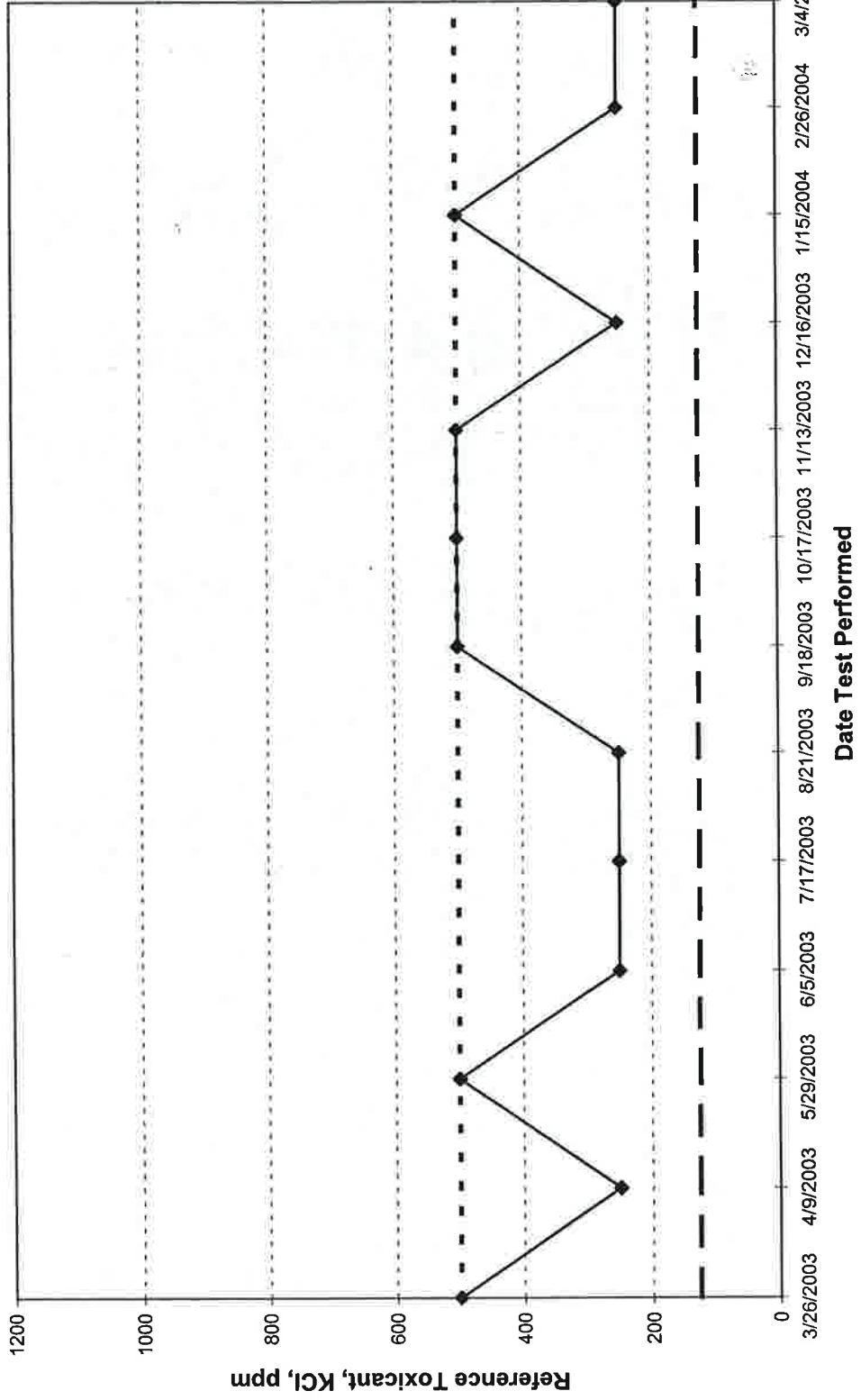
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW SURVIVAL
QUALITY ASSURANCE



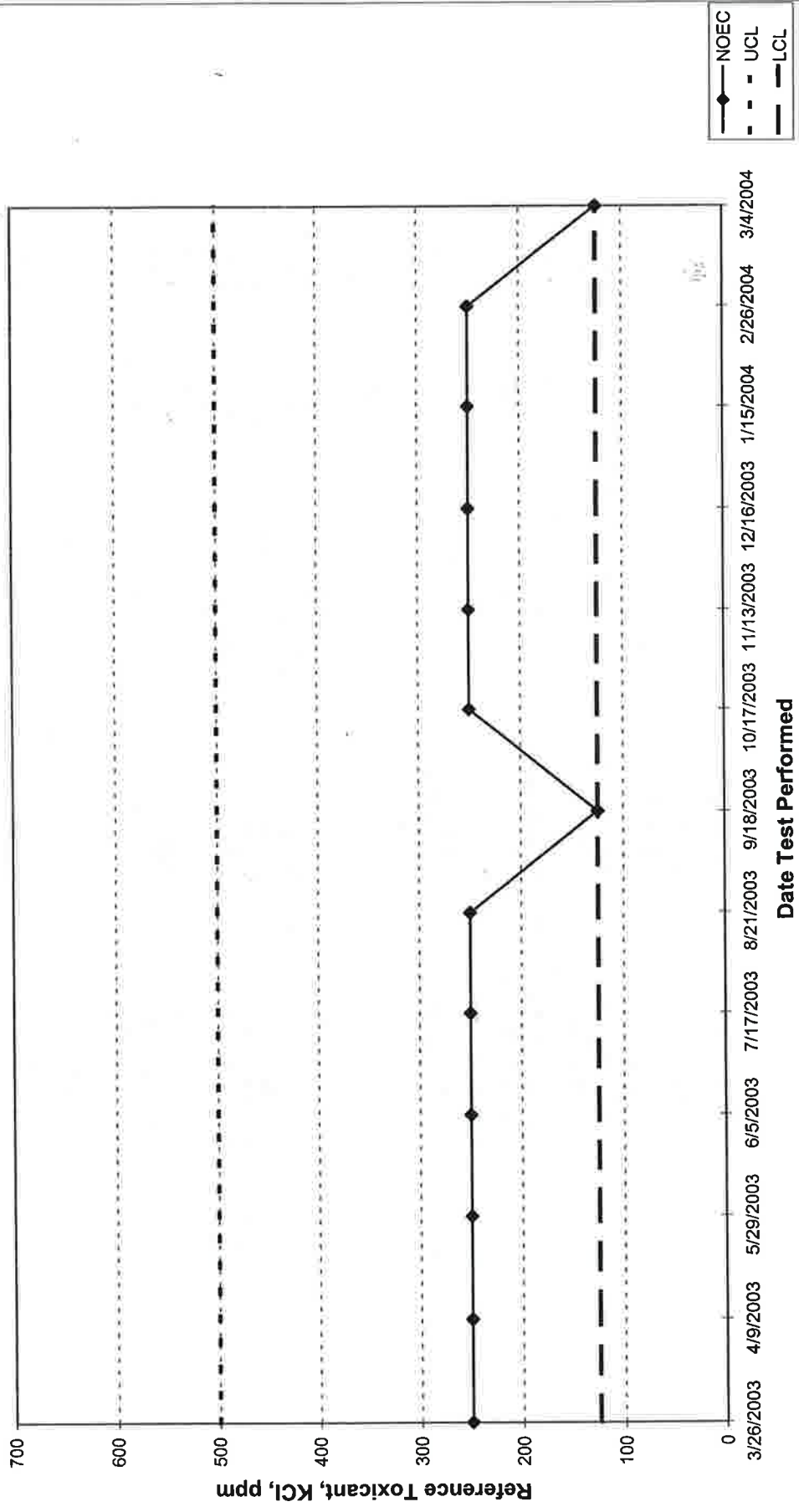
ARKANSAS ANALYTICAL, INC.
FATHEAD MINNOW GROWTH
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA SURVIVAL
QUALITY ASSURANCE



ARKANSAS ANALYTICAL, INC.
CERIODAPHNIA DUBIA REPRODUCTION
QUALITY ASSURANCE



APPENDIX G

Lab Certification



State of Arkansas

Department of Environmental Quality Laboratory Certification Program



Be it known that
Arkansas Analytical, Inc
Little Rock, Arkansas
has earned certification by this Department for the period of
October 30, 2003 to October 30, 2004

Laboratory ID # 60-1754

Certificate # 03-079-0

The following parameters are certified:

Alkalinity	Oil & Grease	Turbidity	Lead	Tin
Ammonia	Orthophosphate	Aluminum	Magnesium	Titanium
BOD	Perchlorate	Antimony	Manganese	Vanadium
Bromide	pH	Arsenic	Mercury	Zinc
CBOD	Phenol	Barium	Molybdenum	Herbicides
Chloride	Sulfate	Beryllium	Nickel	Pesticides & PCBs
Chlorine	Sulfide	Boron	Potassium	Semi-volatiles
COD	Surfactants	Cadmium	Selenium	TPHC
Conductivity	TDS	Calcium	Silver	Volatile Organics
Cyanide	TKN	Chromium	Sodium	Fecal Coliform
Fluoride	TOC	Cobalt	Strontium	Acute Toxicity
Hardness	Total Phosphorus	Copper	Thallium	Chronic Toxicity
Nitrate	Total Solids	Hex. Chromium		
Nitrite	TSS	Iron		

John Sembruski
Quality Assurance Officer

October 24, 2003
Date