

# Arkansas Analytical, Inc.

## Toxicity Test Results

**MAGCOBAR MINE SITE**  
**NPDES PERMIT NUMBER: AR0049794**  
**August, 2009**  
**AFIN# 00-00348**

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

*Ceriodaphnia dubia*, Survival and Reproduction Test  
Test 1002.0

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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 Ouachita River in Segment 2F of the Ouachita River Basin.

The permit requires chronic biomonitoring testing bi-monthly for both *Ceriodaphnia dubia* and *Pimephales promelas*. The test results in this report represent the testing for August of 2009.

## **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:	8-10-09, 1015	8-11-09, 1015
Sample #2:	8-11-09, 0825	8-12-09, 0825
Sample #3:	8-13-09, 0920	8-14-09, 0920

The samples were composites 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	Temperature Upon Receipt (°C)
Sample #1:	8-11-09, 1333	4
Sample #2:	8-12-09, 1349	6
Sample #3:	8-14-09, 1450	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. The alternate method suggested in the method (11.3.4.5) for combating pathogen interference, was run in place of the original fathead minnow test. The test chambers were 30 ml plastic cups with 20 ml of test solution. Each chamber contained 2 organisms. The total number of fish was 40 per test solution. The fish were then combined to perform growth analysis. 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 < 24 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	90%	X	
Average of 15 or more young per surviving female	15.6	X	
At least 60% of surviving females should have produced 3 broods	66.7%	X	
The percent coefficient of variation between replicates must be 40% or less for the young of surviving females	27.3%	X	

#### TEST ACCEPTANCE CRITERIA for *Pimephales promelas*

Control Criteria	Results	Pass	Fail
Greater than or equal to 80% survival	95%	X	
The percent coefficient of variation between replicates must be 40% or less for survival	7.21%	X	
Minimum of 0.25 mg average dry weight of surviving controls	0.451	X	
The percent coefficient of variation between replicates must be 40% or less for growth	10.7%	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> 8/20-27/09		<i>Pimephales promelas</i> 8/20-27/09	
NOEC Survival:	250 ppm KCl	NOEC Survival:	500 ppm KCl
LOEC Survival:	500 ppm KCl	LOEC Survival:	1000 ppm KCl
NOEC Reproduction:	250 ppm KCl	NOEC Growth:	500 ppm KCl
LOEC Reproduction:	500 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)	13.4	%CV survival (critical dilution)	7.21%
%CV Reproduction (critical dilution)	28.2%	Mean dry weight (critical dilution) in milligrams	.510
		%CV growth (critical dilution)	29.9%
PMSD Reproduction	36.9	PMSD Growth	28.3

### 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 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.

Biomonitoring Analysts:



Ken Pigue

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:	8-10-09, 1015	8-11-09, 1015
Sample #2:	8-11-09, 0825	8-12-09, 0825
Sample #3:	8-13-09, 0920	8-14-09, 0920

Test initiated (date, time): 8-12-09, 1530      Test terminated (date, time): 8-19-09, 1445

Dilution water used:      Soft Synthetic

**DATA TABLE FOR FATHEAD MINNOW SURVIVAL**

**Percent Survival in Replicate Chambers**

**Mean Percent Survival**

**DATA TABLE FOR GROWTH OF FATHEAD MINNOWS**

Effluent Conc %	A	B	C	D	E		24 hours	48 hours	7 days	CV %
0%	100	87.5	87.5	100	100		100	100	95	7.21
32%	100	100	87.5	100	100		100	100	97.5	
42%	100	100	100	87.5	100		100	100	97.5	
56%	100	100	100	87.5	100		100	100	97.5	
75%	100	87.5	100	100	100		100	100	97.5	
100%	100	87.5	87.5	100	100		100	100	95	7.21

**SUMMARY**

Effluent Conc %	A	B	C	D	E		Mean Dry Weight	CV%
0%	0.396	0.473	0.407	0.465	0.512		0.451	10.7
32%	0.319	0.373	0.409	0.393	0.425		0.384	
42%	0.475	0.361	0.480	0.382	0.519		0.443	
56%	0.500	0.511	0.494	0.441	0.463		0.482	
75%	0.534	0.370	0.514	0.439	0.666		0.505	
100%	0.374	0.351	0.493	0.664	0.669		0.510	29.9

Coefficient of Variation = standard deviation / mean \* 100

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)= \_\_\_\_\_ 29.9 \_\_\_\_\_ %



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:	8-10-09, 1015	8-11-09, 1015
Sample #2:	8-11-09, 0825	8-12-09, 0825
Sample #3:	8-13-09, 0920	8-14-09, 0920

Test initiated (date, time): 8-12-09, 1420      Test terminated (date, time): 8-19-09, 0955

Dilution water used:    Soft Synthetic

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

Replicate	0%	32%	42%	56%	75%	100%
A	17	5	8	10	13	8
B	8	8	17	15	10	19
C	15	15	14	15	x4	8
D	13	11	15	7	16	13
E	17	13	9	12	9	15
F	15	19	10	18	10	x2
G	17	12	4	12	17	14
H	14	15	16	x2	12	x9
I	24	13	14	16	20	14
J	x3	11	20	24	19	16
Mean	14.3	12.2	12.7	13.1	13.0	11.8
Mean/surviving female	15.6	12.2	12.7	14.3	14.0	13.4
CV%*	27.3					28.2

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	100	100	100	100	100	100
Test termination	90	100	100	90	90	80

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)= 28.2 %

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 1988007

Test Start (Date/Time) 8/12/09

Client Wuxton

Test End (Date/Time) 8/19/09

Day of Test

		1	2	3	4	5	6	7	notes/remarks
<b>Control</b>	<u>SS</u>	<u>8/12</u>	<u>8/13</u>	<u>8/14</u>	<u>8/15</u>	<u>8/16</u>	<u>8/17</u>	<u>8/18</u>	
D.O. (mg/L)	INITIAL	<u>80</u>	<u>78</u>	<u>79</u>	<u>84</u>	<u>85</u>	<u>84</u>	<u>80</u>	
	FINAL	<u>78</u>	<u>76</u>	<u>79</u>	<u>75</u>	<u>77</u>	<u>76</u>	<u>74</u>	
pH (s.u.)	INITIAL	<u>79</u>	<u>75</u>	<u>74</u>	<u>79</u>	<u>78</u>	<u>78</u>	<u>73</u>	
	FINAL	<u>77</u>	<u>78</u>	<u>78</u>	<u>76</u>	<u>75</u>	<u>74</u>	<u>74</u>	
temp (C)	INITIAL	<u>228</u>	<u>228</u>	<u>226</u>	<u>217</u>	<u>226</u>	<u>225</u>	<u>223</u>	
	FINAL	<u>250</u>	<u>250</u>	<u>231</u>	<u>237</u>	<u>250</u>	<u>250</u>	<u>250</u>	
ALKALINITY (mg/L)		<u>38</u>	<u>—</u>	<u>28</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>	
HARDNESS (mg/L)		<u>42</u>	<u>—</u>	<u>56</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>	
CONDUCTIVITY (umhos/cm)		<u>149</u>	<u>—</u>	<u>158</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>	
CHLORINE (mg/L)		<u>0.05</u>	<u>—</u>	<u>0.05</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>	
<b>CONC:</b>	<u>32</u>								
D.O. (mg/L)	INITIAL	<u>80</u>	<u>79</u>	<u>81</u>	<u>84</u>	<u>83</u>	<u>84</u>	<u>83</u>	
	FINAL	<u>78</u>	<u>76</u>	<u>73</u>	<u>74</u>	<u>76</u>	<u>76</u>	<u>73</u>	
pH (s.u.)	INITIAL	<u>79</u>	<u>73</u>	<u>71</u>	<u>76</u>	<u>76</u>	<u>78</u>	<u>70</u>	
	FINAL	<u>74</u>	<u>74</u>	<u>74</u>	<u>73</u>	<u>73</u>	<u>70</u>	<u>73</u>	
temp (C)	INITIAL	<u>229</u>	<u>230</u>	<u>232</u>	<u>219</u>	<u>233</u>	<u>227</u>	<u>223</u>	
	FINAL	<u>250</u>	<u>250</u>	<u>232</u>	<u>238</u>	<u>250</u>	<u>250</u>	<u>250</u>	
<b>CONC:</b>	<u>42</u>								
D.O. (mg/L)	INITIAL	<u>81</u>	<u>80</u>	<u>81</u>	<u>85</u>	<u>83</u>	<u>85</u>	<u>83</u>	
	FINAL	<u>78</u>	<u>75</u>	<u>73</u>	<u>77</u>	<u>77</u>	<u>75</u>	<u>73</u>	
pH (mg/L)	INITIAL	<u>78</u>	<u>73</u>	<u>71</u>	<u>77</u>	<u>76</u>	<u>79</u>	<u>70</u>	
	FINAL	<u>74</u>	<u>74</u>	<u>74</u>	<u>73</u>	<u>72</u>	<u>70</u>	<u>73</u>	
temp (C)	INITIAL	<u>231</u>	<u>230</u>	<u>236</u>	<u>215</u>	<u>239</u>	<u>227</u>	<u>223</u>	
	FINAL	<u>250</u>	<u>250</u>	<u>232</u>	<u>237</u>	<u>250</u>	<u>250</u>	<u>250</u>	
<b>CONC:</b>	<u>56</u>								
D.O. (mg/L)	INITIAL	<u>82</u>	<u>81</u>	<u>82</u>	<u>87</u>	<u>84</u>	<u>86</u>	<u>84</u>	
	FINAL	<u>76</u>	<u>74</u>	<u>72</u>	<u>73</u>	<u>77</u>	<u>76</u>	<u>72</u>	
pH (s.u.)	INITIAL	<u>78</u>	<u>77</u>	<u>71</u>	<u>76</u>	<u>75</u>	<u>74</u>	<u>76</u>	
	FINAL	<u>74</u>	<u>73</u>	<u>73</u>	<u>73</u>	<u>72</u>	<u>6.7</u>	<u>70</u>	
temp (C)	INITIAL	<u>234</u>	<u>230</u>	<u>241</u>	<u>214</u>	<u>239</u>	<u>227</u>	<u>223</u>	
	FINAL	<u>250</u>	<u>250</u>	<u>232</u>	<u>238</u>	<u>250</u>	<u>250</u>	<u>250</u>	
<b>CONC:</b>	<u>75</u>								
D.O. (mg/L)	INITIAL	<u>82</u>	<u>82</u>	<u>84</u>	<u>88</u>	<u>84</u>	<u>86</u>	<u>84</u>	
	FINAL	<u>75</u>	<u>74</u>	<u>73</u>	<u>75</u>	<u>76</u>	<u>75</u>	<u>71</u>	
pH (s.u.)	INITIAL	<u>77</u>	<u>72</u>	<u>71</u>	<u>74</u>	<u>75</u>	<u>74</u>	<u>70</u>	
	FINAL	<u>73</u>	<u>73</u>	<u>73</u>	<u>72</u>	<u>71</u>	<u>6.7</u>	<u>6.8</u>	
temp (C)	INITIAL	<u>235</u>	<u>231</u>	<u>246</u>	<u>209</u>	<u>242</u>	<u>228</u>	<u>223</u>	
	FINAL	<u>250</u>	<u>250</u>	<u>233</u>	<u>238</u>	<u>250</u>	<u>250</u>	<u>250</u>	
<b>CONC:</b>	<u>100</u>								
D.O. (mg/L)	INITIAL	<u>83</u>	<u>82</u>	<u>85</u>	<u>88</u>	<u>84</u>	<u>86</u>	<u>86</u>	
	FINAL	<u>73</u>	<u>74</u>	<u>75</u>	<u>78</u>	<u>75</u>	<u>74</u>	<u>6.8</u>	
pH (s.u.)	INITIAL	<u>76</u>	<u>71</u>	<u>70</u>	<u>74</u>	<u>75</u>	<u>73</u>	<u>6.9</u>	
	FINAL	<u>73</u>	<u>72</u>	<u>72</u>	<u>72</u>	<u>72</u>	<u>6.7</u>	<u>6.7</u>	
temp (C)	INITIAL	<u>238</u>	<u>231</u>	<u>247</u>	<u>202</u>	<u>247</u>	<u>228</u>	<u>224</u>	
	FINAL	<u>250</u>	<u>250</u>	<u>232</u>	<u>238</u>	<u>250</u>	<u>250</u>	<u>250</u>	
<b>CONC:</b>	<u>100%</u>	<u>A</u>	<u>A</u>	<u>A</u>	<u>B</u>	<u>B</u>	<u>C</u>	<u>C</u>	
ALKALINITY (mg/L)		<u>4</u>	<u>—</u>	<u>—</u>	<u>4</u>	<u>—</u>	<u>6</u>	<u>—</u>	
HARDNESS (mg/L)		<u>2600</u>	<u>—</u>	<u>—</u>	<u>2600</u>	<u>—</u>	<u>2600</u>	<u>—</u>	
CONDUCTIVITY (umhos/cm)		<u>1962</u>	<u>—</u>	<u>—</u>	<u>1934</u>	<u>—</u>	<u>1953</u>	<u>—</u>	
CHLORINE (mg/L)		<u>0.05</u>	<u>—</u>	<u>—</u>	<u>0.05</u>	<u>—</u>	<u>0.05</u>	<u>—</u>	



CHEMICAL DATA SHEET FOR CHRONIC TOXICITY TESTING

Cerodaphnia Dubia

Lab # / Sample ID K908007

Test Start (Date/Time) 8/12/09

Client Weston

Test End (Date/Time) 8/19/09

		Day of Test							notes/remarks
		1	2	3	4	5	6	7	
Control	SS	8/12	8/13	8/14	8/15	8/16	8/17	8/18	
D.O. (mg/L)	INITIAL	86	88	79	<del>78</del> 84	85	84	80	
	FINAL	76	75	74	76	74	73	72	
pH (s.u.)	INITIAL	79	75	74	79	78	78	73	
	FINAL	75	74	74	76	79	79	77	
temp (C)	INITIAL	228	228	226	217	216	225	225	
	FINAL	250	250	250	250	250	250	250	
ALKALINITY (mg/L)		38	—	28	—	—	—	—	
HARDNESS (mg/L)		42	—	56	—	—	—	—	
CONDUCTIVITY (umhos/cm)		149	—	158	—	—	—	—	
CHLORINE (mg/L)		0.05	—	0.05	—	—	—	—	
CONC:	32								
D.O. (mg/L)	INITIAL	80	79	81	84	83	84	83	
	FINAL	76	74	74	75	74	74	72	
pH (s.u.)	INITIAL	79	73	71	74	74	74	70	
	FINAL	72	74	72	74	76	76	74	
temp (C)	INITIAL	229	230	232	219	233	227	223	
	FINAL	250	250	250	250	250	250	250	
CONC:	42								
D.O. (mg/L)	INITIAL	81	80	81	85	83	85	83	
	FINAL	75	74	73	75	75	73	72	
pH (mg/L)	INITIAL	78	73	71	77	76	84	70	
	FINAL	72	72	72	73	76	76	74	
temp (C)	INITIAL	231	230	236	215	236	227	223	
	FINAL	250	250	250	250	250	250	250	
CONC:	56								
D.O. (mg/L)	INITIAL	82	81	82	87	84	86	84	
	FINAL	75	74	73	75	76	73	75	
pH (s.u.)	INITIAL	78	72	71	76	75	74	70	
	FINAL	72	71	72	73	76	75	74	
temp (C)	INITIAL	234	230	241	214	239	227	223	
	FINAL	250	250	250	250	250	250	250	
CONC:	75								
D.O. (mg/L)	INITIAL	82	82	84	88	84	86	84	
	FINAL	75	74	74	76	76	72	73	
pH (s.u.)	INITIAL	77	72	71	74	75	74	70	
	FINAL	72	71	72	73	75	75	73	
temp (C)	INITIAL	235	231	246	209	242	228	223	
	FINAL	250	250	250	250	250	250	250	
CONC:	100								
D.O. (mg/L)	INITIAL	83	82	85	88	94	86	80	
	FINAL	76	75	73	76	76	72	73	
pH (s.u.)	INITIAL	76	71	70	74	75	73	69	
	FINAL	70	76	71	73	75	74	72	
temp (C)	INITIAL	238	231	241	202	247	228	224	
	FINAL	250	250	250	250	250	250	250	
CONC:	100%	A	A	A	B	B	C	C	
ALKALINITY (mg/L)		4	—	—	4	—	6	—	
HARDNESS (mg/L)		2600	—	—	2600	—	2600	—	
CONDUCTIVITY (umhos/cm)		1962	—	—	1934	—	1953	—	
CHLORINE (mg/L)		0.05	—	—	0.05	—	0.05	—	

## APPENDIX C

Fathead minnow raw data and statistics

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID 1908007 TEST START DATE 8/12/09 TIME 15:30  
 CLIENT Weston TEST END DATE 8/19/09 TIME 1445  
 AGE AND SOURCE OF MINNOWS

Summary Page

DAY (NUMBER SURVIVING)

SURVIVAL

CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
0%	A	8	8	8	8	8	8	8	100	95	7.21
	B	8	8	8	8	8	8	8	87.5		
	C	8	8	8	8	8	8	8	87.5		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
32	A	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	87.5		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
12	A	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	87.5		
	E	8	8	8	8	8	8	8	100		
50	A	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	8	8	8	8	100		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	87.5		
	E	8	8	8	8	8	8	8	100		
75	A	8	8	8	8	8	8	8	100	97.5	
	B	8	8	8	8	8	8	8	87.5		
	C	8	8	8	8	8	8	8	100		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
100	A	8	8	8	8	8	8	8	100	95	7.21
	B	8	8	8	8	8	8	8	87.5		
	C	8	8	8	8	8	8	8	87.5		
	D	8	8	8	8	8	8	8	100		
	E	8	8	8	8	8	8	8	100		
ANALYST											
DATE:											
TIME:											

CV = PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN \* 100

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START DATE		TIME							
1908007		8/12/09		1530							
CLIENT		TEST END DATE		TIME							
Weston		8/19/09		1445							
AGE AND SOURCE OF MINNOWS		DAY (NUMBER SURVIVING)							SURVIVAL		
REP #	start	1	2	3	4	5	6	7	%	MEAN %	CV
CONC: 0	A	2	3	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1			
CONC: 32	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1			
CONC: 42	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1			
CONC: 56	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1			
CONC: 75	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1			
CONC: 100	A	2	2	2	2	2	2	2			
	B	1	1	1	1	1	1	1			
	C	1	1	1	1	1	1	1			
	D	1	1	1	1	1	1	1			
	E	1	1	1	1	1	1	1			
ANALYST	KP	KP	KP	KP	KP	KP	KP	KP			
DATE:	8/12	8/13/09	8/14/09	8/15	8/16	8/17	8/18/09	8/19/09			
TIME:	1530	1515	1200	1110	1030	1120	1330	1445			

CV = PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN \* 100

SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME						
CLIENT <i>Weston</i>		TEST END	DATE	TIME						
<i>B</i>		AGE AND SOURCE OF MINNOWS								
		DAY (NUMBER SURVIVING)				SURVIVAL				
REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: <i>01</i>	A	2	2	2	2	2	2	2		
	B						1	1		
	C						2	2		
	D						2	2		
	E									
CONC: <i>32</i>	A	2	2	2	2	2	2	2		
	B									
	C									
	D									
	E									
CONC: <i>47</i>	A	2	2	2	2	2	2	2		
	B									
	C									
	D									
	E									
CONC: <i>56</i>	A	2	2	2	2	2	2	2		
	B									
	C									
	D									
	E									
CONC: <i>75</i>	A	2	2	2	2	2	2	2		
	B						2	2		
	C						2	2		
	D									
	E									
CONC: <i>106</i>	A	2	2	2	2	2	2	2		
	B						1	1		
	C						2	2		
	D						2	2		
	E									
ANALYST										
DATE:										
TIME:										

CV = PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN \* 100

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SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME								
CLIENT <i>Winston</i>		TEST END	DATE	TIME								
AGE AND SOURCE OF MINNOWS												
DAY (NUMBER SURVIVING)												
		REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 0	A		2	2	2	2	2	2	2	2		
	B		2	2	2	2	2	2	2	2		
	C		2	2	2	2	2	2	2	2		
	D		2	2	2	2	2	2	2	2		
	E		2	2	2	2	2	2	2	2		
CONC: 3L	A		2	2	2	2	2	2	2	2		
	B		2	2	2	2	2	2	2	2		
	C		2	2	2	2	2	2	2	2		
	D		2	2	2	2	2	2	2	2		
	E		2	2	2	2	2	2	2	2		
CONC: 42	A		2	2	2	2	2	2	2	2		
	B		2	2	2	2	2	2	2	2		
	C		2	2	2	2	2	2	2	2		
	D		2	2	2	2	2	2	2	2		
	E		2	2	2	2	2	2	2	2		
CONC: 56	A		2	2	2	2	2	2	2	2		
	B		2	2	2	2	2	2	2	2		
	C		2	2	2	2	2	2	2	2		
	D		2	2	2	2	2	2	2	2		
	E		2	2	2	2	2	2	2	2		
CONC: 75	A		2	2	2	2	2	2	2	2		
	B		2	2	2	2	2	2	2	2		
	C		2	2	2	2	2	2	2	2		
	D		2	2	2	2	2	2	2	2		
	E		2	2	2	2	2	2	2	2		
CONC: 100	A		2	2	2	2	2	2	2	2		
	B		2	2	2	2	2	2	2	2		
	C		2	2	2	2	2	2	2	2		
	D		2	2	2	2	2	2	2	2		
	E		2	2	2	2	2	2	2	2		
ANALYST												
DATE:												
TIME:												

CV = PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN \* 100

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SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME						
CLIENT <u>Weston</u>		TEST END	DATE	TIME						
AGE AND SOURCE OF MINNOWS										
DAY (NUMBER SURVIVING)										
REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV
CONC: 0	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 32	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 42	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 56	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 75	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
CONC: 100	A	2	2	2	2	2	2	2		
	B	↓	↓	↓	↓	↓	↓	↓		
	C	↓	↓	↓	↓	↓	↓	↓		
	D	↓	↓	↓	↓	↓	↓	↓		
	E	↓	↓	↓	↓	↓	↓	↓		
ANALYST										
DATE:										
TIME:										

CV = PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN \* 100

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SURVIVAL DATA FOR FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

LAB # / SAMPLE ID		TEST START	DATE	TIME									
CLIENT <i>Weston</i>		TEST END	DATE	TIME									
		AGE AND SOURCE OF MINNOWS											
		DAY (NUMBER SURVIVING)							SURVIVAL				
CONC:	REP #	start	1	2	3	4	5	6	7 %	MEAN %	CV		
0	A	2	2	2	3	3	2	2					
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
32	A	2	2	2	3	2	2	2					
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
42	A	2	2	2	2	2	2	2					
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
50	A	2	2	2	2	2	2	2					
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
75	A	2	2	2	2	2	2	2					
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
100	A	2	2	2	2	2	2	2					
	B	1	1	1	1	1	1	1					
	C	1	1	1	1	1	1	1					
	D	1	1	1	1	1	1	1					
	E	1	1	1	1	1	1	1					
ANALYST													
DATE:													
TIME:													

CV = PERCENT COEFFICIENT OF VARIATION: STANDARD DEVIATION/MEAN \* 100

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WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s:		K908007		TEST DATES (BEGIN / END):		8/12-19/09	
CLIENT:		EEMA		WEIGHING DATE / TIME:		8/25/09, 1600	
ANALYSTS:		KP		DRYING TEMP (DEGREES C):		60	
SAMPLE ID:		SEE COC		DRYING TIME (HOURS):		24	
	REP #	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A	1.00160	0.99843	0.00317	8	0.396	AVG DRY
	B	1.00387	1.00009	0.00378	8	0.473	WEIGHT (mg)
	C	1.00602	1.00276	0.00326	8	0.407	0.451
	D	0.98930	0.98558	0.00372	8	0.465	CV
	E	1.00114	0.99704	0.00410	8	0.512	10.7
CONC:	A	1.00198	0.99943	0.00255	8	0.319	AVG DRY
	B	1.00598	1.00300	0.00298	8	0.373	WEIGHT (mg)
	C	0.99869	0.99542	0.00327	8	0.409	0.384
	D	1.00635	1.00321	0.00314	8	0.393	CV
	E	1.00848	1.00508	0.00340	8	0.425	
CONC:	A	1.00896	1.00516	0.00380	8	0.475	AVG DRY
	B	0.97874	0.97585	0.00289	8	0.361	WEIGHT (mg)
	C	1.00546	1.00162	0.00384	8	0.480	0.444
	D	0.99093	0.98787	0.00306	8	0.382	CV
	E	1.00381	0.99966	0.00415	8	0.519	
CONC:	A	1.00011	0.99611	0.00400	8	0.500	AVG DRY
	B	0.97096	0.96687	0.00409	8	0.511	WEIGHT (mg)
	C	0.96198	0.95803	0.00395	8	0.494	0.482
	D	1.00139	0.99786	0.00353	8	0.441	CV
	E	1.00546	1.00176	0.00370	8	0.463	
CONC:	A	0.99919	0.99492	0.00427	8	0.534	AVG DRY
	B	1.00026	0.99730	0.00296	8	0.370	WEIGHT (mg)
	C	0.99854	0.99443	0.00411	8	0.514	0.504
	D	0.99605	0.99254	0.00351	8	0.439	CV
	E	1.00477	0.99944	0.00533	8	0.666	
CONC:	A	1.00453	1.00154	0.00299	8	0.374	AVG DRY
	B	0.99216	0.98935	0.00281	8	0.351	WEIGHT (mg)
	C	0.99446	0.99052	0.00394	8	0.493	0.510
	D	1.00703	1.00172	0.00531	8	0.664	CV
	E	1.01051	1.00516	0.00535	8	0.669	29.9

CV = (STANDARD DEVIATION/MEAN)\*100

REMARKS:

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Pimephales promelas

FATHEAD MINNOW

TEST 1000.0

WEIGHT DATA FOR LARVAL SURVIVAL AND GROWTH TEST

LAB # / #s: K968007	TEST DATES (BEGIN / END): 8/12-19/09
CLIENT: Weston	WEIGHING DATE / TIME: 8/25/09, 1600
ANALYSTS: KP	DRYING TEMP (DEGREES C): 60
SAMPLE ID:	DRYING TIME (HOURS): 24

REP#	FINAL DRY WEIGHT TIN+LARVAE (g)	INITIAL WEIGHT TIN (g)	TOTAL DRY WEIGHT OF LARVAE (g)	NUMBER OF LARVAE	DRY WEIGHT OF LARVAE (mg)	
CONTROL	A	1.00160	0.99843			AVG DRY WEIGHT (mg)
	B	1.00387	1.00009			
	C	1.00602	1.00276			
	D	0.98930	0.98558			
	E	1.00114	0.99704			
CONC:	A	1.00198	0.99943			AVG DRY WEIGHT (mg)
	B	1.00598	1.00300			
	C	0.99869	0.99542			
	D	1.00635	1.00321			
	E	1.00848	1.00508			
CONC:	A	1.00896	1.00516			AVG DRY WEIGHT (mg)
	B	0.97874	0.97585			
	C	1.00546	1.00162			
	D	0.99093	0.98787			
	E	1.00381	0.99966			
CONC:	A	1.00011	0.99611			AVG DRY WEIGHT (mg)
	B	0.97096	0.96687			
	C	0.96198	0.95803			
	D	1.00139	0.99786			
	E	1.00546	1.00176			
CONC:	A	0.99919	0.99492			AVG DRY WEIGHT (mg)
	B	1.00026	0.99730			
	C	0.99854	0.99443			
	D	0.99605	0.99254			
	E	1.00177	0.99944			
CONC:	A	1.00453	1.00154			AVG DRY WEIGHT (mg)
	B	0.99896	0.98935			
	C	0.99446	0.99052			
	D	1.00703	1.00172			
	E	1.01051	1.00516			

CV = (STANDARD DEVIATION/MEAN)\*100

REMARKS:

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AA# K908007, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-12-09  
File: H:/toxstat/monte\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

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D = 0.230

W = 0.701

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# K908007, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-12-09  
File: H:/toxstat/monte\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 0.42

---

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.

AA# K908007, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-12-09  
File: H:/toxstat/monte\FHSURV~1. Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

---

D = 0.189

W = 0.701

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# K908007, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-12-09

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 0.42

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# K908007, FATHEAD MINNOW SURVIVAL, CHRONIC, 8-12-09  
FILE: H:/toxstat/monte\FHSURV~1.  
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.0000	1.3931
1	CONTROL	2	0.8750	1.2094
1	CONTROL	3	0.8750	1.2094
1	CONTROL	4	1.0000	1.3931
1	CONTROL	5	1.0000	1.3931
2	32 % EFFLUENT	1	1.0000	1.3931
2	32 % EFFLUENT	2	1.0000	1.3931
2	32 % EFFLUENT	3	0.8750	1.2094
2	32 % EFFLUENT	4	1.0000	1.3931
2	32 % EFFLUENT	5	1.0000	1.3931
3	42 % EFFLUENT	1	1.0000	1.3931
3	42 % EFFLUENT	2	1.0000	1.3931
3	42 % EFFLUENT	3	1.0000	1.3931
3	42 % EFFLUENT	4	0.8750	1.2094
3	42 % EFFLUENT	5	1.0000	1.3931
4	56 % EFFLUENT	1	1.0000	1.3931
4	56 % EFFLUENT	2	1.0000	1.3931
4	56 % EFFLUENT	3	1.0000	1.3931
4	56 % EFFLUENT	4	0.8750	1.2094
4	56 % EFFLUENT	5	1.0000	1.3931
5	75 % EFFLUENT	1	1.0000	1.3931
5	75 % EFFLUENT	2	0.8750	1.2094
5	75 % EFFLUENT	3	1.0000	1.3931
5	75 % EFFLUENT	4	1.0000	1.3931
5	75 % EFFLUENT	5	1.0000	1.3931
6	100 % EFFLUENT	1	1.0000	1.3931
6	100 % EFFLUENT	2	0.8750	1.2094
6	100 % EFFLUENT	3	0.8750	1.2094
6	100 % EFFLUENT	4	1.0000	1.3931
6	100 % EFFLUENT	5	1.0000	1.3931

## STEEL'S MANY-ONE RANK TEST

- Ho:Control&lt;Treatment

---

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	1.320				
2	32 % EFFLUENT	1.356	30.00	16.00	5.00	
3	42 % EFFLUENT	1.356	30.00	16.00	5.00	
4	56 % EFFLUENT	1.356	30.00	16.00	5.00	
5	75 % EFFLUENT	1.356	30.00	16.00	5.00	
6	100 % EFFLUENT	1.320	27.50	16.00	5.00	

---

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

AA# K908007, FATHEAD MINNOW GROWTH CHRONIC, 8-12-09  
File: H:/toxstat/monte\FHGROWTH. KP1 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's test for normality

---

D = 0.186

W = 0.951

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# K908007, FATHEAD MINNOW GROWTH CHRONIC, 8-12-09  
File: H:/toxstat/monte\FHGROWTH. Transform: ARC SINE(SQUARE ROOT(Y))

---

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 13.58

---

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.

KP2

TITLE: AA# K908007, FATHEAD MINNOW GROWTH CHRONIC, 8-12-09  
 FILE: H:/toxstat/monte\FHGROWTH.  
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.3960	0.6806
1	CONTROL	2	0.4730	0.7584
1	CONTROL	3	0.4070	0.6919
1	CONTROL	4	0.4650	0.7504
1	CONTROL	5	0.5120	0.7974
2	32 % EFFLUENT	1	0.3190	0.6002
2	32 % EFFLUENT	2	0.3730	0.6570
2	32 % EFFLUENT	3	0.4090	0.6939
2	32 % EFFLUENT	4	0.3930	0.6776
2	32 % EFFLUENT	5	0.4250	0.7101
3	42 % EFFLUENT	1	0.4750	0.7604
3	42 % EFFLUENT	2	0.3610	0.6445
3	42 % EFFLUENT	3	0.4800	0.7654
3	42 % EFFLUENT	4	0.3820	0.6663
3	42 % EFFLUENT	5	0.5190	0.8044
4	56 % EFFLUENT	1	0.5000	0.7854
4	56 % EFFLUENT	2	0.5110	0.7964
4	56 % EFFLUENT	3	0.4940	0.7794
4	56 % EFFLUENT	4	0.4410	0.7263
4	56 % EFFLUENT	5	0.4630	0.7484
5	75 % EFFLUENT	1	0.5340	0.8194
5	75 % EFFLUENT	2	0.3700	0.6539
5	75 % EFFLUENT	3	0.5140	0.7994
5	75 % EFFLUENT	4	0.4390	0.7242
5	75 % EFFLUENT	5	0.6660	0.9546
6	100 % EFFLUENT	1	0.3740	0.6580
6	100 % EFFLUENT	2	0.3510	0.6341
6	100 % EFFLUENT	3	0.4930	0.7784
6	100 % EFFLUENT	4	0.6640	0.9525
6	100 % EFFLUENT	5	0.6690	0.9578

AA# K908007, FATHEAD MINNOW GROWTH CHRONIC, 8-12-09  
 File: H:/toxstat/monte\FHGROWTH. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.057	0.011	1.476
within (Error)	24	0.186	0.008	
Total	29	0.244		

Critical F value = 2.62 (0.05,5,24)  
 Since F < Critical F FAIL TO REJECT Ho: All equal

File: H:/toxstat/monte\FHGROWTH.

KP2  
Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	0.736	0.451		
2	32 % EFFLUENT	0.668	0.384	1.220	
3	42 % EFFLUENT	0.728	0.443	0.135	
4	56 % EFFLUENT	0.767	0.482	-0.564	
5	75 % EFFLUENT	0.790	0.505	-0.980	
6	100 % EFFLUENT	0.796	0.510	-1.085	

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

AA# K908007, FATHEAD MINNOW GROWTH CHRONIC, 8-12-09

File: H:/toxstat/monte\FHGROWTH.

Transform: ARC SINE(SQUARE ROOT(Y))

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	0.128	28.3	0.067
3	42 % EFFLUENT	5	0.128	28.3	0.007
4	56 % EFFLUENT	5	0.128	28.3	-0.031
5	75 % EFFLUENT	5	0.128	28.3	-0.054
6	100 % EFFLUENT	5	0.128	28.3	-0.060



APPENDIX D

*Ceriodaphnia dubia* Raw Data and Statistics

Cerodaphnia dubia

**SURVIVAL AND REPRODUCTION TEST**

KP

Discharger: Weston Lab Number/s  
 Location:  
 Date Sample Collected:

Analyst:  
 Test Start - Date/Time: 8/12/09, 1420  
 Test Stop - Date/Time: 8/19/09, 0955

Conc 1		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
0	1	0	0	0	0	0	0	0	0	0	0	0	10	0	KP
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	KP
	3	0	0	0	0	0	0	0	0	0	0	0	10	0	KP
	4	2	1	0	0	0	1	1	3	2		10	10	1.0	KP
	5	2	2	0	5	2	2	4	0	2	0	19	10	1.9	KP
	6	6	5	4	0	9	6	5	4	7	4	45	9	5.2	KP
	7	7	0	1	8	6	7	7	9	12	-	67	9	7.4	KP
	8														
	Total	17	8	15	13	17	15	17	14	24	23	144	15	15.6	KP

Conc 4		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
56	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	0	1	0	0	0	10	0.1	
	4	0	2	1	2	0	3	0	1	5	3	17	9	1.9	
	5	2	5	4	0	5	3	0	-	2	4	25	9	2.8	
	6	4	8	2	2	4	3	4	-	2	5	34	9	3.8	
	7	6	0	8	9	3	9	8	-	11	9	54	9	6.0	
	8														
	Total	10	15	15	7	12	18	12	2	16	24	131			

Conc 2		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
32	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	2	2	0	0	0	0	0	0	0	0	2	10	0.2	
	4	0	0	2	3	0	4	0	3	0	0	12	10	1.2	
	5	0	2	4	1	3	1	1	2	0	1	15	10	1.5	
	6	0	4	1	0	3	5	6	3	2	4	28	10	2.8	
	7	3	2	8	7	7	9	5	7	11	6	65	10	6.5	
	8														
	Total	5	8	15	11	13	19	12	15	13	11	122			

Conc 5		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
75	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	2	0	0	0	0	2	10	0.2	
	4	0	0	1	2	0	0	0	1	0	4	8	10	0.8	
	5	3	2	3	5	0	0	3	0	2	6	24	9	2.7	
	6	7	2	-	5	2	3	5	4	8	1	37	9	4.1	
	7	3	6	-	4	7	5	9	7	10	8	59	9	6.6	
	8														
	Total	13	10	4	16	9	10	17	12	20	19	130			

Conc 3		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
42	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	0	1	0	0	1	1	10	0.1	
	4	2	2	0	7	1	0	0	1	2	2	11	16	1.1	
	5	0	4	3	3	1	4	3	1	1	3	23	10	2.3	
	6	0	4	2	7	0	3	7	6	0	6	29	10	2.9	
	7	6	7	9	4	7	3	6	7	11	9	63	10	6.3	
	8														
	Total	8	17	14	15	9	10	4	16	14	20	127			

Conc 6		Replicate										No. of Young	No. of Adult	Young/Adult	Analyst
%	Day	A	B	C	D	E	F	G	H	I	J				
100	1	0	0	0	0	0	0	0	0	0	0	0	10	0	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0	
	3	0	0	0	0	0	2	0	0	2	0	4	9	0.4	
	4	0	3	0	0	2	-	0	1	0	0	6	9	0.6	
	5	0	5	2	0	0	-	3	1	0	2	13	9	1.4	
	6	1	4	3	3	5	-	7	4	6	5	38	8	4.8	
	7	7	7	3	10	8	-	7	-	6	9	57	8	7.1	
	8														
	Total	8	19	8	13	15	2	14	9	14	16	118			

X= DEAD; Y= MALE

$\bar{X} = 13.4$   
 $CV = 28.2$

AA # K908007 C. DUBIA CHRONIC, REPRODUCTION, 8-12-09  
File: H:/toxstat/monte\C.DUB 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 # K908007 C. DUBIA CHRONIC, REPRODUCTION, 8-12-09  
File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

---

Bartlett's test for homogeneity of variance  
Calculated B1 statistic = 1.91

---

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.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	9	10
32%	0	10	10
TOTAL	1	19	20

CRITICAL FISHER'S VALUE (10,10,1) (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	1	9	10
42%	0	10	10
TOTAL	1	19	20

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

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
56%	9	1	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.  
 Since b is greater than 4 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
75%	9	1	10
TOTAL	18	2	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 9.  
 Since b is greater than 4 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	9	1	10
100%	8	2	10
TOTAL	17	3	20

CRITICAL FISHER'S VALUE (10,10,9) (p=0.05) IS 4. b VALUE IS 8.  
 Since b is greater than 4 there is no significant difference  
 between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
-------	----------------	----------------	-------------	-------------

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

---

TITLE: AA # K908007 C. DUBIA CHRONIC, REPRODUCCION, 8-12-09  
FILE: H:/toxstat/monte\C.DUB  
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

---

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	17.0000	17.0000
1	CONTROL	2	8.0000	8.0000
1	CONTROL	3	15.0000	15.0000
1	CONTROL	4	13.0000	13.0000
1	CONTROL	5	17.0000	17.0000
1	CONTROL	6	15.0000	15.0000
1	CONTROL	7	17.0000	17.0000
1	CONTROL	8	14.0000	14.0000
1	CONTROL	9	24.0000	24.0000
1	CONTROL	10	3.0000	3.0000
2	32 % EFFLUENT	1	5.0000	5.0000
2	32 % EFFLUENT	2	8.0000	8.0000
2	32 % EFFLUENT	3	15.0000	15.0000
2	32 % EFFLUENT	4	11.0000	11.0000
2	32 % EFFLUENT	5	13.0000	13.0000
2	32 % EFFLUENT	6	19.0000	19.0000
2	32 % EFFLUENT	7	12.0000	12.0000
2	32 % EFFLUENT	8	15.0000	15.0000
2	32 % EFFLUENT	9	13.0000	13.0000
2	32 % EFFLUENT	10	11.0000	11.0000
3	42 % EFFLUENT	1	8.0000	8.0000
3	42 % EFFLUENT	2	17.0000	17.0000
3	42 % EFFLUENT	3	14.0000	14.0000
3	42 % EFFLUENT	4	15.0000	15.0000
3	42 % EFFLUENT	5	9.0000	9.0000
3	42 % EFFLUENT	6	10.0000	10.0000
3	42 % EFFLUENT	7	4.0000	4.0000
3	42 % EFFLUENT	8	16.0000	16.0000
3	42 % EFFLUENT	9	14.0000	14.0000
3	42 % EFFLUENT	10	20.0000	20.0000
4	56 % EFFLUENT	1	10.0000	10.0000
4	56 % EFFLUENT	2	15.0000	15.0000
4	56 % EFFLUENT	3	15.0000	15.0000
4	56 % EFFLUENT	4	7.0000	7.0000
4	56 % EFFLUENT	5	12.0000	12.0000
4	56 % EFFLUENT	6	18.0000	18.0000
4	56 % EFFLUENT	7	12.0000	12.0000
4	56 % EFFLUENT	8	2.0000	2.0000
4	56 % EFFLUENT	9	16.0000	16.0000
4	56 % EFFLUENT	10	24.0000	24.0000

5	75 % EFFLUENT	1	13.0000	13.0000
5	75 % EFFLUENT	2	10.0000	10.0000
5	75 % EFFLUENT	3	4.0000	4.0000
5	75 % EFFLUENT	4	16.0000	16.0000
5	75 % EFFLUENT	5	9.0000	9.0000
5	75 % EFFLUENT	6	10.0000	10.0000
5	75 % EFFLUENT	7	17.0000	17.0000
5	75 % EFFLUENT	8	12.0000	12.0000
5	75 % EFFLUENT	9	20.0000	20.0000
5	75 % EFFLUENT	10	19.0000	19.0000
6	100 % EFFLUENT	1	8.0000	8.0000
6	100 % EFFLUENT	2	19.0000	19.0000
6	100 % EFFLUENT	3	8.0000	8.0000
6	100 % EFFLUENT	4	13.0000	13.0000
6	100 % EFFLUENT	5	15.0000	15.0000
6	100 % EFFLUENT	6	2.0000	2.0000
6	100 % EFFLUENT	7	14.0000	14.0000
6	100 % EFFLUENT	8	9.0000	9.0000
6	100 % EFFLUENT	9	14.0000	14.0000
6	100 % EFFLUENT	10	16.0000	16.0000

AA # K908007 C. DUBIA CHRONIC, REPRODUCCION, 8-12-09  
 File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	37.350	7.470	0.286
Within (Error)	54	1412.300	26.154	
Total	59	1449.650		

Critical F value = 2.45 (0.05,5,40)  
 Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal

AA # K908007 C. DUBIA CHRONIC, REPRODUCCION, 8-12-09  
 File: H:/toxstat/monte\C.DUB Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	14.300	14.300		
2	32 % EFFLUENT	12.200	12.200	0.918	
3	42 % EFFLUENT	12.700	12.700	0.700	
4	56 % EFFLUENT	13.100	13.100	0.525	
5	75 % EFFLUENT	13.000	13.000	0.568	
6	100 % EFFLUENT	11.800	11.800	1.093	

Dunnnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

AA # K908007 C. DUBIA CHRONIC, REPRODUCTION, 8-12-09

File: H:/toxstat/monte\C.DUB

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	10			
2	32 % EFFLUENT	10	5.283	36.9	2.100
3	42 % EFFLUENT	10	5.283	36.9	1.600
4	56 % EFFLUENT	10	5.283	36.9	1.200
5	75 % EFFLUENT	10	5.283	36.9	1.300
6	100 % EFFLUENT	10	5.283	36.9	2.500

---

AA # K908007 C. DUBIA CHRONIC, REPRODUCTION, 8-12-09

File: H:/toxstat/monte\C.DUB

Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST

-

Ho:Control<Treatment

---

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	14.300				
2	32 % EFFLUENT	12.200	87.50	75.00	10.00	
3	42 % EFFLUENT	12.700	96.00	75.00	10.00	
4	56 % EFFLUENT	13.100	96.50	75.00	10.00	
5	75 % EFFLUENT	13.000	98.00	75.00	10.00	
6	100 % EFFLUENT	11.800	89.50	75.00	10.00	

---

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



## APPENDIX E

### Organism History

# AQUATOX, INC.

416 Twin Points Road  
Hot Springs, Arkansas 71913  
(501) 520-0560

## TEST ORGANISM HISTORY

DATE SHIPPED 8-12-09 Arkansas Analytical

SPECIES *Pimephales promelas*

QUANTITY SHIPPED 240<sup>+</sup> + 1150<sup>+</sup>

AGE/LIFE STAGE 24 hrs 8/12 <sup>1500<sup>+</sup></sup> + 5 Days old 8/12

BROODSTOCK SOURCE Anderson Farms, AR

CULTURE WATER groundwater

ALKALINITY (Mg/l as CaCO<sub>3</sub>) =180

HARDNESS (Mg/l as CaCO<sub>3</sub>)/Salinity (ppt) =160

FEEDING ATF 1000

COMMENTS \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

PACKAGED BY LMH

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: 6/22/09

SPECIES: Ceriodaphnia dubia

AGE: Variable

LIFE STAGE: Adult

HATCH DATE: Variable

BEGAN FEEDING: Immediately

FOOD: YTC, *Selenastrum* sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	<u>25°C</u>	<u>20-25°C</u>
SALINITY/CONDUCTIVITY:	<u>--</u>	<u>--</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>142 mg/l</u>	<u>86-124 mg/l</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>100 mg/l</u>	<u>65-130 mg/l</u>
pH:	<u>7.92</u>	<u>7.56-8.35</u>

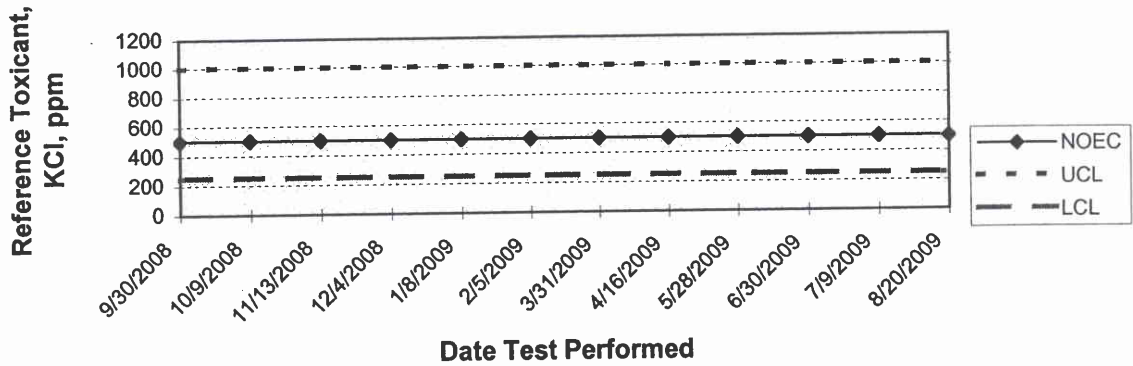
Comments:

  
\_\_\_\_\_  
Facility Supervisor

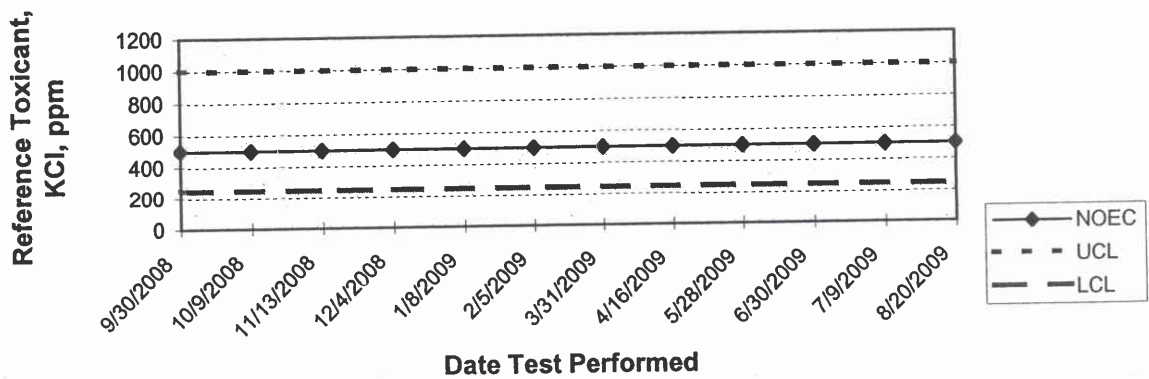
## APPENDIX F

### Quality Assurance Charts

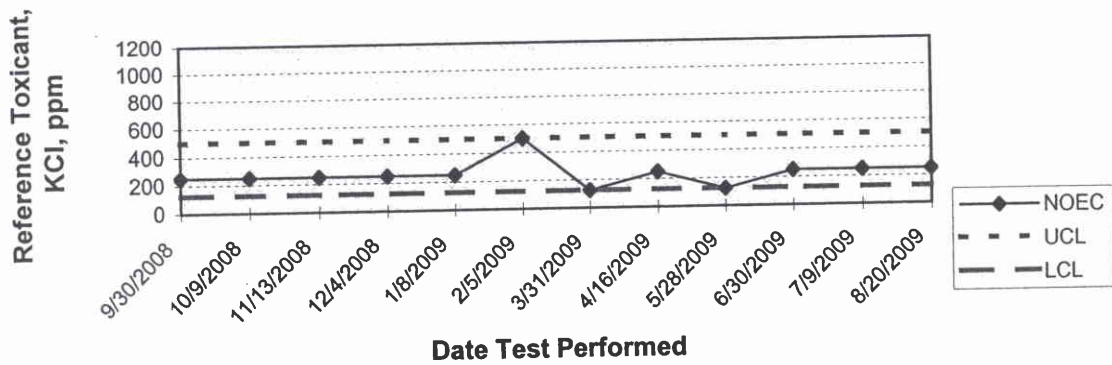
**ARKANSAS ANALYTICAL, INC.**  
**FATHEAD MINNOW SURVIVAL**  
**QUALITY ASSURANCE**



**ARKANSAS ANALYTICAL, INC.**  
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