



CHEMICAL COMPANY

August 31, 2012

Mary Barnett, Ecologist
Water Division
Arkansas Department of Environmental Quality
5301 Northshore Drive
North Little Rock, AR. 72118-5317

Re: Final Report Sub-lethal Response (SLR): Outfall 001
El Dorado Chemical Company (EDCC)
NPDES Permit # AR 00000752; AFIN 70-00040

Dear Ms. Barnett:

As required by the Sub-lethal Response (SLR) Study Plan– Revision 2.1 (dated April 19, 2010) (**Attachment A**), this letter report is submitted in accordance with the SLR Study Plan approval letter dated May 7, 2010 (**Attachment B**). El Dorado Chemical Company (EDCC) initiated the implementation of the SLR study after receipt of ADEQs notice of the SLR plan approval. Activities completed as part of the SLR study included:

- 1) Continued the WET testing and analytical chemistry on a monthly basis (as discharge occurred) with particular attention to the sub-lethal endpoints and the effect of the UV treatment on the 100% effluent exposure;
- 2) Completed toxicity identification evaluations (TIEs) on Outfall 001 effluent samples collected concurrently with the monthly WET testing samples in an effort to identify cause and/or confirm previous results; and
- 3) Continued the assemblage of facility discharge data including: flow, TDS, chloride, sulfate, TOC, storm amounts and pH.

Electronic copies (pdf files) of the individual TIE reports completed during the course of the SLR are provided in **Attachments C1-4, C-5 & 5b, C-6a & 6b, and C-7a & 7b.**

Continued the Routine Baseline Toxicity Testing and Associated Analytical Chemistry

During the SLR Study, the routine WET tests were completed monthly on Outfall 001 effluent. The individual reports have been submitted to ADEQ along with the DMR for the testing period and are not included with this final report. The following table summarizes the results of the monthly WET tests during the SLR study period (April 2010 through July 2012).

Table 1. Summary of EDCC Outfall 001 Effluent Routine WET Testing Results POR April 2010 - July 2012.

Test Period	Water Flea				Fathead Minnow			
	% NOEC		Number of young/female		% NOEC		Growth mg per larvae	
	Lethality	Reproduction	Cnt	100%	Lethality	Growth	Cnt	100% effluent
April 2010	100	100	18.8	18.3	100	100	0.846	0.78
May 2010	100	100	16.3	14.7	100	100	0.559	0.64
June 2010	100	100	22.8	20.8	100	100	0.565	0.63
July 2010	100	100			100	100		
August 2010	NO DISCHARGE							
September 2010	100	100	15.9	19.9	100	100	0.468	0.4
October 2010	NO DISCHARGE							
November 2010	100	32	24	6.6	100	100	0.458	0.36
December 2010	100	32	19.5	4.5	100	100	0.93	0.95
January 2011	100	32	20.5	5.7	100	100	0.63	0.66
February 2011	100	32	19.9	12.2	100	100	0.68	0.87
March 2011	100	100	20.3	19.2	100	100	0.868	0.82
April 2011	NO DISCHARGE							
May 2011	100	56	21.1	15.6	100	100	0.725	0.653
June 2011	100	100	14.2	13.7	100	100	0.615	0.610
July 2011	100	100	16.4	17.5	100	100	0.550	0.480
August 2011	100	<32	22	4.6	100	100	0.27	0.505
September 2011	100	32	17.2	8.1	100	100	0.395	0.403
October 2011*	100	56*	15.7	13.7	100	100	0.410	0.350
November 2011	100	<32	15.6	2.1	100	100	0.260	0.315
December 2011	100	<32	22.9	3.3	100	100	0.293	0.293

Test Period	Water Flea				Fathead Minnow			
	% NOEC		Number of young/female		% NOEC		Growth mg per larvae	
	Lethality	Reproduction	Cnt	100%	Lethality	Growth	Cnt	100% effluent
January 2012**	100	<32	21.7	0.1	100	100	0.570	0.533
February 2012	100	<32	19.6	1.1	100	100	0.553	0.633
March 2012	100	<32	20.1	4.3	100	100	0.383	0.425
April 2012	100	<32	28.8	7.7	100	32	0.465	0.240
May 2012	100	<32	24.5	6.2	100	100	0.490	0.605
June 2012	100	<32	23.5	14.3	100	100	0.558	0.538
July 2012	100	<32	20	5.5	100	100	0.515	0.503

*October WET test had atypical dose response curve, 100% exposure not significantly different from the control but the 75% exposure was, therefore the reported NOEC was 56%.

** January water flea test board had males in test field. Males indication of culture stress.

Monthly WET Testing

Pimephales promelas (Fathead Minnow)

All of the fathead minnow WET tests completed during the SLR study passed both the lethal and sub-lethal endpoints through March 2012. This consecutive string of 23 **PASSED** tests with sub-lethal (growth endpoint) NOECs of 100% effluent extends back to November 2009. However, the April 2012 fathead minnow sub-lethal endpoint failed in the 100% effluent exposure. This was the first and only sub-lethal failure in the fathead minnow WET testing during the SLR study. A detailed review of the WET test report and associated analytical chemistry did not identify a potential cause for the test failure.

Ceriodaphnia dubia (Water Flea)

Like the fathead minnow, the lethality endpoint has **PASSED ALL** the WET testing, prior to and during the SLR study period.

The SLR study was initiated in April 2010, and the sub-lethal endpoint of reproduction **PASSED** in the 100% critical dilutions until the November 2010 WET test. There was a series of sporadic failures from November 2010 until May 2011, followed by a pair of passed tests. Then in August 2011, a consecutive string of sub-lethal failures occurred with NOEC of <32% effluent.

A detailed review of the routine WET tests results failed to verify a direct cause of the sub-lethal test failures. However, there appears to be a relationship with the reference toxicity test performance (sensitivity of test cultures) and the magnitude of the reduced neonate production in the effluent exposures. During the SLR study,

the lab culture performance in the monthly reference toxicant testing was less than the culture means for extended periods. This indicates increased culture sensitivity causing a decreased performance when compared to the long term mean performance. This decrease in sensitivity translated to organisms that are more likely to underperform when exposed to stress (effluents) than those whose sensitivity was normal or above the average. In other words, the cultures used in the WET testing during the SLR were frequently predisposed to decreased reproduction due to increased culture sensitivities.

In addition male water fleas were identified in the test setup for the January 2012 WET tests. The presence of male organisms in the water flea cultures are indicative of culture stress which may have played a role in the tests results.

Summary of Routine WET Testing

The following observations are based on the results of the routine WET testing completed during the SLR study.

- The string of consecutive fathead minnow WET tests that passed the lethality endpoint in the 100% Outfall 001 effluent dating back to December 2009 was continued through the SLR. However, the fathead minnow failed the sub-lethal growth endpoint only once during the SLR study during April 2012.
- The water flea lethality endpoint passed every routine WET test during the SLR study.
- Water fleas passed the sub-lethal endpoint in 1/3 of the monthly WET tests completed during the SLR study.
- Sub-lethal WET test failures of the water flea were consistent during the latter part of the SLR study period;
- On numerous occasions during the SLR study, the effluent samples used in the WET testing had to be filtered to remove native daphnids from the 100% effluent prior to the test initiation, demonstrating that the native daphnia populations are maintained in the 100% effluent;
- At times, the culture sensitivities (and variability in the reference toxicity IC_{25}) are responsible for the sub-lethal WET test performance;
- A cause for the sub-lethal failures could not be confirmed based on the results of the routine WET test chemistry;
- The sub-lethal water flea WET test results triggered the requirement for chronic Toxicity Identification Evaluations (TIE) for the water flea in future monthly monitoring; and
- Ultra-violet (UV) light treatments to kill native pathogens were either not different from the control or the performance of the UV treated effluents were less than the untreated effluents. These results indicate that native pathogens were not contributing to the WET test performance during the SLR study.

Sub-lethal Chronic TIEs Completed During the SLR Study

TIE efforts were initiated in December 2011, after consecutive sub-lethal WET test failures. Seven (7) chronic sub-lethal toxicity identification evaluations (TIEs) were completed on samples collected during the months of December 2011 and February, March, April, May, June and July 2012. The sub-lethal reproduction of the individual TIE manipulations completed during the SLR study is summarized in Table 2. In addition, the following sections provide additional details of the individual TIEs. The TIE reports are provided as Attachment C to this report.

Table 2. EDCC Outfall 001 Reproduction Summary (number of young per adult) for all TIE Samples and the Resulting Chronic Toxic Units (TUc) for Individual Manipulations - POR December 2011- July 2012.

Baseline Tests							
Sample Date	DMW	12.5%	25%	50%	100%	IC25 (%)	TUc
12/14/2011	36.3	11.2	4.5	5	3.3	4.5	22.2
02/15/2012	22.3	11.2	5.7	3.7	5.3	6.3	26.3
03/14/2012	38.2	29.3	24.8	18.2	17.2	15.3	6.5
04/12/2012	27.0	25.0	12.2	7.8	7.3	16.4	6.1
05/23/2012	19.8	18.3	16.2	16.2	6.2	56.4	1.8
06/13/2012	33.6	39.2	31.5	27.2	27.0	>100	<1.0
07/18/2012	38.7	14.3	14.0	10.5	2.3	5.0	20
1.0 µm filtration							
Sample Date	DMW	12.5%	25%	50%	100%	IC25 (%)	TUc
12/14/2011	25.8	2.2	1.8	2.2	3.0	3.5	28.6
02/15/2012	25.2	3.7	5.3	3.0	4.3	3.8	26.3
03/14/2012	32.2	30.7	37.3	42.0	42.5	>100	<1.0
04/12/2012	28.8	36.7	25.3	28.7	7.7	49.4	2.0
05/23/2012	29.5	22.2	24.8	30.5	35.0	>100	<1.0
06/13/2012	36.5	38.5	34.2	36.3	41.5	>100	<1.0
07/18/2012	35.8	39.5	40.6	43.3	42.7	>100	<1.0
C-18 SPE Treatment							
Sample Date	DMW	12.5%	25%	50%	100%	IC25 (%)	TUc
12/14/2011	31.7	38.8	37.5	41.0	40.3	>100	<1.0
02/15/2012	25.2	38.8	36.2	35.2	36.0	>100	<1.0
03/14/2012	39.5	33.2	37.7	39.7	44	>100	<1.0
04/12/2012	17.2	23.5	22.8	27.5	8.8	56.1	0.2
05/23/2012	29.5	26.8	19.0	25.5	34.2	>100	<1.0
06/13/2012	38.8	41.3	41.5	41.7	41.0	>100	<1.0
07/18/2012	35.8	35.0	37.5	35.2	43.7	>100	<1.0

Aeration							
Sample Date	DMW	12.5%	25%	50%	100%	IC25 (%)	TU_c
12/14/2011	29.7	5.5	3.7	3.5	4.3	3.9	25.6
02/15/2011	27.8	8.5	5.2	5.7	6.5	4.5	22.2
03/14/2012	38.2	29.7	23.8	22.5	19.8	15	6.7
04/12/2012	18.2	22.5	20.2	13.7	3.7	23.3	4.3
05/23/2012	33.5	11.0	18.8	17.8	5.8	5.9	16.9
06/13/2012	42.7	37.3	31.5	26.5	22.3	>100	>1.0
07/18/2012	35.2	17.5	11.3	7.2	11.2	6.3	15.9
EDTA 25 mg/l							
Sample Date	DMW^a	12.5%	25%	50%	100%	IC25 (%)	TU_c
12/14/2011	32.2	1.7	2.3	4.0	0	3.4	29.4
02/15/2012	25.0b	7.3	4.2	3.7	0.7	4.5	22.2
03/14/2012	28.0	25.3	17.5	16.5	6.8	19.8	5.1
04/12/2012	34.3	18.2	11.7	10.3	4.2	7.3	13.7
05/23/2012	15.2	12.2	10.5	9.8	0.0	18.4	5.4
06/13/2012	36.0	32.7	31.2	24.7	17.5	<100	>1.0
07/18/2012	36.7	11.0	5.6	14.3	9.2	4.6	21.7
NaThio							
Sample Date	DMW^b	12.5%	25%	50%	100%	IC25 (%)	TU_c
12/14/2011	25	8.0	12.5	11.5	5.5	5.9	16.9
02/15/200	22.5	10.7	7.0	5.7	0.2	64	15.6
3/14/2012	30.2	28.2	29.3	25.2	16.8	64.2	16
4/14/2012	32.5	25.8	17.7	17.7	5.8	15.1	6.6
5/23/2012	27.8	15.0	6.5	11.0	7.3	6.8	14.7
06/13/20	35.3	36.7	31.0	28.3	24.5	<100	>1.0
07/18/2012	29.8	18.6	14.3	15.5	15.2	9.3	10.7

a - Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

b - NaThio treated DMW did not meet control requirement for minimum reproduction (>15.0), therefore the baseline control was substituted for statistical analysis.

The following observations of the individual manipulations are provided below:

- Six (6) of the seven (7) TIEs demonstrate sub-lethal response in the lab WET tests and the toxic units ranged from 26.3 TU to <1TU, indicating a wide range of response in the baseline sub-lethal effects. The June 2012 baseline TIE sample did not demonstrate WET test failure.
- None of the manipulations were successful in removing all of the sub-lethal effects in all test periods; however, the combination of

filtration plus C-18 SPE treatment worked in tandem in all but the April 2012 Sub-lethal TIE;

- The variability in the TIE results indicated that the sub-lethal failures were caused by multiple factors;
- Filtration was the most successful manipulation, removing all sub-lethal effects in 4 of the 7 TIEs. However, this manipulation was not successful in removing any effects during the December 2011 and February 2012 samples;
- The C-18 SPE treatment was successful in removal of effects in the December 2011 and February 2012 samples, indicating a non-polar organic chemical may be responsible to the observed effects. However, the C-18 SPE treatment did not appear to improve sub-lethal performance in subsequent TIE efforts;
- Aeration was not successful in reducing the effects in any of the TIE efforts, indicating volatile chemicals were not responsible for sub-lethal WET test failures;
- EDTA treatments did not routinely improve sub-lethal performance, eliminating chelatable toxic heavy metals as a potential cause for the sub-lethal WET tests failures; and
- Sodium thiosulfate treatments did not improve sub-lethal performance in any of the TIE characterizations, eliminating oxidation/reducing compounds as a potential candidate for the sub-lethal WET test failures.

Additional details of each individual TIE is provided in the following sections.

December 2011 TIE Results

The results of the TIE completed in December 2011 are provided in Table 3. The only manipulation that eliminated the sub-lethal effect (removing 100% of the toxicity in the lab testing) was the C18-SPE treatment. This treatment indicates that the cause of the sub-lethal WET tests failure was a polar or non-polar organic. However, the C-18 SPE column can also act as a filter and reduce the effect of certain "filterable" materials. However, none of the other 5 treatments effectively increased the reproduction of the water flea, including the 1.0 μm filtration treatment (see the 100% column under the reproduction heading). Since the filtration treatment did not improve the reproduction while the C-18 SPE column removed 100% of the effect, the cause for the WET test failure appeared to be an organic (polar or non-polar) compound.

Table 3. Summary of Outfall 001 Chronic TIE Test Results for Sample Collected in December 2011.

<i>Ceriodaphnia dubia</i> TIE Sample Collected on 12/14/11 Sample ID: EDCC Outfall 001 Final Effluent Test Dates: 12/16-23/2011	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TU	TUc% Removed
Baseline Toxicity (No manipulation) – Test 1	100%	67%	50%	100%	50%	NA%	0.0	–
1.0 µm Filtration – Test 2	83%	33%	67%	67%	100%	>100%	0.0	NA
C18-SPE Treatment – Test 3	100%	100%	100%	100%	100%	>100%	0.0	NA
Aeration – Test 4	100%	33%	67%	100%	83%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	100%	17%	33%	67%	67%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	33%	83%	83%	100%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	36.3	11.2	4.5	5.0	3.3	4.5	22.2	NA
1.0 µm Filtration – Test 2	25.8	2.2	1.8	2.2	3.0	3.5	28.6	0
C18-SPE Treatment – Test 3	31.7	38.8	37.5	41.0	40.3	>100	<1.0	100
Aeration – Test 4	29.7	5.5	3.7	3.5	4.3	3.9	25.6	0
Cation Chelation with EDTA – Test 5	32.2	1.7	2.3	4.0	0	3.4	29.4	0
Sodium Thiosulfate Treatment – Test 6	25	8.0	12.5	11.5	5.5	5.9	16.9	23.7

NA- Not Applicable or Not available

TU_c, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

The other treatments completed during this chronic sub-lethal TIE failed to significantly alter results indicating that reduced neonate production were not related to:

- heavy metals (total or dissolved);
- easily filterable components (larger than 1.0 µm);
- a volatile, oxidizable or aeratable compound;
- an oxidant or thiosulfate reducible compound (e.g. chlorine); or
- conventional oxygen demanding waste.

February 2012 TIE results

The results of the TIE completed in February 2012 are summarized in Table 4. The only manipulation that reduced the sub-lethal effect (removing 100% of the toxicity in the lab testing) was the C18-SPE treatment. This result was also demonstrated in the December 2011 TIE effort. The C18-SPE treatment indicates that the cause of the sub-lethal WET tests failure was a polar or non-polar organic. However, the C-18 SPE column can also act as a filter and reduce the effect of certain “filterable” materials. However, none of the other 5 treatments effectively

increased the reproduction of the water flea, including the 1.0 µm filtration treatment (compare 100% column under the reproduction heading). Since the filtration treatment did not improve the reproduction while the C18-SPE column removed 100% of the effect, the cause for the WET test failure appeared to be an organic (polar or non-polar).

Table 4. Summary of EDCC Outfall 001 Chronic TIE Test Results for Sample Collected in February 2012.

<i>Ceriodaphnia dubia</i> TIE Sample Collected on 2/15/12 Sample ID: EDCC Outfall 001 Final Effluent Test Dates: 2/16-23/2012	Percent Survival							
	<i>DMW</i>	12.5%	25%	50%	100%	<i>LC</i> ₅₀	<i>TUc</i>	<i>TUc</i> % Removed
Baseline Toxicity (No manipulation) – Test 1	100%	80%	100%	100%	100%	>100%	0.0	–
1.0 µm Filtration – Test 2	100 %	33%	83%	100%	100%	>100%	0.0	NA
C18-SPE Treatment – Test 3	83%	100%	100%	100%	100%	>100%	0.0	NA
Aeration – Test 4	100%	67%	83%	100%	100%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	100%	67%	83%	100%	83%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	67%	100%	83%	100%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	<i>DMW</i>	12.5%	25%	50%	100%	<i>IC</i> ₂₅	<i>TUc</i>	<i>TUc</i> % Removed
Baseline Toxicity (No manipulation) – Test 1	22.3	11.2	5.7	3.7	5.3	6.3	15.9	NA
1.0 µm Filtration – Test 2	25.2	3.7	5.3	3.0	4.3	3.8	26.3	0
C18-SPE Treatment – Test 3	25.2	38.8	36.2	35.2	36.0	>100	<1.0	100
Aeration – Test 4	27.8	8.5	5.2	5.7	6.5	4.5	22.2	0
Cation Chelation with EDTA – Test 5	25.0 b	7.3	4.2	3.7	0.7	4.5	22.2	0
Sodium Thiosulfate Treatment – Test 6	22.5	10.7	7.0	5.7	0.2	6.4	15.6	1.6

NA- Not Applicable or Not available

a *TUc*, Chronic Toxic Unit: (100/*IC*₂₅), based on reproduction only.

Much like the December 2011 TIE efforts, the February 2012 TIE indicates that the reduced water flea reproduction does not appear to be related to volatile compounds, chelatable metals, filterable particulates larger than 1 µm or oxidative materials.

March 2012 TIE Results

The results of the TIE completed in March 2012 are summarized in Table 5. The results of the March TIE were different from the two previous TIEs (December 2011 and February 2012). In the March 2012 TIE, two manipulations (the filtration and C18-SPE column) reduced the sub-lethal effect, resulting in IC_{25} of >100%. However, since the C18-SPE treatment is completed on filtered samples and there was only marginal improvement in the C18-SPE treatment (mean number of young per adult = 44.0) when compared to the filtered treatment (mean number of young per adult = 42.5), it is likely that the improvement in the C18-SPE treatment was largely due to the filtration treatment and not the C18-SPE treatment.

Table 5. Summary of Outfall 001 Chronic TIE Test Results for Sample Collected in March 2012.

Ceriodaphnia dubia TIE Sample Collected on 3/14/12 Sample ID: EDCC Outfall 001 Final Effluent Test Dates: 3/15-21/2012	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc	TUc% Removed
Baseline Toxicity (No manipulation) – Test 1	100%	100%	83%	83%	100%	>100%	0.0	–
1.0 µm Filtration – Test 2	100 %	100%	100%	100%	100%	>100%	0.0	NA
C18-SPE Treatment – Test 3	100%	100%	100%	100%	100%	>100%	0.0	NA
Aeration – Test 4	100%	100%	83%	100%	100%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	100%	100%	83%	100%	100%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	100%	100%	100%	100%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	38.0	29.3	24.8	18.2	17.2	15.3	6.5	NA
1.0 µm Filtration – Test 2	32.2	30.7	37.3	42.0	42.5	>100	<1.0	100
C18-SPE Treatment – Test 3	39.5	33.2	37.7	39.7	44.0	>100	<1.0	unknown
Aeration – Test 4	38.2	29.7	23.8	22.5	19.8	15.0	6.7	0
Cation Chelation with EDTA – Test 5	28.0	25.3	17.5	16.5	6.8	19.8	5.1	21.5
Sodium Thiosulfate Treatment – Test 6	30.2	28.2	29.3	25.2	16.8	64.2	1.6	76.2

NA- Not Applicable or Not available

a TU_c, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

b The DMW control for test 5 (EDTA treatment) did not receive EDTA treatment because of historical evidence that EDTA causes toxicity in DMW.

None of the other treatments effectively increased the reproduction of the water flea. Although there appeared to be improvements to the reproductive success after sodium thiosulfate treatment, comparisons with the baseline reproduction reveals the actual numbers of young produced is not an improvement over the baseline levels of reproduction. The lower DMW control of the treatment results in the increased percent of TU_c removed, making it appear that the treatment improved the reproductive endpoint without increasing the actual young produced. Like the previous

TIE efforts, the March 2012 TIE indicates that the reduced water flea reproduction does not appear to be related to volatile compounds, chatable metals, or oxidative materials.

April 2012 TIE Results

The results of the TIE completed in April 2012 are summarized in Table 6. The results of the April TIE were different from the previous TIEs (December 2011, February 2012, and March 2012), in that none of the manipulations resulted in an IC₂₅ of >100% effluent, completely removing all sub-lethal effects. In the March 2012 TIE, two manipulations (the filtration and C18-SPE column) reduced the sub-lethal effect, resulting in IC₂₅ of >100%. In the April TIE, the filtration and the C18-SPE manipulations increased the reproduction more than any other manipulation, but were not sufficient to remove all the measures effects (e.g. reduced reproduction when compared to the control/baseline).

Table 6. Summary of Outfall 001 Chronic TIE Test Results for Sample Collected in April 2012

<i>Ceriodaphnia dubia</i> TIE Sample Collected on 4/12/12 Sample ID: EDCC Outfall 001 Final Effluent Test Dates: 3/13-20/2012	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc	TUc% Removed
Baseline Toxicity (No manipulation) – Test 1	83%	83%	17%	50%	50%	>100%	0.0	–
1.0 µm Filtration – Test 2	83%	83%	33%	67%	33%	>100%	0.0	NA
C18-SPE Treatment – Test 3	83%	33%	50%	33%	0%	>100%	0.0	NA
Aeration – Test 4	83%	50%	83%	50%	17%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	83%	33%	17%	33%	33%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	50%	33%	83%	50%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc	TUc% Removed
Baseline Toxicity (No manipulation) – Test 1	27.0	25.0	12.2	7.8	7.3	16.4	6.1	NA
1.0 µm Filtration – Test 2	28.8	36.7	25.3	28.7	7.7	49.4	2.0	66.8
C18-SPE Treatment – Test 3	17.2	23.5	22.8	27.5	8.8	56.1	0.2	unknown
Aeration – Test 4	18.2	22.5	20.2	13.7	3.7	23.3	4.3	29.6
Cation Chelation with EDTA – Test 5	34.3	18.2	11.7	10.3	4.2	7.3	13.7	0
Sodium Thiosulfate Treatment – Test 6	32.5	25.8	17.7	17.7	5.8	15.1	6.6	0

In addition, although not statistically significant, the April 2012 TIE is the first time that aeration treatment was found to remove any measured effect. It is not clear if this indicated a change in the causative agent or is an artifact of the TIE. Future TIEs will be evaluated to determine if this treatment signals a recurring change to the effluent signature.

May 2012 TIE Results

The results of the TIE completed in May 2012 are summarized in Table 7. The May 2012 TIE baseline test resulted in a reproduction IC_{25} of 56.4% effluent indicating there was a reproductive effect in the 100% effluent. The effects were removed completely through filtration. The May 2012 results were similar to the March 2012 results, however, were not repeated in the April 2012 TIE.

Table 7. Summary of Outfall 001 Chronic TIE Test Results for Sample Collected in May 2012

<i>Ceriodaphnia dubia</i> TIE Sample Collected on 5/23/12 Sample ID: EEC9558 Test Dates: 5/25/12-6/1/12	Percent Survival							
	<i>DMW</i>	12.5%	25%	50%	100%	LC_{50}	<i>TUc</i>	<i>TUc% Removed</i>
Baseline Toxicity (No manipulation) – Test 1	83%	83%	83%	100%	100%	>100%	0.0	–
1.0 μ m Filtration – Test 2	100%	100%	100%	100%	100%	>100%	0.0	NA
C18-SPE Treatment – Test 3	100%	100%	67%	100%	100%	>100%	0.0	NA
Aeration – Test 4	100%	50%	100%	100%	100%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	83%	100%	100%	100%	100%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	100%	67%	100%	100%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	<i>DMW</i>	12.5%	25%	50%	100%	IC_{25}	<i>TUc</i>	<i>TUc % Removed</i>
Baseline Toxicity (No manipulation) – Test 1	19.8	18.3	16.2	16.2	6.2	56.4	1.8	–
1.0 μ m Filtration – Test 2	29.5	22.2	24.8	30.5	35.0	>100	<1.0	100%
C18-SPE Treatment – Test 3	29.5	26.8	19.0	25.5	34.2	>100	<1.0	0% ^b
Aeration – Test 4	33.5	11.0	18.8	17.8	5.8	5.9	16.9	0%
Cation Chelation with EDTA – Test 5	15.2	12.2	10.5	9.8	0.0	18.4	5.4	0%
Sodium Thiosulfate Treatment – Test 6	27.8	15.0	6.5	11.0	7.3	6.8	14.7	0%

Filtration did not reduce the sub-lethal effect in the previous TIEs (December 2011 and February 2012). It is not clear if this modification represents a change in the causative agent or is an artifact of the TIE.

June 2012 TIE Results

The results of the TIE completed in June 2012 are summarized in Table 8. The TIE baseline tests **PASSED** both the lethal and sub-lethal endpoints although the routine WET test failed the sub-lethal endpoint with a NOEC of <32% effluent. This was the only time during the SLR study that TIE results did not concur with the routine WET testing.

Since there were no baseline sub-lethal effects, the manipulations failed to indicate improved performance.

Table 8. Summary of Outfall 001 Chronic TIE Test Results for Sample Collected June 2012

<i>Ceriodaphnia dubia</i> TIE Sample Collected on 6/13/2012 Sample ID: EDCC Outfall 001 Final Effluent Test Dates: 6/15-21/12	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc	TUc% Removed
Baseline Toxicity (No manipulation) – Test 1	100%	100%	100%	100%	100%	>100%	0.0	–
1.0 µm Filtration – Test 2	100%	83%	100%	100%	100%	>100%	0.0	NA
C18-SPE Treatment – Test 3	100%	100%	100%	100%	100%	>100%	0.0	NA
Aeration – Test 4	100%	100%	100%	100%	100%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	83%	100%	100%	100%	100%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	100%	100%	100%	100%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	33.6	39.2	31.5	27.2	27.0	>100	<1	NA
1.0 µm Filtration – Test 2	36.5	38.5	34.2	36.3	41.5	>100	<1	66.8
C18-SPE Treatment – Test 3	38.8	41.3	41.5	41.7	41.0	>100	<1	unknown
Aeration – Test 4	42.7	37.3	31.5	26.5	22.3	<100	>1	29.6
Cation Chelation with EDTA – Test 5	36.0	32.7	31.2	24.7	17.5	<100	>1	0
Sodium Thiosulfate Treatment – Test 6	35.3	36.7	31.0	28.3	24.5	<100	>1	0

July 2012 TIE Results

The results of the TIE completed in July 2012 are summarized in Table 9. The results of the July 2012 TIE were different from the previous TIEs in that none of the manipulations resulted in an IC₂₅ of >100% effluent, completely removing all sub-lethal response.

Table 9. Summary of Outfall 001 Chronic TIE Test Results for Sample Collected in July 2012

Ceriodaphnia dubia TIE Sample Collected on 7/18/12 Sample ID: EDCC Outfall 001 Final Effluent Test Dates: 3/13-20/2012	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc	TUc% Removed
Baseline Toxicity (No manipulation) – Test 1	100%	67%	83%	83%	67%	>100%	0.0	–
1.0 µm Filtration – Test 2	100%	100%	100%	100%	100%	>100%	0.0	NA
C18-SPE Treatment – Test 3	100%	100%	100%	100%	100%	>100%	0.0	NA
Aeration – Test 4	100%	100%	83%	50%	83%	>100%	0.0	NA
Cation Chelation with EDTA – Test 5	100%	67%	0%	100%	83%	>100%	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100%	80%	67%	50%	100%	>100%	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	27.0	25.0	12.2	7.8	7.3	16.4	6.1	NA
1.0 µm Filtration – Test 2	28.8	36.7	25.3	28.7	7.7	49.4	2.0	66.8
C18-SPE Treatment – Test 3	17.2	23.5	22.8	27.5	8.8	56.1	0.2	unknown
Aeration – Test 4	18.2	22.5	20.2	13.7	3.7	23.3	4.3	29.6
Cation Chelation with EDTA – Test 5	34.3	18.2	11.7	10.3	4.2	7.3	13.7	0
Sodium Thiosulfate Treatment – Test 6	32.5	25.8	17.7	17.7	5.8	15.1	6.6	0

In addition, although not statistically significant, the July 2012 TIE is the first test period where aeration treatment was found to remove any measured effect. It is not clear if this indicated a change in the causative agent or is an artifact of the TIE.

Summary of TIE Efforts

During the EDCC SLR study, seven (7) TIEs were completed on Outfall 001 effluents, with varying results and only limited success in identifying a potential cause for the routine WET test failures of the water flea sub-lethal endpoint.

Facility Data

Assemblage of facility data included monitoring of routine discharge data with particular attention to facility conditions during the WET monitoring periods. The Outfall 001 effluent data was analyzed to determine if a correlation with individual

effluent discharge parameters and the WET test sub-lethal NOECs could be determined.

Additional Statistical Analyses

Statistical evaluation of the WET test results and the concentration of various Outfall 001 NPDES permit parameters were completed in an effort to identify relationships with the discharge constituent and the sub-lethal WET test performance. The statistical analyses of data associated with routine toxicity test endpoints were completed for effluent data collected since 2006.

The goal of the analysis was to determine if discharge constituents were related to the sub-lethal WET test performance (pass or fail). A summary of the effluent data is presented in Table 10 below.

Table 10. Summary of Data Used in the Statistical Analysis September, 2009 through July 2012.

Statistic	Conductivity (μmhos/cm)	Hardness mg/L	Zinc (μg/L) monthly max	Copper¹ (μg/L) monthly max	Total suspended solids (mg/L) monthly max	Total dissolved solids (mg/L) monthly max	Sulfate (mg/L) daily max	Ammonia (mg/L) daily max
Average	455	40	10.23	2.06	50.29	261	32.77	6.52
Minimum	132	8	0.00	0.00	4.80	120	12.30	0.00
Maximum	688	68	36.00	13.00	641.05	890	57.40	22.90
Standard deviation	93	12	9.17	3.25	118.80	114	10.48	4.82
Median	453	40	8.00	0.00	18.00	240	31.90	5.91
90%tile	567	51	25.40	6.03	34.80	314	46.28	11.06

Statistical analyses were completed to determine if statistically significant relationships existed at the $\alpha=0.05$ level, between the effluent constituents with the *Ceriodaphnia dubia* (water flea) No Observed Effect Concentration (NOEC) sub-lethal endpoint, and number of young produced at 100% effluent. Water flea WET test performance was the focus of the statistical assessment as they exhibited more failures of the WET sub-lethal endpoint than did the fathead minnows.

Linear regression techniques were chosen for the analysis as it demonstrates significant positive or negative relationships (a correlation) that exist in the data, calculates a correlation coefficient to quantify the strength of the relationship, and provides a quantitative measure if the relationship is significant statistically (has a significant line slope) or not. Linear regression analysis provides a visual representation

¹ The copper Method Detection Level (MDL) was modified in September 2011 from 10 microgram per liter (μ g/L) to 2 μ g/L. This change in detection level resulted in reported concentrations typically in the single digit microgram per liter for Copper during the later part of the SLR Study. Copper concentrations prior to September 2011 that were reported as less than detection were recorded as zero. Due to the difference in the detection levels, the regression analyses for copper was an artifact of the change in detection levels and excluded from the linear regression analyses.

of the relationship, depicted as a line best fit to the plotted data. The slope of the line demonstrates the type of relationship, either negative (downward slope) or positive (upward slope) that the variables have to one another. The term "relationship" refers to the reaction of one variable's values to the fluctuation of another variable's values. When a strong relationship exists, one variable's values can be predictive of another variable's values.

In the case of this analysis, the key question is:

Are the effluent constituents and the concentration(s) thereof linked to WET test failures and can these concentrations be used to predict the potential WET test failures of water fleas (e.g. reduced NOEC, and number of young produced).

Regression analysis variables were varied to ensure that likely combinations were examined.

Dependent variables included:

- water flea reproductive NOEC and
- number of young produced at 100% effluent.

Independent variables included:

- IC_{25} ,
- conductivity ($\mu\text{mhos/cm}$),
- water hardness,
- zinc ($\mu\text{g/L}$) monthly maximum,
- total suspended solids (mg/L) monthly maximum,
- total dissolved solids (mg/L) monthly maximum,
- sulfate (mg/L) monthly maximum, and
- ammonia (mg/L) monthly maximum.

The dependent variables were regressed against concentrations of the independent variables as measured in Outfall 001 samples collected concurrent with the WET test from which the dependent variable were generated. The overall period of record utilized in the regression analyses was September 2006 to July 2012.

Linear regression analyses were completed for the multiple pairs of data as presented in Table 11.

Table 11. Linear Regression and Correlation Analyses for the *Ceriodaphnia dubia* (Water Flea) September, 2006 to July, 2009.

<i>Ceriodaphnia dubia</i> (Water Flea) Variables Compared	Correlation Coefficient	R ² Value	Slope	P-value ¹
Reproduction NOEC: Conductivity	-0.17	0.03	-0.08	0.31
Reproductive NOEC: Hardness	-0.24	0.06	-0.97	0.15
Reproductive NOEC: Zinc monthly max	-0.12	0.01	-0.59	0.47
Reproductive NOEC: Total suspended solids monthly max	0.22	0.05	0.08	0.19
Reproductive NOEC: Total dissolved solids monthly max	0.14	0.02	0.05	0.42
Reproductive NOEC: Sulfate monthly max	0.17	0.03	0.73	0.30
Reproductive NOEC: Ammonia monthly max	0.08	0.01	0.71	0.65
Number of young produced: Conductivity	-0.22	0.05	-0.02	0.18
Number of young produced: Hardness	-0.10	0.01	-0.07	0.56
Number of young produced: Zinc monthly max	0.01	0.00	0.01	0.97
Number of young produced: Total suspended solids monthly max				
Number of young produced: Total dissolved solids max	0.12	0.01	0.01	0.46
Number of young produced: Sulfate monthly max	0.09	0.01	0.01	0.58
Number of young produced: Ammonia monthly max	0.03	0.00	0.02	0.85

¹P-value (probability that the line slope is significant) must be below 0.05 for a slope to be considered statistically significant.

The R² value relates the predictive ability of the independent variable, indicating what percent of the time the dependent variable can accurately predict the independent variable. For this analysis, the R² values are low, less than 5%, for all paired variables, indicating that its predictive accuracy is less than 10%.

None of the paired variables displayed a statistically significant slope (relationship) at the $\alpha=0.05$ level,

All pairs had slopes with insignificant p-values and R² values, indicating that no relationship exists for any pair of variables evaluated. Therefore, the lack of a significant relationship indicates none of the independent variables evaluated are having an effect on the periodic WET test failures observed.

The WET test and effluent data were collected over six years, from September, 2006 to July, 2012. Analyses were also conducted to analyze the older data (2006-2010) versus newer data (2011-2012). Results indicate that neither the reproductive NOEC nor the numbers of young produced were related to any variable in either the older or newer data time frames.

Neither reproductive NOEC nor the number of young produced were statistically related to total dissolved solids in the older data. However, the 2011-2012 results had a significant p-value but the R² was too low for both the reproductive NOEC (p=0.02, R²=0.29), and the number of young produced (p=0.02, R²=0.29) to indicate significance.

In summary, the statistical assessment demonstrates there is no correlation between the WET test sub-lethal failures in the water fleas and the Outfall 001 constituents evaluated.

Additional Efforts

Efforts during the SLR Study to identify possible sources that could contribute to the demonstrated WET test sub-lethal failures included:

- Completed additional WET testing on potential sources of non-polar organics;
- Continued evaluation of individual composite samples to determine variability and persistence of effects;
- Continued efforts to identify facility use of non-polar organics through review of MSDS and chemical use inventories; and
- Continued evaluation of discharge conditions that existed during the collection of the TIE samples.

To date, efforts to identify possible sources that could contribute to the sub-lethal WET test failures have not identified a cause for the WET test performance and the failures of the water flea sub-lethal reproduction endpoint.

Please do not hesitate to contact me if you have any questions or require additional information regarding the results of the SLR Study.

Respectfully Submitted,



Kyle Wimsett
EDCC EHS Manager

Attachments

ecc: Craig Uyada, ADEQ NPDES Enforcement, w/attachments
John Carver, LSB Industries w/o attachments
Greg Withrow, EDCC General Manager w/o attachments
Roland McDaniel, GBMc & Associates, w/o attachments

Sub-lethal Response (SLR) Study Plan

Prepared for:

**El Dorado Chemical Company
El Dorado, Arkansas**

Prepared by:

**GBM^c & Associates
219 Brown Lane
Bryant, AR 72022**

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ATTACHMENTS

- Attachment 1 – ADEQ Sub-lethal request letter
- Attachment 2 – EDCC response letter
- Attachment 3 – WET tests summary table
- Attachment 4 – Figures of Historical WET NOECs

1.0 INTRODUCTION

El Dorado Chemical Company (EDCC) (**Figure 1**) was issued a modified National Pollutant Discharge Elimination System (NPDES) permit AR0000752 effective on June 1, 2004 for discharge from multiple outfalls, including Outfall 001. As a condition of the permit modification, the facility was required to conduct routine 7-day chronic Whole Effluent Toxicity (WET) on a monthly basis and report the results of the WET tests. In June 2007, the lethality endpoint of the WET testing became a WET limit. Since June 2007, EDCC has completed monthly WET testing and has maintained compliance with the WET permit limit every month with the exception of a single monthly test failure in March 2009. EDCC has not failed the WET testing since March 2009 and currently is in compliance with the WET limit for lethality in 100% effluent.

On or about January 12, 2010, EDCC received a directive from ADEQ requesting EDCC develop and implement a TRE for sub-lethal effects (**Attachment 1**). This demand letter was based on the reported results of the sub-lethal monitoring and report requirements of the existing NPDES permit. Although there is no requirement in the current permit and as an alternative to reopening the existing EDCC NPDES permit, ADEQ is requesting that EDCC undertake actions to address any future consistent and significant sub-lethal for additional monitoring or TRE-related activities associated with sub-lethal results in the WET testing language of Part III, Other Conditions in EDCC's current NPDES permit.

As an alternative, EDCC is proposing a self-directed assessment of the sub-lethal WET test failures should they recur.

2.0 STUDY OBJECTIVE

The Sub-Lethal Response (SLR) Study Plan objectives are to:

1. Evaluate the cause of significant and consistent reductions to water flea neonate production and/or fathead minnow larval growth in whole effluent toxicity (WET) tests in the laboratory 7-day chronic biomonitoring tests on effluent from Outfall 001; and

2. Identify, where possible, and correct the cause of any significant and consistent failures of the sub-lethal endpoints in WET tests completed on effluent from Outfall 001.

The site specific sub-lethal study will combine routine WET testing and analyses of the physical and chemical characteristics of final effluents to determine, to the extent possible and as appropriate, a cause of significant reductions in neonate production of the water flea and/or larval growth of the fathead minnow. In addition, any available historical data will be evaluated during the SLR study period. The findings of the study will be submitted to ADEQ at the conclusion of the SLR study period.

Should the cause of any reduced neonate production and/or the reduced fathead minnow growth be identified as a result of current facility operations and/or the current water management operations, the final report will provide a Compliance Plan defining subsequent actions to increase WET test performance as measured by the routine monitoring requirements.

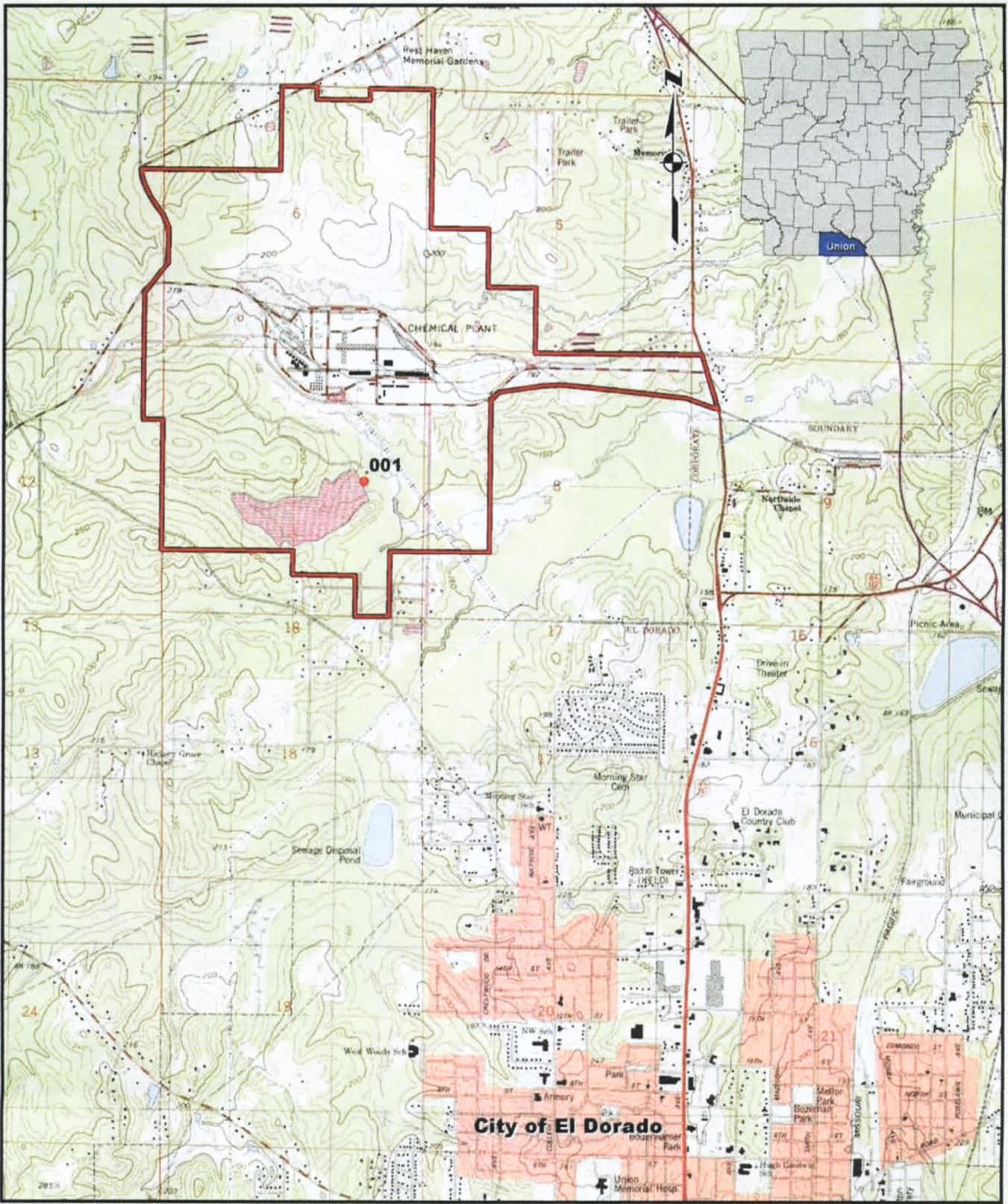


Figure 1. El Dorado Chemical Company facility and Location Outfall 001.

3.0 BACKGROUND

3.1 Historical Summary

ADEQ referenced the historical WET testing completed on Outfall 001 over the period from January 2005 through October 2009 as the basis for the implementation of the sub-lethal TRE on Outfall 001 effluent (Attachment 1). EDCC has completed an additional four (4) monthly WET tests that were not included in the ADEQ request (**Attachment 2**). During that period (January 2005 through February 2010), EDCC had completed 46 WET tests, passing 44 and 42 of the WET tests during 2009 for the lethality endpoint for the water flea and fathead minnow, respectively. Although there have been more sub-lethal endpoints failed than lethality endpoints in the WET tests, EDCC has passed 70 percent of the sub-lethal WET tests during the five-year period, including the last four consecutive WET tests during the period from (November 2009 through February 2010). Therefore, the sub-lethal test failures have not been consistent and, while failing the sub-lethal WET test end-point at the critical dilution of 100% effluent during 2009, the variability in the sub-lethal no observed effect concentration (NOEC) has implicated failures due to procedural considerations and not effluent toxicity.

Outfall 001 discharges water from a 50-acre stabilization basin that is managed to provide final treatment, equalization, storm water control and secondary containment for facility wastewater. In addition to the inflows from the facility, the basin receives storm water from the upstream drainage basin. The sub-lethal record for 2009 was atypical for Outfall 001 when compared to the previous four year period. In comparison, 2009 was the wettest year on record for the state of Arkansas with record rain fall during several months of the year, including the typically drier months of the year.

In addition, given the record for the WET tests results for the fathead minnow (*Pimephales promelas*) as summarized in **Attachment 3**, the SLR Study Plan does not propose to include tasks for evaluating the fathead minnow WET tests in Outfall 001 unless changes in the routine monthly WET testing indicates consistent failures of either test endpoint (lethality and/or growth). However, in order to verify that any proposed modifications will also support the fathead minnow, the final confirmation will include an assessment of the sub-lethal performance of the fathead minnow.

The following sections provide a more detailed accounting of the historical WET testing results for both the water flea and the fathead minnow.

3.2 Water Flea (*Ceriodaphnia dubia*)

As reported in the ADEQ request, there have been 42 7-day chronic WET tests completed using the water flea from January 2005 through October 2009. The WET test record has demonstrated a consistent record of passing the lethality endpoint at the 100% effluent exposure results. There has only been two WET lethality test failure during the 5-year period of record from the January 2005 through October 2009.

In addition, EDCC has completed four additional monthly WET tests (November 2009 through February 2010) that were not included in the period of record (POR) presented in the ADEQ request letter. All four of these tests have also passed the sub-lethal endpoint (**Attachment 4**). Subsequent evaluations of sub-lethal test failures have failed to identify a potential cause-effect relationship of the failures.

3.3 Fathead Minnow (*Pimephales promelas*)

Due to the historical record related to the WET testing of the fathead minnow, the SLR Study Plan will not include assessments of the fathead minnow unless there is a shift in the typical WET test results for Outfall 001. However, should the fathead minnow begin to demonstrate a consistent and definitive failure of WET testing when exposed to Outfall 001 discharge, the application of the SLR Plan will be reevaluated to include the fathead minnow exposure to Outfall 001 as well.

4.0 BIOMONITORING ASSESSMENT

4.1 Objectives

The SLR Plan will be implemented to:

- 1) Determine the cause of any persistent sub-lethal WET test failures in the discharge from Outfall 001 and propose actions to reduce the sub-lethal WET test failures.
- 2) Evaluate the effect of pathogens and/or low water hardness on the sub-lethal WET test failures.
- 3) Evaluate the role that water management plays on the WET test results.

- 4) Document how any unusual operating conditions or unique events within the facility may impact the WET test results.
- 5) Characterize effluent to determine if the sporadic sub-lethal failures of WET testing can be attributed to individual contributors.
- 6) Evaluate WET test results in concert with analytical, rainfall, flow and operation data to determine the role methodology and effluent characteristics may play in any reported significant differences in the sub-lethal endpoints.
- 7) Implement such additional toxicity reduction/identification evaluation (TR/IE) activities as may be appropriate to address any consistent and significant sub-lethal WET test failures in an effort to determine a source of sub-lethal endpoint test failures.

Additional details of each of the study objectives and actions planned to accomplish each of the objectives are provided below.

4.2 Approach

The following activities will be completed, as required, to accomplish the above study objectives as they relate to repeated sub-lethal WET test failures for either test species at effluent concentrations less than 76% effluent.

4.2.1 Further evaluate the effect of pathogens and/or low water hardness on the sub-lethal WET test failures on Outfall 001 effluent

Efforts to determine the role of biological pathogens and/or low water hardness on the sub-lethal test results have been initiated during the 1st Qtr of 2010 and will continue throughout the study period on a monthly basis. These potential sources of sub-lethal WET test performance will be evaluated by continuing the UV treatment of effluents and/or hardness adjustments when effluents are determined to have low levels of hardness. These manipulations will be completed on effluent samples which will run concurrently with the routine un-manipulated effluents. The results of the side-

by-side WET tests will clarify the role natural pathogens and/or low hardness might play in the sub-lethal WET test results.

4.2.2 Evaluate the role flow contributes to the sub-lethal water flea WET testing results

During the three year study period, flow data and daily rainfall data at the facility will be documented. This information will be utilized to develop a water balance estimate of the relative proportional volumes of influent streams making up the Outfall 001 effluent during WET testing; and provide relative strengths of those constituents originating from each source.

Additional analytical chemistry and WET test may be completed on any or all of the influent waste streams as may be necessary to determine if any individual source stream contributes to any future WET test failure. The specific analytical chemistry and WET testing will be determined based on conditions during the specific study period. Additional testing/investigations will be directed at specific issues. The specifics of the approach will be determined by the specific conditions that may lead to any future consistent WET test failure.

4.2.3 Document unusual operating conditions or unique events within the facility

Facility operational information and operating data will be documented with specific attention to unusual operating conditions or events that occurred during the time frame of WET testing. These operational conditions will be evaluated to determine if a specific activity may have contributed to unanticipated results in the WET testing through Outfall 001. Since this is a manufacturing facility, there are conditions that are not controllable or preventable. There are policies in place such as the SWPPP and the SPCC to limit and correct deficiencies once identified. These policies and procedures will be evaluated as they may relate to the WET test results. Modifications to the policies and procedures will be developed as required to address WET test failures to the extent that those modifications improve WET compliance.

4.2.4 Routine chronic biomonitoring

As required by the NPDES permit, monthly 7-day chronic biomonitoring will continue through the study period. The critical dilution is 100% effluent. The results of WET tests will be evaluated for adherence to analytical chemistry, test acceptance criteria, and reference toxicity results evaluating the condition of the organism cultures.

4.2.5 Evaluate WET toxicity test results in concert with analytical, rainfall, flow and operation data

The results of the WET testing will be evaluated in association with the information developed in the tasks above. The objective of the assessment is to determine the existing conditions that result in sub-lethal WET test failure (if it occurs) and those conditions that promote tests success. In the absence of any identified cause effect relationship. This data will be utilized to document conditions just prior to and during the WET testing periods. The specific analyses have not yet been determined and will be dependent on information developed during the implementation of the SLR Study Plan.

4.2.6 Evaluate the potential cause of the significant and persistent sub-lethal WET test failures in Outfall 001 discharge

Due to the historically inconsistent results demonstrating sporadic and variability in level of significance with the sub-lethal test failures, one or more sub-lethal toxicity identification evaluations (TIE) will be designed and implemented on effluent from Outfall 001 should consecutive sub-lethal effects be demonstrated in effluent concentrations less than 76% Outfall 001 effluent.

Initially, TIE actions will be directed at the water flea only. However, should the routine fathead minnow WET test exhibit consistent and significant WET tests failures, TIE manipulations will be implemented in an effort to identify the cause of the fathead minnow WET test failures. This approach is proposed based on the standard TRE language now being utilized in ADEQ NPDES permits as provided below in the excerpt from standard NPDES language defining the application of Whole Effluent Toxicity Limits.

TOXICITY REDUCTION EVALUATIONS (TREs)

TREs for lethal and sub-lethal effects are performed in a very similar manner. EPA Region 6 is currently addressing TREs as follows: a sub-lethal TRE (TRE_{SL}) is triggered based on three sub-lethal test failures while a lethal effects (TRE_L) is triggered based on only two failures for lethality. In addition, EPA Region 6 will consider the magnitude of toxicity and use flexibility when considering a (TRE_{SL}) where there are no effects at effluent dilutions of less than 76% effluent.

4.2.7 Implement such additional QA/QC activities as may be appropriate to determine if an identified source of sub-lethal endpoint test results can be eliminated

Depending on the results of the routine analytical monitoring and WET testing, additional analytical parameters and WET testing may be completed to include but not limited to duplicate sampling and/or split samples to multiple labs. Any additional effort will be designed to answer specific questions generated by the information developed during the initial 24-month period of the routine monitoring.

4.2.8 WET Test Scheduling

At least two (2) WET tests will be conducted within each year which includes rainfall contribution to the effluent and best efforts to schedule WET tests such that the effluent includes rainfall contributions during two WET test events each year. It is the intent of the SLR Plan to accomplish this characterization within the required monthly WET monitoring and may or may not be accomplished in conjunction with other storm water assessments. The specific application of WET test scheduling is to characterize the range of discharge conditions typical of the facility operations.

5.0 SCHEDULE

The SLR Study will be implemented over a 28-month period. Any additional activities to implement controls are outside the scope of this Study Plan. Due to the sporadic nature and the variability demonstrated in the historical water flea sub-lethal test failures when exposed to Outfall 001 effluent, and the nature of the discharge (from a large 50-acre equalization basin with native biotic communities), the following table represents a best estimate of the schedule required to implement the SLR Study. However, the schedule may be modified (compressed or

expanded) as required by developments within the proposed study schedule. The SLR Study is expected to take 28 months to implement. During this period, status reports will be submitted every six months to the Water Quality Planning Branch to the attention of Ms. Mary Barnett.

As indicated in the introduction (Section 1), the SLR Study was to be submitted by April 1, 2010 and therefore serves as the date of initiation for the 28-month study period. Based on the 28-month schedule the final report is due to ADEQ no later than August 31, 2012.

Table1. Proposed schedule for the implementation of the Sub-lethal Response (SLR) Study, EDCC El Dorado, AR. NPDES Permit No. AR0000752.

Tasks	Description	Duration in Months	Dates	
			From	To
Task 1	Study Plan submitted to ADEQ	3	April 2010	May 2010
Task 2	Pathogen & hardness Evaluations	24	May 2010	May 2012
Task 3	Flow monitoring	24	May 2010	May 2012
Task 4	Monitoring of facility conditions	28	May 2010	August 2012
Task 5	Routine chronic toxicity testing	28	March 2010	August 2012
Task 6	Routine assessment of WET results	28	May 2010	August 2012
Task 7	Chronic TIE manipulations, as needed	24	August 2010	August 2012
Task 8	Implementation of additional monitoring	16	May 2011	August 2012
Task 9	Evaluation of monitoring data	24	August 2010	August 2012
Task 10	Status reports	Semi-annual	November 2010 & 2011 and May 2011 & 2012	
Task 11	Final Report (to ADEQ)	1	September 2012	

ATTACHMENT 1

ADEQ Sub-Lethal TRE Request

ADEQ

ARKANSAS
Department of Environmental Quality

Certified Mail No.: 7009 0960 0000 7899 0831

January 12, 2010

David Sartain
El Dorado Chemical Company
P.O. Box 231
El Dorado, AR 71731-0231

RE: Request to begin Toxicity Reduction Evaluation (TRE).
NPDES Permit No. AR0000752
AFIN: 70-00040
Outfall 001

Dear Mr. Sartain:

During a review of the whole effluent toxicity (WET) testing data for the past five years, it was noted that there have been numerous failures reported for sub-lethality for *P. promelas* (growth) and *C. dubia* (reproduction). It is necessary at this time for EDCC to take the appropriate actions to address *P. promelas* and *C. dubia* toxicity at outfall 001. Therefore, the Department requires that EDCC begin a Toxicity Reduction Evaluation (TRE) for *P. promelas* and *C. dubia* sub-lethality.

Reg 2.508 states "Toxic substances shall not be present in receiving waters, after mixing, in such quantities as to be toxic to human, animal, plant or aquatic life or to interfere with the normal propagation, growth and survival of the indigenous aquatic biota."

Below is a summary of the reported WET test failures for NPDES Permit No. AR0000752

Number of tests performed during previous 5 years by species:

Pimephales promelas (Fathead minnow): 42

Ceriodaphnia dubia (water flea): 42

Failed test dates during previous 5 years by species:

Pimephales promelas (Fathead minnow):

Lethal

Sub-lethal

01-05

01-05

02-05

02-05

03-05

03-05

03-09

04-05

05-05

06-05

03-06

09-06

01-07
10-07
11-07
10-08
03-09

Ceriodaphnia dubia (water flea):

Lethal
12-08
02-09

Sub-lethal
01-05
03-05
05-05
09-05
04-06
12-08
02-09
04-09
06-09
07-09
09-09
10-09

Enclosed are the Departments standard requirements for permittee's conducting a TRE.
including guidelines, schedules, and reporting requirements.

If you have any questions, please contact myself or Sarah Clem.

Sincerely,



Mary Barnett
Ecologist
501-682-0666
barnett@adeq.state.ar.us

TOXICITY REDUCTION EVALUATION (TRE)

a. Within ninety (90) days of confirming lethality in the retests, the permittee shall submit a Toxicity Reduction Evaluation (TRE) Action Plan and Schedule for conducting a TRE. The TRE Action Plan shall specify the approach and methodology to be used in performing the TRE. A Toxicity Reduction Evaluation is an investigation intended to determine those actions necessary to achieve compliance with water quality-based effluent limits by reducing an effluent's toxicity to an acceptable level. A TRE is defined as a step-wise process which combines toxicity testing and analyses of the physical and chemical characteristics of a toxic effluent to identify the constituents causing effluent toxicity and/or treatment methods which will reduce the effluent toxicity. The TRE Action Plan shall lead to the successful elimination of effluent toxicity at the critical dilution and include the following:

- i. Specific Activities. The plan shall detail the specific approach the permittee intends to utilize in conducting the TRE. The approach may include toxicity characterizations, identifications and confirmation activities, source evaluation, treatability studies, or alternative approaches. When the permittee conducts Toxicity Characterization Procedures the permittee shall perform multiple characterizations and follow the procedures specified in the documents "Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures" (EPA-600/6-91/003) and "Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I" (EPA-600/6-91/005F), or alternate procedures. When the permittee conducts Toxicity Identification Evaluations and Confirmations, the permittee shall perform multiple identifications and follow the methods specified in the documents "Methods for Aquatic Toxicity Identification Evaluations, Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity" (EPA/600/R-92/080) and "Methods for Aquatic Toxicity Identification Evaluations, Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity" (EPA/600/R-92/081), as appropriate.

The documents referenced above may be obtained through the National Technical Information Service (NTIS) by phone at (800) 553-6847, or by writing:

U.S. Department of Commerce
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161

- ii. Sampling Plan (e.g., locations, methods, holding times, chain of custody, preservation, etc.). The effluent sample volume collected for all tests shall be adequate to perform the toxicity test, toxicity characterization,

identification and confirmation procedures, and conduct chemical specific analyses when a probable toxicant has been identified;

Where the permittee has identified or suspects specific pollutant(s) and/or source(s) of effluent toxicity, the permittee shall conduct, concurrent with toxicity testing, chemical specific analyses for the identified and/or suspected pollutant(s) and/or source(s) of effluent toxicity. Where lethality was demonstrated within 48 hours of test initiation, each composite sample shall be analyzed independently. Otherwise the permittee may substitute a composite sample, comprised of equal portions of the individual composite samples, for the chemical specific analysis;

- iii. Quality Assurance Plan (e.g., QA/QC implementation, corrective actions, etc.); and
- iv. Project Organization (e.g., project staff, project manager, consulting services, etc.).

b. The permittee shall initiate the TRE Action Plan within thirty (30) days of plan and schedule submittal. The permittee shall assume all risks for failure to achieve the required toxicity reduction.

c. The permittee shall submit a quarterly TRE Activities Report, with the Discharge Monitoring Report in the months of January, April, July and October, containing information on toxicity reduction evaluation activities including:

- i. any data and/or substantiating documentation which identifies the pollutant(s) and/or source(s) of effluent toxicity;
- ii. any studies/evaluations and results on the treatability of the facility's effluent toxicity; and
- iii. any data which identifies effluent toxicity control mechanisms that will reduce effluent toxicity to the level necessary to meet no significant lethality at the critical dilution.

d. The permittee shall submit a Final Report on Toxicity Reduction Evaluation Activities no later than twenty-eight (28) months from confirming lethality in the retests, which provides information pertaining to the specific control mechanism selected that will, when implemented, result in reduction of effluent toxicity to no significant lethality at the critical dilution. The report will also provide a specific corrective action schedule for implementing the selected control mechanism.

Quarterly testing during the TRE is a minimum monitoring requirement. EPA recommends that permittees required to perform a TRE not rely on quarterly testing alone to ensure success in the TRE, and that additional screening tests be performed to capture

toxic samples for identification of toxicants. Failure to identify the specific chemical compound causing toxicity test failure will normally result in a permit limit for whole effluent toxicity limits per federal regulations at 40 CFR 122.44(d)(1)(v).

ATTACHMENT 2

EDCC Response to ADEQ Request



CHEMICAL COMPANY

January 26, 2010

Ms. Mary Barnett
Ecologist, Water Division
ADEQ
5301 Northshore Drive
North Little Rock, AR 72118

Re: Sub-lethal TRE El Dorado Chemical Company
NPDES No. AR0000752, AFIN: 70-00040

Dear Ms Barnett:

This letter is in response to your letter dated January 12, 2010 requesting El Dorado Chemical Company (EDCC) initiate a Toxicity Reduction Evaluation (TRE) on effluent from Outfall 001. As specified in your letter, the TRE is to target the sub-lethal effects for both *Pimephales promelas* (fathead minnow) and *Ceriodaphnia dubia* (water flea).

Based on our understanding of the request, we offer the following comments:

First, our existing NPDES permit does have a WET permit limit for lethality, with which EDCC is in compliance, but does not have the language requiring the implementation of a TRE for either lethality or sub-lethal effects for Outfall 001.

Second, the listing of lethal and sub-lethal WET test failures referenced in the January 12th letter date back to January, 2005. However, the historical test results are no longer characteristic of the discharge. Since the beginning of 2005, EDCC has completed numerous actions to reduce concentrations of permitted constituents resulting in significant reductions in permitted discharge parameters thus allowing compliance with EDCC's current permit.

These efforts have also resulted in the reduction of WET test failures in both species, particularly with the fathead minnow. Since March 2005, there has been only one WET test failure (1 failure in 18 quarters of monitoring) of the lethality endpoint. In addition, the incidence of sub-lethal failures has been reduced to only one per year for 2008 and 2009. Clearly, the recent history (last 2 year period of record) does not support the need to implement a sub-lethal TRE even if EDCC's NPDES permit required one. According to typical NPDES language, a TRE is typically triggered only after consecutive WET test failures are demonstrated at the critical dilution. That requirement has not been triggered with the fathead minnow WET test results.

Based on EDCC records, during the most recent 2 year period of record, 2008 - 2009, EDCC has passed all but two 7-day chronic WET tests on the water flea. Subsequent WET tests have passed the lethality endpoint at the 100% critical dilution.

Although the sub-lethal endpoint (i.e. reproduction) failed several tests during the 2009 period, there is evidence that at least some of the test failures were due to natural pathogens (e.g. bacteria) that exist in the wastewater treatment holding pond and were transferred to the test organisms resulting in the sub-lethal test failure.

Although the current NPDES permit does not contain TRE language for Outfall 001, EDCC recognizes the need to determine the cause for the sub-lethal test failures. Therefore, EDCC will voluntarily initiate a self directed investigation to identify and, to the extent possible, correct the cause of the sub-lethal WET test failures as they may occur in future WET tests at dilutions of 75% effluent or below.

Within the next 60 day period, EDCC will develop and submit to ADEQ an approach to evaluate significant sub-lethal WET test failures (LOEC 75% or less), to identify the cause for any sub-lethal test failure (if possible), and develop a corrective action to address significant and consistent sub-lethal effects as indicated by the test results.

EDCC does not agree that there is a regulatory requirement to implement a sub-lethal TRE, Therefore, EDCC respectfully requests clarification of the regulatory basis and the historical WET test performance that would trigger this requirement.

Unless ADEQ stipulates otherwise, EDCC will initiate the development of the study plan within the next 14 days and proceed with the self directed investigation as set forth therein. EDCC welcomes the opportunity to meet with you and water division management to discuss this matter in greater detail. Please do not hesitate to contact me at (870) 863-1414 or Roland McDaniel at (501) 847-7077 should you have any questions or need additional information.

Regards,
El Dorado Chemical Company

Greg Withrow

Greg Withrow
General Manager

cc Steve Drown, Water Division Chief, ADEQ
Teresa Marks, Director, ADEQ
David Sartain, EDCC
John Carver, LSB
Chuck Nestrud, Chisenhall, Nestrud & Julian
Roland McDaniel, GBM^c & Associates.

ATTACHMENT 3

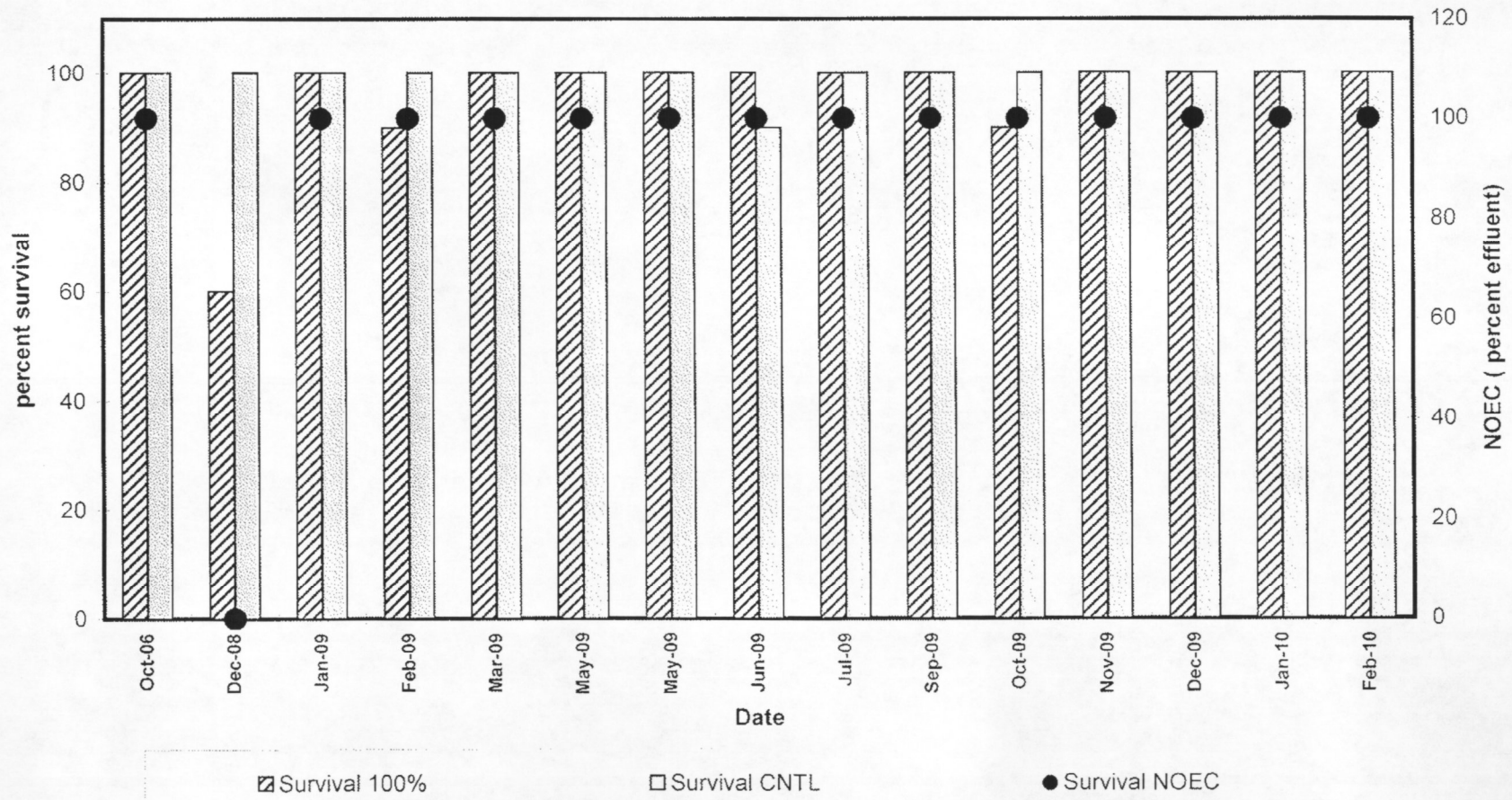
**Outfall 001 Chronic Summary Table
WET NOEC For Fathead Minnow and Water Flea**

Outfall 001 Toxicity Summary (7-day chronic toxicity test).																						
		Ceriodaphnia dubia (Water Flea)							Pimephales promelas (Fathead Minnow)							Maximum concentrations					Min.	
		Lethality			Sub-lethal																	
Report Date	Sample Date	Survival CNTL	Survival 1:100%	Survival NOEC	Young produced CNTL	Young produced in 100%	Repro: NOEC	LC50	Survival CNTL	Survival 1:100%	Survival NOEC	Growth CNTL	Growth 100%	Growth NOEC	LC50	Hardness	Alkalinity	Conductivity	pH	D.O.	NOTES	
10/1/2006	9/18-22/2006	100	100	100	24.3	31.2	100	1.25	100	100	100	0.673	0.57	0	7.71	68	112	533	8.3	7	effluent. Growth reduced in fathead minnow when compared to control but still 0.565 grams But significant to < 32 % effluent exposure.	
12/1/2008	12/15-19/2008	100	60	0	18.4	0	0	1.17	100	92.5	100	0.885	0.78	100	0.949	48	85	445	8.5	4.3	PASSED Fathead minnow endpoints, including the growth, but all effluent concentrations failed lethality endpoint to Ceriodaphnia dubia. Note the DO very low	
1/30/2009	1/19-23/2009	100	100	100	19.2	7.8	0	1.62	87.5	95	100	0.5	0.72	100	6.27	40	76	450	8.2	7.6	PASSED 3 of 4 endpoints. Failed reproduction on C. dubia for critical dilution	
2/28/2009	2/16-20/2009	100	90	100	16.1	18.8	100	1.41	100	47.5	42	0.78	0.46	0	7.16	44	72	455	8.8	7.9	PASSED water Flea endpoints NOEC =100%. Failed fathead minnow lethality in 100%	
3/31/2009	3/16-20/2009	100	100	100	19.3	18.9	100	1.62	95	57.5	56	0.835	0.75	56	5.92	10	20	132.4	7.8	7.7	PASSED water Flea endpoints NOEC =100%. Failed fathead minnow lethality in 100%	
5/1/2009	4/27-5/1/2009	100	100	100	28.4	14.4	0	1.62	90	70	100	0.615	0.48	100	6	48	92	439	8.7	5.3	PASSED 3 of 4 endpoints. Non-lethal test failure were noted for the water flea.	
5/30/2009	5/11-15/2009	100	100	100	20.9	7.2	0	1.41	97.5	55	42	0.783	0.62	42	6.95	40	76	440	8.5	5	*UV treated 100% effluent received 81% survival and 0.687 growth, Non-lethal effects on C. dubia for all effluent concentrations, Failed lethality endpoint to P. promelas in the critical dilutions	
6/30/2009	6/22-26/2009	90	100	100	22.7	6.6	0	1.54	100	97.5	100	0.983	0.82	100	2.45	40	88	414	8.2	7	PASSED 3 of 4 endpoints. Non-lethal test failure were noted for the water flea. UV treatment of 100% fathead minnow implicated fungal infections however VU not completed on Water flea exposure.	
7/31/2009	7/20-24/2009	100	100	100	27.1	8.8	0	1.41	97.5	100	100	0.88	0.85	100	6.56	36	112	395	9.8	7.4	PASSED 3 of 4 endpoints. Non-lethal test failure were noted for the water flea. UV treatment of 100% fathead minnow implicated fungal infections however VU not completed on Water flea exposure.	
9/30/2009	9/21-25/2009	100	100	100	21.2	14.4	32	1.41	87.5	95	100	0.733	0.57	100	6.37	64	80	478	8	4	were noted for the water flea. UV treatment of 100% fathead minnow implicated fungal infections however VU not completed on Water flea exposure.	
10/30/2009	10/19-23/2009	100	90	100	17.8	11.6	42	1.62	97.5	97.5	100	0.973	0.9	100	6.31	40	44	405	7.7	7.6	PASSED 3 of 4 endpoints. Non-lethal test failure were noted for the water flea.	
11/30/2009	11/16-20/2009	100	100	100	19.1	18.1	100	1.54	100	95	100	0.908	0.73	0	7.38	32	48	362	7.9	7.6	PASSED 3 of 4 endpoints. Non-lethal test failure were noted for the P. promelas	
12/30/2009	12/14-18/2009	100	100	100	20.6	21.8	100	1.5	97.5	97.5	100	0.858	0.93	100	1.169	32	48	354	7.9	5.2	PASSED ALL 4 endpoints	
1/31/2010	1/19/26/2010	100	100	100	19.7	23.3	100		97.5	100	100	1.106	1.07	100		28	44	366	7.9	7.5	PASSED ALL 4 endpoints	
2/28/2010	2/14-20/2010	100	100	100	20	22.7	100		94	100	100	0.958	1	100							PASSED ALL 4 endpoints (report not finalized, results verbal)	

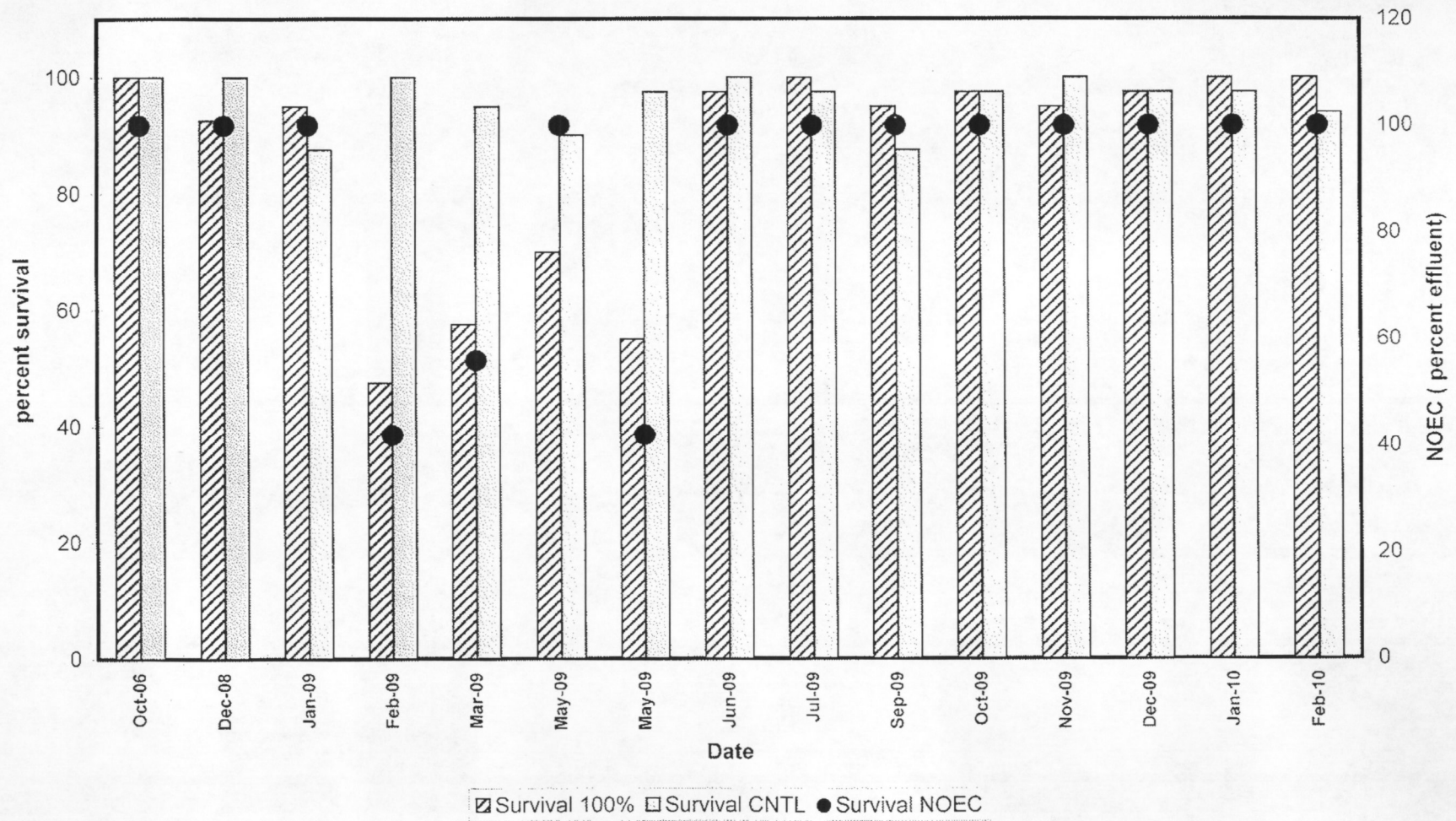
ATTACHMENT 4

**Figures of WET NOEC
for Fathead Minnow and Water Flea**

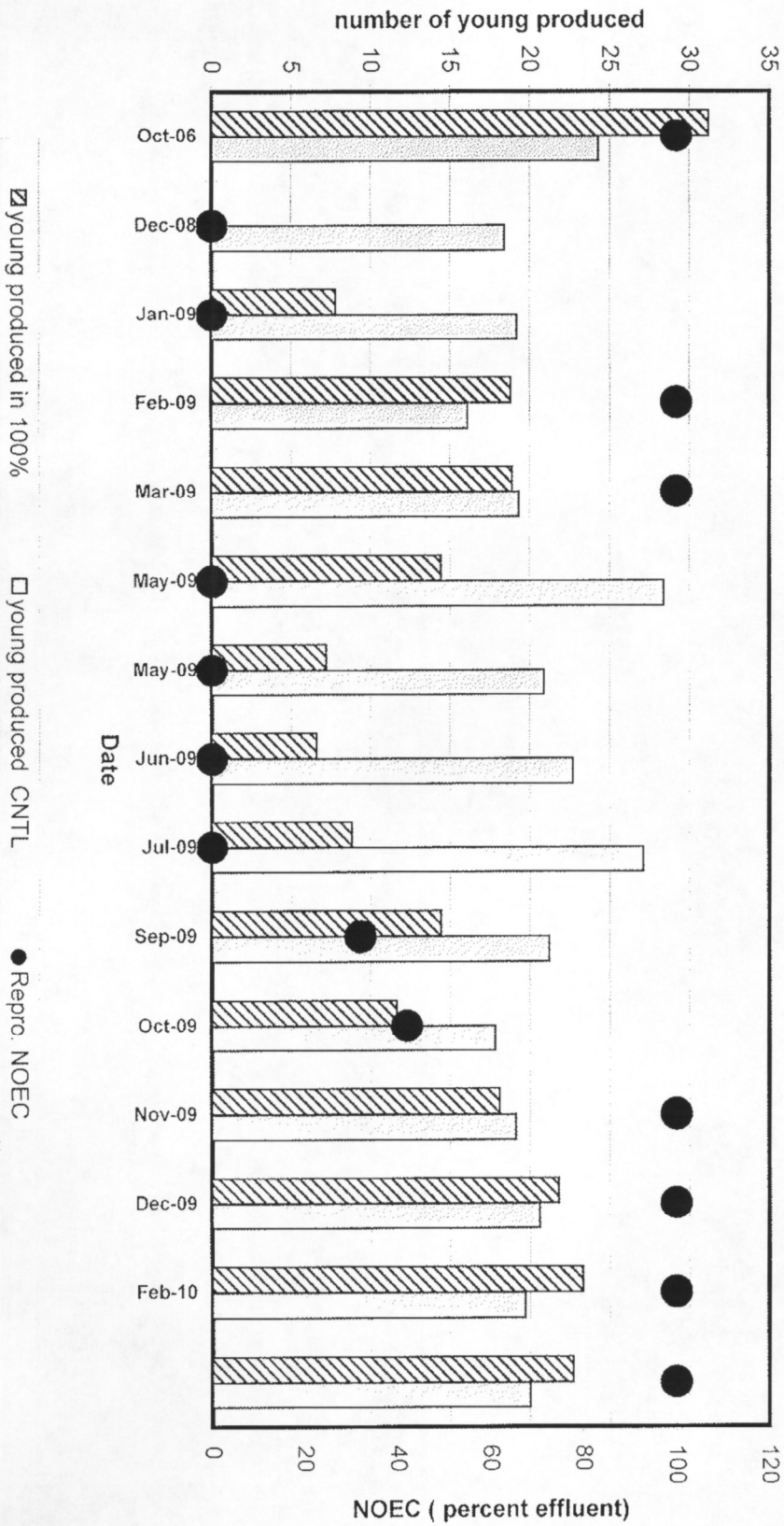
EDCC Outfall 001
7-Day Chronic Water Flea
Survival and NOEC



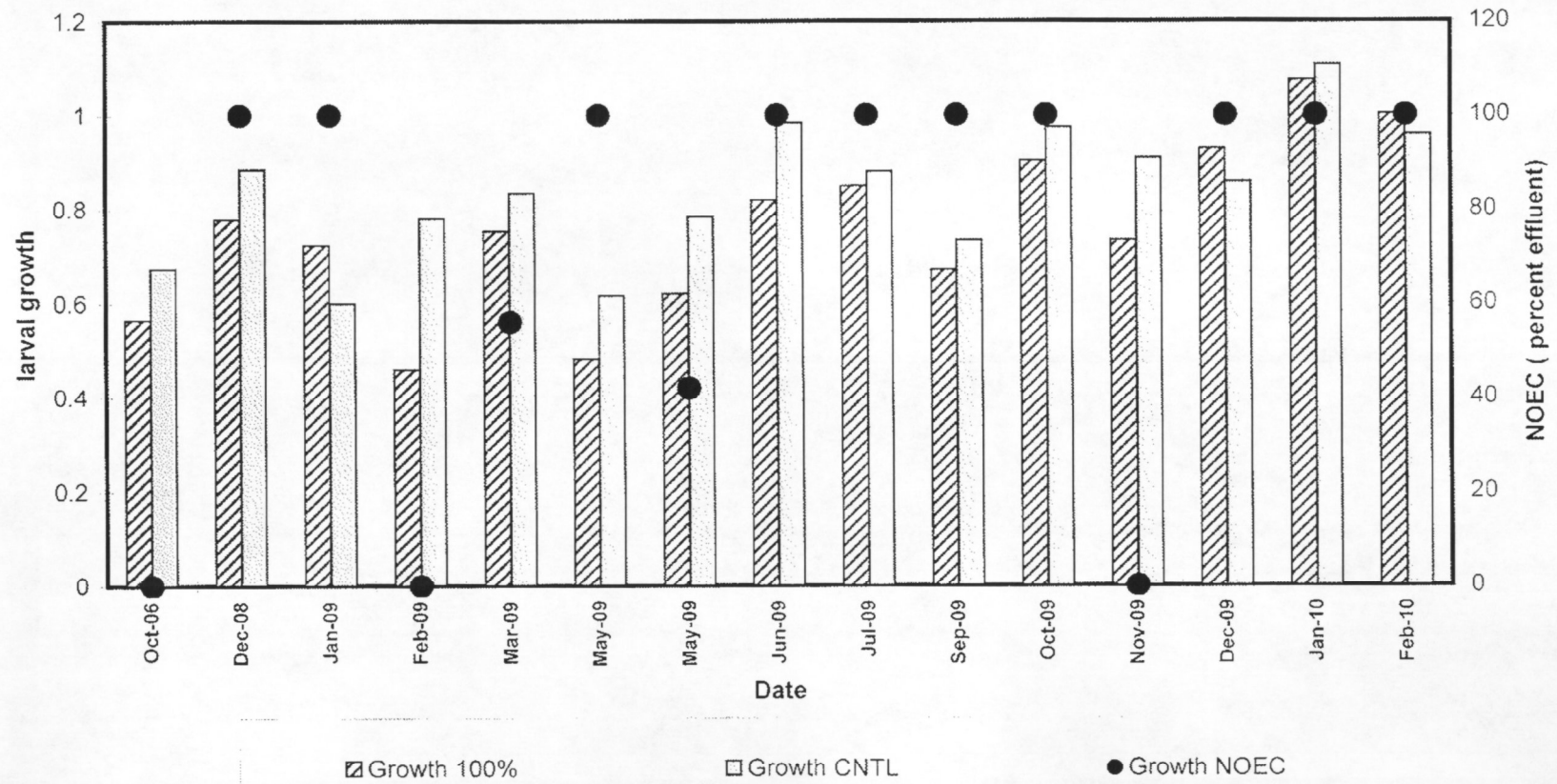
EDCC Outfall 001
7-Day Chronic Fathead Minnow
Survival and NOEC



EDCC Outfall 001
7-Day Chronic Water Flea
Production and NOEC



EDCC Outfall 001
7-Day Chronic Fathead Minnow
Growth and NOEC



ADEQ

ARKANSAS
Department of Environmental Quality

May 7, 2010

Greg Withrow
El Dorado Chemical Company
P.O. Box 231
El Dorado, AR 71731

RE: Approval of EDCC Sub-lethal Response Study Plan
NPDES No. AR0000752
AFIN: 70-00040

Dear Mr. Withrow:

Thank you for your prompt submission of the revised EDCC Sub-lethal Response Study Plan. The Study Plan has been reviewed, and ADEQ approves of the approach outlined in the Sub-lethal Response Study Plan (received April 20, 1010).

Based on the Plan, EDCC–Outfall 001 will begin Sub-lethal Study activities in May 2010. Semi-annual activities reports will be due on the last day of the month for the following months: November 2010, May and November 2011, and May 2012. The final report will be due August 31, 2012.

If you have any questions, please contact myself or Sarah Clem.

Sincerely,



Mary Barnett
Ecologist
501-682-0666
barnett@adeq.state.ar.us

CC: Loretta Reiber, NPDES Permitting
Deb Gerst, NPDES Enforcement
David Sartain, EDCC
Ronald McDaniel, GBM^c



**Great
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Center**

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www.glec-online.com

**Traverse City
Operations**
739 Hastings St.
Traverse City
MI 49686

231-941-2230
231-941-2240 fax

**Columbus
Operations**
1295 King Ave.
Columbus
OH 43212

614-487-1040
614-487-1920 fax

January 24, 2012

Roland McDaniel, Project Manager
GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

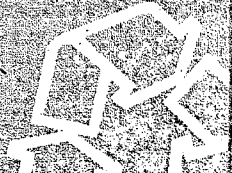
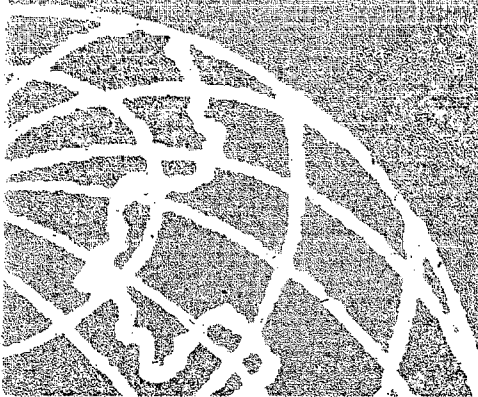
**RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED
DECEMBER 14, 2011 FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL
DORADO, ARKANSAS**

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected December 14, 2011. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tarr
Laboratory Coordinator



PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED DECEMBER 14, 2011
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

December 2011



Great Lakes Environmental Center

Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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APPENDIX B. DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS B-1

INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled December 14, 2011 to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

Ceriodaphnia dubia Static Renewal Chronic Toxicity Tests

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for seven days under static renewal conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one

organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity $10\text{-}20 \mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test (7 days).

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUc) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUc} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1). The baseline toxicity test exhibited 50 percent survival in the 100 percent test concentration; however a 7 day LC_{50} value could not be calculated due to the dose response for survival. The outfall 001 sample was very toxic to *C. dubia* reproduction and exhibited an IC_{25} value of 22.2 percent effluent or 4.5 TUc (Table 1).

1.0 μm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a

Gelman A/E glass fiber filter (1.0 μm).

The *C. dubia* filtration test had an IC_{25} of 3.5 percent or 28.6 TUc. Compared to the baseline toxicity test, filtration did not remove toxicity to *C. dubia* reproduction (Table 1).

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample **after 1.0 μm filtration treatment** was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

After C18 treatment, 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent was removed as the *C. dubia* exhibited an IC_{25} of >100 percent or <1.0 TUc. Therefore, C-18 was very effective as a treatment and the toxicity to *C. dubia* reproduction in the EDCC effluent sample appears to be associated with non-polar and semi-polar organic compounds.

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

Aeration of the outfall 001 sample did not remove toxicity when compared to the concurrent baseline and demonstrated an IC_{25} of 3.9 percent or 25.6 TUc (Table 1). Therefore, the toxicity to *C. dubia* reproduction was not related to volatile, easily oxidizable or aeratable compound(s).

Cation Chelation with EDTA - Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test IC_{25} of 4.5 percent, the addition of EDTA (25 mg/L) did not remove any sample toxicity and exhibited an IC_{25} of 3.4 percent or 29.4 TUc (Table 1).

Sodium Thiosulfate Treatment - Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

The *C. dubia* sodium thiosulfate treatment removed 23.7 percent of the toxicity demonstrated in the EDCC outfall 001 effluent sample and had an IC_{25} value of 5.9 percent or 16.9 TUc (Table 1). However, this difference is not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Also, closer inspection of the data demonstrates that the removal of toxicity after sodium thiosulfate treatment was somewhat of an aberration as the sodium thiosulfate control mean number of young per adult of

25.0 was relatively low compared to the baseline control mean for reproduction of 36.3 young per adult. Because of the relatively low mean number of young per adult (25.0) in the sodium thiosulfate treated control water, the IC25 statistical program calculation for Test 6 was skewed higher which exaggerated the actual TUC value and ultimately the level toxicity removal after sodium thiosulfate treatment when compared to the baseline test (Test 1). Therefore, sodium thiosulfate treatment did not demonstrate a reduction in toxicity.

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected December 14, 2011 did demonstrate removal of chronic toxicity, but the reduction of toxicity to *C. dubia* reproduction was only demonstrated by one of the five TIE treatments performed. Four of the treatments, filtration, aeration, EDTA and sodium thiosulfate were not effective in removing any meaningful toxicity from the outfall 001 effluent sample. Thus, the effluent toxicity does not appear to be related to; a filterable toxicant, an easily oxidizable or aeratable compound, a chelatable metal or thiosulfate reducible compounds or oxidants.

The C-18 treatment removed 100 percent of the toxicity present in the EDCC outfall 001 effluent sample. Therefore, the chronic toxicity to *C. dubia* reproduction present in the ECCC outfall 001 effluent sample appears to be associated with non-polar and semi-polar organic compound. For a summary of all test results, see Table 1.

Summary of the chronic toxicity characterization of the EDCC outfall 001 sample collected December 14, 2011 (EEC 9450):

- The toxicant (s) was not filterable.
- **The toxicant(s) was a non-polar and or a semi-polar organic compound.**
- The toxicant(s) was not a chelatable metal.
- The toxicant(s) was not a volatile, easily oxidizable or aeratable compound.
- The toxicant(s) was not a thiosulfate reducible compound or oxidant.

Table 1. Summary of Chronic TIE Test results

EDCC Outfall 001 final effluent (Collection date: 12/14/11) <i>C. dubia</i> TIE Test Dates 12/16-23/11	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	100	67	50	100	50	NA ^c	0.0	--
1.0 µm Filtration – Test 2	83	33	67	67	100	>100	0.0	NA
C18-SPE Treatment – Test 3	100	100	100	100	100	>100	0.0	NA
Aeration – Test 4	100	33	67	100	83	>100	0.0	NA
Cation Chelation with EDTA – Test 5	100	17	33	67	67	>100	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100	33	83	83	100	>100	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	36.3	11.2	4.5	5.0	3.3	4.5	22.2	--
1.0 µm Filtration – Test 2	25.8	2.2	1.8	2.2	3.0	3.5	28.6	0
C18-SPE Treatment – Test 3	31.7	38.8	37.5	41.0	40.3	>100	<1.0	100
Aeration – Test 4	29.7	5.5	3.7	3.5	4.3	3.9	25.6	0
Cation Chelation with EDTA – Test 5	32.2 ^b	1.7	2.3	4.0	0	3.4	29.4	0
Sodium Thiosulfate Treatment – Test 6	25.0	8.0	12.5	11.5	5.5	5.9	16.9	23.7

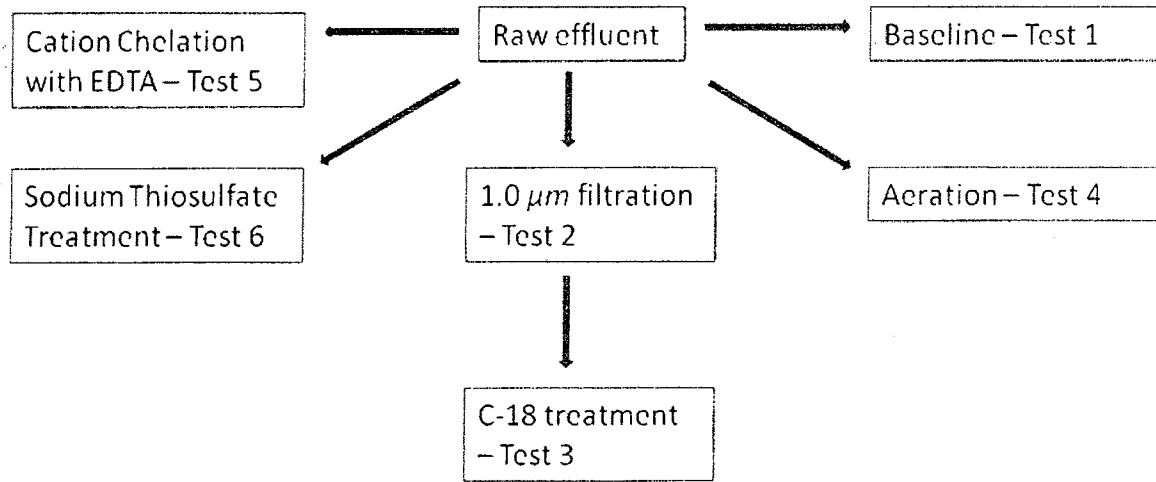
NA – Not applicable or Not available

^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

^c The LC₅₀ could not be calculated due to the lack of a dose response for survival.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC₂₅ value for the most recent *C. dubia* reference toxicant test was 0.78 g/L of sodium chloride which was within the acceptance range of 0.78 to 1.74 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.

U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED DECEMBER 14, 2011



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: GBMe El Dorado PROJECT NUMBER: 1953-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>12-16-11</u>	INITIALS				
EEC NUMBER		<u>9450</u>			
OUTFALL/DESCRIPTION		<u>effluent</u>			
DISSOLVED OXYGEN (mg/L)	<u>YBK</u>	<u>13.4</u>			
TEMPERATURE (°C)	<u>YBK</u>	<u>1.5</u>			
pH	<u>YBK</u>	<u>8.4</u>			
CONDUCTIVITY (µmhos/cm)	<u>YBK</u>	<u>270</u>			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>12/16/11</u>	INITIALS				
EEC NUMBER		<u>9450</u>			
OUTFALL/DESCRIPTION		<u>effluent</u>			
DISSOLVED OXYGEN (mg/L)	<u>ONT</u>	<u>10.6</u>			
TEMPERATURE (°C)	<u>ONT</u>	<u>25.0</u>			
pH	<u>ONT</u>	<u>8.2</u>			
CONDUCTIVITY (µmhos/cm)	<u>ONT</u>	<u>429</u>			
HARDNESS (mg/L CaCO ₂)	<u>85</u>	<u>1.3 × 40</u> <u>= 52</u>	<u>7.5 × 40</u> <u>= 60 *</u>		
ALKALINITY (mg/L CaCO ₂)		<u>1.5 × 40</u> <u>= 60</u>			
TOTAL CHLORINE (mg/L)*					
TOTAL AMMONIA (mg/L)*					

*Check with project manager to see if necessary

* entry error

Test Dates: 12/16-23/11

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	100%	67%	50%	100%	50%
1.0 µm filtration (Test 2)	83%	33%	67%	67%	100%
C-18 SPE treatment (Test 3)	100%	100%	100%	100%	100%
Aeration (Test 4)	100%	33%	67%	100%	83%
EDTA 25 mg/l (Test 5)	100%	17%	33%	67%	67%
NaThio (50 mg/l) (Test 6)	100%	33%	83%	83%	100%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	36.3	11.2	4.5	5.0	3.3	4.5	22.2	--
1.0 µm filtration (Test 2)	25.8	2.2	1.8	2.2	3.0	3.5	28.6	0.0%
C-18 SPE treatment (Test 3)	31.7	38.8	37.5	41.0	40.3	>100	<1.0	100.0%
Aeration (Test 4)	29.7	5.5	3.7	3.5	4.3	3.9	25.6	0.0%
EDTA (25 mg/l) (Test 5)	32.2	1.7	2.3	4.0	0.0	3.4	29.4	0.0%
NaThio (50 mg/l) (Test 6)	25.0	8.0	12.5	11.5	5.5	5.9	16.9	23.7%

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to C.dubia reproduction in DMW

Baseline (Test 1)

El Dorado Chemical outfall 001 (EEC 9450)
(Tested 12/16-23/11)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	3	6	3
DEAD	0	2	3	0	3
% SURV	100.00%	66.67%	50.00%	100.00%	50.00%

Scito River Water 2° Control	5
	1
	83.33%
Scito River 2° Control	47
	40
	36
	42
	41
	43
	6
	41.5
	3.6193922
	8.721427
	249

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	38	4	0	6	6
2	37	12	4	4	0
3	34	3	2	5	5
4	39	16	1	3	2
5	33	13	11	7	3
6	37	19	9	5	4
N	6	6	6	6	6
MEAN	36.333333	11.1666667	4.5	5.0	3.3
SD	2.3380904	6.43169236	4.5055521	1.4142136	2.1602469
CV	6.4351112	57.5972451	100.12338	28.284271	64.807407
Total Young	218	67	27	30	20

1.0 µm filtration (Test 2)

El Dorado Chemical outfall 001 (EEC 9450)
(Tested 12/16-23/11)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	2	4	4	6
DEAD	1	4	2	2	0
% SURV	83.33%	33.33%	66.67%	66.67%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	36	0	1	2	2
2	0	8	2	0	3
3	30	0	2	4	3
4	32	0	0	1	2
5	33	4	6	2	3
6	24	1	0	4	5
N	6	6	6	6	6
MEAN	25.8	2.2	1.8	2.2	3.0
SD	13.272779	3.25064096	2.228602	1.602082	1.0954451
CV	51.3785	150.029583	121.56011	73.942245	36.514837
Total Young	155	13	11	13	18

C-18 SPE treatment (Test 3)
El Dorado Chemical outfall 001 (EEC 9450)
(Tested 12/16-23/11)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	29	36	38	37	44
2	36	38	37	44	36
3	21	42	33	39	37
4	34	39	41	42	43
5	34	35	35	45	46
6	36	43	41	39	36
N	6	6	6	6	6
MEAN	31.666667	38.8333333	37.5	41.0	40.3
SD	5.8195074	3.18852108	3.2093613	3.1622777	4.5018515
CV	18.377392	8.21078389	8.5582968	7.7128723	11.161615
Total					
Young	190	233	225	246	242

Aeration (Test 4)
El Dorado Chemical outfall 001 (EEC 9450)
(Tested 12/16-23/11)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	2	4	6	5
DEAD	0	4	2	0	1
% SURV	100.00%	33.33%	66.67%	100.00%	83.33%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	34	0	2	4	4
2	28	4	2	2	5
3	35	17	4	5	7
4	34	1	6	2	0
5	24	4	8	4	5
6	23	7	0	4	5
N	6	6	6	6	6
MEAN	29.666667	5.5	3.7	3.5	4.3
SD	5.3913511	6.15629759	2.9439203	1.2247449	2.3380904
CV	18.173094	111.932683	80.288735	34.992711	53.955932
Total					
Young	178	33	22	21	26

EDTA 25 mg/l (Test 5)
El Dorado Chemical outfall 001 (EEC 9450)
(Tested 12/16-23/11)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	1	2	4	4
DEAD	0	5	4	2	2
% SURV	100.00%	16.67%	33.33%	66.67%	66.67%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	23	0	4	5	0
2	37	0	0	8	0
3	31	0	0	0	0
4	38	0	0	0	0
5	32	7	0	7	0
6	32	3	10	4	0
N	6	6	6	6	6
MEAN	32.166667	1.66666667	2.3	4.0	0.0
SD	5.3447794	2.87518115	4.0824829	3.4058773	0
CV	16.615894	172.510869	174.96355	85.146932	#DIV/0!
Total Young	193	10	14	24	0

NaThio (50 mg/l) (Test 6)
El Dorado Chemical outfall 001 (EEC 9450)
(Tested 12/16-23/11)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	2	5	5	6
DEAD	0	4	1	1	0
% SURV	100.00%	33.33%	83.33%	83.33%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	18	1	16	16	6
2	20	1	0	16	8
3	22	18	16	0	1
4	35	24	17	14	7
5	22	4	14	9	5
6	33	0	12	14	6
N	6	6	6	6	6
MEAN	25	8	12.5	11.5	5.5
SD	7.1554175	10.3344085	6.3796552	6.1886994	2.4289916
CV	28.62167	129.180107	51.037241	53.814777	44.163483
Total Young	150	48	75	69	33

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	38	4	0	6	6
Response 2	37	12	4	4	0
Response 3	34	3	2	5	5
Response 4	39	16	1	3	2
Response 5	33	13	11	7	3
Response 6	37	19	9	5	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9450 Test #1

Test Start Date: 12/16/11 Test Ending Date: 12/23/11

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	36.333	2.338	36.333
2	6	12.500	11.167	6.432	11.167
3	6	25.000	4.500	4.506	4.750
4	6	50.000	5.000	1.414	4.750
5	6	100.000	3.333	2.160	3.333

The Linear Interpolation Estimate: 4.5116 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 4.5129 Standard Deviation: 0.4028

Original Confidence Limits: Lower: 3.7847 Upper: 5.2719

Resampling time in seconds: 0.06 Random_Seed: -265916568

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	36	0	1	2	2
Response 2	0	8	2	0	3
Response 3	30	0	2	4	3
Response 4	32	0	0	1	2
Response 5	33	4	6	2	3
Response 6	24	1	0	4	5

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9450 Test #2

Test Start Date: 12/16/11 Test Ending Date: 12/23/11

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	25.833	13.273	25.833
2	6	12.500	2.167	3.251	2.292
3	6	25.000	1.833	2.229	2.292
4	6	50.000	2.167	1.602	2.292
5	6	100.000	3.000	1.095	2.292

The Linear Interpolation Estimate: 3.4292 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 3.5064 Standard Deviation: 0.1808

Original Confidence Limits: Lower: 3.3065 Upper: 3.9583

Resampling time in seconds: 0.06 Random_Seed: 1820744184

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	34	0	2	4	4
Response 2	28	4	2	2	5
Response 3	35	17	4	5	7
Response 4	34	1	6	2	0
Response 5	24	4	8	4	5
Response 6	23	7	0	4	5

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9450 Test #4
 Test Start Date: 12/16/11 Test Ending Date: 12/23/11
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	29.667	5.391	29.667
2	6	12.500	5.500	6.156	5.500
3	6	25.000	3.667	2.944	3.833
4	6	50.000	3.500	1.225	3.833
5	6	100.000	4.333	2.338	3.833

The Linear Interpolation Estimate: 3.8362 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 3.9017 Standard Deviation: 0.3991

Original Confidence Limits: Lower: 3.5032 Upper: 4.8452

Resampling time in seconds: 0.00 Random_Seed: 373950600

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	23	0	4	5	0
Response 2	37	0	0	8	0
Response 3	31	0	0	0	0
Response 4	38	0	0	0	0
Response 5	32	7	0	7	0
Response 6	32	3	10	4	0

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9450 Test #5

Test Start Date: 12/16/11 Test Ending Date: 12/23/11

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	32.167	5.345	32.167
2	6	12.500	1.667	2.875	2.667
3	6	25.000	2.333	4.082	2.667
4	6	50.000	4.000	3.406	2.667
5	6	100.000	0.000	0.000	0.000

The Linear Interpolation Estimate: 3.4075 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 3.4129 Standard Deviation: 0.0905

Original Confidence Limits: Lower: 3.2716 Upper: 3.6316

Resampling time in Seconds: 0.00 Random_Seed: -1695909096

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	18	1	16	16	6
Response 2	20	1	0	16	8
Response 3	22	18	16	0	1
Response 4	35	24	17	14	7
Response 5	22	4	14	9	5
Response 6	33	0	12	14	6

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EC 9450 Test #6

Test Start Date: 12/16/11 Test Ending Date: 12/23/11

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	25.000	7.155	25.000
2	6	12.500	8.000	10.334	10.667
3	6	25.000	12.500	6.380	10.667
4	6	50.000	11.500	6.189	10.667
5	6	100.000	5.500	2.429	5.500

The Linear Interpolation Estimate: 5.4506 Entered P value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 5.9523 Standard Deviation: 1.5960

Original Confidence Limits: Lower: 4.3570 Upper: 9.9315

Resampling time in seconds: 0.00 Random_Seed: 941666728

Parental Blockage Map for *C. dubia*

Date: 12/16/11

Time Neonates Pulled: 14.30

Source Board: SR/DMW 12-7-11

Initials: JSW

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: 1953-00 [#] MAN. 1 (baseline)

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3					R5				R6	
2					R3				R4	
1			R1X					R2		

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 12/16/11

Time Neonates Pulled: 14:30

Source Board: SR/DMW 12-7-11

Initials: JSW

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: 1953-00 ^{II} MAN. 2 (FIT.)

	1	2	3	4	5	6	7	8	9	10
6										
5										
4		R2	R3	R4			R5	R6		
3										R1
2										
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 12/16/11

Time Neonates Pulled: 14.30

Source Board: SR/DMW 12-7-11

Initials: JSW

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: 1953-00

3 (C-18)
MAN.

	1	2	3	4	5	6	7	8	9	10
6										
5										
4		R4	R5	R6			R1	R2	R3	
3										
2										
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 12/16/11

Time Neonates Pulled: 14.30

Source Board: SR/DmW 12-7-11

Initials: JSW

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: 1953-00 ^{#4} max. (Aeriation)

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3						R5			R6	
2					R3				R4	
1			R1						R2	

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 12/16/11

Time Neonates Pulled: 14.30

Source Board: SR/DmW 12-7-11

Initials: JSW

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: 1953-00 #5 (EDTA)
MON.

	1	2	3	4	5	6	7	8	9	10
6										
5										
4		R1	R2		R3		R4	R5	R6	
3										
2										
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 12/16/11

Time Neonates Pulled: 14.30

Source Board: SR/DMW 12-7-11

Initials: JSW

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: 1953-00 #6 (+ Nathio)
MAN.

	1	2	3	4	5	6	7	8	9	10
6										
5										
4				R1		R2	R3	R45		
3										
2										
1			R4	R6						

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.



Baseline

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curt

Date: 12/1

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: Dmw

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1700

YOUNG FROM: 58/2mw 12/1/11 < 24 hrs

TECHNICIANS: DAY: 0 1700gs - 11230gs - 2/1430 CAD 3/0945gs - 4 6830 con 58:40 VPK 6 9:10 VPK 7 1130 con

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
Dmw	0	+	+	+	+	+	+	+	+	+	+	8.0		8.7		25.0		167	
	1	+	+	+	+	+	+					7.8	7.9	10.3	8.7	24.9	25.0	170	163
	2	+	+	+	+	+	+					8.0	8.1	7.9	7.9	24.2	24.8	173	172
	3	+e	+e	+e	+e	+e	+e					8.2	7.9	7.8	8.0	24.9	24.5	171	167
	4	te6	te5	te7	te7	te6	te6					8.1	7.9	8.5	8.6	25.1	25.1	167	167
	5	te	te	te	te	te	te ^s					8.0	8.0	8.5	8.4	25.0	24.4	155	173
	6	te12	te11	te10	te13	te11	te12					8.0	7.8	8.6	8.0	25.0	24.6	164	165
	7	te20	te21	te17	te19	te16	te18						7.8		8.2		25.0		168
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.0		9.0		25.0		204	
	1	+	+	+	+	+	+					7.9	8.0	10.5	8.5	24.9	25.0	200	187
	2	+	+	+	+	+	+					8.0	9.2	8.1	7.7	24.2	24.8	206	198
	3	+e	+e	+e	+e	+e	+e					8.2	8.0	8.1	8.0	24.9	24.5	206	197
	4	te4	te2	te3	te4	te2	te5					8.1	8.1	8.8	8.6	25.1	25.1	199	198
	5	te	te	te	te	te	te					8.1	8.1	9.0	8.6	25.0	24.4	196	197
	6	te	te6	te	te5	te5	te6					8.1	8.1	9.2	9.0	25.0	24.6	200	197
	7	te	te4	te	te7	te6	te8						8.0		8.4		25.0		198
25%	0	+	+	+	+	+	+	+	+	+	+	8.1		9.2		25.0		240	
	1	+	+	+	+	+	+									24.9	25.0	243	216
	2	+	+	+	+	+	+									24.2	24.8	241	232
	3	+e	+e	+e	+e	+e	+e									24.9	25.0	240	228
	4	te	te	te2	te*	te2	te1									25.1	25.1	233	229
	5	te	te	te	te	te	te									25.0	24.4	227	228
	6	te	te4	te	te	te4	te4									25.0	24.6	234	224
	7	te	te	te	te	te5	te4									25.0	25.0	226	226

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

certified copies

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1700

YOUNG FROM: SP10MW
1217H 1 < 24 hrs

TECHNICIANS: DAY: 0 1700 1730 21430 CAD 30945 40830 58:40VAK 69:10VAK 71130CAD

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
			0	+	+	+	+	+	+	+	+	+	+	8.1		9.8		25.0	
50%	1	+	+	+	+	+	+									24.9	25.0	305	275
	2	+	+	+	+	+	+									24.2	24.8	307	289
	3	+e	+e	+	+e	+e	+e									24.9	24.5	307	289
	4	te	te	+	te	te	te									25.1	25.1	304	287
	5	tl3*	tl2*	tl1	tl1	tl2	tl									25.0	24.4	284	287
	6	tl3*	tl2*	tl4	tl2	tl3	tl3									25.0	24.6	297	287
	7	te	te	te	te	te	te									25.0			286
100%	0	+	+	+	+	+	+	+	+	+	+	8.2		10.6		25.0		429	
	1	+	+	+	+	+	+					8.0	8.1	10.9	9.0	24.9	25.0	428	389
	2	+	+	+	+	+	+					8.2	8.2	9.6	8.4	24.2	24.8	436	408
	3	+e	+e	+e	+e	+e	+e					8.3	8.1	10.3	8.3	24.9	24.5	433	409
	4	te	+	+	+	te	+					8.2	8.1	10.9	8.8	25.1	25.1	424	406
	5	tl3	+	tl2	tl1	tl2	tl					8.1	8.1	11.7	8.8	25.0	24.4	412	408
	6	tl3*	tl2*	tl2*	tl	tl1	tl4					8.1	8.1	11.2	8.9	25.0	24.6	432	404
7	te	te	te	te	te	te						8.0		8.3		25.0		409	
	0	+	+	+	+	+	+	+	+	+	+					2			
	1	i																	
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EEC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11 1700

YOUNG FROM: SR 12/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1700g - 1 1300g - 2 1430g - 3 0945g - 4 0830g - 5 8:40 AM - 6 9:10 AM - 7 1130 AM

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
SR	0	+	+	+	+	+	+	+	+	+	+	7.8		10.2		25.0		254	
	1	+	+	+	+	+	+									24.9	25.0	255	236
	2	+e	+	+	tl	+	tl									24.8	24.8	266	261
	3	+e	+e	+e	+e	+e	+e									24.9	24.5	261	251
	4	r7	r7	r7	r7	r6	r6									25.1	25.1	273	254
	5	+tl6	+tl9	+tl10	+tl15	+e	+tl17									25.0	24.4	235	266
	6	+e	+e	+e	+e	+tl6	+e									25.0	24.6	250	251
	7	r24	r21	r19	r20	r19	r20												246
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



② 1.0µm filtration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: ELC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: Dmw

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1700

YOUNG FROM: 5/21/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1700, 3-112308 - 2 1440 CRP 3 1030, 5 - 4 0900 CRP 5 9:20 URK 6 10:10 YRK 7 1145 CRP

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
Dmw	0	+	+	+	+	+	+	+	+	+	+	8.2		8.0		25.0		179	
	1	+	+	+	+	+	+					7.8	8.1	10.2	8.7	24.9	25.0	177	164
	2	+e	+	+e	+e	+	+e					7.9	8.2	8.7	7.9	24.2	24.8	178	172
	3	+e	T	+e	+e	+e	+e					7.9	7.9	10.1	8.2	24.9	24.5	171	163
	4	R7		R4	R5	R6	R5					7.9	8.1	10.9	8.6	25.1	25.1	178	179
	5	+e11		+e12	+e10	+e10	+e8					7.8	8.0	11.6	8.7	25.0	24.4	165	170
	6	+e		+e	+e	+e	+e					7.8	8.1	11.1	8.9	25.0	24.6	177	177
	7	+e18		+e14	+e17	+e17	+e11						8.0		8.2		25.0		170
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.4		25.0		211	
	1	+	+	+	+	+	+					7.9	8.1	10.4	8.6	24.9	25.0	215	193
	2	+	+	+	+	+	+					8.0	8.2	8.2	8.1	24.2	24.8	212	202
	3	+e	+e	+*	+	+*	+					8.2	8.0	8.5	8.1	24.9	24.5	206	199
	4	+*	R	T	T	R	R1					8.1	8.1	9.1	8.6	25.1	25.1	209	204
	5	T	+e2			+e	+e					8.1	7.9	9.0	8.6	25.0	24.4	197	197
	6		+e3*			+e4	T					8.1	8.2	9.0	8.1	25.0	24.6	206	199
	7		+e3			R							8.1		8.4		25.0		200
25%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.7		25.0		245	
	1	+	+	+	+	+	+									24.9	25.0	245	227
	2	+	+	+	+	+	+									24.2	24.8	243	234
	3	+e	+*	+	+e	+*	+									24.9	24.5	240	230
	4	R	R	R	+	R	+									25.1	25.1	242	234
	5	+e*	+e*	+e	T	+e	+									25.0	24.4	230	235
	6	+e1*	+e2*	+e2*		+e4*										25.0	24.6	241	239
	7	+	+	+		+e2										25.0			242

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.

d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTAY CAROL YRK

237



② 1.0 µm filtration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1700

YOUNG FROM: SR 10mW
12/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1700 JS - 11230 JS

2 1440 ORD 3 1030 JS 4 0900 GMR 5 9:20 YBL 6 10:10 YBL 7 1145 COB

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
50%	0	+	+	+	+	+	+	+	+	+	+	8.1		8.8		25.0		305	
	1	+	+	+	+	+	+									24.9	25.0	308	280
	2	+	+	+	+	+	+									24.2	24.8	306	293
	3	+	+	+	+	+	+									24.9	24.5	307	283
	4	re	re	re2	+	re	re									25.1	25.1	305	284
	5	re	re*	re	T	re	re									25.0	24.4	288	290
	6	re2*	T	re2*		re2*	re3*									25.0	24.6	300	299
	7	re	↓	re	↓	re	re1										25.0		272
100%	0	+	+	+	+	+	+	+	+	+	+	8.2		8.9		25.0		431	
	1	+	+	+	+	+	+					8.0	8.1	10.4	8.9	24.9	25.0	420	400
	2	+	+	+	+	+	+					8.1	8.1	9.3	8.3	24.2	24.8	433	410
	3	+	+	+	+	+	+					8.2	8.0	10.2	8.4	24.9	24.5	433	407
	4	re	re	re2	re	re2	re1					8.0	8.0	10.9	8.7	25.1	25.1	432	407
	5	re	re*	re	re	re	re					7.9	7.9	11.4	8.8	25.0	24.4	411	411
	6	re2*	re3	re1	re2	re1	re3					7.9	8.1	11.6	8.8	25.0	24.6	430	427
	7	re	re	re	re	re	re1						7.8		8.5		25.0		418
	0	+	+	+	+	+	+	+	+	+									
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



③ C-18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curtin

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EPC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: Dmw

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1700

YOUNG FROM: SP10M W 1 < 24 hrs

TECHNICIANS: DAY: 0 1700 - 1230 - 213 1505 GRD 3 1130 GRS - 49:30 VIK 5 0830 CRT 6 1000 SW 7 11:45 VIK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
Dmw	0	+	+	+	+	+	+	+	+	+	+	8.1		5.6		25.0			
	1	+	+	+	+	+	+					7.9	8.1	10.0	8.7	25.0	24.9	178	178
	2	+	+	+	+	+	+					8.0	8.1	8.3	8.0	24.2	24.8	174	176
	3	+e	+e	+e	+e	+e	+e					7.8	7.9	9.6	8.1	25.0	24.8	174	166
	4	+e5	+e6	+e5	+e5	+e8	+e5					7.8	8.0	11.0	8.3	25.1	25.1	169	175
	5	+e	+e	+e	+e	+e	+e					7.5	7.9	11.4	8.3	25.0	24.4	165	172
	6	+e6	+e12	+e12	+e13	+e12	+e10					7.6	7.9	11.4	8.6	25.0	25.3	167	165
	7	+e18	+e18	+e16	+e16	+e14	+e21						8.0		8.2		25.0		
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.3		25.0			208
	1	+	+	+	+	+	+					7.9	8.1	10.0	8.5	25.0	24.9	213	203
	2	+	+	+	+	+	+					8.0	8.1	8.2	7.8	24.2	24.8	207	203
	3	+e	+e	+e	+e	+e	+e					7.9	7.9	9.3	7.9	25.0	24.8	211	198
	4	+e6	+e7	+e8	+e8	+e8	+e8					8.1	8.0	9.3	8.2	25.1	25.1	202	203
	5	+e	+e	+e	+e	+e	+e					8.0	8.0	9.1	8.2	25.0	24.4	199	202
	6	+e13	+e12	+e13	+e12	+e12	+e14					8.1	8.0	8.9	8.5	25.0	25.3	208	198
	7	+e17	+e19	+e21	+e19	+e15	+e21						8.1		8.2		25.0		
25%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.1		25.0			238
	1	+	+	+	+	+	+									25.0	24.9	243	231
	2	+	+	+	+	+	+									24.2	24.8	240	233
	3	+e	+e	+e	+e	+e	+e									25.0	24.8	246	230
	4	+e7	+e7	+e5	+e6	+e5	+e7									25.1	25.1	237	231
	5	+e	+e	+e	+e	+e	+e									25.0	24.4	231	232
	6	+e11	+e12	+e10	+e14	+e	+e11									25.0	25.3	240	227
	7	+e20	+e18	+e18	+e21	+e19	+e23									25.0			227

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

© 2W+R4 ERROR VIK 12-23-11



(3) C-18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Amr

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1700

YOUNG FROM: 50/10mW
13/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 5-1700 1/23/11 2 1505 CRD 3 1130 gsw 4 8:30 VRK 5 6:30 omr 6 1000 gsw 7 11:45 VRK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
			0	+	+	+	+	+	+	+	+	+	+	8.1		8.4		25.0	
50%	1	+	+	+	+	+	+												
	2	+	+	+	+	+	+									25.0	24.9	303	284
	3	+e	+e	+e	+e	+e	+e									24.2	24.8	303	288
	4	+e8	+e8	+e7	+e7	+e6	+e7									25.0	24.8	311	289
	5	+e	+e	+e	+e	+e	+e									25.1	25.1	302	286
	6	+e11	+e15	+e12	+e14	+e16	+e									25.0	24.4	289	289
	7	+e18	+e21	+e20	+e21	+e23	+e21									25.0	25.3	301	283
100%	0	+	+	+	+	+	+	+	+	+	+	8.1		6.5		25.0		427	
	1	+	+	+	+	+	+					7.8	8.0	10.1	8.6	25.0	24.9	429	400
	2	+	+	+	+	+	+					7.8	8.1	9.1	8.6	24.2	24.8	428	407
	3	+e	+e	+e	+e	+e	+e					7.8	7.9	9.8	8.4	25.0	24.8	443	398
	4	+e6	+e5	+e7	+e8	+e7	+e6					7.7	8.0	11.4	8.4	25.1	25.1	430	407
	5	+e	+e	+e	+e	+e	+e					7.6	8.6	11.1	8.3	25.0	24.4	413	401
	6	+e15	+e14	+e12	+e14	+e15	+e					7.7	7.9	11.0	8.6	25.0	25.3	429	399
7	+e22	+e17	+e18	+e21	+e24	+e22						8.0		8.3		25.0		402	
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 • ENTRY ERROR VRK 12-23-11



④ Aeration
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Em T Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: Dmw

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 12/16/11 11:30

YOUNG FROM: 12/7/11
set DMW 1 < 24 hrs

TECHNICIANS: DAY: 0 1730 gjs - 11300 gjs 2 1515 URP 3 1200 gjs 4 9:05 YBK 5 6830 emt 6 1100 gjs 7 113:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
Dmw	0	+	+	+	+	+	+	+	+	+	+	7.8		8.9		24.8		157	
	1	+	+	+	+	+	+					7.8	8.1	9.7	8.6	25.0	24.9	173	172
	2	+	+	+	+	+	+					8.0	8.1	8.9	8.0	24.2	24.8	173	174
	3	+e	+e	+e	+e	+e	+e					8.0	8.0	8.2	8.2	25.0	24.8	183	170
	4	+e7	+e8	+e8	+e8	+e6	+e6					7.9	7.8	10.9	8.5	25.1	25.1	181	154
	5	+e	+e	+e	+e	+e	+e					7.8	8.1	11.4	8.6	25.0	24.4	157	179
	6	+e3	+e4	+e6	+e6	+e6	+e2					7.7	7.9	11.2	8.6	25.0	25.3	168	177
	7	+e24	+e20	+e21	+e20	+e	+e15						8.1		8.5		25.0		189
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.9		8.8		24.8		193	
	1	+	+	+	+	+	+					7.9	8.1	9.7	8.6	25.0	24.9	213	200
	2	+	+	+	+	+	+					8.0	8.1	8.4	8.0	24.2	24.8	205	206
	3	+e	+	+	+	+	+e					8.0	8.0	8.3	8.1	25.0	24.8	217	202
	4	T	+e4*	+e3*	+e1*	T4	+e2*					8.1	8.0	9.3	8.6	25.1	25.1	209	188
	5	T	T	+e	T	T	+*					8.1	8.2	8.6	8.8	25.0	24.4	194	205
	6	T	T	+e6	T	T	+e2*					8.0	8.0	9.2	8.5	25.0	25.3	202	186
	7	T	T	+e8	T	T	+3						8.2		8.5		25.0		196
25%	0	+	+	+	+	+	+	+	+	+	+					24.8		226	
	1	+	+	+	+	+	+									25.0	24.9	249	233
	2	+	+	+	+	+	+									24.2	24.8	242	234
	3	+e	+	+	+	+	+e									25.0	24.8	251	235
	4	+*	+2*	+*	+*	+3*	T									25.1	25.1	241	220
	5	+e	T	+e	+e	T	T									25.0	24.4	227	234
	6	+e2	T	+e4	+e2	+e1	T									25.0	25.3	236	237
	7	+e	T	+e*	+3*	+4*	T									95.0			234

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 • entry error 12/19/11 gjs - YBK 12-23-11



④ Aeration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: *[Signature]*

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: PEC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1730

YOUNG FROM: SKJDMW
12/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1730gs-1300gs 1 1515 GRD 2 3/200gs 3 9:05 YBK 4 0830 cm 5 1100gs 6 7:28:00 VBL

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
			0	+	+	+	+	+	+	+	+	+	+								
50%	1	+	+	+	+	+	+									24.8		291			
	2	+	+	+	+	+	+									25.0	24.9	313	289		
	3	+	+	+	+	+	+									24.2	24.8	303	294		
	4	+	+	+	tl	+	+									25.0	24.8	316	292		
	5	tl	tl	tl	tl	tl	tl									25.1	25.1	365	278		
	6	te4	te2	te3	te	te4	te2									25.0	24.4	362	293		
	7	tl	tl	+	tl	tl	tl									25.0	25.3	302	295		
100%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.9		24.8		407			
	1	+	+	+	+	+	+					7.9	8.0	10.3	8.8	25.0	24.9	446	409		
	2	+	+	+	+	+	+					8.0	8.1	9.2	8.2	24.2	24.8	433	414		
	3	+	+	+	+	+	+					8.0	8.0	9.1	8.3	25.0	24.8	452	405		
	4	tl	tl	tl	tl	tl	tl					7.9	8.0	11.1	8.8	25.1	25.1	447	391		
	5	tl	tl	tl	tl	tl	tl					7.8	8.1	11.4	8.9	25.0	24.4	430	411		
	6	te2	te3	te5	+	te2	te3					7.7	7.9	11.2	8.6	25.0	25.3	427	419		
7	tl	tl	tl	tl	tl	tl						8.0		8.0		25.0		404			
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
7																					

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



⑤ EDTA

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by Am Tr

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: BFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1730

YOUNG FROM: 38/10 DMW
12/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1730 S - 11400 S - 2 1350 CR2 3 1300 S - 4 0930 S - 5 1000 S - 6 12:00 DMW 7 1200 CR2

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		8.6		24.8		171	
	1	+	+	+	+	+	+					7.8	8.1	9.5	8.2	25.0	24.9	172	165
	2	+	+	+	+	+	+					7.9	7.9	8.0	7.5	24.1	24.9	168	171
	3	te	te	te	te	te	te					7.9	7.8	7.9	8.1	25.0	24.9	173	179
	4	te7	te8	te7	te7	te7	te8					8.1	7.9	8.4	8.5	25.1	25.1	184	166
	5	te	te14	te	te	te	te					8.1	7.9	8.7	8.3	25.1	24.3	172	181
	6	te3 ^d	te	te9	te11	te10	te9					8.1	7.9	8.6	8.5	25.0	24.6	174	170
	7	te3	te5	te5	te20	te15	te15						8.0		8.0		25.0		173
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.9		8.7		24.8		201	
	1	+	+	+	+	+	+					7.9	8.1	10.0	8.2	25.0	24.9	211	197
	2	+*	+	+	+	+	+					8.0	8.0	8.3	7.5	24.1	24.8	203	206
	3	↓	+*	↓	↓	+	+					7.9	8.0	8.1	8.0	25.0	24.9	215	207
	4	↓	↓	↓	↓	te	te3					8.1	8.0	8.9	8.5	25.1	25.1	207	180
	5	↓	↓	↓	↓	te	↓					8.1	8.0	9.0	8.6	25.1	24.3	205	205
	6	↓	↓	↓	↓	te1	↓					8.1	8.0	8.8	8.6	25.0	24.6	208	182
	7	↓	↓	↓	↓	te6	↓						8.1		7.5		25.0		183
2.5%	0	+	+	+	+	+	+	+	+	+	+					24.8		235	
	1	+	+	+	+	+	+									25.0	24.9	242	227
	2	+	+	+	+	+	+									24.1	24.8	236	238
	3	+	↓	↓	+	+	+									25.0	24.9	252	238
	4	te	↓	↓	↓	↓	te									25.1	25.1	243	228
	5	te	↓	↓	↓	↓	te									25.1	24.3	239	229
	6	te4	↓	↓	↓	↓	te5									25.0	24.6	242	234
	7	te	↓	↓	↓	↓	te5									25.0			227

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cm Th Date: 12/23/11

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11 1730

YOUNG FROM: SP10 MW 12/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 5:50 1730 11400 2 1350 3 1300 4 0930 5 1000 6 12:00 YBK 7 1200 CMK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+									24.8		300			
	2	+	+	+	+	+	+									25.0	24.9	316	287		
	3	+	+	+	+	+	+									24.1	24.8	301	300		
	4	te	te	*	+	te	te									25.0	24.9	316	295		
	5	te	te		+	te	te									25.1	25.1	311	284		
	6	te3	te4		+	te4	te3									25.1	24.3	305	298		
	7	te2	te4		+	te3	te1									25.0	24.6	304	307		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					7.7		9.4		24.8		428			
	2	+	+	+	+	+	+					7.8	8.1	10.3	8.3	25.0	24.9	442	401		
	3	+	+	+	+	+	+					8.0	8.0	10.0	7.9	24.1	24.8	433	423		
	4	te	te	te	te	te	te					7.8	8.0	10.0	8.3	25.0	24.9	450	407		
	5	te	te	te	te	te	te					7.7	8.0	10.9	8.7	25.1	25.1	447	405		
	6	+	+	+	+	+	+					7.7	8.0	11.6	8.6	25.1	24.3	432	412		
	7	+	+	+	+	+	+					7.7	8.0	11.5	8.7	25.0	24.6	436	420		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



© No This

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1730

YOUNG FROM: SP/DMW
+2/17/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1730 - 1 1400 - 2 1400 CAD 3 1315 - 4 1000 - 5 1000 - 6 19:30 VLN 7 1230 CR

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		8.7		24.8		192	
	1	+	+	+	+	+	+					7.9	8.1	9.4	8.3	25.0	24.9	208	189
	2	+	+	+	+	+	+					7.9	8.0	9.0	7.6	24.1	24.8	195	210
	3	+e	+e	+e	+e	+e	+e					7.9	8.0	9.3	8.1	25.0	24.9	207	187
	4	+e2	+e6	+e7	+e8	+e6	+e6					7.9	8.0	11.2	8.5	25.1	25.1	203	193
	5	+e8	+e	+e	+e	+e	+e11					7.9	7.9	11.6	8.3	25.1	24.3	197	190
	6	+e	+e3 ^d	+e6 ^d	+e9	+e5 ^d	+e					7.8	8.0	11.2	8.6	25.0	24.6	198	196
	7	+e8	+e11	+e9	+e16	+e11	+e16						8.1		8.0		25.0		194
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.8		24.8		215	
	1	+	+	+	+	+	+					8.0	8.1	9.3	8.0	25.0	24.9	224	210
	2	+	+	+	+	+	+					8.0	8.0	9.4	7.2	24.1	24.8	215	217
	3	+e	+e	+e	+e	+e						8.0	8.0	8.4	8.0	25.0	24.9	225	204
	4	+e1	+e1	+e5	+e7	+e4						8.1	8.1	8.2	8.5	25.1	25.1	225	210
	5	↓	↓	+e	+e2							8.1	8.0	9.4	8.4	25.1	24.3	217	210
	6	↓	↓	+e4	+e4 [@]							8.1	8.1	9.1	8.6	25.0	24.6	216	207
	7	↓	↓	+e9	+e11	↓	↓						8.1		8.3		25.0		207
25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+									24.8		254	
	2	+	+	+	+	+	+									25.0	24.9	270	248
	3	+e	+	+	+	+e	+e									24.1	24.8	255	259
	4	+e2	+e	+e1	+e3	+e3	+e2									25.0	24.9	270	250
	5	+e4	↓	+e7	+e6	+e4	+e4									25.1	25.1	271	248
	6	+e	↓	+e	+e	+e	+e									25.1	24.3	260	253
	7	+e7	↓	+e8	+e8	+e7	+e6									25.0	24.6	256	257

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
* ENTRY ENDS: CAD 12/19/11



DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curtin

Date: 12/23/11

Great Lakes Environmental Center

TEST MATERIAL: EFC 9450

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 1953-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 12/16/11/1730

YOUNG FROM: SR/DMW 1 < 24 hrs
12/17/11

TECHNICIANS: DAY: 0 1730 1 1400 2 1400 3 1315 4 1000 5 1000 6 12:30 7 12:30

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+									24.8		332			
	2	+	+	+	+	+	+									25.0	24.9	354	321		
	3	te	te	te	te	te	te									24.1	24.8	336	335		
	4	te4	te3		te4	te2	te4									25.0	24.9	351	324		
	5	te	te		te	te	te									25.1	25.1	353	318		
	6	te4	te5		te5	te4	te5									25.1	24.3	346	331		
	7	te8	te8	✓	te5	te3	te5									25.0	24.6	333	332		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					8.3		10.4		24.8		486			
	2	+	+	+	+	+	+					8.0	8.1	10.3	8.3	25.0	24.9	516	476		
	3	te	te	te	te	te	te					8.2	7.9	10.3	7.8	24.1	24.8	502	491		
	4	te3	te	te	te3	te1	te					8.0	7.9	10.0	8.3	25.0	24.9	522	476		
	5	te	te	te	te	te	te					8.0	7.9	10.9	8.6	25.1	25.1	521	460		
	6	te3	te5	te1	te4	te4	te4					8.7	7.9	11.1	8.6	25.1	24.3	516	482		
	7	te	te3	te	te	te	te2					7.8	7.9	11.1	8.7	25.0	24.6	503	506		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 # writing error off

APPENDIX B

DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST		FHM
	NO.	C. dubia	
2/10	96	1.39	1.59
3/10	97	0.81	1.80
5/10	98	1.37	2.09
6/10	99	1.48	2.13
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
AVERAGE		1.26	2.11
STD. DEV.		0.24	0.34
RANGE: LOW		0.78	1.42
RANGE: HIGH		1.74	2.80
Coefficient of variation		0.19	0.16
Date of last test		12/13-19/11	12/13-20/11
MSD of most recent test		7.16	0.0417
PMSD of most recent test		22.6	13.8
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

From EPA's Wet Interlaboratory Variability Study

Test Species	GLEC ¹	IC25 Coefficient of Variation National Percentiles ²				
		10th	25th	50th	75th	90th
C. dubia	0.19	0.08	0.17	0.27	0.45	0.62
P. promelas	0.16	0.12	0.21	0.26	0.38	0.45

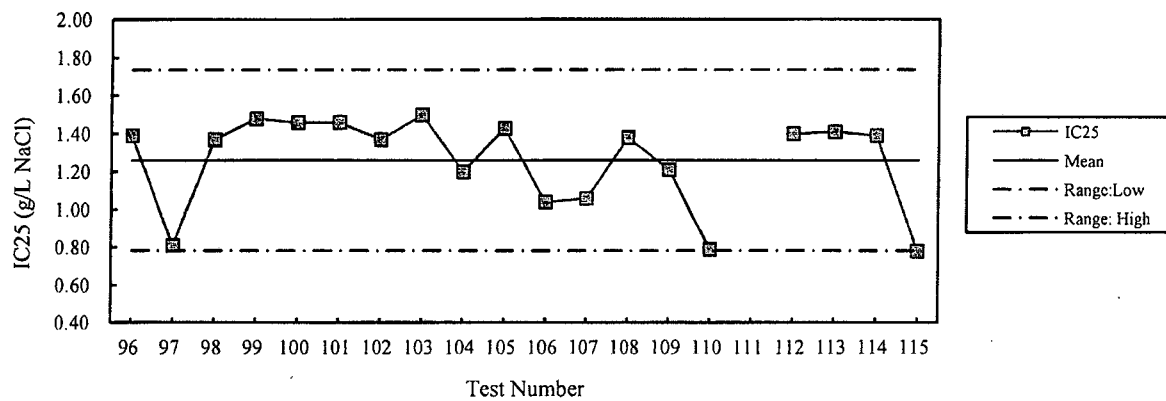
¹ Based on cumulative GLEC data from the most recent 20 tests.

² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

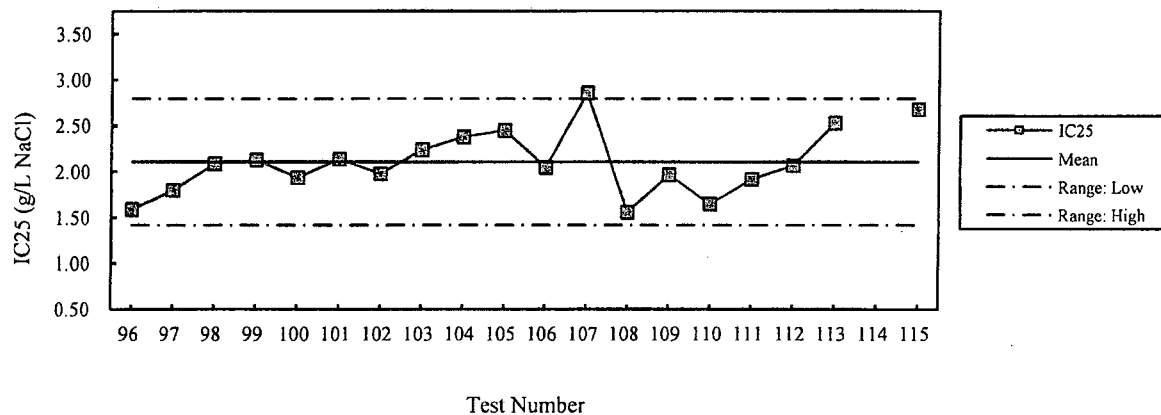
Chronic Reference Toxicant IC25

Ceriodaphnia dubia



Chronic Reference Toxicant IC25

Pimephales promelas





Great
Lakes
Environmental
Center

March 5, 2012

Applied
Environmental
Sciences
www.glec-online.com

Roland McDaniel, Project Manager
GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

Traverse City
Operations
739 Hastings St.
Traverse City
MI 49686

231 941-2230
231 941-2240 fax

Columbus
Operations
1295 King Ave
Columbus
OH 43212

614 487-1040
614 487-1920 fax

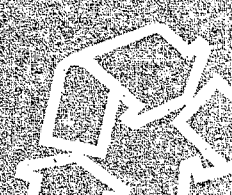
**RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED
FEBRUARY 15, 2012 FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL
DORADO, ARKANSAS**

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected February 15, 2012. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tarr
Laboratory Coordinator



PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED FEBRUARY 15, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

February 2012



Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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APPENDIX B. DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS B-1

INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled February 15, 2012 (Sample ID: EEC 9462) to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

Ceriodaphnia dubia Static Renewal Chronic Toxicity Tests

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for seven days under static renewal conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one

organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity $10\text{-}20 \mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test (7 days).

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUC) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUC} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1). The baseline toxicity test was not toxic to *C. dubia* survival and exhibited 100 percent survival in the 100 percent test concentration, resulting in a 7 day LC_{50} value of >100 percent effluent. The outfall 001 sample was very toxic to *C. dubia* reproduction and exhibited an IC_{25} value of 6.3 percent effluent or 15.9 TUC (Table 1).

1.0 μm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a

Gelman A/E glass fiber filter (1.0 μm).

The *C. dubia* filtration test had an IC_{25} of 3.8 percent or 26.3 TUc. Compared to the baseline toxicity test, filtration did not remove toxicity to *C. dubia* reproduction (Table 1).

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample **after 1.0 μm filtration treatment** was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

After C18 treatment, 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent was removed as the *C. dubia* exhibited an IC_{25} of >100 percent or <1.0 TUc. Therefore, C-18 was very effective as a treatment and the toxicity to *C. dubia* reproduction in the EDCC effluent sample appears to be associated with non-polar and semi-polar organic compounds.

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

Aeration of the outfall 001 sample did not remove toxicity when compared to the concurrent baseline and demonstrated an IC_{25} of 4.5 percent or 22.2 TUc (Table 1).

Cation Chelation with EDTA - Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test IC_{25} of 6.3 percent, the addition of EDTA (25 mg/L) did not remove any sample toxicity and exhibited an IC_{25} of 4.5 percent or 22.2 TUc (Table 1).

Sodium Thiosulfate Treatment - Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

The *C. dubia* sodium thiosulfate treatment removed 1.6 percent of the toxicity demonstrated in the EDCC outfall 001 effluent sample and had an IC_{25} value of 6.4 percent or 15.6 TUc (Table 1). However, this difference is not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Therefore, sodium thiosulfate treatment did not demonstrate a clear reduction in toxicity.

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected February 15, 2012 did demonstrate removal of chronic toxicity, but the reduction of toxicity to *C. dubia* reproduction was only demonstrated by one of the five TIE treatments performed. Four of the treatments, filtration, aeration, EDTA and sodium thiosulfate were not effective in removing meaningful toxicity from the outfall 001 effluent sample. Thus, the effluent toxicity does not appear to be related to; a filterable toxicant, an easily oxidizable or aeratable compound, a chelatable metal or thiosulfate reducible compounds or oxidants.

The C-18 treatment removed 100 percent of the toxicity present in the EDCC outfall 001 effluent sample. Therefore, the chronic toxicity to *C. dubia* reproduction present in the EDCC outfall 001 effluent sample appeared to be associated with non-polar and or semi-polar organic compound(s). For a summary of all test results, see Table 1.

Summary of the chronic toxicity characterization of the EDCC outfall 001 sample collected February 15, 2012 (Sample ID: EEC 9462):

- The toxicant (s) was not filterable.
- **The toxicant(s) was a non-polar and or a semi-polar organic compound.**
- The toxicant(s) was not a chelatable metal.
- The toxicant(s) was not a volatile, easily oxidizable or aeratable compound.
- The toxicant(s) was not a thiosulfate reducible compound or oxidant.

Table 1. Summary of Chronic TIE Test results

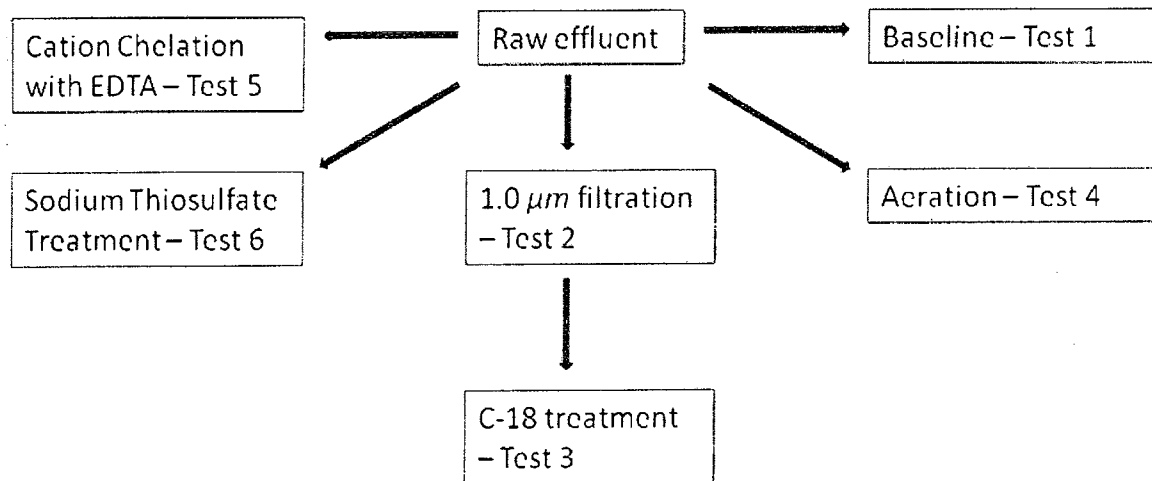
EDCC Outfall 001 final effluent (Collection date: 2/15/12) <i>C. dubia</i> TIE Test Dates 2/16-23/12	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	100	80	100	100	100	>100	0.0	--
1.0 µm Filtration – Test 2	100	33	83	100	100	>100	0.0	NA
C18-SPE Treatment – Test 3	83	100	100	100	100	>100	0.0	NA
Aeration – Test 4	100	67	83	100	100	>100	0.0	NA
Cation Chelation with EDTA – Test 5	100	67	83	100	83	>100	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100	67	100	83	100	>100	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	22.3	11.2	5.7	3.7	5.3	6.3	15.9	--
1.0 µm Filtration – Test 2	25.2	3.7	5.3	3.0	4.3	3.8	26.3	0
C18-SPE Treatment – Test 3	25.5	33.8	36.2	35.2	36.0	>100	<1.0	100
Aeration – Test 4	27.8	8.5	5.2	5.7	6.5	4.5	22.2	0
Cation Chelation with EDTA – Test 5	25.0 ^b	7.3	4.2	3.7	0.7	4.5	22.2	0
Sodium Thiosulfate Treatment – Test 6	22.5	10.7	7.0	5.7	0.2	6.4	15.6	1.6

NA – Not applicable or Not available

^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC_{25} value for the most recent *C. dubia* reference toxicant test was 0.78 g/L of sodium chloride which was within the acceptance range of 0.78 to 1.74 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED FEBRUARY 15, 2012



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: GBME El Dorado

PROJECT NUMBER: 2159-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>2/16/12</u>	INITIALS				
EEC NUMBER		<u>9462</u>			
OUTFALL/DESCRIPTION		<u>outfall-001</u>			
DISSOLVED OXYGEN (mg/L)	<u>ACS</u>	<u>13.4</u>			
TEMPERATURE (°C)	<u>ACS</u>	<u>1.4</u>			
pH	<u>ACS</u>	<u>8.0</u>			
CONDUCTIVITY (µmhos/cm)	<u>ACS</u>	<u>321</u>			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>2/16/12</u>	INITIALS				
EEC NUMBER		<u>9462</u>			
OUTFALL/DESCRIPTION		<u>outfall-001</u>			
DISSOLVED OXYGEN (mg/L)					
TEMPERATURE (°C)					
pH					
CONDUCTIVITY (µmhos/cm)					
HARDNESS (mg/L CaCO ₂)	<u>ACS</u>	<u>1.3 x 40 = 52</u>			
ALKALINITY (mg/L CaCO ₂)	<u>ACS</u>	<u>1.3 x 40 = 52</u>			
TOTAL CHLORINE (mg/L)*					
TOTAL AMMONIA (mg/L)*					

*Check with project manager to see if necessary



Great Lakes Environmental Center
 1295 KING AVE.
 COLUMBUS, OH 43212
 PHONE: (614) 487-1040
 FAX: (614) 487-1920

Two Important Notes for Whole Effluent Toxicity Testing:

- There is a maximum hold time for all samples of 36 hours (Hold time begins when sample is taken off the sampler)
- Samples must be received at 4°C ± 2°C

CHAIN OF CUSTODY FORM

(TO BE COMPLETED ONSITE AND SUBMITTED WITH SAMPLES)

FACILITY: El Dorado Chemical Company
 LOCATION: El Dorado, AR
 CONTACT PERSON: Larken Pennington
 PHONE: 870-312-1752

COLLECTOR: Larken Pennington
 DATE: 2/15/12
 WITNESS: Brent Parker
 DATE: 2/15/12

BEC# (lab only)	SAMPLE ID	SAMPLE SOURCE (Eff/Upstr.)	TYPE (grab or composite)	SAMPLE START DATE	SAMPLE START TIME (24-hr notation)	SAMPLE END DATE	SAMPLE END TIME (24-hr notation)	VOLUME COLLECTED	SAMPLE CONTAINER	SAMPLE COLLECTOR	OTHER COMMENTS
9462	001		grab grab	2/15/12 2/15/12	8:45am 8:45am	2/15/12	8:45am	4 cubitainers	cubitainers	Larken Pennington	

ANALYSIS REQUIRED: Please fill in completely

NAME OF STREAM SAMPLED: outfall 001

Species: *Ceriodaphnia dubia*

Pimephales promelas (fathead minnows)

Other - please specify: _____

Test Type: Acute: 24-hour

Acute: 24-hour 48-hour

96-hour: with 48-hour renewal

Chronic (7-day)

Chronic (7-day)

without 48-hour renewal

Dilutions: Screen (100% only)

Definitive (5 sample concentrations): List test concentrations: _____

Other - please specify: _____

Dilution Water: Receiving Water

Lab water

Other - please specify: _____

TRANSFER OF SAMPLES:

(FIRST SIGNATURE IS SAMPLER, LAST SIGNATURE IS AUTHORIZED LABORATORY REPRESENTATIVE)

SHIPPER	RECEIVER	DATE	TIME
1. <u>Larken Pennington</u>	<u>Andr. Rye</u>	<u>2/16/12</u>	<u>1000</u>
2.			

For Lab Use Only:
 Ice remaining in cooler upon receipt
 Temperature of samples when received:
1.4

FOR SATURDAY DELIVERY??? MARK PACKAGE AS SUCH AND CALL GLEC ON FRIDAY WITH TRACKING NUMBER

Baseline (Test 1)
El Dorado Chemical outfall 001 (EEC 9462)
(Tested 2/16-23/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	6	6	6
DEAD	0	1	0	0	0
% SURV	100.00%	80.00%	100.00%	100.00%	100.00%

Scito River Water 2° Control	5
	0
	100.00%

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	15	9	3	1	1
2	23	16	5	4	7
3	27	7	6	3	7
4	29	12	7	5	5
5	23	12	10	4	6
6	17		3	5	6
N	6	5	6	6	6
MEAN	22.3	11.2	5.7	3.7	5.3
SD	5.4650404	3.42052628	2.6583203	1.5055453	2.2509257
CV	24.47033	30.5404132	46.911534	41.060327	42.204858
Total Young	134	56	34	22	32

Scito River 2° Control	40
	40
	36
	37
	37
	5
	38.0
	1.8708287
	4.9232334
	190

1.0 µm filtration (Test 2)
El Dorado Chemical outfall 001 (EEC 9462)
(Tested 2/16-23/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	2	5	6	6
DEAD	0	4	1	0	0
% SURV	100.00%	33.33%	83.33%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	20	0	4	1	1
2	25	0	8	3	3
3	29	6	7	3	6
4	27	6	7	4	5
5	28	7	6	3	7
6	22	3	0	4	4
N	6	6	6	6	6
MEAN	25.2	3.7	5.3	3.0	4.3
SD	3.5449495	3.14112506	2.9439203	1.0954451	2.1602469
CV	14.085892	85.6670472	55.198505	36.514837	49.851852
Total Young	151	22	32	18	26

C-18 SPE treatment (Test 3)
El Dorado Chemical outfall 001 (EEC 9462)
(Tested 2/16-23/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	6	6	6	6
DEAD	1	0	0	0	0
% SURV	83.33%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	31	39	37	34	38
2	30	33	34	38	33
3	32	31	39	36	29
4	14	35	37	40	39
5	32	33	34	30	37
6	14	32	36	33	40
N	6	6	6	6	6
MEAN	25.5	33.8	36.2	35.2	36.0
SD	8.93868	2.85773803	1.9407902	3.6009258	4.1952354
CV	35.053647	8.44651635	5.3662402	10.239599	11.653432
Total Young	153	203	217	211	216

Aeration (Test 4)
El Dorado Chemical outfall 001 (EEC 9462)
(Tested 2/16-23/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	5	6	6
DEAD	0	2	1	0	0
% SURV	100.00%	66.67%	83.33%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	29	8	0	5	6
2	25	2	8	6	7
3	22	11	7	5	7
4	34	12	9	8	10
5	30	3	1	6	5
6	27	15	6	4	4
N	6	6	6	6	6
MEAN	27.8	8.5	5.2	5.7	6.5
SD	4.1673333	5.16720427	3.7638633	1.3662601	2.0736441
CV	14.972455	60.7906385	72.848966	24.110472	31.902217
Total Young	167	51	31	34	39

EDTA 25 mg/l (Test 5)
El Dorado Chemical outfall 001 (EEC 9462)
(Tested 2/16-23/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	5	6	5
DEAD	0	2	1	0	1
% SURV	100.00%	66.67%	83.33%	100.00%	83.33%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	30	13	4	3	0
2	27	0	7	2	0
3	27	7	2	4	0
4	26	14	4	4	0
5	19	1	0	4	1
6	21	9	8	5	3
N	6	6	6	6	6
MEAN	25	7.33333333	4.2	3.7	0.7
SD	4.1472883	5.88784058	2.9944393	1.0327956	1.2110601
CV	16.589153	80.2887351	71.866543	28.167152	181.65902
Total Young	150	44	25	22	4

NaThio (50 mg/l) (Test 6)
El Dorado Chemical outfall 001 (EEC 9462)
(Tested 2/16-23/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	6	5	6
DEAD	0	2	0	1	0
% SURV	100.00%	66.67%	100.00%	83.33%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	23	17	9	0	0
2	18	14	6	7	1
3	30	0	9	7	0
4	21	15	6	7	0
5	28	0	6	5	0
6	15	18	6	8	0
N	6	6	6	6	6
MEAN	22.5	10.6666667	7.0	5.7	0.2
SD	5.7532599	8.3825215	1.5491933	2.9439203	0.4082483
CV	25.570044	78.586139	22.131333	51.951535	244.94897
Total Young	135	64	42	34	1

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	15	9	3	1	1
Response 2	23	16	5	4	7
Response 3	27	7	6	3	7
Response 4	29	12	7	5	5
Response 5	23	12	10	4	6
Response 6	17		3	5	6

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9462 EDCC Outfall 001 Test / Base line

Test Start Date: 2/16/12 Test Ending Date: 2/23/12

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	22.333	5.465	22.333
2	5	12.500	11.200	3.421	11.200
3	6	25.000	5.667	2.658	5.667
4	6	50.000	3.667	1.506	4.500
5	6	100.000	5.333	2.251	4.500

The Linear Interpolation Estimate: 6.2687 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 6.3218 Standard Deviation: 0.9992

Original Confidence Limits: Lower: 4.9592 Upper: 8.7500

Expanded Confidence Limits: Lower: 4.5664 Upper: 9.4944

Resampling time in seconds: 0.00 Random_Seed: 735715706

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	20	0	4	1	1
Response 2	25	0	8	3	3
Response 3	29	6	7	3	6
Response 4	27	6	7	4	5
Response 5	28	7	6	3	7
Response 6	22	3	0	4	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9462 Test 2
 Test Start Date: 2/16/12 Test Ending Date: 2/23/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	25.167	3.545	25.167
2	6	12.500	3.667	3.141	4.500
3	6	25.000	5.333	2.944	4.500
4	6	50.000	3.000	1.095	3.667
5	6	100.000	4.333	2.160	3.667

The Linear Interpolation Estimate: 3.8054 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 3.8361 Standard Deviation: 0.1655

Original Confidence Limits: Lower: 3.5609 Upper: 4.1480

Resampling time in seconds: 0.00 Random_Seed: -205192174

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	29	8	0	5	6
Response 2	25	2	8	6	7
Response 3	22	11	7	5	7
Response 4	34	12	9	8	10
Response 5	30	3	1	6	5
Response 6	27	15	6	4	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9462 Test 4
 Test Start Date: 2/16/12 Test Ending Date: 2/23/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	27.833	4.167	27.833
2	6	12.500	8.500	5.167	8.500
3	6	25.000	5.167	3.764	5.778
4	6	50.000	5.667	1.366	5.778
5	6	100.000	6.500	2.074	5.778

The Linear Interpolation Estimate: 4.4989 Entered P value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 4.5303 Standard Deviation: 0.4182

Original confidence Limits: Lower: 3.8993 Upper: 5.4768

Resampling time in Seconds: 0.00 Random_Seed: 1784933666

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	30	13	4	3	0
Response 2	27	0	7	2	0
Response 3	27	7	2	4	0
Response 4	26	14	4	4	0
Response 5	19	1	0	4	1
Response 6	21	9	8	5	3

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9462 Test 5
 Test Start Date: 2/16/12 Test Ending Date: 2/23/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	25.000	4.147	25.000
2	6	12.500	7.333	5.888	7.333
3	6	25.000	4.167	2.994	4.167
4	6	50.000	3.667	1.033	3.667
5	6	100.000	0.667	1.211	0.667

The Linear Interpolation Estimate: 4.4222 Entered P value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 4.5343 Standard Deviation: 0.5870
 Original Confidence Limits: Lower: 3.6563 Upper: 5.7566
 Resampling time in seconds: 0.00 Random_Seed: 1178856258

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	23	17	9	0	0
Response 2	18	14	6	7	1
Response 3	30	0	9	7	0
Response 4	21	15	6	7	0
Response 5	28	0	6	5	0
Response 6	15	18	6	8	0

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9462 Test 6
 Test Start Date: 2/16/12 Test Ending Date: 2/23/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	22.500	5.753	22.500
2	6	12.500	10.667	8.383	10.667
3	6	25.000	7.000	1.549	7.000
4	6	50.000	5.667	2.944	5.667
5	6	100.000	0.167	0.408	0.167

The Linear Interpolation Estimate: 5.9419 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 6.4337 Standard Deviation: 2.0191
 Original Confidence Limits: Lower: 4.1440 Upper: 12.0690
 Resampling time in Seconds: 0.06 Random_Seed: -471296126

Test Dates: 2/16-23/12

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	100%	80%	100%	100%	100%
1.0 µm filtration (Test 2)	100%	33%	83%	100%	100%
C-18 SPE treatment (Test 3)	83%	100%	100%	100%	100%
Aeration (Test 4)	100%	67%	83%	100%	100%
EDTA 25 mg/l (Test 5)	100%	67%	83%	100%	83%
NaThio (50 mg/l) (Test 6)	100%	67%	100%	83%	100%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	22.3	11.2	5.7	3.7	5.3	6.3	15.9	--
1.0 µm filtration (Test 2)	25.2	3.7	5.3	3.0	4.3	3.8	26.3	0.0%
C-18 SPE treatment (Test 3)	25.5	33.8	36.2	35.2	36.0	>100	<1.0	100.0%
Aeration (Test 4)	27.8	8.5	5.2	5.7	6.5	4.5	22.2	0.0%
EDTA (25 mg/l) (Test 5)	25.0	7.3	4.2	3.7	0.7	4.5	22.2	0.0%
NaThio (50 mg/l) (Test 6)	22.5	10.7	7.0	5.7	0.2	6.4	15.6	1.6%

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to C.dubia reproduction in DMW



Baseline ①

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by Am

Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 21589-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2-16-12/14:00 YOUNG FROM: SR 2/8 < 24 hrs

TECHNICIANS: DAY: 0 14:00 YBK 1 9:10 YBK 2 12:30 KAM 3 9:45 ACS 4 12:40 YBK 5 13:00 YBK 6 8:10 YBK 7 12:00 em

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.7		9.4		25.0		180	
	1	+	+	+	+	+	+					7.8	7.7	8.0	7.8	24.0	24.5	177	182
	2	te	te	te	te	te	+					8.1	8.1	8.2	8.0	25.0	24.7	189	169
	3	te	te	te	te	te	te					7.9	7.8	8.4	8.2	25.5	24.3	170	177
	4	te6	te6	te6	te6	te5	te4					7.8	8.2	8.2	9.0	24.2	24.5	179	187
	5	te9	te9	te11	te12	te6	te5					8.0	7.8	8.2	8.6	25.0	24.7	176	183
	6	te	te	te	te	te	te					8.1	8.0	8.8	8.5	25.0	24.1	173	178
	7	te8	te8	te10	te11	te12	te8						7.9		8.5		24.7		171
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		9.7		25.0		219	
	1	+	+	+	+	+	+					7.9	8.0	8.1	7.9	24.0	24.5	212	225
	2	te	te	te	te	te	te					8.1	8.2	9.1	8.2	25.0	24.7	228	220
	3	te	te	te	te	te	te					8.0	7.9	8.7	8.3	25.5	24.3	206	215
	4	te2	te4	te2	te1	te2						7.8	8.5	8.4	9.3	24.2	24.5	220	221
	5	te3	te4	te5	te4	te3						8.0	8.0	8.6	8.6	25.0	24.7	210	213
	6	te	te	te	te	te	te					8.0	8.0	9.0	8.5	25.0	24.1	223	220
	7	te4	te8	te7	te7	te7	te7						7.9		8.5		24.7		210
25%	0	+	+	+	+	+	+	+	+	+	+					25.0		261	
	1	+	+	+	+	+	+									24.0	24.5	255	257
	2	te	te	te	te	te	te									25.0	24.7	271	244
	3	te	te	te	te	te	te									25.0	24.3	244	250
	4	te	te	te1	te2	te2	te									24.7	24.5	255	253
	5	te	te	te	te	te	te									25.0	24.7	248	244
	6	te1	te2	te2	te1	te3	te									25.0	24.1	259	240
	7	te2	te3	te3	te4	te5	te5										24.7		243

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 • entry error YBK 1-24-12, KAM 2-18-12 @mem not present ACS 2/19/12



Baseline ①
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Amr Date: 2/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9402

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2-16-12/14:00

YOUNG FROM: SR 2/8 < 24 hrs

TECHNICIANS: DAY: 0 14:00 YBK 1 9:10 YBK 2 12:30 Kom 3 895 4 ACS 5 12:40 YBK 6 13:00 YBK 7 8:10 YBK 8 12:00 Kom

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.							
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old						
50%	0	+	+	+	+	+	+	+	+	+	+														
	1	+	+	+	+	+	+											25.0		336					
	2	+*	+*	+*	+*	+*	+*											24.0	24.5	333	325				
	3	+*	te	te	+*	+*	+											25.0	24.7	339	307				
	4	+*	+e1	+e1*	+e	+e1	+e											25.5	24.3	315	316				
	5	+e*	+e	+e	+e3	+e	+e											24.2	24.5	328	321				
	6	+e1*	+e1	+e2	+e	+e1	+e2											25.0	24.7	321	311				
	7	te1	te2	te	te2	te2	te3											25.0	24.1	320	314				
100%	0	+	+	+	+	+	+	+	+	+	+										24.7		305		
	1	+	+	+	+	+	+							7.8		12.6		25.0		484					
	2	+	te	+	+*	+*	+*							7.7	7.9	8.6	8.0	24.0	24.5	490	463				
	3	x+	te	te	te*	te*	te							7.9	8.2	9.9	8.7	25.0	24.7	493	439				
	4	+e	+e2	+e2	+e2	+e2	+e1							7.7	7.9	10.8	8.4	25.5	24.3	464	450				
	5	+e	+e2	+e	+e	+e	+e							7.7	8.4	9.6	9.7	24.2	24.5	481	462				
	6	+e1	+e	+e2	+e1	+e2	+e2							7.8	8.0	9.7	8.8	25.0	24.7	474	451				
	7	te	te3	te3	te2	te3	te3							7.6	8.0	11.5	8.9	25.0	24.1	479	453				
	0	+	+	+	+	+	+	+	+	+	+														
	1																								
	2																								
	3																								
	4																								
	5																								
	6																								
	7																								

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTRY ERROR YBK 1-24-12, 2/19/12 ACS



Baseline ①
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Ann T... Date: 2/22/12

Great Lakes Environmental Center

TEST MATERIAL: ECC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 21589-00

ANIMALS/CONC.: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2-16-12/14:00

YOUNG FROM: SR 2/8 1 < 24 hrs

TECHNICIANS: DAY: 0 14:00 YRK 1 9:10 YRK 2 12:30 YRK 3 8:45

ACS 12:40 YRK 5 13:00 YRK 6 8:10 YRK 7 12:00 YRK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
SR	0	+	+	+	+	+	+	+	+	+	+	7.8		12.8		25.0		462	
	1	+	+	+	+	+	+					7.9	8.3	10.9	8.0	24.0	24.5	456	447
	2	e	e	e	e	e	e					8.1	8.3	9.8	8.1	25.0	24.7	484	432
	3	e	e	e	@	e	e					8.0	8.3	9.9	8.4	25.5	24.3	456	445
	4	+e6	+e7	+e4		+e6	+e6					8.0	8.4	9.2	9.0	24.2	24.5	475	451
	5	+e13	+e14	+e15		+e14	+e13					8.1	8.2	9.7	9.5	25.0	24.7	453	456
	6	+e	+e19	+e17		+e	+e					7.9	8.2	11.5	8.7	25.0	24.1	470	451
	7	e21	e	e	↓	e17	e18						8.2		8.6		24.7		467
	0	+	+	+	+	+	+	+	+	+									
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	0	+	+	+	+	+	+	+	+	+									
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ENTRY ERROR YRK 24-12, ACS 2/18/12 @ no mom present ACS 2/19/12



1.0MM PF (2) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Th

Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2-16-12/14:20

YOUNG FROM: SR 2/21 < 24 hrs

TECHNICIANS: DAY: 0 14:20 YBK 19:30 YBK 2 13:00 YBK 3 9:10

4 13:40 YBK 5 13:50 YBK 6 9:00 YBK 7 12:15 CW

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		8.8		25.0		185	
	1	+	+	+	+	+	+					7.9	8.0	8.2	7.9	24.0	24.5	179	183
	2	e	e	e	e	e	e					8.0	8.3	8.9	8.4	25.0	24.7	192	178
	3	e	e	e	e	e	e					7.8	8.1	10.1	8.4	25.0	24.3	177	180
	4	+e5	+e6	+e6	+e7	+e7	+e6					7.8	8.3	9.5	8.9	24.2	24.5	185	181
	5	+e9	+e11	+e10	+e12	+e13	+e12					7.9	8.0	9.6	8.8	25.0	24.7	180	181
	6	+e	+e	+e13	+e	+e	+e					7.8	8.0	11.4	8.7	25.0	24.1	183	181
	7	+e3	+e8	+e	+e8	+e8	+e4						e)		8.5		24.7		190
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		9.5		25.0		226	
	1	+	+	+	+	+	+					8.0	8.0	8.2	7.9	24.0	24.5	219	221
	2	-	+*	+	+	+	+					8.0	8.5	8.5	8.4	25.0	24.7	230	209
	3	↓	↓	e*	e	e	e					8.0	8.1	8.9	8.4	25.0	24.3	213	211
	4	↓	↓	+e3	+e3	+e	T3					7.8	8.3	8.5	9.0	24.2	24.5	213	212
	5	↓	↓	T3	+e1	+e2						8.0	8.0	8.6	8.6	25.0	24.7	214	208
	6	↓	↓		+e	+e						8.0	8.0	9.3	8.6	25.0	24.1	219	209
	7	↓	↓	↓	+e2	+e5	↓						8.1		8.6		24.7		212
25%	0	+	+	+	+	+	+	+	+	+	+					25.0		254	
	1	+	+	+	+	+	+									24.0	24.5	255	254
	2	+	+	+*	+	+	+									25.0	24.7	262	245
	3	e*	e*	e	+	e*	+									25.0	24.3	250	247
	4	+1*	+e2	+e1*	+e	+e1	T									24.2	24.5	255	251
	5	+*	+e2	+e2	+e3*	+e										25.0	24.7	248	245
	6	+e	+e	+e	+e	+e										25.0	24.1	255	240
	7	+e3	+e4	+e4	+e4	+e5	↓									24.7			243

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTBY EADOR YBK 1-24-12



1.0µm PF ②

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Great Lakes Environmental Center

Reviewed by: Curt

Date: 2/29/12

TEST MATERIAL: EEP 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 21589-00

ANIMALS/CONC: 10 / CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2-16-12/14:20

YOUNG FROM: SPW/81 <24 hrs

TECHNICIANS: DAY: 0 14:20 YBK 1 9:30 YBK 2 13:00 YBK 3 9:10 MS 4 13:40 YBK 5 13:50 YBK 6 9:00 YBK 7 12:15 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.						
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old					
50%	0	+	+	+	+	+	+	+	+	+	+													
	1	+	+	+	+	+	+										25.0		324					
	2	+*	+*	te*	te*	+*	+*										24.0	24.5	328	323				
	3	+*	te*	te*	te*	+*	+*										25.0	24.7	342	309				
	4	+*	+1*	+*	te*	+*	te*										25.0	24.3	318	316				
	5	+*	+	+	+1	te	+2*										24.2	24.5	329	316				
	6	+*	te	te	te	te	te										25.0	24.7	325	316				
	7	te1	te2	te3	te3	te3	te2										25.0	24.1	324	313				
100%	0	+	+	+	+	+	+	+	+	+	+													
	1	+	+	+	+	+	+										25.0		475					
	2	+*	+*	+*	+*	+*	+*										7.8	7.9	9.6	8.1	24.0	24.5	470	460
	3	+	te*	+	+	+	+										7.8	8.1	9.7	8.5	25.0	24.7	491	448
	4	+	te*	+	te	te	te										7.8	8.0	10.7	8.5	25.0	24.3	465	447
	5	+*	te	te	te	te	te										7.7	8.1	9.4	9.1	24.2	24.5	475	471
	6	te	te	te3	te1	te2	te1										7.8	7.9	9.9	8.8	25.0	24.7	472	463
	7	te1	te3	te3	te4	te5	te3										7.6	7.9	12.0	8.7	25.0	24.1	472	462
	0	+	+	+	+	+	+	+	+	+	+													
	1																							
	2																							
	3																							
	4																							
	5																							
	6																							
	7																							

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ENTRY ERROR YBK 1-24-12 ENTRY ERROR YBK 2-20-12



10mm PF → C18-SPE (3)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): _____

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 2/10/12 1400

YOUNG FROM: 2-8-12 1 < 24 hrs

TECHNICIANS: DAY: 05-1400 1 10:10 YBK 2 12:30 AM 3 09:30 ACS 4 13:30 ACS 5 14:45 YBK 6 8:35 AM 7 11:25 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
DMW	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+													
	2	+	+	+	+	+	+													
	3	+	+	+	+	+	+													
	4	+	+	+	+	+	+													
	5	+	+	+	+	+	+													
	6	+	+	+	+	+	+													
	7	+	+	+	+	+	+													
12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+													
	2	+	+	+	+	+	+													
	3	+	+	+	+	+	+													
	4	+	+	+	+	+	+													
	5	+	+	+	+	+	+													
	6	+	+	+	+	+	+													
	7	+	+	+	+	+	+													
25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+													
	2	+	+	+	+	+	+													
	3	+	+	+	+	+	+													
	4	+	+	+	+	+	+													
	5	+	+	+	+	+	+													
	6	+	+	+	+	+	+													
	7	+	+	+	+	+	+													

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTRY ERROR YBK 1-24-12, ACS 2/20/12



10MM PF → C18 - SPE ③
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Amr

Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2/16/12 1400 YOUNG FROM: 2-8-12 ^{SR} 1 < 24 hrs

TECHNICIANS: DAY: Qsu 1400 1 10:10 YBK 2 12:30 GR 3 8:30 CPO 4 13:30 ACS 5 14:45 YBK 6 8:35 ACS 7 11:25 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+										25.0		324	
	2	+	+	+	+	+	+										24.0	24.4	324	316
	3	+	+	+	+	+	+										24.1	25.1	317	307
	4	+	+	+	+	+	+										25.0	24.3	318	317
	5	+	+	+	+	+	+										24.6	24.6	309	317
	6	+	+	+	+	+	+										24.3	24.6	322	295
	7	+	+	+	+	+	+										25.7	24.4	321	302
100%	0	+	+	+	+	+	+	+	+	+	+									308
	1	+	+	+	+	+	+										25.0		451	
	2	+	+	+	+	+	+										24.0	24.4	461	449
	3	+	+	+	+	+	+										24.1	25.1	453	423
	4	+	+	+	+	+	+										25.0	24.3	458	453
	5	+	+	+	+	+	+										24.2	24.6	462	442
	6	+	+	+	+	+	+										24.3	24.6	471	441
	7	+	+	+	+	+	+										25.5	24.4	467	440
	0	+	+	+	+	+	+	+	+	+	+									442
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTRY ERROR YBK 1-24-12, ACS 2/23/12



AERATION (4) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EE 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2/16/12 1400 YOUNG FROM: 2-8-12 1 < 24 hrs

TECHNICIANS: DAY: 0 1430gs 10: 20 YBK 2 1230 cr 3 1000 4 1405 5 1545 cr 6 850 7 1155 AC

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
DMW	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+													
	2	R	R	R	R	R	R													
	3	te	te	te	te	te	te													
	4	te 6	te 6	te 7*	te 7	te 6	te 4													
	5	R12	R12	te 7	te 11	R12	R10													
	6	te 11*	te	te	te 6	te	te													
	7	te	te 7	te 13	te	te 12	te 13													
12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+													
	2	R	+	+	+	+	+													
	3	+	+	+	+	+	te													
	4	te*	te 2	te 1*	te 3*	te 3	te 4*													
	5	te 3		te 4	te 4		te 7													
	6	te		te	te		te*													
	7	te 5*		te 6	te 5*		te 4													
25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+													
	2	+	+	+	+	+	+													
	3	T	+	+	+	+	+	*	+	*										
	4		te 2	te 1	te 2*	te 1*	te 2*													
	5		te	te	te 3	te	te													
	6		te 2	te 2	te	te*	te 2													
	7		te 4*	te 4*	te 4	te	te 2													

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTRY ERROR YBK 1-24-12, ACS 2/23/12 ENTRY ERROR YBK 2-23-12



Great Lakes Environmental Center

OPERATION (4) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Com Tr

Date: 2/29/12

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

DILUTION WATER: DMW

STARTING DATE/TIME: 2/16/12 1400

YOUNG FROM: 5-8-12 1 < 24 hrs

LIGHT INTENSITY (LUX): 500-1000

PHOTOPERIOD (L:D): 16:8

TECHNICIANS: DAY: 0 1430 gsw 1 110:20 VIK 2 1230 CRT 3 1000 4 LRD 4 5 1405 ACC 6 1545 CRT 7 850 ALS 8 1155 ALS

TEMPERATURE (°C): 25 ± 1°C

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+													
	1	+	+	+	+	+	+	+	+	+										
	2	+	+	+	+	+	+													
	3	+ ^e	+ ^e	+ ^e	+ ^e	+ ^e	+ ^e													
	4	k2*	k3*	k1*	k3	k1*	k2*													
	5	k	k	k	k	k	k													
	6	k1*	k2*	k1	k2	k2	k2													
	7	k2*	k1*	k3	k3	k3*	k													
100%	0	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+										
	2	+	+	+	+	+	+													
	3	+ ^e	+ ^e	+ ^e	+ ^e	+ ^e	+ ^e													
	4	k2*	k1	k*	k2	k1	k													
	5	k1	k	k3	k4	k	k1													
	6	k	k2	k	k	k1	k2													
	7	k3*	k4*	k4	k4	k3*	k1													
	0	+	+	+	+	+	+	+	+	+										
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTRY ERROR VIK 1-24-12
 CRD 2-19-12



EDTA (5) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cum Tu

Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2/16/12 1420

YOUNG FROM: SR 2/8 1 < 24 hrs

TECHNICIANS: DAY: 0 1420 ACS 1 11:00 YBK 2 1330 Km 3 945 ACS 4 1330 Km 5 1530 cs 6 930 ACS 7 1208 km

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		9.5		25.0		188	
	1	+	+	+	+	+	+					8.0	7.8	8.1	7.8	24.0	24.6	179	194
	2	e	e	e	e	e	e					8.0	8.1	8.0	8.2	25.1	25.0	184	@
	3	e	e*	e	e	e*	e					8.1	8.3	8.3	8.6	24.5	24.6	181	186
	4	e6	e5	e4	e6	e2	e5					7.9	8.1	8.0	8.7	25.1	24.7	187	197
	5	e11	e7	e10	e3	e9	e4					7.8	7.9	8.0	8.7	24.8	24.6	180	183
	6	e	e	e	e*	e*	e					8.0	7.8	8.7	8.6	24.6	24.4	176	175
	7	e13	e15	e13	e17	e8	e12						8.0		8.2		24.7		170
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		9.7		25.0		225	
	1	+	+	+	+	+	+					7.9	7.9	8.2	7.8	24.0	24.6	216	220
	2	+	+	+	e	+	e					8.0	8.1	8.3	8.1	25.1	25.0	225	@
	3	e	e*	e*	e	e	e					8.0	8.4	8.5	8.9	24.5	24.6	213	214
	4	e	+	e	e4	e1*	e2*					7.9	8.2	7.8	8.9	25.1	24.7	226	224
	5	e1*	↓	e	e3	↓	e1*					7.8	8.0	8.1	8.6	24.8	24.6	218	212
	6	e4	↓	e4	e*	↓	e					8.0	7.9	8.8	8.6	24.6	24.4	214	212
	7	e8	↓	e3	e7	↓	e6						8.0		8.1		24.7		212
25%	0	+	+	+	+	+	+	+	+	+	+					25.0		261	
	1	+	+	+	+	+	+									24.0	24.6	259	260
	2	+	+	+	e	+	e									25.1	25.0	270	@
	3	e	+	e*	e	e*	e									24.5	24.6	255	255
	4	e1	e	+	e1	↓	e									25.1	24.7	264	264
	5	e	e3*	e*	e	↓	e5									24.8	24.6	257	245
	6	e2	e	e*	e	↓	e									24.7	24.4	254	255
	7	e1	e4	e2	e3	↓	e3										24.7		235

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ENTRY ERROR YBK1-24-12 @conductivities not taken due to tech error: ACS 2/19/12



EDTA (5)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Th Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEP 9462
 PROJECT NO.: 2158900
 STARTING DATE/TIME: 2/16/12 1420

TEST SPECIES: Ceriodaphnia dubia
 ANIMALS/CONC: 10 /CHAMBER: 1
 YOUNG FROM: SK 2/8 1 < 24 hrs

DILUTION WATER: DMW
 LIGHT INTENSITY (LUX): 500-1000

PHOTOPERIOD (L:D): 16:8
 TEMPERATURE (°C): 25 ± 1°C

TECHNICIANS: DAY: 0 1420 ARS 1 11:00 VBR 2 1330 Km 3 945 ACS 4 1330 Km 5 1530 gsm 6 930 ARS 7 1200 gsm

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.							
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old						
50%	0	+	+	+	+	+	+	+	+	+	+														
	1	+*	+*	+	+	+	+										25.0		338						
	2	+	+	+	+	+	+										24.0	24.6	322	326					
	3	+	+	+*	+	+	+										25.1	25.0	350	⊕					
	4	+	+	+	+	+	+										24.6	24.6	323	321					
	5	+	+	+	+	+	+										25.1	24.7	336	331					
	6	+2*	+	+3*	+	+	+										24.8	24.6	330	306					
	7	+	+	+	+	+	+										24.2	24.4	321	320					
100%	0	+	+	+	+	+	+	+	+	+	+														
	1	+*	+*	+	+	+	+										7.3		11.8		25.0		482		
	2	+	+	+	+	+	+										7.6	7.9	10.2	8.9	24.0	24.6	474	474	
	3	+*	+	+	+	+	+										7.6	8.0	9.8	8.4	25.1	25.0	495	⊕	
	4	+	+	+	+	+	+										7.4	8.1	11.2	8.4	24.5	24.6	476	461	
	5	+	+	+	+	+	+										7.5	8.2	9.3	9.4	25.1	24.7	487	492	
	6	+	+	+	+	+	+										7.6	8.0	9.0	9.1	24.8	24.6	483	450	
	7	+	+	+	+	+	+										7.5	7.9	10.6	8.7	24.6	24.4	470	470	
	0	+	+	+	+	+	+	+	+	+	+														
	1																								
	2																								
	3																								
	4																								
	5																								
	6																								
	7																								

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 • ENTRY ERROR 1-24-12
 ⊕ conductivity not taken due to tech error ARS 2/19/12



Sodium Thiosulfate Addition (B)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: and

Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 2/16/12 ACS 1430

YOUNG FROM: SR 2/9 1 < 24 hrs

TECHNICIANS: DAY: 0 1430 ACS

1 11:20 VBR 2 1400 km 3 1000 ACS 4 1400 km 5 1000 ACS 6 950 ACS 7 1200 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.8		9.6		25.0		215	
	1	+	+	+	+	+	+					7.7	8.1	9.7	8.0	24.0	24.6	204	215
	2	te	te	te	te	te	te					7.8	8.1	9.0	8.1	25.1	25.0	215	@
	3	te	te	te	te ⁴	te	te ⁵					7.7	8.4	10.0	8.8	24.5	24.6	206	208
	4	te ¹	te	te ⁴	te	te ⁶	te					7.8	8.2	8.6	8.7	24.9	24.7	221	220
	5	te ⁹	te ⁵	te ¹¹	te ²	te ¹¹	te ^d					7.9	8.1	8.4	8.8	24.8	24.6	217	208
	6	te	te	te	te ¹⁵	te	te ^{10^d}					7.5	8.0	10.9	8.4	25.5	24.4	218	215
	7	te ¹³	te ¹³	te ¹⁵	te	te ^{11^d}	te						7.9		8.3		24.7		206
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		9.8		25.0		223	
	1	+	+	+	+	+	+					8.0	8.1	8.3	8.0	24.0	24.6	227	233
	2	te	+	+	te	+	te					8.0	8.1	8.6	8.1	25.1	25.0	242	@
	3	te	te	te*	te	te*	te ⁴					8.1	8.3	8.6	8.9	24.5	24.6	224	233
	4	te ⁴	te	↓	te ⁴	te*	te					7.9	8.3	8.1	8.8	24.9	24.7	238	238
	5	te ⁷	te ⁶	↓	te ⁶	↓	te*					7.9	8.1	8.1	8.8	24.8	24.6	231	225
	6	te	te	↓	te*	↓	te					7.9	8.0	9.9	8.3	24.4	24.4	221	228
	7	te ^{6^d}	te ⁸	↓	te ^{5^d*}	↓	te ⁷						8.0		8.5		24.7		223
25%	0	+	+	+	+	+	+	+	+	+	+					25.0		279	
	1	+	+	+	+	+	+									24.0	24.6	270	268
	2	+	+	te	te	+	te									25.1	25.0	288	@
	3	te	+	te	te	te	te									24.5	24.6	268	272
	4	te ³	te	te ³	te ^d	te	te									24.4	24.7	284	281
	5	te	te ⁴	te	te ⁴	te	te ⁴									24.8	24.6	279	270
	6	te ²	te*	te ³	te	te ^{3*}	te*									24.5	24.4	267	268
	7	te ⁴	te ^{2*}	te ³	te ²	te ^{3*}	te ^{2*}									24.7			266

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 2MAY ERROR VBR 1-24-12, 2/22/12 ACS @ conductivities not taken due to tech error ACS 2/19/12



Sodium Thiosulfate Addition (6)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: AmT

Date: 2/29/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9462

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2158900

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 2/16/12 1430

YOUNG FROM: SR 2/8 1 < 24 hrs

TECHNICIANS: DAY: 0 1930 ACS

1 11:20 YBK 2 1400 km 3 1000 ACS

4 1400 km 5 1600 km

6 950 ACS

7 1200 km

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+*	+*	+	+	+	+											25.0		373	
	2	+	+	+	te	+	te											24.0	24.6	364 360	
	3	te*	te	te	te	te	te											25.1	25.0	378 @	
	4	T	te	te	te	te	te											24.5	24.6	357 355	
	5	T	te	te	te	te	te											24.9	24.7	382 366	
	6	T	te ³	te ^{2*}	te	te ²	te*											24.8	24.5	370 351	
	7	V	te ^{4*}	te ^{4*}	te ^{3*}	te ³	te ^{3*}											24.3	24.4	361 361	
100%	0	+	+	+	+	+	+	+	+	+	+									24.7 352	
	1	+*	+	+	+	+	+											7.8		11.9 260 562	
	2	+	+	+	te	te	te											7.8 7.9	10.3 8.2	24.0 24.6 537 537	
	3	te	te	te	te	te*	te											7.8 8.0	9.8 8.5	25.1 25.0 561 @	
	4	te	te	te	te	te	te											7.7 8.2	10.5 9.0	24.5 24.6 535 532	
	5	te	te	te	te	te	te											7.7 8.1	9.7 9.2	24.9 24.7 567 556	
	6	te	te ^d	te*	te	te*	te											7.7 7.9	9.0 8.9	24.8 24.6 558 530	
	7	te	te ^d	te ^{d*}	te	te*	te											7.6 7.9	10.2 8.5	24.7 24.4 532 544	
	0	+	+	+	+	+	+	+	+	+	+									8.4 24.7 544	
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

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APPENDIX B

DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST NO.	C. dubia	FHM
2/10	96	1.39	1.59
3/10	97	0.81	1.80
5/10	98	1.37	2.09
6/10	99	1.48	2.13
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
AVERAGE		1.26	2.11
STD. DEV.		0.24	0.34
RANGE: LOW		0.78	1.42
RANGE: HIGH		1.74	2.80
Coefficient of variation		0.19	0.16
Date of last test		12/13-19/11	12/13-20/11
MSD of most recent test		7.16	0.0417
PMSD of most recent test		22.6	13.8
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

From EPA's Wet Interlaboratory Variability Study

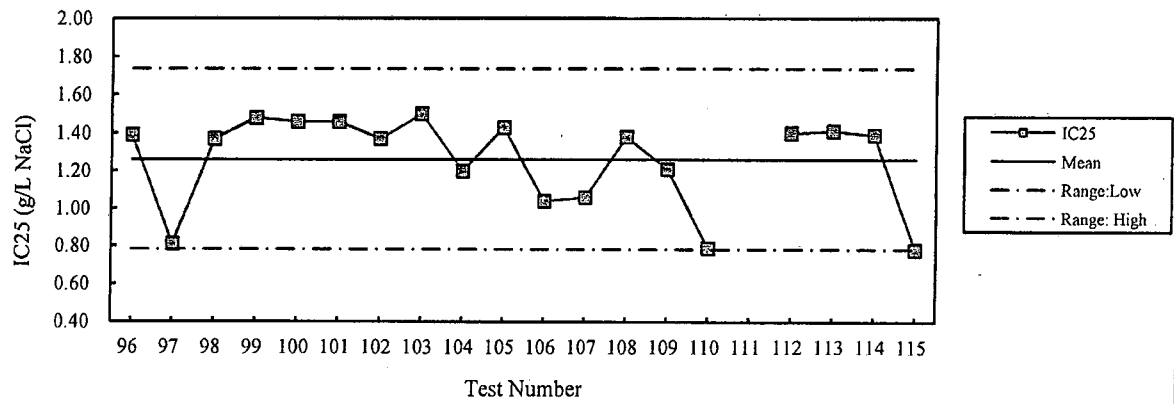
IC25 Coefficient of Variation						
National Percentiles ²						
Test Species	GLEC ¹	10th	25th	50th	75th	90th
C. dubia	0.19	0.08	0.17	0.27	0.45	0.62
P. promelas	0.16	0.12	0.21	0.26	0.38	0.45

¹ Based on cumulative GLEC data from the most recent 20 tests.

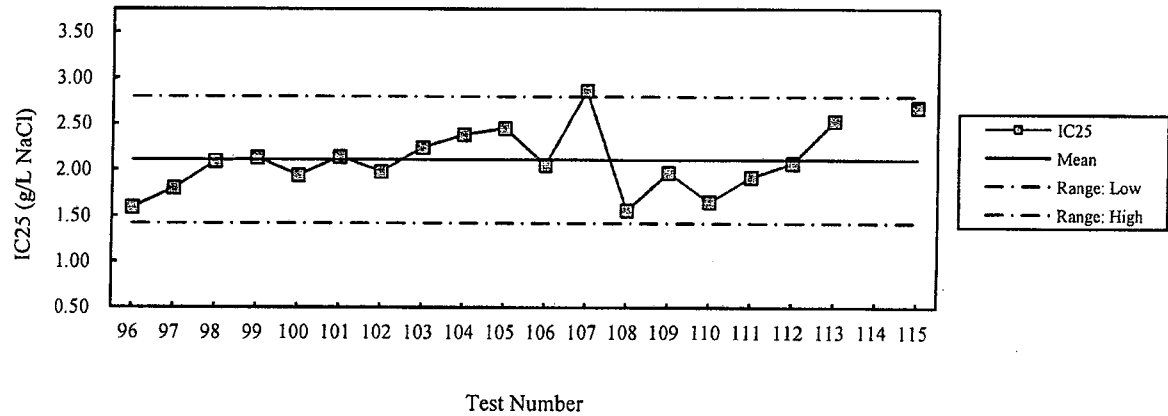
² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

Chronic Reference Toxicant IC25
Ceriodaphnia dubia



Chronic Reference Toxicant IC25
Pimephales promelas





Great
Lakes
Environmental
Center

April 3, 2012

Applied
Environmental
Sciences
www.glec-online.com

Roland McDaniel, Project Manager
GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

Traverse City
Operations
739 Hastings St.
Traverse City
MI 49686

231 941-2230
231 941-2240 fax

Columbus
Operations
1295 King Ave
Columbus
OH 43212

614 487-1040
614 487-1920 fax

**RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED
MARCH 14, 2012 FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL
DORADO, ARKANSAS**

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected March 14, 2012. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tari
Laboratory Coordinator

PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED MARCH 14, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

March 2012



Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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APPENDIX B. DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS B-1

INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled March 14, 2012 (Sample ID: EEC 9501) to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

Ceriodaphnia dubia Static Renewal Chronic Toxicity Tests

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for seven days under static

renewal conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity 10-20 $\mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test (6 days).

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUc) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUc} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1). The baseline toxicity test was not toxic to *C. dubia* survival and exhibited 100 percent survival in the 100 percent test concentration, resulting in a 6 day LC_{50} value of >100 percent effluent. The outfall 001 sample was chronically toxic to *C. dubia* reproduction and exhibited an IC_{25} value of 15.3 percent effluent or 6.5 TUc (Table 1).

1.0 µm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a Gelman A/E glass fiber filter (1.0 µm).

After filtration treatment, 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent was removed as the *C. dubia* exhibited an IC₂₅ of >100 percent or <1.0 TUC. Therefore, filtration was very effective as a treatment and the toxicity to *C. dubia* reproduction in the EDCC effluent sample appears to be associated with a filterable toxicant.

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample **after 1.0 µm filtration treatment** was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

Prior to the C-18 treatment, filtration removed 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent. Therefore, it could not be determined whether C-18 was an effective treatment in removing toxicity to *C. dubia* reproduction in the EDCC effluent sample.

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

Aeration of the outfall 001 sample did not remove toxicity when compared to the concurrent baseline and demonstrated an IC₂₅ of 15.0 percent or 6.7 TUC (Table 1). As a result, the chronic toxicity to *C. dubia* in the EDCC effluent is not related to a volatile, easily oxidizable or aeratable toxicant.

Cation Chelation with EDTA - Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test IC₂₅ of 15.3 percent, the addition of EDTA (25 mg/L) removed 21.5 percent of the sample toxicity and exhibited an IC₂₅ of 19.8 percent or 5.1 TUC (Table 1). However, this difference is not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Therefore, EDTA treatment did not demonstrate a clear reduction in toxicity.

Sodium Thiosulfate Treatment – Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

Sodium thiosulfate treatment removed 76.2 percent of the toxicity to *C. dubia* reproduction when compared to the baseline test suggesting toxicity was removed (Table 1). However, this difference is not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Also, closer inspection of the data demonstrates that the removal of toxicity after sodium thiosulfate treatment was somewhat of an aberration as the sodium thiosulfate control mean number of young per adult of 30.2 was relatively low compared to the baseline control mean for reproduction of 38.0 young per adult. Because of the relatively low mean number of young per adult (30.2) in the sodium thiosulfate treated control water, the IC25 for Test 6 was higher, which exaggerated the differences between the sodium thiosulfate and baseline toxicity tests. Therefore, sodium thiosulfate treatment did not demonstrate a clear reduction in toxicity.

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected March 14, 2012 did demonstrate removal of chronic toxicity, but the reduction of toxicity to *C. dubia* reproduction was only demonstrated by one of the five TIE treatments performed. Three of the treatments, aeration, EDTA and sodium thiosulfate were not effective in removing meaningful toxicity from the outfall 001 effluent sample. Thus, the effluent toxicity does not appear to be associated with easily oxidizable or aeratable compounds, chelatable metals or thiosulfate reducible compounds or oxidants.

The 1.0 µm filtration treatment removed 100 percent of the toxicity present in the EDCC outfall 001 effluent sample. Therefore, the chronic toxicity to *C. dubia* present in the ECCC outfall 001 effluent sample appears to be associated with a filterable toxicant(s). For a summary of all test results, see Table 1.

The effectiveness of C-18 in removing chronic toxicity to *C. dubia* in the EDCC effluent sample is unknown due to the fact that filtration removed 100 percent of the sample toxicity prior to C-18 treatment.

Summary of the chronic toxicity characterization of the EDCC outfall 001 sample collected March 14, 2012 (Sample ID: EEC 9501):

- **The toxicant (s) was filterable.**
- The toxicant(s) was not a chelatable metal.
- The toxicant(s) was not a volatile, easily oxidizable or aeratable compound.
- The toxicant(s) was not a thiosulfate reducible compound or oxidant.

Table 1. Summary of Chronic TIE Test results

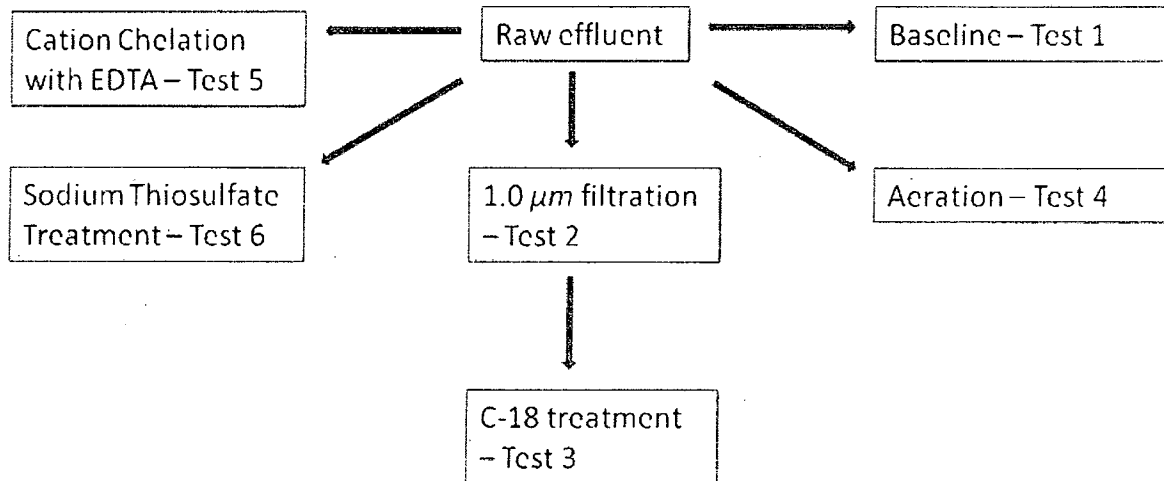
EDCC Outfall 001 final effluent (Collection date: 3/14/12) <i>C. dubia</i> TIE Test Dates 3/15-21/12	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	100	100	83	83	100	>100	0.0	--
1.0 µm Filtration – Test 2	100	100	100	100	100	>100	0.0	NA
C18-SPE Treatment – Test 3	100	100	100	100	100	>100	0.0	NA
Aeration – Test 4	100	100	83	100	100	>100	0.0	NA
Cation Chelation with EDTA – Test 5	100	100	83	100	100	>100	0.0	NA
Sodium Thiosulfate Treatment – Test 6	100	100	100	100	100	>100	0.0	NA
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	38.0	29.3	24.8	18.2	17.2	15.3	6.5	--
1.0 µm Filtration – Test 2	32.2	30.7	37.3	42.0	42.5	>100	<1.0	100
C18-SPE Treatment – Test 3	39.5	33.2	37.7	39.7	44.0	>100	<1.0	Unknown
Aeration – Test 4	38.2	29.7	23.8	22.5	19.8	15.0	6.7	0
Cation Chelation with EDTA – Test 5	28.0 ^b	25.3	17.5	16.5	6.8	19.8	5.1	21.5
Sodium Thiosulfate Treatment – Test 6	30.2	28.2	29.3	25.2	16.8	64.2	1.6	76.2

NA – Not applicable or Not available

^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC₂₅ value for the most recent *C. dubia* reference toxicant test was 1.46 g/L of sodium chloride which was within the acceptance range of 0.78 to 1.74 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

- U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.
- U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.
- U.S. EPA, 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED MARCH 14, 2012

Test Dates: 3/15-21/12

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	100%	100%	83%	83%	100%
1.0 µm filtration (Test 2)	100%	100%	100%	100%	100%
C-18 SPE treatment (Test 3)	100%	100%	100%	100%	100%
Aeration (Test 4)	100%	100%	83%	100%	100%
EDTA 25 mg/l (Test 5)	100%	100%	83%	100%	100%
NaThio (50 mg/l) (Test 6)	100%	100%	100%	100%	100%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	38.0	29.3	24.8	18.2	17.2	15.3	6.5	--
1.0 µm filtration (Test 2)	32.2	30.7	37.3	42.0	42.5	>100	<1.0	100.0%
C-18 SPE treatment (Test 3)	39.5	33.2	37.7	39.7	44.0	>100	<1.0	100.0%
Aeration (Test 4)	38.2	29.7	23.8	22.5	19.8	15.0	6.7	0.0%
EDTA (25 mg/l) (Test 5)	28.0	25.3	17.5	16.5	6.8	19.8	5.1	21.5%
NaThio (50 mg/l) (Test 6)	30.2	28.2	29.3	25.2	16.8	64.2	1.6	76.2%

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to C.dubia reproduction in DMW

Baseline (Test 1)
El Dorado Chemical outfall 001 (EEC 9501)
(Tested 3/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	5	5	6
DEAD	0	0	1	1	0
% SURV	100.00%	100.00%	83.33%	83.33%	100.00%

Scito River Water 2° Control	6
	0
	100.00%

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	40	28	25	9	19
2	45	24	24	17	15
3	38	32	25	22	18
4	29	31	24	19	15
5	37	32	28	21	18
6	39	29	23	21	18
N	6	5	6	6	6
MEAN	38.0	29.3	24.8	18.2	17.2
SD	5.2153619	3.07679487	1.7224014	4.833908	1.7224014
CV	13.724637	10.4890734	6.9358447	26.608668	10.033406
Total Young	228	176	149	109	103

Scito River 2° Control	44
	38
	46
	37
	45
	43
	5
	42.2
	3.7638633
	8.9261579
	253

1.0 µm filtration (Test 2)
El Dorado Chemical outfall 001 (EEC 9501)
(Tested 3/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	37	35	31	42	48
2	40	35	41	45	49
3	29	37	42	46	34
4	18	32	30	34	36
5	38	43	37	40	46
6	31	2	43	45	42
N	6	6	6	6	6
MEAN	32.2	30.7	37.3	42.0	42.5
SD	8.1342896	14.5143607	5.6803756	4.5166359	6.3166447
CV	25.287947	47.3294371	15.215292	10.753895	14.862693
Total Young	193	184	224	252	255

C-18 SPE treatment (Test 3)
El Dorado Chemical outfall 001 (EEC 9501)
(Tested 3/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	42	32	32	42	52
2	33	29	34	32	41
3	36	41	38	44	45
4	41	28	40	42	39
5	45	30	42	37	37
6	40	39	40	41	50
N	6	6	6	6	6
MEAN	39.5	33.2	37.7	39.7	44.0
SD	4.3243497	5.49241902	3.8815804	4.4121046	6.0663004
CV	10.947721	16.5600573	10.305081	11.122953	13.787046
Total Young	237	199	226	238	264

Aeration (Test 4)
El Dorado Chemical outfall 001 (EEC 9501)
(Tested 3/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	5	6	6
DEAD	0	0	1	0	0
% SURV	100.00%	100.00%	83.33%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	42	35	28	21	19
2	34	26	24	20	20
3	36	29	22	23	20
4	36	32	24	24	17
5	43	25	19	22	19
6	38	31	26	25	24
N	6	6	6	6	6
MEAN	38.2	29.7	23.8	22.5	19.8
SD	3.6009258	3.77712413	3.1251667	1.8708287	2.3166067
CV	9.4347401	12.7318791	13.112587	8.3147942	11.68037
Total Young	229	178	143	135	119

EDTA 25 mg/l (Test 5)
El Dorado Chemical outfall 001 (EEC 9501)
(Tested 3/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	5	6	6
DEAD	0	0	1	0	0
% SURV	100.00%	100.00%	83.33%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	33	28	22	18	6
2	35	20	16	17	8
3	12	24	16	10	11
4	35	28	14	18	4
5	26	26	18	16	6
6	27	26	19	20	6
N	6	6	6	6	6
MEAN	28.0	25.3	17.5	16.5	6.8
SD	8.7635609	3.01109061	2.8106939	3.4496377	2.4013885
CV	31.298432	11.885884	16.061108	20.906895	35.142271
Total					
Young	168	152	105	99	41

NaThio (50 mg/l) (Test 6)
El Dorado Chemical outfall 001 (EEC 9501)
(Tested 3/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	24	29	28	20	18
2	37	26	30	25	21
3	26	33	30	24	15
4	29	36	29	28	15
5	38	22	27	26	20
6	27	23	32	28	12
N	6	6	6	6	6
MEAN	30.2	28.2	29.3	25.2	16.8
SD	5.9132619	5.56477014	1.7511901	2.9944393	3.4302575
CV	19.601973	19.7565804	5.9699662	11.898434	20.377767
Total					
Young	181	169	176	151	101

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	40	28	25	9	19
Response 2	45	24	24	17	15
Response 3	38	32	25	22	18
Response 4	29	31	24	19	15
Response 5	37	32	28	21	18
Response 6	39	29	23	21	18

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9501 Test #1
 Test Start Date: 3/15/12 Test Ending Date: 3/21/12
 Test Species: C.dubia
 Test Duration: 6 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	38.000	5.215	38.000
2	6	12.500	29.333	3.077	29.333
3	6	25.000	24.833	1.722	24.833
4	6	50.000	18.167	4.834	18.167
5	6	100.000	17.167	1.722	17.167

The Linear Interpolation Estimate: 14.8148 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 15.2748 Standard Deviation: 4.2664
 Original Confidence Limits: Lower: 9.5806 Upper: 23.2422
 Resampling time in Seconds: 0.00 Random_Seed: 4621455

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	42	35	28	21	19
Response 2	34	26	24	20	20
Response 3	36	29	22	23	20
Response 4	36	32	24	24	17
Response 5	43	25	19	22	19
Response 6	38	31	26	25	24

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9501 Test #4
 Test Start Date: 3/15/12 Test Ending Date: 3/21/12
 Test Species: C.dubia
 Test Duration: 6 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	38.167	3.601	38.167
2	6	12.500	29.667	3.777	29.667
3	6	25.000	23.833	3.125	23.833
4	6	50.000	22.500	1.871	22.500
5	6	100.000	19.833	2.317	19.833

The Linear Interpolation Estimate: 14.7321 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 14.9718 Standard Deviation: 2.7113
 Original Confidence Limits: Lower: 10.6884 Upper: 20.2586
 Resampling time in Seconds: 0.00 Random Seed: 69256927

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	33	28	22	18	6
Response 2	35	20	16	17	8
Response 3	12	24	16	10	11
Response 4	35	28	14	18	4
Response 5	26	26	18	16	6
Response 6	27	26	19	20	6

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9501 Test #5

Test Start Date: 3/15/12 Test Ending Date: 3/21/12

Test Species: C.dubia

Test Duration: 6 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	28.000	8.764	28.000
2	6	12.500	25.333	3.011	25.333
3	6	25.000	17.500	2.811	17.500
4	6	50.000	16.500	3.450	16.500
5	6	100.000	6.833	2.401	6.833

The Linear Interpolation Estimate: 19.4149 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 19.8484 Standard Deviation: 5.5473

Original Confidence Limits: Lower: 12.7467 Upper: 33.5417

Resampling time in Seconds: 0.00 Random_Seed: 1624522031

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	24	29	28	20	18
Response 2	37	26	30	25	21
Response 3	26	33	30	24	15
Response 4	29	36	29	28	15
Response 5	38	22	27	26	20
Response 6	27	23	32	28	12

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9501 Test #6

Test Start Date: 3/15/12 Test Ending Date: 3/21/12

Test Species: C.dubia

Test Duration: 6 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	30.167	5.913	30.167
2	6	12.500	28.167	5.565	28.750
3	6	25.000	29.333	1.751	28.750
4	6	50.000	25.167	2.994	25.167
5	6	100.000	16.833	3.430	16.833

The Linear Interpolation Estimate: 65.2500 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 64.1689 Standard Deviation: 11.5092

Original Confidence Limits: Lower: 38.8393 Upper: 82.9082

Resampling time in seconds: 0.00 Random_Seed: -132782209



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: OBMC - El Dorado Chemical

PROJECT NUMBER: 2159-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>3/15/12</u>	INITIALS	EEC			
EEC NUMBER		<u>9501</u>			
OUTFALL/DESCRIPTION		<u>outfall 001</u>			
DISSOLVED OXYGEN (mg/L)	<u>ONT</u>	<u>11.2</u>			
TEMPERATURE (°C)	<u>ONT</u>	<u>4.0</u>			
pH	<u>ONT</u>	<u>7.3</u>			
CONDUCTIVITY (µmhos/cm)	<u>ONT</u>	<u>286</u>			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>3/15/12</u>	INITIALS				
EEC NUMBER		<u>9501</u>			
OUTFALL/DESCRIPTION		<u>outfall 001</u>			
DISSOLVED OXYGEN (mg/L)	<u>ONT</u>	<u>9.0</u>			
TEMPERATURE (°C)	<u>ONT</u>	<u>24.0</u>			
pH	<u>ONT</u>	<u>7.6</u>			
CONDUCTIVITY (µmhos/cm)	<u>ONT</u>	<u>44</u>			
HARDNESS (mg/L CaCO ₂)	<u>ACS</u>	<u>1.2 x 40 = 48</u>			
ALKALINITY (mg/L CaCO ₂)	<u>ACS</u>	<u>1.5 x 40 = 60</u>			
TOTAL CHLORINE (mg/L)*					
TOTAL AMMONIA (mg/L)*					

*Check with project manager to see if necessary



Great Lakes Environmental Center
 1295 KING AVE.
 COLUMBUS, OH 43212
 PHONE: (614) 487-1040
 FAX: (614) 487-1920

Two Important Notes for Whole Effluent Toxicity Testing:

- There is a maximum hold time for all samples of 36 hours (Hold time begins when sample is taken off the sampler)
- Samples must be received at 4°C ± 2°C

CHAIN OF CUSTODY FORM

(TO BE COMPLETED ONSITE AND SUBMITTED WITH SAMPLES)

FACILITY: El Dorado Chemical Co.
 LOCATION: El Dorado, AR
 CONTACT PERSON: Larken Pennington
 PHONE: 870-312-1752

COLLECTOR: Larken Pennington
 DATE: 3/14/12
 WITNESS: Grant Pecker
 DATE: 3-14-12

EEC# (lab only)	SAMPLE ID	SAMPLE SOURCE (Eff/Upstr.)	TYPE (grab or composite)	SAMPLE START DATE	SAMPLE START TIME (24-hr notation)	SAMPLE END DATE	SAMPLE END TIME (24-hr notation)	VOLUME COLLECTED	SAMPLE CONTAINER	SAMPLE COLLECTOR	OTHER COMMENTS
EEC9501	001		grab	3/14/12	8:30am			4 cubitainers	4 cubitainers	Larken Pennington	

ANALYSIS REQUIRED: Please fill in completely

Species: *Ceriodaphnia dubia* *Pimephales promelas* (fathead minnows)
 Test Type: Acute: 24-hour Acute: 24-hour 48-hour Other - please specify: _____
 48-hour 48-hour 96-hour: with 48-hour renewal
 Chronic (7-day) Chronic (7-day) without 48-hour renewal
 Dilutions: Screen (100% only) Definitive (5 sample concentrations): List test concentrations: _____
 Dilution Water: Receiving Water Lab water Other - please specify: _____

NAME OF STREAM SAMPLED: Outfall 001

TRANSFER OF SAMPLES:

(FIRST SIGNATURE IS SAMPLER, LAST SIGNATURE IS AUTHORIZED LABORATORY REPRESENTATIVE)

SHIPPER	RECEIVER	DATE	TIME
1. <u>Larken Pennington</u>	<u>Kim McDonald</u>	<u>3-15-12</u>	<u>1015</u>
2.			

For Lab Use Only:
 Ice remaining in cooler upon receipt
 Temperature of samples when received:
4.0°C

FOR SATURDAY DELIVERY??? MARK PACKAGE AS SUCH AND CALL GLEC ON FRIDAY WITH TRACKING NUMBER

Parental Blockage Map for *C. dubia*

Date: 3-15-12

Time Neonates Pulled: 0900

Source Board: SR10MW 3-7-12

Initials: Kam

Estimated Age Range of *C. dubia* neonates: 8h

Name and Project # neonates used for:

Test ① & ②

	1	2	3	4	5	6	7	8	9	10
6										
5										
4					R3			R6		
3										R2
2								R1		
1						R4			R5	

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 3-15-12

Time Neonates Pulled: 0900

Source Board: JHDMW 37-12

Initials: Jm

Estimated Age Range of *C. dubia* neonates: 8-h

Name and Project # neonates used for:

Test (3) & (4)

	1	2	3	4	5	6	7	8	9	10
6										
5	R6									
4				R5		R1				
3					R3					
2					R2					
1							R4			

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 3-15-12

Time Neonates Pulled: 0900

Source Board: 52/AMW 37-12

Initials: km

Estimated Age Range of *C. dubia* neonates: 8-h

Name and Project # neonates used for:

Test (5) & (6)

	1	2	3	4	5	6	7	8	9	10
6										
5			R1				R2			
4									R3	
3										
2		R6					R4			
1		R5								

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.



① Baseline
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Th Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: EBL 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12 / 1500

YOUNG FROM: SR/DMW 3/17/12 24 hrs

TECHNICIANS: DAY: 0 1500 AC 1 1030 Kom 2 850 ACS 3 800 ACS 4 1100 Kom 5 1130 Kom 6 1700 Kom 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	8.0		8.3		24.1		173	
	1	+	+	+	+	+	+					8.0	7.9	8.6	8.8	25.1	25.4	179	182
	2	e	e	e	e	e	e					8.0	7.9	8.1	8.3	24.7	24.7	157	175
	3	e	e	e	e	e	e					7.9	8.0	7.5	6.9	25.0	25.0	173	183
	4	e4	e8	e7	e5	e7	e6					7.9	7.9	8.3	8.0	24.5	25.3	172	183
	5	e16	e19	e13	e6	e10	e13					8.0	8.0	8.2	8.6	24.6	25.1	174	175
	6	e20	e18	e18	e18	e20	e20						8.0		8.5		25.0		170
	7																		
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.4		24.1		201	
	1	+	+	+	+	+	+					8.0	8.0	9.0	8.8	25.1	25.4	205	210
	2	e	e	e	e	e	e					8.0	7.9	8.4	8.4	24.0	24.7	190	205
	3	e	e	e	e	e	e					8.0	8.0	7.8	7.3	24.4	25.0	200	215
	4	e6	e5	e7	e6	e6	e6					7.9	8.0	8.7	8.0	24.5	25.3	201	212
	5	e15	e10	e13	e12	e11	e10					8.0	8.1	8.2	8.8	24.6	25.1	208	202
	6	e23	e9	e16	e20	e13	e15						8.1		8.3		25.0		201
	7																		
25%	0	+	+	+	+	+	+	+	+	+	+					24.3		238	
	1	+	+	+	+	+	+									25.1	25.4	239	244
	2	e	e	e	e	e	e									24.0	24.7	228	233
	3	e	e	e	e	e	e									24.7	25.0	231	247
	4	e6	e6	e6	e6	e6	e5									24.5	25.3	233	243
	5	e10	e9	e10	e8	e11	e8									24.6	25.1	241	232
	6	e9	e9	e9	e10	e11	e10										25.0		219
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 @data entered on wrong line 3-20-12 km



① Baseline

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Amr

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: ELC 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 3/15/12 / 1500

YOUNG FROM: SR/DMW 3/17/12 24 hrs

TECHNICIANS: DAY: 0 1500 ACS 1 1030 km 2 950 ACS 3 800 ACS 4 1100 km 5 1130 km 6 1700 ACS 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+										24.7		292	
	2	e	+	+	+	+	+										25.1	25.4	297	298
	3	e	e	e	e	e	e										24.0	24.7	281	288
	4	e5	e5	e5	e4	e4	e4										24.4	25.0	287	300
	5	T4	e6	e8	e6	e7	e8										24.5	25.3	290	302
	6	↓	e6	e9	e9	e10	e9										24.6	25.1	292	288
	7	↓																25.0		280
100%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+					7.6		9.0		24.0		411		
	2	+	+	+	+	+	+					7.6	8.0	10.3	8.8	25.1	25.4	419	405	
	3	e	e	e	e	e	e					7.6	8.0	10.4	8.5	24.1	24.7	387	401	
	4	e4	e1	e4	e3	e4	e5					7.5	8.0	10.2	7.3	25.2	25.0	405	415	
	5	e6	e6	e7	e2	e6	e5					7.6	8.1	11.4	8.3	24.5	25.3	406	413	
	6	e9	e8	e7	e10	e8	e8					7.6	8.2	11.0	9.1	24.6	25.1	398	395	
	7												8.1		8.3		25.0		389	
	0	+	+	+	+	+	+	+	+	+	+									
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



① Baseline

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: EL 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1

STARTING DATE/TIME: 3/15/12 / 1500

YOUNG FROM: SR/DMW 3/17/12 24 hrs

TECHNICIANS: DAY: 0 1500 ACS 1 1030 KDM 2 850 ACS 3 800 ACS 4 1100 KDM 5

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
			0	+	+	+	+	+	+	+	+	+	+	7.7		10.9		25.0	
Scioto River	1	+	+	+	+	+	+					7.8	8.0	10.8	8.5	25.1	25.4	355	344
	2	e	+	e	+	+	+					7.8	8.1	9.4	8.3	25.1	24.7	341	346
	3	e	e	e	e	e	e					7.9	8.1	9.5	7.2	24.5	25.0	336	354
	4	e6	e6	e7	e6	e7	e7					7.9	8.1	10.0	8.1	24.5	25.3	355	364
	5	e15	e16	e19	e15	e16	e17					7.9	8.3	10.1	8.7	24.6	25.1	348	362
	6	e23	e16	e20	e16	e22	e19						8.2		8.4		25.0		360
	7																		
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
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DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: C. Th

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: ENC 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12/1525

YOUNG FROM: SR/DMW 3/7/12 ≤ 24 hrs

TECHNICIANS: DAY: 0 1525 ACS 1 1100 Kcm 2 900 ACS 3 820 ACS 4 1130 Kcm 5 200 Kcm 6 1200 Kcm 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	8.0		7.7		24.6		175	
	1	+	+	+	+	+	+					8.0	8.0	8.7	8.8	25.0	25.2	177	177
	2	e	e	+	+	e	+					8.1	8.1	9.3	8.4	24.2	24.7	174	178
	3	e	e	e	+	e	e					8.0	8.0	9.3	7.0	25.1	25.0	180	182
	4	e4 ^d	e7	e5	e1 ^d	e7	e8					7.9	8.1	10.6	8.1	25.4	25.3	177	191
	5	e14	e18	e10	e8	e15	e13					8.0	8.3	10.7	9.0	24.7	25.1	180	183
	6	e19	e15	e14	e9	e16	e10						7.7		7.8		25.0		175
	7																		
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.0		8.2		24.4		206	
	1	+	+	+	+	+	+					8.0	8.0	8.8	8.8	25.0	25.2	204	209
	2	e	e*	+	e	+	+					8.1	8.0	9.0	8.4	24.3	24.7	202	210
	3	e	e*	e	e	e	+					8.1	8.0	8.9	7.4	24.4	25.0	206	212
	4	e8	e5	e7	e4	e7	+					8.0	8.1	9.0	8.1	25.4	25.3	205	222
	5	e8	e12	e12	e6	e15	e					8.0	8.2	8.8	9.0	24.7	25.1	210	205
	6	e19	e18	e18	e22	e1	e2						7.8		7.8		25.0		209
	7																		
25%	0	+	+	+	+	+	+	+	+	+	+					24.6		237	
	1	+	+	+	+	+	+									25.0	25.2	236	238
	2	e	e	+	+	+	+									24.8	24.7	234	239
	3	e	e	e	e	e	e									24.6	25.0	232	243
	4	e6	e5	e7	e2	e6	e8									25.4	25.3	241	249
	5	e8	e18	e18	e10	e11	e17									24.7	25.1	242	235
	6	e19	e18	e17	e18	e20	e18										25.0		230
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



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DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Con T

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12/1525

YOUNG FROM: SR/DMW 3/7/12 24 hrs

TECHNICIANS: DAY: 0 1525 ACS 1 1100 Kom 2 900 ACS 3 820 AC 4 1130 Kom 5 1200 Kom 6 1200 Kom 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+									24.0		292			
	2	e	e	+	+	+	+									25.0	25.2	298	299		
	3	e	e	e	e	e	e									24.2	24.7	290	294		
	4	e8	e5	e6	e4	e4	e6									24.4	25.0	289	303		
	5	e14	e17	e18	e7	e15	e16									25.4	25.3	294	306		
	6	e20	e23	e22	e23	e21	e23									24.7	25.1	294	289		
	7															25.0			289		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					7.6		9.0		24.4		412			
	2	e	e	+	+	e	+					7.6	7.9	10.0	8.8	25.0	25.2	410	409		
	3	e	e	e	e	e	e					7.7	8.0	10.3	8.3	25.1	24.7	402	403		
	4	e7	e9	e5	e8	e7	e8					7.6	7.9	10.3	7.3	24.7	25.0	405	419		
	5	e15	e18	e13	e10	e17	e14					7.6	8.0	11.1	8.0	25.4	25.3	411	420		
	6	e26	e22	e16	e19	e22	e20					7.7	8.1	11.1	8.8	24.7	25.1	408	400		
	7												7.8		7.8		25.0		398		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



③ C-18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cmth

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: ERC 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: -1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 3/15/12/1515

YOUNG FROM: SR DMW 3/11/12 24 hrs

Initiation: QC - Arc 3/15/12

TECHNICIANS: DAY: 0 1515 CRT 1 1130 KOM 2 930 ACS 3 900 ACS 4 1245 Kms 5 1230 CRT 6 1230 KOM 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
DMW	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+					7.8		7.0		24.7		182		
	2	te	+	L	te	te	te					8.0	7.9	7.6	8.8	25.0	25.1	179	187	
	3	te	te	te	te	te	te					7.8	7.8	8.2	8.4	24.9	25.0	176	178	
	4	te6	te3	te5	te6	te7	te6					7.6	8.0	8.4	6.9	24.7	25.2	170	185	
	5	te14	te13	te15	te14	te16	te14					7.7	7.8	9.0	8.0	24.9	25.3	176	188	
	6	te22	te17	te16	te21	te22	te20					7.7	7.9	10.3	8.4	24.4	25.0	171	179	
	7												7.9		7.8		25.0		185	
12.5%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+					7.9		7.7		24.4		215		
	2	te	+	te	te	te	te					8.0	8.0	8.3	8.7	25.0	25.1	209	213	
	3	te	te	te	te	te3	te					7.9	7.9	8.4	8.3	24.4	25.0	204	208	
	4	te7	te6	te8	te5	te	te8					7.9	8.0	8.1	7.3	24.3	25.2	200	213	
	5	te14	te7	te17	te5	te16	te12					7.9	7.9	8.0	7.9	24.9	25.3	207	217	
	6	te11	te16	te16	te18	te11	te19					7.9	8.0	9.4	8.5	24.4	25.0	203	210	
	7												7.9		7.7		25.0		211	
25%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+									24.1		245		
	2	te	+	+	te	te	te									25.0	25.1	234	240	
	3	te	te	te*	te	te9*	te									24.2	25.0	234	236	
	4	te7	te6	te7	te7	te	te7									24.8	25.2	234	245	
	5	te7	te11	te11	te12	te15	te13									24.9	25.3	235	248	
	6	te18	te17	te20	te21	te19	te20									24.4	25.0	236	233	
	7																25.0		237	

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



③ C-18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cmth

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: ATL 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 206/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12/1515

YOUNG FROM: SR/DMW 3/7/12 24 hrs

TECHNICIANS: DAY: 0 1515 cmr

1 1130 Kdm

2 930

3 900

4 1245 Kdm

5 1230 cmr

6 1230 Kdm

7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+					21.4		301			
	2	e	+	+	+	e	+									25.0	25.1	293	296		
	3	e	e	e	e	e	e									24.7	25.0	291	293		
	4	e3	e4	e7	e6	e	e6									25.8	25.2	292	292		
	5	e18	e8	e15	e15	e9	e13									24.9	25.3	292	304		
	6	e21	e20	e22	e21	e20	e22									24.4	25.0	291	295		
	7																25.0		293		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	7.7		7.9		24.4		414			
	2	e	+	e	+	e	e					7.8	8.0	8.8	8.9	25.0	25.1	417	407		
	3	e	e	e	e	e	e					7.7	7.9	9.4	9.4	25.5	25.0	413	404		
	4	e9	e6	e8	e6	e	e7					7.7	7.9	9.1	9.1	24.7	25.2	403	413		
	5	e21	e14	e17	e13	e13	e19					7.6	7.9	9.6	8.0	24.9	25.3	409	420		
	6	e22	e21	e20	e20	e18	e24					7.8	8.0	9.8	8.6	24.4	25.0	401	401		
	7												7.9		7.8		25.0		407		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



④ Aeration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: C. Smith

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: BSC 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12/0725

YOUNG FROM: SR/DMW 3/7/12 24 hrs

Institution QC - ACS 3/15/12

TECHNICIANS: DAY: 0/15/5 CR 1 1200 Kom 2 290 ACS 3 915 ACS 4 1300 Kom 5 1300 CR 6 1700 CR 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
DMW	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	7.8		8.3		24.4		180			
	2	e	+	e	e	e	e					8.0	8.1	9.4	8.8	25.0	25.1	183	184		
	3	e	e	e	e	e	e					7.9	8.0	9.5	8.3	24.8	25.0	180	182		
	4	te6	te3	te6	te4	te	te6					7.9	8.6	7.7	7.2	24.8	25.2	175	181		
	5	te14	te15	te11	te15	te14	te14					7.9	8.0	9.3	8.0	24.9	25.3	181	193		
	6	te22	te16	te19	te17	te22	te18														
	7												8.0		8.3		24.2		175		
12.5%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	7.8		8.4		24.4		210			
	2	e	+	+	e	e	e					8.1	8.1	9.0	8.8	25.0	25.1	209	214		
	3	e	e	e	e	e	e					8.0	8.0	9.0	8.3	24.1	25.0	204	209		
	4	te6	te5	te6	te4	te4	te5					8.6	8.0	8.4	8.4	24.4	25.2	205	211		
	5	te14	te11	te12	te14	te11	te12					8.0	8.0	8.1	8.1	24.9	25.3	211	220		
	6	te15	te10	te11	te14	te10	te14					7.9	8.1	10.1	8.9	24.4	25.0	204	202		
	7												8.0		8.3		24.2		200		
25%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+					24.4		245			
	2	e	+	+	+	e	e									25.0	25.1	238	240		
	3	e	e	e	e	e	e									24.4	25.0	235	237		
	4	te6	te6	te6	te5	te7	te5									24.2	25.2	233	241		
	5	te10	te9	te8	te9	te12	te9									24.9	25.3	238	246		
	6	te12	te9	te8	te10		te12									24.4	25.0	237	235		
	7																24.2		221		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



④ Aeration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 3/26/12

Lakes Environmental Center

TEST MATERIAL: 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 206/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 3/15/12/1515

YOUNG FROM: SR/DMW 3/17/12 24 hrs

TECHNICIANS: DAY: 0 1515/12

1 1200 Kom 2 940 ACS 3 915 ACS 4 300 Kom 5 1300 cm 6 1700 cm 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+*	+	+									24.4		301			
	2	e	+	+	+	e	e									25.0	25.1	295	295		
	3	e	e	e	e	e	e									24.3	25.0	291	293		
	4	e4	e4	e6	e4	e4	e5									24.7	25.2	287	298		
	5	e8	e7	e7	e8	e7	e9									24.9	25.3	292	311		
	6	e9	e9	e10	e12	e11	e11									24.4	25.0	294	285		
	7																24.2		298		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					7.7		9.0		24.4		412			
	2	+	+	+	+	e	e					8.2	8.0	10.7	8.9	25.0	25.1	415	417		
	3	e	e	e	e	e	e					7.8	8.0	9.7	8.4	24.8	25.0	406	402		
	4	e2	e4	e2	e3	e4	e5					7.8	8.0	9.7	7.4	24.9	25.2	404	412		
	5	e8	e6	e8	e	e7	e8					7.8	7.9	9.6	8.1	24.9	25.3	408	430		
	6	e9	e10	e10	e14	e8	e11					7.8	8.1	9.8	9.0	24.4	25.0	409	412		
	7												8.0		8.3		24.2		401		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



⑤ EDTA

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: EDC 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12/1600 YOUNG FROM: SR 12000 3/11/12 ≤ 24 hrs

TECHNICIANS: DAY: 0 1600 Gm 1 1200 Gm 2 1030 AKS 3 945 AKS 4 1430 CAD 5 1330 Km 6 1700 Cms 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.8		8.4		24.3		175	
	1	+	+	+	+	+	+					7.9	8.1	8.5	8.8	24.8	25.0	174	189
	2	+	+	+	+	+	+					7.9	7.8	8.1	8.3	24.6	24.8	178	178
	3	+	+	+	+	+	+					7.8	7.9	7.5	7.1	25.4	25.1	177	179
	4	te7	te6	te4	te2	te6	te6					7.9	7.9	7.7	8.2	24.8	25.3	183	192
	5	te13	te15	te1	te19	te12	te12					7.9	8.1	8.2	8.6	25.3	25.2	179	180
	6	te13	te14	te8	te14	te8	te9						8.1		8.3		25.0		175
	7																		
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		8.4		24.1		207	
	1	+	+	+	+	+	+					7.9	8.1	8.8	9.0	24.8	25.0	201	219
	2	+	+	+	+	+	+					7.9	7.9	8.1	8.3	24.7	24.8	205	208
	3	+	+	+	+	+	+					7.9	8.0	7.8	7.9	25.1	25.1	207	214
	4	te7	te4	te6	te7	te6	te6					7.9	8.0	7.9	8.2	24.7	25.3	210	220
	5	te12	te9	te10	te13	te11	te10					7.9	8.2	8.4	8.6	25.3	25.2	210	207
	6	te9	te7	te8	te8	te9	te10						8.1		8.3		25.0		206
	7																		
25%	0	+	+	+	+	+	+	+	+	+	+					24.3		236	
	1	+	+	+	+	+	+									24.8	25.0	231	246
	2	+	+	+	+	+	+									24.7	24.8	236	234
	3	+	+	+	+	+	+									25.1	25.1	238	240
	4	te6	te4	te3	te6	te5	te4									24.9	25.3	241	254
	5	te8	te6	te6	te8	te9	te8									25.3	25.2	242	233
	6	te8	te6	te7		te4	te7										25.0		219
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



EDTA

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cruta

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 3/15/12/1600 YOUNG FROM: SR/DMW 3/7/12 24 hrs

TECHNICIANS: DAY: 0 1600CNT 1 1200CUT 2 1030 3 MS 3945 4 MS 5 1430 CAP 6 1330/10M 7 1700CR

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+									24.8		271			
	2	+	+	+	+	+	+									24.8	25.0	287	302		
	3	e	e	e	e	e	e									24.5	24.8	292	289		
	4	te5	te5	te4	te4	te5	te5									24.6	25.1	291	302		
	5	te7	te6	te6	te7	te6	te7									25.3	25.3	302	313		
	6	te6	te6	te	te7	te5	te8									25.3	25.2	298	287		
	7															25.0			267		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					7.2		8.7		24.5		410			
	2	+	+	+	+	+	+					7.4	8.6	9.6	9.2	24.8	25.0	403	411		
	3	e	e	e	e	e	e					7.6	7.9	9.6	8.3	24.5	24.8	407	403		
	4	te2	te2	te3	te	te2	te2					7.5	7.9	9.3	7.4	24.6	25.1	404	411		
	5	te4	te4	te4	te4	te3	te4					7.6	8.0	9.8	8.2	25.3	25.3	419	430		
	6	te	te	te	te	te	te					7.6	8.1	9.9	8.8	25.3	25.2	413	376		
	7												8.1		8.3		25.0		410		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

TEST MATERIAL: ELC 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 206/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 3/15/12/1600

YOUNG FROM: SR/DMW 3/17/12 24 hrs

TECHNICIANS: DAY: 0 1600 1 1230 2 1040 ACS 3 955 ACS 4 1530 5 CRD 6 1415 7 Kom 8 1700

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		8.4		24.2		205	
	1	+	+	+	+	+	+					7.9	8.1	9.1	7.9	25.0	25.0	202	207
	2	+	+	+	+	e	+					7.9	7.8	9.4	9.3	25.2	24.8	203	203
	3	e	e	e	e	e	e					7.9	7.8	9.3	7.3	24.6	25.1	204	209
	4	te7	te8	te6	te7	te8	te5					7.8	7.9	8.9	8.1	25.4	25.3	212	216
	5	te13	te15	te14	te9	te16	te10					7.8	8.1	10.0	8.9	25.3	25.2	207	203
	6	te4	te14	te6	te13	te14	te12						8.1		8.5		25.0		201
	7																		
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.9		8.4		24.6		214	
	1	+	+	+	+	+	+					8.0	8.1	8.7	8.8	25.0	25.0	221	222
	2	+	+	+	+	e	+					8.0	7.9	9.2	8.4	24.5	24.8	216	219
	3	e	e	e	e	e	e					8.0	7.9	7.6	7.4	25.3	25.1	218	223
	4	te7	te6	te6	te5	te6	te4					8.0	8.0	8.1	8.1	25.3	25.3	224	229
	5	te10	te9	te13	te14	te6	te6					8.0	8.2	8.8	8.9	25.3	25.2	225	213
	6	te12	te11	te14	te17	te10	te13						8.2		8.6		25.0		215
	7																		
25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+									24.2		255	
	2	+	+	+	+	+	+									25.0	25.0	248	263
	3	e	e	e	e	e	e									24.6	24.8	252	254
	4	te3	te7	te8	te7	te7	te6									25.5	25.1	255	262
	5	te12	te10	te10	te11	te10	te9									25.4	25.3	262	272
	6	te13	te13	te12	te11	te10	te17									25.3	25.2	262	250
	7																25.0		245

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



© NaThio

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Th

Date: 3/26/12

Great Lakes Environmental Center

TEST MATERIAL: SPZ 9501

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 106/CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 3/15/12/1600

YOUNG FROM: SR/DMW 3/17/12 524 hrs

TECHNICIANS: DAY: 0 1600 CMT 1 1230 CMT 2 1040 ACS 3 955 ACS 4 1530 CRD 5 1415 KOM 6 1700 CMT 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+									24.9		330			
	2	+*	+	+	+	+	+									24.6	25.0	322	317		
	3	e	e	e	e	e	e									24.6	24.8	327	320		
	4	te3	te6	te5	te6	te7	te6									25.5	25.1	328	334		
	5	te7	te8	te6	te9	te8	te8									25.9	25.3	342	360		
	6	te10	te11	te13	te13	te11	te14									25.3	25.2	336	315		
	7																25.0		319		
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					7.6		8.7		24.4		474			
	2	+*	+	+	+	+	+					7.7	8.1	9.6	8.9	24.9	25.0	466	466		
	3	te3	te	te	te	te	te					7.9	7.9	10.7	8.4	24.6	24.8	474	467		
	4	te5	te4	te5	te2	te5	te4					7.7	7.8	9.6	7.3	25.1	25.1	479	489		
	5	te6	te8	te6	te7	te8	te6					7.9	8.0	9.6	8.2	25.8	25.3	495	515		
	6	te7	te9	te4	te6	te7	te2					7.7	8.1	10.0	8.9	25.3	25.2	489	465		
	7												8.1		8.8		25.0		439		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 entry error ACS 3/17/12 CRD 3/14/12

APPENDIX B
DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST NO.	C. dubia	FHM
3/10	97	0.81	1.80
5/10	98	1.37	2.09
6/10	99	1.48	2.13
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
3/12	116	1.46	2.42
AVERAGE		1.26	2.15
STD. DEV.		0.24	0.33
RANGE: LOW		0.78	1.49
RANGE: HIGH		1.74	2.81
Coefficient of variation		0.19	0.15
Date of last test		3/1-8/12	3/20-27/12
MSD of most recent test ¹		5.47	0.0537
PMSD of most recent test		15.2	20.7
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

From EPA's Wet Interlaboratory Variability Study

IC25 Coefficient of Variation						
National Percentiles ²						
Test Species	GLEC ¹	10th	25th	50th	75th	90th
C. dubia	0.19	0.08	0.17	0.27	0.45	0.62
P. promelas	0.15	0.12	0.21	0.26	0.38	0.45

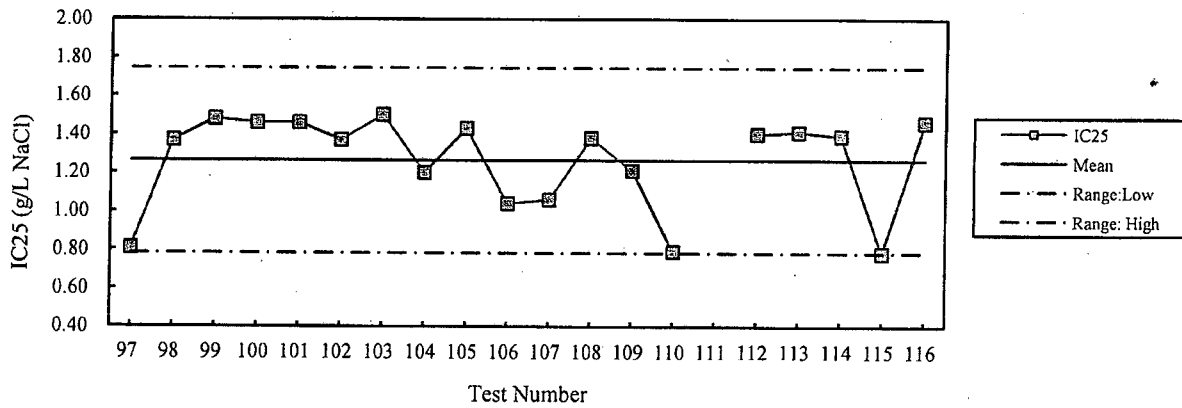
¹ Based on cumulative GLEC data from the most recent 20 tests.

² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

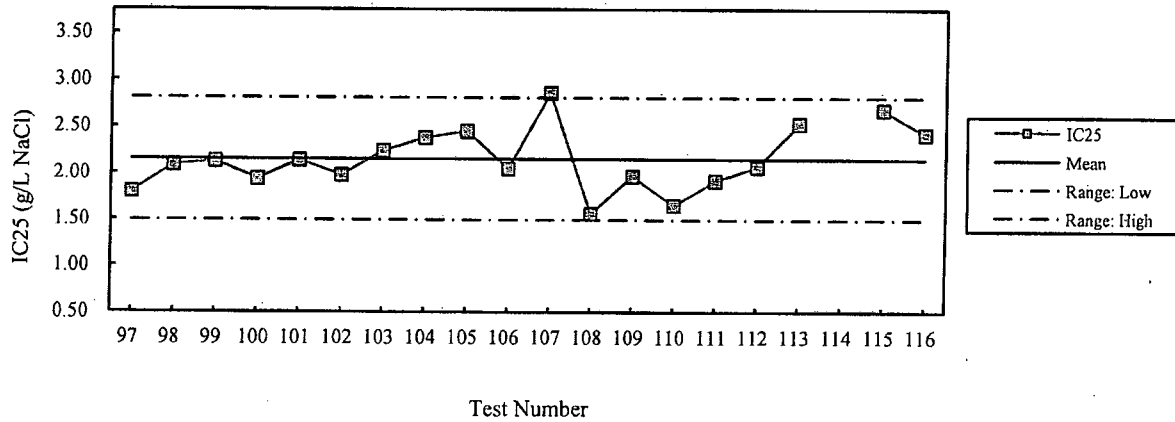
Chronic Reference Toxicant IC25

Ceriodaphnia dubia



Chronic Reference Toxicant IC25

Pimephales promelas





**Great
Lakes
Environmental
Center**

Applied
Environmental
Sciences
www.glec-online.com

April 30, 2012

Roland McDaniel, Project Manager
GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

**RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED
APRIL 11, 2012 FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL
DORADO, ARKANSAS**

Traverse City
Operations
739 Hastings St.
Traverse City
MI 49686

231 941-2230
231 941-2240 fax

Columbus
Operations
1295 King Ave
Columbus
OH 43212

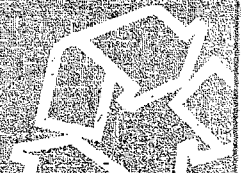
614 487-1040
614 487-1920 fax

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected April 11, 2012. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tarr
Laboratory Coordinator



PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED APRIL 11, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

April 2012



Great Lakes Environmental Center

Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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APPENDIX B. DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS B-1

INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled April 11, 2012 (Sample ID: EEC 9527) to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

Ceriodaphnia dubia Static Renewal Chronic Toxicity Tests

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for seven days under static

renewal conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity 10-20 $\mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test (7 days).

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUc) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUc} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1).

The baseline toxicity test was toxic to *C. dubia* survival and exhibited 50 percent survival in the 100 percent test concentration. However, it was not possible to calculate a reliable LC_{50} value due to the lack of a dose response (test concentrations lower than the 100 percent test concentration exhibited *C. dubia* survival that was lower than 50 percent) exhibited in the baseline test concentrations and all of the subsequent effluent

characteristic tests.

The outfall 001 sample was chronically toxic to *C. dubia* reproduction and exhibited an IC₂₅ value of 16.4 percent effluent or 6.1 TUc (Table 1).

1.0 µm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a Gelman A/E glass fiber filter (1.0 µm).

After filtration treatment, 66.8 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent was removed as the *C. dubia* exhibited an IC₂₅ of 49.4 percent or 2.0 TUc. Filtration was very effective as a treatment in removing some of the toxicity to *C. dubia* reproduction, but was not effective in removing all of the toxicity present. Therefore, a portion of the toxicity in the EDCC effluent sample appears to be associated with a filterable toxicant.

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample **after 1.0 µm filtration treatment** was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

Prior to the C-18 treatment, filtration removed 66.8 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent, while C-18 removed an additional four percent of the baseline toxicity (IC₂₅ of 56.1 or an additional 0.2 TUc's removed after C-18 treatment, post filtration treatment which removed 4.1 TUc's). The removal of four percent of the baseline toxicity is not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Therefore, C-18 treatment did not demonstrate a clear reduction in toxicity and the remaining baseline toxicity after filtration treatment does not appear to be related to non-polar organic compounds.

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

Aeration of the EDCC outfall 001 sample removed 29.6 percent of the toxicity when compared to the concurrent baseline and demonstrated an IC₂₅ of 23.3 percent or 4.3 TUc (Table 1). However, this difference was not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Therefore, aeration did not demonstrate a clear reduction in toxicity.

Cation Chelation with EDTA – Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test IC₂₅ of 16.4 percent, the addition of EDTA (25 mg/L) did not remove any sample toxicity and exhibited an IC₂₅ of 7.3 percent or 13.7 TUC (Table 1). Therefore, EDTA treatment did not demonstrate a reduction in toxicity and as a result the toxicity in the EDCC sample was not related to a metal.

Sodium Thiosulfate Treatment – Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

Sodium thiosulfate treatment of the outfall 001 sample did not remove toxicity when compared to the concurrent baseline and demonstrated an IC₂₅ of 15.1 percent or 6.6 TUC (Table 1). As a result, the toxicity to *C. dubia* reproduction present in the outfall 001 sample is not related to thiosulfate reducible compounds or oxidants.

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected April 11, 2012 did demonstrate removal of chronic toxicity, but the reduction of toxicity to *C. dubia* reproduction was only demonstrated by one of the five TIE treatments performed. Four of the treatments, C-18, aeration, EDTA and sodium thiosulfate were not effective in removing meaningful toxicity from the outfall 001 effluent sample (C-18 did not remove any meaningful baseline toxicity after filtration treatment). Thus, the effluent toxicity does not appear to be associated with non-polar organic compounds, easily oxidizable or aeratable compounds, chelatable metals or thiosulfate reducible compounds or oxidants.

The 1.0 µm filtration treatment removed 66.8 percent of the toxicity present in the EDCC outfall 001 effluent sample. Therefore, a portion the chronic toxicity to *C. dubia* present in the ECCC outfall 001 effluent sample appears to be associated with a filterable toxicant(s). For a summary of all test results, see Table 1.

Summary of the chronic toxicity characterization of the EDCC outfall 001 sample collected April 11, 2012 (Sample ID: EEC 9527):

- **A portion of the toxicity was associated with a filterable toxicant.**
- The remaining baseline toxicity (after filtration treatment) does not appear to be associated with non-polar organic compounds.
- The toxicant(s) did not appear to be associated with volatile, easily oxidizable or aeratable compound.
- The toxicant(s) was not a chelatable metal.
- The toxicant(s) was not a thiosulfate reducible compound or oxidant.

Table 1. Summary of Chronic TIE Test results

<i>C. dubia</i> TIE Test Dates: 4/13/12 – 4/20/12	Percent Survival						Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	7-day LC ₅₀	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	83	83	17	50	50	NA	27.0	25.0	12.2	7.8	7.3	16.4	6.1	--
1.0 µm Filtration – Test 2	83	83	33	67	33	NA	28.8	36.7	25.3	28.7	7.7	49.4	2.0	66.8%
C18-SPE Treatment – Test 3	83	33	50	33	0	NA	17.2 ^b	23.5	22.8	27.5	8.8	56.1	0.2 ^c	4% ^c
Aeration – Test 4	83	50	83	50	17	NA	18.2 ^b	22.5	20.2	13.7	3.7	23.3	4.3	29.6%
Cation Chelation with EDTA – Test 5	83	33	17	33	33	NA	34.3 ^d	18.2	11.7	10.3	4.2	7.3	13.7	0%
Sodium Thiosulfate Treatment – Test 6	100	50	33	83	50	NA	32.5	25.8	17.7	17.7	5.8	15.1	6.6	0%

NA – Not applicable or Not available

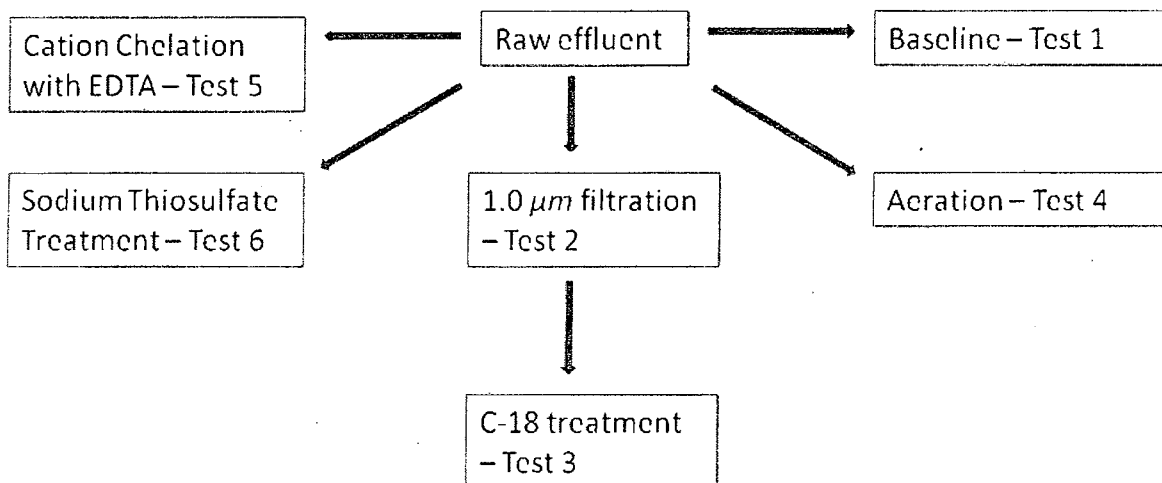
^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b DMW did not meet the control acceptability requirement of 60 percent or more of the female *C. dubia* having three broods, therefore the baseline control result (27.0 mean number of young per adult) was substituted for statistical analysis.

^c Additional baseline toxicity removed by C-18 treatment after filtration treatment.

^d Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC₂₅ value for the most recent *C. dubia* reference toxicant test was 1.46 g/L of sodium chloride which was within the acceptance range of 0.78 to 1.74 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED APRIL 11, 2012

Test Dates: 4/13-20/12

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	83%	83%	17%	50%	50%
1.0 µm filtration (Test 2)	83%	83%	33%	67%	33%
C-18 SPE treatment (Test 3)	83%	33%	50%	33%	0%
Aeration (Test 4)	83%	50%	83%	50%	17%
EDTA 25 mg/l (Test 5)	83%	33%	17%	33%	33%
NaThio (50 mg/l) (Test 6)	100%	50%	33%	83%	50%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	27.0	25.0	12.2	7.8	7.3	16.4	6.1	--
1.0 µm filtration (Test 2)	28.8	36.7	25.3	28.7	7.7	49.4	2.0	66.8%
C-18 SPE treatment (Test 3)	17.2*	23.5	22.8	27.5	8.8	56.1	1.8	4% ^b
Aeration (Test 4)	18.2*	22.5	20.2	13.7	3.7	23.3	4.3	29.6%
EDTA (25 mg/l) (Test 5) ^a	34.3	18.2	11.7	10.3	4.2	7.3	13.7	0.0%
NaThio (50 mg/l) (Test 6)	32.5	25.8	17.7	17.7	5.8	15.1	6.6	0.0%

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to C.dubia reproduction in DMW

b - additional toxicity removed by C-18 treatment after filtration treatment.

***DMW control did not meet control requirement for minimum number of females with 3 broods, therefore the baseline control was substituted for statistical analysis**

Baseline (Test 1)
Dorado Chemical outfall 001 (EEC 9527)
 (Tested 4/13-20/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	5	1	3	3
DEAD	1	1	5	3	3
% SURV	83.33%	83.33%	16.67%	50.00%	50.00%

Scito River Water 2° Control	6
	0
	100.00%

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	30	21	8	17	4
2	30	22	23	0	0
3	26	11	11	9	5
4	24	32	5	3	4
5	34	34	11	0	15
6	18	30	15	18	16
N	6	5	6	6	6
MEAN	27.0	25.0	12.2	7.8	7.3
SD	5.6213877	8.67179336	6.2742861	8.1833164	6.5625198
CV	20.819955	34.6871734	51.569475	104.46787	89.488907
Total Young	162	150	73	47	44

Scito River 2° Control	40
	39
	50
	44
	52
	40
	5
	44.2
	5.6005952
	12.680593
	265

1.0 µm filtration (Test 2)
Dorado Chemical outfall 001 (EEC 9527)
 (Tested 4/13-20/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	5	2	4	2
DEAD	1	1	4	2	4
% SURV	83.33%	83.33%	33.33%	66.67%	33.33%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	10	31	33	43	6
2	27	31	30	23	4
3	37	39	18	44	4
4	33	40	29	25	5
5	35	40	24	29	7
6	31	39	18	8	20
N	6	6	6	6	6
MEAN	28.8	36.7	25.3	28.7	7.7
SD	9.8471654	4.41210456	6.3770422	13.515423	6.1535897
CV	34.152019	12.0330124	25.172535	47.146825	80.264214
Total Young	173	220	152	172	46

C-18 SPE treatment (Test 3)
Dorado Chemical outfall 001 (EEC 9527)
(Tested 4/13-20/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	2	3	2	0
DEAD	1	4	3	4	6
% SURV	83.33%	33.33%	50.00%	33.33%	0.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	19	26	5	24	13
2	19	19	35	20	6
3	28	13	31	19	16
4	16	18	30	32	7
5	16	28	5	26	6
6	5	37	31	44	5
N	6	6	6	6	6
MEAN	17.166667	23.5	22.8	27.5	8.8
SD	7.4139508	8.59651092	13.92001	9.3327381	4.5350487
CV	43.188063	36.5808975	60.963546	33.937229	51.340174
Total Young	103	141	137	165	53

Aeration (Test 4)
Dorado Chemical outfall 001 (EEC 9527)
(Tested 4/13-20/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	3	5	3	1
DEAD	1	3	1	3	5
% SURV	83.33%	50.00%	83.33%	50.00%	16.67%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	27	15	19	20	0
2	15	11	4	4	15
3	25	31	20	18	0
4	20	32	22	8	4
5	22	31	27	23	0
6	0	15	29	9	3
N	6	6	6	6	6
MEAN	18.2	22.5	20.2	13.7	3.7
SD	9.826834	9.79285454	8.8411915	7.6594169	5.8195074
CV	54.092664	43.5237979	43.840619	56.044514	158.71384
Total Young	109	135	121	82	22

EDTA 25 mg/l (Test 5)
Dorado Chemical outfall 001 (EEC 9527)
(Tested 4/13-20/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	2	1	2	2
DEAD	1	4	5	4	4
% SURV	83.33%	33.33%	16.67%	33.33%	33.33%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	40	16	4	19	4
2	33	21	19	5	2
3	44	12	13	19	11
4	41	22	13	4	6
5	44	8	8	13	2
6	4	30	13	2	0
N	6	6	6	6	6
MEAN	34.3	18.2	11.7	10.3	4.2
SD	15.396969	7.85917723	5.1251016	7.6854842	3.920034
CV	44.845542	43.261526	43.929443	74.375653	94.080816
Total Young	206	109	70	62	25

NaThio (50 mg/l) (Test 6)
Dorado Chemical outfall 001 (EEC 9527)
(Tested 4/13-20/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	3	2	5	3
DEAD	0	3	4	1	3
% SURV	100.00%	50.00%	33.33%	83.33%	50.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	20	16	13	22	0
2	33	30	22	20	12
3	36	35	23	22	7
4	34	18	5	2	10
5	37	19	24	19	2
6	35	37	19	21	4
N	6	6	6	6	6
MEAN	32.5	25.8	17.7	17.7	5.8
SD	6.2849025	9.28260021	7.3665913	7.7631609	4.665476
CV	19.338162	35.932646	41.697686	43.94242	79.979589
Total Young	195	155	106	106	35

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	30	21	8	17	4
Response 2	30	22	23	0	0
Response 3	26	11	11	9	5
Response 4	24	32	5	3	4
Response 5	34	34	11	0	15
Response 6	18	30	15	18	16

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC ~~9501~~ Baseline (Test 1)
 Test Start Date: 4-13-12⁷⁵²⁷ Test Ending Date: 4-20-12
 Test Species: C. dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	27.000	5.621	27.000
2	6	12.500	25.000	8.672	25.000
3	6	25.000	12.167	6.274	12.167
4	6	50.000	7.833	8.183	7.833
5	6	100.000	7.333	6.563	7.333

The Linear Interpolation Estimate: 17.1266 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 16.4495 Standard Deviation: 2.6326

Original Confidence Limits: Lower: 9.3750 Upper: 20.0893

Resampling time in seconds: 0.00 Random_Seed: 237178875

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	10	31	33	43	6
Response 2	27	31	30	23	4
Response 3	37	39	18	44	4
Response 4	33	40	29	25	5
Response 5	35	40	24	29	7
Response 6	31	39	18	8	20

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9501 Filtration (Test 2)
 Test Start Date: 4-13-12 ⁹⁵⁰¹ Test Ending Date: 4-20-12
 Test Species: C. dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	28.833	9.847	32.750
2	6	12.500	36.667	4.412	32.750
3	6	25.000	25.333	6.377	27.000
4	6	50.000	28.667	13.515	27.000
5	6	100.000	7.667	6.154	7.667

The Linear Interpolation Estimate: 56.3039 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 49.4077 Standard Deviation: 14.3135
 Original Confidence Limits: Lower: 21.9234 Upper: 66.5472
 Resampling time in Seconds: 0.00 Random_Seed: 1149934907

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	30	26	5	24	13
Response 2	30	19	35	20	6
Response 3	26	13	31	19	16
Response 4	24	18	30	32	7
Response 5	34	28	5	26	6
Response 6	18	37	31	44	5

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 9527 C-18-SPE (Test #3)
 Test Start Date: 4/13/12 Test Ending Date: 4/20/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	27.000	5.621	27.000
2	6	12.500	23.500	8.597	24.611
3	6	25.000	22.833	13.920	24.611
4	6	50.000	27.500	9.333	24.611
5	6	100.000	8.833	4.535	8.833

The Linear Interpolation Estimate: 63.8204 Entered P value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 56.0621 Standard Deviation: 16.7466
 Original Confidence Limits: Lower: 11.0738 Upper: 71.0227
 Resampling time in seconds: 0.00 Random_Seed: -257783669

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	30	15	19	20	0
Response 2	30	11	4	4	15
Response 3	26	31	20	18	0
Response 4	24	32	22	8	4
Response 5	34	31	27	23	0
Response 6	18	15	29	9	3

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 9529 Aeration (Test #4)

Test Start Date: 4/13/12 Test Ending Date: 4/20/12

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	27.000	5.621	27.000
2	6	12.500	22.500	9.793	22.500
3	6	25.000	20.167	8.841	20.167
4	6	50.000	13.667	7.659	13.667
5	6	100.000	3.667	5.820	3.667

The Linear Interpolation Estimate: 24.5536 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 23.3434 Standard Deviation: 10.0149

Original Confidence Limits: Lower: 8.5744 Upper: 43.1641

Resampling time in Seconds: 0.00 Random_Seed: 505987547

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	40	16	4	19	4
Response 2	33	21	19	5	2
Response 3	44	12	13	19	11
Response 4	41	22	13	4	6
Response 5	44	8	8	13	2
Response 6	4	30	13	2	0

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC ~~9501~~ EDTA (Test 5)
 Test Start Date: 4-13-12 ^{7/527} Test Ending Date: 4-20-12
 Test Species: C. dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	34.333	15.397	34.333
2	6	12.500	18.167	7.859	18.167
3	6	25.000	11.667	5.125	11.667
4	6	50.000	10.333	7.685	10.333
5	6	100.000	4.167	3.920	4.167

The Linear Interpolation Estimate: 6.6366 Entered P value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 7.3290 Standard Deviation: 2.8542

Original Confidence Limits: Lower: 4.6266 Upper: 16.0473

Resampling time in seconds: 0.05 Random_Seed: -953410597

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	20	16	13	22	0
Response 2	33	30	22	20	12
Response 3	36	35	23	22	7
Response 4	34	18	5	2	10
Response 5	37	19	24	19	2
Response 6	35	37	19	21	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9501 NaThio (Test 6)
 Test Start Date: 4-13-12 ⁹⁵²⁷ Test Ending Date: 4-20-12
 Test Species: C. dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	32.500	6.285	32.500
2	6	12.500	25.833	9.283	25.833
3	6	25.000	17.667	7.367	17.667
4	6	50.000	17.667	7.763	17.667
5	6	100.000	5.833	4.665	5.833

The Linear Interpolation Estimate: 14.7321 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 15.0718 Standard Deviation: 4.7660

Original Confidence Limits: Lower: 7.7257 Upper: 23.0769

Resampling time in seconds: 0.06 Random_Seed: -213792469



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: GBMC EL DORADO CHEMICAL

PROJECT NUMBER: 2159-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>4-12-12</u>	INITIALS	<u>EEC 9527</u>			
EEC NUMBER					
OUTFALL/DESCRIPTION		<u>outfall 001</u>			
DISSOLVED OXYGEN (mg/L)	<u>Kom</u>	<u>9.6</u>			
TEMPERATURE (°C)	<u>Kom</u>	<u>3.0</u>			
pH	<u>Kom</u>	<u>6.8</u>			
CONDUCTIVITY (µmhos/cm)	<u>Kom</u>	<u>267</u>			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>4-13-12</u>	INITIALS	<u>EEC 9527</u>			
EEC NUMBER					
OUTFALL/DESCRIPTION		<u>outfall 001</u>			
DISSOLVED OXYGEN (mg/L)	<u>Kom</u>	<u>8.0</u>			
TEMPERATURE (°C)	<u>Kom</u>	<u>24.3</u>			
pH	<u>Kom</u>	<u>7.5</u>			
CONDUCTIVITY (µmhos/cm)	<u>Kom</u>	<u>404</u>			
HARDNESS (mg/L CaCO ₂)	<u>Kom</u>	<u>1.3 × 40 = 52</u>			
ALKALINITY (mg/L CaCO ₂)	<u>Kom</u>	<u>1.4 × 40 = 56</u>			
TOTAL CHLORINE (mg/L)*	-	-			
TOTAL AMMONIA (mg/L)*	-	-			

*Check with project manager to see if necessary



Great Lakes Environmental Center
 1295 KING AVE.
 COLUMBUS, OH 43212
 PHONE: (614) 487-1040
 FAX: (614) 487-1920

Two Important Notes for Whole Effluent Toxicity Testing:

- There is a maximum hold time for all samples of 36 hours (Hold time begins when sample is taken off the sampler)
- Samples must be received at 4°C ± 2°C

CHAIN OF CUSTODY FORM

(TO BE COMPLETED ONSITE AND SUBMITTED WITH SAMPLES)

FACILITY: El Dorado Chemical Company
 LOCATION: 4500 Northwest Ave, El Dorado, AR 71730
 CONTACT PERSON: Larken Pennington
 PHONE: 870-312-1752

COLLECTOR: Larken Pennington
 DATE: 4/11/12
 WITNESS: Kim Parker
 DATE: 4/11/12

ECC# (lab only)	SAMPLE ID	SAMPLE SOURCE (Eff/Upstr.)	TYPE (grab or composite)	SAMPLE START DATE	SAMPLE START TIME (24-hr notation)	SAMPLE END DATE	SAMPLE END TIME (24-hr notation)	VOLUME COLLECTED	SAMPLE CONTAINER	SAMPLE COLLECTOR	OTHER COMMENTS
ECC 9527	outfall 001		grab	4/11/12	8:30am	4/11/12	8:45am	4 cubitainers		L. Pennington	

ANALYSIS REQUIRED: Please fill in completely

NAME OF STREAM SAMPLED: Outfall 001

Species: *Ceriodaphnia dubia*

Test Type: Acute: 24-hour 48-hour Chronic (7-day)

Pimephales promelas (fathead minnows)

Acute: 24-hour 48-hour

Chronic (7-day)

Other - please specify: _____
 96-hour: with 48-hour renewal without 48-hour renewal

Other - please specify: _____

Dilutions: Screen (100% only)

Definitive (5 sample concentrations): List test concentrations: _____

Dilution Water: Receiving Water

Lab water

Other - please specify: _____

TRANSFER OF SAMPLES:

(FIRST SIGNATURE IS SAMPLER, LAST SIGNATURE IS AUTHORIZED LABORATORY REPRESENTATIVE)

SHIPPER
 1. Larken Pennington

RECEIVER
Kim McDonald

DATE
4/11/12

TIME
2:30pm

2.

4-12-12

1030

For Lab Use Only:
 Ice remaining in cooler upon receipt
 Temperature of samples when received:
30°C

FOR SATURDAY DELIVERY??? MARK PACKAGE AS SUCH AND CALL GLEC ON FRIDAY WITH TRACKING NUMBER

Parental Blockage Map for *C. dubia*

Date: 4/13/12

Time Neonates Pulled: 1230

Source Board: SR/DMW 4/4/12

Initials: *gsw*

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: EDCC 2159-00 # 1 + 2

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3										
2	R6									
1			R1	R5	R2		R3			R4

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 4/13/12

Time Neonates Pulled: 1230

Source Board: SR/DMW 4/4/12

Initials: JSR

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: EDCC 2159-00 II 3+4

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3										
2		R1	R2	R3	R4	R5	R6			
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 4/13/12

Time Neonates Pulled: 1230

Source Board: SR/DNW 4/4/12

Initials: JSC

Estimated Age Range of *C. dubia* neonates: < 24h

Name and Project # neonates used for: EDCC 2159-00 # 5+6

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3	R3		R4	R5				R6		
2								R1		R2
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.



① Baseline (raw)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KDM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1325

YOUNG FROM: SMW 4/14 1 < 24 hrs

TECHNICIANS: DAY: 0 1325 ACS

1 1045 Km 2 1030 ACS 3 0830

4 0915 SMW 5 0930 SMW

6 8:10 VRK 7 0830 SMW

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
① DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		7.7		24.0		170	
	1	+	+	+	+	+	+	+	+	+	+	8.1	8.0	7.7	8.0	25.4	24.8	176	181
	2	+	+	+	+	+	+	+	+	+	+	7.9	7.9	7.7	8.0	24.2	24.8	171	167
	3	te	te	te	te	te	te	te	te	te	te	7.9	7.9	7.7	7.9	24.8	24.9	167	165
	4	te5	te6	te6 ^d	te7	te7	te7	te	te	te	te	8.0	7.9	8.4	8.3	24.5	25.1	164	177
	5	te8	te	te4	te	te	te	te	te	te	te	8.1	7.9	8.2	8.2	24.9	25.0	172	172
	6	te	te7 ^d	te	te1 ^d	te6 ^d	te	te	te	te	te	8.0	7.8	9.3	8.3	25.0	24.6	179	178
	7	te17	te17	te16	te16	te21	te	te	te	te	te		8.0		7.9		24.6		180
① 125%	0	+	+	+	+	+	+	+	+	+	+	7.8		7.9		24.2		198	
	1	+	+	+	+	+	+	+	+	+	+	7.9	8.1	7.9	8.0	25.4	24.8	211	208
	2	+	+	+	+	+	+	+	+	+	+	7.8	8.2	7.8	8.0	24.2	24.9	200	201
	3	te	te	te	te	te	te	te	te	te	te	7.9	8.1	8.0	7.9	24.8	24.9	195	192
	4	te4	te6	te7*	te6	te8	te7	te	te	te	te	7.9	8.0	8.4	8.3	24.5	25.1	198	197
	5	te	te4*	te*	te	te11	te11*	te	te	te	te	8.1	8.0	8.3	8.2	24.9	25.0	197	199
	6	te11	te	te4	te10	te5 ^s	te	te	te	te	te	7.9	8.0	9.6	8.1	25.0	24.6	205	219
	7	te6*	te12	te	te16	te14	te12	te	te	te	te		8.0		7.6		24.6		208
① 25%	0	+	+	+	+	+	+	+	+	+	+					24.1		228	
	1	+	+	+	+	+	+	+	+	+	+					25.4	24.8	241	232
	2	+	+	+	+	+	+	+	+	+	+					24.1	24.9	230	225
	3	te	te	te	te	te	te	te	te	te	te					24.8	24.9	221	222
	4	te4	te4*	te5*	te5	te6	te4	te	te	te	te					24.5	25.1	225	222
	5	te*	te	te*	te*	te*	te*	te	te	te	te					24.9	25.0	228	221
	6	te4	te10	te6*	te5	te8*	te	te	te	te	te					25.0	24.6	232	232
	7	te9*	te	te	te	te	te3	te	te	te	te					24.6			223

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 country error ACS 4/15/12



① Baseline (Raw)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KOM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 4527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1325

YOUNG FROM: SR/DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1326 ACS 1 1045 KOM 2 1030 ACS 3 0830 COM 4 0915 JEN 5 0930 JEN 6 8:10 YRK 7 0830 JEN

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
① 50%	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+									24.1		287			
	2	+	+	+	+	+	+									25.4	24.8	298	278		
	3	+	+	+	+	+	+									24.7	24.9	291	276		
	4	+	+	+	+	+	+									24.8	24.9	284	272		
	5	+	+	+	+	+	+									24.5	25.1	283	270		
	6	+	+	+	+	+	+									24.9	25.0	288	272		
	7	+	+	+	+	+	+									25.0	24.6	285	286		
① 100%	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+					7.5		8.0		24.3		404			
	2	+	+	+	+	+	+					7.3	7.3	9.8	8.0	25.4	24.8	412	388		
	3	+	+	+	+	+	+					7.2	8.1	10.0	8.0	25.8	24.9	408	378		
	4	+	+	+	+	+	+					7.3	7.9	10.8	7.9	24.8	24.9	394	374		
	5	+	+	+	+	+	+					7.4	7.9	11.5	8.7	24.5	25.1	401	373		
	6	+	+	+	+	+	+					7.6	7.9	9.6	8.6	24.9	25.0	398	374		
	7	+	+	+	+	+	+					7.2	7.7	9.8	8.1	25.0	24.6	405	389		
	0	+	+	+	+	+	+														
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



① Baseline (Raw)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KOM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 4/13/12 1325

YOUNG FROM: SR/DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 B25 ACS 1 1045 Kom 2 1030 ACS 3 0830com 4 0915RSAN 5 0930JSE 6 8:10VHK 7 0830JSE

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
① SR	0	+	+	+	+	+	+	+	+	+	+	8.1	8.1	8.7	8.3	24.1	24.8	527	541
	1	+	+	+	+	+	+	+	+	+	+	8.1	8.1	10.4	8.3	25.4	24.8	533	541
	2	+	+	+	+	+	+	+	+	+	+	8.0	8.6	10.0	7.9	25.4	24.9	529	530
	3	+	+	+	+	+	+	+	+	+	+	8.0	8.4	11.4	7.6	24.8	24.9	537	524
	4	+	+	+	+	+	+	+	+	+	+	8.1	8.4	8.7	8.8	24.5	25.1	527	501
	5	+	+	+	+	+	+	+	+	+	+	8.2	8.4	10.2	8.3	24.9	25.0	528	505
	6	+	+	+	+	+	+	+	+	+	+	8.1	8.2	11.0	8.2	25.0	24.6	507	548
	7	+	+	+	+	+	+	+	+	+	+	8.5	8.5	7.9	7.9	24.6	24.6	521	521
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



② 1.0 um pressure filtration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Korn A-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1335

YOUNG FROM: SA/DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1335 AS 1 1100 Korn 2 1040 AS 3 0900 CM 4 0930 SR 5 1015 SS 6 9:20 YLK 7 0900 SS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
② DMW	0	+	+	+	+	+	+	+	+	+	+	8.0		8.8		24.3		174	
	1	+	+	+	+	+	+	+	+	+	+	8.0	8.0	8.5	7.9	25.4	24.8	178	177
	2	+	+	+	+	+	+	+	+	+	+	7.9	8.2	8.8	8.0	25.5	24.9	177	169
	3	+	+	+	+	+	+	+	+	+	+	7.9	8.1	10.2	7.8	24.8	24.9	172	181
	4	+e7	+e6	+e8	+e7	+e6	+e8	+	+	+	+	7.8	8.2	11.4	8.6	24.5	25.1	177	176
	5	T-3	+e2	+e11	+e8	+e11	+e7	+	+	+	+	7.9	8.1	10.4	8.2	24.9	25.0	174	178
	6	↓	+l	+l	+l	+l	+l	+	+	+	+	8.0	7.9	9.9	8.3	25.0	24.6	175	178
	7	↓	+e19	+e18	+e18	+e18	+e16	+	+	+	+		8.1		8.0		24.6		178
② 12.5%	0	+	+	+	+	+	+	+	+	+	+	7.9		8.2		24.0		203	
	1	+	+	+	+	+	+	+	+	+	+	8.6	8.0	8.1	7.7	25.4	24.8	210	207
	2	+*	+	+	+	+	+	+	+	+	+	7.9	8.2	8.2	8.1	24.1	24.9	203	198
	3	+	+	+	+	+	+	+	+	+	+	8.0	8.1	8.4	7.9	24.2	24.9	199	206
	4	+e4	+e7	+e4	+e7	+e8	+e5	+	+	+	+	8.0	8.1	8.7	8.4	24.5	25.1	202	201
	5	+e9	+e4	+e15	+e15	+e12	+e17	+	+	+	+	8.1	8.0	8.7	8.1	24.9	25.0	204	201
	6	+l	+l	+l	+l	+l	+l	+	+	+	+	7.9	7.9	9.7	8.1	25.0	24.6	202	208
	7	+e18	+e20	+e20	+e18	+e20	↓17	+	+	+	+		8.1		7.9		24.6		203
② 25%	0	+	+	+	+	+	+	+	+	+	+					24.1		228	
	1	+	+	+	+	+	+	+	+	+	+					25.4	24.8	240	231
	2	+	+	+	+	+	+	+	+	+	+					24.2	24.9	232	231
	3	+	+	+	+	+	+	+	+	+	+					24.8	24.9	227	233
	4	+e8	+e6	+e7	+e	+e8	+e8	+	+	+	+					24.5	25.1	231	225
	5	+e8*	+e17*	+e10	+e11	+e14*	+e10*	+	+	+	+					24.9	25.0	232	227
	6	+l	+l	+l*	+l	+l2*	↓	+	+	+	+					25.0	24.6	233	227
	7	↓	+e7	↓1	+e18	↓	↓	+	+	+	+					24.6			229

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(2) 1.0um pressure filtration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: kom 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1335

YOUNG FROM: SR/DMW 4/4 1 <24 hrs

TECHNICIANS: DAY: 0 1335

ACS 1 1100 kom 2 1040 ACS 3 0900 cmf 4 0930 gsr 5 1015 gsw 6 9:20 VSK 7 0900 gsw

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
② 50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+					24.2		290			
	2	te*	+	+	+	+	+	+	+	+	+					25.4	24.8	292	285		
	3	te	te	te	te	te	te	+	+	+	+					25.2	24.9	288	278		
	4	te8	te6	te6	te	te*	te8	+	+	+	+					24.8	24.7	276	283		
	5	te16	te13	te17	te10	te15	↓	+	+	+	+					24.5	25.1	286	277		
	6	te	te*	te	te	te	↓	+	+	+	+					24.9	25.0	287	279		
	7	te19	↓4	te21	te15	te14	↓	+	+	+	+					25.0	24.6	289	283		
② 100%	0	+	+	+	+	+	+	+	+	+	+							24.6			282
	1	+	+	+	+	+	+	+	+	+	+	7.5		7.9		24.2		398			
	2	te	+	+	+	+	te	+	+	+	+	7.5	7.3	8.8	7.9	25.4	24.8	400	389		
	3	te	te	te	te	te	te	+	+	+	+	7.4	7.9	9.3	7.9	24.7	24.9	397	378		
	4	te6	te4*	↓	te4*	te7*	te7	+	+	+	+	7.4	7.8	10.5	7.9	24.8	24.9	390	378		
	5	te*	te*	↓	te1*	te*	te13*	+	+	+	+	7.4	7.9	11.2	8.7	24.5	25.1	396	379		
	6	te*	te*	↓	↓	↓	↓	+	+	+	+	7.5	7.8	10.9	8.2	24.9	25.0	397	380		
	7	te*	te*	↓	↓	↓	↓	+	+	+	+	7.5	7.7	11.6	8.2	25.0	24.6	408	396		
	0	+	+	+	+	+	+	+	+	+	+							24.6			383
	1							+	+	+	+										
	2							+	+	+	+										
	3							+	+	+	+										
	4							+	+	+	+										
	5							+	+	+	+										
	6							+	+	+	+										
	7							+	+	+	+										

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(3) C18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KDM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1355

YOUNG FROM: 9R/DMW 4/4/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1355

ACS 1 1115 KDM 2 1105 ACS 3 0915 SEN 4 1015 CAP 5 1045 CAP 6 0830 KDM 7 0930 SEN

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
③ DMW	0	+	+	+	+	+	+	+	+	+	+	7.7		8.5		24.1		165	
	1	+	+	+	+	+	+	+	+	+	+	7.9	8.0	7.7	8.0	24.2	24.7	167	170
	2	+	+	+	+	+	+	+	+	+	+	7.6	8.1	8.2	7.8	24.9	24.4	166	169
	3	te	te	te	te	te	te	te	te	te	te	7.9	7.9	9.0	8.0	24.9	24.5	177	169
	4	te6	te6	te7	te	te	te5	te	te	te	te	7.7	8.0	10.3	8.6	24.8	24.7	175	174
	5	te	te	te	te	te	te	te	te	te	te	7.8	7.9	9.5	8.1	25.0	24.6	169	179
	6	te	te	te3	te3	te3	te3	te	te	te	te	8.0	7.9	9.3	8.0	25.0	24.8	172	175
	7	te13	te13	te18	te16	te16	te16	te	te	te	te		7.8		7.9		25.0		180
③ 12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		8.1		24.3		195	
	1	+	+	+	+	+	+	+	+	+	+	8.0	8.0	8.3	8.0	24.3	24.7	202	198
	2	+	+	+	+	+	+	+	+	+	+	7.8	8.6	8.1	7.8	24.4	25.4	199	198
	3	te	te	te	te	te	te	te	te	te	te	8.0	7.9	8.0	7.7	24.9	24.5	211	200
	4	te7	te6	te8	te7	te7	te6	te	te	te	te	7.9	8.1	8.5	8.5	24.8	24.7	202	201
	5	te8	te7	te	te10	te9	te2	te	te	te	te	7.9	7.9	9.0	8.1	25.0	24.6	199	204
	6	te	te	te5	te*	te	te	te	te	te	te	8.0	7.9	9.6	8.9	25.0	24.8	202	200
	7	te11	te1	te	te1	te12*	te19*	te	te	te	te		7.9		7.8		25.0		205
③ 25%	0	+	+	+	+	+	+	+	+	+	+					24.6		231	
	1	+	+	+	+	+	+	+	+	+	+					24.2	24.7	233	227
	2	+	+	+	+	+	+	+	+	+	+					24.4	25.4	226	227
	3	te	te	te	te	te	te	te	te	te	te					24.9	24.5	238	224
	4	te5	te3	te6	te5	te5	te7	te	te	te	te					24.8	24.7	222	227
	5	te	te10	te12	te14	te	te12	te	te	te	te					25.0	24.6	228	225
	6	te	te	te	te11	te	te	te	te	te	te					25.0	24.8	233	221
	7	te17	te13	te	te	te12	te12	te	te	te	te					25.0	25.0		223

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 entry error: ACS 4/15/12 @entry error 4/20/12 SEN



③ C18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KOM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1355

YOUNG FROM: SR/DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1355 ACS 1 1115 KOM 21105 ACS 3 0915 JSW 4 1015 CAD 5 1045 JSW 6 0830 KOM 7 0930 JSW

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
③ 50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+					24.3				284	
	2	+	+	+	+	+	+	+	+	+	+					24.2	24.7			285	279
	3	+e	+e	+e	+e	+e	+e	+	+	+	+					24.4	25.4			284	268
	4	+e3	+e8	+e7	+e7	+e6	+e8	+	+	+	+					24.9	24.5			301	275
	5	R11	R12	R12	R12	R12	R15	+	+	+	+					24.8	24.7			284	279
	6	de	T	de*	de	de*	de	+	+	+	+					25.0	24.6			288	274
	7	J5	J	J	+e13	J8	+e21	+	+	+	+					25.0	24.8			287	277
③ 100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	7.6		7.5		24.2				397	
	2	+	+	+	+	+	+	+	+	+	+	7.6	7.9	8.6	8.0	24.3	24.7			393	391
	3	+e	+e	+e	+e	+e	+e	+	+	+	+	7.5	8.0	8.5	7.6	24.6	25.4			394	381
	4	+e6	+e6	+e6	+e7	+e6	+e5	+	+	+	+	7.6	7.9	10.1	7.6	24.9	24.9			413	389
	5	R7	R	R8	R	R	R	+	+	+	+	7.5	7.9	11.3	8.6	24.8	24.7			406	375
	6	T	T	de	T	T	de	+	+	+	+	7.5	7.8	9.8	8.1	25.0	24.6			402	386
	7	J	J	J2	J	J	J	+	+	+	+	7.6	7.8	10.9	7.9	25.0	24.8			410	388
	0	+	+	+	+	+	+	+	+	+	+		7.7		7.9		25.0				363
	1							+	+	+	+										
	2							+	+	+	+										
	3							+	+	+	+										
	4							+	+	+	+										
	5							+	+	+	+										
	6							+	+	+	+										
	7							+	+	+	+										

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(4) Aeration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Kam 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1410

YOUNG FROM: 4/14/12 SR/DMW < 24 hrs

TECHNICIANS: DAY: 0 1410 ACS 1 1130 Kam 2 1115 ACS 3 0930 4 1045 CRT 5 1045 CRT 6 0845 Kam 7 1015 CRT

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
④ DMW	0	+	+	+	+	+	+	+	+	+	+	7.8		8.4		24.1		187	
	1	+	+	+	+	+	+	+	+	+	+	8.0	8.1	8.7	7.8	24.2	24.7	169	171
	2	+	+	+	+	+	+	+	+	+	+	7.8	8.2	8.9	7.9	24.4	25.4	166	185
	3	te	te	te	te	te	te	+	+	+	+	7.9	8.0	10.1	7.9	24.9	24.5	180	171
	4	te3	te2	te3	te3	te2	te	+	+	+	+	7.8	8.2	10.9	8.6	24.8	24.7	171	181
	5	R7	R3	R6	R	te2	te	+	+	+	+	7.8	8.0	10.6	8.0	25.0	24.6	168	166
	6	te	te	te	te	te	te	+	+	+	+	8.0	8.0	9.4	8.0	25.0	24.8	166	180
	7	te17	te10	te19	te17	te18	te	+	+	+	+		8.0		8.0		25.0		181
④ 12.5%	0	+	+	+	+	+	+	+	+	+	+	7.9		8.0		24.3		204	
	1	+	+	+	+	+	+	+	+	+	+	8.0	8.1	8.6	8.0	24.3	24.7	202	201
	2	+	+	+	+	+	+	+	+	+	+	7.9	8.2	8.5	7.8	24.4	25.4	201	209
	3	te	te	te	te	te	te	+	+	+	+	8.0	8.1	8.1	7.8	24.9	24.5	209	200
	4	te3	te3	te4	te4	te4	te6	+	+	+	+	8.0	8.1	8.8	8.7	24.8	24.7	201	204
	5	te12	te6	te14	te11	te12	te	+	+	+	+	8.0	8.6	8.5	8.3	25.0	24.6	206	202
	6	te*	te*2	te*	te	te	te*9	+	+	+	+	7.9	8.0	9.6	8.0	25.0	24.8	204	203
	7	↓	↓	te13*	te17	te15	↓	+	+	+	+		8.0		7.9		25.0		200
④ 25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+	+	+	+	+					24.3		235	
	2	+	+	+	+	+	+	+	+	+	+					24.2	24.7	236	226
	3	te	te	te	te	te	te	+	+	+	+					24.7	25.4	231	232
	4	te3	te	te5	te4	te6	te5	+	+	+	+					24.9	24.5	239	227
	5	te6	te	te6	te8	te9	te10	+	+	+	+					24.8	24.7	228	228
	6	te	te	te	te	te	te	+	+	+	+					25.0	24.6	227	220
	7	te10	te	te9	te10	te12	te14	+	+	+	+					25.0	24.8	234	231

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 & curly arrows



(4) Aeration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KAM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1410

YOUNG FROM: SR/DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1410 ACS 1 1130 KAM 2 1115 ACS 3 0930 KAM 4 1645 CRA 5 1045 CAM 6 0845 KAM 7 1015 GSE

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
④ 50%	0	+	+	+	+	+	+	+	+	+	+							24.0		287	
	1	+	+	+	+	+	+	+	+	+	+							24.2	24.7	287	280
	2	+	+	+	+	+	+	+	+	+	+							24.4	25.4	284	281
	3	te	te	te	te	te	te	te	te	te	te							24.9	24.5	297	277
	4	te4	te4	te3	te3	te4	te3	te	te	te	te							24.8	24.7	287	279
	5	te8	te	te	te	te	te	te	te	te	te							25.0	24.6	286	279
	6	te*	T	te7	T5	te8	te6	te	te	te	te							25.0	24.8	294	278
	7	te8	↓	te8	↓	te11	↓	te	te	te	te							25.0			287
④ 100%	0	+	+	+	+	+	+	+	+	+	+	7.7		8.0		24.1		401			
	1	+	+	+	+	+	+	+	+	+	+	7.9	7.9	8.5	8.2	24.3	24.7	394	392		
	2	+	+	+	+	+	+	+	+	+	+	7.6	8.0	8.8	7.7	24.6	25.4	396	384		
	3	te	te	te*	te	te	+	te	te	te	te	7.6	7.8	10.4	8.0	24.9	24.5	406	374		
	4	*te	te4	T	te	te	te3	te	te	te	te	7.6	7.9	11.6	8.7	24.8	24.7	406	382		
	5	te	te	T	te	te	te	te	te	te	te	7.5	7.8	9.3	8.4	25.0	24.6	401	394		
	6	te*	te4	↓	te*	T	te*	te	te	te	te	7.6	7.8	11.2	8.0	25.0	24.8	418	389		
	7	↓	te7	↓	↓4	↓	↓	te	te	te	te		7.7		7.9		25.0		392		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

printing error cos 4/18/12



⑤ EDTA Addition

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KDM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 4/13/12 1435

YOUNG FROM: SR/PMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1435 ACS 1 1200 Kom 2 1145 ACS 3 0930 Kom 4 0900 Kom 5 1145 gsr 6 0915 gsr 7 10:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
⑤ DMW	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	te	+	+	+													
	3	de	de	de	de	de	de													
	4	te8	te5	te8	te7	te8	te4													
	5	te13	te	te15	te15	te15														
	6	te	te10	te	te	te														
	7	te19	te18	te21	te19	te21	↓													
⑤ 12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+													
	3	de	de	de	de	de	de													
	4	te6	te5	te6	te7	te5*	te6													
	5	te*	te	te6*	te	te*	te*													
	6	↓10	te10	↓13	te13	↓3	te10													
	7	↓	te6*	↓	↓2	↓	te14*													
⑤ 25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+													
	3	de	de	de	de	de	de													
	4	te4 ^d	te4	te5	te5	te5	te6													
	5	↓	te	te	te	te*	te*													
	6	↓	te7*	te8*	te8*	↓3	↓7													
	7	↓	te8*	te8*	te8*	↓	↓													

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 entry error 4/19/12 gsa, 4-20-12 YBK



(5) EDTA Addition

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KAM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1435

YOUNG FROM: SR DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1435 ACS 1 1200 km 2 1145 AKS 3 0930 km 4 0900 km 5 1145 JSE 6 0915 JSE 7 10:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.				
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old			
⑤ 50%	0	+	+	+	+	+	+	+	+	+	+	+	+					24.9		290		
	1	+	+	+	+	+	+	+	+	+	+	+	+					24.1	24.7	287	286	
	2	+	+	+	+	+	+	+	+	+	+	+	+					24.4	25.4	286	261	
	3	de	de	de	de	de	de	de	de	de	de	de	de					25.0	25.2	290	264	
	4	te4	te3	te4	te4	te4	te4	te4	te4	te4	te4	te4	te4					25.0	24.9	294	302	
	5	te	te*	te	te*	te	te	te	te	te	te	te	te					24.8	24.9	287	276	
	6	te7	te2*	te7	te7	te4	te4	te4	te4	te4	te4	te4	te4					25.0	25.1	286	280	
	7	te8	te8	te8	te8	te5	te5	te5	te5	te5	te5	te5	te5					24.7			280	
⑤ 100%	0	+	+	+	+	+	+	+	+	+	+	+	+	7.3		8.1		24.8		408		
	1	+	+	+	+	+	+	+	+	+	+	+	+	7.2	7.9	8.9	8.0	24.1	24.7	398	394	
	2	+	+	+	+	+	+	+	+	+	+	+	+	7.1	8.1	8.6	8.0	24.4	25.4	399	383	
	3	de	de	de	de	de	de	de	de	de	de	de	de	7.2	7.9	10.3	8.1	25.0	25.2	408	378	
	4	te4	te2	te1	te	te2	te	te	te	te	te	te	te	7.2	7.8	11.3	8.7	25.0	24.9	405	432	
	5	te	te*	te	te	te	te	te	te	te	te	te	te	7.3	8.0	10.6	7.8	24.8	24.9	399	385	
	6	te	te*	te4	te5	te	te	te	te	te	te	te	te	7.4	7.5	11.4	8.7	25.0	25.1	405	382	
	7	te	te*	te6	te1	te	te	te	te	te	te	te	te		7.6		8.1		24.7			387
	0	+	+	+	+	+	+	+	+	+	+	+	+									
	1																					
	2																					
	3																					
	4																					
	5																					
	6																					
	7																					

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

0. emy era 4/18/12 JSE



⑥ Nathio Addition

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KOM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1745

YOUNG FROM: SR/DMW 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1445

ACS 1 1230 km 2 1155 ACS 3 0945 Kom 4 0945 Kom 5 1200 JSA 6 1000 JSA 7 10:15 YBL

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
⑥ DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		7.7		24.7		195	
	1	+	+	+	+	+	+					7.8	8.2	8.8	8.0	24.1	24.7	189	198
	2	+	+	+	+	+	+					7.8	8.1	8.6	8.0	24.4	25.4	193	188
	3	de	de	de	de	de	de					7.9	8.1	10.1	8.1	25.0	25.2	207	191
	4	de7	de4	de6	de6	de5	de6					7.9	8.1	10.8	8.4	25.0	24.9	202	191
	5	de2	de12	de13	de13	de15	de12					7.8	8.2	9.4	8.6	24.8	25.0	193	202
	6	+	+	+	+	+	+					8.0	8.0	9.5	8.1	25.0	25.1	175	186
	7	+	+	+	+	+	+						8.1	8.2		24.7			180
⑥ 12.5%	0	+	+	+	+	+	+	+	+	+	+	7.9		7.8		24.4		213	
	1	+	+	+	+	+	+					7.9	8.1	8.0	8.1	24.1	24.7	212	207
	2	+	+	+	+	+	+					7.9	8.1	8.5	7.9	24.4	25.4	213	209
	3	de	de	de	de	de	de					8.0	8.1	8.0	8.1	25.0	25.2	228	203
	4	de5	de8	de6	de7	de4	de7					8.0	8.0	8.5	8.4	25.0	24.9	210	203
	5	+	+	+	+	+	+					7.9	8.2	8.3	8.5	24.8	25.0	214	201
	6	+	+	+	+	+	+					7.9	8.0	9.2	8.0	25.0	25.1	210	209
	7	+	+	+	+	+	+						8.1	8.0		24.7			211
⑥ 25%	0	+	+	+	+	+	+	+	+	+	+					24.3		250	
	1	+	+	+	+	+	+									24.1	24.7	250	249
	2	+	+	+	+	+	+									24.4	25.4	247	244
	3	de	de	de	de	de	de									25.0	25.2	271	239
	4	de7	de6	de5	de3	de6	de3									25.0	24.9	251	227
	5	+	+	+	+	+	+									24.8	25.0	253	244
	6	+	+	+	+	+	+									25.0	25.1	252	244
	7	+	+	+	+	+	+									24.7			246

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(6) Nathio Addition

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: KDM 4-25-12

Date: _____

Great Lakes Environmental Center

TEST MATERIAL: EEC 9527

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2159-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 4/13/12 1445

YOUNG FROM: 52/11hr 4/4 1 < 24 hrs

TECHNICIANS: DAY: 0 1445 AG 1 1230 km 2 1155 AG 3 0945 Kom 4 0945 Kom 5 1200 gsw 6 1000 gsw 7 10:15 YB

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.				
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old			
⑥ 50%	0	+	+	+	+	+	+	+	+	+	+	+	+					24.9		324		
	1	+	+	+	+	+	+	+	+	+	+	+	+					24.1	24.7	318	320	
	2	+	+	+	+	+	+	+	+	+	+	+	+					24.5	25.4	320	309	
	3	e	e	e	e*	e	e											25.0	25.2	330	291	
	4	e4	e4	e5	e2*	e4	e6											25.0	24.9	323	276	
	5	e*	e*	e9	↓	e	e											24.8	24.9	327	318	
	6	e9*	e5*	e*	↓	e5	e9*											25.0	25.1	323	319	
	7	e9*	e10*	e8*	↓	e9	e6*												24.7			312
⑥ 100%	0	+	+	+	+	+	+	+	+	+	+	+	+	7.4		8.0		24.6		468		
	1	+	+	+	+	+	+	+	+	+	+	+	+	7.3	8.1	8.9	8.0	24.1	24.7	469	459	
	2	+	+	+	+	+	+	+	+	+	+	+	+	7.4	8.0	8.7	7.9	24.9	25.4	463	451	
	3	e	e	e	e	e	e							7.4	8.0	10.1	7.9	25.0	25.2	478	436	
	4	e	e2	e3	e1	e2	e1							7.3	7.9	10.9	8.3	25.0	24.9	472	381	
	5	e*	e	e*	e	↓	e							7.5	7.8	9.5	8.6	24.8	24.9	473	464	
	6	↓	e5	e4*	e7	↓	e3							7.5	7.7	11.6	8.2	25.0	25.1	482	465	
	7	↓	e5	↓	e9 ^d	↓	e							7.8		8.2		24.7			445	
	0	+	+	+	+	+	+	+	+	+	+	+	+									
	1																					
	2																					
	3																					
	4																					
	5																					
	6																					
	7																					

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

APPENDIX B
DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST NO.	C. dubia	FHM
3/10	97	0.81	1.80
5/10	98	1.37	2.09
6/10	99	1.48	2.13
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
3/12	116	1.46	2.42
AVERAGE		1.26	2.15
STD. DEV.		0.24	0.33
RANGE: LOW		0.78	1.49
RANGE: HIGH		1.74	2.81
Coefficient of variation		0.19	0.15
Date of last test		3/1-8/12	3/20-27/12
MSD of most recent test		5.47	0.0537
PMSD of most recent test		15.2	20.7
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

From EPA's Wet Interlaboratory Variability Study

Test Species	GLEC ¹	IC25 Coefficient of Variation National Percentiles ²				
		10th	25th	50th	75th	90th
C. dubia	0.19	0.08	0.17	0.27	0.45	0.62
P. promelas	0.15	0.12	0.21	0.26	0.38	0.45

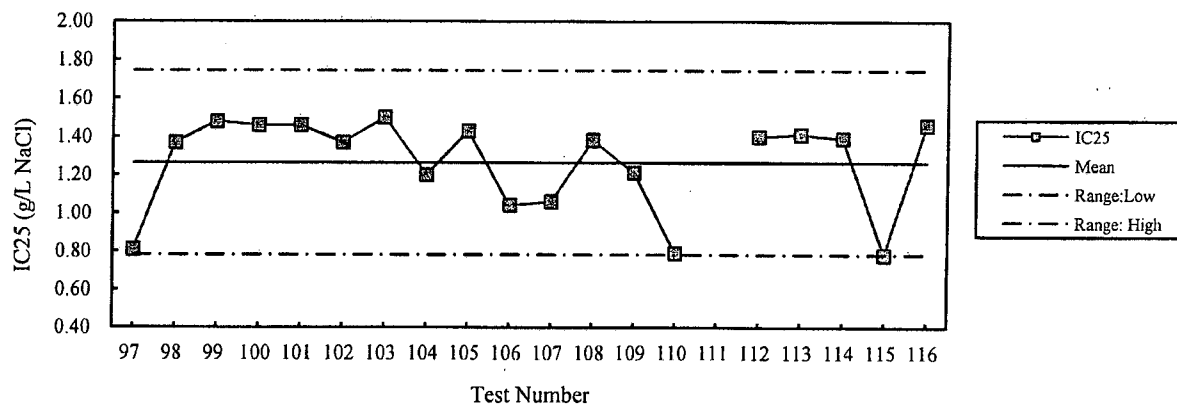
¹ Based on cumulative GLEC data from the most recent 20 tests.

² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

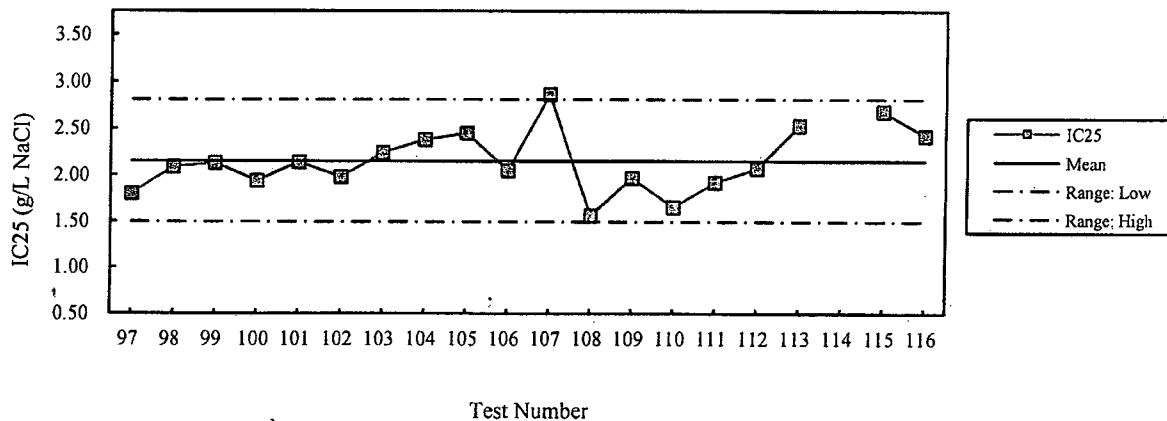
Chronic Reference Toxicant IC25

Ceriodaphnia dubia



Chronic Reference Toxicant IC25

Pimephales promelas





Great
Lakes
Environmental
Center

July 3, 2012

Applied
Environmental
Sciences
www.glec-online.com

Roland McDaniel, Project Manager
GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

Traverse City
Operations
739 Hastings St.
Traverse City
MI 49686

231 941-2230
231 941-2240 fax

Columbus
Operations
1295 King Ave.
Columbus
OH 43212

614 487-1040
614 487-1920 fax

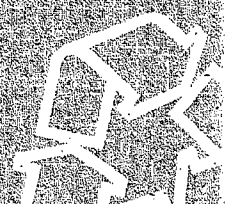
RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED MAY 23, 2012 FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected May 23, 2012. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tarr
Laboratory Coordinator



PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED MAY 23, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

June 2012



Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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APPENDIX B. DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS B-1

INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled May 23, 2012 (Sample ID: EEC 9558) to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

Ceriodaphnia dubia Static Renewal Chronic Toxicity Tests

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for seven days under static

renewal conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity 10-20 $\mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test (7 days).

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUc) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUc} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1). The baseline toxicity test was not toxic to *C. dubia* survival and exhibited 100 percent survival in the 100 percent test concentration resulting in a 7 day LC_{50} value of >100 percent effluent. The outfall 001 sample was chronically toxic to *C. dubia* reproduction and exhibited an IC_{25} value of 56.4 percent effluent or 1.8 TUc (Table 1).

1.0 µm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a Gelman A/E glass fiber filter (1.0 µm).

After filtration treatment, 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent was removed as the *C. dubia* exhibited an IC₂₅ of >100 percent or <1.0 TUc. Therefore, filtration was very effective as a treatment and the toxicity to *C. dubia* reproduction in the EDCC effluent sample appears to be associated with a filterable toxicant.

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample **after 1.0 µm filtration treatment** was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

Prior to the C-18 treatment, filtration removed 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent. Therefore, it could not be determined whether C-18 was an effective treatment in removing toxicity to *C. dubia* reproduction in the EDCC effluent sample.

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

Aeration of the outfall 001 sample did not remove toxicity when compared to the concurrent baseline toxicity test and demonstrated an IC₂₅ of 5.9 percent or 16.9 TUc (Table 1). As a result, the chronic toxicity to *C. dubia* in the EDCC effluent is not related to a volatile, easily oxidizable or aeratable toxicant.

Cation Chelation with EDTA - Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test IC₂₅ of 56.4 percent, the addition of EDTA (25 mg/L) did not remove any of the sample toxicity and exhibited an IC₂₅ of 18.4 percent or 5.4 TUc (Table 1). Therefore, the toxicity to *C. dubia* reproduction does not appear to be associated chelatable metals.

Sodium Thiosulfate Treatment – Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

Sodium thiosulfate treatment of the outfall 001 sample did not remove toxicity when compared to the concurrent baseline toxicity test and demonstrated an IC₂₅ of 6.8 percent or 14.7 TUc (Table 1). As a result, the toxicity to *C. dubia* reproduction present in the outfall 001 sample is not related to thiosulfate reducible compounds or oxidants.

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected May 23, 2012 did demonstrate removal of chronic toxicity, but the reduction of toxicity to *C. dubia* reproduction was only demonstrated by one of the five TIE treatments performed. Three of the treatments, aeration, EDTA and sodium thiosulfate were not effective in removing toxicity from the outfall 001 effluent sample. Thus, the effluent toxicity does not appear to be associated with easily oxidizable or aeratable compounds, chelatable metals or thiosulfate reducible compounds or oxidants.

The 1.0 µm filtration treatment removed 100 percent of the toxicity present in the EDCC outfall 001 effluent sample. Therefore, the chronic toxicity to *C. dubia* present in the EDCC outfall 001 effluent sample appears to be associated with a filterable toxicant(s). For a summary of all test results, see Table 1.

The effectiveness of C-18 in removing chronic toxicity to *C. dubia* in the EDCC effluent sample is unknown due to the fact that filtration removed 100 percent of the sample toxicity prior to C-18 treatment.

Summary of the chronic toxicity characterization of the EDCC outfall 001 sample collected May 23, 2012 (Sample ID: EEC 9558):

- **The toxicant (s) was filterable.**
- The toxicant(s) was not a chelatable metal.
- The toxicant(s) was not a volatile, easily oxidizable or aeratable compound.
- The toxicant(s) was not a thiosulfate reducible compound or oxidant.

Table 1. Summary of Chronic TIE Test results

<i>C. dubia</i> Chronic TIE on EDCC Outfall 001 Sample Collected 5/23/12 GLEC Sample ID: EEC 9558 Test Dates: 5/25/12 – 6/1/12	Percent Survival								
	DMW	12.5%	25%	50%	100%	7-day LC ₅₀			
Baseline Toxicity (No manipulation) – Test 1	83	83	83	100	100	NA			
1.0 µm Filtration – Test 2	100	100	100	100	100	NA			
C18-SPE Treatment – Test 3	100	100	67	100	100	NA			
Aeration – Test 4	100	50	100	100	100	NA			
Cation Chelation with EDTA – Test 5	83	100	100	100	100	NA			
Sodium Thiosulfate Treatment – Test 6	100	100	67	100	100	NA			
	Reproduction – Mean Number of Young per Adult								
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed	
Baseline Toxicity (No manipulation) – Test 1	19.8	18.3	16.2	16.2	6.2	56.4	1.8	--	
1.0 µm Filtration – Test 2	29.5	22.2	24.8	30.5	35.0	>100	<1.0	100%	
C18-SPE Treatment – Test 3	29.5	26.8	19.0	25.5	34.2	>100	<1.0	0% ^b	
Aeration – Test 4	33.5	11.0	18.8	17.8	5.8	5.9	16.9	0%	
Cation Chelation with EDTA – Test 5 ^c	15.2	12.2	10.5	9.8	0.0	18.4	5.4	0%	
Sodium Thiosulfate Treatment – Test 6	27.8	15.0	6.5	11.0	7.3	6.8	14.7	0%	

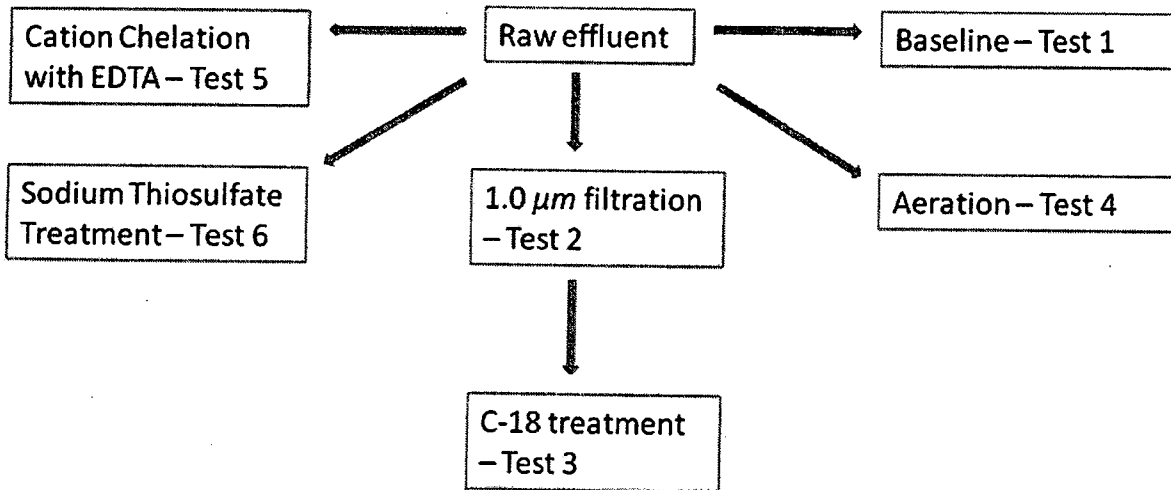
NA – Not applicable or Not available

^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b Additional baseline toxicity removed by C-18 treatment after filtration treatment.

^c Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC₂₅ value for the most recent *C. dubia* reference toxicant test was 1.46 g/L of sodium chloride which was within the acceptance range of 0.86 to 1.74 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED MAY 23, 2012



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: GBMC - EDCC

PROJECT NUMBER: 2179-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>5/24/12</u>	INITIALS				
EEC NUMBER		^{gfc} 9558			
OUTFALL/DESCRIPTION		outfall 801			
DISSOLVED OXYGEN (mg/L)	<u>cm</u>	5.9			
TEMPERATURE (°C)	<u>cm</u>	4.2			
pH	<u>cm</u>	7.0			
CONDUCTIVITY (µmhos/cm)	<u>cm</u>	289			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>5/24/12</u>	INITIALS				
EEC NUMBER		9558			
OUTFALL/DESCRIPTION		outfall-001			
DISSOLVED OXYGEN (mg/L)	<u>cm</u>	8.7			
TEMPERATURE (°C)	<u>cm</u>	24.3			
pH	<u>cm</u>	7.4			
CONDUCTIVITY (µmhos/cm)	<u>cm</u>	300			
HARDNESS (mg/L CaCO ₂)	<u>Km</u>	1.3 x 40 = 52			
ALKALINITY (mg/L CaCO ₂)	<u>Km</u>	1.4 x 40 = 56			
TOTAL CHLORINE (mg/L)*					
TOTAL AMMONIA (mg/L)*					

*Check with project manager to see if necessary



Great Lakes Environmental Center
 1295 KING AVE.
 COLUMBUS, OH 43212
 PHONE: (614) 487-1040
 FAX: (614) 487-1920

Two Important Notes for Whole Effluent Toxicity Testing:

- There is a maximum hold time for all samples of 36 hours (Hold time begins when sample is taken off the sampler)
- Samples must be received at 4°C ± 2°C

CHAIN OF CUSTODY FORM

(TO BE COMPLETED ONSITE AND SUBMITTED WITH SAMPLES)

FACILITY: El Dorado Chemical Co.
 LOCATION: El Dorado, AR
 CONTACT PERSON: Larken Pennington
 PHONE: 870-863-1125

COLLECTOR: Larken Pennington
 DATE: 5/23/12
 WITNESS: Carole Hendricks
 DATE: 5/23/12

EEC# (lab only)	SAMPLE ID	SAMPLE SOURCE (Eff/Upstr.)	TYPE (grab or composite)	SAMPLE START DATE	SAMPLE START TIME (24-hr notation)	SAMPLE END DATE	SAMPLE END TIME (24-hr notation)	VOLUME COLLECTED	SAMPLE CONTAINER	SAMPLE COLLECTOR	OTHER COMMENTS
ELC 9558	001	Effluent outfall 001	Grab	5/23/12	8:30am	5/23/12	8:30am	4 Cubitainers		Pennington	

ANALYSIS REQUIRED: Please fill in completely

NAME OF STREAM SAMPLED: Outfall 001

- Species: *Ceriodaphnia dubia* *Pimephales promelas* (fathead minnows) Other - please specify: _____
- Test Type: Acute: 24-hour Acute: 24-hour 48-hour 96-hour: with 48-hour renewal
 48-hour 48-hour 48-hour without 48-hour renewal
 Chronic (7-day) Chronic (7-day) Other - please specify: _____
- Dilutions: Screen (100% only) Definitive (5 sample concentrations): List test concentrations: _____
- Dilution Water: Receiving Water Lab water Other - please specify: _____

TRANSFER OF SAMPLES:

(FIRST SIGNATURE IS SAMPLER, LAST SIGNATURE IS AUTHORIZED LABORATORY REPRESENTATIVE)

SHIPPER	RECEIVER	DATE	TIME
1. <u>Larken Pennington</u>	<u>[Signature]</u>	<u>5/23/12</u>	<u>2:30pm</u>
2.	<u>[Signature]</u>	<u>5/24/12</u>	<u>1030</u>

For Lab Use Only:
 Ice remaining in cooler upon receipt
 Temperature of samples when received:
4.2

FOR SATURDAY DELIVERY??? MARK PACKAGE AS SUCH AND CALL GLEC ON FRIDAY WITH TRACKING NUMBER

Test Dates: 5/25/12 - 6/1/12

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	83%	83%	83%	100%	100%
1.0 µm filtration (Test 2)	100%	100%	100%	100%	100%
C-18 SPE treatment (Test 3)	100%	100%	67%	100%	100%
Aeration (Test 4)	100%	50%	100%	100%	100%
EDTA 25 mg/l (Test 5)	83%	100%	100%	100%	100%
NaThio (50 mg/l) (Test 6)	100%	100%	67%	100%	100%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	19.8	18.3	16.2	16.2	6.2	56.4	1.8	--
1.0 µm filtration (Test 2)	29.5	22.2	24.8	30.5	35.0	>100	<1.0	100.0%
C-18 SPE treatment (Test 3)	29.5	26.8	19.0	25.5	34.2	>100	<1.0	0% ^b
Aeration (Test 4)	33.5	11.0	18.8	17.8	5.8	5.9	16.9	0.0%
EDTA (25 mg/l) (Test 5)^a	15.2	12.2	10.5	9.8	0.0	18.4	5.4	0.0%
NaThio (50 mg/l) (Test 6)	27.8	15.0	6.5	11.0	7.3	6.8	14.7	0.0%

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to C.dubia reproduction in DMW

b - additional toxicity removed by C-18 treatment after filtration treatment.

***DMW control did not meet control requirement for minimum number of females with 3 broods, therefore the baseline control was substituted for statistical analysis**

Baseline (Test 1)

El Dorado Chemical outfall 001 (EEC 9558)

(Tested 5/25/12 - 6/1/12)

Sample Date: 5/23/12

Sample Received: 5/24/12

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	5	5	6	6
DEAD	1	1	1	0	0
% SURV	83.33%	83.33%	83.33%	100.00%	100.00%

Scito River Water 2° Control	6
	0
	100.00%

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	0	0	0	11	6
2	22	24	24	17	7
3	26	21	16	15	4
4	17	19	22	19	8
5	28	23	21	18	8
6	26	23	14	17	4
N	6	5	6	6	6
MEAN	19.8	18.3	16.2	16.2	6.2
SD	10.476959	9.15787457	8.7730648	2.857738	1.8348479
CV	52.825003	49.9520431	54.26638	17.67673	29.75429
Total Young	119	110	97	97	37

Scito River 2° Control	41
	44
	32
	39
	35
	34
	5
	37.5
	4.5934736
	12.249263
	225

1.0 µm filtration (Test 2)

El Dorado Chemical outfall 001 (EEC 9558)

(Tested 5/25/12 - 6/1/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	31	21	23	26	31
2	36	27	34	33	37
3	33	25	27	30	27
4	35	22	18	32	40
5	23	18	25	32	39
6	19	20	22	30	36
N	6	6	6	6	6
MEAN	29.5	22.2	24.8	30.5	35.0
SD	6.9209826	3.31159579	5.419102	2.5099801	5.0199602
CV	23.460958	14.9395299	21.821887	8.2294429	14.342743
Total Young	177	133	149	183	210

C-18 SPE treatment (Test 3)
El Dorado Chemical outfall 001 (EEC 9558)
(Tested 5/25/12 - 6/1/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	4	6	6
DEAD	0	0	2	0	0
% SURV	100.00%	100.00%	66.67%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	33	34	30	29	39
2	30	30	34	28	35
3	33	18	18	25	31
4	32	28	4	20	32
5	17	31	28	24	35
6	32	20	0	27	33
N	6	6	6	6	6
MEAN	29.5	26.8	19.0	25.5	34.2
SD	6.2209324	6.40052081	14.240786	3.2710854	2.857738
CV	21.087906	23.8528726	74.951508	12.827786	8.3641113
Total Young	177	161	114	153	205

Aeration (Test 4)
El Dorado Chemical outfall 001 (EEC 9558)
(Tested 5/25/12 - 6/1/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	3	6	6	6
DEAD	0	3	0	0	0
% SURV	100.00%	50.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	32	19	20	17	7
2	36	22	21	22	7
3	33	18	17	14	4
4	36	3	16	10	2
5	39	4	20	19	6
6	25	0	19	25	9
N	6	6	6	6	6
MEAN	33.5	11.0	18.8	17.8	5.8
SD	4.8476799	9.6747093	1.9407902	5.419102	2.4832774
CV	14.470686	87.9519027	10.305081	30.387488	42.57047
Total Young	201	66	113	107	35

EDTA 25 mg/l (Test 5)
El Dorado Chemical outfall 001 (EEC 9558)
(Tested 5/25/12 - 6/1/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	6	6	6	6
DEAD	1	0	0	0	0
% SURV	83.33%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	19	14	18	8	0
2	0	15	9	13	0
3	22	16	8	9	0
4	15	13	8	9	0
5	15	12	7	10	0
6	20	3	13	10	0
N	6	6	6	6	6
MEAN	15.2	12.2	10.5	9.8	0.0
SD	7.9351539	4.70814896	4.2308392	1.7224014	0
CV	52.319696	38.6971148	40.293706	17.515947	#DIV/0!
Total Young	91	73	63	59	0

NaThio (50 mg/l) (Test 6)
El Dorado Chemical outfall 001 (EEC 9558)
(Tested 5/25/12 - 6/1/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	4	6	6
DEAD	0	0	2	0	0
% SURV	100.00%	100.00%	66.67%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	37	16	0	11	6
2	34	18	12	17	8
3	18	12	9	11	6
4	29	15	11	8	10
5	17	15	7	10	10
6	32	14	0	9	4
N	6	6	6	6	6
MEAN	27.8	15.0	6.5	11.0	7.3
SD	8.4241716	2	5.3197744	3.1622777	2.4221203
CV	30.266485	13.3333333	81.842684	28.747979	33.028913
Total Young	167	90	39	66	44

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	0	0	0	11	6
Response 2	22	24	24	17	7
Response 3	26	21	16	15	4
Response 4	17	19	22	19	8
Response 5	28	23	21	18	8
Response 6	26	23	14	17	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9558 Test #1
 Test Start Date: 5/25/12 Test Ending Date: 6/1/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	19.833	10.477	19.833
2	6	12.500	18.333	9.158	18.333
3	6	25.000	16.167	8.773	16.167
4	6	50.000	16.167	2.858	16.167
5	6	100.000	6.167	1.835	6.167

The Linear Interpolation Estimate: 56.4583 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 41.5075 Standard Deviation: 20.9172
 Original Confidence Limits: Lower: 7.8683 Upper: 70.2922
 Resampling time in Seconds: 0.05 Random_Seed: 85937216

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	31	21	23	26	31
Response 2	36	27	34	33	37
Response 3	33	25	27	30	27
Response 4	35	22	18	32	40
Response 5	23	18	25	32	39
Response 6	19	20	22	30	36

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9558 Test #2

Test Start Date: 5/25/12 Test Ending Date: 6/1/12

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	29.500	6.921	29.500
2	6	12.500	22.167	3.312	28.125
3	6	25.000	24.833	5.419	28.125
4	6	50.000	30.500	2.510	28.125
5	6	100.000	35.000	5.020	28.125

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	33	34	30	29	39
Response 2	30	30	34	28	35
Response 3	33	18	18	25	31
Response 4	32	28	4	20	32
Response 5	17	31	28	24	35
Response 6	32	20	0	27	33

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9558 Test #3

Test Start Date: 5/25/12 Test Ending Date: 6/1/12

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	29.500	6.221	29.500
2	6	12.500	26.833	6.401	26.833
3	6	25.000	19.000	14.241	26.222
4	6	50.000	25.500	3.271	26.222
5	6	100.000	34.167	2.858	26.222

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	32	19	20	17	7
Response 2	36	22	21	22	7
Response 3	33	18	17	14	4
Response 4	36	3	16	10	2
Response 5	39	4	20	19	6
Response 6	25	0	19	25	9

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9558 Test #4
 Test Start Date: 5/25/12 Test Ending Date: 6/1/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	33.500	4.848	33.500
2	6	12.500	11.000	9.675	15.889
3	6	25.000	18.833	1.941	15.889
4	6	50.000	17.833	5.419	15.889
5	6	100.000	5.833	2.483	5.833

The Linear Interpolation Estimate: 5.9444 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 6.0397 Standard Deviation: 0.5950
 Original Confidence Limits: Lower: 5.1551 Upper: 7.4486
 Resampling time in Seconds: 0.00 Random_Seed: -1520029344

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	19	14	18	8	0
Response 2	0	15	9	13	0
Response 3	22	16	8	9	0
Response 4	15	13	8	9	0
Response 5	15	12	7	10	0
Response 6	20	3	13	10	0

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9558 Test #5
 Test Start Date: 5/25/12 Test Ending Date: 6/1/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	15.167	7.935	15.167
2	6	12.500	12.167	4.708	12.167
3	6	25.000	10.500	4.231	10.500
4	6	50.000	9.833	1.722	9.833
5	6	100.000	0.000	0.000	0.000

The Linear Interpolation Estimate: 18.4375 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 27.1727 Standard Deviation: 17.9696
 Original Confidence Limits: Lower: 7.4306 Upper: 59.0909
 Resampling time in Seconds: 0.00 Random_Seed: 1258326512

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	37	16	0	11	6
Response 2	34	18	12	17	8
Response 3	18	12	9	11	6
Response 4	29	15	11	8	10
Response 5	17	15	7	10	10
Response 6	32	14	0	9	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9558 Test #6
 Test Start Date: 5/25/12 Test Ending Date: 6/1/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	27.833	8.424	27.833
2	6	12.500	15.000	2.000	15.000
3	6	25.000	6.500	5.320	8.750
4	6	50.000	11.000	3.162	8.750
5	6	100.000	7.333	2.422	7.333

The Linear Interpolation Estimate: 6.7776 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 7.2579 Standard Deviation: 1.5020
 Original Confidence Limits: Lower: 5.4688 Upper: 11.0294
 Resampling time in seconds: 0.00 Random_Seed: 1593190016

Parental Blockage Map for *C. dubia*

Date: 5-25-12

Time Neonates Pulled: 1130

Source Board: 5-11-12

Initials: Km

Estimated Age Range of *C. dubia* neonates: 3 1/2 h

Name and Project # neonates used for: tests ① & ②

	1	2	3	4	5	6	7	8	9	10
6										
5	R1						R4	R5		
4										
3						R3				
2								R2		
1										R6

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 5-25-12

Time Neonates Pulled: 1300

Source Board: MH

Initials: *kr*

Estimated Age Range of *C. dubia* neonates: 3 1/2 hours

Name and Project # neonates used for: ~~2~~ ~~3~~ 3 4

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3										
2										
1		R1, R2			R3 R6				R4	R5

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 5-25-12

Time Neonates Pulled: 1400

Source Board: S-1402

Initials: CMJ

Estimated Age Range of *C. dubia* neonates: 3 1/2 hours

Name and Project # neonates used for: ~~5~~ 6

	1	2	3	4	5	6	7	8	9	10
6										
5									R1	
4										
3								R4		
2				R6		R2	R3			
1					R5					

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.



D Busalpa

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curt

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1530CMT

1 9:30YBK 2 915 ACS 3 810 ACS 4 13:30YBK 5 1220 ACS

6 8:45YBK 7 12:30YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.8		8.1		25.1		187	
	1	+	+	+	+	+	+	+	+	+	+	7.8	7.7	7.8	8.0	25.0	24.3	190	192
	2	+	+	+	+	+	+	+	+	+	+	7.8	7.8	7.9	7.7	24.2	24.4	183	189
	3	+	+	+	+	+	+	+	+	+	+	8.0	8.0	7.9	7.8	25.2	24.4	183	181
	4	T	+e4	+e	+e3	+e1	+e5					7.7	8.1	7.8	8.8	24.4	24.3	183	188
	5		+e5	+e5	+e6	+e	+e6					7.9	7.7	8.1	8.4	24.3	24.1	175	174
	6		+e7	+e9	+e	+e11	+e					7.9	7.9	8.1	8.6	25.0	24.5	189	179
	7	V	+e6	+e12	+e8	+e16	+e15						7.8		8.2		24.5		182
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.7		8.4		24.8		211	
	1	+	+	+	+	+	+	+	+	+	+	7.7	7.8	8.0	7.9	25.0	24.3	212	209
	2	+	+	+	+	+	+	+	+	+	+	7.8	7.9	7.9	7.9	24.2	24.4	203	209
	3	T	+e	+e	+e	+e	+e					7.9	8.0	8.1	8.0	24.3	24.4	204	199
	4		+e3	+e2	+e	+e4	+e4					7.7	8.3	7.9	8.7	24.4	24.3	198	199
	5		+e	+e	+e7	+e7	+e6					7.9	7.9	8.1	8.5	24.4	24.6	195	180
	6		+e10	+e8	+e	+e	+e					7.8	7.9	8.4	8.4	25.0	24.5	209	192
	7	V	+e11	+e11	+e12	+e12	+e13						7.9		8.2		24.5		206
25%	0	+	+	+	+	+	+	+	+	+	+					24.3		235	
	1	+	+	+	+	+	+	+	+	+	+					25.0	24.3	235	234
	2	+	+	+	+	+	+	+	+	+	+					24.5	24.4	231	231
	3	T	+e	+e	+e	+e	+e									25.2	24.4	233	227
	4		+e4	+e1	+e2	+e5	+e3									24.4	24.3	224	223
	5		+e	+e	+e2	+e5	+e									24.2	24.6	226	221
	6		+e7	+e6	+e7	+e	+e									25.0	24.5	226	223
	7	V	+e13	+e9	+e11	+e11	+e11									24.5			231

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



① Baseline

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curt

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1530cm

1 9:30 YBK 2 9:15 AS 3 8:10 4 13:30 YBK 5 12:20 AS 6 8:45 YBK 7 12:30 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+							24.3		281	
	1	+*	+	+	+	+	+	~	~	~	~							25.0	24.3	283	278
	2	+*	+	+	+	+	+	~	~	~	~							25.0	24.4	283	276
	3	e*	e	e	e	e	e	~	~	~	~							25.5	24.4	284	273
	4	+e3	+e4	+e4	+e5	+e4	+e5	~	~	~	~							24.4	24.3	276	268
	5	e	e	e	e	e	e	~	~	~	~							24.6	24.6	276	272
	6	+e5	+e6	+e5	+e6	+e6	+e6	~	~	~	~							25.0	24.5	284	271
	7	+e3d	+e7	+e6*	+e8	+e8	+e6	~	~	~	~										
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+*	+	+*	+	+	+	~	~	~	~	7.4		8.7		24.3		380			
	2	+*	+	+	+	+	+	~	~	~	~	7.2	7.9	9.1	8.1	25.0	24.3	381	365		
	3	e	e	+*	e*	e	e*	~	~	~	~	7.3	7.9	9.7	7.9	24.3	24.4	377	377		
	4	+e1	+e3	+e	+e3	+e3	+e3	~	~	~	~	7.5	8.0	10.0	8.2	25.0	24.4	380	369		
	5	e*	e	e2	e	e	e*	~	~	~	~	7.4	8.2	9.0	8.8	24.4	24.3	380	360		
	6	+e5	+e4	+e	+e5	+e2	+e1	~	~	~	~	7.7	7.8	9.8	8.4	24.2	24.6	380	368		
	7	+e	+e	+e2	+e	+3	+e	~	~	~	~	7.3	8.0	11.2	8.6	25.0	24.5	386	373		
	0	+	+	+	+	+	+	+	+	+	+										
	1							~	~	~	~										
	2							~	~	~	~										
	3							~	~	~	~										
	4							~	~	~	~										
	5							~	~	~	~										
	6							~	~	~	~										
	7							~	~	~	~										

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

entry error AS 6/28/12



(i) Baseline
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: C. M. H.

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: E1FC 9558

TEST SPECIES: Ceriodaphnia dubia

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

STARTING DATE/TIME: 5/25/12

YOUNG FROM: 5/25/11 1 < 24 hrs

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

TECHNICIANS: DAY: 0 1530am

1 9:30YBK 2 9:15 ACS 3 9:10 ACS 4 13:30YBK 5 12:20 ACS 6 8:45YBK 7 12:30YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
			0	+	+	+	+	+	+	+	+	+	+	8.1		9.5		24.4	
Scioto River water	1	+	+	+	+	+	+	*	*	*	*					24.4		663	
	2	+	+	+	+	+	+									25.0	24.3	678	622
	3	e	e	e	e	e	e									24.5	24.4	667	653
	4	+e5	+e5	+e6	+e5	+e6	+e6									24.8	24.4	668	628
	5	e	e1@	e9	e	e9	e									24.4	24.3	664	648
	6	+e15	+e17	+e	+e14	+e	+e9									24.0		659	654
	7	+e21	+e21	+e17	+e20	+e20	+e19									25.0	24.5	671	654
	0	+	+	+	+	+	+	+	+	+	+						24.5		673
	1							*	*	*	*								
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	0	+	+	+	+	+	+	+	+	+	+								
	1							*	*	*	*								
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



② 1.0µm filtration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EPEC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SP 5/11 <24 hrs

TECHNICIANS: DAY: 0 1545/12

1 9:50 YBK 2 925 ACS 3 825 ACS

4 14:20 YBK 5 1245 ACS

6 9:30 YBK 7 13:30 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	?	?	?	?	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	?	?	?	?	7.8		7.6		24.7		185	
	2	+	+	+	+	+	+	?	?	?	?	7.8	8.1	9.9	8.3	25.0	24.3	185	180
	3	e	e	e	e	e	e	?	?	?	?	7.6	8.0	10.8	7.9	24.2	24.4	183	184
	4	+e4	+e5	+e6	+e4	+e5	+e5	?	?	?	?	7.7	8.1	11.3	7.8	24.4	24.4	178	178
	5	e	e	e	e	e10	e10	?	?	?	?	7.7	8.1	9.3	8.7	24.4	24.3	182	174
	6	+e13	+e14	+e14	+e12	+e	+e	?	?	?	?	7.6	8.0	12.1	8.4	24.1	24.6	177	183
	7	+e14	+e17	+e13	+e19	+e8	+e4	?	?	?	?	7.7	8.0	11.7	8.6	25.0	24.5	186	182
12.5%	0	+	+	+	+	+	+	?	?	?	?		8.0		8.4		24.5		187
	1	+	+	+	+	+	+	?	?	?	?	7.8		8.3		24.2		211	
	2	+	+	+	+	+	+	?	?	?	?	7.8	7.9	8.1	8.1	25.0	24.3	209	209
	3	+	e	e	e	e	e	?	?	?	?	7.8	8.0	8.2	7.9	24.3	24.4	206	203
	4	+	+e2	+e3	+e4	+e4	+e5	?	?	?	?	7.9	8.0	9.3	8.0	24.1	24.4	203	201
	5	+e7	e	e	e	e	+e4	?	?	?	?	7.8	8.2	8.0	8.6	24.4	24.3	198	199
	6	+e	+e10	+e7	+e8	+e	+e	?	?	?	?	7.8	7.9	10.8	8.2	24.5	24.6	198	195
	7	+e14	+e15	+e15	+e10	+e14	+e11	?	?	?	?	7.9	8.0	8.6	8.5	25.0	24.5	206	198
25%	0	+	+	+	+	+	+	?	?	?	?		7.9		8.3		24.5		207
	1	+	+	+	+	+	+	?	?	?	?					24.4		236	
	2	+	+	+	+	+	+	?	?	?	?					25.0	24.3	233	232
	3	e	e	e*	+	e*	e	?	?	?	?					24.7	24.4	235	227
	4	+e4	+e3	+e6	+e	+e1	+e1	?	?	?	?					24.4	24.4	234	228
	5	e	e	e*	e6*	e10*	e7	?	?	?	?					24.4	24.3	226	224
	6	+e9	+e14	+e8	+e12	+e	+e	?	?	?	?					24.1	24.6	224	222
	7	+e10	+e17	+e13	+e	+e14	+e14	?	?	?	?					25.0	24.5	228	222

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



② 1.0µm filtration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Amr

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SP5/11 <24 hrs

TECHNICIANS: DAY: 0 1545

1 9:50 VIBK 2 925 ACS 3 825 ACS 4 14:20 VIBK 5 1245 ACS

6 9:30 VIBK 7 13:30 VIBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+													
	1	+*	+*	+*	+	+	+	+	+	+							24.2		282	
	2	+	+	+	+	+	+										25.0	24.3	287	273
	3	e	e	e*	e	e*	e*										24.7	24.4	282	281
	4	+e4	+e4	+e1	+e4	+e4	+e3										24.3	24.4	278	275
	5	e	e*	e*	e*	e*	e*										24.4	24.3	276	267
	6	+e9	+e13	+e13	+e9	+e	+e13										24.3	24.6	276	273
	7	+e13	+e16	+e16	+e19	+e17	+e14										25.0	24.5	282	272
100%	0	+	+	+	+	+	+										24.5		279	
	1	+	+	+	+	+	+	+	+	+							7.5		8.4	
	2	+	+	+	+	+	+										7.4	7.8	9.0	8.0
	3	e	e	e	e	e	e										7.4	7.9	9.8	7.8
	4	+e5	+e5	+e2	+e6	+e6	+e7										7.5	7.9	10.3	8.0
	5	e*	e*	e*	e*	e*	e*										7.5	8.0	8.8	8.6
	6	+e10	+e15	+e10	+e13	+e	+e12										7.5	7.8	11.1	8.2
	7	+e16	+e17	+e15	+e2	+e17	+e17										7.5	7.9	11.0	8.4
	0	+	+	+	+	+	+										7.8		8.1	
	1							+	+	+										
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



③C-18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Th

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EPC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: Dmw

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1500 KDM 1 0930 GMS 2 2100 S ACS 3 895 ACS 4 1335 ACS 5 1405 ACS 6 0915 KOM 7 1300 KOM

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
Dmw	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4
	5	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4	e4
	6	e12	e11	e12	e12	e13	e10	e12	e12	e12	e12	e12	e12	e12	e12	e12	e12	e12	e12	e12
	7	e17	e15	e17	e16	e18	e18	e18	e18	e18	e18	e18	e18	e18	e18	e18	e18	e18	e18	e18
12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	4	e5	e2	e	e2	e2	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	5	e	e	e6	e	e	e5	e	e	e	e	e	e	e	e	e	e	e	e	e
	6	e12	e12	e12	e10	e12	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	7	e17	e16	e	e16	e17	e15	e	e	e	e	e	e	e	e	e	e	e	e	e
25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3	e	e	+	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	4	e4	e5	e	e	e2	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	5	e	e	e6	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	6	e9	e12	e12	e	e12	e	e	e	e	e	e	e	e	e	e	e	e	e	e
	7	e17	e17	e	e	e14	e	e	e	e	e	e	e	e	e	e	e	e	e	e

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 entry error: ACS 5/30/12



③ C-18
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Luca

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 < 24 hrs

TECHNICIANS: DAY: 0 1500 RPM 1 0930 AM 2 1005 ACS 3 855 ACS 4 1335 ACS 5 1405 ACS 6 0915 AM 7 1300 KOM

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+	+	+	+	+					24.5		283		
	2	+	+	+	+	+	+	+	+	+	+					25.1	24.3	279	280	
	3	e*	e	e*	e*	e*	+	+	+	+	+					25.0	24.5	282	275	
	4	e7	e6	e3	e1	e2	e	+	+	+	+					25.0	24.3	283	272	
	5	e	e	e	e	e	e8	+	+	+	+					24.9	25.0	277	264	
	6	e10	e10	e10	e8	e9	e8	+	+	+	+					25.0	25.0	281	264	
	7	e12	e12	e12	e11	e13	e11	+	+	+	+					24.6	24.9	278	264	
100%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+	+	+	+	+	7.6		7.8		24.4		379		
	2	+	+	+	+	+	+	+	+	+	+	7.7	7.9	8.4	7.8	25.1	24.3	375	368	
	3	e*	e	e	e	e	e*	+	+	+	+	7.6	7.9	9.7	8.0	24.9	24.5	383	369	
	4	e4	e4	e6	e4	e5	e6	+	+	+	+	7.6	8.0	7.9	8.0	25.3	24.3	385	367	
	5	e*	e*	e9	e	e	e	+	+	+	+	7.6	8.0	9.0	8.2	24.8	25.0	379	359	
	6	e14	e	e	e11	e14	e11	+	+	+	+	7.6	7.9	10.5	8.4	25.0	24.9	385	362	
	7	e21	e19	e16	e17	e16	e16	+	+	+	+	7.6	8.0	11.3	8.4	24.6	24.9	379	364	
	0	+	+	+	+	+	+	+	+	+	+									
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(D) Aeration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curt

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1500 KDM 1 0930 CRT 21015 ACS 3905 ACS 4 1400 ACS 5 1435 ACS

6 1000 Kom 7 1300 Kom

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
DMW	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	e*	e	e	e	e	e*													
	4	e4	e5	e5	e6	e6	e3													
	5	e10	e14	e13	e	e	e11													
	6	e	e	e	e15	e15	e													
	7	e18	e17	e15	e15	e18	e11													
12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3	e*	e	e	e	e*														
	4	e1	e4	e3	e3	e														
	5	e5	e	e	e*	e4														
	6	e	e5	e3	e	e														
	7	e13	e13	e12	e	e														
25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3	e*	e	e*	e*	e*	e*													
	4	e5*	e3	e1	e3*	e3*	e3													
	5	e	e	e	e*	e	e													
	6	e5	e7	e7	e5	e8	e7													
	7	e10	e11	e9	e8	e9	e9													

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



④ Aeration
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cum Th

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 < 24 hrs

TECHNICIANS: DAY: 0 1500 KDM 1 0930 cm 2 1015 ACS 3 905 ACS 4 1400 ACS 5 1435 ACS 6 1000 KDM 7 1300 KDM

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+	+	+	+	+					24.8		283			
	2	+	+	+	+	+	+	+	+	+	+					25.1	24.3	286	279		
	3	e	e	e*	e*	e	e*									24.7	24.5	283	272		
	4	e3	e2	e	e	e4	e5									24.7	24.3	281	273		
	5	e*	e	e6	e2	e	e									24.6	25.0	272	275		
	6	e5	e9	e	e	e7	e10									25.0	24.9	281	262		
	7	e9	e11	e8	e8	e8	e10									25.0	24.9	281	268		
100%	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+	+	+	+	+	7.7		8.6		24.6		378			
	2	+	+	+	+	+	+	+	+	+	+	7.7	7.9	9.5	8.1	25.1	24.3	379	371		
	3	e	e*	e*	e*	e*	e					7.6	7.9	10.1	8.1	25.4	24.5	383	368		
	4	e	e*	e	e*	e	e					7.7	8.0	10.2	8.2	25.8	24.3	383	367		
	5	e3*	e3*	e*	e*	e3	e4					7.7	8.0	9.1	8.3	24.4	25.0	376	361		
	6	e4	e4	e4	e2	e	e5					7.6	7.9	11.2	8.5	25.0	24.9	385	364		
	7	e	e	e	e	e3	e					7.6	8.0	10.9	8.7	25.0	24.9	389	366		
	0	+	+	+	+	+	+														
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



⑤ EDTA

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Th

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EPC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 1 < 24 hrs

TECHNICIANS: DAY: 0 1815 cm

1: 10:45 YBK 2: 1055 ACS

3: 0930 cm 4: 1450 ACS

5: 1545 cm

6: 10:45 YBK 7: 1330 km

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
DMW	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(5) EDTA
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Amr Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EiFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR5/11 <24 hrs

TECHNICIANS: DAY: 0 1815cmr 1 10:45Vrk 2 1055 MS 3 0930cmr 4 1450 MS 5 1545cmr 6 10:45Vrk 7 1330Kmm

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+													
	1	+	+	+	+	+	+	+	+	+							24.2		279	
	2	+	+	+	+	+	+										25.0	24.3	273	278
	3	+	+	+	+	+	+										25.4	24.2	280	273
	4	+	+	+	+	+	+										25.0	25.0	279	265
	5	+	+	+	+	+	+										24.4	25.1	277	268
	6	+	+	+	+	+	+										25.0	24.3	278	267
	7	+	+	+	+	+	+										25.0	24.6	277	277
100%	0	+	+	+	+	+	+											24.5		280
	1	+	+	+	+	+	+	+	+	+			7.2		8.1		24.2		377	
	2	+	+	+	+	+	+						7.2	7.9	8.6	8.2	25.0	24.3	379	373
	3	+	+	+	+	+	+						7.2	8.0	9.1	8.1	26.0	24.2	385	370
	4	+	+	+	+	+	+						7.3	8.0	9.8	8.3	25.0	25.0	383	356
	5	+	+	+	+	+	+						7.5	8.0	8.7	8.3	24.5	25.1	381	360
	6	+	+	+	+	+	+						7.7	8.0	9.5	8.2	25.0	24.3	374	361
	7	+	+	+	+	+	+						7.5	8.0	10.4	8.7	25.0	24.6	379	378
	0	+	+	+	+	+	+							8.1		7.8		24.5		381
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



© NaThio

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SAS/11 < 24 hrs

TECHNICIANS: DAY: 0 1815cmr

1 11:20 YRK 2 1110 ACS 3 6230cmr 4 1505 ACS 5 1600cmr 6 11:30 YRK 7 1400 Kam

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+					New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+					7.7		9.7		24.6		211	
	2	+	+	+	+	+	+					7.6	8.0	9.7	8.2	25.0	24.3	213	207
	3	e	e	e	e	e	e					7.5	8.1	10.1	8.2	24.7	24.2	211	202
	4	e4	e5	e	e	e*	e4					7.6	8.0	10.4	8.3	25.0	25.0	207	198
	5	e	e	e6	e3	e5	e					7.7	8.1	9.7	8.2	24.4	24.1	212	203
	6	+e15	+e13	+e12	+e13	+e12	+e13					7.6	8.0	11.3	8.1	25.0	24.3	210	199
	7	e18	e16	e	e13	e	e15					7.7	8.0	10.4	8.6	25.0	24.6	213	210
12.5%	0	+	+	+	+	+	+						8.0		7.8		24.5		218
	1	+	+	+	+	+	+					7.8		8.4		24.1		217	
	2	+	+	+	+	+	+					7.8	8.0	8.0	8.1	25.0	24.3	215	219
	3	+	+	+	+	+	+					7.7	8.1	8.9	8.2	24.3	24.2	217	210
	4	e	e	e	e	e	e*					7.8	8.1	8.3	8.3	25.0	25.0	212	207
	5	e	e6	e2	e5	e4	e5					7.8	8.1	8.2	8.3	24.7	24.1	211	207
	6	+e4	+e	+e10	+e	+e11	+e					7.9	8.0	9.6	8.1	25.0	24.3	210	202
	7	e12	e12	e	e10	e	e9					7.9	8.1	8.5	8.5	25.0	24.6	212	211
25%	0	+	+	+	+	+	+						8.1		8.0		24.5		219
	1	+	+	+	+	+	+									24.2		252	
	2		+	+	+	+	+									25.0	24.3	251	253
	3		+	e	+	+	+									24.7	24.2	250	245
	4		e*	e	e	e										25.0	25.0	248	245
	5		e3	e4	e4	e2										24.8	24.1	245	234
	6		+e	+e5	+e4	+e5										25.0	24.3	244	231
	7		e9	e	e3	e										25.0	24.6	246	240

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

● ENTAY ERROR YRK 5-31-12



⑥ Nathan
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cum T

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EFC 9558

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: _____

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 5/25/12

YOUNG FROM: SR 5/11 <24 hrs

TECHNICIANS: DAY: 0 1815 CAT

1 11:20 YLK 2 11:10 ACS 3 0930 CAT 4 1505 ACS 5 1600 CAT

6 11:30 YBK 7 1400 KAM

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+*	+	+	+	+	+	+	+	+	+							24.3		314	
	2	+	x	+	+	+	+											25.0	24.3	316	310
	3	+	k	k	k	k	k											25.5	24.2	323	307
	4	k*	k	k	k	k	k*											25.0	25.0	312	301
	5	k3	k4	k5	k4	k5	k3											24.7	24.1	313	298
	6	tl	tl5	tl6	tl4	tl5	tl6											25.0	24.3	310	295
	7	de8	de8	de	de	de	de											25.0	24.6	317	316
100%	0	+	+	+	+	+	+	+	+	+	+								24.5		320
	1	+	+*	+	+	+	+	+	+	+	+	7.5		8.1				24.3		452	
	2	+	+	+	+	+	+	+	+	+	+	7.4	7.9	8.7	8.1	25.0	24.3	453	443		
	3	+	k	k	+	k	+	+	+	+	+	7.5	8.0	8.9	8.1	26.0	24.2	462	437		
	4	k*	k	k	k	k	k*					7.5	8.0	9.9	8.3	25.0	25.0	448	432		
	5	k3	k3	k4	k4	k5	k1					7.6	8.0	8.6	8.2	24.7	24.1	449	431		
	6	tl	tl@	tl9	tl1	tl	tl3					7.6	8.0	11.2	8.2	25.0	24.3	445	437		
	7	de3	de4	de	de5	de5	de					7.6	8.0	10.4	8.5	25.0	24.6	456	456		
	0	+	+	+	+	+	+	+	+	+								24.5		466	
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

APPENDIX B
DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST NO.	C. dubia	FHM
6/10	99	1.48	2.13
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
3/12	116	1.46	2.42
4/12	117	1.37	2.51
5/12	118	1.46	1.66
AVERAGE		1.30	2.17
STD. DEV.		0.22	0.35
RANGE: LOW		0.86	1.47
RANGE: HIGH		1.74	2.86
Coefficient of variation		0.17	0.16
Date of last test		5/3-10/12	5/22-29/12
MSD of most recent test		4.5	0.0635
PMSD of most recent test		13.8	21.5
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

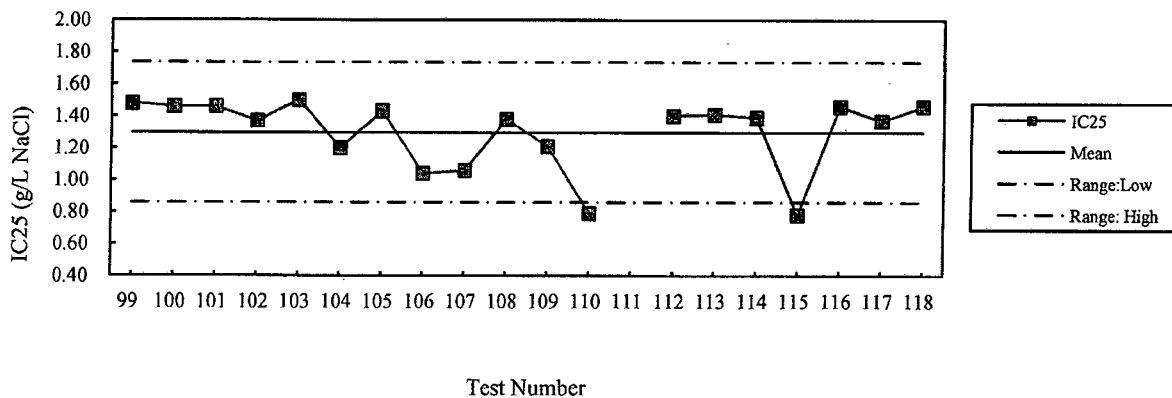
From EPA's Wet Interlaboratory Variability Study

Test Species	GLEC ¹	IC25 Coefficient of Variation National Percentiles ²				
		10th	25th	50th	75th	90th
C. dubia	0.17	0.08	0.17	0.27	0.45	0.62
P. promelas	0.16	0.12	0.21	0.26	0.38	0.45

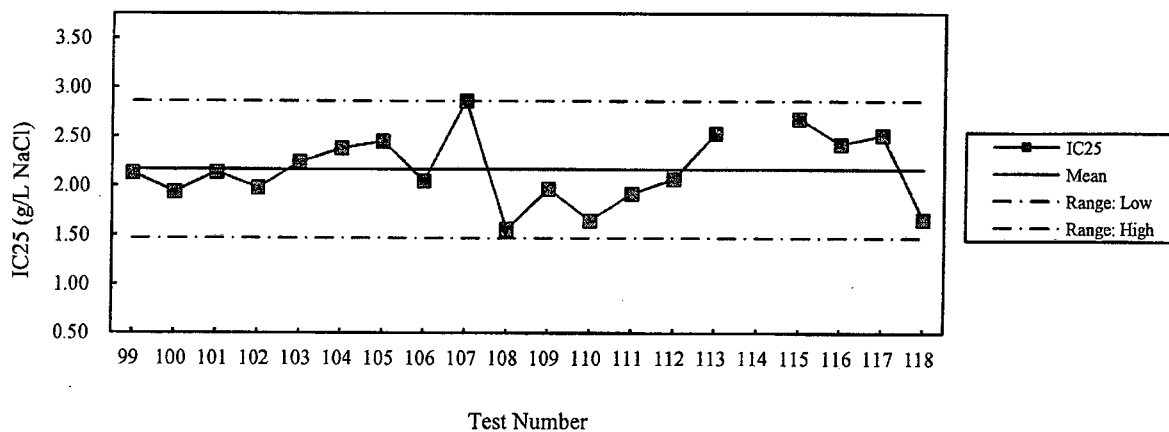
¹ Based on cumulative GLEC data from the most recent 20 tests.
² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

Chronic Reference Toxicant IC25
Ceriodaphnia dubia



Chronic Reference Toxicant IC25
Pimephales promelas





Great
Lakes
Environmental
Center

June 30, 2012

Applied
Environmental
Sciences
www.glec-online.com

Roland McDaniel, Project Manager
GEMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED JUNE 13, 2012 FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

Traverse City
Operations
739 Hastings St.
Traverse City
MI 49686

231 941-2230
231 941-2240 fax

Columbus
Operations
1295 King Ave.
Columbus
OH 43212

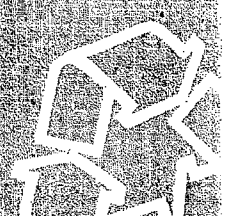
614 487-1040
614 487-1920 fax

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected June 13, 2012. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tarr
Laboratory Coordinator



PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED JUNE 13, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

JUNE 2012



Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled June 13, 2012 (Sample ID: EEC 9585) to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

Ceriodaphnia dubia Static Renewal Chronic Toxicity Tests

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for 6 days under static renewal

conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity 10-20 $\mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test.

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUC) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUC} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1).

The baseline toxicity test conducted concurrently with the TIE manipulations, using EDCC Outfall 001 sample collected June 13, 2012 (EEC 9585), had a LC_{50} value of >100 percent effluent and an IC_{25} value of >100 percent effluent or <1.0 TUC's (Table 1). **Based on the baseline toxicity test result (IC_{25} value of >100 percent) the EDCC Outfall 001 sample did not exhibit toxicity to *C. dubia* survival or reproduction and as**

a result the removal of toxicity by the five subsequent TIE treatments could not be determined.

1.0 µm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a Gelman A/E glass fiber filter (1.0 µm).

After filtration treatment, the *C. dubia* exhibited an IC₂₅ of >100 percent or <1.0 TUc (see Table 1).

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample after 1.0 µm filtration treatment was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

The *C. dubia* C-18 treatment test had an IC₂₅ of >100 percent or <1.0 TUc (see Table 1).

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

Compared to the baseline toxicity test, *C. dubia* reproduction after aeration treatment was lower which resulted in an IC₂₅ value of <100 percent or >1.0 TUc (see Table 1).

Cation Chelation with EDTA - Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test, *C. dubia* reproduction after EDTA treatment was reduced which resulted in an IC₂₅ of <100 percent or >1.0 TUc (see Table 1).

Sodium Thiosulfate Treatment – Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

After thiosulfate treatment, *C. dubia* reproduction was lower when compared to the baseline toxicity test and exhibited an IC₂₅ of <100 percent or >1.0 TUc (see Table 1).

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected June 13 2012 did not exhibit the presence of chronic toxicity to *C. dubia* reproduction in the baseline toxicity test and therefore the TIE treatments did not demonstrate the removal of toxicity (see Table 1 for a summary of all test results).

Table 1. Summary of Chronic TIE Test results

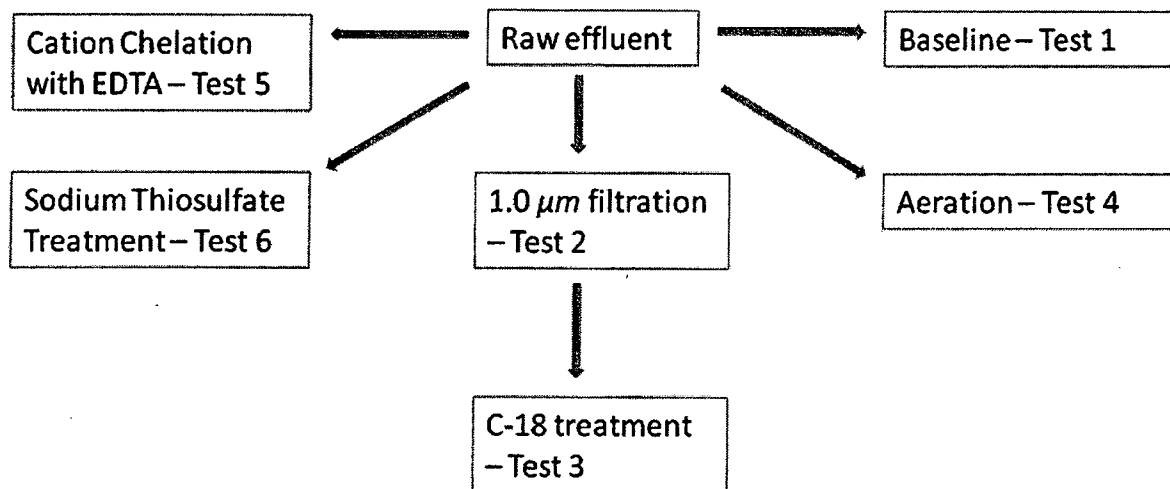
<i>C. dubia</i> Chronic TIE on EDCC Outfall 001 Sample Collected 6/13/12 GLEC Sample ID: EEC 9585 Test Dates: 6/15/12 – 6/21/12	Percent Survival								
	DMW	12.5%	25%	50%	100%	LC ₅₀			
Baseline Toxicity (No manipulation) – Test 1	100	100	100	100	100	>100			
1.0 µm Filtration – Test 2	100	83	100	100	100	>100			
C18-SPE Treatment – Test 3	100	100	100	100	100	>100			
Aeration – Test 4	100	100	100	100	100	>100			
Cation Chelation with EDTA – Test 5	83	100	100	100	100	>100			
Sodium Thiosulfate Treatment – Test 6	100	100	100	100	100	>100			
	Reproduction – Mean Number of Young per Adult								
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed	
Baseline Toxicity (No manipulation)– Test 1	33.6	39.2	31.5	27.2	27.0	>100	<1.0	NA	
1.0 µm Filtration – Test 2	36.5	38.5	34.2	36.3	41.5	>100	<1.0	NA	
C18-SPE Treatment – Test 3	38.8	41.3	41.5	41.7	41.0	>100	<1.0	NA	
Aeration – Test 4	42.7	37.3	31.5	26.5	22.3	<100	>1.0	NA	
Cation Chelation with EDTA – Test 5	36.0	32.7	31.2	24.7	17.5	<100	>1.0	NA	
Sodium Thiosulfate Treatment – Test 6	35.3	36.7	31.0	28.3	24.5	<100	>1.0	NA	

NA – Not applicable or Not available

^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC₂₅ value for the most recent *C. dubia* reference toxicant test was 1.46 g/L of sodium chloride which was within the acceptance range of 0.86 to 1.74 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency. Duluth, MN.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED JUNE 13, 2012



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: EL DORADO CHEMICAL

PROJECT NUMBER: 2179-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>6/14/12</u>	INITIALS				
EEC NUMBER		EEC <u>9585</u>			
OUTFALL/DESCRIPTION		<u>outfall 601</u>			
DISSOLVED OXYGEN (mg/L)	<u>BSL</u>	<u>10.6</u>			
TEMPERATURE (°C)	<u>CR</u>	<u>2.0</u>			
pH	<u>BSL</u>	<u>8.1</u>			
CONDUCTIVITY (µmhos/cm)	<u>BSL</u>	<u>272</u>			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>6/14/12</u>	INITIALS				
EEC NUMBER		<u>9585</u>			
OUTFALL/DESCRIPTION		<u>outfall 001</u>			
DISSOLVED OXYGEN (mg/L)	<u>CR</u>	<u>8.2</u>			
TEMPERATURE (°C)	<u>CR</u>	<u>24.5</u>			
pH	<u>CR</u>	<u>7.9</u>			
CONDUCTIVITY (µmhos/cm)	<u>CR</u>	<u>361</u>			
HARDNESS (mg/L CaCO ₂)	<u>CMV</u>	<u>1.1 x 40 = 44</u>			
ALKALINITY (mg/L CaCO ₂)	<u>CMV</u>	<u>2.6 x 40 = 104</u>			
TOTAL CHLORINE (mg/L)*					
TOTAL AMMONIA (mg/L)*					

*Check with project manager to see if necessary



Great Lakes Environmental Center
 1295 KING AVE.
 COLUMBUS, OH 43212
 PHONE: (614) 487-1040
 FAX: (614) 487-1920

Two Important Notes for Whole Effluent Toxicity Testing:

- There is a **maximum** hold time for all samples of 36 hours (Hold time begins when sample is taken off the sampler)
- Samples must be received at 4°C ± 2°C

CHAIN OF CUSTODY FORM

(TO BE COMPLETED ONSITE AND SUBMITTED WITH SAMPLES)

FACILITY: El Dorado Chemical Co.
 LOCATION: El Dorado, AR 71730
 CONTACT PERSON: Larken Pennington
 PHONE: 870-863-1400

COLLECTOR: Larken Pennington
 DATE: 6-13-12
 WITNESS: Brent Parker
 DATE: _____

ECC# (lab only)	SAMPLE ID	SAMPLE SOURCE (Eff/Upstr.)	TYPE (grab or composite)	SAMPLE START DATE	SAMPLE START TIME (24-hr notation)	SAMPLE END DATE	SAMPLE END TIME (24-hr notation)	VOLUME COLLECTED	SAMPLE CONTAINER	SAMPLE COLLECTOR	OTHER COMMENTS
9585	001		grab	6/13/12	8:30	6/13/12		4 cubitainers	Cubitainer	L. Pennington	

ANALYSIS REQUIRED: Please fill in completely

NAME OF STREAM SAMPLED: _____

Species: *Ceriodaphnia dubia*

Pimephales promelas (fathead minnows)

Other - please specify: _____

Test Type:

Acute: 24-hour
 48-hour

Acute: 24-hour 48-hour

96-hour: with 48-hour renewal
 without 48-hour renewal

Chronic (7-day)

Chronic (7-day)

Other - please specify: _____

Dilutions: Screen (100% only)

Definitive (5 sample concentrations): List test concentrations: _____

Dilution Water: Receiving Water

Lab water

Other - please specify: _____

TRANSFER OF SAMPLES:

(FIRST SIGNATURE IS SAMPLER, LAST SIGNATURE IS AUTHORIZED LABORATORY REPRESENTATIVE)

SHIPPER
 1. Larken Pennington

RECEIVER
 DATE TIME

2. [Signature] 6/14/12 1045

For Lab Use Only:
~~Ice~~ remaining in cooler upon receipt
 Temperature of samples when received:
2.0

FOR SATURDAY DELIVERY??? **MARK PACKAGE AS SUCH AND CALL GLEC ON FRIDAY WITH TRACKING NUMBER**

Parental Blockage Map for *C. dubia*

Date: 6/15/12

Time Neonates Pulled: 1600

Source Board: SR 6/8/12 (7 days)

Initials: CAJ

Estimated Age Range of *C. dubia* neonates: 16-24 hrs

Name and Project # neonates used for: EACC Wound TIE (9585) Tests 1+2

	1	2	3	4	5	6	7	8	9	10
6										
5										
4										
3										
2			R5	R6						
1						R1	R2		R3	R4

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 6/15/12

Time Neonates Pulled: 1600

Source Board: SR 6/8/12 (7 days old)

Initials: CAJ

Estimated Age Range of *C. dubia* neonates: 16-24 hrs

Name and Project # neonates used for: EDCC Chronic TIE (9585) Tests 3+4

	1	2	3	4	5	6	7	8	9	10
6										
5										
4	R3		R4		R5					R6
3	R2									
2								R1		
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 6/15/12

Time Neonates Pulled: 1600

Source Board: SR 6/8/12 (7 days)

Initials: CAT

Estimated Age Range of *C. dubia* neonates: 16-24 hrs

Name and Project # neonates used for: EDCC Chronic TIE (9585) Tests 5+6

	1	2	3	4	5	6	7	8	9	10
6										
5	R1			R2			R3	R4	R5	R6
4										
3										
2										
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

EEC 9585

Test Dates: 6/15-21/12

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	100%	100%	100%	100%	100%
1.0 µm filtration (Test 2)	100%	83%	100%	100%	100%
C-18 SPE treatment (Test 3)	100%	100%	100%	100%	100%
Aeration (Test 4)	100%	100%	100%	100%	100%
EDTA 25 mg/l (Test 5)	83%	100%	100%	100%	100%
NaThio (50 mg/l) (Test 6)	100%	100%	100%	100%	100%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	33.6	39.2	31.5	27.2	27.0	>100	<1.0	--
1.0 µm filtration (Test 2)	36.5	38.5	34.2	36.3	41.5	>100	<1.0	NA
C-18 SPE treatment (Test 3)	38.8	41.3	41.5	41.7	41.0	>100	<1.0	NA ^b
Aeration (Test 4)	42.7	37.3	31.5	26.5	22.3	<100	>1.0	NA
EDTA (25 mg/l) (Test 5) ^a	36.0	32.7	31.2	24.7	17.5	<100	>1.0	NA
NaThio (50 mg/l) (Test 6)	35.3	36.7	31.0	28.3	24.5	<100	>1.0	NA

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to *C.dubia* reproduction in DMW

b - additional toxicity removed by C-18 treatment after filtration treatment.

Baseline (Test 1)
El Dorado Chemical Outfall 001 Sampled 6/13/12 (EEC 9585)
(Tested 6/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

Scito River Water 2° Control	6
	0
	100.00%

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	37	37	33	32	28
2		35	33	24	23
3	42	38	30	29	24
4	36	40	31	31	31
5	40	40	33	24	28
6	13	45	29	23	28
N	5	5	6	6	6
MEAN	33.6	39.2	31.5	27.2	27.0
SD	11.760102	3.43025752	1.7606817	3.9707262	2.9664794
CV	35.000304	8.75810431	5.5894657	14.61617	10.986961
Total Young	168	235	189	163	162

Scito River 2° Control	29
	29
	45
	39
	44
	29
	5
	35.8
	7.7567175
	21.646654
	215

1.0 µm filtration (Test 2)

El Dorado Chemical Outfall 001 Sampled 6/13/12 (EEC 9585)
(Tested 6/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	5	6	6	6
DEAD	0	1	0	0	0
% SURV	100.00%	83.33%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	24	42	38	32	46
2	35	40	25	39	37
3	44	35	34	39	38
4	36	34	36	23	42
5	38	46	35	41	45
6	42	34	37	44	41
N	6	6	6	6	6
MEAN	36.5	38.5	34.2	36.3	41.5
SD	7.0356236	4.96990946	4.708149	7.6332606	3.6193922
CV	19.275681	12.9088557	13.779948	21.008974	8.721427
Total Young	219	231	205	218	249

C-18 SPE treatment (Test 3)
El Dorado Chemical Outfall 001 Sampled 6/13/12 (EEC 9585)
(Tested 6/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	36	46	42	41	41
2	40	39	42	45	43
3	41	37	38	41	40
4	38	38	41	37	38
5	42	46	42	46	42
6	36	42	44	40	42
N	6	6	6	6	6
MEAN	38.833333	41.3	41.5	41.7	41.0
SD	2.5625508	3.98329847	1.9748418	3.32666	1.7888544
CV	6.5988433	9.63701242	4.7586549	7.983984	4.3630595
Total Young	233	248	249	250	246

Aeration (Test 4)
El Dorado Chemical Outfall 001 Sampled 6/13/12 (EEC 9585)
(Tested 6/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	40	35	29	30	22
2	39	38	31	25	23
3	46	39	33	27	24
4	48	36	34	26	18
5	42	35	34	26	24
6	41	41	28	25	23
N	6	6	6	6	6
MEAN	42.7	37.3	31.5	26.5	22.3
SD	3.5590261	2.42212028	2.5884358	1.8708287	2.2509257
CV	8.3414674	6.48782219	8.2172566	7.0597309	10.078772
Total Young	256	224	189	159	134

EDTA 25 mg/l (Test 5)
El Dorado Chemical Outfall 001 Sampled 6/13/12 (EEC 9585)
(Tested 6/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	5	6	6	6	6
DEAD	1	0	0	0	0
% SURV	83.33%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	45	36	33	28	23
2	37	32	28	27	17
3	37	34	31	24	16
4	19	35	31	20	21
5	44	22	32	22	8
6	34	37	32	27	20
N	6	6	6	6	6
MEAN	36.0	32.7	31.2	24.7	17.5
SD	9.3808315	5.50151494	1.7224014	3.204164	5.3197744
CV	26.057865	16.8413723	5.5264217	12.989854	30.398711
Total Young	216	196	187	148	105

NaThio (50 mg/l) (Test 6)
El Dorado Chemical Outfall 001 Sampled 6/13/12 (EEC 9585)
(Tested 6/15-21/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	33	39	13	31	16
2	39	33	35	30	25
3	15	35	36	21	27
4	36	31	32	29	26
5	46	43	36	29	29
6	43	39	34	30	24
N	6	6	6	6	6
MEAN	35.3	36.7	31.0	28.3	24.5
SD	11.00303	4.45720391	8.9442719	3.6696957	4.5055521
CV	31.140651	12.1560107	28.85249	12.951867	18.390009
Total Young	212	220	186	170	147



① BASELINE

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585TEST SPECIES: Ceriodaphnia dubiaDILUTION WATER: DMWPHOTOPERIOD (L:D): 16:8PROJECT NO.: 9179-00ANIMALS/CONC: 10 /CHAMBER: 1LIGHT INTENSITY (LUX): 500-1000TEMPERATURE (°C): 25 ± 1 °CSTARTING DATE/TIME: 6/15/12YOUNG FROM: SR 6/18/12 1 < 24 hrsTECHNICIANS: DAY: 0 1800 GWT 1 825 ACS 2 1030 Kom 3 8:30 YBK 4 1410 ACS 5 8:40 YBK 6 9:00 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.8		8.1		24.5		177	
	1	+	+	+	+	+	+					8.0	7.9	7.9	7.8	25.0	24.7	176	182
	2	te	te	te	te	te	te					7.8	7.9	8.1	7.7	24.5	24.9	171	179
	3	+e7	+e8	+e9	+e10	+e11	+e12					8.0	7.9	8.1	8.4	25.0	24.9	188	184
	4	te	te	te	te	te	te					7.6	7.8	8.0	8.2	24.7	24.8	179	181
	5	+e11	+e12	+e13	+e14	+e15	+e16					7.8	7.8	8.3	8.2	25.0	24.9	191	187
	6	+e19	+e20	+e21	+e22	+e23	+e24					7.8	7.8	8.0	8.1	25.0	24.9	188	186
	7																		
12.5 %	0	+	+	+	+	+	+	+	+	+	+	7.8		8.1		24.5		202	
	1	+	+	+	+	+	+					8.0	8.0	8.1	7.9	25.0	24.7	201	205
	2	te	te	te	te	te	te					7.8	8.0	8.2	7.7	24.5	24.9	197	197
	3	+e7	+e8	+e9	+e10	+e11	+e12					8.0	8.1	8.3	8.4	25.0	24.9	205	199
	4	te	te	te	te	te	te					7.7	7.9	8.1	8.2	24.3	24.8	201	197
	5	+e12	+e13	+e14	+e15	+e16	+e17					7.9	7.9	8.5	8.3	25.0	24.9	208	202
	6	+e20	+e21	+e22	+e23	+e24	+e25					7.9	7.9	8.1	8.0	25.0	24.9	209	199
	7																		
25 %	0	+	+	+	+	+	+	+	+	+	+					24.5		228	
	1	+	+	+	+	+	+									25.0	24.7	222	228
	2	te	te	te	te	te	te									24.5	24.9	220	220
	3	+e7	+e8	+e9	+e10	+e11	+e12									25.0	24.9	228	218
	4	te	te	te	te	te	te									24.6	24.8	225	215
	5	+e11	+e12	+e13	+e14	+e15	+e16									25.0	24.9	229	222
	6	+e15	+e16	+e17	+e18	+e19	+e20									25.0	24.9	232	222
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

ENTAY 2 PPD 4 BK 6-18-12



① Baseline DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 6/27/12

TEST MATERIAL: EEL 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1800 AM 1 825 AGS 2 1030 AM 3 8:30 YBK 4 1410 ACS 5 8:40 YBK 6 9:00 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
50%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+										24.5		265
	2	de	+	de	de	de	de										25.0	24.7	261
	3	+e7	+e3	+e6	+e7	+4	+e6										24.5	24.9	269
	4	te	te	te	te	te	te										25.0	24.9	274
	5	+e10	+e8	+e8	+e10	+e8	+e8										24.6	24.9	266
	6	+e15	+e13	+e15	+e14	+e12	+e9										25.0	24.9	275
	7																25.0	24.9	278
100%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+										7.9		8.2
	2	de	de	de	de	de	de										8.0	8.1	8.9
	3	+e7	+e6	+e5	+e5	+e6	+e7										7.8	8.1	9.6
	4	te	te	te	te	te	te										7.9	8.1	11.3
	5	+e9	+e8	+e7	+e8	+e8	te										7.9	8.1	8.7
	6	+e12	+e9	+e12	+e18	+e14	+e13										8.0	8.1	10.0
	7																8.0	8.0	10.1
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ENTRY ERROR YBK 6-20-12



DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: AmT

Date: 6/27/12

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1800 chr

1 825 ACS 2 1030 AM 3 8:30 YBK 4 1110 ACS 5 8:40 YBK 6 9:00 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
SR	0	+	+	+	+	+	+	+	+	+	+										
	1	+e	+e	+e	+e	+e	+e									24.5		647			
	2	+e	+e	+e	+e	+e	+e									25.0	24.7	646	639		
	3	+e	+e	+e	+e	+e	+e									24.5	24.9	653	623		
	4	+e	+e	+e	+e	+e	+e									25.0	24.9	640	619		
	5	+e	+e	+e	+e	+e	+e									24.3	24.8	639	625		
	6	+e	+e	+e	+e	+e	+e									25.0	24.9	651	617		
	7															25.0	24.9	644	654		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



② 1.0 µm Pressure Filtration at ambient pH

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tu

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 9179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1000 am

1 830 ACS 2 1045/10m 3 8:30 YBK 4 1140 ACS 5 9:15 YBK 6 11:15 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.9		7.4		21.6		178	
	1	+	+	+	+	+	+					7.9		7.4		21.6		178	
	2	de	de	de	de	de	de					7.8	8.0	8.1	7.9	25.2	24.7	183	186
	3	+e	+e7	+e6	+e4	+e6	+e7					7.8	8.0	9.3	7.7	24.5	24.9	185	191
	4	+e5	e	+e16	+e11	e	+e13					7.8	8.0	9.7	8.3	25.0	24.9	182	181
	5	+e9	+e12	+e	+e	+e11	+e					7.7	8.0	8.9	8.3	24.5	24.8	187	179
	6	+e10	+e16	+e22	+e21	+e21	+e22					7.7	7.9	10.6	8.4	25.0	24.9	180	176
	7											7.7	7.9	10.6	8.0	25.0	24.9	184	185
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		7.7		24.6		202	
	1	+	+	+	+	+	+					7.8		7.7		24.6		202	
	2	de	de	de	de	de	de					8.0	8.0	8.2	7.9	25.2	24.7	203	205
	3	+e2	+e4	+e5	+e6	+e8	+e2					7.9	8.0	8.2	7.7	24.5	24.9	204	203
	4	+e	+e13	+e7	+e12	e	+e12					8.0	8.0	8.5	8.3	25.0	24.9	205	197
	5	+e15	+e	+e	+e	+e16	+e					7.8	8.0	8.4	8.4	24.5	24.8	203	198
	6	+e25	+e23	+e23	+e16	+e22	+e20					7.9	8.0	8.7	8.3	25.0	24.9	205	197
	7											7.9	7.9	8.5	8.1	25.0	24.9	206	206
25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+									24.6		219	
	2	de	de	de	de	de	de									25.0	24.7	223	227
	3	+e6	+e5	+e5	+e6	+e6	+e6									24.5	24.9	224	221
	4	+e	e	+e6	e	+e8	+e12									25.0	24.9	226	216
	5	+e10	+e	+e	+e11	+e	+e									24.4	24.8	226	218
	6	+e22	+e20	+e23	+e19	+e21	+e19									25.0	24.9	230	218
	7															25.0	24.9	225	225

Key: ++ = live

- = dead

e = eggs present

▲ = possible male daphnid

■ = confirmed male daphnid

* = daphnid is erratic, pale, etc.

d = dead babies present (not counted)

@ = daphnid was killed or mishandled, which may affect survival

S = split brood, attached number indicates how many were observed

▲ fungus on the dead CERIO YBK 6-21-12



DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

② 1.0µm Filtrate

Reviewed by: Lin Tu

Date: 6/25/12

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 < 24 hrs

TECHNICI/NS: DAY: 0 1800 AM

1 830 ACS

2 1045 Kom

3 8:30 VSK

4 1440 ACS

5 9:15 VSK

6 11:15 VSK

7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.								
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old							
50 %	0	+	+	+	+	+	+	+	+	+	+															
	1	+	+	+	+	+	+	+	+	+	+							24.6		269						
	2	e	e	e	e	e	e											25.0	24.7	261	266					
	3	+e6	+e5	+e4	+e4	+e6	+e5											24.5	24.9	268	262					
	4	e10	e14	e	e8	e15	e18											25.0	24.9	249	253					
	5	+e	+e	+e14	+e	+e	+e											24.3	24.8	269	258					
	6	+e16	+e20	+e21	+e11	+e20	+e21											25.0	24.9	275	259					
	7																	25.0	24.9	275	257					
100 %	0	+	+	+	+	+	+	+	+	+	+															
	1	+	+	+	+	+	+	+	+	+	+															
	2	e	e	e	e	e	e																			
	3	+e6	+e5	+e6	+e6	+e6	+e4											8.0	8.1	8.4	7.8	25.0	24.7	362	351	
	4	e	e	e	e	e	e15											7.9	8.1	8.6	7.9	24.5	24.9	356	350	
	5	+e15	+e13	+e13	+e16	+e17	+e											8.0	8.1	9.7	8.3	25.0	24.9	367	340	
	6	+e25	+e19	+e19	+e20	+e22	+e22											7.9	8.0	9.0	8.4	24.2	24.8	359	346	
	7																	7.8	8.0	10.4	8.3	25.0	24.9	367	374	
	0	+	+	+	+	+	+	+	+	+	+															
	1																									
	2																									
	3																									
	4																									
	5																									
	6																									
	7																									

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



③ 1.0 μm PF → C18

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Lu Tr D. 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 9179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1800 AM 18:50 VRK 2 1100 AM 3 850 AM 4 14:30 VRK 5 10:00 VRK 6 1130 AM 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+	7.9		6.0		24.5		181	
	2	de	de	de	de	de	de					7.8	8.0	7.4	7.9	25.0	24.9	185	180
	3	te6	te	te	te	te	te					7.8	8.1	8.7	7.8	24.5	24.9	177	189
	4	te	te6	te7	te3	te5	te6					7.7	7.8	9.7	7.7	24.7	24.3	179	178
	5	te10	te14	te14	te19	te14	te13					7.6	8.0	8.8	8.5	25.0	24.6	183	183
	6	te20	te20	te20	te23	te23	te17					7.6	7.9	10.2	8.4	25.0	24.9	181	178
	7											7.7	7.8	10.6	7.7	24.7	24.9	179	194
12.5%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+	+	+	+	+	7.9		7.6		24.5		202	
	2	de	de	de	de	de	de					8.0	8.1	7.7	7.8	25.0	24.9	198	202
	3	te8	te	te	te	te7	te					7.9	8.1	8.2	7.9	24.5	24.9	202	202
	4	te	te5	te6	te5	te	te7					7.9	7.8	9.2	8.0	24.5	24.3	198	196
	5	te15	te14	te13	te19	te16	te16					7.7	8.1	8.2	8.4	25.0	24.6	207	196
	6	te23	te20	te18	te21	te23	te19					7.8	8.1	8.5	8.4	25.0	24.9	203	199
	7											7.9	7.9	8.4	7.7	24.7	24.9	209	206
25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+	+	+	+	+					24.5		222	
	2	de	de	de	de	de	te									25.0	24.9	221	221
	3	te7	te	te	te5	te7	te									24.5	24.9	224	219
	4	te	te7	te8	te	te	te7									24.8	24.3	223	214
	5	te14	te15	te13	te15	te14	te15									25.0	24.6	227	217
	6	te21	te20	te17	te21	te21	te22									25.0	24.9	230	218
	7															24.7	24.9	234	226

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



(3) C-18
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: [Signature] Date: 6/27/12

TEST MATERIAL: EEC 9585
 PROJECT NO.: 9179-00
 STARTING DATE/TIME: 6/15/12
 TECHNICIANS: DAY: 0 1800 gm

TEST SPECIES: Ceriodaphnia dubia
 ANIMALS/CONC: 10 /CHAMBER: 1
 YOUNG FROM: SR 6/18/12 1 < 24 hrs

DILUTION WATER: DMW
 LIGHT INTENSITY (LUX): 500-1000

PHOTOPERIOD (L:D): 16:8
 TEMPERATURE (°C): 25 ± 1°C

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50 %	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+									24.5		267			
	2	de	de	de	de	de	de									25.0	24.9	267	260		
	3	te 8	* 8	* 8	* 8	* 7	* 8									24.5	24.9	264	263		
	4	te	te	te 7	te 5	te	te 3									25.3	24.3	269	257		
	5	te 14	te 15	te 11	te 12	te 14	te 14									25.0	24.6	273	257		
	6	te 19	te 22	te 23	te 20	te 25	te 23									25.0	24.9	275	258		
	7															24.7	24.9	271	265		
100	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+					8.1		5.2		24.5		356			
	2	de	de	de	de	de	de					8.0	8.2	7.3	7.9	25.0	24.9	356	341		
	3	te 6	* 6	* 6	* 5	* 7	* 6					7.9	8.2	8.9	7.9	24.5	24.9	352	346		
	4	te	te 5	te 3	te	te	te 8					7.9	8.0	9.9	8.0	25.3	24.3	359	341		
	5	te 14	te 15	te 12	te 13	te 14	te 15					7.8	8.2	8.9	8.6	25.0	24.6	364	334		
	6	te 21	te 23	te 25	te 20	te 21	te 19					7.8	8.1	10.1	8.4	25.0	24.9	358	346		
	7											7.8	8.0	10.3	7.7	24.7	24.9	363	346		
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



④ Aeration at ambient pth
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: lm Date: 6/27/12

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 <24 hrs

TECHNICIANS: DAY: 0 1800 GY

1 9:00 UBB 2 1115 KDM 3 915 ACS 4 14:30 YBK 5 10:45 YBK 6 1145 KDM 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
DMW	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+					7.8		8.1		24.5		180			
	2	de	de	de	de	de	de					7.8	8.1	8.8	8.1	25.0	24.9	179	181		
	3	te5	te	te8	te5	te5	te					7.7	8.1	9.5	8.0	24.5	24.9	179	180		
	4	te	te5	te	te	te	te5					7.8	8.0	10.3	8.0	24.3	24.3	178	174		
	5	te12	te12	te14	te17	te12	te13					7.7	8.2	8.7	8.6	25.0	24.6	183	174		
	6	te23	te22	te24	te26	te25	te23					7.7	8.0	10.2	8.4	25.0	24.9	183	182		
	7											7.7	7.9	10.8	7.9	25.2	24.9	184	181		
12.5%	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+					7.8		8.1		24.5		203			
	2	te	te	te	te	te	te					8.0	8.1	8.3	8.0	25.0	24.9	201	199		
	3	te4	te	te4	te4	te5	te6					7.9	8.2	8.2	8.1	24.5	24.9	199	199		
	4	te	te6	te33	te	te	te					8.0	8.0	8.3	8.1	24.8	24.3	204	195		
	5	te13	te14	te12	te12	te12	te15					7.8	8.2	8.2	8.6	25.0	24.6	204	198		
	6	te18	te18	te20	te20	te18	te20					7.9	8.0	8.6	8.3	25.0	24.9	205	199		
	7											7.9	8.0	8.6	7.9	25.2	24.9	208	203		
25%	0	+	+	+	+	+	+														
	1	+	+	+	+	+	+									24.5		228			
	2	te	te	te	te	te	te									25.0	24.9	219	225		
	3	te6	te	te4	te6	te5	te									24.5	24.9	228	224		
	4	te	te7	te	te	te	te6									25.1	24.3	226	218		
	5	te8	te9	te13	te10	te10	te9									25.0	24.6	228	220		
	6	te8	te5	te6	te18	te19	te13									25.0	24.9	229	224		
	7															25.2	24.9	229	229		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 Entry Error 6-21-12 km



(4) Iteration

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curtin

Date: 6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/13/12 1 <24 hrs

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

TECHNICIANS: DAY: 0 1800am

1 9:00 YB/ 2 11:5 AM 3 9:15

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

ACS 4 14:30 YB/ 5 10:45 YB/ 6 11:45 AM 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+					27.5				265	
	2	de	de	de	de	de	de									25.0	24.9			268	267
	3	te6	te	te6	te4	te4	te									24.5	24.9			268	263
	4	te	te5	te	te	te	te6									24.5	24.3			269	259
	5	te9	te8	te9	te9	te9	te7									25.0	24.6			275	260
	6	te15	te12	te12	te13	te13	te12									25.0	24.9			276	267
	7															25.2	24.9			277	264
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	8.6		8.2		27.5				377	
	2	de	de	de	de	de	de					8.0	8.2	8.9	8.0	25.0	24.9			365	350
	3	te	te	te	te	te	te					7.9	8.2	9.6	8.0	24.5	24.9			364	350
	4	te4	te6	te4	te4	te6	te5					7.9	8.1	10.2	8.2	24.6	24.3			364	342
	5	te8	te8	te9	te7	te8	te8					7.9	8.1	9.6	8.9	25.0	24.6			366	357
	6	te10	te9	te11	te7	te10	te10					7.8	8.1	10.2	8.6	25.0	24.9			371	354
	7											7.8	8.1	10.7	8.0	25.2	24.9			371	360
	0	+	+	+	+	+	+	+	+	+											
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



EDTA Addition

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: cmh

6/28/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 9179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: 5/1 6/15/12 < 24 hrs

TECHNICIANS: DAY: 0 1800cmh

1 955 ACS 2 1130 Kom 3 0900 Kom 4 1430 Kom 5 11:45 YBK 6 11:50 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
DMW	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+													
	2	de	de	de	de	de	de													
	3	de	de3	de3	de4	de	de													
	4	de10	de	de13	de	de8	de10													
	5	+e14	+e14	+e	+e15	+e16	+e12													
	6	+e21	+e20	+e21		+e20	+e16													
	7				↓															
12.5%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+													
	2	de	de	de	de	de	de													
	3	de	de2	de4	de6	de	de													
	4	de7	de12	de	de10	de4	de6													
	5	+e12	+e	+e11	+e	+e18	+e12													
	6	+e17	+e18	+e19	+e19	+e	+e19													
	7																			
25%	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1	+	+	+	+	+	+													
	2	de	de	de	de	de	de													
	3	de	de4	de5	de6	de	de													
	4	de4	de8	de9	de8	de4	de6													
	5	+e12	+e	+e	+e	+e10	+e11													
	6	+e17	+e16	+e17	+e17	+e18	+e15													
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



GLEC @ EDTA

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: C. M. T.

Date: 6/28/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 9179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 6/8/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1800 CMT

1 855 ACS 2 1130 KOM 3 0900 KOM 4 1430 KOM 5 11:45 YBK 6 11:50 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+					24.2				263	
	2	de	de	de	de	+	+									25.0	24.7			268	258
	3	de	de ³	de ⁴	de ³	de	de									24.5	24.9			270	264
	4	de ⁴	de ¹⁰	de	de ⁶	de ⁵	de ⁷									24.8	25.0			267	259
	5	+e ¹¹	+e	+e ⁵	+e	+e	+e ⁹									25.1	24.7			272	261
	6	+e ¹³	+e ¹⁴	+e ¹⁵	+e ¹¹	+e ¹⁷	+e ¹¹									25.0	24.9			277	261
	7																24.9				266
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	7.7		8.1		24.2				361	
	2	de	de	de	de	de	de					7.7	8.1	8.6	8.2	25.0	24.7			361	347
	3	de	de	de ²	de ⁴	de	de					7.7	8.3	9.1	8.3	24.5	24.9			360	349
	4	de ⁵	de ⁶	de	de	de ⁴	de ⁴					7.6	8.1	9.8	8.1	24.8	25.0			363	344
	5	+e ⁸	+e	+e ⁸	+e ⁸	+e ⁴	+e ⁵					7.7	8.3	9.2	8.3	25.1	24.7			368	347
	6	+e ¹⁰	+e ¹¹	+e ⁶	+e ⁹	+e	+e ¹¹					7.7	8.1	9.4	9.6	25.0	24.9			371	352
	7												8.0		7.9		24.9				354
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



Sodium Thiosulfate Addition

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr

6/27/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: 586/18/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1800 cur

1 905 ACS 2 1145 Kom 3 0940 Kom 4 1500 Kom 5 12:154 BK 6 12:204 BK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+					7.8		7.5		24.2		208	
	2	de	de	de	de	de	de					7.8	8.1	8.8	8.2	25.0	24.7	211	205
	3	de	de4	de2	de5	de	de6					7.8	8.2	9.3	8.2	24.5	24.9	211	211
	4	de	de14	de	de9	de7	de					7.8	8.0	9.9	8.0	24.8	25.0	210	206
	5	de13	de	de11	de	de17	de15					7.7	8.1	8.9	8.3	25.1	24.7	215	210
	6	de20	de21	de12	de22	de22	de22					7.7	8.0	9.4	8.5	25.0	24.9	220	209
	7												7.9		7.9		24.9		212
12.5%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+					7.8		7.9		24.2		220	
	2	de	de	de	de	de	de					8.0	8.1	8.4	8.7	25.0	24.7	209	211
	3	de	de4	de4	de3	de	de6					8.0	8.2	8.6	8.0	24.5	24.9	217	212
	4	de8	de9	de12	de10	de8	de13					8.0	8.0	8.4	8.0	24.8	25.0	213	207
	5	de12	de	de	de	de14	de					7.8	8.1	8.6	8.3	25.1	24.7	219	206
	6	de19	de20	de19	de18	de21	de20					7.8	8.0	8.3	8.5	25.0	24.9	216	211
	7												7.8		7.8		24.9		212
25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+									24.2		241	
	2	de	de	de	de	de	de									25.0	24.7	243	240
	3	de	de7	de8	de5	de	de6									24.5	24.9	247	244
	4	de5	de10	de9	de10	de5	de10									24.8	25.0	246	235
	5	de8	de	de	de	de13	de									25.1	24.7	248	237
	6	de	de18	de19	de17	de18	de18									25.0	24.9	252	237
	7																24.9		244

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



© Sodium Thiosulfate

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Curt

Date: 6/27/12

TEST MATERIAL: EEC 9585

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 / CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 6/15/12

YOUNG FROM: SR 1/08/12 1 < 24 hrs

TECHNICIANS: DAY: 0 1800 am

1 905 A/S 2 1145 Kom 3 0940 KOM 4 1500 Kom 5 12:15 YBK 6 12:20 YBK 7

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
50%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+												
	2	de	de	de	de	de	de									24.2		301	
	3	de	de4	de5	de5	de	de5									25.0	24.7	298	292
	4	de5	de10	de	de	de4	de									24.5	24.9	302	302
	5	+e12	+e	+e	+e9	+e11	+e11									24.8	25.0	307	293
	6	+e14	+e16	+e16	+e15	+e14	+e14									25.1	24.7	307	301
	7															25.0	24.9	314	299
100%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+												
	2	de	de	de	de	de	de									8.0	8.1	8.1	8.1
	3	de	de4	de36	de36	de36	de36									24.2		429	
	4	de7	de	de	de	de6	de6									8.0	8.1	8.8	8.1
	5	+e9	+e9	+e10	+e8	+e11	+e8									7.9	8.2	9.8	7.9
	6	de	de12	de11	de12	de12	de10									24.5	24.9	434	426
	7															7.9	8.1	9.9	8.4
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

Entry Error 6-18-12 km

APPENDIX B

DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST NO.	C. dubia	FHM
6/10	99	1.48	2.13
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
3/12	116	1.46	2.42
4/12	117	1.37	2.51
5/12	118	1.46	1.66
AVERAGE		1.30	2.17
STD. DEV.		0.22	0.35
RANGE: LOW		0.86	1.47
RANGE: HIGH		1.74	2.86
Coefficient of variation		0.17	0.16
Date of last test		5/3-10/12	5/22-29/12
MSD of most recent test		4.5	0.0635
PMSD of most recent test		13.8	21.5
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

From EPA's Wet Interlaboratory Variability Study

IC25 Coefficient of Variation						
National Percentiles ²						
Test Species	GLEC ¹	10th	25th	50th	75th	90th
C. dubia	0.17	0.08	0.17	0.27	0.45	0.62
P. promelas	0.16	0.12	0.21	0.26	0.38	0.45

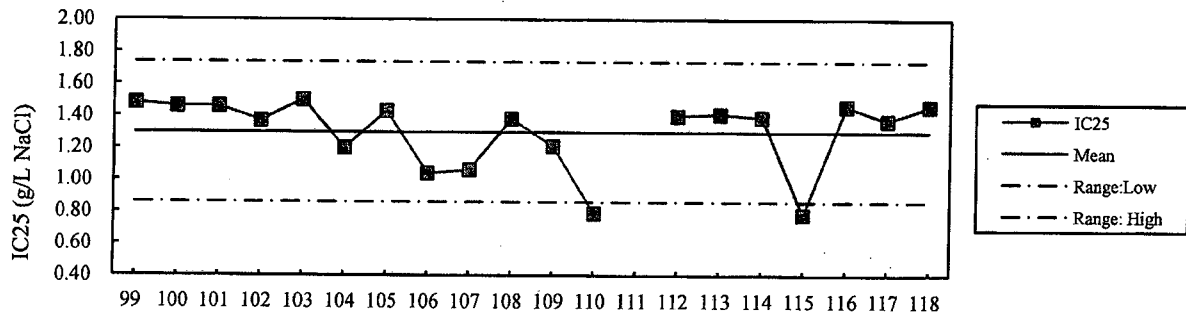
¹ Based on cumulative GLEC data from the most recent 20 tests.

² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

Chronic Reference Toxicant IC25

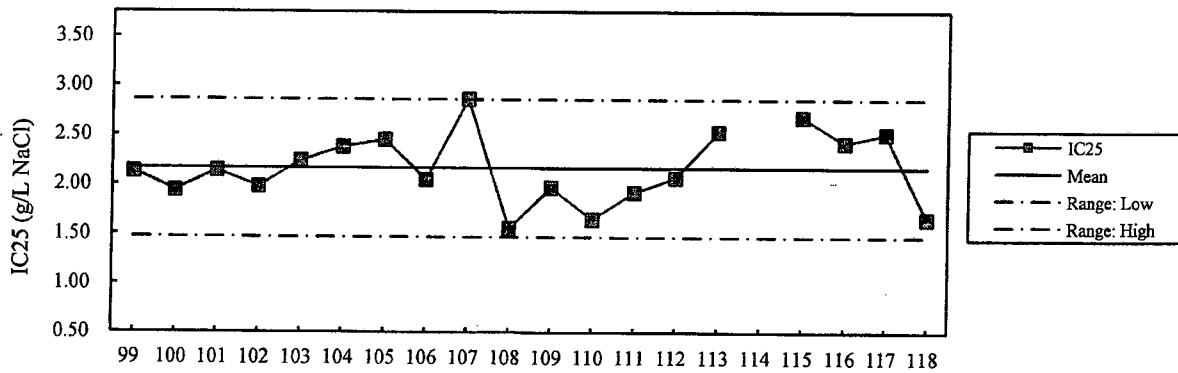
Ceriodaphnia dubia



Test Number

Chronic Reference Toxicant IC25

Pimephales promelas



Test Number



Great Lakes Environmental Center
Columbus Operations 1295 King Avenue
Columbus, OH 43212
614-487-1040

August 16, 2012

Roland McDaniel, Project Manager
GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

**RE: PHASE I CHRONIC TIE OF OUTFALL 001 FINAL EFFLUENT COLLECTED JULY 18, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS**

Dear Roland:

Provided for you is a copy of the report on the results from the *Ceriodaphnia dubia* chronic TIE tests performed on El Dorado Chemical Company Outfall 001 effluent sample collected July 18, 2012. If you have any questions regarding the report please call me or Dennis McIntyre (614) 487-1040.

Regards,

Christopher Tarr
Laboratory Coordinator

PHASE I CHRONIC TIE
OF OUTFALL 001 FINAL EFFLUENT SAMPLE COLLECTED JULY 18, 2012
FROM EL DORADO CHEMICAL COMPANY LOCATED IN EL DORADO, ARKANSAS

to

GBMc & Associates
219 Brown Ln
Bryant, Arkansas 72022

July 2012



Great Lakes Environmental Center

Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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INTRODUCTION

Great Lakes Environmental Center (GLEC) was requested to conduct a chronic Toxicity Identification Evaluation (TIE) of El Dorado Chemical Company (EDCC) outfall 001 final effluent using *Ceriodaphnia dubia*. The chronic TIE was requested based on historic *C. dubia* toxicity of EDCC outfall 001 final effluent samples. The specific objective of the Toxicity Identification Evaluation is:

- To determine the cause of the toxicity of the El Dorado Chemical Company outfall 001 final effluent sampled July 18, 2012 (Sample ID: EEC 9602) to *C. dubia* reproduction.

AQUATIC TOXICITY TEST METHODS

The chronic TIE of the EDCC outfall 001 final effluent was evaluated using *C. dubia*. The *C. dubia* chronic toxicity tests were conducted in accordance with GLEC in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (U.S. EPA, 2002, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013, 4th Ed).

Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the chronic toxicity tests were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth, Minnesota) and were cultured at GLEC in Millipore/Perrier reconstituted laboratory water and natural surface waters in environmental chambers under controlled conditions (temperature, $25 \pm 1^\circ\text{C}$; photoperiod, 16-hours light: 8-hours dark; light intensity, 10-20 $\mu\text{E}/\text{m}^2/\text{s}$). Survival and reproduction of culture animals were checked each time the culture water was changed (a minimum of three times a week). Twenty-four hours before the start of the test, the adults were transferred to clean beakers with food to ensure that only daphnids less than 24-hours old would be used to start the test. All neonates used for testing were within 8 hours of age of one another.

Test Water

Reconstituted Waters

The primary control water for the *C. dubia* TIE static renewal chronic tests was Millipore/Perrier® reconstituted water (20 percent diluted mineral water, DMW). The Millipore/Perrier® reconstituted water was prepared based on instructions cited in U.S. EPA (2002). Base water used in the preparation of the reconstituted water was deionized water from a Millipore Milli-Q™ Plus water system. Bottled Perrier® (a commercially available mineral water) was added in the appropriate amount to deionized water and mixed at room temperature. After preparation, each batch of reconstituted water was aerated and used in the laboratory for up to one month.

Test System

***Ceriodaphnia dubia* Static Renewal Chronic Toxicity Tests**

The specific details of the *C. dubia* static renewal chronic test system are based on EPA guidelines (U.S. EPA, 2002). For the chronic toxicity tests, *C. dubia* were continuously exposed for 6 days under static renewal

conditions to four concentrations of the outfall 001 final effluent (12.5, 25, 50 and 100 percent effluent) and the DMW control. *C. dubia* were exposed in 30-mL plastic cups containing 16 mL of test solution with one organism per beaker and six replicates per concentration (6 animals per concentration). Tests were placed in an environmental chamber under the specified conditions (temperature $25^{\circ} \pm 1^{\circ}\text{C}$; photoperiod, 16 h light and 8 h dark; light intensity 10-20 $\mu\text{E}/\text{m}^2/\text{s}$) and the animals were fed during the test.

Temperature, dissolved oxygen, pH, and specific conductivity were measured in the new and old test solutions daily. Observations on the number of live and dead animals and the number of young per adult were made daily for the duration of the test.

Statistical Analysis

Reproduction data from the *C. dubia* chronic toxicity tests was used to estimate the inhibition concentration (IC_{25}), which is the concentration that causes a 25 percent reduction to test organism reproduction when compared to the test control. Estimates of IC_{25} values were obtained using the ICpin statistical program. Chronic toxic units (TUc) were then calculated for each test by dividing 100 by the IC_{25} value ($\text{TUc} = 100 \div \text{IC}_{25}$).

EFFLUENT TOXICITY CHARACTERIZATION

Chronic TIE Test Methods and Results

The EDCC outfall 001 final effluent sample was characterized to define the characteristics of the constituents that contribute to *C. dubia* chronic toxicity. The effluent sample was characterized to determine if EDCC effluent toxicity is associated with:

- Filterable toxicants
- Non-polar organic compounds
- Volatile, easily oxidizable or aeratable compounds
- Chelatable metals
- Thiosulfate reducible compounds or oxidants

The toxicity characterization procedures generally followed those described by U.S. EPA; *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures* (EPA/600/6-91/003) and *Phase II Toxicity Identification Procedures* (EPA/600/R-92/080).

A summary of the results for each characterization is given in the following sections of this report. Copies of the chronic TIE data sheets, raw effluent chemistry sheets and statistical calculations sheets are provided in Appendix A.

Baseline Toxicity - Test 1

Concurrent with each toxicity characterization, a baseline chronic definitive toxicity test (no sample manipulation) was performed along with the manipulated samples to provide a comparison of the effectiveness of each effluent characterization (Toxicity test 1 in Figure 1).

The baseline toxicity test was not toxic to *C. dubia* survival and exhibited 67 percent survival in the 100 percent test concentration. The EDCC outfall 001 sample was chronically toxic to *C. dubia* reproduction and exhibited an IC_{25} value of 5.0 percent effluent or 20.0 TUc (Table 1).

1.0 µm Filtration - Test 2

In some types of effluents, toxicity can be reduced by filtration which removes certain biologically available toxicants. Therefore, the role of filterable materials as a cause of toxicity in the EDCC outfall 001 effluent sample was examined (Toxicity test 2 in Figure 1). The final effluent sample was filtered using a Gelman A/E glass fiber filter (1.0 µm).

After filtration treatment, 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent was removed as the *C. dubia* exhibited an IC₂₅ of >100 percent or <1.0 TUc. Therefore, filtration was very effective as a treatment and the toxicity to *C. dubia* reproduction in the EDCC effluent sample appears to be associated with a filterable toxicant.

C18-SPE Treatment - Tests 3

Toxicity which is not removed by filtration is usually the result of either organic and/or inorganic toxic constituents which are in solution (although other materials such as colloids may also pass through filters and cause toxicity). The toxicity in effluent samples associated with non-polar and semi-polar organic compounds is generally removed by passing the effluent sample over a C18-SPE pad (although other toxicants such as certain metals and colloids may also be removed by C18-SPE treatment). Therefore, C-18 treatment of the final effluent sample **after 1.0 µm filtration treatment** was performed to determine the specific role that non-polar organic compounds may play in the effluent toxicity (Toxicity test 3 in Figure 1). (In order to isolate the effects of individual treatments, filtration is performed prior to C-18 treatment to determine the presence of filterable toxicants which are also potentially removed by the C18-SPE pad)

Prior to the C-18 treatment, filtration removed 100 percent of the toxicity to *C. dubia* reproduction in the EDCC effluent. Therefore, it could not be determined whether C-18 was an effective treatment in removing toxicity to *C. dubia* reproduction in the EDCC effluent sample.

Aeration - Tests 4

The presence of toxic volatile substances, easily oxidizable substances, and/or surfactants can sometimes be detected by aeration of the effluent sample. The EDCC effluent sample was gently aerated (fine stream of air bubbles) for one hour in a one-liter glass graduated cylinder. A pad of glass wool was placed approximately 1.0 cm above the water surface to capture and retain any foam produced by the aeration (Toxicity test 4 in Figure 1).

The *C. dubia* aeration treatment removed 20.5 percent of the toxicity demonstrated in the EDCC outfall 001 effluent sample and had an IC₂₅ value of 6.3 percent or 15.9 TUc (Table 1). However, this difference is not meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Therefore, aeration treatment did not demonstrate a clear reduction in toxicity.

Cation Chelation with EDTA – Test 5

The EDCC outfall 001 effluent sample was treated with 25 mg/l of EDTA to chelate certain metals in solution, and therefore render them biologically unavailable to the test organisms (Toxicity test 5 in Figure 1).

Relative to the concurrent baseline toxicity test IC₂₅ of 5.0 percent, the addition of EDTA (25 mg/L) did not remove any sample toxicity and exhibited an IC₂₅ of 4.6 percent or 21.7 TUc (Table 1). Therefore, the toxicity to *C. dubia* reproduction does not appear to be associated with chelatable metals.

Sodium Thiosulfate Treatment – Test 6

The final effluent sample was treated with sodium thiosulfate to chemically reduce any oxidants present in the effluent that could contribute to toxicity (Toxicity test 6 in Figure 1). Sodium thiosulfate was added to the final effluent sample at 50 mg/L prior to toxicity testing.

The *C. dubia* sodium thiosulfate treatment removed 46.5 percent of the toxicity demonstrated in the EDCC outfall 001 effluent sample and had an IC₂₅ value of 9.3 percent or 10.7 TUC (Table 1). The removal of 46.5 percent of the sample toxicity cannot be considered very meaningful given the amount of toxicity it represents and the inherent variability of toxicity tests. Therefore, sodium thiosulfate treatment did not demonstrate a clear reduction in toxicity.

CHRONIC TIE DISCUSSION AND RESULTS SUMMARY

The toxicity identification of the EDCC outfall 001 effluent sample collected July 18, 2012 did demonstrate a clear removal of chronic toxicity, but the reduction of toxicity to *C. dubia* reproduction was only demonstrated by one of the five TIE treatments performed. Three of the treatments, aeration, EDTA and sodium thiosulfate, did not demonstrate a clear removal of any meaningful toxicity from the outfall 001 effluent sample. Thus, the effluent toxicity does not appear to be related to; an easily oxidizable or aeratable compound, a chelatable metal or thiosulfate reducible compounds or oxidants.

The 1.0 µm filtration treatment removed 100 percent of the toxicity present in the EDCC outfall 001 effluent sample. Therefore, the chronic toxicity to *C. dubia* present in the EDCC outfall 001 effluent sample collected July 18, 2012 appears to be associated with a filterable toxicant(s).

The effectiveness of the C-18 treatment in removing chronic toxicity to *C. dubia* in the EDCC effluent sample is unknown due to the fact that filtration removed 100 percent of the sample toxicity prior to C-18 treatment. For a summary of all test results, see Table 1.

Summary of the chronic toxicity characterization of the EDCC outfall 001 sample collected July 18, 2012 (Sample ID: EEC 9602):

- **The toxicant (s) was filterable.**
- The toxicant(s) was not a chelatable metal.
- The toxicant(s) was not a volatile, easily oxidizable or aeratable compound.
- The toxicant(s) was not a thiosulfate reducible compound or oxidant.

Table 1. Summary of Chronic TIE Test results

<i>C. dubia</i> Chronic TIE on EDCC Outfall 001 Sample Collected 7/18/12 GLEC Sample ID: EEC 9602 Test Dates: 7/19/12 – 7/26/12	Percent Survival							
	DMW	12.5%	25%	50%	100%	LC ₅₀		
Baseline Toxicity (No manipulation) – Test 1	100	67	83	83	67	>100		
1.0 µm Filtration – Test 2	100	100	100	100	100	>100		
C18-SPE Treatment – Test 3	100	100	100	100	100	>100		
Aeration – Test 4	100	100	83	50	83	>100 ^C		
Cation Chelation with EDTA – Test 5	100	67	0	100	83	>100 ^C		
Sodium Thiosulfate Treatment – Test 6	100	80	67	50	100	>100 ^C		
	Reproduction – Mean Number of Young per Adult							
	DMW	12.5%	25%	50%	100%	IC ₂₅	TUc ^a	TUc % Removed
Baseline Toxicity (No manipulation) – Test 1	38.7	14.3	14.0	10.5	2.3	5.0	20.0	NA
1.0 µm Filtration – Test 2	35.8	39.5	40.6	43.3	42.7	>100	<1.0	100
C18-SPE Treatment – Test 3	35.8	35.0	37.5	35.2	43.7	>100	<1.0	NA ^d
Aeration – Test 4	35.2	17.5	11.3	7.2	11.2	6.3	15.9	20.5%
Cation Chelation with EDTA – Test 5	36.7 ^b	11.0	5.6	14.3	9.2	4.6	21.7	NA
Sodium Thiosulfate Treatment – Test 6	29.8	18.6	14.3	15.5	15.2	9.3	10.7	46.5

NA – Not applicable or Not available

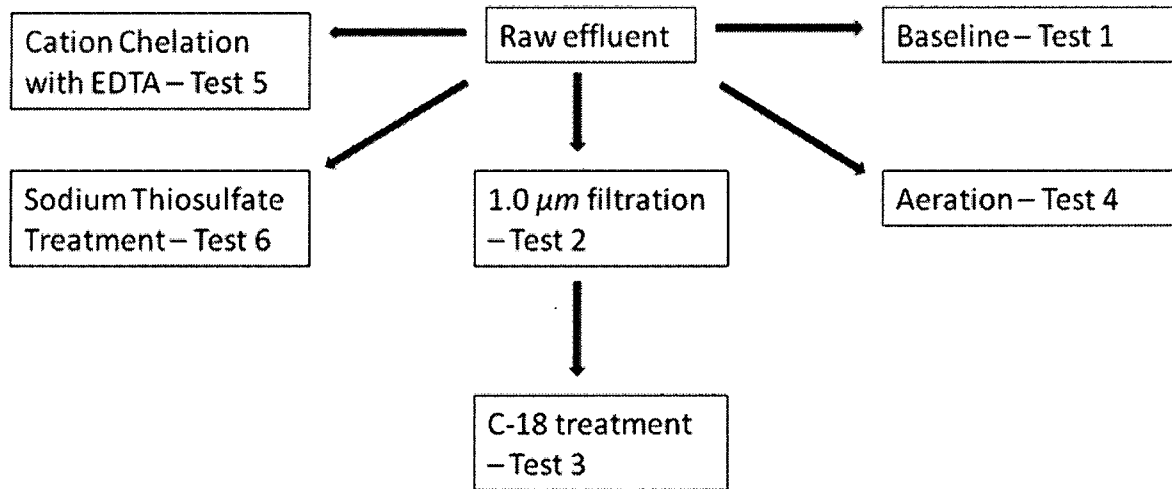
^a TUc, Chronic Toxic Unit: (100/IC₂₅), based on reproduction only.

^b Control water did not receive EDTA treatment due to historical evidence that demonstrates EDTA causes toxicity to *C. dubia* in DMW.

^c Despite the erratic dose response, the LC₅₀ is considered to be >100 since *C. dubia* survival in the 100% concentration was greater than 50 percent.

^d No additional baseline toxicity was removed by C-18 treatment after filtration treatment.

Figure 1. El Dorado Chemical Company Outfall 001 Chronic TIE schematic



CHRONIC REFERENCE TOXICITY TEST RESULTS

Sodium chloride was used as the reference toxicant for *C. dubia*. The 7-day IC₂₅ value for the most recent *C. dubia* reference toxicant test was 1.36 g/L of sodium chloride which was within the acceptance range of 0.86 to 1.72 g/L. For results of the 20 most recent chronic reference toxicity tests, see Appendix B.

REFERENCES

U.S. EPA, 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.

U.S. EPA, 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA/600/R-92/080. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

APPENDIX A

G.L.E.C DATA SHEETS FOR THE *Ceriodaphnia dubia* CHRONIC TOXICITY
CHARACTERIZATION TESTS CONDUCTED WITH EDCC OUTFALL 001 FINAL EFFLUENT
COLLECTED JULY 18, 2012



EFFLUENT AND RECEIVING WATER CHARACTERIZATION FORM

Great Lakes Environmental Center

CLIENT: GBinc - EDC

PROJECT NUMBER: 2179-00

INVESTIGATORS: _____

INITIAL WATER CHEMISTRY

DATE: <u>7/19/12</u>	INITIALS	<u>EEC</u>			
EEC NUMBER		<u>9602</u>			
OUTFALL/DESCRIPTION		<u>outfall 001</u>			
DISSOLVED OXYGEN (mg/L)	<u>CR</u>	<u>10.2</u>			
TEMPERATURE (°C)	<u>CR</u>	<u>10.6</u>			
pH	<u>CR</u>	<u>9.4</u>			
CONDUCTIVITY (µmhos/cm)	<u>CR</u>	<u>271</u>			

WATER CHEMISTRY AT TEST TEMPERATURES

DATE: <u>7/19/12</u>	INITIALS				
EEC NUMBER					
OUTFALL/DESCRIPTION					
DISSOLVED OXYGEN (mg/L)					
TEMPERATURE (°C)					
pH					
CONDUCTIVITY (µmhos/cm)					
HARDNESS (mg/L CaCO ₂)	<u>KOM</u>	<u>0.8 x 40 = 32</u>			
ALKALINITY (mg/L CaCO ₂)	<u>KOM</u>	<u>2.9 x 40 = 116</u>			
TOTAL CHLORINE (mg/L)*					
TOTAL AMMONIA (mg/L)*					

*Check with project manager to see if necessary



Great Lakes Environmental Center
 1295 KING AVE.
 COLUMBUS, OH 43212
 PHONE: (614) 487-1040
 FAX: (614) 487-1920

Two Important Notes for Whole Effluent Toxicity Testing:

- There is a maximum hold time for all samples of 36 hours (Hold time begins when sample is taken off the sampler)
- Samples must be received at 4°C ± 2°C

CHAIN OF CUSTODY FORM

(TO BE COMPLETED ONSITE AND SUBMITTED WITH SAMPLES)

FACILITY: El Dorado Chemical Co.
 LOCATION: El Dorado, AR
 CONTACT PERSON: Larken Pennington
 PHONE: 870-863-1125

COLLECTOR: Larken Pennington
 DATE: 7/18/12
 WITNESS: [Signature]
 DATE: 7/18/12

EECH# (lab only)	SAMPLE ID	SAMPLE SOURCE (Eff/Upstr.)	TYPE (grab or composite)	SAMPLE START DATE	SAMPLE START TIME (24-hr notation)	SAMPLE END DATE	SAMPLE END TIME (24-hr notation)	VOLUME COLLECTED	SAMPLE CONTAINER	SAMPLE COLLECTOR	OTHER COMMENTS
9602	001		grab	7-17-12	8:30am	7-18-12	8:30am	4 cubitainers	4	L Pennington	

ANALYSIS REQUIRED: Please fill in completely

NAME OF STREAM SAMPLED: _____

Species: *Ceriodaphnia dubia*

Pimephales promelas (fathead minnows)

Other - please specify: _____

Test Type: Acute: 24-hour
 48-hour

Acute: 24-hour 48-hour

96-hour: with 48-hour renewal

Chronic (7-day)

Chronic (7-day)

Other - please specify: _____

Dilutions: Screen (100% only)

Definitive (5 sample concentrations): List test concentrations: _____

Dilution Water: Receiving Water

Lab water

Other - please specify: _____

TRANSFER OF SAMPLES:

(FIRST SIGNATURE IS SAMPLER, LAST SIGNATURE IS AUTHORIZED LABORATORY REPRESENTATIVE)

SHIPPER
 1. Larken Pennington

RECEIVER

DATE
7/18/12

TIME

2. [Signature]

DATE
7/19/12 TIME
1000

For Lab Use Only:
 ice remaining in cooler upon receipt → No Ice
 Temperature of samples when received:
10°C

FOR SATURDAY DELIVERY??? MARK PACKAGE AS SUCH AND CALL GLEC ON FRIDAY WITH TRACKING NUMBER

EEC 9602

Test Dates: 7/19-26/12

Survival Summary - (% Survival)

Concentration -% effluent	DMW	12.5%	25%	50%	100%
Baseline (Test 1)	100%	67%	83%	83%	67%
1.0 µm filtration (Test 2)	100%	100%	100%	100%	100%
C-18 SPE treatment (Test 3)	100%	100%	100%	100%	100%
Aeration (Test 4)	100%	100%	83%	50%	83%
EDTA 25 mg/l (Test 5)	100%	67%	0%	100%	83%
NaThio (50 mg/l) (Test 6)	100%	80%	67%	50%	100%

Reproduction Summary - (number of young per adult)

Concentration -% effluent	DMW	12.5%	25%	50%	100%	IC25	TUc	%TUc removed
Baseline (Test 1)	38.7	14.3	14.0	10.5	2.3	5.0	20.0	--
1.0 µm filtration (Test 2)	35.8	39.5	40.6	43.3	42.7	>100	<1.0	100.0%
C-18 SPE treatment (Test 3)	35.8	35.0	37.5	35.2	43.7	>100	<1.0	100.0% ^b
Aeration (Test 4)	35.2	17.5	11.3	7.2	11.2	6.3	15.9	20.5%
EDTA (25 mg/l) (Test 5) ^a	36.7	11.0	5.6	14.3	9.2	4.6	21.7	NA
NaThio (50 mg/l) (Test 6)	29.8	18.6	14.3	15.5	15.2	9.3	10.7	46.5%

a - Control water did not receive EDTA treatment due to historical data that EDTA causes toxicity to C.dubia reproduction in DMW

b - additional toxicity removed by C-18 treatment after filtration treatment.

NA - Not Available

Baseline (Test 1)

El Dorado Chemical Outfall 001 Sampled 7/18/12 (EEC 9602)

(Tested 7/19-26/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	5	5	4
DEAD	0	2	1	1	2
% SURV	100.00%	66.67%	83.33%	83.33%	66.67%

Scito River Water 2° Control	6
	0
	100.00%

OFFSPRING

Concentration-Calculated TDS	DMW	12.5%	25%	50%	100%
1	37	11	8	13	2
2	38	17	18	13	3
3	40	8	16	4	0
4	35	13	11	14	5
5	40	18	18	11	0
6	42	19	13	8	4
N	6	6	6	6	6
MEAN	38.7	14.3	14.0	10.5	2.3
SD	2.5033311	4.36653944	4.0496913	3.8340579	2.0655911
CV	6.4741322	30.4642286	28.926367	36.514837	88.525334
Total Young	232	86	84	63	14

Scito River 2° Control	41
	42
	42
	35
	47
	44
	6
	41.8
	3.9707262
	9.4917758
	251

1.0 µm filtration (Test 2)

El Dorado Chemical Outfall 001 Sampled 7/18/12 (EEC 9602)

(Tested 7/19-26/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	5	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	29	39	42	42	45
2	36	39	41	43	44
3	42	44	42	46	40
4	34	38	38	41	39
5	39	40	40	48	45
6	35	37		40	43
N	6	6	5	6	6
MEAN	35.8	39.5	40.6	43.3	42.7
SD	4.445972	2.42899156	1.6733201	3.0767949	2.5819889
CV	12.407364	6.14934572	4.121478	7.1002959	6.0515365
Total Young	215	237	203	260	256

C-18 SPE treatment (Test 3)

El Dorado Chemical Outfall 001 Sampled 7/18/12 (EEC 9602)

(Tested 7/19-26/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	6	6	6
DEAD	0	0	0	0	0
% SURV	100.00%	100.00%	100.00%	100.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	38	34	38	35	46
2	35	33	41	17	43
3	37	33	39	36	42
4	34	39	32	40	44
5	38	32	38	41	46
6	33	39	37	42	41
N	6	6	6	6	6
MEAN	35.833333	35.0	37.5	35.2	43.7
SD	2.1369761	3.16227766	3.0166206	9.325592	2.0655911
CV	5.9636541	9.03507903	8.0443217	26.518271	4.7303613
Total Young	215	210	225	211	262

Aeration (Test 4)

El Dorado Chemical Outfall 001 Sampled 7/18/12 (EEC 9602)

(Tested 7/19-26/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	6	5	3	5
DEAD	0	0	1	3	1
% SURV	100.00%	100.00%	83.33%	50.00%	83.33%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	37	12	15	0	13
2	39	20	19	17	15
3	33	18	14	2	17
4	37	18	4	5	6
5	31	19	14	11	0
6	34	18	2	8	16
N	6	6	6	6	6
MEAN	35.2	17.5	11.3	7.2	11.2
SD	2.9944393	2.81069386	6.7428975	6.2423286	6.7354782
CV	8.5149932	16.0611078	59.496154	87.10226	60.317715
Total Young	211	105	68	43	67

EDTA 25 mg/l (Test 5)

El Dorado Chemical Outfall 001 Sampled 7/18/12 (EEC 9602)
(Tested 7/19-26/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	0	6	5
DEAD	0	2	5	0	1
% SURV	100.00%	66.67%	0.00%	100.00%	83.33%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	38	16		19	6
2	38	4	4	15	18
3	34	3	4	17	7
4	40	12	7	14	10
5	34	13	9	6	9
6	36	18	4	15	5
N	6	6	6	6	6
MEAN	36.7	11.0	5.6	14.3	9.2
SD	2.4221203	6.19677335	2.3021729	4.4572039	4.708149
CV	6.6057826	56.3343032	41.11023	31.096771	51.361625
Total Young	220	66	28	86	55

NaThio (50 mg/l) (Test 6)

El Dorado Chemical Outfall 001 Sampled 7/18/12 (EEC 9602)
(Tested 7/19-26/12)

SURVIVAL RATIO

Concentration - % effluent	DMW	12.5%	25%	50%	100%
ALIVE	6	4	4	3	6
DEAD	0	1	2	3	0
% SURV	100.00%	80.00%	66.67%	50.00%	100.00%

OFFSPRING

Concentration - % effluent	DMW	12.5%	25%	50%	100%
1	35	22	18	0	17
2	33	19	11	22	13
3	36	20	17	16	12
4	30	21	8	19	14
5	32		17	19	17
6	13	11	15	17	18
N	6	6	6	6	6
MEAN	29.8	18.6	14.3	15.5	15.2
SD	8.5186071	4.39317653	3.9832985	7.8676553	2.4832774
CV	28.55399	23.6192286	27.790454	50.759066	16.373258
Total Young	179	93	86	93	91

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	37	11	8	13	2
Response 2	38	17	18	13	3
Response 3	40	8	16	4	0
Response 4	35	13	11	14	5
Response 5	40	18	18	11	0
Response 6	42	19	13	8	4

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9602 Test #1
 Test Start Date: 7/19/12 Test Ending Date: 7/26/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	38.667	2.503	38.667
2	6	12.500	14.333	4.367	14.333
3	6	25.000	14.000	4.050	14.000
4	6	50.000	10.500	3.834	10.500
5	6	100.000	2.333	2.066	2.333

The Linear Interpolation Estimate: 4.9658 Entered P value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 5.0535 Standard Deviation: 0.2881
 Original Confidence Limits: Lower: 4.5833 Upper: 5.7087
 Resampling time in seconds: 0.00 Random_Seed: 80415580

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	37	12	15	0	13
Response 2	39	20	19	17	15
Response 3	33	18	14	2	17
Response 4	37	18	4	5	6
Response 5	31	19	14	11	0
Response 6	34	18	2	8	16

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9602 Test #4

Test Start Date: 7/19/12 Test Ending Date: 7/26/12

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	35.167	2.994	35.167
2	6	12.500	17.500	2.811	17.500
3	6	25.000	11.333	6.743	11.333
4	6	50.000	7.167	6.242	9.167
5	6	100.000	11.167	6.735	9.167

The Linear Interpolation Estimate: 6.2205 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 6.2756 Standard Deviation: 0.3987

Original Confidence Limits: Lower: 5.5840 Upper: 7.0486

Resampling time in seconds: 0.00 Random_Seed: 393403116

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	38	16	4	19	6
Response 2	38	4	4	15	18
Response 3	34	3	7	17	7
Response 4	40	12	9	14	10
Response 5	34	13	4	6	9
Response 6	36	18		15	5

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9602 Test #5

Test Start Date: 7/19/12 Test Ending Date: 7/26/12

Test Species: C.dubia

Test Duration: 7 days

DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	36.667	2.422	36.667
2	6	12.500	11.000	6.197	11.000
3	5	25.000	5.600	2.302	10.364
4	6	50.000	14.333	4.457	10.364
5	6	100.000	9.167	4.708	9.167

The Linear Interpolation Estimate: 4.4643 Entered P Value: 25

Number of Resamplings: 200

The Bootstrap Estimates Mean: 4.5863 Standard Deviation: 0.3381

Original Confidence Limits: Lower: 4.0959 Upper: 5.2885

Expanded Confidence Limits: Lower: 3.9854 Upper: 5.5357

Resampling time in Seconds: 0.00 Random_Seed: -2055098756

Conc. ID	1	2	3	4	5
Conc. Tested	0	12.5	25	50	100
Response 1	35	22	18	0	17
Response 2	33	19	11	22	13
Response 3	36	20	17	16	12
Response 4	30	21	8	19	14
Response 5	32	11	17	19	17
Response 6	13		15	17	18

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: EEC 9602 Test #6
 Test Start Date: 7/19/12 Test Ending Date: 7/26/12
 Test Species: C.dubia
 Test Duration: 7 days
 DATA FILE:

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	29.833	8.519	29.833
2	5	12.500	18.600	4.393	18.600
3	6	25.000	14.333	3.983	15.000
4	6	50.000	15.500	7.868	15.000
5	6	100.000	15.167	2.483	15.000

The Linear Interpolation Estimate: 8.2993 Entered P Value: 25

Number of Resamplings: 200
 The Bootstrap Estimates Mean: 9.2930 Standard Deviation: 4.6453
 Original Confidence Limits: Lower: 6.2713 Upper: 19.3946
 Expanded Confidence Limits: Lower: 5.6628 Upper: 22.7232
 Resampling time in Seconds: 0.00 Random_Seed: 1607002068

Parental Blockage Map for *C. dubia*

Date: 7-19-12

Time Neonates Pulled: 1015

Source Board: Selpmw 7-11-12

Initials: km

Estimated Age Range of *C. dubia* neonates: 18h

Name and Project # neonates used for: 2179-00 EDCC tests ① & ②

	1	2	3	4	5	6	7	8	9	10
6										
5										
4		R5						R4		
3		R3								
2					R2					
1			R6		R1					

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 7-19-12

Time Neonates Pulled: 1015

Source Board: Selomw 7-11-12

Initials: km

Estimated Age Range of *C. dubia* neonates: ~8h

Name and Project # neonates used for: 2179-00 EDC tests ② & ③

	1	2	3	4	5	6	7	8	9	10
6										
5										
4	R5									
3				R3						
2			R6					R1		
1							R2		R4	

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.

Parental Blockage Map for *C. dubia*

Date: 7-19-12

Time Neonates Pulled: 1015

Source Board: SR10MW 7-11-12

Initials: *Von*

Estimated Age Range of *C. dubia* neonates: ~ 8h

Name and Project # neonates used for: 2179-00 EDCC tests (3) & (4)

	1	2	3	4	5	6	7	8	9	10
6										
5										
4									R3	R1
3	R5		R2							R4
2							R6			
1										

Legend:

R = Repetition

corresponds to the repetition number of the test

for example: "R1" means that all neonates for repetition #1 of the test come from the same adult *C. dubia* indicated by parental blockage map.



Baseline - ①
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cam Tu Date: 8/3/12

Great Lakes Environmental Center

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW 1667

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2178-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/1400

YOUNG FROM: SR/DMW 7-11-12 < 24 hrs

TECHNICIANS: DAY: 0 1400 Kam 1 9:10 YBK 2 0900 Kam 3 900 / AEL 4 1200 Kam 5 0900 Kam 6 8:40 YBK 7 8:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.7		7.0		24.7		176	
	1	+	+	+	+	+	+					7.9	7.9	8.5	8.2	25.0	25.4	185	196
	2	e	e	e	e	e	e					8.0	8.2	8.4	7.8	25.2	25.3	178	189
	3	e	e	e	e	e	e					7.5	7.9	8.8	7.5	25.9	25.1	184	183
	4	e6	e7	e7	e5	e7	e7					7.5	8.0	7.9	6.9	25.0	24.8	178	188
	5	e12	e12	e12	e11	e13	e13					7.7	7.7	9.0	7.6	25.1	25.2	176	185
	6	e	e	e	e	e	e					7.4	7.6	10.5	8.2	25.0	25.2	185	194
	7	e19	e19	e91	e19	e20	e22						7.6		8.4		25.2		198
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.1		7.2		24.7		203	
	1	+	+	+	+	+	+					8.4	7.9	8.2	8.2	25.0	25.4	205	210
	2	e	+	+	+	+	+					8.5	8.3	7.9	7.9	25.2	25.3	206	215
	3	e	e	e	e	e	e					8.1	8.0	9.3	7.6	25.3	25.1	205	202
	4	e6	e4	e4	e5	e5						8.1	8.0	7.6	6.9	25.0	24.8	212	213
	5	T5	+	+	e	e	e					8.3	7.8	7.8	7.7	25.1	25.2	207	212
	6		e	+4	+5	+5	+5					8.1	7.9	8.5	8.3	25.0	25.2	205	215
	7	V	e10	J	+4	+8	+9						7.8		8.5		25.2		211
25%	0	+	+	+	+	+	+	+	+	+	+					24.7		226	
	1	+	+	+	+	+	+									25.0	25.9	227	229
	2	e	e	+	e	e	+									25.2	25.3	237	236
	3	e	e	e	e	e	e									25.4	25.1	229	226
	4	e5	e4	e5	e5	e5	e4									25.0	24.8	231	238
	5	T3	+5	e	e	e	e									25.1	25.2	231	228
	6		e	e4	+6	+5	+4									25.0	25.2	225	237
	7	V	e9	e7	e	e8	e5									25.2		235	

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 Entry Error 7-23-12 Kam



Baseline - ①
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T Date: 8/3/12

TEST MATERIAL: EEL 9602
 PROJECT NO.: 2179-00
 STARTING DATE/TIME: 7-19-12/1400
 TECHNICIANS: DAY: 0 1400 Kom

TEST SPECIES: Ceriodaphnia dubia
 ANIMALS/CONC: 10 /CHAMBER: 1
 YOUNG FROM: SPDMW 7-11-12 24 hrs

DILUTION WATER: DMW
 LIGHT INTENSITY (LUX): 500-1000

PHOTOPERIOD (L:D): 16:8
 TEMPERATURE (°C): 25 ± 1°C

1 9:10 YBLK 2 0900 Kom 3 900 ACS 4 1200 Kom 5 0900 Kom 6 8:40 YBLK 7 8:00 YBLK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+										24.7		265	
	2	te	te	te	te	+	+										25.0	25.4	270	269
	3	te	te	te	te	te	te										25.2	25.3	279	279
	4	te4	te4	te4	te4	te4	te4										25.9	25.1	277	270
	5	te	te 3	+	te	te	+										25.0	24.8	269	283
	6	te1	te	↓	te4	te	te										25.1	25.2	269	268
	7	te8*	te6*	↓	te6	te7	te4										25.0	25.2	269	277
100%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+										24.7		361	
	2	te	+	+	te	+	+										25.0	25.4	374	350
	3	te	te	te	te	+	te										25.2	25.3	368	366
	4	te	te	+	te	↓	te										25.9	25.1	378	348
	5	te	te	+	te	↓	te										25.0	24.8	363	369
	6	te	te	↓	te	↓	te										25.1	25.2	368	350
	7	te2	te3	↓	te5	↓	te4										25.0	25.2	356	365
	0	+	+	+	+	+	+	+	+	+	+									
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ⊕ = after aeration



Baseline - (V)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Great Lakes Environmental Center

Reviewed by: Am T

Date: 8/3/12

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2178-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 7-19-72/1400

YOUNG FROM: SR/DMW 7-11-12 <24 hrs

TECHNICIANS: DAY: 0 1400 km

1 9:10 YBK

2 900 ACS 0900 km

3 900 ACS

4 1200 km

5 0900 km

6 8:40 YBK

7 8:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
SR %	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+	7.9		8.1		24.7		623	
	2	te	te	te	te	te	te					7.8	8.1	9.1	8.2	25.0	25.4	629	638
	3	te	te	te	te	te	te									25.2	25.3	625	664
	4	te6	te6	te7	te7	te7	te8									24.8	25.1	620	621
	5	te14	te14	te14	te14	te17	te16									25.0	24.8	636	620
	6	te	te	te	te	te	te20									25.1	25.2	629	661
	7	te21	te22	te21	te14	te23	te									25.0	25.2	623	635
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	0	+	+	+	+	+	+	+	+	+	+								
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed

entry error ACS 7/22/12



1.0µm PF - (2)

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr

Date: 8/3/12

Great Lakes Environmental Center

TEST MATERIAL: EEC 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 / CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/1400

YOUNG FROM: SP DMW 7-11-12 < 24 hrs

TECHNICIANS: DAY: 0 1400 km

1 9:40 YBK 2 0930 km 3 900

ACS 4 1300 km 5 1000 km

6 9:20 YBK 7 8:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	7.8		7.4		24.5		181	
	1	+	+	+	+	+	+					8.0	7.9	8.4	8.2	25.0	25.4	181	181
	2	e	e	e	e	e	e					8.1	8.3	8.4	8.1	25.2	25.3	183	199
	3	e	e	e	e	e	e					7.9	7.9	8.8	7.5	25.8	25.1	185	179
	4	e5	e5	e7	e5	e8	e6					7.7	7.8	7.8	6.6	25.0	24.8	182	191
	5	e10	e12	e13	e12	e12	e13					7.9	7.9	8.7	7.7	24.9	25.2	181	186
	6	e	e	e	e	e	e					7.8	7.9	10.6	8.3	25.0	25.2	180	186
	7	e14	e19	e22	e17	e19	e16						7.9		8.6		25.2		184
12.5%	0	+	+	+	+	+	+	+	+	+	+	8.3		7.2		24.5		203	
	1	+	+	+	+	+	+					8.4	7.9	8.1	8.1	25.0	25.4	205	205
	2	e	e	e	e	e	e					8.5	8.3	8.0	8.1	25.2	25.3	215	213
	3	e	e	e	e	e	e					8.3	8.0	8.4	7.6	25.3	25.1	209	208
	4	e6	e6	e7	e5	e8	e7					8.2	8.0	7.5	6.6	25.0	24.8	208	217
	5	e13	e14	e14	e13	e10	e14					8.2	7.9	8.4	7.6	24.9	25.2	205	210
	6	e	e	e	e	e	e					8.3	8.0	8.6	8.1	25.0	25.2	206	211
	7	e20	e19	e23	e20	e22	e16						8.0		8.2		25.2		211
25%	0	+	+	+	+	+	+	+	+	+	+					24.5		221	
	1	+	+	+	+	+	+									25.0	25.4	229	224
	2	e	e	e	e	e	e									25.2	25.3	232	236
	3	e	e	e	e	e	e									25.7	25.1	233	228
	4	e6	e6	e6	e4	e7										25.0	24.8	230	242
	5	e14	e13	e15	e13	e12										24.9	25.2	229	233
	6	e	e	e	e	e										25.0	25.2	230	236
	7	e22	e22	e21	e21	e21										25.2			231

Key: + = live

- = dead

e = eggs present

▲ = possible male daphnid

■ = confirmed male daphnid

* = daphnid is erratic, pale, etc.

d = dead babies present (not counted)

@ = daphnid was killed or mishandled, which may affect survival

S = split brood, attached number indicates how many were observed

Ⓢ no animal found 7-21-12 km

* entry error 7-21-12 km



1.0µm PF - (2)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr Date: 8/3/12

Great Lakes Environmental Center

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2178-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/1400

YOUNG FROM: SP/DMW 7-11-12 < 24 hrs

TECHNICIANS: DAY: 01400 Kom 1 9:40 YBK 2 0930 Kom 3 920 ACS 4 1300 Kom 5 1000 Kom 6 9:20 YBK 7 8:00 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.					
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old				
50%	0	+	+	+	+	+	+	+	+	+	+												
	1	+	+	+	+	+	+											24.5		258			
	2	d	d	d	d	d	d											25.0	25.4	271	268		
	3	e	e	e	e	e	e											25.2	25.3	273	277		
	4	e7	e7	e7	e5	e8	e7											25.9	25.1	278	267		
	5	e13	e14	e15	e13	e16	e14											25.0	24.8	273	284		
	6	e	e	e ^(S)	e	e	e											24.9	25.2	271	278		
	7	e22	e22	e23	e23	e24	e19											25.0	25.2	270	279		
100%	0	+	+	+	+	+	+	+	+	+	+												
	1	+	+	+	+	+	+											24.5		364			
	2	d	d	d	d	d	d											8.5	8.1	25.0	25.4	367	366
	3	e	e	e	e	e	e											8.5	8.4	25.2	25.3	368	369
	4	e7	e8	e6	e5	e7	e7											8.4	8.2	25.2	25.1	370	353
	5	e15	e14	e11	e14	e14	e14											8.1	8.3	25.0	24.8	363	377
	6	e	e	e	e	e	e											8.3	8.1	24.9	25.2	358	366
	7	e23	e22	e23	e20	e24	e22											8.5	8.1	25.0	25.2	364	366
0	+	+	+	+	+	+	+	+	+	+													
1																							
2																							
3																							
4																							
5																							
6																							
7																							

Key: ++ live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ⊕ afteracuation



1.0µm PF → C18 (3)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Great Lakes Environmental Center

Reviewed by: Am T

Date: 8/3/12

TEST MATERIAL: EEC 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT I.D.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/13:50

YOUNG FROM: SP/DMW 7/11 < 24 hrs

TECHNICIANS: DAY: 0 13:50 YBK 1 915 ACS 2 1000 KDM 3 945 ACS 4 12:15 YBK 5 9:20 YBK 6 905 ACS 7 810 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+	7.9		5.3		25.0		177	
	2	e	e	e	e	e	e					7.7	7.6	7.6	8.1	25.0	25.7	178	183
	3	e	e	e	e	e	e					8.2	8.2	8.2	7.8	25.1	25.2	181	190
	4	+e7	+e6	+e6	+e5	+e5	+e6					7.6	7.9	8.6	7.5	25.8	25.3	178	182
	5	+e13	+e13	+e13	+e12	+e13	+e12					7.5	7.8	7.3	6.5	25.0	25.0	181	178
	6	e	e	e18	e17	e	e					7.5	7.6	8.6	7.5	25.0	24.8	180	186
	7	e18	e16	e	e	e20	e15					7.5	7.9	10.4	7.9	25.1	25.6	176	187
12.5%	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+	8.1		6.7		25.0		195	
	2	e	e	e	e	e	e					8.1	7.8	7.6	8.1	25.6	25.7	205	203
	3	e	e	e	e	e	e					8.4	8.3	7.9	7.8	25.1	25.2	212	213
	4	+e6	+e4 ^d	+e6	+e6	+e4	+e5					8.0	8.0	8.4	7.6	25.7	25.3	205	207
	5	+e10	+e13	+e9 ^d	+e14	+e10 ^d	+e13					7.8	7.9	6.9	6.5	25.0	25.0	205	203
	6	e	e16 [*]	e18	e19	e	e					7.9	7.8	8.1	7.3	25.0	24.8	205	207
	7	e18	e	e	e	e18	e21					7.8	8.0	8.7	7.9	25.2	25.6	202	210
25%	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+					25.0		216	
	2	e	e	e	e	e	e									25.3	25.7	227	224
	3	e	e	e	e	e	e									25.1	25.2	231	235
	4	+e4	+e6	+e7	+e6	+e7	+e5									25.9	25.3	228	227
	5	+e15	+e15	+e14	+e14	+e11 ^d	+e14									25.0	25.0	228	225
	6	e	e14 [*]	e18	e12	e	e									25.0	24.8	234	228
	7	e19	e2 [⊗]	e	e	e20	e18									25.9	25.6	232	237

Key: + = live

- = dead

e = eggs present

▲ = possible male daphnid

■ = confirmed male daphnid

* = daphnid is erratic, pale, etc.

d = dead babies present (not counted)

@ = daphnid was killed or mishandled, which may affect survival

S = split brood, attached number indicates how many were observed

⊗ No Entry Error 7:24-12pm



1.0 μ m PF \rightarrow C18 (3)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: cm tr Date: 8/3/12

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 / CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 \pm 1

STARTING DATE/TIME: 7-19-12/13:50

YOUNG FROM: SP/DMW 7/11 < 24 hrs

TECHNICIANS: DAY: 0 13:50 YBK 1 9:15 ACS 2 10:00 km 3 9:45 ACS 4 12:15 YBK 5 9:20 YBK 6 9:05 ACS 7 8:10 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.		
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old	
50%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+													
	2	e	e	e	e	e	e													
	3	e	e	e	e	e	e													
	4	+e5	+e2	+e6	+e	+e7	+e7													
	5	+e11	+e9	+e11	+e14	+e13	+e13													
	6	e1	e	e19	e20	e21	e													
	7	e18	e6	e	e	e	e22													
100%	0	+	+	+	+	+	+	+	+	+	+									
	1	+	+	+	+	+	+													
	2	e	e	e	e	e	e													
	3	e	e	e1	e6	e	e													
	4	+e6	+e8	+e5	+e	+e6	+e6													
	5	+e16	+e14	+e15	+e16	+e16	+e14													
	6	e	e20	e21	e22	e	e													
	7	e24	e1	e	e	e24	e21													
	0	+	+	+	+	+	+	+	+	+	+									
	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 ⊕ after aeration ACS >



AERATION (4) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr

Date: 8/3/12

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 7-19-12/14:10

YOUNG FROM: SD/DMW 7/11 < 24 hrs

TECHNICIANS: DAY: 0 14:10 YBK 1 9:25 ACS 2 10:30 YBK 3 10:00 ACS 4 13:15 YBK 5 10:15 YBK 6 9:30 ACS 7 8:25 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+	7.9		7.3		25.0		183	
	2	de	de	de	de	de	de					7.9	7.9	8.4	8.1	25.1	25.7	180	182
	3	te	te	te	te	te	te					8.0	8.3	8.4	7.9	25.1	25.2	162	184
	4	+e7	+e7	+e	+e6	+e6	+e5					7.8	7.8	9.1	7.5	24.8	25.3	180	174
	5	+e11	+e13	+e11	+e13	+e10	+e11					7.8	8.1	7.4	6.6	25.0	25.0	187	176
	6	te	te19	te17	te18	te	te					7.7	7.9	8.6	7.3	25.0	24.8	181	183
	7	te19	te	te	te	te15	te18					7.6	8.0	10.8	8.1	24.8	25.6	181	186
12.5%	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+	7.9		7.1		25.0		203	
	2	+	te	te	te	+	+					8.0	8.0	8.1	8.1	24.7	25.7	205	202
	3	te	te	te	te	te	te					8.1	8.4	8.5	8.0	25.1	25.2	190	210
	4	+e4	+e4	+e5	+e5	+e4	+e4					7.9	8.1	8.9	7.8	24.9	25.3	204	201
	5	+e	+e5	+e5	+e6	+e6	+e5					7.8	8.1	7.2	6.6	25.0	25.0	214	200
	6	te2	te	te	te	te	te					7.9	8.0	8.2	7.3	25.0	24.8	206	208
	7	te6	te11	te8	te7	te9	te9					7.9	8.1	8.6	8.3	24.6	25.1	203	210
25%	0	+	+	+	+	+	+	+	+	+	+	New	Old	New	Old	New	Old	New	Old
	1	+	+	+	+	+	+	+	+	+	+					25.0		225	
	2	+	te	de	de	te	+									24.8	25.7	228	224
	3	te	te	te	te	te	+									25.1	25.2	212	232
	4	+e5	+e6	+e4	+e4	+e3	+									25.1	25.3	231	225
	5	+e4	+e6	+e4	+	+e4	+									25.0	25.0	235	224
	6	te	te	te	te	te	te									25.0	24.8	231	228
	7	te6	te7	te6	te	te7	te2									25.4	25.6	232	239

Key: + = live - = dead e = eggs present Δ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



Aeration (4) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am Tr Date: 8/3/12

TEST MATERIAL: EEC 9602 TEST SPECIES: Ceriodaphnia dubia DILUTION WATER: DMW PHOTOPERIOD (L:D): 16:8
 PROJECT NO.: 2179-00 ANIMALS/CONC: 10 /CHAMBER: 1 LIGHT INTENSITY (LUX): 500-1000 TEMPERATURE (°C): 25 ± 1°C
 STARTING DATE/TIME: 7-19-12/14:10 YOUNG FROM: SD/DMW 7/11 < 24 hrs
 TECHNICIANS: DAY: 0 14:10 YBK 1 9:25 ACS 2 10:30 km 3 10:00 ACS 4 13:15 YBK 5 10:15 YBK 6 9:30 ACS 7 8:25 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+							25.0		273	
	2	+	te	de	de	de	+											24.9	25.7	273	268
	3	+	te	te	te	te	te											25.1	25.2	247	280
	4	+	te 14	te 2	te 15	te 15	te 14*											25.9	25.3	283	269
	5	+	te 6				te 5*	te 1										25.0	25.0	281	263
	6	+	te				te *	te 1										25.0	24.8	274	277
	7	+	te 7	te 7	te 3	te 1*	te 3											24.8	25.6	273	276
100%	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+										
	2	+	te	de	de	+	+														
	3	te	te	te	te	te	te														
	4	te 14	te 2	te 15	te 13		te 14														
	5	te 1	te 4	te 6	te		te 3														
	6	te 2	te	te	te		te														
	7	te 7	te 9	te 6	te 3	te 9															
	0	+	+	+	+	+	+	+	+	+	+										
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed



EDTA (5)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Great Lakes Environmental Center

Reviewed by: Carla

Date: 8/3/12

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/14:40

YOUNG FROM: Sel DMW 7-15-12, 1 < 24 hrs

TECHNICIANS: DAY: 0 14:40 YBL 1 1000 ACS 2 1200 Km 3 1030 ACS 4 1240 ACS 5 1245 Km 6 10:10 YBL 7 855 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
DMW	0	+	+	+	+	+	+	+	+	+	+										
	1	+	+	+	+	+	+	+	+	+	+	7.5		6.8		24.8		179			
	2	de	de	de	de	de	de					7.7	7.9	8.3	7.9	24.8	25.3	179	178		
	3	te	te	te	te	te	te					8.0	8.3	9.0	7.8	25.1	25.3	166	176		
	4	te 7	te 6	te 6	te 7	te 3	te 6					7.5	7.8	9.2	7.4	25.2	25.1	179	184		
	5	te 12	te 10	te 9	te 13	te 13	te 12					7.4	7.7	7.0	6.0	24.5	25.5	181	182		
	6	te 1	te 1	te 1	te 18	te 18	te 18					7.6	7.6	7.1	6.8	25.0	24.8	179	185		
	7	te 19	te 22	te 19	te 19	te 19	te 19					7.5	7.5	10.8	8.2	24.8	25.2	176	182		
12.5%	0	+	+	+	+	+	+	+	+	+	+		7.8		8.1		25.3		181		
	1	+	+	+	+	+	+	+	+	+	+	7.8		6.8		24.8		202			
	2	+	te	+	+	te	te					8.1	8.0	8.1	7.9	24.8	25.3	200	200		
	3	te	te	te	te	te	te					8.3	8.4	8.7	7.9	25.1	25.3	194	201		
	4	te 3	te 4	te 3	te 2	te 5	te 6					8.0	8.0	8.7	7.6	24.6	25.1	201	204		
	5	te 3			te 3	te 4	te 6					7.8	7.8	6.9	6.0	24.5	25.5	206	205		
	6	te 1			te 1	te 4	te 1					7.9	7.8	6.8	6.8	25.0	24.8	211	198		
	7	te 10			te 7	te 6	te 6					8.1	8.0	8.4	8.2	24.6	25.2	201	205		
25%	0	+	+	+	+	+	+	+	+	+	+		8.6		8.3		25.3		208		
	1	+	+	+	+	+	+	+	+	+	+					24.8		224			
	2	+	te	te	te	te	te									24.7	25.3	224	220		
	3	te	te	te	te	te	te									25.1	25.3	212	227		
	4	te 4	te 4	te 4	te 4	te 4	te 4									25.9	25.1	233	230		
	5				te 3	te 5										24.5	25.5	231	227		
	6															25.0	24.8	232	214		
	7															25.0	25.2	229	225		

Key: + = live

- = dead

e = eggs present

▲ = possible male daphnid

■ = confirmed male daphnid

* = daphnid is erratic, pale, etc.

d = dead babies present (not counted)

@ = daphnid was killed or mishandled, which may affect survival

S = split brood, attached number indicates how many were observed



EDTA (5) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cmth

Date: 8/3/12

TEST MATERIAL: EEC 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1 °C

STARTING DATE/TIME: 7-19-12/14:40

YOUNG FROM: SPDMW 7-11-12 < 24 hrs

TECHNICIANS: DAY: 0 14:40 YBK 1 1000 ACS 2 1200 km 3 1030 ACS 4 1240 ACS 5 1245 km 6 10:10 YBK 7 855 ACS

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.								
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old							
50%	0	+	+	+	+	+	+	+	+	+	+															
	1	+	+	+	+	+	+											24.8		269						
	2	+	te	te	te	te	te											24.9	25.3	268	263					
	3	te	te	te	te	te	te											25.1	25.3	255	271					
	4	te 4	te 5	te 4	te 4	te 4	te 5											25.4	25.1	271	272					
	5	te 6	te 6	te 5	te 4	te 2	te 4											25.5	25.5	279	269					
	6	te 2	te 2	te 2	te 2	te 2	te 2											25.0	24.8	279	278					
	7	te 9	te 4	te 4	te 6	te	te 6											25.1	25.2	270	272					
100%	0	+	+	+	+	+	+	+	+	+	+															
	1	+	+	+	+	+	+											24.8		347						
	2	+	te	+	te	te	te											8.9 → 7.9	7.3	25.6	25.3	366	348			
	3	te	te	te	te	te	te											9.3 → 8.0 / 8.3	8.3	7.9	25.1	25.3	329	340		
	4	te 1	te 5	te *	te 4	te 4	te d											9.2 → 8.3	8.6	9.2	8.2	25.7	25.1	361	355	
	5	+	te 6	te *	te 3	te 1	te											9.0 → 8.0	8.2	9.1	7.7	24.5	25.5	361	346	
	6	te	te	te 3	te	te	te											8.9 → 8.0	8.2	7.4	6.0	23.0	24.0	371	359	
	7	te	te 7	te 4	te 3	te 4	te 5											8.8	8.2	11.4	8.3	25.0	25.2	358	355	
0	+	+	+	+	+	+	+	+	+	+	+															
1																										
2																										
3																										
4																										
5																										
6																										
7																										

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
 d = dead babies present (not counted) @ = daphnid was killed or mishandled, which may affect survival S = split brood, attached number indicates how many were observed
 (⊕) after aeration

entry error ACS 7/23/12



Sodium Thiosulfate (6)
DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Am T

Date: 8/3/12

TEST MATERIAL: EEC 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 /CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/14:50

YOUNG FROM: SRDMW 7-14-12 < 24 hrs

TECHNICIANS: DAY: 0 14:50 YBK 1 10:15 ACS 2 12:30 km 3 10:45 ACS 4 13:35 ACS 5 11:30 VAK 6 10:40 YBK 7 9:20 YBK

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.	
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old
DMW	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+					7.6		6.7		25.0		209	
	2	de	de	de	de	de	de					7.9	8.1	8.5	8.1	25.7	25.3	210	202
	3	te	te	te	te	te	te					8.1	8.4	8.2	8.1	25.1	25.3	208	206
	4	te 5	te 5	te 6	te 4	te	te					7.8	7.8	9.2	7.9	25.4	25.1	204	202
	5	te 11	te 8 ^d	te 11	te 11	te 9	te 9					7.8	8.0	7.2	6.3	24.5	25.5	208	200
	6	te 19	te 20	te 19	te 15	te 18	+					7.6	7.5	7.1	6.7	25.0	24.8	213	203
	7	te	te	te	te	te	+					7.8	8.0	10.9	8.1	25.1	25.2	207	203
12.5%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+					7.9		6.8		25.0		217	
	2	+	de	de	de	+	+					8.4	8.1	8.1	8.1	25.5	25.3	218	210
	3	te	te	te	te	+	+					8.4	8.3	8.2	8.1	25.1	25.3	214	213
	4	te 4	te 5	te 4	te 4	+	+					8.2	8.0	8.9	7.8	25.0	25.1	212	212
	5	te 6	te 4	te 5	te 5	+	+					8.1	8.0	6.9	6.3	24.5	25.5	215	209
	6	te	te	te	te	+	+					7.9	7.7	6.8	6.7	25.0	24.8	218	212
	7	te 12	te 10	te 11	te 12	+	+					8.2	8.0	8.8	8.0	25.3	25.2	216	213
25%	0	+	+	+	+	+	+	+	+	+	+								
	1	+	+	+	+	+	+									25.0		244	
	2	+	de	de	de	de	+									25.6	25.3	244	234
	3	te	te	te	te	te	te									25.1	25.3	246	242
	4	te 4	te 5	te 4	te 4	te 5	te 4									25.4	25.1	240	244
	5	te 5	te 6	te 5	te 4	te 7	te 7									24.5	25.5	242	239
	6	te	te	te	te	te	te									25.0	24.8	252	244
	7	te 9	te 8	te 8	te 5	te	te									25.0	25.2	245	238

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
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Sodium Thiosulfate (6) DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST

Reviewed by: Cmtr Date: 8/13/12

TEST MATERIAL: EEL 9602

TEST SPECIES: Ceriodaphnia dubia

DILUTION WATER: DMW

PHOTOPERIOD (L:D): 16:8

PROJECT NO.: 2179-00

ANIMALS/CONC: 10 / CHAMBER: 1

LIGHT INTENSITY (LUX): 500-1000

TEMPERATURE (°C): 25 ± 1°C

STARTING DATE/TIME: 7-19-12/14:50

YOUNG FROM: 5:10 PM W 7-11-12, < 24 hrs

TECHNICIANS: DAY: 0 14:50 VLB 1 10:15 ACS 2 12:30 VM 3 10:45 ACS 4 13:35 ACS 5 11:30 VLB 6 10:40 VLB 7 9:20 VLB

Concentration	Day	Test Chamber										pH		DO		Temperature		Spec. Cond.			
		1	2	3	4	5	6	7	8	9	10	New	Old	New	Old	New	Old	New	Old		
50%	0	+	+	+	+	+	+	+	+	+	+										
	1	+*	+	+	+	+	+									25.0		307			
	2	-	te	+	te	te	te									24.6	25.3	303	290		
	3		te	te	te	te	te									25.1	25.3	301	300		
	4		te 6	te 4	te 5	te 5	te 3									25.7	25.1	304	303		
	5		+17	+15	+16	+17	+17									24.5	25.5	306	303		
	6		te	te*	te	te	+7									25.0	24.8	309	305		
	7	↓	+9	↓7	↓8	+17	te									25.2	25.2	307	303		
100%	0	+	+	+	+	+	+	+	+	+							25.1			309	
	1	+	+	+	+	+	+					9.1 → 7.9		7.1		25.0		426			
	2	te	te	te	te	te	te					9.4 → 8.1/8.2		8.6	8.1	24.7	25.3	435	405		
	3	te	te	te	te	te	te					9.4 → 8.3	8.4	8.3	8.1	25.1	25.3	433	415		
	4	te 5	te 5	te 4	te 5	te 5	te 5					7.2 → 8.3	8.2	7.3	7.7	25.2	25.1	431	420		
	5	+16	+15	+14	+15	+15	+16					9.1 → 8.1	8.3	7.4	6.2	24.5	25.5	435	426		
	6	te	te	te	te	te	te					9.0	8.1	7.4	6.7	25.0	24.8	441	422		
	7	+16	+13	+14	+14	+17	+17					9.0	8.2	11.2	8.2	25.3	25.2	438	421		
	0	+	+	+	+	+	+	+	+	+		8.1		8.4						442	
	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				

Key: + = live - = dead e = eggs present ▲ = possible male daphnid ■ = confirmed male daphnid * = daphnid is erratic, pale, etc.
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 ⊕ = after aeration

APPENDIX B
DATA FOR THE 20 MOST RECENT CHRONIC TOXICITY TESTS

Most Recent 20 Sodium Chloride
Reference Toxicant IC25

TEST DATE	TEST NO.	C. dubia	FHM
7/10	100	1.46	1.94
8/10	101	1.46	2.14
9/10	102	1.37	1.98
10/10	103	1.50	2.24
11/10	104	1.20	2.38
12/10	105	1.43	2.45
2/11	106	1.04	2.05
3/11	107	1.06	2.86
4/11	108	1.38	1.56
5/11	109	1.21	1.97
6/11	110	0.79	1.65
8/11	111		1.92
9/11	112	1.40	2.07
10/11	113	1.41	2.53
11/11	114	1.39	
12/11	115	0.78	2.68
3/12	116	1.46	2.42
4/12	117	1.37	2.51
5/12	118	1.46	1.66
6/12	119	1.36	2.02
AVERAGE		1.29	2.16
STD. DEV.		0.22	0.35
RANGE: LOW		0.86	1.46
RANGE: HIGH		1.72	2.86
Coefficient of variation		0.17	0.16
Date of last test		6/21-28/12	6/20-27/12
MSD of most recent test		6.8	0.0832
PMSD of most recent test		23.7	17.4
Upper and lower bounds ¹		13 - 47	12 - 30

¹ Lower and upper PMSD bounds were determined from the 10th and 90th

From EPA's Wet Interlaboratory Variability Study

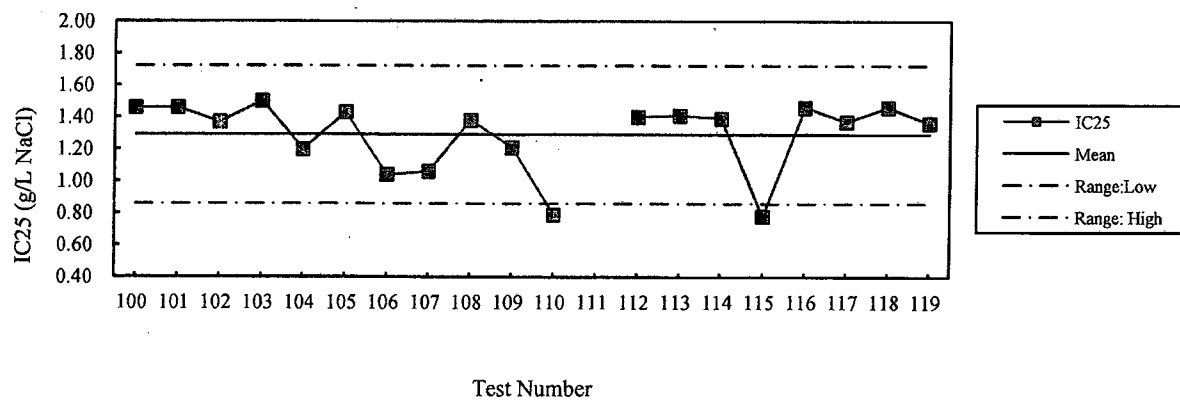
IC25 Coefficient of Variation						
National Percentiles ²						
Test Species	GLEC ¹	10th	25th	50th	75th	90th
C. dubia	0.17	0.08	0.17	0.27	0.45	0.62
P. promelas	0.16	0.12	0.21	0.26	0.38	0.45

¹ Based on cumulative GLEC data from the most recent 20 tests.

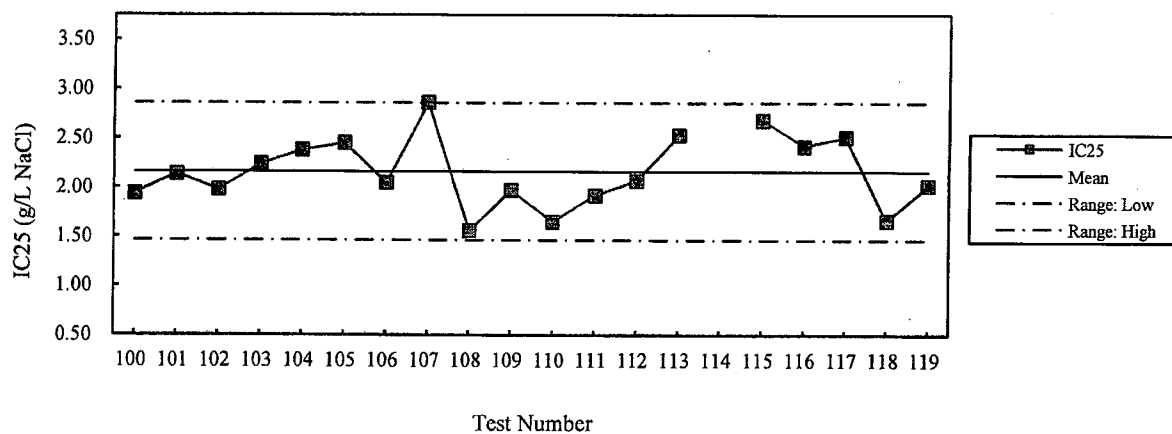
² EPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications.

*Blank spaces indicate that the data is not available for that month

Chronic Reference Toxicant IC25
Ceriodaphnia dubia



Chronic Reference Toxicant IC25
Pimephales promelas



From: (870) 863-1125
Larken Pennington
El Dorado Chemical Co.
4500 Northwest Ave.

Origin ID: ELDA



J12201207160325

El Dorado, AR 71730

Ship Date: 31AUG12
ActWgt: 1.0 LB
CAD: 5887030/NET3300

Delivery Address Bar Code



SHIP TO: (870) 863-1484

BILL SENDER

Mary Barnett
ADEQ - Water Division Enforcement
5301 NORTSHORE DR

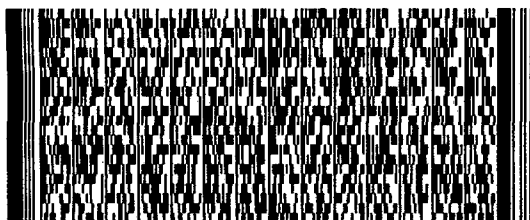
Ref #
Invoice #
PO #
Dept #

NORTH LITTLE ROCK, AR 72118

TUE - 04 SEP A4
PRIORITY OVERNIGHT

TRK# 7988 7162 2313
0201

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