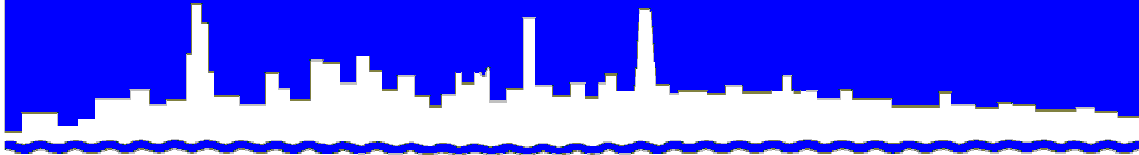


Protecting Our Water Environment



Metropolitan Water Reclamation District of Greater Chicago

***MONITORING AND RESEARCH
DEPARTMENT***

REPORT NO. 09-59

***BIOMONITORING REPORT
2009***

***CHRONIC WHOLE EFFLUENT TOXICITY TEST RESULTS
FOR THE HANOVER PARK WATER RECLAMATION PLANT,
HANOVER PARK, ILLINOIS,***

***NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
PERMIT NUMBER IL0036137, JULY 2009***

SEPTEMBER 2009

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Louis Kollias, P.E., BCEE
Director of Monitoring and Research
louis.kollias@mwr.org

September 9, 2009

Ms. Catherine Siders
Environmental Specialist
Compliance Assurance Section - 19
Illinois Environmental Protection Agency
1021 North Grand Avenue
Springfield, IL 62794-9276

Dear Ms. Siders:

Subject: Biomonitoring Report for 2009 – Chronic Whole Effluent Toxicity Test Results for the Hanover Park Water Reclamation Plant, Hanover Park, Illinois, National Pollutant Discharge Elimination System Permit Number IL0036137, July 2009

The subject Biomonitoring Report is submitted in compliance with the National Pollutant Discharge Elimination System Permit Number IL0036137, Special Condition 11.

The subject report includes copies of all bench sheets, chain-of-custody forms, sample receipt and preparation forms, hard copies of computer generated statistical analyses, control charts, and a certification of accuracy statement.

If you have any questions concerning this report, please contact Dr. Geeta Rijal, Microbiologist IV at (708) 588-4224.

Very truly yours,

Louis Kollias
Director
Monitoring and Research

LK:TCG:GR:cm
Enclosures
cc w/encl.: Jamjun/Gronski/Grabis/Granato
Lazicki/O'Connor/Rijal/Glymph
cc: Cohen (Transmittal letter and report title page)

Metropolitan Water Reclamation District of Greater Chicago
100 East Erie Street Chicago, IL 60611-2803 (312) 751-5600

**BIOMONITORING REPORT
2009**

**CHRONIC WHOLE EFFLUENT TOXICITY TEST RESULTS
FOR HANOVER PARK WATER RECLAMATION PLANT
HANOVER PARK, ILLINOIS**

**NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
PERMIT NUMBER IL0036137, JULY 2009**

**Monitoring and Research Department
Louis Kollias, Director**

SEPTEMBER 2009

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ACKNOWLEDGMENTS

Mrs. Barbara Sanders is acknowledged for typing this report.

DISCLAIMER

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

CHRONIC WHOLE EFFLUENT TOXICITY TEST RESULTS FOR THE HANOVER PARK
WATER RECLAMATION PLANT, HANOVER PARK, ILLINOIS
NATIONAL POLLUTION DISCHARGE ELIMINATION SYSTEM
PERMIT NUMBER IL0036137, JULY 2009

Summary

The chronic toxicity test with *Pimephales promelas* (*P. promelas*) (7-day, static, renewal), was conducted on samples of Hanover Park Water Reclamation Plant (WRP) final effluent collected July 20-25, 2009. The results indicated that the tests were valid. No toxic effect on *P. promelas* larval survival or growth was observed. Results of the quality control chronic toxicity tests with *P. promelas* using the reference toxicant sodium chloride (NaCl) fell within control chart limits prescribed as acceptable by the United States Environmental Protection Agency (USEPA).

The chronic toxicity test with *Ceriodaphnia dubia* (*C. dubia*) (7-day, static, renewal) was conducted on samples of the Hanover Park WRP final effluent collected July 20-25, 2009. The results indicated that the tests were valid. No toxic effect on *C. dubia* survival or reproduction was observed. Results of quality control chronic toxicity tests with *C. dubia* using the reference toxicant NaCl fell within limits prescribed as acceptable by the USEPA.

Sample Information

Tests were performed using 24-hour composite samples of Hanover Park WRP final effluent collected on July 20 through July 25, 2009 for the chronic toxicity tests. The individual grab samples were stored on site at 0.1 – 6°C in a refrigerator. These samples were received in the laboratory within 4 hours of the final grab sample collection. Sample temperatures at the time of receipt were below 6°C. Samples were stored in the laboratory at 4 ± 1°C. Sample collection information is shown in Table 1.

Whole Effluent Toxicity Tests

The chronic toxicity tests with *P. promelas* and *C. dubia* were conducted on the Hanover Park WRP effluent samples collected July 20 through July 25, 2009. Chronic Whole Effluent Toxicity (WET) test methods and procedures were followed in accordance with *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, EPA 821/R-02/013, Fourth Edition, October 2002. *P. promelas* were exposed to 12.5, 25, 50, 75, and 100 percent concentration of final effluent for seven days. *C. dubia* were exposed to the same concentrations of effluent for seven days.

TABLE 1: SAMPLE COLLECTION INFORMATION

Effluent Collection Point:	Hanover Park Water Reclamation Plant Effluent Discharge
----------------------------	---

Effluent Collection Method:	Three 24-hour composite samples. Five 2 1/2 gallon grab samples collected over a 24-hour period were combined to make each 24-hour composite sample. The individual grab samples were collected at 6-hour intervals.
-----------------------------	--

Effluent Collection Times and Dates:	
First Sample Set	0600 July 20, 2009 1200 July 20, 2009 1800 July 20, 2009 2400 July 20, 2009 0600 July 21, 2009
Second Sample Set	0600 July 22, 2009 *1540 July 22, 2009 1800 July 22, 2009 2400 July 22, 2009 0600 July 23, 2009
Third Sample Set	0600 July 24, 2009 1200 July 24, 2009 1800 July 24, 2009 2400 July 24, 2009 0600 July 25, 2009

*Due to a scheduled shut-down there was no flow to outfall 007. The sample was collected after the flow was restored.

The chronic fathead minnow test (*P. promelas*) was set up on 07/22/09 and completed on 07/29/09. The chronic *C. dubia* test was set up on 07/22/09 and completed on 07/29/09. Hard synthetic water with selenium (HSW) was used as control and dilution water for both test species. The laboratory controls met USEPA test acceptability criteria for both test species. Statistical analyses were performed using the CETIS™ Software program version 1.7.0 (Tidepool Scientific Software, California).

Concurrent reference toxicant tests (RTT) using sodium chloride (NaCl) were conducted, and the control charts for the *P. promelas* and *C. dubia* chronic RTT were prepared.

Analysts

WET tests were conducted by G. V. Billett (Laboratory Technician II) and James Kaehn (Laboratory Technician I). Auralene Glymph (Microbiologist III) entered the raw data in an Excel and CETIS™ program. Auralene Glymph and Geeta Rijal (Microbiologist IV) prepared this report.

Results

Results of the chronic *P. promelas* WET test are shown in [Table 2](#). The *P. promelas* test results indicated a valid test. No toxic effect on *P. promelas* larval survival or growth was observed. The HSW control water met the test acceptability criteria (>80% survival) for the *P. promelas* test. Results of the quality control chronic toxicity test with *P. promelas* using the RTT fell within limits prescribed as acceptable by the USEPA, i.e. within ± 2 standard deviations from the mean.

Results of the chronic *C. dubia* WET test are shown in [Table 3](#). The *C. dubia* test results indicated a valid test. No toxic effect on *C. dubia* survival or reproduction was observed. The HSW control water met the test acceptability criteria (>80% survival) for the *C. dubia* test. Results of the quality control, chronic toxicity test with *C. dubia* using the RTT fell within limits prescribed as acceptable by USEPA, i.e. within ± 2 standard deviations from the mean.

The WET test results indicated the absence of chronic toxicity to *P. promelas* and *C. dubia*. Tabulated summaries of the *P. promelas* and *C. dubia* WET tests are presented in [Appendices AI](#) and [AII](#), respectively. Raw data for the *P. promelas* and *C. dubia* WET tests are presented in [Appendices BI](#) and [BII](#), respectively. Chain-of-Custody documentation is provided in [Appendix CI](#). Raw data, statistical calculations, culture data, and control charts for the *P. promelas* and *C. dubia* concurrent RTT are provided in [Appendices DI](#) and [DII](#), respectively.

TABLE 2: CHRONIC *PIMEPHALES PROMELAS* TEST RESULTS

Chronic Test Parameters	Results
NOEC ¹ Value (Survival)	100%
NOEC Value (Growth)	100%
IC ₂₅ (Growth)	>100%
7-Day Survival Rate (Control) ²	>95%
Mean Dry Weight (Control) ³ <i>P. promelas</i> (Growth)	1.18 mg ($\alpha=0.05$)
Minimum Significant Difference (MSD) ⁴ <i>P. promelas</i> (Growth)	17.7
Toxicity Observed	No
Valid Test	Yes
Concurrent Reference Toxicant Test in Control	Yes

¹No observed effect concentration.

²Results within test acceptability criteria (80% - NL) limits.

³Results within test acceptability criteria (0.25 - NL) limits.

⁴Results within test acceptability criteria (12 - 30%) limits.

TABLE 3: CHRONIC *CERIODAPHNIA DUBIA* TEST RESULTS

Chronic Test Parameters	Results
NOEC ¹ Value (Survival)	100%
NOEC Value (Reproduction)	100%
IC ₂₅ (Reproduction)	>100%
7-Day Survival Rate (Control) ²	100%
7-Day Mean Reproduction (Control) ³	31.4
Minimum Significant Difference (MSD) ⁴ <i>C. dubia</i> (Reproduction)	18.7 ($\alpha=0.05$)
Toxicity Observed	No
Valid Test	Yes
Concurrent Reference Toxicant Test in Control	Yes

¹No observed effect concentration.

²Results within test acceptability criteria (80% - NL) limits.

³Results within test acceptability criteria (15 - NL) limits.

⁴Results within test acceptability criteria (12 - 30%) limits.

CERTIFICATION OF ACCURACY

I certify under penalty of law that this document and all appendices were prepared under my supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering data, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations 40 C.F.R. 122.22 (d).

Date

Louis Kollias
Director
Monitoring and Research

If you have any questions concerning this report, please contact Dr. Geeta Rijal, Microbiologist IV, at 708-588-4224.

APPENDIX AI

SUMMARY OF CHRONIC WHOLE EFFLUENT TOXICITY RESULTS
PIMEPHALES PROMELAS
CETIS TEST SUMMARY AND COMPARISON REPORT

CETIS Summary Report

Report Date: 18 Aug-09 08:50 (p 1 of 2)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Batch ID: 09-1102-4977	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 22 Jul-09	Protocol: EPA/821/R-02-013 (2002)	Diluent: Hard Synthetic Water
Ending Date: 29 Jul-09	Species: Pimephales promelas	Brine: Not Applicable
Duration: 7d 0h	Source: Environmental Consult & Test	Age: 48h
Sample ID: 14-7567-2707	Code: 57F4FA83	Client:
Sample Date: 21 Jul-09	Material: POTW Effluent	Project:
Receive Date: 21 Jul-09	Source: Hanover Park WRP	
Sample Age: 24h	Station:	

Comments: Hanover Park Chronic Fish Test on samples collected July 20-25, 2009.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-5708-3935	7d Survival Rate	100	>100	N/A	7.46%	1	Steel Many-One Rank Test
01-1499-8662	Mean Dry Weight-mg	100	>100	N/A	17.7%	1	Dunnett's Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	Conc-%	95% LCL	95% UCL	TU	Method
21-1100-1271	Mean Dry Weight-mg	IC25	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
17-5708-3935	7d Survival Rate	Control Resp	0.95	0.8 - NL	Yes	Result Within Limits
01-1499-8662	Mean Dry Weight-mg	Control Resp	1.18	0.25 - NL	Yes	Result Within Limits
21-1100-1271	Mean Dry Weight-mg	Control Resp	1.18	0.25 - NL	Yes	Result Within Limits
01-1499-8662	Mean Dry Weight-mg	PMSD	0.177	0.12 - 0.3	Yes	Result Within Limits

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	0.95	0.913	0.987	0.8	1	0.0183	0.1	10.5%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	-5.26%
25		4	0.975	0.956	0.994	0.9	1	0.00913	0.05	5.13%	-2.63%
50		4	1	1	1	1	1	0	0	0.0%	-5.26%
75		4	1	1	1	1	1	0	0	0.0%	-5.26%
100		4	1	1	1	1	1	0	0	0.0%	-5.26%

Mean Dry Weight-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	1.18	1.13	1.23	1.06	1.32	0.0251	0.137	11.7%	0.0%
12.5		4	1.33	1.28	1.38	1.13	1.43	0.025	0.137	10.3%	-13.2%
25		4	1.24	1.19	1.29	1.12	1.44	0.0251	0.138	11.1%	-5.52%
50		4	1.38	1.36	1.4	1.32	1.43	0.00935	0.0512	3.72%	-17.0%
75		4	1.29	1.27	1.31	1.21	1.33	0.0102	0.0556	4.3%	-9.77%
100		4	1.36	1.33	1.39	1.28	1.43	0.0143	0.0785	5.78%	-15.3%

CETIS Summary Report

Report Date: 18 Aug-09 08:50 (p 2 of 2)
Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

7d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1	1	1	0.8
12.5		1	1	1	1
25		1	1	1	0.9
50		1	1	1	1
75		1	1	1	1
100		1	1	1	1

Mean Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1.32	1.27	1.06	1.06
12.5		1.38	1.13	1.43	1.39
25		1.2	1.21	1.44	1.12
50		1.35	1.43	1.32	1.41
75		1.33	1.21	1.31	1.32
100		1.28	1.43	1.42	1.3

CETIS Analytical Report

Report Date: 18 Aug-09 08:50 (p 1 of 4)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Analysis-ID: 17-5708-3935	Endpoint: 7d Survival Rate	CETIS-Version: CETISv1.7.0
Analyzed: 18 Aug-09 8:49	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes
Batch ID: 09-1102-4977	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 22 Jul-09	Protocol: EPA/821/R-02-013 (2002)	Diluent: Hard Synthetic Water
Ending Date: 29 Jul-09	Species: Pimephales promelas	Brine: Not Applicable
Duration: 7d 0h	Source: Environmental Consult & Test	Age: 48h
Sample ID: 14-7567-2707	Code: 57F4FA83	Client:
Sample Date: 21 Jul-09	Material: POTW Effluent	Project:
Receive Date: 21 Jul-09	Source: Hanover Park WRP	
Sample Age: 24h	Station:	

Comments: Hanover Park Chronic Fish Test on samples collected July 20-25, 2009.

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)		C > T	Not Run	100	>100	N/A	1	7.46%

Steel Many-One Rank Test

Control	vs Conc-%	Test Stat	Critical	Ties	P-Value	Decision(5%)
Lab Water	12.5	20	10	1	0.9520	Non-Significant Effect
	25	18.5	10	1	0.8730	Non-Significant Effect
	50	20	10	1	0.9520	Non-Significant Effect
	75	20	10	1	0.9520	Non-Significant Effect
	100	20	10	1	0.9520	Non-Significant Effect

Test Acceptability

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.95	0.8 - NL	Yes	Result Within Limits

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.02075619	0.004151239	5	0.834	0.5430	Non-Significant Effect
Error	0.08962759	0.004979311	18			
Total	0.1103838	0.00913055	23			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(5%)
Variances	Mod Levene Equality of Variance	0.834	2.77	0.5430	Equal Variances
Distribution	Shapiro-Wilk Normality	0.644		0.0000	Non-normal Distribution

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	0.95	0.912	0.988	0.8	1	0.0186	0.1	10.5%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	-5.26%
25		4	0.975	0.956	0.994	0.9	1	0.00928	0.05	5.13%	-2.63%
50		4	1	1	1	1	1	0	0	0.0%	-5.26%
75		4	1	1	1	1	1	0	0	0.0%	-5.26%
100		4	1	1	1	1	1	0	0	0.0%	-5.26%

Angular (Corrected) Transformed Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	1.34	1.28	1.39	1.11	1.41	0.0283	0.152	11.4%	0.0%
12.5		4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	-5.71%
25		4	1.37	1.34	1.4	1.25	1.41	0.0151	0.0815	5.94%	-2.66%
50		4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	-5.71%
75		4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	-5.71%
100		4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	-5.71%

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

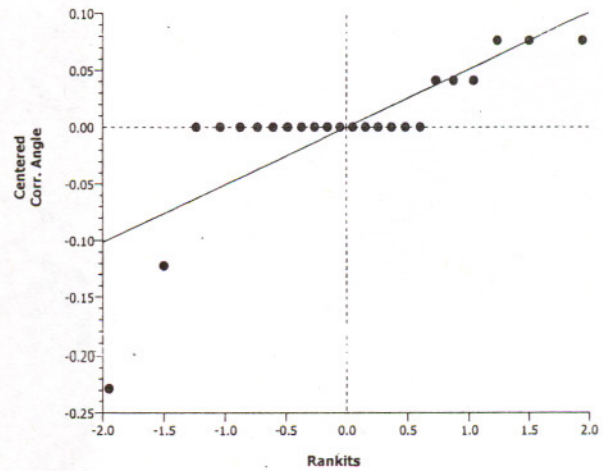
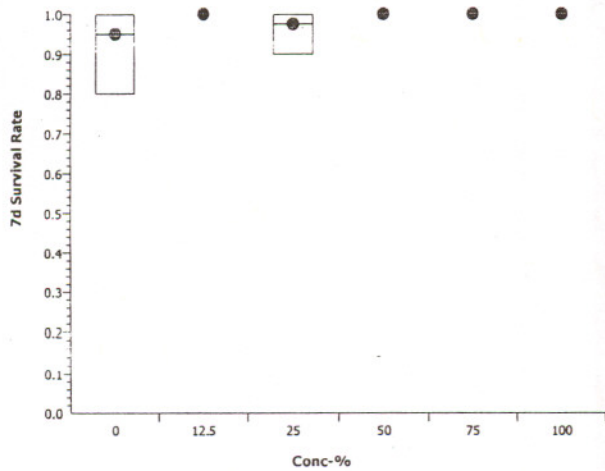
Analysis ID: 17-5708-3935 Endpoint: 7d Survival Rate
 Analyzed: 18 Aug-09 8:49 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.7.0
 Official Results: Yes

7d-Survival Rate-Details

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1	1	1	0.8
12.5		1	1	1	1
25		1	1	1	0.9
50		1	1	1	1
75		1	1	1	1
100		1	1	1	1

Graphics



CETIS Analytical Report

Report Date: 18 Aug-09 08:50 (p 3 of 4)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Analysis ID: 01-1499-8662	Endpoint: Mean Dry Weight-mg	CETIS Version: CETISv1.7.0
Analyzed: 18 Aug-09 8:48	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 09-1102-4977	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 22 Jul-09	Protocol: EPA/821/R-02-013 (2002)	Diluent: Hard Synthetic Water
Ending Date: 29 Jul-09	Species: Pimephales promelas	Brine: Not Applicable
Duration: 7d 0h	Source: Environmental Consult & Test	Age: 48h
Sample ID: 14-7567-2707	Code: 57F4FA83	Client:
Sample Date: 21 Jul-09	Material: POTW Effluent	Project:
Receive Date: 21 Jul-09	Source: Hanover Park WRP	
Sample Age: 24h	Station:	

Comments: Hanover Park Chronic Fish Test on samples collected July 20-25, 2009.

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C <> T	Not Run	100	>100	N/A	1	17.7%

Dunnett's Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Lab Water		12.5	2.05	2.76	0.208	0.1890	Non-Significant Effect
		25	0.861	2.76	0.208	0.8560	Non-Significant Effect
		50	2.65	2.76	0.208	0.0624	Non-Significant Effect
		75	1.52	2.76	0.208	0.4330	Non-Significant Effect
		100	2.38	2.76	0.208	0.1040	Non-Significant Effect

Test Acceptability

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1.18	0.25 - NL	Yes	Result Within Limits
PMSD	0.177	0.12 - 0.3	Yes	Result Within Limits

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.1146722	0.02293445	5	2.01	0.1250	Non-Significant Effect
Error	0.2050553	0.01139196	18			
Total	0.3197276	0.03432641	23			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	4.99	15.1	0.4170	Equal Variances
Distribution	Shapiro-Wilk Normality	0.983		0.9410	Normal Distribution

Mean Dry Weight-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	1.18	1.13	1.23	1.06	1.32	0.0255	0.137	11.7%	0.0%
12.5		4	1.33	1.28	1.38	1.13	1.43	0.0254	0.137	10.3%	-13.2%
25		4	1.24	1.19	1.29	1.12	1.44	0.0256	0.138	11.1%	-5.52%
50		4	1.38	1.36	1.4	1.32	1.43	0.00951	0.0512	3.72%	-17.0%
75		4	1.29	1.27	1.31	1.21	1.33	0.0103	0.0556	4.3%	-9.77%
100		4	1.36	1.33	1.39	1.28	1.43	0.0146	0.0785	5.78%	-15.3%

000-015-170-3

CETIS™ v1.7.0

Analyst: _____ QA: _____

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Analysis ID: 01-1499-8662
 Analyzed: 18 Aug-09 8:48

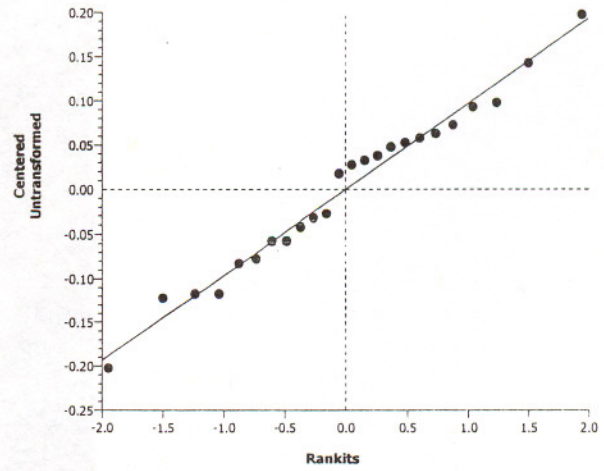
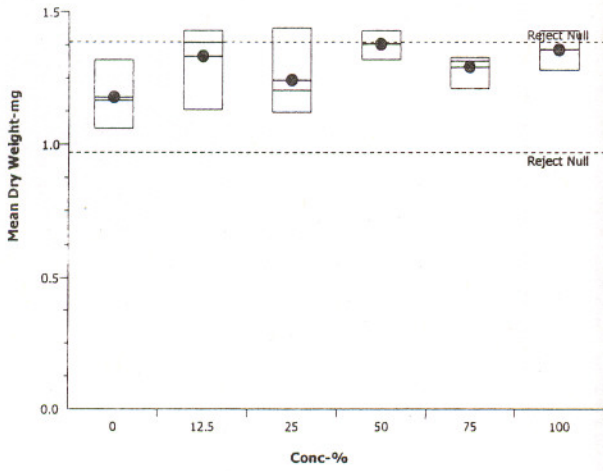
Endpoint: Mean Dry Weight-mg
 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.7.0
 Official Results: Yes

Mean Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1.32	1.27	1.06	1.06
12.5		1.38	1.13	1.43	1.39
25		1.2	1.21	1.44	1.12
50		1.35	1.43	1.32	1.41
75		1.33	1.21	1.31	1.32
100		1.28	1.43	1.42	1.3

Graphics



CETIS Analytical Report

Report Date: 18 Aug-09 08:50 (p 1 of 2)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Analysis ID: 21-1100-1271 **Endpoint:** Mean Dry Weight-mg **CETIS Version:** CETISv1.7.0
Analyzed: 18 Aug-09 8:48 **Analysis:** Linear Interpolation (ICPIN) **Official Results:** Yes

Batch ID: 09-1102-4977 **Test Type:** Growth Survival (7d) **Analyst:**
Start Date: 22 Jul-09 **Protocol:** EPA/821/R-02-013 (2002) **Diluent:** Hard Synthetic Water
Ending Date: 29 Jul-09 **Species:** Pimephales promelas **Brine:** Not Applicable
Duration: 7d 0h **Source:** Environmental Consult & Test **Age:** 48h

Sample ID: 14-7567-2707 **Code:** 57F4FA83 **Client:**
Sample Date: 21 Jul-09 **Material:** POTW Effluent **Project:**
Receive Date: 21 Jul-09 **Source:** Hanover Park WRP
Sample Age: 24h **Station:**

Comments: Hanover Park Chronic Fish Test on samples collected July 20-25, 2009.

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	57951	200	Yes	Two-Point Interpolation

Test Acceptability

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1.18	0.25 - NL	Yes	Result Within Limits

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	N/A	N/A	<1	N/A	N/A

Mean Dry Weight-mg Summary

Calculated Variate

Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	1.18	1.06	1.32	0.0251	0.137	11.7%	0.0%
12.5		4	1.33	1.13	1.43	0.025	0.137	10.3%	-13.2%
25		4	1.24	1.12	1.44	0.0251	0.138	11.1%	-5.52%
50		4	1.38	1.32	1.43	0.00935	0.0512	3.72%	-17.0%
75		4	1.29	1.21	1.33	0.0102	0.0556	4.3%	-9.77%
100		4	1.36	1.28	1.43	0.0143	0.0785	5.78%	-15.3%

Mean Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1.32	1.27	1.06	1.06
12.5		1.38	1.13	1.43	1.39
25		1.2	1.21	1.44	1.12
50		1.35	1.43	1.32	1.41
75		1.33	1.21	1.31	1.32
100		1.28	1.43	1.42	1.3

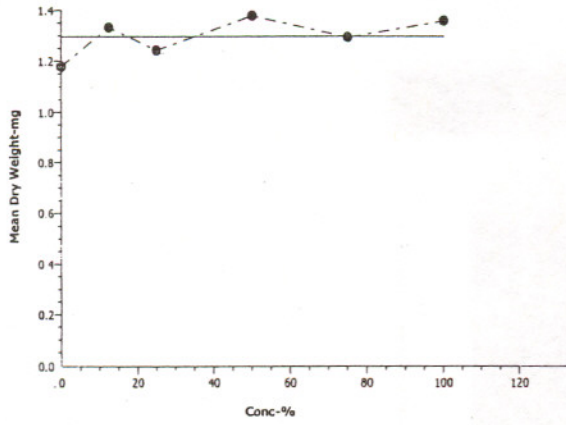
Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Analysis ID: 21-1100-1271 Endpoint: Mean Dry Weight-mg
Analyzed: 18 Aug-09 8:48 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.7.0
Official Results: Yes

Graphics



CETIS Measurement Report

Report Date: 20 Aug-09 09:36 (p 1 of 4)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Batch ID: 09-1102-4977	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 22 Jul-09	Protocol: EPA/821/R-02-013 (2002)	Diluent: Hard Synthetic Water
Ending Date: 29 Jul-09	Species: Pimephales promelas	Brine: Not Applicable
Duration: 7d 0h	Source: Environmental Consult & Test	Age: 48h

Comments: Hanover Park Chronic Fish Test on samples collected July 20-25, 2009.

Parameter Acceptability

Parameter	Min	Max	Acceptability Limits	Overlap	Decision
Total Residual Chlorine-mg/L	0	0	0 - 0	Yes	Results Within Limits
Final Dissolved Oxygen-mg/L	4.34	5.85	4 - NL	Yes	Results Within Limits
Initial Dissolved Oxygen-mg/L	7.04	8.95	4 - NL	Yes	Results Within Limits
Total Ammonia (N)-mg/L	0.18	0.38	NL - 5	Yes	Results Within Limits
Final pH-Units	6.68	7.41	6 - 9	Yes	Results Within Limits
Initial pH-Units	7.27	7.9	6 - 9	Yes	Results Within Limits
Temperature-°C	24.6	25.8	24 - 26	Yes	Results Within Limits

Alkalinity (CaCO3)-mg/L

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	3	113	112	113	112	114	0.192	1.15	1.02%	0
100		3	113	112	114	110	116	0.509	3.06	2.7%	0
Overall		6	113			110	116				0 (0%)

Total Residual Chlorine-mg/L

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	3	0	0	0	0	0	0	0	0	0
Overall		3	0			0	0				0 (0%)

Conductivity-µmhos

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	8	0.573	0.572	0.575	0.567	0.581	0.000839	0.00504	0.88%	0
12.5		8	0.604	0.602	0.606	0.594	0.61	0.000821	0.00493	0.82%	0
25		8	0.639	0.637	0.641	0.63	0.644	0.000745	0.00447	0.7%	0
50		8	0.717	0.714	0.719	0.701	0.725	0.00138	0.00828	1.16%	0
75		8	0.795	0.791	0.799	0.776	0.808	0.00195	0.0117	1.47%	0
100		8	0.871	0.865	0.876	0.84	0.887	0.00287	0.0172	1.98%	0
Overall		48	0.7			0.567	0.887				0 (0%)

Final Dissolved Oxygen-mg/L

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	5.19	5.01	5.37	4.34	5.85	0.0875	0.525	10.1%	0
12.5		7	5.25	5.11	5.39	4.67	5.72	0.0702	0.421	8.02%	0
25		7	4.8	4.74	4.86	4.45	5.03	0.0313	0.188	3.91%	0
50		7	5.22	5.12	5.31	4.7	5.54	0.0476	0.285	5.47%	0
75		7	5.28	5.16	5.39	4.7	5.7	0.0555	0.333	6.31%	0
100		7	5.29	5.19	5.39	4.79	5.62	0.0493	0.296	5.59%	0
Overall		42	5.17			4.34	5.85				0 (0%)

Initial Dissolved Oxygen-mg/L

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	7.37	7.28	7.46	7.08	7.71	0.0453	0.272	3.69%	0
12.5		7	7.57	7.46	7.68	7.04	8.01	0.0527	0.316	4.18%	0
25		7	7.72	7.63	7.82	7.19	8.03	0.0462	0.277	3.59%	0
50		7	8.06	7.98	8.13	7.69	8.41	0.0382	0.229	2.84%	0
75		7	8.39	8.3	8.48	7.96	8.72	0.0427	0.256	3.05%	0
100		7	8.74	8.65	8.83	8.21	8.95	0.0431	0.259	2.96%	0
Overall		42	7.98			7.04	8.95				0 (0%)

CETIS Measurement Report

Report Date: 20 Aug-09 09:36 (p 2 of 4)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Hardness (CaCO3)-mg/L

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	3	167	165	168	164	172	0.77	4.62	2.77%	0
100		3	217	214	221	208	228	1.68	10.1	4.63%	0
Overall		6	192			164	228				0 (0%)

Total Ammonia (N)-mg/L

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	3	0.257	0.22	0.293	0.18	0.38	0.018	0.108	42.0%	0
Overall		3	0.257			0.18	0.38				0 (0%)

Final pH-Units

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	7.2	7.12	7.29	6.68	7.41	0.0402	0.241	3.35%	0
12.5		7	7.35	7.33	7.37	7.25	7.39	0.00822	0.0493	0.67%	0
25		7	7.32	7.31	7.33	7.29	7.37	0.00435	0.0261	0.36%	0
50		7	7.36	7.35	7.37	7.32	7.4	0.00478	0.0287	0.39%	0
75		7	7.35	7.34	7.36	7.29	7.39	0.00604	0.0363	0.49%	0
100		7	7.34	7.33	7.36	7.28	7.4	0.00686	0.0412	0.56%	0
Overall		42	7.32			6.68	7.41				0 (0%)

Initial pH-Units

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	7.79	7.77	7.81	7.69	7.84	0.00825	0.0495	0.64%	0
12.5		7	7.84	7.82	7.85	7.79	7.9	0.00686	0.0412	0.53%	0
25		7	7.74	7.73	7.75	7.69	7.78	0.00539	0.0324	0.42%	0
50		7	7.55	7.55	7.56	7.51	7.59	0.0045	0.027	0.36%	0
75		7	7.44	7.42	7.45	7.38	7.47	0.00592	0.0355	0.48%	0
100		7	7.34	7.32	7.35	7.27	7.38	0.00592	0.0355	0.48%	0
Overall		42	7.62			7.27	7.9				0 (0%)

Temperature-°C

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	8	25.5	25.4	25.6	24.7	25.8	0.0587	0.352	1.38%	0
12.5		8	25.5	25.4	25.6	24.7	25.7	0.0559	0.336	1.32%	0
25		8	25.5	25.3	25.6	24.7	25.8	0.057	0.342	1.34%	0
50		8	25.5	25.3	25.6	24.6	25.7	0.0591	0.354	1.39%	0
75		8	25.5	25.4	25.6	24.6	25.8	0.0636	0.382	1.5%	0
100		8	25.5	25.4	25.6	24.7	25.8	0.0562	0.337	1.32%	0
Overall		48	25.5			24.6	25.8				0 (0%)

CETIS Measurement Report

Report Date: 20 Aug-09 09:36 (p 3 of 4)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Alkalinity (CaCO3)-mg/L

Conc-%	Control Type	1	2	3
0	Lab Water	112	114	112
12.5				
25				
50				
75				
100		110	116	114

Total Residual Chlorine-mg/L

Conc-%	Control Type	1	2	3
0	Lab Water	0	0	0
12.5				
25				
50				
75				
100				

Conductivity-µmhos

Conc-%	Control Type	1	2	3	4	5	6	7	8
0	Lab Water	0.571	0.567	0.57	0.571	0.577	0.581	0.57	0.579
12.5		0.602	0.594	0.602	0.604	0.607	0.61	0.608	0.605
25		0.639	0.63	0.637	0.639	0.644	0.638	0.644	0.641
50		0.707	0.701	0.718	0.717	0.723	0.725	0.721	0.72
75		0.781	0.776	0.792	0.794	0.802	0.808	0.802	0.806
100		0.851	0.84	0.867	0.87	0.882	0.887	0.881	0.886

Final Dissolved Oxygen-mg/L

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	4.77	5.21	4.34	5.52	5.03	5.85	5.62
12.5		4.67	4.98	4.83	5.72	5.36	5.63	5.57
25		4.45	4.86	4.67	5.03	4.91	4.81	4.86
50		4.7	5.31	5.03	5.54	5.14	5.38	5.42
75		5.46	5.7	4.7	5.46	4.99	5.29	5.34
100		4.79	5.51	5.19	5.62	5.03	5.42	5.44

Initial Dissolved Oxygen-mg/L

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	7.18	7.11	7.62	7.27	7.63	7.71	7.08
12.5		8.01	7.04	7.55	7.45	7.83	7.7	7.41
25		8.03	7.19	7.75	7.72	7.93	7.86	7.58
50		8.41	7.69	8.08	8.06	8.21	8.07	7.88
75		8.72	7.96	8.64	8.32	8.49	8.34	8.25
100		8.85	8.21	8.95	8.64	8.86	8.94	8.74

Hardness (CaCO3)-mg/L

Conc-%	Control Type	1	2	3
0	Lab Water	164	164	172
12.5				
25				
50				
75				
100		208	216	228

CETIS Measurement Report

Report Date: 20 Aug-09 09:36 (p 4 of 4)
 Test Code: 12-4437-7691/4A2BB25B

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Total Ammonia (N)-mg/L

Conc-%	Control Type	1	2	3
0	Lab Water	0.21	0.38	0.18
12.5				
25				
50				
75				
100				

Final pH-Units

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	6.68	7.23	7.22	7.25	7.29	7.35	7.41
12.5		7.25	7.33	7.36	7.39	7.35	7.38	7.39
25		7.3	7.32	7.37	7.33	7.31	7.29	7.31
50		7.32	7.37	7.4	7.38	7.33	7.36	7.34
75		7.39	7.39	7.36	7.35	7.29	7.34	7.32
100		7.32	7.34	7.39	7.4	7.28	7.35	7.33

Initial pH-Units

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	7.8	7.83	7.78	7.84	7.69	7.81	7.79
12.5		7.8	7.84	7.79	7.9	7.87	7.85	7.8
25		7.76	7.75	7.72	7.78	7.76	7.69	7.71
50		7.59	7.57	7.57	7.55	7.53	7.51	7.56
75		7.46	7.46	7.47	7.42	7.4	7.38	7.46
100		7.33	7.34	7.38	7.33	7.27	7.37	7.33

Temperature-°C

Conc-%	Control Type	1	2	3	4	5	6	7	8
0	Lab Water	24.7	25.7	25.6	25.4	25.5	25.7	25.7	25.8
12.5		24.7	25.7	25.5	25.6	25.7	25.6	25.6	25.7
25		24.7	25.3	25.6	25.5	25.7	25.5	25.6	25.8
50		24.6	25.5	25.6	25.5	25.6	25.6	25.6	25.7
75		24.6	25.7	25.6	25.4	25.6	25.8	25.7	25.6
100		24.7	25.5	25.5	25.4	25.8	25.6	25.6	25.7

APPENDIX AII

SUMMARY OF CHRONIC WHOLE EFFLUENT TOXICITY RESULTS
CERIODAPHNIA DUBIA
CETIS TEST SUMMARY AND COMPARISON REPORT

CETIS Summary Report

Report Date: 18 Aug-09 06:44 (p 1 of 1)
 Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Batch ID: 18-8218-5685 Test Type: Reproduction-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Ceriodaphnia dubia Brine: Not Applicable
 Duration: 7d 0h Source: MWRD WET Lab In-house Culture Age: <24h

Sample ID: 01-7746-4099 Code: A93E323 Client:
 Sample Date: 21 Jul-09 Material: POTW Effluent Project:
 Receive Date: 21 Jul-09 Source: Hanover Park WRP
 Sample Age: 24h Station:

Comments: Hanover Park Chronic C. dubia test on samples collected July 20-25, 2009.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-8172-8499	7d Survival Rate	100	>100	N/A	N/A	1	Fisher Exact/Bonferroni-Holm Test
15-0917-4297	Reproduction	100	>100	N/A	18.7%	1	Steel Many-One Rank Test

Point Estimate Summary

Analysis ID	Endpoint	Level	Conc-%	95% LCL	95% UCL	TU	Method
00-5927-5234	Reproduction	IC25	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
18-8172-8499	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Result Within Limits
00-5927-5234	Reproduction	Control Resp	31.4	15 - NL	Yes	Result Within Limits
15-0917-4297	Reproduction	Control Resp	31.4	15 - NL	Yes	Result Within Limits
15-0917-4297	Reproduction	PMSD	0.187	0.13 - 0.47	Yes	Result Within Limits

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	10	1	1	1	1	1	0	0	0.0%	0.0%
12.5		10	1	1	1	1	1	0	0	0.0%	0.0%
25		10	1	1	1	1	1	0	0	0.0%	0.0%
50		10	1	1	1	1	1	0	0	0.0%	0.0%
75		10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	0.0%

Reproduction Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	10	31.4	30.1	32.7	24	36	0.634	3.47	11.1%	0.0%
12.5		10	35.3	34.6	36	33	39	0.345	1.89	5.35%	-12.4%
25		10	34.5	33.5	35.5	30	38	0.496	2.72	7.88%	-9.87%
50		10	33.2	32.1	34.3	30	40	0.522	2.86	8.61%	-5.73%
75		10	26.8	23.3	30.3	0	33	1.73	9.5	35.4%	14.6%
100		10	25.4	23.3	27.5	14	35	1.04	5.7	22.4%	19.1%

CETIS Analytical Report

Report Date: 18 Aug-09 06:43 (p 1 of 2)
 Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test **MWRD of Greater Chicago**

Analysis ID: 15-0917-4297 Endpoint: Reproduction CETIS Version: CETISv1.7.0
 Analyzed: 12 Aug-09 9:00 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

Batch ID: 18-8218-5685 Test Type: Reproduction-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Ceriodaphnia dubia Brine: Not Applicable
 Duration: 7d 0h Source: MWRD WET Lab In-house Culture Age: <24h

Comments: Hanover Park Chronic C. dubia test on samples collected July 20-25, 2009.

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C <> T	Not Run	100	>100	N/A	1	18.7%

Steel Many-One Rank Test

Control	vs	Conc-%	Test Stat	Critical	Ties	P-Value	Decision(5%)
Lab Water		12.5*	69	71	3	0.0281	Significant Effect
		25	77	71	5	0.1310	Non-Significant Effect
		50	91	71	4	0.7340	Non-Significant Effect
		75	77.5	71	3	0.1420	Non-Significant Effect
		100	72	71	1	0.0523	Non-Significant Effect

Test Acceptability

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	31.4	15 - NL --	Yes	Result Within Limits
PMSD	0.187	0.13 - 0.47	Yes	Result Within Limits

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	846.8	169.36	5	6.61	0.0001	Significant Effect
Error	1384.6	25.64074	54			
Total	2231.4	195.0007	59			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Bartlett Equality of Variance	31.2	15.1	0.0000	Unequal Variances
Distribution	Shapiro-Wilk Normality	0.755		0.0000	Non-normal Distribution

Reproduction Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	10	31.4	30.1	32.7	24	36	0.644	3.47	11.1%	0.0%
12.5		10	35.3	34.6	36	33	39	0.351	1.89	5.35%	-12.4%
25		10	34.5	33.5	35.5	30	38	0.505	2.72	7.88%	-9.87%
50		10	33.2	32.1	34.3	30	40	0.531	2.86	8.61%	-5.73%
75		10	26.8	23.2	30.4	0	33	1.76	9.5	35.4%	14.6%
100		10	25.4	23.2	27.6	14	35	1.06	5.7	22.4%	19.1%

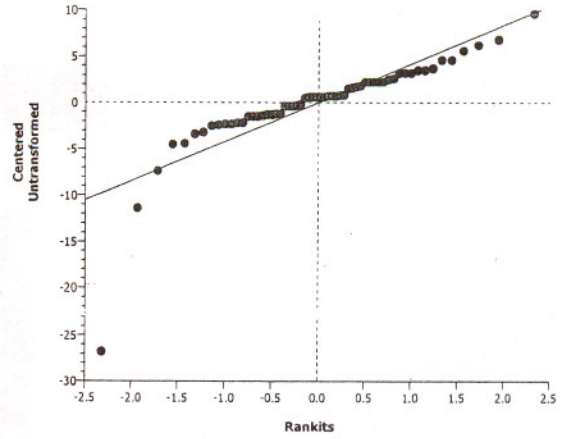
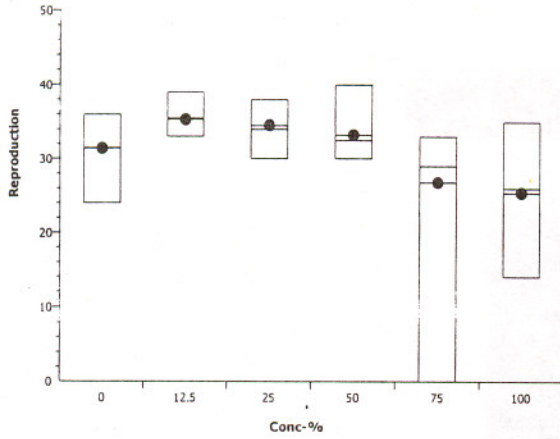
Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Analysis ID: 15-0917-4297 Endpoint: Reproduction
Analyzed: 12 Aug-09 9:00 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.7.0
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 18 Aug-09 06:43 (p 1 of 1)
 Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Analysis ID: 00-5927-5234 Endpoint: Reproduction CETIS Version: CETISv1.7.0
 Analyzed: 12 Aug-09 9:00 Analysis: Linear Interpolation (ICPIN) Official Results: Yes

Batch ID: 18-8218-5685 Test Type: Reproduction-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Ceriodaphnia dubia Brine: Not Applicable
 Duration: 7d 0h Source: MWRD WET Lab In-house Culture Age: <24h

Comments: Hanover Park Chronic C. dubia test on samples collected July 20-25, 2009.

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	57951	200	Yes	Two-Point Interpolation

Test Acceptability

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	31.4	15 - NL	Yes	Result Within Limits

Point Estimates

Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	N/A	N/A	<1	N/A	N/A

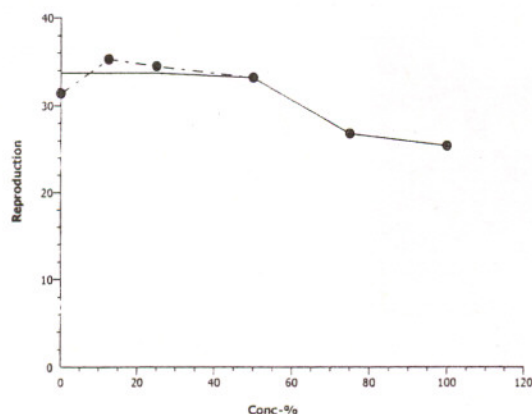
Reproduction Summary

Conc-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	10	31.4	24	36	0.634	3.47	11.1%	0.0%
12.5		10	35.3	33	39	0.345	1.89	5.35%	-12.4%
25		10	34.5	30	38	0.496	2.72	7.88%	-9.87%
50		10	33.2	30	40	0.522	2.86	8.61%	-5.73%
75		10	26.8	0	33	1.73	9.5	35.4%	14.6%
100		10	25.4	14	35	1.04	5.7	22.4%	19.1%

Reproduction Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	31	32	30	33	32	24	36	36	29	31
12.5		33	34	39	33	34	36	37	36	36	35
25		33	32	33	33	30	36	35	38	38	37
50		32	40	35	34	31	30	32	34	33	31
75		30	0	29	29	29	29	29	30	33	30
100		14	35	21	28	31	26	25	26	26	22

Graphics



Daphnid 7-d Survival and Reproduction Test MWRD of Greater Chicago

Analysis ID: 18-8172-8499 Endpoint: 7d Survival Rate CETIS Version: CETISv1.7.0
 Analyzed: 12 Aug-09 8:58 Analysis: STP 2x2 Contingency Tables Official Results: Yes

Batch ID: 18-8218-5685 Test Type: Reproduction-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Ceriodaphnia dubia Brine: Not Applicable
 Duration: 7d 0h Source: MWRD WET Lab In-house Culture Age: <24h

Comments: Hanover Park Chronic C. dubia test on samples collected July 20-25, 2009.

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	100	>100	N/A	1	N/A

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Lab Water		12.5	1	1	Non-Significant Effect
		25	1	1	Non-Significant Effect
		50	1	1	Non-Significant Effect
		75	1	1	Non-Significant Effect
		100	1	1	Non-Significant Effect

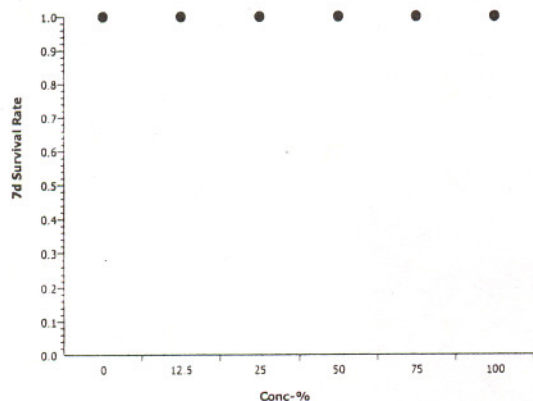
Test Acceptability

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Result Within Limits

Data Summary

Conc-%	Control Type	No-Resp	Resp	Total
0	Lab Water	10	0	10
12.5		10	0	10
25		10	0	10
50		10	0	10
75		10	0	10
100		10	0	10

Graphics



CETIS Measurement Report

Report Date: 18 Aug-09 06:43 (p 1 of 4)
 Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Batch ID: 18-8218-5685 Test Type: Reproduction-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Ceriodaphnia dubia Brine: Not Applicable
 Duration: 7d 0h Source: MWRD WET Lab In-house Culture Age: <24h

Comments: Hanover Park Chronic C. dubia test on samples collected July 20-25, 2009.

Parameter Acceptability

Parameter	Min	Max	Acceptability Limits	Overlap	Decision
Total Residual Chlorine-mg/L	0	0	0 - 0	Yes	Results Within Limits
Final Dissolved Oxygen-mg/L	7.24	8.62	4 - NL	Yes	Results Within Limits
Initial Dissolved Oxygen-mg/L	6.36	8.85	4 - NL	Yes	Results Within Limits
Total Ammonia (N)-mg/L	0.18	0.38	NL - 5	Yes	Results Within Limits
Final pH-Units	7.8	8.39	6 - 9	Yes	Results Within Limits
Initial pH-Units	7.26	8.06	6 - 9	Yes	Results Within Limits
Temperature-°C	24.7	25.3	24 - 26	Yes	Results Within Limits

Total Residual Chlorine-mg/L

Conc.-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	3	0	0	0	0	0	0	0		0
Overall		3	0			0	0				0 (0%)

Final Dissolved Oxygen-mg/L

Conc.-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	7.76	7.63	7.88	7.24	8.25	0.0611	0.366	4.72%	0
12.5		7	7.98	7.86	8.1	7.49	8.42	0.0577	0.346	4.34%	0
25		7	8.07	7.96	8.19	7.58	8.48	0.0578	0.347	4.29%	0
50		7	8.02	7.92	8.13	7.52	8.38	0.053	0.318	3.96%	0
75		7	8.05	7.93	8.17	7.5	8.45	0.0572	0.343	4.26%	0
100		7	8.04	7.91	8.17	7.45	8.62	0.0657	0.394	4.91%	0
Overall		42	7.99			7.24	8.62				0 (0%)

Initial Dissolved Oxygen-mg/L

Conc.-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	7.31	7.14	7.47	6.36	7.74	0.0802	0.481	6.58%	0
12.5		7	7.33	7.21	7.44	7	7.79	0.0568	0.341	4.65%	0
25		7	7.45	7.34	7.56	7.1	7.9	0.0546	0.327	4.39%	0
50		7	7.74	7.59	7.88	7.23	8.22	0.0701	0.42	5.44%	0
75		7	8.02	7.84	8.2	7.43	8.73	0.0886	0.532	6.63%	0
100		7	8.21	8.03	8.4	7.54	8.85	0.0917	0.55	6.7%	0
Overall		42	7.68			6.36	8.85				0 (0%)

Total Ammonia (N)-mg/L

Conc.-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	3	0.257	0.22	0.293	0.18	0.38	0.018	0.108	42.0%	0
Overall		3	0.257			0.18	0.38				0 (0%)

Final pH-Units

Conc.-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	8.05	7.99	8.12	7.8	8.39	0.03	0.18	2.24%	0
12.5		7	8.12	8.08	8.17	8	8.38	0.0224	0.134	1.65%	0
25		7	8.14	8.1	8.19	8	8.35	0.021	0.126	1.55%	0
50		7	8.13	8.09	8.18	7.97	8.35	0.0229	0.138	1.69%	0
75		7	8.12	8.07	8.17	7.93	8.32	0.0258	0.155	1.91%	0
100		7	8.1	8.05	8.14	7.92	8.26	0.0238	0.143	1.76%	0
Overall		42	8.11			7.8	8.39				0 (0%)

CETIS Measurement Report

Report Date: 18 Aug-09 06:43 (p 2 of 4)

Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Initial pH-Units

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	7	7.91	7.86	7.96	7.75	8.06	0.0242	0.145	1.84%	0
12.5		7	7.72	7.66	7.79	7.33	7.89	0.0313	0.188	2.43%	0
25		7	7.72	7.7	7.73	7.64	7.79	0.00772	0.0463	0.6%	0
50		7	7.57	7.55	7.59	7.48	7.63	0.00936	0.0562	0.74%	0
75		7	7.45	7.43	7.46	7.38	7.51	0.00821	0.0493	0.66%	0
100		7	7.36	7.35	7.38	7.26	7.42	0.00843	0.0506	0.69%	0
Overall		42	7.62			7.26	8.06				0 (0%)

Temperature-°C

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	QA Count
0	Lab Water	8	25.1	25	25.1	25	25.3	0.0172	0.103	0.41%	0
12.5		8	25.1	25	25.2	24.9	25.3	0.0267	0.16	0.64%	0
25		8	24.9	24.8	24.9	24.7	25.1	0.0259	0.155	0.62%	0
50		8	25.1	25	25.1	24.8	25.3	0.0236	0.141	0.57%	0
75		8	25.1	25.1	25.2	24.8	25.3	0.0259	0.155	0.62%	0
100		8	25.1	25	25.1	24.8	25.3	0.0252	0.151	0.6%	0
Overall		48	25			24.7	25.3				0 (0%)

CETIS Measurement Report

Report Date: 18 Aug-09 06:44 (p 3 of 4)

Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Total Residual Chlorine-mg/L

Conc-%	Control Type	1	2	3
0	Lab Water	0	0	0
12.5				
25				
50				
75				
100				

Final Dissolved Oxygen-mg/L

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	7.5	8.25	7.57	7.86	7.24	8.18	7.69
12.5		7.73	8.42	7.83	8.16	7.49	8.37	7.85
25		7.76	8.44	7.93	8.31	7.58	8.48	8.02
50		7.71	8.38	7.96	8.29	7.52	8.25	8.06
75		7.68	8.19	8.02	8.45	7.5	8.29	8.22
100		7.65	8.28	7.95	8.62	7.45	8.17	8.16

Initial Dissolved Oxygen-mg/L

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	7.25	6.36	7.74	7.05	7.62	7.61	7.52
12.5		7.79	7	7.5	7.24	7.72	7.02	7.03
25		7.73	7.16	7.73	7.35	7.9	7.19	7.1
50		8.22	7.58	8.06	7.48	8.22	7.36	7.23
75		8.73	7.82	8.51	7.66	8.45	7.52	7.43
100		8.71	7.98	8.85	8.01	8.75	7.64	7.54

Total Ammonia (N)-mg/L

Conc-%	Control Type	1	2	3
0	Lab Water	0.21	0.38	0.18
12.5				
25				
50				
75				
100				

Final pH-Units

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	7.97	8.01	8.11	7.8	8	8.39	8.1
12.5		8.02	8.03	8.2	8	8.09	8.38	8.15
25		8	8.03	8.25	8.07	8.12	8.35	8.19
50		7.97	8	8.26	8.08	8.1	8.35	8.18
75		7.94	7.93	8.28	8.08	8.08	8.32	8.2
100		7.92	7.94	8.26	8.1	8.01	8.25	8.19

Initial pH-Units

Conc-%	Control Type	1	2	3	4	5	6	7
0	Lab Water	7.77	8.06	8.05	8.04	7.92	7.75	7.76
12.5		7.33	7.89	7.78	7.86	7.8	7.71	7.7
25		7.73	7.79	7.71	7.75	7.7	7.64	7.71
50		7.59	7.63	7.57	7.59	7.5	7.48	7.61
75		7.46	7.51	7.47	7.49	7.38	7.4	7.41
100		7.36	7.39	7.39	7.36	7.26	7.42	7.36

CETIS Measurement Report

Report Date: 18 Aug-09 06:44 (p 4 of 4)
Test Code: 13-2360-3083/4EE4948B

Daphnid 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Temperature-°C

Conc-%	Control Type	1	2	3	4	5	6	7	8
0	Lab Water	25	25.3	25	25.1	25.1	25	25.1	25
12.5		24.9	25.3	25.2	25.1	25	25.1	24.9	25.3
25		25	25	24.7	24.7	25.1	24.8	24.8	25
50		25	25.1	25.3	25.1	25	24.8	25	25.1
75		25.1	25.3	25	25.2	25.1	24.8	25.2	25.2
100		25	25.1	25	25.2	25	24.8	25	25.3

APPENDIX BI

RAW DATA FOR *PIMEPHALES PROMELAS*
WHOLE EFFLUENT TOXICITY TEST CONDUCTED ON
HANOVER PARK WATER RECLAMATION PLANT
FINAL EFFLUENT COLLECTED JULY 20-25, 2009

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FATHEAD MINNOW-Pimephales promelas CHRONIC TOXICITY TEST DATA SHEET

MWRD Plant: HANOVER PARK

Analysts: G. V. BILLET

Sample type: Composite Final Effluent

Beginning Test Date 7-22-09 Time 11:00 Am

Sample Dates 7/20-7/21 7/22-7/23 7/24-7/25

Ending Test Date 7-29-09 Time 11:00 Am

Laboratory water (LW): MHSW (HSW WITH SELENIUM (circle one))

Date approved 7-13-09

Species: Pimephales promelas

Age < 24 hrs

Source EC+T

Hatch Date 7/20/09

Incubator I.D. Hotpack Chamber A Test Chamber A

Calibrated Thermometer#: S/N 8031998

BI-1

% Conc.	Dissolved Oxygen- mg/l								pH							
	0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
New 0	7.18	7.11	7.62	7.27	7.63	7.71	7.71	7.08	7.80	7.83	7.78	7.84	7.69	7.81	7.79	
12.5	8.01	7.04	7.55	7.45	7.83	7.70	7.41		7.80	7.84	7.79	7.90	7.87	7.85	7.80	
25	8.03	7.19	7.75	7.72	7.93	7.86	7.58		7.76	7.75	7.72	7.78	7.76	7.67	7.71	
50	8.41	7.69	8.08	8.06	8.21	8.07	7.88		7.59	7.57	7.57	7.55	7.53	7.51	7.56	
75	8.72	7.96	8.64	8.32	8.49	8.34	8.25		7.46	7.46	7.47	7.42	7.40	7.38	7.46	
100	8.85	8.21	8.95	8.64	8.86	8.94	8.74		7.33	7.34	7.38	7.33	7.27	7.37	7.33	
Old 0		4.77	5.21	4.34	5.52	5.03	5.85	5.62		6.68	7.23	7.22	7.25	7.29	7.35	7.41
12.5		4.67	4.98	4.83	5.72	5.36	5.63	5.57		7.25	7.33	7.36	7.39	7.35	7.38	7.39
25		4.45	4.86	4.67	5.03	4.91	4.81	4.86		7.30	7.32	7.37	7.33	7.31	7.29	7.31
50		4.70	5.31	5.03	5.54	5.14	5.38	5.42		7.32	7.37	7.40	7.38	7.33	7.36	7.34
75		5.46	5.70	4.70	5.46	4.99	5.29	5.34		7.34	7.39	7.36	7.35	7.29	7.34	7.32
100		4.79	5.51	5.19	5.62	5.03	5.42	5.44		7.32	7.34	7.39	7.40	7.28	7.35	7.33
Time	10:40 Am	12:45 8:05 pm	10:50 Am 2:25 pm	9:25 pm 12:50 pm	9:05 Am 12:45 pm	9:00 Am 1:15 pm	9:05 Am 12:55 pm	11:00 Am	10:40 Am	12:45 3:05 pm	10:50 Am 2:35 pm	9:25 pm 12:50 pm	9:05 Am 12:45 pm	9:00 Am 1:15 pm	9:05 Am 12:55 pm	11:00 Am
Initials	GVB	GVB	GVB	JWB	JWB	GVB	GVB	GVB	GVB	GVB	GVB	GVB	GVB	GVB	GVB	GVB

Measure DO at the end of each 24 h exposure period in at least one test chamber at each test concentration and in the control. Measure Temperature at the end of each 24 h exposure period in at least one test chamber at each test concentration and in the control. Measure temperature in two test vessels at the end of the test to determine the temperature variation in the environmental chamber. Measure pH each day, at the end of each 24 h exposure period, in at least one test chamber at each test concentration and in the control.

Pimephales promelas CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: HANOVER PARK ANALYST: G.V. BILLET ASSAY DATE: 7-22-09

% Conc.	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
Control LW	7A	10	10	10	10	10	10	10	10	24.7				25.5			
	7B	10	10	10	10	10	10	10	10		25.7				25.7		
	7C	10	10	10	10	10	10	10	10			25.6				25.7	
	7D	10	9	9	9	9	8	8	8				25.4				25.8
TIME		11:00 AM	11:30 AM	1:05 PM	11:15 AM	11:05 AM	11:05 AM	10:50 AM	10:50 AM	2:25 PM	8:20 AM	7:55 AM	8:50 AM	7:55 AM	8:05 AM	8:15 AM	8:35 AM
INITIAL		QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

BI-2

% Conc.	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
EFFLUENT	8A	10	10	10	10	10	10	10	10	24.7				25.7			
	8B	10	10	10	10	10	10	10	10		25.7				25.6		
	8C	10	10	10	10	10	10	10	10			25.5				25.6	
	8D	10	10	10	10	10	10	10	10				25.6				25.7
TIME		11:00 AM	11:30 AM	1:10 PM	11:20 AM	11:10 AM	11:10 AM	10:55 AM	10:55 AM	2:25 PM	8:20 AM	7:55 AM	8:50 AM	7:55 AM	8:05 AM	8:15 AM	8:35 AM
INITIAL		QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

Comments: _____

Pimephales promelas CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: HANOVER PARK ANALYST: G.V. BILLATT ASSAY DATE: 7-22-09

BI-3

% Conc.	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
25%	9A	10	10	10	10	10	10	10	10	24.7				25.7			
	9B	10	10	10	10	10	10	10	10		25.3				25.5		
	9C	10	10	10	10	10	10	10	10			25.6				25.6	
	9D	10	10	10	10	10	10	10	9				25.5				25.8
	TIME	11:00 AM	11:25 AM	1:20 PM	11:25 AM	11:15 AM	11:15 AM	11:00 AM	11:00 AM	2:25 PM	8:20 AM	7:55 AM	8:55 AM	7:55 AM	8:05 AM	8:15 AM	8:35 AM
	INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

% Conc.	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
50%	10A	10	10	10	10	10	10	10	10	24.6				25.6			
	10B	10	10	10	10	10	10	10	10		25.5				25.6		
	10C	10	10	10	10	10	10	10	10			25.6				25.6	
	10D	10	10	10	10	10	10	10	10				25.5				25.7
	TIME	11:00 AM	11:40 AM	1:25 PM	11:30 PM	11:20 AM	11:20 AM	11:05 AM	11:15 AM	2:25 PM	8:25 AM	7:55 AM	8:55 AM	7:55 AM	8:05 AM	8:15 AM	8:35 AM
	INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

Comments: _____

Pimephales promelas CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: HANOVER PARK ANALYST: G.V. BILLIET ASSAY DATE: 7-22-09

B1-4

% Conc.	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
75%	11A	10	10	10	10	10	10	10	10	24.6				25.6			
	11B	10	10	10	10	10	10	10	10		25.7				25.8		
	11C	10	10	10	10	10	10	10	10			25.6				25.7	
	11D	10	10	10	10	10	10	10	10				25.4				25.6
	TIME	11:00 AM	11:45 AM	1:35 PM	11:35 AM	11:25 AM	11:25 AM	11:15 AM	11:20 AM	2:30 PM	8:25 AM	8:00 AM	8:55 AM	8:00 AM	8:10 AM	8:20 AM	8:40 AM
	INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

% Conc.	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
100%	12A	10	10	10	10	10	10	10	10	24.7				25.8			
	12B	10	10	10	10	10	10	10	10		25.5				25.6		
	12C	10	10	10	10	10	10	10	10			25.5				25.6	
	12D	10	10	10	10	10	10	10	10				25.4				25.7
	TIME	11:00 AM	11:45 AM	1:40 PM	11:40 AM	11:30 AM	11:30 AM	11:20 AM	11:30 AM	2:30 PM	8:25 AM	8:00 AM	8:55 AM	8:00 AM	8:10 AM	8:20 AM	8:40 AM
	INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

Comments: _____

Alkalinity, Hardness, and Residual Chlorine Record:

Date/Initials/Time		Burette Reading	Alkalinity (mg/L as CaCO ₃)	Burette Reading	Hardness (mg/L as CaCO ₃)	Residual Chlorine (mg/L)
Date: Sample 1 7 / 22 / '09 GUB.	Control	5.6	112.0	4.1	164.0	
	100% Effluent	5.5	110.0	5.2	208.0	0.0
Date: Sample 2 7 / 24 / '09	Control	5.7	114.0	4.1	164.0	
	100% Effluent	5.8	116.0	5.4	216.0	0.0
Date: Sample 3 7 / 26 / '09 ²⁵ 27 7/25/09 28	Control	5.6	112.0	4.3	172.0	
	100% Effluent	5.7	114.0	5.7	228.0	0.0

BIS

CONTROL/ EFF conc.	CONDUCTIVITY							
	0	24	48	72	96	120	144	168
LW	0.571	0.567 0.64 GUB	0.570	0.571	0.577	0.581	0.570	0.579
12.5	0.602	0.594	0.602	0.604	0.607	0.610	0.608	0.608
25	0.639	0.630	0.637	0.639	0.644	0.638	0.644	0.641
50	0.707	0.701	0.718	0.717	0.723	0.725	0.721	^{GUB} 0.720
75	0.781	0.776	0.792	0.794	0.802	0.808	0.802	0.806
100	0.851 ^{GUB} 10:40 AM	0.840	0.867	0.870	0.882	0.887	0.881	0.886
Date/Time	7-22-09 10:40 AM	7-23-09 12:55 PM	7-24-09 10:50 AM	7-25-09 9:25 AM	7-26-09 9:05 AM	7-27-09 9:05 AM	7-28-09 9:05 AM	7-29-09 9:50 AM
Initials	GUB.	GUB.	GUB.	GUB.	GUB.	GUB.	GUB.	GUB.

Pimephales promelas CHRONIC TOXICITY TEST Fish Feeding Record

MWRD Plant: HANOVER PARK Analysts: G. V. BILLET Test Date 7-22-09

Feeding Times/Type of Food/Initials: Brand Argentina Lot Number BV074A

BS = Brine Shrimp : Shrimp Brine Shrimp set-up (Time) Beaker A 2⁰⁰ AM / (PM) Beaker B 9⁰⁰ (AM) PM

(Fish fed 0.15 mL of concentrated newly hatched baby brine shrimp per test vessel as specified in USEPA Manual EPA/600/4-91/002; 1994)

FEEDING SCHEDULE: If the test is initiated after 12:00 PM, feed fish once; on following days, feed fish at the beginning of the work day, at least 2 h before test solution renewal, and at the end of the work day, after test solution renewal. Do not feed fish during the final 12 h of the test.

FISH FEEDING RECORD

TIME AND INITIALS

Time (h)	0	24 (day 1)	48 (day 2)	72 (day 3)	96 (day 4)	120 (day 5)	144 (day 6)
Morning	9:15 AM GVB	8:05 AM GVB	7:55 AM GVB	8:10 AM GVB	7:40 AM GVB	7:50 AM GVB	8:40 AM GVB
Evening	2:40 PM GVB	3:25 PM GVB	3:25 PM GVB	2:30 PM GVB	1:55 PM GVB	3:25 PM GVB	3:20 PM GVB

TEMPERATURE*/LIGHT RECORD**

	0	24 (day 1)	48 (day 2)	72 (day 3)	96 (day 4)	120 (day 5)	144 (day 6)	168 (day 7)
Tray 1	25.5 °C 73.1	25.0 °C 83.2	25.1 °C 88.1	25.0 °C 78.2	25.0 °C 73.6	24.9 °C 69.4	24.9 °C 74.6	25.0 °C 85.8
Tray 2	25.5 °C 74.8	25.0 °C 68.8	25.1 °C 72.6	25.0 °C 55.4	25.0 °C 60.0	24.9 °C 63.6	24.9 °C 60.9	25.0 °C 62.1

**Light Meter (SPER Scientific Serial No. 031444) Calibrated on MAR. / 04 / 2009

BI-6

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
 FATHEAD MINNOW-*Pimephales promelas* CHRONIC TEST DATA SHEET TEST DATE 7-22-09
 MWRD Plant: HANOVER PARK Analysts: G.Y. BILLATT

Treatment/Replicae	Dry Wt. W/Fish gm	Tare Wt. gm	Wt. Of Fish gm	ORIGINAL # OF FISH (ON)	Avg. Wt Per Fish gm (ON)	Avg. Wt Per Fish mg (ON)
CONTROL	<u>6.63</u>					
7A	2. 1.731 <u>1.731</u>	2.1599	0.0132	10	0.0013	1.3200
7B	2. <u>1.747</u>	2.1620	0.0127	10	0.0013	1.2700
7C	2. <u>0.848</u>	2.0742	0.0106	10	0.0011	1.0600
7D	2. 0.848 <u>0.848</u>	2.0906	0.0106	10	0.0011	1.0600
12.5 % EFFLUENT	2. <u>1.012 FD</u>		<u>0.0138 AY</u>			
8A	2. <u>0.930</u>	2.0792	0.0136	10	0.0014	1.3800
8B	2. <u>1.739</u>	2.1326	0.0113	10	0.0011	1.1300
8C	2. <u>0.888</u>	2.0745	0.0143	10	0.0014	1.4300
8D	2. <u>1.554</u>	2.1415	0.0139	10	0.0014	1.3900
25 % EFFLUENT						
9A	2. <u>0.938</u>	2.0818	0.0120	10	0.0012	1.2000
9B	2. <u>1.552</u>	2.1431	0.0121	10	0.0012	1.2100
9C	2. <u>0.830</u>	2.0686	0.0144	10	0.0014	1.4400
9D	2. <u>0.866</u>	2.0754	0.0112	10	0.0011	1.1200
50 % EFFLUENT						
10A	2. <u>1.727</u>	2.1592	0.0135	10	0.0014	1.3500
10B	2. <u>1.593</u>	2.1450	0.0143	10	0.0014	1.4300
10C	2. <u>1.622</u>	2.1490	0.0132	10	0.0013	1.3200
10D	2. <u>1.462</u>	2.1321	0.0141	10	0.0014	1.4100
75 % EFFLUENT						
11A	2. <u>1.616</u>	2.1483	0.0133	10	0.0013	1.3300
11B	2. <u>0.881</u>	2.0760	0.0121	10	0.0012	1.2100
11C	2. <u>0.864</u>	2.0733	0.0131	10	0.0013	1.3100
11D	2. <u>1.631</u>	2.1499	0.0132	10	0.0013	1.3200
100 % EFFLUENT						
12A	2. <u>1.589</u>	2.1461	0.0128	10	0.0013	1.2800
12B	2. <u>1.594</u>	2.1451	0.0143	10	0.0014	1.4300
12C	2. <u>1.380</u>	2.1238	0.0142	10	0.0014	1.4200
12D	2. <u>1.551</u>	2.1421	0.0130	10	0.0013	1.3000

BI-7

Count the surviving larvae in each test chamber and immediately prepare for dry weight determination. Transfer each group of larvae to a tared glass vial that has been properly labeled. Dry vials at 60°C, for 24 h or at 100°C for a minimum of 6 h. Immediately upon removal from the drying oven, place the glass vials in a dessicator until weighed. Measure all weights to the nearest 0.01 mg and average weights should be expressed to the nearest 0.001 mg. Prepare a summary table as illustrated above.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Fathead Minnow

Water Reclamation Plant: Hanover Park

Collection Date: 07-22-09

Number of treatments, including the Control(s): 6

Number of replicates: 4

Number of organisms per replicate: 10

ORDER OF RANDOM PLACEMENT OF CUPS IN TRAYS

Cup 12A ✓ Cup 11B ✓ Cup 7E ✓ Cup 8D ✓

Cup 7C ✓ Cup 12C ✓ Cup 9C ✓ Cup 9A ✓

Cup 10A ✓ Cup 9B ✓ Cup 11D ✓ Cup 10D ✓

Cup 10B ✓ Cup 11A ✓ Cup 8C ✓ Cup 12E ✓

_____ Front of Tray _____

Cup 8A ✓ Cup 8E ✓ Tray 2 _____

Cup 9D ✓ Cup 11C ✓

Cup 7D ✓ Cup 7A ✓

Cup 10C ✓ Cup 12D ✓

_____ Front of Tray _____

Number of organisms for each cup checked by _____

12:45 PM
JLB

APPENDIX BII

RAW DATA FOR *CERIODAPHNIA DUBIA*
WHOLE EFFLUENT TOXICITY TEST CONDUCTED ON
HANOVER PARK WATER RECLAMATION PLANT
FINAL EFFLUENT COLLECTED JULY 20-25, 2009

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD Plant: Hanover Park Sample type: Effluent Analysts: James Kaehn
 Laboratory water (LW): MHSW/HSW WITH SELENIUM (circle one) Date 7/13/09 Approved by Biologist I
 Sample Dates 7-21-09 7-23-09 7-25-09 Beginning Test Date 7-22-09 Time 10:45am
 Calibrated Thermometer: 80319998 Ending Test Date 7-29-09 Time 10:45am
 Species: C. dubia Source: In-house Age group of neonates 3pm to 11pm 11pm to 7am, or 7am to 3pm
 Incubator I.D. A *Ceriodaphnia* Tray # 10 D Test Tray Location Center Table, Left side (air side)

BII-1

% Conc.	Dissolved Oxygen- mg/l								pH							
	0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
New 0	7.25	6.36	7.74	7.05	7.62	7.61	7.52		7.77	8.06	8.05	8.04	7.92	7.75	7.76	
12.5	7.79	7.00	7.50	7.24	7.72	7.02	7.03		7.33	7.89	7.78	7.86	7.80	7.71	7.70	
25	7.73	7.16	7.73	7.35	7.90	7.19	7.10		7.73	7.79	7.71	7.75	7.70	7.64	7.71	
50	8.22	7.58	8.06	7.48	8.22	7.36	7.23		7.59	7.63	7.57	7.59	7.50	7.48	7.61	
75	8.73	7.82	8.51	7.66	8.45	7.52	7.43		7.46	7.51	7.47	7.49	7.38	7.40	7.41	
100	8.71	7.98	8.85	8.01	8.75	7.64	7.54	NA	7.36	7.39	7.39	7.36	7.26	7.42	7.36	NA
Old 0		7.50	8.25	7.57	7.86	7.24	8.18	7.69		7.97	8.01	8.11	7.80	8.00	8.39	8.10
12.5		7.73	8.42	7.83	8.16	7.49	8.37	JK 7/23/09 8.15 7.85		8.02	8.03	8.20	8.00	8.09	8.38	8.15
25		7.76	8.44	7.93	8.31	7.58	8.48	8.02		8.00	8.03	8.25	8.07	8.12	8.35	8.19
50		7.71	8.38	7.96	8.29	7.52	8.25	8.06		7.97	8.00	8.26	8.08	8.10	8.35	8.18
75		7.68	8.19	8.02	8.45	7.50	8.29	8.22		7.94	7.93	8.28	8.08	8.08	8.32	8.20
100		7.65	8.28	7.95	8.62	7.45	8.17	8.16		7.92	7.94	8.26	8.10	8.01	8.25	8.19
Time		2:45pm	2:45pm	2:45pm	2:45pm	2:45pm	2:45pm	12:20pm		2:55pm	2:55pm	2:55pm	2:55pm	2:55pm	2:55pm	12:20pm
Initials	NA	JK	JK	JK	JK	JK	JK	JK	NA	JK	JK	JK	JK	JK	JK	JK

Measure DO & pH at the beginning and end of each 24 h exposure period in at least one test chamber at each test concentration and in the control.

CHRONIC -CD

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: Hanover Park

ANALYST: James Kaehn

ASSAY DATE: 7-22-09

BII-2

% Conc.	Cup No.	Temperature °C (acceptable temperature : 24-26°C)								% Conc.	Cup No.	Temperature °C (acceptable temperature : 24-26°C)							
		0	24	48	72	96	120	144	168			0	24	48	72	96	120	144	168
	control																		
LW	1A	25.1	25.0							12.5%	2A	24.7	24.9						
	1B			25.3							2B			25.3					
	1C				25.0						2C				25.2				
	1D					25.1					2D					25.1			
	1E						25.1				2E						25.0		
	1F							25.0			2F							25.1	
	1G								25.1		2G								24.9
	1H								25.0		2H								25.3
	1I										2I								
	1J										2J								
	TIME	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am		TIME	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am		
	INITIAL	JK	JK	JK	JK	JK	JK	JK		INITIAL	JK	JK	JK	JK	JK	JK	JK		

% Conc.	Cup No.	Temperature °C (acceptable temperature : 24-26°C)								% Conc.	Cup No.	Temperature °C (acceptable temperature : 24-26°C)							
		0	24	48	72	96	120	144	168			0	24	48	72	96	120	144	168
25%	3A	25.1	25.0	25.0						50%	4A	25.0	25.0						
	3B			25.0							4B			25.1					
	3C				24.7						4C				25.3				
	3D					24.7					4D					25.1			
	3E						25.1				4E						25.0		
	3F							24.8			4F							24.8	
	3G								24.8		4G								25.0
	3H								25.0		4H								25.1
	3I										4I								
	3J										4J								
	TIME	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am		TIME	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am	11:15 am		
	INITIAL	JK	JK	JK	JK	JK	JK	JK		INITIAL	JK	JK	JK	JK	JK	JK	JK		

Measure Temperature at the end of each 24 h exposure period in at least one test chamber at each test concentration and in the control. Measure temperature in two test vessels at the end of the test to determine the temperature variation in the environmental chamber. Comments _____

CHRONIC-CD

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: Hanover Park ANALYST: James Kaehn ASSAY DATE: 7-22-09

% Conc.	Cup No.	Temperature °C (acceptable temperature : 24-26°C)								% Conc.	Cup No.	Temperature °C (acceptable temperature : 24-26°C)								
		0	24	48	72	96	120	144	168			0	24	48	72	96	120	144	168	
75%	5A	24.8	25.1							100%	6A	25.0								
	5B			25.3							6B		25.1							
	5C				25.0						6C			25.0						
	5D					25.2					6D				25.2					
	5E						25.1				6E	24.9				25.0				
	5F							24.8			6F						24.8			
	5G								25.2		6G								25.0	
	5H										6H									25.3
	5I										6I									
	5J										6J									
TIME		11:15am	11:15am	11:15am	11:15am	11:15am	11:15am	11:15am	11:15am	TIME		11:15am	11:15am	11:15am	11:15am	11:15am	11:15am	11:15am		
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	INITIAL		JK	JK	JK	JK	JK	JK	JK		

BII-3

Measure Temperature at the end of each 24 h exposure period in at least one test chamber at each test concentration and in the control. Measure temperature in two test vessels at the end of the test to determine the temperature variation in the environmental chamber.

Comments _____

NEONATES (TIME : 3pm - 11pm) Initials: JK

TRAY # 10D

BIRTH DATE: ~~7-21-09~~ ^{JK} ~~7-22-09~~

WATER SOURCE: LW (DATE: 7-13-09)

CHRONIC -CD

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: Hanover Park ANALYST: James Kaehn ASSAY DATE: 7-22-09

% Conc.	Cup No.	Number of Neonates Produced								Adult Survival								
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
*Parent Cup		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
7	1A	0	0	0	6	10	15	0	31	1	1	1	1	1	1	1	1	1
22	1B	0	0	7	0	11	14	13	32	1	1	1	1	1	1	1	1	1
Control	26	0	0	6	0	10	14	13	30	1	1	1	1	1	1	1	1	1
	27	0	0	6	0	12	15	0	33	1	1	1	1	1	1	1	1	1
	28	0	0	6	0	10	16	0	32	1	1	1	1	1	1	1	1	1
	30	0	0	0	4	7	13	0	24	1	1	1	1	1	1	1	1	1
	34	0	0	3	0	10	18	0	36	1	1	1	1	1	1	1	1	1
	35	0	0	8	0	13	15	14	36	1	1	1	1	1	1	1	1	1
	39	0	0	0	5	9	15	0	29	1	1	1	1	1	1	1	1	1
	42	0	0	0	6	11	14	0	31	1	1	1	1	1	1	1	1	1
TOTAL		0	0	41	21	103	149	40	314	10	10	10	10	10	10	10	10	10
TIME		11:20am	11:20am	11:20am	11:20am	11:20am	11:20am	11:20am	11:20am	10:45am	10:45am	10:45am	10:45am	10:45am	10:45am	10:45am	10:45am	10:45am
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

BII-4

% Conc.	Cup No.	Number of Neonates Produced								Adult Survival								
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
Effluent		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
12.5	2A	0	0	5	0	11	17	0	33	1	1	1	1	1	1	1	1	1
	2B	0	0	6	0	11	17	14	34	1	1	1	1	1	1	1	1	1
	2C	0	0	6	0	13	20	0	39	1	1	1	1	1	1	1	1	1
	2D	0	0	1	4	12	16	0	33	1	1	1	1	1	1	1	1	1
	2E	0	0	5	0	11	18	13	34	1	1	1	1	1	1	1	1	1
	2F	0	0	6	0	14	16	0	36	1	1	1	1	1	1	1	1	1
	2G	0	0	7	0	12	18	0	37	1	1	1	1	1	1	1	1	1
	2H	0	0	7	0	12	17	16	36	1	1	1	1	1	1	1	1	1
	2I	0	0	4	3	13	16	0	36	1	1	1	1	1	1	1	1	1
	2J	0	0	7	0	13	15	0	35	1	1	1	1	1	1	1	1	1
TOTAL		0	0	54	7	122	170	43	353	10	10	10	10	10	10	10	10	10
TIME		11:20am	11:20am	11:20am	11:20am	11:20am	11:20am	11:20am	11:20am	10:45am	10:50am	10:50am	10:50am	10:50am	10:50am	10:50am	10:50am	10:50am
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

At test termination all observations on test organism survival and numbers of offspring should be completed within 2 hours. Any animal not producing young neonates should be examined to determine if it is male after day 7 of the test. Check with Biologist I to observe test organism on a microscope slide.
 *For tracking test organisms to brood board tray.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: Hanover Park ANALYST: James Kaehn ASSAY DATE: 7-22-09

BII-5

% Conc.	Cup No.	Number of Neonates Produced								Adult Survival								
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
25%	3A	0	0	5	0	10	18	0	33	1	1	1	1	0	1	1	1	1
	3B	0	0	4	0	12	16	0	32	1	1	1	1	1	1	1	1	1
	3C	0	0	7	0	12	14	0	33	1	1	1	1	1	1	1	1	1
	3D	0	0	6	0	10	17	11	33	1	1	1	1	1	1	1	1	1
	3E	0	0	4	0	11	15	0	30	1	1	1	1	1	1	1	1	1
	3F	0	0	6	0	12	18	0	36	1	1	1	1	1	1	1	1	1
	3G	0	0	5	0	12	18	0	35	1	1	1	1	1	1	1	1	1
	3H	0	0	4	0	13	21	16	38	1	1	1	1	1	1	1	1	1
	3I	0	0	6	0	13	19	0	38	1	1	1	1	1	1	1	1	1
	3J	0	0	0	8	14	15	0	37	1	1	1	1	1	1	1	1	1
TOTAL		0	0	47	8	119	171	27	345	10	10	10	10	10	10	10	10	
TIME		11:22am	11:22am	11:22am	11:22am	11:22am	11:22am	11:22am	11:22am	10:45am	10:55am	10:55am	10:55am	10:55am	10:55am	10:55am	10:55am	
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	

% Conc.	Cup No.	Number of Neonates Produced								Adult Survival								
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
50%	4A	0	0	6	0	10	16	19	32	1	1	1	1	1	1	1	1	1
	4B	0	0	4	0	11	15	10	40 ³⁰	1	1	1	1	1	1	1	1	1
	4C	0	0	5	0	11	19	16	35	1	1	1	1	1	1	1	1	1
	4D	0	0	0	6	11	17	0	34	1	1	1	1	1	1	1	1	1
	4E	0	0	3	0	11	17	0	31	1	1	1	1	1	1	1	1	1
	4F	0	0	5	0	11	14	11	30	1	1	1	1	1	1	1	1	1
	4G	0	0	3	0	12	17	16	32	1	1	1	1	1	1	1	1	1
	4H	0	0	6	0	10	18	15	34	1	1	1	1	1	1	1	1	1
	4I	0	0	6	0	11	16	0	33	1	1	1	1	1	1	1	1	1
	4J	0	0	5	0	10	16	10	31	1	1	1	1	1	1	1	1	1
TOTAL		0	0	43	6	108	165	97	322	10	10	10	10	10	10	10	10	
TIME		11:22am	11:22am	11:22am	11:22am	11:22am	11:22am	11:22am	11:22am	10:45am	11:00am	11:00am	11:00am	11:00am	11:00am	11:00am	11:00am	
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	

At test termination all observations on test organism survival and numbers of offspring should be completed within 2 hours. Any animal not producing young neonates should be examined to determine if it is male after day 7 of the test. Check with Biologist I to observe test organism on a microscope slide.

CHRONIC -CD

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: _____ ANALYST: _____ ASSAY DATE: _____

BII-6

% Conc.	Cup No.	Number of Neonates Produced								Adult Survival								
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
Effluent		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
75%	5A	0	0	5	0	10	14	1	30	1	1	1	1	1	1	1	1	1
	5B	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0
	5C	0	0	5	0	11	13	0	29	1	1	1	1	1	1	1	1	1
	5D	0	0	5	0	10	14	0	29	1	1	1	1	1	1	1	1	1
	5E	0	0	0	6	10	13	0	29	1	1	1	1	1	1	1	1	1
	5F	0	0	0	6	9	14	0	29	1	1	1	1	1	1	1	1	1
	5G	0	0	5	0	10	14	10	29	1	1	1	1	1	1	1	1	1
	5H	0	0	5	0	12	13	14	30	1	1	1	1	1	1	1	1	1
	5I	0	0	6	0	11	16	0	33	1	1	1	1	1	1	1	1	1
5J	0	0	0	6	10	14	0	30	1	1	1	1	1	1	1	1	1	
TOTAL		0	0	31	18	91	125	25	268	10	10	10	10	9	9	9	9	9
TIME		11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	10:45 _{am}	11:05 _{am}	11:05 _{am}	11:05 _{am}	11:05 _{am}	11:05 _{am}	11:05 _{am}	11:05 _{am}	11:05 _{am}
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

% Conc.	Cup No.	Number of Neonates Produced								Adult Survival								
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
Effluent		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168	Total
100%	6A	0	0	0	5	8	1	0	14	1	1	1	1	1	1	1	1	1
	6B	0	0	0	0	10	13	12	35	1	1	1	1	1	1	1	1	1
	6C	0	0	2	0	8	11	0	21	1	1	1	1	1	1	1	1	1
	6D	0	0	0	0	8	11	9	28	1	1	1	1	1	1	1	1	1
	6E	0	0	1	0	8	11	11	31	1	1	1	1	1	1	1	1	1
	6F	0	0	0	6	10	10	0	26	1	1	1	1	1	1	1	1	1
	6G	0	0	4	0	10	10	1	25	1	1	1	1	1	1	1	1	1
	6H	0	0	5	0	8	13	13	26	1	1	1	1	1	1	1	1	1
	6I	0	0	0	5	10	11	0	26	1	1	1	1	1	1	1	1	1
6J	0	0	0	6	9	7	0	22	1	1	1	1	1	1	1	1	1	
TOTAL		0	0	12	20	89	98	46	254	10	10	10	10	10	10	10	10	10
TIME		11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	11:24 _{am}	10:45 _{am}	11:10 _{am}	11:10 _{am}	11:10 _{am}	11:10 _{am}	11:10 _{am}	11:10 _{am}	11:10 _{am}	11:10 _{am}
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

At test termination all observations on test organism survival and numbers of offspring should be completed within 2 hours. Any animal not producing young neonates should be examined to determine if it is male after day 7 of the test. Check with Biologist I to observe test organism on a microscope slide.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

MWRD PLANT: Hanover Park Amount Algae Fed: 0.1 mL Amount YTC Fed: 0.1 mL

ANALYST: James Kehn, _____ ASSAY DATE: 7-22-09

Date	Algae Type / Harvest Date	Cell Concentration (cells/mL)	YTC/Thaw Date	Time Fed	Initials
7-22-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	10:45am	JK
7-23-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	10:45am	JK
7-24-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	10:45am	JK
7-25-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-24-09	10:45am	JK
7-26-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-24-09	10:45am	JK
7-27-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-26-09	10:45am	JK
7-28-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-26-09	10:45am	JK
NA	<i>S. capricornutum</i> /				JK

BII-7

Incubator Temperature (*Calibrated Thermometer # 43) / Light Record**/Intials:

**Light Meter (SPER Scientific Serial No. 031443) Calibrated on 3 / 4 / 09

Incubator/ Shelf	*Temperature (°C) / Light Reading							
	0	24	48	72	96	120	144	168
air side	25.0°C / 95.0	25.0°C / 88.2	25.5°C / 87.5	24.5°C / 86.7	24.8°C / 86.0	24.8°C / 87.3	24.8°C / 76.1	25.0°C / 66.5
wall side	25.0 / 95.0	25.0°C / 88.2	25.0°C / 80.4	25.0°C / 82.5	24.8°C / 79.1	24.8°C / 80.7	24.8°C / 74.8	25.0°C / 72.5

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia

Water Reclamation Plant: Hannover Park

Collection Date: 07-21-09

Number of treatments, including the Control(s): 6

Number of replicates: 10

Number of organisms per replicate: 1

ORDER OF RANDOM PLACEMENT OF CUPS IN TRAYS

_____ Tray 1 _____

Cup 1A Cup 6F Cup 5C Cup 1G Cup 5E Cup 2C Cup 6C Cup 4H Cup 3B Cup 2I
Cup 4D Cup 5J Cup 3I Cup 5I Cup 4C Cup 4G Cup 6D Cup 6A Cup 5D Cup 1E
Cup 3H Cup 2G Cup 5F Cup 6B Cup 5H Cup 5G Cup 6I Cup 2A Cup 4I Cup 6G
Cup 5B Cup 1D Cup 4B Cup 4A Cup 2J Cup 6E Cup 1J Cup 1I Cup 3G Cup 4E
Cup 5A Cup 6J Cup 4J Cup 1F Cup 2B Cup 4F Cup 3A Cup 2F Cup 6H Cup 3E
Cup 1H Cup 2H Cup 3C Cup 2E Cup 1C Cup 3D Cup 3J Cup 1B Cup 3F Cup 2D

_____ Front of Tray _____

Number of organisms for each cup checked by Erica Collins

APPENDIX CI

CHAIN-OF-CUSTODY FOR WHOLE EFFLUENT TOXICITY TESTS
CONDUCTED ON HANOVER PARK WATER RECLAMATION
PLANT FINAL EFFLUENT COLLECTED JULY 20-25, 2009

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

The WET Laboratory Chain-of-Custody Form

SAMPLE COLLECTION			SAMPLE TYPE	SAMPLE LOCATION	SAMPLE Temp °C	ON-SITE SAMPLE STORAGE (0.1-6 °C)	PRINT NAME & SIGNATURE
DATE	TIME	BY					
07/20/09	0600	TR	GRAB EFFLUENT	HP Final effluent	69°F	<input checked="" type="checkbox"/> YES / <input type="checkbox"/> NO	Tom Rube TR
07/20/09	1200	MS	GRAB EFFLUENT	HP FINAL EFFLUENT	68°F	<input checked="" type="checkbox"/> YES / <input type="checkbox"/> NO	NICUSOR SIRSI MA 6/20
07/20/09	1800	MS	GRAB EFFLUENT	HP FINAL EFFLUENT	70°F	<input checked="" type="checkbox"/> YES / <input type="checkbox"/> NO	NICUSOR SIRSI MA 6/20
07/20/09	2400	AW	GRAB EFFLUENT	HP FINAL EFFLUENT	21°C	<input checked="" type="checkbox"/> YES / <input type="checkbox"/> NO	Cynthia Whithead CWH
07/21/09	0600	MZ	GRAB EFFLUENT	HP FINAL EFFLUENT	68°F	<input checked="" type="checkbox"/> YES / <input type="checkbox"/> NO	MICHAEL ZIDONS MZidons

Note: Sample container should be rinsed and should be filled completely leaving no air space between contents & lid. Preserve samples on ice or refrigerator (0.1-6°C) immediately after collection. Transport samples to the WET Laboratory immediately after the fifth sample is collected. The WET laboratory is located in Room LE-100, Lue-Hing R&D Complex. AFTER CHLORINATION → DECHLORINATION
Indicate if the final effluent was chlorinated: Yes / No / NA
SAMPLES RELINQUISHED BY: Name NICUSOR SIRSI Signature: MA 6/20 Date/Time: 7/21/09 8:24 AM
SAMPLES TRANSPORTED TO WET LAB. BY Name ANANDA SAVERSON Signature: Ananda Saverson Date/Time: 7/21/09 8:24 AM

FOR WET LABORATORY USE ONLY:
Sample Received by: Name G.V. BILLET Signature: G.V. Billett Date/Time 10:00 AM
1. Samples received with prescribed holding time (within 4 h of collection)? (Not Applicable, if chronic test) 1. Yes / No / (NA)
2. Samples logged in by MS Date 7-21-09 Time 10:30 AM 2. Yes / No
3. Each sample container labeled with a unique ID? 3. Yes / No
4. Were collection times for effluent and receiving water within 1 h of each other? 4. Yes / No / (NA)
5. Did samples have sufficient volume for analysis? 5. Yes / No
6. Samples accepted 6. Yes / No
Special Observations _____

LIMS #	Sample Type/ID	Temp °C	pH	Residual Chlorine (mg/L) Initials	Sodium-thio-sulfate Added YES/NO	Sample Custodian Signature
5789786	HPOUTCH1A	3.0	7.01	0.008	Indicate Total ml of 5% Sodium-thio-sulfate added. Initial	<u>G.V. Billett</u>
5789786	HPOUTCH1B	3.0	6.92	Total Ammonia (mg-L) ALD Results	Final Residual Cl ₂ reading = mg/l	
5789786	HPOUTCH1C	3.0	6.86		Initial	
5789786	HPOUTCH1D	3.0	6.84			
5789786	HPOUTCH1E	5.0	7.00	0.21		

Note: Set aside one cubitainer for metals and chemical analyses
Sample Received By: Trace Metals John Schmitt Date 7/21/09 Time 11:26 A
Lachat [Signature] Date 7-21-09 Time 11:28A
Materials [Signature] Date 7-21-09 Time 11:28A
Sample aliquot received by _____ Name _____ Signature _____ Date _____ Time _____

Sample Release for Disposal
Sample released for disposal following analysis on (Date) _____ by _____
Samples Discarded by _____ Date/Time _____

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

The WET Laboratory Chain-of-Custody Form

SAMPLE COLLECTION			SAMPLE TYPE	SAMPLE LOCATION	SAMPLE Temp °C	ON-SITE SAMPLE STORAGE (0.1-6 °C)	PRINT NAME & SIGNATURE
DATE	TIME	BY					
07/22/09	0600	MZ	GRAB	EFFLUENT	21°C	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	MIKE ZIDONIS MZidonis
07/22/09	1200 3:40 PM	NS	GRAB	EFFLUENT	NS 20.5°C	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	NICUSOR SIRBI NSirbi
07/22/09	15:10 1800	NS	GRAB	EFFLUENT	21°C	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	NICUSOR SIRBI NSirbi
07/22/09	2400	MZ	GRAB	EFFLUENT	21°C	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	MIKE ZIDONIS MZidonis
07/23/09	0600	MZ	GRAB	EFFLUENT	20.5°C	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	MIKE ZIDONIS MZidonis

Note: Sample container should be rinsed and should be filled completely leaving no air space between contents & lid. Preserve samples on ice or refrigerator (0.1-6°C) immediately after collection. Transport samples to the WET Laboratory immediately after the fifth sample is collected. The WET laboratory is located in Room LE-100, Luc-Hing R&D Complex. CHLORINATION → DECHLORINATION
 Indicate if the final effluent was chlorinated: Yes / No / NA
 SAMPLES RELINQUISHED BY: Name NICUSOR SIRBI Signature: NSirbi Date/Time: 7/23/09 8:20 A
 SAMPLES TRANSPORTED TO WET LAB BY Name A SAVERS Signature: ASavers Date/Time: 7/23/09 1:20 P

FOR WET LABORATORY USE ONLY:
 Sample Received by: Name G.V. BILLET Signature: GBillet Date/Time 7-27-09
 1. Samples received with prescribed holding time (within 4 h of collection)? Yes / No / (NA)
 (Not Applicable, if chronic test)
 2. Samples logged in by AS Date 7-23-09 Time 10:45 AM
 3. Each sample container labeled with a unique ID? Yes / No
 4. Were collection times for effluent and receiving water within 1 h of each other? Yes / No / (NA)
 5. Did samples have sufficient volume for analysis? Yes / No
 6. Samples accepted Yes / No
 Special Observations _____

LIMS #	Sample Type/ID	Temp °C	pH	Residual Chlorine (mg/L) Initials	Sodium-thio-sulfate Added YES/NO	Sample Custodian Signature
5792025	HPOUTCH2A	1.5°C	7.03	0.0 GVB	Indicate Total _____ ml of 5% Sodium-thio-sulfate added. Initial _____	G.V. Billet
5792025	HPOUTCH2B	2.0°C	7.16	Total Ammonia (mg-L) ALD Results	Final Residual Cl ₂ reading = _____ mg/l	
5792025	HPOUTCH2C	1.0°C	7.01		Initial _____	
5792025	HPOUTCH2D	3.0°C	6.95			
5792025	HPOUTCH2E	6.0°C	6.94	0.38		

Note: Set aside one cubitainer for metals and chemical analyses

Sample Received By: Trace Metals JUDITH SCHWITT Date 7-23-09 Time 11:30
 Lachat [Signature] Date 7-23-09 Time 11:35
 Materials [Signature] Date 7-23-09 Time 11:35

Sample aliquot received by _____ Date _____ Time _____
 Name _____ Signature _____

Sample Release for Disposal

Sample released for disposal following analysis on (Date) _____ by _____

Samples Discarded by _____ Date/Time _____

73
64

21st
Friday OK

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

The WET Laboratory Chain-of-Custody Form

SAMPLE COLLECTION			SAMPLE TYPE	SAMPLE LOCATION	SAMPLE Temp °C	ON-SITE SAMPLE STORAGE (0.1-6 °C) YES/NO	PRINT NAME & SIGNATURE
DATE	TIME	BY					
07/24/09	0600	MZ	GRAB	EFFLUENT	20.5°	<input checked="" type="checkbox"/> YES	MIKE ZIDONIS M Zidonis
07/24/09	1200	CK	GRAB	EFFLUENT	23°	<input checked="" type="checkbox"/> YES	CLAUDE LEDECK C. Ledeck
07/24/09	1800	VMS	GRAB	EFFLUENT	21.5°	<input checked="" type="checkbox"/> YES	VASIG SIDEA M. Sidia
07/24/09	2400	CAW	GRAB	EFFLUENT	22°	<input checked="" type="checkbox"/> YES	CYNTHIA WOODHEAD C. Woodhead
07/25/09	0600	CAW	GRAB	EFFLUENT	22°	<input checked="" type="checkbox"/> YES	CYNTHIA WOODHEAD C. Woodhead

Note: Sample container should be rinsed and should be filled completely leaving no air space between contents & lid. Preserve samples on ice or refrigerator (0.1-6°C) immediately after collection. Transport samples to the WET Laboratory immediately after the fifth sample is collected. The WET laboratory is located in Room LE-100, Lue-Hing R&D Complex. **CHLORINATED / DECHLORINATED**
 Indicate if the final effluent was chlorinated: Yes No NA
 SAMPLES RELINQUISHED BY: Name CLAUDE LEDECK Signature: Claude Ledeck Date/Time: 8:40 - 7/25/09
 SAMPLES TRANSPORTED TO WET LAB. BY Name A. Manoj S. K. S. Signature: A. Manoj S. K. S. Date/Time: 7:51:09 8:40+4

FOR WET LABORATORY USE ONLY:

Sample Received by: Name G.V. Billiet Signature G.V. Billiet Date/Time 7-25-09
 1. Samples received with prescribed holding time (within 14 h of collection)? (Not Applicable, if chronic test)
 2. Samples logged in by AUB Date 7-25-09 Time 10:30 am
 3. Each sample container labeled with a unique ID?
 4. Were collection times for effluent and receiving water within 1 h of each other?
 5. Did samples have sufficient volume for analysis?
 6. Samples accepted
 Special Observations _____

- Yes No (NA)
- Yes No
- Yes No
- Yes No (NA)
- Yes No
- Yes No

LIMS #	Sample Type/ID	Temp °C	pH	Residual Chlorine (mg/L) Initials	Sodium-thio-sulfate Added YES/NO	Sample Custodian Signature
5792032	HPOUTCH3A	1.0	7.00	0.0	Indicate Total ml of 5% Sodium-thio-sulfate added. Initial	G.V. Billiet
5792032	HPOUTCH3B	3.0	6.97	Total Ammonia (mg-L) ALD Results	Initial	
5792032	HPOUTCH3C	2.5	7.07		Final Residual Cl ₂ reading = mg/l	
5792032	HPOUTCH3D	3.0	7.00		Initial	
5792032	HPOUTCH3E	4.0	7.11	0.10		

Note: Set aside one cubitainer for metals and chemical analyses

Sample Received By: Trace Metals CS Date 7-27-09 Time 7:15
 Lachat ST Date 7-27-09 Time 7:20
 Materials ST Date 7-27-09 Time 7:20
 Sample aliquot received by _____ Date _____ Time _____
 Name _____ Signature _____

Sample Release for Disposal
 Sample released for disposal following analysis on (Date) _____ by _____
 Samples Discarded by _____ Date/Time _____

APPENDIX DI

QUALITY ASSURANCE FOR THE *PIMEPHALES PROMELAS*
WHOLE EFFLUENT TOXICITY TEST:
RAW DATA AND STATISTICAL CALCULATIONS
FOR THE CONCURRENT REFERENCE TOXICANT TEST,
CONTROL CHARTS, AND CULTURE DATA

CETIS Summary Report

Report Date: 18 Aug-09 08:58 (p 1 of 2)
 Test Code: 11-5520-6908/44DB0EFC

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

Batch ID: 07-3038-2282 Test Type: Growth-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Pimephales promelas Brine: Not Applicable
 Duration: 7d 0h Source: Environmental Consult & Test Age: 48h

Sample ID: 12-4971-8211 Code: 4A7D2FC3 Client:
 Sample Date: 21 Jul-09 Material: Sodium chloride Project:
 Receive Date: 21 Jul-09 Source: Reference Toxicant
 Sample Age: 24h Station:

Comments: Concurrent RTT with Hanover Park Chronic WET Test.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-8095-1414	7d Survival Rate	3000	6000	4240	33.4%		Dunnett's Multiple Comparison Test
13-0070-9020	Mean Dry Weight-mg	3000	6000	4240	27.3%		Bonferroni Adj t Test

Point Estimate Summary

Analysis ID	Endpoint	Level	Conc-mg/	95% LCL	95% UCL	TU	Method
17-5009-5126	Mean Dry Weight-mg	IC25	3610	3220	4110		Linear Interpolation (ICPIN)

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-8095-1414	7d Survival Rate	Control Resp	0.875	0.8 - NL	Yes	Result Within Limits
13-0070-9020	Mean Dry Weight-mg	Control Resp	1.16	0.25 - NL	Yes	Result Within Limits
17-5009-5126	Mean Dry Weight-mg	Control Resp	1.16	0.25 - NL	Yes	Result Within Limits
13-0070-9020	Mean Dry Weight-mg	PMSD	0.273	0.12 - 0.3	Yes	Result Within Limits

7d Survival Rate Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	0.875	0.839	0.911	0.8	1	0.0175	0.0957	10.9%	0.0%
750		4	0.8	0.77	0.83	0.7	0.9	0.0149	0.0816	10.2%	8.57%
1500		4	0.95	0.928	0.972	0.9	1	0.0105	0.0577	6.08%	-8.57%
3000		4	0.8	0.77	0.83	0.7	0.9	0.0149	0.0816	10.2%	8.57%
6000		4	0.475	0.363	0.587	0.2	0.9	0.0545	0.299	62.9%	45.7%
8000		4	0	0	0	0	0	0	0		100.0%

Mean Dry Weight-mg Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	4	1.16	1.09	1.23	0.88	1.29	0.0344	0.188	16.2%	0.0%
750		4	1.17	1.12	1.22	1.03	1.34	0.0237	0.13	11.1%	-1.08%
1500		4	1.46	1.42	1.5	1.33	1.56	0.0197	0.108	7.37%	-26.1%
3000		4	1.17	1.12	1.21	1.06	1.35	0.0232	0.127	10.9%	-0.65%
6000		4	0.347	0.269	0.426	0.15	0.62	0.0383	0.21	60.4%	70.0%

CETIS Summary Report

Report Date: 18 Aug-09 08:58 (p 2 of 2)

Test Code: 11-5520-6908/44DB0EFC

Larval Fish 7-d Survival and Growth Test

MWRD of Greater Chicago

7d Survival Rate Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1	0.8	0.8	0.9
750		0.8	0.8	0.7	0.9
1500		1	1	0.9	0.9
3000		0.9	0.8	0.8	0.7
6000		0.4	0.9	0.4	0.2
8000		0	0	0	0

Mean Dry Weight-mg Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water	1.24	1.29	0.88	1.23
750		1.19	1.03	1.13	1.34
1500		1.33	1.42	1.56	1.54
3000		1.11	1.15	1.35	1.06
6000		0.22	0.62	0.4	0.15

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FATHEAD MINNOW - *Pimephales promelas* CHRONIC RTT TEST DATA SHEET

Reference Toxicant: NaCl

Analysts: G. V. BILLET

Laboratory water (LW): MHSW/HSW WITH SELENIUM (circle one) Date approved 7-13-09

Beginning Test Date 7-22-09 Time 2:30 pm

Species: *Pimephales promelas*

Ending Test Date 7-29-09 Time 2:30: pm

Age < 24 hrs Source EC + T

Hatch Date _____

Incubator I.D. CHAMBER A

Calibrated Thermometer # S/N 8031 9988

DI-3

NaCl (mg/L)	Dissolved Oxygen- mg/l								pH							
	0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
Old 0	<u>NEW</u> 7.26	5.34	5.16	4.69	5.26	5.12	4.83	5.31	<u>NEW</u> 8.01	7.21	7.04	7.29	7.24	7.28	7.23	7.25
750	7.95	5.24	5.17	5.09	5.72	4.88	5.08	5.10	7.97	7.36	7.26	7.38	7.37	7.27	7.32	7.30
1500	7.99	5.56	5.40	5.42	5.66	5.15	5.55	5.47	7.98	7.43	7.28	7.42	7.39	7.32	7.37	7.33
3000	8.00	5.31	6.02	5.41	5.62	5.17	5.49	5.44	7.96	7.38	7.36	7.43	7.38	7.34	7.36	7.32
6000	7.98	5.38	5.97	5.69	6.05	5.41	6.54	6.31	7.89	7.36	7.30	7.45	7.43	7.36	7.47	7.37
8000	8.01	5.79	6.58	6.62	7.22	/	/	/	7.94	7.41	7.42	7.56	7.64	/	/	/
Time	11:00 am	2:30 pm	3:25 pm	1:45 pm	12:30 pm	2:05 pm	2:15 pm	3:04 pm	11:00 am	2:30 pm	3:25 pm	1:45 pm	1:30 pm	2:05 pm	2:15 pm	3:04 pm
Init.	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

Measure DO at the end of each 24 h exposure period in at least one test chamber at each test concentration and in the control.

Measure Temperature at the end of each 24 h exposure period in at least one test chamber at each test concentration and in the control. Measure temperature in two test vessels at the end of the test to determine the temperature variation in the environmental chamber.

Measure pH each day, at the end of each 24 h exposure period, in at least one test chamber at each test concentration and in the control.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FATHEAD MINNOW - *Pimephales promelas*

CHRONIC RTT TEST DATA SHEET

Reference Toxicant: NaCl

Analysts: G.V. BELLETT

Date: 7-22-09

DI-4

Cup No.	Adult Survival									Temperature(°C)							
	0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168	
Control LW	7A	10	10	10	10	10	10	10	10	24.7				25.3			
	7B	10	10	10	10	9	9	9	8		25.4				25.5		
	7C	10	10	10	10	8	8	8	8			25.4				25.2	
	7D	10	10	10	10	10	10	10	9				25.2				25.6
TIME	2:30 pm	2:30 pm	2:45 pm	1:10 pm	1:05 pm	1:35 pm	1:15 pm	1:40 pm	2:45 pm	8:30 pm	7:45 am	8:45 am	7:45 am	7:55 am	8:10 am	8:25 pm	
INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	

NaCl (mg/L)	Cup No.	Adult Survival									Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168	
750	8A	10	10	10	10	10	10	9	8	24.8				25.6				
	8B	10	10	10	9	9	9	9	8		25.4				25.5			
	8C	10	10	10	10	10	7	7	7			25.4				25.5		
	8D	10	10	10	10	9	9	9	9				25.3				25.6	
TIME	2:30 pm	2:30 pm	2:50 pm	1:15 pm	1:10 pm	1:35 pm	1:20 pm	1:40 pm	2:45 pm	8:30 pm	7:45 am	8:45 am	7:45 am	7:55 am	8:10 am	8:25 pm		
INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB		

Comments: _____

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
 FATHEAD MINNOW - *Pimephales promelas* CHRONIC RTT TEST DATA SHEET
 Reference Toxicant: NaCl Analysts: G.V. BILLET Date: 7-22-09

DI-5

NaCl (mg/L)	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
1500	9A	10	10	10	10	10	10	10	10	24.1				25.4			
	9B	10	10	10	10	10	10	10	10		25.3				25.4		
	9C	10	10	9	9	9	9	9	9			25.7				25.7	
	9D	10	10	9	9	9	9	9	9				25.3				25.6
	TIME	2:30 pm	2:30 pm	2:55 pm	1:20 pm	1:15 pm	1:40 pm	1:25 pm	1:35 pm	2:45 pm	8:30 pm	7:45 am	8:45 pm	7:45 am	7:55 pm	8:10 am	8:50 am
	INITIAL	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB

NaCl (mg/L)	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
3000	10A	10	10	10	10	10	10	9	9	24.0				25.2			
	10B	10	10	10	10	10	10	9	8		25.1				25.2		
	10C	10	10	9	9	8	8	8	8			25.2				25.2	
	10D	10	10	10	10	10	10	8	7				25.4				25.7
	TIME	2:30 pm	2:40 pm	3:00 pm	1:25 pm	1:20 pm	1:40 pm	1:30 pm	1:40 pm	2:45 pm	8:30 pm	7:50 am	8:45 pm	7:45 am	7:55 pm	8:10 am	8:30 pm
	INITIAL	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB	GMB

Comments: _____

FATHEAD MINNOW - *Pimephales promelas*

CHRONIC RTT TEST DATA SHEET

Reference Toxicant: NaCl

Analysts: G.V. BILLET

Date: 7-22-09

NaCl (mg/L)	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
6000	11A	10	7	6	6	6	4	4	4	24.2				25.5			
	11B	10	10	10	10	10	9	9	9		25.4				25.5		
	11C	10	8	8	8	7	5	5	4			25.6				25.6	
	11D	10	8	8	7	5	3	3	2				25.2				25.6
	TIME	2:30 pm	2:40 pm	3:05 pm	1:30 pm	1:25 pm	1:45 pm	1:35 pm	1:40 pm	2:50 pm	8:35 pm	7:50 pm	8:50 am	7:50 pm	8:00 pm	8:15 am	8:30 pm
	INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

DI-6

NaCl (mg/L)	Cup No.	Adult Survival								Temperature(°C)							
		0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
8000	12A	10	2	0	0	0	0	0	0	24.3				25.4			
	12B	10	0	0	0	0	0	0	0		25.5				25.5		
	12C	10	0	0	0	0	0	0	0			25.5				25.5	
	12D	10	4	1	1	0	0	0	0				25.2				25.5
	TIME	2:30 pm	2:40 pm	3:10 pm	1:35 pm	1:30 pm	1:30 pm	1:40 pm	1:40 pm	2:50 pm	8:35 pm	7:50 pm	8:50 am	7:50 pm	8:00 pm	8:15 am	8:30 pm
	INITIAL	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB	QMB

Comments: _____

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
 FATHEAD MINNOW - *Pimephales promelas* CHRONIC RTT TEST DATA SHEET

Fish Feeding Record

Reference Toxicant: NaCl Analysts: G. V. BILLET Test Date 7-22-09
 Feeding Times/Type of Food/Initials: Brand Argentemia Lot Number BVO74A
 BS = Brine Shrimp : Shrimp Brine Shrimp set-up (Time) Beaker A _____ AM / PM Beaker B _____ AM / PM

(Fish fed 0.15 mL of concentrated newly hatched baby brine shrimp per test vessel as specified in USEPA Manual EPA/600/4-91/002; 1994)

FEEDING SCHEDULE: If the test is initiated after 12:00 PM, feed fish once; on following days, feed fish at the beginning of the work day, at least 2 h before test solution renewal, and at the end of the work day, after test solution renewal. Do not feed fish during the final 12 h of the test.

FISH FEEDING RECORD

TIME AND INITIALS

Time (h)	0	24 (day 1)	48 (day 2)	72 (day 3)	96 (day 4)	120 (day 5)	144 (day 6)
Morning	 	8:05 AM GVB	7:55 AM GVB	8:10 AM GVB	7:45 AM GVB	7:50 PM GVB	8:35 AM GVB
Evening	2:40 PM GVB	3:25 PM GVB	3:25 GVB	2:30 PM GVB	1:55 PM GVB	3:25 PM GVB	3:20 PM GVB

TEMPERATURE*/LIGHT RECORD**

	0 GVB	24 (day 1) GVB	48 (day 2) GVB	72 (day 3) GVB	96 (day 4) GVB	120 (day 5) GVB	144 (day 6) GVB	168 (day 7) GVB
Tray 1 *Therm. # 43	24.9°C 64.8	24.9°C 63.1	25.1°C 59.3	24.8°C 71.6	24.9°C 60.1	24.8°C 58.1	24.9°C 60.2	25.0°C 56.5
Tray 2 *Therm. # 43	24.9°C 54.9	24.9°C 62.7	25.1°C 57.4	24.8°C 61.8	24.9°C 59.5	24.8°C 51.5	24.9°C 58.3	25.0°C 52.8

**Light Meter (SPER Scientific Serial No. _____) Calibrated on _____/_____/_____

DI-7

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
 FATHEAD MINNOW - *Pimephales promelas* CHRONIC RTT TEST DATA SHEET

Reference Toxicant: NaCl Analysts: G. V. BILLET Date: 7-22-'09

Date	Day 0 (<u>7 / 22 / '09</u>)	Day 7 (<u>7 / 29 / '09</u>)
CONDUCTIVITY (Initials <u>G.V.B.</u>)		
F7 Minnow Lab water (LW)	(Reference~0.543 mS) <u>0.567</u>	<u>0.571</u>
F8 Minnow 750 mg/l	(Reference~1.914 mS) <u>2.060</u>	<u>2.100</u>
F9 Minnow 1500 mg/l	(Reference~3.257 mS) <u>3.480</u>	<u>3.530</u>
F10 Minnow 3000 mg/l	(Reference ~5.86 mS) <u>6.290</u>	<u>6.400</u>
F11 Minnow 6000 mg/l	(Reference~10.94 mS) <u>11.510</u>	<u>11.660</u>
F12 Minnow 8000 mg/l	(Reference~14.25 mS) <u>14.910</u>	<u>14.920</u>
ALKALINITY mg/L as CaCO ₃ (Initials <u>G.V.B.</u>)		
F7 Minnow Lab water	Titr. Calc. <u>5.7</u> x 20 = <u>114.0</u>	Titr. Calc. <u>5.6</u> x 20 = <u>112.0</u>
HARDNESS mg/L as CaCO ₃ (Initials <u>G.V.B.</u>)		
F7 Minnow Lab Dilution w	Titr. Calc. <u>4.2</u> x 40 = <u>168.0</u>	Titr. Calc. <u>4.2</u> x 40 = <u>168.0</u>

Measure Conductivity, Alkalinity, and Hardness in each new sample (sodium chloride concentrations and in the control).

DI-8

Reference Toxicant: NaCl Analysts: G. V. BILLET DATE 7-22-'09

DI-9

Treatment / Replicate	Dry Wt. W/Fish gm	Tare Wt. gm	Wt. Of Fish gm	ORIGINAL # OF FISH (ON)	Avg. Wt Per Fish gm (ON)	Avg. Wt Per Fish mg (ON)
CONTROL						
7A	2.1469	2.1345	0.0124	10	0.0012	1.2400
7B	2.1585	2.1456	0.0129	10	0.0013	1.2900
7C	2.0863	2.0775	0.0088	10	0.0009	0.8800
7D	2.1120	2.0997	0.0123	10	0.0012	1.2300
750 mg/L						
8A	2.1127	2.1008	0.0119	10	0.0012	1.1900
8B	2.1601	2.1498	0.0103	10	0.0010	1.0300
8C	2.1566	2.1453	0.0113	10	0.0011	1.1300
8D	2.1003	2.0869	0.0134	10	0.0013	1.3400
1500 mg/L						
9A	2.1354	2.1221	0.0133	10	0.0013	1.3300
9B	2.1475	2.1333	0.0142	10	0.0014	1.4200
9C	2.1624	2.1468	0.0156	10	0.0016	1.5600
9D	2.1028	2.0874	0.0154	10	0.0015	1.5400
3000 mg/L						
10A	2.0810	2.0699	0.0111	10	0.0011	1.1100
10B	2.1391	2.1276	0.0115	10	0.0011	1.1500
10C	2.0930	2.0795	0.0135	10	0.0014	1.3500
10D	2.0877	2.0771	0.0106	10	0.0011	1.0600
6000 mg/L						
11A	2.0894	2.0872	0.0022	10	0.0002	0.2200
11B	2.0888	2.0826	0.0062	10	0.0006	0.6200
11C	2.1332	2.1292	0.0040	10	0.0004	0.4000
11D	2.0696	2.0681	0.0015	10	0.0002	0.1500
8000 mg/L						
12A	2.	2.	0.	10	0.0000	0.0000
12B	2.	2.	0.	10	0.0000	0.0000
12C	2.	2.	0.	10	0.0000	0.0000
12D	2.	2.	0.	10	0.0000	0.0000

*
*
*
*

Count the surviving larvae in each test chamber and immediately prepare for dry weight determination. Transfer each group of larvae to a tared glass vial that has been properly labeled. Dry vials at 60°C, for 24 h or at 100°C for a minimum of 6 h. Immediately upon removal from the drying oven, place the glass vials in a desiccator until weighed. Measure all weights to the nearest 0.01 mg and average weights should be expressed to the nearest 0.001 mg. Prepare a summary table as illustrated above.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Fathead Minnow

Water Reclamation Plant:Reftox

Collection Date:07-22-09@

Number of treatments, including the Control(s):6

Number of replicates:4

Number of organisms per replicate:10

ORDER OF RANDOM PLACEMENT OF CUPS IN TRAYS

Tray 1			
Cup 8C	Cup 7B	Cup 12B	Cup 9C
Cup 10C	Cup 11A	Cup 10D	Cup 11C
Cup 7A	Cup 12A	Cup 9D	Cup 11B
Cup 9A	Cup 11D	Cup 8B	Cup 8A
_____ Front of Tray _____			

2:10 pm
JMB

Tray 2	
Cup 12D	Cup 8D
Cup 10B	Cup 12C
Cup 10A	Cup 7D
Cup 7C	Cup 9B
_____ Front of Tray _____	

Number of organisms for each cup checked by _____



Environmental Consulting & Testing

No.

1423 N. 8th St. Suite 118
Superior, Wisconsin 54880
(715)392-6635 fax (715)395-2463

Shipping Slip

Shipping Date: 7/20/2009

Name: Metro Waste Dist.-RND
Address: 6001 Pershing Road
City: Cicero
State/Providence: IL
Zip: 60804
Phone: 708-588-4225

From: ECT Superior, Wisconsin

Species: Fathead Minnow (Pimephales Promelas)
Date of Hatch: 7/20/2009
Number: 600
Temp at Shipping: 24.6

PO# J. Yamanaka 8005771 SEND INVOICE WITH

Shipped By: Federal Express: ECT Account

WATER CHEMISTRY RECORDS

Temperature ----- 24 to 26 As C°
Conductivity ----- 90 to 120 As UMHOS/CM
T. Hardness ----- 50 to 80 As CaCO3
T. Alkalinity ----- 40 to 60 As CACO3
pH ----- 6.9 to 7.9

RECENT REF. TESTS WITH NaCl

(Overall mean (s.d.) = 2.47 (1.03) g/l n=75 (0.41 g/l to 4.53 g/l) n=42 *4.73 (1.02) g/l (2.69 g/l to 6.77 g/l)

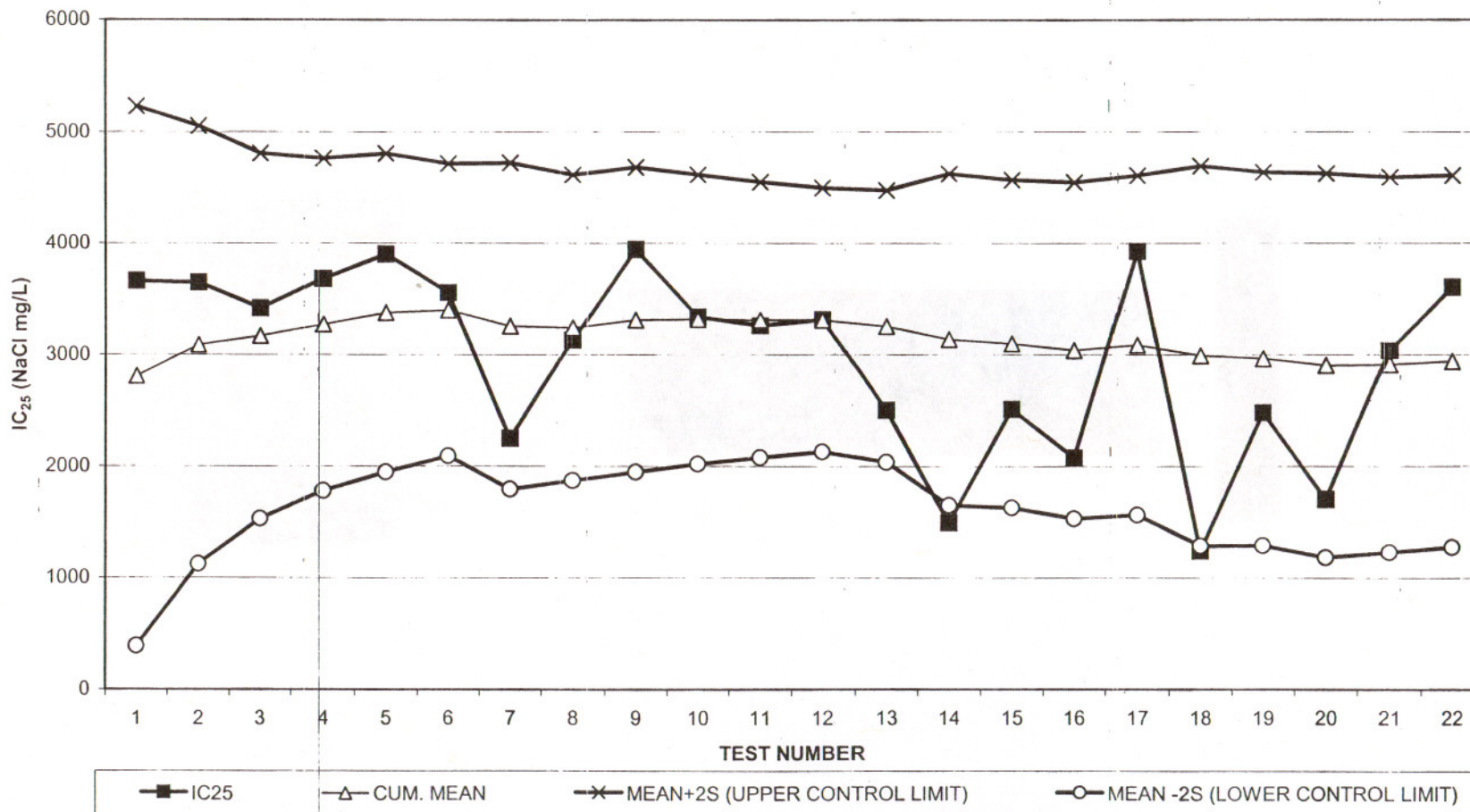
2005

Jan. Chronic IC25 = 1.93 g/l	Acute (01 Day Old) LC50 = 4.02 g/l
Feb. Chronic IC25 = 1.90 g/l	Acute (01 Day Old) LC50 = 4.75 g/l
Mar. Chronic IC25 = 2.13 g/l	Acute (01 Day Old) LC50 = 4.75 g/l
Apr. Chronic IC25 = 1.50 g/l	Acute (01 Day Old) LC50 = 4.44 g/l
May Chronic IC25 = 2.02 g/l	Acute (01 Day Old) LC50 = 4.63 g/l
June Chronic IC25 = 1.60 g/l	Acute (01 Day Old) LC50 = 5.43 g/l
July Chronic IC25 = 2.22 g/l	Acute (01 Day Old) LC50 = 7.92 g/l
Aug. Chronic IC25 = 2.36 g/l	Acute (01 Day Old) LC50 = 7.14 g/l
Sept. Chronic IC25 = 2.41 g/l	Acute (01 Day Old) LC50 = 4.87 g/l
Oct. Chronic IC25 = 2.19 g/l	Acute (01 Day Old) LC50 = 7.14 g/l
Nov. Chronic IC25 = 2.07 g/l	Acute (01 Day Old) LC50 = 6.21 g/l
Dec. Chronic IC25 = 1.22 g/l	Acute (01 Day Old) LC50 = 4.24 g/l

*Acute Toxicity Data extrapolated from Chronic Data

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

CONTROL CHART FOR CHRONIC REFERENCE TOXICITY TESTS
PIMEPHALES PROMELAS (GROWTH-ON) - IC₂₅
 REFERENCE TOXICANT (NaCl)



Note: Test number 22 was conducted concurrently with the chronic test on Hanover Park WRP. Test numbers 1-14, 16, 21 and 22 were conducted with hard synthetic water (HSW) with selenium, and test numbers 15, 17, 18, 19, and 20 were conducted with moderately hard synthetic water (MHSW) with selenium. Test numbers 1-19, 21 and 22 were conducted with fish from outside supplier. Test number 20 was conducted with fish raised in-house.

DI-12

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

REFERENCE TOXICANT TEST RESULTS
 REFERENCE TOXICANT- SODIUM CHLORIDE
 TEST ORGANISMS: *Pimephales promelas*

Reference Toxicant Test Number	Setup Date	IC ₂₅ mg/L	Cumulative Mean mg/L	MEAN+2S (mg/L) (UPPER CONTROL LIMIT)	MEAN - 2S (mg/L) (LOWER CONTROL LIMIT)
1	02/25/04	3663	2808	5227	389
2	03/10/04	3647	3087	5053	1122
3	03/24/04	3420	3170	4810	1531
4	04/07/04	3683	3273	4765	1781
5	04/21/04	3901	3378	4807	1948
6	05/05/04	3560	3404	4716	2092
7	05/19/04	2251	3259	4722	1797
8	07/07/04	3131	3245	4616	1874
9	07/21/04	3944	3315	4681	1949
10	08/18/04	3343	3318	4614	2021
11	09/15/04	3264	3313	4549	2077
12	11/03/04	3319	3314	4497	2130
13	04/13/05	2500	3255	4473	2038
14	06/08/05	1497	3138	4622	1655
15	05/17/06	2511	3099	4566	1632
16	06/23/06	2075	3039	4544	1534
17	05/16/07	3927	3088	4607	1569
18	06/08/07	1244	2991	4692	1290
19	12/04/07	2480	2966	4637	1294
20	07/10/08	1706	2906	4625	1186
21	09/10/08	3037	2912	4591	1232
22	07/22/09	3610	2942	4608	1276

DI-13

APPENDIX DII

QUALITY ASSURANCE FOR THE *CERIODAPHNIA DUBIA*
WHOLE EFFLUENT TOXICITY TEST:
RAW DATA AND STATISTICAL CALCULATIONS
FOR THE CONCURRENT REFERENCE TOXICANT TEST,
CONTROL CHARTS, AND CULTURE DATA

CETIS Summary Report

Report Date: 12 Aug-09 10:37 (p 1 of 2)
 Test Code: 07-2695-2159/2B5468DF

Cladoceran 7-d Survival and Reproduction Test

MWRD of Greater Chicago

Batch ID: 11-9359-7163 Test Type: Reproduction-Survival (7d) Analyst:
 Start Date: 22 Jul-09 Protocol: EPA/821/R-02-013 (2002) Diluent: Hard Synthetic Water
 Ending Date: 29 Jul-09 Species: Ceriodaphnia dubia Brine: Not Applicable
 Duration: 7d 0h Source: MWRD WET Lab In-house Culture Age: <24h

Sample ID: 03-8149-6632 Code: 16BD2D38 Client:
 Sample Date: 21 Jul-09 Material: Sodium chloride Project:
 Receive Date: 21 Jul-09 Source: Reference Toxicant
 Sample Age: 24h Station:

Comments: Hanover Park Concurrent Reference Toxicant Test.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
21-2245-5110	7d Survival Rate	1750	2000	1870	N/A		Fisher Exact/Bonferroni-Holm Test
03-0749-9407	Reproduction	<375	375	N/A	23.6%		Steel Many-One Rank Test

Point Estimate Summary

Analysis ID	Endpoint	Level	Conc-mg/	95% LCL	95% UCL	TU	Method
18-2736-4510	Reproduction	IC25	344	210	487		Nonlinear Regression

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
21-2245-5110	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Result Within Limits
03-0749-9407	Reproduction	Control Resp	32.8	15 - NL	Yes	Result Within Limits
18-2736-4510	Reproduction	Control Resp	32.8	15 - NL	Yes	Result Within Limits
03-0749-9407	Reproduction	PMSD	0.236	0.13 - 0.47	Yes	Result Within Limits

7d Survival Rate Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	10	1	1	1	1	1	0	0	0.0%	0.0%
375		10	0.8	0.643	0.957	0	1	0.077	0.422	52.7%	20.0%
750		10	0.7	0.52	0.88	0	1	0.0882	0.483	69.0%	30.0%
1750		10	0.8	0.643	0.957	0	1	0.077	0.422	52.7%	20.0%
2000		10	0.4	0.207	0.593	0	1	0.0943	0.516	129.0%	60.0%
3000		10	0	0	0	0	0	0	0	100.0%	100.0%

Reproduction Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Water	10	32.8	31.5	34.1	28	36	0.625	3.43	10.4%	0.0%
375		10	21.7	17.5	25.9	0	30	2.06	11.3	51.9%	33.8%
750		10	16.4	12	20.8	0	27	2.13	11.7	71.3%	50.0%
1750		10	8.7	6.93	10.5	1	14	0.865	4.74	54.5%	73.5%
2000		10	1.6	0.665	2.53	0	6	0.457	2.5	156.0%	95.1%
3000		10	0	0	0	0	0	0	0	100.0%	100.0%

CETIS Summary Report

Report Date: 12 Aug-09 10:37 (p 2 of 2)
 Test Code: 07-2695-2159/2B5468DF

Cladoceran 7-d Survival and Reproduction Test

MWRD of Greater Chicago

7d Survival Rate Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	1	1	1	1	1	1	1	1	1	1
375		1	1	0	1	1	1	0	1	1	1
750		0	1	0	1	0	1	1	1	1	1
1750		1	0	1	1	1	1	1	0	1	1
2000		1	1	0	1	0	0	0	0	1	0
3000		0	0	0	0	0	0	0	0	0	0

Reproduction Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Water	28	36	35	28	33	34	35	36	28	35
375		30	28	0	27	26	29	6	30	12	29
750		0	21	0	25	0	26	22	26	17	27
1750		14	2	12	11	14	10	10	4	1	9
2000		6	6	0	1	0	0	0	0	3	0
3000		0	0	0	0	0	0	0	0	0	0

REFERENCE TOXICANT: NaCl Analysts: James Kaehn RTT TEST DATE 7-22-09

Laboratory water (LW): MHSW/HSW WITH SELENIUM (circle one) Date 7-13-09 Approved by Biologist I

Calibrated Thermometer: 80319998 Beginning Test Date 7-22-09 Time 12:45pm

Species: *C. dubia* Source: In-house Ending Test Date 7-29-09 Time 12:45pm

Age group of neonates 3pm to 11pm, 11pm to 7am, or 7am to 3pm

Incubator I.D. A *Ceriodaphnia* Tray # 10 D Test Tray Location Center Table Right side

DII-3

mg/L	Dissolved Oxygen- mg/l								pH							
	0	24	48	72	96	120	144	168	0	24	48	72	96	120	144	168
Old LW	7.25	7.67	8.28	7.82	7.86	7.55	8.00	7.62	7.77	7.87	7.99	8.08	8.03	8.00	7.85	8.06
375	7.25	7.85	8.36	7.96	7.87	7.69	8.01	7.74	7.87	7.99	7.94	8.07	8.04	8.21	7.99	8.14
750	7.63	7.93	8.34	8.05	8.10	7.71	8.09	7.86	8.06	7.99	7.96	8.11	8.01	8.24	8.01	8.19
1750	8.29	7.87	8.33	7.92	7.93	7.70	8.06	7.85	8.06	7.96	7.92	8.14	8.21	8.23	8.00	8.18
2000	7.96	7.82	8.40	8.00	8.21	7.57	8.11	7.68	8.11	7.91	7.98	8.15	8.10	8.26	8.03	8.20
3000	8.21	7.86	8.36	7.98	8.23	7.77	8.12	7.89	8.11	7.94	7.96 JK 7/29/09	8.12	8.03	8.24	8.01	8.23
Time	2:45pm	2:10pm	2:10pm	2:10pm	2:10pm	2:10pm	2:10pm	2:10pm	2:45pm	2:10pm	2:10pm	2:10pm	2:10pm	2:10pm	2:10pm	2:10pm
Initials	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

Measure DO & pH at test set-up and at the ^{end of} ~~end of~~ each 24 h exposure period combining 10 replicates for each test concentration and in the control after neonates are counted.

Ceriodaphnia dubia CHRONIC REFERENCE TOXICITY TEST DATA SHEET

Reference Toxicant : NaCl Analysts: James Kaehn, _____, _____ Assay Date 7-22-09

DII-4

		Temperature °C (acceptable temperature : 24-26°C)										Temperature °C (acceptable temperature : 24-26°C)								
CONTROL		0	24	48	72	96	120	144	168	mg/L	Cup No.	0	24	48	72	96	120	144	168	
lab - Water	1A	25.0	25.1							375	2A	25.3	25.0							
	1B			25.3							2B			24.9						
	1C				25.0						2C				24.7					
	1D					25.3					2D					25.0				
	1E						25.1				2E						25.1			
	1F							25.3			2F							24.8		
	1G								25.1		2G								25.0	
	1H								25.0		2H									25.1
	1I										2I									
	1J										2J									
TIME		1:00pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	TIME		1:00pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	

		Temperature °C (acceptable temperature : 24-26°C)										Temperature °C (acceptable temperature : 24-26°C)								
750		0	24	48	72	96	120	144	168	mg/L	Cup No.	0	24	48	72	96	120	144	168	
750	3A	25.1	25.0							1750	4A	25.1	25.3							
	3B			25.0							4B			25.0						
	3C				25.0						4C				24.8					
	3D					24.8					4D					25.1				
	3E						25.0				4E						25.0			
	3F							25.1			4F							25.1		
	3G								24.8		4G									24.8
	3H								24.8		4H									25.0
	3I										4I									
	3J										4J									
TIME		1:00pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	TIME		1:00pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	

• Comments: _____

Ceriodaphnia dubia CHRONIC REFERENCE TOXICITY TEST DATA SHEET

Reference Toxicant : NaCl Analysts: James Kaehn, _____, _____ Assay Date 7-22-09

DII-5

mg/L	Cup No.	Temperature °C (acceptable temperature : 24-26°C)								mg/L	Cup No.	Temperature °C (acceptable temperature : 24-26°C)									
		0	24	48	72	96	120	144	168			0	24	48	72	96	120	144	168		
2000	5A	25.3	25.1							3000	6A	25.1	25.3								
	5B			25.0							6B			25.2							
	5C				24.8						6C				24.8						
	5D					25.0					6D					25.0					
	5E						24.8				6E						25.1				
	5F							24.8			6F							25.0			
	5G								25.0		6G									25.1	
	5H										25.0		6H								25.0
	5I												6I								
	5J												6J								
TIME		1:00pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:00pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm			
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK			

• Comments:

NEONATES (TIME : 3pm - 11pm)
 TRAY # 10D
 BIRTH DATE: 7-22-09
 WATER SOURCE: LW (DATE: 7-13-09)

~~NEONATES (TIME :)
 TRAY #
 BIRTH DATE:
 WATER SOURCE: LW (DATE:)~~ NA

C. dubia <<NEONATES>>
 Tray No: 10D
 Birthdate: 7-22-09
 Water Source: FW
 HSW Date: Fresh

(CHRONIC RTI-CD) METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO
Ceriodaphnia dubia CHRONIC REFERENCE TOXICITY TEST DATA SHEET

Reference Toxicant: NaCl Analysts: James Koehn Assay Date 7-22-09

D-II-6

mg/l	Cup No.	Number of Neonates Produced								Adult Survival							
		3 Broods															
Parent Cup*		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168
Lab Water	7 1A	0	0	5	0	10	13	0	28	1	1	1	1	1	1	1	1
	22 1B	0	0	7	0	12	17	2	36	1	1	1	1	1	1	1	1
	26 1C	0	0	6	0	13	16	0	35	1	1	1	1	1	1	1	1
	27 1D	0	0	4	0	11	13	0	28	1	1	1	1	1	1	1	1
	28 1E	0	0	5	0	13	15	0	33	1	1	1	1	1	1	1	1
	30 1F	0	0	6	0	11	17	0	34	1	1	1	1	1	1	1	1
	34 1G	0	0	6	0	12	17	6	35	1	1	1	1	1	1	1	1
	35 1H	0	0	6	0	13	17	15	36	1	1	1	1	1	1	1	1
	39 1I	0	0	5	0	11	12	13	28	1	1	1	1	1	1	1	1
	42 1J	0	0	5	0	13	17	0	35	1	1	1	1	1	1	1	1
TOTAL		0	0	55	0	119	154	36	328	10	10	10	10	10	10	10	10
TIME		2:15pm	2:15pm	2:15pm	2:15pm	2:15pm	2:15pm	2:15pm	2:15pm	12:45pm	12:45pm	12:45pm	12:45pm	12:45pm	12:45pm	12:45pm	12:45pm
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

mg/l	Cup No.	Number of Neonates Produced								Adult Survival							
		3 Broods															
NaCl		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168
375	2A	0	0	6	0	10	14	0	30	1	1	1	1	1	1	1	1
	2B	0	0	5	0	9	14	7	28	1	1	1	1	1	1	1	1
	2C	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
	2D	0	0	4	0	11	12	0	27	1	1	1	1	1	1	1	1
	2E	0	0	4	0	10	12	0	26	1	1	1	1	1	1	1	1
	2F	0	0	6	0	11	12	0	29	1	1	1	1	1	1	1	1
	2G	0	0	6	0	0	0	0	6	1	1	1	1	0	0	0	0
	2H	0	0	6	0	10	14	11	30	1	1	1	1	1	1	1	1
	2I	0	0	1	0	0	11	0	12	1	1	1	1	1	1	1	1
	2J	0	0	5	0	10	14	0	29	1	1	1	1	1	1	1	1
TOTAL		0	0	43	0	71	103	18	217	10	10	10	9	8	8	8	8
TIME		2:20pm	2:20pm	2:20pm	2:20pm	2:20pm	2:20pm	2:20pm	2:20pm	12:45pm	12:45pm	12:45pm	12:50pm	12:50pm	12:50pm	12:50pm	12:50pm
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

At test termination all observations on test organism survival and numbers of offspring should be completed within 2 hours. Any animal not producing young neonates should be examined to determine if it is male after day 7 of the test. Check with Biologist 1 to observe test organism on a microscope slide.

*For tracking test organisms to brood board tray.

Reference Toxicant: NaCl Analysts: James Kzehn, _____, _____ Assay Date 7-22-07

DII-7

mg/l	Cup No.	Number of Neonates Produced								Adult Survival									
		24	48	72	96	120	144	168	Total	3 Broods	0	24	48	72	96	120	144	168	
NaCl																			
750	3A	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	
	3B	0	0	3	0	8	10	0	21	1	1	1	1	1	1	1	1	1	
	3C	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	
	3D	0	0	4	0	9	12	1	25	1	1	1	1	1	1	1	1	1	
	3E	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	
	3F	0	0	2	0	10	14	0	26	1	1	1	1	1	1	1	1	1	
	3G	0	0	3	0	10	9	0	22	1	1	1	1	1	1	1	1	1	
	3H	0	0	3	0	10	13	11	26	1	1	1	1	1	1	1	1	1	
	3I	0	0	2	0	7	8	0	17	1	1	1	1	1	1	1	1	1	
	3J	0	0	6	0	10	11	0	27	1	1	1	1	1	1	1	1	1	
TOTAL		0	0	23	0	64	77	12	164	10	10	10	10	7	7	7	7	7	
TIME		2:25 pm	2:25 pm	2:25 pm	2:25 pm	2:25 pm	2:25 pm	2:25 pm	2:25 pm	12:45 pm	1:00 pm	1:00 pm	1:00 pm	1:00 pm	1:00 pm	1:00 pm	1:00 pm	1:00 pm	
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	

mg/l	Cup No.	Number of Neonates Produced								Adult Survival									
		24	48	72	96	120	144	168	Total	3 Broods	0	24	48	72	96	120	144	168	
NaCl																			
1750	4A	0	0	0	4	5	5	0	14	1	1	1	1	1	1	1	1	1	
	4B	0	0	0	2	0	0	0	2	1	1	1	1	1	0	0	0	0	
	4C	0	0	0	2	6	0	4	12	1	1	1	1	1	1	1	1	1	
	4D	0	0	0	2	4	0	5	11	1	1	1	1	1	1	1	1	1	
	4E	0	0	0	2	5	7	0	14	1	1	1	1	1	1	1	1	1	
	4F	0	0	0	2	4	0	4	10	1	1	1	1	1	1	1	1	1	
	4G	0	0	0	1	5	4	0	10	1	1	1	1	1	1	1	1	1	
	4H	0	0	0	0	4	0	0	4	1	1	1	1	1	1	0	0	0	
	4I	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	
	4J	0	0	0	3	6	0	0	9	1	1	1	1	1	1	1	1	1	
TOTAL		0	0	0	18	39	17	13	87	10	10	10	10	10	9	8	8	8	
TIME		2:30 pm	2:30 pm	2:30 pm	2:30 pm	2:30 pm	2:30 pm	2:30 pm	2:30 pm	12:45 pm	1:05 pm	1:05 pm	1:05 pm	1:05 pm	1:05 pm	1:05 pm	1:05 pm	1:05 pm	
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	

At test termination all observations on test organism survival and numbers of offspring should be completed within 2 hours. Any animal not producing young neonates should be examined to determine if it is male after day 7 of the test. Check with Biologist I to observe test organism on a microscope slide.

Reference Toxicant: NaCl Analysts: James Koehn, _____, _____ Assay Date 7-22-09

DII-8

mg/l	Cup No.	Number of Neonates Produced								Adult Survival							
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168
2000	5A	0	0	0	0	2	0	4	6	1	1	1	1	1	1	1	1
	5B	0	0	0	0	3	0	3	6	1	1	1	1	1	1	1	1
	5C	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	2
	5D	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1
	5E	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	5F	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
	5G	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	5H	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	5I	0	0	0	0	0	0	3	3	1	1	1	1	1	1	1	1
	5J	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
TOTAL		0	0	0	0	5	1	10	16	10	9	7	7	5	5	4	4
TIME		2:35pm	2:35pm	2:35pm	2:35pm	2:35pm	2:35pm	2:35pm	2:35pm	12:45pm	1:10pm	1:10pm	1:10pm	1:10pm	1:10pm	1:10pm	1:10pm
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

mg/l	Cup No.	Number of Neonates Produced								Adult Survival							
		24	48	72	96	120	144	168	Total	0	24	48	72	96	120	144	168
3000	6A	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6B	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6C	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6D	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6E	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6F	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6G	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6H	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	6I	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	6J	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
TOTAL		0	0	0	0	0	0	0	0	10	9	1	1	0	0	0	0
TIME		2:40pm	2:40pm	2:40pm	2:40pm	2:40pm	2:40pm	2:40pm	2:40pm	12:45pm	12:45pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm	1:15pm
INITIAL		JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK	JK

At test termination all observations on test organism survival and numbers of offspring should be completed within 2 hours. Any animal not producing young neonates should be examined to determine if it is male after day 7 of the test. Check with Biologist I to observe test organism on a microscope slide.

Toxicant: NaCl Amount Algae Fed: 0.1 mL Amount YTC Fed: 0.1 mL DATE: 7-22-09
 JK 7/22/09

Date	Algae Type / Harvest Date	Cell Concentration (cells/mL)	YTC/Thaw Date	Time Fed	Initials
7-22-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	12:45pm	JK
7-23-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	12:45pm	JK
7-24-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	12:45pm	JK
7-25-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-21-09	12:45pm	JK
7-26-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-24-09	12:45pm	JK
7-27-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-26-09	12:45pm	JK
7-28-09	<i>S. capricornutum</i> / 7-7-09	3.39×10^7	4-22-09 / 7-26-09	12:45pm	JK
NA	<i>S. capricornutum</i> /				JK

DII-9

Gray Metal Table Location	*Temperature (°C) / Light Reading**							
	0	24	48	72	96	120	144	168
Wall	25.0°C / 84.7	25.0°C / 86.0	25.0°C / 80.4	25.0°C / 82.5	24.8°C / 79.1	24.8°C / 81.7	24.8°C / 74.8	25.0°C / 72.5
Aisle	25.0°C / 84.7	25.0°C / 86.0	25.5°C / 87.3	24.5°C / 86.7	24.8°C / 86.0	24.8°C / 89.3	24.8°C / 76.1	25.0°C / 66.5

*Incubator Temperature (*Calibrated Thermometer # 45) / Light Record**/Intials:

**Light Meter (SPER Scientific Serial No. 031443) Calibrated on 3 / 4 / 09

Ceriodaphnia dubia CHRONIC TOXICITY TEST DATA SHEET

Assay Date 7-22-09

DII-10

Date	Day 0 (<u>7</u> / <u>22</u> / <u>09</u>)	Day 7 (<u>7</u> / <u>29</u> / <u>09</u>)
CONDUCTIVITY (Initials <u>JK</u>)		
F1 Dilution water...	(Reference ~0.534 mS) <u>0.566 mS</u>	<u>0.566 mS</u> ^{* 7/22/09} <u>0.568 mS</u> <u>0.525 mS</u>
F7 <i>C. dubia</i>		
<i>C. dubia</i> 375 mg/l	(Reference ~1.210 mS) <u>1.233 mS</u>	<u>1.251 mS</u>
<i>C. dubia</i> 750 /mg/l	(Reference ~1.910 mS) <u>1.993 mS</u>	<u>2.04 mS</u>
<i>C. dubia</i> 1,750 mg/l	(Reference ~3.63 mS) <u>3.61 mS</u>	<u>3.61 mS</u>
<i>C. dubia</i> 2,000 mg/l	(Reference ~4.11 mS) <u>4.11 mS</u>	<u>4.21 mS</u>
<i>C. dubia</i> 3,000 mg/l	(Reference ~5.79 mS) <u>5.89 mS</u>	<u>6.07 mS</u>
ALKALINITY mg/L as CaCO ₃ (Initials <u>JK</u>)		
Date	Day 0 (<u>7</u> / <u>22</u> / <u>09</u>)	Day 7 (<u>7</u> / <u>29</u> / <u>09</u>)
Lab Dilution water	Titr. <u>5.60</u> Calc. <u>112</u> <u>5.60</u> x 20 = <u>112</u>	Titr. <u>4.10</u> Calc. <u>114</u> ^{* 7-22-09} <u>4.10</u> x 20 = <u>114</u>
HARDNESS mg/L as CaCO ₃ (Initials <u>JK</u>)		
Date	Day 0 (<u>7</u> / <u>22</u> / <u>09</u>)	Day 7 (<u>7</u> / <u>29</u> / <u>09</u>)
Lab Dilution water	Titr. <u>4.10</u> Calc. <u>164</u> <u>4.10</u> x 40 = <u>164</u>	Titr. <u>4.30</u> Calc. <u>172</u> <u>4.30</u> x 40 = <u>172</u>

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Ceriodaphnia

Water Reclamation Plant: Reftox

Collection Date: 07-21-09A

Number of treatments, including the Control(s): 6

Number of replicates: 10

Number of organisms per replicate: 1

ORDER OF RANDOM PLACEMENT OF CUPS IN TRAYS

Tray J

Cup 1C	Cup 4A	Cup 3B	Cup 2G	Cup 6A	Cup 1H	Cup 4B	Cup 2H	Cup 3I	Cup 6F
Cup 5J	Cup 3D	Cup 3F	Cup 1D	Cup 3G	Cup 4C	Cup 5C	Cup 1G	Cup 2F	Cup 5I
Cup 6G	Cup 1E	Cup 4J	Cup 6E	Cup 6D	Cup 2C	Cup 2J	Cup 3A	Cup 4I	Cup 5H
Cup 3J	Cup 1I	Cup 2B	Cup 4F	Cup 5D	Cup 1A	Cup 5B	Cup 6H	Cup 6C	Cup 3A
Cup 1J	Cup 5F	Cup 4E	Cup 5A	Cup 5E	Cup 3H	Cup 1B	Cup 4G	Cup 2E	Cup 4D
Cup 6J	Cup 3C	Cup 5G	Cup 6J	Cup 4H	Cup 3E	Cup 1F	Cup 2I	Cup 2D	Cup 6B

Front of Tray

Number of organisms for each cup checked by Erica Collins

⊕ 8 or more neonates
reservoirs

- NO NEONATE

CERIODAPHNIA DUBIA

CULTURE DATA

Date 7-10-09 Tray# 10C Mean Average Birth/Day _____ Total Deaths over _____ Days: _____

Control Water Source FTW Generation Birth Date 7-10-09 Initials PM

Cup No.	NEONATES PRODUCED							Tot	ADULT SURVIVAL							LIPIDS						EGGS						APPEARANCE
	Sa	S	M	T	W	Th	F		Sa	S	M	T	W	Th	F	192	216	240	264	288	312	336	192	216	240	264	288	
1	⊕	⊕	1	⊕	15			A	A	A	A	A																
2	⊕	⊕	⊕	-	16																							
3	⊕	⊕	⊕	-	14																							
4	⊕	⊕	⊕	-	17																							
5	2	⊕	⊕	-	15																							
6	⊕	⊕	⊕	-	16																							
7	⊕	⊕	⊕	-	15																							
8																												
9	⊕	⊕	⊕	-	13																							
10	⊕	⊕	⊕	-	14																							
11	⊕	⊕	⊕	-	16																							
12	⊕	⊕	⊕	-	17																							
13	⊕	⊕	⊕	-	15																							
14	⊕	2	⊕	-	13																							
15	⊕	⊕	⊕	-	15																							
16	⊕	⊕	⊕	-	14																							
17	⊕	⊕	⊕	-	16																							
18	⊕	⊕	⊕	-	15																							
19	⊕	⊕	⊕	-	16																							
20	⊕	⊕	⊕	-	15																							
21	⊕	⊕	⊕	-	14																							
22	⊕	⊕	⊕	-	14																							
23	⊕	⊕	⊕	-	13																							
24	-	⊕	⊕	-	0																							
25	⊕	-	⊕	-																								
26	⊕	⊕	⊕	-	17																							
27	⊕	⊕	⊕	-	14																							
28	⊕	⊕	⊕	-	13																							
29	⊕	⊕	⊕	-	15																							
30	⊕	⊕	⊕	-	16																							
31	⊕	⊕	⊕	-	2	3																						
32	⊕	⊕	⊕	-	13																							
33	⊕	⊕	⊕	-	14																							
34	⊕	⊕	⊕	-	16																							
35	-	⊕	⊕	-	14																							
36	-	⊕	⊕	-	0																							
37	⊕	-	⊕	-	15																							
38	⊕	⊕	⊕	-	13																							
39	⊕	⊕	⊕	-	13																							
40	⊕	⊕	⊕	-	14																							
41	⊕	⊕	⊕	-	12																							
42	⊕	⊕	⊕	-	13																							
43	⊕	⊕	⊕	-	13																							
44	⊕																											
45	⊕	⊕	⊕	-	17																							
46	⊕	⊕	⊕	-	15																							
47	⊕	⊕	⊕	-	14																							
48	⊕	⊕	⊕	-	15																							
49	⊕	⊕	⊕	-	14																							
50	⊕	⊕	⊕	-	16																							
51	⊕	⊕	⊕	-	15																							
52	⊕	⊕	⊕	-	16																							
53	⊕	⊕	⊕	-	17																							
54	⊕	⊕	⊕	-	15																							
55	⊕	⊕	⊕	-	14																							
56	⊕	⊕	⊕	-	15																							
57	⊕	⊕	⊕	-	14																							
58	⊕	⊕	⊕	-	15																							
59	⊕	⊕	⊕	-	17																							
60	⊕	⊕	⊕	-	0																							
TOTALS								56	57	57	57	59																

PM PM PM PM PM

uf PM PM PM PM

Week 2

CERIODAPHNIA DUBIA
CULTURE
DATA

Date 7-10-09 Tray # 10E Amt. Algae fed 0.1 mL Amt. YTC fed 0.1

Date	Algae type	Harvest Date	Cell concentration cells/mL	YTC	Thaw date	time	initials
7-10-09	S. CAPRICORNUTUM	7-7-09	3.39×10^7	4-22-09	7-7-09	2:32 PM	DM
7-11-09	"	7-7-09	"	4-22-09	7-10-09	2:14 PM	DM
7-12-09	"	7-7-09	"	4-22-09	7-10-09	11:20 AM	SR
7-13-09	"	7-7-09	"	4-22-09	7-12-09	11:05 AM	DM
7-14-09	"	7-7-09	"	4-22-09	7-12-09	9:18 AM	DM
7-15-09	"	7-7-09	"	4-22-09	7-14-09	10:50 AM	DM
7-16-09	"	7-7-09	"	4-22-09	7-14-09	11:00 AM	DM
7-17-09	S. capricornutum	7-7-09	3.39×10^7	4-22-09	7-16-09	8:50 AM	AG
7-18-09	"	"	"	4-22-09	7-16-09	9:00 AM	AG
7-19-09	"	7-7-09	"	4-22-09	7-17-09	1:48 PM	DM
7-20-09	"	7-7-09	"	4-22-09	7-17-09	11:00 AM	DM
7-21-09	"	7-7-09	"	4-22-09	7-17-09	2:35 PM	DM
7-22-09	"	7-7-09	"	4-22-09	7-21-09	9:18 AM	DM

checked for
nematodes @ 3:08

TIME / TEMPERATURE (°C) / LIGHT READING
6302 27781

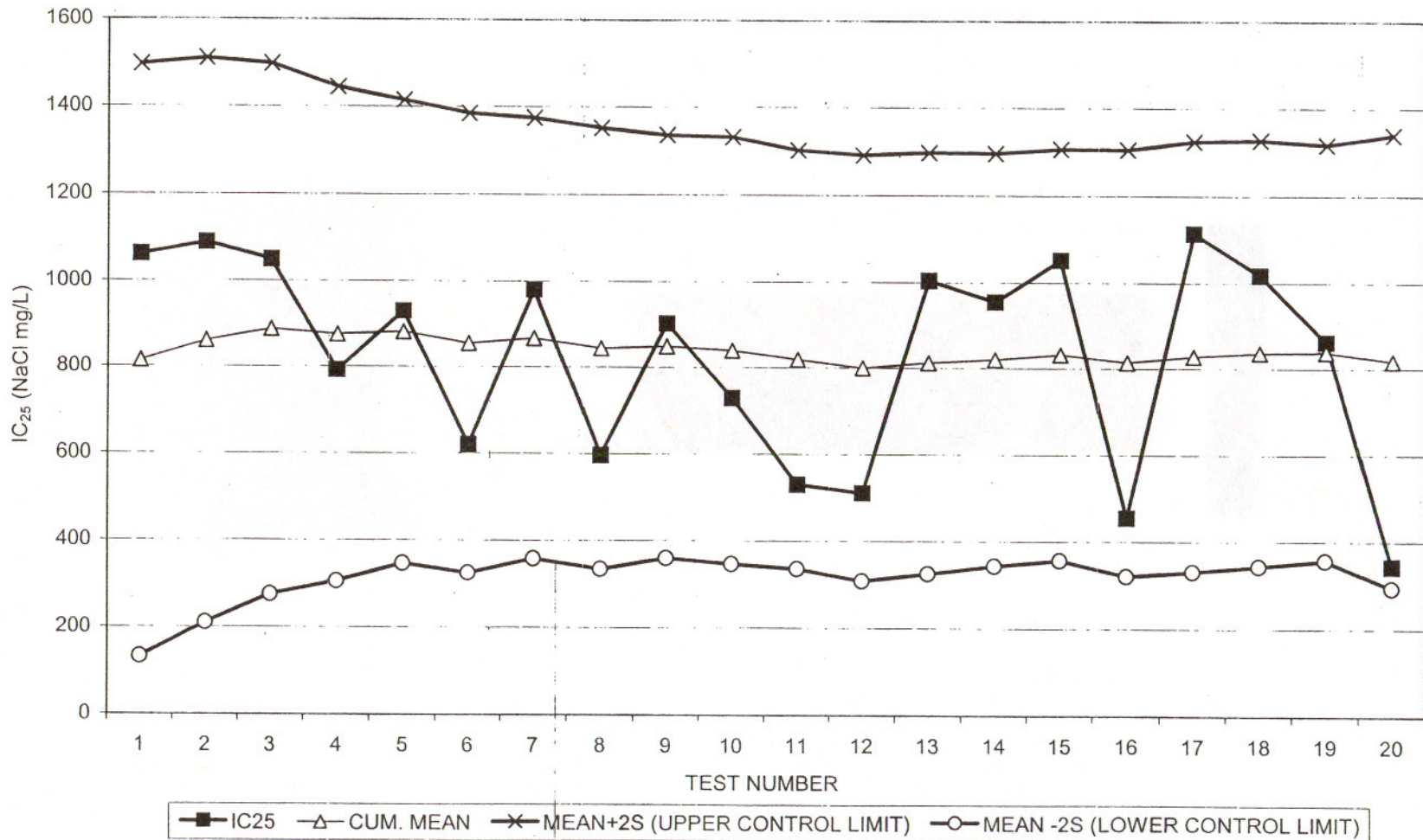
FRI 0 DM	SAT 24 DM	SUN 48 SR	MON 72 DM	TUE 96 DM	WED 120 DM	THUR 44 DM
2:32 PM 24.5°C 63.7	2:14 PM 24.5°C 60.3	11:40 AM 23.5°C 68.7	11:05 AM 24.5°C 62.2	9:18 AM 24.5°C 66.4	10:50 AM 24.5°C 63.8	11:00 AM 24.5°C 61.6
FRI 168	SAT 192	SUN 216 DM	MON 240 DM	TUE 264 DM	WED 288 DM	THUR 312
8:50 AM 25°C 63.6	9:00 AM 25°C 68.5	1:48 PM 24.7°C 66.4	11:00 AM 24.5°C 62.8	2:35 PM 24.5°C 64.3	9:18 AM 24.5°C 60.7	
336	360	384	408	432	456	480
/ / / / / / / /						
504	528	552				
/ / / / / / / /						

ALTERNATE FOOD

DAILY COMMENTS Marked cups for neonates 7:20 am 7/22/09
Fed 7:30 AM

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

CONTROL CHART FOR CHRONIC REFERENCE TOXICITY TEST
 CERIODAPHNIA DUBIA (REPRODUCTION) - IC₂₅
 REFERENCE TOXICANT (NaCl)



Note: Test number 20 was conducted concurrently with the chronic test for Hanover Park WRP. Test numbers 1-10, 12, 14, 17-18 and 20 were conducted with hard synthetic water (HSW) with selenium. Test numbers 11, 13-14 and 17 were conducted with moderately hard synthetic water (MHSW) with selenium.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

REFERENCE TOXICANT TEST RESULTS
 REFERENCE TOXICANT- SODIUM CHLORIDE
 TEST ORGANISMS: *Ceriodaphnia dubia*

Reference Toxicant Test Number	Setup Date	IC ₂₅ mg/L	Cumulative Mean mg/L	MEAN+2S (mg/L) (UPPER CONTROL LIMIT)	MEAN - 2S (mg/L) (LOWER CONTROL LIMIT)
1	04/07/04	1062	814	1497	132
2	04/21/04	1089	860	1510	210
3	05/05/04	1049	887	1498	276
4	05/19/04	792	875	1445	306
5	07/07/04	930	881	1415	347
6	07/21/04	620	855	1385	325
7	08/18/04	981	867	1375	358
8	09/15/04	597	844	1353	335
9	11/03/04	902	849	1337	360
10	04/13/05	729	840	1333	347
11	06/08/05	531	819	1303	336
12	05/17/06	512	800	1292	309
13	07/19/06	1004	812	1298	326
14	05/16/07	955	820	1297	344
15	06/09/07	1052	832	1307	357
16	06/17/08	456	814	1306	321
17	09/10/08	1114	828	1325	331
18	03/13/09	1018	836	1328	344
19	06/03/09	862	838	1318	357
20	07/21/09	344	817	1340	294

DII-16