

Effluent Quality Assessment

City of Clearwater East AWWTF

NPDES #FL0021865

City of Clearwater Northeast AWWTF

NPDES #FL0128937

Pinellas County

Sampled June 20, 22, 24, and July 25, 27, and 29, 2011

October 2011

Biology Section
Bureau of Laboratories
Division of Environmental Assessment and Restoration

Quality Manual No. 870346G
NELAC Certification No. E31780

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City of Clearwater East Advanced Wastewater Treatment Facility (AWWTF), 3141 Gulf to Bay Boulevard, Clearwater, Pinellas County, Florida, 34619. NPDES #FL0021865

City of Clearwater Northeast AWWTF, 3290 State Road 580, Clearwater, Pinellas County, Florida, 34695. NPDES #FL0128937

Effluent grab and composite samples for chemical analyses for this facility were collected on June 20, 2011. Effluent grab samples for biological analysis for this facility were collected on June 20, 22, and 24; and July 25, 27, and 29, 2011.

Introduction

City of Clearwater East AWWTF and City of Clearwater Northeast AWWTF are Type 1 advanced wastewater treatment facilities which use the Bardenpho BNR Process to treat domestic wastewater. Both facilities share a common outfall, D-001, which is located at the Clearwater East AWWTF facility. (See Appendix F for detailed facility information provided by Jason Cohen of FDEP Southwest District.)

Methods

Samples were collected from the EFD-01 NPDES sampling point for Outfall D-001 by Jason Cohen, Josephine Molé, Peter Wenner, and Michele Duggan (FDEP Southwest District) following DEP-SOP-001/01 FS 2400 Wastewater Sampling. All DEP SOPs are available on the web at: http://www.floridadep.org/labs/bars/sas/sop/index.htm.

See Appendix A for the completed chain of custody form.

The 7- day chronic definitive toxicity tests and algal growth potential test were performed following internal DEP SOPs TA07_05, TA07_07, and TA08_01 through TA08_09. All internal DEP SOPs are available on the web at: http://www.floridadep.org/labs/cgi-bin/sop/biosop.asp.

Bioassay samples 1358734, 1359816, 1360234, 1367426, and 1367740 were aerated for 30 minutes at a rate of approximately 100 bubbles per minute to decrease the dissolved oxygen (DO) from 11.6 mg/L, 9.9 mg/L, 10.6 mg/L, 10.1 mg/L, and 9.3 mg/L to 8.3 mg/L, 7.8 mg/L, 7.9 mg/L, 8.2 mg/L, and 8.3 mg/L, respectively.

An aliquot of the AGP sample was salinity adjusted by the addition of artificial sea salts to meet the salinity requirements of the test species.

Due to the *Ceriodaphnia dubia* bioassay being reported as No Result for the June (20th, 22nd and 24th) sampling event, the test was repeated with bioassay samples collected during the July (25th, 27th and 29th) sampling event.

Results and Discussion

- **Toxicity Test Results**
  Tests performed June 21 - 28, 2011.

Ceriodaphnia dubia 7-day chronic definitive bioassay –	NOEC = No Result IC25 = No Result
Pimephales promelas 7-day chronic definitive bioassay –	NOEC = 100% IC25 = > 100%

The bioassay samples were not chronically toxic to the *Pimephales promelas* test species. The *Ceriodaphnia dubia* chronic definitive test was repeated on July 26, 2011, due to an interrupted dose response at the 6.25% and 12.5% concentrations. See NCR 3815 for additional details.

Test performed July 26- August 2, 2011

Ceriodaphnia dubia 7-day chronic definitive bioassay – NOEC = 100%
IC25 = > 100%

The bioassay samples were not chronically toxic to the *Ceriodaphnia dubia* test species.

See Appendix B for bioassay bench sheets. See Appendix C for results of the last 20 standard reference toxicant (SRT) tests and the bench sheets and statistical analyses of the SRT tests that correspond to these facility tests. See Appendix D for test organism receipts.

- **Algal Growth Potential**
  The effluent Algal Growth Potential (AGP) was 5.7 mg dry wt/L of the unicellular green alga, *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*. Raschke and Schultz (1987) determined that AGP values above 5.0 mg dry weight/L represent a "problem" threshold for fresh receiving waters, implying nutrient enrichment. The analytical chemistry suggests that the effluent is nitrogen-limited. To determine actual nutrient limitation, a limiting-nutrient algal growth potential test with associated nutrient chemistry should be performed (Miller et al. 1978).

  The salinity adjusted effluent Algal Growth Potential (AGP) was 15.5 mg dry wt/L of the saltwater species, *Dunaliella tertiolecta.* Raschke and Schultz determined that AGP values above 10.0 mg dry weight/L represent a "problem" threshold for marine receiving waters, implying nutrient enrichment (personal communication, Marshall Faircloth, et. al. FDEP, with Ron Raschke USEPA Region 4 1987).

  See Table 1 for AGP results and qualifiers.

- **Chemistry Results**
  Total residual chlorine was detected (0.04 mg/L) in the bioassay sample (LIMS ID 1360234) collected on June 24, 2011, but was not detected in the other five bioassay samples. Total ammonia was not detected in any of the six bioassay samples in the laboratory. The total ammonia concentration in the composite sample collected and preserved for chemical analysis was 0.036 mg N/L. Based on the pH, salinity, and temperature of the effluent as collected, the calculated unionized ammonia concentration was < 0.01 mg/L.

  Bromodichloromethane, aluminum, copper, and zinc were detected in the effluent at levels that comply with Class II marine water quality criteria (62-302.530, F.A.C.) and /or permitted limits (NPDES# FL0021865). Arsenic and nickel were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL). The facility has a mixing zone for bromdichloromethane (limit ≤ 43 µg/L).

  Total suspended solids (TSS) and carbonaceous biochemical oxygen demand (CBOD) were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL) and complied with permit limits.

  See Table 2 for results of analytes detected in the effluent, corresponding limits, and qualifiers. See Appendix E for a complete list of chemical analyses performed. See Appendix B for bioassay lab equipment.

Conclusion

The effluent samples collected from the EFD-01 NPDES sampling point for Outfall D-001 for this facility on June 20, 22, and 24, 2011; were not chronically toxic to the *Pimephales promelas* test organisms. The *Ceriodaphnia dubia* test was reported as "No Result" due to an unexplained interrupted dose response. The *Ceriodaphnia dubia* bioassay, repeated with samples collected on July 25, 27 and 29, 2011, did not show chronic toxicity. The effluent AGP results were greater than the "problem" threshold for fresh and

marine receiving waters, suggesting that nutrient enrichment may be an issue. Effluent water quality samples collected on June 20, 2011, did not exceed any applicable water quality standards or violate any permit conditions.

Literature Cited

Miller, W. E., T. E. Maloney, and J. C. Greene. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA-600/9-78-018. U. S. EPA, Cincinnati, Ohio. 126 p.

Raschke, R. L. and D. A. Schultz. 1987. The use of the algal growth potential test for data assessment. J. Wat. Poll. Cont. Fed. 59(4): 222-227.

Table 1a. Measured and predicted algal growth potential (AGP; mg dry weight/L) for total soluble inorganic nitrogen (TSIN) and total nitrogen (TN) limitation of the freshwater species *Pseudokirchneriella subcapitata* and ratios of nitrogen to phosphorus for samples collected from the City of Clearwater East and Northeast AWWTFs common Outfall D-001 on June 20, 2011.

City of Clearwater East AWWTF NPDES# FL0021865 City of Clearwater Northeast AWWTF NPDES# FL0128937				
Location	**AGP (measured)**	**Predicted AGP (TSIN) ± 20%**	**Predicted AGP (TN) ± 20%**	**Total N:P ratio**
Effluent	5.7	16.6 ± 3.3	44.8 ± 9.0	1.6

Table 1b. Measured algal growth potential (AGP; mg dry weight/L) of the saltwater species *Dunaliella tertiolecta* and ratios of nitrogen to phosphorus for samples collected from the City of Clearwater East and Northeast AWWTFs common Outfall D-001 on June 20, 2011.

City of Clearwater East AWWTF NPDES # FL0021865 City of Clearwater Northeast AWWTF NPDES# FL0128937			
Location	**AGP (measured)**	**Total N:P ratio**	**Inorganic N:P ratio**
Effluent*	15.5	1.6	N/A

* - sample salinity adjusted to 7ppt

Table 2. Effluent limits, Class II Criteria for predominantly marine waters, chemical and microbiological data, and qualifiers for the City of Clearwater East and Northeast AWWTFs common Outfall D-001 on June 20, 2011.

City of Clearwater East AWWTF NPDES# FL0021865 City of Clearwater Northeast AWWTF NPDES# FL0128937	Class II Criteria	Effluent Limits	Effluent	
Organic Constituents (µg/L)				
Bromodichloromethane	≤ 22.0 a	≤ 43.0 a	39	
Metals (µg/L unless otherwise noted)				
Aluminum	≤ 1500	-	66	A
Arsenic	≤ 50	-	2.2	I
Cadmium	≤ 8.8	-	0.15	U
Chromium-III	-	-	0.9	U
Copper	≤ 3.7	≤ 3.7 s	3.2	A
Iron	≤ 300	-	30	U
Lead	≤ 8.5	-	0.6	U
Magnesium (mg/L)	-	-	10.8	A
Nickel	≤ 8.3	-	1.1	I
Selenium	≤ 71	-	0.6	U
Silver	≤ 2.3	-	0.075	U
Zinc	≤ 86	-	34	A
Nutrients (mg/L)				
Total Phosphorus	-	-	0.74	
Total Ammonia	-	-	0.036	
Un-ionized Ammonia	-	-	< 0.01	c
Nitrate and Nitrite	-	-	0.4	
Total Kjeldahl Nitrogen	-	-	0.78	
Organic Nitrogen	-	-	0.744	c
Total Nitrogen	-	6.0 p	1.18	c
General Physical and Chemical Parameters				
Dissolved Oxygen (mg/L)	≥ 4.0	≥ 4.0 s	8.6	
pH (SU)	6.5 - 8.5	6.5 - 8.5 s	7.8	
Conductivity (µmhos/cm)	-	-	1199	
Temperature (°C)	-	-	29.0	
Salinity	-	-	< 0.55	c
Total Residual Chlorine (mg/L)	≤ 0.01	≤ 0.01 s	0.03	UQ£
Chlorophyll *a* (µg/L) - Corrected	-	-	2.7	
Phaeophytin (µg/L)	-	-	0.4	U
Total Suspended Solids (mg/L)	-	10.0 p	3	I
CBOD, 5 day (mg/L), N - inhibited	-	10.0 p	0.91	I
Chloride (mg/L)	-	-	190	
Fluoride (mg/L)	≤ 5.0	-	0.62	
Sulfate (mg/L)	-	-	86	
Flow (MGD) (East)	-	5.0 a	2.23	a*
Flow (MGD) (Northeast)	-	13.0 a	2.23	a*
Hardness (mg $CaCO_3$)	-	-	261.71	c
Microbiology (Number of colony forming units/100ml)				
Fecal Coliforms ¥	≤ 400s #	-	1	U

Value exceeds the Class III Water Quality Criteria or permit limits

a - Annual average
c - Value is calculated
p - 24 hour flow proportioned composite
s - Single sample
A - Value reported is the mean of two or more determinations
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit
Q - Sample held beyond normal holding time
U - Material analyzed for but not detected; value reported is the method detection limit
¥ - Fecal coliform analysis performed by Diversified Environmental Laboratories
- If 10 or fewer samples are taken within a month, the single sample limit is 400 CFU (62-302.530(6), F.A.C.)
£ - measurement taken in the FDEP laboratory
* - Reported from 6-30-2010 to 5-31-2011

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-06-20-42
FYI5 Sampling Inspection for Clearwater East WWTF and

Requester: Edward Smith
Field Report Prepared By:

Customer: SW-DIST
Project ID: FYI5
PMAS: 1143

Collected By:
Send Final Report To: Joe Squiteri/ Michele Duggan/
Sampling Agency:

Lab ID *	Location		Collection (begin)	Collection (end)	Bottle Group(s)**
38703	D-001		☑ Comp ☐ Grab Date 6/19/11 Time 0530 Eastern Central	Date 6/20/11 Time 0530 Eastern Central	A
	Field ID		Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C) / pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Lab ID *	Location		Collection (begin)	Collection (end)	Bottle Group(s)**
38706	D-001		☐ Comp ☑ Grab Date 6/20/11 Time 0955 Eastern Central	Date Time Eastern Central	A
	Field ID		Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L) 8.63	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C) 29.64 / pH 7.78	Sample Depth ☐ m ☐ ft 0.59	☑ Salinity (PPTh) ☑ Sp Conductance (umho/cm) 1199	NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Lab ID *	Location		Collection (begin)	Collection (end)	Bottle Group(s)**
38707	BLANK		☐ Comp ☑ Grab Date 6/20/11 Time 1010 Eastern Central	Date Time Eastern Central	A
	Field ID		Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C) / pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Lab ID *	Location		Collection (begin)	Collection (end)	Bottle Group(s)**
38708	EFB-01		☐ Comp ☑ Grab Date 6/20/11 Time 0935 Eastern Central	Date Time Eastern Central	E
	Field ID		Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C) / pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments 2 Dioxsay Receive Tor by JD 9:58 6.21.11		

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
	6/20/11 1400	FED EX						6-21-11 9:20

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-06-20-42
FYI5 Sampling Inspection for Clearwater East WWTF and Northeast

Requester: Edward Smith
Field Report Prepared By:

Customer: SW-DIST
Project ID: FYI5
PMAS: 1143

Collected By: J Colen
Send Final Report To: Joe Squiteri/ Michele Duggan/

Sampling Agency: FDEP SWD

38704

Lab ID *	Location D-001			☑ Comp ☐ Grab	Collection (begin) Date 6/21/11 Time 0530 Eastern Central		Collection (end) Date 6/22/11 Time 0530 Eastern Central		Bottle Group(s)** A
	Field ID			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)		Storet Station Number		
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft		☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)		NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude	☐ dd ☐ dms	Comments					
Lab ID *	Location			☐ Comp ☐ Grab	Collection (begin) Date Time Eastern Central		Collection (end) Date Time Eastern Central		Bottle Group(s)**
	Field ID			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)		Storet Station Number		
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft		☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)		NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude	☐ dd ☐ dms	Comments					
Lab ID *	Location			☐ Comp ☐ Grab	Collection (begin) Date Time Eastern Central		Collection (end) Date Time Eastern Central		Bottle Group(s)**
	Field ID			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)		Storet Station Number		
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft		☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)		NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude	☐ dd ☐ dms	Comments					
Lab ID *	Location			☐ Comp ☐ Grab	Collection (begin) Date Time Eastern Central		Collection (end) Date Time Eastern Central		Bottle Group(s)**
	Field ID			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)		Storet Station Number		
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft		☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)		NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude	☐ dd ☐ dms	Comments 2 Biscayne Reid in for by JD 10:04 6.23.11					

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
	6/22/11 1200	FED Ex	ABB	6/23/11 09:20				

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** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-06-20-42
FYI5 Sampling Inspection for Clearwater East WWTF and

Requester: Edward Smith
Field Report Prepared By:

Customer: SW-DIST
Project ID: FYI5
PMAS: 1143

Collected By: M DUGGAN
Sampling Agency: FDEP SWD
Send Final Report To: Joe Squiteri/ Michele Duggan/

38705

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	D-001	☑ Comp ☐ Grab	Date 6/23/11 Time 0530 Eastern Central	Date 6/24/11 Time 0530 Eastern Central	14
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
		—	—	—	
	Matrix (include type e.g. Salt, Fresh, etc) Temp (C) pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Effluent — —				
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
		☐ Comp ☐ Grab			
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (include type e.g. Salt, Fresh, etc) Temp (C) pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
		☐ Comp ☐ Grab			
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (include type e.g. Salt, Fresh, etc) Temp (C) pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
		☐ Comp ☐ Grab			
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (include type e.g. Salt, Fresh, etc) Temp (C) pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments		

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
M Duggan	06/24/11 0930	Fed Ex						

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** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Recieved 6 25 11 9:45 temp: 1.3

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-06-20-42
FYI5 Sampling Inspection for Clearwater East WWTF and [illegible]

Requester: Edward Smith
Field Report Prepared By:

Customer: SW-DIST
Project ID: FYI5
PMAS: 1143

Collected By: J Cohen, P Wenner
Send Final Report To: Joe Squiteri/ Michele Duggan/

Sampling Agency: FDEP SWD

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
	D-001	☑ Comp ☐ Grab Date 7/24/11 Time 500 Eastern Central	Date 7/25/11 Time 500 Eastern Central	A
	Field ID: 38904	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc): FRESH	Temp (C)	pH	Sample Depth ☐ m ☐ ft; ☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm); NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments	

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
		☐ Comp ☐ Grab Date Time Eastern Central	Date Time Eastern Central	
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft; ☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm); NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments	

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
		☐ Comp ☐ Grab Date Time Eastern Central	Date Time Eastern Central	
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft; ☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm); NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments	

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
		☐ Comp ☐ Grab Date Time Eastern Central	Date Time Eastern Central	
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft; ☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm); NPDES Number
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments: 2 bioassay Rec'd in for by JD/KC 7.26.11 9:59	

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
	7/25/11 1300	FEDEX	[illegible]	7/26/11 09:25				

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** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-06-20-42
FYI5 Sampling Inspection for Clearwater East WWTF and

Requester: Edward Smith
Field Report Prepared By:

Customer: SW-DIST
Project ID: FYI5
PMAS: 1143

Collected By: PETE WENNER
Sampling Agency: FDEP/SWD
Send Final Report To: Joe Squiteri/ Michele Duggan/

Lab ID *	Location / Field ID	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Latitude	Longitude	Collection (begin)	Collection (end)	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	Sample Depth	Salinity (PPTh) / Sp Conductance (umho/cm)	NPDES Number	Bottle Group(s)**	Comments
38905	D-001	FRESH			☐ dd ☐ dms	☐ dd ☐ dms	☑ Comp ☐ Grab Date 7/26/11 Time 0530 Eastern Central	Date 7/27/11 Time 0530 Eastern Central				☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)		A	2-1L AMBER BOTTLES COLLECTED FOR RERUN OF CD TEST
					☐ dd ☐ dms	☐ dd ☐ dms	☐ Comp ☐ Grab Date Time Eastern Central	Date Time Eastern Central				☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)			
					☐ dd ☐ dms	☐ dd ☐ dms	☐ Comp ☐ Grab Date Time Eastern Central	Date Time Eastern Central				☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)			
					☐ dd ☐ dms	☐ dd ☐ dms	☐ Comp ☐ Grab Date Time Eastern Central	Date Time Eastern Central				☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)			2 bionomy Rec'd in for by J.D. 10:28 7.28.11

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
	7/27/11:1700	FEDEX						7.28.11/9:25

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-06-20-42
FYI5 Sampling Inspection for Clearwater East WWTF and

Customer: SW-DIST
Project ID: FYI5
PMAS: 1143

Requester: Edward Smith
Collected By: PETE WENNER
Sampling Agency: FDEP-SWD

Field Report Prepared By:
Send Final Report To: Joe Squiteri/ Michele Duggan/

38906

*Lab ID *	Location / Field ID	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Latitude	Longitude	Comp / Grab	Collection (begin) Eastern Central	Collection (end) Eastern Central	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	Sample Depth m / ft	Salinity (PPTh) / Sp Conductance (umho/cm)	NPDES Number	Bottle Group(s)**	Comments
	D001	FRESH (EFFLUENT)			dd / dms	dd / dms	☑ Comp ☐ Grab	Date 7/28/11 Time 0530	Date 7/28/11 Time 0530							A	FINAL TOX SAMPLE FOR FAILED CTD TEST
					dd / dms	dd / dms	☐ Comp ☐ Grab	Date Time	Date Time								
					dd / dms	dd / dms	☐ Comp ☐ Grab	Date Time	Date Time								
					dd / dms	dd / dms	☐ Comp ☐ Grab	Date Time	Date Time								

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
	7/29/11; 1700	FEDEX	M. Fairclath	7/30/11 0905				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

1 Blue Cooler - Temp 0.7°C M. Fairclath 07/30/11

Appendix B. Bench Sheets

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia

Facility: Clearwater east AWWTF and Northeast AWWTF (Combined)
SOP#: TA07_05 EPA 821-R-02-013 Method 1002.0
Address: 3141 Gulf to Bay Blvd, Clearwater, FL, (EAST)
County: Pinellas FL0128937 BA
NPDES Permit #: FL0021865 & ~~FL0031607~~
Contact/District: Pete Wenner/Southwest
Add 1855 Northeast-3290 State Road 580 Clearwater, FL MF
LIMS entry: 7-13-11MF verified: 7-13-11KC

Diluent/ Batch: 20% DMW / #6.15.11 Test Days Used: 0-1
20% DMW / 6.17.11 Test Days Used: 2-7

Organism: *Ceriodaphnia dubia*
Organism Batch #: 6.21.11
Organism age : <24 hours
Feeding: Prior to Test and Daily
Food: ☑ YCT ☑ *P. subcapitata*
Food Batch: 5.5.11 4.18.11
Chamber size : 30 ml
Sample volume : 20 ml

Sample Received	LAB Sample #	LIMS JOB #	LIMS SAMPLE #	Collection Date/Time	Received Date/Time
Test Day 0	1	TLH-2011-06-21-11	1358734	06.20.11/1730	06.21.11/0920
Test Day 2	2	TLH-2011-06-23-100	1359816	06.22.11/0530A	06.23.11/0920
Test Day 4	3	TLH-2011-06-27-18	1360234	06.24.11/1730	06.25.11/0945

Batch #	6.15.11	6.17.11	NA	NA	NA			
Water Quality Parameters	20% DMW	20% DMW	Sample 1	Sample 2	Sample 3	Other:	Measured By:	Verified by:
Field Total Residual Cl_2 (mg/L):	NA	NA	not measured	—	—		n/a	KC
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03	<0.03	<0.03	0.04		KC/JD	KC
Alkalinity (mg/L as $CaCO_3$):	85	86	208	212	200		KC/BA	KC
Hardness (mg/L as $CaCO_3$):	82	82	268	266	268		KC/BA	KC
Total Ammonia (mg/L as N):	<0.017	<0.017	<0.017	<0.017	<0.017		BA	KC
Conductivity: ~~µmhos~~ mmhos:	181	164	1167	1122	1215		BA/JD	KC
Salinity (ppth):	–	–	–	–	–		n/a	KC

Test Incubated in: Incubator #: 2

Temperature Shipped ≤ 6° C (all samples) (yes) no
Holding Time ≤ 36 hours (yes) no
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 25.6 max. 27.5 mean —
Room B246 min. ____ max. ____ mean ____
Test min. 22.5 max. 24.5 mean —

Loaded by: KC

Test Replicates	Loaded from culture vial
1	B26
2	B6
3	B3
4	A25
5	A29
6	A24
7	A17
8	A9
9	C26
10	C21

Results	
Control % CV:	42.7
Dunnetts PMSD:	49.6
Reproduction NOEC:	25%
Reproduction LOEC:	MF 6.25%
Survival NOEC:	100 %
Survival LOEC:	>100%
ICP_{20}:	55%
ICP_{25}:	>100 %

Investigators' signatures
Kara Cox
Bruce Abel
James Dangl
Marshall Faircloth
REVIEWER

Comments: (A) 1730 - KC -

MF 7-13-11 Result reported as no result due to PMSD exceeding upper bound and interrupted dose response at 6.25% and 12.5% sample. Calculated IC 25 was >100%. NCR 3815 created 7-13-11 MF

Test Page 1 of 6 PAGE 000055 revised 07/15/08 MF

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

SOP TA07_05

Facility: Clearwater East + Northeast

LIMS Sample #: 1358734 for lab sample #1

Start Date: 6.21.11 Time: 1415

End Date: 6.28.11 Time: 1230

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving adults	Neonates per adult
Control	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	0	0	0	0	0	0	0	0	0	10	0
	4	5	4	4	3	5	5	4	0	4	6	40	10	4.0
	5	0	0	5	4	5	8	6	2	6	8	44 46 BA	10	4.4 46 BA
	6	0	12	0	15	10	9	10	15	5	10	58 86	10	5.8 86 BA
	7	6	0	6	0	*	*	*	0	*	*	12	10	1.2
	8													
	Total	11	16	15	8	20	22	20	3	15	24	154	10	15.4

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving adults	Neonates per adult
6.25%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	4	0	0	0	0	0	0	3	0	7	10	0.7
	4	0	3	4	4	1	5	3	0	0	0	17 19 BA	10	1.7 1.9 BA
	5	0	0	15	0	0	15	0	1	7	0	10 8 BA	10	1.0 0.8 BA
	6	3	2	8	10	2	0	5	0	3	10	33	10	3.3 MF
	7	0	0	0	0	0	0	0	0	4*	12	13 MF	10	1.3
	8									13 MF		MF		MF
	Total	3	6	13	14	3	6	8	1	17	13	80	10	8.0

Organisms loaded by: KC		
Day	Counted/ verified by:	Concentrations prepared by:
0	KC KC	BA
1	KC	KC
2	KC	KC
3	BA	BA
4	BA	SD
5	KC	KC
6	KC	KC
7	KC	
8		

Concentration	Mean neonates /adult	% survival
Control	15.4	100%
6.25%	8.0	100%
12.50%	6.6	100%
25%	13.7	100%
50%	14.2	90%
100%	17.0	100%

Test acceptability	
Mean control reproduction 15/female	(yes) no
3rd brood in 60% of surviving females	(yes) no
Control survival ≥ 80%:	(yes) no

Comments: * Already had 3 broods. 4th brood doesn't count. -KC-

(5) Indicates split brood
Arrow indicates day split counted on 6.26.11

*Day 0 is test initiation day
X = Dead (original organism)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

SOP TA07_05

Facility: Clearwater East & Northeast

LIMS Sample # : 1358734 for lab sample #1

Start Date: 6.21.11 Time: 1415

End Date: 6.28.11 Time: 1230

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
12.50%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	0	0	0	0	0	0	0	0	0	10	0
	4	1	1	5	4	3	2	4	0	0	0	20	10	2.0
	5	0	0	16	16	16	0	4	0	0	1	86	10	8.6
	6	1	0	5	0	5	8	13	3	0	2	24	10	2.4
	7	0	0	0	0	0	13	0	0	0	0	13	10	1.3
	8													
	Total	2	1	11	5	9	23	9	3	0	3	56	10	6.6

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
25% 0.6	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	0	0	0	0	0	0	1	0	1	10	0.1
	4	0	0	3	3	0	0	0	0	0	0	6	10	0.6
	5	0	21	0	4	0	6	0	3	2	8	24	10	2.4
	6	2	9	9	8	9	12	40	94	9	0	63	10	6.3
	7	0	16	0	0	0	0	15	0	0	13	44	10	4.4
	8													
	Total	2	26	12	15	9	18	15	7	12	21	137	10	13.7

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
50%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	X	0	0	0	0	0	0	0	0	0	9(10)	0
	4	0		3	0	0	0	1	0	0	0	4	9(10)	0.4
	5	8		9	16	0	10	7	10	5	7	32	9(10)	3.2
	6	10		7	9	5	13	8	4	10	10	55	9(10)	5.5
	7	6		0	0	0	0	0	15	12	12	45	9(10)	4.5
	8													
	Total	15	0	19	10	5	14	16	29	23	20	142	9(10)	14.2

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
100%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	0	0	0	0	0	0	0	3	5	8	10	0.8
	4	0	1	5	0	0	5	3	0	0	0	14	10	1.4
	5	10	9	2	1	1	0	2	0	9	0	34	10	3.4
	6	12	10	8	11	2	12	9	0	12	13	90	10	9.0
	7	12	0	0	0	0	0	0	0	8	13	25	10	2.5
	8													
	Total	34	20	15	12	3	17	14	0	24	31	170	10	17.0

Comments: (A) Total Neonates, 39 Neonates/10 adults, 3.9 BA 6.30.11

F = Female

*Day 0 is test initiation day

X = Dead (original organism)

Appendix B. (continued)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Parameters

Facility: Clearwater East + Northeast SOP TA07_05

LIMS Sample #: 1358734 for lab sample 1

Circle µmhos-cm or mmhos-cm

Test Day ↓		Concentration	New Sample					Old Sample				
			Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 0	sample	Control	M	7.9	24.3	7.5	181					
	1	6.25%	M	7.9	25.8	8.0	253					
	1	12.5%	M	8.0	25.8	8.1	316					
	1	25%	M	8.2	25.6	8.0	439					
	1	50%	M	8.3	25.5	7.8	687					
	1	100%	M	8.4	25.2	7.7	1167					
	Measured by:			BA	BA	BA	BA					
	Recorded by:			BA	BA	BA	BA					
Day 1	sample:	Control	M	8.0	24.0	8.2	169	M	8.0	24.0	7.7	188
	1	6.25%	M	8.1	24.0	8.0	231	M	8.1	24.0	7.7	260
	1	12.5%	M	8.3	24.3	8.0	294	M	8.1	24.0	7.8	325
	1	25%	M	8.4	24.7	8.0	416	M	8.2	24.0	7.7	463
	1	50%	M	8.5	25.4	8.0	654	M	8.3	24.0	7.7	718
	1	100%	M	8.6	26.4	8.0	1124	M	8.5	24.0	7.5	1184
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
(A) Day 2 BA	sample:	Control	M	8.1	~~24.0~~	7.9	~~175~~	M	8.0	24.0	7.5	190
	2	6.25%	M	8.2	~~25.1~~	7.8	~~348~~	M	8.2	24.0	7.8	247
	2	12.5%	M	8.2	~~25.4~~	7.8	~~521~~	M	8.2	24.0	7.8	328
	2	25%	M	8.2	~~25.2~~	7.9	~~837~~	M	8.3	24.0	7.9	438
	2	50%	M	8.3	~~24.9~~	7.8	+	M	8.3	24.0	7.8	679
	2	100%	M	8.4	~~25.1~~	7.8		M	8.5	24.0	7.8	1152
	Measured by:			~~KC BA~~	~~BA~~	~~KC BA~~	~~BA~~		KC	KC	KC	KC
	Recorded by:			~~KC BA~~	~~BA~~	~~KC BA~~	~~BA~~		KC	KC	KC	KC
Day 3	sample:	Control	M	7.9	24.3	7.1	173	M	7.8	24.0	7.5	182
	2	6.25%	M	8.0	24.9	7.4	246	M	8.0	24.0	7.5	248
	2	12.5%	M	8.2	24.9	7.5	312	M	8.1	24.0	7.5	321
	2	25%	M	8.3	24.6	7.5	436	M	8.2	24.0	7.5	453
	2	50%	M	8.5	24.4	7.5	666	M	8.3	24.0	7.5	696
	2	100%	M	8.6	24.3	7.5	1137	M	8.4	24.0	7.4	1221
	Measured by:			BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:			BA	BA	BA	BA		BA	BA	BA	BA

Comments: (A) Day "2" parameters written in wrong spot BA 2.23.11

Day "2" parameters: Temp: Control 25.1 6.25% 24.8 12.5% 24.5 25% 24.0 50% 24.0 100% 25.3

conductivity: ctrl 164 6.25% 228 12.5% 291 25% 414 50% 652 100% 1122

Initials KC

Replicate M= "Metered" vial, a surrogate vial for parameters

Appendix B. (continued)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Parameters

Facility: Clearwater East & Northeast SOP TA07_05

LIMS Sample #: 1358734 for lab sample 1

Circle µmhos-cm or mmhos-cm

Test Day ↓		Concentration	New Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Old Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 4	sample:	Control	M	7.8	24.8	8.4	184	M	8.1	24.0	7.2	185
	3	6.25%	M	7.8	25.4	8.5	259	M	8.1	24.0	7.3	273
	3	12.5%	M	7.9	25.4	8.4	328	M	8.0	24.0	7.2	346
	3	25%	M	8.0	25.2	8.5	457	M	8.1	24.0	7.2	478
	3	50%	M	8.1	25.1	8.5	680	M	8.2	24.0	7.3	742
	3	100%	M	8.1	25.6	8.7	1215	M	8.4	24.0	7.3	1248
	Measured by:			SD	SD	SD	SD		BA	BA	BA	BA
	Recorded by:			BA	BA	BA	BA		BA	BA	BA	BA
Day 5	sample:	Control	M	8.1	24.9	8.6	168	M	7.9	24.0	7.9	190
	3	6.25%	M	8.2	24.9	8.5	234	M	7.9	24.0	8.0	270
	3	12.5%	M	8.2	25.1	8.4	299	M	8.1	24.0	8.0	349
	3	25%	M	8.3	25.0	8.6	417	M	8.2	24.0	8.2	458
	3	50%	M	8.4	25.3	8.5	648	M	8.3	24.0	8.2	691
	3	100%	M	8.4	25.8	8.6	1110	M	8.5	24.0	8.3	1218
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
Day 6	sample:	Control	M	7.9	25.3	7.5	179	M	7.9	24.0	8.4	180
	3	6.25%	M	8.0	25.9	8.1	244	M	7.9	24.0	8.5	253
	3	12.5%	M	8.1	25.8	8.4	308	M	7.9	24.0	8.2	315
	3	25%	M	8.2	25.7	8.5	431	M	8.1	24.0	8.2	438
	3	50%	M	8.5	25.6	8.6	674	M	8.2	24.0	8.1	682
	3	100%	M	8.6	25.8	8.6	1162	M	8.4	24.0	8.3	1117
	Measured by:			BA	BA	BA	BA		KC	KC	KC	KC
	Recorded by:			BA	BA	BA	BA		KC	KC	KC	KC
Day 7	sample:	Control	M					M	8.1	24.0	7.0	191
	3	6.25%	M					M	8.3	24.0	7.4	263
	3	12.5%	M					M	8.3	24.0	7.7	318
	3	25%	M					M	8.3	24.0	7.5	438
	3	50%	M					M	8.4	24.0	7.3	672
	3	100%	M					M	8.5	24.0	7.3	1170
	Measured by:								KC	KC	KC	KC
	Recorded by:								KC	KC	KC	KC

Comments:

Replicate M= "Metered" vial, a surrogate vial for parameters

Test Page 5 of 6

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revised 07/15/08 MF

Appendix B. (continued)

Test ID: Clearwater East + Northeast Dates: 12.21.11 - 12.28.11

METER / DEVICE	ID	SOP	Parameter	Method	√ used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 #5 SN61990624	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓ #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

Entered By: KC by MF 1/3/11

V 1.1 9/21/10 MF/jd

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Appendix B. (continued)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia

Facility: Clearwater (East + Northeast Annex)
Address: EAST - 3141 Gulf to Bay Blvd, Clearwater, FL. 34619
County: Pinellas Northeast - 3290 SR 580 clearwater, fl 34695
NPDES Permit #: FL DO21865(EAST) FL0128937 (northeast)
Contact/District: Peter Wenner / Southwest

LIMS entry: 8/19/11 MF verified: 8/19/11 BA by MF

Diluent/ Batch: 20% DMW 7.21.11 Test Days Used: 0-3
20% DMW 7.28.11 Test Days Used: 4-6

SOP#: TA07_05 EPA 821-R-02-013 Method 1002.0

Organism: *Ceriodaphnia dubia*
Organism Batch #: 7.26.11
Organism age : <24 hours
Feeding: Prior to Test and Daily
Food: ☑ YCT ☑ *P. subcapitata*
Food Batch: 5.5.11 5.5.11
Chamber size : 30 ml
Sample volume : 20 ml

Sample Received	LAB Sample #	LIMS JOB #	LIMS SAMPLE #	Collection Date/Time	Received Date/Time
Test Day 0	1	TLH- 2011-07-26-64	1366569	7.25.11 / 0500	7.26.11 / 0925
Test Day 2	2	TLH- 2011-07-28-51	1367426	7-27-11 / 0530	7-28-11 / 0925
Test Day 4	3	TLH- 2011-08-01-01	1367740	7-29-11 / 0530	7-30-11 / 0905

7.21.11 7.28.11

Batch #	7.21.11 KC	7.27.11 KC	NA	NA	NA			
Water Quality Parameters	20% DMW	20% DMW	Sample 1	Sample 2	Sample 3	Other:	Measured By: MF	Verified by:
Field Total Residual Cl_2 (mg/L):	NA	NA	—	—	—	Not measured	Not measured	KC
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03	<0.03	<0.03	<0.03		KC/MF	KC
Alkalinity (mg/L as $CaCO_3$):	80	KS 178	166	168	181		KC	KC
Hardness (mg/L as $CaCO_3$):	81	80	217	224	228		KC	KC
Total Ammonia (mg/L as N):	<0.017	<0.017	<0.017	<0.017	<0.017		MF/BA	KC
Conductivity: ~~µmhos~~ mmhos:	182	176	941	953	990		KC/MF	KC
Salinity (ppth):								

Test Incubated in: Incubator #: 2

Loaded by: KC

Temperature Shipped ≤ 6° C (all samples) (yes) no
Holding Time ≤ 36 hours (yes) no
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 24.4 max 27.7 mean —
Room B246 min. 24.0 max 25.7 mean —
Test min. ____ max. ____ mean ____

Test Replicates	Loaded from culture vial
1	B18
2	A18
3	A28
4	B10
5	A22
6	A21
7	B17
8	A29
9	B2
10	B8

Results	
Control % CV:	22.8%
Dunnetts PMSD:	35.1%
Reproduction NOEC:	100%
Reproduction LOEC:	>100%
Survival NOEC:	100%
Survival LOEC:	>100%
ICP_{20}:	>100%
ICP_{25}:	>100%

Investigators' signatures
KARA COX
Marshall Faircloth
Becca Alexander
Tom Smith
Liz Miller
REVIEWER

Comments:

Test Page 1 of 6 PAGE 13 revised 07/15/08 MF

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

SOP TA07_05

Facility: ClearWater East + Northeast AWWTP

LIMS Sample #: 1366569 for lab sample #1

Start Date: 7.26.11 Time: 1130

End Date: 8.2.11 Time: 1000

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving adults	Neonates per adult
Control	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	3	4	0	1	0	0	0	0	3	11	10	1.1
	4	0	0	0	0	5	8	2	8	0	7	30	10	3.0
	5	9	8	9	7	0	0	6	0	8	0	47	10	4.7
	6	11	11	12	9	2	11	0	7	0	7	78	10	7.8
	7	0	0	0	0	5	8	8	11	4	FC	23	10	2.3
	8				6 mf									18.9
	Total	20	22	25	17	16	19	16	26	12	17	198 mf	10	~~18.0~~ mf

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving adults	Neonates per adult
6.25%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0		10	—
	3	0	2	1	2	0	0	0	0	0	2	7	10	0.7
	4	4	0	0	X*	0	6	2	0	0	0	12 mf	9	1.2
	5	8	8	8		8	0	7	7	0	2	50 ~~48~~ mf	9	5.0
	6	8	15	10			11	0	15	5	0	45	9	4.5
	7	0	0	0			11	2	0	9	2	24	9	2.4
	8													
	Total	20	11	19	2	30	8	10	21	2	15	138	9	13.8

Organisms loaded by: _____

Day	Counted/ verified by:	Concentrations prepared by:
0	KC	KC
1	KC	KC
2	KC	KC
3	BA	BA
4	mf	mf
5	mf	mf
6	mf	mf
7	mf	—
8		

Concentration	Mean neonates /adult	% survival
Control	18.9	100
6.25%	13.8	90
12.50%	17.7	100
25%	17.6	100
50%	20.3	100
100%	24.4	100

Test acceptability	
Mean control reproduction 15/female	(yes) no
3rd brood in 60% of surviving females	(yes) no
Control survival ≥ 80%:	(yes) no

Comments: S= split brood. mf 8-11 *4th broods excluded from counts mf 9-28-mf 11

*Day 0 is test initiation day
X = Dead (original organism)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

SOP TA07_05

Facility: Clearwater East + Northeast AWWTP

LIMS Sample #: 1366569 for lab sample #1

Start Date: 7.26.11 Time: 1130

End Date: 8-2-11 Time: 1000

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
12.50%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	4	2	0	0	0	2	0	1	9	10	0.9
	4	0	0	0	0	5	1	4	0	4	0	14	10	1.4
	5	7	5	6	7	6	0	7	9	8	3	58 mF	10	5.8
	6	11	7	6	6	0	10	0	12	0	12	64A5	10	6.5
	7	0	0	0	S1	4	9	9	0	9	0	31	10	3.1
	8									MF				
	Total	18	12	16	16	15	20	20	23	21	16	177	10	17.7

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
25%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	3	0	0	0	0	0	0	0	3	10	0.3
	4	5	4	0	0	8	5	2	0	0	0	24	10	2.4
	5	6	9	7	5	0	0	7	8	6	6	54	10	5.4
	6	10	0	8	0	7	6	4	4	0	11	50	10	5.0 mF
	7	0	0	0	0	9	10	0	11	5	10	~~45~~ mF	10	4.5
	8													
	Total	21	13	18	5	24	21	13	23	11	27	176	10	17.6

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
50%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	1	3	0	0	0	0	0	0	4	10	0.4
	4	0	0	0	0	0	9	0	0	1	0	11	10	1.1
	5	8	9	9	6	7	S1	8	8	7	7	69	10	6.9
	6	6	9	6	6	13	11	0	8	0	5	64	10	6.4
	7	0	8	0	0	10	13	1	8	6	9	55	10	5.5
	8													
	Total	14	26	16	15	30	34	9	24	14	21	203	10	20.3

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Surviving Adults	Neonates per adult
100%	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	2	0	1	0	0	0	0	0	0	3	10	0.3
	4	2	0	0	0	9	10	5	0	7	0	33	10	3.3
	5	8	9	6	9	0	0	9	9	0	11	61	10	6.1
	6	8	13	10	10	8	12	12	12	6	7	98	10	9.8
	7	0	*11	0	*7	12	10	0	11	8	8	49	10	4.9
	8													
	Total	18	24	16	20	29	32	26	32	21	26	244	10	24.4

Comments: S= Split brood 7-31-11 MF

*4th brood not counted MF 8-2-11

*Day 0 is test initiation day
X = Dead (original organism)

Appendix B. (continued)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Parameters

Facility: ClearWater East of Northeast SOP TA07_05

LIMS Sample #: 1366569 for lab sample 1 AWWTP

Circle µmhos-cm or mmhos-cm

Test Day ↓		Concentration	New Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Old Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 0	sample	Control	M	8.1	25.4	8.5	182					
	1	6.25%	M	8.1	25.5	8.0	237					
	1	12.5%	M	8.2	25.4	7.8	288					
	1	25%	M	8.2	24.8	7.8	385					
	1	50%	M	8.1	24.5	7.9	575					
	1	100%	M	8.0	24.0	8.5	941					
	Measured by:			KC	KC	KC	KC					
	Recorded by:			KC	KC	KC	KC					
Day 1	sample:	Control	M	8.1	25.3	8.0	178	M	8.2	24.0	8.3	189
	1	6.25%	M	8.3	25.3	8.0	230	M	8.2	24.0	8.3	253
	1	12.5%	M	8.3	25.0	8.0	279	M	8.3	24.0	8.3	308
	1	25%	M	8.4	24.7	8.0	378	M	8.3	24.0	8.2	411
	1	50%	M	8.4	24.3	8.0	571	M	8.3	24.0	8.1	619
	1	100%	M	8.5	24.0	8.0	946	M	8.4	24.0	8.0	970
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
Day 2	sample:	Control	M	8.0	24.0	8.2	172	M	8.0	24.0	8.4	195
	2	6.25%	M	8.1	24.0	8.3	224	M	8.0	24.0	8.1	240
	2	12.5%	M	8.1	24.0	8.4	275	M	8.1	24.0	7.9	290
	2	25%	M	8.2	24.0	8.5	373	M	8.2	24.0	7.9	390
	2	50%	M	8.2	24.0	8.5	562	M	8.2	24.0	7.9	584
	2	100%	M	8.3	24.0	8.5	953	M	8.4	24.0	8.0	991
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
Day 3	sample:	Control	M	8.0	24.4	8.0	181	M	7.9	24.0	7.5	194
	2	6.25%	M	8.0	24.7	8.0	237	M	7.9	24.0	8.0	241
	2	12.5%	M	8.1	24.8	7.8	288	M	8.0	24.0	8.3	286
	2	25%	M	8.1	24.7	8.0	387	M	8.0	24.0	8.1	385
	2	50%	M	8.1	24.5	8.0	583	M	8.1	24.0	8.2	576
	2	100%	M	8.3	24.5	8.2	968	M	8.3	24.0	8.1	1007
	Measured by:			BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:			BA	BA	BA	BA		BA	BA	BA	BA

Comments:

Replicate M= "Metered" vial, a surrogate vial for parameters

Test Page 4 of 6

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revised 07/15/08 MF

Appendix B. (continued)

FDEP Biology Laboratory - Chronic Definitive Bench Sheet

Ceriodaphnia dubia Parameters

Facility: Clearwater East + Northeast SOP TA07_05

LIMS Sample #: 1366569 for lab sample 1 AWWTP

Circle µmhos-cm or mmhos-cm

Test Day ↓		Concentration	New Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Old Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 4	sample:	Control	M	8.1	24.4	8.3	176	M	8.2	24.3	8.3 MF	192
	3	6.25%	M	8.2	24.4	8.2	228	M	8.2	24.3	8.25 MF	239
	3	12.5%	M	8.2	24.3	8.1	279	M	8.2	24.4	8.16 MF	289
	3	25%	M	8.1	24.3	8.1	383	M	8.2	24.3	8.16 MF	394
	3	50%	M	8.1	24.4	8.0	585	M	8.2	24.2	8.06 MF	607
	3	100%	M	8.1	24.5	8.0	990	M	8.3	24.4	8.07 MF	1004
	Measured by:			MF	MF	MF	MF		MF MF	MF	MF	MF
	Recorded by:			MF	MF	MF	MF		MF	MF	MF	MF
Day 5	sample:	Control	M	8.0	24.2	7.9	179	M	8.0	24.6	7.9	200
	3	6.25%	M	8.0	24.3	8.0	233	M	8.1	25.0	8.0	246
	3	12.5%	M	8.0	24.2	8.0	288	M	8.1	25.4	8.1	296
	3	25%	M	8.0	24.3	8.0	394	M	8.1	25.6	8.1	405
	3	50%	M	8.1	24.4	8.0	605	M	8.1	25.4	8.1	620
	3	100%	M	8.1	24.5	8.0	1004	M	8.3	24.8	8.0	1070
	Measured by:			MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:			MF	MF	MF	MF		MF	MF	MF	MF
Day 6	sample:	Control	M	8.1	24.8	7.6	179	M	8.2	24.4	7.6	200
	3	6.25%	M	8.1	25.2	7.5	232	M	8.2	24.4	7.5	253
	3	12.5%	M	8.1	25.4	7.4	285	M	8.2	24.6	7.7	298
	3	25%	M	8.0	25.5	7.5	389	M	8.2	24.9	7.5	405
	3	50%	M	8.0	25.7	7.5	592	M	8.2	25.0	7.5	620
	3	100%	M	8.1	25.7	7.5	996	M	8.4	24.7	7.7	1037
	Measured by:			MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:			MF	MF	MF	MF		MF	MF	MF	MF
Day 7	sample:	Control	M					M	8.2	24.3	7.9	199
	3	6.25%	M					M	8.2	24.4	8.0	244
	3	12.5%	M					M	8.2	25.2	8.2	294
	3	25%	M					M	8.2	25.2	8.2	397
	3	50%	M					M	8.2	25.0	8.3	621
	3	100%	M					M	8.4	24.7	8.5	1050
	Measured by:								MF	MF	MF	MF
	Recorded by:								MF	MF	MF	MF

Comments:

Replicate M= "Metered" vial, a surrogate vial for parameters

Test Page 5 of 6 PAGE 77 revised 07/15/08 MF

Appendix B. (continued)

Test ID: Clearwater, East + Northwest C. dubia Dates: 7-26-11 — 8-2-11

METER / DEVICE	ID	SOP	Parameter	Method	v used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	√
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	√
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	√
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	√
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	√
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 #5 SN61990624	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	QC #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 Cl G	√
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	√
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	√
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT–Chronic SRT)

entered by MF

Pg. 78

V 1.1 9/21/10 MF/jd

Appendix B. (continued)

FDEP Biology Laboratory - Chronic Bioassay Bench Sheet

Pimphales promelas or Menidia beryllina

Facility: Clearwater East and Northeast (combined) (AWWTF) (AWWTF)
Address: 3141 Gulf to Bay Boulevard, Clearwater, FL (east)
County: Pinellas
NPDES Permit #: FL0021865 (EAST) — FL0128937 (north east)
Contact/District: Pete Wenner / Southwest
Address: Northeast - 3290 State Rd. 580 Clearwater, fl.
Diluent/ Batch:
MHW / 6.10.11 Test Days Used: 0-3
MHW / 6.22.11 Test Days Used: 4-7
ASW / _____ Test Days Used: _____
ASW / _____ Test Days Used: _____

Test Species (circle one)
(P. promelas) SOP#: TA07_09 EPA-821-R-02-013 Method 1000.0
M. beryllina SOP#: TA07_14 EPA-821-R-02-014 Method 1006.0

Start Date: 6.21.11 Time: 1315
End Date: 06.28.11 Time: 1030

Organism age : <24hrs days
Feeding Prior to Test and Daily
Artemia Lot # : 23613
Chamber size : 1000 ml
Test volume : 500 ml

Artemia Batch #	Day 3 115-11
Day 0 112-11	Day 4 116-11
Day 1 113-11	Day 5 ~~Not used~~ (*) KC
Day 2 114-11	Day 6 118-11

Sample Received	Lab Sample #	LIMS Job #	LIMS Sample #	Collection Date/Time	Received Date/Time
Test Day 0	1	TLH-2011-06-21-11	1358734	06/20/11 @1730	06/21/11 @0920
Test Day 2	2	TLH-2011-06-23-100	1359816	06/22/11 @0536	06/23/11 @0920
Test Day 4	3	TLH-2011-06-27-18	1360234	06/24/11 @1730	06/25/11 @0945

(A) 1730 -KC-

Water Quality Parameters	Field Chlorine (mg/L)			Lab Chlorine (mg/L)			Alkalinity (mg/L as $CaCO_3$)			Hardness (mg/L as $CaCO_3$)		
Lab Sample #:	1	2	3	1	2	3	1	2	3	1	2	3
Sample:	not measured			<0.03	<0.03	0.04	208	212	200	268	266	268
Diluent/Control:				<0.03	<0.03	<0.03	78	78	79	96	96	90
Method:	—	—	—	Hach	Hach	Hach	Hach	Hach	Hach	Hach	Hach	Hach
Measured by:	n/a	n/a	n/a	KC/JD	KC/JD	JD/KC	KC	KC	BA/KC	KC	KC	BA/KC
Verified by:	KC	KC	KC	KC	KC	KC	KC	KC	KC	KC	KC	KC

	Conductivity (circle one) (µmhos-cm / mmhos-cm)			Ammonia (mg/L)			Salinity (ppth)		
Lab Sample#:	1	2	3	1	2	3	1	2	3
Sample:	1212	1150	1190	<0.017	<0.017	<0.017			
Diluent/Control:	333	321	317	<0.017	<0.017	<0.017			
Method:	Mettler	Mettler	Mettler	Denver	Denver	Denver	Mettler	Mettler	Mettler
Measured by:	JD	JD	JD	JD	JD	JD			
Verified by:	KC	KC	KC	KC	KC	KC			

LIMS Entry: 7-13-11 MF
LIMS Verification: 7/13/11 KC

Statistics	
Growth NOEC:	100%
Growth LOEC:	>100%
Growth IC_{20}:	>100%
Growth IC_{25}:	>100%
Ctrl % CV:	9.14%
Dunnetts PMSD:	20.83
Survival NOEC:	100%
Survival LOEC:	>100%

Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 5 min 25.1 max 26.6 mean —
Water bath # ____ min. _____ max. _____ mean _____
Room B246 min 22.5 max 24.5 mean —

Investigators' signatures
James Daniels
Kate Lior
Brad Blackmon
Marshall Faircloth
Liz Nielsen
REVIEWER

Comments: (*) Artemia #117-11 was dead on day 5 so 116-11 was used to feed on day 5. -KC-

revised 08/24/06 MF/SP

Appendix B. (continued)

Chronic Bioassay Survival Benchsheet

Facility: Clearwater East + Northeast

LIMS Sample Number: 1358734
(for Lab Sample # 1)

Department of Environmental Protection
Biology Laboratory Chronic Screening Bioassay
***P. promelas* SOP TA07_09 *M. beryllina* SOP TA07_14**

Organism: (*P. promelas*) *M.beryllina* (circle one)

		TEST DAY							
Concentration	Replicate	0	1	2	3	4	5	6	7
Control	A	10	9 1D	9	9	9	9	9	9
	B	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	10	10
	D	10	9 1D	9	9	7 2D	7	7	7
Sample 6.25 %	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
	C	10	9 1D	9	9	8 1D	8	8	8
	D	10	10	10	10	10	10	10	10
Sample 12.5 %	A	10	10	10	10	9 1D	9	9	9
	B	10	9 1D	9	9	9	9	8 1D	8
	C	10	10	10	10	10	10	10	10
	D	10	10	9 1D	9	9	9	9	9
Sample 25 %	A	10	9 1D	9	9	9	9	9	9
	B	10	10	9 1D	9	9	9	9	9
	C	10	10	10	10	10	10	10	10
	D	10	10	10	10	10	10	10	10
Sample 50 %	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	9 1D	9
	D	10	10	10	10	10	10	10	10
Sample 100 %	A	10	10	10	10	10	10	9 1D	9
	B	10	10	10	10	10	10	10	10
	C	10	8 2D	8	8	8	8	8	8
	D	10	10	10	10	10	10	10	10
Sample %	A	10							
	B	10							
	C	10							
	D	10							
Loaded by: JD	Checked by:		BA	JD	JD	JD	KC	JD	JD
Verified by: BA									

Comments:

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Appendix B. (continued)

Chronic Bioassay Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg 1

Sample Source: Clearwater East & Northeast — Start Date: 6.21.11 — End Date: 6.28.11

LIMS Sample #: 1358734 (for Lab sample #1) — Start Time: 1315 — End Time: 1030

conc = concentration — rep = replicate — circle umhos or mmhos

Test Day ↓		Conc.	Before Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos/mmhos	Salinity ppth	Test Start and After Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos/mmhos	Salinity ppth
Day 0	sample:	Control							A	8.1	25.9	8.1	333	
	1	6.25%							A	8.2	25.8	8.0	386	
	1	12.5%							A	8.3	25.4	8.2	422	
	1	25%							A	8.3	25.9	8.4	551	
	1	50%							A	8.4	25.7	8.2	758	
	1	100%							A	8.4	25.9	8.3	1212	
	1								A					
	Measured by:									JD	JD	JD	JD	
	Recorded by:									JD	JD	JD	JD	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 1	sample:	Control	B	7.9	25.3	6.5	323		B	7.9	25.4	6.6	325	
	1	6.25%	B	7.9	24.9	6.6	371		B	7.9	25.3	6.9	388	
	1	12.5%	B	7.9	24.7	6.5	416		B	7.9	25.4	7.1	442	
	1	25%	B	7.9	24.7	6.6	528		B	8.2	25.3	7.1	535	
	1	50%	B	8.4	24.8	6.6	744		B	8.4	25.3	7.2	736	
	1	100%	B	8.5	24.7	6.6	1141		B	8.4	24.9	7.2	1154	
	1		B						B					
	Measured by:			BA	BA	BA	BA			BA	BA	BA	BA	
	Recorded by:			BA	BA	BA	BA			BA	BA	BA	BA	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 2	sample:	Control	C	7.9	24.6	7.0	319		C	8.1	25.8	7.3	321	
	2	6.25%	C	7.8	24.8	7.2	378		C	8.0	25.6	7.4	372	
	2	12.5%	C	7.9	24.9	7.2	427		C	8.1	25.7	7.6	427	
	2	25%	C	7.9	25.0	7.3	528		C	8.2	25.6	7.6	539	
	2	50%	C	8.1	24.6	7.3	732		C	8.2	25.3	7.6	723	
	2	100%	C	8.4	25.0	7.2	1146		C	8.2	25.8	7.5	1150	
	2		C						C					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 3	sample:	Control	D	7.7	24.6	6.6	322		D	7.8	25.3	7.4	318	
	2	6.25%	D	7.6	24.7	6.5	378		D	7.8	25.2	7.4	362	
	2	12.5%	D	7.7	24.8	7.4	432		D	7.9	25.1	7.7	413	
	2	25%	D	7.8	24.7	7.5	550		D	8.2	24.9	7.9	527	
	2	50%	D	8.0	24.5	7.3	744		D	8.3	24.5	7.7	717	
	2	100%	D	8.1	24.5	6.6	1159		D	8.4	24.2	7.3	1107	
	2		D						D					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	

Comments:

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional

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Appendix B. (continued)

Chronic Bioassay Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg 2

Sample Source: Clearwater East + Northcest Start Date: 6.21.11 End Date: 6.28.11

LIMS Sample #: 1558734 (for Lab sample #1) Start Time: 1315 End Time: 1030

conc = concentration rep = replicate

Test Day			Before Renewal						Test Start and After Renewal					
	Conc.		Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos/mmhos	Salinity ppth	Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos/mmhos	Salinity ppth
Day 4	sample:	Control	A	7.9	24.0	6.9	328		A	7.9	24.0	7.9	317	
	3	6.25%	A	8.0	24.1	7.4	373		A	8.1	24.0	8.3	362	
	3	12.5%	A	8.0	24.0	7.4	425		A	8.1	24.0	8.5	415	
	3	25%	A	8.0	24.1	7.3	541		A	8.1	24.0	8.5	531	
	3	50%	A	8.0	24.2	7.4	739		A	8.2	24.3	8.6	733	
	3	100%	A	8.3	24.2	6.7	1168		A	8.1	25.6	8.1	1190	
	3		A						A					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 5	sample:	Control	B	7.9	24.2	8.1	327		B	7.8	24.6	8.1	321	
	3	6.25%	B	7.9	24.3	8.0	375		B	8.0	24.7	8.0	376	
	3	12.5%	B	7.9	24.2	7.9	427		B	8.1	24.7	8.0	429	
	3	25%	B	8.0	24.1	8.1	542		B	8.1	24.8	8.1	536	
	3	50%	B	8.2	24.5	8.0	742		B	8.2	24.8	8.2	737	
	3	100%	B	8.3	24.3	7.8	1180		B	8.4	24.4	8.3	1105	
	3		B						B					
	Measured by:			KC	KC	KC	KC			KC	KC	KC	KC	
	Recorded by:			KC	KC	KC	KC			KC	KC	KC	KC	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 6	sample:	Control	C	8.0	24.6	7.8	322		C	7.9	25.4	8.2	315	
	3	6.25%	C	8.1	24.5	7.9	372		C	7.9	25.2	7.7	368	
	3	12.5%	C	8.1	24.8	8.0	428		C	8.0	25.2	7.5	421	
	3	25%	C	8.1	25.0	8.0	536		C	8.2	25.0	7.3	526	
	3	50%	C	8.2	24.6	7.8	744		C	8.3	24.8	7.2	714	
	3	100%	C	8.3	24.7	7.8	1096		C	8.5	24.9	7.6	1105	
	3		C						C					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 7	sample:	Control	D	7.8	25.4	7.3	318							
	3	6.25%	D	7.7	25.0	6.9	374							
	3	12.5%	D	7.7	25.1	7.0	429							
	3	25%	D	7.8	24.8	7.2	538							
	3	50%	D	8.0	24.6	7.1	729							
	3	100%	D	8.2	24.5	7.3	1138							
	3		D											
	Measured by:			KC	JD	KC	KC							
	Recorded by:			KC	JD	KC	KC							

Comments:

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional.

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Appendix B. (continued)

Chronic Bioassay Weighing Benchsheet

Facility: Clearwater East + Northeast

LIMS Sample Number: B58734 (for Lab Sample # 1)

P. promelas and *M. beryllina* Bench Sheet
SOP TA06_21

Concentration	Replicate	Pan #	Pan wt. g	Pan + Fish g	Fish wt. g	Original # Fish	Mean wt. mg
			mean wt = fish wt divided by 10 convert g to mg: move decimal 3 places to right				
Control	A	52	0.02266	0.02761	0.00495	10	.495
	B	53	0.02284	0.02802	0.00518	10	.518
	C	54	0.02349	0.02888	0.00539	10	.539
	D	55	0.02231	0.02665	0.00434	10	.434
sample 6.25 %	A	56	0.02224	0.02689	0.00465	10	.465
	B	57 (A)	~~0.02268~~	0.02748	0.00423	10	.423
	C	58 (B)	~~0.02325~~	0.02815	0.00479	10	.479
	D	59 (C)	~~0.02336~~	0.02765	0.00544	10	.544
sample 12.5 %	A	60	0.02241	0.02777	0.00536	10	.536
	B	61	0.02300	0.02813	0.00513	10	.513
	C	62	0.02263	0.02681	0.00418	10	.418
	D	63	0.02291	0.02812	0.00521	10	.521
sample 25 %	A	64	0.02271	0.02838	0.00567	10	.567
	B	65	0.02273	0.02820	0.00547	10	.547
	C	66	0.02295	0.02783	0.00488	10	.488
	D	67	0.02337	0.02898	0.00561	10	.561
sample 50 %	A	68	0.02371	0.03054	0.00683	10	.683
	B	69	0.02209	0.02901	0.00692	10	.692
	C	70	0.02099	0.02619	0.00520	10	.520
	D	71	0.02370	0.03006	0.00636	10	.636
sample 100 %	A	72	0.02275	0.02693	0.00418	10	.418
	B	73	0.02388	0.02987	0.00599	10	.599
	C	74	0.02318	0.02887	0.00569	10	.569
	D	75	0.02249	0.02837	0.00588	10	.588
sample %	A					10	
	B					10	
	C					10	
	D					10	
	Weighed by initials:		JD	JD		revised 7/16/08 mf	

Comments:
(A) 0.02325
(B) 0.02336 -KC-
(C) 0.02221

Balance: Ohaus 250D Ser# 1125481084
S-Weights: BIO-S-Weights-01

Note: Pan number (#) refers to actual Pan number.

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Appendix B. (continued)

Chronic Bioassay Weighing Benchsheet

Quality Control

Facility: Clearwater East + Northeast

LIMS Sample Number: 1358734 (for Lab Sample # 1)

P. promelas and M. beryllina Bench sheet

SOPs TA07_09, TA07_14 and TA06_21

Drying Oven ID:	ER034000
Temperature °C:	100
Hours:	8

Tare weighed by: JD

Final weighed by: JS

Tare Weighing Date: 6.27.11 Final Weighing Date: 6.30.11

			Measured Value (g)		S-weight Value (g)
Tare Pan 1 after Pan 5 (g)	0.02268	@ 0.02266	S-weights	@	0.01
Tare Pan 1 after Pan 10 (g)	0.02267	@ 0.02266	at tare 0.03004	@	0.03
Tare Pan 1 after Pan 15 (g)	0.02268	@ 0.02266	S-weights	@	0.01
Tare Pan 1 after Pan 20 (g)	0.02269	@ 0.02266	at final 0.05003	@	0.05
Tare Pan 1 after Pan 25 (g)	0.02269	@ 0.02266			
Tare Pan 1 after Pan 30 (g)		@	Balance: Ohaus 250D Ser# 1125481084		
Tare Pan 1 after Pan 35 (g)		@	S-Weights: BIO-S-Weights-01		
Tare Pan 1 after Pan 40 (g)		@			
Tare Pan 1 after Pan 45 (g)		@			
Tare Pan 1 after Pan 48 (g)		@			
Final Pan 1 after Pan 5 (g)	0.02763	@ 0.02761			
Final Pan 1 after Pan 10 (g)	0.02762	@ 0.02761			
Final Pan 1 after Pan 15 (g)	0.02761	@ 0.02761			
Final Pan 1 after Pan 20 (g)	0.02760	@ 0.02761			
Final Pan 1 after Pan 25 (g)	0.02764	@ 0.02761			
Final Pan 1 after Pan 30 (g)		@			
Final Pan 1 after Pan 35 (g)		@			
Final Pan 1 after Pan 40 (g)		@			
Final Pan 1 after Pan 45 (g)		@			
Final Pan 1 after Pan 48 (g)		@			

Note: Pan numbers (5, 10, etc.) for Tare and Final refers to sequence of weighing as actual Pan #s may differ.

Comments:

revised 7/16/08 MF

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Appendix B. (continued)

Test ID: Clearwater East + Northeast Dates: 6.21.11 – 6.28.11

METER / DEVICE	ID	SOP	Parameter	Method	v used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 #5 SN61990624	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓ #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	✓
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	✓

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

Karen Clay 6/28/11

V 1.1 9/21/10 MF/jd

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Appendix C. SRT Bench Sheets and Statistical Analysis

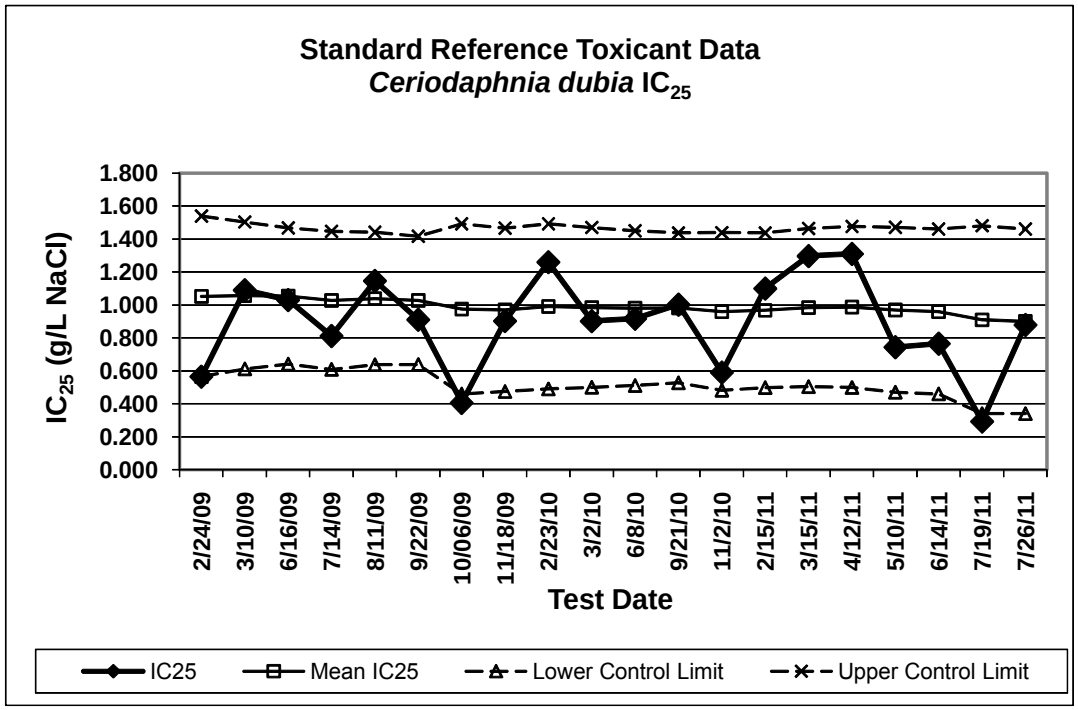
Florida Department of Environmental Protection
Bureau of Laboratories

Standard Reference Toxicant (SRT) Test Data - 7-Day Chronic Toxicity
Ceriodaphnia dubia (water flea) - Sodium Chloride (NaCl)

Date	IC25	Mean IC25	Std Dev	Lower Control Limit	Upper Control Limit
2/24/09	0.565	1.05	0.24	0.57	1.54
3/10/09	1.090	1.06	0.22	0.61	1.50
6/16/09	1.030	1.05	0.21	0.64	1.47
7/14/09	0.812	1.03	0.21	0.61	1.45
8/11/09	1.146	1.04	0.20	0.64	1.44
9/22/09	0.911	1.03	0.19	0.64	1.42
10/06/09	0.406	0.98	0.26	0.46	1.49
11/18/09	0.903	0.97	0.25	0.47	1.47
2/23/10	1.260	0.99	0.25	0.49	1.49
3/2/10	0.902	0.98	0.24	0.50	1.47
6/8/10	0.917	0.98	0.23	0.51	1.45
9/21/10	1.002	0.98	0.23	0.53	1.44
11/2/10	0.590	0.96	0.24	0.48	1.44
2/15/11	1.100	0.97	0.23	0.50	1.44
3/15/11	1.297	0.98	0.24	0.50	1.46
4/12/11	1.310	0.99	0.24	0.50	1.48
5/10/11	0.744	0.97	0.25	0.47	1.47
6/14/11	0.765	0.96	0.25	0.46	1.46
7/19/11	0.293	0.91	0.28	0.34	1.48
7/26/11	0.879	0.90	0.28	0.34	1.46

Means are of the last 20 SRT tests.
Lower Confidence Limit = mean - 2 * Std Dev
Upper Confidence Limit = mean + 2 * Std Dev

EPA 75th Percentile %CV for Reproduction **45%**
Lab %CV Reproduction **24** **31.30%**

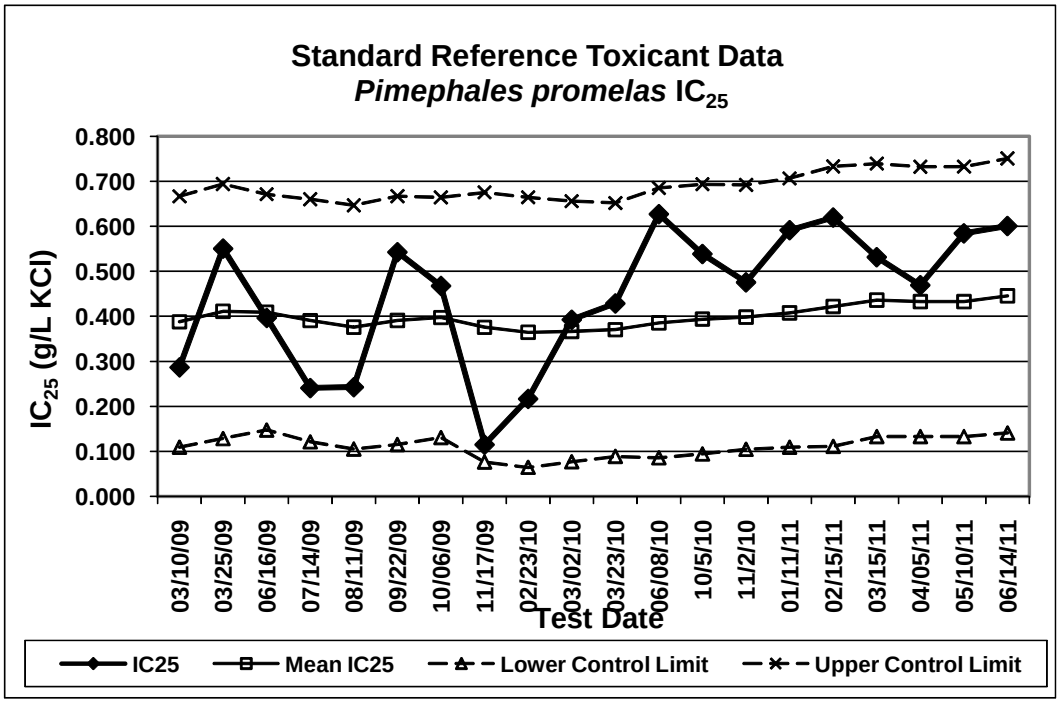


Appendix C. (continued)

Florida Department of Environmental Protection
Bureau of Laboratories
Standard Reference Toxicant (SRT) Test Data - 7-Day Chronic Toxicity
Pimephales promelas (fathead minnow) - Potassium Chloride (KCl)

Date	IC25	Mean IC25	Std Dev	Lower Control Limit	Upper Control Limit
03/10/09	0.287	0.388	0.14	0.109	0.667
03/25/09	0.551	0.411	0.14	0.129	0.694
06/16/09	0.397	0.410	0.13	0.148	0.671
07/14/09	0.241	0.391	0.13	0.121	0.660
08/11/09	0.243	0.376	0.14	0.105	0.647
09/22/09	0.543	0.391	0.14	0.115	0.667
10/06/09	0.468	0.398	0.13	0.131	0.664
11/17/09	0.115	0.376	0.15	0.076	0.676
02/23/10	0.217	0.365	0.15	0.064	0.665
03/02/10	0.393	0.366	0.14	0.077	0.656
03/23/10	0.429	0.370	0.14	0.089	0.652
06/08/10	0.628	0.385	0.15	0.086	0.685
10/05/10	0.539	0.394	0.15	0.094	0.694
11/02/10	0.476	0.398	0.15	0.105	0.692
01/11/11	0.592	0.408	0.15	0.109	0.707
02/15/11	0.620	0.422	0.16	0.111	0.733
03/15/11	0.532	0.436	0.15	0.133	0.740
04/05/11	0.470	0.433	0.15	0.133	0.733
05/10/11	0.585	0.433	0.15	0.133	0.733
06/14/11	0.601	0.446	0.15	0.141	0.751

Means are from the last 20 SRTs.
Lower Confidence Limit = mean - 2 * Std Dev
Upper Confidence Limit = mean + 2 * Std Dev



FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia

Diluent/ Batch: 20% DMW / 7-21-11 Test Days Used: D-3
20% DMW / 7.28.11 Test Days Used: 4-6
Toxicant/ Batch: NaCl / 7-26-11

SOP # : TA 07_05
EPA-821-R-02-013 Method 1002.0

Organism: *Ceriodaphnia dubia*
Organism Batch #/ Age: 7.26.11 / <24 hr.
Feeding: Prior to Test and Daily
Food: ☑ YCT ☑ *P. subcapitata*
Food Batch: 5.5.11 ~~#18~~ 5.5.11 KC
Chamber size: 30 mL
Test Volume: 20 mL

Start Date: 7-26-11 Time: 1230
End Date: 8-1-11 Time: 1000

Batch:	7.21.11	7.28.11			
Water Quality Parameters	20% DMW	20% DMW	Other	Measured by:	Verified by:
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03		KC	KC
Alkalinity (mg/L as $CaCO_3$):	80	78		KC	KC
Hardness (mg/L as $CaCO_3$):	81	80		KC	KC
Total Ammonia (mg/L as N):	<0.017	<0.017		BA/MF	KC
Conductivity ~~µmhos-cm~~ mmhos-cm :	180	179		MF/BA	KC

Loaded by: KC

Day	Concentrations prepared by	Counted by
0	MF	KC
1	KC	KC/BA
2	BA	~~BA~~ BA
3	KC/BA	KC
4	KC/BA	BA
5	KC/BA	MF
6	MF	MF
7		
8		

Test Replicate	Loaded from culture vial
1	B8
2	A18
3	B4
4	A19
5	B17
6	B10
7	A22
8	A21
9	B4
10	A19

Results	
Control % CV:	15.8%
Dunnetts PMSD:	19.1%
Reproduction NOEC:	0.59 g/L
Reproduction LOEC:	0.89 g/L
Survival NOEC:	3 g/L
Survival LOEC:	3 g/L
0.766 g/L ICP_{20}:	MF ~~0.564~~ g/L*
0.879 g/L ICP_{25}:	MF ~~0.656~~ g/L*

* Calculated 2 ways MF 8-1-11

Comments:

Investigators' signatures

Marshall Faircloth
Kara Cox
Bree Best
James Daniels

Liz Miller
REVIEWER

Test Incubated in: Incubator: # 2
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 24.4 max 27.7 mean –
Room B248 ~~21.6~~ 22 min. 24.0 max. 25.7 mean –
Test min. ____ max. ____ mean ____

Appendix C. (continued)

Ceriodaphnia dubia Survival and Reproduction

Start Date: 7.26.11 Time: 1230 Loaded by: KC
End Date: 8-1-11 Time: 1000 Verified by: MF

Concentration	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Live adults	Neonates per adult
Control	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	3	0	0	3	0	0	4	5	15	10	1.5
	4	0	0	0	8	4	0	0	9	0	10	31	10	3.1
	5	7	6	8	2	7	7	7	0	8	0	52	10	5.2
	6	11	9	8	12	9	7	12	9	10	11	98	10	9.8
	7													
	8													
	Total	18	15	19	22	20	17	19	18	22	26	196	10	19.6
0.59 g/L	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	0	0	0	2	1	20	2	0	5	10	0.6
	4	0	0	0	7	3	0	1	9	0	0	19	10	1.9
	5	7	6	0	0	6	5	6	7	7	6	43	10	4.3
	6	11	10	0	10	13	9	10	9	8	7	87	10	8.7
	7			MAY										
	8													17.2
	Total	18	16	0	17	22	16	18	18	17	13	155	10(9)	15.5
0.89 g/L	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	0	0	0	0	3	0	0	0	3	10	0.3
	4	6	0	0	0	0	0	0	0	8	7	21	10	2.1
	5	0	7	0	7	4	4	5	8	10	0	45	10	4.5
	6	9	9	7	9	7	5	13	9	0	9	77	10	7.7
	7													
	8													
	Total	15	16	7	16	11	9	21	17	18	16	146	10	14.6

Comments: S = split brood MF 7-30-11
0.59 g/L Rep 3 F Died as Male by M. Heyn 8-1-11 MF

Test Page 2 of 6 PAGE 14 07/15/08 MF

Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

Concentration	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Live adults	Neonates per adult
1.3 g/L	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	0	1	0	4	0	0	0	1	6	10	0,6
	4	0	4	0	0	0	0	0	6	3	0	13	10	1.3
	5	7	0	1	0	3	5	6	5	0	7	34	10	3.4
	6	2	6	6	0	6	5	6	7	6	7	51	10	5.1
	7													
	8													
	Total	9	10	7	1	9	14	12	18	9	15	104	10	10,4
2.0 g/L	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	—
	3	0	0	0	0	0	0	0	2	0	0	2	10	0.2
	4	0	0	0	0	0	0	0	0	0	0	0	10	0
	5	2 X out	1	2	1	4	2	2	1	0	3	18	10	1.8
	6	2	4	0	3	0	2	6	4	0	4	25	10	2.5
	7									male				
	8													5
	Total	4	5	2	4	4	4	8	7	0	7	45	10 9	~~4,5~~
3.0 g/L	0												10	
	1		\				X						8	
	2	X		X	X	X		X	X	X	X	0	0	
	3													
	4													
	5													
	6													
	7													
	8													
	Total	0	0	0	0	0	0	0	0	0	0	0	0	0

Comments: 2.0 g/L Rep 9 Ided as male by M. Hoyn 8-1-11 MF

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07/15/08 MF

Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia Parameters

Test Day		New Sample				Conductivity	Old Sample				Conductivity
↓	Conc.	Replicate	pH (su)	Temp (°C)	DO (mg/L)	(µmhos/cm) (mmhos/cm)	Replicate	pH (su)	Temp (°C)	DO (mg/L)	(µmhos/cm) (mmhos/cm)
Day 0	Control	M	7.9	24.7	7.9	180					
	0.59	M	7.9	24.7	7.8	1324					
	0.89	M	7.9	24.5	7.8	1882					
	1.3	M	7.9	24.5	7.9	2670					
	2.0	M	8.0	24.4	7.8	3830					
	3.0	M	8.0	24.2	7.8	5550					
	Measured by:		MF	MF	MF	MF					
	Recorded by:		MF	MF	MF	MF					
Day 1	Control	M	8.2	24.6	7.9	180	A	8.2	25.1	8.3	187
	0.59	M	8.2	24.4	7.9	1334	A	8.2	25.2	8.1	1352
	0.89	M	8.2	24.3	7.9	1892	A	8.2	25.1	8.0	1894
	1.3	M	8.2	24.2	8.0	2700	A	8.2	25.1	8.0	2690
	2.0	M	8.2	24.1	8.0	3870	A	8.2	25.0	8.0	3920
	3.0	M	8.2	24.0	8.0	5680	A	8.2	25.0	7.9	5790
	Measured by:		KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:		KC	KC	KC	KC		KC	KC	KC	KC
Day 2	Control	M	7.8	24.3	6.8	179	A	8.0	24.0	7.8	194
	0.59	M	7.8	25.2	7.1	1353	A	8.0	24.0	7.7	1450
	0.89	M	7.9	24.2	7.2	1902	A	8.0	24.2	7.6	1921
	1.3	M	7.9	24.1	6.7	2710	A	8.0	24.3	7.6	2730
	2.0	M	7.9	24.4	7.2	3950	A	8.0	24.2	7.7	3960
	3.0	M	7.9	24.4	7.3	5660	A	8.0	24.1	7.7	5950
	Measured by:		BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:		BA	BA	BA	BA		BA	BA	BA	BA
Day 3	Control	M	8.1	24.5	8.5	178	A	8.0	24.0	8.5	196
	0.59	M	8.1	24.3	8.4	1319	A	8.1	24.0	8.5	1384
	0.89	M	8.1	24.1	8.4	1863	A	8.1	24.0	8.5	1937
	1.3	M	8.1	24.0	8.4	2640	A	8.1	24.0	8.3	2770
	2.0	M	8.1	24.0	8.5	3790	A	8.1	24.0	8.3	3980
		M									
	Measured by:		KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:		KC	KC	KC	KC		KC	KC	KC	KC

Comments: Test Terminated on DAY 6 after New Sample concentrations poured and parameters measured. MF 8-1-11

M= "Metered" vial, a surrogate vial for parameters

Test Page 4 of 6 PAGE 16 07/15/08 MF

Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia Parameters

Test Day ↓	Conc.	Replicate	New Sample pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Replicate	Old Sample pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 4	Control	M	7.9	25.1	7.5	179	A	7.9	24.0	7.6	187
	0.59	M	7.9	25.6	7.7	1353	A	7.9	24.0	7.6	1324
	0.89	M	7.9	26.0	7.8	1921	A	7.9	24.0	7.7	1882
	1.3	M	7.9	25.7	7.9	2590	A	7.9	24.0	7.7	2660
	2.0	M	8.0	25.6	8.0	3800	A	7.9	24.0	7.5	3950
		M									
	Measured by:		BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:		BA	BA	BA	BA		BA	BA	BA	BA
Day 5	Control	M	8.0	25.4	7.7	179	A	8.1	24.8	7.9	196
	0.59	M	8.0	25.3	7.6	1293	A	8.1	25.1	7.9	1370
	0.89	M	8.0	25.1	7.5	1850	A	8.1	25.4	7.8	1930
	1.3	M	8.0	25.3	7.6	2500	A	8.1	25.6	7.9	2640
	2.0	M	8.0	25.5	7.6	3790	A	8.1	25.7	7.9	3910
		M									
	Measured by:		MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:		MF	MF	MF	MF		MF	MF	MF	MF
Day 6	Control	M	8.2	25.2	7.7	180	A	8.1	25.0	7.7	193
	0.59	M	8.2	25.2	7.7	1303	A	8.2	25.1	7.7	1380
	0.89	M	8.2	24.8	7.9	1832	A	8.2	25.4	7.8	1949
	1.3	M	8.2	24.6	7.9	2600	A	8.2	25.6	7.8	2650
	2.0	M	8.2	24.7	7.9	3750	A	8.2	24.9	7.8	3980
		M									
	Measured by:		MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:		MF	MF	MF	MF		MF	MF	MF	MF
Day 7	Control	M									
		M									
		M									
		M									
		M									
		M									
	Measured by:										
	Recorded by:										
Day 8	Control	M									
		M									
		M									
		M									
		M									
		M									
	Measured by:										
	Recorded by:										

Replicate M= "Metered" vial, a surrogate vial for parameters

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Appendix C. (continued)

Test ID: C. dubia Chronic SRT July 11 Report Dates: 7-26-11 — 8-1-11

METER / DEVICE	ID	SOP	Parameter	Method	v used	
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓	
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	✓	
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓	
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓	
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓	
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 #5 (SN61990624	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓	EC #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓	
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓	
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓	
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-		
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-		

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

entered by M. Faucheux 8-1-11

V 1.1 9/21/10 MF/jd

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Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or *Menidia beryllina*

Test Species (circle one)

(*P. promelas*)	SOP#: TA07_09	EPA-821-R-02-013	Method 1000.0
M. beryllina	SOP#: TA07_14	EPA-821-R-02-014	Method 1006.0

Diluent/ Batch: MHW/6.10.11 Test Days Used: 0-7

Diluent/ Batch: _____ Test Days Used: _____

Toxicant/ Batch # : KCl / ~~KBr 6.11~~ 6.13.11

Artemia Lot # : 23613

Start Date: 6.14.11 Time: 1300

End Date: 6.21.11 Time: 0830

Organism Batch #/ Age: 6.14.11 / < 24 HRS.

Artemia Batch #	Day 2 107-11	Day 5 110-11
Day 0 105-11	Day 3 108-11	Day 6 111-11
Day1 106-11	Day 4 109-11	

Chamber size : 1000 ml

Test volume : 500 ml

Feeding: *P. promelas* 0.15 g wet wt. 2X day 6 hour interval

M. beryllina 0.10 g wet wt days 0-2 0.15g wet wt. days 3-7

Water Quality Parameters	Diluent	Diluent (or other)	Method	Measured by	Verified by
Total Residual Cl_2 (mg/L):	<0.03		HACH	JD	KC
Alkalinity (mg/L as $CaCO_3$):	78		HACH	KC	KC
Hardness (mg/L as $CaCO_3$):	96		HACH	KC	KC
Total Ammonia (mg/L as N):	<0.017		DENVER	BA	KC
Conductivity (µmhos-cm mmhos-cm):	325		METTLER	JD	KC

Statistics	(calculated?)	(IC_{25} calculated)
Growth NOEC:	0.5 g/L	
Growth LOEC:	~~1.0~~ 1.0 g/L	BA
Growth IC_{20}:	0.5756	0.573
Growth IC_{25}:	0.6012	0.5977
Ctrl % CV:	13%	
Dunnetts PMSD:	20.8%	14.9 %
Survival NOEC:	0.5 g/L	
Survival LOEC:	1.0 g/L	

Test incubated in : ☑ Incubator # 5

☐ Waterbath #____

Light Intensity : 50 - 100 ft. candles

Photoperiod : 16 light 8 dark

Temperature Range °C:

Incubator # 5 min. 24.8 max. 26.3 mean –

Water bath # ____ min.____ max.____ mean____

Room B246 min. 22.5 max 23.4 mean –

P. promelas PMSD Bounds for Growth: Lower 12 Upper 30

M. beryllina PMSD Bounds for Growth: Lower 11 Upper 28

Investigators' signatures

Kara Lov

Marshall Faircloth

Bruce Noble

James Daniel

Liz Miller

REVIEWER

Comments:

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revised 7/16/08 MF

Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or Menidia beryllina Survival

Organism: (*P. promelas*) *M.beryllina* (circle one) D = dead M = missing

Concentration: µg/L (g/L) (circle one)

			TEST DAY							
Concentration	Replicate	Chamber	0	1	2	3	4	5	6	7
Control	A	A1	10	10	10	10	9 JD	9	9	9
	B	A2	10	10	10	10	10	8 JD	8	8
	C	A3	10	10	10	10	10	10	10	10
	D	A4	10	10	10	10	10	10	10	10
.125	A	A5	10	10	10	9 JD	9	9	9	~~8~~ 9 JD
	B	A6	10	10	10	10	10	10	10	10
	C	A7	10	10	10	10	10	10	10	10
	D	A8	10	10	10	10	10	10	10	10
.25	A	A9	10	10	10	10	10	10	10	10
	B	A10	10	10	10	10	10	10	10	9 JD
	C	A11	10	10	10	10	10	10	10	10
	D	A12	10	10	10	10	10	10	10	10
.5	A	A13	10	10	9 JD	9	9	9	9	8 JD
	B	A14	10	9 JD	9	9	9	9	9	9
	C	A15	10	10	9 JD	9	9	9	9	9
	D	A16	10	10	10	10	10	10	10	10
1	A	A17	10	~~0~~ 3 JD	3	1 JD	1	1	1	1
	B	A18	10	~~0~~ 2 JD	2	2	1 JD	0 JD	—	—
	C	A19	10	~~0~~ 2 JD	2	2	2	2	0 JD	—
	D	A20	10	~~0~~ 4 JD	4	4	4	~~8~~ 5 JD	2 JD	~~2~~ 2 JD
2	A	A21	10	0 JD						
	B	A22	10	0 JD						
	C	A23	10	0 JD						
	D	A24	10	0 JD						
	A		10							
	B		10							
	C		10							
	D		10							
Loaded by (0 hr): JD		Checked by:		JD	JD	JD	JD	JD	JD	JD
Verified by (0 hr): KC										
		Concentrations prepared by:		JD	JD	JD	JD	JD	JD	JD

Comments:

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revised 7/16/08 MF

Appendix C. (continued)

Chronic SRT Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg. 1

Start Date: 6.14.11 End Date: 6.21.11

Start Time: 1300 End Time: 0830

conc = concentration rep = replicate circle umhos or mmhos

Test Day		Before Renewal							After Renewal						
	Conc.	Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth		Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth
Day 0	Control	A	8.0	25.4	7.3	325								
	.125	A	8.0	25.8	7.4	537								
	.25	A	8.1	24.9	7.6	739								
	.5	A	8.1	24.0	7.6	1218								
	1	A	8.1	24.9	7.7	2150								
	2	A	8.1	25.9	7.7	3960								
		A												
	Measured by		KC	JD	KC	KC								
	Recorded by		KC	JD	KC	KC								
		Rep	pH	Temp	DO	Conductivity	Salinity		Rep	pH	Temp	DO	Conductivity	Salinity
Day 1	Control	B	7.7	24.4	7.3	331			B	7.8	25.0	7.5	328	
	.125	B	7.8	24.2	7.3	534			B	7.9	24.6	7.0	548	
	.25	B	7.8	24.2	7.4	743			B	7.9	24.4	7.3	753	
	.5	B	7.9	24.1	7.5	1234			B	7.9	24.4	7.4	1264	
	1	B	7.9	24.8	7.2	2130			B	8.0	24.6	7.6	2150	
	2	B	7.9	24.3	7.3	3880			B					
		B							B					
	Measured by		JD	JD	JD	JD				JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD				JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity		Rep	pH	Temp	DO	Conductivity	Salinity
Day 2	Control	C	8.0	25.7	6.2	330			C	8.1	24.6	7.4	321	
	.125	C	7.9	25.6	6.3	541			C	8.0	24.6	7.4	544	
	.25	C	7.9	25.4	7.7	780			C	8.0	24.5	7.4	784	
	.5	C	7.9	25.5	6.9	1235			C	8.0	24.4	7.5	1222	
	1	C	7.9	25.2	7.2	2070			C	8.0	24.3	7.4	2100	
		C							C					
		C							C					
	Measured by		JD	JD	JD	JD				JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD				JD	KC	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity		Rep	pH	Temp	DO	Conductivity	Salinity
Day 3	Control	D	7.8	25.5	5.8	346			D	7.6	25.3	7.1	330	
	.125	D	7.8	25.3	5.9	578			D	7.7	24.0	7.0	547	
	.25	D	7.8	25.5	5.8	835			D	7.7	24.0	7.1	780	
	.5	D	7.8	25.3	5.3	1280			D	7.7	24.0	7.0	1224	
	1	D	7.8	25.1	6.5	2180			D	7.8	24.0	7.1	2120	
		D							D					
		D							D					
	Measured by		JD	JD	JD	JD				JD	KC/JD	JD	JD	
	Recorded by		JD	JD	JD	JD				JD	KC/JD	JD	JD	

Comments:

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional.

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Appendix C. (continued)

Chronic SRT Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg 2

Start Date: 6.14.11 End Date: 6.21.11

Start Time: 1300 End Time: 0830

conc = concentration rep = replicate

Test Day	Conc.	Before Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (umhos/mmhos)	Salinity ppth	After Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (umhos/mmhos)	Salinity ppth
Day 4	Control	A	7.8	25.9	6.6	339		A	7.7	25.4	7.5	333	
	.125	A	7.8	25.7	6.4	569		A	7.7	25.5	7.8	572	
	.25	A	7.8	25.4	6.3	811		A	7.8	25.7	8.0	800	
	.5	A	7.8	25.9	6.6	1277		A	7.8	25.7	8.0	1275	
	1	A	7.9	25.7	7.1	2180		A	7.9	25.9	8.3	2210	
		A						A					
		A						A					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 5	Control	B	7.8	25.8	6.2	349		B	8.0	25.9	7.7	340	
	.125	B	7.8	25.4	6.3	591		B	8.0	25.5	7.8	572	
	.25	B	7.8	25.2	6.1	826		B	8.0	25.8	8.0	827	
	.5	B	7.8	25.0	5.8	1296		B	8.0	25.8	7.9	1296	
	1	B	7.8	25.4	6.3	2250		B/C	8.0	25.9	8.0	2210	
		B						B					
		B						B					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 6	Control	C	7.8	25.9	6.8	339		C	7.6	25.4	7.5	373	
	.125	C	7.7	25.7	6.8	573		C	7.8	25.7	7.7	~~407~~ JD 568	
JD	~~.25~~ .25	C	7.8	25.8	6.7	815		C	7.8	25.5	7.7	~~462~~ JD 785	
	.5	C	7.8	25.7	6.6	1294		C	7.8	25.4	7.7	1240	
	1	C	7.8	25.9	6.6	2230		E.D	7.9	25.9	7.8	2140	
		C						C					
		C						C					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 7	Control	D	7.6	25.9	7.5	336		D					
	.125	D	7.6	25.9	7.4	570		D					
	.25	D	7.6	25.9	6.7	801		D					
	.5	D	7.6	25.9	6.8	1271		D					
	1	D	7.7	25.9	7.5	2190		D					
								D					
								D					
	Measured by		KC	JD	KC	KC							
	Recorded by		KC	JD	KC	KC							

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional

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Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or *Menidia beryllina* Weights

SOP TA06_21

Organism: (*P. promelas*) *M.beryllina* (circle one)

Concentration: µg/L (g/L) (circle one)

mean wt = fish wt divided by 10

convert g to mg: move decimal 3 places to right

Concentration	Replicate	Pan #	Pan wt. (g)	Pan + Fish (g)	Fish wt. (g)	Original # of fish	Mean wt. (mg)
Control	A	25	0.02126	0.02666	0.00540	10	.540
	B	26	0.02248	0.02771	0.00523	10	.523
	C	27	0.02170	0.02862	0.00692	10	.692
	D	28	0.02337	0.02941	0.00604	10	.604
.125	A	29	0.02275	0.02752	0.00477	10	.477
	B	30	0.02240	0.02827	0.00587	10	.587
	C	31	0.02365	0.02988	0.00623	10	.623
	D	32	0.02266	0.02821	0.00555	10	.555
.25	A	33	0.02319	0.02916	0.00597	10	.597
	B	34	0.02301	0.02881	0.00580	10	.580
	C	35	0.02204	0.02698	0.00494	10	.494
	D	36	0.02188	0.02696	0.00508	10	.508
.5	A	37	0.02240	0.02771	0.00531	10	.531
	B	38	0.02208	0.02796	0.00588	10	.588
	C	39	0.02181	0.02791	0.00610	10	.610
	D	40	0.02249	0.02837	0.00588	10	.588
1	A	41	0.02317	0.02351	0.00034	10	0.034
	B	—	—	—	—	10	—
	C	—	—	—	—	10	—
	D	42	0.02260	0.02299	0.00039	10	.039
2	A					10	
	B					10	
	C					10	
	D					10	
	A					10	
	B					10	
	C					10	
	D					10	
		Weighed by:	JD	JD			

Comments:

Note: Pan number (#) refers to actual Pan number.

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revised 8/24/06 MF/SP

Appendix C. (continued)

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or *Menidia beryllina* QC

SOPs TA07_09, TA07_14 and TA06_21

all weights in grams

(A) Fish + Pan

~~Empty~~ Pan Weights Date: 6.24.11 Weighed by: JD

	Pan 1 wt.	Pan 1 initial wt.
after Pan 5	0.02663	@ 0.2666
after Pan 10	0.02661	@ 0.2666
after Pan 15	0.02661	@ 0.2666
after Pan 20	0.02662	@ 0.2666
after Pan 25		@
after Pan 30		@
after Pan 35		@
after Pan 40		@
after Pan 45		@
after Pan 48		@

	Measured Value	S-weight Value
S-weights	0.01	@ 0.01
	0.03003	@ 0.03

Balance: Ohaus 250D Ser# 1125481084

S-Weights: BIO-S-Weights-01

(A) Empty

~~Fish + Pan~~ Weights Date: 6.20.11 Weighed by: JD

	Pan 1 wt.	Pan 1 initial wt.
after Pan 5	0.02124	@ 0.02126
after Pan 10	0.02127	@ 0.02126
after Pan 15	0.02125	@ 0.02126
after Pan 20	0.02126	@ 0.02126
after Pan 25		@
after Pan 30		@
after Pan 35		@
after Pan 40		@
after Pan 45		@
after Pan 48		@

	Measured Value	S-weight Value
S-weights	0.00999	@ 0.01
	0.05006	@ 0.05

Drying Oven ID: ER034000

Temperature °C: 100

Drying Hours: 8

Note: Pan number (5, 10, etc.) refers to sequence of weighing as actual Pan #s may differ.

Comments:

(A) Transcription ERROR. -KC-
↳ Table at the top is the "Fish + Pan" weights & the bottom table lists the "Empty Pan" weights.

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revised 7/16/08 MF

Test ID: P. promelas SRT Dates: 6.14.11 – 6.21.11

METER / DEVICE	ID	SOP	Parameter	Method	v used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 (#5) SN61990624	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓ #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	✓
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	✓

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT–Chronic SRT)

entered 6/21/11 by Kara Cox

V 1.1 9/21/10 MF/jd

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Appendix D. Test Organism Receipts

MARINCO BIOASSAY LABORATORY
The Aquatic Toxicology Specialists

NELAP Certification # E84191

Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Shipping Date: 6/13/11

Ship to: FDEP

P.O. No: _____

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (‰)
Mysidopsis bahia						
Menidia beryllina						
						Hardness mg/L
Cyprinella leedsi	200 ac	7-10 DAYS	CL 110GE1	25	7.8	60
Pimephales promelas	600	< 24 Hrs	FM 110612-1715	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: PM

Shipped Via: Fed Ex

Notes: _____

Thank you for your order.

4569 Samuel Street · Sarasota, FL 34233 · Tel: 941-925-3594 · Fax: 941-922-3874 · Web: www.biologylab.com

Appendix D. (continued)

MARINCO BIOASSAY LABORATORY
The Aquatic Toxicology Specialists

NELAP Certification # E84191

Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Shipping Date: 6/20/11

Ship to: FDEP

P.O. No: _____

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (‰)
Mysidopsis bahia						
Menidia beryllina						
						Hardness mg/L
Cyprinella leedsi						
Pimephales promelas	600	<24Hrs	FM 110619-1620	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: PM

Shipped Via: Fed Ex

Notes: _____

Thank you for your order.

4569 Samuel Street · Sarasota, FL 34233 · Tel: 941-925-3594 · Fax: 941-922-3874 · Web: www.biologylab.com

Appendix E. Chemical Analyses performed on the combined effluent from the City of Clearwater East and Northeast AWWTFs common Outfall D-001, sampled on June 20, 2011.

Date Sampled	Sample Location	Analysis Group	Components	Result	Remark	Units	MDL	PQL
6/20/2011 9:55	D-001	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
6/20/2011 9:55	D-001	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	2.7		ug/L	0.56	1.7
6/20/2011 9:55	D-001	Overflow	Fecal Coliforms-Membrane Filter	1	U	cfu/100 mL	1	
6/20/2011 9:55	D-001	Pesticides	Metribuzin	0.1	U	ug/L	0.1	0.4
6/20/2011 9:55	D-001	Pesticides	Ethion	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Metolachlor	0.5	U	ug/L	0.5	2
6/20/2011 9:55	D-001	Pesticides	Naled	0.81	UJ	ug/L	0.81	3.2
6/20/2011 9:55	D-001	Pesticides	Simazine	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Phorate	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Prometryn	0.15	U	ug/L	0.15	0.6
6/20/2011 9:55	D-001	Pesticides	Ametryn	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Atrazine	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Fonofos	0.1	U	ug/L	0.1	0.4
6/20/2011 9:55	D-001	Pesticides	Malathion	0.15	U	ug/L	0.15	0.6
6/20/2011 9:55	D-001	Pesticides	Parathion Ethyl	0.15	U	ug/L	0.15	0.6
6/20/2011 9:55	D-001	Pesticides	Norflurazon	0.15	U	ug/L	0.15	0.6
6/20/2011 9:55	D-001	Pesticides	Parathion Methyl	0.1	U	ug/L	0.1	0.4
6/20/2011 9:55	D-001	Pesticides	Bromacil	0.2	U	ug/L	0.2	0.81
6/20/2011 9:55	D-001	Pesticides	Chlorpyrifos Ethyl	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Fenamiphos	0.2	U	ug/L	0.2	0.81
6/20/2011 9:55	D-001	Pesticides	Hexazinone	0.1	U	ug/L	0.1	0.4
6/20/2011 9:55	D-001	Pesticides	Azinphos Methyl	0.2	U	ug/L	0.2	0.81
6/20/2011 9:55	D-001	Pesticides	Chlorpyrifos Methyl	0.1	U	ug/L	0.1	0.4
6/20/2011 9:55	D-001	Pesticides	Ethoprop	0.1	U	ug/L	0.1	0.4
6/20/2011 9:55	D-001	Pesticides	Metalaxyl	0.25	U	ug/L	0.25	1
6/20/2011 9:55	D-001	Pesticides	Diazinon	0.05	U	ug/L	0.05	0.2
6/20/2011 9:55	D-001	Pesticides	Mevinphos	0.2	U	ug/L	0.2	0.81
6/20/2011 9:55	D-001	Pesticides	Alachlor	0.6	U	ug/L	0.6	2.4
6/20/2011 9:55	D-001	Pesticides	Butylate	0.2	U	ug/L	0.2	0.81
6/20/2011 9:55	D-001	Priority Organic Pollutants	2,4-Dimethylphenol	10	U	ug/L	10	40
6/20/2011 9:55	D-001	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	3	U	ug/L	3	12

Appendix E. (continued)

Date Sampled	Sample Location	Analysis Group	Components	Result	Remark	Units	MDL	PQL
6/20/2011 9:55	D-001	Priority Organic Pollutants	Butyl benzyl phthalate	5	U	ug/L	5	20
6/20/2011 9:55	D-001	Priority Organic Pollutants	Chrysene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	2	U	ug/L	2	8
6/20/2011 9:55	D-001	Priority Organic Pollutants	2-Chloronaphthalene	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	4-Bromophenyl phenyl ether	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	4-Nitrophenol	15	U	ug/L	15	60
6/20/2011 9:55	D-001	Priority Organic Pollutants	N-Nitrosodimethylamine	2	U	ug/L	2	8
6/20/2011 9:55	D-001	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	3	U	ug/L	3	12
6/20/2011 9:55	D-001	Priority Organic Pollutants	Pyrene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	2,4,6-Trichlorophenol	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Di-n-butyl phthalate	5	U	ug/L	5	20
6/20/2011 9:55	D-001	Priority Organic Pollutants	Pentachlorophenol	3	U	ug/L	3	12
6/20/2011 9:55	D-001	Priority Organic Pollutants	2-Chlorophenol	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	3	U	ug/L	3	12
6/20/2011 9:55	D-001	Priority Organic Pollutants	Acenaphthene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Fluorene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Phenol	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	2,4-Dinitrotoluene	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	2-Nitrophenol	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Benzidine	100	U	ug/L	100	400
6/20/2011 9:55	D-001	Priority Organic Pollutants	Bis(2-chloroethyl)ether	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Di-n-octyl phthalate	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	2	U	ug/L	2	8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Acenaphthylene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Benzo(a)anthracene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Benzo(a)pyrene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Diethyl phthalate	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Dimethyl phthalate	10	U	ug/L	10	40
6/20/2011 9:55	D-001	Priority Organic Pollutants	Fluoranthene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Hexachlorobutadiene	3	U	ug/L	3	12
6/20/2011 9:55	D-001	Priority Organic Pollutants	Hexachloroethane	3	U	ug/L	3	12
6/20/2011 9:55	D-001	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	2,4-Dichlorophenol	1	U	ug/L	1	4

Appendix E. (continued)

Date Sampled	Sample Location	Analysis Group	Components	Result	Remark	Units	MDL	PQL
6/20/2011 9:55	D-001	Priority Organic Pollutants	Anthracene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Benzo(b)fluoranthene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Hexachlorocyclopentadiene	3	U	ug/L	3	12
6/20/2011 9:55	D-001	Priority Organic Pollutants	Phenanthrene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	1,2,4-Trichlorobenzene	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	2,4-Dinitrophenol	15	U	ug/L	15	60
6/20/2011 9:55	D-001	Priority Organic Pollutants	2,6-Dinitrotoluene	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	3,3'-Dichlorobenzidine	40	UJ	ug/L	40	160
6/20/2011 9:55	D-001	Priority Organic Pollutants	4-Chloro-3-methylphenol	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Benzo(k)fluoranthene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	15	U	ug/L	15	60
6/20/2011 9:55	D-001	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Hexachlorobenzene	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Isophorone	1	U	ug/L	1	4
6/20/2011 9:55	D-001	Priority Organic Pollutants	Naphthalene	0.2	U	ug/L	0.2	0.8
6/20/2011 9:55	D-001	Priority Organic Pollutants	Nitrobenzene	2	U	ug/L	2	8
6/20/2011 9:55	D-001	Field Parameter	Temperature	29.04		C		
6/20/2011 9:55	D-001	Field Parameter	pH	7.78				
6/20/2011 9:55	D-001	Field Parameter	Specific Conductance	1199		umhos/cm		
6/20/2011 9:55	D-001	Field Parameter	Dissolved Oxygen	8.63		mg/L		
6/20/2011 9:35	EFB-01	Nutrients	TSS	2	U	mg/L	2	10
6/20/2011 17:30	D-001	AGP/LN	Algal Growth Potential	5.72		mg DryWt/L	0.3	0.9
6/20/2011 17:30	D-001	AGP/LN	Algal Growth Potential-Salinity Adjusted	15.5		mg DryWt/L	3	3
6/20/2011 17:30	D-001	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	0.91	I	mg/L	0.2	2
6/20/2011 17:30	D-001	Toxicology	Bioassay-Chron-Def-FW-C.dubia, IC25	No Result		IC25		
6/20/2011 17:30	D-001	Toxicology	Bioassay-Chron-Def-FW-Fish, NOEC	100		NOEC		
6/20/2011 17:30	D-001	Toxicology	Bioassay-Chron-Def-FW-Fish, IC25	100	L	IC25		
6/20/2011 17:30	D-001	Toxicology	Bioassay-Chron-Def-FW-C.dubia, NOEC	No Result		NOEC		
6/20/2011 17:30	D-001	Metals	Iron	30	U	ug/L	30	120
6/20/2011 17:30	D-001	Metals	Calcium	87	A	mg/L	0.075	0.3
6/20/2011 17:30	D-001	Metals	Magnesium	10.8	A	mg/L	0.04	0.16
6/20/2011 17:30	D-001	Metals	Chromium	0.9	U	ug/L	0.9	3.6
6/20/2011 17:30	D-001	Metals	Selenium	0.6	U	ug/L	0.6	2.4

Appendix E. (continued)

Date Sampled	Sample Location	Analysis Group	Components	Result	Remark	Units	MDL	PQL
6/20/2011 17:30	D-001	Metals	Arsenic	2.2	I	ug/L	0.75	3
6/20/2011 17:30	D-001	Metals	Sodium	136	A	mg/L	0.5	2
6/20/2011 17:30	D-001	Metals	Zinc	34	A	ug/L	5	20
6/20/2011 17:30	D-001	Metals	Cadmium	0.15	U	ug/L	0.15	0.6
6/20/2011 17:30	D-001	Metals	Lead	0.6	U	ug/L	0.6	2.4
6/20/2011 17:30	D-001	Metals	Silver	0.075	U	ug/L	0.075	0.3
6/20/2011 17:30	D-001	Metals	Aluminum	66	A	ug/L	15	60
6/20/2011 17:30	D-001	Metals	Copper	3.2	A	ug/L	1.5	3
6/20/2011 17:30	D-001	Metals	Nickel	1.1	I	ug/L	0.75	3
6/20/2011 17:30	D-001	Nutrients	Organic Carbon	6.8		mg C/L	1	5
6/20/2011 17:30	D-001	Nutrients	Kjeldahl Nitrogen	0.78		mg N/L	0.08	0.2
6/20/2011 17:30	D-001	Nutrients	Ammonia-N	0.036		mg N/L	0.01	0.02
6/20/2011 17:30	D-001	Nutrients	Chloride	190		mg Cl/L	2	5
6/20/2011 17:30	D-001	Nutrients	Fluoride	0.62		mg F/L	0.035	0.1
6/20/2011 17:30	D-001	Nutrients	Total-P	0.74		mg P/L	0.005	0.05
6/20/2011 17:30	D-001	Nutrients	NO2NO3-N	0.4		mg N/L	0.004	0.01
6/20/2011 17:30	D-001	Nutrients	Alkalinity	195		mg CaCO3/L	0.65	2.5
6/20/2011 17:30	D-001	Nutrients	TSS	3	I	mg/L	2	10
6/20/2011 17:30	D-001	Nutrients	Color (true)	6.6		PCU	2	6
6/20/2011 17:30	D-001	Nutrients	Sulfate	86		mg SO4/L	2	5
6/20/2011 17:30	D-001	Priority Organic Pollutants	Bromodichloromethane	39		ug/L	0.2	1
6/20/2011 10:10	BLANK	Priority Organic Pollutants	Bromodichloromethane	0.2	U	ug/L	0.2	1
6/19/2011 17:30	D-001	Priority Organic Pollutants	Bromodichloromethane	0.2	U	ug/L	0.2	1
7/25/2011 5:00	D-001	Toxicology	Bioassay-Chron-Def-FW-C.dubia, IC25	100	L	IC25		
7/25/2011 5:00	D-001	Toxicology	Bioassay-Chron-Def-FW-C.dubia, NOEC	100		NOEC		

Appendix F. Facility Summary, Effluent Limits, and Permit Violations Recorded on Discharge Monitoring Report

State of Florida
Department of Environmental Protection
Facility Introduction & Summary

PART I: Information gathered prior to sampling event	
Prepared By: Jason Cohen Jason.Cohen@dep.state.fl.us	**Contact Number:** 813-632-7600 x361
Facility Name (as it appears on permit): City of Clearwater East AWWTF City of Clearwater Northeast AWWTF	**Former Names:**
NPDES Permit Number: FL0021865 (East) FL~~0128~~37 (Northeast) 0128937	**Permit Expiration Date:** March 18, 2012 (both)
Physical Address: 3141 Gulf to Bay Boulevard Clearwater, Fl 34619 (East) 3290 State Road 580 Clearwater, Fl 34695 (Northeast)	
District: Southwest	**County:** Pinellas
Function of Facility: Type I domestic AWWTF	
Facility Type: Major	**Permitted Capacity (MGD):** 5.0 (East) 13.5 (Northeast)
Mean Flow for previous 12 months for all EXTERNAL outfalls listed in permit (list date range including months): May 2010 – April 2011 D-001 : 2.8 MGD	
If applicable, Mean Flow for previous 12 months for REUSE outfall(s) (list date range including months):	
Description of type of Discharge: ☒**Continuous** ☐ **Intermittent** ☐ **Seasonal** ☐ **Rainfall Dependent** ☐ **Other, please specify**	

Appendix F. (continued)

Description of Sampling Location(s) - actual permit designation of permitted sampling point(s): **These facilities share a common outfall point, D-001 as described below. This is information from the East facility only.** EFD- 01 After dechlorination and prior to final discharge
Description of Permitted Outfall(s): **These facilities share a common outfall point, D-001 at the East facility. This is information from the East facility only.** An existing 5.0 MGD AADF permitted discharge to Old Tampa Bay (Class II marine waters and Outstanding Florida Water) at Discharge Location D-001, which is approximately 1,400 feet in length and discharges at a depth of approximately 10 feet. The point of discharge is located approximately at latitude 27 ° 57' 19"N and longitude 82 ° 42' 24"W

Appendix F. (continued)

Description of Treatment Process (if multiple discharge points, include a detailed map or diagram of facility):

These facilities share a common outfall point, D-001 as described above.

EAST:
Type I domestic advanced wastewater treatment facility (AWWTF), using the Bardenpho BNR process, consisting of the following components: an onsite influent pumping station with four variable rate submersible pumps; a transit time dopples meter attached to the influent pipeling to measure flow; preliminary treatment consisting of two mechanically cleaned fine bar screens, a three unit vortex-cyclonic grit removal sustem with associated grits classifier, and an influent flow measurement via a 24-inch Parshall flume with an ultrasonic flow meter, a biological treatment process consisting of a five stage Bardenpho BNR process that includes four 83, 580 gallon fermentation basins, eight 81,985 gallon anoxic reactors, a screw-lift pumping station comprised of three 60-inch Archimedes screws, one 1.967 MG Carrousel oxidation ditch aeration basin, seven 84, 615 gallon second-anoxic basins, and one 84,300-gallon re-aeration basin; two 100-foot diameter secondary clarifiers; polishing filtration consisting of a four unit filter pumping station and six rapid, single-media, gravity-type, automatic backwash filters with a total surface area of 2,160 square feet; an effluent disinfection sustem using gaseous chlorination consisting of a 10,016-gallonchlorine dosing chamber, a 115,379 gallon, dual channel chlorine contact basin..

Chlorinated effluent from the chlorine contact basin is directed to the on-site 5 MG reclaimed water storage tank and the Master Reuse System (R-001) or piped to Clearwater's East AWWTF and directed to a mixing basin and combined with chlorinated effluent from the East AWWTF. The combined chlorinated effluent streams then flow through a 118,000 gallon dechlorination basin that uses flow-paced sulfur dioxide to eliminate the remaining chlorine residual, then through a 143,000 gallon dissolved oxygen boost re-aeration basin, to an outfall junction/sampling box and finally through 1,400-foot long, 48-inch diameter outfall pipe that discharges to Old Tampa Bay.

Waste sludge from the primary clarifiers is pumped to two 108,000 gpd rotary drum thickeners equipped with polymer injection. Thickened sludge is then pumped to a 55,000 gallon sludge holding tank. Thickened sludge is transferred to the Northeast AWWTF for final treatment and dewatering.

NORTHEAST:
An existing 13.5 MGD annual average daily flow (AADF) Type I domestic advanced wastewater treatment facility (AWWTF), using the Bardenpho BNR process, consisting of the following components: preliminary treatment consisting of three mechanically cleaned fine bar screens and an influent flow measurement via a 48-inch Parshall flume with an ultrasonic flow meter; primary treatment consisting of sedimentation in two 75-foot diameter basins; a two unit hydro-cyclone grit removal system with associated grit classifier; four primary sludge gravity thickeners; a biological treatment process consisting of a five stage Bardenpho BNR process that includes two 589,000 gallon fermentation basins, two 945,200 gallon first anoxic reactors, a screw lift pumping station with five 84-inch diameter Archimedes screw lifts, two 1,700,000 gallon Carrousel oxidation ditch aeration basins, ten 190,740 gallon second anoxic basins, and ten 33,660 gallon reaeration basins; eight 75-foot diameter secondary clarifiers configured in two sets of four basins; polishing filtration consisting of 12 rapid sand, pulsed filtration gravity-type, automatic backwash filters with a total surface area of 4,320 square feet; an effluent disinfection system using liquid sodium hypochlorite and a 402,000 gallon, dual channel chlorine contact basin. Also on-site are a 5 MG reclaimed water storage tank and a 3.5 MG reject water storage tank.

Chlorinated effluent from the chlorine contact basin is directed to the on-site 5 MG reclaimed water storage tank and the Master Reuse System (R-001) or piped to Clearwater's East AWWTF and directed to a mixing basin and combined with chlorinated effluent from the East AWWTF. The combined chlorinated effluent streams then flow through a 118,000 gallon dechlorination basin that uses flow-paced sulfur dioxide to eliminate the remaining chlorine residual, then through a 143,000 gallon dissolved oxygen boost re-aeration basin, to an outfall junction/sampling box and finally through 1,400-foot long, 48-inch diameter outfall pipe that discharges to Old Tampa Bay.

Waste sludge from the primary clarifiers is pumped to four 18-foot diameter gravity thickeners. The thickened primary sludge is then transferred to two anaerobic digesters with a combined capacity of 1,944,800 gallons. Waste sludge from the secondary clarifiers is pumped to two rotating drum thickeners equipped with polymer injection, then to four 224,600 gallon aerobic digesters where it is combined with thickened sludge trucked in from the East AWWTF. Thickened sludge from the anaerobic and aerobic digesters is sent to two 160,000 gallon sludge blend tanks. The blended sludge is then dewatered using three 2-meter belt filter presses.

Appendix F. (continued)

PART I: Continued

List current Effluent Limits of Outfall(s) to be Sampled: This should include any AO or CO limits the facility may be under (Leave cell blank if not applicable)

			Effluent Limitations				Monitoring Requirements			
Parameter	Units	Max/Min	Annual Average	Monthly Average	Weekly Average	Single Sample	Monitoring Frequency	Sample Type	Monitoring Location Site Number	Notes
Flow (D-001)	MGD	Maximum	13.5	Report	-	-	Continuous	Recording flow meters and totalizers	FLW-02	See Cond.I.A.4
BOD, Carbonaceous 5 day, 20C	MG/L	Maximum	5.0	6.25	7.5	10.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
Solids, Total Suspended	MG/L	Maximum	5.0	6.25	7.5	10.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
Nitrogen, Total (as N)	MG/L	Maximum	3.0	3.75	4.5	6.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
Solids, Total Suspended	MG/L	Maximum	-	-	-	5.0	7 Days/Week	Grab	EFB-01	
pH	SU	Range	-	-	-	6.5 to 8.5	Continuous	Meter	EFD-01	See Cond.I.A.3
Coliform, Fecal, % less than detection	PERCENT	Minimum		75			Monthly	Calculation	EFA-01	See Cond.I.A.5
Coliform, Fecal	#/100ML	Maximum				25	7 Days/Week	Grab	EFA-01	See Cond.I.A.5
Oxygen, Dissolved (DO)	MG/L	Minimum	-	-	-	4.0	Daily	Grab	EFD-01	
Total Residual Chlorine (For Disinfection)	MG/L	Minimum	-	-	-	1.0	Continuous	Meter	EFA-01	See Cond.I.A. 6
Total Residual Chlorine (For Dechlorination)	MG/L	Maximum	-	-	-	0.01	Continuous	Meter	EFD-01	
Copper, Total Recoverable	UG/L	Maximum	-	-	-	3.7	Monthly	Grab	EFD-01	
Dichlorobromomethane	UG/L	Maximum	43	-	-	-	Monthly	Grab	EFD-01	See Cond.I.A.9
Chronic Whole Effluent Toxicity	Per-cent	Minimum	-	-	-	100	Quarterly	24-hour flow proportioned composite	EFD-01	See Cond. I. A. 7.

Appendix F. (continued)

PART I: Continued

Receiving Waters:	Classification (indicate whether fresh or marine):
Old Tampa Bay	Class II OFW

Toxicity Test Requirements (routine and/or additional test language, test species, salinity adjustment, etc.):

Chronic Whole Effluent Toxicity Testing

The permittee shall comply with the following whole effluent toxicity testing requirements and initiate the series of tests described below within 60 days of permit issuance, unless otherwise directed by the Department in writing, to evaluate chronic whole effluent toxicity of the discharge from outfall D-001 to Old Tampa Bay.

a. Effluent Limitation

1. A No Observed Effect Concentration (NOEC) of greater than or equal to 100% effluent in any routine test or any additional follow-up test shall constitute compliance with these permit conditions and Rule 62-302.530(62), F.A.C.
2. A NOEC of less than 100% effluent in any routine test or additional test shall constitute a failure of the test. The permittee shall notify the Department via telephone or e-mail within 24 hours of notification that a routine or additional test failed.

b. Monitoring Frequency

The "routine" toxicity tests specified shall be conducted *once every three months.*

c. Routine Test Requirements

1. **The permittee shall conduct a daphnid, *Ceriodaphnia dubia*, Survival and Reproduction Test and a fathead minnow, *Pimephales promelas*, Larval Survival and Growth Test, concurrently.**
2. **All test species, procedures and quality assurance criteria used shall be in accordance with Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th ed., EPA-821-R-02-013, or the most current edition. Any deviation of the toxicity procedures outlined herein shall be submitted in writing to the Department for review and approval prior to use.**
3. **The control water and dilution water used shall be moderately hard water as described in EPA-821-R-02-013, Section 7, or the most current edition.**
4. All routine tests shall be conducted using a control (0% effluent) and a minimum of five test concentrations: 100%, 50%, 25%, 12.5%, and 6.25% final effluent.

d. Sampling Requirements

1. **For each routine test or additional test required, a total of three 24-hour, flow-proportioned composite samples of final effluent shall be collected and used per the sampling protocol discussed in EPA-821-R-02-013, Section 8, or the most current edition. The first sample shall be used to initiate the test. The remaining two composite samples shall be collected according to the protocol and used as renewal solutions on Day 3 (48 hours) and Day 5 (96 hours) of the test. If the duration of the discharge is less than 24-hours, the duration of discharge shall be documented on the chain of custody.**

e. Additional Testing Requirements, if required

1. **If chronic toxicity (an NOEC of less than 100% effluent) is found in any routine test, the permittee shall conduct two additional definitive tests on each species indicating an NOEC of less than 100% effluent.**
2. **Each additional test shall be conducted using a control (0% effluent) and a minimum of five dilutions: 100%, 50%, 25%, 12.5% and 6.25% effluent. The dilution series may be modified in the second test to more accurately bracket the toxicity, such that at least two dilutions above (not to exceed 100% effluent) and two dilutions below the target concentration and a control (0% effluent) are run. All test results shall be statistically analyzed according to the Appendices in EPA-821-R-02-013, or the most current edition.**
3. **The first additional test shall be initiated within two weeks of the end of the failed routine test. The second valid additional test shall be conducted one week thereafter. The additional tests will be used to evaluate the persistence of the observed toxicity.**

Administrative or Consent Orders (date executed, specific requirements, interim limit dates-if applicable, etc.):

Facility Mixing Zone Details (if applicable):

The permittee is granted a mixing zone for Dichlorobromomethane consisting of a distance of two meters in circumference around the entire centerline of the outfall.

List Permit Violations (DMR data) and plant upsets that occurred at the plant within the last year:

Appendix F. (continued)

Monitoring Date	Parameter Description	Concentration (1)	Concentration Limit (1)	Concentration Statistical Base (1)	Concentration Units
8/31/2007	NOEL STATRE 7DAY CHR CERIODAPHNIA	<100	100	MINIMUM	PER- CENT
9/30/2007	NOEL STATRE 7DAY CHR CERIODAPHNIA	<100	100	MINIMUM	PER- CENT
9/30/2007	NOEL STATRE 7DAY CHR CERIODAPHNIA	<100	100	MINIMUM	PER- CENT
2/29/2008	NITROGEN, TOTAL	2.80	3.75	MO AVG	MG/L
10/31/2008	COPPER, TOTAL RECOVERABLE	4.6	3.7	MAXIMUM	UG/L
10/31/2008	NOEL STATRE 7DAY CHR PIMEPHALES	<100	100	MINIMUM	PER- CENT
1/31/2010	NOEL STATRE 7DAY CHR CERIODAPHNIA	100	100	MINIMUM	%
7/31/2010	NOEL STATRE 7DAY CHR CERIODAPHNIA	100	100	MINIMUM	%
8/31/2010	NOEL STATRE 7DAY CHR CERIODAPHNIA	100	100	MINIMUM	%

Describe previous FYI and WQBEL study:

Describe previous or current Enforcement Actions (including WLs):

Describe any violations or problems noted in previous inspection:

Discuss whether DMR trends within previous five years are improving or declining:

Additional Comments (this should include a trend of the Effluent, i.e. if the facility is now Reuse):

Appendix F. (continued)

State of Florida
Department of Environmental Protection
Facility Introduction & Summary

PART II: Sampling Event Information	
Date(s) sample collected: 6/19/11 6/21/11 6/23/11	**Sampler(s):** Jason Cohen Josephine Molé Michele Duggan
Temperature (Deg C): 29.64*	**pH (SU):** 7.78*
Dissolved Oxygen (mg/L): 8.63*	**Conductivity (umhos/cm):** 1199*
Total Residual Chlorine (mg/L):	**Total Residual Chlorine - after Disinfection (mg/L):**
Method of Chlorination: Sodium hypochlorite	**Method of De-chlorination:** Sodium bisulfate
Flow During Sample Collection for each outfall(MGD): D-001: 2.5 MGD*	
Mean Flow for each outfall for previous 12 months based on sampling date (If samples are taken on more than one day, indicate Mean Flow for each day): D-001: 2.8 MGD	
Describe Discharge during Sampling Event: ☒ **Continuous** ☐ **Intermittent** ☐ **Seasonal** ☐ **Rainfall Dependent** ☐ **Other, please specify** *: values only pertain to the 6/19/2011 sampling event	

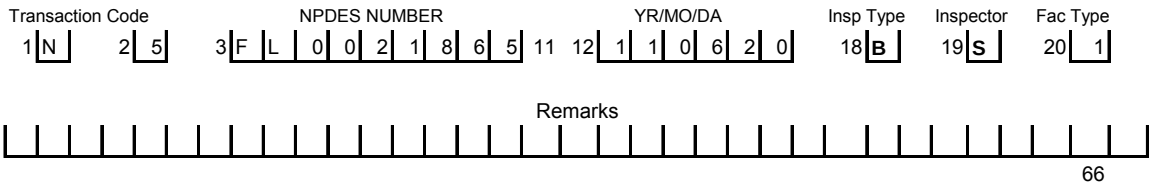
Appendix F. (continued)

Describe any conditions, difficulties or abnormal events that occurred during the sample collection and any other additional comments here:

Appendix G. EPA End Sheet

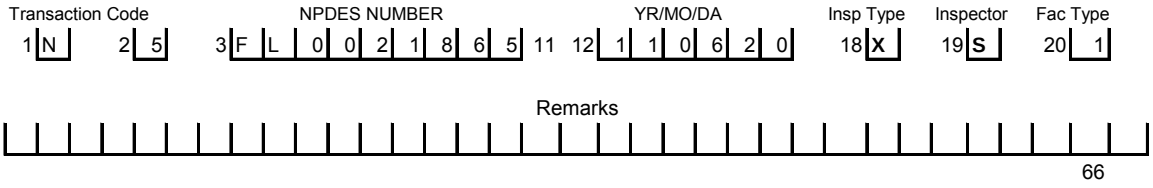
The Bioassay of the combined outfall of City of Clearwater East and Northeast AWWTFs, discharging from the East facility and sampled on June 20, 22, and 24, and July 25, 27, and 29, 2011. NPDES #FL0021865 and FL0128937.

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)



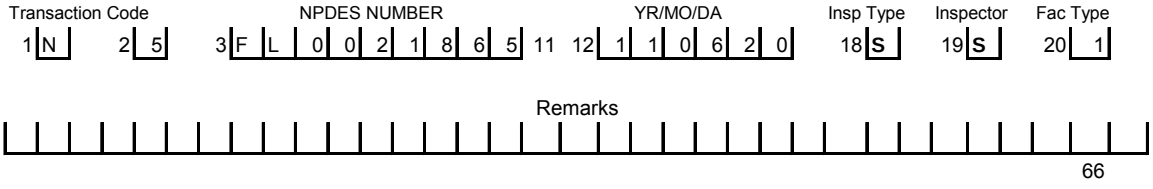
The Priority Pollutants Analysis of the combined outfall of City of Clearwater East and Northeast AWWTFs, sampled on June 20, 2011. NPDES #FL0021865 and FL0128937.

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)



Biological Analyses of the combined outfall of City of Clearwater East and Northeast AWWTFs, sampled on June 20, 2011. NPDES #FL0021865 and FL0128937.

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)



Appendix H. EPA End Sheet for *Ceriodaphnia dubia* test repeat.

The Bioassay of the combined outfall of City of Clearwater East and Northeast AWWTFs, discharging from the East facility and sampled on July 25, 27, and 29, 2011. NPDES #FL0021865 and FL0128937.

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)

Transaction Code	NPDES NUMBER	YR/MO/DA	Insp Type	Inspector	Fac Type
1 N 2 5	3 F L 0 0 2 1 8 6 5 11	12 1 1 0 7 2 5	18 **B**	19 **S**	20 1

Remarks

C e r i o d a p h n i a d u b i a t e s t r e p e a t

66

Priority Pollutants Samples Not Collected.

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)

Transaction Code	NPDES NUMBER	YR/MO/DA	Insp Type	Inspector	Fac Type
1 N 2 5	3 F L 0 0 2 1 8 6 5 11	12 1 1 0 7 2 5	18 **X**	19 **S**	20 1

Remarks

66

Biological Samples Not Collected.

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)

Transaction Code	NPDES NUMBER	YR/MO/DA	Insp Type	Inspector	Fac Type
1 N 2 5	3 F L 0 0 2 1 8 6 5 11	12 1 1 0 7 2 5	18 **S**	19 **S**	20 1

Remarks

66