

Effluent Quality Assessment

Winter Garden WWTF

Orange County

NPDES #FL0020109

Sampled August 22, 2011

February 2012

Biology Section
Bureau of Laboratories
Division of Environmental Assessment and Restoration

Quality Manual No. 870346G
NELAC Certification No. E31780

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Winter Garden Wastewater Treatment Facility (WWTF), 101 East Crest Avenue, Winter Garden, Orange County, Florida, 34777. NPDES #FL0020109. Effluent grab samples for biological and chemical analyses for this facility were collected on August 22, 2011.

Introduction

Winter Garden WWTF is a 3.5 MGD annual average daily flow design capacity advanced domestic wastewater treatment facility consisting of flow equalization, influent screening, grit removal, biological nutrient removal (via a 5-stage Bardenpho process), chemical feed facilities, clarification, filtration, disinfection by chlorination, with thickening, aerobic digestion, and dewatering of residuals. (See Appendix F for detailed facility information provided by Blake Vahlsing of FDEP Central District.)

Methods

Samples were collected from the EFD-1 NPDES sampling point for Outfall D-001 by Phil Kane (FDEP Central 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 96-hour acute definitive toxicity tests and algal growth potential (AGP) test were performed following internal DEP SOPs TA07_01, TA07_02, 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.

Results and Discussion

- **Toxicity Test Results**
  Tests were performed August 23 - August 27, 2011.

Ceriodaphnia dubia 96-hour acute definitive bioassay –	LC_{50} > 100%*, 0%** mortality in 100% sample at 96 hours**
Cyprinella leedsi 96-hour acute definitive bioassay –	LC_{50} > 100%, 20%** mortality in 100% sample at 96 hours

  The bioassay samples were not acutely toxic to the *Ceriodaphnia dubia* or the *Cyprinella leedsi* test organisms.
  *The *Ceriodaphnia dubia* test showed an interrupted dose response, producing 50% mortality in both the 12.5% and 25% concentration. A cause for this response was not determined and is thought to be a Type I error (assuming that the treatment is significantly different from the control when it is not (the treatment is considered anomalous)).

  **NOTE: In a 96-hour acute definitive test of 100% effluent, mortality of <20% provides confidence that the effluent meets the acute toxicity criterion of Florida's Surface Water Quality Standards (Rule 62-302.500 (1) (a) 4, F.A.C.). Mortality between 20-50% indicates low to moderate levels of toxicity, and further action may be required. Mortality of >50% indicates that the effluent fails to meet the minimum requirement to discharge to waters of the state (Rule 62-4.244(3) (a), F.A.C.).

  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 78.9 mg dry wt/L of the unicellular green alga, *Pseudokirchneriella subcapitata*. 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).

See Table 1 AGP results.

- **Chemistry Results**
  Total residual chlorine and total ammonia were not detected in the bioassay sample in the bioassay laboratory. The total ammonia concentration in the sample collected and preserved for chemical analysis was 0.088 mg N/L. Based on the pH, salinity, and temperature of the effluent as collected, the calculated unionized ammonia concentration was < 0.02 mg N/L

  Copper, iron and nickel were detected in the effluent at levels that comply with Class III freshwater quality criteria (62-302.530, F.A.C.) and/or NPDES permit # FL0020109 limits. Arsenic, chromium-III, selenium, and zinc were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL). Aluminum was also detected in the effluent.

  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-1 NPDES sampling point for Outfall D-001 for this facility on August 22, 2011, were not acutely toxic to the *Ceriodaphnia dubia* (water flea) or the *Cyprinella leedsi* (fish) test organisms during the 96-hour acute definitive bioassays. The effluent AGP exceeded the “problem” threshold for freshwaters, suggesting nutrient enrichment may be an issue. Effluent water quality samples did not exceed Class III freshwater criteria and/or permitted limits.

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 1. 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,* for samples collected from the Winter Garden WWTF on August 22, 2011.

Winter Garden WWTF					
Location	AGP (measured)	Predicted AGP (TSIN) ± 20%	Predicted AGP (TN) ± 20%	Inorganic N:P ratio	Total N:P ratio
Effluent	78.9	56.5 ± 11.3	77.1 ± 15.4	3.4	4.2

Table 2. Effluent limits, Class III Freshwater Criteria and chemical data for samples collected from the Winter Garden WWTF on August 22, 2011.

Winter Garden WWTF NPDES# FL0020109	Class III Criteria	Effluent Limits	Effluent	
Organic Constituents (µg/L)				
None Detected	-	-	None Detected	
Metals (µg/L unless otherwise noted)				
Aluminum	-	-	112	
Arsenic	≤ 50	-	0.75	I
Cadmium	≤ 0.4 b	-	0.05	U
Calcium (mg/L)	-	-	44.5	
Chromium-III	≤ 117.1 b	-	0.57	I
Copper	≤ 12.8 b	-	1.48	
Iron	≤ 1,000	-	170	
Lead	≤ 5.1 b	≤ 5.1 b	0.2	U
Magnesium (mg/L)	-	-	8.34	
Mercury	≤ 0.012	0.2 s	0.12	U
Nickel	≤ 71.6 b	-	2.1	
Selenium	≤ 5.0	-	0.35	I
Silver	≤ 0.07	-	0.025	U
Zinc	≤ 164.6 b	-	12	I
Nutrients (mg/L)				
Ortho-phosphate	-	-	0.44	
Total Phosphorus	-	1.0 p	0.48	
Total Ammonia	-	-	0.088	
Un-ionized Ammonia	≤ 0.02 s	-	≤ 0.02	c
Nitrate and Nitrite	-	-	1.4	
Total Kjeldahl Nitrogen	-	-	0.63	
Organic Nitrogen	-	-	0.542	c
Total Nitrogen	-	18.3 p	2.03	c
General Physical and Chemical Parameters				
Dissolved Oxygen (mg/L)	≥ 5.0	≥ 5.0 s	6.4	
pH (SU)	6.0 - 8.5	6.0 - 8.5 s	6.9	
Conductivity (µmhos/cm)	≤ 1,275	-	620	
Temperature (°C)	-	-	27.4	
Total Residual Chlorine (mg/L)	≤ 0.01	-	0.03	UQ£
Chlorophyll *a* (µg/L) - Corrected	-	-	4.7	
Phaeophytin (µg/L)	-	-	0.5	I
CBOD, 5 day (mg/L), N - inhibited	-	-	17	J
Cyanide (µg/L)	≤ 5.2	≤ 5.2 p	0.005	U
Flow (MGD) (9/1/2010 - 8/31/2011)	-	4.0 a	0.845 a	
Hardness (mg $CaCO_3$)	-	Report	145.46	c

Value exceeds the Class III Water Quality Criteria or permit limits

a - Annual average
b - Value is calculated based on hardness
c - Value is calculated
p - 24 hour flow proportioned composite
s - Single sample
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit
J - Estimated value
Q - Sample held beyond normal holding time
U - Material analyzed for but not detected; value reported is the method detection limit
£ - measurement taken in the FDEP laboratory

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-08-22-34
FYI3 Sampling Inspection for City of Winter Gardens

Requester: Edward Smith
Field Report Prepared By: KANE
Collected By: KANE,
Send Final Report To: ~~Gary Miller~~ Phil Kane
Sampling Agency: FDEP CENTRAL

Customer: CEN-DIST
Project ID: FYI3
PMAS: 1143

Lab ID *	Location WINTER GARDEN WWTF 8 N. HIGHLAND AVE. WINTER GARDEN, FL.			☐ Comp ☑ Grab	Collection (begin) Date ~~PK~~ Time ~~Eastern Central~~		Collection (end) Date 8/22/11 Time 850AM Eastern Central		Bottle Group(s)**
	Field ID WINT			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L) 6.41		Storet Station Number		A
	Matrix (include type e.g. Salt, Fresh, etc) EFFLUENT	Temp (C) 27.37	pH 6.94	Sample Depth ☐ m ☐ ft SURFACE	620	☐ Salinity (PPTh) ☑ Sp Conductance (umho/cm)		NPDES Number FL0020109	
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments NO CHLORINE, NO SULPHIDE pH < 2.5 NO pH CN					
Lab ID *	Location WINTER GARDEN WWTF 8 N. HIGHLAND AVE. WINTER GARDEN, FL.			☐ Comp ☑ Grab	Collection (begin) Date ~~PK~~ Time ~~Eastern Central~~		Collection (end) Date 8/22/11 Time 850AM Eastern Central		Bottle Group(s)**
	Field ID WINT			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L) 6.41		Storet Station Number		A
	Matrix (include type e.g. Salt, Fresh, etc) EFFLUENT	Temp (C) 27.37	pH 6.94	Sample Depth ☐ m ☐ ft SURFACE	620	☐ Salinity (PPTh) ☑ Sp Conductance (umho/cm)		NPDES Number FL0020109	
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments BIOASSAY					
Lab ID *	Location ~~WINTER GARDEN WWTF~~ 8 N. HIGHLAND AVE. WINTER GARDEN, FL.			☑ Comp ☑ Grab	Collection (begin) Date ~~PK~~ Time ~~Eastern Central~~		Collection (end) Date 8/22/11 Time 0930 Eastern Central		Bottle Group(s)**
	Field ID WINT			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)		Storet Station Number		B
	Matrix (include type e.g. Salt, Fresh, etc) BLANK	Temp (C)	pH	Sample Depth ☐ m ☐ ft N/A		☐ Salinity (PPTh) ☑ Sp Conductance (umho/cm)		NPDES Number FL0020109	
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments BLANK NO CHLORINE NO SULPHIDE NO pH CN pH < 2.5					
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 4 Bioassay Rec'd in Tox by JD 10:14 8.23.11					

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
KANE	8/22/11	FEDEX	ATP	08/23/11 09:15				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

FDEP Biology Laboratory - Acute Bioassay Bench Sheets

Facility: Wintergarden, City of

Address : 101 East Crest Avenue

City: Winter Garden **County:** Orange

Contact/District: Phil Kane/ Central

NPDES Permit #: FL 0020109

LIMS Job #: TLH-2011-08-23-54 **LIMS Sample #:** 1372725

Lims Data Entry: 09-01-11 MF Data Entry Verification: 09.01.11 KC

Sample Collection: Date: 08.22.11 Time: 0850

Hold Time Start: Date: 08.22.11 Time: 0850

Comments:

Initial sample handling:

Instructions: Check yes or no. If yes is checked, complete blanks

PH adjustment : ☐ yes ☑ no Initial pH: ____ NaOH ____ N HCl ____ N ____ Drops mL Final pH: ____

Aeration : ☐ yes ☑ no Initial DO: ____mg/L Final DO: ____mg/L Duration: ____minutes Rate: ~____bubbles/min

Salinity adjusted (Test 1): ☐ yes ☑ no Initial Salinity: ____ Final Salinity: ____ Salts Hypersaline brine

Salinity adjusted (Test 2): ☐ yes ☑ no Initial Salinity: ____ Final Salinity: ____ Salts Hypersaline brine

Dechlorination: ☐ yes ☑ no ____mL of 0.025N Sodium Thiosulfate per liter of sample. Final TRC: ____

Sample Validation:

Instructions: Check yes or no. Fill in temperatures.

Temperature: Shipped ≤ 6°C ☑ Yes ☐ No — IF Hand Delivered: Cooling ☐ Yes ☐ No (received ____°C collected____°C)

Holding Time: ≤ 36 Hours ☑ Yes ☐ No (Composite-end of collection; grab-when collected; 4 in 24 - time last sample collected)

Investigators' Signatures:	Initials:
Brad Richter	BA
Kara Cox	KC
Marshall Faircloth	MF
Jenna Jones	JJ
REVIEWER: Liz Miller	EM

Diluent/Control Batch#:	8.18.11	~~8.23.11~~ KC 8.23.11				Other:	Original Sample	Measured by	Verified by
Water Quality Parameters	20% DMW	Well Water	Moderately Hard Water	Salt Water ASW NSW Test 1	Salt Water ASW NSW Test 2				
Field Total Residual Cl_2 (mg/L):	N/A	N/A	N/A	N/A	N/A	N/A	not measured	n/a	KC
Lab Total Residual Cl_2 (mg/L):	<0.03		<0.03				<0.03	JD	KC
Alkalinity (mg/L as $CaCO_3$):	86		82				155	KC	KC/MF
Hardness (mg/L as $CaCO_3$):	85		90				139	KC	KC/MF
Total Ammonia (mg/L as N):	<0.017	KC	<0.017				<0.017	BA	KC
Conductivity: ☑ µmhos ☐ mmhos	174	~~340~~	340				lole 1	BA	KC
Salinity (ppth):	—		—				0.2 ppt	JD	MF

V 1.8 1/13/10

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample # 1312725 SOP#: TA 07 01 Test # 1 of 2

Test Species:
Batch #: D8 23.11 Age: <24hrs.
☑ *Ceriodaphnia dubia*
☐ *Cyprinella leedsi*
☐ *Pimephales promelas*
☐ *Menidia beryllina*
☐ *Americamysis bahia*
☐ Other:

Test Validation
Control Survival ≥ 90%: ☑ Yes ☐ No
Temperature Range ≤ 3° C: ☑ Yes ☐ No

Diluent (Batch # 8.18.11)
☐ ASW ☑ 20% DMW
☐ MHW ☐ Well

Test Type: ☑ Acute ☑ Definitive
☐ Static ☑ Static Renewal ☐ Flow-Through

Test Duration:
☐ 48 hr
☑ 96 hr
☐ ___hr

Test Started: Date 8.23.11 Time 13:00

Test Ended: Date 8.27.11 Time 1200

Chamber Size 30 ml
Test Volume: 20 ml

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Ctrl	A	A	5	5	5	5	5	5
	B	B	5	5	5	5	5	5
	C	C	5	5	5	5	5	5
	D	D	5	5	5	5	5	5
6.25%	A	A	5	5	5	5	4D	2 3D
	B	B	5	5	5	5	5	5
	C	C	5	5	5	5	5	5
	D	D	5	5	5	5	5	5
12.5%	A	A	5	5	4D	4	4	1 3D
	B	B	5	5	5	5	5	5
	C	C	5	5	5	5	5	3 2D
	D	D	5	5	5	5	5	1 4D
25%	A	A	5	5	5	5	4D	3 1D
	B	B	5	5	5	5	3 2D	0 3D
	C	C	5	5	5	5	4D	2 2D
	D	D	5	5	5	5	5	5
50%	A	A	5	5	5	5	5	5
	B	B	5	5	5	5	5	5
	C	C	5	5	5	5	5	5
	D	D	5	5	5	5	5	5
100%	A	A	5	5	5	5	5	5
	B	B	5	5	5	5	5	5
	C	C	5	5	5	5	5	5
	D	D	5	5	5	5	5	5
Checked or Verified by:			BA	KC	KC	KC	KC	BA by MF

Food
☑ YCT Batch# 5.5.11
☑ P. subcapitata Batch# 5.5.11
☐ Artemia Lot#_____

Feeding and Artemia Batch #
☑ 0 hr___ ☑ Prior to Test
☐ 24 hr___
☑ 48 hr___ ☑ Prior to Renewal
☐ 72 hr___

Temperature Range (°C)
Incubator # 2
min. 24.8 max. 27.5 mean –

Incubator #___
min.___ max.___ mean___

Waterbath #___
min.___ max.___ mean___

Room B246
min. 23.6 max. 24.8 mean –

Light intensity (ft. candles): 50-100

Photoperiod (hours): 16 light, 8 dark

Loaded/ Verified by: BA / KC
m = missing, d = dead, BR/AR Before/ After Renewal

Test Results:
Screening Tests: Report LC50 as >100%, =100%, or <100%.
% mortality in 100% sample: 0% LC50: ~~>100%~~ >100%
Calculated LC50 (Definitive) 95% CI_____ Method EPA (TOXCalc)

Comments: LC50 not reported ~~interpolated done~~ ~~response in/at 12.5% concentration~~ on 8/30/11 Corrected Statmost. Results of ~~EAF~~ MF EPA methods in TOXCalc ~~2006~~ See comment on B. Biology Lims report 9/9/11

V. 2.5 1-13-10

Substitute highest test concentration used if other than 100% (example: Ocean outfall tested at 30% concentration)

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Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample # 1372725 SOP#: TA 07-02 Test # 2 of 2

Test Species:
Batch #: 8.23.11 Age: 8 days
- ☐ *Ceriodaphnia dubia*
- ☐ *Cyprinella leedsi*
- ☒ *Pimephales promelas*
- ☐ *Menidia beryllina*
- ☐ *Americamysis bahia*
- ☐ Other:

Test Validation
Control Survival ≥ 90%: ☑ Yes ☐ No
Temperature Range ≤ 3° C: ☑ Yes ☐ No

Diluent (Batch # 8.23.11)
☐ ASW ☐ 20% DMW ☒ MHW ☐ Well

Test Type: ☑ Acute ☑ Definitive ☐ Static ☑ Static Renewal ☐ Flow-Through

Test Duration: ☐ 48 hr ☑ 96 hr ☐ ___hr

Test Started: Date 8.23.11 Time 1315

Test Ended: Date 8.27.11 Time 1200

Chamber Size 300 ml
Test Volume: 1-00 ml

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Ctrl	A	D1	10	10	10	10	10	10
	B	D2	10	10	10	10	10	10
6.25%	A	D3	10	8 2D	8	8	8	8
	B	D4	10	10	10	10	9 1D	8 1D
12.5%	A	D5	10	9 1D	9	9	9	9
	B	D6	10	10	10	10	9 1D	9
25%	A	D7	10	10	10	10	8 2D	8
	B	D8	10	9 1D	9	9	9	9
50%	A	D9	10	10	10	10	10	10
	B	D10	10	10	10	10	10	10
100%	A	D11	10	9 1D	9	9	8 1D	8
	B	D12	10	8 2D	8	8	8	8
Checked or Verified by:			JD	JD	JS	JD	JS	BA

Food
- ☐ YCT Batch#_____
- ☐ P. subcapitata Batch#_____
- ☑ Artemia Lot# 23613

Feeding and Artemia Batch #
- ☑ 0 hr 145-11 ☑ Prior to Test
- ☐ 24 hr_____
- ☑ 48 hr 147-11 ☑ Prior to Renewal
- ☐ 72 hr____

Temperature Range (°C)
Incubator # 3
min. 24.6 max. 26.7 mean —
Incubator #___
min.____max.____mean____
Waterbath #___
min.____max.____mean____
Room B246
min. 23.6 max. 24.8 mean —
Light Intensity (ft. candles): 50-100
Photoperiod (hours): 16 light, 8 dark

Loaded/ Verified by: JD 1KC
m = missing, d = dead, BR/AR Before/ After Renewal

Test Results:
Screening Tests: Report LC50 as >100%, =100%, or <100%.
% mortality in 100% sample: 20% LC50: >100%
Calculated LC50 (Definitive) 95% CI_____ Method_____

Comments:

V. 2.5 1-13-10

Substitute highest test concentration used if other than 100% (example: Ocean outfall tested at 30% concentration)

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Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Parameter Sheet

LIMS Sample # 1312725 Test # 1 of 2

Test Species:

☒ *Ceriodaphnia dubia* ☐ *Pimephales promelas* ☐ *Americamysis bahia*
☐ *Cyprinella leedsi* ☐ *Menidia beryllina* ☐ Other:_____

Test aerated: (if DO is < 4.0 mg/L)
☐ Yes ☒ No

Concentration	Replicate	Test Hour	pH (SU)	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity ☒ µmhos ☐ mmhos	Salinity* (ppth)
	M	0 hr	7.9	24.0	7.4	174	
	—	24 hr	—	—	—	—	
ctrl	A	48 hr BR	8.1	24.0	8.3	180	
	M	48 hr AR	8.0	24.0	8.1	174	
	—	72 hr	—	—	—	—	
	A	96 hr	8.1	24.8	7.6	205	
	M	0 hr	7.9	24.2	7.3	211	
	—	24 hr	—	—	—	—	
6.25%	A	48 hr BR	8.4	24.0	8.9	213	
	M	48 hr AR	8.1	24.0	8.3	206	
	—	72 hr	—	—	—	—	
	A	96 hr	8.5	24.7	8.2	229	
	M	0 hr	7.8	24.2	7.3	239	
	—	24 hr	—	—	—	—	
12.5%	A	48 hr BR	8.5	24.0	8.9	247	
	M	48 hr AR	8.1	24.0	8.2	235	
	—	72 hr	—	—	—	—	
	A	96 hr	8.7	24.7	8.5	251	
	M	0 hr	7.8	24.3	7.2	299	
	—	24 hr	—	—	—	—	
25%	A	48 hr BR	8.6	24.0	8.9	300	
	M	48 hr AR	8.1	24.0	8.2	290	
	—	72 hr	—	—	—	—	
	A	96 hr	8.6	24.6	8.5	322	
	M	0 hr	7.6	24.3	7.5	422	
	—	24 hr	—	—	—	—	
50%	A	48 hr BR	8.6	24.0	8.9	426	
	M	48 hr AR	7.9	24.0	8.1	401	
	—	72 hr	—	—	—	—	
	A	96 hr	8.6	24.6	8.3	472	
	M	0 hr	7.3	24.2	7.3	661	
	—	24 hr	—	—	—	—	
100%	A	48 hr BR	8.7	24.0	8.9	648	
	M	48 hr AR	7.6	24.0	7.7	634	
	—	72 hr	—	—	—	—	
	A	96 hr	8.6	24.5	8.1	747	
		0 hr					
		24 hr					
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					
		0 hr					
		24 hr					
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					

*Measure/ record salinity for marine tests

Comments:

Measured By:
0 hr: BA
24 hr: —
48 hr BR: KC
48 hr AR: KC
72 hr: —
96 hr: BA

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V. 2.3 1-13-10

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Parameter Sheet

LIMS Sample # 1372725 Test # 2 of 2

Test Species:

☐ *Ceriodaphnia dubia* ☒ *Pimephales promelas* ☐ *Americamysis bahia*

☐ *Cyprinella leedsi* ☐ *Menidia beryllina* ☐ Other:_____

Test aerated: (if DO is < 4.0 mg/L) ☐ Yes ☒ No

Concentration	Replicate	Test Hour	pH (SU)	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity ☒ µmhos ☐ mmhos	Salinity* (ppth)
Ctrl	A	0 hr	8.0	25.8	7.1	340	
	B	24 hr	7.8	24.0	7.3	335	
	A	48 hr BR	8.1	24.1	7.5	345	
	A	48 hr AR	7.9	24.3	7.6	336	
	B	72 hr	7.8	24.5	6.9	329	
	A	96 hr	7.8	24.0	6.3	333	
6.25%	A	0 hr	8.1	25.9	7.8	360	
	B	24 hr	7.8	24.5	7.3	357	
	A	48 hr BR	8.0	24.3	7.8	366	
	A	48 hr AR	7.9	24.0	7.8	353	
	B	72 hr	7.9	24.4	6.5	347	
	A	96 hr	7.9	24.2	6.7	355	
12.5%	A	0 hr	8.0	26.0	8.1	375	
	B	24 hr	7.8	24.4	7.3	368	
	A	48 hr BR	8.0	24.5	7.8	376	
	A	48 hr AR	7.9	25.0	7.8	372	
	B	72 hr	7.8	24.1	6.4	369	
	A	96 hr	8.0	24.2	6.6	379	
25%	A	0 hr	8.0	25.9	8.2	417	
	B	24 hr	7.8	24.4	7.1	404	
	A	48 hr BR	8.0	24.5	7.8	413	
	A	48 hr AR	7.8	25.0	7.8	410	
	B	72 hr	7.9	24.3	6.6	411	
	A	96 hr	7.9	24.1	6.7	421	
50%	A	0 hr	7.8	25.6	8.3	495	
	B	24 hr	7.9	24.5	7.1	487	
	A	48 hr BR	8.1	24.5	8.0	494	
	A	48 hr AR	7.8	24.9	7.7	482	
	B	72 hr	8.0	24.6	6.8	484	
	A	96 hr	8.1	24.4	7.1	497	
100%	A	0 hr	7.5	25.6	8.3	654	
	B	24 hr	8.0	24.7	7.3	637	
	A	48 hr BR	8.2	24.4	8.0	647	
	A	48 hr AR	7.6	24.9	7.1	649	
	B	72 hr	8.0	24.3	6.8	651	
	A	96 hr	8.1	24.0	7.3	670	
		0 hr					
		24 hr					
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					
		0 hr					
		24 hr					
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					

*Measure/ record salinity for marine tests

Comments:

Measured By:
0 hr: BA
24 hr: JD
48 hr BR: JD
48 hr AR: JD
72 hr: JD
96 hr: BA

Page 000077

V. 2.3 1-13-10

Appendix B. (continued)

Test ID: Winter Garden Dates: 8.23.11 – 8.27.11

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 by Kara Cop 8/29/11

V 1.1 9/21/10 MF/jd

Appendix C. SRT Bench Sheets and Statistical Analysis

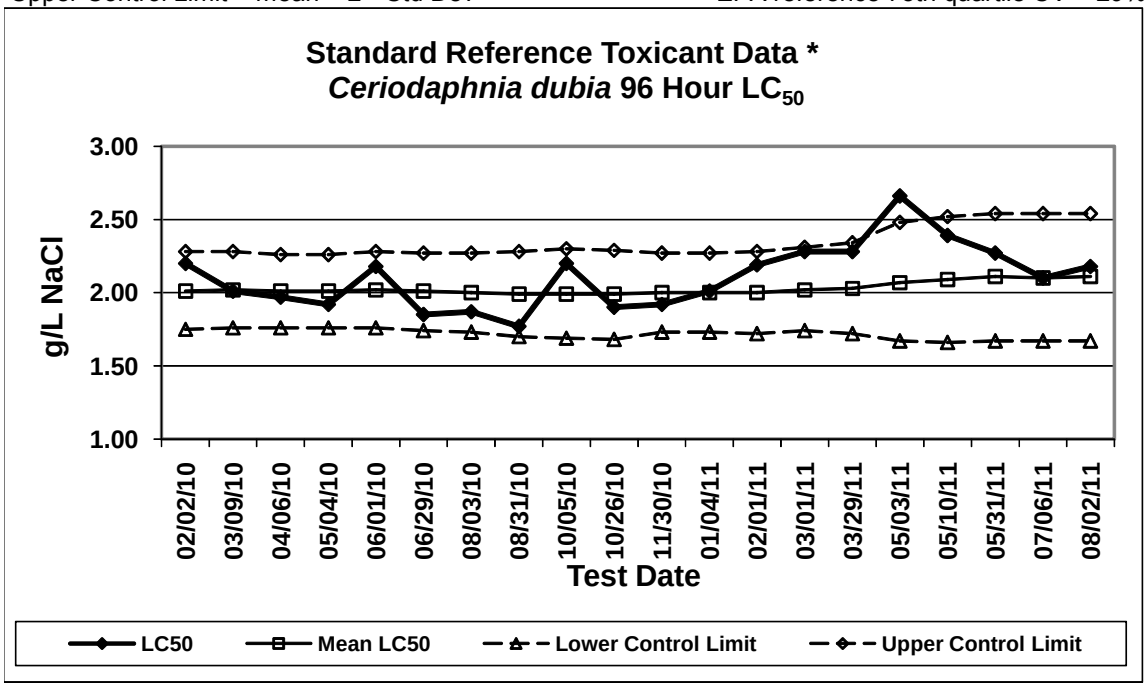
Florida Department of Environmental Protection
Bureau of Laboratories

Standard Reference Toxicant (SRT) Test Data - 96-hour Acute Toxicity
Ceriodaphnia dubia (water flea) - Sodium Chloride (NaCl)

Date	LC50	Mean LC50	Std Dev	Lower Control Limit	Upper Control Limit
02/02/10	2.20	2.01	0.13	1.75	2.28
03/09/10	2.01	2.02	0.13	1.76	2.28
04/06/10	1.97	2.01	0.12	1.76	2.26
05/04/10	1.92	2.01	0.12	1.76	2.26
06/01/10	2.18	2.02	0.13	1.76	2.28
06/29/10	1.85	2.01	0.13	1.74	2.27
08/03/10	1.87	2.00	0.14	1.73	2.27
08/31/10	1.77	1.99	0.14	1.70	2.28
10/05/10	2.20	1.99	0.15	1.69	2.30
10/26/10	1.90	1.99	0.15	1.68	2.29
11/30/10	1.92	2.00	0.14	1.73	2.27
01/04/11	2.01	2.00	0.14	1.73	2.27
02/01/11	2.19	2.00	0.14	1.72	2.28
03/01/11	2.28	2.02	0.14	1.74	2.31
03/29/11	2.28	2.03	0.15	1.72	2.34
05/03/11	2.66	2.07	0.20	1.67	2.48
05/10/11	2.39	2.09	0.21	1.66	2.52
05/31/11	2.27	2.11	0.22	1.67	2.54
07/06/11	2.10	2.10	0.22	1.67	2.54
08/02/11	2.18	2.11	0.22	1.67	2.54

Lower Control Limit = mean - 2 * Std Dev
Upper Control Limit = mean + 2 * Std Dev

CV = 10.29
EPA reference 75th quartile CV = 29%



Appendix C. (continued)

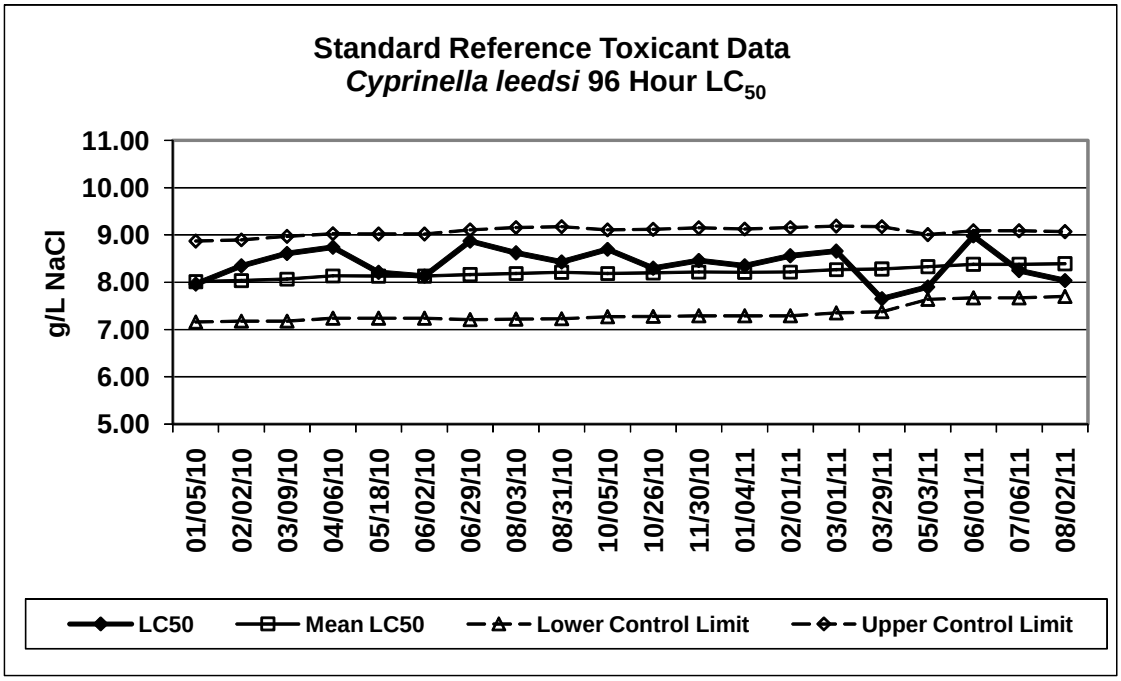
Florida Department of Environmental Protection
Bureau of Laboratories

Standard Reference Toxicant (SRT) Test Data - 96-hour Acute Toxicity
Cyprinella leedsi (bannerfin shiner) - Sodium Chloride (NaCl)

Date	LC50	Mean LC50	Std Dev	Lower Control Limit	Upper Control Limit
01/05/10	7.96	8.01	0.43	7.16	8.87
02/02/10	8.35	8.04	0.43	7.18	8.90
03/09/10	8.61	8.07	0.45	7.18	8.97
04/06/10	8.74	8.14	0.45	7.24	9.03
05/18/10	8.21	8.13	0.45	7.24	9.02
06/02/10	8.13	8.13	0.45	7.24	9.02
06/29/10	8.87	8.16	0.47	7.21	9.11
08/03/10	8.62	8.19	0.48	7.22	9.16
08/31/10	8.43	8.21	0.49	7.23	9.18
10/05/10	8.70	8.19	0.46	7.27	9.11
10/26/10	8.30	8.20	0.46	7.28	9.12
11/30/10	8.46	8.22	0.46	7.29	9.15
01/04/11	8.35	8.21	0.46	7.29	9.13
02/01/11	8.56	8.22	0.47	7.29	9.16
03/01/11	8.66	8.27	0.46	7.35	9.19
03/29/11	7.65	8.28	0.45	7.38	9.18
05/03/11	7.90	8.33	0.34	7.64	9.01
06/01/11	8.98	8.38	0.36	7.67	9.09
07/06/11	8.25	8.38	0.36	7.67	9.09
08/02/11	8.04	8.39	0.34	7.70	9.07

Lower Confidence Limit = mean - 2 * Std Dev
Upper Confidence Limit = mean + 2 * Std Dev

CV = 4.09
EPA reference 75th quartile CV = 19%



Appendix C. (continued)

FDEP Biology Laboratory - Acute SRT Bench Sheet

Method from EPA-821-R-02-012 except *H. azteca*

Test Organism: (check one)
- ☐ *Americamysis bahia* Method 2007.0 TA07.03
- ☐ *Menidia Beryllina* Method 2006.0 TA07.04
- ☐ *Hyalella azteca* Method 100.1 EPA-600-R-99-064
- ☑ *Ceriodaphnia dubia* Method 2002.0 TA07.01
- ☐ *Cyprinella leedsi* Method 2000.0 TA07.02
- ☐ *Pimephales promelas* Method 2000.0 TA07.02

Test Type: ☐ non-renewal ☑ renewal

Test Page 1 of 2

Diluent/ Batch #: DMW 17.28.11 **Organism Batch:** 8.2.11 **Test Beginning:** Date: 8.2.11 Time: 1015

Toxicant/ Batch#: NaCl 18.1.11 Age: <24 hrs **Test Ending:** Date: 8.6.11 Time: 1030

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<0.03	TA06_22	BA
Alkalinity (mg/L as $CaCO_3$):	78	TA06_08	KC
Hardness (mg.L as $CaCO_3$):	80	TA06_09	KC
Total Ammonia (mg/L as N):	<0.017	TA06_17	MF
Conductivity: µmhos mmhos	173	TA06_25	KC
Salinity (ppth):	—	TA06_25	—

Food:	Batch
☑ YCT	5.5.11
☑ *P. subcapitata*	5.5.11
☐ Artemia Lot #	

Artemia Batch:

Chamber size: 30 mL

Test volume: 20 mL per replicate

0 hr:	
24 hr:	
48 hr:	
72 hr:	

Concentrations prepared by: KC

Test Incubated in: Incubator # 15 Waterbath # —

Light Intensity: 50-100 Ft. candles

Photoperiod: 16 hours Light / 8 hours dark

Temperature Range °C:(if used)

Incubator # KC 13 min 24.7 max 26.9 mean —

Waterbath # ___ min. ___ max. ___ mean ___

Room B246 min 24.8 max 25.9 mean —

Feeding: √as needed
☑ Before test
☑ 0 hr
☐ 24 hr
☑ 48 hr
☐ 72 hr

all species 2 hrs prior to test

mysids daily

all species except *H. azteca* 2 hr prior to renewal

H. azteca 0 hr and 48 hr

Concentration µg/L	mg/L	g/L	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control			A	A	5	5	5	5	5	5
			B	B	5	5	5	5	5	5
			C	C	5	5	5	5	5	5
			D	D	5	5	5	5	5	5
1.5			A	A	5	4 1D	4	4	4	4
			B	B	5	5	5	5	5	5
			C	C	5	5	5	5	5	5
			D	D	5	5	5	5	5	5
2.0			A	A	5	6	5	5	5	5
			B	B	5	5	4 1D	4 1D KC	2 2D	2
			C	C	5	5	4 1D	4	4	4
			D	D	5	5	5	5	5	5
2.5			A	A	5	5	3 2D	3	2 1D	2
			B	B	5	4 1D	3 1D	3	1 2D	1
			C	C	5	4 1D	1 3D	1	0 1D	—
			D	D	5	5	1 4D	1	0 1D	—
3.0			A	A	5	BA 3^{2D}	0 3D	0	—	—
			B	B	5	5	1 4D	1	1	0 1D
			C	C	5	4 1D	0 4D	0	—	—
			D	D	5	4 1D	0 4D	0	—	—
4.0			A	A	5	0 5D				
			B	B	5	0 5D				
			C	C	5	0 5D				
			D	D	5	0 3D				
Organisms loaded by: KC			Checked by:		BA	BA	KC	KC	BA	JD

H. azteca		
0 hr	Alk	Hrd
Well		
100%		
96 hr	Alk	Hrd
Well		
100%		

Loading Verified by: BA AR = after renewal

m = missing d = dead BR = before renewal hr = hour

Investigators' Signatures

Kara Cox

Brea Addo

James Daniel

Marshall Faircloth

Reviewer: Liz Miller

Comments:

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour: 2.55	2.35	2.77
96 hour: 2.18	2.05	2.33

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Revised 7/15/2008 mf

Appendix C. (continued)

FDEP Biology Laboratory - Acute SRT Bench Sheet
0-96 hour parameters
Methods from EPA-821-R-02-012 except *H. azteca*
Test Page 2 of 2

Test Organism: (check one)
- ☐ *Americamysis bahia* Method 2007.0 TA07.03
- ☐ *Menidia Beryllina* Method 2006.0 TA07.04
- ☐ *Hyalella azteca* Method 100.1 EPA-600-R-99-064
- ☑ *Ceriodaphnia dubia* Method 2002.0 TA07.01
- ☐ *Cyprinella leedsi* Method 2000.0 TA07.02
- ☐ *Pimephales promelas* Method 2000.0 TA07.02

Test Beginning: Date: 8.2.11 Time: 1015
Test Ending: Date: 8.6.11 Time: 1030
Test Type: ☐ non-renewal ☑ renewal

hr = hour AR = after renewal BR = before renewal

Concentration µg/L mg/L g/L	Replicate	Test Hour	pH (SU)	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity ☐ µmhos ☑ mmhos	Salinity (ppth)
Control	M	0 hr	8.0	25.7	KC 8.8 8.4	0.173	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.0	24.0	7.8	0.199	
	M	48 hr AR	8.1	24.0	8.2	0.179	
	—	72 hr	—	—	—	—	
	M	96 hr	8.1	24.0	7.6	0.220	
1.5	M	0 hr	8.1	25.75 KC	8.5	2.87	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.2	24.0	7.7	3.03	
	M	48 hr AR	8.2	24.0	7.9	2.90	
	—	72 hr	—	—	—	—	
	M	96 hr	8.1	24.1	7.7	3.06	
2.0	M	0 hr	8.1	25.2	8.5	3.77	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.2	24.0	8.1	4.17	
	M	48 hr AR	8.2	24.0	7.9	3.76	
	—	72 hr	—	—	—	—	
	M	96 hr	8.1	24.1	7.8	4.03	
2.5	M	0 hr	8.1	24.8	8.5	4.69	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.2	24.0	8.2	4.96	
	M	48 hr AR	8.2	24.0	7.8	4.64	
	—	72 hr	—	—	—	—	
	M	96 hr	8.1	24.0	8.0	5.12	
3.0	M	0 hr	8.1	24.5	8.5	5.49	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.2	24.0	8.1	6.44	
	M	48 hr AR	8.2	24.0	7.8	5.46	
		72 hr	—	—	—	—	
	M	96 hr	8.1	24.1	7.7	6.29	
4.0	M	0 hr	8.1	24.0	8.6	7.14	
	A	24 hr	8.1	24.0	8.6	8.07	
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					

Investigators Signatures
Kaila Cook
Bruce Alsobrook
for Sarah Marshall Faircloth

Reviewed By: Shirley Allen

Measured by:
0 hr: KC
24 hr: BA
48 hr BR: KC
48 hr AR: KC
72 hr:
96 hr: JD

Measure/record salinity for marine tests

Comments: Parameters for Replicate C in the 2.5 g/L concentration were inadvertently not measured @ 72 hours, MF 8-5-11 MF

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Revised 07/15/2008 MF

Appendix C. (continued)

FDEP Biology Laboratory - Acute SRT Bench Sheet

Method from EPA-821-R-02-012 except *H. azteca*

Test Organism: (check one)
- ☐ *Americamysis bahia* Method 2007.0 TA07.03
- ☐ *Menidia Beryllina* Method 2006.0 TA07.04
- ☐ *Hyalella azteca* Method 100.1 EPA-600-R-99-064
- ☐ *Ceriodaphnia dubia* Method 2002.0 TA07.01
- ☒ *Cyprinella leedsi* Method 2000.0 TA07.02
- ☐ *Pimephales promelas* Method 2000.0 TA07.02

Test Type: ☐ non-renewal ☒ renewal

Test Page 1 of 2

Diluent/ Batch #: Well / B.1.11
Toxicant/ Batch#: NaCl / B.1.11
Organism Batch: B.2.11 Age: 14 days
Test Beginning: Date: 8.2.11 Time: 1315
Test Ending: Date: 8.6.11 Time: 1100

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<0.03	TA06_22	KC
Alkalinity (mg/L as $CaCO_3$):	143	TA06_08	KC
Hardness (mg/L as $CaCO_3$):	134	TA06_09	KC
Total Ammonia (mg/L as N):	<0.017	TA06_17	BA
Conductivity: µmhos mmhos	278	TA06_25	JD
Salinity (ppth):	—	TA06_25	—

Food:	Batch
☐ YCT	
☐ *P. subcapitata*	
☒ Artemia Lot #	23b13

Artemia Batch:
0 hr: 1330-11
24 hr:
48 hr: 1332-11
72 hr:

Chamber size: 1000 mL
Test volume: 400 mL per replicate

Concentrations prepared by: JD
Test Incubated in: Incubator # 3 Waterbath # —
Incubator # 3 min. 24.7 max. 26.9 mean —
Waterbath # ___ min. ___ max. ___ mean ___
Room B246 min. 24.8 max. 25.9 mean —
Light Intensity: 50-100 Ft. candles
Photoperiod: 16 hours Light / 8 hours dark
Temperature Range °C: (if used)

Feeding: √as needed
- ☑ Before test
- ☐ 0 hr
- ☐ 24 hr
- ☑ 48 hr
- ☐ 72 hr

all species 2 hrs prior to test
mysids daily
all species except *H. azteca* 2 hr prior to renewal
H. azteca 0 hr and 48 hr

H. azteca		
0 hr	Alk	Hrd
Well		
100%		
96 hr	Alk	Hrd
Well		
100%		

Concentration µg/L mg/L (50)	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	A1	10	9 JD	9	9	9	9
	B	A2	10	10	10	10	10	10
6	A	A3	10	9 JD	8 JD	8	7 JD	7
	B	A4	10	7 JD	7	7	7	7
7	A	A5	10	7 JD	7	7	6 JD	6
	B	A6	10	8 JD	8	8	8	7 JD
8	A	A7	10	6 JD	6	6	6	6
	B	A8	10	7 JD	6 JD	6	6	6
9	A	A9	10	1 JD	1	1	1	1
	B	A10	10	3 JD	2 JD	2	2	2
10	A	A11	10	0 JD	—	—	—	—
	B	A12	10	~~1 JD~~ 2 JD	2	2	2	1 JD
Organisms loaded by: JD		Checked by:	JD	JD	JD	JD	JD	JD

Loading Verified by: BA
AR = after renewal
m = missing d = dead BR = before renewal hr = hour

Investigators' Signatures
James Daniels
Brooke Hilmer
Kara Cox
Marshall Faircloth

Reviewer: Liz Miller

Comments:

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour: 8.16	7.74	8.60
96 hour: ~~7.79~~ 8.04 KC	~~7.10~~ 7.34	~~8.56~~ 8.80

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Revised 7/15/2008 mf

Appendix C. (continued)

FDEP Biology Laboratory - Acute SRT Bench Sheet
0-96 hour parameters

Methods from EPA-821-R-02-012 except *H. azteca*

Test Page 2 of 2

Test Organism: (check one)
- ☐ *Americamysis bahia* Method 2007.0 TA07.03
- ☐ *Menidia Beryllina* Method 2006.0 TA07.04
- ☐ *Hyalella azteca* Method 100.1 EPA-600-R-99-064
- ☐ *Ceriodaphnia dubia* Method 2002.0 TA07.01
- ☒ *Cyprinella leedsi* Method 2000.0 TA07.02
- ☐ *Pimephales promelas* Method 2000.0 TA07.02

Test Beginning: Date: 8.2.11 Time: 1315
Test Ending: Date: 8.6.11 Time: 1100

Test Type: ☐ non-renewal ☒ renewal

hr = hour AR = after renewal BR = before renewal

Concentration µg/L mg/L (circle)	Replicate	Test Hour	pH (SU)	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity ☐ µmhos ☒ mmhos	Salinity (ppth)
Control	A	0 hr	7.8	25.2	8.2	0.278	
	B	24 hr	7.7	24.5	7.6	0.288	
	A	48 hr BR	8.2	25.4	7.0	0.308	
	A	48 hr AR	7.8	25.8	7.3	0.292	
	B	72 hr	8.0	25.1	6.5	0.281	
	A	96 hr	8.0	25.9	6.4	0.353	
6	A	0 hr	8.0	25.9	7.7	10.8	
	B	24 hr	7.9	24.5	7.4	10.5	
	A	48 hr BR	8.2	25.6	6.7	11.1	
	A	48 hr AR	7.9	25.9	7.1	10.9	
	B	72 hr	8.0	25.4	6.3	11.2	
	A	96 hr	8.1	25.8	6.9	9.92	
7	A	0 hr	8.0	25.9	7.8	13.0	
	B	24 hr	7.9	24.9	7.3	12.7	
	A	48 hr BR	8.2	25.5	6.8	13.3	
	A	48 hr AR	8.0	25.8	6.8	12.9	
	B	72 hr	8.0	24.9	6.4	12.6	
	A	96 hr	8.1	25.8	6.9	13.0	
8	A	0 hr	8.0	25.9	7.8	14.4	
	B	24 hr	7.9	24.7	7.0	14.2	
	A	48 hr BR	8.2	25.9	6.7	14.6	
	A	48 hr AR	8.0	25.7	6.8	14.4	
	B	72 hr	7.9	25.9	6.2	14.5	
	A	96 hr	8.1	25.9	6.7	14.6	
9	A	0 hr	8.0	25.9	7.7	16.1	
	B	24 hr	7.9	24.6	6.9	15.9	
	A	48 hr BR	8.2	25.4	6.6	16.7	
	A	48 hr AR	8.0	25.6	6.9	16.1	
	B	72 hr	7.9	25.8	5.7	15.9	
	A	96 hr	8.1	25.9	6.7	16.3	
10	A	0 hr	8.0	25.9	7.5	18.0	
	B	24 hr	7.9	25.3	6.2	17.9	
	B	48 hr BR	8.2	24.9	6.8	18.1	
	B	48 hr AR	8.0	25.9	7.0	18.1	
	B	72 hr	8.1	25.6	6.4	17.8	
	B	96 hr	8.2	25.6	6.8	18.1	

Investigators Signatures: James Daniel, Brooke Hilyard, Kara Lidy, Marshall Faircloth

Reviewed By: Steve Miller

Measured by:
- 0 hr: JD
- 24 hr: JD
- 48 hr BR: JD
- 48 hr AR: JD
- 72 hr: JD
- 96 hr: JD

Measure/record salinity for marine tests

Comments:

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Revised 07/15/2008 MF

Appendix C. (continued)

***Ceriodaphnia dubia* Acute Standard Reference Toxicant Test**

DATE: 8/02/201 DURATION: 96 hr TOXICANT : NaCl
SPECIES: *Ceriodaphnia dubia*

RAW DATA: Concentration (g/L)	Number Exposed	Mortalities
.00	20	0
1.50	20	1
2.00	20	4
2.50	20	17
3.00	20	20
4.00	20	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 2.18
95% LOWER CONFIDENCE: 2.05
95% UPPER CONFIDENCE: 2.33

***Cyprinella leedsi* Acute Standard Reference Toxicant Test**

DATE: 8/2/11 DURATION: 96 h TOXICANT : NaCl
SPECIES: *Cyprinella leedsi*

RAW DATA: Concentration (g/L)	Number Exposed	Mortalities
.00	20	1
6.00	20	6
7.00	20	7
8.00	20	8
9.00	20	17
10.00	20	19

SPEARMAN-KARBER TRIM: 26.32%

SPEARMAN-KARBER ESTIMATES: LC50: 8.04
95% LOWER CONFIDENCE: 7.34
95% UPPER CONFIDENCE: 8.80

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

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: 8/1/11

Ship to: FDEP

P.O. No:

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (°/₀₀)
Mysidopsis bahia	150 ac	4 DAYS	MS 110728	25	7.9	20
Menidia beryllina	150 ac	12 DAYS	SS 110720	25	7.9	20
						Hardness mg/L
Cyprinella leedsi	150 ac	13 DAYS	CL 110719	25	7.8	80
Pimephales promelas	150 ac	7 DAYS	FM 110725	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: PR

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: 8/22/11

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	150 ac	12 DAYS	CL 110810	25	7.8	80
Pimephales promelas	300	<24 HRS	FM 110821-1830	25	7.8	80
	150 ac	7 DAYS	FM 110815	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: DM

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 effluent from the Winter Garden WWTF Outfall D-001, sampled on August 22, 2011.

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 8:50	Effluent	AGP/LN	Algal Growth Potential	78.9		mg DryWt/L	0.3	0.9
8/22/2011 8:50	Effluent	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	17	J	mg/L	0.2	2
8/22/2011 8:50	Effluent	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	4.7		ug/L	0.55	1.7
8/22/2011 8:50	Effluent	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.5	I	ug/L	0.4	1.2
8/22/2011 8:50	Effluent	Toxicology	Bioassay-Acute-Def-FW-C.dubia, LC50	100	L	LC50		
8/22/2011 8:50	Effluent	Toxicology	Bioassay-Acute-Def-FW-Fish, LC50	100	L	LC50		
8/22/2011 8:50	Effluent	Metals	Aluminum	112		ug/L	5	20
8/22/2011 8:50	Effluent	Metals	Calcium	44.5		mg/L	0.075	0.3
8/22/2011 8:50	Effluent	Metals	Lead	0.2	U	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Metals	Arsenic	0.75	I	ug/L	0.25	1
8/22/2011 8:50	Effluent	Metals	Silver	0.025	U	ug/L	0.025	0.1
8/22/2011 8:50	Effluent	Metals	Selenium	0.35	I	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Metals	Nickel	2.1		ug/L	0.25	1
8/22/2011 8:50	Effluent	Metals	Iron	170		ug/L	30	120
8/22/2011 8:50	Effluent	Metals	Copper	1.48		ug/L	0.5	1
8/22/2011 8:50	Effluent	Metals	Chromium	0.57	I	ug/L	0.3	1.2
8/22/2011 8:50	Effluent	Metals	Magnesium	8.34		mg/L	0.04	0.16
8/22/2011 8:50	Effluent	Metals	Zinc	12	I	ug/L	5	20
8/22/2011 8:50	Effluent	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Metals	Mercury	0.12	U	ug/L	0.12	0.5
8/22/2011 8:50	Effluent	Nutrients	Total-P	0.48		mg P/L	0.005	0.05
8/22/2011 8:50	Effluent	Nutrients	O-Phosphate-P	0.44		mg P/L	0.02	0.05
8/22/2011 8:50	Effluent	Nutrients	Ammonia-N	0.088		mg N/L	0.01	0.02
8/22/2011 8:50	Effluent	Nutrients	Cyanide	0.005	U	mg CN/L	0.005	0.02
8/22/2011 8:50	Effluent	Nutrients	Kjeldahl Nitrogen	0.63		mg N/L	0.08	0.2
8/22/2011 8:50	Effluent	Nutrients	NO2NO3-N	1.4		mg N/L	0.04	0.1
8/22/2011 8:50	Effluent	Pesticides	Diazinon	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Pesticides	Ethion	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Pesticides	Simazine	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Pesticides	Ametryn	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Pesticides	Chlorpyrifos Methyl	0.1	U	ug/L	0.1	0.4
8/22/2011 8:50	Effluent	Pesticides	Malathion	0.15	U	ug/L	0.15	0.6
8/22/2011 8:50	Effluent	Pesticides	Atrazine	0.05	U	ug/L	0.05	0.2

Appendix E. continued

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 8:50	Effluent	Pesticides	Ethoprop	0.1	UJ	ug/L	0.1	0.4
8/22/2011 8:50	Effluent	Pesticides	Azinphos Methyl	0.2	U	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Pesticides	Fenamiphos	0.2	UJ	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Pesticides	Metribuzin	0.1	U	ug/L	0.1	0.4
8/22/2011 8:50	Effluent	Pesticides	Naled	0.8	U	ug/L	0.8	3.2
8/22/2011 8:50	Effluent	Pesticides	Phorate	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Pesticides	Norflurazon	0.15	U	ug/L	0.15	0.6
8/22/2011 8:50	Effluent	Pesticides	Bromacil	0.2	UJ	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Pesticides	Fonofos	0.1	U	ug/L	0.1	0.4
8/22/2011 8:50	Effluent	Pesticides	Hexazinone	0.1	U	ug/L	0.1	0.4
8/22/2011 8:50	Effluent	Pesticides	Metalaxyl	0.25	U	ug/L	0.25	1
8/22/2011 8:50	Effluent	Pesticides	Metolachlor	0.5	U	ug/L	0.5	2
8/22/2011 8:50	Effluent	Pesticides	Butylate	0.2	U	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Pesticides	Chlorpyrifos Ethyl	0.05	U	ug/L	0.05	0.2
8/22/2011 8:50	Effluent	Pesticides	Parathion Ethyl	0.15	U	ug/L	0.15	0.6
8/22/2011 8:50	Effluent	Pesticides	Parathion Methyl	0.1	U	ug/L	0.1	0.4
8/22/2011 8:50	Effluent	Pesticides	Alachlor	0.6	U	ug/L	0.6	2.4
8/22/2011 8:50	Effluent	Pesticides	Mevinphos	0.2	U	ug/L	0.2	0.8
8/22/2011 8:50	Effluent	Pesticides	Prometryn	0.15	U	ug/L	0.15	0.6
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2-Nitrophenol	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Di-n-butyl phthalate	4.8	U	ug/L	4.8	19
8/22/2011 8:50	Effluent	Priority Organic Pollutants	N-Nitrosodimethylamine	1.9	U	ug/L	1.9	7.6
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2,4-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Benzidine	95	U	ug/L	95	380
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Benzo(a)pyrene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Fluoranthene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Isophorone	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Naphthalene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Pentachlorophenol	2.9	U	ug/L	2.9	11
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Pyrene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2,4-Dinitrophenol	14	U	ug/L	14	57
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2-Chlorophenol	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	4-Bromophenyl phenyl ether	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Anthracene	0.19	U	ug/L	0.19	0.76

Appendix E. continued

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Benzo(a)anthracene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Benzo(b)fluoranthene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Bis(2-chloroethyl)ether	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Dimethyl phthalate	9.5	U	ug/L	9.5	38
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Hexachlorocyclopentadiene	2.9	U	ug/L	2.9	11
8/22/2011 8:50	Effluent	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	2.9	U	ug/L	2.9	11
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Phenol	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	2.9	U	ug/L	2.9	11
8/22/2011 8:50	Effluent	Priority Organic Pollutants	4-Chloro-3-methylphenol	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	1.9	U	ug/L	1.9	7.6
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Hexachlorobutadiene	2.9	U	ug/L	2.9	11
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Hexachloroethane	2.9	U	ug/L	2.9	11
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Phenanthrene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2,4,6-Trichlorophenol	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2,4-Dichlorophenol	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2,4-Dimethylphenol	9.5	U	ug/L	9.5	38
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Acenaphthylene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Chrysene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Nitrobenzene	1.9	U	ug/L	1.9	7.6
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2-Chloronaphthalene	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	4-Nitrophenol	14	U	ug/L	14	57
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Butyl benzyl phthalate	4.8	U	ug/L	4.8	19
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Di-n-octyl phthalate	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	1,2,4-Trichlorobenzene	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	2,6-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Benzo(k)fluoranthene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	14	U	ug/L	14	57
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Hexachlorobenzene	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	3,3'-Dichlorobenzidine	38	UJ	ug/L	38	150
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Acenaphthene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	2.9	U	ug/L	2.9	11

Appendix E. continued

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Diethyl phthalate	0.95	U	ug/L	0.95	3.8
8/22/2011 8:50	Effluent	Priority Organic Pollutants	Fluorene	0.19	U	ug/L	0.19	0.76
8/22/2011 8:50	Effluent	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	1.9	U	ug/L	1.9	7.6
8/22/2011 8:50	Effluent	Field Parameter	Dissolved Oxygen	6.41		mg/L		
8/22/2011 8:50	Effluent	Field Parameter	Temperature	27.37		C		
8/22/2011 8:50	Effluent	Field Parameter	pH	6.94				
8/22/2011 8:50	Effluent	Field Parameter	Specific Conductance	620		umhos/cm		
8/22/2011 9:30	Equip. Blank	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
8/22/2011 9:30	Equip. Blank	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
8/22/2011 9:30	Equip. Blank	Metals	Lead	0.2	U	ug/L	0.2	0.8
8/22/2011 9:30	Equip. Blank	Metals	Nickel	0.65	I	ug/L	0.25	1
8/22/2011 9:30	Equip. Blank	Metals	Copper	0.5	U	ug/L	0.5	1
8/22/2011 9:30	Equip. Blank	Metals	Chromium	0.3	U	ug/L	0.3	1.2
8/22/2011 9:30	Equip. Blank	Metals	Magnesium	0.04	U	mg/L	0.04	0.16
8/22/2011 9:30	Equip. Blank	Metals	Silver	0.025	U	ug/L	0.025	0.1
8/22/2011 9:30	Equip. Blank	Metals	Selenium	0.2	U	ug/L	0.2	0.8
8/22/2011 9:30	Equip. Blank	Metals	Arsenic	0.25	U	ug/L	0.25	1
8/22/2011 9:30	Equip. Blank	Metals	Aluminum	5	U	ug/L	5	20
8/22/2011 9:30	Equip. Blank	Metals	Calcium	0.075	U	mg/L	0.075	0.3
8/22/2011 9:30	Equip. Blank	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
8/22/2011 9:30	Equip. Blank	Metals	Mercury	0.12	U	ug/L	0.12	0.5
8/22/2011 9:30	Equip. Blank	Metals	Iron	30	U	ug/L	30	120
8/22/2011 9:30	Equip. Blank	Metals	Zinc	5	U	ug/L	5	20
8/22/2011 9:30	Equip. Blank	Nutrients	Kjeldahl Nitrogen	0.08	U	mg N/L	0.08	0.2
8/22/2011 9:30	Equip. Blank	Nutrients	Total-P	0.004	U	mg P/L	0.004	0.01
8/22/2011 9:30	Equip. Blank	Nutrients	O-Phosphate-P	0.004	U	mg P/L	0.004	0.01
8/22/2011 9:30	Equip. Blank	Nutrients	Cyanide	0.005	U	mg CN/L	0.005	0.02
8/22/2011 9:30	Equip. Blank	Nutrients	NO2NO3-N	0.004	U	mg N/L	0.004	0.01
8/22/2011 9:30	Equip. Blank	Nutrients	Ammonia-N	0.01	U	mg N/L	0.01	0.02
8/22/2011 9:30	Equip. Blank	Pesticides	Azinphos Methyl	0.19	U	ug/L	0.19	0.76
8/22/2011 9:30	Equip. Blank	Pesticides	Norflurazon	0.14	U	ug/L	0.14	0.57
8/22/2011 9:30	Equip. Blank	Pesticides	Parathion Methyl	0.095	U	ug/L	0.095	0.38
8/22/2011 9:30	Equip. Blank	Pesticides	Ametryn	0.048	U	ug/L	0.048	0.19

Appendix E. continued

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 9:30	Equip. Blank	Pesticides	Ethion	0.048	U	ug/L	0.048	0.19
8/22/2011 9:30	Equip. Blank	Pesticides	Metalaxyl	0.24	U	ug/L	0.24	0.95
8/22/2011 9:30	Equip. Blank	Pesticides	Ethoprop	0.095	UJ	ug/L	0.095	0.38
8/22/2011 9:30	Equip. Blank	Pesticides	Chlorpyrifos Methyl	0.095	U	ug/L	0.095	0.38
8/22/2011 9:30	Equip. Blank	Pesticides	Metolachlor	0.48	U	ug/L	0.48	1.9
8/22/2011 9:30	Equip. Blank	Pesticides	Atrazine	0.048	U	ug/L	0.048	0.19
8/22/2011 9:30	Equip. Blank	Pesticides	Diazinon	0.048	U	ug/L	0.048	0.19
8/22/2011 9:30	Equip. Blank	Pesticides	Fenamiphos	0.19	UJ	ug/L	0.19	0.76
8/22/2011 9:30	Equip. Blank	Pesticides	Fonofos	0.095	U	ug/L	0.095	0.38
8/22/2011 9:30	Equip. Blank	Pesticides	Hexazinone	0.095	U	ug/L	0.095	0.38
8/22/2011 9:30	Equip. Blank	Pesticides	Malathion	0.14	U	ug/L	0.14	0.57
8/22/2011 9:30	Equip. Blank	Pesticides	Parathion Ethyl	0.14	U	ug/L	0.14	0.57
8/22/2011 9:30	Equip. Blank	Pesticides	Phorate	0.048	U	ug/L	0.048	0.19
8/22/2011 9:30	Equip. Blank	Pesticides	Prometryn	0.14	U	ug/L	0.14	0.57
8/22/2011 9:30	Equip. Blank	Pesticides	Alachlor	0.57	U	ug/L	0.57	2.3
8/22/2011 9:30	Equip. Blank	Pesticides	Metribuzin	0.095	U	ug/L	0.095	0.38
8/22/2011 9:30	Equip. Blank	Pesticides	Simazine	0.048	U	ug/L	0.048	0.19
8/22/2011 9:30	Equip. Blank	Pesticides	Chlorpyrifos Ethyl	0.048	U	ug/L	0.048	0.19
8/22/2011 9:30	Equip. Blank	Pesticides	Bromacil	0.19	UJ	ug/L	0.19	0.76
8/22/2011 9:30	Equip. Blank	Pesticides	Butylate	0.19	U	ug/L	0.19	0.76
8/22/2011 9:30	Equip. Blank	Pesticides	Mevinphos	0.19	U	ug/L	0.19	0.76
8/22/2011 9:30	Equip. Blank	Pesticides	Naled	0.76	U	ug/L	0.76	3.1
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Diethyl phthalate	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Hexachloroethane	2.9	U	ug/L	2.9	12
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Isophorone	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Phenanthrene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Pyrene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2,4-Dichlorophenol	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2,4-Dinitrophenol	14	U	ug/L	14	58
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2,6-Dinitrotoluene	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Anthracene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Benzo(k)fluoranthene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	2.9	U	ug/L	2.9	12

Appendix E. continued

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Fluoranthene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Nitrobenzene	1.9	U	ug/L	1.9	7.7
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2,4-Dinitrotoluene	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2-Nitrophenol	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	4-Nitrophenol	14	U	ug/L	14	58
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Hexachlorobenzene	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Hexachlorobutadiene	2.9	U	ug/L	2.9	12
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Bis(2-chloroethyl)ether	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Dimethyl phthalate	9.6	U	ug/L	9.6	38
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	1.9	U	ug/L	1.9	7.7
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Pentachlorophenol	2.9	U	ug/L	2.9	12
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	3,3'-Dichlorobenzidine	38	UJ	ug/L	38	150
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	4-Bromophenyl phenyl ether	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Acenaphthylene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Benzidine	96	U	ug/L	96	380
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	14	U	ug/L	14	58
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Chrysene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Di-n-butyl phthalate	4.8	U	ug/L	4.8	19
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Fluorene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Naphthalene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	4-Chloro-3-methylphenol	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Butyl benzyl phthalate	4.8	U	ug/L	4.8	19
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Hexachlorocyclopentadiene	2.9	U	ug/L	2.9	12
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	N-Nitrosodimethylamine	1.9	U	ug/L	1.9	7.7
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	2.9	U	ug/L	2.9	12
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Phenol	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2,4-Dimethylphenol	9.6	U	ug/L	9.6	38
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2-Chlorophenol	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	2.9	U	ug/L	2.9	12
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	1.9	U	ug/L	1.9	7.7
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Acenaphthene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Benzo(a)pyrene	0.19	U	ug/L	0.19	0.77

Appendix E. continued

Date	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	1,2,4-Trichlorobenzene	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2,4,6-Trichlorophenol	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	2-Chloronaphthalene	0.96	U	ug/L	0.96	3.8
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Benzo(a)anthracene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Benzo(b)fluoranthene	0.19	U	ug/L	0.19	0.77
8/22/2011 9:30	Equip. Blank	Priority Organic Pollutants	Di-n-octyl phthalate	0.96	U	ug/L	0.96	3.8

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: Blake Vahlsing	**Contact Number:** (407) 897-4172
Facility Name (as it appears on permit): Winter Garden WWTF	**Former Names:** N/A
NPDES Permit Number: FL0020109	**Permit Expiration Date:** December 11, 2012
Physical Address: 101 East Crest Avenue, Winter Garden, FL 34777	
District: Central	**County:** Orange
Function of Facility: Domestic Wastewater Treatment Facility	
Facility Type: Domestic Wastewater	**Permitted Capacity (MGD):** 3.5
Mean Flow for previous 12 months for all EXTERNAL outfalls listed in permit (list date range including months): D-001 outfall flow: 1.28 million gallons per day (MGD) annual average daily flow (aadf), as reported on the February 2011 DMR (March 2010 through February 2011 reporting period).	
If applicable, Mean Flow for previous 12 months for REUSE outfall(s) (list date range including months): R-001 rapid infiltration basins (RIBs) flow: 1.47 MGD aadf; R-002 public access irrigation flow: 0.94 MGD aadf, as reported on the February 2011 DMR (March 2010 through February 2011 reporting period).	
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):
Surface water discharge (D-001) EDF-1 sampling location is at the Parshall flume discharge point. The RIBs (R-001) and public access reuse (R-002) EFA-1 sampling location is after the chlorine contact chambers. Reuse continuous turbidity

Description of Permitted Outfall(s):
D-001 outfall is a 1.75 MGD aafd to Lake Apopka (Class III fresh waters) via an underdrain system of land application system R-001 to an unnamed ditch at outfall D-001, thence through approximately one (1) mile of wetlands and swamp to Lake Apopka. Outfall D-001 is approximately 9 feet in length, discharges at ground level and the point of discharge (D-001) is located approximately at latitude 28° 36' 26" N, longitude 81° 32' 56" W.

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

Winter Garden WWTF is a 4.0 MGD aadf design capacity advanced domestic wastewater treatment facility consisting of flow equalization, influent screening, grit removal, biological nutrient removal (via a 5-stage Bardenpho process advanced waste water treatment system), chemical feed facilities for total suspended solids control, clarification, filtration, disinfection by chlorination, with thickening, aerobic digestion, and dewatering of residuals.

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 (to surface water)	MGD	Maximum	1.75	-	-	-	Continuous	Recording flow meters and totalizers	FLW-2	See Cond. I.A.4
pH	SU	Range	-	-	-	6.0 to 8.5	Continuous	Meter	EFD-1	See Cond. I.A.3
Nitrogen, Total (as N)	MG/L	Maximum	9.17	9.17	-	18.3	Weekly	24-hour flow proportioned composite	EFD-1	See Cond. I.A.6.
Phosphorus, Total (as P)	MG/L	Maximum	0.5	0.5	-	1.0	Weekly	24-hour flow proportioned composite	EFD-1	See Cond. I.A.6.
Cyanide, Total (as CN)	UG/L	Maximum		-	-	5.2	Monthly	24-hour flow proportioned composite	EFD-1	
Hardness, Total (as CaCO3)	MG/L	Maximum	Report	-	-	-	Monthly	24-hour flow proportioned composite	EFD-1	
Mercury, Total Recoverable	UG/L	Maximum		-	-	0.2	Monthly	Grab	EFD-1	
Lead, Total Recoverable	UG/L	Maximum	Hardness	-	-	-	Monthly	Grab	EFD-1	See Cond. I.A.9.
Oxygen, Dissolved (DO)	MG/L	Minimum	-	-	-	5.0	Daily	Grab	EFD-1	
Whole Effluent Toxicity (Chronic)	See Permit Condition I.A.5								EFD-1	

Appendix F. (continued)

PART I: Continued	
Receiving Waters: Lake Apopka	**Classification (indicate whether fresh or marine):** Class III fresh water

Appendix F. (continued)

Toxicity Test Requirements (routine and/or additional test language, test species, salinity adjustment, etc.):
The permittee shall comply with the following requirements to evaluate chronic whole effluent toxicity of the discharge from outfall D-001.

Effluent Limitation:

- Whole effluent chronic toxicity shall not exceed a No Observed Effect Concentration (NOEC) of less than 100% effluent in any test. [Rule 62-302.530(62), F.A.C.]

Monitoring Frequency

- "Routine" toxicity tests shall be conducted once every six months, the first starting within 60 days of permit issuance, and lasting for the duration of this permit unless a reduction in the frequency of monitoring is granted in writing by the Department.

Test Requirements

- Routine Tests: 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
- Additional Follow-up Testing Requirements, if required:

If a routine test does not meet the chronic toxicity limitation in 5.a. (1) above, the permittee shall conduct two additional definitive tests on each species that failed the test.

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 additional follow-up 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.

The first additional test shall be initiated within two weeks of the end of the failed routine test and weekly thereafter until a total of two valid additional follow-up tests are completed.

- The permittee shall conduct a daphnid, Ceriodaphnia dubia, Survival and Reproduction Test and a fathead minnow, Pimephales promelas, Larval Survival and Growth Test, concurrently.
- 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. Any deviation of the bioassay procedures outlined herein shall be submitted in writing to the Department for review and approval prior to use. In the event the above method is revised, the permittee shall conduct chronic toxicity testing in accordance with the revised method.
- The control water and dilution water used shall be moderately hard water as described in EPA-821-R-02-013, Section 7.

Sampling Requirements

- For each routine test or additional follow-up test conducted, a total of three 24-hour composite samples of final effluent shall be collected and used per the sampling protocol discussed in EPA-821-R-02-013, Section 8.
- 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.

Quality Assurance Requirements

- A standard reference toxicant (SRT) quality assurance (QA) chronic toxicity test shall be conducted with each species used in the required toxicity tests either concurrently or started no more than 30 days before the date of each routine or additional follow-up test conducted. The SRT-QA data shall be submitted with each companion routine or additional follow-up test required.
- If the mortality in the control (0% effluent) exceeds 20% for either species in any test or does not meet "test acceptability criteria", the test for that species (including the control) shall be invalidated and the test repeated. Test acceptability criteria for each species are defined in EPA-821-R-02-013, Section 13.12 (C. dubia) and Section 11.12 (P. promelas).

Appendix F. (continued)

Administrative or Consent Orders (date executed, specific requirements, interim limit dates-if applicable, etc.):
Administrative Order No.:07-0004 was issued to evaluate potential sources of elevated mercury concentrations. The AO established the interim limit of 0.200 ug/L. Modifications were to be completed within 36 months of permit issuance. On August 8, 2011, the DEP issued a letter requesting the facility provide a revised time frame for complying with the water quality standard for mercury that will be met prior to the expiration date of the permit.

Facility Mixing Zone Details (if applicable):
Not Applicable

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

Date	Parameter and Units	Limit	Result
December 2010	Total Cyanide Max (D-001)	5.2 ug/L	10 ug/L
January 2011	Total Cyanide Max (D-001)	5.2 ug/L	<10 ug/L*
January 2011	TSS Max (R-002)	5.0 mg/L	7.0 mg/L

***January 2011 Total Cyanide exceedance occurred because the laboratory did not use the proper MDL/**

Describe previous FYI and WQBEL study:
A third year inspection and bioassay was performed August 28, 2006. The effluent sample collected from EFD-1 NPDES sampling point for Outfall D-001 for this facility was not acutely toxic to either test species during the 48-hour acute definitive bioassays. The effluent freshwater AGP result exceeded the "problem" threshold for fresh receiving waters, suggesting nutrient enrichment may be an issue. Effluent water quality samples did not violate Class III freshwater quality criteria nor exceed any permit limits.

Describe previous or current Enforcement Actions (including WLs):
Not Applicable

Describe any violations or problems noted in previous inspection:
Through August 2009 through July 2010, the facility had exceeded the permitted flow annual average limit of 1.75 MGD for D-001 (the highest exceedance was 2.14 MGD in April 2010). Since the facility has been creating reuse water, flow annual average to D-001 has returned to compliance, and was reported as 1.28 MGD on the February 2011 DMR.

Discuss whether DMR trends within previous five years are improving or declining:
Flow to D-001 was consistently increasing over the past five years, but has steadily decreased over the past year. Nitrogen and Phosphorus annual averages have remained consistent over the past five years.

Additional Comments (this should include a trend of the Effluent, i.e. if the facility is now Reuse):
As mentioned above, the facility has been increasing its output of reuse water, and flow annual average to D-001 has been steadily dropping. D-001 annual average flow has been in compliance since August 2010, and was reported as 1.28 MGD on the February 2011 DMR. The facility has a permitted flow annual average of 4.0 MGD, and was reported as 0.94 MGD on the February 2011 DMR.

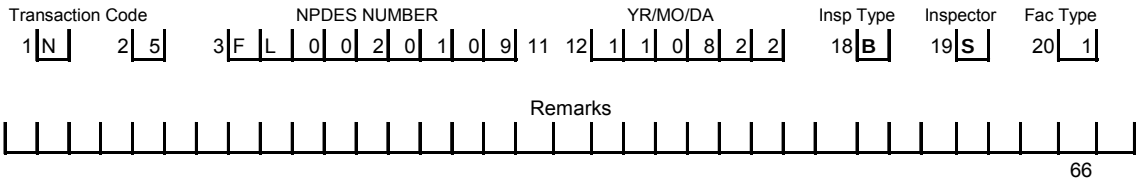
Appendix F. (continued)

PART II: Sampling Event Information	
Date(s) sample collected: August 22, 2011	**Sampler(s):** Phil Kane, Steve Kent, Blake Vahlsing
Temperature (Deg C): 27.37 ˚C	**pH (SU):** 6.94 SU
Dissolved Oxygen (mg/L): 6.41 mg/L	**Conductivity (umhos/cm):** 620 umhos/cm
Total Residual Chlorine (mg/L): 1.88 mg/L	**Total Residual Chlorine - after Dechlorination (mg/L):** N/A
Method of Chlorination: Liquid chlorine	**Method of De-chlorination:** The under-drain de-chlorinates
Flow During Sample Collection for each outfall(MGD): The flow from the under-drain was continuous into the wetlands/swamp. At the time of sample, the flow rate was 0.733 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):	
Describe Discharge during Sampling Event: ☒ **Continuous** ☐ **Intermittent** ☐ **Seasonal** ☐ **Rainfall Dependent** ☐ **Other, please specify**	
Describe any conditions, difficulties or abnormal events that occurred during the sample collection and any other additional comments here: The sampling location was difficult to reach the flume. Samples were collected from inside the sampling building using the pump used by the continuous pH meter.	

Appendix G. EPA End Sheet

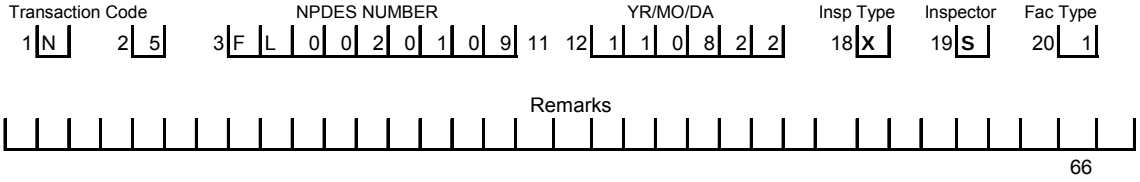
The Bioassay of the Winter Garden WWTF effluent sampled on August 22, 2011. NPDES #FL0020109.

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



The Priority Pollutants Analysis of Winter Garden WWTF effluent sampled on August 22, 2011. NPDES #FL0020109.

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



Biological Analyses of Winter Garden WWTF effluent sampled on August 22, 2011. NPDES #FL0020109.

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

