

Effluent Quality Assessment

Holly Hill Wastewater Treatment Facility (WWTF)

Volusia County

NPDES #FL0027677

Sampled January 30, 2012

April 2012

Biology Section
Bureau of Laboratories
Division of Environmental Assessment and Restoration

Quality Manual No. 870346G
NELAC Certification No. E31780

CONTENTS

Introduction

Methods

Results and Discussion

Literature Cited

Tables

Table 1 Measured and predicted algal growth potential (AGP)

Table 2 Effluent limits, Class III Marine Criteria, chemical data, and qualifiers

Appendices

Appendix A Chain of Custody

Appendix B Bioassay Bench Sheets

Appendix C SRT Bench Sheets and Statistical Analyses

Appendix D Organism Receipts

Appendix E Chemical Analyses of Effluent

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

Appendix G EPA End Sheet

Holly Hill Wastewater Treatment Facility (WWTF), 453 LPGA Boulevard, Holly Hill, Volusia County, Florida, 32117. NPDES #FL0027677. Effluent grab samples for biological and chemical analyses for this facility were collected on January 30, 2012.

Introduction

Holly Hill WWTF is an advanced wastewater treatment facility which utilizes an oxidation ditch system to treat domestic wastewater prior to discharge of effluent to the Class III Marine waters of the Halifax River or to reuse through a slow-rate public access reuse system. (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, Blake Vahlsing, and Steve Kent (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 test (AGP) 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 January 31- February 4, 2012.

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

  Pimephales promelas 96-hour acute definitive bioassay – LC_{50} > 100%, 5%* mortality in 100% sample at 96 hours

  The bioassay samples were not acutely toxic to the test organisms.

  *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 27.1 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 phosphorus-limited (Table 1; Miller et al. 1978).

Normally, when a freshwater effluent discharges to marine receiving water, the laboratory splits the sample and runs a salinity adjusted effluent sample as well. However, in this case, not enough sample was received to perform an additional analysis.

See Table 1 for AGP results and qualifiers.

- **Chemistry Results**
  Total residual chlorine and total ammonia were not detected in the bioassay sample in the laboratory. The total ammonia concentration in the grab sample collected and preserved for chemical analysis was 0.043 mg N/L. Based on the pH, salinity, and temperature of the effluent as collected, the calculated unionized ammonia concentration was ≤ 0.02 mg/L.

  The effluent's nickel (21.7 µg/L) exceeded the Class III marine criterion (62-305.530(44) F.A.C.). Aluminum, bromodichloromethane, copper and zinc were detected in the effluent at levels that comply with Class III marine water quality criteria (62-302.530, F.A.C.) and/or the facility's permitted limit. Lead and Carbonaceous Biochemical Oxygen Demand (CBOD) were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL). Isophorone was also detected in the effluent, but there is currently no water quality criterion for this organic constituent. Copper and nickel were detected between the MDL and PQL in the equipment blank.

  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 sample collected from the EFD-1 NPDES sampling point for this facility on January 30, 2012, was not acutely toxic to either test species during the 96-hour acute definitive bioassays. Although a salinity-adjusted AGP test was not performed, the effluent AGP result was significantly greater than the "problem" threshold for fresh receiving waters suggesting that nutrient enrichment could be an issue in marine receiving waters. However, a marine AGP test should be performed for verification of potential nutrient enrichment. The effluent's nickel (21.7 µg/L) concentration exceeded the Class III marine criterion for nickel. Although nickel and copper were detected between the MDL and PQL in the equipment blank, the effluent value for nickel was more than 10 times that of the equipment blank. Additional effluent water quality samples collected on January 30, 2012, did not exceed Class III water quality standards 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) orthophosphate (OP) and total phosphorus (TP) limitation of the freshwater species *Pseudokirchneriella subcapitata* and ratios of nitrogen to phosphorus for samples collected from the Holly Hill WWTF on January 30, 2012.

Holly Hill WWTF NPDES# FL0027677					
Location	AGP (measured)	Predicted AGP (OP) ± 20%	Predicted AGP (TP) ± 20%	Inorganic N:P ratio	Total N:P ratio
Effluent	27.1	12.0 ± 2.4	19.4 ± 3.9	28.7	34.4

Table 2. Effluent limits, Class III Criteria for predominantly marine waters, chemical data, and qualifiers for the Holly Hill WWTF sampled on January 30, 2012 .

Holly Hill WWTF NPDES# FL0027677	Class III Criteria	Effluent Limits	Effluent	
Organic Constituents (µg/L)				
Bromodichloromethane	≤ 22.0 a	≤ 44.0 ap	19	
Isophorone	-	-	5.5	
Metals (µg/L unless otherwise noted)				
Aluminum	≤ 1500	-	82	
Arsenic	≤ 50	-	0.25	U
Cadmium	≤ 8.8	-	0.05	U
Calcium (mg/L)	-	-	75.9	
Chromium-III	-	-	0.3	U
Copper	≤ 3.7	-	1.11	J
Iron	≤ 300	-	30	U
Lead	≤ 8.5	-	0.34	I
Magnesium (mg/L)	-	-	6.67	
Nickel	≤ 8.3	-	21.7	
Selenium	≤ 71	-	0.2	U
Silver	≤ 2.3	-	0.025	U
Zinc	≤ 86	-	60	
Nutrients (mg/L)				
Ortho-phosphate	-	-	0.028	
Total Phosphorus	-	2.0 p	0.045	
Total Ammonia	-	-	0.043	
Un-ionized Ammonia	-	-	≤ 0.02	c
Nitrate and Nitrite	-	-	0.76	
Total Kjeldahl Nitrogen	-	-	0.79	
Organic Nitrogen	-	-	0.747	c
Total Nitrogen	-	6.0 p	1.55	c
General Physical and Chemical Parameters				
Dissolved Oxygen (mg/L)	≥ 4.0	≥ 2.0	9.3	
pH (SU)	6.5 - 8.5	6.5 - 8.5	7.4	
Conductivity (µmhos/cm)	-	-	721	
Temperature (°C)	-	-	20.4	
Salinity	-	-	≤ 0.39	c
Total Residual Chlorine (mg/L)	≤ 0.01	≤ 0.01	≤ 0.03	UQ£
Chlorophyll *a* (µg/L) - Corrected	-	-	0.55	U
Phaeophytin (µg/L)	-	-	0.4	U
Total Suspended Solids (mg/L)	-	10.0 p	2	U
CBOD, 5 day (mg/L), N - inhibited	-	20.0 p	1.4	I
Flow (MGD)	-	3	0.966	a*
Hardness (mg $CaCO_3$)	-	-	216.99	c

Value exceeds the Class III Water Quality Criteria or permit limits

a - Annual average

c - Value is calculated

p - 16 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

* - Measured From 11/2010 to 10/2011 and recorded on the October 2011 DMR

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2012-01-30-22
FYI3 City of Holly Hill Sampling Inspection

Requester: Michelle Fish

Field Report Prepared By: KANE

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

Collected By: KANE, KENT, VAHLSING

Sampling Agency: FDEP CENTRAL DISTRICT

Send Final Report To: ~~Gary Miller~~ PK Phil Kane

Lab ID *	Location: HOLLY HILL WWTF 453 LPGA BLVD. HOLLY HILL, FL. 32117			☐ Comp ☑ Grab	Collection (begin) ~~Date~~ ~~Time~~ PK ~~Eastern Central~~		Collection (end) Date 1/30/12 Time 9:17 ~~AM~~ PK Eastern Central		Bottle Group(s)** A
	Field ID: HOLL			Tot Res Chlorine (mg/L): 0.00 surf meter		Diss Oxygen (mg/L): 9.3	Stored Station Number		
	Matrix (include type e.g. Salt, Fresh, etc): EFFLUENT	Temp (C): 20.4	pH: 7.4	Sample Depth ☐ m ☐ ft: SURFACE	721	☐ Salinity (PPTh) ☑ Sp Conductance (umho/cm)	NPDES Number: FL0027677		
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: pH checked: <2 SU					
Lab ID *	Location: HOLLY HILL WWTF 453 LPGA BLVD. HOLLY HILL, FL. 32117			☐ Comp ☑ Grab	Collection (begin) ~~Date~~ ~~Time~~ PK ~~Eastern Central~~		Collection (end) Date 1/30/12 Time 9:17 ~~AM~~ PK Eastern Central		Bottle Group(s)** A
	Field ID: HOLL			Tot Res Chlorine (mg/L): 0.00 surf meter		Diss Oxygen (mg/L): 9.3	Stored Station Number		
	Matrix (include type e.g. Salt, Fresh, etc): EFFLUENT	Temp (C): 20.4	pH: 7.4	Sample Depth ☐ m ☐ ft: SURFACE	721	☐ Salinity (PPTh) ☑ Sp Conductance (umho/cm)	NPDES Number: FL0027677		
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: BIOASSAY pH					
Lab ID *	Location: HOLLY HILL WWTF 453 LPGA BLVD. HOLLY HILL, FL. 32117			☐ Comp ☑ Grab	Collection (begin) ~~Date~~ ~~Time~~ PK ~~Eastern Central~~		Collection (end) Date 1/30/12 Time 9:17 ~~AM~~ PK Eastern Central		Bottle Group(s)** B
	Field ID: HOLL			Tot Res Chlorine (mg/L):		Diss Oxygen (mg/L):	Stored Station Number		
	Matrix (include type e.g. Salt, Fresh, etc): BLANK	Temp (C):	pH:	Sample Depth ☐ m ☐ ft: SURFACE		☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number: FL0027677		
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: BLANK pH checked: <2 SU					
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)	Stored 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: 4bioassay Rec'd in Tox by J.D. 10:44 1/30/12					

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
KANE	1/30/12	FEDEX	ABB	01-31-12 10:00				

* 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: City of Holly Hill WWTF
Address: 453 LPGA Boulevard
City: Holly Hill **County:** Volusia 32117
Contact/District: Phil Kane / Central
NPDES Permit #: FL 0027677
LIMS Job #: T44-2012-01-31-48 **LIMS Sample #:** 1405627
Lims Data Entry: 2-7-12 MF Data Entry Verification: 2-7-12 MF

Sample Collection: Date: 1-30-12 Time: 0917
Hold Time Start: Date: 1-30-12 Time: 0917

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:
Bruce Albright	BA
James Daniels	JD
Marshall Faircloth	MF
Willie Day	AD

REVIEWER: Lisa Miller LM

Diluent/Control Batch#:	1.10.12		1.3.12						
Water Quality Parameters	SDMW ~~20% DMW~~	Well Water	Moderately Hard Water	Salt Water ASW NSW Test 1	Salt Water ASW NSW Test 2	Other:	Original Sample 1405627	Measured by	Verified by
Field Total Residual Cl_2 (mg/L):	N/A	N/A	N/A	N/A	N/A	N/A	0.00	facility meter	MF
Lab Total Residual Cl_2 (mg/L):	<0.03		<0.03				<0.03	AD/JD	MF
Alkalinity (mg/L as $CaCO_3$):	84		87				164	AD/JD	MF
Hardness (mg/L as $CaCO_3$):	75		97				233	AD/JD	MF
Total Ammonia (mg/L as N):	<0.017		<0.017				<0.017	MF	BA
Conductivity: ☑ µmhos ☐ mmhos	320		345				773	~~AD~~ JD	MF
Salinity (ppth):									

V 1.8 1/13/10

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample # 1405627 SOP#: TA 07 01 Test # 1 of 2

Test Species:
Batch #: 1-31-12 Age: <24hrs
- [x] *Ceriodaphnia dubia*
- [ ] *Cyprinella leedsi*
- [ ] *Pimephales promelas*
- [ ] *Menidia beryllina*
- [ ] *Americamysis bahia*
- [ ] Other:_____

Test Validation
Control Survival ≥ 90%: [x] Yes [ ] No
Temperature Range ≤ 3° C: [x] Yes [ ] No

Diluent (Batch # 1,10,12)
- [ ] ASW
- [x] 20% DMW
- [ ] MHW
- [ ] Well

Test Type: [x] Acute [x] Definitive
[ ] Static [x] Static Renewal [ ] Flow-Through

Test Duration:
- [ ] 48 hr
- [x] 96 hr
- [ ] ___hr

Test Started: Date 1.31.12 Time 1300

Test Ended: Date 2.4.12 Time 10.00

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	5	5
	B	B	5	5	5	5	5	5
	C	C	5	5	4 1D	4	4	4
	D	D	5	5	5	5	5	5
12.5%	A	A	5	5	5	5	4 1D	4
	B	B	5	5	4 1D	4	3 1D	3
	C	C	5	5	4 1D	4	4	4
	D	D	5	5	5	5	5	5
25%	A	A	5	2 3D	2	2	2	2
	B	B	5	5	3 2D	3	3	3
	C	C	5	5	5	5	4 1D	4
	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	4 1D	4	4	4
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	BA	BA	BA	BA	BA

Food
- [x] YCT Batch# 8.26.11 10.31 MF
- [x] P. subcapitata Batch# 8.4.11
- [ ] Artemia Lot#_____

Feeding and Artemia Batch #
- [x] 0 hr____ [ ] Prior to Test
- [ ] 24 hr____
- [x] 48 hr____ [ ] Prior to Renewal
- [ ] 72 hr____

Temperature Range (°C)
Incubator # 2
min. 24.4 max. 26.3 mean ___
Incubator #___
min.____max.____mean____
Waterbath #___
min.____max.____mean____
Room B246
min. 23.6 max. 25.5 mean ___
Light intensity (ft. candles): 50-100
Photoperiod (hours): 16 light, 8 dark

Loaded/ Verified by: BA/ AD
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%
Calculated LC50 (Definitive) 95% CI_____ Method_____

Comments:

V. 2.5 1-13-10

Page 38

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

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample # 1405627 SOP#: TA 07 02 Test # 2 of 2

Test Species:
Batch #: 1.31.12 Age: 8days
- ☐ *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 # 1.3.12)
☐ ASW ☐ 20% DMW ☒ MHW ☐ Well

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

Test Duration: ☐ 48 hr ☒ 96 hr ☐ ___hr

Test Started: Date 1.31.12 Time 1330

Test Ended: Date 2.4.12 Time 9:45

Chamber Size 300 ml
Test Volume: 400 ml

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Ctrl	A	B1	10	10	10	10	10	10
	B	B2	10	10	10	10	10	10
6.25%	A	B3	10	10	10	10	10	10
	B	B4	10	10	10	10	10	10
12.5%	A	B5	10	10	10	10	10	10
	B	B6	10	10	10	10	10	10
25%	A	B7	10	10	10	10	10	10
	B	B8	10	10	10	10	10	10
50%	A	B9	10	10	10	10	10	10
	B	B10	10	10	10	10	10	10
100%	A	B11	10	10	10	10	10	10
	B	B12	10	10	10	10	9 10	9
Checked or Verified by:			JD	JD	AD	AD	JD	BA

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

Feeding and Artemia Batch #
- ☒ 0 hr 9-12 ☐ Prior to Test
- ☐ 24 hr___
- ☒ 48 hr 10-12 ☐ Prior to Renewal
- ☐ 72 hr___

Temperature Range (°C)
Incubator # 3
min. 24.6 max. 26.4 mean ~
Incubator #___
min.___ max.___ mean___
Waterbath #___
min.___ max.___ mean___
Room B246
min. 23.6 max. 25.5 mean ~

Light Intensity (ft. candles): 50-100

Photoperiod (hours): 16 light, 8 dark

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

Test Results:
Screening Tests: Report LC50 as >100%, =100%, or <100%.
% mortality in 100% sample: 5% 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)

Page 39

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Parameter Sheet

LIMS Sample # 1405627 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)
Cntl	M	0 hr	7.8	24.0	7.6	0.320	
	–	24 hr	–	–	–	–	
	A	48 hr BR	7.5	23.8	7.0	0.358	
	M	48 hr AR	~~8.7~~	24.0	7.5	0.306	
		72 hr BA	7.45				
	A	96 hr	7.6	24.0	6.6	0.368	
6.25%	M	0 hr	7.8	24.1	8.2	0.353	
	–	24 hr	–	–	–	–	
	A	48 hr BR	7.8	23.9	7.3	0.383	
	M	48 hr AR	7.5	24.3	7.5	0.373	
		72 hr					
	A	96 hr	7.6	24.0	6.7	0.416	
12.5%	M	0 hr	7.8	24.2	7.8	0.369	
	–	24 hr	–	–	–	–	
	A	48 hr BR	8.0	23.8	7.8	0.405	
	M	48 hr AR	7.7	24.1	7.6	0.312	
		72 hr					
	A	96 hr	7.7	24.0	6.7	0.362	
25%	M	0 hr	7.8	24.0	8.0	0.407	
	–	24 hr	–	–	–	–	
	A	48 hr BR	8.2	23.8	7.8	0.467	
	M	48 hr AR	7.6	24.3	7.9	0.430	
		72 hr					
	A	96 hr	7.7	24.0	6.8	0.485	
50%	M	0 hr	7.7	24.1	7.9	0.551	
	–	24 hr	–	–	–	–	
	A	48 hr BR	8.3	23.9	8.0	0.607	
	M	48 hr AR	7.5	24.7	8.1	0.543	
		72 hr					
	A	96 hr	7.8	24.0	6.9	0.557	
100%	M	0 hr	7.5	24.3	7.8	0.778	
	–	24 hr	–	–	– ME	–	
	A	48 hr BR	8.4	23.3	~~7.8~~ 7.8	1.160	
	M	48 hr AR	7.4	25.0	8.2	0.785	
		72 hr					
	A	96 hr	7.8	24.0	7.0	0.941	
		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: AD
24 hr:
48 hr BR: AD
48 hr AR: AD
72 hr:
96 hr: BA

Page 40

V. 2.3 1-13-10

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Parameter Sheet

LIMS Sample # 1405627 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)
	A	0 hr	7.7	25.9	8.0	345	
	B	24 hr	7.5	24.0	5.8	334	
4.1	A	48 hr BR	7.4	24.3	7.0	335	
	A	48 hr AR	7.3	25.1	7.2	336	
	B	72 hr	7.4	24.0	6.1	335	
	A	96 hr	8.0	24.0	6.8	354	
	A	0 hr	7.7	25.9	8.1	374	
	B	24 hr	7.6	24.1	6.7	365	
6.25%	A	48 hr BR	7.6	24.1	7.2	365	
	A	48 hr AR	7.7	25.3	7.2	368	
	B	72 hr	7.5	24.0	6.6	365	
	A	96 hr	8.0	24.1	6.7	384	
	A	0 hr	7.7	25.9	7.8	391	
	B	24 hr	7.7	24.0	6.7	382	
12.5%	A	48 hr BR	7.6	24.1	6.0	383	
	A	48 hr AR	7.8	24.9	7.5	394	
	B	72 hr	7.4	24.2	6.7	390	
	A	96 hr	7.9	24.2	6.7	409	
	A	0 hr	7.7	25.9	7.7	452	
	B	24 hr	7.8	24.0	6.6	439	
25%	A	48 hr BR	7.6	24.2	7.2	441	
	A	48 hr AR	7.8	25.2	7.2	449	
	B	72 hr	7.5	24.2	6.7	444	
	A	96 hr	7.8	24.3	6.8	464	
	A	0 hr	7.7	25.6	7.10	542	
	B	24 hr	7.9	24.0	6.7	539	
50%	A	48 hr BR	7.8	24.2	7.3	542	
	A	48 hr AR	7.8	25.2	7.3	558	
	B	72 hr	7.6	24.0	6.5	561	
	A	96 hr	7.8	24.3	6.7	584	
	A	0 hr	7.5	25.9	7.9	773	
	B	24 hr	8.0	24.0	6.6	761	
100%	A	48 hr BR	8.1	24.8	7.2	773	
	A	48 hr AR	7.6	25.5	7.4	773	
	B	72 hr	7.7	24.5	6.2	769	
	A	96 hr	7.8	24.1	6.6	804	
		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: JD/AD
24 hr: JD/AD
48 hr BR: AD/JD
48 hr AR: JD/AD
72 hr: AD/JD
96 hr: BA

Page 41

V. 2.3 1-13-10

Appendix B. (continued)

Test ID: Holly Hill WWTF Dates: 1-31-12 — 2-1-12

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	✓
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 on 2-6-12 JH

V 1.1 9/21/10 MF/jd

Page 42

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)

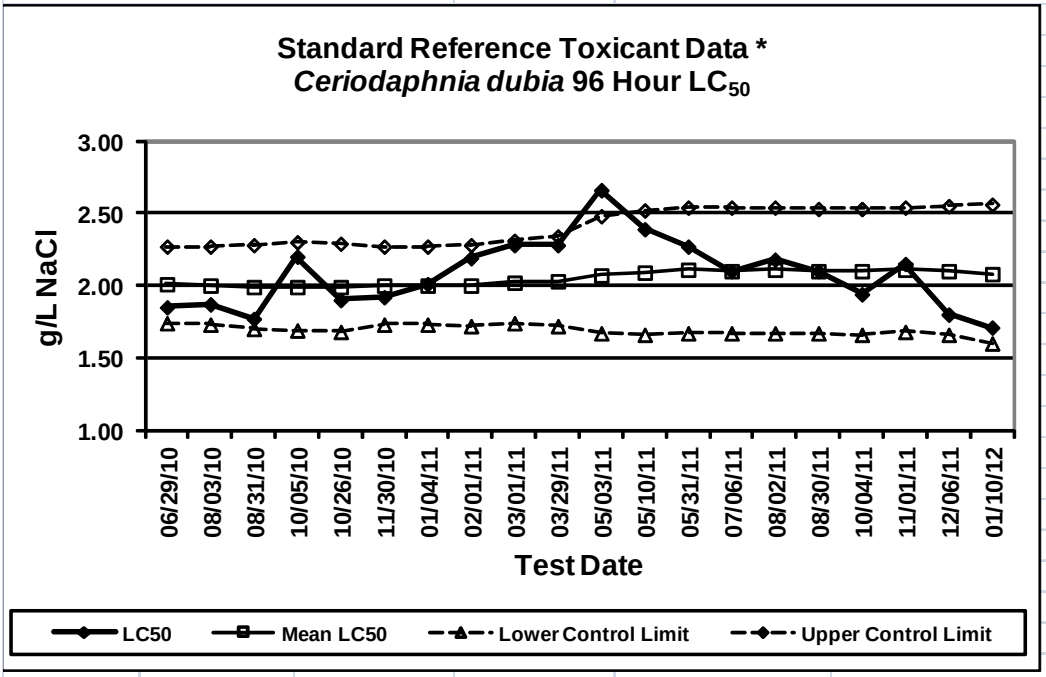
Date	LC50	Mean LC50	Std Dev	Lower Control Limit	Upper Control Limit
06/29/10	1.85	2.01	0.13	1.74	2.27
08/03/10	1.87	2.00	0.14	1.73	2.27
08/31/10	1.77	1.99	0.14	1.70	2.28
10/05/10	2.20	1.99	0.15	1.69	2.30
10/26/10	1.90	1.99	0.15	1.68	2.29
11/30/10	1.92	2.00	0.14	1.73	2.27
01/04/11	2.01	2.00	0.14	1.73	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
08/30/11	2.10	2.10	0.22	1.67	2.53
10/04/11	1.94	2.10	0.22	1.66	2.53
11/01/11	2.15	2.11	0.22	1.68	2.54
12/06/11	1.80	2.10	0.22	1.66	2.55
01/10/12	1.71	2.08	0.24	1.60	2.56

Lower Control Limit = mean - 2 * Std Dev

Upper Control Limit = mean + 2 * Std Dev

CV = 11.48

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

Pimephales promelas (fathead minnow) - Sodium Chloride (NaCl)

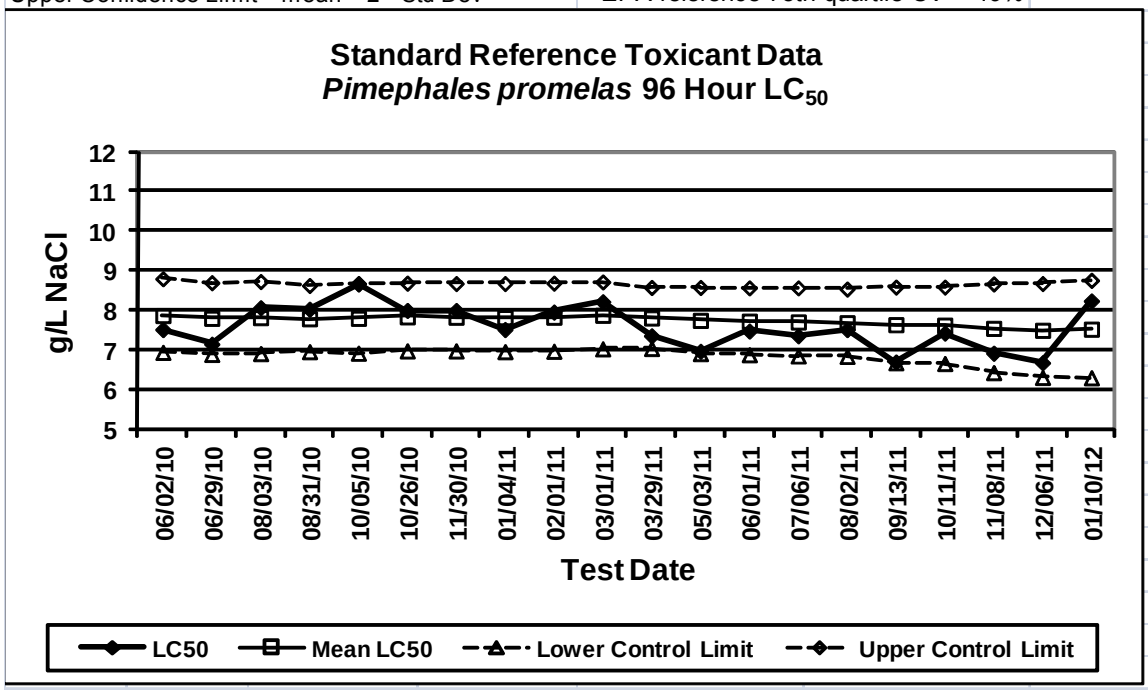
Date	LC50	Mean LC50	Std Dev	Lower Control Limit	Upper Control Limit
06/02/10	7.51	7.86	0.46	6.93	8.79
06/29/10	7.14	7.79	0.45	6.88	8.69
08/03/10	8.06	7.81	0.45	6.9	8.72
08/31/10	8.02	7.78	0.42	6.95	8.62
10/05/10	8.66	7.79	0.44	6.91	8.67
10/26/10	7.99	7.83	0.43	6.97	8.68
11/30/10	7.99	7.82	0.42	6.97	8.67
01/04/11	7.51	7.81	0.43	6.95	8.67
02/01/11	7.95	7.82	0.43	6.96	8.68
03/01/11	8.21	7.87	0.42	7.02	8.71
03/29/11	7.35	7.8	0.39	7.03	8.57
05/03/11	6.96	7.74	0.42	6.9	8.57
06/01/11	7.47	7.72	0.42	6.87	8.56
07/06/11	7.35	7.7	0.43	6.84	8.56
08/02/11	7.51	7.68	0.43	6.83	8.53
09/13/11	6.69	7.63	0.48	6.67	8.58
10/11/11	7.42	7.62	0.48	6.65	8.58
11/08/11	6.91	7.54	0.56	6.42	8.66
12/06/11	6.67	7.49	0.59	6.3	8.67
01/10/12	8.23	7.52	0.61	6.29	8.75

Lower Confidence Limit = mean - 2 * Std Dev

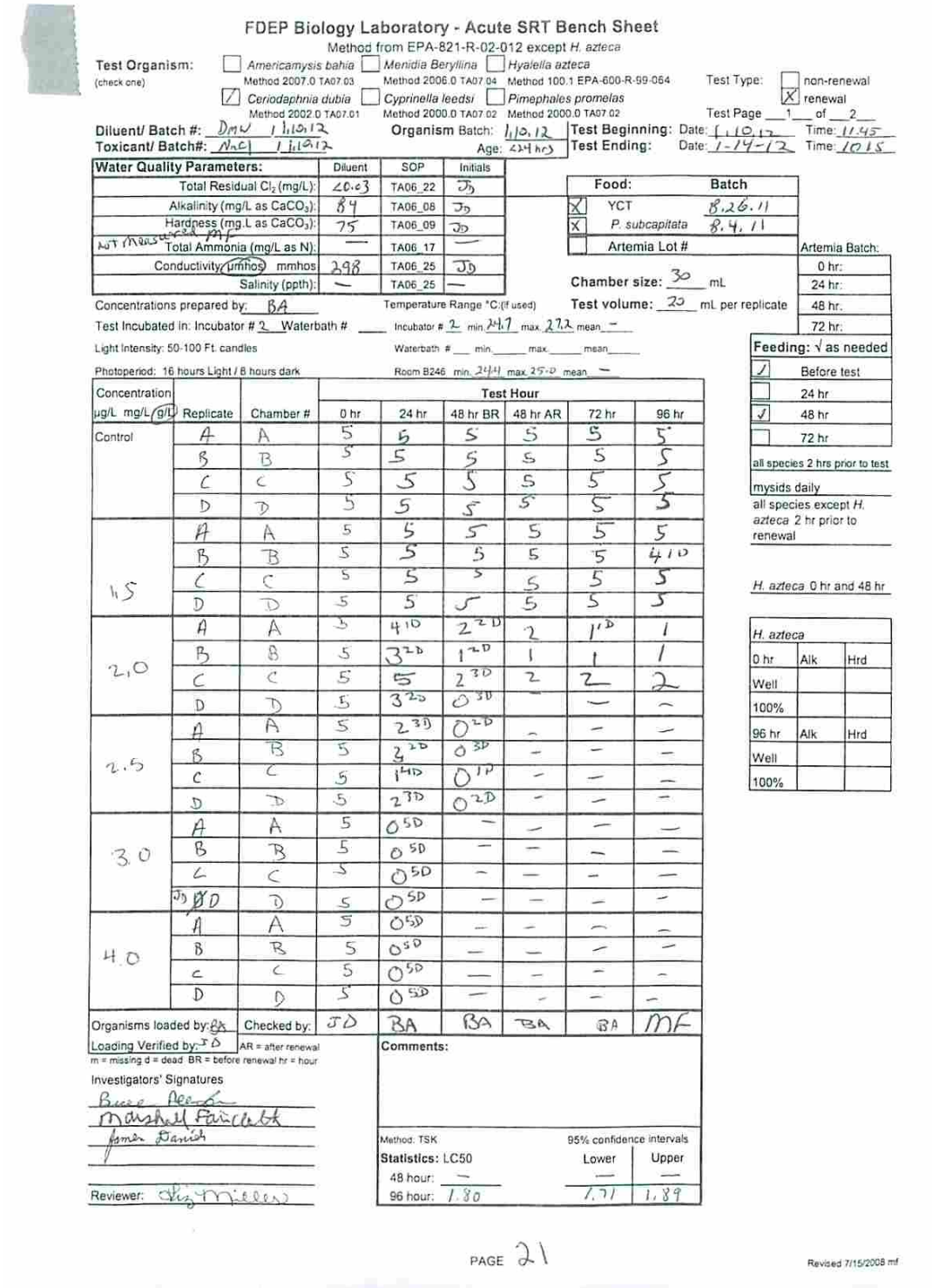
Upper Confidence Limit = mean + 2 * Std Dev

CV = 8.16

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 1.10.12 **Organism Batch:** 1.10.12 **Test Beginning:** Date: 1.10.12 Time: 11.45

Toxicant/ Batch#: NaCl 1.10.12 Age: 24 hrs **Test Ending:** Date: 1-14-12 Time: 10.15

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<0.03	TA06_22	JD
Alkalinity (mg/L as $CaCO_3$):	84	TA06_08	JD
Hardness (mg.L as $CaCO_3$):	75	TA06_09	JD
NOT measured MF Total Ammonia (mg/L as N):	—	TA06_17	—
Conductivity (umhos) mmhos:	298	TA06_25	JD
Salinity (ppth):	—	TA06_25	—

Food:	Batch
☒ YCT	8.26.11
☒ *P. subcapitata*	8.4.11
☐ Artemia Lot #	Artemia Batch:

Chamber size: 30 mL

Test volume: 25 mL per replicate

Concentrations prepared by: BA

Temperature Range °C:(if used)

Test Incubated In: Incubator # 2 Waterbath # ___ Incubator # 2 min. 24.7 max. 27.2 mean. —

Light Intensity: 50-100 Ft. candles Waterbath # ___ min. ___ max. ___ mean. ___

Photoperiod: 16 hours Light / 8 hours dark Room B246 min. 24.4 max. 25.0 mean. —

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

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 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	5	5	5	5	5
	B	B	5	5	5	5	5	4 1D
	C	C	5	5	5	5	5	5
	D	D	5	5	5	5	5	5
2.0	A	A	5	4 1D	2 2D	2	1 1D	1
	B	B	5	3 2D	1 2D	1	1	1
	C	C	5	5	2 3D	2	2	2
	D	D	5	3 2D	0 3D	—	—	—
2.5	A	A	5	2 3D	0 2D	—	—	—
	B	B	5	3 2D	0 3D	—	—	—
	C	C	5	1 4D	0 1D	—	—	—
	D	D	5	2 3D	0 2D	—	—	—
3.0	A	A	5	0 5D	—	—	—	—
	B	B	5	0 5D	—	—	—	—
	C	C	5	0 5D	—	—	—	—
	JD ~~BD~~	D	5	0 5D	—	—	—	—
4.0	A	A	5	0 5D	—	—	—	—
	B	B	5	0 5D	—	—	—	—
	C	C	5	0 5D	—	—	—	—
	D	D	5	0 5D	—	—	—	—
Organisms loaded by: BA	Checked by:		JD	BA	BA	BA	BA	MF

Loading Verified by: JD AR = after renewal

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

Comments:

Investigators' Signatures

Bruce Aeo

Marshall Faircloth

Jomar Davis

Reviewer: Amy Millsaps

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour:	—	—
96 hour: 1.80	1.71	1.89

PAGE 21

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: 1/10/12 Time: 1145
Test Ending: Date: 1-14-12 Time: 1015
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	7.6	24.9	6.9	298	
	—	24 hr	—	—	—	—	
	A	48 hr BR	7.7	24.9	6.8	350	
	M	48 hr AR	7.6	26.0	7.0	371	
		72 hr					
	A	96 hr	7.7	24.3	7.8	380	
1.5	M	0 hr	7.6	24.8	6.7	3010	
	—	24 hr	—	—	—	—	
	A	48 hr BR	7.8	24.6	6.6	3450	
	M	48 hr AR	7.7	26.0	6.8	3170	
		72 hr					
	A	96 hr	7.7	24.4	8.0	3380	
2.0	M	0 hr	7.7	24.8	6.6	3920	
	—	24 hr	—	—	—	—	
	A	48 hr BR	7.8	24.5	6.3	4500	
	M	48 hr AR	7.7	26.0	6.9	4020	
		72 hr					
	A	96 hr	7.7	24.6	8.0	4210	
2.5	M	0 hr	7.7	24.7	6.8	4790	
	—	24 hr	—	—	—	—	
	A	48 hr BR	7.8	24.5	6.0	5150	
	M	48 hr AR	7.7	26.0	6.7	4850	
		72 hr					
		96 hr					
3.0	M	0 hr	7.8	24.7	6.7	5540	
	A	24 hr	7.6	24.7	7.3	5770	
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					
4.0	M	0 hr	7.8	24.6	6.8	7200	
	A	24 hr	7.7	24.6	7.1	7300	
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					

Investigators Signatures
Bruce Alexander
Marshall Faucett
Jenn Tomb

Reviewed By: Lisa Miller

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

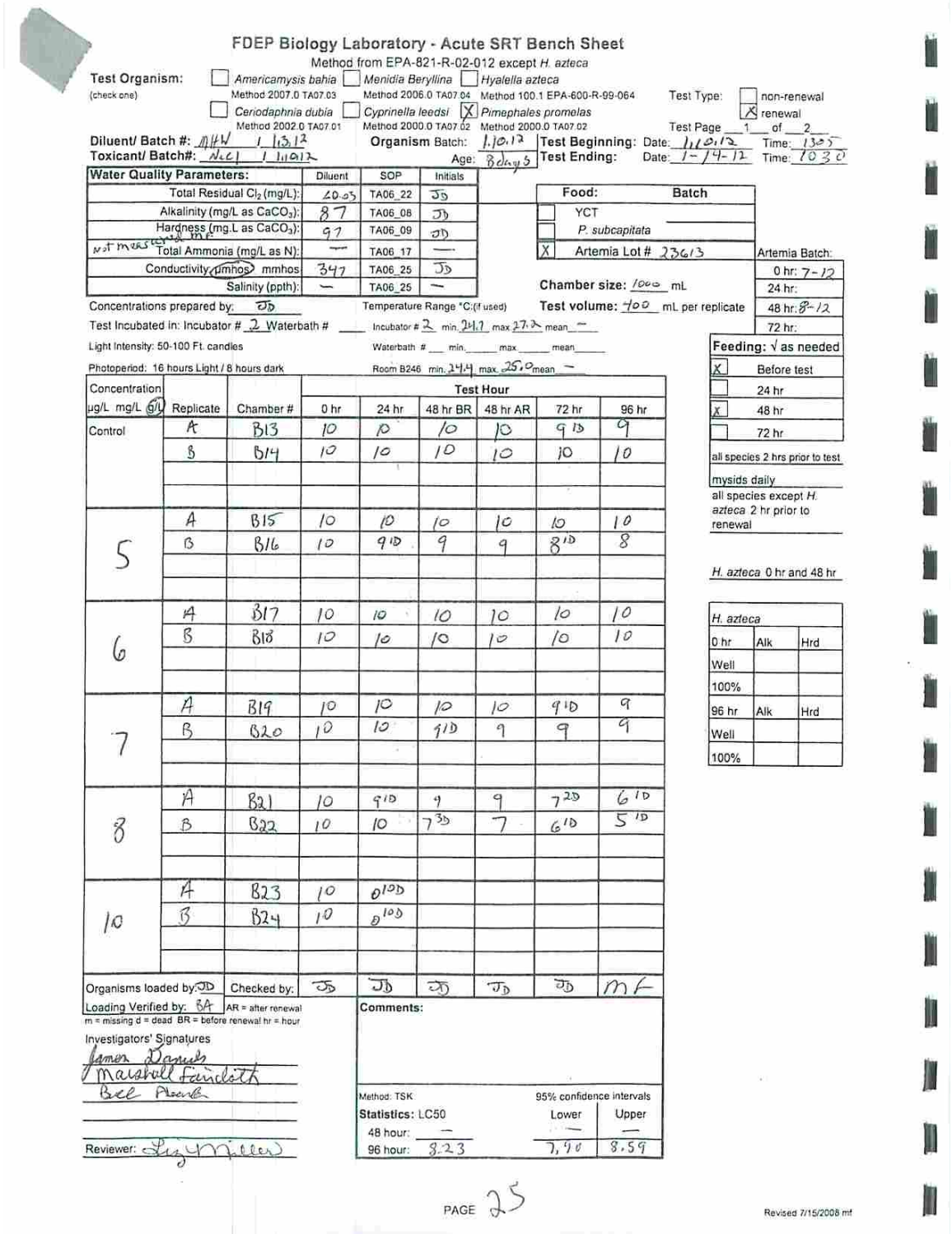
Measure/record salinity for marine tests

Comments:

PAGE 22

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 #: MHW 1.13.12

Toxicant/ Batch#: NaCl 1.11.012

Organism Batch: 1.10.12 Age: 8 days

Test Beginning: Date: 1/10/12 Time: 1305

Test Ending: Date: 1-14-12 Time: 1030

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<0.03	TA06_22	JD
Alkalinity (mg/L as $CaCO_3$):	87	TA06_08	JD
Hardness (mg.L as $CaCO_3$):	97	TA06_09	JD
not measured Total Ammonia (mg/L as N):	—	TA06_17	—
Conductivity (µmhos) mmhos	347	TA06_25	JD
Salinity (ppth):	—	TA06_25	—

Food:	Batch
☐ YCT	
☐ *P. subcapitata*	
☒ Artemia Lot # 23613	

Artemia Batch:

0 hr:	7-12
24 hr:	
48 hr:	8-12
72 hr:	

Chamber size: 1000 mL

Test volume: 400 mL per replicate

Concentrations prepared by: JD

Temperature Range °C: (if used)

Test Incubated in: Incubator # 2 Waterbath # ___ Incubator # 2 min. 24.7 max 27.2 mean —

Light Intensity: 50-100 Ft. candles Waterbath # ___ min. ___ max ___ mean ___

Photoperiod: 16 hours Light / 8 hours dark Room B246 min. 24.4 max. 25.0 mean —

Feeding: √ as needed

☒	Before test
☐	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 %	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	B13	10	10	10	10	9 D	9
	B	B14	10	10	10	10	10	10
5	A	B15	10	10	10	10	10	10
	B	B16	10	9 D	9	9	8 D	8
6	A	B17	10	10	10	10	10	10
	B	B18	10	10	10	10	10	10
7	A	B19	10	10	10	10	9 D	9
	B	B20	10	10	9 D	9	9	9
8	A	B21	10	9 D	9	9	7 2D	6 D
	B	B22	10	10	7 3D	7	6 D	5 D
10	A	B23	10	0 10D				
	B	B24	10	0 10D				
Organisms loaded by: JD		Checked by:	JD	JD	JD	JD	JD	MF

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

James Daniels

Marshall Faircloth

Bec Pearce

Reviewer: Lisa Miller

Comments:

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour: —	—	—
96 hour: 8.23	7.90	8.59

PAGE 25

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: 1/10/12 Time: 1305
Test Ending: Date: 1-14-12 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	A	0 hr	7.8	25.9	8.5	0.347	
	B	24 hr	7.4	25.9	7.2	0.364	
	A	48 hr BR	7.9	25.9	7.1	0.399	
	A	48 hr AR	7.7	24.8	7.3	0.335	
	B	72 hr	7.5	25.0	7.1	0.357	
	A	96 hr	7.8	24.3	7.2	0.380	
5	A	0 hr	7.9	25.9	7.9	9.9	
	B	24 hr	7.6	25.9	7.0	9.8	
	A	48 hr BR	7.9	25.9	7.1	9.7	
	A	48 hr AR	7.8	24.7	7.4	9.7	
	B	72 hr	7.6	24.4	6.9	9.9	
	A	96 hr	7.8	24.4	7.1	10.1	
6	A	0 hr	7.9	25.9	7.7	11.9	
	B	24 hr	7.6	25.9	7.0	11.3	
	A	48 hr BR	7.9	25.9	7.1	11.3	
	A	48 hr AR	7.8	24.5	7.3	11.5	
	B	72 hr	7.6	25.2	7.6	12.0	
	A	96 hr	7.8	24.8	7.0	12.1	
7	A	0 hr	7.9	25.9	7.5	13.6	
	B	24 hr	7.7	25.9	7.0	13.2	
	A	48 hr BR	7.9	25.9	7.1	13.0	
	A	48 hr AR	7.8	24.3	7.3	13.3	
	B	72 hr	7.6	24.8	7.3	13.8	
	A	96 hr	7.8	24.9	7.0	14.0	
8	A	0 hr	7.9	25.9	7.5	15.6	
	JD A B	24 hr	7.7	25.9	7.1	14.9	
	A	48 hr BR	7.9	25.9	7.0	14.7	
	A	48 hr AR	7.8	24.3	7.2	14.7	
	B	72 hr	7.6	24.6	7.3	15.0	
	A	96 hr	7.8	24.6	7.0	15.2	
10	A	0 hr	7.9	25.9	7.6	18.9	
	B	24 hr	7.7	25.9	7.1	18.5	
		48 hr BR			7.3		
		48 hr AR					
		72 hr					
		96 hr					

Investigators Signatures
James Stanish
Marshall Faircloth
Bruce Ashley

Reviewed By: Lisa Mitchell

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

Measure/record salinity for marine tests

Comments:

PAGE 26

Revised 07/15/2008 MF

Appendix C. (continued)

***Ceriodaphnia dubia* Acute Standard Reference Toxicant Test**
DATE: 01/10/12 DURATION: 96 H TOXICANT: NaCl
SPECIES: *Ceriodaphnia dubia*

RAW DATA: Concentration (g/L)	Number Exposed	Mortalities
.00	20	0
1.50	20	1
2.00	20	16
2.50	20	20
3.00	20	20
4.00	20	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.80
95% LOWER CONFIDENCE: 1.71
95% UPPER CONFIDENCE: 1.89

***Pimephales promelas* Acute Standard Reference Toxicant Test**
DATE: 01/10/12 DURATION: 96 H TOXICANT : NaCl
SPECIES: *Pimephales promelas*

RAW DATA: Concentration (g/L)	Number Exposed	Mortalities
.00	20	1
5.00	20	2
6.00	20	0
7.00	20	2
8.00	20	9
10.00	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 8.23
95% LOWER CONFIDENCE: 7.90
95% UPPER CONFIDENCE: 8.59

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: 1/9/12

Ship to: FDEP

P.O. No: N/A

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (‰)
Mysidopsis bahia						
Menidia beryllina						
						Hardness mg/L
Cyprinella leedsi	150 ac	10 DAYS	CL 111230	25	7.8	80
Pimephales promelas	150 ac	7 DAYS	FM 120102	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: 1/30/12

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 120118	25	7.8	80
Pimephales promelas	300 ac	7 DAYS	FM 120123	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 effluent from the Holly Hill WWTF Outfall D-001, sampled on January 30, 2012.

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	HOLL	AGP/LN	Algal Growth Potential	27.1		mg DryWt/L	0.3	0.9
1/30/2012 9:17	HOLL	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	1.4	I	mg/L	0.2	2
1/30/2012 9:17	HOLL	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
1/30/2012 9:17	HOLL	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
1/30/2012 9:17	HOLL	Toxicology	Bioassay-Acute-Def-FW-Fish, LC50	100	L	LC50		
1/30/2012 9:17	HOLL	Toxicology	Bioassay-Acute-Def-FW-C.dubia, LC50	100	L	LC50		
1/30/2012 9:17	HOLL	Metals	Calcium	75.9		mg/L	0.075	0.3
1/30/2012 9:17	HOLL	Metals	Silver	0.025	U	ug/L	0.025	0.1
1/30/2012 9:17	HOLL	Metals	Iron	30	U	ug/L	30	120
1/30/2012 9:17	HOLL	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
1/30/2012 9:17	HOLL	Metals	Zinc	60		ug/L	5	20
1/30/2012 9:17	HOLL	Metals	Arsenic	0.25	U	ug/L	0.25	1
1/30/2012 9:17	HOLL	Metals	Copper	1.11		ug/L	0.5	1
1/30/2012 9:17	HOLL	Metals	Magnesium	6.67		mg/L	0.04	0.16
1/30/2012 9:17	HOLL	Metals	Nickel	21.7		ug/L	0.25	1
1/30/2012 9:17	HOLL	Metals	Chromium	0.3	U	ug/L	0.3	1.2
1/30/2012 9:17	HOLL	Metals	Lead	0.34	I	ug/L	0.2	0.8
1/30/2012 9:17	HOLL	Metals	Selenium	0.2	U	ug/L	0.2	0.8
1/30/2012 9:17	HOLL	Metals	Aluminum	82		ug/L	5	20
1/30/2012 9:17	HOLL	Nutrients	TSS	2	U	mg/L	2	10
1/30/2012 9:17	HOLL	Nutrients	Kjeldahl Nitrogen	0.79		mg N/L	0.08	0.2
1/30/2012 9:17	HOLL	Nutrients	O-Phosphate-P	0.028		mg P/L	0.004	0.01
1/30/2012 9:17	HOLL	Nutrients	Total-P	0.045		mg P/L	0.004	0.01
1/30/2012 9:17	HOLL	Nutrients	NO2NO3-N	0.76		mg N/L	0.04	0.1
1/30/2012 9:17	HOLL	Nutrients	Ammonia-N	0.043		mg N/L	0.01	0.02
1/30/2012 9:17	HOLL	Pesticides	Phorate	0.047	U	ug/L	0.047	0.19
1/30/2012 9:17	HOLL	Pesticides	Metribuzin	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	HOLL	Pesticides	Metolachlor	0.47	U	ug/L	0.47	1.9
1/30/2012 9:17	HOLL	Pesticides	Hexazinone	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	HOLL	Pesticides	Ethoprop	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	HOLL	Pesticides	Diazinon	0.047	U	ug/L	0.047	0.19
1/30/2012 9:17	HOLL	Pesticides	Azinphos Methyl	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Pesticides	Ametryn	0.047	U	ug/L	0.047	0.19

Appendix E. (continued)

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	HOLL	Pesticides	Alachlor	0.57	U	ug/L	0.57	2.3
1/30/2012 9:17	HOLL	Pesticides	Parathion Methyl	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	HOLL	Pesticides	Simazine	0.047	U	ug/L	0.047	0.19
1/30/2012 9:17	HOLL	Pesticides	Naled	0.76	UJ	ug/L	0.76	3
1/30/2012 9:17	HOLL	Pesticides	Chlorpyrifos Methyl	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	HOLL	Pesticides	Chlorpyrifos Ethyl	0.047	U	ug/L	0.047	0.19
1/30/2012 9:17	HOLL	Pesticides	Bromacil	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Pesticides	Prometryn	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	HOLL	Pesticides	Norflurazon	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	HOLL	Pesticides	Malathion	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	HOLL	Pesticides	Fenamiphos	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Pesticides	Atrazine	0.047	U	ug/L	0.047	0.19
1/30/2012 9:17	HOLL	Pesticides	Mevinphos	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Pesticides	Metalaxyl	0.24	U	ug/L	0.24	0.95
1/30/2012 9:17	HOLL	Pesticides	Ethion	0.047	U	ug/L	0.047	0.19
1/30/2012 9:17	HOLL	Pesticides	Butylate	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Pesticides	Fonofos	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	HOLL	Pesticides	Parathion Ethyl	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2,4-Dimethylphenol	9.5	U	ug/L	9.5	38
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2,4-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2,6-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Benzo(a)anthracene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Benzo(a)pyrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Butyl benzyl phthalate	4.8	U	ug/L	4.8	19
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Di-n-butyl phthalate	4.8	U	ug/L	4.8	19
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Di-n-octyl phthalate	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Pyrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	1,2,4-Trichlorobenzene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2,4,6-Trichlorophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	4-Chloro-3-methylphenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	4-Nitrophenol	14	U	ug/L	14	57
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Benzo(k)fluoranthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Chrysene	0.19	U	ug/L	0.19	0.76

Appendix E. (continued)

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Fluorene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Hexachlorocyclopentadiene	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Phenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2,4-Dinitrophenol	14	U	ug/L	14	57
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Acenaphthylene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Diethyl phthalate	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Hexachlorobenzene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	N-Nitrosodimethylamine	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Benzidine	95	U	ug/L	95	380
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Bis(2-chloroethyl)ether	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Dimethyl phthalate	9.5	U	ug/L	9.5	38
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Hexachlorobutadiene	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Hexachloroethane	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Nitrobenzene	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Phenanthrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Bromodichloromethane	19		ug/L	0.2	1
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2,4-Dichlorophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2-Nitrophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Anthracene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Pentachlorophenol	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2-Chlorophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	3,3'-Dichlorobenzidine	38	U	ug/L	38	150
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Acenaphthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Isophorone	5.5		ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	2.9	U	ug/L	2.9	11
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Naphthalene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	2-Chloronaphthalene	0.95	U	ug/L	0.95	3.8

Appendix E. (continued)

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	HOLL	Priority Organic Pollutants	4-Bromophenyl phenyl ether	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Benzo(b)fluoranthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	14	U	ug/L	14	57
1/30/2012 9:17	HOLL	Priority Organic Pollutants	Fluoranthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	HOLL	Field Parameter	Total Residual Chlorine	0		mg/L		
1/30/2012 9:17	HOLL	Field Parameter	pH	7.4				
1/30/2012 9:17	HOLL	Field Parameter	Temperature	20.4		C		
1/30/2012 9:17	HOLL	Field Parameter	Specific Conductance	721		umhos/cm		
1/30/2012 9:17	HOLL	Field Parameter	Dissolved Oxygen	9.3		mg/L		
1/30/2012 9:17	EQUIPMENT BLANK	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
1/30/2012 9:17	EQUIPMENT BLANK	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Copper	0.51	I	ug/L	0.5	1
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Silver	0.025	U	ug/L	0.025	0.1
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Calcium	0.075	U	mg/L	0.075	0.3
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Magnesium	0.04	U	mg/L	0.04	0.16
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Iron	30	U	ug/L	30	120
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Nickel	0.58	I	ug/L	0.25	1
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Chromium	0.3	U	ug/L	0.3	1.2
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Aluminum	5	U	ug/L	5	20
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Lead	0.2	U	ug/L	0.2	0.8
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Selenium	0.2	U	ug/L	0.2	0.8
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Zinc	5	U	ug/L	5	20
1/30/2012 9:17	EQUIPMENT BLANK	Metals	Arsenic	0.25	U	ug/L	0.25	1
1/30/2012 9:17	EQUIPMENT BLANK	Nutrients	O-Phosphate-P	0.004	U	mg P/L	0.004	0.01
1/30/2012 9:17	EQUIPMENT BLANK	Nutrients	NO2NO3-N	0.004	U	mg N/L	0.004	0.01
1/30/2012 9:17	EQUIPMENT BLANK	Nutrients	Kjeldahl Nitrogen	0.08	U	mg N/L	0.08	0.2
1/30/2012 9:17	EQUIPMENT BLANK	Nutrients	Total-P	0.004	U	mg P/L	0.004	0.01
1/30/2012 9:17	EQUIPMENT BLANK	Nutrients	Ammonia-N	0.01	U	mg N/L	0.01	0.02
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Simazine	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Bromacil	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Alachlor	0.57	U	ug/L	0.57	2.3
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Azinphos Methyl	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Metolachlor	0.48	UJ	ug/L	0.48	1.9

Appendix E. (continued)

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Prometryn	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Parathion Methyl	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Ametryn	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Fenamiphos	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Butylate	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Parathion Ethyl	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Norflurazon	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Metribuzin	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Naled	0.76	UJ	ug/L	0.76	3
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Mevinphos	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Malathion	0.14	U	ug/L	0.14	0.57
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Atrazine	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Diazinon	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Ethion	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Ethoprop	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Hexazinone	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Metalaxyl	0.24	U	ug/L	0.24	0.95
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Phorate	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Fonofos	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Chlorpyrifos Ethyl	0.048	U	ug/L	0.048	0.19
1/30/2012 9:17	EQUIPMENT BLANK	Pesticides	Chlorpyrifos Methyl	0.095	U	ug/L	0.095	0.38
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Di-n-octyl phthalate	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Fluoranthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Pentachlorophenol	2.9	U	ug/L	2.9	11
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2,4,6-Trichlorophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2,4-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Benzo(k)fluoranthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	2.9	U	ug/L	2.9	11
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2,4-Dinitrophenol	14	U	ug/L	14	57
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Benzidine	95	U	ug/L	95	380
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Fluorene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Hexachloroethane	2.9	U	ug/L	2.9	11

Appendix E. (continued)

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Nitrobenzene	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2,4-Dichlorophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2-Chlorophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	4-Nitrophenol	14	U	ug/L	14	57
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Hexachlorobenzene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Hexachlorocyclopentadiene	2.9	U	ug/L	2.9	11
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Naphthalene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Pyrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2-Chloronaphthalene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Benzo(a)pyrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Benzo(b)fluoranthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Bis(2-chloroethyl)ether	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	14	U	ug/L	14	57
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Isophorone	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Bromodichloromethane	0.2	U	ug/L	0.2	1
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2,4-Dimethylphenol	9.5	U	ug/L	9.5	38
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2-Nitrophenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Acenaphthylene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Anthracene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Butyl benzyl phthalate	4.8	U	ug/L	4.8	19
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Chrysene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Diethyl phthalate	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	1,2,4-Trichlorobenzene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	4-Bromophenyl phenyl ether	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Acenaphthene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Benzo(a)anthracene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	N-Nitrosodimethylamine	1.9	U	ug/L	1.9	7.6
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Phenanthrene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2,6-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	2.9	U	ug/L	2.9	11
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	3,3'-Dichlorobenzidine	38	U	ug/L	38	150

Appendix E. (continued)

Date Sampled	Field ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	4-Chloro-3-methylphenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.19	U	ug/L	0.19	0.76
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Di-n-butyl phthalate	4.8	U	ug/L	4.8	19
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Dimethyl phthalate	9.5	U	ug/L	9.5	38
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Hexachlorobutadiene	2.9	U	ug/L	2.9	11
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	2.9	U	ug/L	2.9	11
1/30/2012 9:17	EQUIPMENT BLANK	Priority Organic Pollutants	Phenol	0.95	U	ug/L	0.95	3.8
1/30/2012 9:17	TRIP BLANK	Priority Organic Pollutants	Bromodichloromethane	0.2	U	ug/L	0.2	1

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	**Contact Number:** (407) 897-4172
Facility Name (as it appears on permit): Holly Hilly WWTF	**Former Names:**
NPDES Permit Number: FL0027677	**Permit Expiration Date:** September 15, 2013
Physical Address: 453 LPGA Boulevard, Holly Hill, FL 32117	
District: Central	**County:** Volusia
Function of Facility: Domestic Wastewater Treatment Plant	
Facility Type: Domestic Wastewater	**Permitted Capacity (MGD):** 3.0
Mean Flow for previous 12 months for all EXTERNAL outfalls listed in permit (list date range including months): D-001 outfall flow: 0.966 million gallons daily (MGD) annual average daily flow (aadf), as reported on the October 2011 DMR (November 2010 through October 2011 reporting period).	
If applicable, Mean Flow for previous 12 months for REUSE outfall(s) (list date range including months): R-001 outfall flow: 0.408 MGD aadf, as reported on the October 2011 DMR (November 2010 through October 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): The sampling location of D-001 is EFD-1, located after effluent dechlorination. EFA-1 is located at the end of the chlorine contact chamber, and samples most of the parameters for R-001. EFB-1 is located after the filters but prior to chlorination for R-001's turbidity and TSS samples.
Description of Permitted Outfall(s): The facility discharges to the Halifax River (Class III marine waters) at Discharge Location (D-001) which is approximately 510 feet in length and discharges at a depth of approximately 6 feet. The point of discharge is located approximately at latitude 29 ° 14' 44" N, longitude 81 ° 02' 03" W.
Description of Treatment Process (if multiple discharge points, include a detailed map or diagram of facility): Holly Hill WWTF is an advanced domestic wastewater treatment plant consisting of oxidation ditches (based on the previously approved construction to expand the plant from 2.4 MGD to 3.0 MGD) flow equalization, influent screening, grit removal, aeration, denitrification, secondary clarification, chemical feed, filtration, chlorination, postaeration, dechlorination and aerobic digestion of residuals followed by thickening, blending and dewatering.

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,	MGD	Maximum	2.4	-	-	-	Continuous	Recording flow meters and totalizers	FLW-1	See Cond. I.A.4.
BOD, Carbonaceous 5 day, 20C	MG/L	Maximum	10.0	10.0	15.0	20.0	Weekly	16-hour flow proportioned composite	EFD-1	
Solids, Total Suspended	MG/L	Maximum	5.0	6.25	7.5	10.0	Weekly	16-hour flow proportioned composite	EFD-1	
pH	SU	Range	-	-	-	6.5 to 8.5	Continuous	Meter	EFD-1	See Cond. I.A.3.
Coliform, Fecal	#/100 ML	Maximum	See Permit Condition I.A.5.				Weekly	Grab	EFA-1	
Total Residual Chlorine (For Disinfection)	MG/L	Minimum	-	-	-	0.5	Continuous	Meter	EFA-1	See Cond. I.A.3., 6.
Total Residual Chlorine (For Dechlorination)	MG/L	Maximum	-	-	-	0.01	Daily	Grab	EFD-1	
Nitrogen, Total (as N)	MG/L	Maximum	**6.0^**	3.0	4.5	6.0	Weekly	16-hour flow proportioned composite	EFD-1	
Phosphorus, Total (as P)	MG/L	Maximum	1.0	1.0	1.5	2.0	Weekly	16-hour flow proportioned composite	EFD-1	
Whole Effluent Toxicity (Chronic)	See Permit Condition I.A.7								EFD-1	
Oxygen, Dissolved (DO)	MG/L	Minimum	-	-	-	2.0	Daily	Grab	EFD-1	
Dichlorobromomethane	UG/L	Maximum	44*	-	-	-	Monthly	16-hour flow proportioned composite	EFD-1	See Cond. I.A.11.
Enterococci	#/100 ML	Maximum	-	35 (Mo. Geo. Mean)	-	-	5 Days/Week	Grab	EFD-1	Enterococci

Table above: * reflects Administrative Order No. 08-0011
^reflects Consent Order No. 10-0843

Appendix F. (continued)

PART I: Continued	
Receiving Waters: Halifax River	**Classification (indicate whether fresh or marine):** Class III marine waters

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.

1. Effluent Limitation
   - Whole effluent chronic toxicity shall not exceed a No Observed Effect Concentration (NOEC) of less than 100% effluent in any test.
   - Whole effluent acute toxicity shall not exceed a 96 hour LC50 of less than 100% effluent in any test. [Rule 62-302.500(1)(a)4., F.A.C.]
2. Monitoring Frequency
   - Routine toxicity tests shall be conducted once every three months, the first starting no later than 3 months from the last whole effluent toxicity test in the previous permit cycle.
   - Upon completion of four consecutive, valid routine tests that demonstrate compliance with the effluent limitation in 7.a.(1) above, the permittee may submit a written request to the Department for a reduction in monitoring frequency to once every six months. The request shall include a summary of the data and the complete bioassay laboratory reports for each test used to demonstrate compliance. The Department shall act on the request within 45 days of receipt. Reductions in monitoring shall only become effective upon the Department's written confirmation that the facility has completed four consecutive valid routine tests that demonstrate compliance with the effluent limitation in 7.a.(1) above.
   - If a test within the sequence of the four is deemed invalid based on the acceptance criteria in EPA-821-R-02-013, but is replaced by a repeat valid test initiated within 21 days after the last day of the invalid test, the invalid test will not be counted against the requirement for four consecutive valid tests for the purpose of evaluating the reduction of monitoring frequency.
3. Sampling Requirements
   - For each routine test or additional follow-up test conducted, a total of three grab samples of final effluent shall be collected and used in accordance with 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 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.
   - Samples for routine and additional follow-up tests shall not be collected on the same day.
4. Test Requirements
   - Routine Tests: Routine tests shall be conducted using a control (0% effluent) & a minimum of 5 test dilutions: **100%, 50%, 25%, 12.5%,** and **6.25%**.
   - 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 shall be moderately hard water as described in EPA-821-R-02-013, Section 7.
5. 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 initiated no more than 30 days before the date of each routine or additional follow-up test conducted. Additionally, the SRT test must be conducted concurrently if the test organisms are obtained from outside the test laboratory unless the test organism supplier provides control chart data from at least the last five monthly chronic toxicity tests using the same reference toxicant and test conditions. If the organism supplier provides the required SRT data, the organism supplier's SRT data and the test laboratory's monthly SRT-QA data shall be included in the reports for 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 (**Ceriodaphnia dubia**) and Section 11.11 (**Pimephales promelas**). The repeat test shall begin within 21 days after the last day of the invalid test.
   - If 100% mortality occurs in all effluent concentrations prior to the end of any test and the control mortality is less than 20% at that time, the test (including the control) shall be terminated with the conclusion that the test fails and constitutes non-compliance.
   - Additional follow-up tests shall be evaluated for acceptability based on the observed dose-response relationship as required by EPA-821-R-02-013, Section 10.2.6., and the evaluation shall be included with the bioassay laboratory reports.
6. Reporting Requirements
   - Results from all required tests shall be reported on the Discharge Monitoring Report (DMR) as follows:
     Routine and Additional Follow-up Test Results: The calculated NOEC for each test species shall be entered on the DMR.
   - A bioassay laboratory report for each routine test shall be prepared according to EPA-821-R-02-013, Section 10, Report Preparation and Test Review, and mailed to the Department at the address below within 30 days after the last day of the test.
   - For additional follow-up tests, a single bioassay laboratory report shall be prepared according to EPA-821-R-02-013, Section 10, and mailed within 30 days after the last day of the second valid additional follow-up test.
   - Data for invalid tests shall be included in the bioassay laboratory report for the repeat test.
   - The same bioassay data shall not be reported as the results of more than one test.
   - All bioassay laboratory reports shall be sent to: DEP, 3319 Maguire Boulevard Suite 232, Orlando, Florida 32803-3767
7. Test Failures
   - A test fails when the test results do not meet the limits in 12.a.(1).
   - Additional Follow-up Tests:
   (a) If routine test does not meet the chronic toxicity limitation in 12.a.(1) above, the permittee shall notify the DEP at the address above within 21 days after the last day of the failed routine test and conduct two additional follow-up tests on each species that failed the test in accordance with 12.d.
   (b) The first test shall be initiated within 28 days after the last day of the failed routine test. The remaining additional follow-up tests shall be conducted weekly thereafter until a total of two valid additional follow-up tests are completed.
   (c) The first additional follow-up 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 permittee may modify the dilution series in the second additional follow-up test to more accurately bracket the toxicity such that at least two dilutions above 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.
   - In the event of three valid test failures (whether routine or additional follow-up tests) within a 12-month period, the permittee shall notify the Department within 21 days after the last day of the third test failure.
   (a) The permittee shall submit a plan for correction of the effluent toxicity within 60 days after the last day of the third test failure.
   (b) The Department shall review and approve the plan before initiation.
   (c) The plan shall be initiated within 30 days following the Department's written approval of the plan.
   (d) Progress reports shall be submitted quarterly to the Department at the address above.
   (e) During the implementation of the plan, the permittee shall conduct quarterly routine whole effluent toxicity tests in accordance with 12.d. Additional follow-up tests are not required while the plan is in progress. Following completion or termination of the plan, the frequency of monitoring for routine and additional follow-up tests shall return to the schedule established in 12.b.(1). If a routine test is invalid according to the acceptance criteria in EPA-821-R-02-013, a repeat test shall be initiated within 21 days after the last day of the invalid routine test.
   (f) Upon completion of four consecutive, valid routine tests that demonstrate compliance with the effluent limitation in 12.a.(1) above, the permittee may submit a written request to the Department to terminate the plan. The plan shall be terminated upon written verification by the Department that the facility has passed at least four consecutive valid routine whole effluent toxicity tests.
   (g) If a test within the sequence of the four is deemed invalid, but is replaced by a repeat valid test initiated within 21 days after the last day of the invalid test, the invalid test will not be counted against the requirement for four consecutive valid tests for the purpose of terminating the plan.
   - If chronic toxicity test results indicate greater than 50% mortality within 96 hours in an effluent concentration equal to or less than the effluent concentration specified as the acute toxicity limit in 12.(1)(b), the Department may revise this permit to require acute definitive whole effluent toxicity testing.
   - The additional follow-up testing and the plan do not preclude the Department taking enforcement action for acute or chronic whole effluent toxicity permit limit violations.

Appendix F. (continued)

Administrative or Consent Orders (date executed, specific requirements, interim limit dates-if applicable, etc.):
Consent Order No. 10-0843 was issued February 26, 2010. The CO raised the Total Nitrogen (TN) annual average limit from 3.0 mg/L to 6.0 mg/L.
Administrative Order No. 08-0011 was issued September 16, 2008. The AO raised the dichlorobromomethane (DCBM) annual average limit from 22 ug/L to 44 ug/L.

Facility Mixing Zone Details (if applicable):
N/A

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

Date	Parameter, Units, and Location	Limit	Result
October 2010	TSS Weekly Avg. mg/L (D-001)	7.5	7.83
October 2010	TSS Monthly Avg. mg/L (D-001)	6.25	6.8

Describe previous FYI and WQBEL study:
The last FYI of Holly Hill WWTF was performed in May 2002. Tests were performed on the 14 to 16 May 2002. The FDEP Biology Section performed two 48-hour static acute screening toxicity bioassays on a sample of effluent collected from the facility. The freshwater test species used were the waterflea, *Ceriodaphnia dubia*, and the bannerfin shiner, *Cyprinella leedsi*. The sample of effluent collected May 13, 2002, was not acutely toxic to either test species during the 48-hour bioassay tests.

Describe previous or current Enforcement Actions (including WLs):
The facility is still under Consent Order No. 10-0843 for Total Nitrogen exceedances and Administrative Order 08-0011 for CDBM exceedances, both of which are described above.
The facility was issued Warning Letter No. 09-0019 on December 11, 2009. The Warning Letter was issued due to numerous effluent exceedances including Total Nitrogen, Total Suspended Solids, and Total Chlorine Residual minimum. The facility also failed to submit a six-month progress report required by the current AO.

Describe any violations or problems noted in previous inspection:
Other than the deficiencies noted above, the facility has not had any major compliance issues noted in the noted in the last five inspection reports.

Discuss whether DMR trends within previous five years are improving or declining:
Over the past five years, the facility has decreased the amount of flow to D-001. Total Nitrogen and Total Phosphorus concentrations spiked in 2008, but have declined back to the values reported in 2006. Dissolved oxygen, pH, and chlorine residual values have remained consistent.

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

Appendix F. (continued)

PART II: Sampling Event Information	
Date(s) sample collected: January 30, 2012	**Sampler(s):** Phil Kane, Steve Kent, Blake Vahlsing
Temperature (Deg C): 20.4	**pH (SU):** 7.4
Dissolved Oxygen (mg/L): 9.3	**Conductivity (umhos/cm):** 721
Total Residual Chlorine (mg/L): 2.98	**Total Residual Chlorine - after Disinfection (mg/L):** 0.00
Method of Chlorination: Gas chlorine	**Method of De-chlorination:** Gas sulfur dioxide
Flow During Sample Collection for each outfall(MGD): D-001: Flow was 0.201 MGD at the time of sampling.	
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: N/A	

Appendix G. EPA End Sheet

The Bioassay of Holly Hill WWTF effluent sampled on January 30, 2012. NPDES #FL0027677.

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	7	6	7	7	11	12	1	2	0	1	3	0	18 **B**	19 **S**	20 1

Remarks

66

The Priority Pollutants Analysis of Holly Hill WWTF effluent sampled on January 30, 2012. NPDES #FL0027677.

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	7	6	7	7	11	12	1	2	0	1	3	0	18 **X**	19 **S**	20 1

Remarks

66

Biological Analyses of Holly Hill WWTF effluent sampled on January 30, 2012. NPDES #FL0027677.

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	7	6	7	7	11	12	1	2	0	1	3	0	18 **S**	19 **S**	20 1

Remarks

66