

Effluent Quality Assessment

City of Fort Myers South WWTP

Lee County

NPDES #FL0021270

Sampled October 11, 2010

December 2010

Biology Section
Bureau of Laboratories
Division of Environmental Assessment and Restoration

Quality Manual No. 870346G
NELAC Certification No. E31780

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City of Fort Myers South Wastewater Treatment Plant (WWTP), 1618 South Drive, Fort Myers, Lee County, Florida, 33907. NPDES #FL0021270. Effluent samples for algal growth potential, bioassay, chemical and microbiological analyses for this facility were collected on October 11, 2010.

Introduction

City of Fort Myers South WWTP is a domestic wastewater treatment facility which uses the Bardenpho Process to treat domestic wastewater. (See Appendix F for facility information found on pages 1-6 of the Facility's NPDES permit provided by Alyssa Mork of FDEP South District.)

Methods

Samples were collected from the EFD-01and EFA-01 NPDES sampling points for Outfall D-001 by Caitlyn Eck and Alyssa Mork (FDEP South 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 screening toxicity tests and algal growth potential (AGP) tests 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.

Both a freshwater AGP test and a marine AGP test were performed. An aliquot of the AGP sample was salinity-adjusted with artificial sea salts from an apparent salinity of approximately 0.47 parts per thousand (ppth) to a salinity of approximately 7.05 ppth in order to meet the minimum salinity requirement of the marine test species.

Results and Discussion

- **Toxicity Test Results**
  Tests were performed October 12 - 16, 2010.

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

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

  *NOTE: In a 96-hour acute screening 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 19.9 A (A – Value reported is the mean of two or more determinations) 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 ratio of total nitrogen to total phosphorus in the sample (7.8:1) suggests that the effluent is nitrogen limited; however, the ratio of inorganic nitrogen to orthophosphate (14.7:1) suggests that the effluent is co-limited by both nitrogen and phosphorus. The predicted AGP based on inorganic nitrogen and phosphorus concentrations was 14.5 (±20%) mg dry wt/L and 11.2 (±20%) mg dry wt/L, respectively (Table 1a; Miller et al. 1978).

The salinity-adjusted effluent AGP was 10.3 mg dry wt/L of the saltwater species *Dunaliella tertiolecta.* Raschke and Schultz determined that AGP values above 10.0 mg dry weight/L represent a “problem” threshold for marine receiving waters, implying nutrient enrichment (personal communication, Marshall Faircloth, et. al. FDEP, with Ron Raschke USEPA Region 4 1987).

See Tables 1a and 1b for AGP results.

- **Chemistry Results**
  Total residual chlorine (TRC) (0.05 mg/L) was detected in the bioassay sample in the laboratory. Total ammonia was not detected in the bioassay sample in the laboratory. The total ammonia concentration in the sample collected and preserved for chemical analysis was 0.031 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.

  Atrazine, bromoform, chloroform, nickel and zinc were detected in the effluent at levels that comply with Class III marine water quality criteria (62-302.530, F.A.C.). Antimony, arsenic, chromium - III, copper, iron, selenium and silver were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL). Bromodichloromethane (32.0 µg/L; limit ≤ 22.0 µg/L, 62.302.500 (35) (b) 5, F.A.C. and permit # FL0021270), dibromochloromethane (53.0 µg/L, limit ≤ 34.0 µg/L, 62.302.500 (35) (b) 2, F.A.C. and permit # FL0021270), and TRC (0.05 mg/L; limit ≤ 0.01 mg/L, 62-302.500 (18), F.AC. and permit # FL0021270), exceeded the Class III criteria for marine waters and the permit limitations. Aluminum and chloroform were detected in the effluent and the field blank. Fecal Coliform bacteria (45 colony forming units/100 mL) were also detected in the effluent.

  See Table 2 for results of analytes detected in the effluent, and corresponding limits. 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-01 NPDES sampling point for Outfall D-001 for this facility on October 11, 2010, was not acutely toxic to either test species during the 96-hour acute screening bioassays. The effluent AGP result and the salinity adjusted AGP result were greater than the "problem” thresholds, respectively, for fresh and marine receiving waters suggesting that nutrient enrichment may be an issue. Effluent water quality samples collected on October 11, 2010, exceeded Class III Marine water quality standards and permit conditions for bromodichloromethane, dibromochloromethane, and TRC.

Literature Cited

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

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

Table 1a. Measured and predicted algal growth potential (AGP; mg dry weight/L) for total soluble inorganic nitrogen (TSIN), orthophosphate (OP), total nitrogen (TN) and total phosphorus (TP) limitation of the freshwater species *Pseudokirchneriella subcapitata* and ratios of nitrogen to phosphorus for samples collected from the City of Fort Myers South Wastewater Treatment Plant on October 11, 2010.

City of Fort Myers South WWTP NPDES #FL0021270												
Location	AGP (measured)		Predicted AGP (TSIN) ± 20%			Predicted AGP (OP) ± 20%			Predicted AGP (TN) ± 20%			Predicted AGP (TP) ± 20%			Inorganic N:P ratio	Total N:P ratio
Effluent 2	19.9	A	14.5	±	2.9	11.2	±	2.2	50.2	±	10.0	73.1	±	14.6	14.7	7.8

A - Value reported is the mean of two or more determinations

Table 1b. Measured algal growth potential (AGP; mg dry weight/L) of the saltwater species *Dunaliella tertiolecta* and ratios of nitrogen to phosphorus for samples collected from the City of Fort Myers South Wastewater Treatment Plant on October 11, 2010.

City of Fort Myers South WWTP NPDES #FL0021270				
Location	AGP (measured)		Inorganic N:P ratio	Total N:P ratio
Effluent 2	10.3	*	14.7	7.8

* - sample salinity adjusted to 7ppt

Table 2. Effluent limits, Class III Criteria for predominantly marine waters and chemical data for samples collected from the City of Fort Myers South WWTP on October 11, 2010.

City of Fort Myers South WWTP NPDES # FL0021270	Class III Criteria	Effluent Limits	Effluent 2	
Organic Constituents (µg/L)				
Atrazine	-	-	0.21	
Bromodichloromethane	≤ 22.0 a	≤ 22.0 a	32	
Bromoform	≤ 360.0 a	-	28	
Chloroform	≤ 470.8 a	Report	17	F
Dibromochloromethane	≤ 34.0 a	≤ 34.0 a	53	
Metals (µg/L unless otherwise noted)				
Aluminum	≤ 1,500	Report	390	F
Antimony	≤ 4,300	Report	0.37	I
Arsenic	≤ 50	Report	0.89	I
Beryllium	≤ 0.13 a	≤ 0.13 a	0.4	U
Cadmium	≤ 8.8	-	0.03	U
Calcium (mg/L)	-	-	67.2	
Chromium-III	-	-	0.59	I
Copper	≤ 3.7	≤ 3.7	0.82	I
Iron	≤ 300	Report	120	I
Lead	≤ 8.5	Report	0.2	U
Magnesium (mg/L)	-	-	9.46	
Mercury	≤ 0.025	Report	-	
Nickel	≤ 8.3	Report	1.11	
Selenium	≤ 71	-	0.22	I
Silver	≤ 2.3	Report	0.051	I
Zinc	≤ 86	Report	20	
Nutrients (mg/L)				
Ortho-phosphate	-	-	0.026	
Total Phosphorus	-	≤ 1.0 f	0.17	
Total Ammonia	-	-	0.031	
Un-ionized Ammonia	-	-	≤ 0.02	c
Nitrate and Nitrite	-	-	0.35	
Total Kjeldahl Nitrogen	-	-	0.97	
Organic Nitrogen	-	-	0.939	c
Total Nitrogen	-	≤ 6.0 f	1.32	c
General Physical and Chemical Parameters				
Dissolved Oxygen (mg/L)	≥ 4.0	≥ 4.0	13.4	
pH (SU)	6.5 - 8.5	6.5 - 8.5	7.4	
Temperature (°C)	-	-	29.0	
Salinity	-	-	0.52	
Total Residual Chlorine (mg/L)	≤ 0.01	≤ 0.01	0.05	£
CBOD, 5 day (mg/L), N - inhibited	-	≤ 60.0 f	-	
Oils and Greases (mg/L)	≤ 5.0	Report	1.4	U
Cyanide (µg/L)	≤ 1.0	≤ 1.0 s	0.005	U
Flow (MGD)	-	12.0 a	*	
Hardness (mg $CaCO_3$)	-	-	206.75	c
Microbiology (Number of colony forming units/100ml)				
Fecal Coliforms ¥	≤ 400s [#]	-	45	

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
f - 24-hour flow proportioned composite sample
s - Single sample
F - Analyte was detected in both sample and field blank
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit
U - Material analyzed for but not detected; value reported is the method detection limit
¥ - Fecal coliform analysis performed by Central lab
- If 10 or fewer samples are taken within a month, the single sample limit is 400 CFU (62-302.530(6), F.A.C.)
£ - Analysis performed in Central labs and entered into Tox sheets
* - Flow information not provided by FDEP South District

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2010-10-11-33
Fort Myers South FYI3

Requester: Cari Butler
Field Report Prepared By: Alyssa Mork

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

Collected By: Clarke Eck and Alyssa Mork
Send Final Report To: Keith Kleinmann

Sampling Agency: DEP
Alyssa Mork

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Fort Myers South WWTP - EFD-01	[X] Comp [ ] Grab	Date 10-11-10 Time 11:00		A
	Field ID: DW-101110-1	Tot Res Chlorine (mg/L): 0.00	Diss Oxygen (mg/L): 15.87	Storet Station Number:	
	Matrix (include type e.g. Salt, Fresh, etc): Wastewater	Temp (C): 10.09°	pH: 7.42	Sample Depth [ ] m [ ] ft: 0	Salinity (PPTh): .58 [X] Sp Conductance (umho/cm)	NPDES Number:
	Latitude [ ] dd [ ] dms	Longitude [ ] dd [ ] dms	Comments:		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Fort Myers South WWTP - EFD-01	[ ] Comp [X] Grab	Date 10-11-10 Time 11:10		A
	Field ID: DW-101110-2	Tot Res Chlorine (mg/L): .05	Diss Oxygen (mg/L): 13.91	Storet Station Number:	
	Matrix (include type e.g. Salt, Fresh, etc): Wastewater	Temp (C): 29	pH: 7.41	Sample Depth [ ] m [ ] ft: 0	Salinity (PPTh): .52 [X] Sp Conductance (umho/cm)	NPDES Number:
	Latitude [ ] dd [ ] dms	Longitude [ ] dd [ ] dms	Comments:		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Fort Myers South WWTP - Blank	[ ] Comp [X] Grab	Date Time 1:00 Central		B
	Field ID: DW-101110-3	Tot Res Chlorine (mg/L):	Diss Oxygen (mg/L): 9.460	Storet Station Number:	
	Matrix (include type e.g. Salt, Fresh, etc): Blank	Temp (C): 25.30	pH: 6.89	Sample Depth [ ] m [ ] ft: 0	Salinity (PPTh): 0.00 [ ] Sp Conductance (umho/cm)	NPDES Number:
	Latitude [ ] dd [ ] dms	Longitude [ ] dd [ ] dms	Comments: Time taken from Bottle 10-12-10 @ 12:30		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Fort Myers South WWTP - EFA-01	[ ] Comp [X] Grab	Date 10-11-10 Time 11:30		A
	Field ID: DW-101110-4	Tot Res Chlorine (mg/L): 1.80	Diss Oxygen (mg/L): ~~26.4~~ 11.20	Storet Station Number:	
	Matrix (include type e.g. Salt, Fresh, etc): Wastewater	Temp (C): 29.	pH: 7.27	Sample Depth [ ] m [ ] ft: 0	Salinity (PPTh): .52 [ ] Sp Conductance (umho/cm)	NPDES Number:
	Latitude [ ] dd [ ] dms	Longitude [ ] dd [ ] dms	Comments: 2 bleach Need to Tox by JD. 10.40 10.12.10		

Relinquished By	Date/Time	Shipping Method	Received By	Date/Time	Relinquished By	Date/Time	Received By	Date/Time
	10-11-10 10:00						MC	10-12-10 9:30

* 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 FT Myers South AWWTP

Address : 1618 South Drive

City: Fort Myers **County:** Lee

Contact/District: Alyssa Mork / South

NPDES Permit #: FL 0021270

LIMS Job #: TLH-2010-10-12-69 **LIMS Sample #:** 1303955

Lims Data Entry: 10-29-10 MF Data Entry Verification: 10-29-10 JD by MF

Sample Collection: Date: 10-11-10 Time: 1130

Hold Time Start: Date: 10-11-10 Time: 1130

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:
James Bagwell	JB
Dave Powers	DP
Bruce Alder	BA
Marshall Faircloth	MF
Amalia Parkin	ABP

REVIEWER: Liz Miller

Diluent/Control Batch#:	10.4.10		10.1.10							
Water Quality Parameters	20% DMW	Well Water	Moderately Hard Water	Salt Water ASW NSW Test 1	Salt Water ASW NSW Test 2	Other:	Original Sample	Measured by	Verified by
Field Total Residual Cl_2 (mg/L):	N/A	N/A	N/A	N/A	N/A	N/A	0.05 @ EFD-01 AM		MF
Lab Total Residual Cl_2 (mg/L):	<0.03		<0.03				0.05	DP/AR	JD
Alkalinity (mg/L as $CaCO_3$):	40		77				151	DP/AR	JD
Hardness (mg/L as $CaCO_3$):	100		96				200	DP/AR	JD
Total Ammonia (mg/L as N):	<0.017		<0.017				<0.017	BA	MF
Conductivity: ☒ µmhos ☐ mmhos	180		332				1091	JD	TH
Salinity (ppth):									

V 1.8 1/13/10

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample # 1303155 | SOP#: TA 07_01 | Test # 1 of 2

Test Species:
Batch #: 10.11.10 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 # 10-4-10)
[ ] ASW [x] 20% DMW
[ ] MHW [ ] Well

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

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

Test Started: Date 10.12.10 Time 1350

Test Ended: Date 10.16.10 Time 12:15

Chamber Size: 30 ml
Test Volume: 25 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
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	DP	BA

Food
- [x] YCT Batch# 5.19.10
- [x] P. subcapitata Batch# 6 2% 10
- [ ] Artemia Lot#_____

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

Temperature Range (°C)
Incubator # 2
min. 26.2 max. 26.7 mean ___
Incubator #___
min.___ max.___ mean___
Waterbath #___
min.___ max.___ mean___
Room B246
min. 24.8 max. 26.0 mean ___
Light intensity (ft. candles): 50-100
Photoperiod (hours): 16 light, 8 dark

Loaded/ Verified by: BA / DP
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

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 # 1303555 **SOP#: TA 07** 02 **Test #** 2 **of** 2

Test Species:
Batch #: 10.12.10 Age: 11days
- [ ] *Ceriodaphnia dubia*
- [x] *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 # 10.1.10 **)**
[ ] ASW [ ] 20% DMW
[x] MHW [ ] Well

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

Test Duration:
[ ] 48 hr
[x] 96 hr
[ ] __hr

Chamber Size 1000 **ml**
Test Volume: 400 **ml**

Test Started:
Date 10.12.10 Time 1300

Test Ended:
Date 10.16.10 Time 12.30

Concentration	Replicate	Chamber #	0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Ctrl	A	A1	5	5	5	5	5	5
	B	A2	5	5	5	5	5	5
	C	A3	5	5	5	5	5	5
	D	A4	5	5	5	5	5	5
100%	A	A5	5	5	5	5	5	5
	B	A6	5	5	5	5	5	5
	C	A7	5	5	5	5	5	5
	D	A8	5	5	5	5	5	5
		Checked or Verified by:	JD	DP	DP	MF	DP	BA

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

Food
- [ ] YCT Batch#______
- [ ] P. subcapitata Batch#______
- [x] Artemia Lot# 23613

Feeding and Artemia Batch #
- [x] 0 hr 138.10 [x] Prior to Test
- [ ] 24 hr____
- [x] 48 hr 139.10 [x] Prior to Renewal
- [ ] 72 hr____

Temperature Range (°C)

Incubator # 2
min. 26.2 max. 26.7 mean —

Incubator #___
min.______max.______mean______

Waterbath #___
min.______max.______mean______

Room B246
min. 24.8 max. 26.0 mean —

Light intensity (ft. candles): 50-100

Photoperiod (hours): 16 light, 8 dark

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:

000003

V. 2.5 1-13-10

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

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Parameter Sheet

LIMS Sample # 1303955 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	8.0	24.2	7.9	180	—
	—	24 hr	—	—	—	—	—
Ctrl	A	48 hr BR	7.9	24.0	7.5	196	—
	M	48 hr AR	8.0	24.3	7.9	178	—
	—	72 hr	—	—	—	—	—
	A	96 hr	7.9	24.3	8.0	207	—
	M	0 hr	7.4	25.2	7.9	1091	—
	~~B~~	24 hr	—	—	—	—	—
100%	A	48 hr BR	8.3	24.0	7.8	1234	—
	M	48 hr AR	7.5	25.9	7.8	1087	—
	—	72 hr	—	—	—	—	—
	A	96 hr	8.3	24.5	8.1	1171	—
		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					
		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					
		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

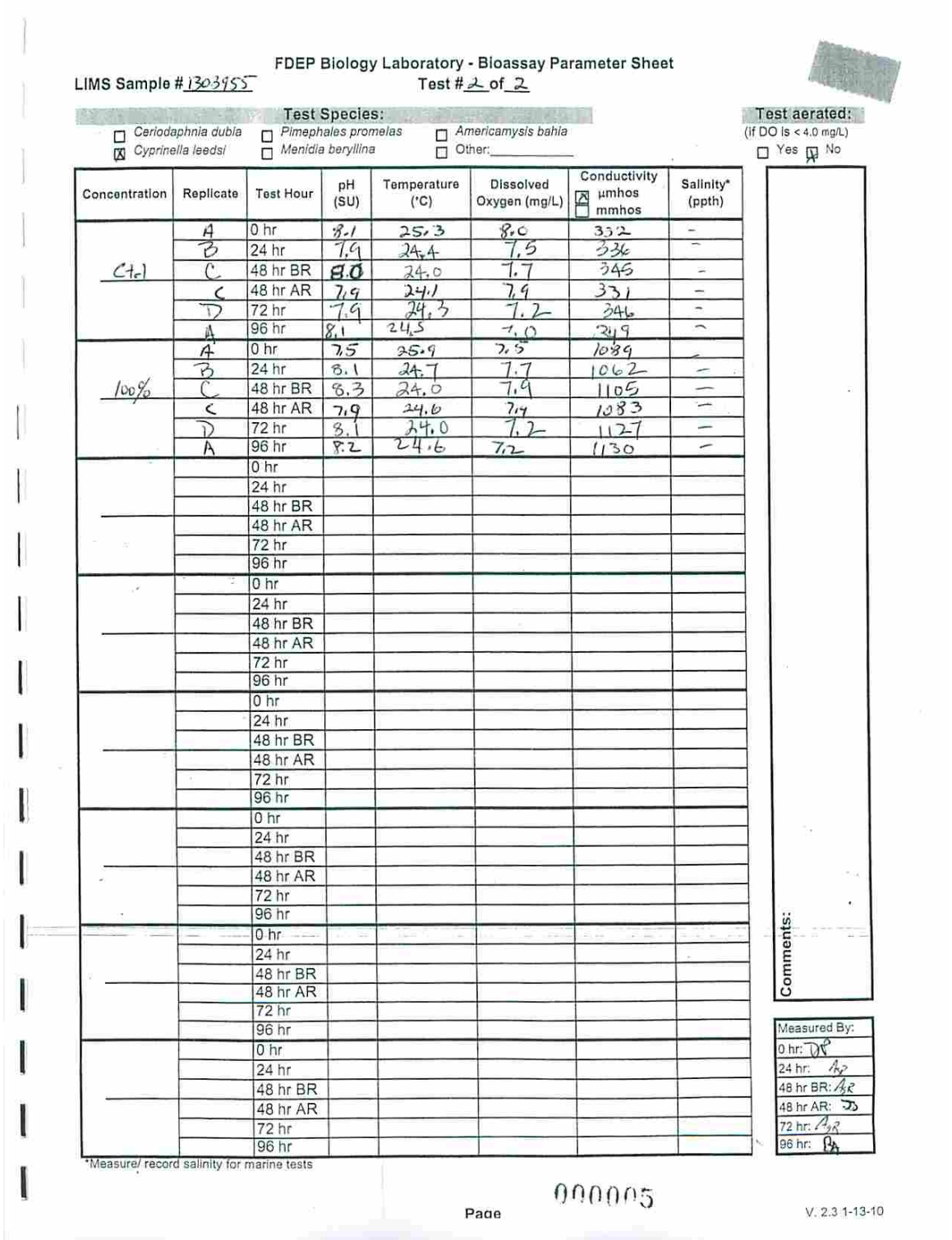
Comments:

Measured By:
0 hr: BA
24 hr: —
48 hr BR: BA
48 hr AR: BA
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 # 1303955 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)
Ctl	A	0 hr	8.1	25.3	8.0	332	–
	B	24 hr	7.9	24.4	7.5	336	–
	C	48 hr BR	8.0	24.0	7.7	345	–
	C	48 hr AR	7.9	24.1	7.9	331	–
	D	72 hr	7.9	24.3	7.2	346	–
	A	96 hr	8.1	24.5	7.0	349	–
100%	A	0 hr	7.5	25.9	7.5	1089	–
	B	24 hr	8.1	24.7	7.7	1062	–
	C	48 hr BR	8.3	24.0	7.9	1105	–
	C	48 hr AR	7.9	24.6	7.4	1083	–
	D	72 hr	8.1	24.0	7.2	1127	–
	A	96 hr	8.2	24.6	7.2	1130	–
		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					
		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					
		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: DP
24 hr: AR
48 hr BR: AR
48 hr AR: DS
72 hr: AR
96 hr: BA

000005

Page

V. 2.3 1-13-10

Appendix B. (continued)

Test ID: City of Ft. Myers South AWWTP Dates: 10-12-10 — 10-15-10

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	#3 √ Backup
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)

Cathy 10-12 9:10 JD

V 1.1 9/21/10 MF/jd

000006

Appendix C. SRT Bench Sheets and Statistical Analysis

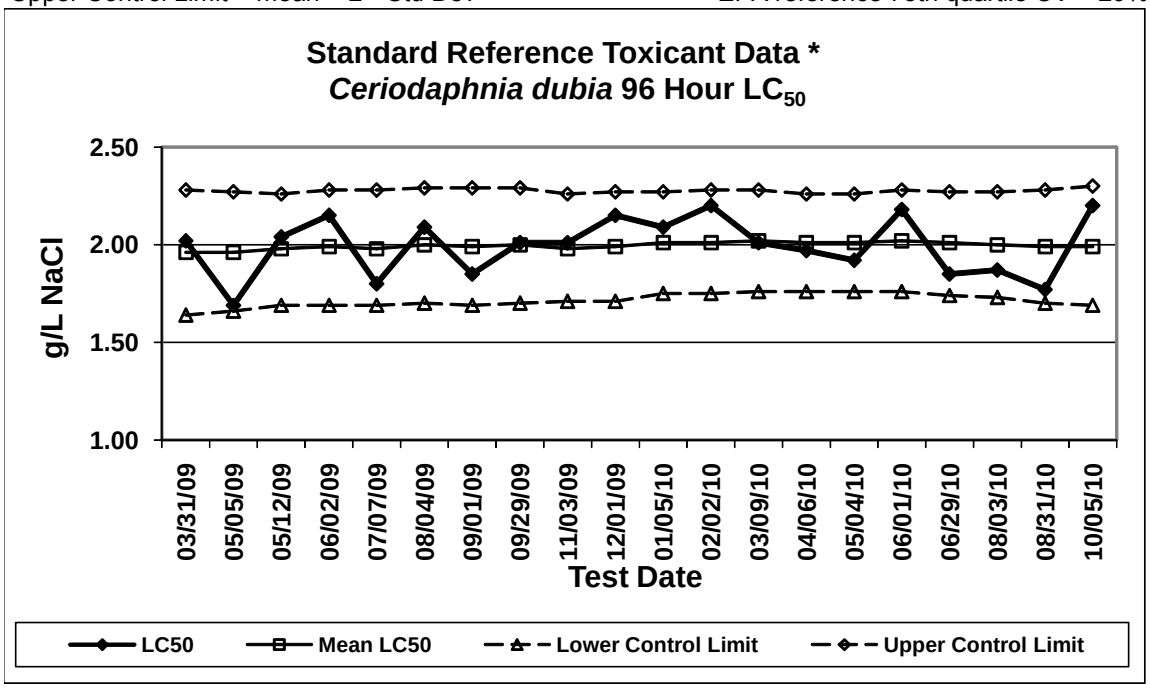
Florida Department of Environmental Protection
Bureau of Laboratories

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
03/31/09	2.02	1.96	0.16	1.64	2.28
05/05/09	1.69	1.96	0.15	1.66	2.27
05/12/09	2.04	1.98	0.14	1.69	2.26
06/02/09	2.15	1.99	0.15	1.69	2.28
07/07/09	1.80	1.98	0.15	1.69	2.28
08/04/09	2.09	2.00	0.15	1.70	2.29
09/01/09	1.85	1.99	0.15	1.69	2.29
09/29/09	2.01	2.00	0.15	1.70	2.29
11/03/09	2.01	1.98	0.14	1.71	2.26
12/01/09	2.15	1.99	0.14	1.71	2.27
01/05/10	2.09	2.01	0.13	1.75	2.27
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

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

CV = 7.57
EPA reference 75th quartile CV = 29%



Appendix C. (continued)

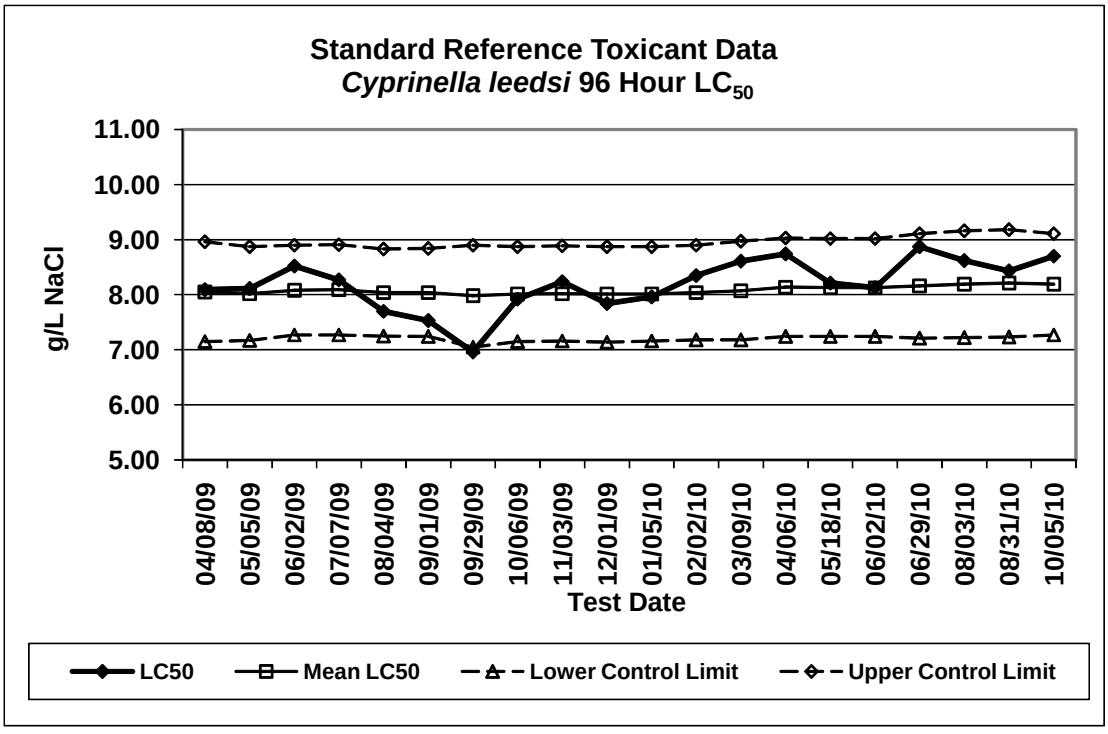
Florida Department of Environmental Protection
Bureau of Laboratories

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
04/08/09	8.09	8.05	0.45	7.15	8.96
05/05/09	8.11	8.02	0.43	7.17	8.87
06/02/09	8.52	8.08	0.41	7.27	8.90
07/07/09	8.27	8.09	0.41	7.27	8.91
08/04/09	7.70	8.04	0.40	7.25	8.83
09/01/09	7.53	8.04	0.40	7.24	8.84
09/29/09	6.96	7.98	0.46	7.05	8.90
10/06/09	7.92	8.01	0.43	7.15	8.87
11/03/09	8.24	8.02	0.43	7.16	8.89
12/01/09	7.84	8.01	0.43	7.14	8.87
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

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

CV = 5.63
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 110410 **Organism Batch:** 104.10 **Test Beginning:** Date: 10.5.10 Time: 1100

Toxicant/ Batch#: NaCl 110410 **Age:** 6 22 hr **Test Ending:** Date: 10-9-10 Time: 0845

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<0.03	TA06_22	BA AMC
Alkalinity (mg/L as $CaCO_3$):	40	TA06_08	DP
Hardness (mg.L as $CaCO_3$):	100	TA06_09	DP
Total Ammonia (mg/L as N):	<0.017	TA06_17	BA
Conductivity: µmhos mmhos	179	TA06_25	BA
Salinity (ppth):	—	TA06_25	—

Food:	Batch
☒ YCT	5.19.10
☒ *P. subcapitata*	6.24.10
☐ Artemia Lot #	Artemia Batch:
	0 hr:
	24 hr:
	48 hr:
	72 hr:

Chamber size: 30 mL **Test volume:** 20 mL per replicate

Concentrations prepared by: BA Temperature Range °C:(if used)

Test Incubated in: Incubator # 2 Waterbath # ____ Incubator # 2 min 25.7 max 26.5 mean ____

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

Photoperiod: 16 hours Light / 8 hours dark Room B246 min 24.3 max 25.3 mean 25.0

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	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	5
		C	C	5	5	5	5	5	5
		D	D	5	5	5	5	5	5
2.0		A	A	5	5	5	5	5	5
		B	B	5	5	4 1D	4	4	4
		C	C	5	4 1D	4	4	4	4
		D	D	5	5	5	5	5	5
2.5		A	A	5	5	1 4D	1	1	1
		B	B	5	5	2 3D	2	2	0 2D
		C	C	5	5	0 5D	—	—	—
		D	D	5	5	2 3D	2	2	0 2D
3.0		A	A	5	5 (A)	0 (5D)	0	—	—
		B	B	5	[illegible]	0 (4D)	0	—	—
		C	C	5	4 1D	0 (4D)	0	—	—
		D	D	5	5	0 (5D)	0	—	—
4.0		A	A	5	2 3D	0 2D	0	—	—
		B	B	5	1 4D	0 1D	0	—	—
		C	C	5	0 5D	—	—	—	—
		D	D	5	0 5D	—	—	—	—
Organisms loaded by: BA		Checked by: BA		BA	MF	MF	MF/AR	MF	MF

Loading Verified by: AR AR = after renewal

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

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

Comments: (A) Transcription error BA 10.4.10

Investigators' Signatures

Bruce Neale

James Dowell

Marshall Faircloth

Amelia Rankin

Diane Power

Reviewer: Lisa Miller

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour: 2.29	2.18	2.42
96 hour: 2.20	2.12	2.29

00 63

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: 10-5-10 Time: 1100
Test Ending: Date: 10-9-10 Time: 0845

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	M	0 hr	8.0	24.0	7.6	0.179	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.1	24.0	8.0	0.232	
	m	48 hr AR	8.1	25.0	7.8	0.179	
	—	72 hr	—	—	—	—	
	A	96 hr	8.1	24.0	7.7	0.193	
1.5	M	0 hr	8.1	24.0	8.0	2.91	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.1	24.1	7.9	3.45	
	m	48 hr AR	8.1	25.0	7.8	~~2.86~~ 2.48 RM	
	—	72 hr	—	—	—	—	
	A	96 hr	8.1	23.9	7.9	3.20	
2.0	M	0 hr	8.1	24.0	7.9	3.92	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.1	24.0	8.0	4.53	
	m	48 hr AR	8.1	25.2	7.9	3.93	
	—	72 hr	—	—	—	—	
	A	96 hr	8.1	23.8	8.0	4.07	
2.5	M	0 hr	8.1	24.0	7.9	4.80	
	—	24 hr	—	—	—	—	
	A	48 hr BR	8.1	24.0	8.1	5.61	
	m	48 hr AR	8.1	24.8	8.0	4.81	
	—	72 hr	~~8.1~~ RM	—	—	—	
	A	96 hr	8.1	24.1	8.0	4.86	
3.0	M	0 hr	8.1	24.0	8.0	5.70	
	—	24 hr	—	—	—	~~6.69~~ RM	
	—	48 hr BR	8.1	24.1	8.0	6.69	
	—	48 hr AR	—	~~24.1~~ MF	—	—	
	—	72 hr	—	—	—	—	
	—	96 hr	—	—	—	—	
4.0	M	0 hr	8.1	24.0	7.9	7.45	
	—	24 hr	—	—	—	~~9.05~~ RM	
	—	48 hr BR	8.1	24.0	8.0	9.05	
	—	48 hr AR	—	—	—	—	
	—	72 hr	—	—	—	—	
	—	96 hr	—	—	—	—	

Investigators Signatures
Becca Alvelo
Jamie Dewitt
Marshall Faircloth
Brooke Howe
Amanda Perkins
Reviewed By: Liz Miller

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

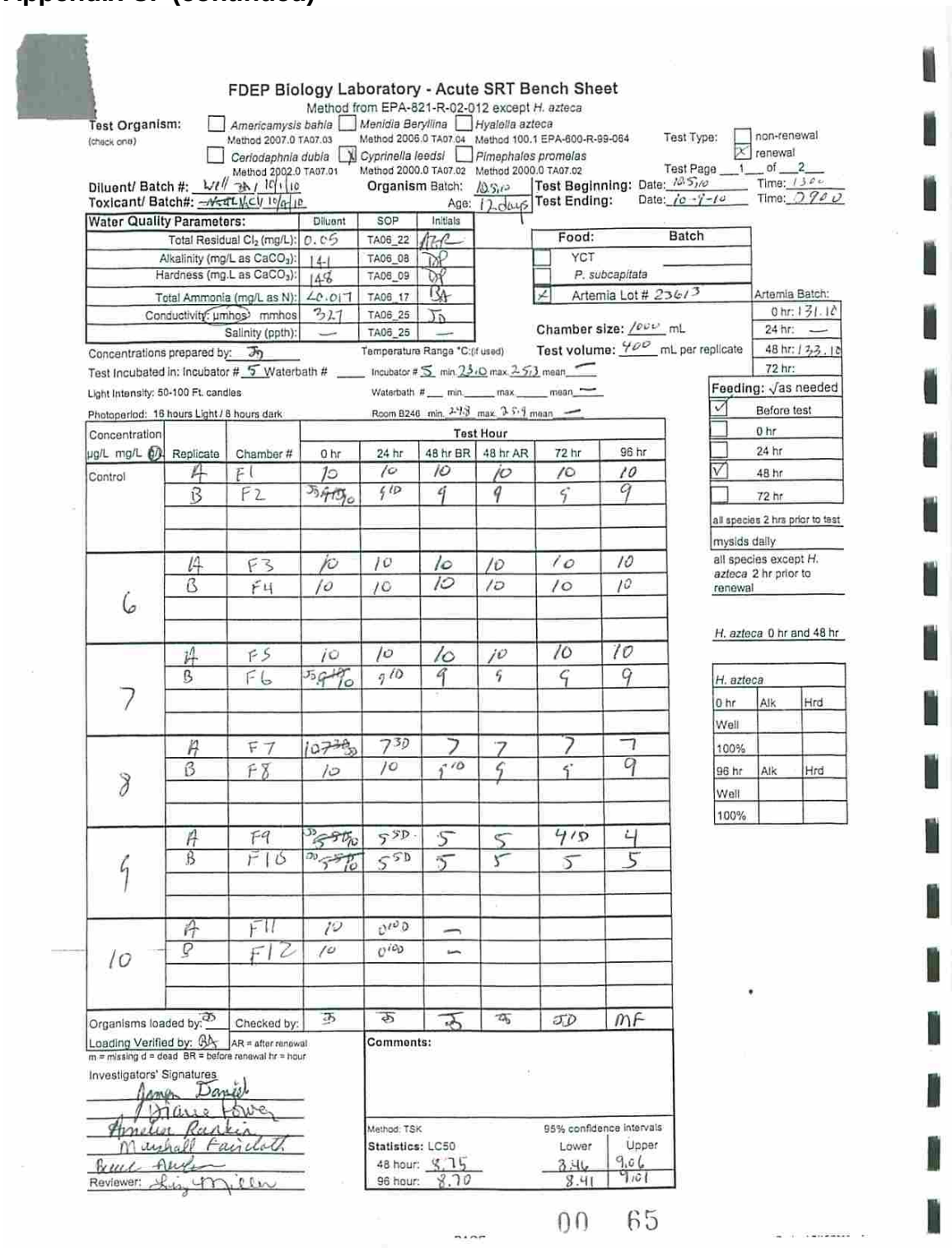
Measure/record salinity for marine tests Parameter

Comments: 24 hour ~~measurements~~ MF IN
4.0 Rep C NOT measured MF 10-9-10

PAGE 00 64

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 2A / 10/1/10
Toxicant/ Batch#: ~~NaCl~~ NaCl 10/4/10
Organism Batch: 10/5/10 Age: 12 days
Test Beginning: Date: 10/5/10 Time: 1300
Test Ending: Date: 10-9-10 Time: 0900

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	0.05	TA06_22	
Alkalinity (mg/L as $CaCO_3$):	141	TA06_08	
Hardness (mg/L as $CaCO_3$):	148	TA06_09	
Total Ammonia (mg/L as N):	<0.017	TA06_17	
Conductivity: µmhos mmhos	327	TA06_25	
Salinity (ppth):	—	TA06_25	—

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

Artemia Batch: 0 hr: 131.18 24 hr: — 48 hr: 133.18 72 hr:

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

Concentrations prepared by:
Test Incubated in: Incubator # 5 Waterbath # ____ Incubator # 5 min 23.0 max 25.3 mean —
Light Intensity: 50-100 Ft. candles Waterbath # ___ min. ___ max. ___ mean —
Photoperiod: 16 hours Light / 8 hours dark Temperature Range °C:(if used) 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

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

Concentration µg/L mg/L (circle)	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	F1	10	10	10	10	10	10
	B	F2	~~10~~ 9	9D	9	9	9	9
6	A	F3	10	10	10	10	10	10
	B	F4	10	10	10	10	10	10
7	A	F5	10	10	10	10	10	10
	B	F6	~~10~~ 9	9D	9	9	9	9
8	A	F7	~~10~~ 9	7SD	7	7	7	7
	B	F8	10	10	9D	9	9	9
9	A	F9	~~5~~ 4	5SD	5	5	4D	4
	B	F10	~~5~~ 4	5SD	5	5	5	5
10	A	F11	10	0D	—			
	B	F12	10	0D	—			
Organisms loaded by:		Checked by:						
Loading Verified by:		AR = after renewal						

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

Comments:

Investigators' Signatures
James Daniel
Diane Howe
Amelia Rankin
Marshall Faircloth
Bruce Ambler
Reviewer: Liz Miller

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour: 8.75	8.46	9.06
96 hour: 8.70	8.41	9.01

00 65

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: 10.5.10 Time: 1300
Test Ending: Date: 10-9-10 Time: 0900

Test Type: ☐ non-renewal ☒ renewal

hr = hour AR = after renewal BR = before renewal

Concentration µg/L mg/L	Replicate	Test Hour	pH (SU)	Temperature (° C)	Dissolved Oxygen (mg/L)	Conductivity ☐ µmhos ☒ mmhos	Salinity (ppth)
Control	A	0 hr	7.8	24.7	8.3	0.327	
	B	24 hr	8.1	25.1	7.1	0.285	
	A	48 hr BR	8.3	24.4	7.6	0.298	
	A	48 hr AR	8.2	24.4	7.5	0.281	
	B	72 hr	8.2	25.2	7.3	0.294	
	A	96 hr	8.3	24.5	7.2	0.287	
6	A	0 hr	7.9	24.5	8.6	10.9	
	B	24 hr	8.0	25.0	7.2	10.93 DP	
	A	48 hr BR	8.2	24.4	7.5	11.5	
	A	48 hr AR	8.1	24.7	7.9	10.2	
	B	72 hr	8.1	25.2	7.2	11.2	
	A	96 hr	8.1	24.8	7.4	10.3	
7	A	0 hr	7.9	24.4	8.7	13.1	
	B	24 hr	8.0	24.9	7.1	13.1	
	A	48 hr BR	8.1	24.3	7.5	13.3	
	A	48 hr AR	8.0	24.5	7.8	13.2	
	B	72 hr	8.1	24.0	7.3	13.5	
	A	96 hr	8.1	24.9	7.2	13.6	
8	A	0 hr	8.0	24.2	8.7	14.7	
	B	24 hr	8.0	24.3	7.1	14.7	
	A	48 hr BR	8.2	24.4	7.7	15.1	
	A	48 hr AR	8.0	24.4	7.7	14.8	
	B	72 hr	8.1	24.6	7.1	15.0	
	A	96 hr	8.2	24.6	7.5	15.1	
9	A	0 hr	8.0	24.5	8.7	16.2	
	B	24 hr	8.0	24.2	7.0	16.3	
	A	48 hr BR	8.2	24.1	7.6	16.4	
	A	48 hr AR	8.0	24.5	7.8	16.1	
	B	72 hr	8.1	24.0	7.4	16.5	
	A	96 hr	8.1	24.5	7.2	16.5	
10	A	0 hr	7.9	24.5	8.9	18.3	
	B	24 hr	7.9	24.1	6.7	15.1	
	A or F	48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					

Investigators Signatures
James Daniel
Diane Tatom
Amelia Rankin
Marshall Trinkle
Bruce Nauman
Reviewed By: Liz Millan

Measured by:
0 hr: JD
24 hr: DP/DT
48 hr BR: AR/DP
48 hr AR: DP
72 hr: DP
96 hr: DP/MT

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: 10/5/10 DURATION: 96 H TOXICANT : NaCl
SPECIES: *Ceriodaphnia dubia*

RAW DATA: Concentration (g/L)	Number Exposed	Mortalities
.00	20	0
1.50	20	0
2.00	20	2
2.50	20	19
3.00	20	20
4.00	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 2.20
95% LOWER CONFIDENCE: 2.12
95% UPPER CONFIDENCE: 2.29

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

DATE: 10/5 201 DURATION: 96 H TOXICANT : NaCl
SPECIES: *Cyprinella leedsi*

RAW DATA: Concentration (g/L)	Number Exposed	Mortalities
.00	20	1
6.00	20	0
7.00	20	1
8.00	20	4
9.00	20	11
10.00	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 8.70
95% LOWER CONFIDENCE: 8.41
95% UPPER CONFIDENCE: 9.01

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: 10/4/10

Ship to: FDEP

P.O. No: _____

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (‰)
Mysidopsis bahia	150 ac	3 DAYS	MS101001	25	7.9	20
Menidia beryllina	150 ac	12 DAYS	SS100922	25	7.9	20
						Hardness mg/L
Cyprinella leedsi	150 ac	11 DAYS	CL100923	25	7.8	80
Pimephales promelas	150 ac	7 DAYS	FM100927	25	7.8	80
	250	<24 HRS	FM101004-0830	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: _____

Shipped Via: FedEx

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: 10/11/10

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	100	10 DAYS	CL101001	25	7.8	80
Pimephales promelas						
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: _____

Shipped Via: FedEx

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 City of Fort Myers South Wastewater Treatment Plant Outfall D-001, sampled on October 11, 2010.

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 11:00	Blank	Priority Organic Pollutants	Dibromochloromethane	0.2	U	ug/L	0.2	1
10/11/2010 11:00	Blank	Priority Organic Pollutants	Chloroform	0.2	U	ug/L	0.2	1
10/11/2010 11:00	Blank	Priority Organic Pollutants	Bromoform	0.5	U	ug/L	0.5	2
10/11/2010 11:00	Blank	Priority Organic Pollutants	Bromodichloromethane	0.2	U	ug/L	0.2	1
10/11/2010 11:00	Effluent 1	Nutrients	TSS	5	I	mg/L	4	16
10/11/2010 11:00	Effluent 1	Nutrients	Chloride	230		mg Cl/L	2	5
10/11/2010 11:00	Effluent 1	Nutrients	Fluoride	0.44		mg F/L	0.035	0.1
10/11/2010 11:00	Effluent 1	Field Parameter	pH	7.42				
10/11/2010 11:00	Effluent 1	Field Parameter	Total Residual Chlorine	0		mg/L		
10/11/2010 11:00	Effluent 1	Field Parameter	Sample Depth	0		m		
10/11/2010 11:00	Effluent 1	Field Parameter	Salinity	0.58		PPTh		
10/11/2010 11:00	Effluent 1	Field Parameter	Dissolved Oxygen	1.87		mg/L		
10/11/2010 11:00	Effluent 1	Field Parameter	Specific Conductance			umhos/cm		
10/11/2010 11:00	Effluent 1	Field Parameter	Temperature	10.09		C		
10/11/2010 11:10	Effluent 2	AGP/LN	AGP	19.9	A	mg DryWt/L	0.3	0.9
10/11/2010 11:10	Effluent 2	AGP/LN	AGP-Salinity Adjusted	10.3		mg DryWt/L	3	3
10/11/2010 11:10	Effluent 2	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
10/11/2010 11:10	Effluent 2	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
10/11/2010 11:10	Effluent 2	Overflow	Fecal Coliforms-Membrane Filter	45		#/100 mL		
10/11/2010 11:10	Effluent 2	Metals	Iron	120	I	ug/L	30	120
10/11/2010 11:10	Effluent 2	Metals	Silver	0.051	I	ug/L	0.025	0.1
10/11/2010 11:10	Effluent 2	Metals	Magnesium	9.46		mg/L	0.04	0.16
10/11/2010 11:10	Effluent 2	Metals	Chromium	0.59	I	ug/L	0.3	1.2
10/11/2010 11:10	Effluent 2	Metals	Calcium	67.2		mg/L	0.075	0.3
10/11/2010 11:10	Effluent 2	Metals	Arsenic	0.89	I	ug/L	0.25	1
10/11/2010 11:10	Effluent 2	Metals	Lead	0.2	U	ug/L	0.2	0.8
10/11/2010 11:10	Effluent 2	Metals	Nickel	1.11		ug/L	0.25	1
10/11/2010 11:10	Effluent 2	Metals	Selenium	0.22	I	ug/L	0.2	0.8
10/11/2010 11:10	Effluent 2	Metals	Zinc	20		ug/L	5	20
10/11/2010 11:10	Effluent 2	Metals	Antimony	0.37	I	ug/L	0.25	1
10/11/2010 11:10	Effluent 2	Metals	Copper	0.82	I	ug/L	0.5	1
10/11/2010 11:10	Effluent 2	Metals	Aluminum	390		ug/L	60	240
10/11/2010 11:10	Effluent 2	Metals	Beryllium	0.4	U	ug/L	0.4	1.6

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 11:10	Effluent 2	Metals	Cadmium	0.03	U	ug/L	0.03	0.12
10/11/2010 11:10	Effluent 2	Nutrients	Kjeldahl Nitrogen	0.97		mg N/L	0.08	0.2
10/11/2010 11:10	Effluent 2	Nutrients	Organic Carbon	8.3		mg C/L	1	5
10/11/2010 11:10	Effluent 2	Nutrients	NO2NO3-N	0.35		mg N/L	0.004	0.01
10/11/2010 11:10	Effluent 2	Nutrients	O-Phosphate-P	0.026		mg P/L	0.004	0.01
10/11/2010 11:10	Effluent 2	Nutrients	Total-P	0.17		mg P/L	0.004	0.01
10/11/2010 11:10	Effluent 2	Nutrients	Ammonia-N	0.031		mg N/L	0.01	0.02
10/11/2010 11:10	Effluent 2	Nutrients	Cyanide	0.005	U	mg CN/L	0.005	0.02
10/11/2010 11:10	Effluent 2	Overflow	Oil and Grease	1.4	U	mg/L	1.4	5
10/11/2010 11:10	Effluent 2	Overflow	MBAS	0.12	I	mg/l LAS	0.12	0.2
10/11/2010 11:10	Effluent 2	Pesticides	Hexazinone	0.096	U	ug/L	0.096	0.38
10/11/2010 11:10	Effluent 2	Pesticides	Metalaxyl	0.24	UJ	ug/L	0.24	0.96
10/11/2010 11:10	Effluent 2	Pesticides	Prometryn	0.14	U	ug/L	0.14	0.56
10/11/2010 11:10	Effluent 2	Pesticides	Aldrin	0.01	U	ug/L	0.0096	0.038
10/11/2010 11:10	Effluent 2	Pesticides	Beta-BHC	0.01	U	ug/L	0.0096	0.038
10/11/2010 11:10	Effluent 2	Pesticides	Delta-BHC	0.01	U	ug/L	0.0096	0.038
10/11/2010 11:10	Effluent 2	Pesticides	Toxaphene	0.72	U	ug/L	0.72	2.9
10/11/2010 11:10	Effluent 2	Pesticides	Fonofos	0.096	U	ug/L	0.096	0.38
10/11/2010 11:10	Effluent 2	Pesticides	Metribuzin	0.096	U	ug/L	0.096	0.38
10/11/2010 11:10	Effluent 2	Pesticides	Norflurazon	0.14	U	ug/L	0.14	0.56
10/11/2010 11:10	Effluent 2	Pesticides	Parathion Methyl	0.096	U	ug/L	0.096	0.38
10/11/2010 11:10	Effluent 2	Pesticides	DDD-p,p'	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	Heptachlor Epoxide	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	Naled	0.77	UJ	ug/L	0.77	3.1
10/11/2010 11:10	Effluent 2	Pesticides	Endrin	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Atrazine	0.21		ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Butylate	0.19	UJ	ug/L	0.19	0.76
10/11/2010 11:10	Effluent 2	Pesticides	Chlorpyrifos Ethyl	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Mevinphos	0.19	U	ug/L	0.19	0.76
10/11/2010 11:10	Effluent 2	Pesticides	Phorate	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	DDT-p,p'	0.029	U	ug/L	0.029	0.12
10/11/2010 11:10	Effluent 2	Pesticides	Methoxychlor	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Ametryn	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Bromacil	0.19	U	ug/L	0.19	0.76

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 11:10	Effluent 2	Pesticides	Chlorpyrifos Methyl	0.096	U	ug/L	0.096	0.38
10/11/2010 11:10	Effluent 2	Pesticides	Fenamiphos	0.19	U	ug/L	0.19	0.76
10/11/2010 11:10	Effluent 2	Pesticides	Parathion Ethyl	0.14	U	ug/L	0.14	0.56
10/11/2010 11:10	Effluent 2	Pesticides	Dieldrin	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	Endosulfan I	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	Endosulfan II	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	Heptachlor	0.01	U	ug/L	0.0096	0.038
10/11/2010 11:10	Effluent 2	Pesticides	Alachlor	0.58	UJ	ug/L	0.58	2.3
10/11/2010 11:10	Effluent 2	Pesticides	Malathion	0.14	U	ug/L	0.14	0.56
10/11/2010 11:10	Effluent 2	Pesticides	Alpha-BHC	0.01	U	ug/L	0.0096	0.038
10/11/2010 11:10	Effluent 2	Pesticides	Chlordane	0.19	U	ug/L	0.19	0.76
10/11/2010 11:10	Effluent 2	Pesticides	Ethoprop	0.096	U	ug/L	0.096	0.38
10/11/2010 11:10	Effluent 2	Pesticides	Azinphos Methyl	0.19	U	ug/L	0.19	0.76
10/11/2010 11:10	Effluent 2	Pesticides	Endrin Aldehyde	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	DDE-p,p'	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Pesticides	Simazine	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Metolachlor	0.48	UJ	ug/L	0.48	1.9
10/11/2010 11:10	Effluent 2	Pesticides	Ethion	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Gamma-BHC	0.01	U	ug/L	0.0096	0.038
10/11/2010 11:10	Effluent 2	Pesticides	Diazinon	0.048	U	ug/L	0.048	0.19
10/11/2010 11:10	Effluent 2	Pesticides	Endosulfan Sulfate	0.019	U	ug/L	0.019	0.076
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2,6-Dinitrotoluene	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	3,3'-Dichlorobenzidine	40	UJ	ug/L	40	160
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Benzo(b)fluoranthene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Bis(2-chloroethyl)ether	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	3	U	ug/L	3	12
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Phenol	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	4-Bromophenyl phenyl ether	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	4-Chloro-3-methylphenol	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Acenaphthylene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Chrysene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Di-n-butyl phthalate	5	U	ug/L	5	20
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.2	U	ug/L	0.2	0.79

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Dimethyl phthalate	9.9	U	ug/L	9.9	40
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Hexachlorobenzene	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	N-Nitrosodimethylamine	2	U	ug/L	2	7.9
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Nitrobenzene	2	U	ug/L	2	7.9
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2-Chloronaphthalene	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	3	U	ug/L	3	12
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	2	U	ug/L	2	7.9
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Benzidine	99	U	ug/L	99	400
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Fluoranthene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Fluorene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	2	U	ug/L	2	7.9
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2-Nitrophenol	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Diethyl phthalate	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Isophorone	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Pyrene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Bromoform	28		ug/L	0.5	2
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Di-n-octyl phthalate	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Chloroform	17		ug/L	0.2	1
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Dibromochloromethane	53		ug/L	0.2	1
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2,4-Dichlorophenol	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2,4-Dinitrotoluene	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	4-Nitrophenol	15	U	ug/L	15	59
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Anthracene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Benzo(a)anthracene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	15	U	ug/L	15	59
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Bromodichloromethane	32		ug/L	0.2	1
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	1,2,4-Trichlorobenzene	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2,4,6-Trichlorophenol	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2-Chlorophenol	0.99	U	ug/L	0.99	4
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Benzo(k)fluoranthene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Hexachlorobutadiene	3	U	ug/L	3	12
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Hexachlorocyclopentadiene	3	U	ug/L	3	12

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2,4-Dimethylphenol	9.9	U	ug/L	9.9	40
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	2,4-Dinitrophenol	15	U	ug/L	15	59
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Acenaphthene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Benzo(a)pyrene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Butyl benzyl phthalate	5	U	ug/L	5	20
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Hexachloroethane	3	U	ug/L	3	12
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	3	U	ug/L	3	12
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Naphthalene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Pentachlorophenol	3	U	ug/L	3	12
10/11/2010 11:10	Effluent 2	Priority Organic Pollutants	Phenanthrene	0.2	U	ug/L	0.2	0.79
10/11/2010 11:10	Effluent 2	Field Parameter	Sample Depth	0		m		
10/11/2010 11:10	Effluent 2	Field Parameter	Salinity	0.52		PPTh		
10/11/2010 11:10	Effluent 2	Field Parameter	Temperature	29		C		
10/11/2010 11:10	Effluent 2	Field Parameter	pH	7.41				
10/11/2010 11:10	Effluent 2	Field Parameter	Dissolved Oxygen	13.41		mg/L		
10/11/2010 11:10	Effluent 2	Field Parameter	Total Residual Chlorine	0.05		mg/L		
10/11/2010 13:00	Effluent 3	Metals	Nickel	0.25	U	ug/L	0.25	1
10/11/2010 13:00	Effluent 3	Metals	Magnesium	0.04	U	mg/L	0.04	0.16
10/11/2010 13:00	Effluent 3	Metals	Aluminum	180	I	ug/L	60	240
10/11/2010 13:00	Effluent 3	Metals	Silver	0.025	U	ug/L	0.025	0.1
10/11/2010 13:00	Effluent 3	Metals	Lead	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Metals	Cadmium	0.03	U	ug/L	0.03	0.12
10/11/2010 13:00	Effluent 3	Metals	Zinc	5	U	ug/L	5	20
10/11/2010 13:00	Effluent 3	Metals	Iron	30	U	ug/L	30	120
10/11/2010 13:00	Effluent 3	Metals	Calcium	0.31		mg/L	0.075	0.3
10/11/2010 13:00	Effluent 3	Metals	Selenium	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Metals	Beryllium	0.4	U	ug/L	0.4	1.6
10/11/2010 13:00	Effluent 3	Metals	Chromium	0.3	U	ug/L	0.3	1.2
10/11/2010 13:00	Effluent 3	Metals	Antimony	0.25	U	ug/L	0.25	1
10/11/2010 13:00	Effluent 3	Metals	Arsenic	0.25	U	ug/L	0.25	1
10/11/2010 13:00	Effluent 3	Metals	Copper	0.5	U	ug/L	0.5	1
10/11/2010 13:00	Effluent 3	Nutrients	O-Phosphate-P	0.004	U	mg P/L	0.004	0.01
10/11/2010 13:00	Effluent 3	Nutrients	Total-P	0.004	U	mg P/L	0.004	0.01
10/11/2010 13:00	Effluent 3	Nutrients	Cyanide	0.005	U	mg CN/L	0.005	0.02

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 13:00	Effluent 3	Nutrients	Kjeldahl Nitrogen	0.08	U	mg N/L	0.08	0.2
10/11/2010 13:00	Effluent 3	Nutrients	NO2NO3-N	0.004	U	mg N/L	0.004	0.01
10/11/2010 13:00	Effluent 3	Nutrients	Chloride	0.2	U	mg Cl/L	0.2	0.5
10/11/2010 13:00	Effluent 3	Nutrients	Fluoride	0.035	U	mg F/L	0.035	0.1
10/11/2010 13:00	Effluent 3	Nutrients	Ammonia-N	0.01	U	mg N/L	0.01	0.02
10/11/2010 13:00	Effluent 3	Nutrients	TSS	4	U	mg/L	4	16
10/11/2010 13:00	Effluent 3	Overflow	Oil and Grease	1.4	U	mg/L	1.4	5
10/11/2010 13:00	Effluent 3	Overflow	MBAS	0.12	U	mg/l LAS	0.12	0.2
10/11/2010 13:00	Effluent 3	Pesticides	Atrazine	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Diazinon	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Metolachlor	0.48	UJ	ug/L	0.48	1.9
10/11/2010 13:00	Effluent 3	Pesticides	Phorate	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Aldrin	0.01	U	ug/L	0.0097	0.039
10/11/2010 13:00	Effluent 3	Pesticides	Alpha-BHC	0.01	U	ug/L	0.0097	0.039
10/11/2010 13:00	Effluent 3	Pesticides	Delta-BHC	0.01	U	ug/L	0.0097	0.039
10/11/2010 13:00	Effluent 3	Pesticides	Alachlor	0.58	UJ	ug/L	0.58	2.3
10/11/2010 13:00	Effluent 3	Pesticides	Malathion	0.15	U	ug/L	0.15	0.6
10/11/2010 13:00	Effluent 3	Pesticides	DDD-p,p'	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	DDE-p,p'	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Chlorpyrifos Ethyl	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Ethion	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Fenamiphos	0.19	U	ug/L	0.19	0.76
10/11/2010 13:00	Effluent 3	Pesticides	Fonofos	0.097	U	ug/L	0.097	0.39
10/11/2010 13:00	Effluent 3	Pesticides	Mevinphos	0.19	U	ug/L	0.19	0.76
10/11/2010 13:00	Effluent 3	Pesticides	Endrin	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Hexazinone	0.097	U	ug/L	0.097	0.39
10/11/2010 13:00	Effluent 3	Pesticides	Beta-BHC	0.01	U	ug/L	0.0097	0.039
10/11/2010 13:00	Effluent 3	Pesticides	Chlordane	0.19	U	ug/L	0.19	0.76
10/11/2010 13:00	Effluent 3	Pesticides	Endosulfan Sulfate	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Ametryn	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Azinphos Methyl	0.19	U	ug/L	0.19	0.76
10/11/2010 13:00	Effluent 3	Pesticides	Chlorpyrifos Methyl	0.097	U	ug/L	0.097	0.39
10/11/2010 13:00	Effluent 3	Pesticides	Ethoprop	0.097	U	ug/L	0.097	0.39
10/11/2010 13:00	Effluent 3	Pesticides	Metalaxyl	0.24	UJ	ug/L	0.24	0.96

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 13:00	Effluent 3	Pesticides	Norflurazon	0.15	U	ug/L	0.15	0.6
10/11/2010 13:00	Effluent 3	Pesticides	Parathion Ethyl	0.15	U	ug/L	0.15	0.6
10/11/2010 13:00	Effluent 3	Pesticides	Prometryn	0.15	U	ug/L	0.15	0.6
10/11/2010 13:00	Effluent 3	Pesticides	Simazine	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Pesticides	Dieldrin	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Gamma-BHC	0.01	U	ug/L	0.0097	0.039
10/11/2010 13:00	Effluent 3	Pesticides	Toxaphene	0.73	U	ug/L	0.73	2.9
10/11/2010 13:00	Effluent 3	Pesticides	Bromacil	0.19	U	ug/L	0.19	0.76
10/11/2010 13:00	Effluent 3	Pesticides	Metribuzin	0.097	U	ug/L	0.097	0.39
10/11/2010 13:00	Effluent 3	Pesticides	DDT-p,p'	0.029	U	ug/L	0.029	0.12
10/11/2010 13:00	Effluent 3	Pesticides	Endosulfan I	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Butylate	0.19	UJ	ug/L	0.19	0.76
10/11/2010 13:00	Effluent 3	Pesticides	Naled	0.78	UJ	ug/L	0.78	3.1
10/11/2010 13:00	Effluent 3	Pesticides	Endrin Aldehyde	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Heptachlor Epoxide	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Parathion Methyl	0.097	U	ug/L	0.097	0.39
10/11/2010 13:00	Effluent 3	Pesticides	Endosulfan II	0.019	U	ug/L	0.019	0.076
10/11/2010 13:00	Effluent 3	Pesticides	Heptachlor	0.01	U	ug/L	0.0097	0.039
10/11/2010 13:00	Effluent 3	Pesticides	Methoxychlor	0.048	U	ug/L	0.048	0.19
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2,4,6-Trichlorophenol	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Anthracene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Isophorone	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2-Chloronaphthalene	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2-Nitrophenol	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	4-Bromophenyl phenyl ether	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Acenaphthylene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Benzo(a)anthracene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Di-n-butyl phthalate	5	U	ug/L	5	20
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Pyrene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2-Chlorophenol	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Benzo(a)pyrene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	15	U	ug/L	15	60
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Hexachlorocyclopentadiene	3	U	ug/L	3	12
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Pentachlorophenol	3	U	ug/L	3	12

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Phenanthrene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	3,3'-Dichlorobenzidine	40	UJ	ug/L	40	160
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	2	U	ug/L	2	8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Benzidine	100	U	ug/L	100	400
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Benzo(k)fluoranthene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Di-n-octyl phthalate	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Hexachlorobenzene	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	N-Nitrosodimethylamine	2	U	ug/L	2	8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	3	U	ug/L	3	12
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Dibromochloromethane	0.2	U	ug/L	0.2	1
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	1,2,4-Trichlorobenzene	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2,4-Dimethylphenol	10	U	ug/L	10	40
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	4-Nitrophenol	15	U	ug/L	15	60
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Chrysene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Diethyl phthalate	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Dimethyl phthalate	10	U	ug/L	10	40
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Hexachloroethane	3	U	ug/L	3	12
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2,6-Dinitrotoluene	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	4-Chloro-3-methylphenol	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	3	U	ug/L	3	12
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Butyl benzyl phthalate	5	U	ug/L	5	20
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Phenol	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Bromoform	0.5	U	ug/L	0.5	2
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Chloroform	0.37	I	ug/L	0.2	1
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2,4-Dichlorophenol	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	3	U	ug/L	3	12
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Acenaphthene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Bis(2-chloroethyl)ether	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Hexachlorobutadiene	3	U	ug/L	3	12
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	2	U	ug/L	2	8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Bromodichloromethane	0.2	U	ug/L	0.2	1

Appendix E. (continued)

Date Sampled	Sample ID	Analysis Group	Components	Result	Remark	Units	MDL	PQL
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2,4-Dinitrophenol	15	U	ug/L	15	60
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	2,4-Dinitrotoluene	1	U	ug/L	1	4
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Benzo(b)fluoranthene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Fluoranthene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Fluorene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Naphthalene	0.2	U	ug/L	0.2	0.8
10/11/2010 13:00	Effluent 3	Priority Organic Pollutants	Nitrobenzene	2	U	ug/L	2	8
10/11/2010 13:00	Effluent 3	Field Parameter	Temperature	25.3		C		
10/11/2010 13:00	Effluent 3	Field Parameter	Sample Depth	0		m		
10/11/2010 13:00	Effluent 3	Field Parameter	Dissolved Oxygen	9.46		mg/L		
10/11/2010 13:00	Effluent 3	Field Parameter	pH	6.89				
10/11/2010 13:00	Effluent 3	Field Parameter	Specific Conductance	0		umhos/cm		
10/11/2010 11:30	Effluent 4	Overflow	ENTEROCOCCI-ENTEROLERT	1		#/100 mL		
10/11/2010 11:30	Effluent 4	Toxicology	BSY-FW-C.dubia, LC50	100	L	LC50		
10/11/2010 11:30	Effluent 4	Toxicology	BSY-FW-Fish, LC50	100	L	LC50		
10/11/2010 11:30	Effluent 4	Field Parameter	Total Residual Chlorine	1.86		mg/L		
10/11/2010 11:30	Effluent 4	Field Parameter	Specific Conductance	0.52		umhos/cm		
10/11/2010 11:30	Effluent 4	Field Parameter	Temperature	29		C		
10/11/2010 11:30	Effluent 4	Field Parameter	pH	7.27				
10/11/2010 11:30	Effluent 4	Field Parameter	Dissolved Oxygen	11.26		mg/L		
10/11/2010 11:30	Effluent 4	Field Parameter	Sample Depth	0		m		

Appendix F. Excerpt from Permit



Department of Environmental Protection

Jeb Bush
Governor

South District
P.O. Box 2549
Fort Myers, Florida 33902-2549

Colleen M. Castille
Secretary

STATE OF FLORIDA
DOMESTIC WASTEWATER FACILITY PERMIT

PERMITTEE:

City of Fort Myers

RESPONSIBLE AUTHORITY:

Mr. Albert Abdo, Jr.
Public Works Director
P.O. Drawer 2217
Fort Myers, FL 33902

(239) 332-6854

PERMIT NUMBER: FL0021270 (Major)
PA FILE NUMBER: FL0021270-010-DW1P
ISSUANCE DATE: July 13, 2006
EXPIRATION DATE: July 12, 2011

FACILITY:

City of Fort Myers South WWTP
1618 South Drive
Fort Myers, FL 33907
Lee County
Latitude: 26° 35' 16" N Longitude: 81° 51' 44" W

This permit is issued under the provisions of Chapter 403, Florida Statutes, and applicable rules of the Florida Administrative Code and constitutes authorization to discharge to waters of the state under the National Pollutant Discharge Elimination System. This permit is accompanied by an Administrative Order, pursuant to paragraphs 403.088(2)(e) and (f), Florida Statutes. Compliance with Administrative Order, AO-042-SD is a specific requirement of this permit. The above named permittee is hereby authorized to operate and construct substantial modification to the facilities shown on the application and other documents attached hereto or on file with the Department and made a part hereof and specifically described as follows:

TREATMENT FACILITIES:

Operate an existing 12.0 mgd annual average daily flow (AADF) Bardenpho process domestic wastewater treatment plant consisting of influent screening, grit removal, fermentation, first anoxic, aeration, second anoxic, reaeration, secondary clarification, chemical feed, chlorination, and dechlorination for the surface water discharge. A substantial modification to this facility for the production of 9.0 MGD annual average daily flow, AADF, reuse quality water is projected during this permit cycle and consists of constructing and installing three new disk filters with associated backwash pumps, automatic valves, two new disinfection contact basins for high level disinfection, two new 5 mg covered reuse storage tanks, four new transfer pumps to pump reuse water to the storage tanks and four new high service pumps (variable frequency drives) to pump into the distribution system. Residuals handling includes aerobic digestion and belt press thickening with residuals disposed of in accordance with Part II of this permit.

Page 1 of 31

Appendix F. (continued)

FACILITY:	City of Fort Myers South AWWT Plant	PERMIT NUMBER:	FL0021270
PERMITTEE:	City of Fort Myers P.O. Drawer 2217 Fort Myers, FL 33902	PA FILE NUMBER:	FL0021270-010-DW1P

DISPOSAL:

Surface Water Discharge: An existing 12.0 MGD monthly average daily flow (MADF) permitted discharge to Caloosahatchee River Estuary (Class III marine waters) at Discharge Location (D-001) which is a 48 inch diameter line from the plant and approximately 1760 feet in length from the shoreline and discharges at a depth of approximately 19.8 feet. The point of discharge is located approximately at latitude 26 ° 35' 35" N, longitude 81 ° 54' 15" W.

REUSE:

Land Application:

The reuse distribution service area is currently defined as that identified in permit number FL0021261 for the Fort Myers Central WWTP. At this time, the City of Fort Myers South wastewater treatment facility does not have treatment capability to produce public access reuse quality water. It also does not have the ability to distribute reuse quality water to the irrigation distribution system.

IN ACCORDANCE WITH: The limitations, monitoring requirements and other conditions set forth in Pages 1 through 31 of this permit.

FACILITY: City of Fort Myers South AWWT Plant
PERMITTEE: City of Fort Myers
P.O. Drawer 2217
Fort Myers, FL 33902

PERMIT NUMBER: FL0021270
PA FILE NUMBER: FL0021270-010-DW1P

I. RECLAIMED WATER AND EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

A. Surface Water Discharges

1. During the period beginning on the issuance date and lasting through the expiration date of this permit, the permittee is authorized to discharge effluent from Outfall D-001 to the Caloosahatchee River Estuary. Such discharge shall be limited and monitored by the permittee as specified below:

			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	Report		12.0	-	-	Continuous	Recording flow meters and totalizers	FLW-1,FLW-2 & CAL-1	See Cond I.A.4
BOD, Carbonaceous 5 day, 20C	MG/L	Maximum	20.0	25.0	40.0	60.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
Solids, Total Suspended	MG/L	Maximum	20.0	30.0	45.0	60.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
pH	SU	Range	-	-	-	6.5 to 8.5	Continuous	Meter	EFA-01	See Cond I.A.3
Coliform, Fecal	#/100ML	Maximum	See Permit Condition I.A.5				5 Days/Week	Grab	EFA-01	
Total Residual Chlorine (For Disinfection)	MG/L	Minimum	-	-	-	0.5	Continuous	Meter	EFA-01	See Cond I.A.3, 6
Total Residual Chlorine (For Dechlorination)	MG/L	Maximum	-	-	-	0.01	Daily	Grab	EFD-01	
Nitrogen, Total (as N)	MG/L	Maximum	3.0	3.0	4.5	6.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
Phosphorus, Total (as P)	MG/L	Maximum	0.5	0.5	0.75	1.0	5 Days/Week	24-hour flow proportioned composite	EFD-01	
Oxygen, Dissolved (DO)	MG/L	Minimum	5.0 Daily Average			4.0	Daily	Grab	EFD-01	See Cond I.A.7
Enterococci	#/100ml	Maximum	-	35.0	-	-	5/month	Grab	EFD-01	See Cond I.A.17
Whole Effluent Toxicity	See Permit Condition I.A.13								EFD-01	

Appendix F. (continued)

2. Effluent samples shall be taken at the monitoring site locations listed in Permit Condition I. A. 1. and as described below:

Monitoring Location Site Number	Description of Monitoring Location
EFA-01	At slide gate No. 3 after disinfection.
EFD-01	At slide gate No. 4 downstream of sodium bisulfite addition and pumped to a flow proportioned sampler.
FLW-1	Ultrasonic flow meter (1), at chlorine contact basin 1. Measures, totalizes and records flows through chlorine contact chamber basin one.
FLW-2	Ultrasonic flow meter (2), at chlorine contact basin 2. Measures, totalizes and records flows through chlorine contact chamber 2.
CAL-1	Total of FLW-1 and FLW-2 calculated at the PLC.

3. Hourly measurement of pH and total residual chlorine for disinfection during the period of required operator attendance may be substituted for continuous measurement. The pH values shall not deviate outside the required range more than 7 hours and 26 minutes in any calendar month and no individual excursion shall exceed 60 minutes. An "excursion" is an unintentional and temporary incident in which the pH value of discharge wastewater exceeds the range set forth in this permit.*[Chapter 62-601, Figure 2, 40 CFR, Part 401.17]*Recording flow meters and totalizers shall be utilized to measure flow and calibrated at least annually. *[62-601.200(17) and .500(6)]*

4. Recording flow meters and totalizers shall be utilized to measure flow and calibrated at least annually. *[62-601.200(17) and .500(6)]*

5. The arithmetic mean of the monthly fecal coliform values collected during an annual period shall not exceed 200 per 100 mL of effluent sample. The geometric mean of the fecal coliform values for a minimum of 10 samples of effluent each collected on a separate day during a period of 30 consecutive days (monthly), shall not exceed 200 per 100 mL of sample. No more than 10 percent of the samples collected (the 90th percentile value) during a period of 30 consecutive days shall exceed 400 fecal coliform values per 100 mL of sample. Any one sample shall not exceed 800 fecal coliform values per 100 mL of sample. Note: To report the 90th percentile value, list the fecal coliform values obtained during the month in ascending order. Report the value of the sample that corresponds to the 90th percentile (multiply the number of samples by 0.9). For example, for 30 samples, report the corresponding fecal coliform number for the 27th value of ascending order. *[62-600.440(4)(c)]*

6. A minimum of 0.5 mg/L total residual chlorine must be maintained for a minimum contact time of 15 minutes based on peak hourly flow. *[62-600.440(4)(b)]*

7. In predominantly marine waters, the discharge concentration of the Dissolved Oxygen shall not average less than 5.0 milligrams per liter (mg/L) in a 24-hour period and shall never be less than 4.0 mg/L. Normal daily and seasonal fluctuations above these levels shall be maintained in both predominantly fresh waters and predominantly marine waters, in accordance with F.A.C. Rule 62-302.530(31).

8. Florida water quality criteria and standards shall not be violated as a result of the discharge. *[Chapter 62-600.500]*

9. The SO_2 **feed rate** shall be monitored daily to assure other water quality parameters such as D.O. are not violated in accordance with Chapter 62-302, F.A.C. *[62-620.620(3), 62-4.070(1) & (3)]*

10. There shall be no discharge of floating solids or visible foam in other than trace amounts. *[62-620.620(3), 62-4.070(1) & (3)]*

11. The effluent shall not cause a visible sheen on the receiving water. *[62-620.620(3), 62-4.070(1) & (3)]*

Appendix F. (continued)

12. Testing for total residual chlorine (TRC) after dechlorination shall be conducted according to either the low-level amperometric titration method, or the DPD colorimetric method or the iodometric electrode method as specified in Section 4500-C1 E., 4500-C1 G. or 4500-C1 I, respectively, Standard Methods for the Examination of Water and Wastewater, 18th or most recent Edition. *[62-620.620(3), 62-601.400]*

13. The permittee shall comply with the following requirements to evaluate acute whole effluent toxicity of the discharge from outfall EFD-01.

    a. Effluent limitation

        (1) Whole effluent acute toxicity shall not exceed in any "routine" or in any "additional follow-up" test an LC50 of less than 100% effluent. [Rule 62-302.200(1), Rule 62-302.500(1)(a)4., and Rule 62-4.244(3)(a), F.A.C.]

    b. Monitoring frequency

        (1) "Routine" toxicity tests shall be conducted once every six months lasting for the duration of this permit unless notified otherwise in writing by the Department. The initial testing shall be due with the permittee's current six month sample and testing sequence for toxicity.

    c. Test Requirements:
        (1) Routine Tests: All routine tests shall be conducted using a control (0% effluent) and a minimum of five dilutions: **100%**, **50%**, **25%**, **12.5%** and **6.25%** effluent
        (2) Additional Follow-up Tests, if required:
            (a) If a routine test does not meet the acute toxicity limitation in 1.a. (1) above, the permittee shall conduct two additional follow-up tests on each species that failed the test.
            (b) The first and second 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. All test results shall be statistically analyzed according to the Appendices in EPA-821-R-02-012.
            (c)The first test shall be initiated within two weeks of the end of the failed routine test. The remaining additional follow-up test shall be conducted weekly thereafter until a total of two valid additional follow-up tests are completed.
        (3) **Freshwater Discharges**: When the effluent salinity is < 1.0 ppt, the permittee shall conduct 96-hour acute static renewal multi-concentration toxicity tests using the daphnid, **Ceriodaphnia dubia**, and the bannerfin shiner, **Cyprinella leedsi**, concurrently.

        (4) **Saltwater Discharges**: When the effluent salinity is ≥ 1.0 ppt, the permittee shall conduct 96-hour acute static renewal toxicity tests using the mysid, **Americamysis (Mysidopsis) bahia**, and the inland silverside, **Menidia beryllina**, concurrent.

        (5) All test species, procedures and quality assurance criteria used shall be in accordance with **Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms**, 5th edition, EPA-821-R-02-012. 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 acute toxicity testing in accordance with the revised method.

        (6) **Freshwater Discharges:** The control water and dilution water used will be moderately hard water as described in the EPA-821-R-02-012, Table 7, or the most current edition.

        (7) **Saltwater Discharges:** The control water and dilution water used shall be artificial seawater and diluted to the test salinity as described in EPA-821-R-02-012, Section 7.2.4. The test salinity shall be determined as follows:

            (a) When the salinity of the effluent is between 1 and 7 parts per thousand (ppt), the following salinity adjustment shall be used. For the **A. bahia** bioassays, the effluent and the control (0% effluent) shall be adjusted to a salinity of 6-7 ppt for the 100% effluent test using artificial sea salts. No salinity adjustment shall be done for the **Menidia beryllina** bioassay test. The salinity of the control/ dilution water shall match the test salinity of the effluent.

Appendix F. (continued)

(b) When the salinity of the effluent is greater than 7 ppt, no salinity adjustment shall be made to the effluent and the test shall be run at the effluent salinity. The salinity of the control/ dilution water shall match the test salinity of the effluent.

d. Sampling Requirements

(1) Routine tests shall be conducted on four separate grab samples collected at evenly-spaced (6-hr) intervals over a 24-hour period and used in four separate tests in order to catch any peaks of toxicity and to account for daily variations in effluent quality. If the duration of the discharge is less than 24-hours, the duration of discharge shall be documented on the chain of custody.

(2) For additional follow-up tests, the first test shall be conducted on four separate grab samples collected at evenly-spaced (6-hr) intervals over a 24-hour period. Each sample shall be run as a separate test. The second additional follow-up test shall be run on a single grab sample collected on the day and time when the greatest toxicity was identified in the first additional follow-up test. If toxicity was not identified in the first additional follow-up test, the second additional follow-up test shall be run on a single grab sample collected on the day and time when the greatest toxicity was identified in the routine test. Results for each additional test shall include the determination of LC50 values with 95% confidence limits.

e. Quality Assurance Requirements

(1) A standard reference toxicant (SRT) quality assurance (QA) acute 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 included in the reports for each companion routine or additional follow-up test required.

(2) If the mortality in the control (0% effluent) exceeds 10% for either species in any test, the test for that species (including the control) shall be invalidated and the test repeated.

(3) If during any routine separate grab sample test, 100% mortality occurs prior to the end of the test and control mortality is less than 10% at that time, that test (including the control) shall be terminated with the conclusion that the test fails and constitutes non-compliance.

(4) Additional follow-up tests shall be evaluated for acceptability based on the concentration-response relationship as required by EPA-821-R-02-012, Section 12.2.6.2. and included with the bioassay reports.

f. Reporting Requirements

(1) Results from all required tests shall be reported on the Discharge Monitoring Report (DMR) as follows:

(a) Routine Test Results: If an LC50> 100% effluent occurs in all four separate grab sample tests for the test species, ">100%" should be entered on the DMR for that test species. If in any of the four separate grab sample tests for the test species a LC50 < 100% effluent occurs, the lowest calculated LC50 effluent concentration shall be entered on the DMR for that test species.

(b) Additional Follow-up Test Results: Report the calculated LC50 value for that test species and the 95%confidence limits.

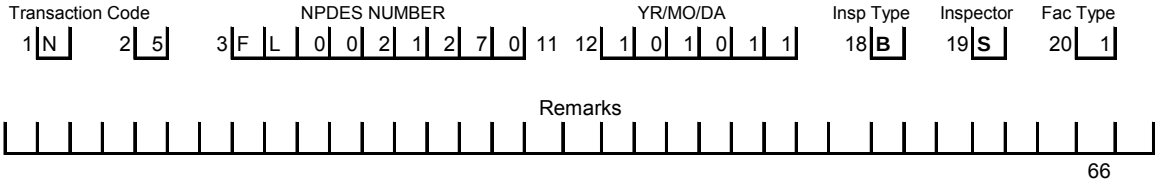
(2) A bioassay laboratory report for the routine test shall be prepared according to EPA-821-R-02-012, Section 12, Report Preparation and Test Review and mailed to the Department at the address below within 30 days of the completion of the test.

(3) For additional follow-up tests, a single bioassay laboratory report shall be prepared according to EPA-821-R-02-012, Section 12, and mailed within 45 days of completion of the second valid additional follow-up test. If any additional follow-up tests, or two consecutive routine tests, do not meet the effluent limitation specified in 1.a. (1) above, the permittee shall contact with the Department within 30 days of the laboratory report submittal to discuss the corrective actions necessary to remedy the observed acute toxicity.

Appendix G. EPA End Sheet

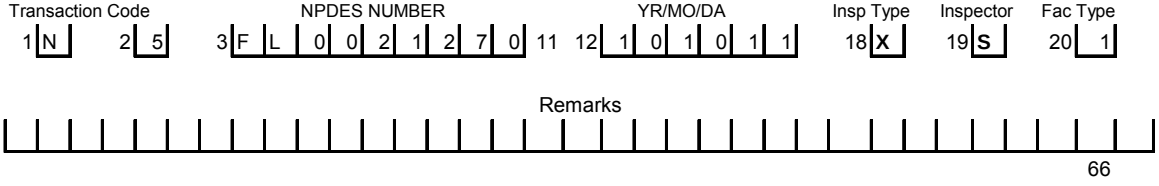
The Bioassay of City of Fort Myers South Wastewater Treatment Plant effluent sampled on October 11, 2010. NPDES #FL0021271.

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



The Priority Pollutants Analysis of City of Fort Myers South Wastewater Treatment Plant effluent sampled on October 11, 2010. NPDES #FL0021271.

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



Biological Analyses of City of Fort Myers South Wastewater Treatment Plant effluent sampled on October 11, 2010. NPDES #FL0021271.

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

