

Effluent Quality Assessment

Fernandina Beach WWTF

Nassau County

NPDES #FL0027260

Sampled September 28 - 29, 2008

February 2009

Biology Section
Bureau of Laboratories
Division of Environmental Assessment and Restoration

Quality Manual No. 870346G
NELAC Certification No. E31780

Fernandina Beach Wastewater Treatment Facility (WWTF), 1007 South Fifth Street Extended, Fernandina Beach, Nassau County, Florida, 32034. NPDES #FL0027260. Effluent samples for this facility were collected on September 28-29, 2008.

Introduction

The Fernandina Beach WWTF is an existing 3.5 million gallons per day (MGD) annual average daily flow (AADF) permitted capacity domestic wastewater treatment facility. Raw wastewater from the influent pumping station is directed to a pretreatment unit and a flow splitter box for separation to the facility's two treatment trains. These two treatment trains consist of:

- a 2.50 MGD AADF permitted capacity oxidation-ditch treatment train consisting of;
  - an aeration / anoxic basin;
  - a secondary clarifier; and,
  - a chlorine contact chamber.

- a 1.000 MGD AADF permitted capacity contact-stabilization treatment train consisting of;
  - a contact tank;
  - a return activated sludge stabilization tank;
  - a secondary clarifier; and,
  - a chlorine contact chamber.

The flows of treated and disinfected effluent from the chlorine contact tanks are combined in an on-site effluent gravity sewer and directed to dechlorination equipment and a Parshall flume for flow measurement. Effluent then flows to Outfall D-001 for discharge into the Class III marine waters of the Amelia River. The outfall is approximately 22.9 m (75 feet) long and discharges at a depth of approximately 8.1 m (26.7 feet). The point of discharge is located approximately at latitude 30º 39' 47" N and longitude 81º 28' 08" W. The AADF through Outfall D-001 from October 2007 through October 2008 was 1.84 MGD.

Waste activated-sludge is treated in two aerobic digesters that operate in series. Treated residuals are dewatered with a rotary press and are then hauled to Camden County Landfill for disposal.

The facility has no Administrative Orders, Consent Orders or mixing zones. A review of Discharge Monitoring Report (DMR) data for the past year, revealed no violations of permit conditions. Effluent quality has improved since the facility stopped using the third treatment train (activated-sludge). (Facility information provided by Eesa Ali and Brian Kirts of FDEP Northeast District.)

Methods

Samples were collected from the EFD-1 NPDES sampling point for Outfall D-001 by Eesa Ali and Lisa Crepso (FDEP Northeast District) following DEP-SOP-001/01 FS 2400 Wastewater Sampling. All DEP SOPs are available on the web at: http://www.floridadep.org/labs/qa/sops.htm.

See Appendix A for the completed chain of custody form.

The 96-hour screening toxicity tests and algal growth potential test were performed following internal DEP SOPs TA07_03, TA07_04, 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. Bioassays were performed on each of four samples which were collected over a 24-hour period (one sample every six hours).

The bioassay samples were salinity-adjusted from apparent salinities of approximately 0.5 parts per thousand (ppth) to salinities of approximately 20 ppth per permit conditions. Forty-Fathoms® artificial sea salts were used for the salinity adjustments. Salt controls were prepared by diluting 20 ppth artificial seawater (ASW) with well water to a salinity of approximately 0.5 ppth then salting this solution with artificial sea salts to match the salinity of the test solution (approximately 20 ppth). The purpose of the salt

controls was to confirm that there was no effect of adding artificial sea salts to the samples. There was 5% mortality in the salt control for the *Americamysis bahia* test for sample # 2 and there was 5% mortality in the salt controls for the *Menidia beryllina* tests for sample #2 and sample #4. There was no mortality in the remaining salt controls for either test species.

Prior to test initiation, bioassay sample #3 was aerated for 20 minutes at approximately 100 bubbles per minute, to decrease the dissolved oxygen concentration from 9.5 mg/L to 6.6 mg/L (final DO measured in salinity adjusted sample).

An aliquot of the AGP sample was salinity adjusted to meet the minimum salinity requirements of the test species. Both a freshwater and a marine AGP test were performed.

Toxicity Test Results

Tests were performed September 30 – October 4, 2008. Samples are arranged in order of collection.

Sample # 1

Americamysis bahia 96-hour acute screening bioassay –	LC_{50} > 100%, 0% mortality in 100% sample at 96 hours
Menidia beryllina 96-hour acute screening bioassay –	No Result - test invalidated due to excess control mortality at 96 hours

Sample # 2

Americamysis bahia 96-hour acute screening bioassay –	LC_{50} > 100%, 0% mortality in 100% sample at 96 hours
Menidia beryllina 96-hour acute screening bioassay –	LC_{50} > 100%, 20% mortality in 100% sample at 96 hours

Sample # 3

Americamysis bahia 96-hour acute screening bioassay –	LC_{50} > 100%, 0% mortality in 100% sample at 96 hours
Menidia beryllina 96-hour acute screening bioassay –	No Result - test invalidated due to excess control mortality at 96 hours

Sample # 4

Americamysis bahia 96-hour acute screening bioassay –	LC_{50} > 100%, 0% mortality in 100% sample at 96 hours
Menidia beryllina 96-hour acute screening bioassay –	LC_{50} > 100%, 25% mortality in 100% sample at 96 hours

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

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 273 mg dry wt/L of the unicellular green alga, *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*. Raschke and Shultz (1987) found that AGP values above 5.0 mg dry weight/L represent a “problem” threshold for fresh receiving waters, implying nutrient enrichment. The analytical chemistry suggests that the effluent is

nitrogen-limited. The predicted AGP based on inorganic nitrogen concentrations was 237 (±20%) mg dry wt/L (Table 1; Miller et al. 1978).

The salinity-adjusted effluent AGP was 140 mg dry wt/L of the saltwater species *Dunaliella tertiolecta.* Raschke and Shultz found 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). One potential reason for less growth in the salinity-adjusted sample is precipitation of orthophosphate by the added salts (Manahan 1994).

See Table 1 for AGP results.

Chemistry Results

Total residual chlorine and total ammonia were not detected in the bioassay samples in the laboratory. The total ammonia concentration in the sample collected and preserved for chemical analysis was 0.036 mg/L. Based on the pH, salinity, and temperature of the effluent as collected, the calculated unionized ammonia concentration was < 0.02 mg/L.

Arsenic and zinc were detected in the effluent at levels that comply with Class III marine water quality criteria (62-302.530, F.A.C.). Copper, iron and nickel were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL).

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.

Conclusion

The effluent samples collected from the EFD-1 NPDES sampling point for Outfall D-001 for this facility on September 28 and 29, 2008, were not acutely toxic to either test species in the valid tests during the 96-hour acute screening bioassays. The *Menidia beryllina* (inland silverside – marine fish species) bioassays for effluent sample #1 and effluent sample #3 were invalidated due to excessive control mortality. The effluent AGP result and the AGP result of the salinity adjusted effluent both exceeded the "problem" thresholds, respectively, for fresh and marine receiving waters. The AGP results suggest nutrient enrichment may be an issue. Effluent water quality samples did not exceed any applicable water quality standards or violate any permit conditions.

Literature Cited

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

Manahan, S. E. 1994. Environmental Chemistry, 6th edition. Lewis Publishers, USA. 811 pp

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 the nitrogen to phosphorus for samples collected from the Fernandina Beach WWTF on September 29, 2008.

Fernandina Beach WWTF							
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	273.0	237.0 ± 47.4	731.0 ± 146.2	285.0 ± 57.0	817.0 ± 163.4	3.7	3.9

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 Fernandina Beach WWTF on September 29, 2008.

Fernandina Beach WWTF			
Location	AGP (measured)	Inorganic N:P ratio	Total N:P ratio
Effluent*	140.0	3.7	3.9

* - sample salinity adjusted to 7ppt

Table 2. Effluent limits, Class III Criteria for predominantly marine waters and chemical and microbiological data for samples collected from the Fernandina Beach WWTF on September 28 and 29, 2008.

Fernandina Beach WWTF NPDES# FL0027260	Class III Criteria	Effluent Limits	Effluent Sampled 9/28/08 1800	Effluent Sampled 9/28/08 2400	Effluent Sampled 9/29/08 0600	Effluent Sampled 9/29/08 1200	
Organic Constituents (µg/L)							
Not Sampled*	-	-	-	-	-	-	
Metals (µg/L unless otherwise noted)							
Aluminum	≤ 1,500	-	-	-	-	60	U
Arsenic	≤ 50	-	-	-	-	2.5	
Cadmium	≤ 8.8	-	-	-	-	0.02	U
Calcium (mg/L)	-	-	-	-	-	68.1	
Chromium-III	-	-	-	-	-	0.5	U
Copper	≤ 3.7	-	-	-	-	1.5	I
Iron	≤ 300	-	-	-	-	60	I
Lead	≤ 8.5	-	-	-	-	0.2	U
Magnesium (mg/L)	-	-	-	-	-	26.6	
Nickel	≤ 8.3	-	-	-	-	0.9	I
Selenium	≤ 71	-	-	-	-	0.5	U
Silver	≤ 2.3	-	-	-	-	0.025	UF
Zinc	≤ 86	-	-	-	-	21	
Nutrients (mg/L)							
Ortho-phosphate	-	Report	-	-	-	1.7	
Total Phosphorus	-	Report	-	-	-	1.9	
Total Ammonia	-	Report	-	-	-	0.036	
Un-ionized Ammonia	-	-	-	-	-	≤ 0.02	c
Nitrate and Nitrite	-	Report	-	-	-	6.2	F
Total Kjeldahl Nitrogen	-	Report	-	-	-	1.3	
Organic Nitrogen	-	Report	-	-	-	1.264	c
Total Nitrogen	-	Report	-	-	-	7.5	c

Value exceeds the Class III Water Quality Criteria or permit limits

c - Value is calculated

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

* - Samples for organic analyses were not collected due to a priority laboratory event

Table 2. (continued)

Fernandina Beach WWTF NPDES# FL0027260	Class III Criteria	Effluent Limits	Effluent Sampled 9/28/08 1800	Effluent Sampled 9/28/08 2400	Effluent Sampled 9/29/08 0600	Effluent Sampled 9/29/08 1200
Dissolved Oxygen (mg/L)	≥ 4.0	≥ 5.0 s	7.2	7.1	7.3	8.0
pH (SU)	6.5 - 8.5	6.5 - 8.5 s	7.0	7.0	7.1	7.1
Conductivity (µmhos/cm)	-	-	817	806	797	790
Temperature °C	-	-	26.4	26.4	26.3	26.5
Total Residual Chlorine (mg/L)	≤ 0.01	≤ 0.01 s	0.005	0.007	0.03 UQ£	0.03 UQ£
Chlorophyll a (µg/L) - Corrected	-	-	-	-	-	0.6 I
Phaeophytin (µg/L)	-	-	-	-	-	0.24 U
Total Suspended Solids (mg/L)	-	≤ 60 s	-	-	-	4 U
CBOD, 5 day (mg/L), N - inhibited	-	≤ 60 s	-	-	-	2.4
Flow (MGD)	-	≤ 3.5 a	-	-	-	1.84 a
Hardness (mg $CaCO_3$)	-	-	268 £	273 £	282 £	279.58 c
Microbiology (Number of colony forming units/100ml)						
Fecal Coliforms	≤ 400s #	≤ 800 s	-	-	-	2 B

Value exceeds the Class III Water Quality Criteria or permit limits

a - Annual average
c - Value is calculated
s - Single sample
B - Results based on colony counts outside the acceptance range
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit
Q - Sample held beyond normal holding time
U - Material analyzed for but not detected; value reported is the method detection limit
- If 10 or fewer samples are taken within a month, the single sample limit is 400 CFU (62-302.530(6), F.A.C.)
£ - Bioassay laboratory measurement

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2008-08-25-34
Fernandina Beach WWTP

Customer: NE-DIST

Project ID: FYI3

PMAS: 1143

Requester: Jennifer Paris

Collected By: JE/DL

Sampling Agency: DEP

Field Report Prepared By: JE

Send Final Report To: Tom Kallemeyn

Lab ID *	Location: Fernandina WTF (Blanks)	☐ Comp ☐ Grab	Collection (begin) Date 9-25-08 Time 0800 Eastern Central	Collection (end) Date 9-29-08 Time 0800 Eastern Central	Bottle Group(s)**: B		
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude o ' " .	Longitude o ' " .	Comments				
Lab ID *	Location	☐ Comp ☐ Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**		
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude o ' " .	Longitude o ' " .	Comments				
Lab ID *	Location	☐ Comp ☐ Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**		
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude o ' " .	Longitude o ' " .	Comments				
Lab ID *	Location	☐ Comp ☐ Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**		
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number	
	Latitude o ' " .	Longitude o ' " .	Comments				

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
								9-30-08 / 840

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

FDEP Biology Laboratory - Acute Screen 4/24 Bioassay Bench Sheets

Facility: Fernandina WWTP 1

Address: 1007 South 5th Street

City: Fernandina Beach County: Nassau

Contact/District: Rosa Ali / Northeast

NPDES Permit #: FL0027260

LIMS Job #: FLH-2008-09-30-03 LIMS Sample #: 1136318

Lims Data Entry: 10-9-08 MF Data Entry Verification: BK 10-9-08

Sample Collection: Date: 9-28-08 Time: 1800

Hold Time Start: Date: 9-29-08 Time: 1133

Sample: 1 of 4

Comments:
Meridia beryllium test invalidated due to excess control mortality MF 10-7-08
Test organisms did not look "good"
NCR 2952 created

Instructions (for below): Circle appropriate wording. If yes is circled, complete blanks.

Test Type: (Screen)
Static / (Static Renewal) / Flow-through
Test Duration: 48 (96) Hours.
Light Intensity : 50 - 100 ft. candles.
Photoperiod : 16 hours light 8 hours dark.

Test 1 validation: Test 1: SOP TA 07_03
Control survival ≥90%: (Yes) No MF after 10-7-08
Temperature Range ≤3°C: (Yes) No

Test 2 validation: Test 2: SOP TA 07_04
Control survival ≥90%: Yes (No) 15% mortality MF 10-9-08
Temperature Range ≤3°C: (Yes) No

(√one)
Test 1 incubated in: ☑ Incubator #: 2 ☐ Waterbath #:_____
Test 2 incubated in: ☑ Incubator #: 2 ☐ Waterbath #:_____

Initial sample handling:
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: 0.44 ppt Final Salinity: 20.7 (Salts) Hypersaline brine
Salinity adjusted (Test 2): (yes) no Initial Salinity: 0.44 ppt Final Salinity: 20.7 (Salts) Hypersaline brine
Dechlorination: yes (no) _____mL of 0.025N Sodium Thiosulfate per liter of sample. Final TRC: _____

Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C: (If used)
Incubator # 2 min. 25.3 max. 26.4 mean 25.9
Incubator # ____ min. _____ max. _____ mean _____
Waterbath # ____ min. _____ max. _____ mean _____
Room B246 min. 25.0 max. 35.8 mean 25.4

Sample Validation:
Temperature: Shipped ≤6°C (Yes) No ----- If Hand Delivered: Cooling (received °C < collected °C) Yes No
Holding Time: ≤36 Hours (Yes) No (Composite-end of collection; grab-when collected; 4 in 24 - time last sample collected)

Investigators' Signatures
James Daniel
Marshall Faircloth
Bert Hardy

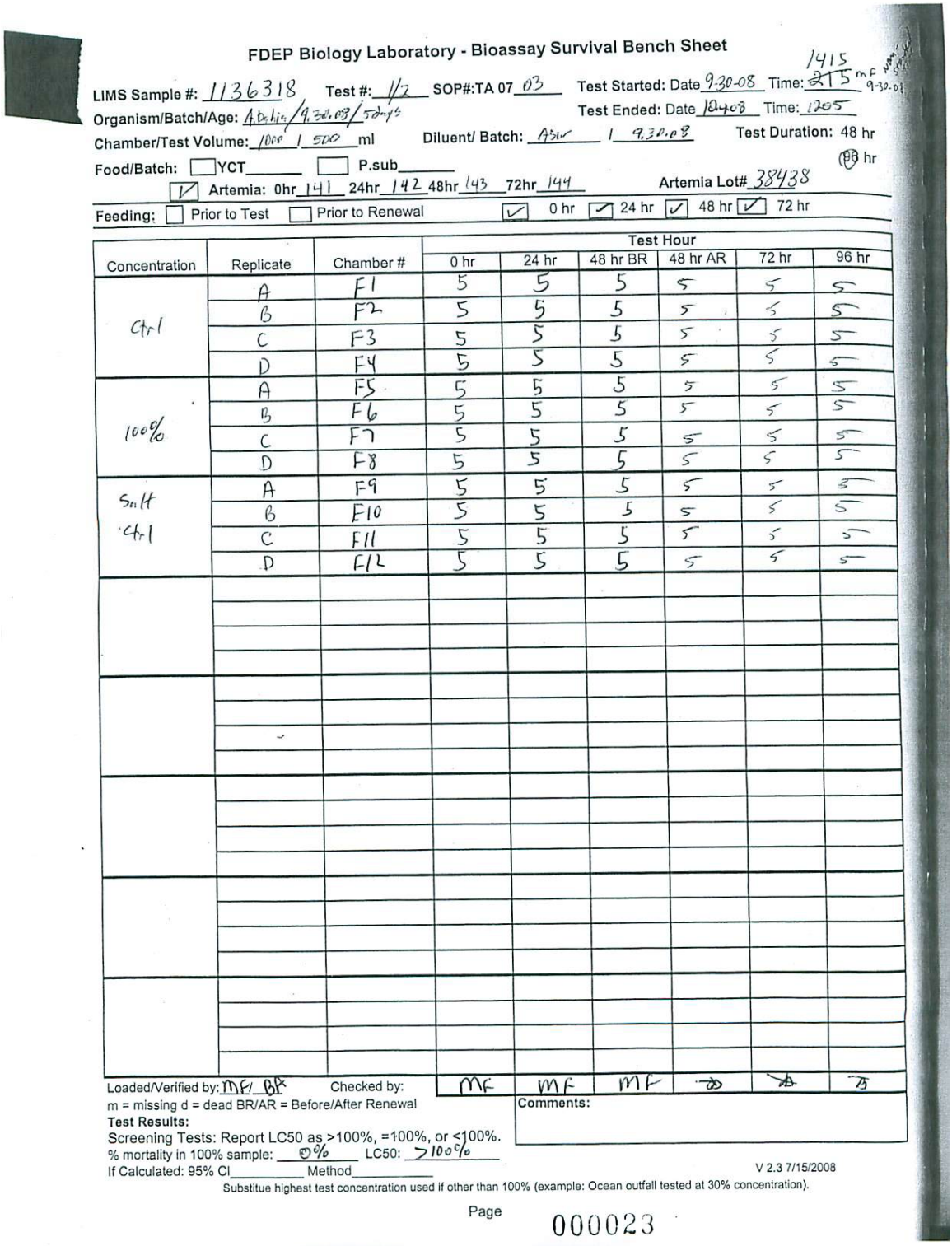
Loretta C. Wolfe
REVIEWER

Batch #				9-29-08	9-30-08				
Water Quality Parameters	20% DMW	Well Water	Moderately Hard Water	Salt Water (ASW) NSW Test 1	Salt Water (ASW) NSW Test 2	Other: SA 1424 Sample	Original Sample	Measured by	Verified by
Field Total Residual Cl_2 (mg/L):	N/A	N/A	N/A	N/A	N/A	—	0.005	Ezzati	MF
Lab Total Residual Cl_2 (mg/L):				<0.03	<0.03	<0.03	<0.03	JD	MF
Alkalinity (mg/L as $CaCO_3$):				224	224	200	142	BA	MF
Hardness (mg/L as $CaCO_3$):				—	—	—	268	BA	MF
Total Ammonia (mg/L as N):				<0.017	(A) <0.017	<0.034	<0.017	MF	BK
Conductivity (umhos) mmhos:				—	—	31,800	990 umhos	JD	MF
Salinity (ppth):				20	20	20.7	0.44 ppt	JD	MF

(A) <0.034 MF 10-1-08

Separation line MF 10-9-08

Appendix B. (continued)



FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample #: 1136318 Test #: 1/2 SOP#:TA 07 03 Test Started: Date 9-30-08 Time: 1415 (215) MF 9-30-08

Organism/Batch/Age: A.bahia / 9.30.08 / 5days Test Ended: Date 10408 Time: 1205

Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9.30.08 Test Duration: 48 hr (96) hr

Food/Batch: ☐ YCT ☐ P.sub

☑ Artemia: 0hr 141 24hr 142 48hr 143 72hr 144 Artemia Lot# 38438

Feeding: ☐ Prior to Test ☐ Prior to Renewal ☑ 0 hr ☑ 24 hr ☑ 48 hr ☑ 72 hr

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Ctrl	A	F1	5	5	5	5	5	5
	B	F2	5	5	5	5	5	5
	C	F3	5	5	5	5	5	5
	D	F4	5	5	5	5	5	5
100%	A	F5	5	5	5	5	5	5
	B	F6	5	5	5	5	5	5
	C	F7	5	5	5	5	5	5
	D	F8	5	5	5	5	5	5
Salt Ctrl	A	F9	5	5	5	5	5	5
	B	F10	5	5	5	5	5	5
	C	F11	5	5	5	5	5	5
	D	F12	5	5	5	5	5	5
Loaded/Verified by: MF/BR		Checked by:	MF	MF	MF	→	→	→

m = missing d = dead BR/AR = Before/After Renewal

Comments:

Test Results:

Screening Tests: Report LC50 as >100%, =100%, or <100%.

% mortality in 100% sample: 0% LC50: >100%

If Calculated: 95% CI_____ Method_____

V 2.3 7/15/2008

Substitue 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 #: 1136318 Test #: 2/2 SOP#:TA 07_04 Test Started: Date 9-30-08 Time: 1430

Organism/Batch/Age: m.beryllina/9.30.08/13days Test Ended: Date 10-4-08 Time: 1205

Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9.30.08 Test Duration: 48 hr / (96) hr

Food/Batch: ☐ YCT____ ☐ P.sub____

☑ Artemia: 0hr 141 24hr____ 48hr 143 72hr____ Artemia Lot# 38438

Feeding: ☑ Prior to Test ☑ Prior to Renewal ☐ 0 hr ☐ 24 hr ☐ 48 hr ☐ 72 hr

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Ctrl	A		10	10	8 2D	~~7~~ Ⓐ 8	8	8
	B		10	10	10	10	10	9 1D
		wrong cell MF 9-30-08	~~10~~					
			~~10~~					
100%	A		10	10	9 1D	7 2D	7	7
	B		10	10	9 1D	8 1D	8	8
Salt Ctrl	A		10	10	10	10	10	10
	B		10	10	10	10	10	10
Loaded/Verified by: BA/MF		Checked by:	MF	JD	JD	JD	JD	JD

m = missing d = dead BR/AR = Before/After Renewal

Test Results:

Screening Tests: Report LC50 as >100%, =100%, or <100%.

% mortality in 100% sample: 25% LC50: >100%

If Calculated: 95% CI________ Method________

Comments: Ⓐ Transcription error. JD 10.2.08
test invalidated - excess Control mortality MF 10-7-08

V 2.3 7/15/2008

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

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FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

.IMS Sample # : 1136318 Test #: 1 of 2

'EST SOP #: TA07 03 Test Species: ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

est aerated: yes (no) (circle one) Check 1: ☒ *Americamysis bahia* ☐ *Menidia beryllina* ☐ Other:_____

Concentration Ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
H (S.U.)	8.3	8.4	8.1	8.4	8.3	8.2
emperature °C	24.0	24.2	24.0	24.0	24.3	24.5
Dissolved Oxygen mg/L erate if < 4	6.5	6.4	4.8	5.9	6.1	4.5
Conductivity µmhos (mmhos)	31.9	31.7	32.4	31.9	32.2	32.5
alinity ppth	19.8	19.9	20.2	19.8	20.1	20.3
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration Salt (trl)	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.4	8.2	8.3	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.4	24.7
Dissolved Oxygen mg/L aerate if < 4	6.7	5.9	4.0	6.0	5.8	4.8
Conductivity µmhos (mmhos)	32.1	31.9	32.9	32.2	32.6	32.9
Salinity ppth	19.8	19.9	20.6	20.1	20.4	20.6
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Salinity only needs measuring if saltwater.

Concentration 100	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
H (S.U.)	8.2	8.2	8.0	8.2	8.1	8.0
emperature °C	24.0	24.0	24.0	24.0	24.4	24.7
Dissolved Oxygen mg/L erate if < 4	6.6	5.8	4.5	6.2	5.4	4.8
Conductivity µmhos (mmhos)	31.9	31.6 MF 31.6 (A)	32.6	32.1	32.3	32.1
alinity ppth	19.8	19.7	20.3	20.0	20.2	20.2
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments: (A) Clarified 31.6 NOT 4.6 MF 10-7-08

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

025

FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 1136318 **Test #:** 2 of 2

TEST SOP #: TA07_04 **Test Species:** ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

Test aerated: yes (no) (circle one) Check 1: ☐ *Americamysis bahia* ☒ *Menidia beryllina* ☐ Other:_____

Concentration ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate (letter error — BA)	BA M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.2	8.4	8.3	8.3
Temperature °C	24.0	24.0	24.0	24.0	24.2	24.7
Dissolved Oxygen mg/L aerate if < 4	6.5	5.7	5.6	5.9	5.3	6.6
Conductivity µmhos (mmhos)	31.8	31.9	32.2	32.1	32.2	32.6
Salinity ppth	19.8	19.9	20.0	20.0	20.1	20.5
(initials) Measured by:	BA	~~BA~~	~~BA~~	~~BA~~	~~BA~~	~~BA~~
(initials) Recorded by:	BA	~~BA~~	~~BA~~	~~BA~~	~~BA~~	~~BA~~

Comments:

Concentration Salt ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.3
Temperature °C	24.0	24.0	24.0	24.0	24.2	24.7
Dissolved Oxygen mg/L aerate if < 4	6.4	~~5.7~~ (A)	5.7	6.4	5.6	6.3
Conductivity µmhos (mmhos)	32.1	32.0	32.4	32.0	32.6	33.3
Salinity ppth	19.9	19.9	20.1	20.0	20.3	20.8
(initials) Measured by:	BA	~~BA~~	~~BA~~	~~BA~~	~~BA~~	~~BA~~
(initials) Recorded by:	BA	~~BA~~	~~BA~~	~~BA~~	~~BA~~	~~BA~~

Comments: (A) clarified 5.7 mf 10-7-08

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.3	8.2	8.0	8.2	8.1	8.2
Temperature °C	24.0	24.1	24.0	24.0	24.1	24.6
Dissolved Oxygen mg/L aerate if < 4	6.3	5.7	5.7	6.5	5.7	6.2
Conductivity µmhos (mmhos)	31.8	31.8	32.4	32.2	32.1	32.9
Salinity ppth	19.8	19.7	20.1	20.1	20.1	20.5
(initials) Measured by:	BA	~~BA~~	~~BA~~	~~BA~~	~~BA~~	~~BA~~
(initials) Recorded by:	BA	~~BA~~	~~BA~~	~~BA~~	~~BA~~	~~BA~~

Comments:

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

FDEP Biology Laboratory - Acute Screen 4/24 Bioassay Bench Sheets

Facility: Fernandina WWTP 2
Address: 1007 South 5th Street
City: Fernandina Beach **County:** Nassau
Contact/District: Tessa Otis / Northeast
NPDES Permit #: FL 0027260
LIMS Job #: TLH-2008-09-30-03 **LIMS Sample #:** 1136319
Lims Data Entry: 10-9-08 ML Data Entry Verification: BA 10-9-08

Sample Collection: Date: 9-29-08 Time: 0000
Hold Time Start: Date: 9-29-08 Time: 1133
Sample: 2 of 4

Comments: Menidia beryllina test organisms did not look good - MF 10-7-08

Instructions (for below): Circle appropriate wording. If yes is circled, complete blanks.

Test 1 validation:
Control survival ≥90%: (Yes) No
Temperature Range ≤3°C: (Yes) No
Test 1: SOP TA 07_ 03

Test 2 validation:
Control survival ≥90%: (Yes) No
Temperature Range ≤3°C: (Yes) No
Test 2: SOP TA 07_ 04

(√one)
Test 1 incubated in: ☑ Incubator #: 2 ☐ Waterbath #:_____
Test 2 incubated in: ☑ Incubator #: 2 ☐ Waterbath #:_____

Initial sample handling:
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: 0.43 ppt Final Salinity: 19.8 (Salts) Hypersaline brine
Salinity adjusted (Test 2): (yes) no Initial Salinity: 0.43 ppt Final Salinity: 19.8 (Salts) Hypersaline brine
Dechlorination: yes (no) _____mL of 0.025N Sodium Thiosulfate per liter of sample. Final TRC: _____

Sample Validation:
Temperature: Shipped ≤6°C (Yes) No — If Hand Delivered: Cooling (received °C < collected °C) Yes No
Holding Time: ≤36 Hours (Yes) No (Composite-end of collection; grab-when collected; 4 in 24 - time last sample collected)

Investigators' Signatures
Kristi Jewell
Marshall Faircloth
Bree Neal

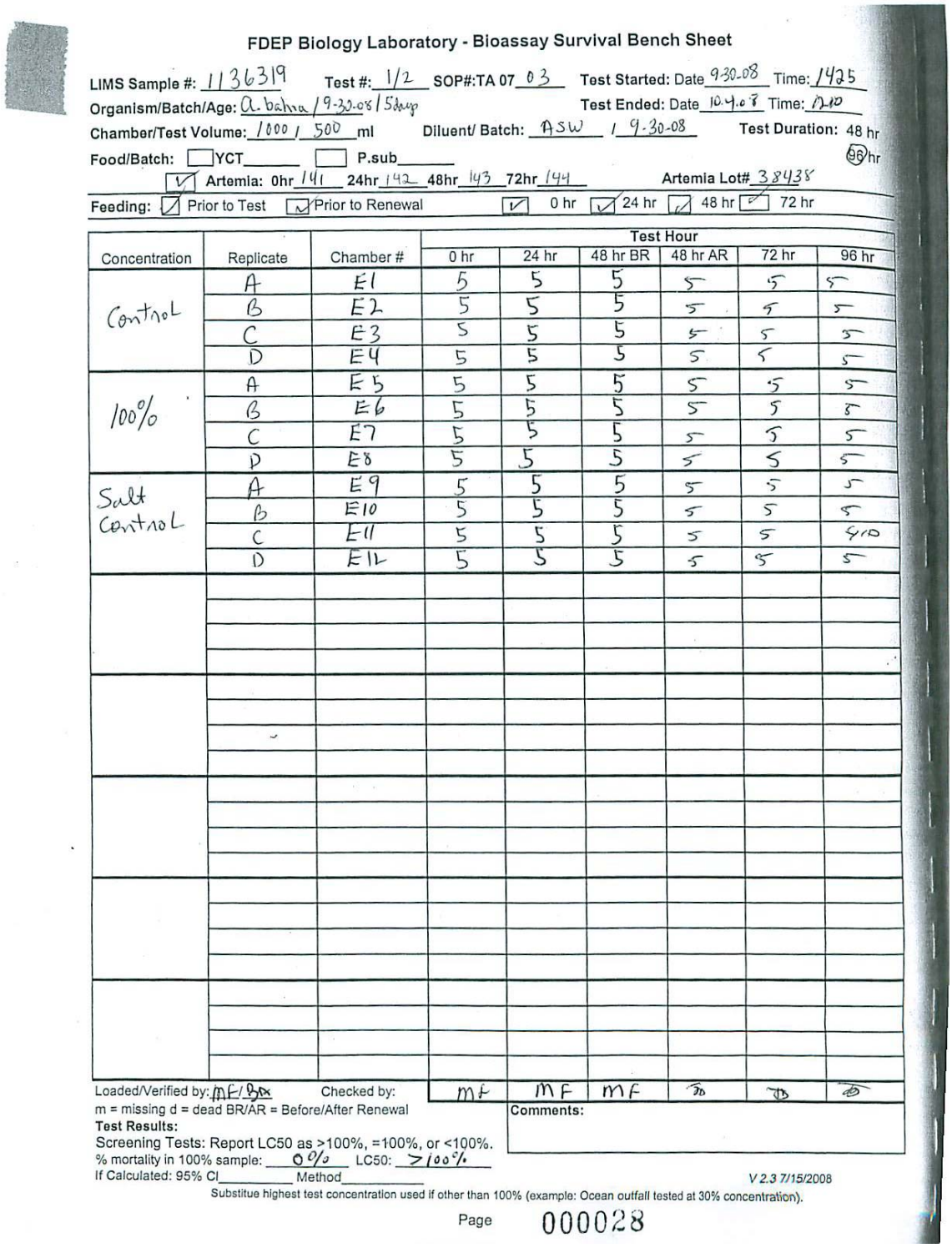
Loretta C. Wolfe
REVIEWER

Water Quality Parameters	Original Sample	Other Salty sample	Measured by	Verified by
Field Total Residual Cl_2 (mg/L):	~~<0.03~~ (A)	—	Plant Operator	MF
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03	~~BA~~ ML	ML
Alkalinity (mg/L as $CaCO_3$):	142	217	BA	MF
Hardness (mg/L as $CaCO_3$):	273	—	BA	MF
Total Ammonia (mg/L as N):	<0.017	<0.034	MF	BA
Conductivity: (umhos) mmhos	971	31,800	~~BA~~	MF
Salinity (ppth):	0.43	19.8	~~BA~~	MF

(A) lab Cl_2 recorded incorrect, field value is 0.006 mg/L MF 10-1-08

V 2.2 7/8/08

Appendix B. (continued)



FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample #: 1136319 Test #: 1/2 SOP#:TA 07 03 Test Started: Date 9-30-08 Time: 1425
Organism/Batch/Age: A. bahia / 9-30-08 / 5 days Test Ended: Date 10-4-08 Time: 1210
Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9-30-08 Test Duration: 48 hr (96) hr
Food/Batch: ☐ YCT ☐ P.sub
☑ Artemia: 0hr 141 24hr 142 48hr 143 72hr 144 Artemia Lot# 38438
Feeding: ☑ Prior to Test ☑ Prior to Renewal ☑ 0 hr ☑ 24 hr ☑ 48 hr ☑ 72 hr

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	E1	5	5	5	5	5	5
	B	E2	5	5	5	5	5	5
	C	E3	5	5	5	5	5	5
	D	E4	5	5	5	5	5	5
100%	A	E5	5	5	5	5	5	5
	B	E6	5	5	5	5	5	5
	C	E7	5	5	5	5	5	5
	D	E8	5	5	5	5	5	5
Salt Control	A	E9	5	5	5	5	5	5
	B	E10	5	5	5	5	5	5
	C	E11	5	5	5	5	5	4/D
	D	E12	5	5	5	5	5	5
Loaded/Verified by: MF/BA		Checked by:	MF	MF	MF	BA	BA	BA

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%
If Calculated: 95% CI Method
Substitue highest test concentration used if other than 100% (example: Ocean outfall tested at 30% concentration).

Comments:

V 2.3 7/15/2008

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Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample #: 1136319 Test #: 2/2 SOP#:TA 07_04 Test Started: Date 9-30-08 Time: 1440

Organism/Batch/Age: M. beryllina / 9-30-08 / 13 days Test Ended: Date 10-4-08 Time: 1210

Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9-30-08 Test Duration: 48 hr / 96 hr

Food/Batch: ☐ YCT____ ☐ P.sub____

☑ Artemia: 0hr 141 24hr____ 48hr 143 72hr____ Artemia Lot# 38438

Feeding: ☑ Prior to Test ☑ Prior to Renewal ☑ 0 hr ☐ 24 hr ☑ 48 hr ☐ 72 hr

Concentration	Replicate	Chamber #	Test Hour: 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	10 F13	10	10	10	10	9 1D	9
	B	10 F14	10	10	10	10	9 1D	9
100%	A	10 F17	10	10	9 1D	9	8 1D	8
	B	10 F18	10	10	10	10	7 3D	7
Salt Control	A	10 F21	10	10	10	10	10	10
	B	10 F22	10	10	10	10	10	10
Loaded/Verified by: BL / MF		Checked by:	MF	JJ	JJ	JJ	JJ	JJ

m = missing d = dead BR/AR = Before/After Renewal

Comments:

Test Results:

Screening Tests: Report LC50 as >100%, =100%, or <100%.

% mortality in 100% sample: 25% LC50: >100%

If Calculated: 95% CI________ Method________

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

V 2.3 7/15/2008

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FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 1136319 Test #: 1 of 2

TEST SOP #: TA07_03 Test Species: ☐ Ceriodaphnia dubia ☐ Cyprinella leedsi ☐ Pimephales promelas

Test aerated: yes (no) (circle one) Check 1: ☒ Americamysis bahia ☐ Menidia beryllina ☐ Other:_____

Concentration Ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.4	8.2	8.3	8.3	8.1
Temperature °C	24.0	24.0	24.0	24.0	24.2	24.6
Dissolved Oxygen mg/L aerate if < 4	6.2	5.5	4.0	6.5	5.8	4.5
Conductivity µmhos (mmhos)	31.9	31.7	32.7	32.1	32.7	32.8
Salinity ppth	19.9	19.7	20.2	19.9	20.1	20.5
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:		JD	JD	JD	JD	JD

Comments:

Concentration Salt Ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.4	8.2	8.4	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.2	24.7
Dissolved Oxygen mg/L aerate if < 4	6.3	5.5	4.9	6.0	5.7	4.4
Conductivity µmhos (mmhos)	32.1	31.9	32.5	32.1	32.5	33.2
Salinity ppth	20.0	19.9	20.3	20.0	20.2	20.6
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.2	8.2	~~8.4~~ A	8.2	8.0	8.0
Temperature °C	24.0	24.0	24.0	24.0	24.4	24.7
Dissolved Oxygen mg/L aerate if < 4	6.4	5.5	~~6.0~~ A	6.7	5.6	4.7
Conductivity µmhos (mmhos)	31.9	31.4	~~32.7~~ A	32.7	33.0	33.0
Salinity ppth	19.8	19.7	20.1	20.4	20.6	20.9
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments: @ pH: 8.1, Diss. O: 5.1, cond: 32.3 Transcription error. JD 10.2.08

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

030

FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 1136319 Test #: 2 of 2

TEST SOP #: TA07 04 Test Species: ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

Test aerated: yes (no) (circle one) Check 1: ☐ *Americamysis bahia* ☒ *Menidia beryllina* ☐ Other:_____

Concentration ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.2	8.2	8.3	8.3
Temperature °C	24.0	24.1	24.0	24.0	24.4	24.6
Dissolved Oxygen mg/L aerate if < 4	6.4	5.6	4.9	6.7	5.1	6.2
Conductivity µmhos (mmhos)	31.9	32.0	32.3	32.6	32.5	33.3
Salinity ppth	19.7	19.9	20.3	20.4	20.2	20.8
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration Salt ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.3
Temperature °C	24.0	24.0	24.0	24.0	24.5	24.8
Dissolved Oxygen mg/L aerate if < 4	6.5	5.6	5.6	6.6	5.5	5.9
Conductivity µmhos (mmhos)	32.0	32.1	32.6	32.1	32.5	32.9
Salinity ppth	19.7	19.9	20.3	20.0	20.3	20.6
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.3	8.2	8.2	8.2	8.2	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.3	24.8
Dissolved Oxygen mg/L aerate if < 4	6.5	5.5	6.7 → 5.6 (4)	6.7	5.6	5.9
Conductivity µmhos (mmhos)	31.8	31.8	32.6	32.6	32.6	33.8
Salinity ppth	19.6	19.7	20.4	20.4	20.4	21.2
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments: (4) Transcription error S.D 10.2.08

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

FDEP Biology Laboratory - Acute Screen 4/24 Bioassay Bench Sheets

Facility: Fernandina WWTF 3
Address: 1007 South 5th Street
City: Fernandina Beach County: Nassau
Contact/District: Resa Ali / Northeast
NPDES Permit #: FL 0027260
LIMS Job #: TLH-2008-09-30-03 LIMS Sample #: 1136320
Lims Data Entry: 10-9-08 MF Data Entry Verification: BA 10-9-08

Sample Collection: Date: 9-29-08 Time: 0600
Hold Time Start: Date: 9-29-08 Time: 1133
Sample: 3 of 4

Comments: Menidia beryllina to ST 7N unlrdated due to excess control mortality MF 10-7-08 test organisms (M. beryllina) did not ~~look~~ look good MF 10-7-08
NCR 2952 created

Instructions (for below): Circle appropriate wording. If yes is circled, complete blanks.

Test 1 validation:
Control survival ≥90%: (Yes) No
Temperature Range ≤3°C: (Yes) No
Test 1: SOP TA 07_03

Test 2 validation:
Control survival ≥90%: Yes (No) 20% mortality
Temperature Range ≤3°C: (Yes) No
Test 2: SOP TA 07_04

(√one)
Test 1 incubated in: [√] Incubator #: 2 [ ] Waterbath #:____
Test 2 incubated in: [√] Incubator #: 2 [ ] Waterbath #:____

Initial sample handling:
PH adjustment: yes (no) Initial pH: ____ NaOH N HCl N ____ Drops mL Final pH: ____
Aeration: ~~Yes~~ no Initial DO: 9.5 mg/L Final DO: 6.6 mg/L Duration: 20 minutes Rate: ~100 bubbles/min
Salinity adjusted (Test 1): (yes) no Initial Salinity: 0.42 Final Salinity: 19.8 (Salts) Hypersaline brine
Salinity adjusted (Test 2): (yes) no Initial Salinity: 0.42 Final Salinity: 19.8 (Salts) Hypersaline brine
Dechlorination: yes (no) ____mL of 0.025N Sodium Thiosulfate per liter of sample. Final TRC: ____

Sample Validation:
Temperature: Shipped ≤6°C (Yes) No — If Hand Delivered: Cooling (received °C < collected °C) Yes No
Holding Time: ≤36 Hours (Yes) No (Composite-end of collection; grab-when collected; 4 in 24 - time last sample collected)

Investigators' Signatures
James Daniel
Marshall Faischlich
Bruce Abel

Loretta C. Wolfe
REVIEWER

Water Quality Parameters	Original Sample	Other Sa Lite/4 SAmp/4	Measured by	Verified by
Field Total Residual Cl_2 (mg/L):	0.006	—	Plant Operator	MF
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03	JD	MF
Alkalinity (mg/L as $CaCO_3$):	150	200	BA	MF
Hardness (mg/L as $CaCO_3$):	282	—	BA	MF
Total Ammonia (mg/L as N):	<0.217	<0.034	MF	BA
Conductivity: (umhos) mmhos	964	(A) ~~37,900~~	JD	MF
Salinity (ppth):	0.42	19.8	JD	MF

(A) 31,900 umhos MF 10-9-08

V 2.2 7/8/08

Appendix B. (continued)

FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample #: 1136320 Test #: 1/2 SOP#:TA 07 03 Test Started: Date 9-30-08 Time: 1435
Organism/Batch/Age: A bahia / 9-30-07 / 5 days Test Ended: Date 10-4-08 Time: 1215
Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9-30-08 Test Duration: 48 hr 96 hr
Food/Batch: ☐ YCT_____ ☐ P.sub_____
☑ Artemia: 0hr 141 24hr 142 48hr 143 72hr 144 Artemia Lot# 38438
Feeding: ☑ Prior to Test ☐ Prior to Renewal ☑ 0 hr ☐ 24 hr ☐ 48 hr ☐ 72 hr

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	G1	5	5	5	5	5	5
	B	G2	5	5	5	5	5	5
	C	G3	5	5	5	5	5	5
	D	G4	5	5	5	5	5	5
100%	A	G5	5	5	5	5	5	5
	B	G6	5	5	5	5	5	5
	C	G7	5	5	5	5	5	5
	D	G8	5	5	5	5	5	5
Salt Control	A	G9	5	5	5	5	5	5
	B	G10	5	5	5	5	5	5
	C	G11	5	5	5	5	5	5
	D	G12	5	5	5	5	5	5
Loaded/Verified by: MF/BK		Checked by:	mf	mf	mf	JD	JD	JD

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%
If Calculated: 95% CI_____ Method_____

Comments:

V 2.3 7/15/2008

Substitue 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 #: 1136320 Test #: 2/2 SOP#:TA 07 04 Test Started: Date 9-30-08 Time: 1450

Organism/Batch/Age: M. beryllina / 9-30-08 / 13days Test Ended: Date 10-4-08 Time: 1215

Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9-30-08 Test Duration: 48 hr 96 hr

Food/Batch: ☐ YCT___ ☐ P.sub___

☑ Artemia: 0hr 141 24hr___ 48hr 143 72hr___ Artemia Lot# 38438

Feeding: ☑ Prior to Test ☑ Prior to Renewal ☑ 0 hr ☐ 24 hr ☑ 48 hr ☐ 72 hr

Concentration	Replicate	Chamber #	0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	A 913	10	10	9 1D	9	8 1D	8
	B	B 914	10	10	9 1D	9	9	8 1D
100%	A	A 917	10	10	10	10	8 2D	7 1D
	B	B 918	10	10	9 1D	9	7 2D	7
Salt Control	A	A 921	10	10	10	10	10	10
	B	B 922	10	10	9 1D	9	9	9
Loaded/Verified by: BR / MF		Checked by:	MF	JD	JD	JD	JD	JD

m = missing d = dead BR/AR = Before/After Renewal

Test Results:

Screening Tests: Report LC50 as >100%, =100%, or <100%.

% mortality in 100% sample: 30% LC50: > 100

If Calculated: 95% CI___ Method___

Comments: test invalidated due to Control failure MF 10-7-08

V 2.3 7/15/2008

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

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FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 1136320 Test #: 1 of 2

TEST SOP #: TA07 03 Test Species: ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

Test aerated: yes (no) (circle one) Check 1: ☒ *Americamysis bahia* ☐ *Menidia beryllina* ☐ Other:

Concentration Ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.4	8.2	8.4	8.2	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.3	24.6
Dissolved Oxygen mg/L aerate if < 4	6.8	6.1	5.3	6.5	6.0	4.7
Conductivity µmhos (mmhos)	31.9	31.7	32.7	32.2	32.4	32.9
Salinity ppth	19.8	19.9	20.4	20.0	20.2	20.5
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration Salt ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.4	24.6
Dissolved Oxygen mg/L aerate if < 4	6.6	5.5	5.3	6.5	5.6	4.6
Conductivity µmhos (mmhos)	32.1	32.0	32.2	32.1	32.6	32.9
Salinity ppth	20.0	19.9	20.3	20.0	20.5	20.5
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.3	8.3	8.2	8.3	8.1	8.0
Temperature °C	24.0	24.0	24.0	24.0	24.4	24.6
Dissolved Oxygen mg/L aerate if < 4	6.6	5.4	5.6	6.6	5.3	4.6
Conductivity µmhos (mmhos)	31.8	31.4	32.3	32.5	32.7	33.4
Salinity ppth	19.9	19.2	20.2	20.2	20.5	20.8
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

035

FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 113 6320 Test #: 2 of 2

TEST SOP #: TA07_ 04 Test Species: ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

Test aerated: yes (no) (circle one) Check 1: ☐ *Americamysis bahia* ☒ *Menidia beryllina* ☐ Other:_____

Concentration Ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.3
Temperature °C	24.0	24.0	24.0	24.0	24.2	24.0
Dissolved Oxygen mg/L aerate if < 4	6.6	5.5	5.6	6.7	5.4	5.8
Conductivity µmhos (mmhos)	32.0	32.0	33.0	32.3	32.5	33.1
Salinity ppth	19.9	19.7	20.3	20.0	20.4	20.7
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration 5.1+ Ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.3
Temperature °C	24.0	24.0	24.0	24.0	24.1	24.2
Dissolved Oxygen mg/L aerate if < 4	6.6	5.7	5.8	6.7	5.8	5.9
Conductivity µmhos (mmhos)	32.2	32.1	32.6	32.2	32.5	33.0
Salinity ppth	20.0	20.0	20.3	20.0	20.3	20.7
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.3	8.2	8.2	8.3	8.2	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.2	24.2
Dissolved Oxygen mg/L aerate if < 4	6.6	5.7	5.8	6.7	5.9	6.0
Conductivity µmhos (mmhos)	31.9	33.0	32.5	32.5	32.9	33.2
Salinity ppth	19.8	20.6	20.2	20.3	20.5	20.5
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

FDEP Biology Laboratory - Acute Screen 4/24 Bioassay Bench Sheets

Facility: Fernandina WWTF 4
Address: 1007 South 5th Street
City: Fernandina Beach County: Nassau
Contact/District: East Ali / Northeast
NPDES Permit #: FL 0027260
LIMS Job #: TLH-2008-09-30-07 LIMS Sample #: 1136326
Lims Data Entry: 10-9-08 MF Data Entry Verification: BA 10-9-08

Sample Collection: Date: 9-29-09 Time: 1133
Hold Time Start: Date: 9-29-09 Time: 1133
Sample: 4 of 4

Comments: Monidia beryllina organisms did not look good. MF 10-7-08

Instructions (for below): Circle appropriate wording. If yes is circled, complete blanks.

Test 1 validation:
Control survival ≥90%: (Yes) No
Temperature Range ≤3°C: (Yes) No
Test 1: SOP TA 07_03

Test 2 validation:
Control survival ≥90%: (Yes) No
Temperature Range ≤3°C: (Yes) No
Test 2: SOP TA 07_04

(√one)
Test 1 incubated in: [√] Incubator #: 2 [ ] Waterbath #:____
Test 2 incubated in: [√] Incubator #: 2 [ ] Waterbath #:____

Initial sample handling:
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: 0.43 Final Salinity: 20.7 (Salts) Hypersaline brine
Salinity adjusted (Test 2): (yes) no Initial Salinity: 0.43 Final Salinity: 20.7 (Salts) Hypersaline brine
Dechlorination: yes (no) ____mL of 0.025N Sodium Thiosulfate per liter of sample. Final TRC: ____

Sample Validation:
Temperature: Shipped ≤6°C (Yes) No — If Hand Delivered: Cooling (received °C < collected °C) Yes No
Holding Time: ≤36 Hours (Yes) No (Composite-end of collection; grab-when collected; 4 in 24 - time last sample collected)

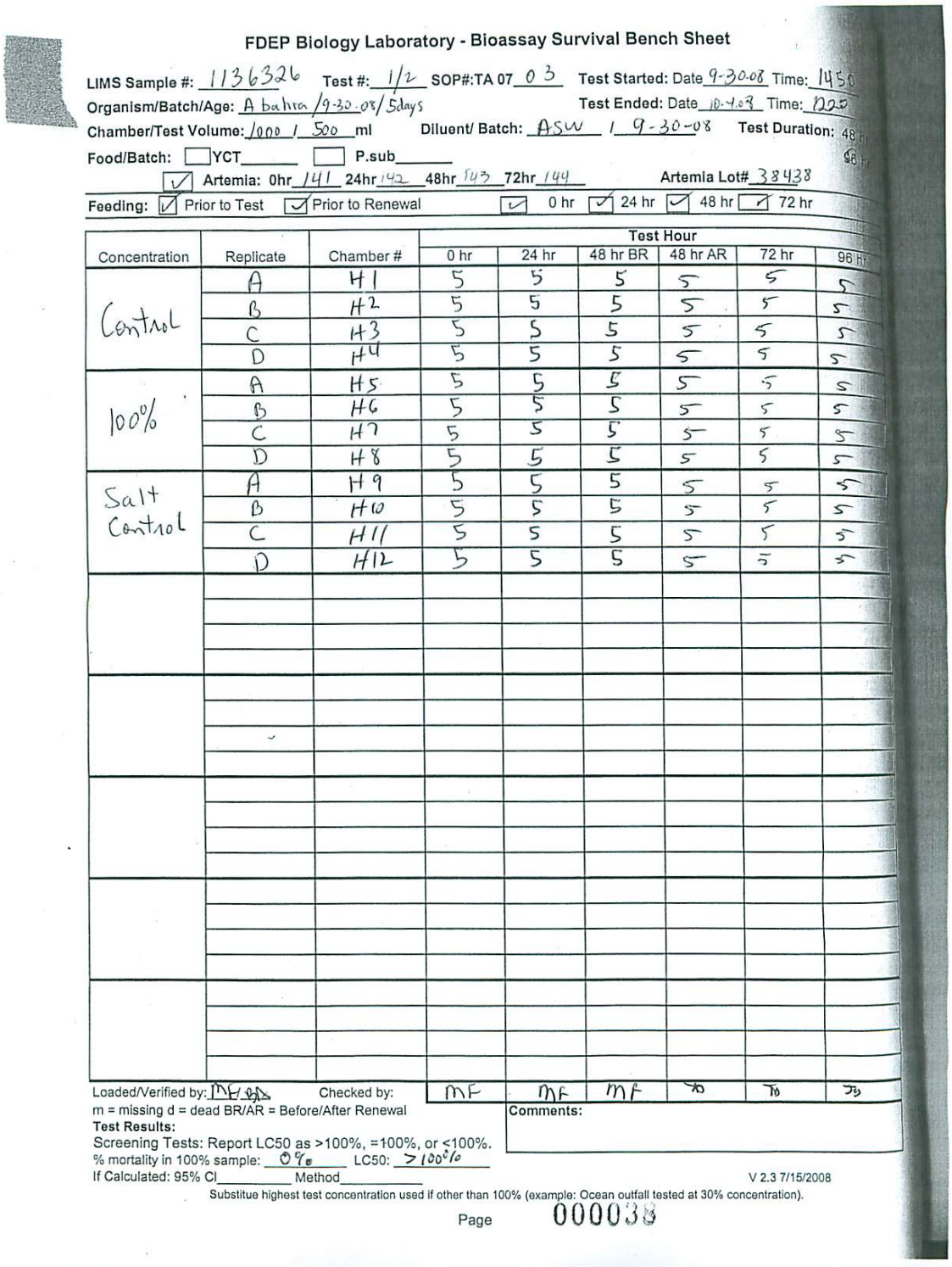
Investigators' Signatures
Marshall Faircloth
James Dimmick
Bruce Hall

Loretta Culotta
REVIEWER

Water Quality Parameters	Original Sample MF strong blank 10.3.08	Saltwater Sample MF	Measured by	Verified by
Field Total Residual Cl_2 (mg/L):	<0.006	—	Pipe Operator	MF
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03	JS	MF
Alkalinity (mg/L as $CaCO_3$):	149	219	BA	MF
Hardness (mg/L as $CaCO_3$):	282	—	BA	MF
Total Ammonia (mg/L as N):	<0.017	<0.034 (Strong Blank 9-30-08)	MF	BA
Conductivity: ~~mmhos~~ mmhos	972	33.1 mmhos	JS	ML
Salinity (ppth):	0.43	20.7	JS	MF

V 2.2 7/8/08

Appendix B. (continued)



FDEP Biology Laboratory - Bioassay Survival Bench Sheet

LIMS Sample #: 1136326 Test #: 1/2 SOP#:TA 07 03 Test Started: Date 9-30-08 Time: 1450
Organism/Batch/Age: A bahia / 9-30-08 / 5days Test Ended: Date 10-4-08 Time: 1225
Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9-30-08 Test Duration: 48 hr 96 hr
Food/Batch: ☐ YCT_____ ☐ P.sub_____
☑ Artemia: 0hr 141 24hr 142 48hr 143 72hr 144 Artemia Lot# 38438
Feeding: ☑ Prior to Test ☑ Prior to Renewal ☑ 0 hr ☑ 24 hr ☑ 48 hr ☑ 72 hr

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	H1	5	5	5	5	5	5
	B	H2	5	5	5	5	5	5
	C	H3	5	5	5	5	5	5
	D	H4	5	5	5	5	5	5
100%	A	H5	5	5	5	5	5	5
	B	H6	5	5	5	5	5	5
	C	H7	5	5	5	5	5	5
	D	H8	5	5	5	5	5	5
Salt Control	A	H9	5	5	5	5	5	5
	B	H10	5	5	5	5	5	5
	C	H11	5	5	5	5	5	5
	D	H12	5	5	5	5	5	5
Loaded/Verified by: MF/BA		Checked by:	MF	MF	MF	TO	TO	TO

m = missing d = dead BR/AR = Before/After Renewal
Comments:
Test Results:
Screening Tests: Report LC50 as >100%, =100%, or <100%.
% mortality in 100% sample: 0% LC50: >100%
If Calculated: 95% CI_____ Method_____
V 2.3 7/15/2008
Substitue 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 #: 1136326 Test #: 2/2 SOP#:TA 07 04 Test Started: Date 9-30-08 Time: 1500
Organism/Batch/Age: M. bahia liver / 9-30-08 / 13 days Test Ended: Date 10-4-08 Time: 1220
Chamber/Test Volume: 1000 / 500 ml Diluent/ Batch: ASW / 9-30-08 Test Duration: 48 hr 96 hr
Food/Batch: ☐ YCT ☐ P.sub
☑ Artemia: 0hr 141 24hr 142 48hr 143 72hr 144 Artemia Lot# 38438
Feeding: ☑ Prior to Test ☑ Prior to Renewal ☑ 0 hr ☐ 24 hr ☑ 48 hr ☐ 72 hr

Concentration	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	H13	10	10	9/D	9	9	9
	B	H14	10	10	9/D	9	9	9
100%	A	H17	10	10	9/D	9	7/D	7
	B	H18	10	10	9/D	9	8/D	8
	9/30/08							
Salt Control	A (mf wrong plate)	H21	10	10	10	10	10	10
	B	H22	10	10	10	10	9/D	9

Loaded/Verified by: BR/MF Checked by: MF
m = missing d = dead BR/AR = Before/After Renewal
Test Results:
Screening Tests: Report LC50 as >100%, =100%, or <100%.
% mortality in 100% sample: 25% LC50: >100%
If Calculated: 95% CI_____ Method_____

Comments:

V 2.3 7/15/2008

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

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FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 1136326 Test #: 1 of 2

TEST SOP #: TA07 03 Test Species: ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

Test aerated: yes (no) (circle one) Check 1: ☒ *Americamysis bahia* ☐ *Menidia beryllina* ☐ Other:

Concentration CTRL	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.4	8.2	8.4	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.0	24.7
Dissolved Oxygen mg/L aerate if < 4	6.8	6.1	5.8	6.6	5.9	4.6
Conductivity µmhos (mmhos)	31.9	31.8	32.5	32.2	32.4	32.8
Salinity ppth	20.0	19.9	20.2	19.9	20.2	20.5
(initials) Measured by:	BA	"	"	"	"	"
(initials) Recorded by:	BA	"	"	"	"	"

Comments:

Concentration Salt (trl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.3	8.5	8.3	8.4	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.0	24.8
Dissolved Oxygen mg/L aerate if < 4	6.8	6.0	5.0	6.5	5.5	5.0
Conductivity µmhos (mmhos)	32.1	32.0	32.7	32.2	32.7	32.8
Salinity ppth	20.0	20.0	20.4	20.1	20.4	20.5
(initials) Measured by:	BA	"	"	"	"	"
(initials) Recorded by:	BA	"	"	"	"	"

Comments:

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	C	C	D	A
pH (S.U.)	8.4	8.3	8.2	8.4	8.1	8.0
Temperature °C	24.0	24.0	24.0	24.0	24.1	24.6
Dissolved Oxygen mg/L aerate if < 4	6.7	5.7	5.8	6.6	5.1	5.1
Conductivity µmhos (mmhos)	33.1	33.0	33.4	32.8	33.2	33.7
Salinity ppth	20.7	20.7	21.3	20.4	20.7	21.1
(initials) Measured by:	BA	"	"	"	"	"
(initials) Recorded by:	BA	"	"	"	"	"

Comments:

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

040

FDEP Biology Laboratory - Bioassay Parameter Bench Sheet

LIMS Sample # : 1136326 **Test #:** 2 of 2

TEST SOP #: TA07_64 **Test Species:** ☐ *Ceriodaphnia dubia* ☐ *Cyprinella leedsi* ☐ *Pimephales promelas*

Test aerated: yes (no) (circle one) Check 1: ☐ *Americamysis bahia* ☒ *Menidia beryllina* ☐ Other:_____

Concentration (+C)	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.1	24.3
Dissolved Oxygen mg/L aerate if < 4	6.5	5.6	5.9	6.7	5.2	5.9
Conductivity µmhos (mmhos)	32.0	31.9	32.5	32.2	32.6	32.7
Salinity ppth	19.9	19.9	20.2	20.0	20.3	20.4
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration Salt ctrl	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.4	8.4	8.3	8.4	8.3	8.2
Temperature °C	24.0	24.0	24.0	24.0	24.1	24.4
Dissolved Oxygen mg/L aerate if < 4	6.5	5.7	5.9	6.3	5.6	5.8
Conductivity µmhos (mmhos)	32.1	32.1	32.6	32.2	32.4	33.3
Salinity ppth	19.9	20.0	20.3	20.0	20.2	20.8
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Salinity only needs measuring if saltwater.

Concentration 100%	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate	M	B	A	A	B	A
pH (S.U.)	8.3	8.3	8.2	8.3	8.2	8.2
Temperature °C	~~24.0~~ 24.0	24.0	24.0	24.0	24.2	24.5
Dissolved Oxygen mg/L aerate if < 4	6.6	5.7	5.8	6.8	5.8	5.5
Conductivity µmhos (mmhos)	33.1	33.1	32.5	32.2	32.5	33.8
Salinity ppth	20.7	20.7	20.2	20.6	20.9	21.1
(initials) Measured by:	BA	JD	JD	JD	JD	JD
(initials) Recorded by:	BA	JD	JD	JD	JD	JD

Comments:

Concentration	0 Hr.	24 Hr.	48 Hr. before renewal	48 Hr. after renewal	72 Hr.	96 Hr.
Replicate						
pH (S.U.)						
Temperature °C						
Dissolved Oxygen mg/L aerate if < 4						
Conductivity µmhos mmhos						
Salinity ppth						
(initials) Measured by:						
(initials) Recorded by:						

Comments:

Appendix B. (continued)

Bioassay Instrument ID

SRTs 3-7 September and 9-13 September 08

Test ID: Fernandina WWTP Dates: 9-30-08 — 10-4-08

METER / DEVICE	ID	SOP	Parameter	Method	√used
YSI Model 58 Dissolved Oxygen Meter	90H018262	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	SM 2510 B	√
			Salinity	SM 2520 B	√
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	√
Thermometer (DO probe)	YSI 5750 SN 05J1720	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)

Marshall Faircloth 1-21-09

V 1.1 6/12/08 MF

PAGE

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Appendix C. SRT Bench Sheets and Statistical Analysis

Florida Department of Environmental Protection
Bureau of Laboratories

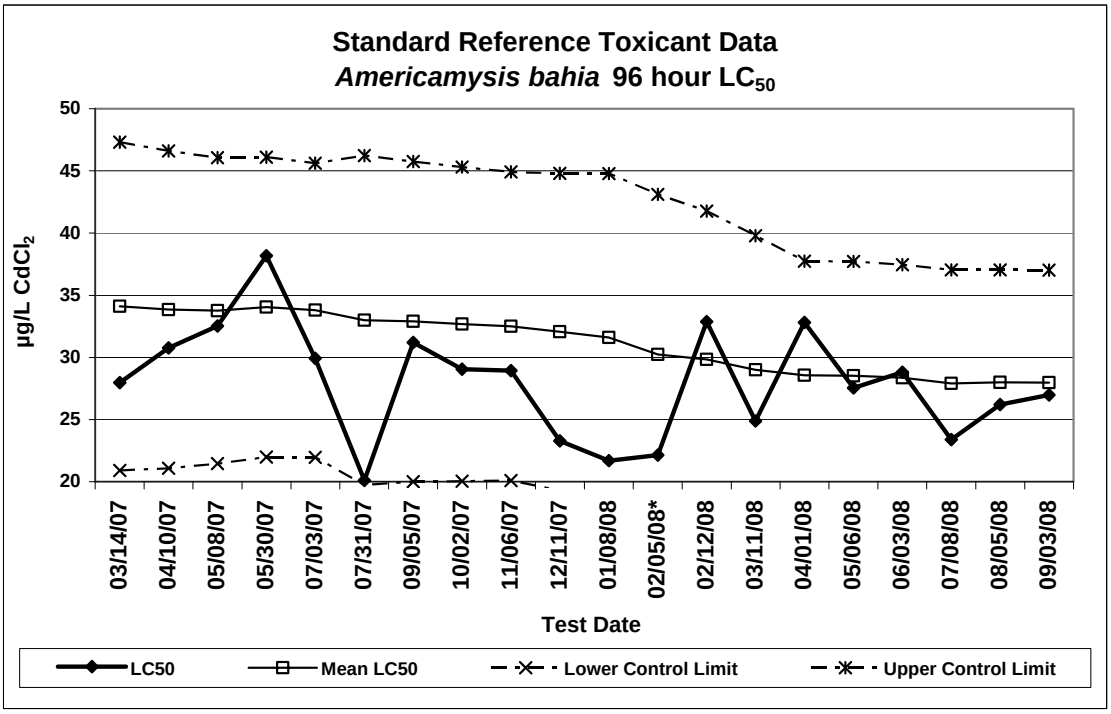
Standard Reference Toxicant (SRT) Test Data - 96-hour Acute Toxicity
Americamysis bahia (mysid shrimp) - Cadmium Chloride ($CdCl_2$)

Date	LC50	Mean LC50	Std Dev	Lower Control Limit	Upper Control Limit
03/14/07	27.97	34.10	6.60	20.90	47.31
04/10/07	30.77	33.85	6.39	21.07	46.62
05/08/07	32.52	33.75	6.15	21.46	46.05
05/30/07	38.18	34.05	6.03	21.98	46.11
07/03/07	29.92	33.79	5.92	21.95	45.63
07/31/07	20.10	32.98	6.62	19.74	46.23
09/05/07	31.20	32.89	6.44	20.01	45.76
10/02/07	29.04	32.68	6.32	20.04	45.32
11/06/07	28.93	32.50	6.21	20.08	44.91
12/11/07	23.28	32.06	6.38	19.30	44.81
01/08/08	21.69	31.59	6.60	18.38	44.79
02/05/08*	22.13	30.23	6.44	17.35	43.12
02/12/08	32.87	29.83	5.96	17.90	41.76
03/11/08	24.89	29.00	5.39	18.22	39.78
04/01/08	32.81	28.56	4.60	19.36	37.75
05/06/08	27.55	28.52	4.60	19.31	37.72
06/03/08	28.81	28.37	4.54	19.29	37.46
07/08/08	23.40	27.91	4.56	18.80	37.03
08/05/08	26.21	27.98	4.52	18.93	37.03
09/03/08	26.98	27.96	4.53	18.91	37.02

CV = 16.19

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

EPA reference 75th quartile CV = 26%



*Forty-eight hour results exceeded control limits. Follow up tests performed on 2/12/08 were carried out to 96-hours.

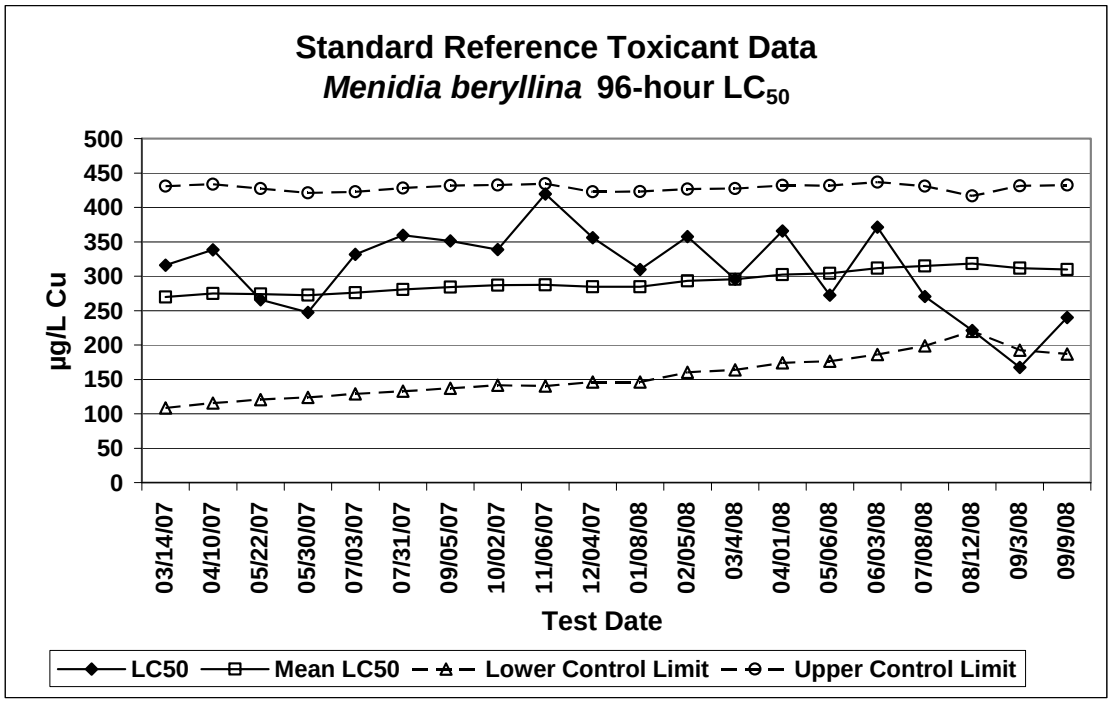
Appendix C. (continued)

Florida Department of Environmental Protection
Bureau of Laboratories

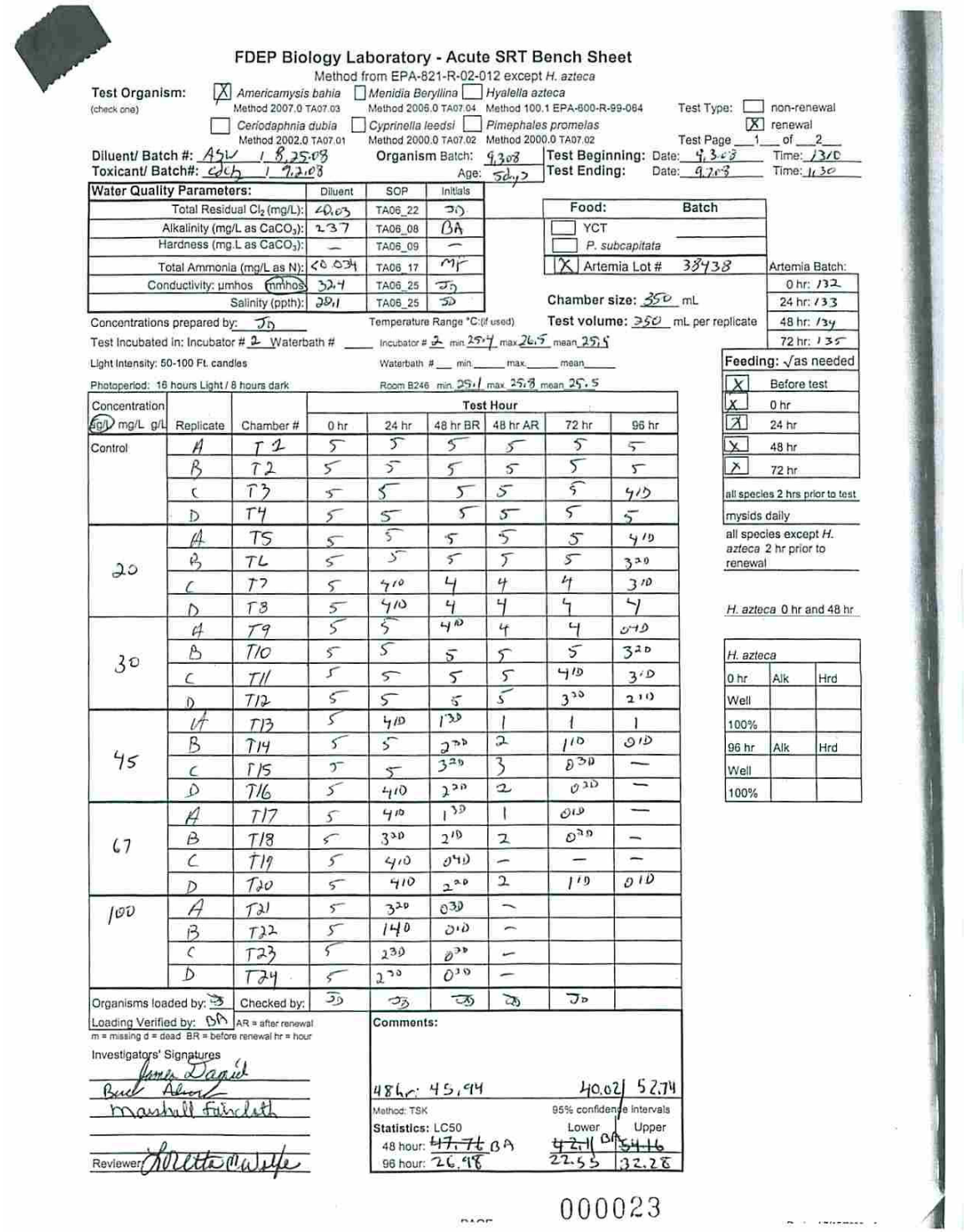
Standard Reference Toxicant (SRT) Test Data - 96-hour Acute Toxicity
Menidia beryllina (inland silverside) - Copper Chloride ($CuCl_2$) as Cu

Date	LC50	Mean LC50	Std Dev	Lower Control Limit	Upper Control Limit
03/14/07	316.16	269.88	80.54	108.80	430.96
04/10/07	338.35	274.77	79.51	115.75	433.79
05/22/07	265.69	274.17	76.66	120.85	427.49
05/30/07	247.36	272.49	74.36	123.77	421.21
07/03/07	331.66	275.97	73.41	129.15	422.80
07/31/07	359.44	280.61	73.89	132.83	428.39
09/05/07	351.35	284.33	73.62	137.10	431.57
10/02/07	338.91	287.06	72.69	141.69	432.44
11/06/07	419.55	287.50	73.50	140.50	434.51
12/04/07	356.02	284.51	69.07	146.36	422.65
01/08/08	309.80	284.71	69.14	146.43	423.00
02/05/08	357.47	293.49	66.51	160.48	426.50
03/4/08	295.52	295.53	65.88	163.77	427.28
04/01/08	365.9	302.28	64.49	174.29	432.27
05/06/08	272.5	304.29	63.81	176.66	431.91
06/03/08	371.38	311.63	62.59	186.45	436.80
07/08/08	270.47	315.01	58.07	198.87	431.14
08/12/08	221.17	318.46	49.27	219.93	416.99
09/3/08	167.46	311.77	59.70	192.36	431.18
09/9/08	240.07	309.81	61.44	186.93	432.70

CV = 19.83
EPA reference 75th quartile CV = 21%



Appendix C. (continued)



FDEP Biology Laboratory - Acute SRT Bench Sheet

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

Test Organism: (check one) [X] *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 [X] renewal

Test Page 1 of 2

Diluent/ Batch #: ASW 1 8.25.08

Toxicant/ Batch#: EDTA 1 9.2.08

Organism Batch: 9308 Age: 5dy3

Test Beginning: Date: 9.3.08 Time: 1310

Test Ending: Date: 9.7.08 Time: 1130

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<0.03	TA06_22	JD
Alkalinity (mg/L as $CaCO_3$):	237	TA06_08	BA
Hardness (mg.L as $CaCO_3$):	—	TA06_09	—
Total Ammonia (mg/L as N):	<0.034	TA06_17	MF
Conductivity: µmhos (mmhos)	32.4	TA06_25	JD
Salinity (ppth):	28.1	TA06_25	JD

Food:	Batch
[ ] YCT	
[ ] *P. subcapitata*	
[X] Artemia Lot #	38438

Artemia Batch:

0 hr:	132
24 hr:	133
48 hr:	134
72 hr:	135

Concentrations prepared by: JD

Temperature Range °C:(if used)

Chamber size: 350 mL

Test volume: 350 mL per replicate

Test Incubated in: Incubator # 2 Waterbath # ____ Incubator # 2 min 25.4 max 26.5 mean 25.9

Light Intensity: 50-100 Ft. candles

Waterbath # ___ min ____ max ____ mean ____

Photoperiod: 16 hours Light / 8 hours dark

Room B246 min 25.1 max 25.8 mean 25.5

Feeding: √as needed

[X]	Before test
[X]	0 hr
[X]	24 hr
[X]	48 hr
[X]	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	T1	5	5	5	5	5	5
	B	T2	5	5	5	5	5	5
	C	T3	5	5	5	5	5	4D
	D	T4	5	5	5	5	5	5
20	A	T5	5	5	5	5	5	4D
	B	T6	5	5	5	5	5	3D
	C	T7	5	4D	4	4	4	3D
	D	T8	5	4D	4	4	4	4
30	A	T9	5	5	4D	4	4	0HD
	B	T10	5	5	5	5	5	3D
	C	T11	5	5	5	5	4D	3D
	D	T12	5	5	5	5	3D	2D
45	A	T13	5	4D	1D	1	1	1
	B	T14	5	5	2D	2	1D	0D
	C	T15	5	5	3D	3	0D	—
	D	T16	5	4D	2D	2	0D	—
67	A	T17	5	4D	1D	1	0D	—
	B	T18	5	3D	2D	2	0D	—
	C	T19	5	4D	0HD	—	—	—
	D	T20	5	4D	2D	2	1D	0D
100	A	T21	5	3D	0D	—		
	B	T22	5	1HD	0D	—		
	C	T23	5	2D	0D	—		
	D	T24	5	2D	0D	—		
Organisms loaded by: JD		Checked by:	JD	JD	JD	JD	JD	

Loading Verified by: BA AR = after renewal

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

Investigators' Signatures

James Dagid

Brad Alvon

Marshall Faircloth

Reviewer: Loretta M Wolfe

Comments:

48hr: 45.94 40.02 52.74

Method: TSK

Statistics: LC50

48 hour: ~~47.76~~ BA ~~42.11~~ BA ~~54.16~~

96 hour: 26.98 22.55 32.28

95% confidence Intervals: Lower / Upper

000023

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)
- [x] *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: 4.30.09 Time: 1310
Test Ending: Date: 5.7.09 Time: 1130

Test Type: [ ] non-renewal [x] renewal

hr = hour AR = after renewal BR = before renewal

Concentration (100) mg/L g/L	Replicate	Test Hour	pH (SU)	Temperature (° C)	Dissolved Oxygen (mg/L)	Conductivity [ ] µmhos [x] mmhos	Salinity (ppth)
Control	A	0 hr	8.4	24.0	~~6.8~~ 6.8	32.4	20.1
	B	24 hr	8.1	24.0	4.1	33.0	20.5
	C	48 hr BR	8.1	24.2	5.2	33.5	20.9
	C	48 hr AR	8.4	24.0	6.4	32.9	20.4
	D	72 hr	8.3	24.2	5.9	33.0	20.6
	A	96 hr	8.2	24.2	6.1	34.3	21.5
20	A	0 hr	8.4	24.0	6.4	32.6	20.3
	B	24 hr	8.2	24.2	4.3	33.2	20.8
	C	48 hr BR	8.1	24.2	5.1	33.2	20.7
	C	48 hr AR	8.4	24.0	6.5	33.1	20.5
	D	72 hr	8.3	24.2	5.8	33.3	20.9
	A	96 hr	8.3	24.2	6.1	34.3	21.4
30	A	0 hr	8.4	24.1	6.7	32.6	20.3
	B	24 hr	8.3	24.2	4.6	33.0	20.7
	C	48 hr BR	8.2	24.4	5.1	33.4	20.7
	C	48 hr AR	8.4	24.0	6.5	33.1	20.6
	D	72 hr	8.3	24.4	5.7	33.4	20.9
	A	96 hr	8.3	24.2	6.1	33.9	21.2
45	A	0 hr	8.4	24.2	6.9	32.6	20.3
	B	24 hr	8.2	24.2	4.7	33.1	20.7
	C	48 hr BR	8.2	24.7	5.2	33.1	20.7
	C	48 hr AR	8.4	24.0	6.5	33.0	20.5
	D	72 hr	8.3	24.4	5.6	33.3	20.9
	A	96 hr	8.3	24.2	6.0	34.0	21.2
67	A	0 hr	8.4	24.2	7.0	32.6	20.3
	B	24 hr	8.2	24.3	4.8	33.0	20.6
	C	48 hr BR	8.2	24.5	5.1	33.8	20.7
	D	48 hr AR	8.4	24.0	6.5	32.6	20.3
	D	72 hr	8.4	24.5	5.8	33.3	20.9
	D	96 hr	8.3	24.4	6.2	33.8	21.1
100	A	0 hr	8.4	24.2	7.0	32.6	20.3
	B	24 hr	8.2	24.3	4.9	33.1	20.7
	C	48 hr BR	8.2	24.3	5.5	33.1	20.7
		48 hr AR					
		72 hr					
		96 hr					

Investigators Signatures
James Parish
Brian Adams
Marshall Faulatt

Reviewed By: Loretta C Wolfe

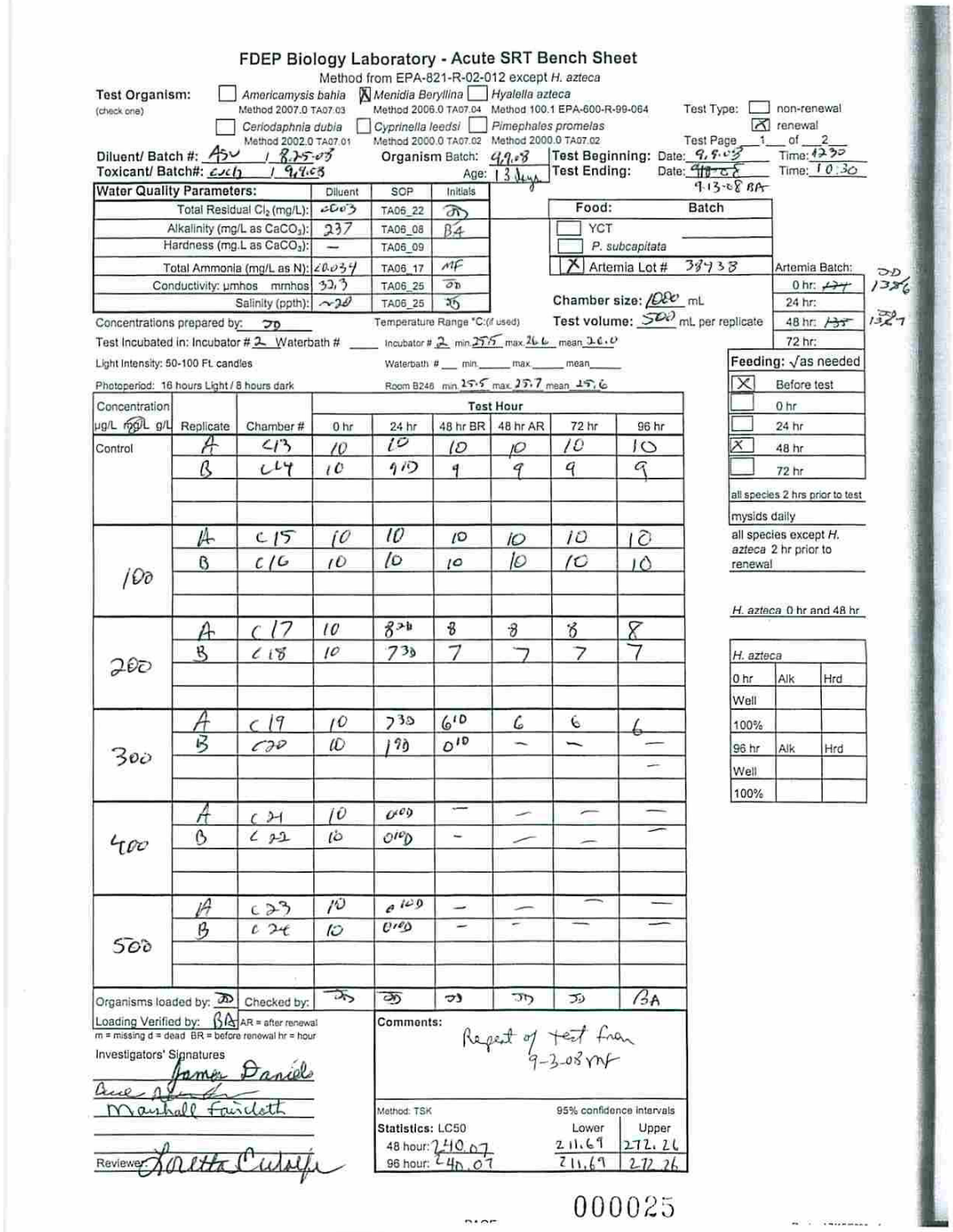
Measured by:
0 hr: JP
24 hr: JP
48 hr BR: JP
48 hr AR: JP
72 hr: JP
96 hr: JP

Measure/record salinity for marine tests

Comments:

000024

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 #: ASW 1 8.25.08 Organism Batch: 49.08 Test Beginning: Date: 9.5.08 Time: 1230
Toxicant/ Batch#: CuCl2 1 9.4.08 Age: 13 days Test Ending: Date: 9.13.08 BA Time: 10:30

Water Quality Parameters:	Diluent	SOP	Initials
Total Residual Cl_2 (mg/L):	<.003	TA06_22	JD
Alkalinity (mg/L as $CaCO_3$):	237	TA06_08	BA
Hardness (mg.L as $CaCO_3$):	—	TA06_09	
Total Ammonia (mg/L as N):	<0.034	TA06_17	MF
Conductivity: µmhos mmhos	32.3	TA06_25	JD
Salinity (ppth):	~28	TA06_25	JD

Food:	Batch
☐ YCT	
☐ *P. subcapitata*	
☒ Artemia Lot #	38438

Artemia Batch: DD 13Sep
0 hr: ~~127~~
24 hr:
48 hr: ~~135~~ DD 13Sep
72 hr:

Chamber size: 1000 mL
Test volume: 500 mL per replicate

Concentrations prepared by: JD
Test Incubated in: Incubator # 2 Waterbath # ____
Light Intensity: 50-100 Ft. candles
Photoperiod: 16 hours Light / 8 hours dark
Temperature Range °C:(if used)
Incubator # 2 min 25.5 max 26.6 mean 26.0
Waterbath # ___ min.____ max.____ mean____
Room B246 min 25.5 max 25.7 mean 25.6

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

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

Concentration µg/L mg/L g/L	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr BR	48 hr AR	72 hr	96 hr
Control	A	C13	10	10	10	10	10	10
	B	C14	10	9 D	9	9	9	9
100	A	C15	10	10	10	10	10	10
	B	C16	10	10	10	10	10	10
200	A	C17	10	8 2D	8	8	8	8
	B	C18	10	7 3D	7	7	7	7
300	A	C19	10	7 3D	6 1D	6	6	6
	B	C20	10	1 9D	0 1D	—	—	—
400	A	C21	10	0 10D	—	—	—	—
	B	C22	10	0 10D	—	—	—	—
500	A	C23	10	0 10D	—	—	—	—
	B	C24	10	0 10D	—	—	—	—
Organisms loaded by: DD		Checked by:	JD	JD	JD	JD	JD	BA

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

Reviewer: Loretta Cutsinger

Comments: Repeat of test from 9-3-08 MF

Method: TSK

Statistics: LC50	95% confidence intervals Lower	Upper
48 hour: 240.07	211.69	272.26
96 hour: 240.07	211.69	272.26

000025

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: 9.4.08 Time: 1230
Test Ending: Date: 9-13-08 Time: 10:30

Test Type: ☐ non-renewal ☒ renewal

hr = hour AR = after renewal BR = before renewal

Concentration µg/L g/L	Replicate	Test Hour	pH (SU)	Temperature (° C)	Dissolved Oxygen (mg/L)	Conductivity ☐ µmhos ☒ mmhos	Salinity (ppth)
Control	A	0 hr	8.4	24.1	6.8	32.7	20.4
	B	24 hr	8.3	24.2	5.9	33.1	20.9
	A	48 hr BR	8.3	24.2	6.0	33.8	21.2
	A	48 hr AR	8.4	24.0	6.6	33.3	20.8
	B	72 hr	8.3	24.4	5.6	33.9	21.1
	A	96 hr	8.2	~~24HR~~ 24.3 BA	5.3	34.4	21.6
100	A	0 hr	8.4	24.2	6.7	33.1	20.7
	B	24 hr	8.3	24.1	5.8	33.6	21.0
	A	48 hr BR	8.3	24.2	6.0	33.8	21.2
	A	48 hr AR	8.4	24.0	6.5	33.2	20.8
	B	72 hr	8.3	24.5	5.7	33.8	21.2
	A	96 hr	8.3	24.4	5.4	34.3	21.5
200	A	0 hr	8.4	24.2	6.8	33.0	20.7
	B	24 hr	8.3	24.0	5.9	33.4	20.9
	A	48 hr BR	8.3	24.0	6.0	33.7	21.1
	A	48 hr AR	8.4	24.0	6.6	33.3	20.8
	B	72 hr	8.3	24.4	5.7	33.7	21.2
	A	96 hr	8.3	24.6	5.5	34.3	21.5
300	A	0 hr	8.4	24.4	6.9	33.1	20.7
	B	24 hr	8.3	24.1	6.0	33.5	21.0
	A	48 hr BR	8.3	24.0	6.2	33.7	21.1
	A	48 hr AR	8.4	24.0	6.7	33.1	20.7
	A	72 hr	8.3	24.2	5.8	33.4	21.0
	A	96 hr	8.3	24.5	5.5	34.1	21.4
400	A	0 hr	8.4	24.8	6.8	33.3	20.7
	B	24 hr	8.3	24.3	5.5	33.7	21.0
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					
500	A	0 hr	8.4	24.7	6.8	32.7	20.5
	B	24 hr	8.3	24.6	5.6	33.5	21.0
		48 hr BR					
		48 hr AR					
		72 hr					
		96 hr					

Investigators Signatures
James Daniels
Brad Acker
Marshall Faircloth

Reviewed By: Loretta C Wolfe

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

Measure/record salinity for marine tests

Comments:

Appendix C. (continued)
***Americamysis bahia* Acute Standard Reference Toxicant Test**

DATE: 9-3-08 DURATION: 96 hr TOXICANT : $CdCl_2$
SPECIES: *Americamysis bahia*

RAW DATA: Concentration (ug/L)	Number Exposed	Mortalities
.00	20	1
20.00	20	6
30.00	20	12
45.00	20	19
67.00	20	20
100.00	20	20

SPEARMAN-KARBER TRIM: 26.32%

SPEARMAN-KARBER ESTIMATES: **LC50: 26.98**
95% LOWER CONFIDENCE: 22.55
95% UPPER CONFIDENCE: 32.28

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

***Menidia beryllina* Acute Standard Reference Toxicant Test**

DATE: 9-9-08 DURATION: 96 hr TOXICANT : $CuCl_2$
SPECIES: *Menidia beryllina*

RAW DATA: Concentration (ug/L)	Number Exposed	Mortalities
.00	20	1
100.00	20	0
200.00	20	5
300.00	20	14
400.00	20	20
500.00	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: **LC50: 240.07**
95% LOWER CONFIDENCE: 211.69
95% UPPER CONFIDENCE: 272.26

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

Appendix D. Test Organism Receipt(s)

MARINCO BIOASSAY LABORATORY
The Aquatic Toxicology Specialists

NELAP Certification # E84191

Organism Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Date: 9/2/08

Shipped to: FDEP

P.O. No: N/A

Species	Quantity	Age	Brood Number	Temp.	pH	Salinity
Mysidopsis bahia	150	4 DAYS	M30808 29	25°C	7.9 s.u.	20‰
Menidia beryllina	150	12 DAYS	SS080821	25°C	7.9 s.u.	20‰
						Hardness
Cyprinella leedsi	150	12 DAYS	CL080821	25°C	7.8 s.u	80mg/L
Pimephales promelas	150	9 DAYS	FM080824	25°C	7.8 s.u	80mg/L

Packed by: MP

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

Organism Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Date: 9/8/08

Shipped to: FDEP

P.O. No: N/A

Species	Quantity	Age	Brood Number	Temp.	pH	Salinity
Mysidopsis bahia						
Menidia beryllina	150	12 DAYS	SS080827	25°C	7.9 s.u.	19 ‰
						Hardness
Cyprinella leedsi						
Pimephales promelas	100 acute	8 DAYS	FM080831	25°C	7.8 s.u.	84 mg/L

Packed by:

Shipped Via: FedEx

Notes: 11 AM's

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

Organism Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Date: 9/29/08

Shipped to: FDEP

P.O. No: N/A

Species	Quantity	Age	Brood Number	Temp.	pH	Salinity
Mysidopsis bahia	350	4 DAYS	MS080925	25°C	7.9	20‰
Menidia beryllina	350	12 DAYS	SS080917	25°C	7.8	20‰
						Hardness
Cyprinella leedsi						
Pimephales promelas						

Packed by: [signature]

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 Fernandina Beach Wastewater Treatment Facility Outfall D-001, sampled on September 28 and 29, 2008.

Date Sampled	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
9/29/2008 12:00	Effluent	AGP/LN	Algal Growth Potential-Salinity Adjusted	140		mg DryWt/L	3	3
9/29/2008 12:00	Effluent	AGP/LN	Algal Growth Potential	273		mg DryWt/L	0.3	0.9
9/29/2008 12:00	Effluent	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.24	U	ug/L	0.24	0.71
9/29/2008 12:00	Effluent	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.6	I	ug/L	0.55	1.7
9/29/2008 12:00	Effluent	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	2.4		mg/L	0.2	2
9/29/2008 12:00	Effluent	Overflow	Fecal Coliforms-Membrane Filter	13	B	#/100 mL	2	
9/28/2008 18:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Mysid, LC50	100	L	LC50		
9/29/2008 0:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Mysid, LC50	100	L	LC50		
9/28/2008 18:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Fish, LC50	No Result	O	LC50		
9/29/2008 6:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Fish, LC50	No Result	O	LC50		
9/29/2008 12:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Mysid, LC50	100	L	LC50		
9/29/2008 0:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Fish, LC50	100	L	LC50		
9/29/2008 12:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Fish, LC50	100	L	LC50		
9/29/2008 6:00	Effluent	Toxicology	Bioassay-Acute-Screen-SW-Mysid, LC50	100	L	LC50		
9/29/2008 12:00	Effluent	Metals	Selenium	0.5	U	ug/L	0.5	2
9/29/2008 12:00	Effluent	Metals	Aluminum	60	U	ug/L	60	240
9/29/2008 12:00	Effluent	Metals	Lead	0.2	U	ug/L	0.2	0.8
9/29/2008 12:00	Effluent	Metals	Magnesium	26.6		mg/L	0.04	0.16
9/29/2008 12:00	Effluent	Metals	Cadmium	0.02	U	ug/L	0.02	0.08
9/29/2008 12:00	Effluent	Metals	Silver	0.025	U	ug/L	0.025	0.1
9/29/2008 12:00	Effluent	Metals	Nickel	0.9	I	ug/L	0.25	1
9/29/2008 12:00	Effluent	Metals	Arsenic	2.5		ug/L	0.5	2
9/29/2008 12:00	Effluent	Metals	Zinc	21		ug/L	5	20
9/29/2008 12:00	Effluent	Metals	Iron	60	I	ug/L	30	120
9/29/2008 12:00	Effluent	Metals	Copper	1.5	I	ug/L	0.5	2
9/29/2008 12:00	Effluent	Metals	Chromium	0.5	U	ug/L	0.5	2
9/29/2008 12:00	Effluent	Metals	Calcium	68.1		mg/L	0.075	0.3
9/29/2008 12:00	Effluent	Nutrients	TSS	4	U	mg/L	4	16
9/29/2008 12:00	Effluent	Nutrients	Ammonia-N	0.036		mg N/L	0.01	0.02
9/29/2008 12:00	Effluent	Nutrients	O-Phosphate-P	1.7		mg P/L	0.04	0.1
9/29/2008 12:00	Effluent	Nutrients	Kjeldahl Nitrogen	1.3		mg N/L	0.08	0.2
9/29/2008 12:00	Effluent	Nutrients	Total-P	1.9		mg P/L	0.1	0.3
9/29/2008 12:00	Effluent	Nutrients	NO2NO3-N	6.2		mg N/L	0.04	0.1

Appendix E. (continued)

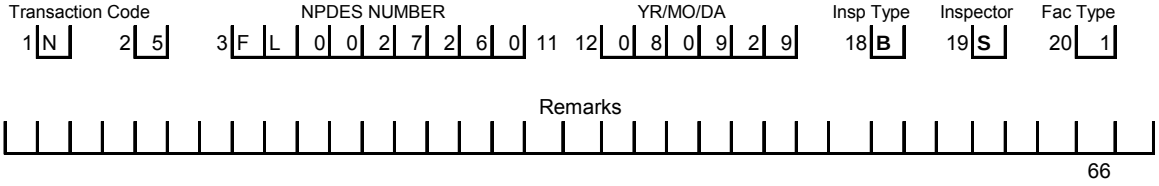
Date Sampled	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
9/29/2008 6:00	Effluent	Field Parameter	Temperature	26.28		C		
9/29/2008 0:00	Effluent	Field Parameter	Specific Conductance	806		umhos/cm		
9/28/2008 18:00	Effluent	Field Parameter	Dissolved Oxygen	7.15		mg/L		
9/28/2008 18:00	Effluent	Field Parameter	Temperature	26.41		C		
9/29/2008 0:00	Effluent	Field Parameter	Sample Depth	0.2		m		
9/29/2008 12:00	Effluent	Field Parameter	Dissolved Oxygen	7.97		mg/L		
9/29/2008 12:00	Effluent	Field Parameter	Specific Conductance	200		umhos/cm		
9/29/2008 6:00	Effluent	Field Parameter	Sample Depth	0.2		m		
9/29/2008 0:00	Effluent	Field Parameter	Dissolved Oxygen	7.13		mg/L		
9/29/2008 0:00	Effluent	Field Parameter	Temperature	26.4		C		
9/29/2008 12:00	Effluent	Field Parameter	Temperature	26.54		C		
9/29/2008 6:00	Effluent	Field Parameter	Specific Conductance	797		umhos/cm		
9/29/2008 0:00	Effluent	Field Parameter	pH	7.02				
9/28/2008 18:00	Effluent	Field Parameter	pH	7.03				
9/29/2008 12:00	Effluent	Field Parameter	pH	7.08				
9/29/2008 6:00	Effluent	Field Parameter	Dissolved Oxygen	7.32		mg/L		
9/28/2008 18:00	Effluent	Field Parameter	Sample Depth	0.15		m		
9/29/2008 6:00	Effluent	Field Parameter	pH	7.07				
9/28/2008 18:00	Effluent	Field Parameter	Specific Conductance	817		umhos/cm		
9/29/2008 12:01	Blank	Metals	Nickel	0.25	U	ug/L	0.25	1
9/29/2008 12:01	Blank	Metals	Calcium	0.075	U	mg/L	0.075	0.3
9/29/2008 12:01	Blank	Metals	Copper	0.5	U	ug/L	0.5	2
9/29/2008 12:01	Blank	Metals	Arsenic	0.5	U	ug/L	0.5	2
9/29/2008 12:01	Blank	Metals	Lead	0.2	U	ug/L	0.2	0.8
9/29/2008 12:01	Blank	Metals	Selenium	0.5	U	ug/L	0.5	2
9/29/2008 12:01	Blank	Metals	Zinc	5	U	ug/L	5	20
9/29/2008 12:01	Blank	Metals	Magnesium	0.04	U	mg/L	0.04	0.16
9/29/2008 12:01	Blank	Metals	Silver	0.083	I	ug/L	0.025	0.1
9/29/2008 12:01	Blank	Metals	Chromium	0.5	U	ug/L	0.5	2
9/29/2008 12:01	Blank	Metals	Cadmium	0.02	U	ug/L	0.02	0.08
9/29/2008 12:01	Blank	Metals	Iron	30	U	ug/L	30	120
9/29/2008 12:01	Blank	Metals	Aluminum	60	U	ug/L	60	240
9/29/2008 12:01	Blank	Nutrients	Ammonia-N	0.02	U	mg N/L	0.02	0.04
9/29/2008 12:01	Blank	Nutrients	O-Phosphate-P	0.006	I	mg P/L	0.004	0.01

Appendix E. (continued)

Date Sampled	Field ID	Analysis	Component	Result	Remark	Units	MDL	PQL
9/29/2008 12:01	Blank	Nutrients	NO2NO3-N	0.009	I	mg N/L	0.004	0.01
9/29/2008 12:01	Blank	Nutrients	Kjeldahl Nitrogen	0.08	U	mg N/L	0.08	0.2
9/29/2008 12:01	Blank	Nutrients	Total-P	0.02	U	mg P/L	0.02	0.06

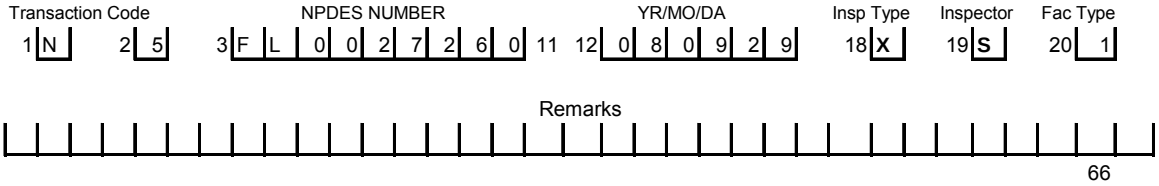
The Bioassay of the Fernandina Beach WWTF effluent sampled on September 28-29, 2008. NPDES #FL0027260.

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



The Priority Pollutants Analysis of the Fernandina Beach WWTF effluent sampled on September 29, 2008. NPDES #FL0027260.

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



Biological Analyses of the Fernandina Beach WWTF effluent sampled on September 29, 2008. NPDES #FL0027260.

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

