


**Effluent Quality Assessment**

# Bayou Marcus Water Reclamation Facility

(formerly Avalon WWTF)

Escambia County

NPDES #FL0031801

Sampled June 7, 9 and 11, 2010

September 2010

**Biology Section**
**Bureau of Laboratories**
**Division of Environmental Assessment and Restoration**

Quality Manual No. 870346G
NELAC Certification No. E31780

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Bayou Marcus Water Reclamation Facility, 3050 Fayal Drive, Pensacola, Escambia County, Florida, 32526. NPDES #FL0031801. Effluent composite samples for the bioassays were collected on June 7th, 9th and 11th, 2010, and grab samples for chemical analyses for this facility were collected on June 7, 2010.

## Introduction

Bayou Marcus Water Reclamation Facility (WRF) (formerly Avalon WWTF) is an advanced domestic wastewater treatment facility which uses the extended aeration-activated sludge treatment process to treat domestic wastewater. (See Appendix F for detailed facility information provided by Kim Allen of FDEP Northwest District.)

## Methods

Samples were collected from the EFF-01 NPDES sampling point for Outfall D-002 by Rick Abad and Nathan Mulkey (FDEP Northwest 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 acute screening fish bioassay, the chronic screening water-flea bioassay, and the algal growth potential (AGP) test were performed following internal DEP SOPs TA07_05, 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.

Due to a shipping issue, the third set of bioassay samples were not received until the sample hold time before first use (36 hours) had expired. The water-flea chronic test was renewed on test day 4 and test day 5 with the second bioassay sample (within 72 hours of first use) and was terminated on test day 6, having met termination criteria. The fish test was not renewed on test day 4 because test renewal on test day 6 would have been with expired sample (sample 2 – 96 hours after first use not allowed); therefore, the fish bioassay was changed from chronic to acute status.

An aliquot of the AGP sample was salinity adjusted with artificial sea salts to meet the salinity requirement of the marine test species.

## Results and Discussion

- **Toxicity Test Results**
  Tests were performed June 8 - 14, 2010.

  *Ceriodaphnia dubia* chronic screening bioassay – $ICP_{20}$ > 100%, 0% mortality in 100% sample at 6 days

  *Pimephales promelas* 96-hour acute screening bioassay – $LC_{50}$ > 100%, 0%* mortality in 100% sample at 96 hours

  The bioassay samples were not acutely or chronically 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 34.6 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 (9.4:1) of inorganic nitrogen to orthophosphate in the effluent suggests that the effluent is nitrogen-limited; whereas the ratio (12.5:1) of total nitrogen to total phosphorus suggests that the effluent is co-limited by both nitrogen and phosphorus (Table 1; Miller et al. 1978).

  The AGP of the salinity-adjusted effluent was 12.2 mg dry wt/L of the saltwater species *Dunaliella tertiolecta.*

  See Table 1a for freshwater AGP results and predicted AGP values. See Table 1b for salinity-adjusted AGP results.

- **Chemistry Results**
  Total residual chlorine was not detected in the bioassay samples in the laboratory. Total ammonia (0.0409 mg N/L) was detected in the second bioassay sample in the laboratory. The total ammonia concentration in the composite sample collected and preserved for chemical analysis was 0.039 mg N/L. Based on the pH, salinity, and temperature of the effluent as collected, the calculated unionized ammonia concentrations in both the bioassay sample and the sample collected for chemical analyses sample were < 0.02 mg/L.

  Copper, 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.). Aluminum, arsenic, atrazine, chromium, lead, and selenium were detected between the laboratory method detection limits (MDL) and practical quantitation limits (PQL). Effluent water samples did not exceed any applicable water quality standards or violate any permit conditions.

  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 EFF-01 for this facility on June 7 and 9, 2010, were not acutely or chronically toxic to either test species during the 96-hour acute or 7-day chronic screening bioassays. The effluent AGP result was greater than the "problem" threshold for both fresh and marine receiving waters, suggesting that nutrient enrichment may be an issue. Effluent water quality samples collected on June 7, 2010, did not exceed any applicable water quality standards or violate any permit conditions.

## Literature Cited

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

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

Maloney, T. E., W. E. Miller, and D. T. Specht. 1974. The Marine Algal Assay Procedure: Bottle Test. National Environmental Research Center Office of Research and Development. U. S. EPA, Corvallis, Oregon. 43 p.

**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 Bayou Marcus WRF on June 7, 2010.**

| Bayou Marcus Water Reclamation Facility<br>NPDES # FL0031801 | | | | | | | |
|---|---|---|---|---|---|---|---|
| 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 | 34.6 | 19.0 ± 3.8 | 22.8 ± 4.6 | 52.1 ± 10.4 | 47.3 ± 9.5 | 9.4 | 12.5 |

**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 Bayou Marcus WRF on June 7, 2010.**

| Bayou Marcus Water Reclamation Facility<br>NPDES # FL0031801 | | | |
|---|---|---|---|
| Location | AGP (measured) | Inorganic N:P ratio | Total N:P ratio |
| Effluent | 12.2 * | 9.4 | 12.5 |

* - 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 Bayou Marcus WRF on June 7, 2010.**

| Bayou Marcus Water Reclamation Facility NPDES# FL0031801 | Class III Criteria | Effluent Limits | Effluent | |
|---|---|---|---|---|
| **Organic Constituents (µg/L)** | | | | |
| Atrazine | - | - | 0.12 | I |
| **Metals (µg/L unless otherwise noted)** | | | | |
| Aluminum | ≤ 1500 | - | 77 | I |
| Arsenic | ≤ 50 | - | 0.55 | I |
| Cadmium | ≤ 8.8 | - | 0.03 | U |
| Calcium (mg/L) | - | - | 22.8 | |
| Chromium-III | - | - | 0.3 | I |
| Copper | ≤ 3.7 | Report | 1.06 | |
| Iron | ≤ 300 | - | 30 | U |
| Lead | ≤ 8.5 | - | 0.26 | I |
| Magnesium (mg/L) | - | - | 12.9 | |
| Nickel | ≤ 8.3 | - | 2.17 | |
| Selenium | ≤ 71 | - | 0.22 | I |
| Silver | ≤ 2.3 | - | 0.025 | U |
| Zinc | ≤ 86 | Report | 39 | |
| **Nutrients (mg/L)** | | | | |
| Ortho-phosphate | - | - | 0.053 | |
| Total Phosphorus | - | ≤ 2.0 f | 0.11 | A |
| Total Ammonia | - | ≤ 3.2 f | 0.039 | |
| Un-ionized Ammonia | - | - | ≤ 0.02 | c |
| Nitrate and Nitrite | - | - | 0.46 | |
| Total Kjeldahl Nitrogen | - | - | 0.91 | |
| Organic Nitrogen | - | - | 0.871 | c |
| Total Nitrogen | - | ≤ 6.0 f | 1.37 | c |
| **General Physical and Chemical Parameters** | | | | |
| Dissolved Oxygen (mg/L) | ≥ 4.0 | ≥ 5.0 | 8.5 | |
| pH (SU) | 6.5 - 8.5 | 6.5 - 8.5 | 7.6 | |
| Conductivity (µmhos/cm) | - | - | 473 | |
| Temperature (°C) | - | - | 27.6 | |
| Salinity | - | - | ≤ 0.22 | c |
| Sample Depth (m) | - | - | 0.15 | |
| Total Residual Chlorine (mg/L) | ≤ 0.01 | - | 0 | |
| Chlorophyll *a* (µg/L) - Corrected | - | - | 1.7 | I |
| Phaeophytin (µg/L) | - | - | 2.4 | |
| CBOD, 5 day (mg/L), N - inhibited | - | ≤ 10.0 f | 0.92 | I |
| Flow (MGD) | - | 8.2 a | 6.27 | a* |
| Hardness (mg $CaCO_3$) | - | Report | 110.05 | c |
| **Microbiology (Number of colony forming units/100ml)** | | | | |
| Fecal Coliforms ¥ | ≤ 400s # | Report | 4 | B |

Value exceeds the Class III Water Quality Criteria or permit limits

a - Annual average
c - Value is calculated
f - 24-hour flow-proportioned composite
s - Single sample
A - Value reported is the mean of two or more determinations
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
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.)
* - AADF measured from 4-1-09 to 5-31-10

# Florida Department of Environmental Protection

## Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2010-05-03-26
Bayou Marcus Water Reclamation Facility

Requester: Michael Tanski
Field Report Prepared By: N. Malkey
Collected By: Rick Bland
Send Final Report To: Toni Edwards
Sampling Agency: FDEP

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

| Lab ID * | Location / Field ID / Matrix / Temp (C) / pH / Latitude / Longitude | Collection (begin) / Collection (end) / Eastern Central / Tot Res Chlorine (mg/L) / Diss Oxygen (mg/L) / Storet Station Number / Sample Depth / Salinity (PPTh) / Sp Conductance (umho/cm) / NPDES Number / Comments | Bottle Group(s)** |
|---|---|---|---|
| | Location: Bayou Marcus Water Facility<br>Field ID: TOX 2 | ☑ Comp ☐ Grab<br>Collection (begin) Date 6/8/10 Time 0640 Eastern Central<br>Collection (end) Date 6/9/10 Time 0845 Eastern Central | C |
| | Location | ☐ Comp ☐ Grab<br>Collection (begin) Date Time Eastern Central<br>Collection (end) Date Time Eastern Central | |
| | Location | ☐ Comp ☐ Grab<br>Collection (begin) Date Time Eastern Central<br>Collection (end) Date Time Eastern Central | |
| | Location | ☐ Comp ☐ Grab<br>Collection (begin) Date Time Eastern Central<br>Collection (end) Date Time Eastern Central | |

Comments: 2 Bio-Assay Rec'd in Tox by JD. 9:37 6.10.10

| Relinquished By | Date/Time | Shipping Method | Received By | Date/Time | Relinquished By | Date/Time | Received By | Date/Time |
|---|---|---|---|---|---|---|---|---|
| Erin Rueda | 6/9/10 0845 | Hand Fed Ex | | | | | | 6-10-10/9:05 |

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

# Florida Department of Environmental Protection

## Central Laboratory Sample Submittal Form

Event ID *

**Request Number: RQ-2010-05-03-26**
Bayou Marcus Water Reclamation Facility

Requester: Michael Tanski
Field Report Prepared By: N. Mulkey

**Customer: NW-DIST**
Collected By: Nathan Mulkey / Rick Abad
Send Final Report To: Toni Edwards

**Project ID: FYI3**
Sampling Agency: NWFDEP

**PMAS: 1143**

| Lab ID * | Location | Collection (begin) | Collection (end) | Bottle Group(s)** |
|---|---|---|---|---|
| | Bayou Marcus Water Facility | ☑ Comp ☐ Grab Date 6/16/10 Time 0600 Eastern Central | Date 6/17/10 Time 0600 Eastern Central | C |
| | Field ID: TOX 1 | Tot Res Chlorine (mg/L) | Diss Oxygen (mg/L) | Storet Station Number |
| | Matrix (Include type e.g. Salt, Fresh, etc): Fresh | Temp (C) | pH | Sample Depth ☐ m ☐ ft |
| | | Salinity (PPTh) ☐ Sp Conductance (umho/cm) | NPDES Number | |
| | Latitude o ' " | Longitude o ' " | Comments | |

| Lab ID * | Location | Collection (begin) | Collection (end) | Bottle Group(s)** |
|---|---|---|---|---|
| | Bayou Marcus Water Facility | ☐ Comp ☐ Grab Date 6/17/10 Time 1020 Eastern Central | Date Time Eastern Central | B |
| | Field ID: Blank | 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 | Collection (begin) | Collection (end) | Bottle Group(s)** |
|---|---|---|---|---|
| | Bayou Marcus Water Facility | ☐ Comp ☑ Grab Date 6/17/10 Time 1040 Eastern Central | Date Time Eastern Central | A |
| | Field ID: Effluent | Tot Res Chlorine (mg/L) | Diss Oxygen (mg/L) 8.46 | Storet Station Number |
| | Matrix (Include type e.g. Salt, Fresh, etc): Fresh | Temp (C) 27.59 | pH 7.64 | Sample Depth 0.15 ☑ m ☐ ft |
| | | Salinity (PPTh) ☑ Sp Conductance (umho/cm) 473 | NPDES Number | |
| | Latitude o ' " | Longitude o ' " | Comments | |

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

| Relinquished By: | Date/Time | Shipping Method | Received By: | Date/Time | Relinquished By | Date/Time | Received By: | Date/Time |
|---|---|---|---|---|---|---|---|---|
| Field Staff | 6/17/10 | Fed Ex | Tahmoores Thahim | 9:10 06-08-10 | | | | |

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information

last revised October 1, 2003

## Appendix B. Bench Sheets

### FDEP Biology Laboratory - Chronic Screen Bench Sheet
*Ceriodaphnia dubia*

Facility: Bayou Marcus
Address: Muldoon Road and Fayal Drive, Pensacola 32501
County: Escambia
NPDES Permit #: FL 0031801
Contact District: Nathan Nruby / NS, TK West

LIMS entry: 7/13/10 MF verified: 7/13/10 BA

Diluent/Batch: 20% DMW / 6-7-10 Test Days Used: 0 – 7
20% DMW / ____ Test Days Used: ____

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

Organism: *Ceriodaphnia dubia*
Organism Batch #: 17-10
Organism age : <24hr hours
Feeding: Prior to Test and Daily
Food: ☑ YCT ☐ *P. subcapitata*
Food Batch: 5-19-10 12-23-09
Chamber size : 30 ml
Sample volume : 20 ml

| Sample Received | LAB Sample # | LIMS JOB # | LIMS SAMPLE # | Collection Date/Time | Received Date/Time |
|---|---|---|---|---|---|
| Test Day 0 | 1 | TLH-2010-06-08-56 | 1276499 | 6/7/10 0800 | 6/8/10 0910 |
| Test Day 2 | 2 | TLH-2010-06-10-52 | 1277489 | 6/9/10 0845 MF | 6/10/10 0947 |
| Test Day 4 | 3 | TLH- NOT Received on Saturday | | MF | |

Control Time initially written MF 6/9/10

| Batch # | 6-7-10 | | NA | NA | NA | | | |
|---|---|---|---|---|---|---|---|---|
| **Water Quality Parameters** | 20% DMW | 20% DMW | Sample 1 | Sample 2 | Sample 3 | Other: | Measured By: | Verified by: |
| Field Total Residual $Cl_2$ (mg/L): | NA | NA | Not measured | not measured | | | – | MF |
| Lab Total Residual $Cl_2$ (mg/L): | <0.03 | | <0.03 | R3.04 | | | kms/JD | MF / MF |
| Alkalinity (mg/L as $CaCO_3$): | 81 | | 137 | 139 | | | BA | MF |
| Hardness (mg/L as $CaCO_3$): | 84 | | 119 | 134 | | | BA | MF |
| Total Ammonia (mg/L as N): | <0.017 | | <0.017 | 0.0409 | | | MF | BA |
| Conductivity (umhos) mmhos: | 178 | | 465 | 467 | | | BA/JD | MF |
| Salinity (ppth): | — | | — | — | | | | |

Test Incubated in: Incubator #: 2
Samples 1 and 2
Temperature Shipped = 6° C (~~all samples~~) MF (yes) no
Holding Time = 36 hours (yes) no
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 24.4 max. 26.6 mean 25.5
Room B246 min. 24.9 max. 25.8 mean ~~25.4~~ 25.4
Test min. 24.0 max. 24.9 mean — MF

Loaded by:

| Test Replicates | Loaded from culture vial |
|---|---|
| 1 | B18 |
| 2 | B21 |
| 3 | B13 |
| 4 | B16 |
| 5 | B27 |
| 6 | B5 |
| 7 | B23 |
| 8 | B17 |
| 9 | B9 |
| 10 | B14 |

| Results | |
|---|---|
| Control % CV: | 21% |
| Dunnetts PMSD: | 20.8% |
| Reproduction NOEC: | 100% |
| Reproduction LOEC: | – |
| Survival NOEC: | 100% |
| Survival LOEC: | – |
| $ICP_{20}$: | >100% |
| $ICP_{25}$: | >100% |

Investigators' signatures
Bree Alexander
Marshall Faircloth
Anne Danish
Kim Spencer
Liz Miller
REVIEWER

**Comments:** The third set of samples for this bioassay was not received on Saturday 6-12-10. Samples arrived on 6/14/10 and were cancelled by receiving since they were out of hold. MF 6-15-10 The second sample was used for 96 hours. MF 7-13-10
CETIS TEST CODE: 5B9DE133

Test Page 1 of 4 PAGE 00 09 Revised 07/15/2008 MF

## FDEP Biology Laboratory - Chronic Screen Bench Sheet

*Ceriodaphnia dubia* Survival and Reproduction

SOP TA07_05

Facility: Bayou Marcus

LIMS Sample # 1276499 for lab sample 1

Start Date: 6.8.10 Time: 10:35

End Date: 6-14-10 Time: 1400

| Conc. | Day | Replicate Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total Neonates | Surviving adults | Neonates per adult |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 0* | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 3 | 0 | ~~4~~ 4 | ~~0~~ | 0 | 4 | 2 | 3 | 0 | 0 | 0 | (A) 17 | 10 | 1.7 |
| | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 4 | 2 | 5 | 13 | 10 | 1.3 |
| | 5 | 5 | 5 | 9 | 7 | 9 | 2 | 7 | 8 | 6 | 9 | 67 | 10 | 6.7 |
| | 6 | 11 | 13 | 11 | 8 | 12 | 8 | 14 | 12 | 12 | 6 | 107 | 10 | 10.7 |
| | 7 | | | | | | | | | | | | | |
| | 8 | | | | | | | | | | | | | |
| | Total | 16 | 22 | 24 | 17 | 25 | 12 | 24 | 24 | 20 | 20 | 204 | 10 | 20.4 |

| Conc. | Day | Replicate Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total Neonates | Surviving adults | Neonates per adult |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100% sample | 0* | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 4 | 0 | 2 | 0 | 0 | 7 | 3 | 0 | 5 | 3 | 4 | 24 | ~~MF 9~~ 10 | 2.4 |
| | 5 | 0 | 0 | 5 | 8 | 3 | 5 | 10 | 6 | 7 | 8 | 52 | 10 | 5.2 |
| | 6 | 11 | 10 | 15 | 0 | 11 | 9 | 14 | 16 | 5 | 13 | 104 | 10 | 10.4 |
| | 7 | | | | | | | | | | | | | |
| | 8 | | | | | | | | | | | | | |
| | Total | 11 | 12 | 20 | 8 | 21 | 17 | 24 | 27 | 15 | 25 | 180 | 10 | 18.0 |

Organisms loaded by: BA

| Day | Counted/ verified by: |
|---|---|
| 0 | MF |
| 1 | BA |
| 2 | BA |
| 3 | BA |
| 4 | MF |
| 5 | JD |
| 6 | MF |
| 7 | |
| 8 | |

| Concentration | Mean neonates /adult | % survival |
|---|---|---|
| Control | 20.4 | 100 |
| 100% | 18.0 | 100 |

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

revised 7/15/2008 MF

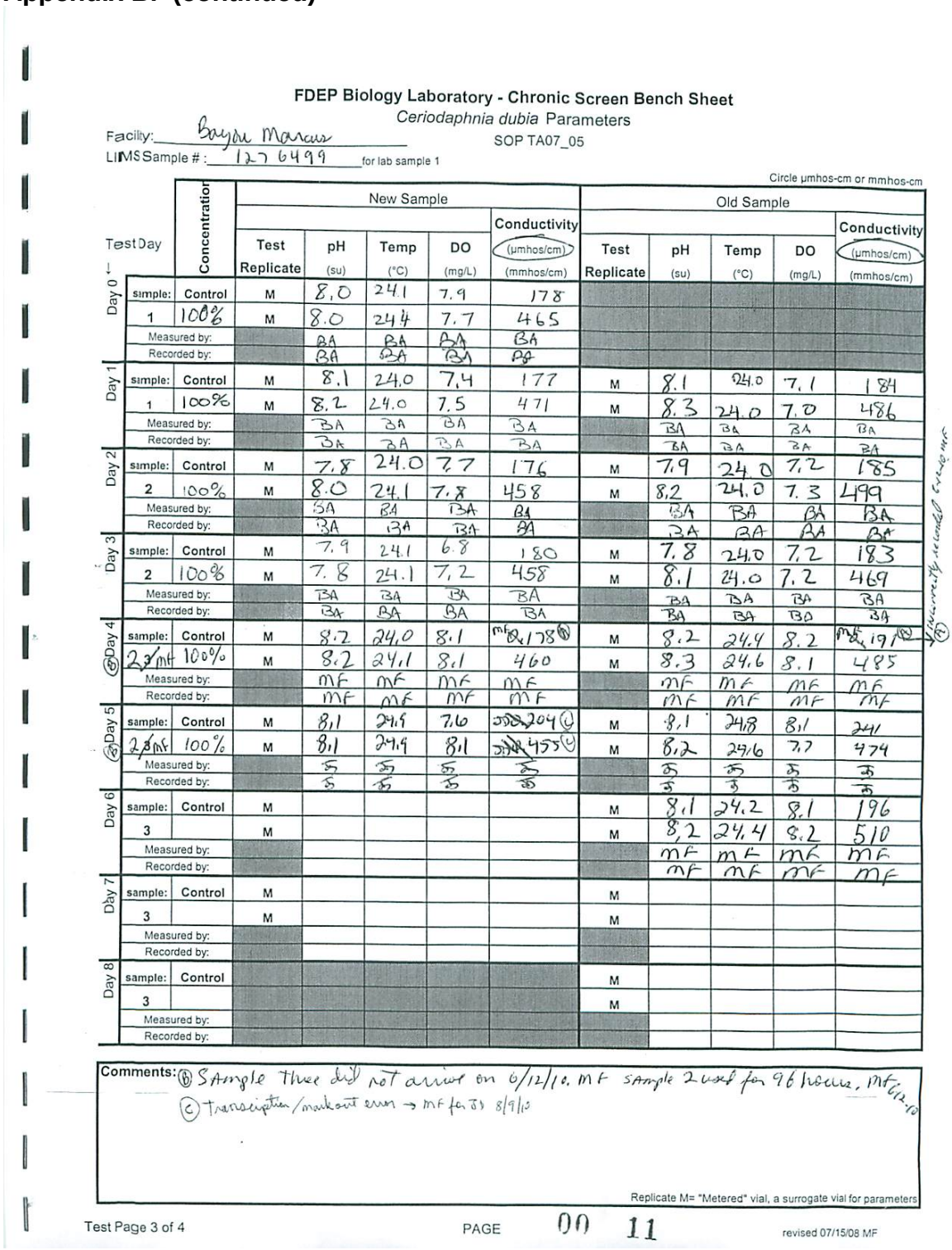
**Comments:** (A) Transcription error Day 3, Replicates 2 & 3 BA

00 10

*Day 0 is test initiation day

X = Dead (original organism)

## Appendix B. (continued)



**FDEP Biology Laboratory - Chronic Screen Bench Sheet**

*Ceriodaphnia dubia* Parameters

SOP TA07_05

Facility: Bayou Marcus

LIMS Sample #: 127 6499 for lab sample 1

Circle µmhos-cm or mmhos-cm

| Test Day ↓ | | Concentration | New Sample: Test Replicate | pH (su) | Temp (°C) | DO (mg/L) | Conductivity (µmhos/cm) (mmhos/cm) | Old Sample: Test Replicate | pH (su) | Temp (°C) | DO (mg/L) | Conductivity (µmhos/cm) (mmhos/cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 0 | sample: | Control | M | 8.0 | 24.1 | 7.9 | 178 | | | | | |
| | 1 | 100% | M | 8.0 | 24.4 | 7.7 | 465 | | | | | |
| | Measured by: | | | BA | BA | BA | BA | | | | | |
| | Recorded by: | | | BA | BA | BA | BA | | | | | |
| Day 1 | sample: | Control | M | 8.1 | 24.0 | 7.4 | 177 | M | 8.1 | 24.0 | 7.1 | 184 |
| | 1 | 100% | M | 8.2 | 24.0 | 7.5 | 471 | M | 8.3 | 24.0 | 7.0 | 486 |
| | Measured by: | | | BA | BA | BA | BA | | BA | BA | BA | BA |
| | Recorded by: | | | BA | BA | BA | BA | | BA | BA | BA | BA |
| Day 2 | sample: | Control | M | 7.8 | 24.0 | 7.7 | 176 | M | 7.9 | 24.0 | 7.2 | 185 |
| | 2 | 100% | M | 8.0 | 24.1 | 7.8 | 458 | M | 8.2 | 24.0 | 7.3 | 499 |
| | Measured by: | | | BA | BA | BA | BA | | BA | BA | BA | BA |
| | Recorded by: | | | BA | BA | BA | BA | | BA | BA | BA | BA |
| Day 3 | sample: | Control | M | 7.9 | 24.1 | 6.8 | 180 | M | 7.8 | 24.0 | 7.2 | 183 |
| | 2 | 100% | M | 7.8 | 24.1 | 7.2 | 458 | M | 8.1 | 24.0 | 7.2 | 469 |
| | Measured by: | | | BA | BA | BA | BA | | BA | BA | BA | BA |
| | Recorded by: | | | BA | BA | BA | BA | | BA | BA | BO | BA |
| (B)Day 4 | sample: | Control | M | 8.2 | 24.0 | 8.1 | mf 8.178 (B) | M | 8.2 | 24.4 | 8.2 | mf 8.191 (B) |
| | 2 mf | 100% | M | 8.2 | 24.1 | 8.1 | 460 | M | 8.3 | 24.6 | 8.1 | 485 |
| | Measured by: | | | MF | MF | MF | MF | | MF | MF | MF | MF |
| | Recorded by: | | | MF | MF | MF | MF | | MF | MF | MF | MF |
| (B)Day 5 | sample: | Control | M | 8.1 | 24.9 | 7.6 | 204 (C) | M | 8.1 | 24.8 | 8.1 | 241 |
| | 2 mf | 100% | M | 8.1 | 24.9 | 8.1 | 455 (C) | M | 8.2 | 24.6 | 7.7 | 474 |
| | Measured by: | | | ~~BA~~ | ~~BA~~ | ~~BA~~ | ~~BA~~ | | ~~BA~~ | ~~BA~~ | ~~BA~~ | ~~BA~~ |
| | Recorded by: | | | ~~BA~~ | ~~BA~~ | ~~BA~~ | ~~BA~~ | | ~~BA~~ | ~~BA~~ | ~~BA~~ | ~~BA~~ |
| Day 6 | sample: | Control | M | | | | | M | 8.1 | 24.2 | 8.1 | 196 |
| | 3 | | M | | | | | M | 8.2 | 24.4 | 8.2 | 510 |
| | Measured by: | | | | | | | | MF | MF | MF | MF |
| | Recorded by: | | | | | | | | MF | MF | MF | MF |
| Day 7 | sample: | Control | M | | | | | M | | | | |
| | 3 | | M | | | | | M | | | | |
| | Measured by: | | | | | | | | | | | |
| | Recorded by: | | | | | | | | | | | |
| Day 8 | sample: | Control | | | | | | M | | | | |
| | 3 | | | | | | | M | | | | |
| | Measured by: | | | | | | | | | | | |
| | Recorded by: | | | | | | | | | | | |

(A) Incorrectly recorded 6/12/10 MF

Comments: (B) Sample three did not arrive on 6/12/10. MF sample 2 used for 96 hours. MF 6/12/10

(C) Transcription/makeup error → MF for BS 8/9/10

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

Test Page 3 of 4 PAGE 00 11 revised 07/15/08 MF

**FDEP Biology Laboratory - Bioassay Instrument Identification**

Test ID: Bayou Marcus Dates: 6-8-10 – 6-14-10

| METER / DEVICE | ID | SOP | Parameter | Method | v 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 electronic and non-mercury filled thermometers.
Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

Comments: Entered by: M.F 6-14-10

V 1.4 8/21/08 BA pg 6 of 6

00 12

## FDEP Biology Laboratory - Chronic Bioassay Bench Sheet

*Pimphales promelas or Menidia beryllina*

Facility: Bayou Marcus
Address: McLaoon Road and Fayal Drive, Pensacola 32501
County: Escambia
NPDES Permit #: FL 0021801
Contact/District: Nathan Mulky / Northwest

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

Start Date: 6/8/10 Time: 1345
End Date: 6-12-10 Time: 0930

Diluent/ Batch:
MHW / 6.8.10 Test Days Used: 0-3
MHW / _____ Test Days Used: _____
ASW / _____ Test Days Used: _____
ASW / _____ Test Days Used: _____

Organism Batch # : 6-8-10
Feeding Prior to Test and Daily
Artemia Lot # : 23613
Chamber size : 1000 ml
Test volume : 400 ml

Organism age : 22hrs days

| Artemia Batch # | Day 3 9.2-10 |
|---|---|
| Day 0 8.9-10 | Day 4 |
| Day 1 9.0-10 | Day 5 |
| Day 2 9.1-10 | Day 6 |

| LIMS: | Date | Initials |
|---|---|---|
| Entry: | 7/13/10 | MF |
| Verification: | 7.15.10 | BA |

| Sample Received | Lab Sample # | LIMS Job # | LIMS Sample # | Collection Date/Time | Received Date/Time |
|---|---|---|---|---|---|
| Test Day 0 | 1 | TLH-2010-06-08-56 | 1276499 | 6-7-10 ~~0600~~ (A) | 6-8-10 |
| Test Day 2 | 2 | TLH-2010-06-10-52 | 1277489 | 6-9-10 0645 (A) | 6-10-10 0947 |
| Test Day 4 | 3 | TLH- Not Received MF | | 6-15-10 | |

(A) → 0700 + 0745 post-set time MF 8/9/10

| Water Quality Parameters | Batch # 6-8-10 | | NA Sample 1 | NA Sample 2 | NA Sample 3 | Other: | Measured by: | Verified by: |
|---|---|---|---|---|---|---|---|---|
| | ☑ MHW ☐ ASW | ☐ MHW ☐ ASW | | | | | | |
| Field Total Residual $Cl_2$ (mg/L): | NA | NA | Not Required | Not Received | | | - | MF |
| Lab Total Residual $Cl_2$ (mg/L): | <0.03 | | <0.03 | 0.04 | | | KMS/JD | MF |
| Alkalinity (mg/L as $CaCO_3$): | 77 | | 137 | 139 | | | BA | MF |
| Hardness (mg/L as $CaCO_3$): | 94 | | 119 | 134 | | | BA | MF (5) → Initialed wrong space MF 6-15-10 |
| Total Ammonia (mg/L as N): | <0.017 | | <0.017 | 0.0409 | | | ME | ~~MF~~ BA |
| Conductivity: (umhos) mmhos: | 326 | | 479 | 467 | | | JD | MF |
| Salinity (ppth): | — | | — | — | | | — | — |

Temperature Shipped ≤6° C (all samples) (yes) no #1 + #2
Holding Time ≤36 hours (yes) no #1 + #2
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark

Temperature Range °C: (if used)
Incubator # 2 min. 24.4 max. 26.6 mean 25.5
Water bath #____ min.____ max.____ mean____
Room B246 min. 24.9 max 25.7 mean 25.3

Test Incubated in: ☑ Incubator #: 2 ☐ Waterbath #:____

Investigators' signatures
James Daniels
Marshall Fairchild
Kim M Spencer
Bree Arden
REVIEWER Lynn Miller

| Statistics | |
|---|---|
| Control %cv: | |
| Dunnetts PMSD: | |
| Survival NOEC: | |
| Survival LOEC: | |
| Growth NOEC: | |
| Growth LOEC: | |

Comments: Third of 3 samples not received on 6-12-10. Samples out of hold on 6/14/10 so # 2 sample could not be used for renewals on 6/13 - 6/14. Test Terminated MF 6/15/10
Data entered into Lims as ~~Definitive~~ MF acute screen 96 hour data. MF 7-13-10
LC50 > 100%

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revised 7/15/08 MF

## Appendix B. (continued)

**FDEP Biology Laboratory - Chronic Screen Bench Sheet**

*Pimphales promelas or Menidia beryllina* Survival

Facility: Bayou Marcus

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

LIMS Sample Number: 1276499 for lab sample #1

Start Date: 6.8.10 Time: 1345

End Date: 6-12-10 Time: 0930

| | | TEST DAY | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | Replicate | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | A | 10 | 10 | 10 | 10 | 10 | | | |
| | B | 10 | 10 | 10 | 10 | 10 | | | |
| | C | 10 | 10 | 10 | 10 | 10 | | | |
| | D | 10 | 9 10 | 9 | 9 | 9 | | | |
| 100 % sample concentration | A | 10 | 10 | 10 | 10 | 10 | | | |
| | B | 10 | 10 | 10 | 10 | 10 | | | |
| | C | 10 | 10 | 10 | 10 | 10 | | | |
| | D | 10 | 10 | 10 | 10 | 10 | | | |
| Control | A | 10 | | | | | | | |
| | B | 10 | | | | | | | |
| | C | 10 | | | | | | | |
| | D | 10 | | | | | | | |
| % sample concentration | A | 10 | | | | | | | |
| | B | 10 | | | | | | | |
| | C | 10 | | | | | | | |
| | D | 10 | | | | | | | |
| Loaded by: [illegible] | Checked by: | | [illegible] | [illegible] | [illegible] | [illegible] | | | |
| Verified by: MF | | | | | | | | | |

Comments:

Test Page 2 of 5

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revised 07/15/08 MF

## Appendix B. (continued)

**Biology Laboratory Chronic Screening Bioassay Benchsheet**

Parameters

*P. promelas* SOP TA-07_09 *M. beryllina* SOP TA-07_14

Sample Source: Bayou Marcus

LIMS Sample #: 1276499 (for Lab sample #1)

| Test Day ↓ | | Conc. | Old Sample: Test Rep. | pH (su) | Temp (°C) | DO (mg/L) | Cond (µmhos/cm) (mmhos/cm) | Salinity ppth | New Sample: Test Rep. | pH (su) | Temp (°C) | DO (mg/L) | Cond (µmhos/cm) (mmhos/cm) | Salinity ppth |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 0 | sample: | Control | | | | | | | A | 7.8 | 24.6 | 7.5 | 326 | — |
| | 1 | 100% | | | | | | | A | 7.8 | 25.9 | 8.9 | 479 | — |
| | Measured by: | | | | | | | | | ″ | ″ | ″ | ″ | — |
| | Recorded by: | | | | | | | | | ″ | ″ | ″ | ″ | — |
| Day 1 | sample: | Control | B | 7.9 | 25.0 | 7.5 | 335 | — | B | 7.9 | 24.0 | 7.7 | 331 | — |
| | 1 | 100% | B | 8.2 | 25.5 | 7.3 | 479 | — | B | 8.1 | 24.3 | 7.9 | 480 | — |
| | Measured by: | | | ″ | ″ | ″ | ″ | ″ | | ″ | ″ | ″ | ″ | ″ |
| | Recorded by: | | | ″ | ″ | ″ | ″ | ″ | | ″ | ″ | ″ | ″ | ″ |
| Day 2 | sample: | Control | C | 7.8 | 25.6 | 7.7 | 373 | — | C | 8.1 | 24.0 | 7.8 | 362 | — |
| | 2 | 100% | C | 7.9 | 25.9 | 6.6 | 495 | — | C | 8.2 | 24.6 | 7.3 | 467 | — |
| | Measured by: | | | KMS | KMS | KMS | KMS | KMS | | KMS | KMS | KMS | KMS | KMS |
| | Recorded by: | | | KMS | KMS | KMS | KMS | KMS | | KMS | KMS | KMS | KMS | KMS |
| Day 3 | sample: | Control | D | 7.9 | 25.3 | 7.6 | 368 | — | D | 7.8 | 24.0 | 8.0 | 356 | — |
| | 2 | 100% | D | 8.1 | 25.4 | 7.2 | 478 | — | D | 7.8 | 24.7 | 8.1 | 467 | — |
| | Measured by: | | | KMS | JD | KMS | KMS | KMS | | KMS | KMS | KMS | KMS | KMS |
| | Recorded by: | | | KMS | KMS | KMS | KMS | KMS | | KMS | KMS | KMS | KMS | KMS |
| Day 4 | sample: | Control | A | 7.7 | 25.8 | 7.3 | 407 | — | A | ~~7.4~~ 7.3 | ~~24.7~~ 24.7 | ~~7.7~~ 7.7 | ~~444~~ 444 IV | — |
| | 3 | 100% | A | 8.1 | 25.9 | 7.2 | 485 | — | A | | | | | — |
| | Measured by: | | | ″ | ″ | ″ | ″ | — | | ″ | ″ | ″ | ″ | — |
| | Recorded by: | | | ″ | ″ | ″ | ″ | — | | ″ | ″ | ″ | ″ | — |
| Day 5 | sample: | Control | B | | | | | — | B | | | | | — |
| | 3 | 100% | B | | | | | — | B | | | | | — |
| | Measured by: | | | | | | | — | | | | | | — |
| | Recorded by: | | | | | | | — | | | | | | — |
| Day 6 | sample: | Control | C | | | | | — | C | | | | | — |
| | 3 | 100% | C | | | | | — | C | | | | | — |
| | Measured by: | | | | | | | — | | | | | | — |
| | Recorded by: | | | | | | | — | | | | | | — |
| Day 7 | sample: | Control | D | | | | | — | | | | | | |
| | 3 | 100% | D | | | | | — | | | | | | |
| | Measured by: | | | | | | | — | | | | | | |
| | Recorded by: | | | | | | | — | | | | | | |

Test Rep. = Replicate  Record Salinity < 1 if freshwater  Circle µmhos-cm or mmhos-cm

Comments:

revised 7/15/08 mf

## Appendix B. (continued)

**Bioassay Instrument ID**

Test ID: Boydin Marcus Dates: June 8 — June 12-2010

| 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)

V 1.1 6/12/08 MF

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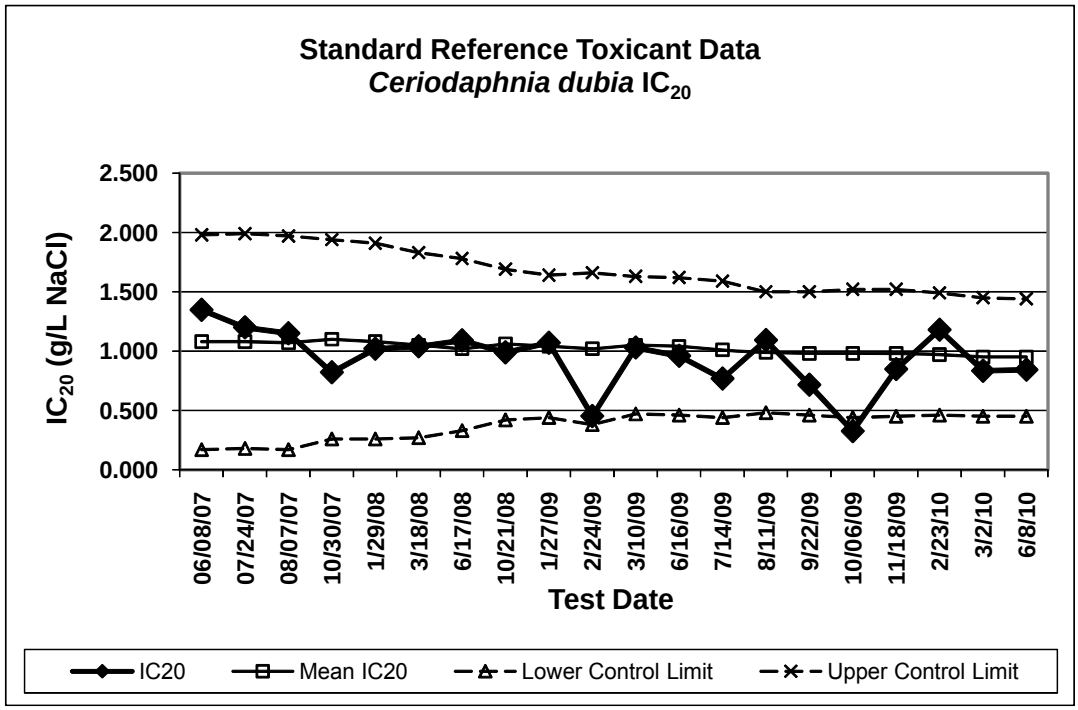
## Appendix C. SRT Bench Sheets and Statistical Analysis

Florida Department of Environmental Protection
Bureau of Laboratories

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

| Date | IC20 | Mean IC20 | Std Dev | Lower Control Limit | Upper Control Limit |
|---|---|---|---|---|---|
| 06/08/07 | 1.347 | 1.08 | 0.45 | 0.17 | 1.98 |
| 07/24/07 | 1.200 | 1.08 | 0.45 | 0.18 | 1.99 |
| 08/07/07 | 1.150 | 1.07 | 0.45 | 0.17 | 1.97 |
| 10/30/07 | 0.821 | 1.10 | 0.42 | 0.26 | 1.94 |
| 1/29/08 | 1.020 | 1.08 | 0.41 | 0.26 | 1.91 |
| 3/18/08 | 1.040 | 1.05 | 0.39 | 0.27 | 1.83 |
| 6/17/08 | 1.090 | 1.02 | 0.36 | 0.33 | 1.78 |
| 10/21/08 | 0.989 | 1.06 | 0.32 | 0.42 | 1.69 |
| 1/27/09 | 1.070 | 1.04 | 0.30 | 0.44 | 1.64 |
| 2/24/09 | 0.452 | 1.02 | 0.32 | 0.38 | 1.66 |
| 3/10/09 | 1.030 | 1.05 | 0.29 | 0.47 | 1.63 |
| 6/16/09 | 0.960 | 1.04 | 0.29 | 0.46 | 1.62 |
| 7/14/09 | 0.768 | 1.01 | 0.29 | 0.44 | 1.59 |
| 8/11/09 | 1.093 | 0.99 | 0.26 | 0.48 | 1.50 |
| 9/22/09 | 0.716 | 0.98 | 0.26 | 0.46 | 1.50 |
| 10/06/09 | 0.325 | 0.98 | 0.27 | 0.44 | 1.52 |
| 11/18/09 | 0.848 | 0.98 | 0.27 | 0.45 | 1.52 |
| 2/23/10 | 1.180 | 0.97 | 0.26 | 0.46 | 1.49 |
| 3/2/10 | 0.834 | 0.95 | 0.25 | 0.45 | 1.45 |
| 6/8/10 | 0.843 | 0.95 | 0.25 | 0.45 | 1.44 |

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



## Appendix C. (continued)

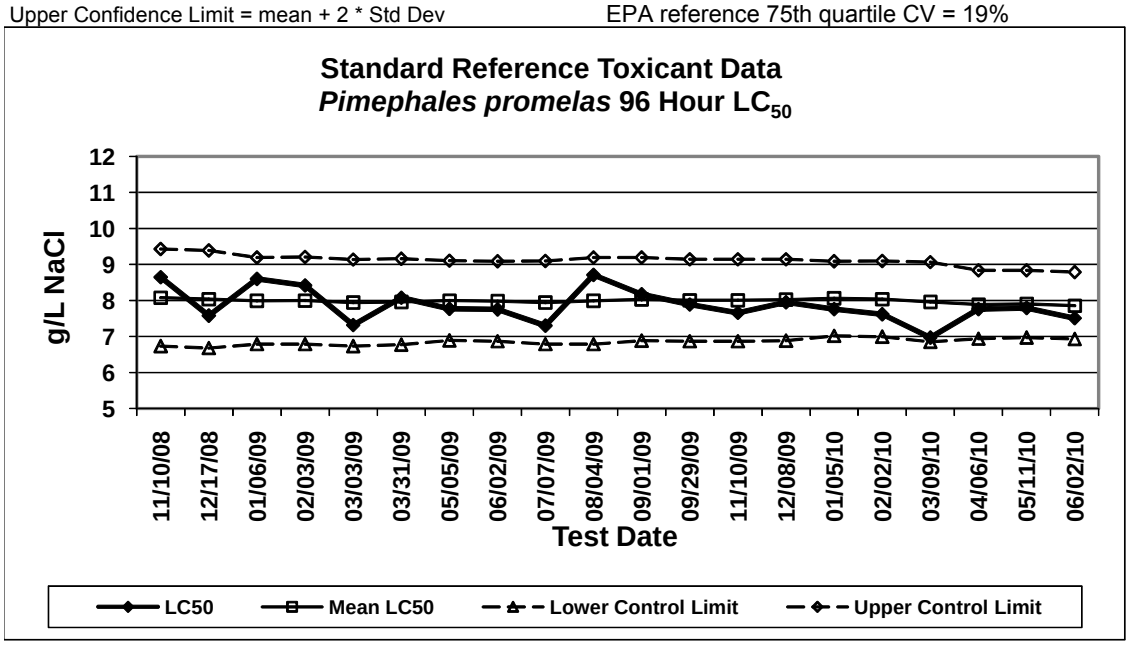
Florida Department of Environmental Protection
Bureau of Laboratories

Standard Reference Toxicant (SRT) Test Data - 96-hour Acute Toxicity
*Pimephales promelas* (fathead minnow) - Sodium Chloride (NaCl)

| Date | LC50 | Mean LC50 | Std Dev | Lower Control Limit | Upper Control Limit |
|---|---|---|---|---|---|
| 11/10/08 | 8.65 | 8.08 | 0.67 | 6.73 | 9.43 |
| 12/17/08 | 7.58 | 8.04 | 0.68 | 6.68 | 9.39 |
| 01/06/09 | 8.60 | 7.99 | 0.6 | 6.79 | 9.19 |
| 02/03/09 | 8.42 | 8 | 0.6 | 6.79 | 9.21 |
| 03/03/09 | 7.32 | 7.94 | 0.6 | 6.73 | 9.14 |
| 03/31/09 | 8.08 | 7.96 | 0.6 | 6.77 | 9.16 |
| 05/05/09 | 7.77 | 8 | 0.55 | 6.89 | 9.11 |
| 06/02/09 | 7.75 | 7.98 | 0.56 | 6.87 | 9.09 |
| 07/07/09 | 7.30 | 7.94 | 0.58 | 6.79 | 9.10 |
| 08/04/09 | 8.71 | 7.99 | 0.60 | 6.79 | 9.19 |
| 09/01/09 | 8.17 | 8.03 | 0.58 | 6.88 | 9.19 |
| 09/29/09 | 7.89 | 8.01 | 0.57 | 6.87 | 9.15 |
| 11/10/09 | 7.66 | 8.01 | 0.57 | 6.87 | 9.15 |
| 12/08/09 | 7.95 | 8.02 | 0.57 | 6.88 | 9.15 |
| 01/05/10 | 7.76 | 8.06 | 0.52 | 7.02 | 9.09 |
| 02/02/10 | 7.62 | 8.04 | 0.53 | 6.99 | 9.10 |
| 03/09/10 | 6.97 | 7.96 | 0.56 | 6.85 | 9.07 |
| 04/06/10 | 7.76 | 7.89 | 0.48 | 6.94 | 8.84 |
| 05/11/10 | 7.79 | 7.9 | 0.47 | 6.97 | 8.84 |
| 06/02/10 | 7.51 | 7.86 | 0.46 | 6.93 | 8.79 |

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

**CV = 5.91**
EPA reference 75th quartile CV = 19%



## FDEP Biology Laboratory - Chronic SRT Bench Sheet

*Ceriodaphnia dubia*

Diluent/ Batch: 20% DMW / 6-7-10 Test Days Used: 0-6
20% DMW / _____ Test Days Used: _____
Toxicant/ Batch: NaCl / 6.7.10

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

Organism: *Ceriodaphnia dubia*
Organism Batch #/ Age: 17-10 / <24 hr.
Feeding: Prior to Test and Daily
Food: ☑ YCT ☑ *P. subcapitata*
Food Batch: ~~10~~ 5-19-10 20 MF 12-23-09
Chamber size: 30 mL
Test Volume: 20 mL

Start Date: 6-8-10 Time: 10:00
End Date: 6-14-10 Time: 1500

| Batch: | 6-7-10 | | | | |
|---|---|---|---|---|---|
| **Water Quality Parameters** | 20% DMW | 20% DMW | Other | Measured by: | Verified by: |
| Lab Total Residual $Cl_2$ (mg/L): | <0.03 | | | KMS | MF |
| Alkalinity (mg/L as $CaCO_3$): | 81 | | | BA | MF |
| Hardness (mg/L as $CaCO_3$): | 84 | | | BA | MF |
| Total Ammonia (mg/L as N): | <0.017 | | | MF ~~MF~~ BA | |
| Conductivity (µmhos-cm) mmhos-cm : | 178 | | | ~~BA~~ BA | MF |

| Results | |
|---|---|
| Control % CV: | 15.5% |
| Dunnetts PMSD: | 18.8% |
| Reproduction NOEC: | 0.59 g/L |
| Reproduction LOEC: | 0.89 g/L |
| Survival NOEC: | 2 g/L |
| Survival LOEC: | 3 g/L |
| $ICP_{20}$: | 0.843 |
| $ICP_{25}$: | 0.917 |

| Day | Concentrations prepared by | Counted by |
|---|---|---|
| 0 | BA | MF |
| 1 | BA | ~~BA~~ |
| 2 | BA | ~~BA~~ |
| 3 | BA | BA |
| 4 | BA | MF |
| 5 | BA | ~~JD~~ |
| 6 | – | MF |
| 7 | | |
| 8 | | |

Loaded by:

| Test Replicate | Loaded from culture vial |
|---|---|
| 1 | B18 |
| 2 | B21 |
| 3 | B13 |
| 4 | B16 |
| 5 | B27 |
| 6 | B5 |
| 7 | B23 |
| 8 | ~~B~~ B17 |
| 9 | B9 |
| 10 | 5 + B14 BA 6.8.10 |

**Comments:** CETIS Test code # 465577446

**Investigators' signatures**

Brad Alexander
Marshall Faircloth
James Daniels
Erin M. Spencer

Liz Miller
REVIEWER

Test Incubated in: Incubator: # 2
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 24.4 max. 26.6 mean 25.5
Room B246 ~~24.9~~ min. ~~25.8~~ max. 25.8 mean 25.4
Test min. 24.0 max. 24.9 mean — MF

## Appendix C. (continued)

*Ceriodaphnia dubia* Survival and Reproduction

Start Date: 6-8-10 Time: 10:00 Loaded by: BA
End Date: 6-14-10 Time: 1500 Verified by: MF

| Concentration | Day | Replicate Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total neonates | Live adults | Neonates per adult |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 3 | 4 | 4 | 3 | 1 | 4 | 3 | 0 | 0 | 0 | 0 | 19 | 10 | 1.9 |
| | 4 | 11 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 4 | 4 | 27 | 10 | 2.7 |
| | 5 | 0 | 8 | 2 | 7 | 8 | 9 | 7 | 8 | 8 | 7 | 64 | 10 | 6.4 |
| | 6 | 10 | 10 | 10 | 13 | 9 | 13 | 12 | 10 | 12 | 6 | 105 | 10 | 10.5 |
| | 7 | | | | | | | | | | | | | |
| | 8 | | | | | | | | | | | | | |
| | Total | 25 | 22 | 15 | 21 | 21 | 25 | 24 | 21 | 24 | 17 | 215 | 10 | 21.5 |
| 0.59 | 0 | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 MF Exhibit | 10 | 0 |
| | 3 | 4 | 4 | 5 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 17 MF | 10 | 1.9 |
| | 4 | 11 | 1(5) | 1(5) | 2 | 0 | 4 | 4 | 4 | 3 | 4 | 32 | 10 | 3.2 |
| | 5 | 0 | 7 | 9 | 5 | 9 | 9 | 3 | 5 | 7 | 7 | 61 | 10 | 6.1 |
| | 6 | 11 | 11 | 11 | 11 | 11 | 12 | 9 | 9 | 10 | 10 | 105 | 10 | 10.5 |
| | 7 | | | | | | | | | | | | | |
| | 8 | CMF folded upon counted 6/21/10 | | | | | | | | | | 7 MF folded incorrectly 7/6 MF | | |
| | Total | 25 | 23 | 26 | 18 | 24 | 25 | 16 | 18 | 20 | 21 | 21.7 MF | 10 | 21.7 |
| 0.89 | 0 | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 3 | 4 | 4 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 14 | 10 | 1.4 |
| | 4 | 0 | 0 | 0 | 2 | 0 | 3 | 4 | 3 | 1 | 0 | 13 | 10 | 1.3 |
| | 5 | 9 | 9 | 7 | 5 | 6 | 8 | 6 | 3 | 7 | 5 | 62 | 10 | 6.2 |
| | 6 | 8 | 10 | 8 | 9 | 9 | 10 | 8 | 9 | 5 | 0 | 76 | 10 | 7.6 |
| | 7 | | | | | | | | | | | | | |
| | 8 | | | | | | | | MF | | | | | |
| | Total | 21 | 23 | 15 | 16 | 18 | 21 | 18 | 215 | 13 | 5 | 165 | 10 | 16.5 |

**Comments:** (5) = Split Brood MF 6-12-10 counted to Day 3

000002

## Appendix C. (continued)

**FDEP Biology Laboratory - Chronic SRT Bench Sheet**

*Ceriodaphnia dubia* Survival and Reproduction

| Concentration | Day | Replicate Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total neonates | Live adults | Neonates per adult |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.3 | 0 | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 3 | 3 | 3 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 10 | 10 | 1.0 |
| | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 2 | 13 | 10 | 1.3 |
| | 5 | 0 | 6 | 7 | 0 | 3 | 8 | 5 | 2 | 4 | 4 | 39 | 10 | 3.9 |
| | 6 | 4 | 8 | 8 | 0 | 7 | 7 | 8 | 5 | 8 | 1 | 56 | 10 | 5.6 |
| | 7 | | | | | | | | | | | | | |
| | 8 | | | | | | | | | | | | | |
| | Total | 14 | 17 | 18 | 0 | 10 | 16 | 16 | 8 | 12 | 7 | 118 | 10 | 11.8 |
| 2.0 | 0 | | | | | | | | | | | | 10 | |
| | 1 | | | | | | | | | | | | 10 | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| | 3 | 0 | 0 | 0 | X | 0 | X | X | 0 | 0 | 0 | 0 | 7 | 0 |
| | 4 | 0 | 0 | 0 | \| | 0 | \| | \| | 0 | 0 | 0 | 0 | 7 | 0 |
| | 5 | 0 | 0 | 1 | \| | 0 | \| | \| | 1 | 0 | 0 | 2 | 7 | 0.2 |
| | 6 | 0 | 0 | 2 | \| | 0 | \| | \| | 0 | 0 | 2 | 4 | 7 | 0.4 |
| | 7 | | | | \| | | \| | \| | | | | | | |
| | 8 | | | | \| | | \| | \| | | | | | | |
| | Total | 0 | 0 | 3 | X | 0 | X | X | 1 | 0 | 2 | 6 | 7(10) | 0.6 |
| 3.0 | 0 | | | | | | | | | | | | 10 | |
| | 1 | X | X | X | X | X | X | X | X | 0 | X | | 1 | |
| | 2 | \| | \| | \| | \| | \| | \| | \| | \| | X | \| | 0 | 0 | 0 |
| | 3 | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| |
| | 4 | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| |
| | 5 | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| |
| | 6 | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| |
| | 7 | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| |
| | 8 | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | ↓ | ↓ | ↓ |
| | Total | X | X | X | X | X | X | X | X | X | X | 0 | 0 | 0 |

**Comments:**

000003

## Appendix C. (continued)

**FDEP Biology Laboratory - Chronic SRT Bench Sheet**

*Ceriodaphnia dubia* Parameters

| Test Day ↓ | Conc. | New Sample Replicate | pH (su) | Temp (°C) | DO (mg/L) | Conductivity (µmhos/cm) (mmhos/cm) | Old Sample Replicate | pH (su) | Temp (°C) | DO (mg/L) | Conductivity (µmhos/cm) (mmhos/cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 0 | Control | M | 7.9 | 24.0 | 7.8 | 0.178 | | | | | |
| | 0.59 | M | 8.0 | 24.0 | 7.8 | 1.32 | | | | | |
| | 0.89 | M | 8.1 | 24.0 | 7.8 | 1.87 | | | | | |
| | 1.3 | M | 8.1 | 24.1 | 7.7 | 2.66 | | | | | |
| | 2.0 | M | 8.1 | 24.2 | 7.6 | 3.82 | | | | | |
| | 3.0 | M | 8.1 | 24.5 | 7.7 | 5.58 | | | | | |
| | Measured by: | | BA | BA | BA | BA | | | | | |
| | Recorded by: | | BA | BA | BA | BA | | | | | |
| Day 1 | Control | M | 7.9 | 24.0 | 7.1 | 0.176 | A | 7.8 | 24.0 | 7.4 | 0.183 |
| | 0.59 | M | 8.0 | 24.0 | 7.2 | 1.29 | A | 7.9 | 24.0 | 7.4 | 1.35 |
| | 0.89 | M | 8.1 | 24.1 | 7.2 | 1.86 | A | 8.0 | 24.0 | 7.3 | 2.10 |
| | 1.3 | M | 8.1 | 24.1 | 7.2 | 2.64 | A | 8.0 | 24.0 | 7.4 | 2.82 |
| | 2.0 | M | 8.1 | 24.1 | 7.3 | 3.80 | A | 8.0 | 24.0 | 7.4 | 4.18 |
| | 3.0 | M | 8.1 | 24.1 | 7.3 | 5.53 | A | 8.0 | 24.0 | 7.4 | 5.98 |
| | Measured by: | | BA | BA | BA | BA | | BA | BA | BA | BA |
| | Recorded by: | | BA | BA | BA | BA | | BA | BA | BA | BA |
| Day 2 | Control | M | 8.0 | 24.0 | 6.9 | 0.176 | A | 7.9 | 24.0 | 6.5 | 0.199 |
| | 0.59 | M | 8.0 | 24.2 | 7.0 | 1.29 | A | 8.0 | 24.0 | 6.6 | 1.43 |
| | 0.89 | M | 8.1 | 24.4 | 7.1 | 1.85 | A | 8.1 | 24.0 | 6.8 | 1.94 |
| | 1.3 | M | 8.1 | 24.5 | 7.1 | 2.64 | A | 8.1 | 24.0 | 6.8 | 2.73 |
| | 2.0 | M | 8.1 | 24.6 | 7.1 | 3.79 | A | 8.1 | 24.0 | 6.9 | 3.88 |
| | 3.0 | M | 8.1 | 24.8 | 7.2 | 5.51 | BA I | 8.1 | 24.0 | 7.0 | 5.61 |
| | Measured by: | | BA | BA | BA | BA | | BA | BA | BA | BA |
| | Recorded by: | | BA | BA | BA | BA | | BA | BA | BA | BA |
| Day 3 | Control | M | 7.6 | 24.0 | 7.4 | 0.179 | A | 7.9 | 24.0 | 7.0 | 0.185 |
| | 0.59 | M | 7.8 | ~~24~~ 24.0 BA | 7.4 | 1.32 | A | 7.9 | 24.0 | 7.1 | 1.35 |
| | 0.89 | M | 7.9 | 24.3 | 7.4 | 1.87 | A | 8.0 | 24.0 | 7.2 | 2.00 |
| | 1.3 | M | 8.0 | 24.1 | 7.4 | 2.60 | A | 8.0 | 24.0 | 7.2 | 2.74 |
| | 2.0 | M | 8.0 | 24.2 | 7.4 | 3.84 | A | 8.0 | 24.0 | 7.2 | 3.88 |
| | | M | | | | | | | | | |
| | Measured by: | | BA | BA | BA | BA | | BA | BA | BA | BA |
| | Recorded by: | | BA | BA | BA | BA | | BA | BA | BA | BA |

**Comments:**

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

Test Page 4 of 6 PAGE 000004 07/15/08 MF

## Appendix C. (continued)

**FDEP Biology Laboratory - Chronic SRT Bench Sheet**

*Ceriodaphnia dubia* Parameters

| Test Day ↓ | Conc. | New Sample Replicate | pH (su) | Temp (°C) | DO (mg/L) | Conductivity (µmhos/cm) (mmhos/cm) | Old Sample Replicate | pH (su) | Temp (°C) | DO (mg/L) | Conductivity (µmhos/cm) (mmhos/cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 4 | Control | M | 7.9 | 24.8 | 8.0 | 0.200 | A | 8.1 | 24.7 | 7.8 | 0,194 |
| | 0.59 | M | 8.0 | 25.4 | 8.2 | 1.27 | A | 8.1 | 24.6 | 7.8 | 1.38 |
| | 0.89 | M | 8.1 | 25.6 | 8.1 | 1.85 | A | 8.1 | 24.9 | 7.9 | 2.01 |
| | 1.3 | M | 8.1 | 25.7 | 8.3 | 2.65 | A | 8.1 | 24.8 | 8.0 | 2.78 |
| | 2.0 | M | 8.1 | 25.7 | 8.1 | 3.78 | A | 8.0 | 24.8 | 7.7 | 3.85 |
| | | M | | | | | | | | | |
| | Measured by: | | [illegible] | [illegible] | [illegible] | [illegible] | | MF | MF | MF | MF |
| | Recorded by: | | [illegible] | [illegible] | [illegible] | [illegible] | | MF | MF | MF | MF |
| Day 5 | Control | M | 8.2 | 24.9 | 7.9 | 0.201 | A | 7.8 | 24.1 | 7.7 | 0,320 |
| | .59 | M | 8.2 | 24.9 | 7.9 | 1.27 | A | 8.0 | 24.0 | 7.8 | 1.32 |
| | .89 | M | 8.2 | 24.9 | 8.0 | 1.85 | A | 8.0 | 24.0 | 7.8 | 2.04 |
| | 1.3 | M | 8.2 | 24.8 | 7.9 | 2.64 | A | 8.1 | 24.0 | 7.9 | 2.72 |
| | 2.0 | M | 8.2 | 24.7 | 8.2 | 3.81 | A | 8.1 | 24.0 | 8.1 | 3.82 |
| | | M | | | | | | | | | |
| | Measured by: | | [illegible] | [illegible] | [illegible] | [illegible] | | [illegible] | [illegible] | [illegible] | [illegible] |
| | Recorded by: | | [illegible] | [illegible] | [illegible] | [illegible] | | [illegible] | [illegible] | [illegible] | [illegible] |
| Day 6 | Control | M | | | | | A | 8.2 | 24.5 | 8.1 | 0.205 |
| | 0.59 | M | | | | | A | 8.1 | 24.4 | 8.0 | 1.39 |
| | 0.89 | M | | | | | A | 8.1 | 24.2 | 8.0 | 1.85 |
| | 1.3 | M | | | | | A | 8.1 | 24.3 | 8.0 | 2.83 |
| | 2.0 | M | | | | | A | 8.1 | 24.3 | 8.0 | 3.87 |
| | | M | | | | | | | | | |
| | Measured by: | | | | | | | MF | MF | MF | MF |
| | Recorded by: | | | | | | | MF | MF | MF | MF |
| Day 7 | Control | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | Measured by: | | | | | | | | | | |
| | Recorded by: | | | | | | | | | | |
| Day 8 | Control | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | | M | | | | | | | | | |
| | Measured by: | | | | | | | | | | |
| | Recorded by: | | | | | | | | | | |

Day 6: Avg Rinsed 6-7 MF

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

000005

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

## Appendix C. (continued)

**Bioassay Instrument ID**

Test ID: Cdbo Chronic SRT  Dates: 6-8 - 6/14/2010

| 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 | - | ~ |
| | | | | | |

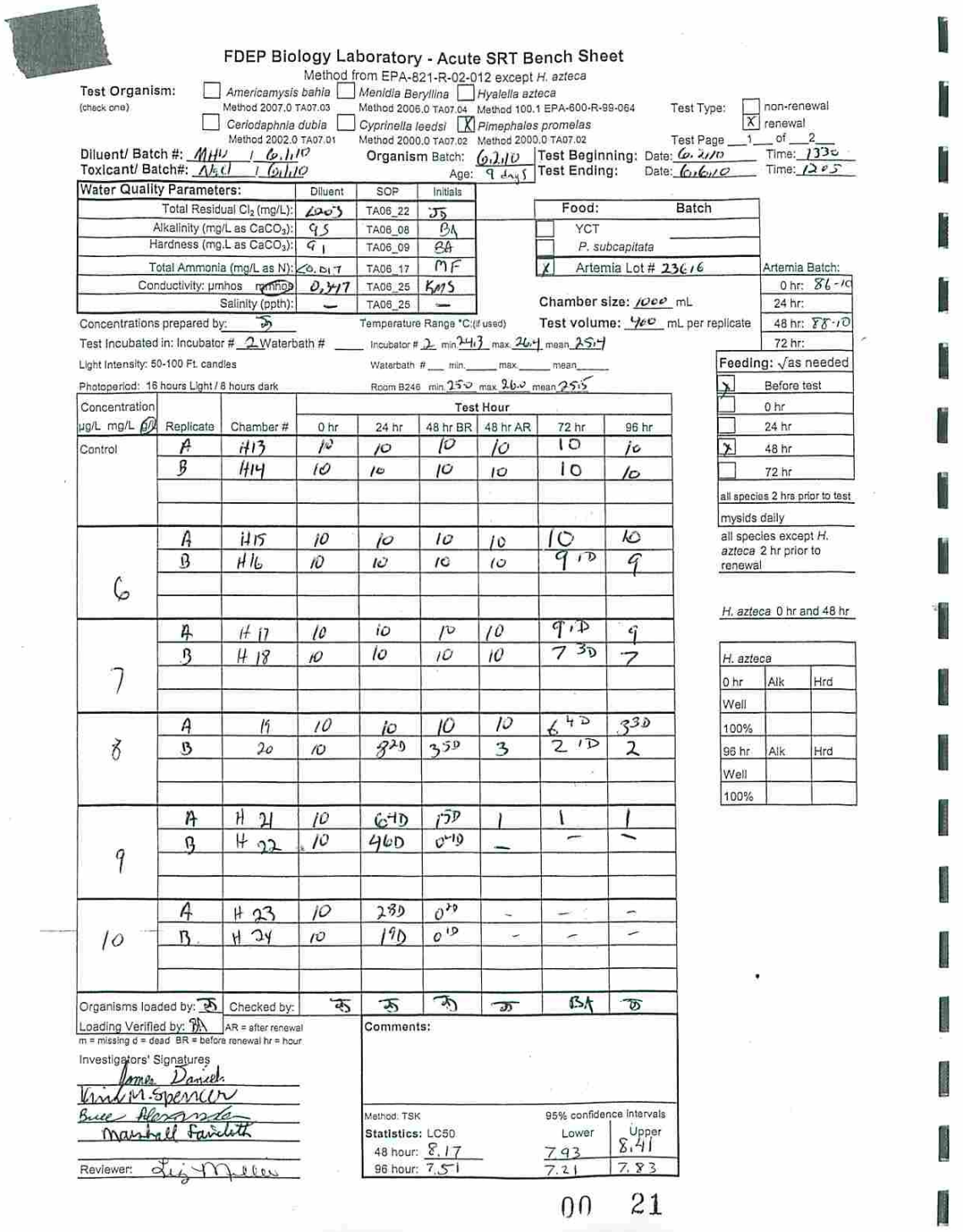
SM-2530B modified to allow use of electronic and non-mercury filled thermometers.
Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT–Chronic SRT)

entered by BA 6-14-10

V 1.1 6/12/08 MF

PAGE

## Appendix C. (continued)



FDEP Biology Laboratory - Acute SRT Bench Sheet

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

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

Test Type: ☐ non-renewal ☒ renewal

Test Page 1 of 2

Diluent/ Batch #: MHW / 6.1.10

Toxicant/ Batch#: NaCl / 6.1.10

Organism Batch: 6.2.10 Age: 9 days

Test Beginning: Date: 6.2.10 Time: 1330

Test Ending: Date: 6.6.10 Time: 1205

| Water Quality Parameters: | Diluent | SOP | Initials |
|---|---|---|---|
| Total Residual $Cl_2$ (mg/L): | 0.003 | TA06_22 | JS |
| Alkalinity (mg/L as $CaCO_3$): | 95 | TA06_08 | BA |
| Hardness (mg.L as $CaCO_3$): | 91 | TA06_09 | BA |
| Total Ammonia (mg/L as N): | <0.017 | TA06_17 | MF |
| Conductivity: µmhos mmhos | 0.347 | TA06_25 | KMS |
| Salinity (ppth): | — | TA06_25 | — |

| Food: | Batch |
|---|---|
| ☐ YCT | |
| ☐ *P. subcapitata* | |
| ☒ Artemia Lot # 23616 | |

Artemia Batch:
- 0 hr: 86-10
- 24 hr:
- 48 hr: 88-10
- 72 hr:

Chamber size: 1000 mL

Test volume: 400 mL per replicate

Concentrations prepared by: JS

Temperature Range °C:(if used)

Test Incubated in: Incubator # 2 Waterbath # ____ Incubator # 2 min 24.3 max 26.4 mean 25.4

Light Intensity: 50-100 Ft. candles

Waterbath # ___ min.____ max.____ mean____

Photoperiod: 16 hours Light / 8 hours dark

Room B246 min 25.0 max 26.0 mean 25.5

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 (circle) | Replicate | Chamber # | Test Hour 0 hr | 24 hr | 48 hr BR | 48 hr AR | 72 hr | 96 hr |
|---|---|---|---|---|---|---|---|---|
| Control | A | H13 | 10 | 10 | 10 | 10 | 10 | 10 |
| | B | H14 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | A | H15 | 10 | 10 | 10 | 10 | 10 | 10 |
| | B | H16 | 10 | 10 | 10 | 10 | 9 1D | 9 |
| 7 | A | H 17 | 10 | 10 | 10 | 10 | 9 1D | 9 |
| | B | H 18 | 10 | 10 | 10 | 10 | 7 3D | 7 |
| 8 | A | 19 | 10 | 10 | 10 | 10 | 6 4D | 3 3D |
| | B | 20 | 10 | 8 2D | 3 3D | 3 | 2 1D | 2 |
| 9 | A | H 21 | 10 | 6 4D | 1 3D | 1 | 1 | 1 |
| | B | H 22 | 10 | 4 6D | 0 4D | — | — | — |
| 10 | A | H 23 | 10 | 2 8D | 0 2D | - | — | — |
| | B | H 24 | 10 | 1 9D | 0 1D | - | — | — |

| *H. azteca* | | |
|---|---|---|
| 0 hr | Alk | Hrd |
| Well | | |
| 100% | | |
| 96 hr | Alk | Hrd |
| Well | | |
| 100% | | |

Organisms loaded by: JS Checked by: JS JS JS JS BA JS

Loading Verified by: BA AR = after renewal

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

Comments:

Investigators' Signatures

James Daniels

Kristi M. Spencer

Bruce Alexander

Marshall Faircloth

Reviewer: Liz Miller

| Method: TSK | 95% confidence intervals | |
|---|---|---|
| Statistics: LC50 | Lower | Upper |
| 48 hour: 8.17 | 7.93 | 8.41 |
| 96 hour: 7.51 | 7.21 | 7.83 |

00 21

## 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: 6.2.10 Time: 1330
**Test Ending:** Date: 6.6.10 Time: 1205

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 | 8.1 | 24.1 | 7.7 | 347 | — |
| | B | 24 hr | 8.1 | 24.4 | 7.3 | 0.371 | - |
| | A | 48 hr BR | 8.2 | 24.7 | 7.7 | 0.378 | - |
| | A | 48 hr AR | 7.9 | 24.0 | 8.0 | 0.410 | - |
| | B | 72 hr | 7.6 | 24.9 | 6.5 | 0.347 | - |
| | A | 96 hr | 7.8 | 24.5 | 8.1 | 0.370 | - |
| 6 | A | 0 hr | 8.0 | 25.2 | 7.8 | 10.8 | — |
| | B | 24 hr | 7.9 | 24.2 | 7.1 | 11.3 | - |
| | A | 48 hr BR | 7.9 | 25.2 | 7.7 | 11.3 | — |
| | A | 48 hr AR | 7.9 | 24.3 | 7.8 | 10.9 | - |
| | B | 72 hr | 7.6 | 24.5 | 6.5 | 10.9 | - |
| | A | 96 hr | 7.7 | 24.6 | 7.9 | 11.2 | - |
| 7 | A | 0 hr | 8.0 | 25.4 | 7.6 | 12.9 | — |
| | B | 24 hr | 7.8 | 24.2 | 7.1 | 12.9 | - |
| | A | 48 hr BR | 7.9 | 25.0 | 7.6 | 12.9 | - |
| | A | 48 hr AR | 7.9 | 24.4 | 7.7 | 12.8 | - |
| | B | 72 hr | 7.6 | 24.3 | 6.4 | 12.9 | - |
| | A | 96 hr | 7.7 | 24.5 | 7.9 | 13.3 | - |
| 8 | A | 0 hr | 8.0 | 25.6 | 7.7 | 14.6 | — |
| | B | 24 hr | 7.8 | 24.2 | 7.2 | 14.7 | - |
| | A | 48 hr BR | 7.7 | 25.0 | 7.8 | 14.4 | - |
| | A | 48 hr AR | 7.8 | 24.5 | 7.5 | 14.4 | - |
| | B | 72 hr | 7.6 | 24.5 | (A) ~~7.7~~ 6.4 | 14.4 | - |
| | | 96 hr | 7.8 | 24.5 | 8.1 | 14.8 | - |
| 9 | A | 0 hr | 8.0 | 25.8 | 7.6 | 16.2 | — |
| | B | 24 hr | 7.7 | 24.5 | 7.0 | 16.1 | - |
| | A | 48 hr BR | 7.9 | 25.1 | 7.5 | 16.2 | - |
| | A | 48 hr AR | 7.9 | 24.9 | 7.7 | 16.3 | - |
| | A | 72 hr | 7.7 | 24.5 | 5.7 | 16.3 | - |
| | | 96 hr | 7.7 | 24.5 | 7.6 | 16.8 | - |
| 10 | A | 0 hr | 8.0 | 25.8 | 7.7 | 17.9 | — |
| | B | 24 hr | 7.7 | 24.2 | 6.8 | 17.8 | - |
| | A | 48 hr BR | 7.9 | 24.9 | 7.4 | 18.0 | - |
| | | 48 hr AR | | | | | |
| | | 72 hr | | | | | |
| | | 96 hr | | | | | |

Investigators Signatures
James Daniels
Kristi Spencer
Bruce Alexander
Marshall Fairchild

Reviewed By: Liz Miller

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

Measure/record salinity for marine tests

Comments: (A) Transcription error PSA 6.4.10

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Revised 07/15/2008 MF

## Appendix C. (continued)

**CETIS Summary Report**

Report Date: 19 Aug-10 08:19 (p 1 of 1)
Test Code: 11-8000-5446/46557446

**Ceriodaphnia 7-d Survival and Reproduction Test** — DEP-Bureau of Labs

| | | | | | |
|---|---|---|---|---|---|
| Batch ID: | 11-3950-2904 | Test Type: | Survival-Reproduction | Analyst: | |
| Start Date: | 08 Jun-10 10:00 | Protocol: | EPA/821/R-02-013 (2002) | Diluent: | 20% DMW |
| Ending Date: | 14 Jun-10 15:00 | Species: | Ceriodaphnia dubia | Brine: | Not Applicable |
| Duration: | 6d 5h | Source: | In-House Culture | Age: | < 24 |
| Sample ID: | 10-2100-4086 | Code: | 3CDB4936 | Client: | Internal Lab |
| Sample Date: | 08 Jun-10 | Material: | Sodium chloride | Project: | Concurrent SRT |
| Receive Date: | 08 Jun-10 | Source: | Reference Toxicant | | |
| Sample Age: | 10h | Station: | In House | | |

**Comparison Summary**

| Analysis ID | Endpoint | NOEL | LOEL | TOEL | PMSD | TU | Method |
|---|---|---|---|---|---|---|---|
| 10-6062-6550 | Reproduction | 0.59 | 0.89 | 0.7246 | 18.79% | | Steel Many-One Rank Test |
| 17-0072-3927 | | 0.59 | 0.89 | 0.7246 | 18.79% | | Steel Many-One Rank Test |

**Point Estimate Summary**

| Analysis ID | Endpoint | Level | gm/L | 95% LCL | 95% UCL | TU | Method |
|---|---|---|---|---|---|---|---|
| 15-7814-3506 | Reproduction | IC5 | 0.6497 | 0.2189 | 0.7229 | | Linear Interpolation (ICPIN) |
| | | IC10 | 0.7117 | 0.4856 | 0.8954 | | |
| | | IC15 | 0.776 | 0.6337 | 0.9529 | | |
| | | IC20 | 0.8427 | 0.7026 | 1.023 | | |
| | | IC25 | 0.9168 | 0.7636 | 1.112 | | |
| | | IC40 | 1.194 | 0.9797 | 1.39 | | |
| | | IC50 | 1.357 | 1.142 | 1.486 | | |

**Reproduction Summary**

| Conc-gm/L | Control Type | Count | Mean | 95% LCL | 95% UCL | Min | Max | Std Err | Std Dev | CV% | Diff% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Dilution Water | 10 | 21.5 | 20.25 | 22.75 | 15 | 25 | 0.6101 | 3.342 | 15.54% | 0.0% |
| 0.59 | | 10 | 21.6 | 20.29 | 22.91 | 16 | 26 | 0.6394 | 3.502 | 16.21% | -0.47% |
| 0.89 | | 10 | 16.5 | 14.59 | 18.41 | 5 | 23 | 0.9359 | 5.126 | 31.07% | 23.26% |
| 1.3 | | 10 | 11.8 | 9.697 | 13.9 | 0 | 18 | 1.028 | 5.633 | 47.74% | 45.12% |
| 2 | | 10 | 0.6 | 0.1986 | 1.001 | 0 | 3 | 0.1963 | 1.075 | 179.2% | 97.21% |
| 3 | | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 100.0% |

**Reproduction Detail**

| Conc-gm/L | Control Type | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 | Rep 9 | Rep 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Dilution Water | 25 | 22 | 15 | 21 | 21 | 25 | 24 | 21 | 24 | 17 |
| 0.59 | | 25 | 23 | 26 | 18 | 24 | 25 | 16 | 18 | 20 | 21 |
| 0.89 | | 21 | 23 | 15 | 16 | 18 | 21 | 18 | 15 | 13 | 5 |
| 1.3 | | 14 | 17 | 18 | 0 | 10 | 16 | 16 | 8 | 12 | 7 |
| 2 | | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## Appendix C. (continued)

***Pimephales promelas* Acute Standard Reference Toxicant Test**

DATE: 6-2-10 DURATION: 96 h TOXICANT: NaCl
SPECIES: *Pimephales promelas*

| RAW DATA: Concentration (g/L) | Number Exposed | Mortalities |
|---|---|---|
| .00 | 20 | 0 |
| 6.00 | 20 | 1 |
| 7.00 | 20 | 4 |
| 8.00 | 20 | 15 |
| 9.00 | 20 | 19 |
| 10.00 | 20 | 20 |

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: **7.51**
95% LOWER CONFIDENCE: **7.21**
95% UPPER CONFIDENCE: **7.83**

## Appendix D. Test Organism Receipts

MARINCO BIOASSAY LABORATORY
The Aquatic Toxicology Specialists

NELAP Certification # E84191

### Shipment Record

**State of Florida Aquaculture Certificate Number AQ0668007**

Shipping Date: 6/1/10

Ship to: FDEP

P.O. No: _____

| Species | Quantity | Age | Brood/Lot Number | Temp. (°C) | pH (S.U.) | Salinity (‰) |
|---|---|---|---|---|---|---|
| *Mysidopsis bahia* | 150 | 4 DAYS | MS100528 | 25 | 80 | 20 |
| *Menidia beryllina* | 150 | 11 DAYS | SS100521 | 25 | 8.0 | 20 |
| | | | | | | Hardness mg/L |
| *Cyprinella leedsi* | 150 | 7 DAYS | CL100525 | 25 | 7.8 | 80 |
| *Pimephales promelas* | 150 | 9 DAYS | FM100523 | 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: 6/7/10

Ship to: FDEP

P.O. No: _____

| Species | Quantity | Age | Brood/Lot Number | Temp. (°C) | pH (S.U.) | Salinity (‰) |
|---|---|---|---|---|---|---|
| *Mysidopsis bahia* | 150 | 4 DAYS | MS100603 | 25 | 8.0 | 20 |
| *Menidia beryllina* | 150 ac | 12 DAYS | SS100528 | 25 | 8.0 | 20 |
| | | | | | | Hardness mg/L |
| *Cyprinella leedsi* | | | | | | |
| *Pimephales promelas* | 350 | <24 HRS | ~~PM~~ FM100607-Dec | 25 | 7.8 | 80 |
| *Ceriodaphnia dubia* | | | | 25 | 7.8 | 80 |
| *Daphnia magna* | | | | | | |
| YCT | | | | | | |
| *P. subcapitata* | | | | | | |

Packed by: BM

Shipped Via: FedEx

Notes: No charge for mysids

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 Bayou Marcus Water Reclamation Facility Outfall D-002, sampled on June 7, 2010.**

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:30 | BLANK | Metals | Magnesium | 0.04 | U | mg/L | 0.04 | 0.16 |
| 6/7/2010 11:30 | BLANK | Metals | Copper | 0.5 | U | ug/L | 0.5 | 1 |
| 6/7/2010 11:30 | BLANK | Metals | Aluminum | 60 | U | ug/L | 60 | 240 |
| 6/7/2010 11:30 | BLANK | Metals | Lead | 0.2 | U | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:30 | BLANK | Metals | Nickel | 0.25 | U | ug/L | 0.25 | 1 |
| 6/7/2010 11:30 | BLANK | Metals | Silver | 0.025 | U | ug/L | 0.025 | 0.1 |
| 6/7/2010 11:30 | BLANK | Metals | Calcium | 0.075 | U | mg/L | 0.075 | 0.3 |
| 6/7/2010 11:30 | BLANK | Metals | Cadmium | 0.03 | U | ug/L | 0.03 | 0.12 |
| 6/7/2010 11:30 | BLANK | Metals | Zinc | 5 | U | ug/L | 5 | 20 |
| 6/7/2010 11:30 | BLANK | Metals | Arsenic | 0.25 | U | ug/L | 0.25 | 1 |
| 6/7/2010 11:30 | BLANK | Metals | Chromium | 0.3 | U | ug/L | 0.3 | 1.2 |
| 6/7/2010 11:30 | BLANK | Metals | Iron | 30 | U | ug/L | 30 | 120 |
| 6/7/2010 11:30 | BLANK | Metals | Selenium | 0.2 | U | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:30 | BLANK | Nutrients | Ammonia-N | 0.01 | U | mg N/L | 0.01 | 0.02 |
| 6/7/2010 11:30 | BLANK | Nutrients | Total-P | 0.004 | U | mg P/L | 0.004 | 0.01 |
| 6/7/2010 11:30 | BLANK | Nutrients | NO2NO3-N | 0.004 | U | mg N/L | 0.004 | 0.01 |
| 6/7/2010 11:30 | BLANK | Nutrients | O-Phosphate-P | 0.004 | U | mg P/L | 0.004 | 0.01 |
| 6/7/2010 11:30 | BLANK | Nutrients | Kjeldahl Nitrogen | 0.08 | U | mg N/L | 0.08 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Chlorpyrifos Methyl | 0.099 | U | ug/L | 0.099 | 0.4 |
| 6/7/2010 11:30 | BLANK | Pesticides | Prometryn | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:30 | BLANK | Pesticides | Atrazine | 0.049 | U | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Azinphos Methyl | 0.2 | UJ | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:30 | BLANK | Pesticides | Diazinon | 0.049 | UJ | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Ethion | 0.049 | U | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Malathion | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:30 | BLANK | Pesticides | Chlorpyrifos Ethyl | 0.049 | U | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Norflurazon | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:30 | BLANK | Pesticides | Ametryn | 0.049 | U | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Naled | 0.79 | UJ | ug/L | 0.79 | 3.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Butylate | 0.2 | UJ | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:30 | BLANK | Pesticides | Bromacil | 0.2 | UJ | ug/L | 0.2 | 0.8 |

## Appendix E. (continued)

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:30 | BLANK | Pesticides | Parathion Ethyl | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:30 | BLANK | Pesticides | Fonofos | 0.099 | U | ug/L | 0.099 | 0.4 |
| 6/7/2010 11:30 | BLANK | Pesticides | Alachlor | 0.59 | U | ug/L | 0.59 | 2.4 |
| 6/7/2010 11:30 | BLANK | Pesticides | Simazine | 0.049 | U | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Parathion Methyl | 0.099 | U | ug/L | 0.099 | 0.4 |
| 6/7/2010 11:30 | BLANK | Pesticides | Mevinphos | 0.2 | U | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:30 | BLANK | Pesticides | Metolachlor | 0.49 | UJ | ug/L | 0.49 | 2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Fenamiphos | 0.2 | U | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:30 | BLANK | Pesticides | Metalaxyl | 0.25 | UJ | ug/L | 0.25 | 1 |
| 6/7/2010 11:30 | BLANK | Pesticides | Hexazinone | 0.099 | U | ug/L | 0.099 | 0.4 |
| 6/7/2010 11:30 | BLANK | Pesticides | Metribuzin | 0.099 | U | ug/L | 0.099 | 0.4 |
| 6/7/2010 11:30 | BLANK | Pesticides | Phorate | 0.049 | U | ug/L | 0.049 | 0.2 |
| 6/7/2010 11:30 | BLANK | Pesticides | Ethoprop | 0.099 | U | ug/L | 0.099 | 0.4 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2,4-Dinitrotoluene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 4-Nitrophenol | 15 | U | ug/L | 15 | 58 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Acenaphthylene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Anthracene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Benzidine | 97 | U | ug/L | 97 | 390 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Benzo(a)anthracene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Benzo(a)pyrene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Chrysene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2,4-Dinitrophenol | 15 | U | ug/L | 15 | 58 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Benzo(g,h,i)perylene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Bis(2-chloroethoxy)methane | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Bis(2-chloroisopropyl)ether | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Bis(2-ethylhexyl)phthalate | 15 | U | ug/L | 15 | 58 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Di-n-octyl phthalate | 0.97 | UJ | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | N-Nitrosodimethylamine | 1.9 | U | ug/L | 1.9 | 7.8 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 1,2,4-Trichlorobenzene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2-Nitrophenol | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 3,3'-Dichlorobenzidine | 39 | U | ug/L | 39 | 160 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Hexachlorobutadiene | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Hexachlorocyclopentadiene | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Indeno(1,2,3-cd)pyrene | 0.97 | U | ug/L | 0.97 | 3.9 |

## Appendix E. (continued)

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | N-Nitrosodi-n-propylamine | 1.9 | U | ug/L | 1.9 | 7.8 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Nitrobenzene | 1.9 | U | ug/L | 1.9 | 7.8 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | N-Nitrosodiphenylamine/ Diphenylamine | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2,6-Dinitrotoluene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 4-Chloro-3-methylphenol | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 4-Chlorophenyl phenyl ether | 1.9 | UJ | ug/L | 1.9 | 7.8 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Butyl benzyl phthalate | 4.9 | U | ug/L | 4.9 | 19 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Diethyl phthalate | 0.97 | UJ | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Dimethyl phthalate | 9.7 | U | ug/L | 9.7 | 39 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Isophorone | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2,4,6-Trichlorophenol | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2,4-Dichlorophenol | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2,4-Dimethylphenol | 9.7 | U | ug/L | 9.7 | 39 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 4-Bromophenyl phenyl ether | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Di-n-butyl phthalate | 4.9 | UJ | ug/L | 4.9 | 19 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Hexachlorobenzene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Hexachloroethane | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Pentachlorophenol | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Phenanthrene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Benzo(k)fluoranthene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Phenol | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Pyrene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2-Chloronaphthalene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2-Chlorophenol | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | 2-Methyl-4,6-dinitrophenol | 2.9 | U | ug/L | 2.9 | 12 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Acenaphthene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Benzo(b)fluoranthene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Bis(2-chloroethyl)ether | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Dibenzo(a,h)anthracene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Fluoranthene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Fluorene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:30 | BLANK | Priority Organic Pollutants | Naphthalene | 0.97 | U | ug/L | 0.97 | 3.9 |
| 6/7/2010 11:40 | EFFLUENT | AGP/LN | Algal Growth Potential | 34.6 | | mg DryWt/L | 0.3 | 0.9 |
| 6/7/2010 11:40 | EFFLUENT | AGP/LN | Algal Growth Potential-Salinity Adjusted | 12.2 | | mg DryWt/L | 3 | 3 |

## Appendix E. (continued)

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:40 | EFFLUENT | Chlorophyll/Grain Size/BOD | Phaeophytin-a | 2.4 | | ug/L | 0.4 | 1.2 |
| 6/7/2010 11:40 | EFFLUENT | Chlorophyll/Grain Size/BOD | Biochemical Oxygen Demand-5 Day,N-Inhib | 0.92 | I | mg/L | 0.2 | 2 |
| 6/7/2010 11:40 | EFFLUENT | Chlorophyll/Grain Size/BOD | Chlorophyll-a, Corrected | 1.7 | I | ug/L | 0.55 | 1.7 |
| 6/7/2010 11:40 | EFFLUENT | Overflow | Fecal Coliforms-Membrane Filter | 4 | B | #/100 mL | 1 | 1 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Iron | 30 | U | ug/L | 30 | 120 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Lead | 0.26 | I | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Arsenic | 0.55 | I | ug/L | 0.25 | 1 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Copper | 1.06 | | ug/L | 0.5 | 1 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Aluminum | 77 | I | ug/L | 60 | 240 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Chromium | 0.3 | I | ug/L | 0.3 | 1.2 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Calcium | 22.8 | | mg/L | 0.075 | 0.3 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Selenium | 0.22 | I | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Nickel | 2.17 | | ug/L | 0.25 | 1 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Magnesium | 12.9 | | mg/L | 0.04 | 0.16 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Zinc | 39 | | ug/L | 5 | 20 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Silver | 0.025 | U | ug/L | 0.025 | 0.1 |
| 6/7/2010 11:40 | EFFLUENT | Metals | Cadmium | 0.03 | U | ug/L | 0.03 | 0.12 |
| 6/7/2010 11:40 | EFFLUENT | Nutrients | Ammonia-N | 0.039 | | mg N/L | 0.01 | 0.02 |
| 6/7/2010 11:40 | EFFLUENT | Nutrients | Total-P | 0.11 | A | mg P/L | 0.005 | 0.05 |
| 6/7/2010 11:40 | EFFLUENT | Nutrients | Kjeldahl Nitrogen | 0.91 | | mg N/L | 0.08 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Nutrients | O-Phosphate-P | 0.053 | | mg P/L | 0.004 | 0.01 |
| 6/7/2010 11:40 | EFFLUENT | Nutrients | NO2NO3-N | 0.46 | | mg N/L | 0.004 | 0.01 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Atrazine | 0.12 | I | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Azinphos Methyl | 0.2 | UJ | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Butylate | 0.2 | UJ | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Diazinon | 0.05 | UJ | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Ethoprop | 0.1 | U | ug/L | 0.1 | 0.4 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Fonofos | 0.1 | U | ug/L | 0.1 | 0.4 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Naled | 0.8 | UJ | ug/L | 0.8 | 3.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Metalaxyl | 0.25 | UJ | ug/L | 0.25 | 1 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Parathion Ethyl | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Prometryn | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Ethion | 0.05 | U | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Parathion Methyl | 0.1 | U | ug/L | 0.1 | 0.4 |

## Appendix E. (continued)

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Simazine | 0.05 | U | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Metolachlor | 0.5 | UJ | ug/L | 0.5 | 2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Alachlor | 0.6 | U | ug/L | 0.6 | 2.4 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Bromacil | 0.2 | UJ | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Fenamiphos | 0.2 | U | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Norflurazon | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Hexazinone | 0.1 | U | ug/L | 0.1 | 0.4 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Malathion | 0.15 | U | ug/L | 0.15 | 0.6 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Metribuzin | 0.1 | U | ug/L | 0.1 | 0.4 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Phorate | 0.05 | U | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Ametryn | 0.05 | U | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Chlorpyrifos Ethyl | 0.05 | U | ug/L | 0.05 | 0.2 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Chlorpyrifos Methyl | 0.1 | U | ug/L | 0.1 | 0.4 |
| 6/7/2010 11:40 | EFFLUENT | Pesticides | Mevinphos | 0.2 | U | ug/L | 0.2 | 0.8 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2,4-Dinitrophenol | 15 | U | ug/L | 15 | 61 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Nitrobenzene | 2 | U | ug/L | 2 | 8.2 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2-Nitrophenol | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Benzo(g,h,i)perylene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Indeno(1,2,3-cd)pyrene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | N-Nitrosodi-n-propylamine | 2 | U | ug/L | 2 | 8.2 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | N-Nitrosodiphenylamine/ Diphenylamine | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2-Chlorophenol | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Hexachlorobenzene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Dibenzo(a,h)anthracene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Di-n-butyl phthalate | 5.1 | UJ | ug/L | 5.1 | 20 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Acenaphthylene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 4-Nitrophenol | 15 | U | ug/L | 15 | 61 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2-Chloronaphthalene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2,4,6-Trichlorophenol | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2,4-Dinitrotoluene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 4-Chloro-3-methylphenol | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Acenaphthene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Bis(2-chloroisopropyl)ether | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Bis(2-ethylhexyl)phthalate | 15 | U | ug/L | 15 | 61 |

## Appendix E. (continued)

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Chrysene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Fluoranthene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Phenanthrene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Benzo(a)anthracene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Benzo(k)fluoranthene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Bis(2-chloroethyl)ether | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Dimethyl phthalate | 10 | U | ug/L | 10 | 41 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Fluorene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Hexachlorocyclopentadiene | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Isophorone | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Benzo(a)pyrene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Hexachlorobutadiene | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Pentachlorophenol | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2-Methyl-4,6-dinitrophenol | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 4-Chlorophenyl phenyl ether | 2 | UJ | ug/L | 2 | 8.2 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Butyl benzyl phthalate | 5.1 | U | ug/L | 5.1 | 20 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Di-n-octyl phthalate | 1 | UJ | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Hexachloroethane | 3.1 | U | ug/L | 3.1 | 12 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Naphthalene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 3,3'-Dichlorobenzidine | 41 | U | ug/L | 41 | 160 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 4-Bromophenyl phenyl ether | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Anthracene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Benzidine | 100 | U | ug/L | 100 | 410 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Benzo(b)fluoranthene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Diethyl phthalate | 1 | UJ | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Pyrene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 1,2,4-Trichlorobenzene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2,4-Dichlorophenol | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2,4-Dimethylphenol | 10 | U | ug/L | 10 | 41 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | 2,6-Dinitrotoluene | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Bis(2-chloroethoxy)methane | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | N-Nitrosodimethylamine | 2 | U | ug/L | 2 | 8.2 |
| 6/7/2010 11:40 | EFFLUENT | Priority Organic Pollutants | Phenol | 1 | U | ug/L | 1 | 4.1 |
| 6/7/2010 11:40 | EFFLUENT | Field Parameter | Specific Conductance | 473 | | umhos/cm | | |

## Appendix E. (continued)

| Date Sampled | Field ID | Analysis Group | Components | Result | Remark | Units | MDL | PQL |
|---|---|---|---|---|---|---|---|---|
| 6/7/2010 11:40 | EFFLUENT | Field Parameter | Dissolved Oxygen | 8.46 | | mg/L | | |
| 6/7/2010 11:40 | EFFLUENT | Field Parameter | pH | 7.64 | | | | |
| 6/7/2010 11:40 | EFFLUENT | Field Parameter | Temperature | 27.59 | | C | | |
| 6/7/2010 11:40 | EFFLUENT | Field Parameter | Sample Depth | 0.15 | | m | | |
| 6/7/2010 7:00 | TOX 1 | Toxicology | Bioassay-Acute-Screen-FW-Fish, LC50 | 100 | L | LC50 | | |
| 6/7/2010 7:00 | TOX 1 | Toxicology | Bioassay-Chronic-Scrn-FW-C.dubia, NOEC | 100 | | NOEC | | |

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

**State of Florida**
**Department of Environmental Protection**
**Facility Introduction & Summary**

| **PART I: Information gathered prior to sampling event** | |
|---|---|
| **Prepared By:**<br>Kim Allen | **Contact Number:**<br>(850)595-8300, ext. 1185 |
| **Facility Name (as it appears on permit):**<br>Bayou Marcus Water Reclamation Facility | **Former Names:**<br>Avalon WWTF |
| **NPDES Permit Number:**<br>FL0031801 | **Permit Expiration Date:**<br>November 15, 2012 |
| **Physical Address:**<br>3050 Fayal Drive<br>Pensacola, FL 32526 | |
| **District:**<br>NWD | **County:**<br>Escambia |
| **Function of Facility:**<br>To treat wastewater for a portion of Escambia County. | |
| **Facility Type:**<br>Domestic WWTF | **Permitted Capacity (MGD):**<br>8.2 mgd AADF |
| **Mean Flow for previous 12 months for all EXTERNAL outfalls listed in permit (list date range including months):**<br>4/1/09-05/31/10 = 6.27 mgd annual average (D-002) | |
| **If applicable, Mean Flow for previous 12 months for REUSE outfall(s) (list date range including months):**<br>N/A | |
| **Description of type of Discharge:**<br>☒ **Continuous** ☐ **Intermittent** ☐ **Seasonal** ☐ **Rainfall Dependent** ☐ **Other, please specify** | |

## Appendix F. (continued)

**Description of Sampling Location(s) - actual permit designation of permitted sampling point(s):**

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 |
|---|---|
| EFF-01 | Effluent monitoring point prior to discharge to the wetlands. |
| FLW-02 | Flow to Northern Portion of Wetlands. |
| FLW-03 | Flow to Southern Portion of Wetlands. |
| FLW-04 | Total Flow to the Wetlands. |
| PPI-02 | Internal Monitoring at UV Unit. |

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

| Monitoring Location Site Number | Description of Monitoring Location |
|---|---|
| WEP-N4 ~~WIM-06~~ | Northern System Quadrant N-4 Sampling Station. Point of discharge (exit) from the northern wetland. |
| WIM-S2 ~~WIM-07~~ | Southern System Quadrant S-2 Sampling Station. Southern wetland internal monitoring station. |
| WEP-S3 ~~WIM-08~~ | Southern System Quadrant S-3 Sampling Station. Point of discharge (exit) from the southern wetland. |
| WIM-N1 ~~WIM-09~~ | Northern System Quadrant N-1 Sampling Station. Northern wetland internal monitoring station. |
| ~~WIM-10~~ | ~~Southern System Quadrant S-1 Sampling Station. Southern wetland internal monitoring station.~~ |

7. The following monitoring wells shall be sampled quarterly and shall be sampled in accordance with the monitoring frequencies specified in Permit Condition III.8 for Disposal System D-002. Quarterly sampling must be reasonably spaced to be representative of potentially changing conditions.

| Well Name | Monitoring Location Site Number | Depth (Feet) | Aquifer Monitored | Well Type | New or Existing |
|---|---|---|---|---|---|
| ~~MWC - 9~~ | ~~26977~~ | ~~7.00~~ | ~~Sand & Gravel~~ | ~~Compliance~~ | ~~Existing~~ |
| MWC - 10 | 26978 | 9.00 | Sand & Gravel | Compliance | Existing |
| MWC - 11 | 26979 | TBD | Sand & Gravel | Compliance | New |
| MWB - 12 | 26980 | TBD | Sand & Gravel | Background | New |
| MWB - 13 | 26981 | TBD | Sand & Gravel | Background | New |
| MWB - 14 | 31193 | 12.00 | Sand & Gravel | Background | Existing |
| MWB - 15 | 31194 | 41.00 | Sand & Gravel | Background | Existing |
| **MWC - 16** | | **TBD** | **Sand & Gravel** | **Compliance** | **New** |

*[62-522.600, 8-21-00]*

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

| Monitoring Location Site Number | Description of Monitoring Location |
|---|---|
| SWU-01 | Bayou Marcus Creek Upstream Monitoring Station - BMC |
| SWD-02 | Mouth of Bayou Marcus Creek (Southern System) - BMP |
| SWA-03 | Perdido Bay Monitoring Station (Northern System) - PB |

## Appendix F. (continued)

**Description of Permitted Outfall(s):**
Disposal: Surface Water Discharge D-002: Existing total flow to both wetlands is 10.25 MGD AADF. The flow is proportioned to the northern wetlands - 7.35 MGD AADF and the southern wetlands – 2.9 MGD AADF.

The D-002 discharge structures are PVC pipe with diffusers, attached to wooden boardwalk with seven (7) zones in use in the Northern portion of the wetlands. The discharge structure for the Southern portion of the wetlands will now be placed in the ground instead of attached to a boardwalk (10/22/08 revision). The Southern portion of the wetland will have four (4) zones. The Northern portion of the wetlands point of discharge is located approximately at latitude 30 ◦ 26' 10" N, longitude 87 ◦20' 00" W and discharges to Bayou Marcus Creek (WBID 697) and upper Perdido Bay (WBID 797).

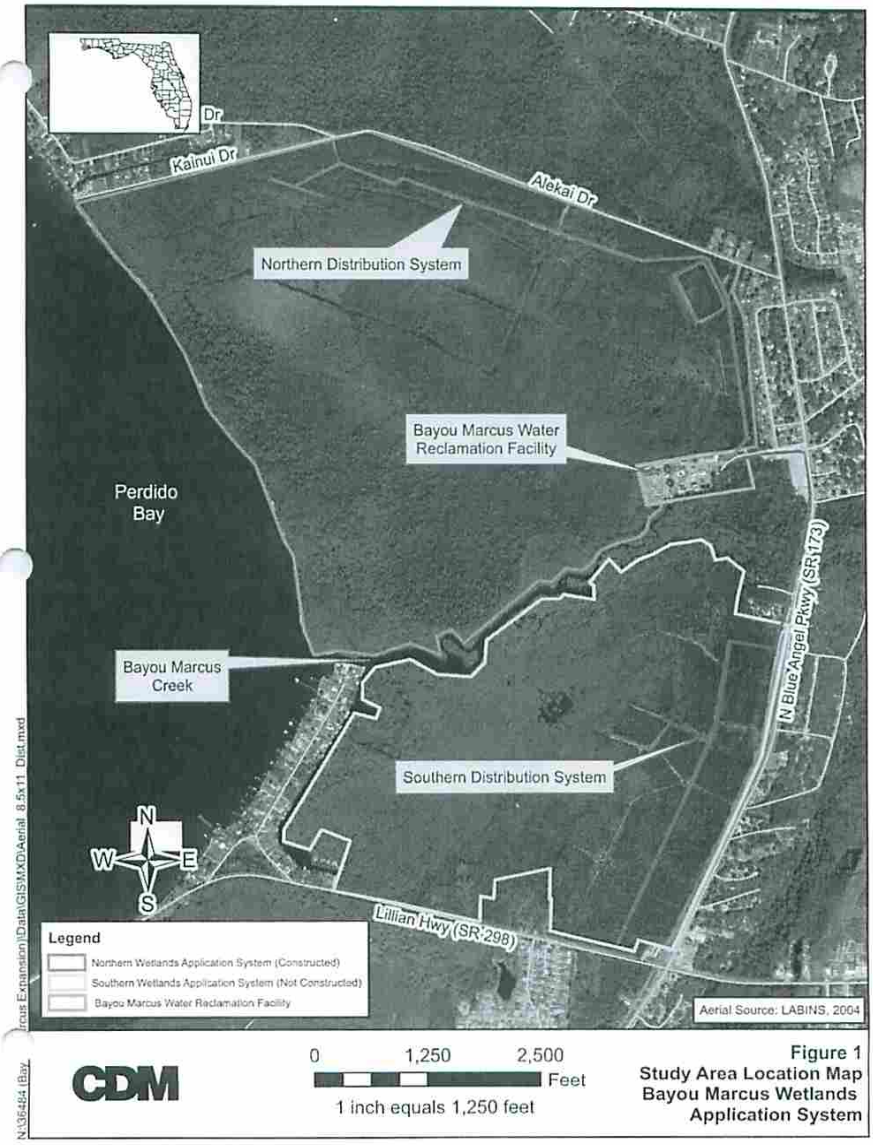
The WWTP discharge outfall line (D002) is approximately:
24" PVC F/M 11,295 linear feet
18 " PVC F/M 4,650 linear feet
12" PVC F/M 185 linear feet
8" PVC Diffuser Pipe 7,800 linear feet

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

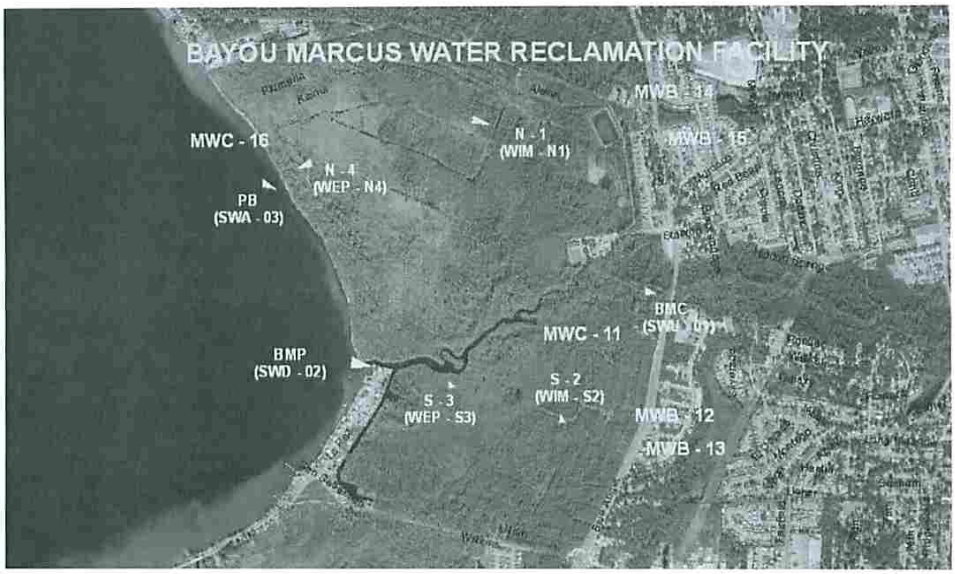
An existing 10.25 MGD annual average daily flow (AADF) permitted capacity activated sludge system. The mode of treatment operation of the wastewater treatment plant (WWTP) is conventional plug flow. The treatment facility is an extended aeration advanced domestic WWTP. The WWTP consist of pretreatment processes that include influent pumping with four (4) submersible pumps and four (4) raw wastewater rotary fine mesh drum screens. The biological treatment process include bioreactors – oxidation ditches (two parallel four-stage EIMCO carrousel aeration basins with anoxic with caustic soda addition, oxic, anoxic with alum addition and re-aeration chambers), three (3) secondary clarifiers. Secondary effluent management includes two (2) traveling bridge cloth media filters, two (2) parallel ultraviolet irradiation disinfection channels, effluent lift station with four (4)submersible pumps which pumps the effluent to the Bayou Marcus wetlands and two (2) turbine pumps for reuse water. An 8.2 MG lined reject holding pond is provided with recirculation.

The biosolids management facilities include one (1) circular plant tankage (aerated sludge holding tank) for sludge processing with four (4) cells, secondary scum and return activated sludge (RAS) pumping, waste activated sludge (WAS) pumping, One (1) rotary drum thickener to thicken sludge and facilities to transfer the thickened sludge to a tanker truck. All residual solids generated at the Bayou Marcus plant are transported by ECUA-owned and operated trucks, to the Main Street plant for treatment in one of two sludge dryers, at that plant, resulting in a Class AA residuals or other residual management facility (RMF).

## Appendix F. (continued)



## Appendix F. (continued)



## Appendix F. (continued)

**PART I: Continued**

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

| | | | Effluent Limitations | | | | Monitoring Requirements | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Units | Max/Min | Annual Average | Monthly Average | Weekly Average | Single Sample | Monitoring Frequency | Sample Type | Monitoring Location Site Number | Notes | |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

Pensacola, Florida 32502

## 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-002 to receiving wetlands. Such discharge shall be limited and monitored by the permittee as specified below and reported in accordance with condition I.B.8:

| | | | 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 |
| Total Flow to Wetlands | MGD | Maximum | 10.25 | Report | - | - | Continuous | Calculated | FLW-04 | |
| Flow to Northern portion of the Wetland. | MGD | Maximum | 7.35 5.3 | Report | - | - | Continuous | Recording flow meters and totalizers | FLW-02 | See Cond.I.B.4 |
| Flow to Southern portion of the Wetland. | MGD | Maximum | 2.9 | Report | - | - | Continuous | Recording flow meters and totalizers | FLW-03 | See Cond.I.B.4 |
| BOD, Carbonaceous 5 day, 20C | MG/L | Maximum | 5.0 | 6.3 | 7.5 | 10.0 | 5 Days/Week | 24-hour flow proportioned composite | EFF-01 | |
| Solids, Total Suspended | MG/L | Maximum | 5.0 | 6.3 | 7.5 | 10.0 | 5 Days/Week | 24-hour flow proportioned composite | EFF-01 | |
| Nitrogen, Ammonia, Total as NH4 | MG/L | Maximum | 1.6 | 2.0 | 2.4 | 3.2 | 5 Days/Week | 24-hour flow proportioned composite | EFF-01 | |
| Phosphorus, Total (as P) | MG/L (LBS/DAY) | Maximum | 1.0 (68.4) | 1.3 | 1.5 | 2.0 | 5 Days/Week | 24-hour flow proportioned composite (Calculated) | EFF-01 | |
| Phosphorus, Total (as P) (Northern portion of the Wetland) | LBS/DAY | Maximum | 44.2 | | | | 5 Days/Week | Calculated | EFF-01 | |
| Phosphorus, Total (as P) (Southern portion of the Wetland) | LBS/DAY | Maximum | 24.2 | | | | 5 Days/Week | Calculated | EFF-01 | |
| Nitrogen, Total | MG/L (LBS/DAY) | Maximum | 3.0 (205.2) | 3.8 | 4.5 | 6.0 | 5 Days/Week | 24-hour flow proportioned composite (Calculated) | EFF-01 | |
| Nitrogen, Total (Northern portion of the Wetland) | LBS/DAY | Maximum | 132.6 | | | | 5 Days/Week | Calculated | EFF-01 | |
| Nitrogen, Total (Southern portion of the Wetland) | LBS/DAY | Maximum | 72.6 | | | | 5 Days/Week | Calculated | EFF-01 | |
| pH | SU | Range | - | - | - | 6.0 to 8.5 | Continuous | Grab | EFF-01 | See Cond.I.A.3 |
| Coliform, Fecal | #/100ML | Maximum | See Permit Condition I.A.5. | | | | 5 Days/Week | Grab | EFF-01 | |
| Oxygen, Dissolved (DO) | MG/L | Minimum | - | - | - | 5.0 | Daily | Grab | EFF-01 | |

## Appendix F. (continued)

| Parameter | Units | Max/Min | Effluent Limitations | | | | Monitoring Requirements | | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Annual Average | Monthly Average | Weekly Average | Single Sample | Monitoring Frequency | Sample Type | Monitoring Location Site Number | |
| Ultraviolet Light Intensity | MW/CM2 | Minimum | - | - | - | Report | Continuous | Meter | PPI-02 | |
| Ultraviolet Light Dosage | MW-S/CM2 | Minimum | - | - | - | 25 | Continuous | Calculated | PPI-02 | |
| Whole Effluent Toxicity (Chronic) | See Permit Condition I.A.6 | | | | | | | | EFF-01 | |
| Zinc, Total Recoverable | UG/L | Maximum | See Permit Condition I.A.7 & 10 | | | | Monthly | 24-hour flow proportioned composite | EFF-01 | |
| Copper, Total Recoverable | UG/L | Maximum | See Permit Condition I.A.9 & 10 | | | | Monthly | 24-hour flow proportioned composite | EFF-01 | |
| Hardness, Total (as $CaCO_3$) | MG/L | Maximum | See Permit Condition I.A.8 | | | | Monthly | 24-hour flow proportioned composite | EFF-01 | |

C. Surface Water

1. During the period beginning on the issuance date and lasting through the expiration date of this permit, the permittee shall monitor water quality from the surface water(s). Surface water monitoring shall be performed in accordance with the following:

| | | | 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 |
| Nitrogen, Nitrate, Total (as N) | MG/L | Maximum | - | | | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Total Nitrogen | MG/L | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| pH | SU | Range | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Dissolved Oxygen (DO) | MG/L | Minimum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Temperature, Water | DEG C | Maximum | - | - | - | Report | Quarterly | Field Thermometer | SWU-01, SWD-02, SWA-03 | |
| Coliform, Fecal | #/100ML | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Nitrogen, Ammonia, Total as NH4 | MG/L | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Specific conductance, Field | UMHOS/CM | Maximum | - | - | - | Report | Quarterly | Field Probe | SWU-01, SWD-02, SWA-03 | |
| BOD, Carbonaceous 5 day, 20C | MG/L | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Solids, Total Suspended | MG/L | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Phosphorus, Total (as P) | MG/L | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Chlorophyll-A, Phytoplankton,Fluorometric Method | MG/CU. METER | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Water Depth | Feet | Maximum | - | - | - | Report | Quarterly | Field Instrument | SWU-01, SWD-02, SWA-03 | |
| Wind Direction | Degrees from North | Maximum | - | - | - | Report | Quarterly | Field Instrument | SWU-01, SWD-02, SWA-03 | |
| Wind Force | MPH | Maximum | - | - | - | Report | Quarterly | Field Instrument | SWU-01, SWD-02, SWA-03 | |

## Appendix F. (continued)

| Parameter | Units | Max/Min | Effluent Limitations | | | | Monitoring Requirements | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Annual Average | Monthly Average | Weekly Average | Single Sample | Monitoring Frequency | Sample Type | Monitoring Location Site Number | Notes |
| Color | PT-CO | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Turbidity | NTU | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| D.O. % Saturation | % | Minimum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Salinity | PPT | Maximum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |
| Secchi Depth | Inches | Minimum | - | - | - | Report | Quarterly | Grab | SWU-01, SWD-02, SWA-03 | |

## Appendix F. (continued)

| PART I: Continued | |
|---|---|
| **Receiving Waters:**<br><br>Receiving wetlands; upper Perdido Bay (WBID 797); Bayou Marcus Creek (WBID 697) | **Classification (indicate whether fresh or marine):**<br><br>Receiving wetlands - Class III<br>Perdido Bay – Class III |

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

**Permit mod 4/9/10:**

6. The permittee shall comply with the following requirements to evaluate chronic whole effluent toxicity of the discharge from outfall D-001.

   a. Effluent Limitation

      (1) In any routine or additional follow-up test for chronic whole effluent toxicity, the 25 percent inhibition concentration (IC25) shall not be less than 100% effluent. [Rules 62-302.530(20) and 62-4.241(1)(b), F.A.C.]
      (2) For acute whole effluent toxicity, the 96-hour LC50 shall not be less than 100% effluent in any test. [Rule 62-302.500(1)(a)4. and 62-4.241(1)(a), F.A.C.]

   c. Sampling Requirements

      (1) For each routine test or additional follow-up test conducted, a total of three samples of final effluent shall be collected and used in accordance with the sampling protocol discussed in EPA-821-R-02-013, Section 8.
      (2) The first sample shall be used to initiate the test. The remaining two samples shall be collected according to the protocol and used as renewal solutions on Day 3 (48 hours) and Day 5 (96 hours) of the test.
      (3) Samples for routine and additional follow-up tests shall not be collected on the same day.

   d. Test Requirements

      (1) Routine Tests: All routine tests shall be conducted using a control (0% effluent) and a minimum of five test dilutions: **100%, 50%, 25%, 12.5%**, and **6.25%** final effluent.
      (2) The permittee shall conduct a daphnid, **Ceriodaphnia dubia**, Survival and Reproduction Test and a fathead minnow, **Pimephales promelas**, Larval Survival and Growth Test, concurrently.
      (3) All test species, procedures and quality assurance criteria used shall be in accordance with **Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms**, 4th Edition, EPA-821-R-02-013. Any deviation of the bioassay procedures outlined herein shall be submitted in writing to the Department for review and approval prior to use. In the event the above method is revised, the permittee shall conduct chronic toxicity testing in accordance with the revised method.
      (4) The control water and dilution water shall be moderately hard water as described in EPA-821-R-02-013, Section 7.2.3.

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

AO048NW issued 11/16/07 gave the facility a limit of "Report Only" for flow from D-002 (northern wetlands) for 2 years and gave a schedule to construct the discharge structure for the Southern Wetland. The AO was modified on 5/13/10 to extend the construction date for the southern wetland structure to 4/30/11 and the report only flow to the northern wetlands to 4/9/10.

Consent Order OGC File No. 09-0276 was executed on 6/26/09 for sewer spills along a portion of the collection/transmission system that extends along Gulf Beach Highway. The CO requires the submittal and implementation of a Master Plan to minimize sewer overflows and improve and rehabilitate the collection system in the Southwest Service Area.

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

## Appendix F. (continued)

| List Permit Violations (DMR data) and plant upsets that occurred at the plant within the last year: | | | |
|---|---|---|---|
| Date | Parameter and Units | Limit | Result |
| 4/09 | Copper (ug/L) | 8.8 ug/L | 47 ug/ |

**Describe previous FYI and WQBEL study:**
The WQBEL Level I Study was conducted pursuant to Rules 62-600.430 and 62-650, Florida Administrative Code (F.A.C.). The WQBEL Level I Study confirmed the previous final effluent limits for this receiving wetland permit of:

Flow 8.2 MGD (Annual Average)
$CBOD_5$ 5 mg/L (Annual Average)
TSS 5 mg/L (Annual Average)
TN 3 mg/L (Annual Average)
Ammonia Nitrogen 1.6 mg/L (Annual Average)
TP 1 mg/L (Annual Average)
DO 5 mg/L (minimum)

**Describe previous or current Enforcement Actions (including WLs):**

Consent Order OGC File No. 09-0276 was executed on 6/26/09 for sewer spills along a portion of the collection/transmission system that extends along Gulf Beach Highway. The CO requires the submittal and implementation of a Master Plan to minimize sewer overflows and improve and rehabilitate the collection system in the Southwest Service Area.

**Describe any violations or problems noted in previous inspection:**

MWC-09 was noted as having a TDS, chlorine and fecal violation in the 4th quarter of 2008. The facility stated that they continue to see effects from hurricanes submerging the well.

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

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

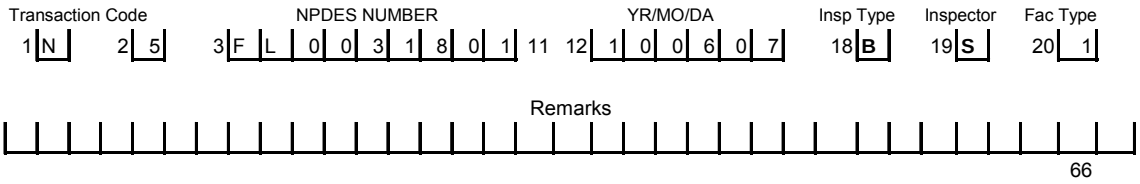
## Appendix F. (continued)

| PART II: Sampling Event Information | |
|---|---|
| **Date(s) sample collected:**<br>6/7/2010 | **Sampler(s):**<br>Nathan Mulkey/Rick Abad |
| **Temperature (Deg C):**<br>27.59 | **pH (SU):**<br>7.64 |
| **Dissolved Oxygen (mg/L):**<br>8.46 | **Conductivity (umhos/cm):**<br>473 |
| **Total Residual Chlorine (mg/L):**<br>N/A | **Total Residual Chlorine - after Disinfection (mg/L):**<br>N/A |
| **Method of Chlorination:**<br>UV Disinfection | **Method of De-chlorination:**<br>N/A |
| **Flow During Sample Collection for each outfall(MGD):** | |
| **Mean Flow for each outfall for previous 12 months based on sampling date (If samples are taken on more than one day, indicate Mean Flow for each day):**<br><br>6.27 mgd annual average | |
| **Describe Discharge during Sampling Event:**<br>☒ Continuous ☐ Intermittent ☐ Seasonal ☐ Rainfall Dependent ☐ Other, please specify | |
| **Describe any conditions, difficulties or abnormal events that occurred during the sample collection and any other additional comments here:** | |

## Appendix G. EPA End Sheet

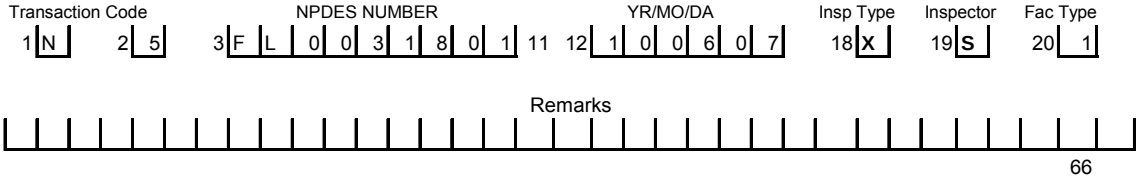
### The Bioassay of Bayou Marcus Wastewater Reclaimation Facility effluent sampled on June 7 & 9, 2010. NPDES #FL0031801.

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




### The Priority Pollutants Analysis of Bayou Marcus Wastewater Reclaimation Facility effluent sampled on June 7, 2010. NPDES #FL0031801.

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




### Biological Analyses of Bayou Marcus Wastewater Reclaimation Facility effluent sampled on June 7, 2010. NPDES #FL0031801.

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

