Client: Environmental Monitoring, Inc.
Project ID: EMIN1114
Client Sample ID: Holston WWTP Outfall 001/EMI #1534.25
Permit No: VA0067351
Sample Period: 9/26/11 to 9/29/11

Received
OCT 13 2011
DEQ-SWRO

Coastal Bioanalysts, Inc.

## Report of Analysis: Whole Effluent Toxicity (WET)

| Submitted To: | Prepared By: |
|---|---|
| Mr. Randy Porter<br>Environmental Monitoring, Inc.<br>P.O. Box 1190<br>Norton, VA 24273 | Coastal Bioanalysts, Inc.<br>6400 Enterprise Court<br>Gloucester, VA 23061<br>(804) 694-8285<br>www.coastalbio.com<br>Contact: Peter F. De Lisle, Technical Director |

**Acute Test Results***

| Species-Test Method | 48-h LC50 | 95% C.L. | $T.U._{Ac}$ | NOAEC |
|---|---|---|---|---|
| *C. dubia* EPA 2002.0 | >100 | N/A | <1.00 | N/A |
| *P. promelas* EPA 2000.0 | >100 | N/A | <1.00 | N/A |

**Chronic Test Results***

| Species-Test Method | Endpoint | NOEC | LOEC | ChrV | PMSD | $T.U._C$ | IC25 | 48-h LC50 | LC50 95% C.L. | $T.U._{Ac}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *C. dubia* EPA 1002.0 | Survival | 100 | >100 | >100 | N/A | 1.00 | N/A | >100 | N/A | <1.00 |
| | Reproduction | 100 | >100 | >100 | 28 | 1.00 | >100 | N/A | N/A | N/A |
| *P. promelas* EPA 1000.0 | Survival | 100 | >100 | >100 | N/A | 1.00 | N/A | >100 | N/A | <1.00 |
| | Biomass | 100 | >100 | >100 | 18 | 1.00 | >100 | N/A | N/A | N/A |

*Note: Details regarding test conduct and data analysis provided in attached bench sheets and printouts as applicable.

| AcuteTest Biological Summary Data | | Sample Concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|
| Species-Method | Endpoint | Control | 13.0 | 21.6 | 36.0 | 60.0 | 100 |
| *C. dubia* EPA 2002.0 | Survival (%): | 100 | 95 | 100 | 100 | 100 | 100 |
| *P. promelas* EPA 2000.0 | Survival (%): | 100 | 100 | 100 | 100 | 100 | 100 |

| Chronic Test Biological Summary Data | | Sample Concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|
| Species-Method | Endpoint | Control | 4.60 | 10.0 | 22.0 | 46.0 | 100 |
| *C. dubia* EPA 1002.0 | Survival (%): | 100 | 100 | 100 | 90 | 100 | 100 |
| | Repro (# young): | 24.5 | 24.1 | 22.5 | 18.7 | 25.0 | 20.8 |
| *P. promelas* EPA 1000.0 | Survival (%): | 100 | 100 | 100 | 100 | 100 | 98 |
| | Biomass (mg): | 0.800 | 0.859 | 0.730 | 0.814 | 0.741 | 0.752 |

| Test Information Species-Method | Start Date/Time End Date/Time | Organism Source | Hatch/Harvest Date/Time | Acclimation Temp. | Acclimation Water | Test Aerated? |
|---|---|---|---|---|---|---|
| *C. dubia* EPA 2002.0 | 9/28/11 1420<br>9/30/11 1415 | CBI Stock | 9/27/11 1625<br>9/28/11 1005 | 25° C | Mod. Hard Syn. FW | No |
| *P. promelas* EPA 2000.0 | 9/28/11 1430<br>9/30/11 1440 | CBI Stock | 9/23/11 0800<br>9/23/11 1300 | 25° C | Mod. Hard Syn. FW | No |
| *C. dubia* EPA 1002.0 | 9/27/11 1130<br>10/3/11 1110 | CBI Stock | 9/26/11 1215<br>9/26/11 1410 | 25° C | Mod. Hard Syn. FW | No |
| *P. promelas* EPA 1000.0 | 9/27/11 1205<br>10/4/11 1205 | CBI Stock | 9/26/11 1630<br>9/26/11 0930 | 25° C | Mod. Hard Syn. FW | No |



VELAP# 460030
EPA# VA01116

NELAP ACCREDITED LABORATORY

Client: Environmental Monitoring, Inc.
Project ID: EMIN1114
Client Sample ID: Holston WWTP Outfall 001/EMI #1534.25
Permit No: VA0067351
Sample Period: 9/26/11 to 9/29/11

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Coastal Bioanalysts, Inc.

| Sample/Dilution Water Data | Acute Test | | Chronic Test | | | |
|---|---|---|---|---|---|---|
| | | Dilution* | Sample | | Dilution Water* | |
| Water Quality Parameter (Units) | Sample | Water | Mean | Std. Dev. | Mean | Std. Dev. |
| Arrival Temperature (°C) | 1 | N/A | 1 | 0 | N/A | N/A |
| Use Temperature (°C) | 25 | 25 | 25 | 0 | 25 | 0 |
| Conductivity (µS/cm) | 658 | 293 | 646 | 14 | 294 | 3.4 |
| pH (S.U.) | 7.84 | 7.64 | 7.81 | 0.05 | 7.65 | 0.02 |
| Dissolved Oxygen (mg/l) | 8.2 | 8.2 | 8.2 | 0 | 8.2 | 0 |
| Total Hardness (mg/l as $CaCO_3$) | 220 | 84 | 221 | 13 | 90 | 8.4 |
| Alkalinity (mg/l as $CaCO_3$) | 131 | 57 | 128 | 4.6 | 58 | 1.3 |
| Total Residual Chlorine (mg/l) | <Q.L. | N/A | <Q.L. | 0 | N/A | N/A |
| Ammonia (mg/l $NH_3$-N) | <1.0 | N/A | <1.0 | 0 | N/A | N/A |

*Dilution water = Moderately hard synthetic freshwater

| Sample Aging/Use/Pretreatment | | | | |
|---|---|---|---|---|
| CBI Sample I.D. | Collection Date/Time | Date(s)/Time(s) 1st Used in Tests | Date(s)/Time(s) Used in Renewals | Sample Adjustments |
| EMIN1114-A | 9/26/11 0845 | 9/27/11 1130, 1205 | N/A | Aerated 2 min |
| EMIN1114-B | 9/27/11 0851 | 9/28/11 1145, 1220<br>9/28/11 1420, 1430* | 9/29/11 1120, 1200 | Aerated 3 min |
| EMIN1114-C | 9/29/11 0856 | 9/30/11 1255, 1330 | 10/1/11 1115, 1145<br>10/2/11 1200, 1320<br>10/3/11 1040 | Aerated 3-3.5 min |

*Acute tests

| Acute Test Water Quality (Mean/Std. Dev.) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test: | *C. dubia* 2002.0 | | | | | | *P. promelas* 2000.0 | | | | | |
| % Conc: | Cont. | 13.0 | 21.6 | 36.0 | 60.0 | 100 | Cont. | 13.0 | 21.6 | 36.0 | 60.0 | 100 |
| Temp. | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| (°C) | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| D.O. | 7.9 | 7.9 | 7.9 | 7.8 | 7.8 | 7.8 | 7.7 | 7.6 | 7.5 | 7.5 | 7.4 | 7.4 |
| (mg/l) | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.5 | 0.5 | 0.6 | 0.6 | 0.7 | 0.7 | 0.7 |
| pH | 7.61 | 7.66 | 7.68 | 7.77 | 7.85 | 7.95 | 7.46 | 7.50 | 7.56 | 7.62 | 7.75 | 7.84 |
| (S.U.) | 0.15 | 0.15 | 0.16 | 0.18 | 0.16 | 0.20 | 0.19 | 0.25 | 0.22 | 0.18 | 0.14 | 0.07 |

| Chronic Test Water Quality (Mean/Std. Dev.) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test: | *C. dubia* 1002.0 | | | | | | *P. promelas* 1000.0 | | | | | |
| % Conc: | Cont. | 4.60 | 10.0 | 22.0 | 46.0 | 100 | Cont. | 4.60 | 10.0 | 22.0 | 46.0 | 100 |
| Temp. | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| (°C) | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| D.O. | 8.3 | 8.4 | 8.5 | 8.5 | 8.6 | 8.7 | 7.6 | 7.5 | 7.6 | 7.5 | 7.6 | 7.6 |
| (mg/l) | 0.2 | 0.2 | 0.3 | 0.4 | 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | 0.6 | 0.6 | 0.6 |
| pH | 7.80 | 7.88 | 7.93 | 7.97 | 8.04 | 8.09 | 7.42 | 7.38 | 7.44 | 7.49 | 7.63 | 7.78 |
| (S.U.) | 0.23 | 0.28 | 0.31 | 0.28 | 0.30 | 0.29 | 0.17 | 0.21 | 0.19 | 0.21 | 0.21 | 0.10 |
| Cond. | 294 | 313 | 332 | 371 | 459 | 641 | 296 | 314 | 333 | 373 | 453 | 644 |
| (µS/cm) | 3.3 | 1.9 | 1.9 | 3.3 | 8.6 | 14 | 3.2 | 2.6 | 2.6 | 5.6 | 8.0 | 14 |



VELAP# 460030
EPA# VA01116

NELAP ACCREDITED TNI LABORATORY

Client: Environmental Monitoring, Inc.
Project ID: EMIN1114
Client Sample ID: Holston WWTP Outfall 001/EMI #1534.25
Permit No: VA0067351
Sample Period: 9/26/11 to 9/29/11

Received
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Coastal Bioanalysts, Inc.

| Acute Test QA/QC Reference Toxicant: KCl Units: mg/l Test Organism Source: CBI Stock Cultures | | | | | |
|---|---|---|---|---|---|
| Species-Method (Ref. Test Date) | Data Source | % Control Survival | 48-h LC50 | 95% C.L./A.L. For LC50 | RTT in Control? |
| *C. dubia* 2002.0 | RTT | 100 | 658 | 620-698 | Yes |
| (9/2/11-9/4/11) | CC | 99 | 601 | 536-666 | |
| *P. promelas* 2000.0 | RTT | 100 | 910 | 841-986 | Yes |
| (9/3/11-9/5/11) | CC | 100 | 967 | 846-1087 | |

| Chronic Test QA/QC Reference Toxicant: KCl Units: mg/l Test Organism Source: CBI Stock Cultures | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Species-Method | Data | % Survival | | Reproduction (# Young) or Biomass (mg) | | | | | RTT in |
| (Ref. Test Date) | Source | Cont. | NOEC | Cont. | NOEC | PMSD | IC25 | IC25 A.L. | Control? |
| *C. dubia* 1002.0 | RTT | 100 | 500 | 31.1 | 250 | 17 | 367 | N/A | Yes |
| (9/6/11-9/12/11) | CC | 99 | 500 | 24.6 | 250 | 24 | 316 | 236-397 | |
| *P. promelas* 1000.0 | RTT | 98 | 500 | 0.82 | 500 | 15 | 606 | N/A | Yes |
| (9/6/11-9/13/11) | CC | 98 | 500 | 0.77 | 500 | 16 | 607 | 559-654 | |

Note: RTT = Reference Toxicant Test, CC = Control Chart, Cont. = Control group.

The results of analysis contained within this report relate only to the sample as received in the laboratory. This report shall not be reproduced except in full without written approval from the laboratory. Unless noted below, these test results meet all requirements of NELAP.

APPROVED:

Peter F. De Lisle, Ph.D.
Technical Director

10/5 /11
Date

Deviations from, additions to, or exclusions from the test method, non-standard conditions or data qualifiers and, as appropriate, a statement of compliance/non-compliance: **NONE**

**GLOSSARY OF TERMS AND ABBREVIATIONS**

**A.L. (Acceptance Limits):** The results of a given reference toxicant test are compared to the control chart mean value ± 2 standard deviations. These limits approximate the 95% probability limits for the "true" reference toxicant value.

**Chronic Value (ChrV):** The geometric mean of the NOEC and LOEC. Units are same as test concentration units.

**C.L. (Confidence Limits):** These are the probability limits, based on the data set and statistical model employed, that the "true value" lies within the limits specified. Typically limits are based on 95% or 99% probabilities.

**Control chart:** A cumulative summary chart of results from QC tests with reference toxicants. The results of a given reference toxicant test are compared to the control chart mean value and 95% Acceptance Limits (A.L.) (mean ± 2 standard deviations).

**IC25:** The concentration of sample or chemical, calculated from the data set using statistical models, causing a 25% reduction in test organism growth, reproduction, etc. The lower the IC25, the more toxic the chemical or sample. Units are same as test concentration units.

**LC50:** The concentration of sample or chemical, calculated from the data set using statistical models, causing a 50% reduction in test organism survival. The lower the LC50, the more toxic the chemical or sample. Units are same as test concentration units. Note: The LC50 value must always be associated with the duration of exposure. Thus 48-h LC50, 96-h LC50, etc. are calculated.



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Permit No: VA0067351
Sample Period: 9/26/11 to 9/29/11

Coastal Bioanalysts, Inc.

**LOEC:** Lowest-observable-effect-concentration. The lowest concentration of sample or chemical in a chronic test dilution series in which the test organisms exhibit a statistically significant reduction in any of the test end points (e.g. growth, survival, reproduction) compared to control organisms. Units are same as test concentration units.

**PMSD:** Percent Minimum Significant Difference: The minimum difference which can exist between a test treatment and the controls in a particular test and be statistically significant; a measure of test sensitivity. The lower the PMSD the more sensitive the test.

**N/A:** Not applicable.

**N/D:** Not determined or measured.

**NOAEC:** No-observable-acute-effect-concentration. The highest concentration of sample or chemical in an acute test dilution series in which the test organisms exhibit no statistically significant reduction in the test end point (e.g. survival) compared to control organisms. Units are same as test concentration units.

**NOEC:** No-observable-effect-concentration. The highest concentration of sample or chemical in a chronic test dilution series in which the test organisms exhibit no statistically significant reduction in any of the test end points (e.g. growth, survival, reproduction) compared to control organisms. Some regulatory definitions also require that the NOEC be less than the LOEC. Units are same as test concentration units.

**Q.L.:** Quantitation Limit. Level, concentration, or quantity of a target variable (analyte) that can be reported at a specified degree of confidence.

**T.U.:** Toxic units. Expresses the relative toxicity of an effluent in such a manner that the larger the toxic unit value the more toxic the effluent. $T.U._{Ac} = 100/LC50$. $T.U._{Chr} = 100/NOEC$. A dimensionless unit.



VELAP# 460030
EPA# VA01116

*CERIODAPHNIA DUBIA* STATIC ACUTE WET TEST
(ACD) FORM ETF1051D

COASTAL BIOANALYSTS, INC
EFFECTIVE DATE: 2/1/09

| % Effluent | I.D. | Day 0 Live | Day 1 Live | Day 2 Live | Final % Survival | % Effluent | I.D. | Day 0 Live | Day 1 Live | Day 2 Live | Final % Survival |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | C-a | 5 | 5 | 5 | 100 | 36.0 | 3-a | 5 | 5 | 5 | 100 |
| | C-b | 5 | 5 | 5 | | | 3-b | 5 | 5 | 5 | |
| | C-c | 5 | 5 | 5 | | | 3-c | 5 | 5 | 5 | |
| | C-d | 5 | 5 | 5 | | | 3-d | 5 | 5 | 5 | |
| 13.0 | 1-a | 5 | 5 | 5 | 95 | 60.0 | 4-a | 5 | 5 | 5 | 100 |
| | 1-b | 5 | 5 | 4 | | | 4-b | 5 | 5 | 5 | |
| | 1-c | 5 | 5 | 5 | | | 4-c | 5 | 5 | 5 | |
| | 1-d | 5 | 5 | 5 | | | 4-d | 5 | 5 | 5 | |
| 21.6 | 2-a | 5 | 5 | 5 | 100 | 100 | 5-a | 5 | 5 | 5 | 100 |
| | 2-b | 5 | 5 | 5 | | | 5-b | 5 | 5 | 5 | |
| | 2-c | 5 | 5 | 5 | | | 5-c | 5 | 5 | 5 | |
| | 2-d | 5 | 5 | 5 | | | 5-d | 5 | 5 | 5 | |
| | | | | | | | Initials: | AG | PB | CB | |
| | | | | | | | Count Time: | 1420 | 0915 | 1445 | *Test end time |

**NOTES:**

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| Parameter | Treatment I.D. | Day 0 | Day 1 | Day 2 |
|---|---|---|---|---|
| Temp. (°C) | C | 25 | 25 | 25 |
| | 1 | 25 | 25 | 25 |
| | 2 | 25 | 25 | 25 |
| | 3 | 25 | 25 | 25 |
| | 4 | 25 | 25 | 25 |
| | 5 | 25 | 25 | 25 |
| pH (S.U.) | C | 7.63 | 7.45 | 7.75 |
| | 1 | 7.72 | 7.49 | 7.76 |
| | 2 | 7.76 | 7.50 | 7.78 |
| | 3 | 7.80 | 7.58 | 7.97 |
| | 4 | 7.86 | 7.69 | 8.01 |
| | 5 | 7.87 | 7.81 | 8.18 |
| D.O. (mg/l) | C | 8.2 | 7.7 | 7.9 |
| | 1 | 8.2 | 7.7 | 7.9 |
| | 2 | 8.2 | 7.6 | 7.8 |
| | 3 | 8.2 | 7.5 | 7.8 |
| | 4 | 8.2 | 7.4 | 7.8 |
| | 5 | 8.2 | 7.3 | 7.9 |
| Conduct. (uS/cm) | C | 294 | | 290 |
| | 1 | 343 | | |
| | 2 | 372 | | |
| | 3 | 422 | | |
| | 4 | 510 | | |
| | 5 | 657 | | 647 |
| Replicate Meas.: | | S | S | B |
| Initials: | | AG | PB | CB |
| TRC (mg/l) in highest conc. at end of test: | | | | NA |

**Species:** *Ceriodaphnia dubia*

**Source: CBI stock cultures** ✓

**Other:** _____

**Brood Release:** **Date/time start:** 9/27/11 1625

**Date /time end:** 9/28/11 1005

**Acclimation:** **Water:** Mod. hard syn. FW ✓

Other _____

**Temperature (°C):** 25

**Feeding:** **Prior to test:** YCT/*Selenastrum*
**During test:** Not Fed

**Illumination:** 16L:8D 10-20 uE/m²/s

**Test chamber size:** ✓ **30 ml**

**Solution volume:** ✓ **15 ml** _____ **ml**

**Number of replicates/treatment: 4**

**Initial number of daphnids/replicate: 5**

**Template number:** 21

**Set up: Date (Day 0):** 9/28/11

**Time water added:** 1410

**Time daphnids added:** 1420

**Set up by (initials):** AG

Peer Rev. by: CB / PB Date: 9/30/11 TEST I.D. EMIN11R ACD

*PIMEPHALES PROMELAS* STATIC ACUTE WET TEST
48-H TEST (APP) FORM ETF1041E

COASTAL BIOANALYSTS, INC
EFFECTIVE DATE: 2/1/09

| % Effluent | I.D. | Day 0 Live | Day 1 Live | Day 2 Live | Final % Survival |
|---|---|---|---|---|---|
| Lab Control | C-A | 10 | 10 | 10 | 100 |
| | C-B | 10 | 10 | 10 | |
| 13.0 | 1-A | 10 | 10 | 10 | 100 |
| | 1-B | 10 | 10 | 10 | |
| 21.6 | 2-A | 10 | 10 | 10 | 100 |
| | 2-B | 10 | 10 | 10 | |
| 36.0 | 3-A | 10 | 10 | 10 | 100 |
| | 3-B | 10 | 10 | 10 | |
| 60.0 | 4-A | 10 | 10 | 10 | 100 |
| | 4-B | 10 | 10 | 10 | |
| 100 | 5-A | 10 | 10 | 10 | 100 |
| | 5-B | 10 | 10 | 10 | |
| Initials: | | AG | PB | CM | |
| Count Time: | | 1430 | 0905 | 1440 | *Test End Time |

| Parameter | Treatment I.D. | Day 0 | Day 1 | Day 2 |
|---|---|---|---|---|
| Temp. (°C) | C | 25 | 25 | 24 |
| | 1 | 25 | 25 | 24 |
| | 2 | 25 | 25 | 24 |
| | 3 | 25 | 25 | 24 |
| | 4 | 25 | 25 | 24 |
| | 5 | 25 | 25 | 24 |
| pH (S.U.) | C | 7.63 | 7.50 | 7.26 |
| | 1 | 7.72 | 7.55 | 7.22 |
| | 2 | 7.76 | 7.59 | 7.33 |
| | 3 | 7.80 | 7.60 | 7.45 |
| | 4 | 7.86 | 7.80 | 7.54 |
| | 5 | 7.87 | 7.90 | 7.76 |
| D.O. (mg/l) | C | 8.2 | 7.7 | 7.2 |
| | 1 | 8.2 | 7.6 | 7.0 |
| | 2 | 8.2 | 7.4 | 7.0 |
| | 3 | 8.2 | 7.3 | 6.9 |
| | 4 | 8.2 | 7.1 | 6.9 |
| | 5 | 8.2 | 7.1 | 7.0 |
| Conduct. (uS/cm) | C | 294 | | 294 |
| | 1 | 343 | | |
| | 2 | 372 | | |
| | 3 | 422 | | |
| | 4 | 570 | | |
| | 5 | 1057 | | 665 |
| Replicate Measured: | | A | B | B |
| Initials: | | AG | PB | CM |
| TRC (mg/l) in highest conc. at end of test: | | | | NA |

**Species:** *Pimephales promelas*

**Source: CBI stock cultures** ✓

**Other:**

**Hatch: Date/time start:** 9/23/11 0800

**Date /time end:** 9/23/11 1300

**Acclimation: Water:** Mod. hard syn. FW ✓

Other

**Temperature (°C):** 25

**Feeding: Prior to test:** *Artemia ad libitum*
**During test:** Not fed

**Illumination:** 16L:8D 10-20 uE/m²/s

**Test chamber size:** ✓ **400 ml** _____ **ml**

**Solution volume:** ✓ **400 ml** _____ **ml**

**Number of replicates/treatment: 2**

**Initial number of fish/replicate: 10**

**Set up: Date (Day 0):** 9/28/11

**Time water added:** 1400

**Time fish added:** 1430

**Set up by (initials):** AG

**NOTES:**

Received
OCT 13 2011
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Peer Rev. by: DBJ PB Date: 9/30/11 TEST I.D. EMIN1114 -APP

OCT 13 2011

DEQ-SWRO

BASELINE TEST INFO – *Ceriodaphnia* 3-BROOD TEST

Coastal Bioanalysts, Inc
Form ETF0051E
Effective Date: 2/1/09

**TEST ORGANISM INFO**

**Species:** *Ceriodaphnia dubia*

**Source:** CBI Stock Cultures

**Acclimation:** **Water:** Mod. hard syn. FW ✓

Other: _____

**Temp. (°C):** 25

**Brood Release: (start date/time)** 9/26/11 1215

**Brood Release: (end date/time)** 9/26/11 1410

**Feeding Prior to Test:** *Selenastrum* + YCT mix[1]

**Feeding During Test:** *Selenastrum* + YCT mix[1]

**TEST DESIGN**

**Test Chamber:** ~30 ml glass vial ✓

Other _____

**Solution Vol:** 15 ml ✓

Other _____

**Illumination:** 16:8 L:D 10-20 uE/m$^2$/s

**Number of Replicates/Concentration:** 10

**Initial Number of Daphnids/Replicate:** 1

**Template No.** 4

**TEST SET UP (Day 0)**

**Set Up Date:** 9/27/11

**Set Up By:** AG

**Time Water Added:** 1115

**Time Animals Added:** 1130

**NOTES**

[1]Food added daily to dilution water and test solutions at renewal for a final feeding level of 0.2 ml of YCT + algae mix per chamber (chamber algae concentration 2-2.3E5 cells/ml)

Peer Review by: PB Date: 10/4/11

Test I.D. EM INFLY -CCD

WATER QUALITY DATA – *Ceriodaphnia dubia* 3-BROOD TEST COASTAL BIOANALYSTS, INC FORM ETF0054D EFFECTIVE DATE: 2/12/10

| Parameter | Treatment | Day 0 Initial | Day 1 Final | Day 1 Initial | Day 2 Final | Day 2 Initial | Day 3 Final | Day 3 Initial | Day 4 Final | Day 4 Initial | Day 5 Final | Day 5 Initial | Day 6 Final | Day 6 Initial | Day 7 Final |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMP (°C) | C | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | |
| | 1 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | |
| | 2 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | |
| | 3 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | |
| | 4 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | |
| | 5 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | |
| pH (S.U.) | C | 7.52 | 7.84 | 7.45 | 8.05 | 7.57 | 7.99 | 7.60 | 7.94 | 7.64 | 8.15 | 7.58 | 8.03 | | |
| | 1 | 7.56 | 8.19 | 7.45 | 8.19 | 7.58 | 8.15 | 7.45 | 8.11 | 7.47 | 8.28 | 7.62 | 7.94 | | |
| | 2 | 7.59 | 8.32 | 7.48 | 8.32 | 7.62 | 8.14 | 7.47 | 8.17 | 7.49 | 8.34 | 7.65 | 8.00 | | |
| | 3 | 7.67 | 8.26 | 7.73 | 8.31 | 7.68 | 8.26 | 7.72 | 8.15 | 7.71 | 8.25 | 7.69 | 8.19 | | |
| | 4 | 7.76 | 8.29 | 7.77 | 8.34 | 7.77 | 8.39 | 7.78 | 8.27 | 7.73 | 8.44 | 7.75 | 8.14 | | |
| | 5 | 7.86 | 8.39 | 7.84 | 8.38 | 7.86 | 8.39 | 7.83 | 8.34 | 7.73 | 8.41 | 7.80 | 8.29 | | |
| D.O. (mg/l) | C | 8.2 | 8.3 | 8.2 | 8.5 | 8.2 | 8.4 | 8.2 | 8.0 | 8.2 | 8.5 | 8.2 | 8.5 | | |
| | 1 | 8.2 | 8.6 | 8.2 | 8.7 | 8.2 | 8.5 | 8.2 | 8.3 | 8.2 | 8.9 | 8.2 | 8.5 | | |
| | 2 | 8.2 | 8.9 | 8.2 | 8.8 | 8.2 | 8.7 | 8.2 | 8.5 | 8.2 | 9.1 | 8.2 | 8.6 | | |
| | 3 | 8.2 | 8.9 | 8.2 | 9.1 | 8.2 | 8.8 | 8.2 | 8.5 | 8.2 | 9.0 | 8.2 | 8.7 | | |
| | 4 | 8.2 | 9.0 | 8.2 | 9.2 | 8.2 | 9.0 | 8.2 | 8.5 | 8.2 | 9.5 | 8.2 | 8.7 | | |
| | 5 | 8.2 | 9.1 | 8.2 | 9.2 | 8.2 | 9.2 | 8.2 | 8.7 | 8.2 | 9.5 | 8.2 | 9.1 | | |
| COND (uS/cm) | C | 300 | | 291 | | 292 | | 294 | | 296 | | 293 | | | |
| | 1 | 313 | | 312 | | 313 | | 316 | | 315 | | 311 | | | |
| | 2 | 332 | | 333 | | 332 | | 333 | | 333 | | 328 | | | |
| | 3 | 372 | | 371 | | 377 | | 371 | | 370 | | 367 | | | |
| | 4 | 458 | | 454 | | 461 | | 470 | | 464 | | 445 | | | |
| | 5 | 656 | | 650 | | 652 | | 630 | | 626 | | 629 | | | |
| Replicate: | | S | E | S | B | S | F | S | G | S | J | S | A | S | |
| Initials: | | AG | GB | | AG | | GA | | GB | | AG | | GA | | |

NOTE: D.O. values > 8.3 mg/L due to photosynthetic activity of algal doses. SA

| | | |
|---|---|---|
| C= 0 % | 2= 10.0 % | 4= 46.0 % |
| 1= 4.60 % | 3= 22.0 % | 5= 100 % |

TEST I.D. EMINILIY -CCD

REPRODUCTION/SURVIVAL DATA – *Ceriodaphnia* 3-BROOD TEST

Coastal Bioanalysts, Inc
Form ETF0053E
Effective Date: 2/12/10

| Treatment | REP | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| C | A | 0 | 0 | 1 | 4 | 0 | 12 | |
| | B | 0 | 0 | 5 | 6 | 0 | 14 | |
| | C | 0 | 0 | 5 | 10 | 0 | 12 | |
| | D | 0 | 0 | 4 | 5 | 0 | 12 | |
| Lab | E | 0 | 0 | 2 | 0 | 12 | 14 | |
| Control | F | 0 | 0 | 3 | 0 | 10 | 10 | |
| | G | 0 | 0 | 4 | 10 | 0 | 14 | |
| | H | 0 | 0 | 4 | 5 | 0 | 12 | |
| | I | 0 | 0 | 5 | 10 | 0 | 14 | |
| | J | 0 | 0 | 4 | 8 | 0 | 14 | |
| 1 | A | 0 | 0 | 2 | 9 | 0 | 14 | |
| | B | 0 | 0 | 3 | 10 | 0 | 16 | |
| | C | 0 | 0 | 3 | 8 | 0 | 8 | |
| | D | 0 | 0 | 2 | 10 | 0 | 14 | |
| 4.6 Dil | E | 0 | 0 | 0 | 0 | 12 | 10 | |
| Conc. | F | 0 | 0 | 0 | 3 | 0 | 10 | |
| | G | 0 | 0 | 3 | 8 | 0 | 12 | |
| | H | 0 | 0 | 4 | 12 | 0 | 16 | |
| 9.2 | I | 0 | 0 | 2 | 7 | 0 | 14 | |
| Spl. Vol. | J | 0 | 0 | 5 | 10 | 0 | 14 | |
| 2 | A | 0 | 0 | 3 | 10 | 0 | 12 | |
| | B | 0 | 0 | 3 | 9 | 22 | 0 | |
| | C | 0 | 0 | 3 | 12 | 0 | 14 | |
| | D | 0 | 0 | 2 | 7 | 0 | 16 | |
| 10.0 Dil | E | 0 | 0 | 2 | 8 | 0 | 12 | |
| Conc. | F | 0 | 0 | 2 | 7 | 0 | 0 | |
| | G | 0 | 0 | 0 | 0 | 0 | 14 | |
| | H | 0 | 0 | 2 | 10 | 0 | 14 | |
| 20 | I | 0 | 0 | 0 | 10 | 0 | 8 | |
| Spl. Vol. | J | 0 | 0 | 2 | 9 | 0 | 12 | |

| Treatment | REP | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| 3 | A | 0 | 0 | 2 | 8 | 0 | 8 | |
| | B | 0 | 0 | 0 | 14 | 0 | 14 | |
| | C | 0 | 0 | 5 | 10 | 0 | 10 | |
| | D | 0 | 0 | 2 | 9 | 0 | 12 | |
| 22.0 Dil | E | 0 | 0 | 4 | 4 | 0 | 6 | |
| Conc. | F | 0 | 0 | 0 | 0 | 0 | 2 | |
| | G | 0 | 0 | 3 | 10 | 0 | 12 | |
| | H | 0 | 0 | 0X | - | - | - | |
| 44 | I | 0 | 0 | 3 | 10 | 0 | 14 | |
| Spl. Vol. | J | 0 | 0 | 3 | 0 | 10 | 12 | |
| 4 | A | 0 | 0 | 2 | 10 | 0 | 8 | |
| | B | 0 | 0 | 3 | 8 | 0 | 16 | |
| | C | 0 | 0 | 0 | 8 | 0 | 14 | |
| | D | 0 | 0 | 4 | 9 | 0 | 12 | |
| 46.0 Dil | E | 0 | 0 | 4 | 10 | 0 | 14 | |
| Conc. | F | 0 | 0 | 2 | 0 | 10 | 10 | |
| | G | 0 | 0 | 4 | 7 | 0 | 16 | |
| | H | 0 | 0 | 4 | 10 | 0 | 10 | |
| 92 | I | 0 | 0 | 4 | 10 | 14 | 0 | |
| Spl. Vol. | J | 0 | 0 | 3 | 10 | 0 | 14 | |
| 5 | A | 0 | 0 | 5 | 12 | 0 | 10 | |
| | B | 0 | 0 | 4 | 10 | 0 | 12 | |
| | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | D | 0 | 0 | 2 | 10 | 0 | 14 | |
| 100 Dil | E | 0 | 0 | 3 | 7 | 0 | 12 | |
| Conc. | F | 0 | 0 | 2 | 0 | 8 | 10 | |
| | G | 0 | 0 | 0 | 8 | 0 | 12 | |
| | H | 0 | 0 | 2 | 8 | 0 | 8 | |
| 200 | I | 0 | 0 | 5 | 6 | 0 | 14 | |
| Spl. Vol. | J | 0 | 0 | 2 | 8 | 0 | 14 | |

| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| Count/Renewal Time*: | 1145 | 1200 | 1255 | 1115 | 1200 | 1110 | |
| Initials: | CB | AG | CB | CB | AG | CB | |

*Counts are number of live offspring at renewal; dead adults indicated by a "X" with dashes (-) for subsequent test days. Time of final count (day 6 or 7) = test end time. Males, if present, identified (M) at test end. See printout of statistical analyses for total number of offspring by replicate.

Note: "Spl. Vol." = Volume sample added to total volume of 200 ml for preparation of dilutions.

**NOTES:**

Test I.D. EMIN1114 -CCD

## Ceriodaphnia Survival and Reproduction Test-6 Day Survival

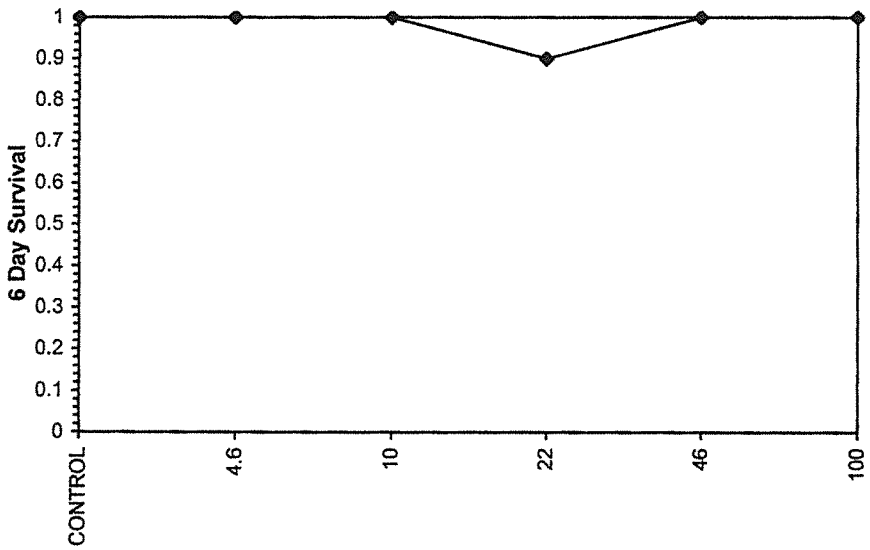
| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 11:30 | Test ID: | EMIN1114CD | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/3/2011 11:10 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | DATA ENTERED BY PB | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 4.6 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 10 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 22 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.0000 | 1.0000 | 1.0000 |
| 46 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 100 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |

| Conc-% | Mean | N-Mean | Resp | Not Resp | Total | N | Fisher's Exact P | 1-Tailed Critical |
|---|---|---|---|---|---|---|---|---|
| CONTROL | 1.0000 | 1.0000 | 0 | 10 | 10 | 10 | | |
| 4.6 | 1.0000 | 1.0000 | 0 | 10 | 10 | 10 | 1.0000 | 0.0500 |
| 10 | 1.0000 | 1.0000 | 0 | 10 | 10 | 10 | 1.0000 | 0.0500 |
| 22 | 0.9000 | 0.9000 | 1 | 9 | 10 | 10 | 0.5000 | 0.0500 |
| 46 | 1.0000 | 1.0000 | 0 | 10 | 10 | 10 | 1.0000 | 0.0500 |
| 100 | 1.0000 | 1.0000 | 0 | 10 | 10 | 10 | 1.0000 | 0.0500 |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Fisher's Exact Test | 100 | >100 | | 1 |

**Dose-Response Plot**






ToxCalc v5.0.23

Reviewed by: PD

## Ceriodaphnia Survival and Reproduction Test-Reproduction

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 11:30 | Test ID: | EMIN1114CD | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/3/2011 11:10 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | DATA ENTERED BY PB | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL | 17.000 | 25.000 | 27.000 | 21.000 | 28.000 | 23.000 | 28.000 | 21.000 | 29.000 | 26.000 |
| 4.6 | 25.000 | 29.000 | 19.000 | 26.000 | 22.000 | 13.000 | 23.000 | 32.000 | 23.000 | 29.000 |
| 10 | 25.000 | 34.000 | 29.000 | 25.000 | 22.000 | 9.000 | 14.000 | 26.000 | 18.000 | 23.000 |
| 22 | 18.000 | 28.000 | 25.000 | 23.000 | 14.000 | 2.000 | 25.000 | 0.000 | 27.000 | 25.000 |
| 46 | 20.000 | 27.000 | 22.000 | 25.000 | 28.000 | 22.000 | 27.000 | 24.000 | 28.000 | 27.000 |
| 100 | 27.000 | 26.000 | 0.000 | 26.000 | 22.000 | 20.000 | 20.000 | 18.000 | 25.000 | 24.000 |

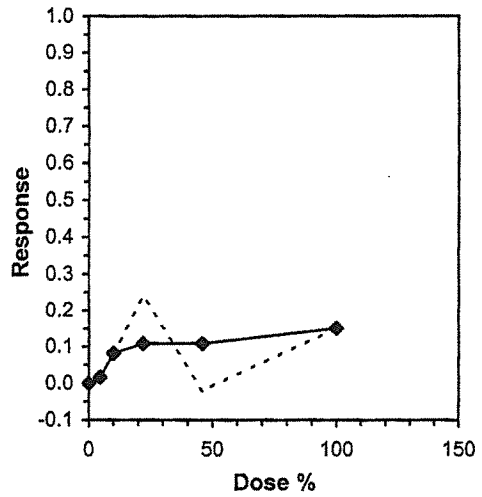
| | | | Transform: Untransformed | | | | | Rank | 1-Tailed | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Mean | N-Mean |
| CONTROL | 24.500 | 1.0000 | 24.500 | 17.000 | 29.000 | 15.896 | 10 | | | 24.500 | 1.0000 |
| 4.6 | 24.100 | 0.9837 | 24.100 | 13.000 | 32.000 | 22.765 | 10 | 105.00 | 75.00 | 24.100 | 0.9837 |
| 10 | 22.500 | 0.9184 | 22.500 | 9.000 | 34.000 | 32.271 | 10 | 96.50 | 75.00 | 22.500 | 0.9184 |
| 22 | 18.700 | 0.7633 | 18.700 | 0.000 | 28.000 | 54.770 | 10 | 87.50 | 75.00 | 21.850 | 0.8918 |
| 46 | 25.000 | 1.0204 | 25.000 | 20.000 | 28.000 | 11.470 | 10 | 106.00 | 75.00 | 21.850 | 0.8918 |
| 100 | 20.800 | 0.8490 | 20.800 | 0.000 | 27.000 | 38.045 | 10 | 87.00 | 75.00 | 20.800 | 0.8490 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | 1.03889 | 1.035 | -1.2982 | 2.14633 |
| Bartlett's Test indicates unequal variances (p = 5.09E-03) | 16.7087 | 15.0863 | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 100 | >100 | | 1 |

### Linear Interpolation (200 Resamples)

| Point | % | SD | 95% CL | Skew |
|---|---|---|---|---|
| IC05 | 7.384 | | | |
| IC10 | 18.308 | | | |
| IC15 | 98.714 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |





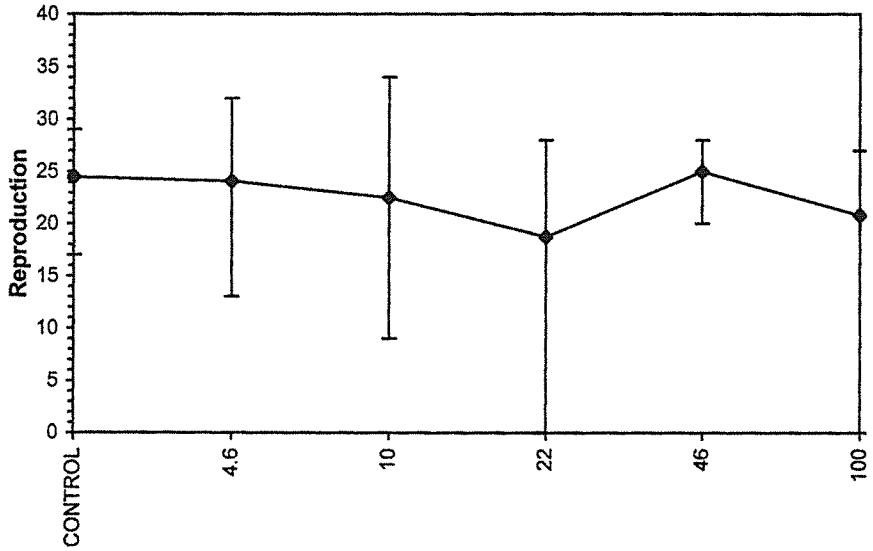

ToxCalc v5.0.23

Reviewed by: PD

## Ceriodaphnia Survival and Reproduction Test-Reproduction

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 11:30 | Test ID: | EMIN1114CD | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/3/2011 11:10 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | DATA ENTERED BY PB | | | | |

### Dose-Response Plot






ToxCalc v5.0.23

Reviewed by: PD

## Ceriodaphnia Survival and Reproduction Test-Reproduction

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 11:30 | Test ID: | EMIN1114CD | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/3/2011 11:10 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | DATA ENTERED BY PB | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL | 17.000 | 25.000 | 27.000 | 21.000 | 28.000 | 23.000 | 28.000 | 21.000 | 29.000 | 26.000 |
| 4.6 | 25.000 | 29.000 | 19.000 | 26.000 | 22.000 | 13.000 | 23.000 | 32.000 | 23.000 | 29.000 |
| 10 | 25.000 | 34.000 | 29.000 | 25.000 | 22.000 | 9.000 | 14.000 | 26.000 | 18.000 | 23.000 |
| 22 | 18.000 | 28.000 | 25.000 | 23.000 | 14.000 | 2.000 | 25.000 | 0.000 | 27.000 | 25.000 |
| 46 | 20.000 | 27.000 | 22.000 | 25.000 | 28.000 | 22.000 | 27.000 | 24.000 | 28.000 | 27.000 |
| 100 | 27.000 | 26.000 | 0.000 | 26.000 | 22.000 | 20.000 | 20.000 | 18.000 | 25.000 | 24.000 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| CONTROL | 24.500 | 1.0000 | 24.500 | 17.000 | 29.000 | 15.896 | 10 | | | |
| 4.6 | 24.100 | 0.9837 | 24.100 | 13.000 | 32.000 | 22.765 | 10 | 0.132 | 2.287 | 6.907 |
| 10 | 22.500 | 0.9184 | 22.500 | 9.000 | 34.000 | 32.271 | 10 | 0.662 | 2.287 | 6.907 |
| 22 | 18.700 | 0.7633 | 18.700 | 0.000 | 28.000 | 54.770 | 10 | 1.920 | 2.287 | 6.907 |
| 46 | 25.000 | 1.0204 | 25.000 | 20.000 | 28.000 | 11.470 | 10 | -0.166 | 2.287 | 6.907 |
| 100 | 20.800 | 0.8490 | 20.800 | 0.000 | 27.000 | 38.045 | 10 | 1.225 | 2.287 | 6.907 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | 1.03889 | 1.035 | -1.2982 | 2.14633 |
| Bartlett's Test indicates unequal variances (p = 5.09E-03) | 16.7087 | 15.0863 | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 100 | >100 | | 1 | 6.90726 | 0.28193 | 60.16 | 45.6222 | 0.27009 | 5, 54 |

FOR PMSD ONLY IPD



ToxCalc v5.0.23

Reviewed by: PP

BASELINE TEST INFO – FATHEAD 7 DAY TEST

Coastal Bioanalysts, Inc
Form ETF0041C
Effective Date: 2/1/09

**TEST ORGANISM INFO**

**Species:** *Pimephales promelas*

**Source:** CBI Stock Cultures: ✓

Other: _____

**Hatch Date/Time:** 09/26/11 1630
(start)

**Hatch Date/Time:** 09/27/11 0930
(end)

**Acclimation:** **Water:** Mod. hard syn. FW ✓

Other: _____

**Temp. (°C):** 25

**Feeding Prior to Test:** *Artemia ad libitum* 2X/day

**Feeding During Test:** *Artemia* 0.15 g/rep 2X/day

**Arrival Date:** NA
**(non-CBI)**

**TEST DESIGN**

**Test Chamber:** 1000 ml Tri-pour beaker ✓

Other _____

**Solution Vol:** 500 ml ✓

Other _____

**Illumination:** 16:8 L:D 10-20 uE/m$^2$/s

**Number of Replicates/Concentration:** 4

**Initial Number of Fish/Replicate:** 10

**TEST SET UP (Day 0)**

**Set Up Date:** 09/27/11

**Set Up By:** bja

**Time Water Added:** 1150

**Time Animals Added:** 1205

**NOTES**

Peer Review by PB/GR Date 10/5/11

Test I.D. EMIN1114 -CPP

WATER QUALITY DATA – *Pimephales promelas* 7-day Larval Test COASTAL BIOANALYSTS, INC FORM ETF0042C EFFECTIVE DATE: 2/15/10

| Parameter | Treatment | Day 0 Initial | Day 1 Final | Day 1 Initial | Day 2 Final | Day 2 Initial | Day 3 Final | Day 3 Initial | Day 4 Final | Day 4 Initial | Day 5 Final | Day 5 Initial | Day 6 Final | Day 6 Initial | Day 7 Final |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMP (°C) | C | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 25 | 25 | 25 | 24 |
| | 1 | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 25 | 25 | 25 | 24 |
| | 2 | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 25 | 25 | 25 | 24 |
| | 3 | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 25 | 25 | 25 | 24 |
| | 4 | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 25 | 25 | 25 | 24 |
| | 5 | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 24 | 25 | 25 | 25 | 24 |
| pH (S.U.) | C | 7.58 | 7.24 | 7.53 | 7.34 | 7.61 | 7.31 | 7.56 | 7.27 | 7.55 | 7.29 | 7.63 | 7.16 | 7.52 | 7.22 |
| | 1 | 7.64 | 7.23 | 7.54 | 7.19 | 7.53 | 7.26 | 7.59 | 7.11 | 7.59 | 7.19 | 7.63 | 7.06 | 7.54 | 7.24 |
| | 2 | 7.67 | 7.27 | 7.55 | 7.17 | 7.62 | 7.20 | 7.63 | 7.25 | 7.61 | 7.33 | 7.65 | 7.28 | 7.62 | 7.36 |
| | 3 | 7.73 | 7.29 | 7.60 | 7.09 | 7.68 | 7.31 | 7.68 | 7.29 | 7.64 | 7.32 | 7.73 | 7.35 | 7.70 | 7.42 |
| | 4 | 7.81 | 7.45 | 7.71 | 7.21 | 7.78 | 7.48 | 7.77 | 7.33 | 7.74 | 7.54 | 7.77 | 7.53 | 7.76 | 7.51 |
| | 5 | 7.90 | 7.46 | 7.84 | 7.61 | 7.88 | 7.46 | 7.88 | 7.67 | 7.74 | 7.81 | 7.83 | 7.71 | 7.84 | 7.82 |
| D.O. (mg/l) | C | 8.2 | 7.4 | 8.2 | 7.4 | 8.1 | 6.9 | 8.1 | 7.0 | 8.0 | 7.2 | 8.0 | 6.6 | 8.0 | 7.0 |
| | 1 | 8.2 | 7.4 | 8.2 | 7.4 | 8.1 | 6.9 | 8.1 | 6.8 | 8.0 | 6.4 | 8.0 | 6.7 | 8.0 | 7.2 |
| | 2 | 8.2 | 7.3 | 8.2 | 7.3 | 8.1 | 7.0 | 8.1 | 6.7 | 7.9 | 7.1 | 7.8 | 6.9 | 8.0 | 7.4 |
| | 3 | 8.2 | 7.3 | 8.2 | 7.3 | 8.0 | 6.8 | 8.1 | 6.6 | 7.9 | 6.6 | 7.9 | 7.0 | 8.1 | 7.5 |
| | 4 | 8.2 | 7.4 | 8.2 | 7.2 | 8.1 | 6.6 | 8.1 | 6.6 | 7.9 | 6.9 | 8.0 | 7.1 | 8.1 | 7.5 |
| | 5 | 8.2 | 7.5 | 8.2 | 7.2 | 8.2 | 6.4 | 8.1 | 6.6 | 7.9 | 7.4 | 8.2 | 7.2 | 8.1 | 7.6 |
| COND (uS/cm) | C | 302 | | 296 | | 294 | | 298 | | 295 | | 293 | | 293 | |
| | 1 | 317 | | 315 | | 313 | | 316 | | 313 | | 313 | | 309 | |
| | 2 | 336 | | 334 | | 332 | | 335 | | 332 | | 330 | | 329 | |
| | 3 | 377 | | 376 | | 376 | | 376 | | 371 | | 372 | | 361 | |
| | 4 | 461 | | 459 | | 459 | | 454 | | 444 | | 453 | | 440 | |
| | 5 | 661 | | 661 | | 653 | | 641 | | 635 | | 634 | | 625 | |
| Replicate: | | D | A | C | A | B | D | A | A | C | B | D | D | C | A |
| Initials: | | bja | CA | bja | RB | AG | CA | bja | CA | CA | AG | AG | CA | CA | CA |

| | | |
|---|---|---|
| C= 0 % | 2= 10.0 % | 4= 46.0 % |
| 1= 8.60 % | 3= 22.0 % | 5= 100 % |

TEST I.D. EMINI/14 -CPP

SURVIVAL & GROWTH DATA – FATHEAD MINNOW 7 DAY TEST  Coastal Bioanalysts, Inc  Form ETF0043F  Effective Date: 11/26/07

| Treatment[1] | | Rep Ltr | Number of Live Fish | | | | | | | Fish Dry Weight Data (mg)[2] | | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Pan # | Tot. Wt. | Tare Wt. | |
| C | CONTROL | A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 1 | 16.70 | 8.86 | |
| | | B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 2 | 15.36 | 8.10 | |
| | | C | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 3 | 17.56 | 8.48 | |
| | | D | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 4 | 16.32 | 8.50 | |
| 1 | C: 4.60% | A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 5 | 18.20 | 10.62 | |
| | | B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 6 | 17.28 | 8.01 | |
| | | C | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7 | 17.87 | 8.71 | |
| | V: 73.6 | D | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 16.89 | 8.53 | |
| 2 | C: 10.0% | A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 15.17 | 8.77 | |
| | | B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 18.53 | 10.12 | |
| | | C | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 11 | 15.69 | 8.61 | |
| | V: 160 | D | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 12 | 17.29 | 9.47 | |
| 3 | C: 22.0% | A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 13 | 16.92 | 9.46 | |
| | | B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 14 | 18.34 | 9.37 | |
| | | C | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 15 | 16.76 | 9.62 | |
| | V: 352 | D | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 16 | 18.93 | 9.46 | |
| 4 | C: 46.0% | A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 17 | 18.12 | 10.76 | |
| | | B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 18 | 16.89 | 8.67 | |
| | | C | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 19 | 15.46 | 8.76 | |
| | V: 736 | D | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 20 | 15.51 | 8.14 | |
| 5 | C: 100% | A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 21 | 14.00 | 9.00 | |
| | | B | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 22 | 17.03 | 10.31 | |
| | | C | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 23 | 17.84 | 10.51 | |
| | V: 1600 | D | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 24 | 19.46 | 10.44 | |
| Renewal/Count Time: | | | 1200 | 1120 | 1330 | 1145 | 1320 | 1040 | 1205[3] | Tare Wt: Date: 10/1 Cal. Chk (100.00 mg[4]): 99.99 Init: GB | | | |
| Initials: | | | bja | AG | bja | GB | AG | GB | bja | Tot. Wt: Date: 10/5 Cal. Chk (100.00 mg[4]): 100.00 Init: PB | | | |

[1] C = Concentration; V = Volume (ml) sample added to total volume of 1600 ml for preparation of solutions. [2] See printout of statistical analyses for biomass weights. [3] Time final count = test end time. [4] True value ± estimated uncertainty of calib. weight (NIST traceable annual certification) = 100.00 ± 0.01 mg

Test ID EMIN1114 -CPP

## Larval Fish Growth and Survival Test-7 Day Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 12:05 | Test ID: | EMIN1114PP | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/4/2011 12:05 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | PP-Pimephales promelas |
| Comments: | DATA ENTERED BY PB | | | | |

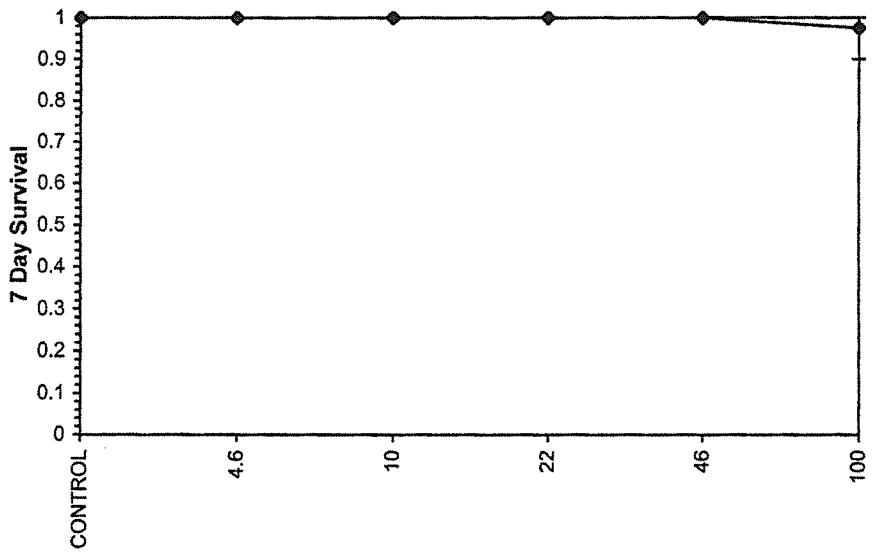
| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| CONTROL | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 4.6 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 10 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 22 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 46 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 100 | 1.0000 | 0.9000 | 1.0000 | 1.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed |
|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical |
| CONTROL | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | | |
| 4.6 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 |
| 10 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 |
| 22 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 |
| 46 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 |
| 100 | 0.9750 | 0.9750 | 1.3713 | 1.2490 | 1.4120 | 5.942 | 4 | 16.00 | 10.00 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.46508 | 0.884 | -3.0206 | 13.9892 |
| Equality of variance cannot be confirmed | | | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 100 | >100 | | 1 |

### Dose-Response Plot






ToxCalc v5.0.23

Reviewed by: 10

## Larval Fish Growth and Survival Test-7 Day Biomass

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 12:05 | Test ID: | EMIN1114PP | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/4/2011 12:05 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | PP-Pimephales promelas |
| Comments: | DATA ENTERED BY PB | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| CONTROL | 0.7840 | 0.7260 | 0.9080 | 0.7820 |
| 4.6 | 0.7580 | 0.9270 | 0.9160 | 0.8360 |
| 10 | 0.6400 | 0.8410 | 0.7080 | 0.7320 |
| 22 | 0.7460 | 0.8970 | 0.7140 | 0.8970 |
| 46 | 0.7360 | 0.8220 | 0.6700 | 0.7370 |
| 100 | 0.7000 | 0.6720 | 0.7330 | 0.9020 |

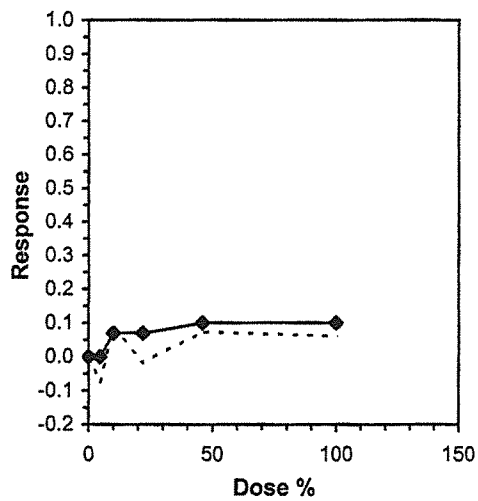
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| CONTROL | 0.8000 | 1.0000 | 0.8000 | 0.7260 | 0.9080 | 9.607 | 4 | | | | 0.8296 | 1.0000 |
| 4.6 | 0.8593 | 1.0741 | 0.8593 | 0.7580 | 0.9270 | 9.164 | 4 | -0.989 | 2.410 | 0.1444 | 0.8296 | 1.0000 |
| 10 | 0.7303 | 0.9128 | 0.7303 | 0.6400 | 0.8410 | 11.432 | 4 | 1.164 | 2.410 | 0.1444 | 0.7719 | 0.9304 |
| 22 | 0.8135 | 1.0169 | 0.8135 | 0.7140 | 0.8970 | 11.960 | 4 | -0.225 | 2.410 | 0.1444 | 0.7719 | 0.9304 |
| 46 | 0.7413 | 0.9266 | 0.7413 | 0.6700 | 0.8220 | 8.404 | 4 | 0.981 | 2.410 | 0.1444 | 0.7465 | 0.8998 |
| 100 | 0.7518 | 0.9397 | 0.7518 | 0.6720 | 0.9020 | 13.731 | 4 | 0.805 | 2.410 | 0.1444 | 0.7465 | 0.8998 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.92748 | 0.884 | 0.41393 | -0.9999 |
| Bartlett's Test indicates equal variances (p = 0.97) | 0.83804 | 15.0863 | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 100 | >100 | | 1 | 0.1444 | 0.1805 | 0.01003 | 0.00718 | 0.27234 | 5, 18 |

### Linear Interpolation (200 Resamples)

| Point | % | SD | 95% CL(Exp) | Skew |
|---|---|---|---|---|
| IC05 | 8.479 | | | |
| IC10 | 45.846 | | | |
| IC15 | >100 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |

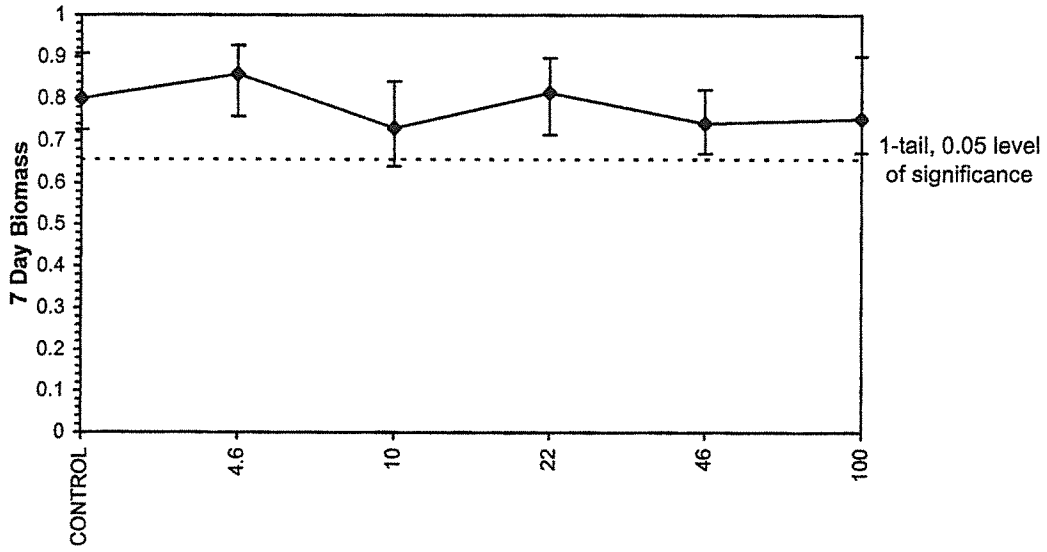



 ToxCalc v5.0.23 Reviewed by: PD

## Larval Fish Growth and Survival Test-7 Day Biomass

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/27/2011 12:05 | Test ID: | EMIN1114PP | Sample ID: | HOLSTON WWTP 001 |
| End Date: | 10/4/2011 12:05 | Lab ID: | CBI | Sample Type: | WW |
| Sample Date: | | Protocol: | EPAF 94-EPA Freshwater | Test Species: | PP-Pimephales promelas |
| Comments: | DATA ENTERED BY PB | | | | |

### Dose-Response Plot






ToxCalc v5.0.23

Reviewed by: PD

EFFLUENT SAMPLE & DILUTION WATER CHARACTERISTICS
**FRESHWATER TESTS** FORM ETF2031E

COASTAL BIOANALYSTS, INC
EFFECTIVE DATE: 1/14/10

| INITIAL SAMPLE CHARACTERIZATION[1] | | | | |
|---|---|---|---|---|
| Sample Bottle[2] | A-1 | B-1 | C-1 | |
| Tot. Res. Chlorine (mg/l) | <QL | <QL | <QL | |
| Hardness (mg/l $CaCO_3$) | 234 | 220 | 208 | |
| Alkalinity (mg/l $CaCO_3$) | 123 | 131 | 131 | |
| $NH_3$-N (mg/l) | <1.0 | <1.0 | <1.0 | |
| Color/Appearance[3] | C | C | C | |
| Obvious Odor? | ND | ND | ND | |
| Date/Time | 9/22 1045 | 9/28 0950 | 9/30 1015 | |
| Initials | GB | GB | GB | |

NOTES:

____ TRC corrected for potential positive interference by Mn or Cr with KI & $NaAsO_2$

| SAMPLE PREPARATION MEASUREMENTS (100% concentration) | | | | | | | | Acute #1 |
|---|---|---|---|---|---|---|---|---|
| Sample Bottle[2] | A-1 | B-1 | B-1 | C-1,2 | C-1,2 | C-1,2 | C-1,2 | A-1 |
| Prep Temperature (°C) | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| Conductivity (uS/cm)[4] | 654 | 654 | NA | 430 | NA | NA | NA | 658 |
| D.O. (mg/l) After Warming | 9.1 | 10.3 | 10.5 | 10.8 | 10.7 | 10.1 | 10.6 | 10.7 |
| Aeration Time (min) | 2.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.5 | 3.0 |
| Adjusted D.O. | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 |
| Final pH (S.U.) | 7.88 | 7.84 | 7.81 | 7.83 | 7.71 | 7.79 | 7.79 | 7.84 |
| Tot. Res. Chlorine (mg/l)[5] | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Sample Filtered (60 um)? | YES | YES | YES | YES | YES | YES | YES | NO |
| Date/Time | 9/27 1100 | 9/28 1120 | 9/29 1115 | 9/30 1025 | 10/1 1055 | 10/2 1155 | 10/3 1025 | 9/28 1400 |
| Initials | AG | LM | AG | GB | GB | AG | LB | AG |

| DILUTION WATER CHARACTERISTICS | | | | | | | | Acute |
|---|---|---|---|---|---|---|---|---|
| Vat Number | 1 | 2 | 2 | 3 | 3 | 1 | 1 | 2 |
| Temperature (°C) | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| Conductivity (uS/cm) | 294 | 293 | 294 | 292 | 301 | 295 | 290 | 293 |
| D.O. (mg/l) | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 | 8.2 |
| pH (S.U.) | 7.64 | 7.64 | 7.65 | 7.60 | 7.63 | 7.68 | 7.60 | 7.64 |
| Hardness (mg/l $CaCO_3$) | 100 | 84 | 84 | 82 | 82 | 98 | 98 | 84 |
| Alkalinity (mg/l $CaCO_3$) | 54 | 57 | 57 | 60 | 60 | 58 | 58 | 57 |
| Date/Time | 9/27 0825 | 9/28 0825 | 9/29 0815 | 9/30 0850 | 10/1 0855 | 10/2 0930 | 10/3 0840 | 9/28 0825 |
| Initials | GB | GB | PB | GB | GB | AG | GB | GB |

[1]Q.L. = Quantification Limit, N.D. = Not Determined/Measured, NA = Not Applicable
[2]Ninth character of Laboratory Sample I.D. (on chain of custody form) and bottle number in collection series (e.g. "A-2" is sample bottle number 2 from "A" collection). Together with project ID below constitutes entire sample bottle ID.
[3]C-Clear, O-Opaque, T-Turbid, S-Solids (Sl-Slight, M-Moderate, H-Heavy), Y-Yellow, B-Brown, Bl-Black, G-Green
[4]Conductivity measured on first use of sample only
[5]Total residual chlorine measured after sample prep only if present in initial sample characterization

Peer Rev by GB / PB Date 10/3/11 PROJECT I.D. EMIN1114
(First 8 characters of Laboratory Sample ID)

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Coastal Bioanalysis
6400 Enterprise Court, Gloucester, VA 23061
PH: 804-694-8285, FAX: 804-695-1129
www.coastalbio.com

EMI
1534.25

# SAMPLE INFORMATION/CHAIN-OF-CUSTODY (FORM ETF2011H Rev. 1/14/11)

**Lab Sample ID** (Lab Use Only): E M I N 1 1 1 4 - A
A A A A Y Y N N — Project ID; A — Spl

**CBI Login #** 11-0735

po# 508122

## FACILITY INFORMATION

| | |
|---|---|
| CLIENT/FACILITY NAME: Holston WWTP | CONTACT & PHONE #: EMI 276.679.6544 |
| NPDES PERMIT NO: VA 0067351 | OUTFALL # OR LOCATION: 001 |
| SAMPLE CHLORINATED? | SAMPLE DECHLORINATED? / IF CHLORINE PRESENT UPON ARRIVAL AT LAB, DOES PERMIT SPECIFY DECHLORINATION OF SAMPLES? |

| TESTS REQUESTED: | SPECIES OR EPA METH # | | | |
|---|---|---|---|---|
| | | C. dubia | ACUTE ☐ | CHRONIC ☑ |
| | | P. promelas | ACUTE ☐ | CHRONIC ☑ |

OTHER TESTS:

A SPECIFIC DILUTION SERIES MAY BE REQUIRED IN THE PERMIT. A DEFAULT SERIES OF 100, 50, 25, 12.5 AND 6.3%, OR CONCENTRATIONS USED IN PRIOR TESTING, WILL BE USED UNLESS INDICATED OTHERWISE. **IF IN DOUBT PLEASE ATTACH A COPY OF APPLICABLE PERMIT PAGES.**

## GRAB SAMPLE INFORMATION

| SAMPLE DATE | SAMPLE TIME | SAMPLE VOLUME |
|---|---|---|
| | | |

## COMPOSITE SAMPLE INFORMATION

| | | |
|---|---|---|
| SAMPLE START DATE & TIME: 09-25-11 at 10:00 | SAMPLE END DATE & TIME: 09-26-11 at 0845 | AUTOSAMPLER TEMP. (°C): 3°C |
| TIME OR FLOW PROPORTIONAL COMPOSITE INFORMATION: NUMBER SUBSAMPLES ____ | VOL (ml) SUBSAMPLES ____ | TIME INCREMENT ____ |
| SET VOLUME SUBSAMPLE: 385ml/sample | SET VOLUME FLOW: sample per 10K gal. | TOTAL VOLUME: 0.21 MG |

FOR VARIABLE VOLUME SUBSAMPLES BASED ON FLOW (COMPOSITING "BY HAND") ATTACH SAMPLE AND FLOW INFORMATION ON SEPARATE SHEET

## FIELD MEASUREMENTS

| DISCHARGE TEMP (°C) | DISCHARGE pH (S.U.) | SAMPLE TEMP (°C) | SAMPLE TRC (mg/l) | DATE/TIME (e.g. 02/23/00 1835) | INITIALS |
|---|---|---|---|---|---|
| 21 | 7.3 | 3°C | ND | 9-26-11 at 0850 | DWP |

MEASUREMENTS MUST BE TAKEN WITHIN 15 MINUTES OF SAMPLE OR LAST SUBSAMPLE COLLECTION.

**COMMENTS:**

David Porter - FieldTech [signature] 9-26-11
(PRINTED NAME/AFFILIATION SAMPLER/ANALYST) (SIGNATURE) (DATE)

| RELINQUISHED BY | DATE | TIME | RECEIVED BY |
|---|---|---|---|
| [signature] | 9-26-11 | 1140 | LAB Cooler |
| Cooler [signature] | 9/26/11 | 1445 | [signature] |
| [signature] | 9/27/11 | 1000 | [signature] |

**SHIPPING METHOD:** UPS ____ FEDEX X HAND DELIVERY ____

DO NOT SHIP FEDEX STANDARD OVERNIGHT. SAMPLES MUST ARRIVE AT LAB BY NOON.

**CONDITION ON ARRIVAL:** ACCEPTABLE ✓ OTHER ____

SAMPLE TEMP: (°C) 1 ARRIVED ON ICE? Y ✓ N ___ CUSTODY SEAL: INTACT ✓ BROKEN ___ ABSENT ___

**NOTE: It is the responsibility of the sampler to insure that samples are properly collected, preserved (>0-6° C) and shipped. Sample hold time is 36 h. Additional costs may be incurred by improper preservation, shipping or receipt of samples after 3 p.m. or on weekends and holidays.**

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Coastal Bioanalysts
6400 Enterprise Court, Gloucester, VA 23061
PH: 804-694-8285, FAX: 804-695-1129
www.coastalbio.com

EMI
1534.25
PO#508122

# SAMPLE INFORMATION/CHAIN-OF-CUSTODY (FORM ETF2011H Rev. 1/14/11)

**Lab Sample ID** (Lab Use Only): E M I N 1 1 1 4 - B
A A A A Y Y N N (Project ID) — A (Spl)

**CBI Login #** 11-0741

## FACILITY INFORMATION

| | |
|---|---|
| CLIENT/FACILITY NAME: Holston Regional WWTP | CONTACT & PHONE #: EMI 276 679 6544 |
| NPDES PERMIT NO: VA 0067351 | OUTFALL # OR LOCATION: 001 |
| SAMPLE CHLORINATED? UV Light | SAMPLE DECHLORINATED? / IF CHLORINE PRESENT UPON ARRIVAL AT LAB, DOES PERMIT SPECIFY DECHLORINATION OF SAMPLES? |

TESTS REQUESTED:

| SPECIES OR EPA METH # | | | |
|---|---|---|---|
| C. dubia | ACUTE ☑ | CHRONIC ☑ | |
| P. promelas | ACUTE ☑ | CHRONIC ☑ | |

OTHER TESTS:

A SPECIFIC DILUTION SERIES MAY BE REQUIRED IN THE PERMIT. A DEFAULT SERIES OF 100, 50, 25, 12.5 AND 6.3%, OR CONCENTRATIONS USED IN PRIOR TESTING, WILL BE USED UNLESS INDICATED OTHERWISE. **IF IN DOUBT PLEASE ATTACH A COPY OF APPLICABLE PERMIT PAGES.**

## GRAB SAMPLE INFORMATION

| SAMPLE DATE | SAMPLE TIME | SAMPLE VOLUME |
|---|---|---|
| | | |

## COMPOSITE SAMPLE INFORMATION

| | | |
|---|---|---|
| SAMPLE START DATE & TIME: 9-26-11 at 0905 | SAMPLE END DATE & TIME: 9-27-11 at 0851 | AUTOSAMPLER TEMP. (°C): 3°C |

TIME OR FLOW PROPORTIONAL COMPOSITE INFORMATION: NUMBER SUBSAMPLES ____ VOL (ml) SUBSAMPLES ____ TIME INCREMENT ____
SET VOLUME SUBSAMPLE: 375 ml/sample SET VOLUME FLOW: sample per 12K GAL. TOTAL VOLUME: 0.302 MG

FOR VARIABLE VOLUME SUBSAMPLES BASED ON FLOW (COMPOSITING "BY HAND") ATTACH SAMPLE AND FLOW INFORMATION ON SEPARATE SHEET

## FIELD MEASUREMENTS

| DISCHARGE TEMP (°C) | DISCHARGE pH (S.U.) | SAMPLE TEMP (°C) | SAMPLE TRC (mg/l) | DATE/TIME (e.g. 02/23/00 1835) | INITIALS |
|---|---|---|---|---|---|
| 21 | 7.3 | 3°C | ND | 9-27-11 at 0855 | DWP |

MEASUREMENTS MUST BE TAKEN WITHIN 15 MINUTES OF SAMPLE OR LAST SUBSAMPLE COLLECTION.

**COMMENTS:** 2 pumps used

DAVID PORTER · Field Tech — (PRINTED NAME/AFFILIATION SAMPLER/ANALYST)
[signature] — (SIGNATURE)
9-27-11 — (DATE)

| RELINQUISHED BY | DATE | TIME | RECEIVED BY |
|---|---|---|---|
| [signature] | 9-27-11 | 1040 | LAB Cooler (EMI) |
| Cooler | 9/27/11 | 1445 | [signature] |
| | 9/28/11 | 0940 | |

**SHIPPING METHOD:** UPS X FEDEX ____ HAND DELIVERY ____

DO NOT SHIP FEDEX STANDARD OVERNIGHT. SAMPLES MUST ARRIVE AT LAB BY NOON.

**CONDITION ON ARRIVAL:** ACCEPTABLE ✓ OTHER ____

SAMPLE TEMP: (°C) 1 ARRIVED ON ICE? Y ✓ N ___ CUSTODY SEAL: INTACT ✓ BROKEN ___ ABSENT ___

**NOTE: It is the responsibility of the sampler to insure that samples are properly collected, preserved (>0-6° C) and shipped. Sample hold time is 36 h. Additional costs may be incurred by improper preservation, shipping or receipt of samples after 3 p.m. or on weekends and holidays.**

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PO# 508122

# SAMPLE INFORMATION/CHAIN-OF-CUSTODY (FORM ETF2011H Rev. 1/14/11)

**Lab Sample ID** (Lab Use Only): E M I N I I I Y - C
A A A A Y Y N N (Project ID) — A (Spl)

**CBI Login #** 11-0754

## FACILITY INFORMATION

| | |
|---|---|
| CLIENT/FACILITY NAME: HOLSTON REGIONAL WWTP | CONTACT & PHONE #: EMI 276.679.6544 |
| NPDES PERMIT NO: VA0067351 | OUTFALL # OR LOCATION: 001 |
| SAMPLE CHLORINATED? UV Light / SAMPLE DECHLORINATED? | IF CHLORINE PRESENT UPON ARRIVAL AT LAB, DOES PERMIT SPECIFY DECHLORINATION OF SAMPLES? |

TESTS REQUESTED:
- SPECIES OR EPA METH #: C. dubia — ACUTE ☐ CHRONIC ☑
- SPECIES OR EPA METH #: P. promelas — ACUTE ☐ CHRONIC ☑

OTHER TESTS:

A SPECIFIC DILUTION SERIES MAY BE REQUIRED IN THE PERMIT. A DEFAULT SERIES OF 100, 50, 25, 12.5 AND 6.3%, OR CONCENTRATIONS USED IN PRIOR TESTING, WILL BE USED UNLESS INDICATED OTHERWISE. **IF IN DOUBT PLEASE ATTACH A COPY OF APPLICABLE PERMIT PAGES.**

## GRAB SAMPLE INFORMATION

| SAMPLE DATE | SAMPLE TIME | SAMPLE VOLUME |
|---|---|---|
| | | |

## COMPOSITE SAMPLE INFORMATION

| | | |
|---|---|---|
| SAMPLE START DATE & TIME: 09-28-11 at 0900 | SAMPLE END DATE & TIME: 9-29-11 at 0856 | AUTOSAMPLER TEMP. (°C): 3°C |

TIME OR FLOW PROPORTIONAL COMPOSITE INFORMATION:
- NUMBER SUBSAMPLES ____
- VOL (ml) SUBSAMPLES ____
- TIME INCREMENT ____
- SET VOLUME SUBSAMPLE: 375 ML/SAMPLE
- SET VOLUME FLOW: PER 10K GAL.
- TOTAL VOLUME: 0.252 MG

FOR VARIABLE VOLUME SUBSAMPLES BASED ON FLOW (COMPOSITING "BY HAND") ATTACH SAMPLE AND FLOW INFORMATION ON SEPARATE SHEET

## FIELD MEASUREMENTS

| DISCHARGE TEMP (°C) | DISCHARGE pH (S.U.) | SAMPLE TEMP (°C) | SAMPLE TRC (mg/l) | DATE/TIME (e.g. 02/23/00 1835) | INITIALS |
|---|---|---|---|---|---|
| 20° | 7.4 | + 3°C | ND | 9-29-11 at 0902 | DWP |

MEASUREMENTS MUST BE TAKEN WITHIN 15 MINUTES OF SAMPLE OR LAST SUBSAMPLE COLLECTION.

**COMMENTS:** 2 PUMPS

DAVID PORTER - FIELD TECH (PRINTED NAME/AFFILIATION SAMPLER/ANALYST) — [signature] (SIGNATURE) — 9-29-11 (DATE)

| RELINQUISHED BY | DATE | TIME | RECEIVED BY |
|---|---|---|---|
| [signature] | 9-29-11 | 1102 | LAB Cooler-EMI |
| Cooler [signature] | 9/29/11 9/30/11 | 1445 1000 | [signature] |

**SHIPPING METHOD:** UPS X FEDEX ____ HAND DELIVERY ____

DO NOT SHIP FEDEX STANDARD OVERNIGHT. SAMPLES MUST ARRIVE AT LAB BY NOON.

**CONDITION ON ARRIVAL:** ACCEPTABLE ✓ OTHER ____

SAMPLE TEMP: (°C) 1 ARRIVED ON ICE? Y ✓ N ___ CUSTODY SEAL: INTACT ✓ BROKEN ___ ABSENT ___

**NOTE: It is the responsibility of the sampler to insure that samples are properly collected, preserved (>0-6° C) and shipped. Sample hold time is 36 h. Additional costs may be incurred by improper preservation, shipping or receipt of samples after 3 p.m. or on weekends and holidays.**

BIN#02 9/29/11 @ 1140 in Cooler JW