# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Facility Bench Sheet

Method: EPA-821-R-02-012



**Sample Collection:** **Date:**_____ **Time:**_____
**Hold Time Start:** **Date:**_____ **Time:**_____

**Test 1 of 2 Start Date:**_____ **Time:**_____ **Test 1 of 2 End Date:**_____ **Time:**_____
**Test 2 of 2 Start Date:**_____ **Time:**_____ **Test 2 of 2 End Date:**_____ **Time:**_____

**Facility:**_____
_____
**Address:**_____
_____
**County:**_____
**NPDES Permit #: FL** _____
**Contact:** _____ **District:**_____
**LIMS Job #:**_____ **LIMS Sample #:**_____
**LIMS Entry:**_____ **Verified:**_____
**Authorized:**_____

| Test Type |
|---|
| ☐ Screen |
| ☐ Definitive |
| ☐ Static |
| ☐ Flow-through |

| Test Duration |
|---|
| ☐ 48 hours |
| ☐ 96 hours |
| ☐ ___ hours |

### Initial Sample Handling:

| pH adjustment: | | Aeration: | | Dechlorination: | | Salinity adjustment: | |
|---|---|---|---|---|---|---|---|
| Yes | No | Yes | No | Yes | No | Yes | No |
| **Initial pH:** _____ | | **Initial DO:** _____mg/L | | **Initial TRC:** _____ mg/L | | **Initital Salinity:** _____ ppt | |
| **NaOH:** _____N | | **Final DO:** _____mg/L | | _____ mL of 0.025 N Sodium Thiosulfate per liter of sample. | | **Final Salinity:** _____ ppt | |
| **Hcl:** _____N | | **Duration:** _____minutes | | **Final TRC:** _____mg/L | | **Salts Hypersaline brine** | |
| _____ mL added | | **Rate:** ~ _____ bubbles per min | | | | | |
| **Final pH:** _____ | | | | | | | |

### Sample Validation:

| | | |
|---|---|---|
| **Temperature Shipped ≤ 6°C:** | **Yes** | **No** |
| **If Hand Delivered, Cooling:** | **Yes** | **No** |
| **Received:** _____ °C **Collected:** _____ °C | | |
| **Holding Time ≤ 36 Hours:** | **Yes** | **No** |

**(Composite: End of collection; Grab: When collected; 4 in 24: Time last sample collected)**

Light Intensity: 50-100 ft candles (recommended) Photoperiod:16 hr light, 8 hr dark (recommended)

### *Artemia* Batch #'s

**0 hr** _____
**24 hr** _____
**48 hr** _____
**72 hr** _____
***Artemia* Lot #** _____

| Food | Days Used |
|---|---|
| **YCT Batch #** _____ | |
| **YCT Batch #** _____ | |
| ***P. subcapitata* Batch #** _____ | |
| ***P. subcapitata* Batch #** _____ | |

*C. dubia* fed 0.2 ml of *YCT and and *P. subcapitata* in a 1:1 ratio**

C. leedsi, P. promelas, M. beryllina, and A. bahia fed 0.2 ml of Artemia.

*YCT= yeast, cereal leaves and trout chow

** Pipet P1 used for C. dubia feeding

**Reviewer**_____

**Comments:**

# FDEP Biology Laboratory- Toxicity Section
## Acute Bioassay Water Quality Information Sheet

Method: EPA-821-R-02-012

| Water Quality Parameters | MHWCD<br>LIMS ID # _____<br>Batch # _____ | MHW<br>LIMS ID # _____<br>Batch # _____ | MHWCD/ MHW<br>LIMS ID # _____<br>Batch # _____ | ASW<br>LIMS ID # _____<br>Batch # _____ | Sample<br>LIMS # _____ | SOP | Measured By: | Transcription Checked by: |
|---|---|---|---|---|---|---|---|---|
| **Field Total Residual $Cl_2$** (mg/L) | N/A | N/A | N/A | N/A | | | | |
| **Lab Total Residual $Cl_2$** (mg/L) | | | | | | TA-06.22 | | |
| **Alkalinity** (mg/L as $CaCO_3$) | | | | | | TA-06.08 | | |
| **Hardness** (mg/L as $CaCO_3$) | | | | | | TA-06.08 | | |
| **Total Ammonia** (mg/L as N) | | | | | | TA-06.35 or TA-06.33 | | |
| **Conductivity** □ µmhos □ mmhos | | | | | | TA-06.36 | | |
| **Salinity** (ppt) | | | | | | TA-06.36 | | |
| **Days Used** | | | | | | | | |

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Information Sheet

Method: EPA-821-R-02-012



**Test 1 of 2**

**LIMS Sample #:** _____

**Start Date:** _____ **Time:** _____

**End Date:** _____ **Time:** _____

| Concentrations Prepared by:_____ | Diluent | Batch #/LIMS # | Days Used |
|---|---|---|---|
| | | | |
| **Test Loaded by:_____** | | | |
| | | | |
| **Loading Verified by:_____** | | | |

**Chamber Size _____mL**

**Test Volume _____mL**

| Test Species |
|---|
| ☐ *Ceriodaphnia dubia* (SOP: TA-07.01) |
| ☐ *Cyprinella leedsi* (SOP: TA-07.02) |
| ☐ *Pimephales promelas* (SOP TA-07.02) |
| ☐ *Menidia beryllina* (SOP TA-07.04) |
| ☐ *Americamysis bahia* (SOP TA-07.03) |
| **Batch #:_____ Age: _____** |
| **Randomizer # _____** |

| Feeding Times | |
|---|---|
| **AM** | **PM** |
| **0 hr * _____** | **0 hr _____** |
| **24 hr_____** | **24 hr_____** |
| **48 hr * _____** | **48 hr _____** |
| **72 hr _____** | **72 hr _____** |

***0 hr AM is prior to test and 48 hr AM is prior to renewal**

| Temperature Range (°C) |
|---|
| **Incubator #_____** |
| **Min._____ Max._____** |
| **Test** |
| **Min._____ Max._____** |
| **Room B246** |
| **Min._____ Max._____** |

| Test Results | | | | |
|---|---|---|---|---|
| **Method: EPA** | | **95% Confidence Intervals** | | **% mortality in 100%** |
| **Statistics:** | **LC50** | **Lower** | **Upper** | |
| **48 hour:** | | | | |
| **96 hour:** | | | | |

| Test Validation | | |
|---|---|---|
| **Control Survival ≥ 90%:** | **Yes** | **No** |
| **Temperature Range ≤ 3° C:** | **Yes** | **No** |

**Stats Calculated by/Date:**_____ **Stats Verified by/Date:**_____

**Comments:**

# FDEP Biology Laboratory -Toxicity Section
## Acute Bioassay Survival Sheet

Method: EPA-821-R-02-012



**LIMS Sample #: ____________________**

| | | | **Test Hour** | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration (%)** | **Replicate** | **Chamber #** | **0 hr** | **24 hr** | **48 hr BR** | **48 hr AR** | **72 hr** | **96 hr** |
| Control | | | | | | | | |
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| **Organisms loaded by: _____** | | **Checked by:** | | | | | | |

**Verified by: _____**

**Concentrations at 48 hrs by: _______**

**BR = before renewal** **AR = after renewal** **hr = hour** **M = missing**

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Parameter Sheet



Method: EPA-821-R-02-012

**Test 1 of 2**

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) **circle one** | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 0 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 24 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 48 Hours Before Renewal | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Parameter Sheet

Method: EPA-821-R-02-012



**Test 1 of 2**

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) **Circle one** | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 48 Hours After Renewal | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 72 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 96 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Test Instrument Page
Method: EPA-821-R-02-012



**Test ID:** **Dates:**

| METER / DEVICE | Instrument Code |
|---|---|
| **DO Meter** | |
| **pH Meter** | |
| **Conductivity Meter** | |
| **Ammonia Kit** | |
| **Traceable Thermometer** | |
| **Chlorine Kit** | |
| **Alkalinity Kit** | |
| **Hardness Kit** | |
| | |
| | |

**Entered by and date:**

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Information Sheet

Method: EPA-821-R-02-012



**Test 1 of 2**

**LIMS Sample #:** _____

**Start Date:** _____ **Time:** _____

**End Date:** _____ **Time:** _____

| Concentrations Prepared by:_____ | Diluent | Batch #/LIMS # | Days Used |
|---|---|---|---|
| | | | |
| Test Loaded by:_____ | | | |
| Loading Verified by:_____ | | | |

Chamber Size _____mL
Test Volume _____mL

| Test Species | |
|---|---|
| ☐ | *Ceriodaphnia dubia* (SOP: TA-07.01) |
| ☐ | *Cyprinella leedsi* (SOP: TA-07.02) |
| ☐ | *Pimephales promelas* (SOP TA-07.02) |
| ☐ | *Menidia beryllina* (SOP TA-07.04) |
| ☐ | *Americamysis bahia* (SOP TA-07.03) |

Batch #:_____ Age: _____

Randomizer # _____

| Feeding Times | |
|---|---|
| AM | PM |
| 0 hr * _____ | 0 hr _____ |
| 24 hr_____ | 24 hr_____ |
| 48 hr * _____ | 48 hr _____ |
| 72 hr _____ | 72 hr _____ |

*0 hr AM is prior to test and 48 hr AM is prior to renewal

**Temperature Range** (°C)

Incubator #_____

Min._____ Max._____

Test

Min._____ Max._____

Room B246

Min._____ Max._____

**Test Results**

| Method: EPA | | 95% Confidence Intervals | | % mortality in 100% |
|---|---|---|---|---|
| Statistics: | LC50 | Lower | Upper | |
| 48 hour: | | | | |
| 96 hour: | | | | |

**Test Validation**

| | | |
|---|---|---|
| Control Survival ≥ 90%: | Yes | No |
| Temperature Range ≤ 3° C: | Yes | No |

**Stats Calculated by/Date:**_____ **Stats Verified by/Date:**_____

**Comments:**

# FDEP Biology Laboratory -Toxicity Section
## Acute Bioassay Survival Sheet

Method: EPA-821-R-02-012



**Test 2 of 2**

**LIMS Sample #:** ____________________

| | | | **Test Hour** | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration (%)** | **Replicate** | **Chamber #** | **0 hr** | **24 hr** | **48 hr BR** | **48 hr AR** | **72 hr** | **96 hr** |
| Control | | | | | | | | |
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| **Organisms loaded by:** ____ | | **Checked by:** | | | | | | |

**Verified by:** _____

**Concentrations at 48 hrs by:** _______

**BR = before renewal** **AR = after renewal** **hr = hour** **M = missing**

| **Comments:** |
|---|
| |

# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Parameter Sheet



Method: EPA-821-R-02-012

**Test 2 of 2**

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) **Circle one** | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 0 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 24 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 48 Hours Before Renewal | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Acute Bioassay Parameter Sheet

Method: EPA-821-R-02-012



| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) **Circle one** | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 48 Hours After Renewal | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 72 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

| | **Concentration (%)** | **Test Replicate** | **pH** (SU) | **Temperature** (°C) | **DO** (mg/L) | **Conductivity** (umhos/mmhos) | **Salinity** (ppt) |
|---|---|---|---|---|---|---|---|
| 96 Hours | Control | | | | | | |
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| | **Measured by:** | | | | | | |
| | **Recorded by:** | | | | | | |

**Comments:**

# FDEP Biology Laboratory - Toxicity Section
## Test Instrument Page
Method: EPA-821-R-02-012



**Test ID:** **Dates:**

| METER / DEVICE | Instrument Code |
|---|---|
| DO Meter | |
| pH Meter | |
| Conductivity Meter | |
| Ammonia Kit | |
| Traceable Thermometer | |
| Chlorine Kit | |
| Alkalinity Kit | |
| Hardness Kit | |
| | |
| | |

**Entered by and date:**

**Comments:**