Effective Date: 09/24/2025
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Revision Author(s): A. Terrane

FDEP Biology Laboratory - Toxicity Section
Acute Bioassay Facility Bench Sheet

Method: EPA-821-R-02-012

TA-ACUTE-BENCH-1.8.xlsx

***Sample Collection (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 / Date:_____
Authorized / Date:_____

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:

Holding Time ≤ 36 Hours:	Yes	No

*** Composite Sample time is end of collection;**
Grab Sample time is when sample was collected;
4 in 24 sampling time is when the last sample was 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* LIMS #** _____

Food	Days Used
YCT LIMS # _____	
YCT LIMS # _____	
R. subcapitata LIMS # _____	
R. subcapitata LIMS # _____	

C. dubia fed 0.2 mL of YCT and and *R. subcapitata* in a 1:1 ratio

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

YCT= yeast, cereal leaves, and trout chow

Pipet P1 used for *C. dubi* a feeding

Pipet P2 used for *Artemia* feeding

Reviewer_____

Comments:

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

Method: EPA-821-R-02-012

LIMS Sample #: _____

Water Quality Parameters	LIMS ID # _____ Batch # _____	LIMS ID # _____ Batch # _____	LIMS ID # _____ Batch # _____	LIMS ID # _____ Batch # _____	LIMS ID # _____ Batch # _____	Sample LIMS # _____	SOP	Transcribed By:	Transcription Checked by:
pH (SU)							TA-06.43		
DO (mg/L)							TA--06.39		
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		
Conductivity □ µmhos □ mmhos							TA-06.36		
Salinity (ppt)							TA-06.36		
Days Used									

Comments:

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

Method: EPA-821-R-02-012

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	
First*	**Second**
0 hr _____	**0 hr _____**
48 hr _____	**48 hr _____**

Temperature Range (°C)

Incubator #_____

Min._____ Max._____

Test

Min._____ Max._____

***"First" is 2 hours before initiation/renewal.**

Test Results					Test Validation		
Method: EPA-821-R-02-012		**95% Confidence Intervals**		**% mortality in 100%**	**Control Survival ≥ 90%:**	**Yes**	**No**
Statistics:	**LC50**	**Lower**	**Upper**				
48 hour:					**Test Temperature Range ≤ 3° C:**	**Yes**	**No**
96 hour:							

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 #: _____

Concentration (%)	Replicate	Chamber #	Test Hour 0 hr	24 hr	48 hr (BR)	48 hr (AR)	72 hr	96 hr
Control	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
Organisms loaded by: _____			**Checked by:**					
Verified by: _____			**Initial Concentrations 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** (µmhos/mmhos) **circle one**	**Salinity** (ppt)
0 Hours	Control						
	Measured by:						
	Recorded by:						

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

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

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

	Concentration (%)	Test Replicate	pH (SU)	Temperature (°C)	DO (mg/L)	Conductivity (µmhos/mmhos)	Salinity (ppt)
96 Hours	Control						
	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 2 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	
*First	Second
0 hr _____	0 hr _____
48 hr _____	48 hr _____

*"First" is 2 hours prior to test initiation/renewal

Temperature Range (°C)

Incubator #_____

Min._____ Max._____

Test

Min._____ Max._____

Test Results					Test Validation		
Method: EPA-821-R-02-012		95% Confidence Intervals		% mortality in 100%			
Statistics:	LC50	Lower	Upper				
48 hour:					Control Survival ≥ 90%:	Yes	No
96 hour:					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 #: _____

Concentration (%)	Replicate	Chamber #	Test Hour: 0 hr	24 hr	48 hr (BR)	48 hr (AR)	72 hr	96 hr
Control	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
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	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							
Organisms loaded by: _____			**Checked by:**					
Verified by: _____			**Initial Concentrations 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** (µmhos/mmhos) **circle one**	**Salinity** (ppt)
0 Hours	Control						
	Measured by:						
	Recorded by:						

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

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

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** (µmhos/mmhos) **circle one**	**Salinity** (ppt)
48 Hours (After Renewal)	Control						
	Measured by:						
	Recorded by:						

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

	Concentration (%)	**Test Replicate**	**pH** (SU)	**Temperature** (°C)	**DO** (mg/L)	**Conductivity** (µmhos/mmhos)	**Salinity** (ppt)
96 Hours	Control						
	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: