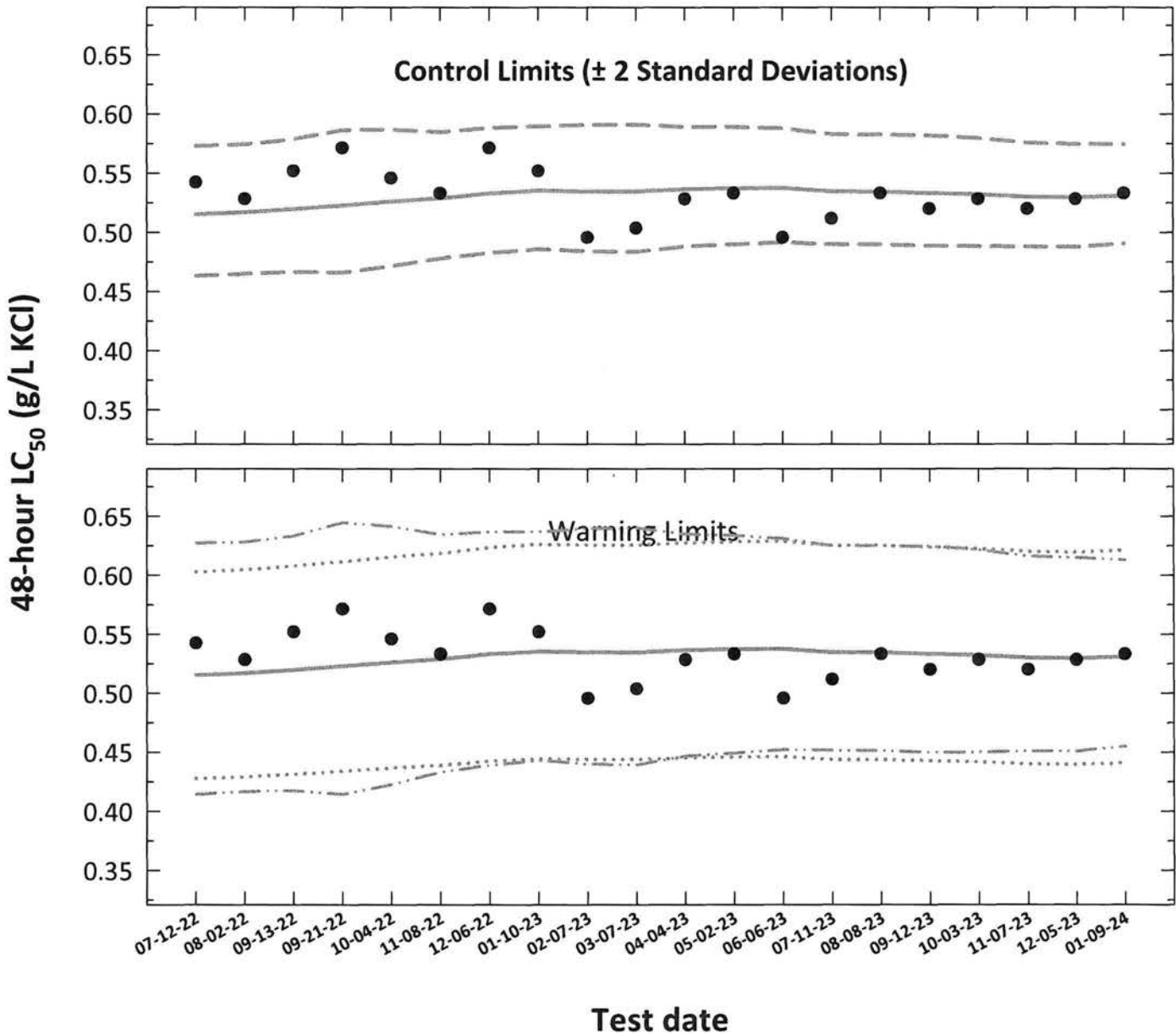


Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ \pm 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic LC₅₀ \pm 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ \pm S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

**Americamysis (*Mysidopsis bahia*)
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.**

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	07-12-22	0.5424	-0.2657	-0.2879	0.0231	0.5153	0.4633	0.5732	0.4144	0.6276	0.4277	0.6029
2	08-02-22	0.5283	-0.2771	-0.2866	0.0229	0.5169	0.4651	0.5744	0.4167	0.6282	0.4290	0.6048
3	09-13-22	0.5519	-0.2581	-0.2843	0.0234	0.5196	0.4665	0.5787	0.4174	0.6334	0.4313	0.6079
4	09-21-22	0.5714	-0.2431	-0.2817	0.0250	0.5227	0.4660	0.5864	0.4141	0.6446	0.4339	0.6116
5	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415	0.4367	0.6155
6	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347	0.4389	0.6187
7	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368	0.4424	0.6236
8	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369	0.4443	0.6263
9	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399	0.6396	0.4439	0.6257
10	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392	0.6402	0.4438	0.6255
11	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350	0.4452	0.6275
12	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338	0.4459	0.6286
13	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312	0.4462	0.6290
14	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252	0.4437	0.6255
15	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250	0.4436	0.6253
16	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244	0.4426	0.6239
17	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217	0.4417	0.6226
18	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161	0.4400	0.6202
19	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151	0.4395	0.6195
20	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550	0.6131	0.4407	0.6212

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.17).

CV = Coefficient of variation.

**Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia
EPA-821-R-02-012, Method 2007.0**

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 274

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	2.5	3.75	5.0	7.5	10.0
mL Dilution water	497.5	496.25	495.0	492.5	490.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2249

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Concentration			
	Analyst	✓	JPBL	✓ JP
	pH (S.U.)	7.06	7.92	7.02
	Dissolved oxygen (mg/L)	7.0	7.4	7.1
	*Salinity (ppt)	25.0	25.5	25.7
250 mg/L	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	25.1	25.0	25.3
	pH (S.U.)	7.02	7.93	7.03
	Dissolved oxygen (mg/L)	7.5	7.4	7.2
375 mg/L	*Salinity (ppt)	25.1	25.5	25.5
	*Temperature (°C)	25.3	25.2	25.2
	pH (S.U.)	7.02	7.96	7.04
	Dissolved oxygen (mg/L)	7.5	7.6	7.4
500 mg/L	*Salinity (ppt)	25.2	25.7	25.6
	*Temperature (°C)	25.3	25.2	25.0
	pH (S.U.)	7.03	7.96	7.04
	Dissolved oxygen (mg/L)	7.6	7.6	7.3
750 mg/L	*Salinity (ppt)	25.2	25.5	25.9
	*Temperature (°C)	25.0	25.2	25.4
	pH (S.U.)	7.04	7.96	
	Dissolved oxygen (mg/L)	7.8	7.7	
1000 mg/L	*Salinity (ppt)	25.5	25.7	
	*Temperature (°C)	25.2	25.0	
	pH (S.U.)	7.05	7.96	
	Dissolved oxygen (mg/L)	7.9	7.9	
	*Salinity (ppt)	25.6	26.0	
	*Temperature (°C)	25.0	25.3	
	pH (S.U.)			
	Dissolved oxygen (mg/L)			

*Analyst identified for each day, performed pH and dissolved oxygen measurements only. Temperature and salinity performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity performed by the analyst identified on the test specific bench sheet and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Americamysis bahia*

EPA-821-R-02-012, Method 2007.0

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 274

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-09-24	1200	J	1400	J	1C	Yellow	01-02-24 B
24	01-10-24			1358	J			
48 Termination	01-11-24			1351	J			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	01-08-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	01-07-24 1200 TO 01-09-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.46 Temperature (°C) 24.5°C

Survival Data (number of living organisms):

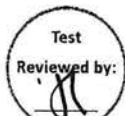
Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	6 ^{4d}	6 ^{4d}	0	0	0	0
Mean Survival	100%		100%		100%		60%		0%		0%	

Comment codes: d = dead, u = unhealthy, s = stressed

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	494.1
Upper 95% confidence limit (mg KCl/L)	575.2
48-hour LC ₅₀ (mg KCl/L)	533.1

Comments:



Acute Mysid Test-48 Hr Survival

Start Date:	1/9/2024	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	1/11/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanmysid bahia

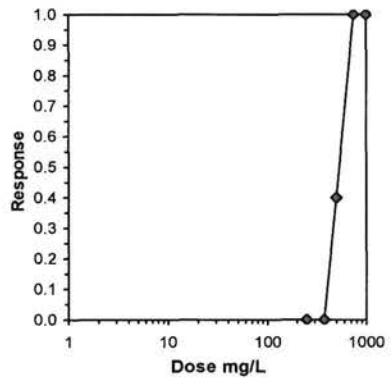
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.6000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	52.594	2.850	0.0285	8	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

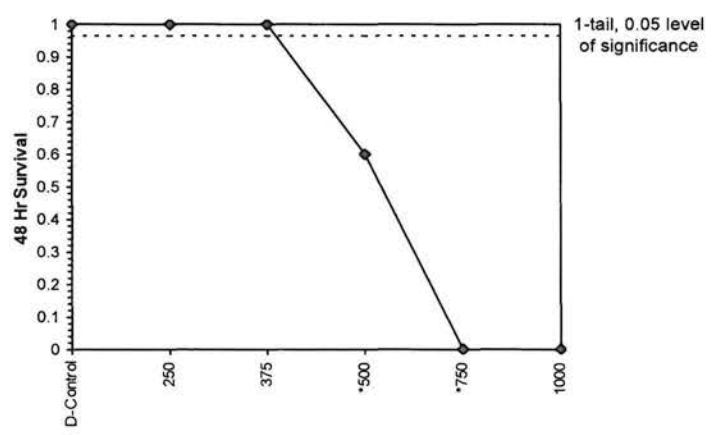
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	375	500	433.013	0.00967
Treatments vs D-Control	MSDu	MSDp	MSB	MSE
	0.00991	0.60706	0.0001	2.1E-09
	df			4, 5

Trim Level	EC50	95% CL	
0.0%	533.10	494.12	575.16
5.0%	533.38	490.12	580.45
10.0%	533.65	484.88	587.32
20.0%	534.18	468.38	609.23
Auto-0.0%	533.10	494.12	575.16

Trimmed Spearman-Kärber



Dose-Response Plot



Acute Mysid Test-24 Hr Survival

Start Date: 1/9/2024	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/11/2024	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	1.0000	1.0000
750	0.0000	0.0000
1000	0.0000	0.0000

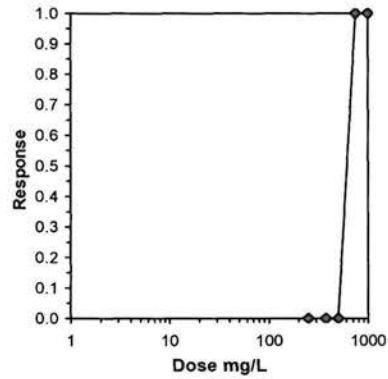
Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	500	750	612.372	0.00967
Treatments vs D-Control				0.00991
				0.62824
				0.0001
				2.0E-09
				4, 5

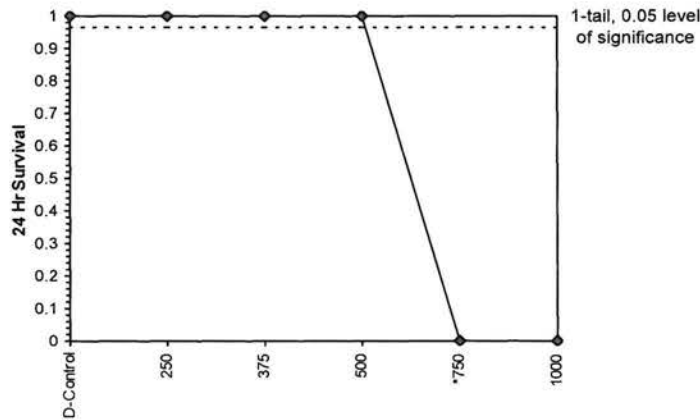
Graphical Method

Trim Level	EC50
0.0%	612.37

612.37



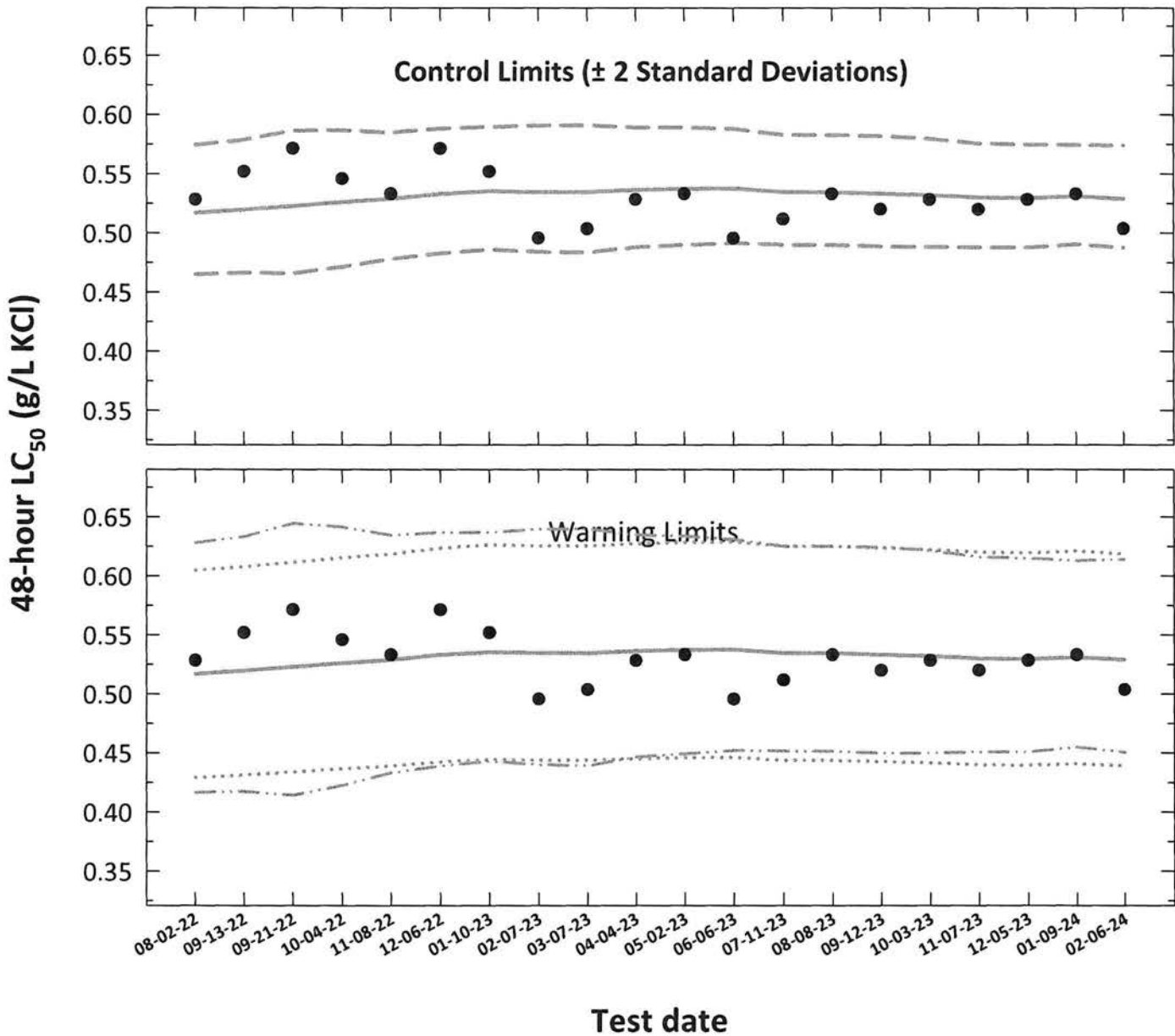
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A,10}$ converted to anti-logarithmic values, $S_{A,10}$ = 10th percentile of CVs reported nationally by USEPA)

Americamysis (*Mysidopsis*) bahia
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	08-02-22	0.5283	-0.2771	-0.2866	0.0229	0.5169	0.4651	0.5744	0.4167	0.6282	0.4290	0.6048
2	09-13-22	0.5519	-0.2581	-0.2843	0.0234	0.5196	0.4665	0.5787	0.4174	0.6334	0.4313	0.6079
3	09-21-22	0.5714	-0.2431	-0.2817	0.0250	0.5227	0.4660	0.5864	0.4141	0.6446	0.4339	0.6116
4	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415	0.4367	0.6155
5	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347	0.4389	0.6187
6	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368	0.4424	0.6236
7	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369	0.4443	0.6263
8	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399	0.6396	0.4439	0.6257
9	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392	0.6402	0.4438	0.6255
10	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350	0.4452	0.6275
11	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338	0.4459	0.6286
12	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312	0.4462	0.6290
13	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252	0.4437	0.6255
14	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250	0.4436	0.6253
15	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244	0.4426	0.6239
16	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217	0.4417	0.6226
17	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161	0.4400	0.6202
18	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151	0.4395	0.6195
19	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550	0.6131	0.4407	0.6212
20	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875	0.5740	0.4505	0.6141	0.4391	0.6189

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCal).

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.17).

CV = Coefficient of variation.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Americamysis bahia*
EPA-821-R-02-012, Method 2007.0

***Americamysis bahia* Potassium Chloride Acute Reference Toxicant Test**

AbKCIAC # 275

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	2.5	3.75	5.0	7.5	10.0
mL Dilution water	497.5	496.25	495.0	492.5	490.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2261

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	JP	JPL	JP
	pH (S.U.)	7.89	7.95	7.88
	Dissolved oxygen (mg/L)	7.7	7.7	7.4
	*Salinity (ppt)	25.1	25.1	25.1
	*Alkalinity (mg/L CaCO ₃)	120		
	*Temperature (°C)	25.4	25.3	25.3
250 mg/L	pH (S.U.)	7.90	7.97	7.90 (7.89)
	Dissolved oxygen (mg/L)	7.7	7.7	7.5
	*Salinity (ppt)	25.2	25.3	25.2
	*Temperature (°C)	25.4	25.0	25.2
375 mg/L	pH (S.U.)	7.90	7.96	7.84
	Dissolved oxygen (mg/L)	7.7	7.7	7.5
	*Salinity (ppt)	25.3	25.3	25.1
	*Temperature (°C)	25.3	25.0	25.2
500 mg/L	pH (S.U.)	7.91	7.96	7.84
	Dissolved oxygen (mg/L)	7.7	7.8	7.4
	*Salinity (ppt)	25.3	25.5	25.6
	*Temperature (°C)	25.3	25.2	25.2
750 mg/L	pH (S.U.)	7.91	7.96	
	Dissolved oxygen (mg/L)	7.7	7.8	
	*Salinity (ppt)	25.6	25.7	
	*Temperature (°C)	25.6	25.2	
1000 mg/L	pH (S.U.)	7.91	7.96	
	Dissolved oxygen (mg/L)	7.7	7.8	
	*Salinity (ppt)	25.8	25.8	
	*Temperature (°C)	25.8	25.2	

*Analyst identified for each day, performed pH and dissolved oxygen measurements only. Temperature and salinity performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity performed by the analyst identified on the test specific bench sheet and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

Handwritten note: 11-02-11-14

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Americamysis bahia*

EPA-821-R-02-012, Method 2007.0

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 275

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-06-21	1140	J	1340	J	6D	Light Green	02-24-21B
24	02-07-21			1340	K			
48 Termination	02-08-21			1335	J			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	02-05-21
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	02-04-21 1200 TO 02-05-21 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.99 Temperature (°C): 24.8

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	1 ^u 9	2 ^u 8	10 ^d 0	10 ^d 0	10 ^d 0	10 ^d 0
48 Termination	10	10	10	10	9 ^u 10	10	5 ^u 5	2 ^u 6	0	0	0	0
Mean Survival	100%		100%		95%		55%		0%		0%	

Comment codes: d = dead, u = unhealthy, s = stressed

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	465.3
Upper 95% confidence limit (mg KCl/L)	557.0
48-hour LC ₅₀ (mg KCl/L)	503.6

Comments:



Statistical Analyses

Acute Mysid Test-24 Hr Survival

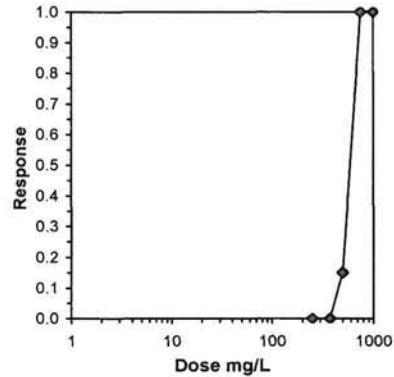
Start Date: 2/6/2024	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 2/8/2024	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.9000	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

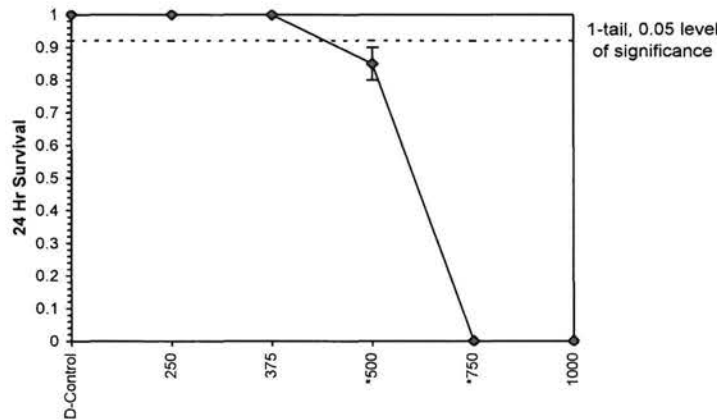
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*500	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	5.213	2.850	0.1279	3	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	27.929	2.850	0.1279	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.05495	0.05636	0.5915	0.00201	4.1E-06	4, 5

Trim Level	EC50	95% CL	
0.0%	581.35	550.05	614.43
5.0%	586.14	549.69	625.01
10.0%	589.52	543.45	639.50
20.0%	590.85	564.96	617.93
Auto-0.0%	581.35	550.05	614.43



Dose-Response Plot



Checked and
Approved by
Jim Sumner
JS

Statistical Analyses

Acute Mysid Test-48 Hr Survival

Start Date: 2/6/2024	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant	
End Date: 2/8/2024	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia	

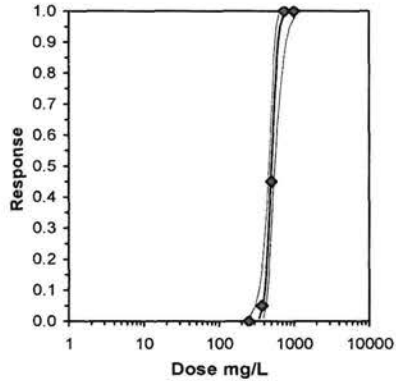
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	1.0000
500	0.5000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20	
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.345	2.850	0.1726	1	20	
*500	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20	
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20.688	2.850	0.1726	20	20	
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

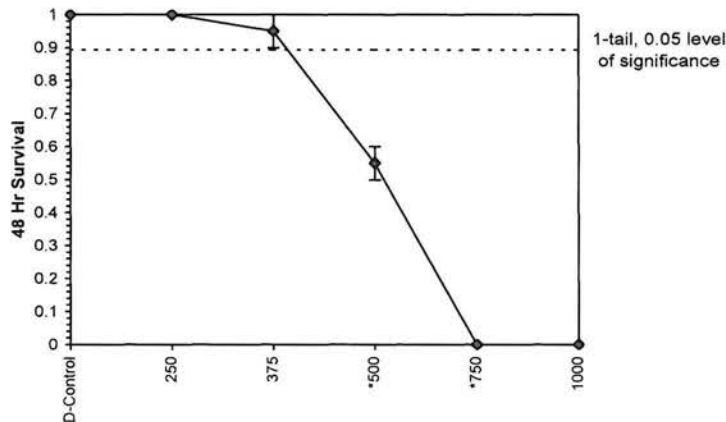
Auxiliary Tests		Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed					
Equality of variance cannot be confirmed					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU
Dunnett's Test		375	500	433.013	
Treatments vs D-Control					

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.9938	3.31443	7.4975	20.4901	0	0.34385	7.81472	0.95158	2.70209	0.07146	4
Intercept	-32.812	8.90228	-50.261	-15.364							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	343.437	250.566	389.808
EC05	3.355	384.19	306.605	423.985
EC10	3.718	407.857	340.45	444.696
EC15	3.964	424.643	364.587	460.226
EC20	4.158	438.476	384.252	473.857
EC25	4.326	450.701	401.218	486.778
EC40	4.747	483.041	442.565	526.574
EC50	5.000	503.603	465.286	557.021
EC60	5.253	525.04	486.136	592.91
EC75	5.674	562.714	517.856	664.144
EC80	5.842	578.403	529.87	696.226
EC85	6.036	597.244	543.691	736.307
EC90	6.282	621.825	560.982	790.894
EC95	6.645	660.131	586.743	880.657
EC99	7.326	738.465	636.526	1080.43



Dose-Response Plot

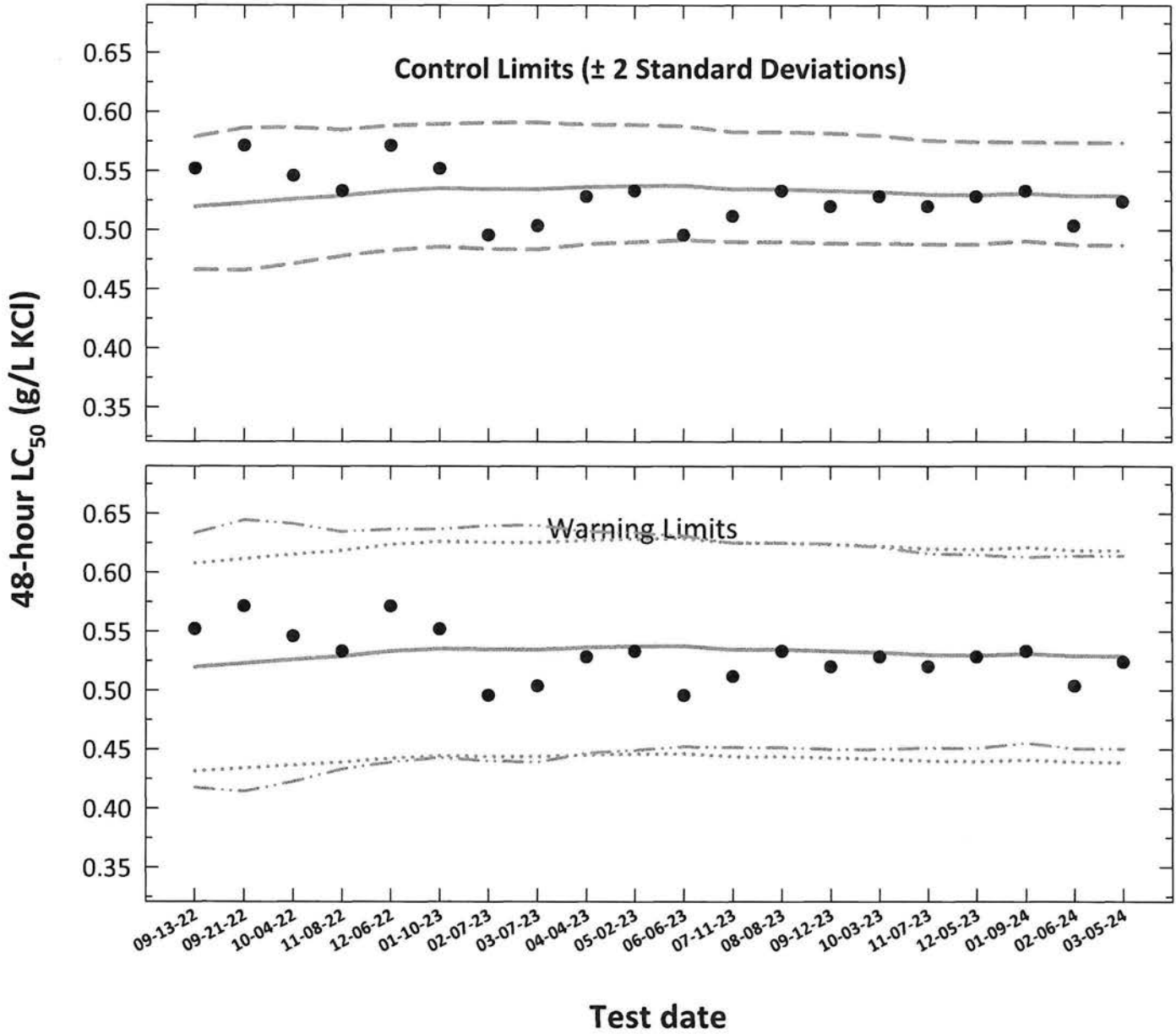


Reviewed and
Approved by
Jim Turner
JT

Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ ± S_{A.10} converted to anti-logarithmic values, S_{A.10} = 10th percentile of CVs reported nationally by USEPA)

Americamysis (Mysidopsis) bahia
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	09-13-22	0.5519	-0.2581	-0.2843	0.0234	0.5196	0.4665	0.5787	0.4174	0.6334	0.4313	0.6079
2	09-21-22	0.5714	-0.2431	-0.2817	0.0250	0.5227	0.4660	0.5864	0.4141	0.6446	0.4339	0.6116
3	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415	0.4367	0.6155
4	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347	0.4389	0.6187
5	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368	0.4424	0.6236
6	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369	0.4443	0.6263
7	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399	0.6396	0.4439	0.6257
8	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392	0.6402	0.4438	0.6255
9	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350	0.4452	0.6275
10	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338	0.4459	0.6286
11	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312	0.4462	0.6290
12	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252	0.4437	0.6255
13	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250	0.4436	0.6253
14	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244	0.4426	0.6239
15	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217	0.4417	0.6226
16	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161	0.4400	0.6202
17	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151	0.4395	0.6195
18	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550	0.6131	0.4407	0.6212
19	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875	0.5740	0.4505	0.6141	0.4391	0.6189
20	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872	0.5738	0.4502	0.6140	0.4389	0.6187

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.17).

CV = Coefficient of variation.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

EPA-821-R-02-012, Method 2007.0

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 276

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	2.5	3.75	5.0	7.5	10.0
mL Dilution water	497.5	496.25	495.0	492.5	490.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2275

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, SaltSW	pH (S.U.)	7.88	7.86	7.75
	Dissolved oxygen (mg/L)	7.5	7.6	7.5
	*Salinity (ppt)	25.0	25.4	25.2
	*Alkalinity (mg/L CaCO ₃)	91		
	*Temperature (°C)	24.8	24.7	25.0
250 mg/L	pH (S.U.)	7.89	7.86	7.73
	Dissolved oxygen (mg/L)	7.6	7.7	7.4
	*Salinity (ppt)	25.0	25.5	25.4
	*Temperature (°C)	24.9	24.5	24.7
375 mg/L	pH (S.U.)	7.89	7.86	7.73
	Dissolved oxygen (mg/L)	7.7	7.7	7.3
	*Salinity (ppt)	25.1	25.7	25.6
	*Temperature (°C)	24.9	24.6	24.6
500 mg/L	pH (S.U.)	7.90	7.84	7.73
	Dissolved oxygen (mg/L)	7.8	7.6	7.4
	*Salinity (ppt)	25.3	25.9	25.7
	*Temperature (°C)	25.0	24.6	24.7
750 mg/L	pH (S.U.)	7.90	7.84	
	Dissolved oxygen (mg/L)	7.8	7.6	
	*Salinity (ppt)	25.4	26.0	
	*Temperature (°C)	25.0	24.3	
1000 mg/L	pH (S.U.)	7.91	7.84	
	Dissolved oxygen (mg/L)	7.9	7.5	
	*Salinity (ppt)	25.5	26.1	
	*Temperature (°C)	25.0	24.7	

*Analyst identified for each day, performed pH and dissolved oxygen measurements only. Temperature and salinity performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity performed by the analyst identified on the test specific bench sheet and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066465

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Americamysis bahia*

EPA-821-R-02-012, Method 2007.0

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 276

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-05-14	1020	JL	1220	JL	1E	PINX	022121A
24	03-06-14			1221	JL			
48 Termination	03-08-14			1222	JL			

*Test organisms were held in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	03-04-14
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	03-03-14 1200 TO 03-04-14 1300
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.81 Temperature (°C): 24.5

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^d	9 ^d	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	5 ^{4d}	6 ^{3d}	0	0	0	0
Mean Survival	100%		100%		100%		55%		0%		0%	

Comment codes: d = dead, u = unhealthy, s = stressed

Statistics:

Method	Trimmed Spearman-Kärber
Lower 95% confidence limit (mg KCl/L)	485.1
Upper 95% confidence limit (mg KCl/L)	565.9
48-hour LC ₅₀ (mg KCl/L)	523.9

Comments:	Karber
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Acute Mysid Test-24 Hr Survival

Start Date: 3/5/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/7/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.9000	0.9000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests **Statistic** **Critical** **Skew** **Kurt**

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

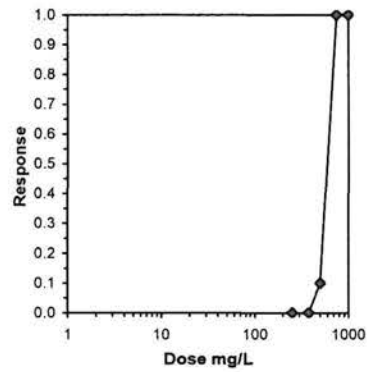
Hypothesis Test (1-tail, 0.05) **NOEC** **LOEC** **ChV** **TU** **MSDu** **MSDp** **MSB** **MSE** **F-Prob** **df**

Dunnett's Test 375 500 433.013 0.00967 0.00991 0.59802 0.0001 2.2E-09 4, 5

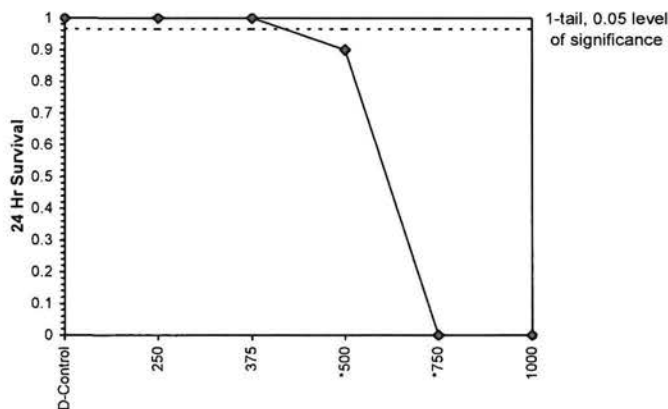
Treatments vs D-Control

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	591.51	564.64	619.67
5.0%	596.72	563.70	631.68
10.0%	598.73	578.96	619.18
20.0%	598.73	578.96	619.18
Auto-0.0%	591.51	564.64	619.67



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 3/5/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/7/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.5000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*500	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.101	2.850	0.0907	9	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests Statistic Critical Skew Kurt

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

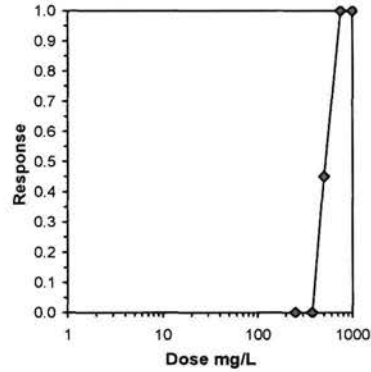
Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU MSDu MSDp MSB MSE F-Prob df

Dunnett's Test 375 500 433.013 0.03598 0.0369 0.61664 0.00101 6.7E-07 4, 5

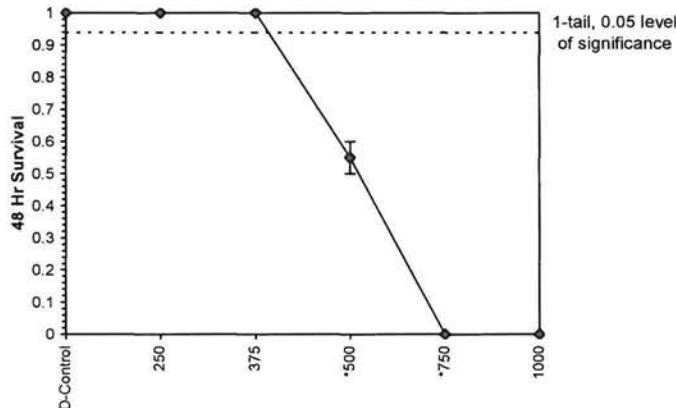
Treatments vs D-Control

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	523.94	485.06 565.94
5.0%	523.31	480.33 570.13
10.0%	522.68	474.60 575.63
20.0%	521.43	458.27 593.29
Auto-0.0%	523.94	485.06 565.94



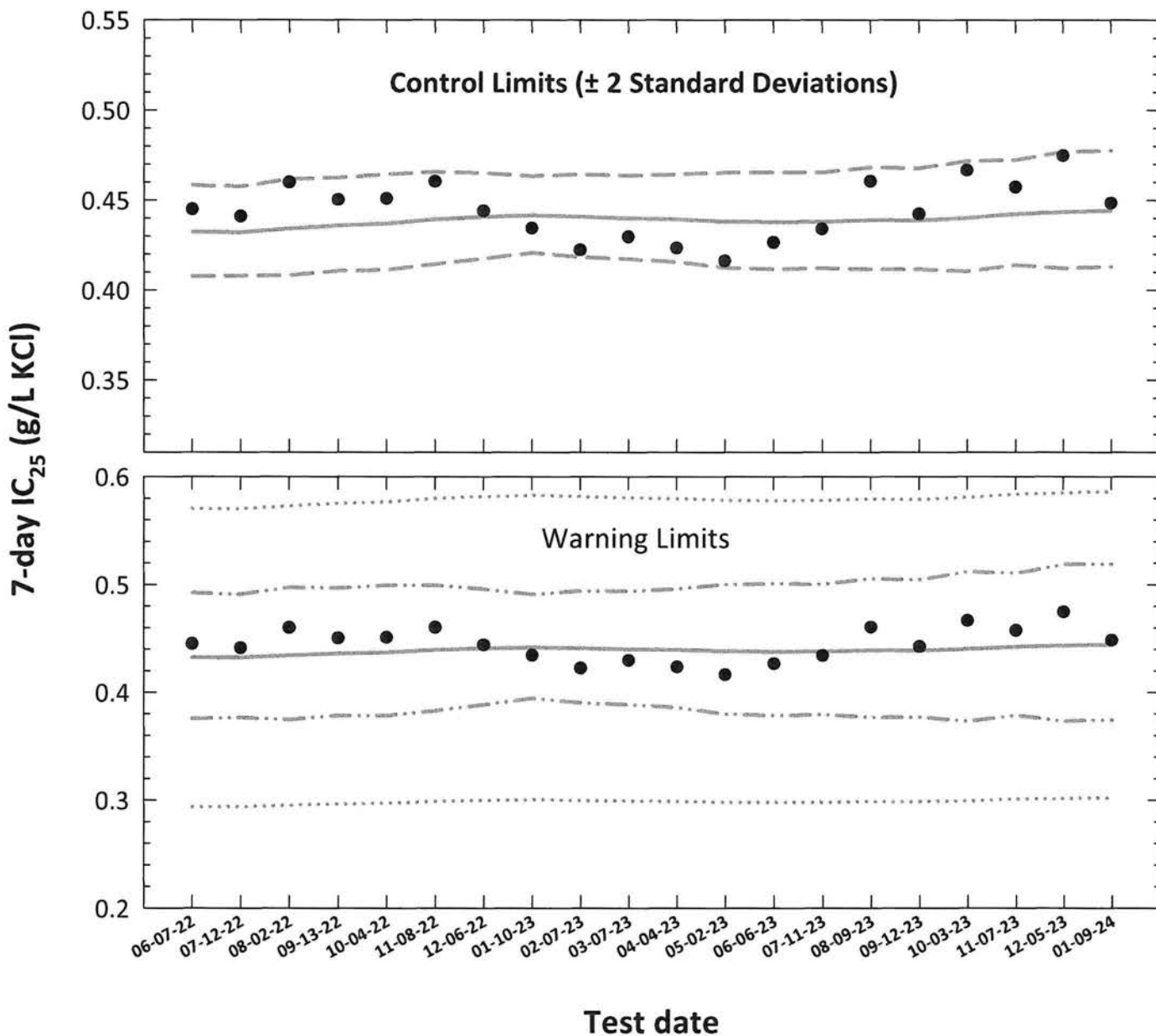
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A,75}$ converted to anti-logarithmic values,
 $S_{A,75}$ = 75th percentile of CVs reported nationally by USEPA)

Americamysis (Mysidopsis) bahia Chronic Reference Toxicant Control Chart Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	06-07-22	0.4450	-0.3516	-0.3641	0.0127	0.4324	0.4078	0.4584	0.3756	0.4925	0.2940	0.5707
2	07-12-22	0.4410	-0.3556	-0.3644	0.0124	0.4321	0.4081	0.4576	0.3764	0.4911	0.2938	0.5704
3	08-02-22	0.4599	-0.3373	-0.3623	0.0134	0.4342	0.4083	0.4617	0.3745	0.4976	0.2952	0.5731
4	09-13-22	0.4502	-0.3466	-0.3605	0.0129	0.4360	0.4109	0.4626	0.3785	0.4970	0.2965	0.5755
5	10-04-22	0.4509	-0.3459	-0.3595	0.0132	0.4371	0.4113	0.4644	0.3782	0.4995	0.2972	0.5769
6	11-08-22	0.4604	-0.3368	-0.3571	0.0127	0.4395	0.4146	0.4658	0.3829	0.4995	0.2988	0.5801
7	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4408	0.4177	0.4651	0.3884	0.4960	0.2997	0.5818
8	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208	0.4635	0.3944	0.4911	0.3003	0.5829
9	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185	0.4644	0.3901	0.4943	0.2998	0.5820
10	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174	0.4638	0.3886	0.4941	0.2992	0.5807
11	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158	0.4643	0.3857	0.4961	0.2988	0.5800
12	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126	0.4654	0.3798	0.5003	0.2980	0.5785
13	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118	0.4655	0.3784	0.5011	0.2977	0.5780
14	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124	0.4655	0.3794	0.5006	0.2979	0.5783
15	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116	0.4683	0.3766	0.5056	0.2985	0.5795
16	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116	0.4677	0.3769	0.5047	0.2983	0.5791
17	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.3730	0.5122	0.2993	0.5810
18	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105	0.3008	0.5838
19	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3732	0.5190	0.3015	0.5853
20	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3744	0.5192	0.3020	0.5863

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

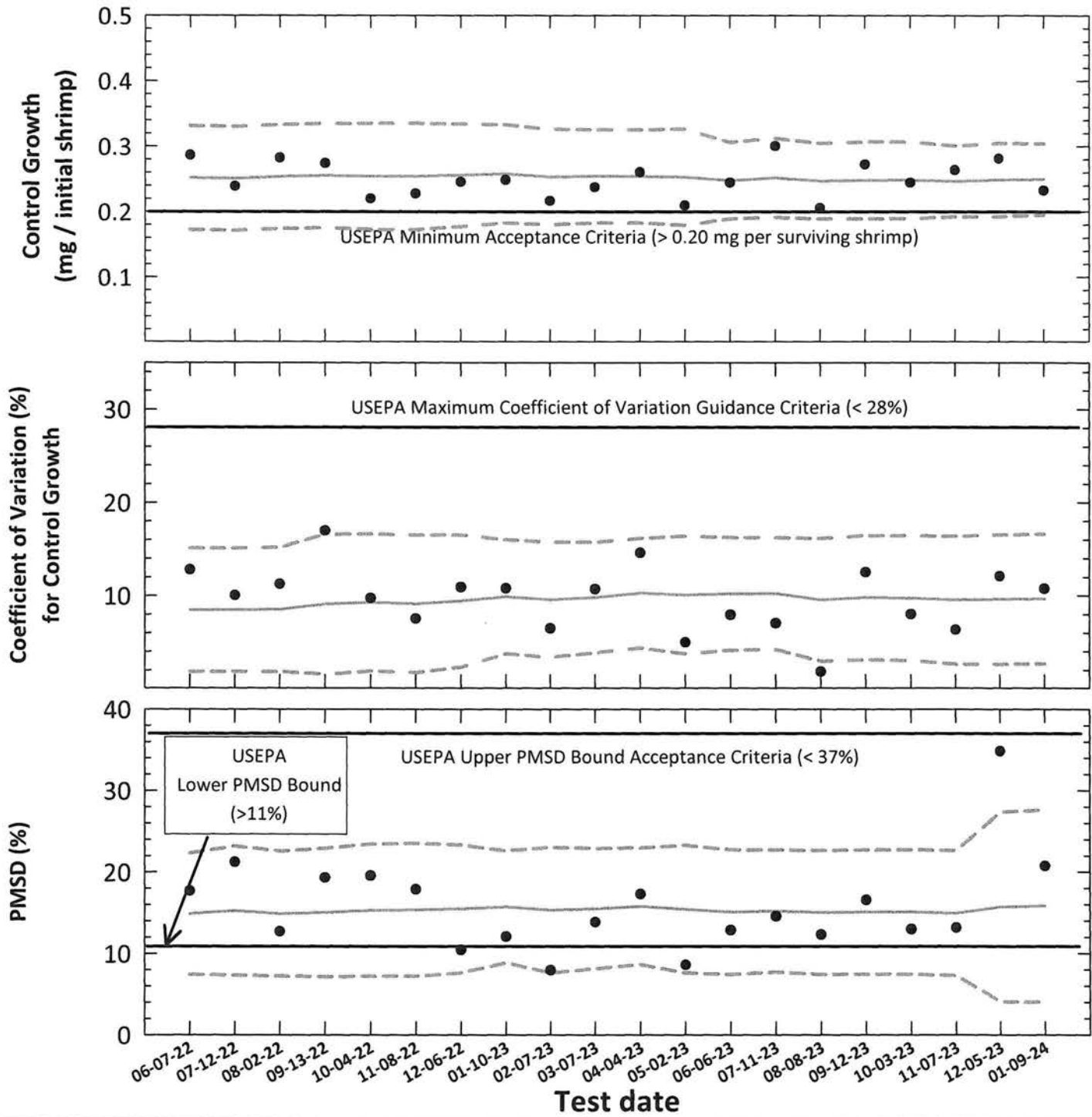
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

CV = Coefficient of variation.

Americamysis (Mysidopsis) bahia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: Aquatic Indicators, Inc.



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency (mean Control Growth, CV or PMSD)**
- - - **95% Confidence Interval (mean Control Growth, CV or PMSD ± 2 Standard Deviations)**

Americamysis (Mysidopsis) bahia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: Aquatic Indicators, Inc.

Test number	Test date	ToxCal Determination					Control Growth		Control Growth (mg/initial shrimp)		Control Growth CV (%)		Test PMSD (%)		
		Control Survival (%)	Control Growth		MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial shrimp)	CV (%)											
1	06-07-22	100	0.287	12.8	0.0506	17.7	0.252	0.173	0.331	8.5	1.8	15.1	14.9	7.4	22.3
2	07-12-22	100	0.239	10.0	0.0507	21.2	0.251	0.171	0.330	8.5	1.8	15.1	15.2	7.3	23.2
3	08-02-22	100	0.282	11.3	0.0358	12.7	0.254	0.174	0.333	8.5	1.8	15.2	14.9	7.2	22.5
4	09-13-22	100	0.274	17.0	0.0529	19.3	0.255	0.175	0.335	9.1	1.6	16.6	15.0	7.1	22.9
5	10-04-22	100	0.220	9.7	0.0430	19.5	0.254	0.173	0.335	9.3	1.9	16.7	15.3	7.2	23.4
6	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	0.335	9.1	1.7	16.5	15.4	7.2	23.5
7	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3
8	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6
9	02-07-23	100	0.217	6.5	0.0172	7.9	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0
10	03-07-23	100	0.238	10.7	0.0329	13.9	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9
11	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
12	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
13	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
14	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
15	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
16	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
17	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
18	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
19	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
20	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6

Note: Control Survival = USEPA minimum test acceptability criteria \geq 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria \geq 0.20 mg/surviving shrimp.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 37%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-014, Method 1007.0)

Species: *Americamysis (Mysidopsis) bahia*

AbKCICR Test Number: 247

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2249				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	GREY
Date and times organisms were born between:	01-02-24 1200 to 01-03-24 1130	Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 01-03-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 7.80 S.U. Temperature = 25.0 °C		Date / Time in oven:	01-16-24 1200
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	01-17-24 1200
		*Final oven temperature:	60 °C
		Total drying time:	24 Hours

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	01-09-24	1200	JL	1430	JL	1250	JL	01-02-24 B
1	01-10-24	0500	JL	1100	JL	0950	JL	J
2	01-11-24	0500	JL	1200	JL	0950	JL	01-09-24
3	01-12-24	0500	JL	1150	JL	0950	JL	J
4	01-13-24	0615	JL	1215	JL	1010	JL	01-11-24
5	01-14-24	0600	JL	1100	JL	0830	JL	01-12-24
6	01-15-24	0600	JL	1200	JL	0830	JL	J
7	01-16-24					1050	JL	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130604685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	01.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	276.8 514.5
Average weight per initial shrimp:	0.233		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.233	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	448.4

AbKCICR Test Number: 247

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac	/															
# females with developing ova in oviducts	/															
# immature females	/															
# males	/															1
*A = Pan weight (mg) Tray color code: <u>R064</u> Analyst: <u>JP</u> Date: <u>12-15-23</u>	14.14	14.00	13.79	15.12	13.81	14.70	14.28	14.66	12.12	12.73	12.91	12.81	14.52	15.35	13.38	14.46
*B = Pan + Shrimp weight (mg) Analyst: <u>JJ</u> Date: <u>01-21-24</u>	15.33	15.28	14.96	16.13	14.84	15.97	15.31	15.98	13.74	13.76	14.28	14.34	15.11	16.65	14.80	15.4
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JJ</u>	1.19	1.28	1.17	1.01	1.03	1.27	1.03	1.32	1.62	1.03	1.37	1.53	1.29	1.30	1.12	1.03
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JJ</u>	0.238	0.256	0.234	0.202	0.206	0.254	0.206	0.264	0.324	0.206	0.274	0.306	0.258	0.260	0.224	0.206
Average weight per initial number of shrimp (mg)	0.233								0.257							
Percent reduction from control (%)	-10.67															

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

AbKCICR Test Number: 247

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	4 nd	S	4 th	4 th	4 th	S	S
3	S	S	S	S	S	S	S	S	S	4	S	4	4	4	4 th	4 th
4	S	S	S	6	S	S	S	S	2 nd	4	4 th	3 rd	4	4	3 rd	3 rd
5	S	S	4 th	S	S	S	S	S	2	4	3 rd	3	4	4	3	3
6	S	S	4	S	S	S	S	S	2	4	3	3	4	4	3	3
7	S	S	4	S	S	S	S	S	2	3 rd	3	3	4	3 rd	3	3
# females with eggs in brood sac	_____															
# females with developing ova in oviducts	_____															
# immature females	_____															
# males	_____															
*A = Pan weight (mg) Tray color code: <u>RVB4</u> Analyst: <u>JP</u> Date: <u>12-15-23</u>	14.65	14.65	13.48	14.47	15.42	13.79	13.14	14.92	15.79	15.39	11.74	15.21	14.02	15.64	13.94	11.59
*B = Pan + Shrimp weight (mg) Analyst: <u>JK</u> Date: <u>12-21-23</u>	16.05	15.72	14.39	15.69	16.58	14.95	14.42	16.28	16.20	16.27	12.51	16.09	14.93	16.22	14.62	12.71
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JK</u>	1.40	1.07	0.91	1.22	1.16	1.16	1.28	1.36	0.41	0.88	0.77	0.88	0.91	0.58	0.68	0.68
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JK</u>	0.280	0.214	0.182	0.244	0.232	0.232	0.256	0.272	0.082	0.176	0.154	0.176	0.182	0.116	0.136	0.134
Average weight per initial number of shrimp (mg)	0.239								0.145							
Percent reduction from control (%)	- 2.87								37.7?							

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

AbKCICR Test Number: **247**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VI
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <i>Ruby and Brown</i> Analyst: <i>JD</i> Date: <i>12-15-23</i>	13.38	15.97	15.38	15.69	12.80	13.24	12.96	14.19	12.73	14.16	15.25	16.14	13.94	13.80	12.79	14.57
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____																
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average weight per initial number of shrimp (mg)	0								0							
Percent reduction from control (%)	100%								100%							

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

**Americamysis bahia Chronic Reference Toxicant Test
EPA-821-R-02-014, Method 1007.0**

**Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses**

Test number: _____

Test dates: January 09-16, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.14	15.33	1.19	0.238	100.0	0.233	10.8	Not applicable
	B	5	5	14.00	15.28	1.28	0.256				
	C	5	5	13.79	14.96	1.17	0.234				
	D	5	5	15.12	16.13	1.01	0.202				
	E	5	5	13.81	14.84	1.03	0.206				
	F	5	5	14.70	15.97	1.27	0.254				
	G	5	5	14.28	15.31	1.03	0.206				
	H	5	5	14.66	15.98	1.32	0.264				
250	I	5	5	12.12	13.74	1.62	0.324	100.0	0.257	17.1	-10.6
	J	5	5	12.73	13.76	1.03	0.206				
	K	5	5	12.91	14.28	1.37	0.274				
	L	5	5	12.81	14.34	1.53	0.306				
	M	5	5	14.52	15.81	1.29	0.258				
	N	5	5	15.35	16.65	1.30	0.260				
	O	5	5	13.38	14.50	1.12	0.224				
	P	5	5	14.45	15.48	1.03	0.206				
375	Q	5	5	14.65	16.05	1.40	0.280	97.5	0.239	13.3	-2.8
	R	5	5	14.65	15.72	1.07	0.214				
	S	5	4	13.48	14.39	0.91	0.182				
	T	5	5	14.47	15.69	1.22	0.244				
	U	5	5	15.42	16.58	1.16	0.232				
	V	5	5	13.79	14.95	1.16	0.232				
	W	5	5	13.14	14.42	1.28	0.256				
	X	5	5	14.92	16.28	1.36	0.272				
500	Y	5	2	15.79	16.20	0.41	0.082	60.0	0.145	23.9	37.7
	Z	5	3	15.39	16.27	0.88	0.176				
	AA	5	3	11.74	12.51	0.77	0.154				
	BB	5	3	15.21	16.09	0.88	0.176				
	CC	5	4	14.02	14.93	0.91	0.182				
	DD	5	3	15.64	16.22	0.58	0.116				
	EE	5	3	13.94	14.62	0.68	0.136				
	FF	5	3	11.59	12.27	0.68	0.136				
750	GG	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	HH	5	0	0.00	0.00	0.00	0.000				
	II	5	0	0.00	0.00	0.00	0.000				
	JJ	5	0	0.00	0.00	0.00	0.000				
	KK	5	0	0.00	0.00	0.00	0.000				
	LL	5	0	0.00	0.00	0.00	0.000				
	MM	5	0	0.00	0.00	0.00	0.000				
	NN	5	0	0.00	0.00	0.00	0.000				
1000	OO	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	PP	5	0	0.00	0.00	0.00	0.000				
	QQ	5	0	0.00	0.00	0.00	0.000				
	RR	5	0	0.00	0.00	0.00	0.000				
	SS	5	0	0.00	0.00	0.00	0.000				
	TT	5	0	0.00	0.00	0.00	0.000				
	UU	5	0	0.00	0.00	0.00	0.000				
	VV	5	0	0.00	0.00	0.00	0.000				

Dunnett's MSD value: 0.0482
PMSD: 20.7

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 11%.

Upper PMSD bound determined by USEPA (90th percentile) = 37%.

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Mysid Survival and Growth Test-7 Day Survival

Start Date: 1/9/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 1/16/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americamysis bahia
 Comments:

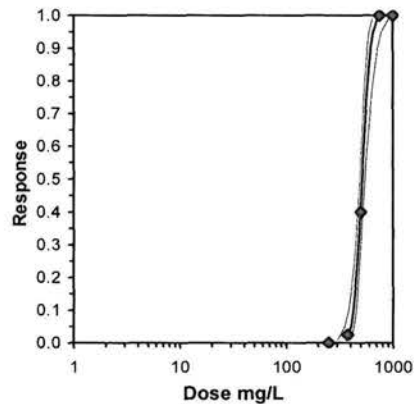
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.4000	0.6000	0.6000	0.6000	0.8000	0.6000	0.6000	0.6000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.6000	0.6000	0.8885	0.6847	1.1071	12.716	8	36.00	48.00	16	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

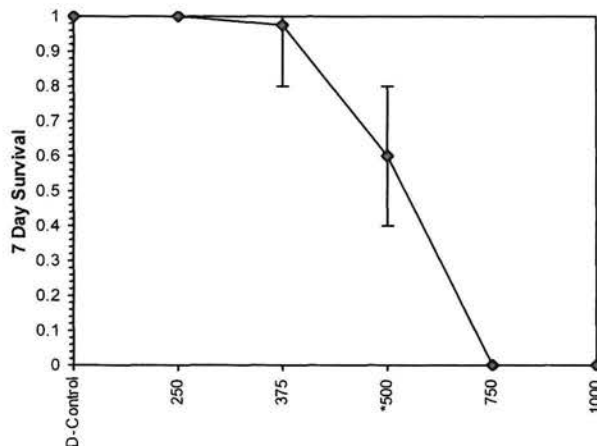
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.57001	0.904	-0.7888	8.17872
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.5616	2.89683	9.88378 21.2393	0	0.49767	7.81472	0.9194	2.71137	0.06426	4
Intercept	-37.193	7.80198	-52.485 -21.901							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	364.646	303.159 399.881
EC05	3.355	403.334	353.522 432.734
EC10	3.718	425.61	382.934 452.248
EC15	3.964	441.329	403.541 466.61
EC20	4.158	454.236	420.146 478.987
EC25	4.326	465.609	434.376 490.497
EC40	4.747	495.547	468.997 524.531
EC50	5.000	514.476	488.334 549.268
EC60	5.253	534.128	506.545 577.357
EC75	5.674	568.471	535.262 630.861
EC80	5.842	582.705	546.414 654.27
EC85	6.036	599.746	559.382 683.051
EC90	6.282	621.897	575.763 721.536
EC95	6.645	656.243	600.391 783.304
EC99	7.326	725.869	648.375 915.325



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date: 1/9/2024	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant	
End Date: 1/16/2024	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanysis bahia	

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2380	0.2560	0.2340	0.2020	0.2060	0.2540	0.2060	0.2640
250	0.3240	0.2060	0.2740	0.3060	0.2580	0.2600	0.2240	0.2060
375	0.2800	0.2140	0.1820	0.2440	0.2320	0.2320	0.2560	0.2720
500	0.0820	0.1760	0.1540	0.1760	0.1820	0.1160	0.1360	0.1360
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

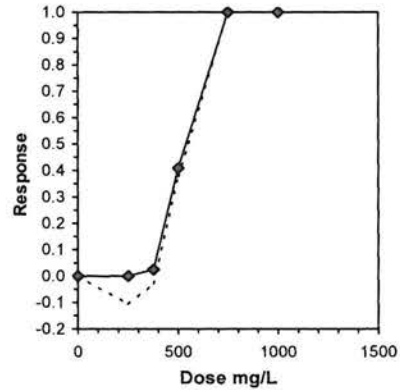
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.2325	1.0000	0.2325	0.2020	0.2640	10.751	8				0.2449	1.0000
250	0.2573	1.1065	0.2573	0.2060	0.3240	17.058	8	-1.437	2.799	0.0482	0.2449	1.0000
375	0.2390	1.0280	0.2390	0.1820	0.2800	13.284	8	-0.377	2.799	0.0482	0.2390	0.9760
500	0.1448	0.6226	0.1448	0.0820	0.1820	23.851	8				0.1448	0.5911
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97594	0.884	0.01217	-0.5947
Bartlett's Test indicates equal variances (p = 0.35)	2.1009	9.21035		

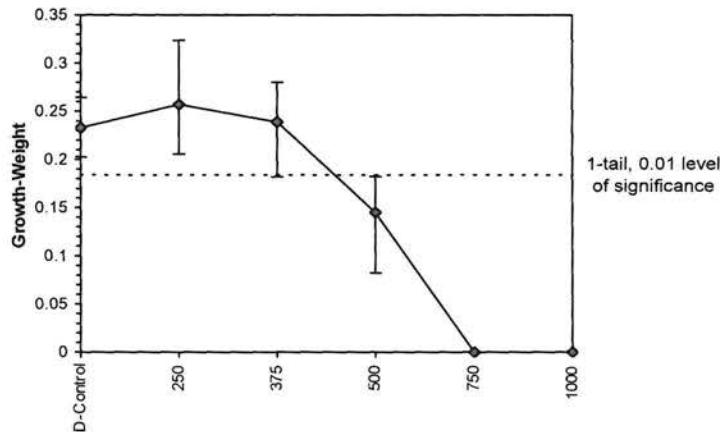
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	>375			0.04819	0.20728	0.00132	0.00119	0.348	2, 21

Treatments vs D-Control

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	383.45	31.28	294.72	393.18	-1.3988
IC10	399.69	17.14	341.44	411.91	-2.2668
IC15	415.92	13.61	380.95	432.29	-1.4534
IC20	432.16	12.57	402.52	451.57	-0.6443
IC25	448.40	12.52	422.94	471.39	0.0153
IC40	497.12	16.03	469.93	530.70	0.0902
IC50	538.54	17.82	497.27	567.25	-0.5851



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKCICR Test Number: 247

Daily Chemistry:

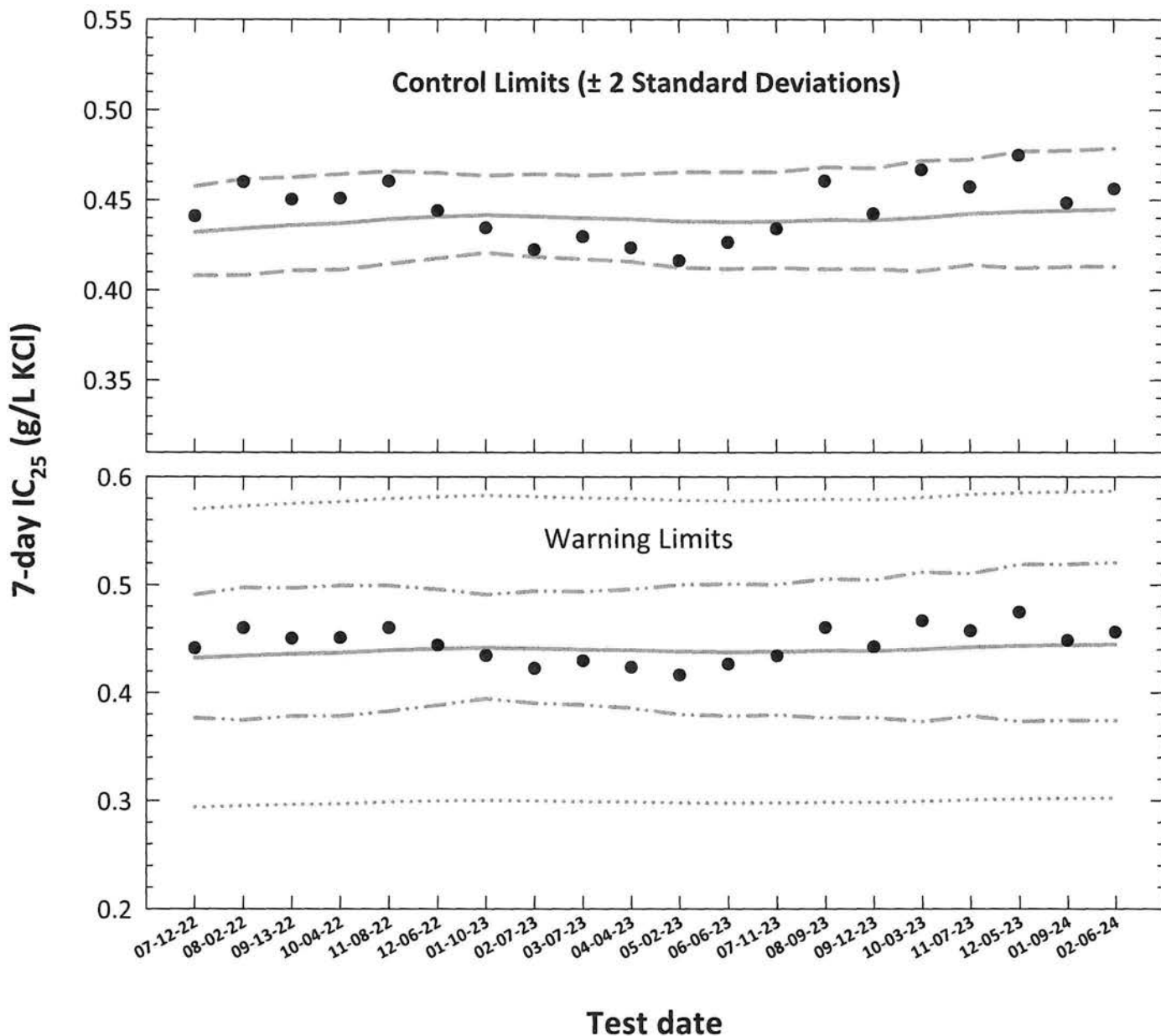
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	R	APBL	PBL	N JP	V JP	BZ
CONTROL, Salt SW	pH (S.U.)	7.86	7.87	8.04	7.02	7.99	7.63
	DO (mg/L)	7.6	7.6	7.8	7.1	7.6	7.5
	Salinity (ppt)	25.0	25.1	25.0	25.1	25.3 (25.0)	24.9
	Alkalinity (mg CaCO ₃ /L)	110				110	
	Temperature (°C)	25.3	26.1	25.2	26.0	25.1	26.2
250 mg KCl/L	pH (S.U.)	7.82	7.89	7.99	7.03	7.98	7.65
	DO (mg/L)	7.5	7.6	7.9	7.1	7.6	7.5
	Salinity (ppt)	25.3	25.5	25.6	25.6	25.6	25.5
	Temperature (°C)	25.4	26.4	25.2	26.3	25.0	26.2
375 mg KCl/L	pH (S.U.)	7.82	7.91	7.99	7.03	7.98	7.66
	DO (mg/L)	7.5	7.6	7.9	7.2	7.6	7.4
	Salinity (ppt)	25.4	25.7	25.5	25.9	25.6	25.8
	Temperature (°C)	25.6	26.2	25.2	25.9	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.83	7.87	8.00	7.04	7.98	7.69
	DO (mg/L)	7.6	7.7	7.8	7.2	7.4	7.4
	Salinity (ppt)	25.2	25.6	25.6	25.9	25.7	25.9
	Temperature (°C)	25.3	26.2	25.2	26.2	25.2	26.2
750 mg KCl/L	pH (S.U.)	7.84	7.92				
	DO (mg/L)	7.8	7.9				
	Salinity (ppt)	25.6	26.0				
	Temperature (°C)	25.6	26.5				
1000 mg KCl/L	pH (S.U.)	7.85	7.92				
	DO (mg/L)	7.8	7.7				
	Salinity (ppt)	25.8	26.2				
	Temperature (°C)	25.6	26.3				
		Initial	Final	Initial	Final	Initial	Final

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ \pm 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ \pm 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ \pm S_{A,75} converted to anti-logarithmic values,
S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

Americamysis (Mysidopsis) bahia
Chronic Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
1	07-12-22	0.4410	-0.3556	-0.3644	0.0124	0.4321	0.4081	0.4576	0.3764	0.4911	0.2938	0.5704
2	08-02-22	0.4599	-0.3373	-0.3623	0.0134	0.4342	0.4083	0.4617	0.3745	0.4976	0.2952	0.5731
3	09-13-22	0.4502	-0.3466	-0.3605	0.0129	0.4360	0.4109	0.4626	0.3785	0.4970	0.2965	0.5755
4	10-04-22	0.4509	-0.3459	-0.3595	0.0132	0.4371	0.4113	0.4644	0.3782	0.4995	0.2972	0.5769
5	11-08-22	0.4604	-0.3368	-0.3571	0.0127	0.4395	0.4146	0.4658	0.3829	0.4995	0.2988	0.5801
6	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4408	0.4177	0.4651	0.3884	0.4960	0.2997	0.5818
7	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208	0.4635	0.3944	0.4911	0.3003	0.5829
8	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185	0.4644	0.3901	0.4943	0.2998	0.5820
9	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174	0.4638	0.3886	0.4941	0.2992	0.5807
10	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158	0.4643	0.3857	0.4961	0.2988	0.5800
11	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126	0.4654	0.3798	0.5003	0.2980	0.5785
12	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118	0.4655	0.3784	0.5011	0.2977	0.5780
13	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124	0.4655	0.3794	0.5006	0.2979	0.5783
14	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116	0.4683	0.3766	0.5056	0.2985	0.5795
15	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116	0.4677	0.3769	0.5047	0.2983	0.5791
16	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.3730	0.5122	0.2993	0.5810
17	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105	0.3008	0.5838
18	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3732	0.5190	0.3015	0.5853
19	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3744	0.5192	0.3020	0.5863
20	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3740	0.5208	0.3024	0.5870

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

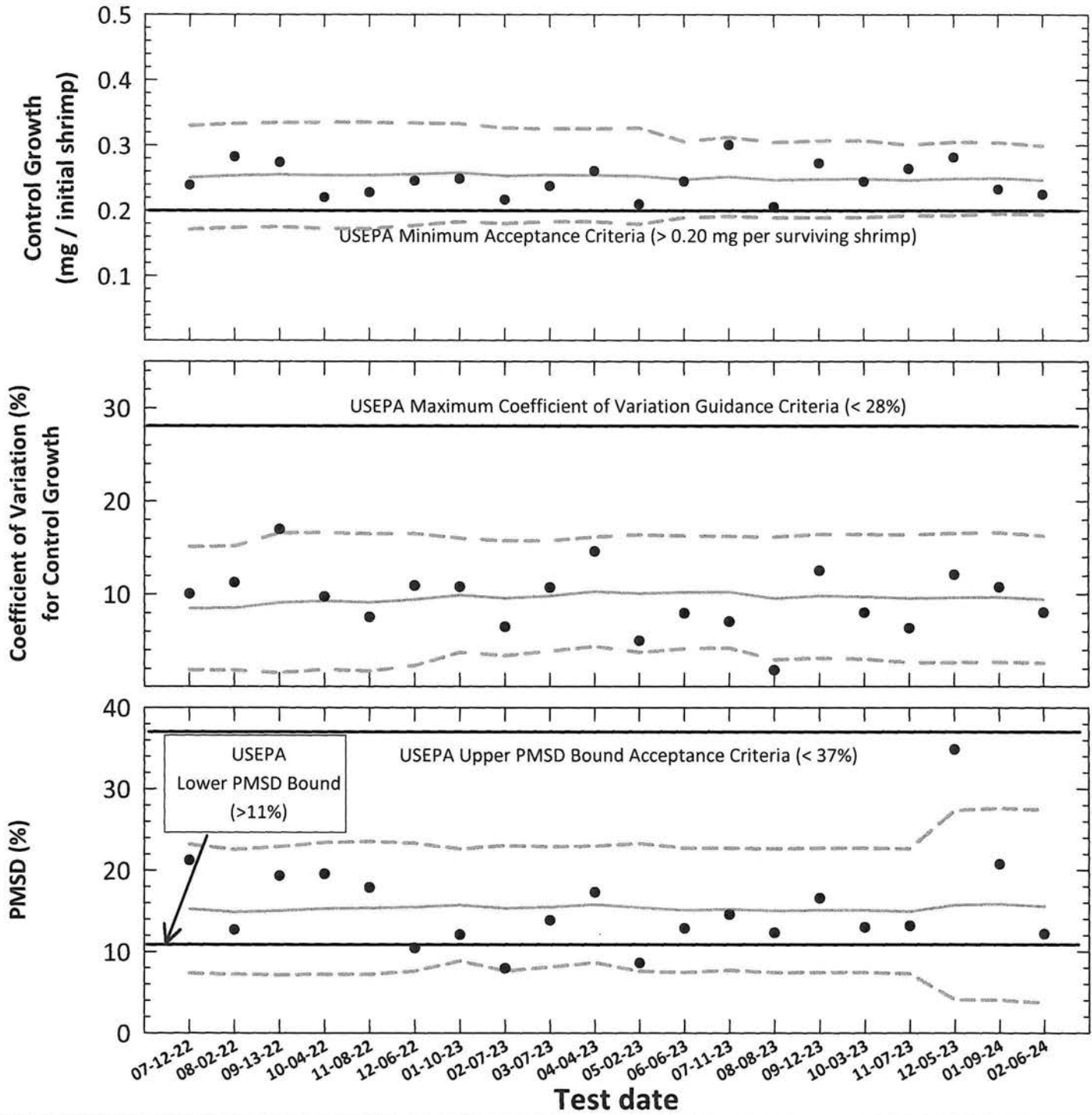
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

CV = Coefficient of variation.

Americamysis (Mysidopsis) bahia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: Aquatic Indicators, Inc.



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
 PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Growth, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Growth, CV or PMSD \pm 2 Standard Deviations)

Entered and Reviewed by
 Jim Sumner

Americamysis (Mysidopsis) bahia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: Aquatic Indicators, Inc.

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD				
		Control Survival (%)	Control Growth		PMSD (%)	CT	95% Confidence Interval	CT	95% Confidence Interval	CT	95% Confidence Interval			
			Mean (mg/initial shrimp)	CV (%)								MSD	Test	
1	07-12-22	100	0.239	10.0	0.0507	21.2	0.251	0.171	0.330	8.5	1.8	15.2	7.3	23.2
2	08-02-22	100	0.282	11.3	0.0358	12.7	0.254	0.174	0.333	8.5	1.8	14.9	7.2	22.5
3	09-13-22	100	0.274	17.0	0.0529	19.3	0.255	0.175	0.335	9.1	1.6	15.0	7.1	22.9
4	10-04-22	100	0.220	9.7	0.0430	19.5	0.254	0.173	0.335	9.3	1.9	15.3	7.2	23.4
5	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	0.335	9.1	1.7	15.4	7.2	23.5
6	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	0.334	9.4	2.3	15.5	7.6	23.3
7	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	0.333	9.9	3.7	15.7	8.8	22.6
8	02-07-23	100	0.217	6.5	0.0172	7.9	0.253	0.180	0.326	9.6	3.4	15.3	7.6	23.0
9	03-07-23	100	0.238	10.7	0.0329	13.9	0.254	0.183	0.326	9.8	3.9	15.5	8.1	22.9
10	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	15.8	8.6	23.0
11	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	15.4	7.6	23.3
12	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	15.1	7.4	22.7
13	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	15.2	7.7	22.7
14	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	15.0	7.4	22.6
15	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	15.1	7.4	22.7
16	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	15.1	7.4	22.7
17	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	14.9	7.3	22.6
18	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	4.1	27.4
19	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	15.7	4.0	27.6
20	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	15.5	3.7	27.4

Note: Control Survival = USEPA minimum test acceptability criteria \geq 80% survival.

Control Mean Growth = USEPA minimum test acceptability criteria \geq 0.20 mg/surviving shrimp.

CV = Coefficient of variation for control growth.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

CT = Central tendency of the growth, CV or PMSD values.

S = Standard deviation of the growth, CV or PMSD values.

The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 37%.

Lower PMSD bound determined by USEPA (10th percentile) > 11%.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

CT = Central tendency of the growth, CV or PMSD values.

S = Standard deviation of the growth, CV or PMSD values.



Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-014, Method 1007.0)
Species: Americamysis (Mysidopsis) bahia

AbKCICR Test Number: 248

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2261				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	GREY
Date and times organisms were born between:	01-30-24 1200 to 01-31-24 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 01-31-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 7.40 S.U. Temperature = 25.0 °C		Date / Time in oven:	02-13-24 1145
Average transfer volume:		*Initial oven temperature:	60°C
< 0.25 mL		Date / Time out of oven:	02-14-24 1145
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-98585

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	02-06-24	1140	JL	1400	JL	1300	JL	01-29-24 B
1	02-07-24	0500	JL	1100	JL	0900	JL	↓
2	02-08-24	0500	JL	1200	JL	0900	JL	02-05-24
3	02-09-24	0500	JL	1100	JL	0910	JL	↓
4	02-10-24	0600	JL	1200	JL	0920	JL	02-07-24
5	02-11-24	0600	JL	1200	JL	0910	JL	↓
6	02-12-24	0600	JL	1200	JL	0910	JL	↓
7	02-13-24					1100	JL	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13064685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	446.44
Average weight per initial shrimp:	0.225		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.225	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	473.0
			IC ₂₅ (mg/L KCl)	456.1

02-19-24
496.4

AbKCICR Test Number: 248

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac	_____															
# females with developing ova in oviducts	_____															
# immature females	_____															
# males	_____															
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>JP</u> Date: <u>01-25-24</u>	14.52	13.90	12.55	14.35	15.08	14.73	15.53	15.01	14.49	14.99	13.46	12.42	11.87	11.77	12.33	13.69
*B = Pan + Shrimp weight (mg) Analyst: <u>H</u> Date: <u>01-16-24</u>	15.84	14.98	13.58	15.49	16.24	15.80	16.60	16.12	16.54	16.01	14.50	13.49	13.02	12.91	13.58	14.71
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.32	1.08	1.03	1.14	1.16	1.07	1.07	1.11	1.05	1.02	1.04	1.07	1.15	1.14	1.25	1.08
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.264	0.216	0.206	0.228	0.232	0.214	0.214	0.222	0.210	0.204	0.208	0.214	0.230	0.228	0.250	0.216
Average weight per initial number of shrimp (mg)	0.225								0.220							
Percent reduction from control (%)	2.0%															

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

AbKCICR Test Number: 248

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	4 ^d	5	4 ^d	4 ^d	5	5
3	5	5	5	5	5	5	5	5	4 ^{1d}	5	4	4 ^{1d}	4	4	5	5
4	5	5	5	5	4 ^{1d}	5	5	5	4	5	4	1 ^{3d}	4	3 ^{1d}	5	5
5	5	5	5	5	4	5	5	5	3 ^{1d}	4 ^{1d}	4	0 ^{1d}	4	3	5	5
6	5	4 ^{1d}	5	5	4	4 ^{1d}	5	5	3	4	4	0	4	3	4 ^{1d}	3 ²
7	5	4	5	5	4	4	5	5	3	3 ^{1d}	3 ^{1d}	0	4	3	2 ^{1d}	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>AP</u> Date: <u>01-25-24</u>	13.93	12.42	12.03	13.39	14.72	14.67	11.49	13.25	13.72	13.12	14.10	15.21	14.57	14.04	12.74	13.21
*B = Pan + Shrimp weight (mg) Analyst: <u>H</u> Date: <u>02-15-24</u>	15.03	13.42	13.24	14.66	15.91	15.63	12.56	14.49	14.36	13.83	14.94	→	15.37	14.71	13.25	13.98
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>AP</u>	1.10	1.00	1.26	1.27	1.19	0.96	1.07	1.24	0.64	0.71	0.84	→	0.80	0.67	1.11	0.77
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>AP</u>	0.220	0.200	0.252	0.254	0.238	0.192	0.214	0.248	0.128	0.142	0.168	0	0.160	0.134	0.222	0.154
Average weight per initial number of shrimp (mg)	0.227								Percent reduction from control (%)							
									-1.2%							
Average weight per initial number of shrimp (mg)									0.134							
									38.3%							

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

AbKCICR Test Number: 248

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VI
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code:																
Analyst: _____																
Date: _____																
*B = Pan + Shrimp weight (mg) Analyst: _____																
Date: _____																
C = Shrimp weight (mg) = B - A																
Hand calculated Analyst: _____																
Weight per initial number of shrimp (mg) = C / Initial number of shrimp																
Hand calculated Analyst: _____																
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Average weight per initial number of shrimp (mg) 0								Percent reduction from control (%) 100%							
	Average weight per initial number of shrimp (mg) 0								Percent reduction from control (%) 100%							

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:



**Americamysis bahia Chronic Reference Toxicant Test
EPA-821-R-02-014, Method 1007.0**

**Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses**

Test number: _____
Test dates: February 06-13, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction in control (%)
Control	A	5	5	14.52	15.84	1.32	0.264	100.0	0.225	8.0	Not applicable
	B	5	5	13.90	14.98	1.08	0.216				
	C	5	5	12.55	13.58	1.03	0.206				
	D	5	5	14.35	15.49	1.14	0.228				
	E	5	5	15.08	16.24	1.16	0.232				
	F	5	5	14.73	15.80	1.07	0.214				
	G	5	5	15.53	16.60	1.07	0.214				
	H	5	5	15.01	16.12	1.11	0.222				
250	I	5	5	14.49	15.54	1.05	0.210	100.0	0.220	6.9	2.0
	J	5	5	14.99	16.01	1.02	0.204				
	K	5	5	13.46	14.50	1.04	0.208				
	L	5	5	12.42	13.49	1.07	0.214				
	M	5	5	11.87	13.02	1.15	0.230				
	N	5	5	11.77	12.91	1.14	0.228				
	O	5	5	12.33	13.58	1.25	0.250				
	P	5	5	13.69	14.77	1.08	0.216				
375	Q	5	5	13.93	15.03	1.10	0.220	92.5	0.227	10.6	-1.2
	R	5	4	12.42	13.42	1.00	0.200				
	S	5	5	12.03	13.29	1.26	0.252				
	T	5	5	13.39	14.66	1.27	0.254				
	U	5	4	14.72	15.91	1.19	0.238				
	V	5	4	14.67	15.63	0.96	0.192				
	W	5	5	11.49	12.56	1.07	0.214				
	X	5	5	13.25	14.49	1.24	0.248				
500	Y	5	3	13.72	14.36	0.64	0.128	52.5	0.139	45.6	38.3
	Z	5	3	13.12	13.83	0.71	0.142				
	AA	5	3	14.10	14.94	0.84	0.168				
	BB	5	0	0.00	0.00	0.00	0.000				
	CC	5	4	14.57	15.37	0.80	0.160				
	DD	5	3	14.04	14.71	0.67	0.134				
	EE	5	2	12.14	13.25	1.11	0.222				
	FF	5	3	13.21	13.98	0.77	0.154				
750	GG	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	HH	5	0	0.00	0.00	0.00	0.000				
	II	5	0	0.00	0.00	0.00	0.000				
	JJ	5	0	0.00	0.00	0.00	0.000				
	KK	5	0	0.00	0.00	0.00	0.000				
	LL	5	0	0.00	0.00	0.00	0.000				
	MM	5	0	0.00	0.00	0.00	0.000				
	NN	5	0	0.00	0.00	0.00	0.000				
1000	OO	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	PP	5	0	0.00	0.00	0.00	0.000				
	QQ	5	0	0.00	0.00	0.00	0.000				
	RR	5	0	0.00	0.00	0.00	0.000				
	SS	5	0	0.00	0.00	0.00	0.000				
	TT	5	0	0.00	0.00	0.00	0.000				
	UU	5	0	0.00	0.00	0.00	0.000				
	VV	5	0	0.00	0.00	0.00	0.000				

Dunnett's MSD value: 0.0273
PMSD: 12.2

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 11%.

Upper PMSD bound determined by USEPA (90th percentile) = 37%.

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.



Mysid Survival and Growth Test-7 Day Survival

Start Date: 2/6/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 2/13/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanmysid bahia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	0.8000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
500	0.6000	0.6000	0.6000	0.0000	0.8000	0.6000	0.4000	0.6000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

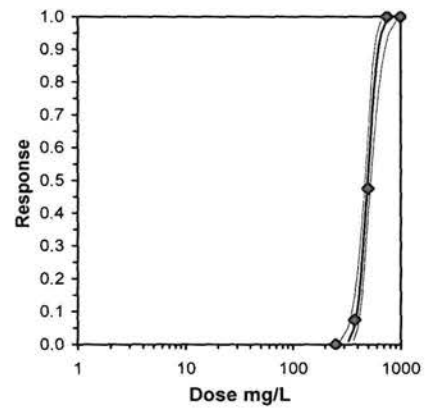
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9250	0.9250	1.2560	1.1071	1.3453	9.813	8	56.00	48.00	3	40
*500	0.5250	0.5250	0.8060	0.2255	1.1071	32.301	8	36.00	48.00	19	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.71928	0.904	-2.3322	10.5159

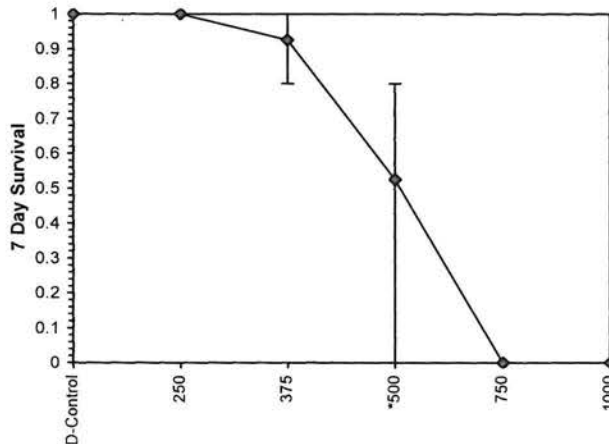
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	12.9873	2.06991	8.93022	17.0443	0	0.92306	7.81472	0.81986	2.69587	0.077	4
Intercept	-30.012	5.55033	-40.891	-19.133							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	328.655	273.441	364.12
EC05	3.355	370.863	324.291	401.301
EC10	3.718	395.538	354.489	423.447
EC15	3.964	413.106	375.93	439.668
EC20	4.158	427.624	393.435	453.527
EC25	4.326	440.485	408.65	466.279
EC40	4.747	474.633	446.851	503.156
EC50	5.000	496.438	469.091	529.471
EC60	5.253	519.246	490.581	559.271
EC75	5.674	559.5	525.217	616.4
EC80	5.842	576.327	538.841	641.596
EC85	6.036	596.581	554.782	672.736
EC90	6.282	623.079	575.059	714.634
EC95	6.645	664.533	605.819	782.439
EC99	7.326	749.879	666.655	929.372



Dose-Response Plot



Entered and Reviewed by Jim Sommer

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

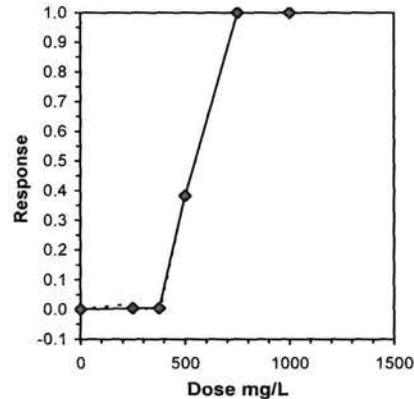
Start Date: 2/6/2024	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant	
End Date: 2/13/2024	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americamysis bahia	
Comments:			

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2640	0.2160	0.2060	0.2280	0.2320	0.2140	0.2140	0.2220
250	0.2100	0.2040	0.2080	0.2140	0.2300	0.2280	0.2500	0.2160
375	0.2200	0.2000	0.2520	0.2540	0.2380	0.1920	0.2140	0.2480
500	0.1280	0.1420	0.1680	0.0000	0.1600	0.1340	0.2220	0.1540
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

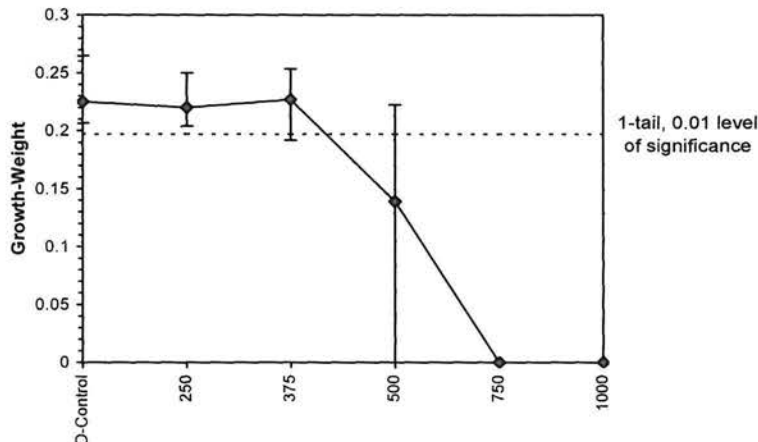
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2245	1.0000	0.2245	0.2060	0.2640	8.021	8				0.2245	1.0000
250	0.2200	0.9800	0.2200	0.2040	0.2500	6.906	8	0.462	2.799	0.0273	0.2236	0.9961
375	0.2273	1.0122	0.2273	0.1920	0.2540	10.633	8	-0.282	2.799	0.0273	0.2236	0.9961
500	0.1385	0.6169	0.1385	0.0000	0.2220	45.559	8				0.1385	0.6169
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000

Auxiliary Tests					Statistic	Critical	Skew	Kurt					
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.96586	0.884	0.38183	-0.2772					
Bartlett's Test indicates equal variances (p = 0.48)					1.48009	9.21035							
Hypothesis Test (1-tail, 0.01)				NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test				375	>375			0.02727	0.12145	0.00011	0.00038	0.7569	2, 21
Treatments vs D-Control													

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL		Skew
IC05	390.20	56.01	193.97	401.10	-2.8999
IC10	406.68	17.98	386.07	428.94	-4.7135
IC15	423.16	16.32	399.95	455.98	1.0284
IC20	439.65	20.01	413.87	487.00	0.8762
IC25	456.13	23.16	427.75	509.41	0.6545
IC40	506.86	27.62	460.97	557.53	0.0675
IC50	547.38	29.17	482.46	589.61	-0.4601



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

AbKCICR Test Number: 248

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	JBL	JBL	JBL	JBL	JBL	L
CONTROL, Salt SW	pH (S.U.)	7.89	7.97	7.96	7.80	7.92	7.83
	DO (mg/L)	7.7	7.5	7.9	7.6	7.7	7.0
	Salinity (ppt)	25.0	25.1	25.0	25.1	25.3 (25.3)	25.1
	Alkalinity (mg CaCO ₃ /L)	120				110	
	Temperature (°C)	25.5	26.1	25.1	25.8	25.3	25.7
250 mg KCl/L	pH (S.U.)	7.90	7.93	7.97	7.88	7.95	7.04
	DO (mg/L)	7.7	7.4	7.9	7.6	7.7	7.1
	Salinity (ppt)	25.4	25.5	25.4	25.5	25.5 (25.5)	25.4
	Temperature (°C)	25.4	25.7	25.2	25.7	25.4	25.6
375 mg KCl/L	pH (S.U.)	7.90	7.93	7.98	7.86	7.96	7.06
	DO (mg/L)	7.7	7.4	7.9	7.6	7.9	7.1
	Salinity (ppt)	25.5	25.8	25.7	26.0	25.8	26.1
	Temperature (°C)	25.5	25.7	25.2	25.9	25.4	25.6
500 mg KCl/L	pH (S.U.)	7.91	7.93	7.99	7.87	7.97	7.08
	DO (mg/L)	7.7	7.4	8.0	7.6	7.9	7.1
	Salinity (ppt)	25.5	25.6	25.7	26.1	25.8	26.2
	Temperature (°C)	25.5	26.2	25.2	25.9	25.2	25.6
750 mg KCl/L	pH (S.U.)	7.91	7.94				
	DO (mg/L)	7.7	7.4				
	Salinity (ppt)	25.6	25.8				
	Temperature (°C)	25.5	25.7				
1000 mg KCl/L	pH (S.U.)	7.91	7.92				
	DO (mg/L)	7.7	7.4				
	Salinity (ppt)	25.7	26.1				
	Temperature (°C)	25.4	25.6				
		Initial	Final	Initial	Final	Initial	Final

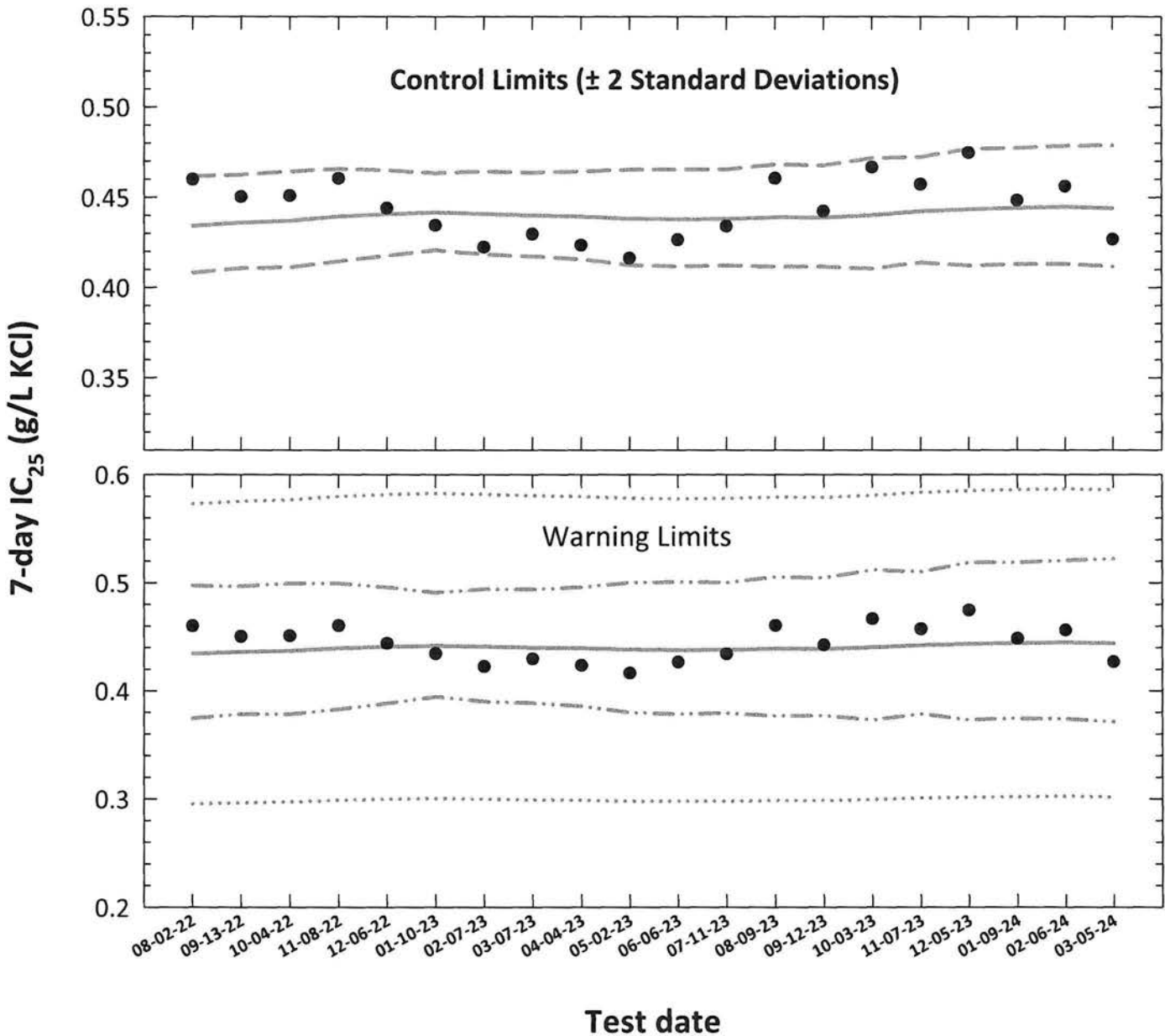
AbKCICR Test Number: 248

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	<i>JK</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>JOBL</i>	<i>JOBL</i>	<i>JK</i>
CONTROL, Salt SW	pH (S.U.)	8.01	7.84	7.97	7.79	7.99	7.79	7.99	7.53
	DO (mg/L)	7.9	7.1	7.5	7.4	7.9	7.4	7.9	6.1
	Salinity (ppt)	25.1	25.2	25.0	25.2	25.0	25.3	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	→	100	100					100
	Temperature (°C)	25.2	26.4	25.2	25.9	25.2	26.1	25.1	26.1
250 mg KCl/L	pH (S.U.)	8.01	7.84	7.98	7.84	7.99	7.78	7.99	7.57
	DO (mg/L)	7.9	7.2	7.5	7.4	7.9	7.3	7.9	6.0
	Salinity (ppt)	25.2	25.4	25.3	25.4	25.4	25.3	25.1	25.3
	Temperature (°C)	25.2	26.1	25.2	25.8	25.2	26.2	25.2	26.4
375 mg KCl/L	pH (S.U.)	8.01	7.85	7.98	7.83	7.99	7.83	7.99	7.62
	DO (mg/L)	8.0	7.2	7.5	7.4	7.8	7.3	7.9	6.0
	Salinity (ppt)	25.2	25.6	25.5	25.6	25.4	25.5	25.5	25.7
	Temperature (°C)	25.1	26.0	25.2	25.8	25.4	26.2	25.2	26.4
500 mg KCl/L	pH (S.U.)	8.01	7.83	7.99	7.83	7.99	7.82	7.99	7.63
	DO (mg/L)	8.0	7.1	7.4	7.3	7.8	7.3	7.9	6.4
	Salinity (ppt)	25.3	25.7	25.6	25.9	25.5	25.6	25.6	25.8
	Temperature (°C)	25.2	26.0	25.2	25.8	25.4	26.0	25.2	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ \pm 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic IC₂₅ \pm 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ \pm S_{A,75} converted to anti-logarithmic values,
S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

Americamysis (*Mysidopsis bahia*)
Chronic Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}			
1	08-02-22	0.4599	-0.3373	-0.3623	0.0134	0.4342	0.4083	0.4617	0.3745	0.4976	0.2952	0.5731
2	09-13-22	0.4502	-0.3466	-0.3605	0.0129	0.4360	0.4109	0.4626	0.3785	0.4970	0.2965	0.5755
3	10-04-22	0.4509	-0.3459	-0.3595	0.0132	0.4371	0.4113	0.4644	0.3782	0.4995	0.2972	0.5769
4	11-08-22	0.4604	-0.3368	-0.3571	0.0127	0.4395	0.4146	0.4658	0.3829	0.4995	0.2988	0.5801
5	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4408	0.4177	0.4651	0.3884	0.4960	0.2997	0.5818
6	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208	0.4635	0.3944	0.4911	0.3003	0.5829
7	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185	0.4644	0.3901	0.4943	0.2998	0.5820
8	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174	0.4638	0.3886	0.4941	0.2992	0.5807
9	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158	0.4643	0.3857	0.4961	0.2988	0.5800
10	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126	0.4654	0.3798	0.5003	0.2980	0.5785
11	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118	0.4655	0.3784	0.5011	0.2977	0.5780
12	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124	0.4655	0.3794	0.5006	0.2979	0.5783
13	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116	0.4683	0.3766	0.5056	0.2985	0.5795
14	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116	0.4677	0.3769	0.5047	0.2983	0.5791
15	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.3730	0.5122	0.2993	0.5810
16	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105	0.3008	0.5838
17	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3732	0.5190	0.3015	0.5853
18	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3744	0.5192	0.3020	0.5863
19	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3740	0.5208	0.3024	0.5870
20	03-05-24	0.4268	-0.3698	-0.3527	0.0164	0.4440	0.4117	0.4788	0.3712	0.5224	0.3019	0.5860

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

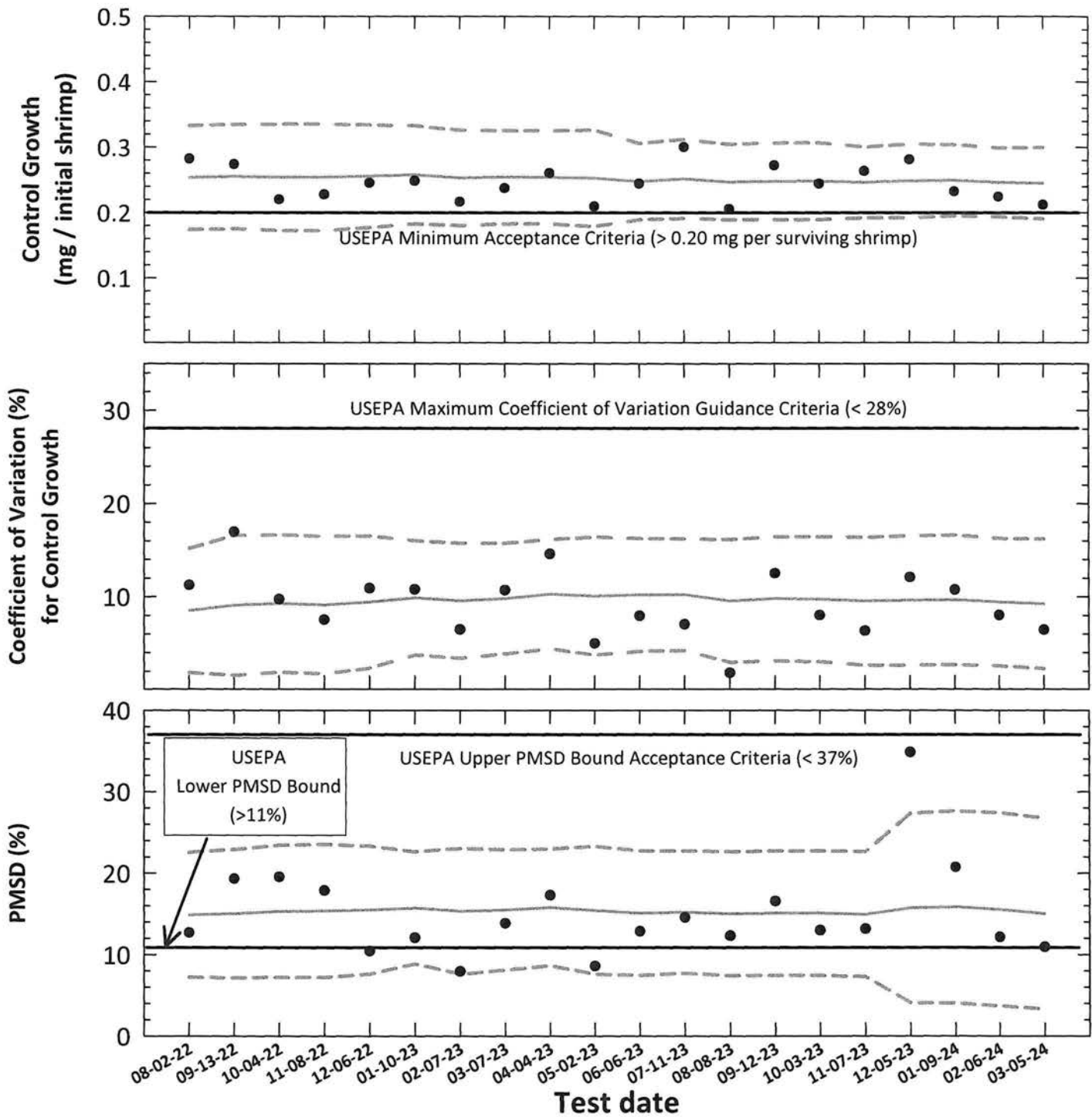
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

CV = Coefficient of variation.

Americamysis (Mysidopsis) bahia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: Aquatic Indicators, Inc.



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
 PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency** (mean Control Growth, CV or PMSD)
- - - **95% Confidence Interval** (mean Control Growth, CV or PMSD \pm 2 Standard Deviations)

Americamysis (*Mysidopsis bahia*)
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: Aquatic Indicators, Inc.

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD					
		Control Survival (%)	Control Growth		CT	95% Confidence Interval	CT	95% Confidence Interval	CT	95% Confidence Interval	CT + 2S				
			Mean (mg/initial shrimp)	CV (%)								MSD	PMSD (%)		
1	08-02-22	100	0.282	11.3	0.0358	12.7	0.254	0.174	0.333	8.5	1.8	15.2	14.9	7.2	22.5
2	09-13-22	100	0.274	17.0	0.0529	19.3	0.255	0.175	0.335	9.1	1.6	16.6	15.0	7.1	22.9
3	10-04-22	100	0.220	9.7	0.0430	19.5	0.254	0.173	0.335	9.3	1.9	16.7	15.3	7.2	23.4
4	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	0.335	9.1	1.7	16.5	15.4	7.2	23.5
5	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3
6	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6
7	02-07-23	100	0.217	6.5	0.0172	7.9	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0
8	03-07-23	100	0.238	10.7	0.0329	13.9	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9
9	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
10	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
11	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
12	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
13	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
14	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
15	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
16	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
17	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
18	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6
19	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4
20	03-05-24	100	0.212	6.5	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.20 mg/surviving shrimp.

CV = Coefficient of variation for control growth.

USEPA maximum CV guidance criteria (90th percentile) < 28%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) < 37%.

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) > 11%.

CT = Central tendency of the growth, CV or PMSD values.

S = Standard deviation of the growth, CV or PMSD values.



Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-014, Method 1007.0)
Species: Americamysis (Mysidopsis) bahia

AbKCICR Test Number: 249

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2275					
Stock preparation:	50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water					
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	Grey
Date and times organisms were born between:	02-27-24 1200 to 02-28-24 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 02-28-24	Artemia CHM number:	CHM1294
Transfer bowl information:	pH = 7.75 S.U. Temperature = 25.0 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	03-12-24 1140
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	03-13-24 1140
		*Final oven temperature:	60 °C
		Total drying time:	24-600/25

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	03-05-24	1020	J	1400	J	1300	J	02-28-24A
1	03-06-24	0500	J	1110	J	0940	J	↓
2	03-07-24	0500	J	1330	J	0940	J	02-28-24B
3	03-08-24	0500	J	1100	J	1000	J	↓
4	03-09-24	0600	J	1200	J	1000	J	03-06-24
5	03-10-24	0600	J	1200	J	0940	J	↓
6	03-11-24	0600	J	1200	J	0950	J	↓
7	03-12-24					1038	J	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	482.0
Average weight per initial shrimp:	0.212		NOEC (mg/L KCl)	250
Average weight per surviving shrimp:	0.212	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	375
			ChV (mg/L KCl)	306.2
			IC ₂₅ (mg/L KCl)	426.8

AbKCICR Test Number: 2

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>Magenta 2</u> Analyst: <u>OL</u> Date: <u>02-20-24</u>	13.81	14.23	12.09	13.83	14.10	14.16	14.55	12.76	14.70	14.84	14.26	14.72	14.48	13.95	12.58	13.1
*B = Pan + Shrimp weight (mg) Analyst: <u>J</u> Date: <u>02-14-24</u>	14.86	15.45	13.14	14.91	15.11	15.18	15.58	13.78	15.83	15.97	15.48	15.97	15.63	16.09	13.96	14.1
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.05	1.22	1.05	1.08	1.01	1.02	1.03	1.02	1.13	1.13	1.22	1.25	1.15	1.14	1.38	1.01
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.210	0.244	0.210	0.216	0.202	0.204	0.206	0.204	0.226	0.226	0.244	0.250	0.230	0.228	0.276	0.204
Average weight per initial number of shrimp (mg)	0.212								0.236							
Percent reduction from control (%)	-11.2%															

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

AbKCICR Test Number: 245

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	I
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	4 ^d	4 ^d	4 ^d	4 ^d	4 ^d	4 ^d	4 ^d	4
3	5	5	5	5	5	5	5	5	4	4	4	4	4	4	3 ^{id}	4
4	4 ^{id}	5	5	5	4 ^{id}	5	4 ^{id}	5	2 ^{2d}	3 ^{id}	3 ^{id}	3 ^{id}	4	2 ^{2d}	3	4
5	4	5	4 ^{id}	5	4	4 ^{id}	4	5	2	2 ^{id}	3	3	3 ^{id}	2	2 ^{id}	2 ²
6	4	5	4	5	4	4	4	5	2	2	3	3	3	2	2	2
7	4	5	4	5	4	4	4	5	2	2	3	3	3	2	2	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>DL</u> Date: <u>02-20-24</u>	15.61	13.35	13.89	14.02	14.56	13.80	12.16	14.20	14.94	14.37	14.83	12.20	13.58	15.24	13.84	12.21
*B = Pan + Shrimp weight (mg) Analyst: <u>DL</u> Date: <u>02-24-24</u>	16.68	14.64	14.77	15.12	15.56	14.83	13.21	15.25	15.43	14.82	15.46	12.86	14.24	15.72	14.18	12.71
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>DL</u>	1.07	1.29	0.88	1.10	1.00	1.03	1.05	1.05	0.49	0.45	0.63	0.66	0.66	0.48	0.34	0.49
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>DL</u>	0.214	0.258	0.176	0.220	0.200	0.206	0.210	0.210	0.098	0.090	0.130	0.136	0.132	0.096	0.063	0.098
Average weight per initial number of shrimp (mg) 0.212				Percent reduction from control (%) 0.17				Average weight per initial number of shrimp (mg) 0.106				Percent reduction from control (%) 50.0%				

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

AbKCICR Test Number: 249

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	V
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	!
1	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: _____																
Analyst: _____ Date: _____																
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____																
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average weight per initial number of shrimp (mg)	0								0							
Percent reduction from control (%)	100%								100%							

KL 03-06-24

03-06-24
77 81

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:



**Americamysis bahia Chronic Reference Toxicant Test
EPA-821-R-02-014, Method 1007.0**

**Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses**

Test number: _____
Test dates: March 05-12, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.81	14.86	1.05	0.210	100.0	0.212	6.5	Not applicable
	B	5	5	14.23	15.45	1.22	0.244				
	C	5	5	12.09	13.14	1.05	0.210				
	D	5	5	13.83	14.91	1.08	0.216				
	E	5	5	14.10	15.11	1.01	0.202				
	F	5	5	14.16	15.18	1.02	0.204				
	G	5	5	14.55	15.58	1.03	0.206				
	H	5	5	12.76	13.78	1.02	0.204				
250	I	5	5	14.70	15.83	1.13	0.226	100.0	0.236	8.9	-11.2
	J	5	5	14.84	15.97	1.13	0.226				
	K	5	5	14.26	15.48	1.22	0.244				
	L	5	5	14.72	15.97	1.25	0.250				
	M	5	5	14.48	15.63	1.15	0.230				
	N	5	5	13.95	15.09	1.14	0.228				
	O	5	5	12.58	13.96	1.38	0.276				
	P	5	5	13.44	14.47	1.03	0.206				
375	Q	5	4	15.61	16.68	1.07	0.214	87.5	0.212	10.8	0.1
	R	5	5	13.35	14.64	1.29	0.258				
	S	5	4	13.89	14.77	0.88	0.176				
	T	5	5	14.02	15.12	1.10	0.220				
	U	5	4	14.56	15.56	1.00	0.200				
	V	5	4	13.80	14.83	1.03	0.206				
	W	5	4	12.16	13.21	1.05	0.210				
	X	5	5	14.20	15.25	1.05	0.210				
500	Y	5	2	14.94	15.43	0.49	0.098	47.5	0.106	22.8	50.0
	Z	5	2	14.37	14.82	0.45	0.090				
	AA	5	3	14.83	15.48	0.65	0.130				
	BB	5	3	12.20	12.88	0.68	0.136				
	CC	5	3	13.58	14.24	0.66	0.132				
	DD	5	2	15.24	15.72	0.48	0.096				
	EE	5	2	13.84	14.18	0.34	0.068				
	FF	5	2	12.21	12.70	0.49	0.098				
750	GG	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	HH	5	0	0.00	0.00	0.00	0.000				
	II	5	0	0.00	0.00	0.00	0.000				
	JJ	5	0	0.00	0.00	0.00	0.000				
	KK	5	0	0.00	0.00	0.00	0.000				
	LL	5	0	0.00	0.00	0.00	0.000				
	MM	5	0	0.00	0.00	0.00	0.000				
	NN	5	0	0.00	0.00	0.00	0.000				
1000	OO	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	PP	5	0	0.00	0.00	0.00	0.000				
	QQ	5	0	0.00	0.00	0.00	0.000				
	RR	5	0	0.00	0.00	0.00	0.000				
	SS	5	0	0.00	0.00	0.00	0.000				
	TT	5	0	0.00	0.00	0.00	0.000				
	UU	5	0	0.00	0.00	0.00	0.000				
	VV	5	0	0.00	0.00	0.00	0.000				

Dunnett's MSD value: 0.0232
PMSD: 10.9

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 11%.
Upper PMSD bound determined by USEPA (90th percentile) = 37%.

Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.



Mysid Survival and Growth Test-7 Day Survival

Start Date: 3/5/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 3/12/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americamysis bahia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	0.8000	1.0000	0.8000	1.0000	0.8000	0.8000	0.8000	1.0000
500	0.4000	0.4000	0.6000	0.6000	0.6000	0.4000	0.4000	0.4000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

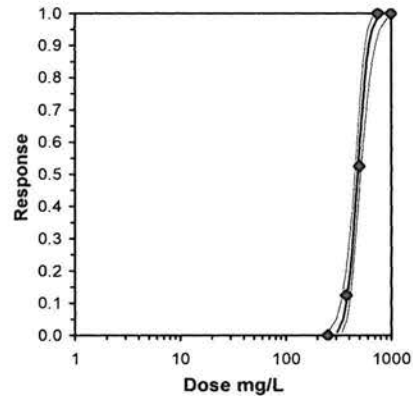
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*375	0.8750	0.8750	1.1964	1.1071	1.3453	10.301	8	48.00	48.00	5	40
*500	0.4750	0.4750	0.7602	0.6847	0.8861	13.708	8	36.00	48.00	21	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.80385	0.904	0.77467	-0.2527

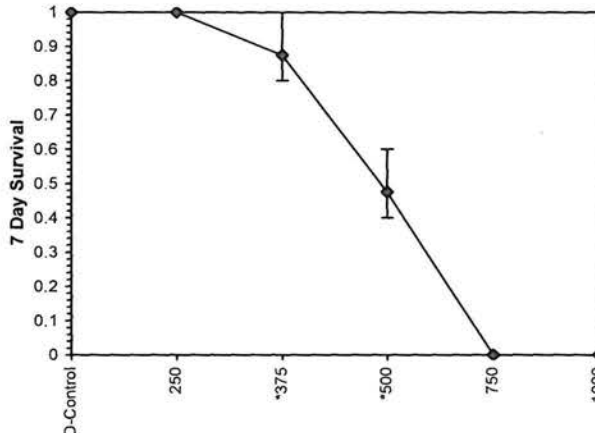
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	250	375	306.186	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	11.7608	1.80417	8.22459	15.2969	0	1.18185	7.81472	0.75736	2.68301	0.08503	5
Intercept	-26.554	4.82175	-36.005	-17.104							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	305.639	250.977	341.829
EC05	3.355	349.265	302.208	380.671
EC10	3.718	375.013	333.067	403.874
EC15	3.964	393.448	355.194	420.861
EC20	4.158	408.745	373.411	435.351
EC25	4.326	422.341	389.369	448.655
EC40	4.747	458.641	430.002	487.012
EC50	5.000	481.964	454.008	514.413
EC60	5.253	506.473	477.368	545.617
EC75	5.674	550.003	515.206	606.034
EC80	5.842	568.298	530.142	632.896
EC85	6.036	590.392	547.657	666.251
EC90	6.282	619.416	570.005	711.369
EC95	6.645	665.08	604.059	784.904
EC99	7.326	760.012	671.965	946.152



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Mysid Survival and Growth Test-Growth-Weight

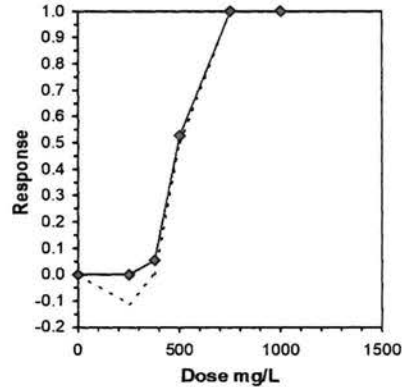
Start Date:	3/5/2024	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/12/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americanysis bahia

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2100	0.2440	0.2100	0.2160	0.2020	0.2040	0.2060	0.2040
250	0.2260	0.2260	0.2440	0.2500	0.2300	0.2280	0.2760	0.2060
375	0.2140	0.2580	0.1760	0.2200	0.2000	0.2060	0.2100	0.2100
500	0.0980	0.0900	0.1300	0.1360	0.1320	0.0960	0.0680	0.0980
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

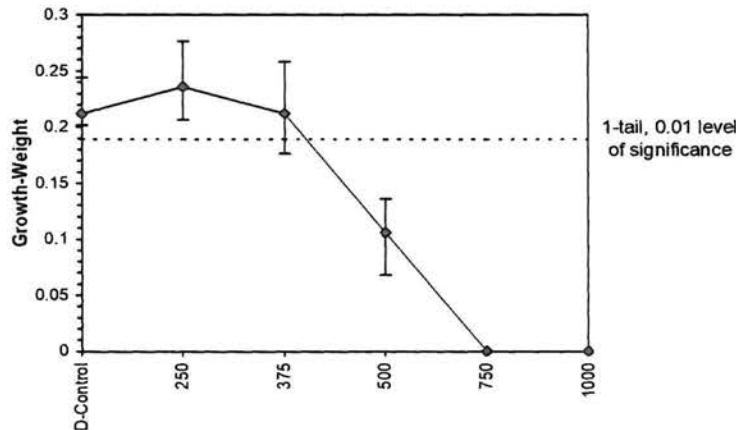
Conc-mg/L			Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	0.2120	1.0000	0.2120	0.2020	0.2440	6.458	8	-2.688	2.624	0.0232	0.2239	1.0000	
250	0.2358	1.1120	0.2358	0.2060	0.2760	8.868	8				0.2239	1.0000	
375	0.2118	0.9988	0.2118	0.1760	0.2580	10.810	8				0.2118	0.9458	
500	0.1060	0.5000	0.1060	0.0680	0.1360	22.776	8				0.1060	0.4735	
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000	
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000	

Auxiliary Tests	Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8634	0.844	1.07809	1.62278		
F-Test indicates equal variances (p = 0.29)	2.33194	8.88531				
Hypothesis Test (1-tail, 0.01)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs D-Control	0.02319	0.10938	0.00226	0.00031	0.01766	1, 14

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	365.40	27.88	301.11 387.34	-0.6564	
IC10	387.13	11.29	352.22 399.96	-1.9043	
IC15	400.36	7.64	382.69 413.34	-0.5068	
IC20	413.59	7.17	398.26 426.77	-0.2641	
IC25	426.82	6.97	413.91 440.21	-0.0167	
IC40	466.52	7.92	451.11 481.10	0.4894	
IC50	492.98	10.85	476.42 517.76	0.9377	



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKCICR Test Number: 249

Daily Chemistry:

Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	KP	BL	APBL	PBL	APBL	BL
CONTROL, Salt SW	pH (S.U.)	7.88	7.80	7.85	7.71	7.80	7.67
	DO (mg/L)	7.5	7.6	7.9	7.6	7.8	7.3
	Salinity (ppt)	25.0	25.4	25.0	25.1	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	91		— vs. 1	24.1	92	
	Temperature (°C)	25.7	25.5	25.2	26.2	25.1	25.9
250 mg KCl/L	pH (S.U.)	7.89	7.82	7.83	7.73	7.80	7.67
	DO (mg/L)	7.6	7.9	7.9	7.7	7.8	7.2
	Salinity (ppt)	25.1	25.4	25.1	25.5	25.0	25.6
	Temperature (°C)	25.6	25.7	25.1	26.1	25.1	26.1
	375 mg KCl/L	pH (S.U.)	7.89	7.82	7.83	7.74	7.80
DO (mg/L)		7.7	7.9	7.9	7.7	7.8	7.3
Salinity (ppt)		25.2	25.6	25.2	26.0	25.4	25.8
Temperature (°C)		25.6	25.5	25.1	26.0	25.1	26.0
500 mg KCl/L		pH (S.U.)	7.90	7.82	7.85	7.73	7.81
	DO (mg/L)	7.8	7.9	8.0	7.8	7.9	7.5
	Salinity (ppt)	25.3	25.8	25.2	26.0	25.4	25.8
	Temperature (°C)	25.7	25.8	25.1	26.3	25.1	26.3
	750 mg KCl/L	pH (S.U.)	7.90	7.82			
DO (mg/L)		7.8	7.9				
Salinity (ppt)		25.5	25.8				
Temperature (°C)		25.7	25.8				
1000 mg KCl/L		pH (S.U.)	7.91	7.81			
	DO (mg/L)	7.9	7.8				
	Salinity (ppt)	25.6	26.0				
	Temperature (°C)	25.7	25.7				
			Initial	Final	Initial	Final	Initial

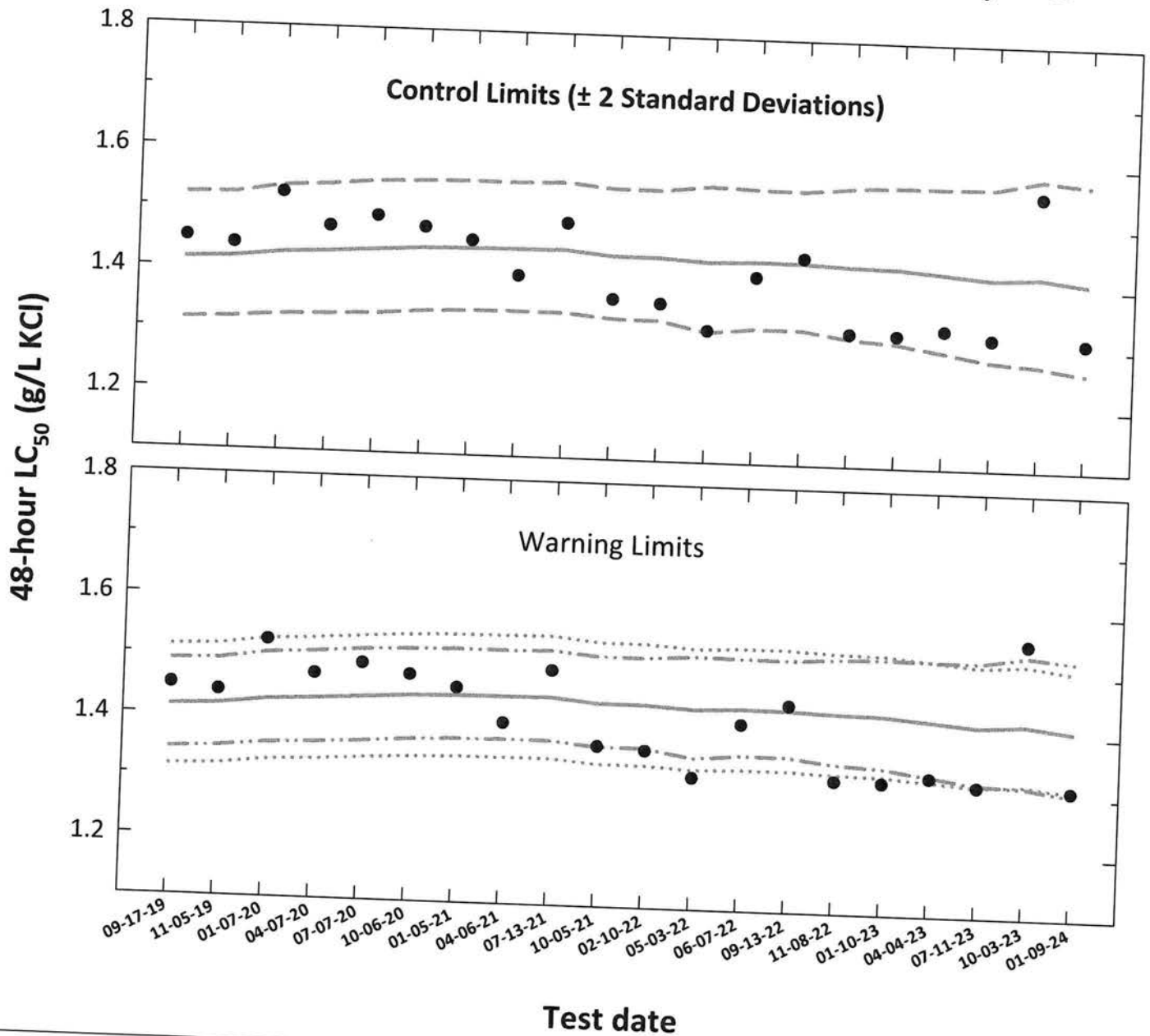
AbKCICR Test Number: 249

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
Analyst	DL	BSL	BSL	BSL	BSL	POBL	POBL	DL	
CONTROL, Salt SW	pH (S.U.)	7.78	7.76	7.90	7.73	7.95	7.74	7.97	7.36
	DO (mg/L)	8.0	7.5	7.7	7.2	7.7	7.7	7.8	6.7
	Salinity (ppt)	24.9	25.3	25.0	25.3	25.2	25.3	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	—	—	85	—	—	—	—	—
	Temperature (°C)	25.2	26.2	25.2	26.1	25.2	26.2	25.2	26.2
250 mg KCl/L	pH (S.U.)	7.77	7.74	7.87	7.76	7.93	7.77	7.96	7.36
	DO (mg/L)	8.0	7.5	7.7	7.2	7.7	7.6	7.8	6.7
	Salinity (ppt)	25.1	25.3	25.2	25.3	25.2	25.6	25.1	25.3
	Temperature (°C)	25.2	26.0	25.1	26.2	25.4	26.2	25.2	26.0
	375 mg KCl/L	pH (S.U.)	7.78	7.65	7.87	7.74	7.93	7.73	7.95
DO (mg/L)		8.0	7.5	7.7	7.2	7.7	7.6	7.9	6.6
Salinity (ppt)		25.2	25.4	25.3	25.4	25.3	25.6	25.5	25.6
Temperature (°C)		25.2	26.2	25.1	26.2	25.4	26.0	25.2	26.0
500 mg KCl/L		pH (S.U.)	7.78	7.76	7.87	7.84	7.93	7.78	7.95
	DO (mg/L)	8.1	7.3	7.7	7.2	7.7	7.6	8.0	6.7
	Salinity (ppt)	25.2	25.6	25.3	25.6	25.3	25.9	25.6	25.9
	Temperature (°C)	25.2	26.1	25.3	26.0	25.3	26.2	25.3	26.0
	750 mg KCl/L	pH (S.U.)							
DO (mg/L)									
Salinity (ppt)									
Temperature (°C)									
1000 mg KCl/L		pH (S.U.)							
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
			Initial	Final	Initial	Final	Initial	Final	Initial

Menidia beryllina

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Menidia beryllina
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	09-17-19	1.4497	0.1504	0.0159	1.4139	1.3143	1.5212	1.3434	1.4898	1.3150	1.5129	
2	11-05-19	1.4402	0.1514	0.0157	1.4172	1.3182	1.5235	1.3474	1.4922	1.3180	1.5164	
3	01-07-20	1.5253	0.1542	0.0162	1.4264	1.3242	1.5365	1.3547	1.5036	1.3266	1.5262	
4	04-07-20	1.4716	0.1554	0.0163	1.4302	1.3269	1.5414	1.3580	1.5079	1.3300	1.5303	
5	07-07-20	1.4906	0.1569	0.0165	1.4351	1.3302	1.5483	1.3620	1.5139	1.3347	1.5356	
6	10-06-20	1.4741	0.1583	0.0162	1.4397	1.3362	1.5513	1.3678	1.5172	1.3390	1.5405	
7	01-05-21	1.4546	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189	1.3411	1.5430	
8	04-06-21	1.3988	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187	1.3415	1.5434	
9	07-13-21	1.4877	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207	1.3424	1.5444	
10	10-05-21	1.3650	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132	1.3353	1.5363	
11	02-10-22	1.3599	0.1570	0.0163	1.4356	1.3320	1.5473	1.3635	1.5134	1.3351	1.5361	
12	05-03-22	1.3180	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182	1.3305	1.5308	
13	06-07-22	1.4081	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169	1.3332	1.5339	
14	09-13-22	1.4415	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163	1.3328	1.5335	
15	11-08-22	1.3190	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205	1.3296	1.5298	
16	01-10-23	1.3180	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227	1.3284	1.5283	
17	04-04-23	1.3283	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213	1.3221	1.5211	
18	07-11-23	1.3153	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209	1.3158	1.5139	
19	10-03-23	1.5515	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331	1.3197	1.5184	
20	01-09-24	1.3113	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256	1.3107	1.5081	

Note: 48-hour LC₅₀ = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
 CT = Central tendency of the LC₅₀ values.
 S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.
Warning Limits = Mean logarithmic LC₅₀ ± 2CV or S_{A,10} converted to anti-logarithmic values.

S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. (S_{A,10} = 0.07).
 CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Menidia beryllina
 EPA-821-R-02-012, Method 2006.0

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 92

Dilution Preparation:

Test concentrations (mg/L KCl)	1000	1250	1500	1750	2000
mL Stock solution	10.0	12.5	15.0	17.5	20.0
mL Dilution water	490.0	487.50	485.0	482.5	480.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2249

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Concentration			
	Analyst	<u>N</u>	<u>JP BL</u>	<u>N JP</u>
	pH (S.U.)	<u>7.06</u>	<u>7.91</u>	<u>7.84</u>
	Dissolved oxygen (mg/L)	<u>7.4</u>	<u>7.6</u>	<u>7.2</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.2</u>	<u>25.3</u>
1000 mg/L	pH (S.U.)	<u>7.07</u>	<u>7.87</u>	<u>7.01</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.6</u>	<u>7.2</u>
	*Salinity (ppt)	<u>25.3</u>	<u>25.9</u>	<u>25.9</u>
	*Temperature (°C)	<u>25.2</u>	<u>25.0</u>	<u>25.3</u>
	1250 mg/L	pH (S.U.)	<u>7.88</u>	<u>7.89</u>
Dissolved oxygen (mg/L)		<u>7.7</u>	<u>7.7</u>	<u>7.3</u>
*Salinity (ppt)		<u>25.7</u>	<u>26.5</u>	<u>26.2</u>
*Temperature (°C)		<u>25.2</u>	<u>25.4</u>	<u>25.3</u>
1500 mg/L		pH (S.U.)	<u>7.91</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.7</u>	<u>7.4</u>
	*Salinity (ppt)	<u>26.1</u>	<u>26.3</u>	<u>26.3</u>
	*Temperature (°C)	<u>25.1</u>	<u>25.1</u>	<u>25.3</u>
	1750 mg/L	pH (S.U.)	<u>7.91</u>	<u>7.90</u>
Dissolved oxygen (mg/L)		<u>7.8</u>	<u>7.8</u>	
*Salinity (ppt)		<u>26.1</u>	<u>26.2</u>	
*Temperature (°C)		<u>25.1</u>	<u>25.1</u>	
2000 mg/L		pH (S.U.)	<u>7.91</u>	<u>7.89</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.9</u>	
	*Salinity (ppt)	<u>26.0</u>	<u>26.4</u>	
	*Temperature (°C)	<u>25.3</u>	<u>25.4</u>	

*Analyst identified for each day, performed pH and dissolved oxygen measurements only. Temperature and salinity performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity performed by the analyst identified on the test specific bench sheet and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	<u>13066468</u>

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 92

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-09-24	1200	JL	1410	JL	1C	Yellow	01-02-24B
24	01-10-24			1405	JL			
48 Termination	01-11-24			1408	JL			

*Test organisms were fed in holding 2 to 5 hours prior to test initiation. Test organisms were not fed during the test.

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch AB):	Mb 12-30-23
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	12-29-23 1200 TO 12-30-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.48 Temperature (°C) 24.5°C

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	7 ^{3d}	7 ^{3d}	1 ^{9d}	2 ^{8d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	9 ^{1d}	10	10	10	6 ^{1d}	6 ^{1d}	1	2	0	0	0	0
Mean Survival	95%		100%		60%		15%		0%		0%	

Comment codes: d = dead, u = unhealthy, s = stressed

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	1214.9
Upper 95% confidence limit (mg KCl/L)	1381.8
48-hour LC ₅₀ (mg KCl/L)	1311.3

Comments:

Test Reviewed by:

Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date: 1/9/2024 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 1/11/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina

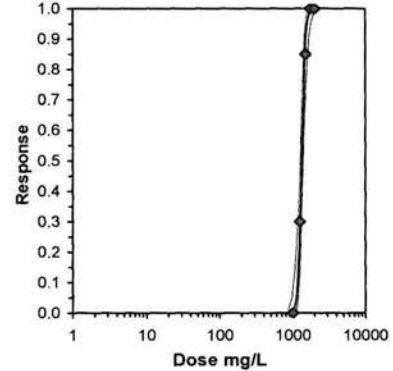
Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.7000	0.7000
1500	0.1000	0.2000
1750	0.0000	0.0000
2000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*1250	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	9.379	2.850	0.1279	6	20
*1500	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	22.716	2.850	0.1279	17	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	27.929	2.850	0.1279	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

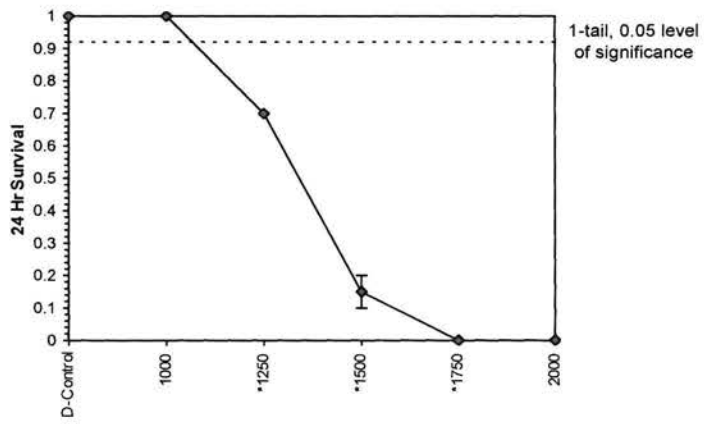
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	1000	1250	1118.03		0.05495	0.05636	0.66792	0.00201	3.0E-06	4, 5
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	21.7203	4.43241	13.0328	30.4078	0	0.29662	7.81472	0.96066	3.12396	0.04604	4
Intercept	-62.853	13.8762	-90.051	-35.656							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1039.58	865.139	1130.96
EC05	3.355	1117.46	972.632	1194.78
EC10	3.718	1161.34	1034.24	1231.5
EC15	3.964	1191.91	1077.26	1257.78
EC20	4.158	1216.78	1112.08	1279.81
EC25	4.326	1238.53	1142.22	1299.73
EC40	4.747	1295.08	1217.75	1355.84
EC50	5.000	1330.34	1261.42	1395.34
EC60	5.253	1366.55	1302.55	1440.5
EC75	5.674	1428.94	1364.67	1529.13
EC80	5.842	1454.49	1387.53	1568.74
EC85	6.036	1484.84	1413.3	1617.76
EC90	6.282	1523.93	1444.82	1683.45
EC95	6.645	1583.76	1490.59	1788.47
EC99	7.326	1702.42	1576.08	2008.93



Dose-Response Plot



Control and
Reviewed by
[Signature]

Statistical Analyses

Acute Silverside Test-48 Hr Survival

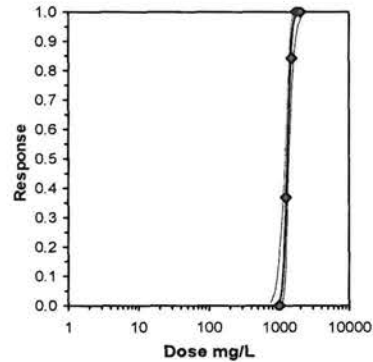
Start Date: 1/9/2024	Test ID: MbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/11/2024	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: MB-Menidia beryllina

Conc-mg/L	1	2
D-Control	0.9000	1.0000
1000	1.0000	1.0000
1250	0.6000	0.6000
1500	0.1000	0.2000
1750	0.0000	0.0000
2000	0.0000	0.0000

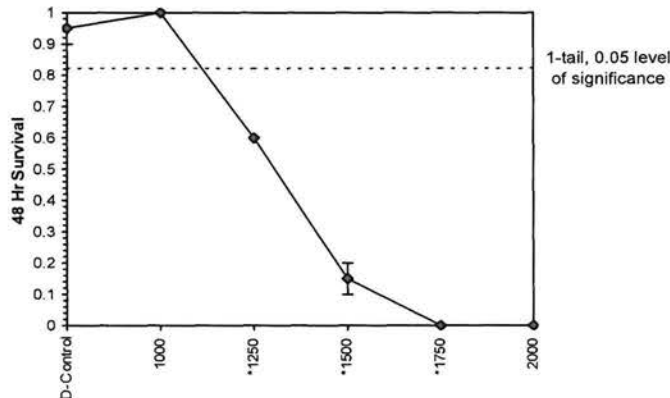
Conc-mg/L	Transform: Arcsin Square Root							1-Tailed			Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
D-Control	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2				1	20
1000	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-1.192	2.850	0.1947	0	20
*1250	0.6000	0.6316	0.8861	0.8861	0.8861	0.000	2	6.504	2.850	0.1947	8	20
*1500	0.1500	0.1579	0.3927	0.3218	0.4636	25.550	2	13.724	2.850	0.1947	17	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	17.148	2.850	0.1947	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	1000	1250	1118.03		0.12097	0.12823	0.617	0.00467	3.0E-05	4, 5

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	20.0815	4.87012	10.5361	29.627	0.05	1.22305	7.81472	0.74748	3.11771	0.0498	12
Intercept	-57.608	15.2556	-87.509	-27.707							
TSCR	0.02622	0.02876	-0.0301	0.08259							
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	1004.3	752.948	1119.14							
EC05	3.355	1085.93	871.371	1183.39							
EC10	3.718	1132.12	941.145	1220.17							
EC15	3.964	1164.39	990.786	1246.34							
EC20	4.158	1190.69	1031.6	1268.15							
EC25	4.326	1213.73	1067.44	1287.77							
EC40	4.747	1273.78	1159.78	1342.75							
EC50	5.000	1311.32	1214.87	1381.78							
EC60	5.253	1349.97	1267.29	1427.87							
EC75	5.674	1416.76	1344.08	1525.19							
EC80	5.842	1444.17	1370.87	1571.3							
EC85	6.036	1476.79	1400.09	1629.91							
EC90	6.282	1518.89	1434.75	1710.34							
EC95	6.645	1583.5	1483.67	1841.89							
EC99	7.326	1712.2	1573.03	2125.94							



Dose-Response Plot

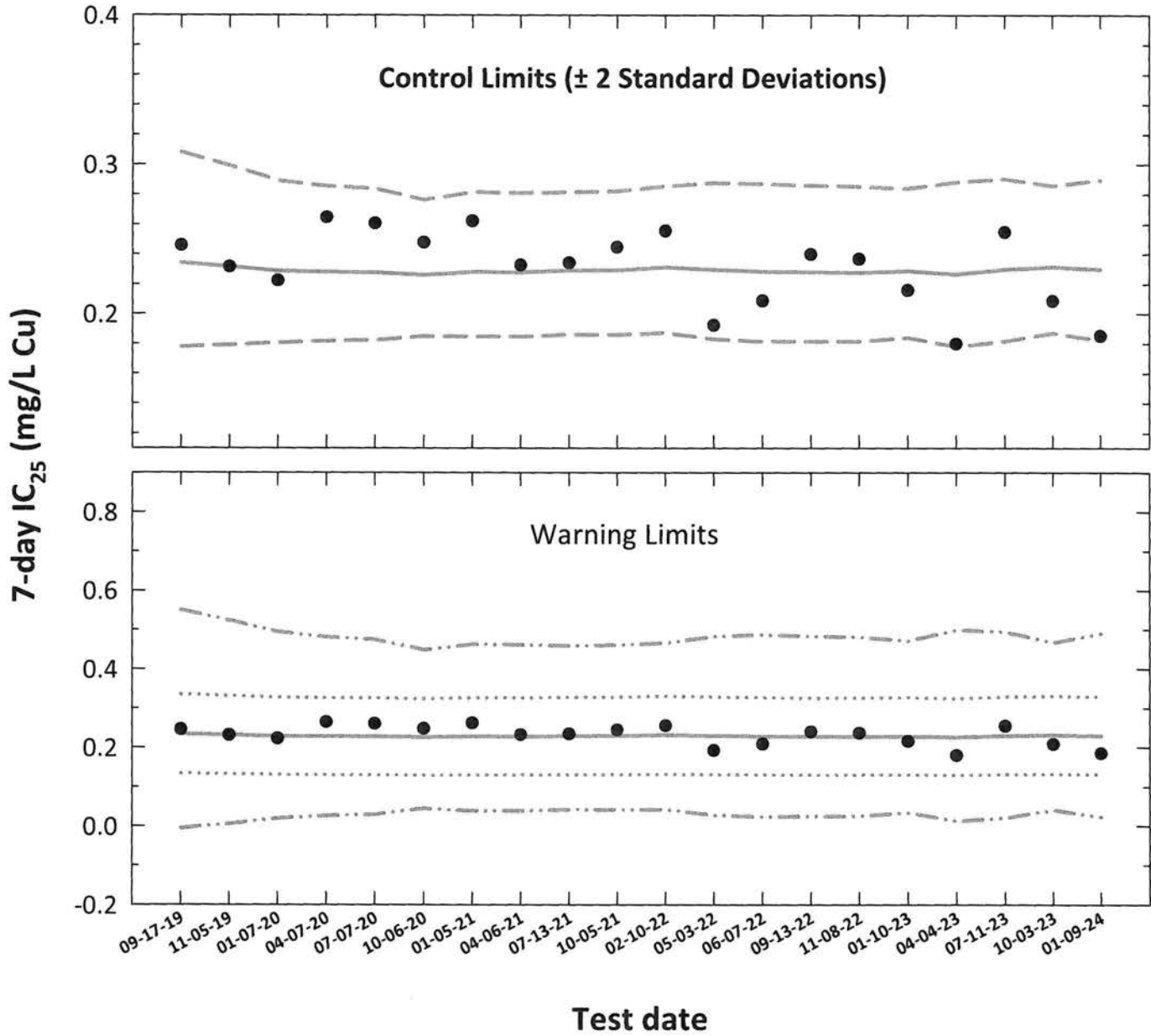


Checked and
Reviewed by
JMS 1/11/2024
jt

Menidia beryllina

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the copper concentration which would cause a 25% reduction in *Menidia* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ ± S_{A,75} converted to anti-logarithmic values, S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

Menidia beryllina

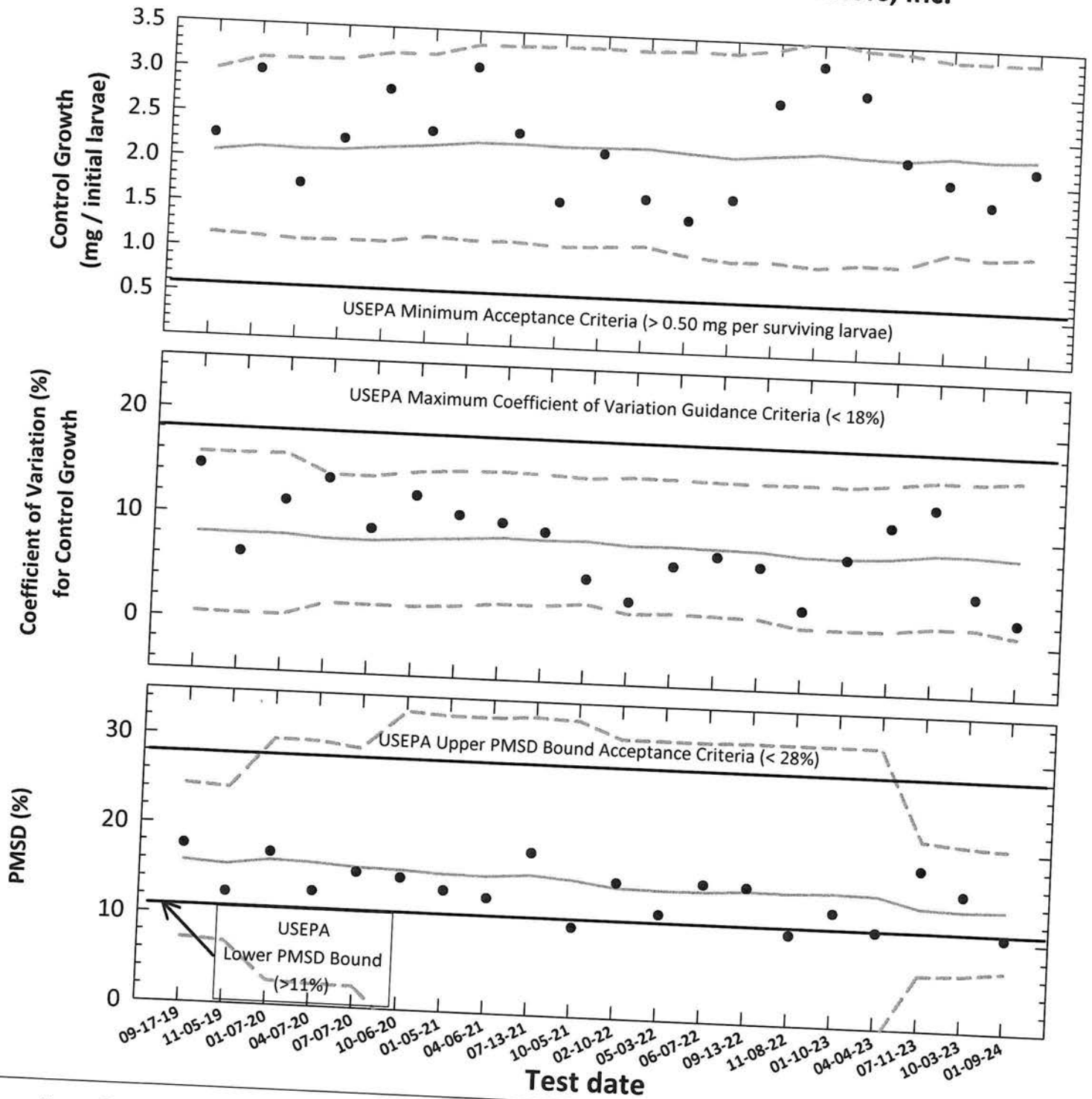
Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion			Anti-logarithmic Values (mg/L Cu)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	09-17-19	0.2458	-0.6094	-0.6304	0.0597	0.2342	0.1779	0.3083	-0.0061	0.5505	0.1335	0.3349
2	11-05-19	0.2315	-0.6354	-0.6352	0.0557	0.2316	0.1792	0.2994	0.0054	0.5240	0.1320	0.3312
3	01-07-20	0.2222	-0.6533	-0.6408	0.0511	0.2286	0.1807	0.2893	0.0189	0.4941	0.1303	0.3270
4	04-07-20	0.2646	-0.5774	-0.6422	0.0490	0.2279	0.1819	0.2856	0.0261	0.4809	0.1299	0.3260
5	07-07-20	0.2606	-0.5840	-0.6429	0.0479	0.2276	0.1825	0.2838	0.0295	0.4745	0.1297	0.3254
6	10-06-20	0.2479	-0.6057	-0.6457	0.0436	0.2261	0.1850	0.2763	0.0443	0.4483	0.1289	0.3233
7	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.2281	0.1848	0.2816	0.0382	0.4625	0.1300	0.3262
8	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.2278	0.1847	0.2810	0.0385	0.4612	0.1298	0.3258
9	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.2290	0.1862	0.2817	0.0420	0.4590	0.1305	0.3274
10	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.2292	0.1861	0.2822	0.0414	0.4604	0.1306	0.3277
11	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.2313	0.1874	0.2855	0.0414	0.4656	0.1318	0.3307
12	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.2296	0.1832	0.2877	0.0276	0.4827	0.1309	0.3283
13	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.2283	0.1815	0.2872	0.0233	0.4861	0.1301	0.3265
14	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.2279	0.1816	0.2861	0.0246	0.4831	0.1299	0.3259
15	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.2276	0.1816	0.2853	0.0254	0.4811	0.1297	0.3255
16	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.2287	0.1841	0.2841	0.0337	0.4708	0.1304	0.3270
17	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.2266	0.1781	0.2884	0.0125	0.4991	0.1292	0.3241
18	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.2298	0.1818	0.2905	0.0208	0.4939	0.1310	0.3286
19	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.2314	0.1873	0.2859	0.0408	0.4669	0.1319	0.3309
20	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.2298	0.1824	0.2896	0.0233	0.4900	0.1310	0.3286

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the copper concentration that would cause a 25% reduction in *Menidia* growth (calculated using ToxCalc).
 CT = Central tendency of the IC₂₅ values.
 S = Standard deviation of the IC₂₅ values.
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.
 S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.43).
 CV = Coefficient of variation.

Menidia beryllina
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: Aquatic Indicators, Inc.



- Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- Central Tendency (mean Control Growth, CV or PMSD)
- - - 95% Confidence Interval (mean Control Growth, CV or PMSD \pm 2 Standard Deviations)

Menidia beryllina
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: Aquatic Indicators, Inc.

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD					
		Control Survival (%)	Control Growth		CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S	CT + 2S				
			Mean (mg/initial larvae)	CV (%)								MSD	PMSD (%)		
1	09-17-19	100	2.259	14.7	0.4004	17.7	2.0666	1.151	2.982	8.1	0.5	15.8	15.8	7.3	24.4
2	11-05-19	100	2.983	6.4	0.3722	12.5	2.125	1.132	3.119	8.1	0.4	15.8	15.8	6.9	24.2
3	01-07-20	100	1.732	11.4	0.2954	17.1	2.112	1.105	3.120	8.1	0.4	15.9	15.9	2.7	29.7
4	04-07-20	100	2.244	13.6	0.2889	12.9	2.123	1.114	3.131	7.8	1.7	14.0	14.0	2.5	29.6
5	07-07-20	100	2.809	9.0	0.4264	15.2	2.166	1.117	3.215	7.8	1.7	14.0	14.0	2.4	29.0
6	10-06-20	100	2.361	12.3	0.3484	14.8	2.205	1.188	3.221	8.1	1.7	14.6	14.6	-2.1	33.3
7	01-05-21	100	3.093	10.6	0.4186	13.5	2.253	1.163	3.343	8.3	1.9	14.8	14.8	-2.3	33.0
8	04-06-21	100	2.377	10.0	0.3062	12.9	2.258	1.167	3.349	8.6	2.2	14.9	14.9	-2.3	33.0
9	07-13-21	100	1.631	9.3	0.2956	18.1	2.247	1.136	3.359	8.5	2.2	14.8	14.8	-2.0	33.3
10	10-05-21	100	2.189	5.0	0.2192	10.0	2.258	1.153	3.363	8.6	2.6	14.7	14.7	-2.5	33.1
11	02-10-22	100	1.701	3.0	0.2583	15.2	2.266	1.180	3.351	8.4	1.8	14.9	14.9	-2.0	31.2
12	05-03-22	100	1.483	6.5	0.1756	11.8	2.232	1.092	3.372	8.4	2.0	14.9	14.9	-2.1	31.2
13	06-07-22	100	1.733	7.6	0.2665	15.4	2.201	1.041	3.361	8.3	1.9	14.8	14.8	-2.1	31.2
14	09-13-22	100	2.822	6.8	0.4290	15.2	2.243	1.057	3.430	8.3	1.8	14.7	14.7	-1.7	31.3
15	11-08-22	100	3.255	2.7	0.3294	10.1	2.280	1.014	3.545	7.9	1.0	14.8	14.8	-1.8	31.3
16	01-10-23	100	2.945	7.8	0.3755	12.8	2.257	1.062	3.453	7.9	1.0	14.8	14.8	-1.4	31.3
17	04-04-23	100	2.220	11.0	0.2389	10.8	2.243	1.053	3.433	8.1	1.1	15.1	15.1	-1.6	31.3
18	07-11-23	100	1.992	12.9	0.3549	17.8	2.287	1.213	3.360	8.5	1.5	15.6	15.6	6.2	21.1
19	10-03-23	100	1.763	4.5	0.2673	15.2	2.270	1.174	3.366	8.5	1.5	15.5	15.5	6.3	20.6
20	01-09-24	100	2.156	2.2	0.2257	10.5	2.287	1.211	3.364	8.4	0.9	15.8	15.8	6.8	20.4

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.50 mg/surviving larvae.

CV = Coefficient of variation for control growth.

USEPA maximum CV guidance criteria (90th percentile) < 18%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) > 11%.

Upper PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

CT = Central tendency of the growth, CV or PMSD values.

S = Standard deviation of the growth, CV or PMSD values.



Copper Sulfate Chronic Reference Toxicant Test (EPA-821-R-02-014, Method 1006.0)

Species: Menidia beryllina

MbCuCR Test Number: 145

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS <u>263</u>				
Stock preparation:		100 mg Cu/L: Dissolve 0.1965 g CuSO ₄ in 500-mL deionized water				
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Test information:

Organism source:	Aquatic Indicators, Inc.	Randomizing template:	<u>ORANGE</u>
Age:	10-days old	Incubator number and shelf location:	<u>1 B</u>
Batch:	Al Mb 12-30-23	Artemia CHM number:	CHM1294
Hatch dates and times:	12-29-23 1200 to	Drying information for weight determination:	
	12-30-23 1130	Date / Time in oven:	<u>01-16-24 1040</u>
Transfer vessel information:	pH (S.U.) = <u>7.48</u>	*Initial oven temperature:	<u>60°C</u>
	Temperature (°C) = <u>24.4°C</u>	Date / Time out of oven:	<u>01-17-24 1040</u>
Average transfer volume (mL):	< 0.25 mL	*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24-HOURS</u>

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	01-09-24	1200	JL	1430	JL	1240	JL	NA	0102-24B
1	01-10-24	0500	JL	1100	JL	0940	JL	↓	↓
2	01-11-24	0500	JL	1100	JL	0942	JL		01-09-24
3	01-12-24	0500	JL	1150	JL	0940	JL		↓
4	01-13-24	0615	JL	1215	JL	1000	JL		01-11-24
5	01-14-24	0600	JL	1100	JL	0940	JL		01-12-24
6	01-15-24	0600	JL	1200	JL	0940	JL		↓
7	01-16-24					1040	JL		

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07</u>	≤ 20%	7-day LC ₅₀ (%)	<u>0.23</u>
Average weight per initial larvae:	<u>2.156</u>		NOEC (%)	<u>0.10</u>
Average weight per surviving larvae:	<u>2.156</u>	≥ 0.25mg/larvae	LOEC (%)	<u>0.20</u>
			ChV (%)	<u>0.14</u>
			IC ₂₅ (%)	<u>0.185</u>

Species: Menidia beryllina

MbCuCR Test Number: 145

Day	Survival and Growth Data												
	CONTROL				0.025 mg/L				0.05 mg/L				
	A	B	C	D	E	F	G	H	I	J	K	L	
0	10	10	10	10	10	10	10	10	10	10	10	10	
1	10	10	10	10	10	10	10	10	10	10	10	10	
2	10	10	10	10	10	10	10	10	10	10	10	10	
3	10	10	10	10	10	10	10	10	10	10	10	10	
4	10	10	10	10	10	10	10	10	10	10	10	10	
5	10	10	10	10	10	10	10	10	10	10	10	10	
6	10	10	10	10	10	10	10	10	10	10	10	10	
7	10	10	10	10	10	10	10	10	10	10	10	10	
*A = Pan weight (mg) Tray color code: <u>purple</u> Analyst: <u>AP</u> Date: <u>12-15-23</u>		12.2524	14.65	14.41	13.33	15.28	14.41	14.60	11.91	12.78	12.92	15.21	13.86
*B = Pan + Larvae weight (mg) Analyst: <u>J</u> Date: <u>01-21-24</u>		34.50	36.43	36.09	35.24	33.92	36.50	38.01	33.81	36.07	35.31	35.92	37.08
C = Larvae weight (mg) = B - A Analyst: <u>J</u>		20.87	21.78	21.68	21.91	18.64	22.09	23.41	21.90	23.29	22.39	20.71	23.22
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>		2.087	2.178	2.168	2.191	1.864	2.209	2.341	2.190	2.329	2.239	2.071	2.322
Average weight per initial number of larvae (mg)		2.156				2.151		0.27		2.240		-3.97	
Percent reduction from control (%)													

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: Menidia beryllina

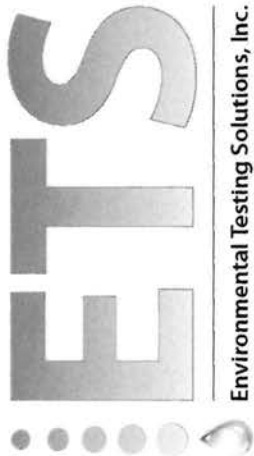
MbCuCR Test Number: 145

Day	Survival and Growth Data											
	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	7 ^{3d}	5 ^{5d}	6 ^{4d}	6 ^{4d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
2	10	10	10	10	7	5	6	6				
3	10	10	10	10	7	5	6	6				
4	10	10	10	10	7	5	6	6				
5	10	10	10	10	7	5	6	6				
6	10	10	10	10	7	5	6	6				
7	10	10	10	10	7	5	6	6				
*A = Pan weight (mg) Tray color code: <u>purple</u> Analyst: <u>JP</u> Date: <u>12-16-23</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>J</u> Date: <u>01-21-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>J</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>												
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		2.101	2.67	1.556	27.87	0	100%			

*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:



Menidia beryllina Chronic Whole Effluent Toxicity Test
EPA-821-R-02-014, Method 1006.0

Quality Control
Verification of Data Entry, Calculations, and Statistical Analyses

Environmental Testing Solutions, Inc.

Test number: 145

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)		B = Pan + Larvae weight (mg)		B - A		Not for Compliance Assessment, Internal Laboratory QC		Weight / initial number of larvae (mg)	Mean survival (%)	Mean weight / initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)	
				Weight / surviving number of larvae (mg)	Surviving number of larvae	Weight / surviving number of larvae (mg)	Surviving number of larvae	Weight / surviving number of larvae (mg)	Surviving number of larvae	Mean weight per surviving number of larvae (%)	Coefficient of variation (%)						
Control	A	10	10	13.63	34.50	20.87	2.087					2.087					
	B	10	10	14.65	36.43	21.78	2.178					2.178					
	C	10	10	14.41	36.09	21.68	2.168		2.156	2.2	100.0	2.156		2.2		Not applicable	
	D	10	10	13.33	35.24	21.91	2.191					2.191					
0.025	E	10	10	15.28	33.92	18.64	1.864					1.864					
	F	10	10	14.41	36.50	22.09	2.209		2.151	9.4	100.0	2.151		9.4		0.2	
	G	10	10	14.60	38.01	23.41	2.341					2.341					
	H	10	10	11.91	33.81	21.90	2.190					2.190					
0.050	I	10	10	12.78	36.07	23.29	2.329					2.329					
	J	10	10	12.92	35.31	22.39	2.239		2.240	5.4	100.0	2.240		5.4		-3.9	
	K	10	10	15.21	35.92	20.71	2.071					2.071					
	L	10	10	13.86	37.08	23.22	2.322					2.322					
0.100	M	10	10	13.77	35.53	21.76	2.176					2.176					
	N	10	10	15.42	36.75	21.33	2.133		2.101	6.7	100.0	2.101		6.7		2.6	
	O	10	10	13.93	32.86	18.93	1.893					1.893					
	P	10	10	14.03	36.04	22.01	2.201					2.201					
0.200	Q	10	7	11.70	29.89	18.19	2.599					1.819					
	R	10	5	15.01	28.80	13.79	2.758		2.600	5.1	60.0	2.600		12.3		27.8	
	S	10	6	13.44	28.03	14.59	2.432					1.459					
	T	10	6	14.17	29.84	15.67	2.612					1.567					
0.500	U	10	0	0.00	0.00	0.00	0.000					0.000					
	V	10	0	0.00	0.00	0.00	0.000		0.000	0.0	0.0	0.000		0.000		100.0	
	W	10	0	0.00	0.00	0.00	0.000					0.000					
	X	10	0	0.00	0.00	0.00	0.000					0.000					

Dunnett's MSD value: $\frac{0.2257}{10.5}$ Minimum Significant Difference
 PMSD: $\frac{0.2257}{10.5}$ Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 11%.
 Upper PMSD bound determined by USEPA (90th percentile) = 28%.
 Lower and upper PMSD bounds were determined from the 10th and 90th percentile of the test method and is not a minimum acceptance criteria.
 The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

USEPA, 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.



Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 1/9/2024 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
 End Date: 1/16/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUSO4
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina

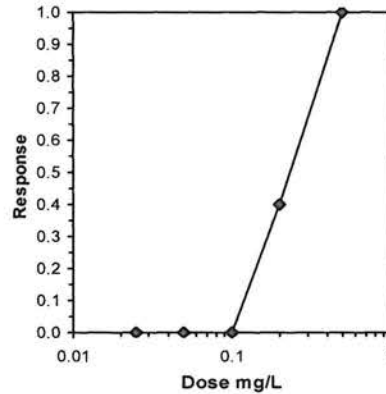
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.025	1.0000	1.0000	1.0000	1.0000
0.05	1.0000	1.0000	1.0000	1.0000
0.1	1.0000	1.0000	1.0000	1.0000
0.2	0.7000	0.5000	0.6000	0.6000
0.5	0.0000	0.0000	0.0000	0.0000

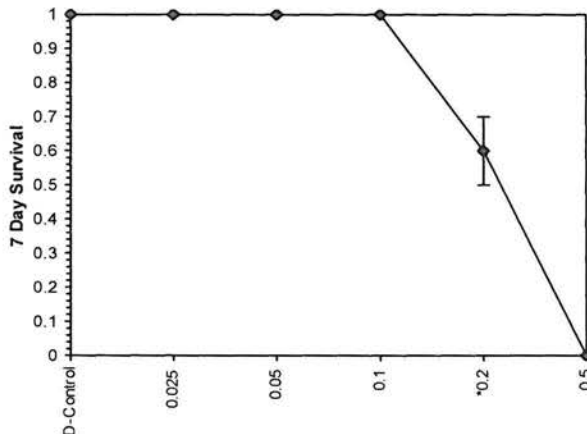
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.2	0.6000	0.6000	0.8872	0.7854	0.9912	9.469	4	10.00	10.00	16	40
0.5	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.45398	0.868	0.10983	9.50298
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Trim Level	Trimmed Spearman-Kärber		
	EC50	95% CL	
0.0%	0.2292	0.2023	0.2596
5.0%	0.2298	0.2000	0.2640
10.0%	0.2303	0.1967	0.2696
20.0%	0.2314	0.1863	0.2875
Auto-0.0%	0.2292	0.2023	0.2596



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

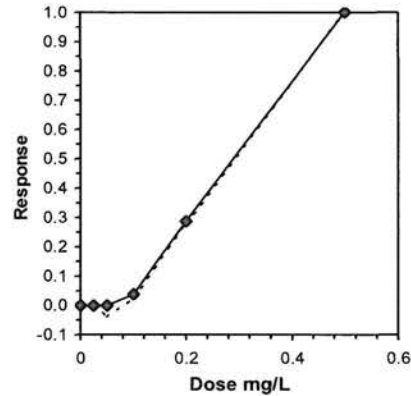
Start Date:	1/9/2024	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	1/16/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2	3	4
D-Control	2.0870	2.1780	2.1680	2.1910
0.025	1.8640	2.2090	2.3410	2.1900
0.05	2.3290	2.2390	2.0710	2.3220
0.1	2.1760	2.1330	1.8930	2.2010
0.2	1.8190	1.3790	1.4590	1.5670
0.5	0.0000	0.0000	0.0000	0.0000

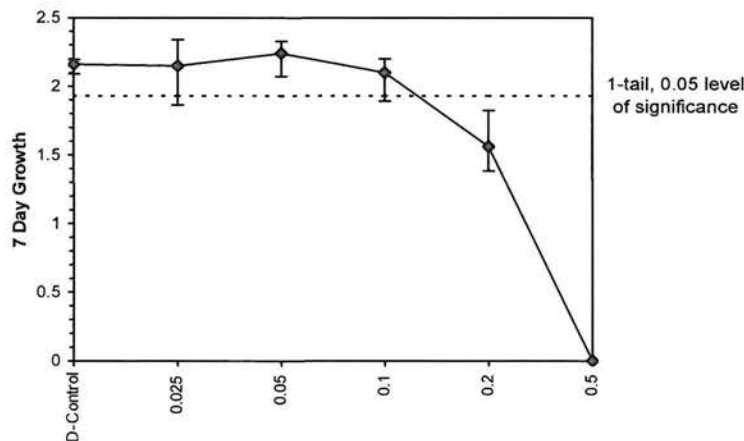
Conc-mg/L	Transform: Untransformed						N	1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%		t-Stat	Critical	MSD	Mean	N-Mean
D-Control	2.1560	1.0000	2.1560	2.0870	2.1910	2.178	4				2.1824	1.0000
0.025	2.1510	0.9977	2.1510	1.8640	2.3410	9.427	4	0.051	2.290	0.2257	2.1824	1.0000
0.05	2.2403	1.0391	2.2403	2.0710	2.3290	5.357	4	-0.855	2.290	0.2257	2.1824	1.0000
0.1	2.1008	0.9744	2.1008	1.8930	2.2010	6.727	4	0.561	2.290	0.2257	2.1008	0.9626
0.2	1.5560	0.7217	1.5560	1.3790	1.8190	12.308	4				1.5560	0.7130
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88246	0.844	-1.0975	0.85072						
Bartlett's Test indicates equal variances (p = 0.21)	4.47106	11.3449								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.1	>0.1			0.22568	0.10467	0.01338	0.01942	0.57619	3, 12
Treatments vs D-Control										

Point	Linear Interpolation (200 Resamples)				
	mg/L	SD	95% CL(Exp)	Skew	
IC05	0.1050	0.0134	0.0572	0.1288	-0.4978
IC10	0.1251	0.0108	0.0875	0.1535	-0.4099
IC15	0.1451	0.0113	0.1115	0.1846	0.4533
IC20	0.1651	0.0129	0.1340	0.2178	0.9355
IC25	0.1852	0.0139	0.1529	0.2371	0.7068
IC40	0.2475	0.0136	0.2043	0.2901	0.1268
IC50	0.2896	0.0113	0.2535	0.3251	0.1268



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Menidia beryllina

MbCuCR Test Number: 145

Daily Chemistry:

Temperature and salinity performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity and chlorine (total residual) performed by the analyst identified on the bench sheet specific for each analysis and transcribed to this bench sheet.

Concentration		Parameter		Day					
				(Analyst identified for each day, performed pH and D.O. measurements only.)					
				0		1		2	
Analyst		J	QOBL	QOBL	N	N	BL		
CONTROL, SaltSW	pH (S.U.)	7.86	7.88	8.04	7.88	7.99	7.69		
	Dissolved oxygen (mg/L)	7.6	7.4	7.8	7.4	7.4	7.3		
	Salinity (ppt)	25.1	25.3	25.0	25.6	25.0	25.2		
	Alkalinity (mg CaCO ₃ /L)	110				110			
	Temperature (°C)	25.0	25.2	24.9	25.0	24.8	25.1		
0.025 mg/L	pH (S.U.)	7.86	7.87	8.00	7.87	8.01	7.68		
	Dissolved oxygen (mg/L)	7.8	7.4	7.6	7.4	7.8	7.9		
	Salinity (ppt)	25.3	25.7	25.0	25.8	25.1	25.4		
	Temperature (°C)	25.2	25.0	25.0	24.8	24.7	25.0		
	0.05 mg/L	pH (S.U.)	7.86	7.87	8.00	7.88	8.02	7.67	
Dissolved oxygen (mg/L)		7.8	7.4	7.6	7.4	7.8	7.4		
Salinity (ppt)		25.6	26.5	25.3	25.8	25.1	25.3		
Temperature (°C)		25.2	25.0	25.0	25.0	24.7	25.0		
0.1 mg/L		pH (S.U.)	7.87	7.88	8.01	7.85	8.02	7.66	
	Dissolved oxygen (mg/L)	7.8	7.5	7.6	7.6	7.8	7.5		
	Salinity (ppt)	25.4	25.8	25.3	25.7	25.1	25.4		
	Temperature (°C)	25.0	25.3	24.9	25.1	24.7	25.2		
	0.2 mg/L	pH (S.U.)	7.88	7.86	8.00	7.84	8.02	7.69	
Dissolved oxygen (mg/L)		7.9	7.4	7.7	7.3	7.8	7.5		
Salinity (ppt)		25.4	26.6	25.3	25.6	25.1	25.4		
Temperature (°C)		25.0	25.3	24.9	25.2	24.8	25.0		
0.5 mg/L		pH (S.U.)	7.86	7.86	8.00				
	Dissolved oxygen (mg/L)	8.0	7.7	7.8					
	Salinity (ppt)	25.4	26.5	25.2					
	Alkalinity (mg CaCO ₃ /L)	NA							
	Temperature (°C)	25.0	25.1	24.9					
		Initial	Final	Initial	Final	Initial	Final		

Species: *Menidia beryllina*

MbCuCR Test Number: 145

		Day							
		(Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
Analyst	BL	JHC / BSL	JHC / BSL	BSL	BSL	JPBL	JPBL	IL	
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.79	7.92	7.97	7.92	8.02	7.99	8.11	7.42
	Dissolved oxygen (mg/L)	7.0	7.6	7.7 (7.6)	7.8	7.7	7.3	7.8	6.9
	Salinity (ppt)	25.1	25.3	24.8	25.3	24.7	25.1	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)			110		110			
	Temperature (°C)	25.0	24.9	24.7	25.1	24.7	25.0	24.7	24.9
0.025 mg/L	pH (S.U.)	7.81	7.88	8.07	7.89	8.11	7.99	8.13	7.52
	Dissolved oxygen (mg/L)	7.0	7.6	7.7	7.8	7.9	7.2	7.8	7.1
	Salinity (ppt)	25.2	25.4	25.2	25.5	24.8	25.0	25.1	25.1
	Temperature (°C)	24.7	25.2	24.7	24.7	24.7	25.0	24.7	24.8
0.05 mg/L	pH (S.U.)	7.80	7.87	8.08	7.90	8.11	7.99	8.14	7.58
	Dissolved oxygen (mg/L)	7.7	7.5	7.8	7.8	7.9	7.3	7.9	7.1
	Salinity (ppt)	25.0	25.4	25.1	25.6	24.8	25.1	25.1	25.3
	Temperature (°C)	24.7	24.8	24.6	24.8	24.6	25.2	24.7	25.0
0.1 mg/L	pH (S.U.)	7.80	7.87	8.08	7.92	8.11	7.99	8.14	7.50
	Dissolved oxygen (mg/L)	7.7	7.5	7.8	7.8	8.0	7.2	7.9	6.9
	Salinity (ppt)	25.1	25.3	25.1	25.5	24.8	24.9	25.1	25.2
	Temperature (°C)	24.6	24.8	24.6	24.8	24.6	24.8	24.7	24.8
0.2 mg/L	pH (S.U.)	7.80	7.88	8.08	7.92	8.11	7.98	8.14	7.60
	Dissolved oxygen (mg/L)	7.7	7.4	7.7	7.8	8.0	7.2	7.7	6.9
	Salinity (ppt)	25.1	25.6	25.0	25.6	24.8	25.0	25.1	25.2
	Temperature (°C)	24.6	24.9	24.6	25.0	24.6	24.7	24.6	24.8
0.5 mg/L	pH (S.U.)								
	Dissolved oxygen (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final