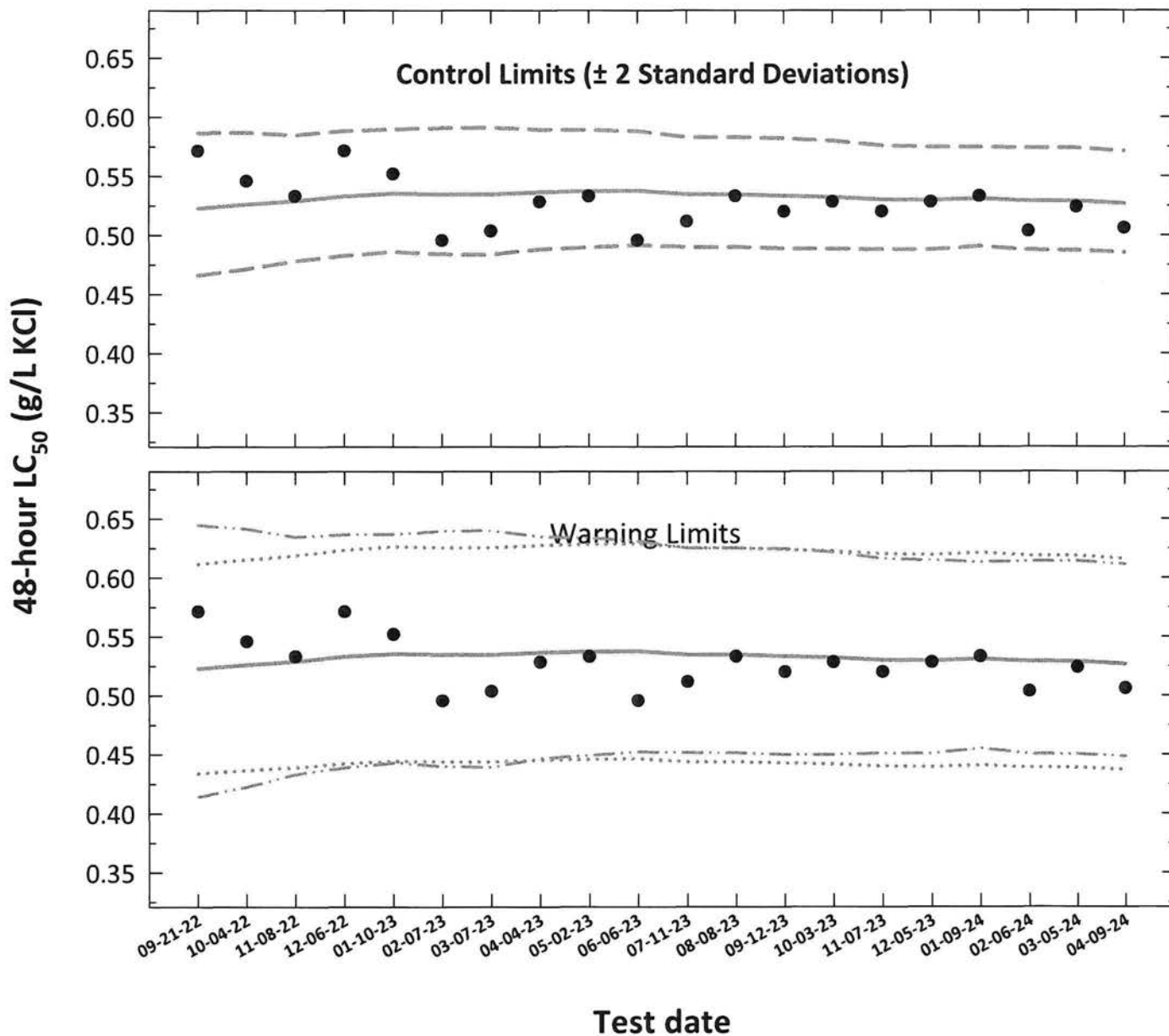


Americamysis (Mysidopsis) bahia Acute Reference Toxicant Control Chart Source: Aquatic Indicators, Inc.



- **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 Warning Limits CT - S _{A,10} CT + S _{A,10}	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853	0.5711	0.4483	0.6113	0.4370	0.6160

Environmental Testing Solutions, Inc.

Calculations in 48 hours (calculated using ToxCalc).

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 # 277

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:

		Hours		
		0	24	48
Control, SaltSW	Analyst	JP N	2P N	N
	pH (S.U.)	7.75	7.48	7.57
	Dissolved oxygen (mg/L)	7.8	7.0	7.4
	*Salinity (ppt)	25.0	25.3	25.3
	*Alkalinity (mg/L CaCO ₃)	75		
	*Temperature (°C)	25.0	25.3	25.5
250 mg/L	pH (S.U.)	7.68	7.67	7.54
	Dissolved oxygen (mg/L)	7.9	7.8	7.3
	*Salinity (ppt)	25.1	25.5	25.5
	*Temperature (°C)	25.2	25.0	25.6
375 mg/L	pH (S.U.)	7.68	7.68	7.53
	Dissolved oxygen (mg/L)	7.9	7.8	7.2
	*Salinity (ppt)	25.4	25.7	25.7
	*Temperature (°C)	25.2	25.2	25.2
500 mg/L	pH (S.U.)	7.69	7.70	7.53
	Dissolved oxygen (mg/L)	8.0	7.8	7.2
	*Salinity (ppt)	25.4	25.6	25.9
	*Temperature (°C)	25.0	25.0	25.5
750 mg/L	pH (S.U.)	7.70	7.69	
	Dissolved oxygen (mg/L)	8.0	7.8	
	*Salinity (ppt)	25.5	25.7	
	*Temperature (°C)	25.0	25.0	
1000 mg/L	pH (S.U.)	7.71	7.69	
	Dissolved oxygen (mg/L)	8.1	7.9	
	*Salinity (ppt)	25.6	26.0	
	*Temperature (°C)	25.0	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	13066461

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 # 277

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-04-24	1045	H	1245	H	1C	WHITE	04-U-24A
24	04-10-24			1242	J			
48 Termination	04-11-24			1242	H			

*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):	AI Ab 04-06-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	04-01-24 1200 TO 04-08-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.76 Temperature (°C): 24.7

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	7 ^{3d}	7 ^{3d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	5 ^{2d}	4 ^{3d}	0	0	0	0
Mean Survival	100%		100%		100%		45%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	468.5
Upper 95% confidence limit (mg KCl/L)	546.7
48-hour LC ₅₀ (mg KCl/L)	506.1

Comments:

Acute Mysid Test-24 Hr Survival

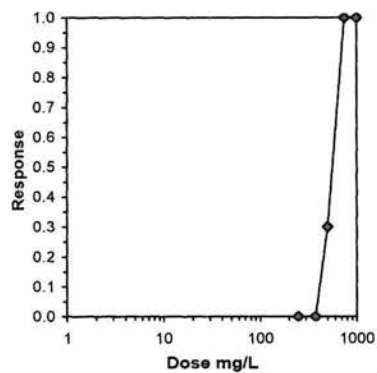
Start Date: 4/9/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/11/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.7000	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

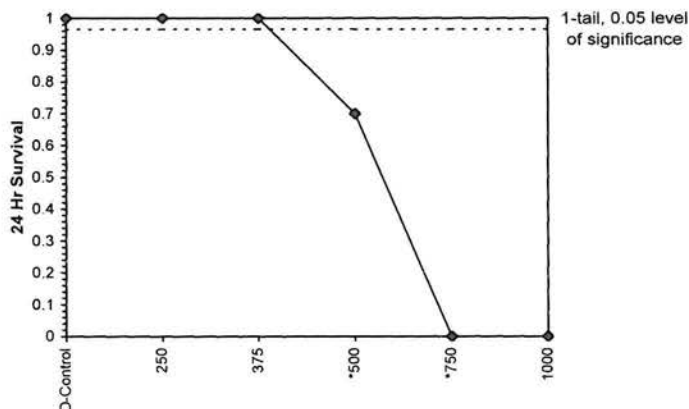
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	42.086	2.850	0.0285	6	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	0.5936	0.0001	2.3E-09	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	551.90	514.06	592.53
5.0%	554.06	511.67	599.96
10.0%	556.11	507.32	609.59
20.0%	559.64	489.18	640.25
Auto-0.0%	551.90	514.06	592.53



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

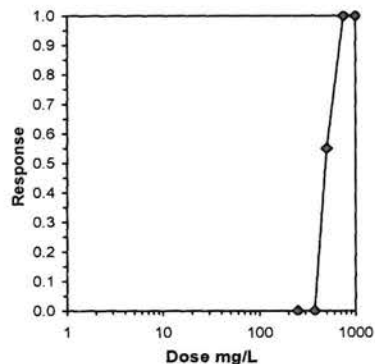
Start Date: 4/9/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/11/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.5000	0.4000
750	0.0000	0.0000
1000	0.0000	0.0000

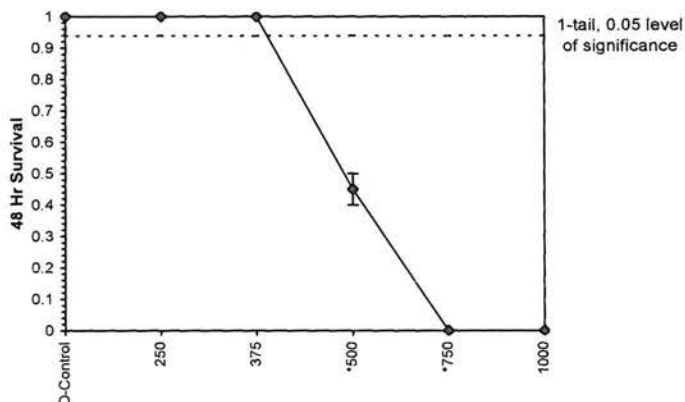
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2					0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907		0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907		0	20
*500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	21.263	2.850	0.0907		11	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	0.64187	0.00101	6.0E-07	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber				
Trim Level	EC50	95% CL		
0.0%	506.10	468.54	546.66	
5.0%	503.74	462.53	548.62	
10.0%	501.39	455.99	551.32	
20.0%	496.78	440.29	560.52	
Auto-0.0%	506.10	468.54	546.66	



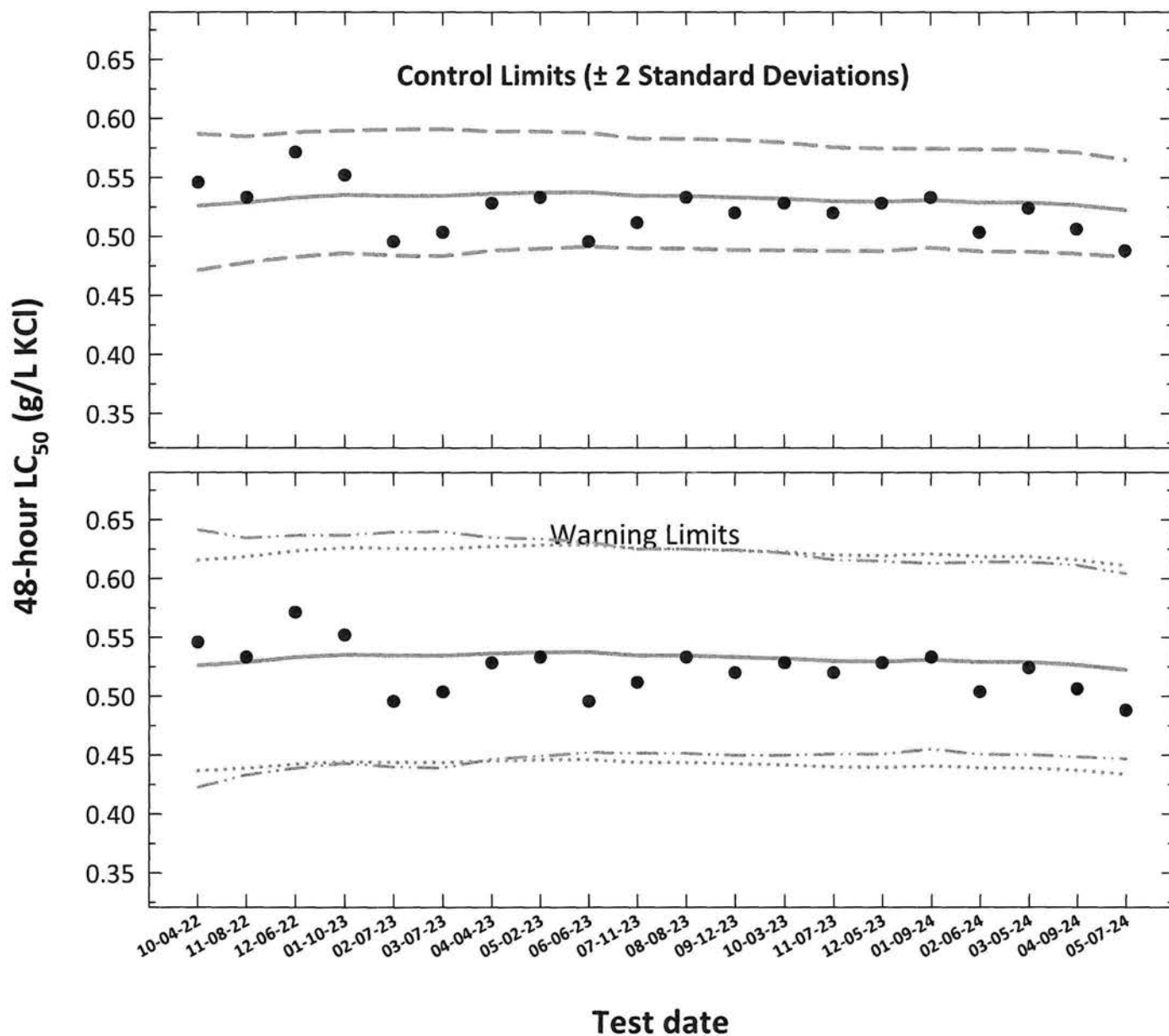
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour LC_{50}** = 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 LC_{50} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A,10}$ 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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415
2	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347
3	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368
4	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369
5	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399	0.6396
6	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392	0.6402
7	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350
8	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338
9	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312
10	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252
11	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250
12	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244
13	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217
14	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161
15	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151
16	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550	0.6131
17	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875	0.5740	0.4505	0.6141
18	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872	0.5738	0.4502	0.6140
19	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853	0.5711	0.4483	0.6113
20	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829	0.5651	0.4467	0.6041

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 # 278

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:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>NP</i>	<i>N</i>	<i>NP</i>
	pH (S.U.)	<i>7.54</i>	<i>7.44</i>	<i>7.40</i>
	Dissolved oxygen (mg/L)	<i>7.7</i>	<i>7.2</i>	<i>7.4</i>
	*Salinity (ppt)	<i>25.0</i>	<i>25.2</i>	<i>25.3</i>
	*Alkalinity (mg/L CaCO ₃)	<i>83</i>		
250 mg/L	*Temperature (°C)	<i>25.2</i>	<i>24.8</i>	<i>24.5</i>
	pH (S.U.)	<i>7.54</i>	<i>7.43</i>	<i>7.44</i>
	Dissolved oxygen (mg/L)	<i>7.7</i>	<i>7.7</i>	<i>7.3</i>
	*Salinity (ppt)	<i>25.2</i>	<i>25.4</i>	<i>25.9</i>
	*Temperature (°C)	<i>25.0</i>	<i>24.9</i>	<i>24.7</i>
375 mg/L	pH (S.U.)	<i>7.55</i>	<i>7.43</i>	<i>7.45</i>
	Dissolved oxygen (mg/L)	<i>7.7</i>	<i>7.7</i>	<i>7.3</i>
	*Salinity (ppt)	<i>25.2</i>	<i>25.4</i>	<i>26.0</i>
	*Temperature (°C)	<i>25.1</i>	<i>24.9</i>	<i>24.3</i>
500 mg/L	pH (S.U.)	<i>7.57</i>	<i>7.42</i>	<i>7.45</i>
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>7.7</i>	<i>7.5</i>
	*Salinity (ppt)	<i>25.3</i>	<i>25.5</i>	<i>25.9</i>
	*Temperature (°C)	<i>25.1</i>	<i>25.1</i>	<i>24.3</i>
750 mg/L	pH (S.U.)	<i>7.59</i>	<i>7.43</i>	
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>7.8</i>	
	*Salinity (ppt)	<i>25.4</i>	<i>25.7</i>	
	*Temperature (°C)	<i>25.1</i>	<i>25.1</i>	
1000 mg/L	pH (S.U.)	<i>7.61</i>	<i>7.42</i>	
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.8</i>	
	*Salinity (ppt)	<i>25.6</i>	<i>25.8</i>	
	*Temperature (°C)	<i>25.0</i>	<i>24.8</i>	

*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 # 278

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05-07-24	1045	JL	1255	JL	05-07-24 21F	PINK	05-07-24A
24	05-08-24			1253	JL			
48 Termination	05-09-24			1255	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):	05-06-24
Age (1 to 5 days old):	2-3 DAYS
Date organisms were born:	05-05-24 1200 TO 05-06-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.85 Temperature (°C): 24.4

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 ^u	8 ^u	0 ^u	0 ^u	0 ^u	0 ^u
48 Termination	10	10	10	10	9 ^u	10	5 ^u	4 ^u	0	0	0	0
Mean Survival	100%		100%		95%		45%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	452.2
Upper 95% confidence limit (mg KCl/L)	537.7
48-hour LC ₅₀ (mg KCl/L)	487.9

Comments:

Acute Mysid Test-24 Hr Survival

Start Date: 5/7/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 5/9/2024 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	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

Transform: Arcsin Square Root								1-Tailed	Number	Total		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
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

Normality of the data set cannot be confirmed

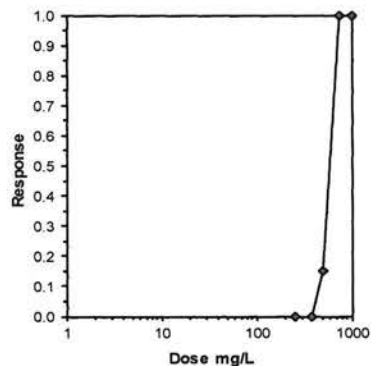
Equality of variance cannot be confirmed

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

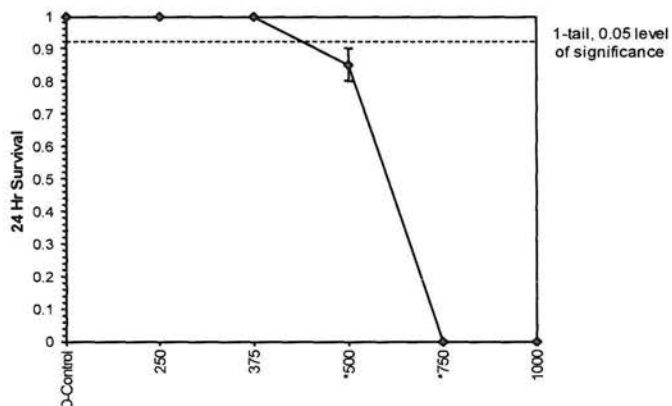
Treatments vs D-Control

Trimmed Spearman-Kärber

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



Acute Mysid Test-48 Hr Survival

Start Date: 5/7/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 5/9/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

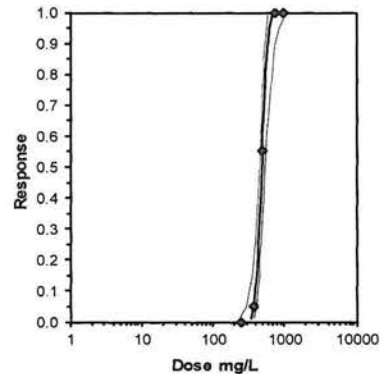
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	1.0000
500	0.5000	0.4000
750	0.0000	0.0000
1000	0.0000	0.0000

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	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	11.175	2.850	0.1726	11	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

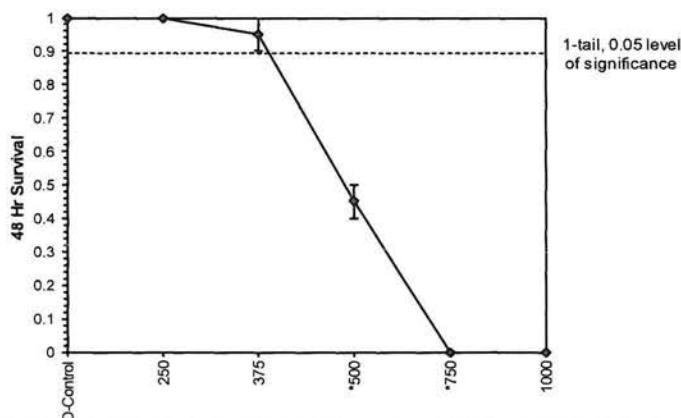
Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.08088	0.08295	0.61307	0.00367	1.7E-05	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	14.8727	3.75808	7.50681 22.2385	0	0.08234	7.81472	0.99387	2.68834	0.06724	3
Intercept	-34.983	10.0528	-54.686 -15.279							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	340.351	242.9 385.416
EC05	3.355	378.224	297.39 416.351
EC10	3.718	400.107	330.403 434.985
EC15	3.964	415.583	354.014 448.925
EC20	4.158	428.308	373.293 461.165
EC25	4.326	439.535	389.946 472.803
EC40	4.747	469.148	430.366 509.197
EC50	5.000	487.915	452.169 537.73
EC60	5.253	507.433	471.728 571.893
EC75	5.674	541.621	500.734 640.38
EC80	5.842	555.819	511.557 671.321
EC85	6.036	572.838	523.936 709.999
EC90	6.282	594.994	539.343 762.687
EC95	6.645	629.42	562.177 849.319
EC99	7.326	699.459	606.016 1042.05



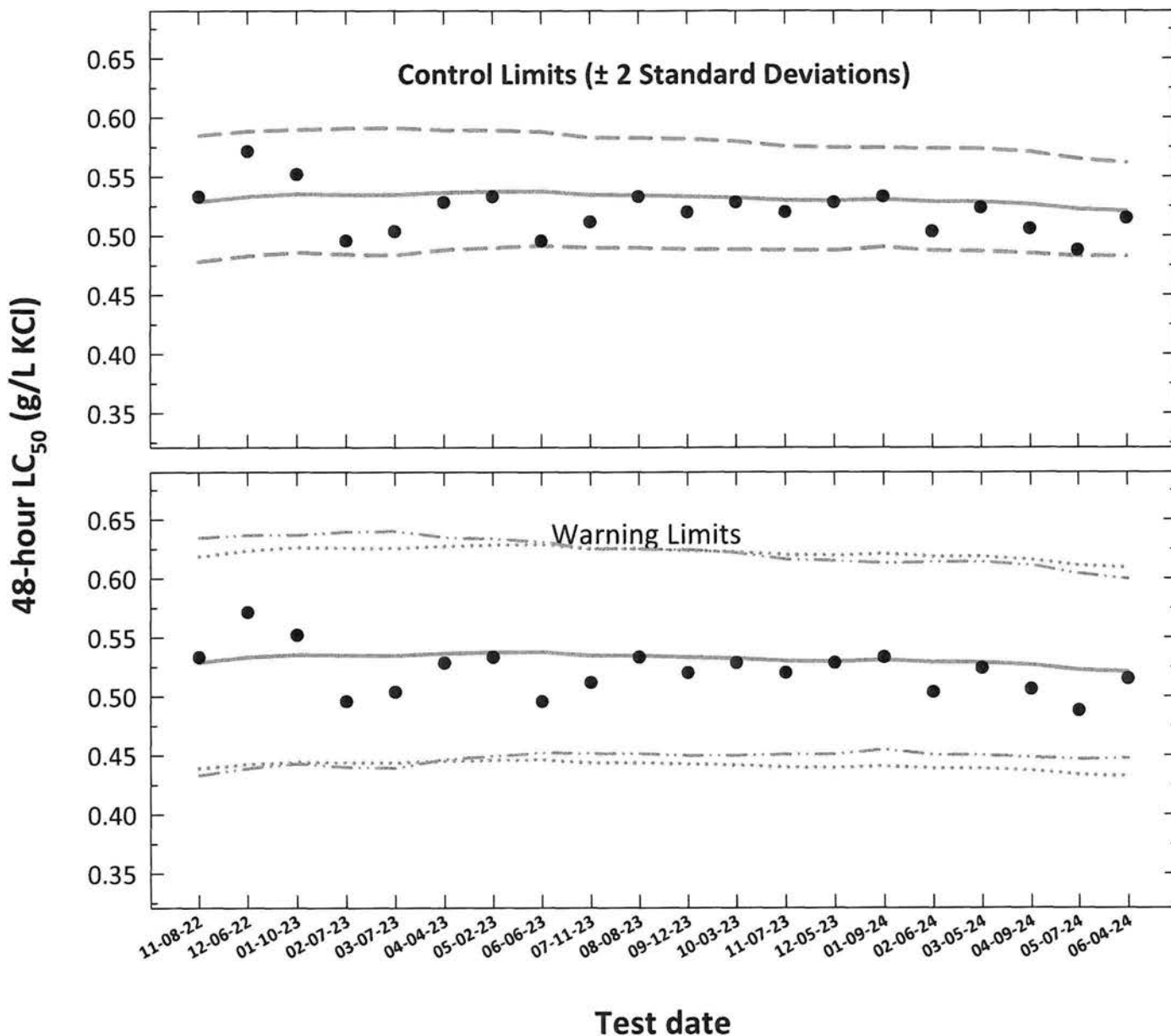
Dose-Response Plot



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		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853	0.5711	0.4483	0.6113	0.4370	0.6160
19	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829	0.5651	0.4467	0.6041	0.4335	0.6111
20	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4827	0.5620	0.4476	0.5998	0.4323	0.6094

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 # 279

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 #: 2288

Chemical Analyses:

		Hours		
		0	24 h	48 h
Control, SaltSW	Analyst	<u>JP</u>	<u>JP</u>	<u>JP</u>
	pH (S.U.)	<u>7.71</u>	<u>7.55</u>	<u>7.32</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.9</u>	<u>7.6</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.2</u>	<u>25.3</u>
	*Alkalinity (mg/L CaCO ₃)	<u>72</u>		
	*Temperature (°C)	<u>25.2</u>	<u>25.0</u>	<u>25.0</u>
250 mg/L	pH (S.U.)	<u>7.88</u>	<u>7.55</u>	<u>7.33</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.9</u>	<u>7.6</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.2</u>	<u>25.6</u>
	*Temperature (°C)	<u>25.0</u>	<u>25.2</u>	<u>24.9</u>
375 mg/L	pH (S.U.)	<u>7.37</u>	<u>7.55</u>	<u>7.33</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.9</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.1</u>	<u>25.2</u>	<u>25.6</u>
	*Temperature (°C)	<u>25.0</u>	<u>25.2</u>	<u>24.9</u>
500 mg/L	pH (S.U.)	<u>7.37</u>	<u>7.56</u>	<u>7.33</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.9</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.2</u>	<u>25.3</u>	<u>25.2</u>
	*Temperature (°C)	<u>25.2</u>	<u>25.1</u>	<u>25.2</u>
750 mg/L	pH (S.U.)	<u>7.36</u>	<u>7.55</u>	
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.8</u>	
	*Salinity (ppt)	<u>25.3</u>	<u>25.3</u>	
	*Temperature (°C)	<u>25.2</u>	<u>25.3</u>	
1000 mg/L	pH (S.U.)	<u>7.37</u>	<u>7.54</u>	
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.8</u>	
	*Salinity (ppt)	<u>25.4</u>	<u>25.5</u>	
	*Temperature (°C)	<u>25.2</u>	<u>25.0</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+ 8-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	<u>13066468</u>

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 # 279

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	06-04-21	* 1110	J	1313	J	1D	WHITE	05-29-21 B
24	06-05-21			1322	J			
48 Termination	06-06-21			1324	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):	AI Ab 06-03-21
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	06-02-21 1100 TO 06-03-21 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.77
	Temperature (°C) 24.6

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	6 ^d	7 ^d	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	5 ^{cd}	5 ^{2d}	0	0	0	0
Mean Survival	100%		100%		100%		50%		0%		0%	

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

Statistics:

Method	SW
Lower 95% confidence limit (mg KCl/L)	476.5
Upper 95% confidence limit (mg KCl/L)	556.4
48-hour LC ₅₀ (mg KCl/L)	514.9

Comments:

Start Date:	6/4/2024	Test ID:	AbKCIAC	Sample ID:	REF-RefToxicant
End Date:	6/6/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanmysis bahia
Comments:					

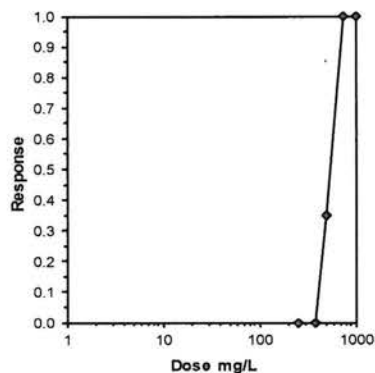
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0947	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0947	0	20
*500	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	14.247	2.850	0.0947	7	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	37.715	2.850	0.0947	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Kurt

Hypothesis Test (1-tail, 0.05)	NO
--------------------------------	----

Treatments vs D-Control

Trim Level	EC50	95%CL	
0.0%	542.42	503.77	584.04
5.0%	543.63	500.55	590.41
10.0%	544.81	495.80	598.66
20.0%	547.01	478.84	624.87
Auto-0.0%	542.42	503.77	584.04



Dose (mg/kg)	24 Hr Survival
Control	1.0
250	1.0
375	1.0
500	~0.65
750	0.0
1000	0.0

Acute Mysid Test-48 Hr Survival

Start Date: 6/4/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 6/6/2024 Lab ID: ETS-Envr. 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	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

Transform: Arcsin Square Root								1-Tailed	Number	Total		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
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	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	62.662	2.850	0.0285	10	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

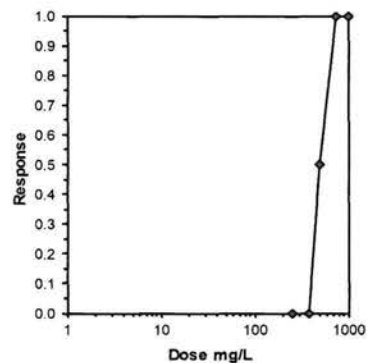
Equality of variance cannot be confirmed

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	0.62824	0.0001	2.0E-09	4, 5

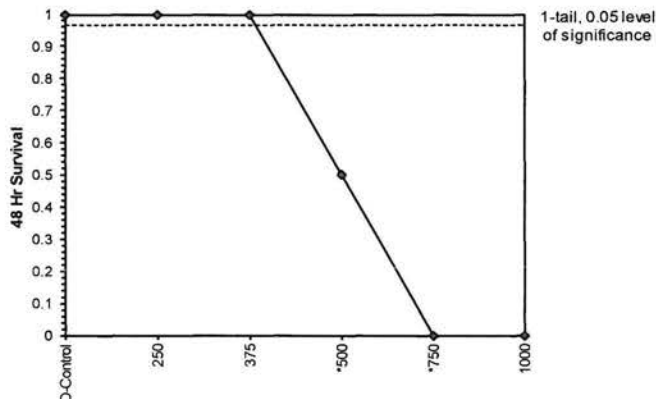
Treatments vs D-Control

Trimmed Spearman-Kärber

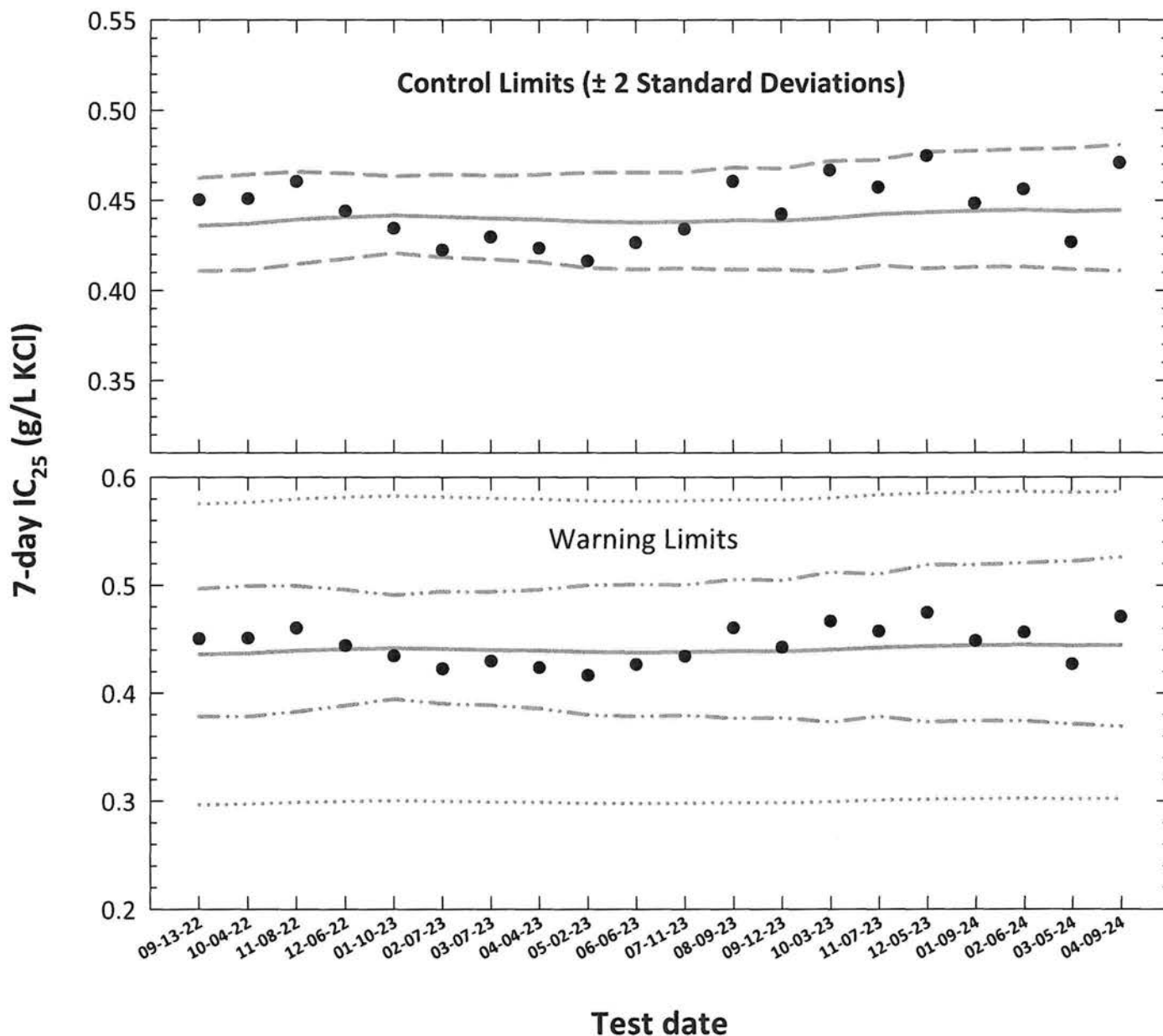
Trim Level	EC50	95% CL
0.0%	514.94	476.54 556.43
5.0%	513.43	471.14 559.51
10.0%	511.92	464.96 563.62
20.0%	508.91	448.82 577.05
Auto-0.0%	514.94	476.54 556.43



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 Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109	0.4808	0.3690	0.5261	0.3022	0.5867

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.

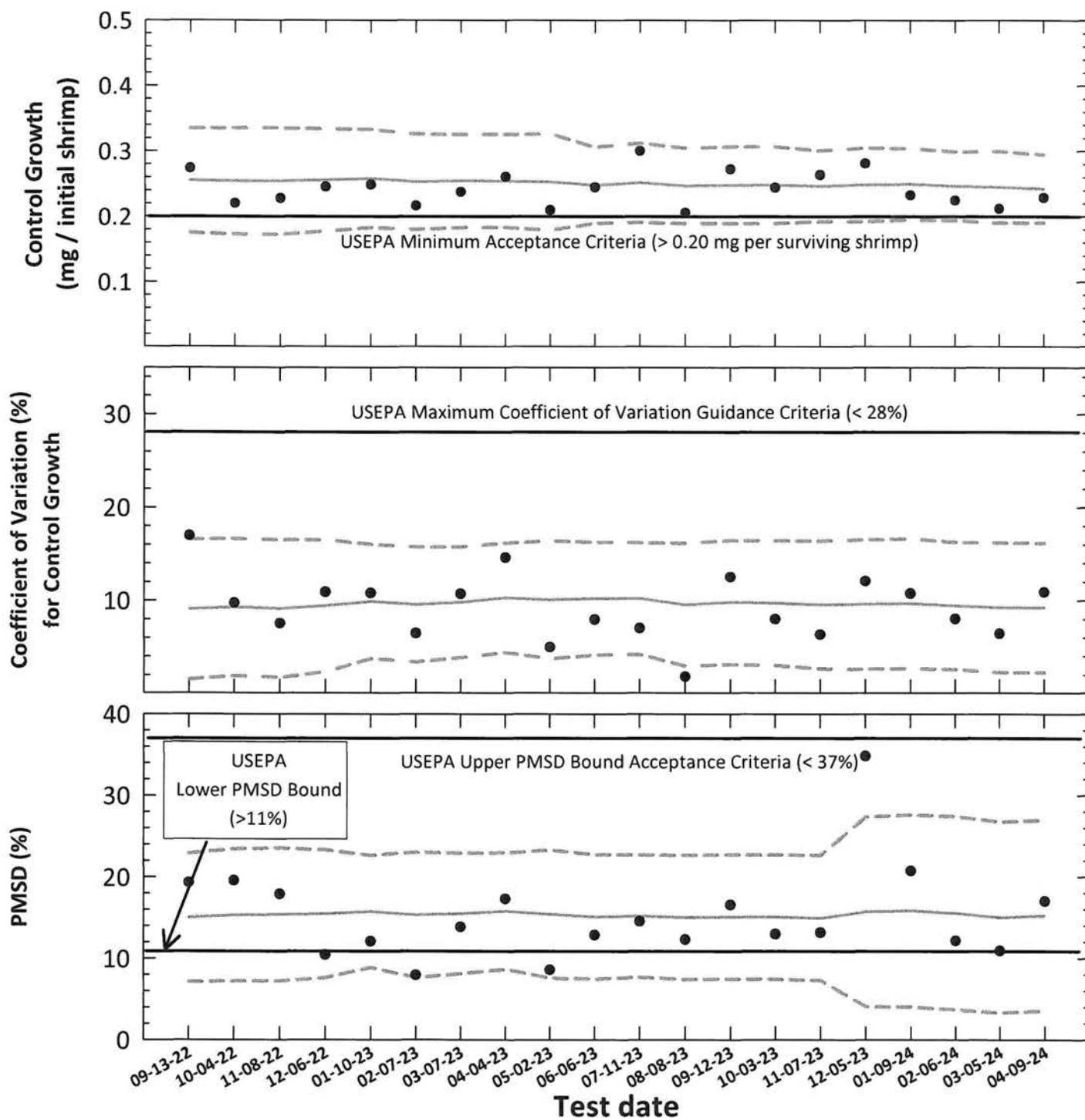
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	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	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial shrimp)	CV (%)													
1	09-13-22	100	0.274	17.0	0.0529	0.255	0.175	0.335	0.255	0.175	0.335	9.1	1.6	16.6	15.0	7.1	22.9
2	10-04-22	100	0.220	9.7	0.0430	0.254	0.173	0.335	0.254	0.173	0.335	9.3	1.9	16.7	15.3	7.2	23.4
3	11-08-22	100	0.228	7.5	0.0407	0.254	0.173	0.335	0.254	0.173	0.335	9.1	1.7	16.5	15.4	7.2	23.5
4	12-06-22	100	0.246	10.9	0.0256	0.256	0.177	0.334	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3
5	01-10-23	100	0.249	10.8	0.0300	0.258	0.183	0.333	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6
6	02-07-23	100	0.217	6.5	0.0172	0.253	0.180	0.326	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0
7	03-07-23	100	0.238	10.7	0.0329	0.254	0.183	0.326	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9
8	04-04-23	100	0.261	14.6	0.0450	0.254	0.183	0.325	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
9	05-02-23	100	0.210	5.0	0.0180	0.253	0.179	0.327	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
10	06-06-23	100	0.245	8.0	0.0314	0.248	0.189	0.306	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
11	07-11-23	100	0.300	7.0	0.0437	0.252	0.192	0.313	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
12	08-08-23	100	0.206	1.8	0.0253	0.247	0.189	0.305	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
13	09-12-23	100	0.273	12.5	0.0451	0.248	0.189	0.307	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
14	10-03-23	100	0.244	8.0	0.0317	0.248	0.190	0.307	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
15	11-07-23	100	0.264	6.3	0.0347	0.247	0.192	0.301	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
16	12-05-23	100	0.282	12.1	0.0981	0.249	0.192	0.305	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
17	01-09-24	100	0.233	10.8	0.0482	0.250	0.195	0.304	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6
18	02-06-24	100	0.225	8.0	0.0273	0.247	0.194	0.299	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4
19	03-05-24	100	0.212	6.5	0.0232	0.245	0.190	0.300	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8
20	04-09-24	100	0.229	10.9	0.0389	0.243	0.190	0.295	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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) > 11%.

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%.

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: 250

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:	04-02-24 1200 to 04-03-24 1130	Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 04-03-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 7.57 S.U. Temperature = 25.0 °C		Date / Time in oven:	04-16-24 1105
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	04-17-24 1105
		*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	04-09-24	1045	JL	1330	JL	1200	JL	04-01-24 A
1	04-10-24	0500	JL	1100	JL	0915	JL	↓
2	04-11-24	0500	JL	1300	JL	1230	JL	04-08-24
3	04-12-24	0500	JL	1100	JL	0913	JL	↓
4	04-13-24	0615	JL	1215	JL	0945	JL	04-10-24
5	04-14-24	0600	JL	1200	JL	0920	JL	04-13-24
6	04-15-24	0500	JL	1100	JL	0910	JL	↓
7	04-16-24					1000	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	130664665

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	01.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	537.7
Average weight per initial shrimp:	0.229		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.229	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	506
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	470.8

AbKCICR Test Number: **250**

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>Fresh Green</u> Analyst: <u>BL</u> Date: <u>03-18-24</u>	13.20	14.73	13.70	15.63	14.10	14.50	13.06	13.56	13.92	15.00	14.72	13.92	14.10	12.78	12.78	15.59
*B = Pan + Shrimp weight (mg) Analyst: <u>SL</u> Date: <u>04-18-24</u>	14.45	15.75	14.82	16.77	15.50	15.59	14.14	14.61	15.23	16.46	15.94	15.39	15.62	14.01	14.41	16.88
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.25	1.02	1.12	1.14	1.40	1.09	1.08	1.05	1.35	1.46	1.22	1.47	1.52	1.23	1.63	1.29
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.250	0.204	0.224	0.228	0.280	0.218	0.216	0.210	0.270	0.292	0.244	0.294	0.304	0.246	0.326	0.258
Average weight per initial number of shrimp (mg) 0.229								Average weight per initial number of shrimp (mg) 0.279				Percent reduction from control (%) -22.1%				

*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: **250**

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	4 ^{1d}	4 ^{1d}	4 ^{1d}	S	S
2	S	S	S	S	S	S	S	S	S	S	S	4	4	4	S	S
3	S	S	S	S	S	S	S	S	S	S	4 ^{1d}	4	4	4	3 ^{2d}	4 ^{1d}
4	S	S	S	S	S	S	S	S	3 ^{2d}	4 ^{1d}	4	3 ^{1d}	3 ^{1d}	3 ^{1d}	3	4
5	S	S	S	S	S	S	S	S	3	4	4	3	3	3	3	3 ^{1d}
6	S	S	S	S	S	S	S	S	3	4	4	3	3	3	3	3
7	S	S	S	S	S	S	S	S	3	3 ^{1d}	4	3	3	3	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>Forest Green</u> Analyst: <u>PL</u> Date: <u>03-18-24</u>	15.01	14.58 <u>15.23</u> 031824	14.89	14.14	14.55	12.90	14.30	14.48	14.23	14.54	13.82	14.46	12.32	14.63	14.98	15.72
*B = Pan + Shrimp weight (mg) Analyst: <u>PL</u> Date: <u>04-18-24</u>	16.47	15.87	16.33	15.27	15.77	14.26	15.66	15.54	15.12	15.37	14.86	15.29	13.13	15.52	15.91	16.38
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>AP</u>	1.46	1.29	1.44	1.13	1.22	1.36	1.36	1.06	0.89	0.83	1.04	0.83	0.81	0.89	0.93	0.66
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>AP</u>	0.292	0.258	0.288	0.226	0.244	0.272	0.272	0.212	0.178	0.166	0.208	0.166	0.162	0.178	0.186	0.132
Average weight per initial number of shrimp (mg) 0.258				Percent reduction from control (%) -12.8%				Average weight per initial number of shrimp (mg) 0.172				Percent reduction from control (%) 24.8%				

*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: **250**

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	VV
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: _____																
Average weight per initial number of shrimp (mg)		Percent reduction from control (%)		Average weight per initial number of shrimp (mg)		Percent reduction from control (%)										
0		100%		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:

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: 250

Test dates: April 09-16, 2024

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	13.20	14.45	1.25	0.250	100.0	0.229	10.9	Not applicable
	B	5	5	14.73	15.75	1.02	0.204				
	C	5	5	13.70	14.82	1.12	0.224				
	D	5	5	15.63	16.77	1.14	0.228				
	E	5	5	14.10	15.50	1.40	0.280				
	F	5	5	14.50	15.59	1.09	0.218				
	G	5	5	13.06	14.14	1.08	0.216				
	H	5	5	13.56	14.61	1.05	0.210				
250	I	5	5	13.92	15.27	1.35	0.270	100.0	0.279	10.5	-22.1
	J	5	5	15.00	16.46	1.46	0.292				
	K	5	5	14.72	15.94	1.22	0.244				
	L	5	5	13.92	15.39	1.47	0.294				
	M	5	5	14.10	15.62	1.52	0.304				
	N	5	5	12.78	14.01	1.23	0.246				
	O	5	5	12.78	14.41	1.63	0.326				
	P	5	5	15.59	16.88	1.29	0.258				
375	Q	5	5	15.01	16.47	1.46	0.292	100.0	0.258	11.1	-12.8
	R	5	5	14.58	15.87	1.29	0.258				
	S	5	5	14.89	16.33	1.44	0.288				
	T	5	5	14.14	15.27	1.13	0.226				
	U	5	5	14.55	15.77	1.22	0.244				
	V	5	5	12.90	14.26	1.36	0.272				
	W	5	5	14.30	15.66	1.36	0.272				
	X	5	5	14.48	15.54	1.06	0.212				
500	Y	5	3	14.23	15.12	0.89	0.178	62.5	0.172	12.7	24.8
	Z	5	3	14.54	15.37	0.83	0.166				
	AA	5	4	13.82	14.86	1.04	0.208				
	BB	5	3	14.46	15.29	0.83	0.166				
	CC	5	3	12.32	13.13	0.81	0.162				
	DD	5	3	14.63	15.52	0.89	0.178				
	EE	5	3	14.98	15.91	0.93	0.186				
	FF	5	3	15.72	16.38	0.66	0.132				
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: 0.0389
PMSD: 17.0

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: 4/9/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 4/16/2024 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:

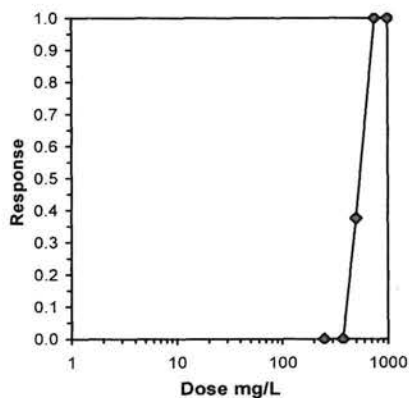
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.6000	0.8000	0.6000	0.6000	0.6000	0.6000	0.6000
750	0.0000	0.0000	0.0000	0.0000	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				N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%					
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	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.6250	0.6250	0.9137	0.8861	1.1071	8.554	8	36.00	48.00	15	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

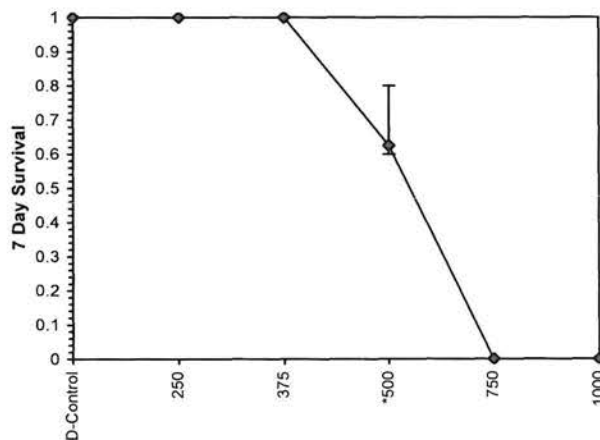
Auxiliary Tests **Statistic** **Critical** **Skew** **Kurt**
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$) 0.37758 0.904 4.76175 25.5788
Equality of variance cannot be confirmed

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

Trimmed Spearman-Kärber				
Trim Level	EC50	95% CL		
0.0%	537.74	509.95	567.04	
5.0%	538.48	507.54	571.30	
10.0%	539.21	504.12	576.74	
20.0%	540.60	492.31	593.63	
Auto-0.0%	537.74	509.95	567.04	



Dose-Response Plot



Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

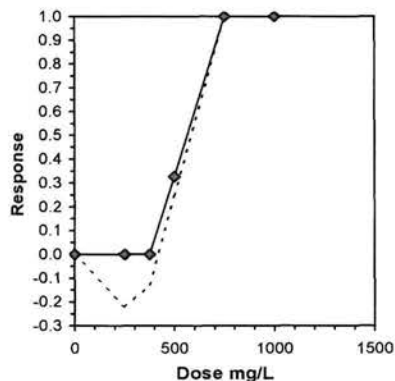
Start Date: 4/9/2024 Test ID: AbKClCR Sample ID: REF-Ref Toxicant
End Date: 4/16/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2500	0.2040	0.2240	0.2280	0.2800	0.2180	0.2160	0.2100
250	0.2700	0.2920	0.2440	0.2940	0.3040	0.2460	0.3260	0.2580
375	0.2920	0.2580	0.2880	0.2260	0.2440	0.2720	0.2720	0.2120
500	0.1780	0.1660	0.2080	0.1660	0.1620	0.1780	0.1860	0.1320
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

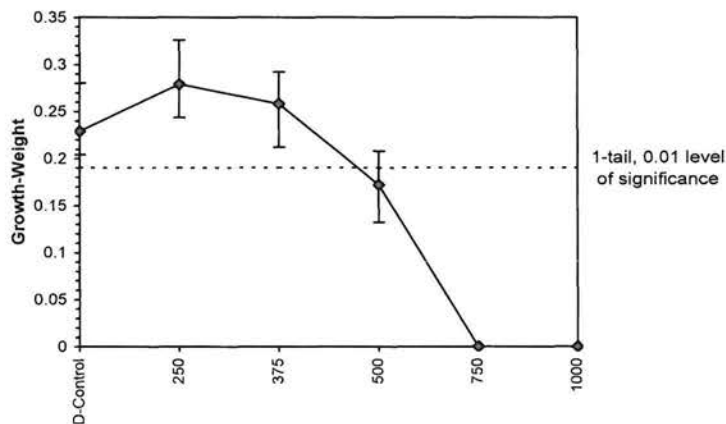
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	1-Tailed			Isotonic	
			Mean	Min	Max	CV%		t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2288	1.0000	0.2288	0.2040	0.2800	10.890	8				0.2553	1.0000
250	0.2793	1.2208	0.2793	0.2440	0.3260	10.539	8	-3.637	2.799	0.0389	0.2553	1.0000
375	0.2580	1.1279	0.2580	0.2120	0.2920	11.142	8	-2.107	2.799	0.0389	0.2553	1.0000
500	0.1720	0.7519	0.1720	0.1320	0.2080	12.692	8				0.1720	0.6736
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 ($p > 0.01$)					0.97364	0.884	0.22765	-0.7423
Bartlett's Test indicates equal variances ($p = 0.90$)					0.20712	9.21035		
Hypothesis Test (1-tail, 0.01)					NOEC	LOEC	ChV	TU
Dunnett's Test					375	>375		
Treatments vs D-Control					0.03885	0.16985	0.00514	0.00077
					0.00572	2, 21		

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	394.15	15.01	335.00	398.65	-3.0869
IC10	413.30	8.60	387.40	422.29	-1.6729
IC15	432.45	8.78	408.45	445.94	-0.7472
IC20	451.60	9.66	427.85	469.58	-0.0956
IC25	470.75	10.96	449.28	493.23	0.2404
IC40	527.33	11.10	504.74	546.10	-0.2430
IC50	564.44	9.30	545.61	580.09	-0.2758



Dose-Response Plot



Controlled and
Reviewed by
Jim Sumner

AbKCICR Test Number: 250

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.

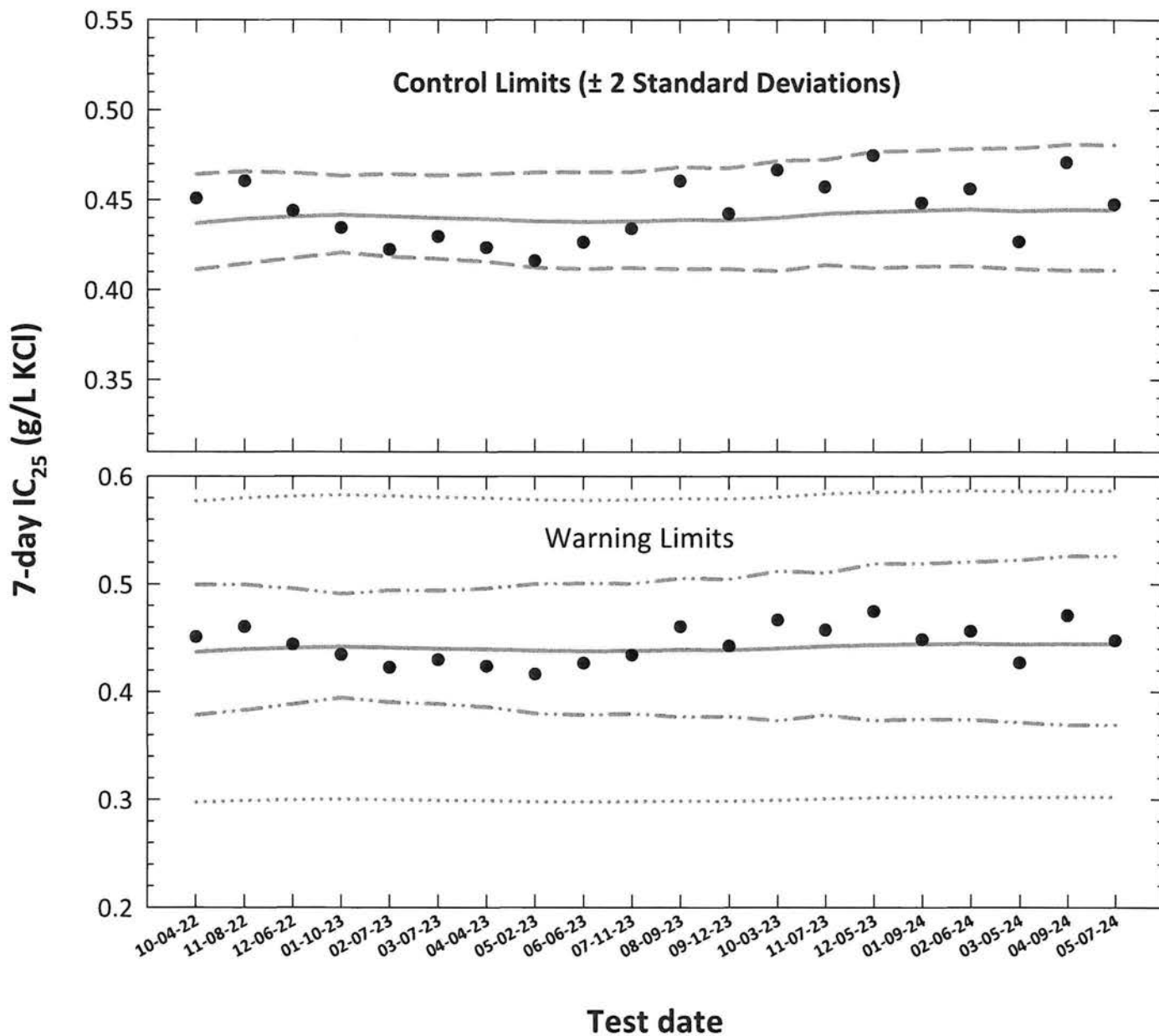
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	<i>RL</i>	<i>2P RL</i>	<i>2P W</i>	<i>RL</i>	<i>RL</i>	<i>RL 2P</i>
CONTROL, Salt SW	pH (S.U.)	7.75	7.65	7.70	7.62	7.67	7.53
	DO (mg/L)	7.0	7.7	7.7	7.2	7.0	6.9
	Salinity (ppt)	25.0	25.1	25.0	25.5	24.9	25.2
	Alkalinity (mg CaCO ₃ /L)	75		—	81-15-14 H	81	
	Temperature (°C)	25.2	25.7	25.2	26.0	25.1	26.1
250 mg KCl/L	pH (S.U.)	7.68	7.62	7.67	7.59	7.66	7.51
	DO (mg/L)	7.9	7.7	7.7	7.3	7.0	7.1
	Salinity (ppt)	25.7	26.5	25.3	25.4	25.2	25.6
	Temperature (°C)	25.4	25.6	25.3	25.9	25.0	26.0
375 mg KCl/L	pH (S.U.)	7.68	7.63	7.66	7.57	7.66	7.49
	DO (mg/L)	7.9	7.7	7.8	7.3	7.0	7.2
	Salinity (ppt)	25.2	25.7	25.6	25.6	25.5	26.0
	Temperature (°C)	25.4	25.7	25.3	25.8	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.69	7.62	7.71	7.60	7.72	7.53
	DO (mg/L)	8.0	7.0	7.8	7.4	7.0	7.2
	Salinity (ppt)	25.4	25.7	25.5	25.7	25.6	26.0
	Temperature (°C)	25.4	25.9	25.1	26.1	25.0	26.2
750 mg KCl/L	pH (S.U.)	7.70	7.63				
	DO (mg/L)	8.0	7.0				
	Salinity (ppt)	25.6	26.1				
	Temperature (°C)	25.4	25.6				
1000 mg KCl/L	pH (S.U.)	7.71	7.63				
	DO (mg/L)	8.1	8.0				
	Salinity (ppt)	25.7	26.2				
	Temperature (°C)	25.3	25.6				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 250

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	7.70	7.66	7.78	7.51	7.76	7.59	7.00	7.30
	DO (mg/L)	7.7	7.5	7.6	7.2	8.0	6.9	7.0	(25.1) 7.1
	Salinity (ppt)	25.0	25.1	24.9	25.3	25.2	25.5	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)	—	—	7.4	—	7.8	—	—	—
	Temperature (°C)	25.2	25.9	25.2	25.5	25.2	25.9	25.2	26.2
250 mg KCl/L	pH (S.U.)	7.70	7.65	7.75	7.56	7.75	7.61	7.79	7.34
	DO (mg/L)	7.7	7.6	7.7	7.2	8.0	6.9	7.0	7.1
	Salinity (ppt)	25.1	25.3	25.1	25.4	25.4	25.7	25.4	25.5
	Temperature (°C)	25.2	26.2	25.2	25.7	25.2	25.9	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.77	7.63	7.76	7.53	7.75	7.60	7.79	7.34
	DO (mg/L)	7.7	7.5	7.7	7.2	8.0	6.8	7.7	(24.0) 6.8
	Salinity (ppt)	25.3	25.4	25.5	25.7	25.6	25.8	25.9	25.9
	Temperature (°C)	25.1	26.3	25.2	25.8	25.2	26.1	25.2	26.4
500 mg KCl/L	pH (S.U.)	7.73	7.67	7.77	7.63	7.75	7.60	7.79	7.40
	DO (mg/L)	7.7	7.4	7.7	7.2	8.0	7.1	7.0	(24.9) 6.8
	Salinity (ppt)	25.3	25.4	25.5	25.7	25.6	26.0	25.8	25.8
	Temperature (°C)	25.2	26.2	25.2	25.9	25.2	26.0	25.2	25.9
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

— 04/10/24

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)			75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109 0.4808	0.3690 0.5261	0.3022 0.5867
20	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109 0.4805	0.3690 0.5258	0.3022 0.5865

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 ToxCal).
CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

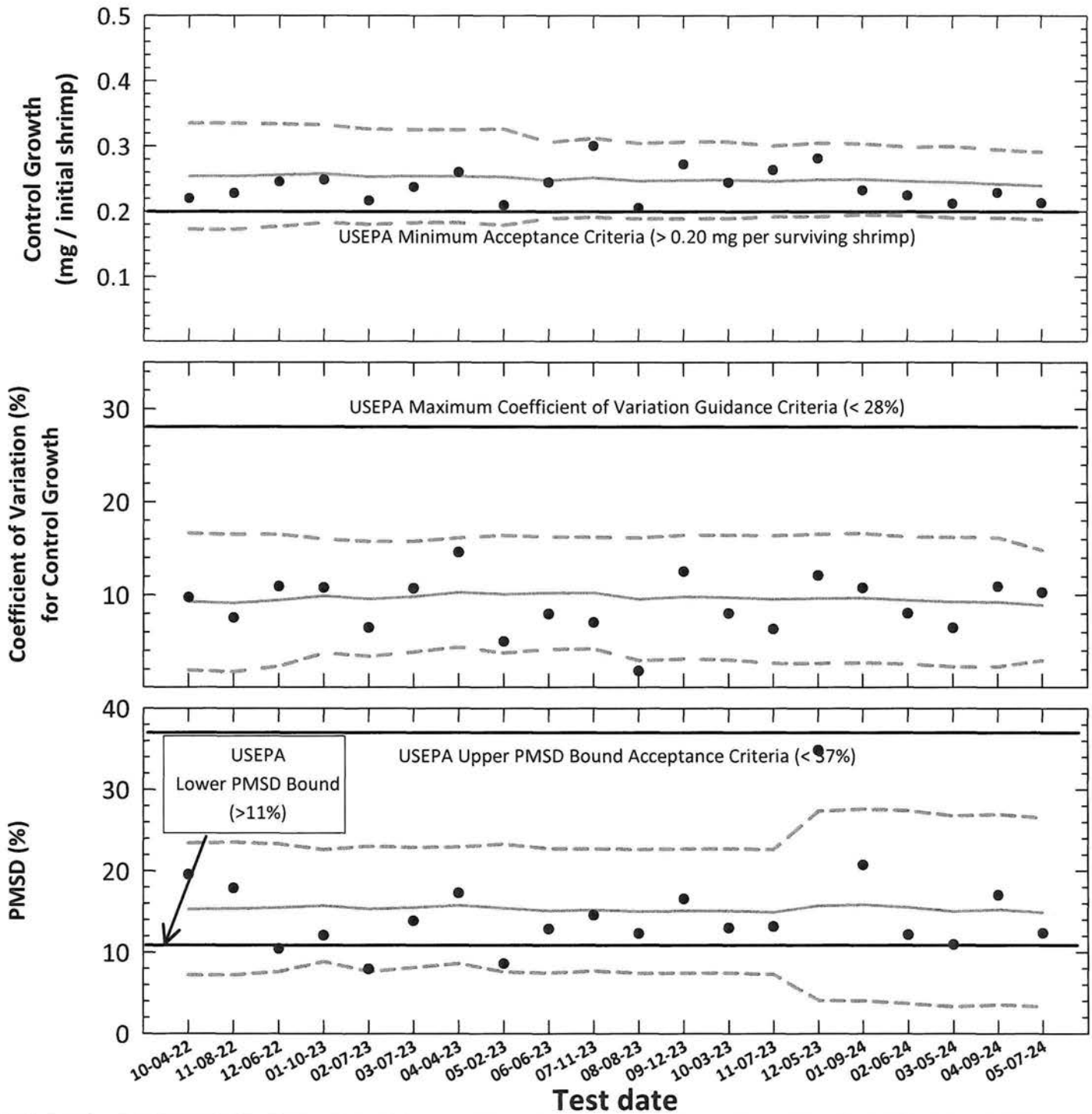
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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		Test	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	CV (%)										
1	10-04-22	100	0.220	9.7	0.0430	0.254	0.173	0.335	9.3	1.9	16.7	15.3	7.2	23.4
2	11-08-22	100	0.228	7.5	0.0407	0.254	0.173	0.335	9.1	1.7	16.5	15.4	7.2	23.5
3	12-06-22	100	0.246	10.9	0.0256	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3
4	01-10-23	100	0.249	10.8	0.0300	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6
5	02-07-23	100	0.217	6.5	0.0172	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0
6	03-07-23	100	0.238	10.7	0.0329	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9
7	04-04-23	100	0.261	14.6	0.0450	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
8	05-02-23	100	0.210	5.0	0.0180	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
9	06-06-23	100	0.245	8.0	0.0314	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
10	07-11-23	100	0.300	7.0	0.0437	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
11	08-08-23	100	0.206	1.8	0.0253	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
12	09-12-23	100	0.273	12.5	0.0451	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
13	10-03-23	100	0.244	8.0	0.0317	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
14	11-07-23	100	0.264	6.3	0.0347	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
15	12-05-23	100	0.282	12.1	0.0981	0.249	0.195	0.305	9.6	2.6	16.6	15.7	4.1	27.4
16	01-09-24	100	0.233	10.8	0.0482	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6
17	02-06-24	100	0.225	8.0	0.0273	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4
18	03-05-24	100	0.212	6.5	0.0232	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8
19	04-09-24	100	0.229	10.9	0.0389	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9
20	05-07-24	100	0.213	10.3	0.0263	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5

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.
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.

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

AbKCICR Test Number: **251**

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:	04-30-24 1200 to 05-01-24 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 05-01-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
Transfer bowl information:	pH = 7.84 S.U. Temperature = 25.0 °C	Date / Time in oven:	05-14-24 1055
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	60°C
		Date / Time out of oven:	05-15-24 1100
		*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	05-07-24	1045	JH	1300	JH	1235	JH	05-01-24A
1	05-08-24	0500	JH	1230	JH	1035	JH	↓
2	05-09-24	0500	JH	1330	JH	1100	JH	05-01-24B
3	05-10-24	0500	JH	1100	JH	1004	JH	↓
4	05-11-24	0600	JH	1240	JH	1100	JH	05-06-24
5	05-12-24	0600	JH	1200	JH	0912	JH	↓
6	05-13-24	0500	JH	1100	JH	0914	JH	↓
7	05-14-24					1040	JH	

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:	0%	≤ 20%	7-day LC ₅₀ (mg/L KCl)	519.42
Average weight per initial shrimp:	0.213		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.213	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.01
			IC ₂₅ (mg/L KCl)	447.49

AbKCICR Test Number: **251**

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>Grey</u> Analyst: <u>JP</u> Date: <u>01-24-24</u>	14.46	12.62	13.22	14.63	14.09	13.27	12.79	11.89	13.03	13.60	13.24	12.24	13.75	13.46	14.46	14.87
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>05-16-24</u>	15.73	13.57	14.24	15.69	15.11	14.30	13.99	12.91	14.21	14.83	14.52	13.26	14.88	14.76	15.56	16.00
C = Shrimp weight (mg) = B - A Hand calculated: <u>JP</u> Analyst: <u>JP</u>	1.27	0.95	1.02	1.01	1.02	1.03	1.20	1.02	1.18	1.23	1.28	1.02	1.13	1.30	1.10	1.13
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>JP</u> Analyst: <u>JP</u>	0.254	0.190	0.204	0.202	0.204	0.206	0.240	0.204	0.236	0.246	0.256	0.204	0.226	0.260	0.220	0.226
Average weight per initial number of shrimp (mg)										Average weight per initial number of shrimp (mg)					Percent reduction from control (%)	
0.213										0.234					-10.0%	

*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: 251

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	4 ^d	4 ^d	4 ^d	4 ^d	5	4 ^d	4 ^d	4 ^d
3	5	5	5	5	5	5	5	5	4	4	4	4	5	4	4	4
4	5	5	5	5	5	4 ^d	5	5	4	3 ^d	4	4	5	4	4	4
5	5	5	5	5	5	4	5	5	3 ^d	3	4	4	4 ^d	3 ^d	4	4
6	5	5	5	5	5	4	5	5	2 ^d	3	3 ^d	4	1	3	4	4
7	5	5	5	5	5	4	5	5	2	3	3	4	1	3	3 ^d	3 ^d
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>grey</u> Analyst: <u>gp</u> Date: <u>01-24-24</u>	14.09	14.33	14.01	14.70	15.07	14.57	14.07	14.57	14.85	14.11	13.02	14.27	12.72	12.13	13.59	12.99
*B = Pan + Shrimp weight (mg) Analyst: <u>X</u> Date: <u>05-16-24</u>	15.23	15.46	15.25	15.73	16.16	15.71	15.08	15.74	15.29	14.81	13.55	15.18	13.04	12.83	14.41	13.66
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>gp</u>	1.14	1.13	1.24	1.03	1.09	1.14	1.01	1.17	0.44	0.70	0.53	0.91	0.32	0.70	0.82	0.67
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>gp</u>	0.228	0.226	0.248	0.206	0.218	0.228	0.202	0.234	0.088	0.140	0.106	0.182	0.064	0.140	0.164	0.134
Average weight per initial number of shrimp (mg) 0.224				Percent reduction from control (%) - 5.0%				Average weight per initial number of shrimp (mg) 0.127				Percent reduction from control (%) 40.3%				

*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: **251**

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	VV
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								0							
Percent reduction from control (%)	100%								100%							
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: 251

Test dates: May 07-14, 2024

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	14.46	15.73	1.27	0.254	100.0	0.213	10.3	Not applicable
	B	5	5	12.62	13.57	0.95	0.190				
	C	5	5	13.22	14.24	1.02	0.204				
	D	5	5	14.68	15.69	1.01	0.202				
	E	5	5	14.09	15.11	1.02	0.204				
	F	5	5	13.27	14.30	1.03	0.206				
	G	5	5	12.79	13.99	1.20	0.240				
	H	5	5	11.89	12.91	1.02	0.204				
250	I	5	5	13.03	14.21	1.18	0.236	100.0	0.234	8.1	-10.0
	J	5	5	13.60	14.83	1.23	0.246				
	K	5	5	13.24	14.52	1.28	0.256				
	L	5	5	12.24	13.26	1.02	0.204				
	M	5	5	13.75	14.88	1.13	0.226				
	N	5	5	13.46	14.76	1.30	0.260				
	O	5	5	14.46	15.56	1.10	0.220				
	P	5	5	14.87	16.00	1.13	0.226				
375	Q	5	5	14.09	15.23	1.14	0.228	97.5	0.224	6.7	-5.0
	R	5	5	14.33	15.46	1.13	0.226				
	S	5	5	14.01	15.25	1.24	0.248				
	T	5	5	14.70	15.73	1.03	0.206				
	U	5	5	15.07	16.16	1.09	0.218				
	V	5	4	14.57	15.71	1.14	0.228				
	W	5	5	14.07	15.08	1.01	0.202				
	X	5	5	14.57	15.74	1.17	0.234				
500	Y	5	2	14.85	15.29	0.44	0.088	55.0	0.127	30.7	40.3
	Z	5	3	14.11	14.81	0.70	0.140				
	AA	5	3	13.02	13.55	0.53	0.106				
	BB	5	4	14.27	15.18	0.91	0.182				
	CC	5	1	12.72	13.04	0.32	0.064				
	DD	5	3	12.13	12.83	0.70	0.140				
	EE	5	3	13.59	14.41	0.82	0.164				
	FF	5	3	12.99	13.66	0.67	0.134				
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: 0.0263
 PMSD: 12.3

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.



Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date: 5/7/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 5/14/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanmysis 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	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
500	0.4000	0.6000	0.6000	0.8000	0.2000	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	0.5500	0.5500	0.8357	0.4636	1.1071	22.503	8	36.00	48.00	18	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

Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$) 0.65828 0.904 -1.5831 8.20343
Equality of variance cannot be confirmed

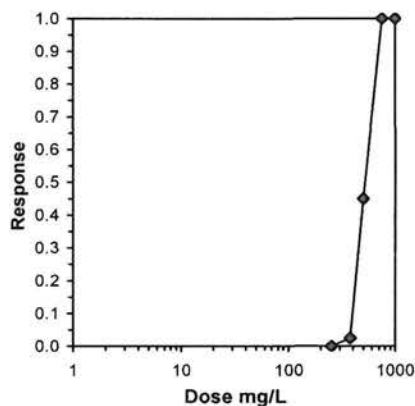
Hypothesis Test (1-tail, 0.05)

Steel's Many-One Rank Test NOEC LOEC ChV TU

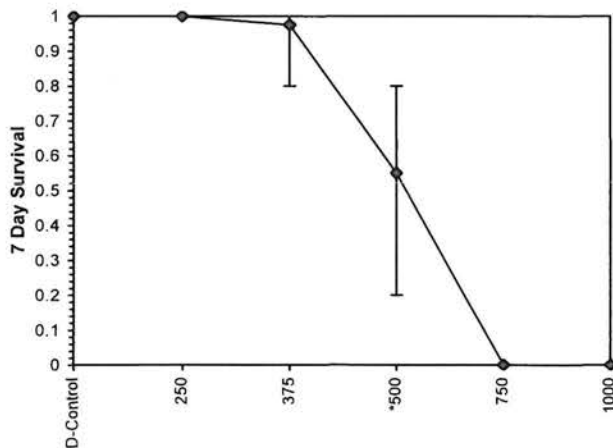
Treatments vs D-Control 375 500 433.013

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	519.42	490.57 549.97
5.0%	521.56	490.81 554.25
10.0%	521.18	486.92 557.84
20.0%	520.41	475.46 569.60
Auto-0.0%	519.42	490.57 549.97



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

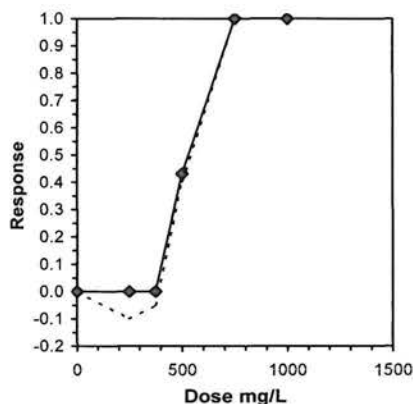
Start Date: 5/7/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 5/14/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2540	0.1900	0.2040	0.2020	0.2040	0.2060	0.2400	0.2040
250	0.2360	0.2460	0.2560	0.2040	0.2260	0.2600	0.2200	0.2260
375	0.2280	0.2260	0.2480	0.2060	0.2180	0.2280	0.2020	0.2340
500	0.0880	0.1400	0.1060	0.1820	0.0640	0.1400	0.1640	0.1340
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

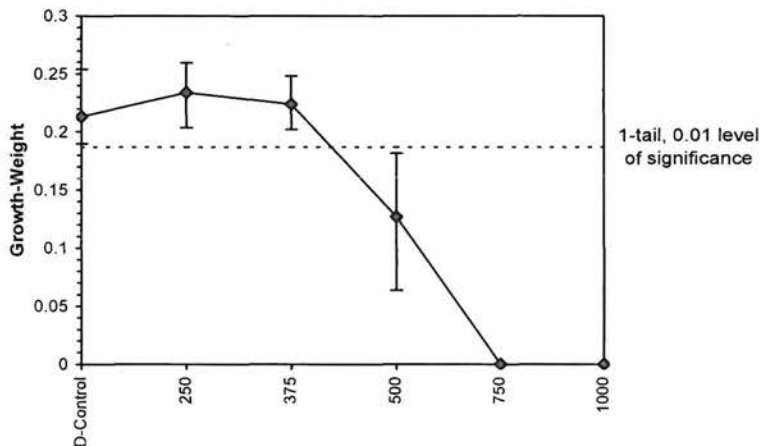
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	Critical			MSD	Mean	N-Mean	
D-Control	0.2130	1.0000	0.2130	0.1900	0.2540	10.274	8					0.2237	1.0000
250	0.2343	1.0998	0.2343	0.2040	0.2600	8.118	8	-2.258	2.799	0.0263		0.2237	1.0000
375	0.2238	1.0505	0.2238	0.2020	0.2480	6.662	8	-1.142	2.799	0.0263		0.2237	1.0000
500	0.1273	0.5974	0.1273	0.0640	0.1820	30.741	8					0.1273	0.5689
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)					0.95345		0.884	0.56509	-0.2365					
Bartlett's Test indicates equal variances (p = 0.62)					0.94918		9.21035							
Hypothesis Test (1-tail, 0.01)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					375	>375			0.02634	0.12364	0.0009	0.00035	0.10203	2, 21
Treatments vs D-Control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL		Skew
IC05	389.50	8.09	364.73	394.26	-3.6519
IC10	404.00	6.10	390.44	413.52	0.0526
IC15	418.50	7.50	404.36	432.78	0.6362
IC20	432.99	9.27	417.49	452.04	0.8463
IC25	447.49	11.23	431.16	471.30	0.9095
IC40	490.99	17.18	466.71	529.65	0.6992
IC50	530.29	21.64	489.67	566.38	0.0386



Dose-Response Plot



AbKCICR Test Number: **251**

Daily Chemistry:

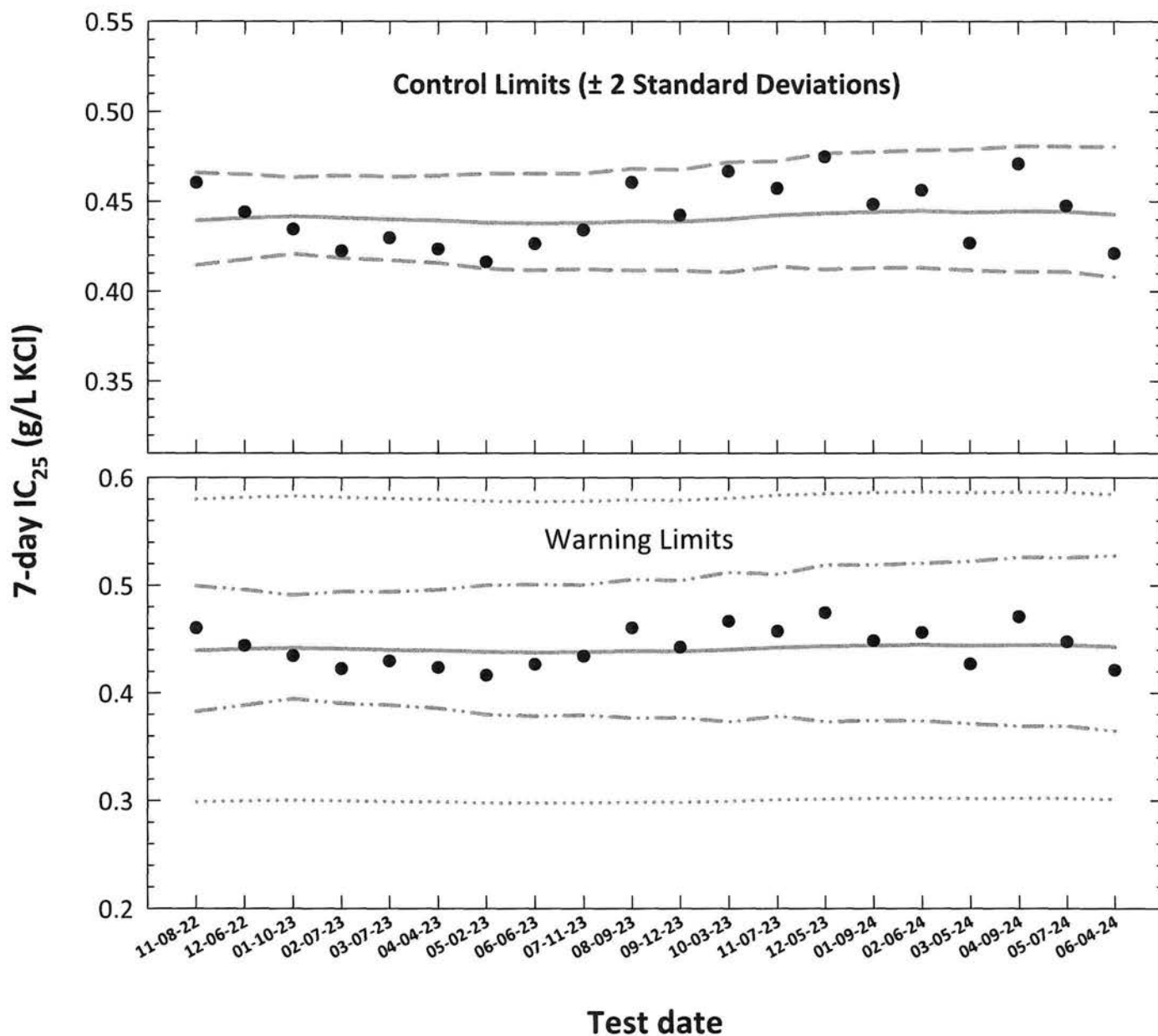
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	<i>RL</i>	<i>RL</i>	<i>RL</i>	<i>RL</i>	<i>RL</i>	<i>RL</i>
CONTROL, Salt SW	pH (S.U.)	7.54	7.44	7.47	7.50	7.40	7.32
	DO (mg/L)	7.7	7.7	7.0	7.4	7.0	7.2
	Salinity (ppt)	25.0	25.6	25.0	25.2	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	83			25-13-2481	78	
	Temperature (°C)	25.3	26.0	25.1	26.0	25.0	26.0
250 mg KCl/L	pH (S.U.)	7.54	7.45	7.47	7.50	7.51	7.33
	DO (mg/L)	7.7	7.7	7.0	7.0	7.9	7.2
	Salinity (ppt)	25.0	25.6	25.2	25.5	25.3	25.5
	Temperature (°C)	25.2	26.2	25.2	25.8	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.55	7.45	7.40	7.55	7.54	7.35
	DO (mg/L)	7.7	7.7	7.0	7.7	7.9	7.4
	Salinity (ppt)	25.3	25.7	25.3	25.9	25.7	25.8
	Temperature (°C)	25.2	26.0	25.2	25.8	25.0	26.0
500 mg KCl/L	pH (S.U.)	7.57	7.45	7.49	7.54	7.57	7.40
	DO (mg/L)	7.8	7.7	7.0	7.7	7.0	7.4
	Salinity (ppt)	25.3	26.0	25.6	26.1	25.7	26.1
	Temperature (°C)	25.2	26.0	25.1	25.8	25.0	26.0
750 mg KCl/L	pH (S.U.)	7.59	7.40				
	DO (mg/L)	7.0	7.0				
	Salinity (ppt)	25.7	26.1				
	Temperature (°C)	25.1	25.9				
1000 mg KCl/L	pH (S.U.)	7.41	7.49				
	DO (mg/L)	7.9	7.9				
	Salinity (ppt)	25.7	26.2				
	Temperature (°C)	25.3	25.9				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 251

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	<i>N</i>	<i>BSC</i>	<i>BSC</i>	<i>BSC</i>	<i>BSC</i>	<i>N</i>	<i>N</i>	<i>N</i>
	DO (mg/L)	7.42	7.40	7.74	7.53	7.70	7.50	7.64	7.03
	Salinity (ppt)	29	7.7	8.0	7.5	8.0	7.4	7.8	6.4
	Alkalinity (mg CaCO ₃ /L)	25.0	25.1	25.1	25.3	25.2	25.5	24.6	25.0
	Temperature (°C)	—	<i>05-13-24 JH</i>	82		—		—	<i>05-13-24 JH</i>
250 mg KCl/L	pH (S.U.)	25.2	26.1	25.0	26.2	25.2	26.2	25.1	26.0
	DO (mg/L)	7.42	7.40	7.73	7.56	7.72	7.52	7.64	7.19
	Salinity (ppt)	7.9	7.4	8.0	7.5	8.0	7.4	7.9	6.5
	Temperature (°C)	25.1	25.3	25.2	25.3	25.3	25.6	25.5	25.4
375 mg KCl/L	pH (S.U.)	25.2	26.0	25.2	26.0	25.2	26.1	25.3	26.1
	DO (mg/L)	7.43	7.41	7.74	7.53	7.73	7.53	7.66	7.26
	Salinity (ppt)	7.9	7.7	8.0	7.7	8.0	7.4	8.0	6.5
	Temperature (°C)	25.1	25.5	25.3	25.3	25.4	25.8	25.8	25.7
500 mg KCl/L	pH (S.U.)	25.3	26.0	25.2	26.0	25.3	26.0	25.3	26.0
	DO (mg/L)	7.46	7.43	7.74	7.64	7.76	7.60	7.68	7.36
	Salinity (ppt)	7.9	7.7	8.0	7.6	8.0	7.4	8.0	7.2
	Temperature (°C)	25.3	25.6	25.4	25.9	25.5	25.7	26.0	25.9
750 mg KCl/L	pH (S.U.)	25.1	26.1	25.1	26.2	25.3	26.2	25.3	26.0
	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 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109	0.4808	0.3690	0.5261	0.3022	0.5867
19	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109	0.4805	0.3690	0.5258	0.3022	0.5865
20	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082	0.4804	0.3645	0.5278	0.3011	0.5845

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.

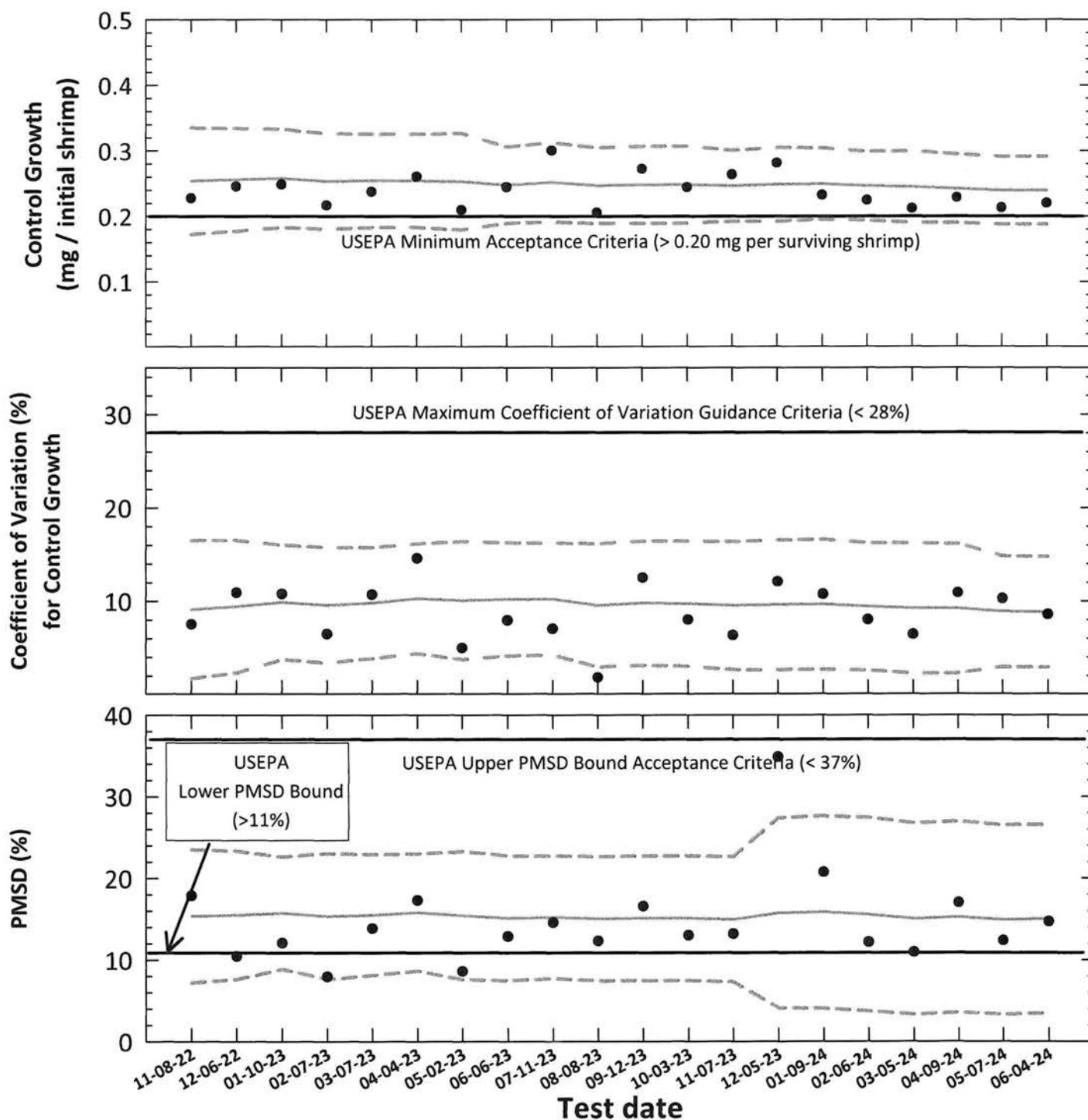
S_{A.75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A.75} = 0.32).

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		Test	PMSD (%)	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S
			Mean (mg/Initial shrimp)	CV (%)								
1	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	9.1	1.7	15.4	7.2
2	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	9.4	2.3	15.5	7.6
3	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	9.9	3.7	15.7	8.8
4	02-07-23	100	0.217	6.5	0.0172	7.9	0.253	0.180	9.6	3.4	15.3	7.6
5	03-07-23	100	0.238	10.7	0.0329	13.9	0.254	0.183	9.8	3.9	15.5	8.1
6	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	10.3	4.4	15.8	8.6
7	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	10.1	3.7	15.4	7.6
8	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	10.2	4.1	15.1	7.4
9	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	10.2	4.2	15.2	7.7
10	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	9.6	2.9	15.0	7.4
11	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	9.8	3.1	15.1	7.4
12	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	9.7	3.0	15.1	7.4
13	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	9.5	2.7	14.9	7.3
14	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	9.6	2.6	15.7	4.1
15	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	9.7	2.7	15.8	4.0
16	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	9.4	2.6	15.5	3.7
17	03-05-24	100	0.212	6.5	0.0232	10.9	0.245	0.190	9.3	2.3	15.0	3.3
18	04-09-24	100	0.229	10.9	0.0389	17.0	0.243	0.190	9.2	2.3	15.2	3.5
19	05-07-24	100	0.213	10.3	0.0263	12.3	0.239	0.188	8.9	2.9	14.9	3.3
20	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	8.8	2.9	15.0	3.5

Note:

Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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) > 11%.

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%.

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: **252**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2288					
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:	05-28-24 1200 to 05-29-24 1130		Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 05-29-24		Artemia CHM number:	CHM1294
			Drying information for weight determination:	
Transfer bowl information:	pH = 7.67 S.U. Temperature = 25.0 °C		Date / Time in oven:	06-11-24 1205
			*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL		Date / Time out of oven:	06-12-24 1205
			*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	06-04-24	1110	<i>jl</i>	1300	<i>jl</i>	1235	<i>jl</i>	05-28-24 A
1	06-05-24	0500	<i>jl</i>	1100	<i>jl</i>	0915	<i>jl</i>	↓
2	06-06-24	0500	<i>jl</i>	1200	<i>jl</i>	0915	<i>jl</i>	06-03-24 A 05-29-24 A 06-06-24 <i>jl</i>
3	06-07-24	0500	<i>jl</i>	1200	<i>jl</i>	0915	<i>jl</i>	06-03-24 B
4	06-08-24	0610	<i>jl</i>	1210	<i>jl</i>	0922	<i>jl</i>	06-06-24 A
5	06-09-24	0600	<i>jl</i>	1150	<i>jl</i>	0910	<i>jl</i>	06-06-24 B
6	06-10-24	0500	<i>jl</i>	1100	<i>jl</i>	0910	<i>jl</i>	06-08-24
7	06-11-24					1133	<i>jl</i>	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130064625

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	510.5
Average weight per initial shrimp:	0.220		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.220	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	421.1

AbKCICR Test Number: **252**

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: Analyst: <u>EM</u> Date: <u>05-29-24</u>	12.79	13.85	13.63	13.30	12.91	14.60	12.98	12.80	12.14	13.25	13.76	14.72	12.67	14.38	13.91	12.31
*B = Pan + Shrimp weight (mg) Analyst: <u>EM</u> Date: <u>06-13-24</u>	13.82	14.90	14.87	14.52	13.99	15.76	13.97	13.83	13.32	14.34	14.90	15.85	13.72	15.42	14.99	13.31
C = Shrimp weight (mg) = B - A Hand calculated Analyst:	1.03	1.05	1.24	1.22	1.08	1.16	0.99	1.03	1.18	1.09	1.14	1.13	1.05	1.04	1.08	1.04
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst:	0.206	0.210	0.248	0.244	0.216	0.232	0.198	0.206	0.236	0.218	0.228	0.226	0.210	0.208	0.216	0.208
Average weight per initial number of shrimp (mg) 0.220												Average weight per initial number of shrimp (mg) 0.219				Percent reduction from control (%) 0.67

*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:

Light pink pans initially massed by Jayden (A-BB) on 4-29-24
LL-FF massed by EM on 05-29-24

AbKCICR Test Number: **252**

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	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	4 ^{1d}	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	4 ^{1d}	4	4 ^{1d}	3 ^{2d}	3 ^{1d}	3 ^{2d}	3 ^{2d}	3 ^{2d}
5	S	S	S	S	S	S	S	S	4	4	4	3	3	2 ^{1d}	2 ^{1d}	2 ^{1d}
6	S	S	S	S	S	S	S	S	3 ^{1d}	3 ^{1d}	4	3	3	2	2	2
7	S	S	S	S	S	S	S	S	3	2 ^{1d}	2 ^{2d}	3	3	2	2	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																1
*A = Pan weight (mg) Tray color code: Analyst: <u>Light pink</u> Date: <u>05-29-24</u>	13.65	13.39	12.67	14.88	12.33	14.50	13.71	14.00	13.80	12.01	13.76	13.79	14.44	15.55	14.85	14.22
*B = Pan + Shrimp weight (mg) Analyst: <u>JK</u> Date: <u>06-19-24</u>	14.65	14.54	13.76	16.06	13.71	15.54	14.77	15.47	14.18	12.29	14.10	14.28	14.96	15.84	15.10	14.56
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JK</u>	1.00	1.15	1.09	1.18	1.38	1.04	1.06	1.47	0.38	0.28	0.34	0.49	0.52	0.29	0.25	0.34
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JK</u>	0.200	0.230	0.218	0.236	0.276	0.208	0.212	0.294	0.076	0.056	0.068	0.098	0.104	0.058	0.050	0.068
	Average weight per initial number of shrimp (mg) 0.234				Percent reduction from control (%) - 6.57				Average weight per initial number of shrimp (mg) 0.072				Percent reduction from control (%) 67.27			

*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: **252**

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	VV												
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5												
1	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	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: 252

Test dates: June 04-11, 2024

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	12.79	13.82	1.03	0.206	100.0	0.220	8.6	Not applicable
	B	5	5	13.85	14.90	1.05	0.210				
	C	5	5	13.63	14.87	1.24	0.248				
	D	5	5	13.30	14.52	1.22	0.244				
	E	5	5	12.91	13.99	1.08	0.216				
	F	5	5	14.60	15.76	1.16	0.232				
	G	5	5	12.98	13.97	0.99	0.198				
	H	5	5	12.80	13.83	1.03	0.206				
250	I	5	5	12.14	13.32	1.18	0.236	100.0	0.219	4.7	0.6
	J	5	5	13.25	14.34	1.09	0.218				
	K	5	5	13.76	14.90	1.14	0.228				
	L	5	5	14.72	15.85	1.13	0.226				
	M	5	5	12.67	13.72	1.05	0.210				
	N	5	5	14.38	15.42	1.04	0.208				
	O	5	5	13.91	14.99	1.08	0.216				
	P	5	5	12.31	13.35	1.04	0.208				
375	Q	5	5	13.65	14.65	1.00	0.200	100.0	0.234	14.4	-6.5
	R	5	5	13.39	14.54	1.15	0.230				
	S	5	5	12.67	13.76	1.09	0.218				
	T	5	5	14.88	16.06	1.18	0.236				
	U	5	5	12.33	13.71	1.38	0.276				
	V	5	5	14.50	15.54	1.04	0.208				
	W	5	5	13.71	14.77	1.06	0.212				
	X	5	5	14.00	15.47	1.47	0.294				
500	Y	5	3	13.80	14.18	0.38	0.076	47.5	0.072	27.1	67.2
	Z	5	2	12.01	12.29	0.28	0.056				
	AA	5	2	13.76	14.10	0.34	0.068				
	BB	5	3	13.79	14.28	0.49	0.098				
	CC	5	3	14.44	14.96	0.52	0.104				
	DD	5	2	15.55	15.84	0.29	0.058				
	EE	5	2	14.85	15.10	0.25	0.050				
	FF	5	2	14.22	14.56	0.34	0.068				
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: 0.0323
PMSD: 14.7

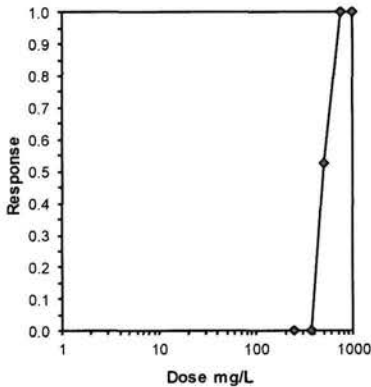
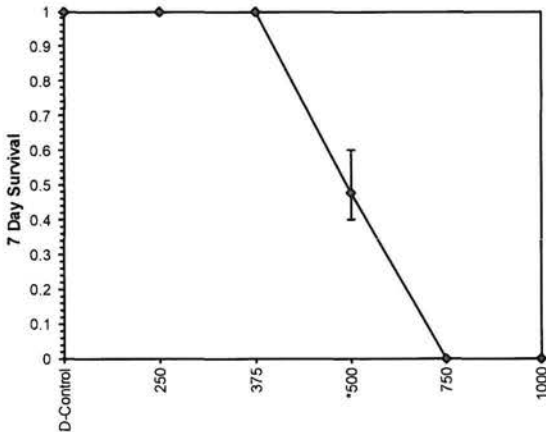
MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

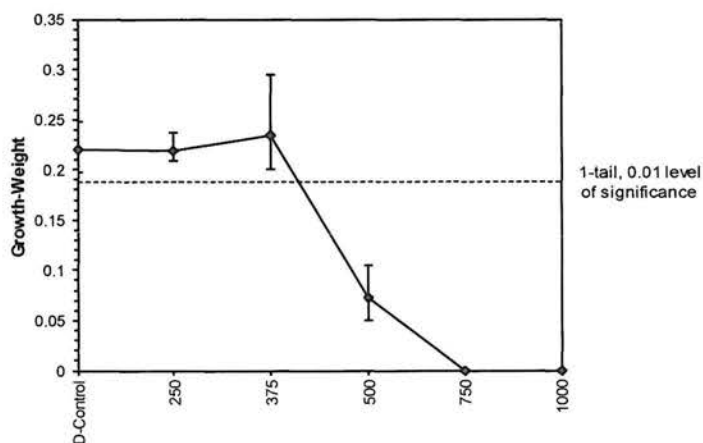
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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:	6/4/2024	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant						
End Date:	6/11/2024	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						
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	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
500	0.6000	0.4000	0.4000	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			
Transform: Arcsin Square Root											
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
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	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	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)								0.65182	0.904	1.0843	2.64391
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											
Trimmed Spearman-Kärber											
Trim Level	EC50	95% CL									
0.0%	510.50	483.31	539.22								
5.0%	508.56	478.63	540.35								
10.0%	506.63	473.48	542.09								
20.0%	502.79	460.78	548.63								
Auto-0.0%	510.50	483.31	539.22								
											
Dose-Response Plot											
											



AbKCICR Test Number: **252**

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.

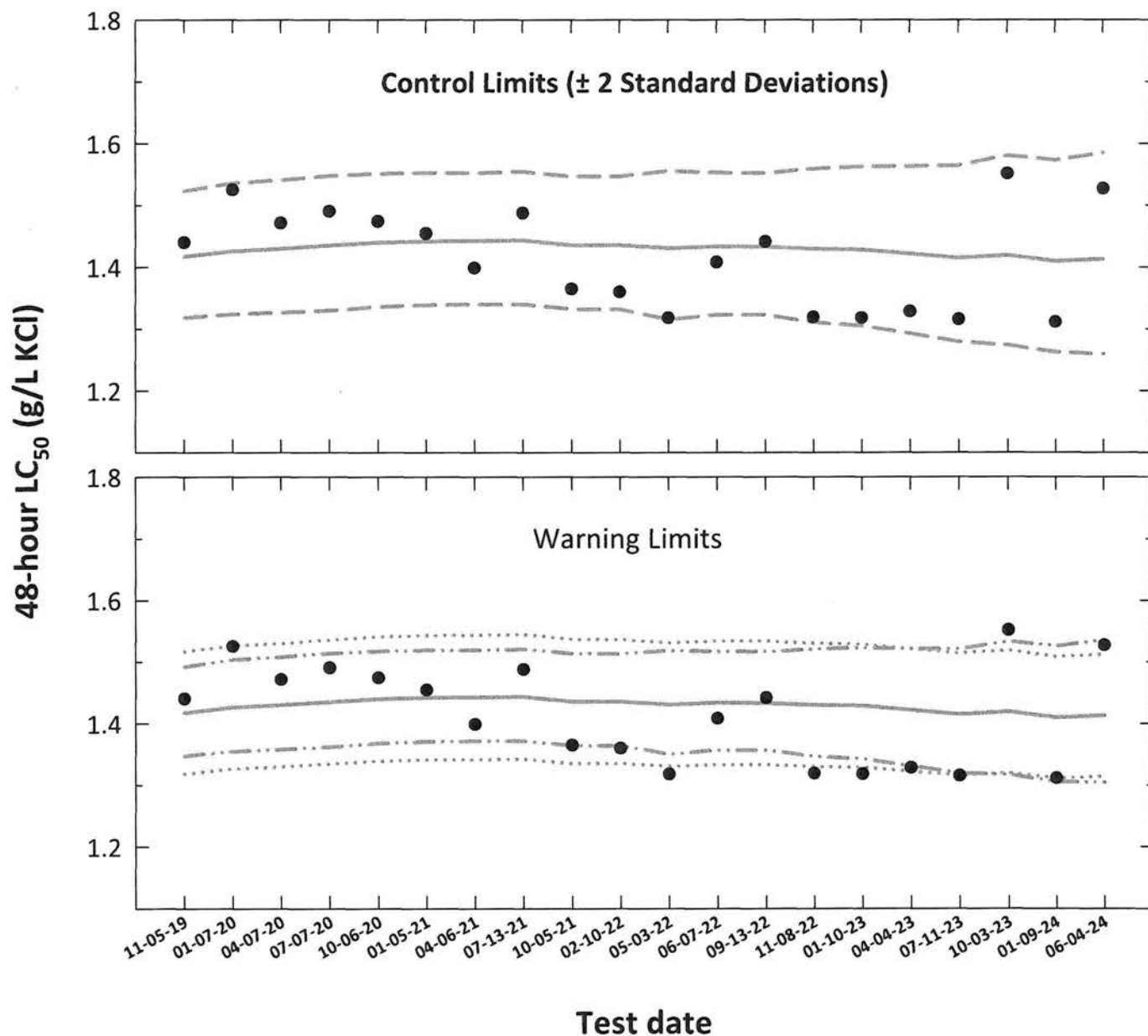
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst		Analyst		Analyst	
CONTROL, Salt SW	pH (S.U.)	N JP	7.71	N JP	7.65	N JP	7.58
	DO (mg/L)	N JP	7.0	N JP	7.0	N JP	6.7
	Salinity (ppt)	N JP	25.0	N JP	24.9	N JP	25.3
	Alkalinity (mg CaCO ₃ /L)	N JP	72	N JP	150	N JP	
	Temperature (°C)	N JP	25.0	N JP	25.2	N JP	25.8
250 mg KCl/L	pH (S.U.)	N JP	7.30	N JP	7.52	N JP	7.20
	DO (mg/L)	N JP	7.0	N JP	7.7	N JP	6.5
	Salinity (ppt)	N JP	25.0	N JP	25.1	N JP	25.0
	Temperature (°C)	N JP	25.1	N JP	25.9	N JP	26.0
375 mg KCl/L	pH (S.U.)	N JP	7.37	N JP	7.52	N JP	7.22
	DO (mg/L)	N JP	7.0	N JP	7.7	N JP	6.4
	Salinity (ppt)	N JP	25.0	N JP	25.4	N JP	25.4
	Temperature (°C)	N JP	25.0	N JP	25.7	N JP	26.0
500 mg KCl/L	pH (S.U.)	N JP	7.37	N JP	7.55	N JP	7.23
	DO (mg/L)	N JP	7.9	N JP	7.7	N JP	7.1
	Salinity (ppt)	N JP	25.1	N JP	25.6	N JP	25.7
	Temperature (°C)	N JP	25.0	N JP	25.9	N JP	26.0
750 mg KCl/L	pH (S.U.)	N JP	7.30	N JP	7.54	N JP	
	DO (mg/L)	N JP	7.9	N JP	7.7	N JP	
	Salinity (ppt)	N JP	25.3	N JP	25.4	N JP	
	Temperature (°C)	N JP	25.2	N JP	25.9	N JP	
1000 mg KCl/L	pH (S.U.)	N JP	7.37	N JP	7.52	N JP	
	DO (mg/L)	N JP	7.9	N JP	7.0	N JP	
	Salinity (ppt)	N JP	25.5	N JP	25.6	N JP	
	Temperature (°C)	N JP	25.2	N JP	26.0	N JP	
		Initial	Final	Initial	Final	Initial	Final

NEW BATCH OF SALT

AbKCICR Test Number: **252**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	<i>RM</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>APKD</i>	<i>APKD</i>	<i>RM</i>
	DO (mg/L)	7.52	7.82	7.55(7.13)	7.94	7.64	8.07	7.99	7.71
	Salinity (ppt)	24.9	25.0	24.9	25.2	25.1	25.6	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	160		160		150		150	
	Temperature (°C)	25.2	26.1	25.2	26.1	25.2	26.1	25.2	26.2
250 mg KCl/L	pH (S.U.)	7.74	7.82	7.94	7.86	7.98	8.07	8.18	7.72
	DO (mg/L)	7.0	7.6	7.8	7.5	7.7	7.6	7.9	7.4
	Salinity (ppt)	25.1	25.1	25.1	25.2	25.3	25.7	25.2	25.5
	Temperature (°C)	25.1	25.9	25.3	26.2	25.2	26.0	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.75	7.78	7.94	7.87	8.00	8.11	8.19	7.73
	DO (mg/L)	7.9	7.6	7.9	7.5	7.8	7.6	7.9	7.4
	Salinity (ppt)	25.2	25.3	25.3	25.5	25.3	25.8	25.7	25.8
	Temperature (°C)	25.1	25.9	25.3	25.9	25.2	26.0	25.1	26.2
500 mg KCl/L	pH (S.U.)	7.76	7.79	7.95	7.73	8.00	8.11	8.19	7.76
	DO (mg/L)	7.9	7.6	8.0	7.5	7.8	7.6	7.9	7.5
	Salinity (ppt)	25.3	25.4	25.4	25.6	25.7	26.0	25.8	25.9
	Temperature (°C)	25.1	26.1	25.2	26.2	25.1	26.2	25.1	26.1
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

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 ToxCat 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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	11-05-19	1.4402	0.1584	0.1514	0.0157	1.4172	1.3182	1.5235	1.3474	1.4922
2	01-07-20	1.5253	0.1834	0.1542	0.0162	1.4264	1.3242	1.5365	1.3547	1.5036
3	04-07-20	1.4716	0.1678	0.1554	0.0163	1.4302	1.3269	1.5414	1.3580	1.5079
4	07-07-20	1.4906	0.1734	0.1569	0.0165	1.4351	1.3302	1.5483	1.3620	1.5139
5	10-06-20	1.4741	0.1685	0.1583	0.0162	1.4397	1.3362	1.5513	1.3678	1.5172
6	01-05-21	1.4546	0.1627	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189
7	04-06-21	1.3988	0.1458	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187
8	07-13-21	1.4877	0.1725	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207
9	10-05-21	1.3650	0.1351	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132
10	02-10-22	1.3599	0.1335	0.1570	0.0163	1.4356	1.3320	1.5473	1.3635	1.5134
11	05-03-22	1.3180	0.1199	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182
12	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169
13	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163
14	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205
15	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227
16	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213
17	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209
18	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331
19	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256
20	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351

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 ToxCat).
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 # 93

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 #: 2288

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>KL JP</i>	<i>LD</i>	<i>KL JP</i>
	pH (S.U.)	<i>7.71</i>	<i>7.62</i>	<i>7.44</i>
	Dissolved oxygen (mg/L)	<i>7.0</i>	<i>7.8</i>	<i>7.5</i>
	*Salinity (ppt)	<i>25.0</i>	<i>24.8</i>	<i>24.6</i>
	*Alkalinity (mg/L CaCO ₃)	<i>72</i>		
1000 mg/L	*Temperature (°C)	<i>25.2</i>	<i>25.2</i>	<i>25.0</i>
	pH (S.U.)	<i>7.53</i>	<i>7.57</i>	<i>7.40</i>
	Dissolved oxygen (mg/L)	<i>7.7</i>	<i>7.9</i>	<i>7.6</i>
	*Salinity (ppt)	<i>24.7</i>	<i>25.4</i>	<i>25.5</i>
	*Temperature (°C)	<i>25.0</i>	<i>25.2</i>	<i>25.2</i>
1250 mg/L	pH (S.U.)	<i>7.53</i>	<i>7.57</i>	<i>7.39</i>
	Dissolved oxygen (mg/L)	<i>7.0</i>	<i>7.8</i>	<i>7.4</i>
	*Salinity (ppt)	<i>25.6</i>	<i>25.8</i>	<i>26.0</i>
	*Temperature (°C)	<i>25.0</i>	<i>25.2</i>	<i>25.4</i>
1500 mg/L	pH (S.U.)	<i>7.53</i>	<i>7.57</i>	<i>7.39</i>
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>7.8</i>	<i>7.5</i>
	*Salinity (ppt)	<i>25.7</i>	<i>25.7</i>	<i>26.2</i>
	*Temperature (°C)	<i>25.0</i>	<i>25.0</i>	<i>25.1</i>
1750 mg/L	pH (S.U.)	<i>7.53</i>	<i>7.59</i>	<i>7.42</i>
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.7</i>	<i>7.6</i>
	*Salinity (ppt)	<i>25.4</i>	<i>25.8</i>	<i>26.1</i>
	*Temperature (°C)	<i>25.0</i>	<i>25.2</i>	<i>25.4</i>
2000 mg/L	pH (S.U.)	<i>7.91</i>	<i>7.52</i>	
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.9</i>	
	*Salinity (ppt)	<i>26.0</i>	<i>26.1</i>	
	*Temperature (°C)	<i>25.0</i>	<i>25.1</i>	

*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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066464

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 # 93

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	06-04-24	1110	JL	1330	JL	1E	PINK	05-24-06
24	06-05-24			1329	JL			
48 Termination	06-06-24			1328	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 Mb):	05-15-24
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	05-24-24 1200 TO 05-15-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.27 Temperature (°C) 24.7

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	10	9 ^{1d}	9 ^{1d}	8 ^{2d}	3 ^{7d}	3 ^{7d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	8 ^{2d}	9	7 ^{2d}	7 ^{1d}	1 ^{2d}	2 ^{1d}	0	0
Mean Survival	100%		100%		85%		70%		15%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1444.7
Upper 95% confidence limit (mg KCl/L)	1607.4
48-hour LC ₅₀ (mg KCl/L)	1521.1

Comments:

Acute Silverside Test-24 Hr Survival

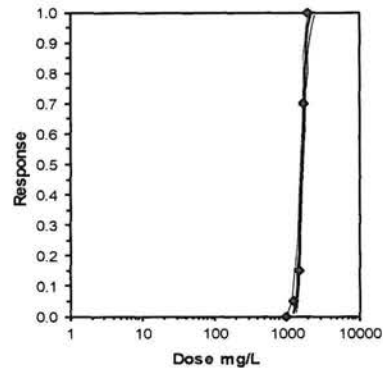
Start Date: 6/4/2024 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 6/6/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	0.9000
1500	0.9000	0.8000
1750	0.3000	0.3000
2000	0.0000	0.0000

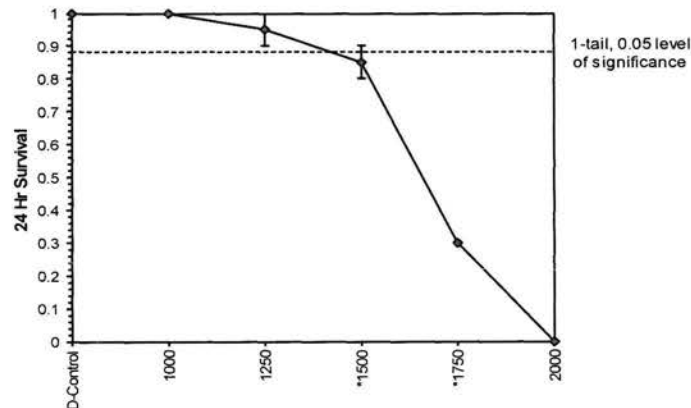
Transform: Arcsin Square Root							1-Tailed		Number		Total	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1947	0	20
1250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.192	2.850	0.1947	1	20
*1500	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.423	2.850	0.1947	3	20
*1750	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	2	12.181	2.850	0.1947	14	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
Dunnett's Test	1250	1500	1369.31		0.09486	0.09729	0.24536	0.00467	2.8E-04	4, 5
Treatments vs D-Control										

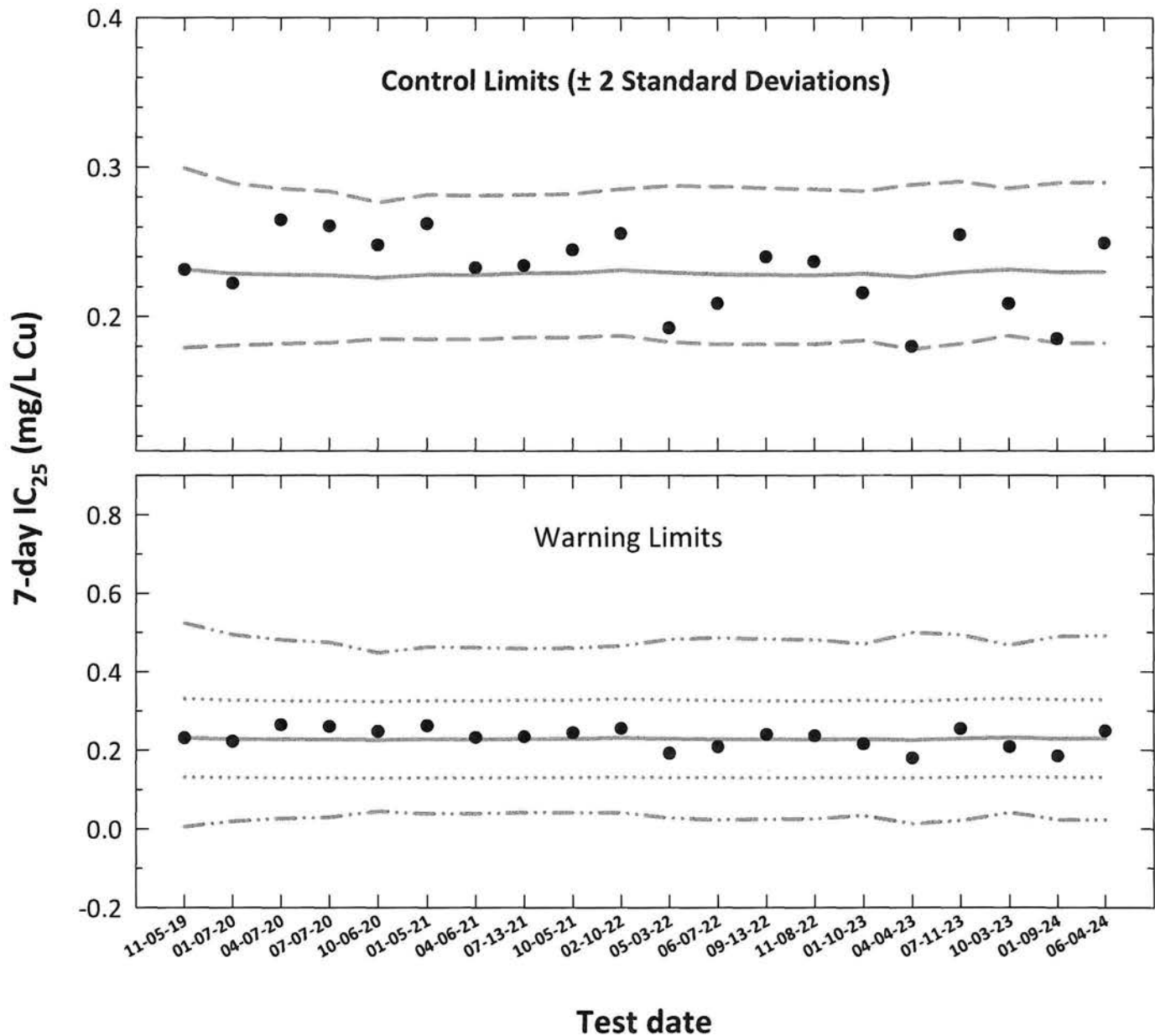
Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	19.7665	3.645	12.6223 26.9107	0	4.45334	7.81472	0.21649	3.21246	0.05059	4
Intercept	-58.499	11.727	-81.484 -35.514							
TSCR										
Point	Probits	mg/L	95% Fiducial Limits							
EC01	2.674	1243.84	1051.24 1352.49							
EC05	3.355	1346.61	1186.63 1438.27							
EC10	3.718	1404.82	1264.51 1487.7							
EC15	3.964	1445.51	1319.01 1523.06							
EC20	4.158	1478.69	1363.19 1552.68							
EC25	4.326	1507.76	1401.48 1579.44							
EC40	4.747	1583.58	1497.63 1654.62							
EC50	5.000	1631.01	1553.4 1707.28							
EC60	5.253	1679.86	1606.25 1767.11							
EC75	5.674	1764.33	1687.22 1883.3							
EC80	5.842	1799.01	1717.52 1934.81							
EC85	6.036	1840.31	1752.03 1998.36							
EC90	6.282	1893.61	1794.69 2083.3							
EC95	6.645	1975.47	1857.38 2218.83							
EC99	7.326	2138.69	1976.21 2503.27							



Dose-Response Plot



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 $\pm$ 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)

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.2300	0.1823	0.2901	0.0227	0.4914	0.1311	0.3288

Note: 7-day IC_{25} = 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 IC_{25} values.

S = Standard deviation of the IC_{25} values.

Control Limits = Mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

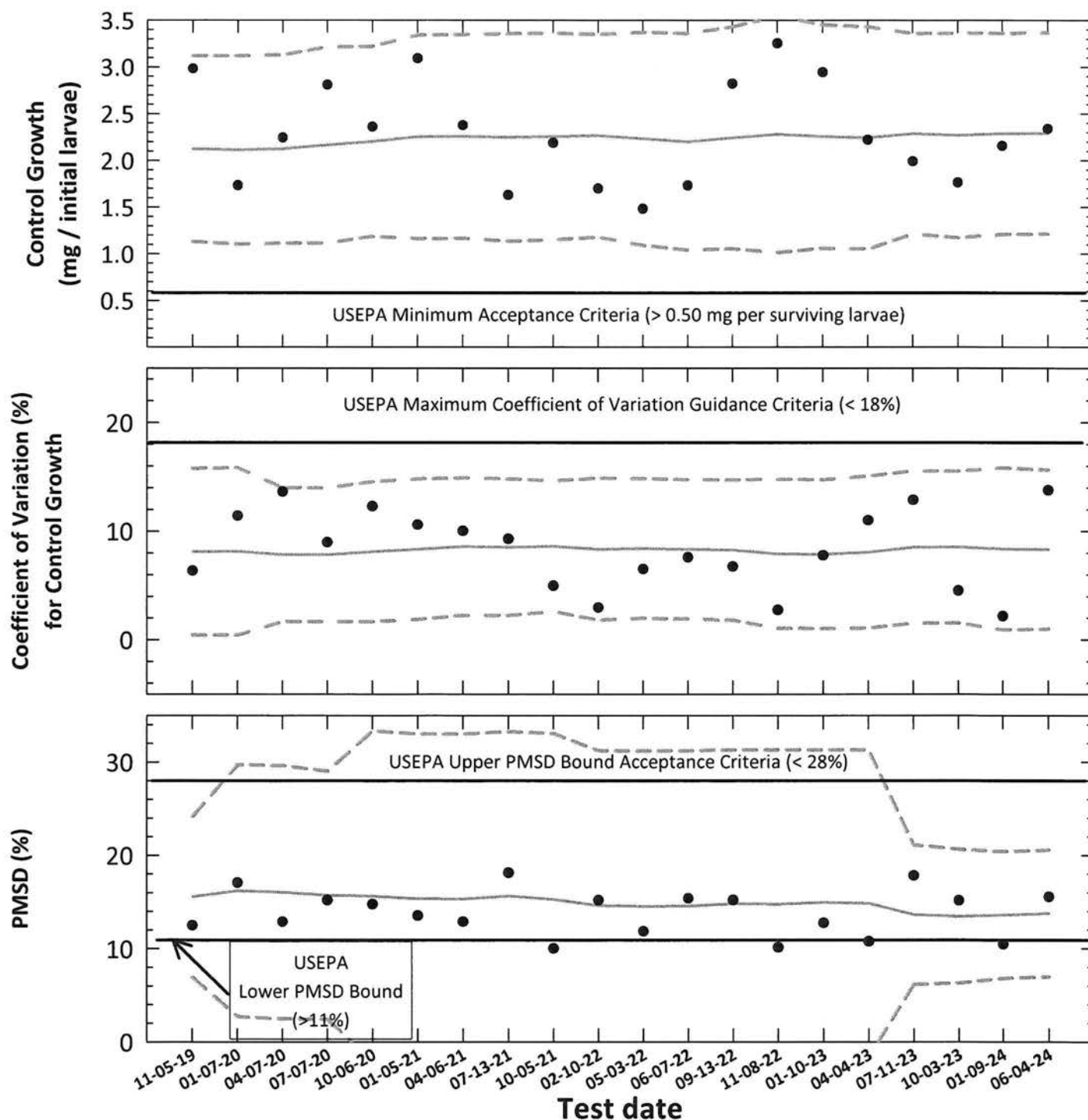
Warning Limits = Mean logarithmic $IC_{25} \pm 2CV$ or $S_{A,75}$ converted to anti-logarithmic values.

$S_{A,75}$ = 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		Test	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S
			Mean (mg/initial larvae)	CV (%)							
1	11-05-19	100	2.983	6.4	0.3722	2.1254	1.132	8.1	0.4	15.5	6.9
2	01-07-20	100	1.732	11.4	0.2954	2.112	1.105	8.1	0.4	16.2	2.7
3	04-07-20	100	2.244	13.6	0.2889	2.123	1.114	7.8	1.7	16.1	2.5
4	07-07-20	100	2.809	9.0	0.4264	2.166	1.117	7.8	1.7	15.7	2.4
5	10-06-20	100	2.361	12.3	0.3484	2.205	1.188	8.1	1.7	15.6	-2.1
6	01-05-21	100	3.093	10.6	0.4186	2.253	1.163	8.3	1.9	15.4	-2.3
7	04-06-21	100	2.377	10.0	0.3062	2.258	1.167	8.6	2.2	15.3	-2.3
8	07-13-21	100	1.631	9.3	0.2956	2.247	1.136	8.5	2.2	15.7	-2.0
9	10-05-21	100	2.189	5.0	0.2192	2.258	1.153	8.6	2.6	15.3	-2.5
10	02-10-22	100	1.701	3.0	0.2583	2.266	1.180	8.4	1.8	14.6	-2.0
11	05-03-22	100	1.483	6.5	0.1756	2.232	1.092	8.4	2.0	14.5	-2.1
12	06-07-22	100	1.733	7.6	0.2665	2.201	1.041	8.3	1.9	14.6	-2.1
13	09-13-22	100	2.822	6.8	0.4290	2.243	1.057	8.3	1.8	14.8	-1.7
14	11-08-22	100	3.255	2.7	0.3294	2.280	1.014	7.9	1.0	14.8	-1.8
15	01-10-23	100	2.945	7.8	0.3755	2.257	1.062	7.9	1.0	15.0	-1.4
16	04-04-23	100	2.220	11.0	0.2389	2.243	1.053	8.1	1.1	14.9	-1.6
17	07-11-23	100	1.992	12.9	0.3549	2.287	1.213	8.5	1.5	13.6	6.2
18	10-03-23	100	1.763	4.5	0.2673	2.270	1.174	8.5	1.5	13.5	6.3
19	01-09-24	100	2.156	2.2	0.2257	2.287	1.211	8.4	0.9	13.6	6.8
20	06-04-24	100	2.339	13.8	0.3637	2.291	1.215	8.3	1.0	13.8	7.0

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

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) < 28%.

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: 146

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS <u>2295</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:

Organism source:	Aquatic Indicators, Inc.	Randomizing template:	<u>Yellow</u>
Age:	10-days old	Incubator number and shelf location:	<u>1C</u>
Batch:	AI Mb 05-25-24	Artemia CHM number:	CHM1294
Hatch dates and times:	05-24-24 1200 to 05-25-24 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = <u>7.27</u> Temperature (°C) = <u>24.7</u>	Date / Time in oven:	<u>06-11-24 1205</u>
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>06-12-24 1205</u>
		*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	06-04-24	1110	<u>JK</u>	1300	<u>JK</u>	1220	<u>JK</u>	NA	05-24-24 B
1	06-05-24	0500	<u>JK</u>	1100	<u>JK</u>	0900	<u>JK</u>	↓	↓
2	06-06-24	0500	<u>JK</u>	1200	<u>JK</u>	0900	<u>JK</u>	↓	06-05-24 A
3	06-07-24	0500	<u>JK</u>	1200	<u>JK</u>	0900	<u>JK</u>	↓	06-05-24 B
4	06-08-24	0610	<u>JK</u>	1210	<u>JK</u>	0910	<u>JK</u>	↓	06-06-24 A
5	06-09-24	0600	<u>JK</u>	1150	<u>JK</u>	0900	<u>JK</u>	↓	06-06-24 B
6	06-10-24	0500	<u>JK</u>	1100	<u>JK</u>	0850	<u>JK</u>	↓	06-06-24
7	06-11-24					1110	<u>JK</u>		

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664645

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07</u>	≤ 20%	7-day LC ₅₀ (%)	<u>0.242</u>
Average weight per initial larvae:	<u>2.339</u>		NOEC (%)	<u>0.1</u>
Average weight per surviving larvae:	<u>2.339</u>	≥ 0.25mg/larvae	LOEC (%)	<u>0.2</u>
			ChV (%)	<u>0.14</u>
			IC ₂₅ (%)	<u>0.249</u>

Species: Menidia beryllina

MbCuCR Test Number: 146

Survival and Growth Data

Day	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>BROWN</u> Analyst: <u>EM</u> Date: <u>05-29-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>sl</u> Date: <u>06-13-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>sl</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>sl</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
2.339 2.552 -9.17 2.558 -9.37												

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

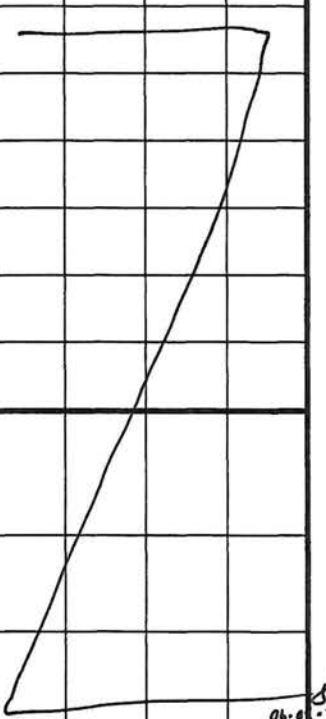
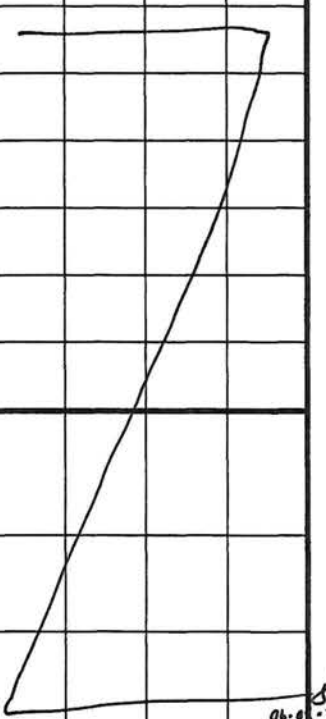
Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: Menidia beryllina

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Survival and Growth Data

Day	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	10	10	10	10	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
2	10	10	10	10	10	10	10	10				
3	10	10	10	10	10	10	10	10				
4	10	10	10	10	10	10	10	10				
5	10	10	10	10	10	10	10	10				
6	10	10	10	10	10	10	10	10				
7	10	10	10	10	9 ^{1d}	9 ^{1d}	9 ^{1d}	9 ^{1d}				
*A = Pan weight (mg) Tray color code: <u>Brown</u> Analyst: <u>gm</u> Date: <u>05-29-24</u>	13.14	14.32	13.95	14.32	13.59	15.28	14.19	14.51				
*B = Pan + Larvae weight (mg) Analyst: <u>jl</u> Date: <u>06-13-24</u>	34.16	38.48	36.80	35.21	34.48	37.88	36.86	37.50				
C = Larvae weight (mg) = B - A Analyst: <u>jl</u>	21.02	24.66	22.85	20.89	20.89	22.60	22.67	22.99				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>jl</u>	2.102	2.466	2.285	2.089	2.089	2.260	2.267	2.299	0	0	0	0
Average weight per initial number of larvae (mg)	2.236				2.229				0			
Percent reduction from control (%)	4.47				4.77				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

Test number: 146

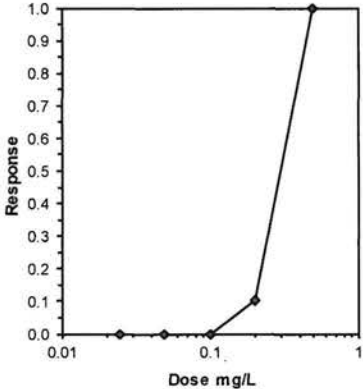
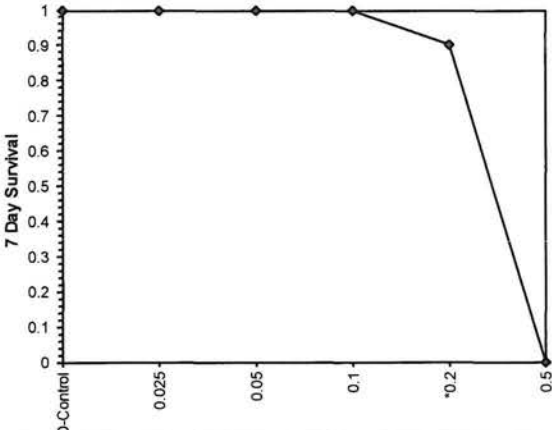
Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mass weight per surviving number of larvae) (%)	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 (%)
Control	A	10	10	14.47	37.29	22.82	2.282	2.339	13.8	2.282	2.282	100.0	2.339	13.8	Not applicable
	B	10	10	12.95	40.54	27.59	2.759			2.759					
	C	10	10	15.16	38.57	23.41	2.341			2.341					
	D	10	10	13.26	33.01	19.75	1.975			1.975					
0.025	E	10	10	13.11	37.25	24.14	2.414	2.552	8.1	2.414	2.414	100.0	2.552	8.1	-9.1
	F	10	10	13.32	37.56	24.24	2.424			2.424					
	G	10	10	13.07	41.63	28.56	2.856			2.856					
	H	10	10	15.16	40.31	25.15	2.515			2.515					
0.050	I	10	10	13.64	38.70	25.06	2.506	2.558	5.9	2.506	2.506	100.0	2.558	5.9	-9.3
	J	10	10	14.04	39.97	25.93	2.593			2.593					
	K	10	10	14.79	42.25	27.46	2.746			2.746					
	L	10	10	12.54	36.39	23.85	2.385			2.385					
0.100	M	10	10	13.14	34.16	21.02	2.102	2.236	8.0	2.102	2.102	100.0	2.236	8.0	4.4
	N	10	10	14.32	38.98	24.66	2.466			2.466					
	O	10	10	13.95	36.80	22.85	2.285			2.285					
	P	10	10	14.32	35.21	20.89	2.089			2.089					
0.200	Q	10	9	13.59	34.48	20.89	2.321	2.476	4.2	2.089	2.089	90.0	2.229	4.2	4.7
	R	10	9	15.28	37.88	22.60	2.511			2.260					
	S	10	9	14.19	36.86	22.67	2.519			2.267					
	T	10	9	14.51	37.50	22.99	2.554			2.299					
0.500	U	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.0	100.0
	V	10	0	0.00	0.00	0.00	0.000			0.000					
	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					

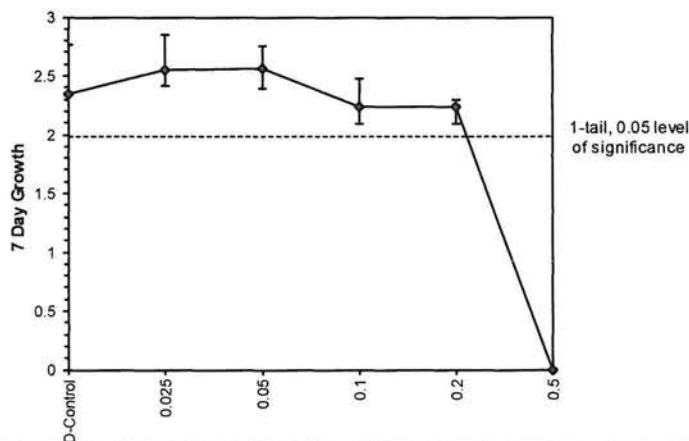
Dunnett's MSD value:
PMDS:

MSD = 0.3637
PMDS = 15.5

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

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

Larval Fish Growth and Survival Test-7 Day Survival											
Start Date:	6/4/2024	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant						
End Date:	6/11/2024	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	0.9000	0.9000	0.9000	0.9000							
0.5	0.0000	0.0000	0.0000	0.0000							
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
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	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	4	10.00	10.00	4	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 normal distribution (p > 0.01)								1	0.868		
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											
Trimmed Spearman-Kärber											
Trim Level	EC50	95% CL									
0.0%	0.2918	0.2703	0.3149								
5.0%	0.2981	0.2714	0.3274								
10.0%	0.3005	0.2848	0.3171								
20.0%	0.3005	0.2848	0.3171								
Auto-0.0%	0.2918	0.2703	0.3149								
											
Dose-Response Plot											
											



Species: Menidia beryllina

MbCuCR Test Number: 146

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.

Analyst		Day (Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
		KL	KD	KL	KD	KL	KD
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	7.71	7.62	7.65	7.77	7.92	7.34
	Dissolved oxygen (mg/L)	7.0	7.7	7.8	7.6	7.9	6.6
	Salinity (ppt)	25.0	24.5	24.9	24.6	25.0	24.9
	Alkalinity (mg CaCO ₃ /L)	72			150		
	Temperature (°C)	25.0	25.1	24.8	25.2	24.7	25.0
0.025 mg/L	pH (S.U.)	7.46	7.58	7.67	7.51	7.91	7.33
	Dissolved oxygen (mg/L)	7.0	7.6	7.9	7.5	7.9	6.6
	Salinity (ppt)	24.8	24.9	25.0	25.0	25.0	25.1
	Temperature (°C)	25.1	24.9	24.9	25.0	24.7	25.2
0.05 mg/L	pH (S.U.)	7.46	7.58	7.65	7.49	7.91	7.32
	Dissolved oxygen (mg/L)	7.0	7.7	7.9	7.6	7.9	6.6
	Salinity (ppt)	24.9	24.9	24.8	25.0	25.0	25.1
	Temperature (°C)	25.1	25.2	24.9	25.0	24.6	25.2
0.1 mg/L	pH (S.U.)	7.47	7.58	7.66	7.49	7.91	7.33
	Dissolved oxygen (mg/L)	7.0	7.7	7.9	7.7	8.0	6.6
	Salinity (ppt)	24.9	25.0	24.8	25.1	25.0	25.2
	Temperature (°C)	25.0	25.2	24.9	25.1	24.6	25.0
0.2 mg/L	pH (S.U.)	7.47	7.58	7.66	7.50	7.91	7.30
	Dissolved oxygen (mg/L)	7.0	7.8	8.0	7.8	8.0	6.7
	Salinity (ppt)	24.9	25.1	24.7	25.1	25.0	25.4
	Temperature (°C)	25.0	25.2	24.9	25.1	24.6	25.0
0.5 mg/L	pH (S.U.)	7.47	7.54	7.64			
	Dissolved oxygen (mg/L)	7.0	7.8	8.0			
	Salinity (ppt)	24.9	25.1	24.5			
	Alkalinity (mg CaCO ₃ /L)	NA					
	Temperature (°C)	25.0	25.0	24.9			
		Initial	Final	Initial	Final	Initial	Final

* NEW BATCH OF SALT.

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		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
		Analyst	BSL	BSL	BSL	BSL	BSL	BSL	BSL
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.52	7.71	7.55 (8.33)	7.74	7.64	7.94	7.99	7.54
	Dissolved oxygen (mg/L)	7.6	7.7	8.0	7.6	7.8	7.6	7.9	7.2
	Salinity (ppt)	24.9	25.1	24.9	25.0	25.1	25.2	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	150		150		160		160	
	Temperature (°C)	24.7	24.7	24.8	25.2	24.6	25.0	24.8	25.0
0.025 mg/L	pH (S.U.)	7.51	7.68	7.83	7.72	7.62	7.96	8.02	7.50
	Dissolved oxygen (mg/L)	7.5	7.7	8.0	7.6	7.8	7.5	7.9	7.4
	Salinity (ppt)	25.0	25.1	25.0	25.1	25.0	25.6	25.5	25.7
	Temperature (°C)	24.6	24.9	24.7	25.0	24.7	25.1	24.8	24.8
0.05 mg/L	pH (S.U.)	7.53	7.64	7.72	7.68	7.64	7.97	8.04	7.57
	Dissolved oxygen (mg/L)	7.5	7.7	8.0	7.6	7.8	7.4	7.9	7.3
	Salinity (ppt)	25.0	25.1	25.0	25.1	25.0	25.6	25.5	25.7
	Temperature (°C)	24.6	25.0	24.7	25.0	24.7	24.9	24.9	24.8
0.1 mg/L	pH (S.U.)	7.54	7.65	7.75	7.67	7.75	7.98	8.06	7.58
	Dissolved oxygen (mg/L)	7.6	7.6	8.1	7.5	7.8	7.3	7.7	7.2
	Salinity (ppt)	25.0	25.1	24.9	25.1	25.1	25.5	25.5	25.6
	Temperature (°C)	24.6	25.0	24.9	25.3	24.7	24.9	24.9	25.1
0.2 mg/L	pH (S.U.)	7.55	7.61	7.78	7.66	7.78	7.97	8.07	7.60
	Dissolved oxygen (mg/L)	7.6	7.5	8.1	7.5	7.8	7.3	8.0	7.2
	Salinity (ppt)	24.9	25.3	25.0	25.3	25.1	25.7	25.5	25.6
	Temperature (°C)	24.6	24.8	24.9	25.1	24.7	25.1	24.9	24.9
0.5 mg/L	pH (S.U.)								
	Dissolved oxygen (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final