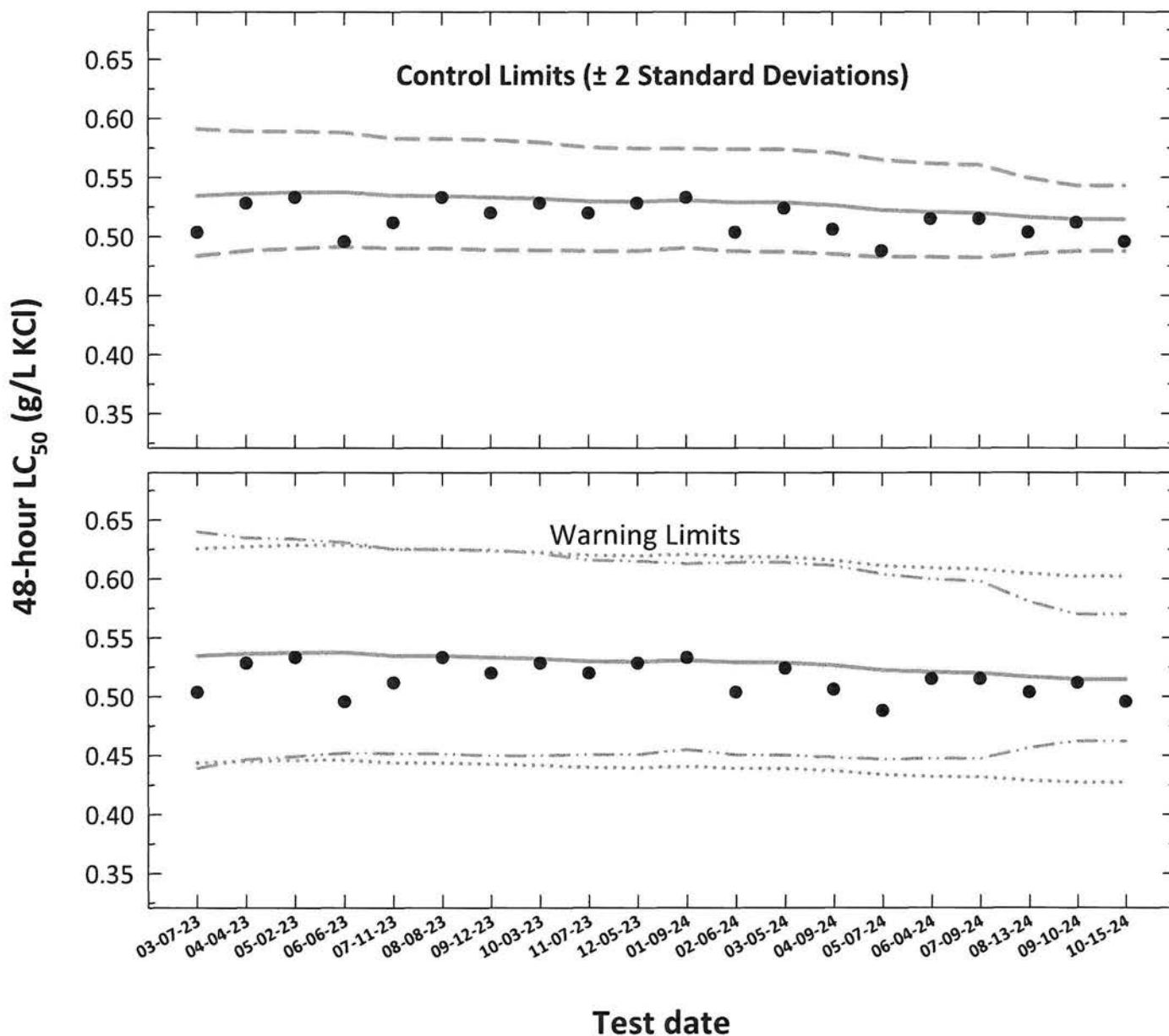


Americamysis (Mysidopsis) bahia Acute Reference Toxicant Control Chart Source: Aquatic Indicators, Inc.



- **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, $S_{A,10} = 10^{\text{th}}$ 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 Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392	0.6402
2	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350
3	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338
4	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312
5	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252
6	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250
7	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244
8	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217
9	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161
10	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151
11	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550	0.6131
12	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875	0.5740	0.4505	0.6141
13	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872	0.5738	0.4502	0.6140
14	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853	0.5711	0.4483	0.6113
15	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829	0.5651	0.4467	0.6041
16	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4827	0.5620	0.4476	0.5998
17	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822	0.5606	0.4473	0.5982
18	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4856	0.5497	0.4565	0.5807
19	09-10-24	0.5117	-0.2910	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700
20	10-15-24	0.4957	-0.3048	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700
									CT - S _{A,10}	CT + S _{A,10}
									0.4438	0.6255
									0.4452	0.6275
									0.4459	0.6286
									0.4462	0.6290
									0.4437	0.6255
									0.4436	0.6253
									0.4426	0.6239
									0.4417	0.6226
									0.4400	0.6202
									0.4395	0.6195
									0.4407	0.6212
									0.4391	0.6189
									0.4389	0.6187
									0.4370	0.6160
									0.4335	0.6111
									0.4323	0.6094
									0.4315	0.6083
									0.4288	0.6045
									0.4272	0.6022
									0.4272	0.6022

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 # 283

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 #: 2319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.72	7.72	7.66
	Dissolved oxygen (mg/L)	7.8	7.6	7.5
	*Salinity (ppt)	24.8	25.0	25.2
	*Alkalinity (mg/L CaCO ₃)	76		
	*Temperature (°C)	24.7	25.1	25.0
250 mg/L	pH (S.U.)	7.73	7.72	7.65
	Dissolved oxygen (mg/L)	7.8	7.5	7.5
	*Salinity (ppt)	25.2	25.3	25.5
	*Temperature (°C)	24.7	25.1	24.9
375 mg/L	pH (S.U.)	7.73	7.71	7.64
	Dissolved oxygen (mg/L)	7.9	7.5	7.6
	*Salinity (ppt)	25.3	25.3	25.7
	*Temperature (°C)	24.6	25.2	24.9
500 mg/L	pH (S.U.)	7.75	7.70	7.67
	Dissolved oxygen (mg/L)	7.9	7.5	7.6
	*Salinity (ppt)	25.4	25.5	25.9
	*Temperature (°C)	24.6	25.2	25.2
750 mg/L	pH (S.U.)	7.75	7.70	
	Dissolved oxygen (mg/L)	7.9	7.3	
	*Salinity (ppt)	25.5	25.7	
	*Temperature (°C)	24.6	25.0	
1000 mg/L	pH (S.U.)	7.75	7.70	
	Dissolved oxygen (mg/L)	7.9	7.1	
	*Salinity (ppt)	25.6	25.9	
	*Temperature (°C)	24.6	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-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	1306246

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 # 283

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-15-24	1215	jl	1415	jl	1B	ORANGE	10-13-24A
24	10-16-24			1420	jl			
48 Termination	10-17-24			1417	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):	AIAB 10-14-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	10-13-24 1200 TO 10-14-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86 Temperature (°C) 24.9

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	9 ^u	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^u	10	5 ^u	5 ^u	0	0	0	0
Mean Survival	100%		100%		95%		50%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	458.6
Upper 95% confidence limit (mg KCl/L)	547.7
48-hour LC ₅₀ (mg KCl/L)	495.7

Comments:



Acute Mysid Test-24 Hr Survival

Start Date: 10/15/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 10/17/2024 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	1.0000	0.9000
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	0.1469	0	20	
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20	
500	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20	
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20	
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

Auxiliary Tests Statistic Critical Skew Kurt

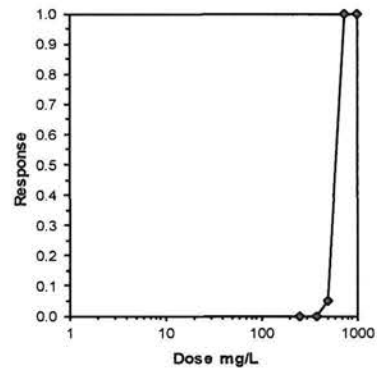
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

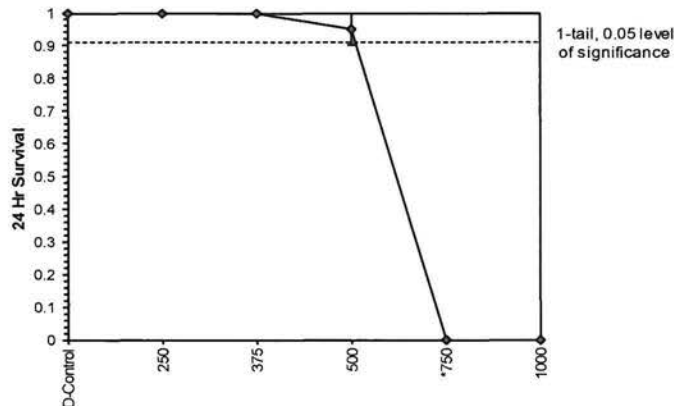
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.06555	0.06723	0.61047	0.00266	7.5E-06	4, 5

Treatments vs D-Control

Trimmed Spearman-Kärber			
Trim Level	EC50	95%CL	
0.0%	601.85	581.86	622.53
5.0%	605.87	592.75	619.28
10.0%	605.87	592.75	619.28
20.0%	605.87	592.75	619.28
Auto-0.0%	601.85	581.86	622.53



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 10/15/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 10/17/2024 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	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	1.0000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

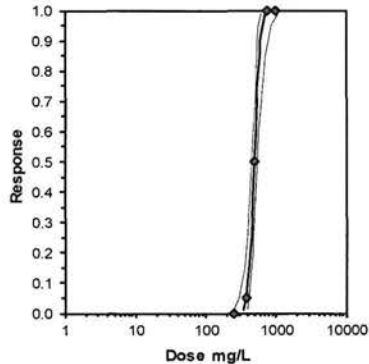
Transform: Arcsin Square Root								1-Tailed		Number		Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	12.159	2.850	0.1469	10	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

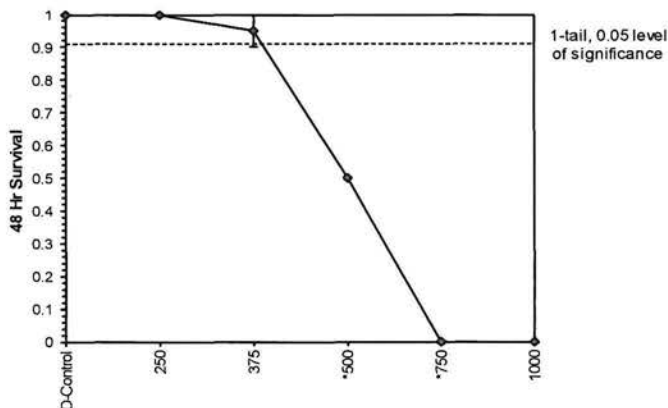
Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.06555	0.06723	0.60026	0.00266	7.8E-06	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	14.3605	3.51976	7.4618	21.2592	0	0.17806	7.81472	0.98105	2.69519	0.06964	4
Intercept	-33.704	9.43318	-52.193	-15.215							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	341.348	245.982	387.152							
EC05	3.355	380.762	301.411	419.772							
EC10	3.718	403.601	334.964	439.493							
EC15	3.964	419.779	358.932	454.273							
EC20	4.158	433.099	378.48	467.26							
EC25	4.326	444.862	395.35	479.6							
EC40	4.747	475.939	436.335	517.953							
EC50	5.000	495.671	458.62	547.68							
EC60	5.253	516.22	478.836	582.992							
EC75	5.674	552.283	509.223	653.418							
EC80	5.842	567.283	520.655	685.187							
EC85	6.036	585.283	533.773	724.894							
EC90	6.282	608.744	550.147	778.99							
EC95	6.645	645.258	574.488	867.975							
EC99	7.326	719.763	621.393	1066.13							



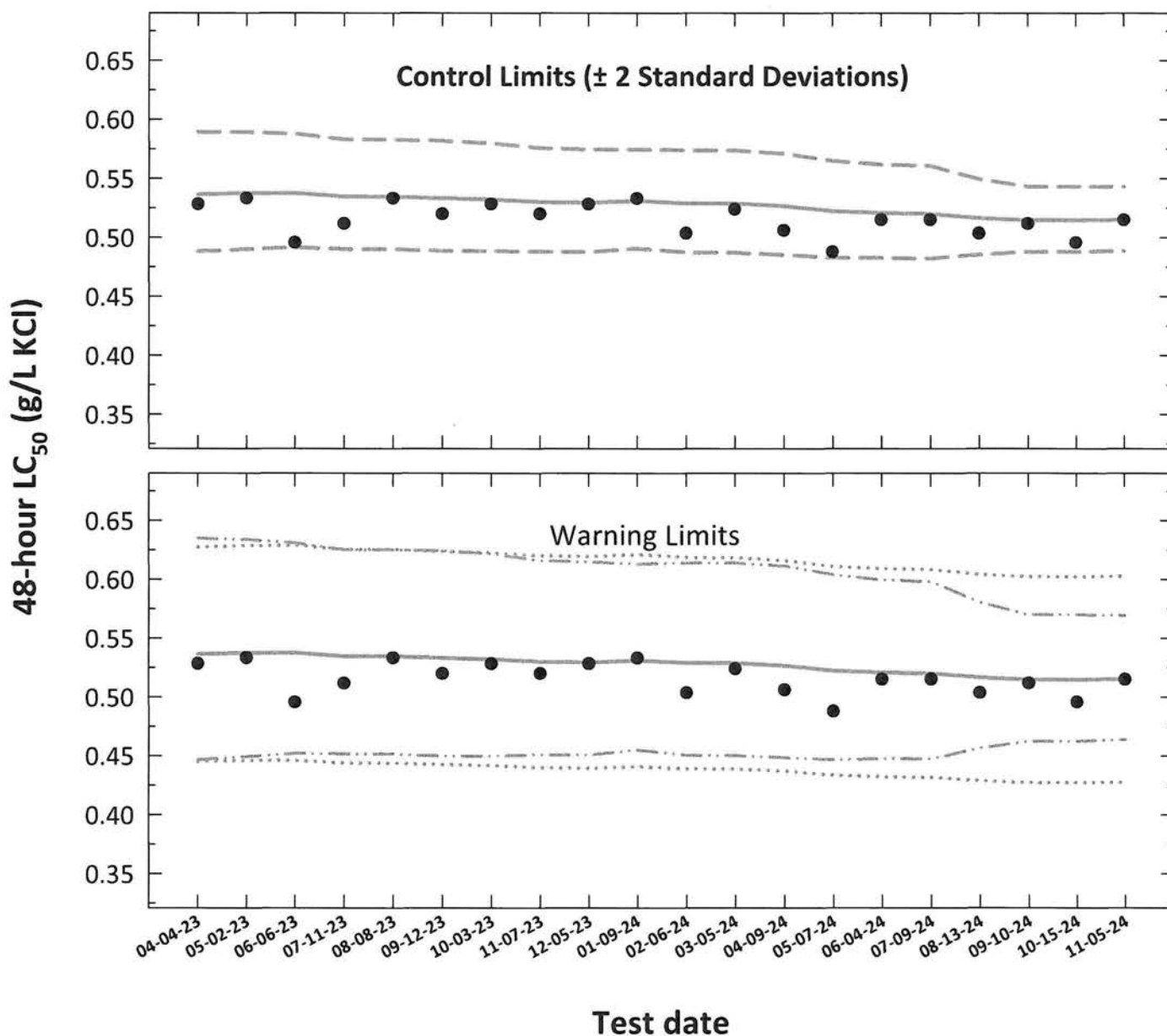
Dose-Response Plot



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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822	0.5606	0.4473	0.5982	0.4315	0.6083
17	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4856	0.5497	0.4565	0.5807	0.4288	0.6045
18	09-10-24	0.5117	-0.2910	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700	0.4272	0.6022
19	10-15-24	0.4957	-0.3048	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700	0.4272	0.6022
20	11-05-24	0.5149	-0.2882	-0.2880	0.0115	0.5153	0.4888	0.5432	0.4638	0.5695	0.4277	0.6029

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 # 284

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 #: 2319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.09	7.44	7.55
	Dissolved oxygen (mg/L)	7.7	7.6	7.5
	*Salinity (ppt)	25.1	25.0	25.1
	*Alkalinity (mg/L CaCO ₃)	94		
	*Temperature (°C)	25.0	25.2	25.3
250 mg/L	pH (S.U.)	7.70	7.45	7.75
	Dissolved oxygen (mg/L)	7.7	7.6	7.6
	*Salinity (ppt)	25.1	25.1	25.3
	*Temperature (°C)	25.1	25.0	25.0
375 mg/L	pH (S.U.)	7.72	7.48	7.75
	Dissolved oxygen (mg/L)	7.7	7.6	7.5
	*Salinity (ppt)	25.1	25.2	25.5
	*Temperature (°C)	24.9	25.2	25.0
500 mg/L	pH (S.U.)	7.73	7.49	7.77
	Dissolved oxygen (mg/L)	7.8	7.6	7.5
	*Salinity (ppt)	25.3	25.2	25.4
	*Temperature (°C)	24.9	25.2	25.2
750 mg/L	pH (S.U.)	7.74	7.52	
	Dissolved oxygen (mg/L)	7.8	7.7	
	*Salinity (ppt)	25.4	25.6	
	*Temperature (°C)	24.9	25.2	
1000 mg/L	pH (S.U.)	7.75	7.81	
	Dissolved oxygen (mg/L)	7.9	7.8	
	*Salinity (ppt)	25.4	25.6	
	*Temperature (°C)	25.2	25.1	

*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	130664685

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 # 284

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-05-24	* 1130	J	1330	J	6C	LGHT GREEN	10-30-24B
24	11-06-24			1320	K			
48 Termination	11-07-24			1325	K			

*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 11-04-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	11-03-24 1200 TO 11-04-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.79 Temperature (°C) 24.8

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	7 ^{3d}	9 ^{4d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	5 ^{2d}	5 ^{4d}	0	0	0	0
Mean Survival	100%		100%		100%		50%		0%		0%	

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

Statistics:

Method	SK
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:

Acute Mysid Test-24 Hr Survival

Start Date: 11/5/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/7/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.7000	0.9000
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	0.2324	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2324	0	20
*500	0.8000	0.8000	1.1201	0.9912	1.2490	16.280	2	3.580	2.850	0.2324	4	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.367	2.850	0.2324	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

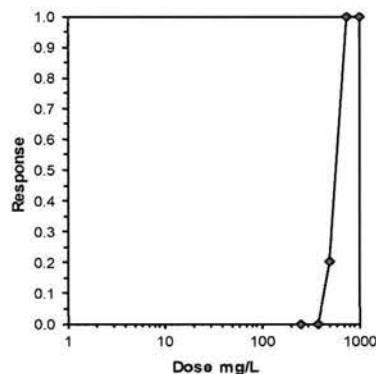
Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.12039	0.12348	0.58916	0.00665	7.9E-05	4, 5

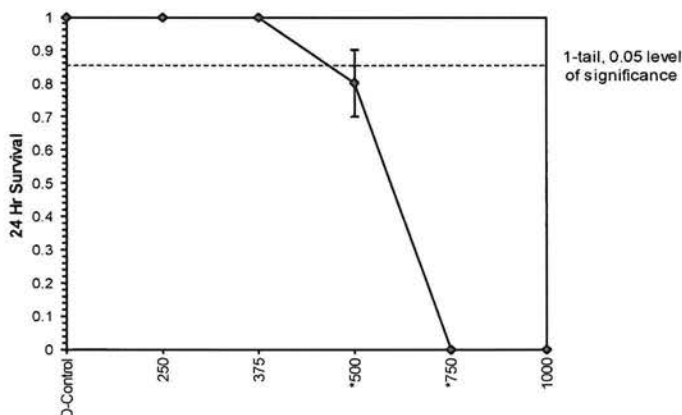
Treatments vs D-Control

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	571.36	537.02 607.91
5.0%	575.37	536.19 617.41
10.0%	578.73	531.66 629.96
20.0%	582.11	550.04 616.05
Auto-0.0%	571.36	537.02 607.91



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 11/5/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/7/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis 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

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	0.5000	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

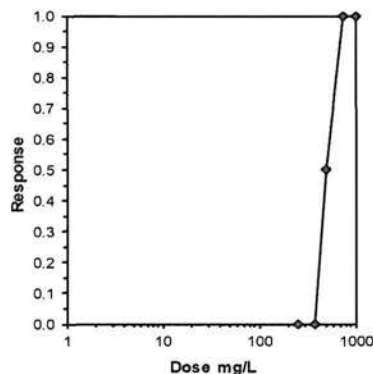
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

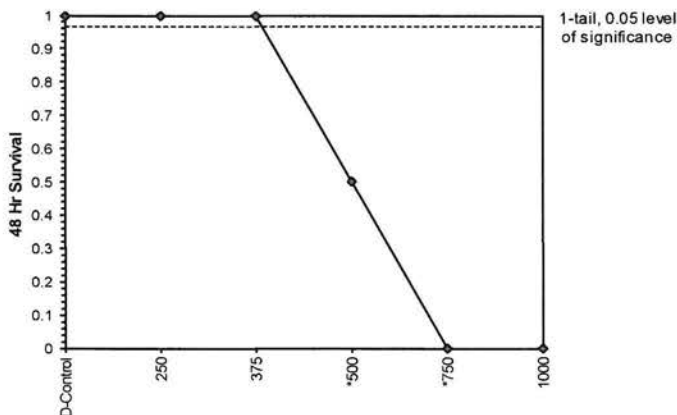
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.62824	0.0001	2.0E-09	4, 5

Treatments vs D-Control

Trim Level	EC50	95% CL	
		Lower	Upper
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



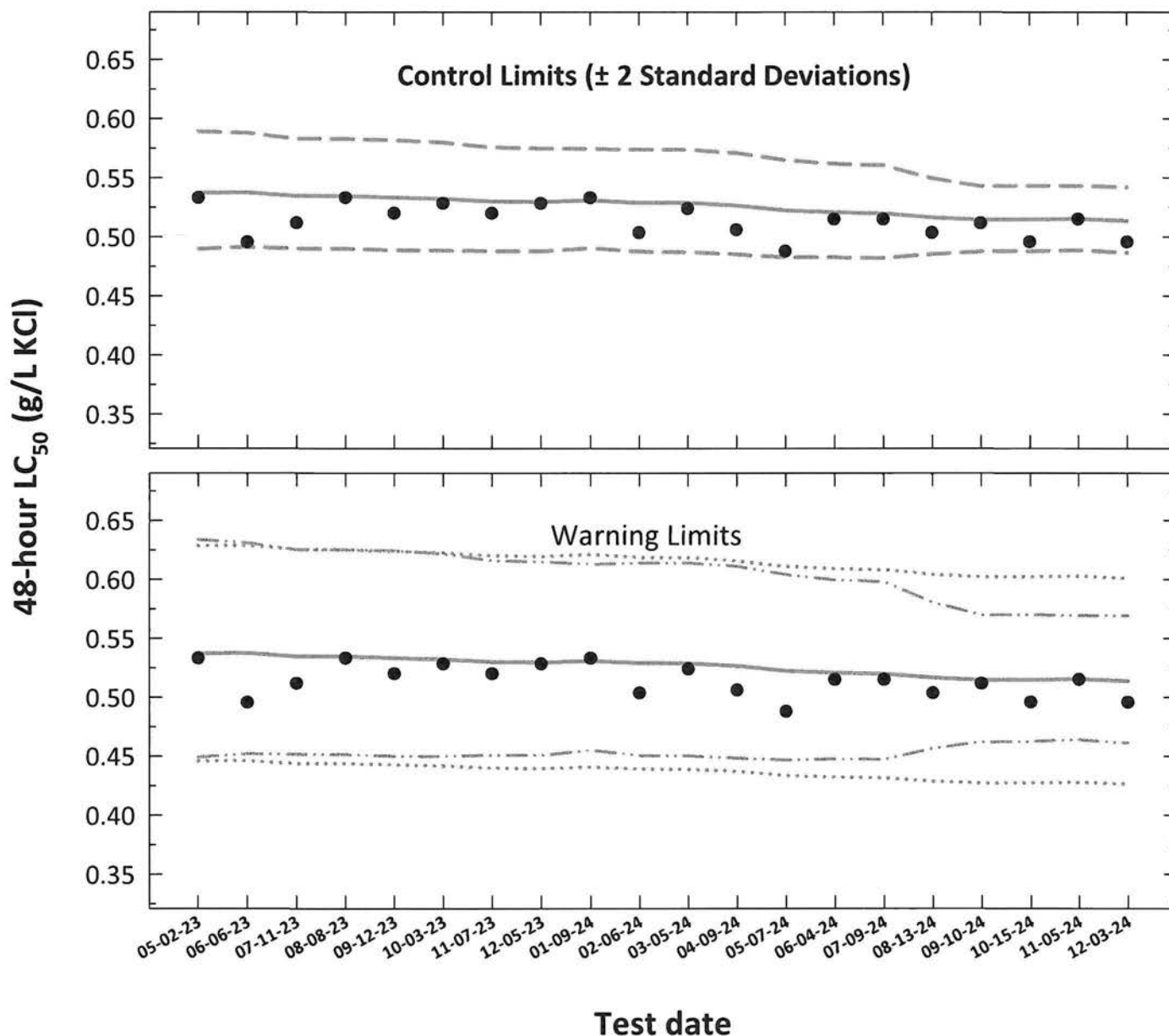
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822 0.5606	0.4473 0.5982	0.4315 0.6083
16	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4856 0.5497	0.4565 0.5807	0.4288 0.6045
17	09-10-24	0.5117	-0.2910	-0.2884	0.0117	0.5147	0.4877 0.5431	0.4623 0.5700	0.4272 0.6022
18	10-15-24	0.4957	-0.3048	-0.2884	0.0117	0.5147	0.4877 0.5431	0.4623 0.5700	0.4272 0.6022
19	11-05-24	0.5149	-0.2882	-0.2880	0.0115	0.5153	0.4888 0.5432	0.4638 0.5695	0.4277 0.6029
20	12-03-24	0.4957	-0.3048	-0.2893	0.0118	0.5136	0.4866 0.5422	0.4609 0.5693	0.4263 0.6009

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 # 285

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 #: 2334

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.76	7.87	7.82
	Dissolved oxygen (mg/L)	7.8	7.4	7.4
	*Salinity (ppt)	25.0	25.4	25.4
	*Alkalinity (mg/L CaCO ₃)	120		
	*Temperature (°C)	25.6	25.3	25.3
250 mg/L	pH (S.U.)	7.74	7.88	7.82
	Dissolved oxygen (mg/L)	7.8	7.5	7.4
	*Salinity (ppt)	25.3	25.5	25.6
	*Temperature (°C)	25.3	25.1	25.2
375 mg/L	pH (S.U.)	7.75	7.89	7.83
	Dissolved oxygen (mg/L)	7.8	7.5	7.4
	*Salinity (ppt)	25.4	25.7	25.7
	*Temperature (°C)	25.4	25.1	25.2
500 mg/L	pH (S.U.)	7.76	7.90	7.85
	Dissolved oxygen (mg/L)	7.8	7.6	7.4
	*Salinity (ppt)	25.4	25.9	25.9
	*Temperature (°C)	25.4	25.4	25.2
750 mg/L	pH (S.U.)	7.77	7.88	
	Dissolved oxygen (mg/L)	7.8	7.6	
	*Salinity (ppt)	25.6	26.0	
	*Temperature (°C)	25.5	25.4	
1000 mg/L	pH (S.U.)	7.78	7.88	
	Dissolved oxygen (mg/L)	7.8	7.6	
	*Salinity (ppt)	25.7	26.2	
	*Temperature (°C)	25.5	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-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	13066466

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 # 285

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-03-24	* 1145	h	1345	h	6C	Yellow	11-27-24 A
24	12-04-24			1340	h			
48 Termination	12-05-24			1340	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):	12-02-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	12-01-24 1200 TO 12-02-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.89 Temperature (°C) 24.2

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 ^{sd}	6 ^{sd}	0 ^{100d}	0 ^{100d}	0 ^{100d}	0 ^{100d}
48 Termination	10	10	10	10	10	9 ^{sd}	4 ^{sd}	6	0	0	0	0
Mean Survival	1007.		1007.		957.		507.		07.		07.	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	458.6
Upper 95% confidence limit (mg KCl/L)	547.7
48-hour LC ₅₀ (mg KCl/L)	495.7

Comments:



Acute Mysid Test-24 Hr Survival

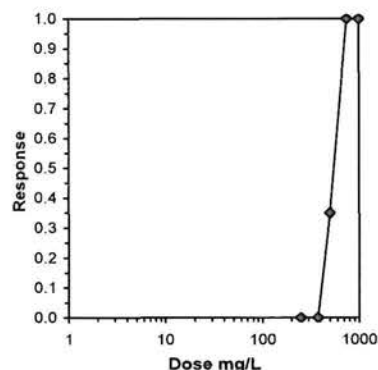
Start Date: 12/3/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 12/5/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.7000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

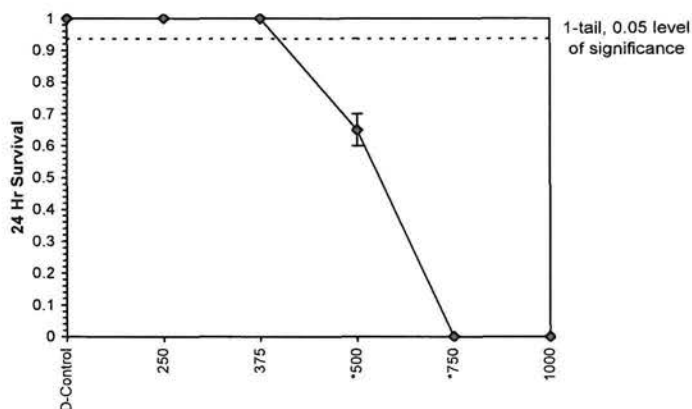
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0947	0	20
375	1.0000	1.0000	1.4120	1.4120	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

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		0.03789	0.03886	0.59923	0.0011	8.8E-07	4, 5
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-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 12/3/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 12/5/2024 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	1.0000	0.9000
500	0.4000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%					
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	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.995	2.850	1	20
*500	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	7.649	2.850	10	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.299	2.850	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2			20	20

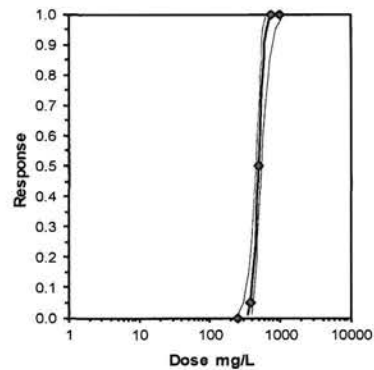
Auxiliary Tests

Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

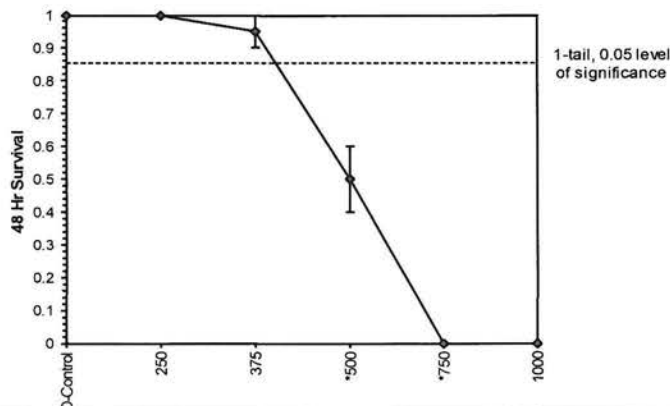
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.12113	0.12423	0.60026	0.00671	7.7E-05	4,5
Treatments vs D-Control										

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.3605	3.51976	7.4618	21.2592	0	0.17806	7.81472	0.98105	2.69519	0.08964	4
Intercept	-33.704	9.43318	-52.193	-15.215							

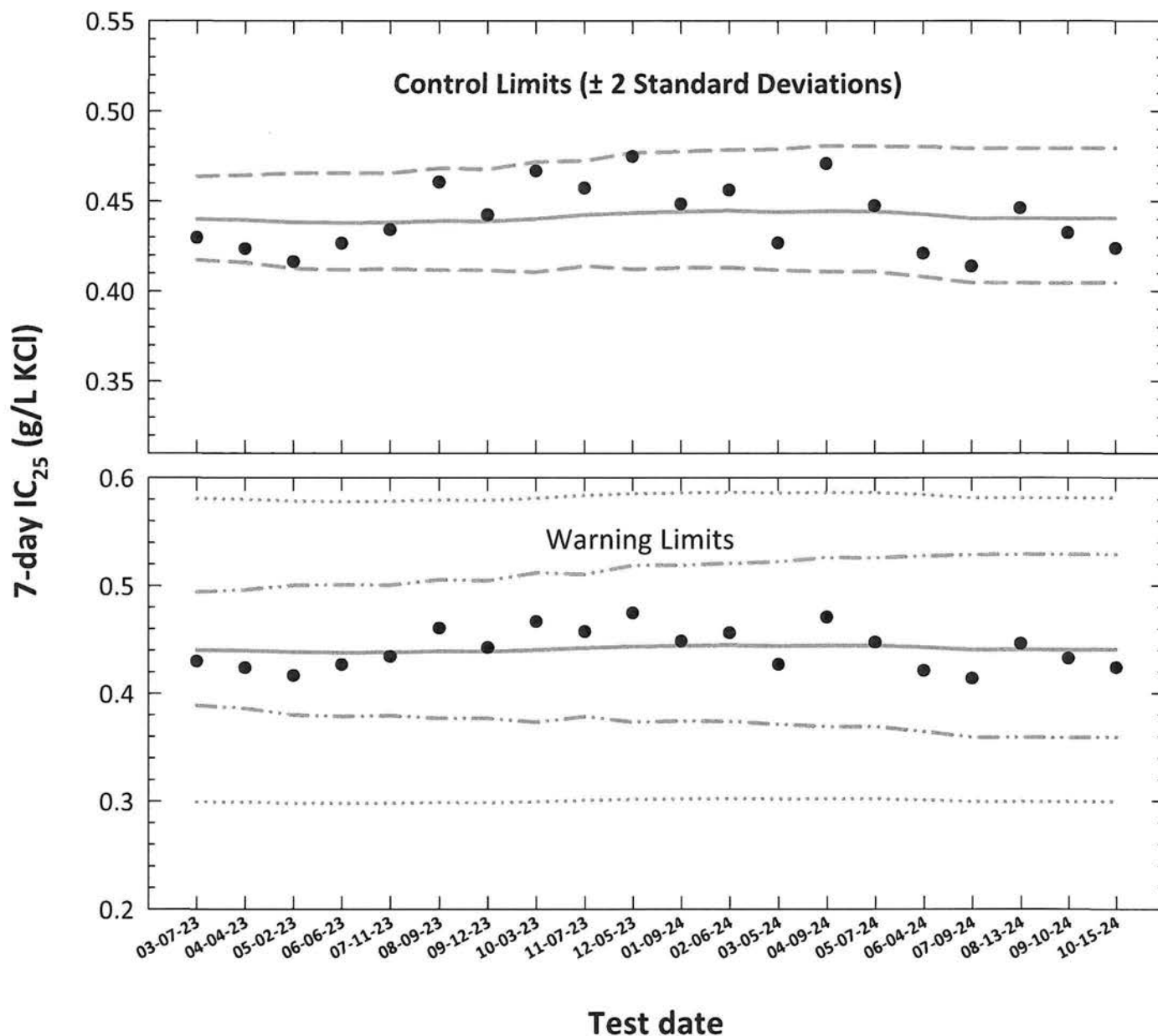
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	341.348	245.982	387.152
EC05	3.355	380.762	301.411	419.772
EC10	3.718	403.601	334.964	439.493
EC15	3.964	419.779	358.932	454.273
EC20	4.158	433.099	378.48	467.26
EC25	4.326	444.862	395.35	479.6
EC40	4.747	475.939	436.335	517.953
EC50	5.000	495.671	458.62	547.68
EC60	5.253	516.22	478.836	582.992
EC75	5.674	552.283	509.223	653.418
EC80	5.842	567.283	520.655	685.187
EC85	6.036	585.283	533.773	724.894
EC90	6.282	608.744	550.147	778.99
EC95	6.645	645.258	574.488	867.975
EC99	7.326	719.763	621.393	1066.13



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 CT - 2S CT + 2S
1	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174 0.4638
2	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158 0.4643
3	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126 0.4654
4	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118 0.4655
5	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124 0.4655
6	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116 0.4683
7	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116 0.4677
8	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106 0.4719
9	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140 0.4725
10	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123 0.4769
11	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131 0.4775
12	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133 0.4785
13	03-05-24	0.4268	-0.3698	-0.3527	0.0164	0.4440	0.4117 0.4788
14	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109 0.4808
15	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109 0.4805
16	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082 0.4804
17	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047 0.4794
18	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048 0.4796
19	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046 0.4796
20	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048 0.4795
						Laboratory Calculated CV Warning Limits	
						CT - 2CV	CT + 2CV
						0.3886	0.4941
						0.3857	0.4961
						0.3798	0.5003
						0.3784	0.5011
						0.3794	0.5006
						0.3766	0.5056
						0.3769	0.5047
						0.3730	0.5122
						0.3784	0.5105
						0.3732	0.5190
						0.3744	0.5192
						0.3740	0.5208
						0.3712	0.5224
						0.3690	0.5261
						0.3690	0.5258
						0.3645	0.5278
						0.3593	0.5288
						0.3593	0.5291
						0.3590	0.5292
						0.3594	0.5289
						75th Percentile CV Warning Limits	
						CT - S _{A,75}	CT + S _{A,75}
						0.2992	0.5807
						0.2988	0.5800
						0.2980	0.5785
						0.2977	0.5780
						0.2979	0.5783
						0.2985	0.5795
						0.2983	0.5791
						0.2993	0.5810
						0.3008	0.5838
						0.3015	0.5853
						0.3020	0.5863
						0.3024	0.5870
						0.3019	0.5860
						0.3022	0.5867
						0.3022	0.5865
						0.3011	0.5845
						0.2995	0.5814
						0.2996	0.5816
						0.2995	0.5814
						0.2996	0.5815

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.

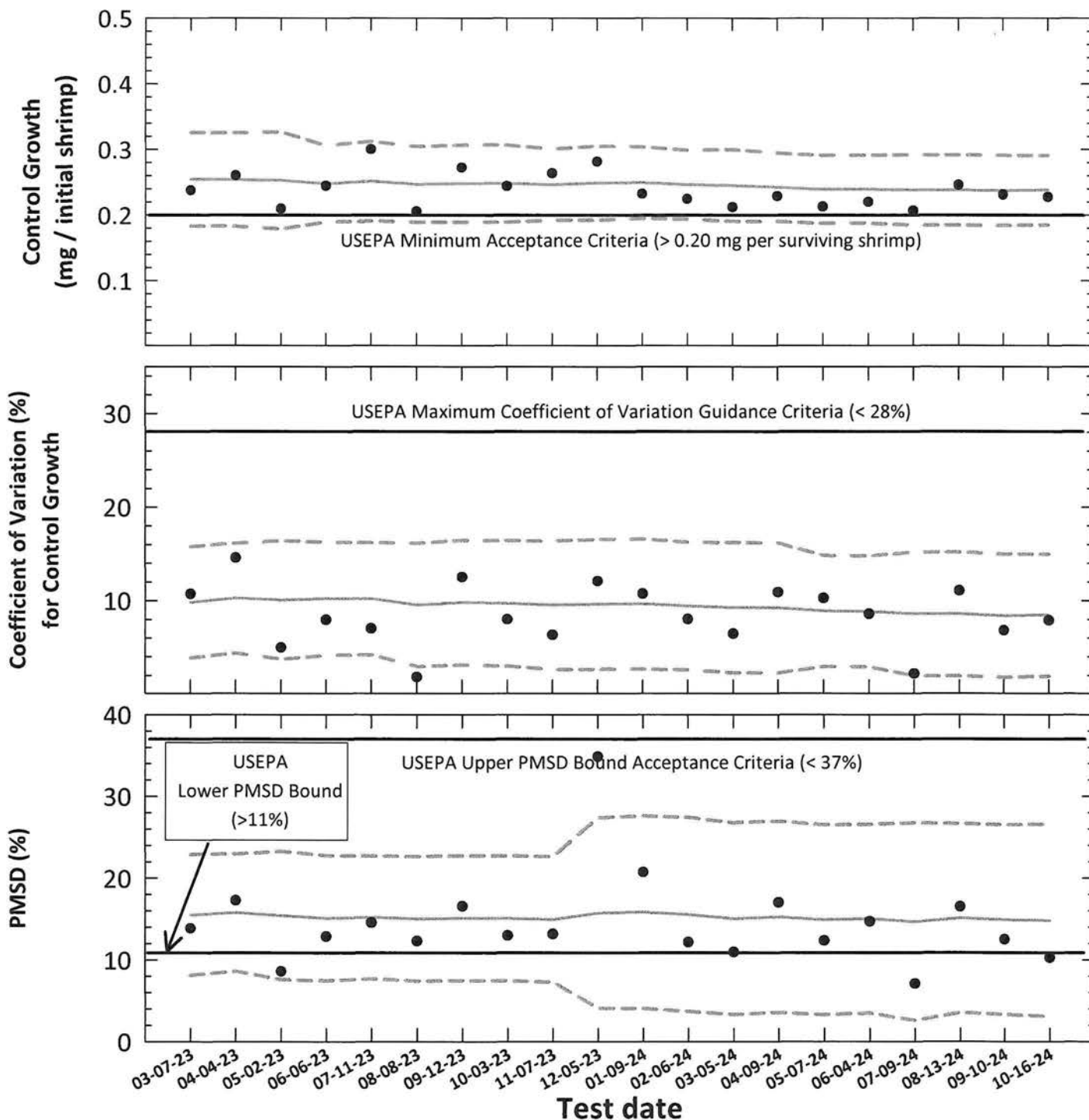
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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 (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Growth		Test	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial shrimp)	CV (%)														
1	03-07-23	100	0.238	10.7	0.0329	13.9	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9	15.5	8.1	22.9
2	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0	15.8	8.6	23.0
3	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3	15.4	7.6	23.3
4	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7	15.1	7.4	22.7
5	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7	15.2	7.7	22.7
6	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6	15.0	7.4	22.6
7	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7	15.1	7.4	22.7
8	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7	15.1	7.4	22.7
9	11-07-23	100	0.264	6.3	0.0347	13.2	0.249	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6	14.9	7.3	22.6
10	12-05-23	100	0.282	12.1	0.0581	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4	15.7	4.1	27.4
11	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6	15.8	4.0	27.6
12	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4	15.5	3.7	27.4
13	03-05-24	100	0.212	6.5	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8	15.0	3.3	26.8
14	04-09-24	100	0.229	10.9	0.0389	17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9	15.2	3.5	26.9
15	05-07-24	100	0.213	10.3	0.0263	12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5	14.9	3.3	26.5
16	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6	15.0	3.5	26.6
17	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7	14.6	2.6	26.7
18	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	15.1	3.5	26.7	15.1	3.5	26.7
19	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.9	3.3	26.5	14.9	3.3	26.5
20	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.8	3.1	26.5	14.8	3.1	26.5

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: **256**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2319					
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:	6/24/24
Date and times organisms were born between:	10-08-24 1200 to 10-09-24 1130	Incubator number and shelf location:	75B
Organism source:	AI Batch Ab: 10-09-24	Artemia CHM number:	CHM1294
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.93 S.U. Temperature = 25.1 °C	Date / Time in oven:	10-22-24 1215
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	10-23-24 1215
		*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	10-15-24	1215	H	1500	H	1300	H	10-13-24 A
1	10-16-24	0500	H	1100	H	0700	H	↓
2	10-17-24	0500	H	1400	H	1200	H	10-15-24
3	10-18-24	0500	H	1100	H	0700	H	↓
4	10-19-24	0500	H	1230	H	1100	H	10-17-24
5	10-20-24	0500	H	1100	H	0710	H	↓
6	10-21-24	0500	H	1100	H	0700	H	↓
7	10-22-24					1100	H	

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	150004665

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07	≤ 20%	7-day LC ₅₀ (mg/L KCl)	502.4
Average weight per initial shrimp:	0.228		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.228	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	423.8

AbKCICR Test Number: **256**

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																10-11-1
*A = Pan weight (mg) Tray color code: <u>Turquoise</u> Analyst: <u>km</u> Date: <u>10-25-24</u>	13.53	15.35	14.81	15.30 15.29 15.27	14.59	12.38	13.77	14.47	13.88	12.94	13.83	12.56	13.04	13.30	13.90	13.55
*B = Pan + Shrimp weight (mg) Analyst: <u>kl</u> Date: <u>10-24-24</u>	14.62	16.13	15.95	16.62	15.80	13.52	14.84	15.52	15.04	14.13	14.93	13.84	14.16	14.49	14.91	14.57
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>kl</u>	1.09	1.08	1.14	1.32	1.21	1.14	1.07	1.05	1.16	1.19	1.10	1.28	1.12	1.19	1.01	1.02
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>kl</u>	0.216	0.216	0.228	0.264	0.212	0.228	0.214	0.210	0.232	0.238	0.220	0.256	0.224	0.238	0.202	0.204
Average weight per initial number of shrimp (mg) 0.228								Average weight per initial number of shrimp (mg) 0.221								Percent reduction from control (%) 0.37.

*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:

AbKCICR Test Number: **256**

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	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	S	S
3	S	S	S	S	S	S	S	S	4	3 ^{1d}	4	4	4	3 ^{1d}	3 ^{2d}	S
4	S	S	S	S	S	S	S	S	4	3	4	4	4	3	3	S
5	S	4 ^{1d} 3 ^{2d} 11-24-24	S	S	S	S	S	S	2 ^{2d}	3	3 ^{1d}	4	4	3	2 ^{1d}	S
6	S	4	S	S	S	S	S	S	2	2	3	4	4	2 ^{1d}	2	4 ^{1d}
7	S	4	S	S	S	S	S	S	2	2	3	4	4	2	2	2 ^{2d}
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																10-26-24
*A = Pan weight (mg) Tray color code: <u>turquoise</u> Analyst: <u>EM</u> Date: <u>04-25-24</u>	14.88	14.58	13.99	13.74	13.34	11.55	13.07	15.41	15.11	14.19	13.81	14.22	12.70	14.74	13.82	13.3 ^{1d}
*B = Pan + Shrimp weight (mg) Analyst: <u>K</u> Date: <u>10-24-24</u>	15.93	15.62	15.07	14.78	14.50	12.51	14.13	16.57	15.58	14.58	14.49	14.96	13.36	15.09	14.21	13.7 ^{1d}
C = Shrimp weight (mg) = B - A Hand calculated: <u>Y</u> Analyst: <u>Y</u>	1.05	1.04	1.08	1.04	1.16	0.96	1.06	1.16	0.47	0.39	0.68	0.74	0.66	0.35	0.39	0.45
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>Y</u> Analyst: <u>Y</u>	0.210	0.208	0.216	0.208	0.232	0.192	0.212	0.232	0.094	0.078	0.136	0.148	0.132	0.070	0.078	0.091
Average weight per initial number of shrimp (mg)					Percent reduction from control (%)					Average weight per initial number of shrimp (mg)					Percent reduction from control (%)	
0.214					6.01.					0.103					54.67.	

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

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

Comments:

AbKCICR Test Number: 256

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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hand calculated Analyst: _____																
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: 256
Test dates: October 15-22, 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	13.53	14.62	1.09	0.218	100.0	0.228	7.9	Not applicable
	B	5	5	15.35	16.43	1.08	0.216				
	C	5	5	14.81	15.95	1.14	0.228				
	D	5	5	15.30	16.62	1.32	0.264				
	E	5	5	14.59	15.80	1.21	0.242				
	F	5	5	12.38	13.52	1.14	0.228				
	G	5	5	13.77	14.84	1.07	0.214				
	H	5	5	14.47	15.52	1.05	0.210				
250	I	5	5	13.88	15.04	1.16	0.232	100.0	0.227	8.0	0.3
	J	5	5	12.94	14.13	1.19	0.238				
	K	5	5	13.83	14.93	1.10	0.220				
	L	5	5	12.56	13.84	1.28	0.256				
	M	5	5	13.04	14.16	1.12	0.224				
	N	5	5	13.30	14.49	1.19	0.238				
	O	5	5	13.90	14.91	1.01	0.202				
	P	5	5	13.55	14.57	1.02	0.204				
375	Q	5	5	14.88	15.93	1.05	0.210	97.5	0.214	6.2	6.0
	R	5	4	14.58	15.62	1.04	0.208				
	S	5	5	13.99	15.07	1.08	0.216				
	T	5	5	13.74	14.78	1.04	0.208				
	U	5	5	13.34	14.50	1.16	0.232				
	V	5	5	11.55	12.51	0.96	0.192				
	W	5	5	13.07	14.13	1.06	0.212				
	X	5	5	15.41	16.57	1.16	0.232				
500	Y	5	2	15.11	15.58	0.47	0.094	52.5	0.103	29.6	54.6
	Z	5	2	14.19	14.58	0.39	0.078				
	AA	5	3	13.81	14.49	0.68	0.136				
	BB	5	4	14.22	14.96	0.74	0.148				
	CC	5	4	12.70	13.36	0.66	0.132				
	DD	5	2	14.74	15.09	0.35	0.070				
	EE	5	2	13.82	14.21	0.39	0.078				
	FF	5	2	13.32	13.77	0.45	0.090				
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: 0.0233
PMSD: 10.2

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) = 11%.
Upper PMSD bound determined by USEPA (90th percentile) = 37%.
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Mysid Survival and Growth Test-7 Day Survival

Start Date: 10/15/2024 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 10/22/2024 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	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.4000	0.4000	0.6000	0.8000	0.8000	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								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	1.3453	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	0.5250	0.5250	0.8155	0.6847	1.1071	23.661	8	36.00	48.00	19	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) 0.76302 0.904 1.02843 3.66412

Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)

Steel's Many-One Rank Test 375 500 433.013

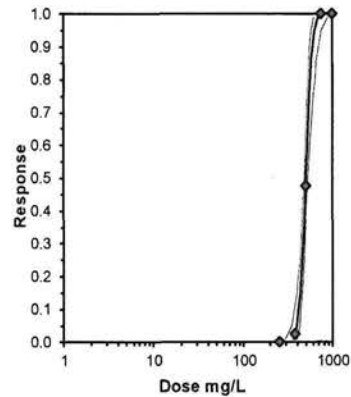
Treatments vs D-Control

Maximum Likelihood-Probit

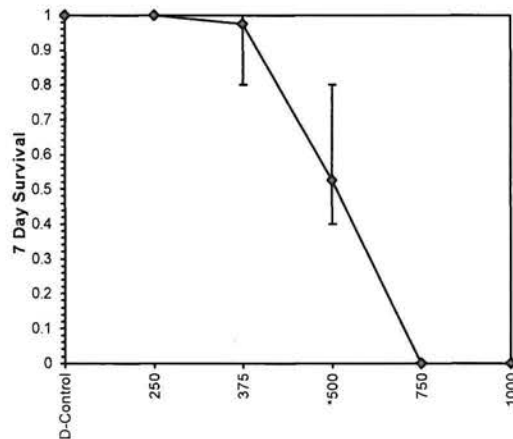
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.0976	3.15413	9.91553 22.2797	0	0.16507	7.81472	0.98302	2.70101	0.06212	3
Intercept	-38.48	8.47091	-55.083 -21.877							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	360.157	296.293 395.164
EC05	3.355	397.033	345.519 425.936
EC10	3.718	418.211	374.335 444.122
EC15	3.964	433.134	394.571 457.468
EC20	4.158	445.373	410.907 468.956
EC25	4.326	456.149	424.921 479.651
EC40	4.747	484.472	458.919 511.532
EC50	5.000	502.35	477.669 535.061
EC60	5.253	520.888	495.078 562.055
EC75	5.674	553.231	522.13 613.832
EC80	5.842	566.616	532.551 636.537
EC85	6.036	582.627	544.632 664.467
EC90	6.282	603.417	559.852 701.819
EC95	6.645	635.604	582.671 761.765
EC99	7.326	700.683	626.978 889.837

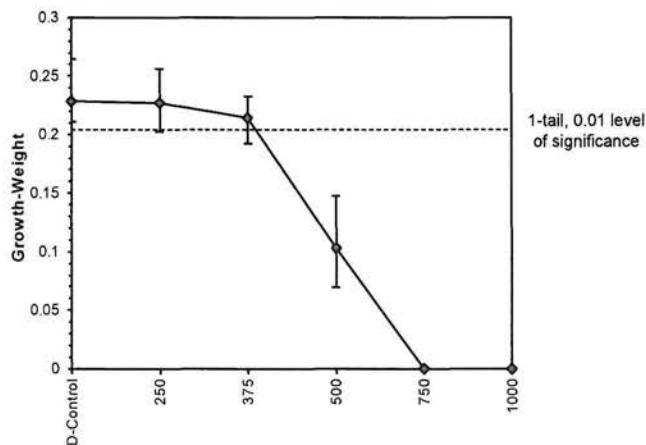
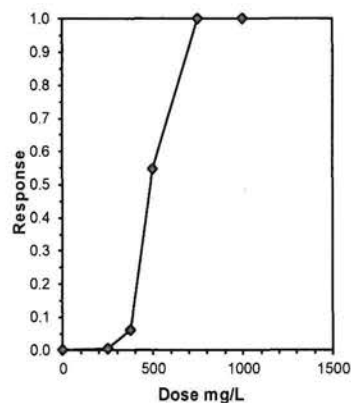


Dose-Response Plot



Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.96677	0.884	0.51714	-0.0356			
Bartlett's Test indicates equal variances (p = 0.68)					0.78469	9.21035					
Hypothesis Test (1-tail, 0.01)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		375	>375			0.02326	0.10223	0.00048	0.00028	0.20144	2, 21
Treatments vs D-Control											

Point	mg/L	SD	95% CL		Skew
IC05	352.16	53.05	172.42	382.66	-1.777
IC10	385.18	13.33	343.56	395.35	-4.6003
IC15	398.05	6.32	384.71	408.33	-0.3569
IC20	410.92	6.47	397.09	422.99	-0.1144
IC25	423.78	6.91	410.53	438.84	0.1782
IC40	462.39	9.45	449.54	486.69	0.7519
IC50	488.12	13.11	471.74	526.47	1.1912



AbKCICR Test Number: 256

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	K	K	K	B	B	K
CONTROL, Salt SW	pH (S.U.)	7.72	7.59	7.61	7.59	7.71	7.40
	DO (mg/L)	7.0	7.5	7.8	7.5	7.7	6.6
	Salinity (ppt)	24.9	25.3	25.0	25.2	25.0	24.9
	Alkalinity (mg CaCO ₃ /L)	76		10-16-24 K		80	
	Temperature (°C)	25.2	25.5	25.2	25.3	25.2	25.4
250 mg KCl/L	pH (S.U.)	7.73	7.59	7.61	7.63	7.73	7.46
	DO (mg/L)	7.0	7.6	7.8	7.5	7.7	7.2
	Salinity (ppt)	25.2	25.4	25.1	25.3	25.1	25.8
	Temperature (°C)	25.2	25.4	25.2	25.3	25.2	25.2
375 mg KCl/L	pH (S.U.)	7.73	7.56	7.62	7.64	7.73	7.46
	DO (mg/L)	7.9	7.6	7.9	7.6	7.6	7.2
	Salinity (ppt)	25.3	25.6	25.1	25.3	25.1	25.9
	Temperature (°C)	25.1	25.2	25.2	25.5	25.2	25.2
500 mg KCl/L	pH (S.U.)	7.75	7.56	7.63	7.64	7.73	7.52
	DO (mg/L)	7.9	7.5	7.9	7.5	7.7	7.6
	Salinity (ppt)	25.4	25.6	25.2	25.4	25.2	24.2
	Temperature (°C)	25.3	25.2	25.2	25.3	25.3	25.2
750 mg KCl/L	pH (S.U.)	7.75	7.57				
	DO (mg/L)	7.9	7.5				
	Salinity (ppt)	25.5	25.7				
	Temperature (°C)	25.2	25.6				
1000 mg KCl/L	pH (S.U.)	7.75	7.60				
	DO (mg/L)	7.9	7.4				
	Salinity (ppt)	25.6	25.9				
	Temperature (°C)	25.2	25.2				
		Initial	Final	Initial	Final	Initial	Final

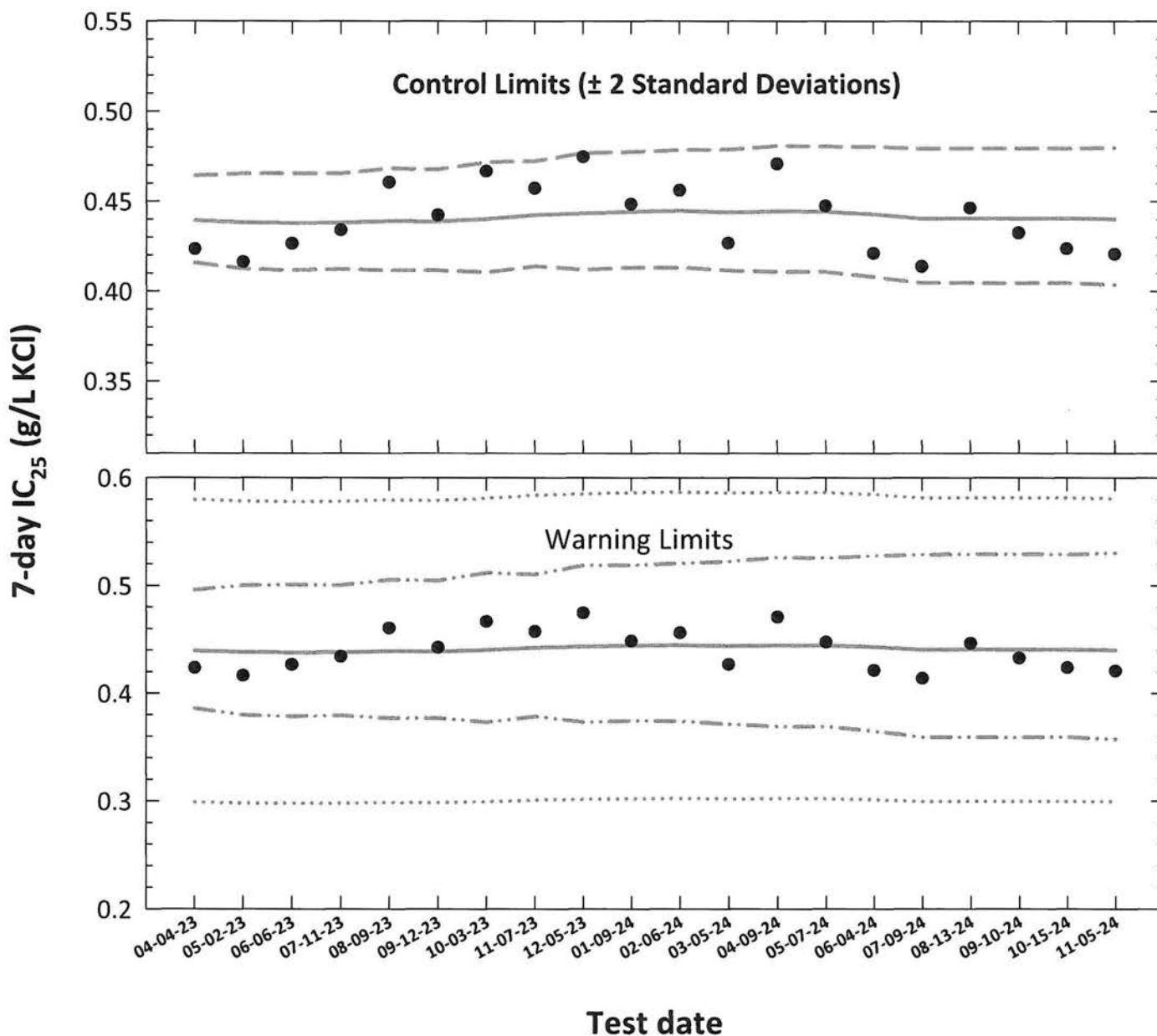
AbKCICR Test Number: **256**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	<i>u</i>	<i>u</i>	<i>u</i>	<i>u</i>	<i>u</i>	<i>u</i>	<i>u</i>	<i>u</i>
CONTROL, Salt SW	pH (S.U.)	7.73	7.44	7.63	7.43	7.63	7.27	7.42	7.29
	DO (mg/L)	7.8	6.9	7.8	7.2	7.8	7.7	7.8	5.9
	Salinity (ppt)	24.9	25.0	24.9	25.0	25.0	25.4	24.9	25.2
	Alkalinity (mg CaCO ₃ /L)	<i>n</i> 10-11-21		7.8			10.2124		<i>n</i> 10-11-21
	Temperature (°C)	25.2	25.5	25.2	25.6	25.2	25.6	25.2	25.7
250 mg KCl/L	pH (S.U.)	7.74	7.45	7.64	7.41	7.61	7.30	7.43	7.38
	DO (mg/L)	7.9	7.4	7.8	7.2	7.8	7.0	7.8	6.1
	Salinity (ppt)	25.0	25.2	25.0	25.7	25.0	25.4	25.0	26.8
	Temperature (°C)	25.2	25.6	25.2	25.6	25.2	25.6	25.3	25.7
375 mg KCl/L	pH (S.U.)	7.74	7.45	7.64	7.41	7.62	7.33	7.43	7.35
	DO (mg/L)	7.9	7.4	7.8	7.3	7.8	7.0	7.9	6.4
	Salinity (ppt)	25.0	25.4	25.1	25.3	25.1	25.6	25.1	25.8
	Temperature (°C)	25.2	25.6	25.3	25.4	25.3	25.5	25.3	25.9
500 mg KCl/L	pH (S.U.)	7.74	7.48	7.65	7.48	7.62	7.41	7.48	7.46
	DO (mg/L)	8.0	7.5	7.9	7.6	7.8	7.2	7.9	7.2
	Salinity (ppt)	25.1	25.5	25.2	26.2	25.1	25.9	25.2	25.8
	Temperature (°C)	25.2	25.3	25.3	25.4	25.3	25.5	25.1	25.7
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)							<i>u</i> 10.1824	
		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 ToxCat 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047	0.4794	0.3593	0.5288	0.2995	0.5814
17	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048	0.4796	0.3593	0.5291	0.2996	0.5816
18	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046	0.4796	0.3590	0.5292	0.2995	0.5814
19	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048	0.4795	0.3594	0.5289	0.2996	0.5815
20	11-05-24	0.4206	-0.3761	-0.3565	0.0188	0.4401	0.4036	0.4798	0.3572	0.5304	0.2993	0.5809

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 ToxCat).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

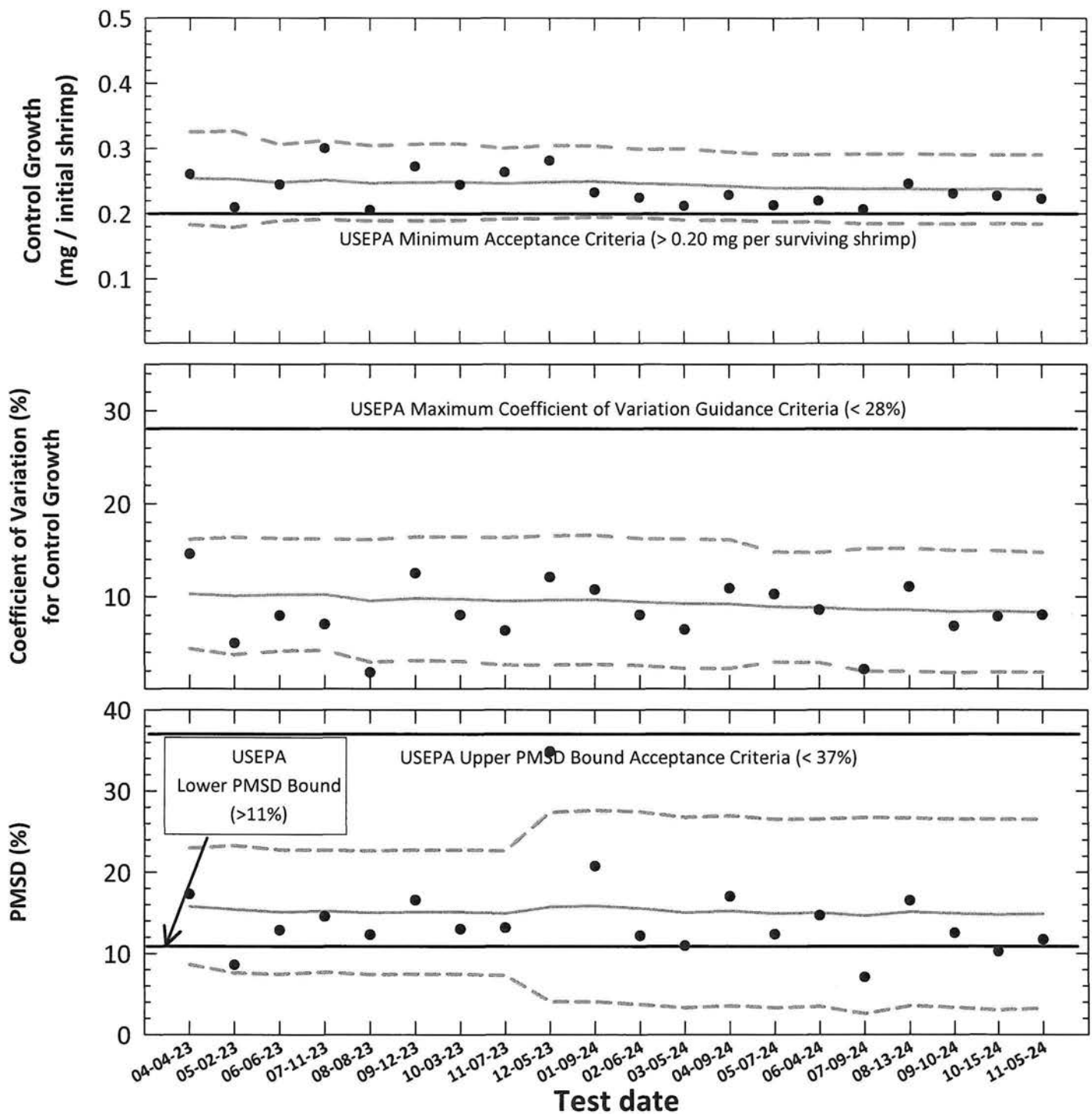
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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		PMSD (%)	Test	CT	Control Growth (mg/Initial shrimp)		CT	Control Growth (%)		CT	Test PMSD (%)	
			Mean (mg/Initial shrimp)	CV (%)				MSD	95% Confidence Interval CT - 2S		95% Confidence Interval CT + 2S	95% Confidence Interval CT - 2S		95% Confidence Interval CT + 2S	
1	04-04-23	100	0.261	14.6	17.3	0.0450	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
2	05-02-23	100	0.210	5.0	8.6	0.0180	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
3	06-06-23	100	0.245	8.0	12.8	0.0314	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
4	07-11-23	100	0.300	7.0	14.6	0.0437	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
5	08-08-23	100	0.206	1.8	12.3	0.0253	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
6	09-12-23	100	0.273	12.5	16.6	0.0451	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
7	10-03-23	100	0.244	8.0	13.0	0.0317	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
8	11-07-23	100	0.264	6.3	13.2	0.0347	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
9	12-05-23	100	0.282	12.1	34.8	0.0981	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
10	01-09-24	100	0.233	10.8	20.7	0.0482	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6
11	02-06-24	100	0.225	8.0	12.2	0.0273	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4
12	03-05-24	100	0.212	6.5	10.9	0.0232	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8
13	04-09-24	100	0.229	10.9	17.0	0.0389	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9
14	05-07-24	100	0.213	10.3	12.3	0.0263	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5
15	06-04-24	100	0.220	8.6	14.7	0.0323	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6
16	07-09-24	100	0.207	2.2	7.1	0.0146	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7
17	08-13-24	100	0.246	11.1	16.5	0.0406	0.238	0.185	0.292	8.6	1.9	15.2	15.1	3.5	26.7
18	09-10-24	100	0.231	6.8	12.5	0.0288	0.238	0.184	0.291	8.4	1.8	15.0	14.9	3.3	26.5
19	10-15-24	100	0.228	7.9	10.2	0.0233	0.238	0.185	0.291	8.5	1.9	15.0	14.8	3.1	26.5
20	11-05-24	100	0.223	8.0	11.7	0.0261	0.237	0.184	0.291	8.3	1.9	14.8	14.9	3.2	26.5

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%.
Upper PMSD bound 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: 257

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2319					
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:	ONE
Date and times organisms were born between:	10-29-24 1200 to 10-30-24 1130		Incubator number and shelf location:	5 B
Organism source:	AI Batch Ab: 10-30-24		Artemia CHM number:	CHM1294
Transfer bowl information:	pH = 7.84 S.U. Temperature = 25.1 °C		Drying information for weight determination:	
Average transfer volume:	< 0.25 mL		Date / Time in oven:	11-24 1245
			*Initial oven temperature:	60 °C
			Date / Time out of oven:	11-24 1245
			*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	11-05-24	1130	JL	1500	JL	1350	JL	10-30-24 B
1	11-06-24	0500	JL	1130	JL	0912	JL	11-04-24 A
2	11-07-24	0500	JL	1350	JL	1312	JL	11-06-24
3	11-08-24	0500	JL	1100	JL	0910	JL	↓
4	11-09-24	0500	JL	1300	JL	1230	JL	11-07-24
5	11-10-24	0500	JL	1100	JL	0910	JL	11-08-24
6	11-11-24	0500	JL	1100	JL	0913	JL	↓
7	11-12-24					1150	JL	

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	130641665

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	523.9
Average weight per initial shrimp:	0.223		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.223	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	420.6

AbKCICR Test Number: **257**

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	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
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>Magenta</u> Analyst: <u>JP</u> Date: <u>11-2-24</u>	13.29	10.41	12.19	14.27	13.06	13.09	13.04	13.11	12.07	12.54	14.47	13.13	15.16	14.10	13.95	10.95
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>11-14-24</u>	14.02	11.52	13.43	15.28	14.12	14.32	14.12	14.28	13.34	13.69	15.82	14.44	16.28	15.19	14.96	12.0
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.02	1.11	1.24	1.01	1.06	1.23	1.08	1.17	1.27	1.16	1.35	1.31	1.12	1.09	1.01	1.05
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.204	0.222	0.248	0.202	0.212	0.246	0.216	0.234	0.234	0.232	0.270	0.262	0.224	0.218	0.202	0.210
Average weight per initial number of shrimp (mg) 0.223								Average weight per initial number of shrimp (mg) 0.234				Percent reduction from control (%) -4.97				

*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: **257**

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	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}
3	S	S	S	S	S	S	S	S	4	4	4	3 ^{1d}	4	3 ^{1d}	3 ^{1d}	3 ^{1d}
4	S	S	S	S	S	S	S	S	4	4	4	3	4	3	3	3
5	S	S	S	S	S	S	S	S	3 ^{1d}	4	4	3	4	3	3	3
6	S	S	S	S	S	S	S	S	3	2 ^{1d}	4	3	3 ^{1d}	3	3	2 ^{1d}
7	S	S	S	S	S	S	S	S	3	2	3 ^{1d}	3	3	3	3	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>magenta</u> Analyst: <u>JP</u> Date: <u>11-21-24</u>	12.85	13.74	13.02	13.86	13.53	14.09	14.03	13.39	13.46	12.37	15.410	14.98	14.12	14.06	13.35	14.72
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>11-14-24</u>	13.92	14.80	14.01	14.96	14.55	15.16	14.76	14.40	14.03	12.96	15.83	15.52	14.56	14.58	13.94	15.04
C = Shrimp weight (mg) = B - A Hand calculated: <u>JP</u> Analyst: <u>JP</u>	1.07	1.06	0.99	1.10	1.02	1.07	1.13	1.01	0.57	0.64	0.43	0.54	0.46	0.52	0.59	0.37
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>JP</u> Analyst: <u>JP</u>	0.214	0.212	0.198	0.220	0.204	0.214	0.226	0.202	0.114	0.128	0.086	0.108	0.092	0.104	0.118	0.062
Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.211				5.3%				0.102				54.4%				

*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: **257**

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 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}	0 ^{Scl}
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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hand calculated Analyst: _____																
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: 25

Test dates: November 05-12, 202

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	13.00	14.02	1.02	0.204	100.0	0.223	8.0	Not applicable
	B	5	5	10.41	11.52	1.11	0.222				
	C	5	5	12.19	13.43	1.24	0.248				
	D	5	5	14.27	15.28	1.01	0.202				
	E	5	5	13.06	14.12	1.06	0.212				
	F	5	5	13.09	14.32	1.23	0.246				
	G	5	5	13.04	14.12	1.08	0.216				
	H	5	5	13.11	14.28	1.17	0.234				
250	I	5	5	12.07	13.34	1.27	0.254	100.0	0.234	10.8	-4.9
	J	5	5	12.54	13.70	1.16	0.232				
	K	5	5	14.47	15.82	1.35	0.270				
	L	5	5	13.13	14.44	1.31	0.262				
	M	5	5	15.16	16.28	1.12	0.224				
	N	5	5	14.10	15.19	1.09	0.218				
	O	5	5	13.95	14.96	1.01	0.202				
	P	5	5	10.95	12.00	1.05	0.210				
375	Q	5	5	12.85	13.92	1.07	0.214	100.0	0.211	4.5	5.3
	R	5	5	13.74	14.80	1.06	0.212				
	S	5	5	13.02	14.01	0.99	0.198				
	T	5	5	13.86	14.96	1.10	0.220				
	U	5	5	13.53	14.55	1.02	0.204				
	V	5	5	14.09	15.16	1.07	0.214				
	W	5	5	14.03	15.16	1.13	0.226				
	X	5	5	13.39	14.40	1.01	0.202				
500	Y	5	3	13.46	14.03	0.57	0.114	55.0	0.102	20.1	54.4
	Z	5	2	12.32	12.96	0.64	0.128				
	AA	5	3	15.40	15.83	0.43	0.086				
	BB	5	3	14.98	15.52	0.54	0.108				
	CC	5	3	14.12	14.58	0.46	0.092				
	DD	5	3	14.06	14.58	0.52	0.104				
	EE	5	3	13.35	13.94	0.59	0.118				
	FF	5	2	14.72	15.04	0.32	0.064				
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: 0.0261
PMSD: 11.7

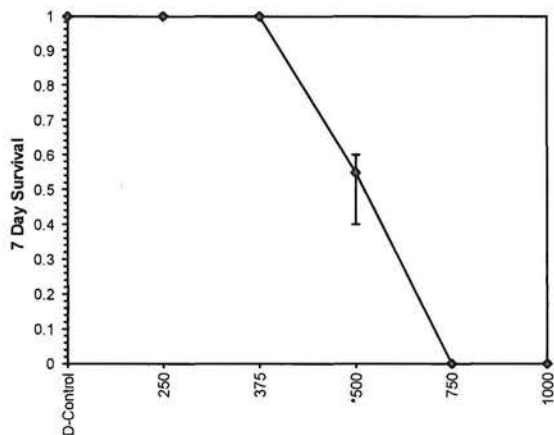
MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

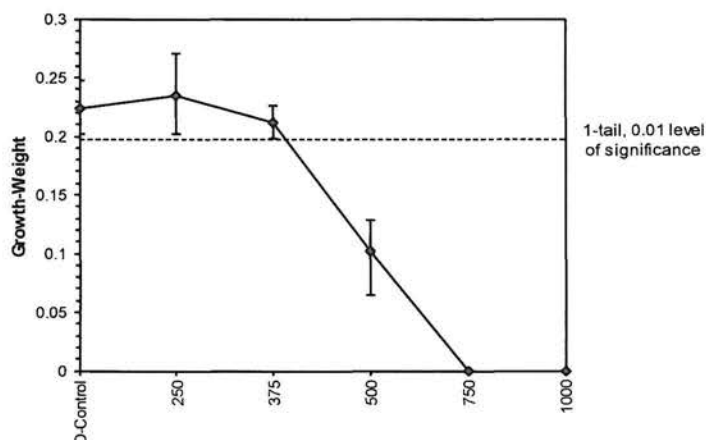
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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.





AbKCICR Test Number: 257

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.

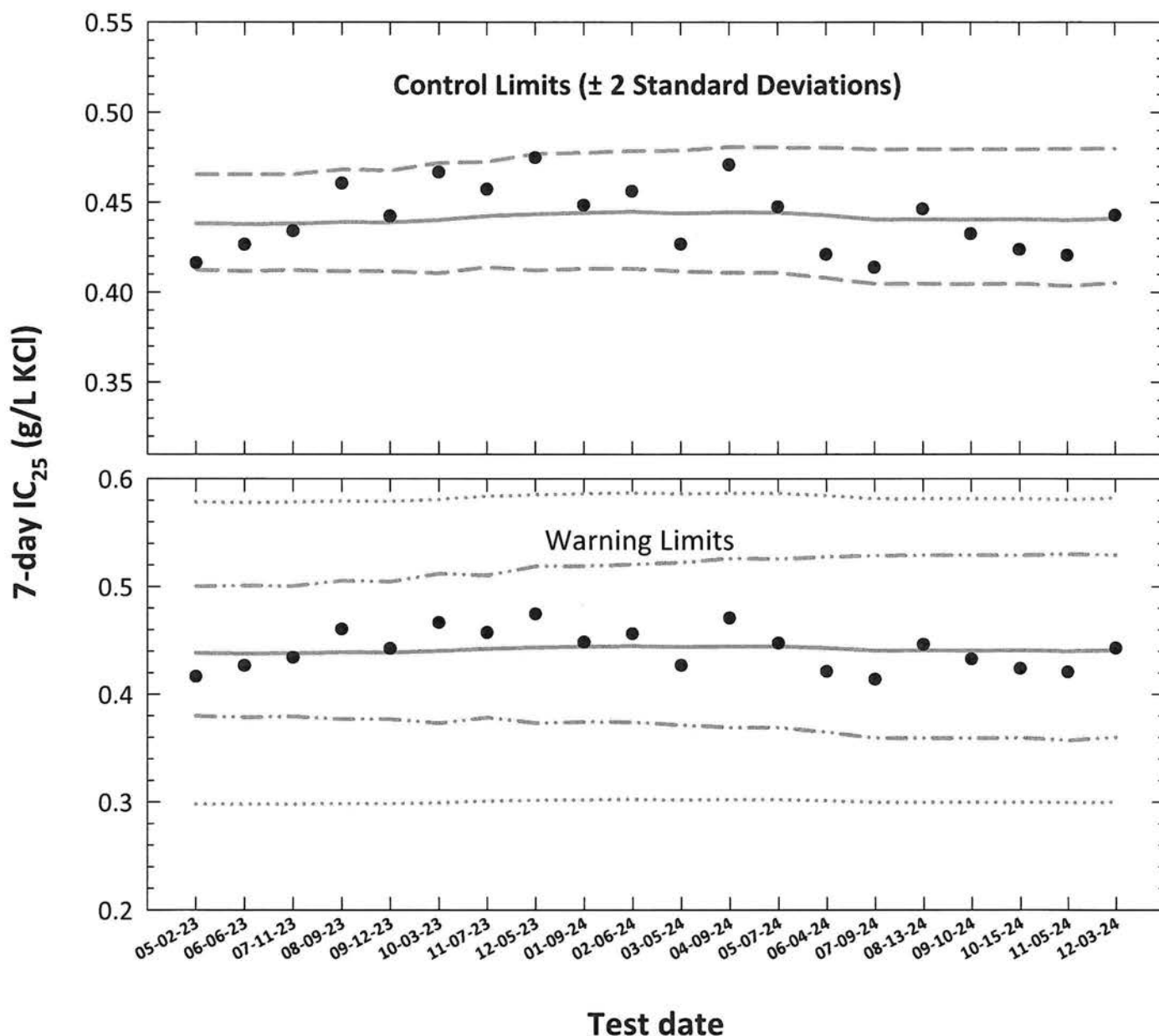
Conc.	Parameter	Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	<i>W</i>	<i>JP</i>	<i>JP</i>	<i>JP</i>	<i>JP</i>	<i>W</i>
CONTROL, Salt SW	pH (S.U.)	<i>7.84</i>	<i>7.86</i>	<i>8.05</i>	<i>7.70</i>	<i>7.25</i>	<i>7.47</i>
	DO (mg/L)	<i>7.7</i>	<i>7.6</i>	<i>7.7</i>	<i>7.4</i>	<i>7.7</i>	<i>7.2</i>
	Salinity (ppt)	<i>25.1</i>	<i>24.9</i>	<i>25.0</i>	<i>25.1</i>	<i>24.9</i>	<i>25.2</i>
	Alkalinity (mg CaCO ₃ /L)	<i>94</i>		<i>* 130</i>		<i>130</i>	
	Temperature (°C)	<i>25.4</i>	<i>25.6</i>	<i>25.2</i>	<i>25.7</i>	<i>25.2</i>	<i>25.8</i>
250 mg KCl/L	pH (S.U.)	<i>7.70</i>	<i>7.88</i>	<i>7.91</i>	<i>7.64</i>	<i>7.64</i>	<i>7.42</i>
	DO (mg/L)	<i>7.7</i>	<i>7.6</i>	<i>7.7</i>	<i>7.5</i>	<i>7.8</i>	<i>7.2</i>
	Salinity (ppt)	<i>25.1</i>	<i>25.1</i>	<i>24.9</i>	<i>25.0</i>	<i>25.0</i>	<i>25.2</i>
	Temperature (°C)	<i>25.2</i>	<i>25.7</i>	<i>25.3</i>	<i>25.7</i>	<i>25.2</i>	<i>25.5</i>
375 mg KCl/L	pH (S.U.)	<i>7.72</i>	<i>7.90</i>	<i>7.92</i>	<i>7.65</i>	<i>7.68</i>	<i>7.42</i>
	DO (mg/L)	<i>7.7</i>	<i>7.6</i>	<i>7.7</i>	<i>7.5</i>	<i>7.8</i>	<i>7.0</i>
	Salinity (ppt)	<i>25.1</i>	<i>25.1</i>	<i>25.4</i>	<i>25.5</i>	<i>25.6</i>	<i>25.9</i>
	Temperature (°C)	<i>25.2</i>	<i>25.7</i>	<i>25.3</i>	<i>25.6</i>	<i>25.2</i>	<i>25.5</i>
500 mg KCl/L	pH (S.U.)	<i>7.73</i>	<i>7.90</i>	<i>7.93</i>	<i>7.68</i>	<i>7.69</i>	<i>7.47</i>
	DO (mg/L)	<i>7.8</i>	<i>7.7</i>	<i>7.7</i>	<i>7.5</i>	<i>7.8</i>	<i>7.5</i>
	Salinity (ppt)	<i>25.3</i>	<i>25.2</i>	<i>25.5</i>	<i>25.6</i>	<i>25.2</i>	<i>26.0</i>
	Temperature (°C)	<i>25.3</i>	<i>25.7</i>	<i>25.4</i>	<i>25.6</i>	<i>25.2</i>	<i>25.7</i>
750 mg KCl/L	pH (S.U.)	<i>7.74</i>	<i>7.90</i>				
	DO (mg/L)	<i>7.8</i>	<i>7.8</i>				
	Salinity (ppt)	<i>25.4</i>	<i>25.4</i>				
	Temperature (°C)	<i>25.3</i>	<i>25.6</i>				
1000 mg KCl/L	pH (S.U.)	<i>7.75</i>	<i>7.89</i>				
	DO (mg/L)	<i>7.9</i>					
	Salinity (ppt)	<i>25.4</i>	<i>25.6</i>				
	Temperature (°C)	<i>25.5</i>	<i>25.6</i>				
		Initial	Final	Initial	Final	Initial	Final

* NEW BATCH OF SALT

AbKCICR Test Number: **257**

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.)	N	N	N	N	N	EM	EM	N
	DO (mg/L)	7.62	7.44	7.60	7.50	7.74	7.72	7.75	7.50
	Salinity (ppt)	25.0	25.3	24.8	25.1	25.2	25.0	25.2	25.4
	Alkalinity (mg CaCO ₃ /L)	---	---	120	---	130	---	---	---
	Temperature (°C)	25.3	25.7	25.3	25.6	25.2	25.8	25.4	26.0
250 mg KCl/L	pH (S.U.)	7.62	7.53	7.65	7.59	7.74	7.74	7.81	7.52
	DO (mg/L)	7.6	7.2	7.9	6.8	7.9	7.4	7.9	7.2
	Salinity (ppt)	25.3	25.3	25.2	25.4	25.2	25.3	25.1	25.4
	Temperature (°C)	25.4	25.7	25.2	25.1	25.3	25.7	25.4	26.2
375 mg KCl/L	pH (S.U.)	7.63	7.54	7.67	7.59	7.75	7.79	7.83	7.50
	DO (mg/L)	7.7	7.2	7.9	7.2	7.9	7.4	7.9	7.3
	Salinity (ppt)	25.2	25.5	25.6	25.7	25.2	25.3	25.3	25.5
	Temperature (°C)	25.4	25.7	25.3	25.4	25.4	25.9	25.3	26.2
500 mg KCl/L	pH (S.U.)	7.65	7.62	7.60	7.64	7.70	7.80	7.83	7.50
	DO (mg/L)	7.7	7.7	8.0	7.5	7.9	7.4	7.9	7.5
	Salinity (ppt)	25.5	25.5	25.6	25.7	25.3	25.4	25.4	25.7
	Temperature (°C)	25.4	25.6	25.3	25.6	25.4	25.9	25.3	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

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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126	0.4654	0.3798	0.5003
2	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118	0.4655	0.3784	0.5011
3	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124	0.4655	0.3794	0.5006
4	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116	0.4683	0.3766	0.5056
5	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116	0.4677	0.3769	0.5047
6	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.3730	0.5122
7	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105
8	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3732	0.5190
9	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3744	0.5192
10	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3740	0.5208
11	03-05-24	0.4268	-0.3698	-0.3527	0.0164	0.4440	0.4117	0.4788	0.3712	0.5224
12	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109	0.4808	0.3690	0.5261
13	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109	0.4805	0.3690	0.5258
14	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082	0.4804	0.3645	0.5278
15	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047	0.4794	0.3593	0.5288
16	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048	0.4796	0.3593	0.5291
17	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046	0.4796	0.3590	0.5292
18	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048	0.4795	0.3594	0.5289
19	11-05-24	0.4206	-0.3761	-0.3565	0.0188	0.4401	0.4036	0.4798	0.3572	0.5304
20	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053	0.4800	0.3600	0.5294

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.

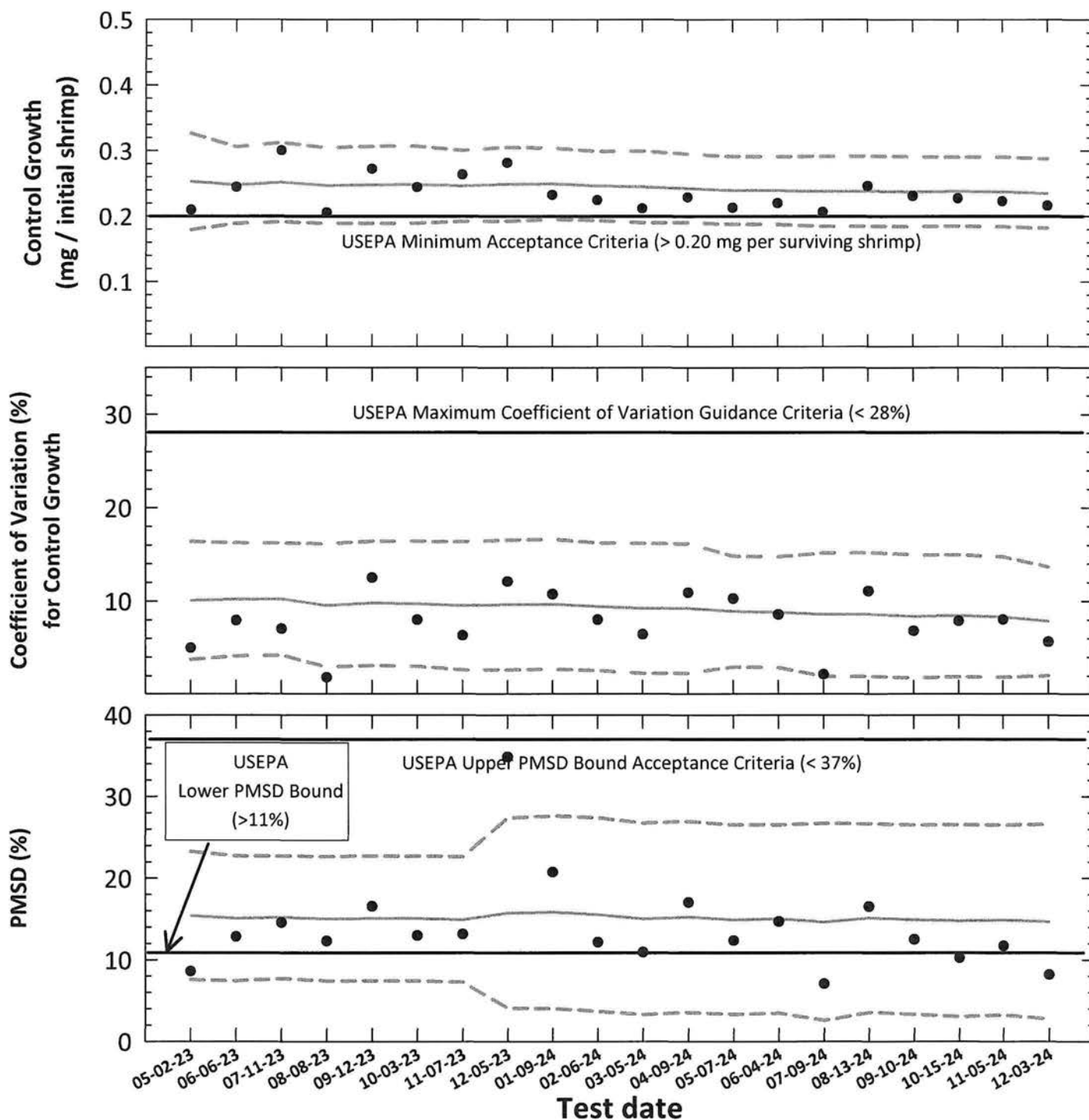
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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		Test		Control Survival (%)	Control Growth (mg/initial shrimp)			Control Growth CV (%)		Test PMSD (%)	
		Control Growth (%)	CV (%)	Mean (mg/initial shrimp)	MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	05-02-23	100	5.0	0.210	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3	15.4	7.6	23.3
2	06-06-23	100	8.0	0.245	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7	15.1	7.4	22.7
3	07-11-23	100	7.0	0.300	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7	15.2	7.7	22.7
4	08-08-23	100	1.8	0.206	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6	15.0	7.4	22.6
5	09-12-23	100	12.5	0.273	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7	15.1	7.4	22.7
6	10-03-23	100	8.0	0.244	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7	15.1	7.4	22.7
7	11-07-23	100	6.3	0.264	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6	14.9	7.3	22.6
8	12-05-23	100	12.1	0.282	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4	15.7	4.1	27.4
9	01-09-24	100	10.8	0.233	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6	15.8	4.0	27.6
10	02-06-24	100	8.0	0.225	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4	15.5	3.7	27.4
11	03-05-24	100	6.5	0.212	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8	15.0	3.3	26.8
12	04-09-24	100	10.9	0.229	0.0389	17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9	15.2	3.5	26.9
13	05-07-24	100	10.3	0.213	0.0263	12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5	14.9	3.3	26.5
14	06-04-24	100	8.6	0.220	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6	15.0	3.5	26.6
15	07-09-24	100	2.2	0.207	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7	14.6	2.6	26.7
16	08-13-24	100	11.1	0.246	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	15.1	3.5	26.7	15.1	3.5	26.7
17	09-10-24	100	6.8	0.231	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.9	3.3	26.5	14.9	3.3	26.5
18	10-15-24	100	7.9	0.228	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.8	3.1	26.5	14.8	3.1	26.5
19	11-05-24	100	8.0	0.223	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.9	3.2	26.5	14.9	3.2	26.5
20	12-03-24	100	5.7	0.216	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.7	2.7	26.6	14.7	2.7	26.6

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%.
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: 258

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2334					
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:	11-26-24 1200 to 11-27-24 1130		Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 11-27-24		Artemia CHM number:	CHM1294
Transfer bowl information:	pH = 7.84 S.U. Temperature = 25.0 °C		Drying information for weight determination:	
			Date / Time in oven:	12-10-24 1105
Average transfer volume:	< 0.25 mL		*Initial oven temperature:	60 °C
			Date / Time out of oven:	12-12-24 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	12-03-24	1145	H	1340	H	1230	H	11-21-24 A
1	12-04-24	0500	H	1415	H	0900	K	↓
2	12-05-24	0500	H	1310	H	0900	H	11-27-24 B
3	12-06-24	0500	H	1100	H	0850	H	12-03-24 11-27-24
4	12-07-24	0530	H	1130	H	1100	H	12-03-24
5	12-08-24	0500	H	1100	H	0803	H	↓
6	12-09-24	0500	H	1210	H	0850	H	↓
7	12-10-24					1030	H	

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	

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	506.3
Average weight per initial shrimp:	0.216		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.216	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.6
			IC ₂₅ (mg/L KCl)	442.8

AbKCICR Test Number: **258**

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>Green</u> Analyst: <u>JP</u> Date: <u>11-27-24</u>	14.45	15.06	11.14	14.60	11.55	13.89	15.03	13.05	13.62	12.78	13.06	13.48	13.86	14.60	14.34	11.92
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>12-11-24</u>	15.47	16.18	12.19	15.81	12.59	14.93	16.12	14.13	14.78	13.95	14.21	14.56	14.93	15.60	15.45	13.08
C = Shrimp weight (mg) = B - A Hand calculated: <u>JP</u> Analyst: <u>JP</u>	1.02	1.12	1.05	1.21	1.04	1.04	1.09	1.08	1.16	1.17	1.15	1.08	1.07	1.00	1.11	1.06
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>JP</u> Analyst: <u>JP</u>	0.204	0.224	0.210	0.242	0.208	0.208	0.218	0.216	0.232	0.234	0.230	0.216	0.214	0.200	0.222	0.216
	Average weight per initial number of shrimp (mg) 0.216								Average weight per initial number of shrimp (mg) 0.211				Percent reduction from control (%) -2.07			

*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: **258**

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	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}
3	S	S	S	S	S	S	S	S	3 ^{1d}	4	4	4	4	3 ^{1d}	3 ^{1d}	4
4	S	S	S	S	S	S	S	S	3	4	3 ^{1d}	4	4	3	3	3 ^{1d}
5	S	S	S	S	S	S	S	S	3	3 ^{1d}	3	4	4	2 ^{1d}	3	3
6	S	S	S	S	S	S	S	4 ^{1d}	3	3	3	3 ^{1d}	4	2	3	3
7	S	S	S	S	S	S	S	4	3	3	3	2 ^{1d}	3 ^{1d}	2	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>Grey</u> Analyst: <u>JP</u> Date: <u>11-27-24</u>	12.96	13.21	13.11	13.52	15.05	13.65	14.42	13.03	13.92	15.82	14.06	12.80	13.71	13.83	14.37	13.54
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>11-27-24</u>	14.01	14.41	14.22	14.53	16.16	14.72	15.53	14.00	14.44	16.40	14.84	13.29	14.47	14.23	14.98	14.10
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.05	1.20	1.11	1.01	1.11	1.07	1.11	0.97	0.62	0.58	0.78	0.49	0.76	0.40	0.61	0.56
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.210	0.240	0.222	0.202	0.222	0.214	0.222	0.194	0.124	0.116	0.156	0.098	0.152	0.060	0.122	0.112
Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.216				0.27				0.120				44.57				

*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: **258**

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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hand calculated Analyst: _____																
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: 25
Test dates: December 03-10, 202

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	14.45	15.47	1.02	0.204	100.0	0.216	5.7	Not applicable
	B	5	5	15.06	16.18	1.12	0.224				
	C	5	5	11.14	12.19	1.05	0.210				
	D	5	5	14.60	15.81	1.21	0.242				
	E	5	5	11.55	12.59	1.04	0.208				
	F	5	5	13.89	14.93	1.04	0.208				
	G	5	5	15.03	16.12	1.09	0.218				
	H	5	5	13.05	14.13	1.08	0.216				
250	I	5	5	13.62	14.78	1.16	0.232	100.0	0.221	5.2	-2.0
	J	5	5	12.78	13.95	1.17	0.234				
	K	5	5	13.06	14.21	1.15	0.230				
	L	5	5	13.48	14.56	1.08	0.216				
	M	5	5	13.86	14.93	1.07	0.214				
	N	5	5	14.60	15.60	1.00	0.200				
	O	5	5	14.34	15.45	1.11	0.222				
	P	5	5	11.92	13.00	1.08	0.216				
375	Q	5	5	12.96	14.01	1.05	0.210	97.5	0.216	6.6	0.2
	R	5	5	13.21	14.41	1.20	0.240				
	S	5	5	13.11	14.22	1.11	0.222				
	T	5	5	13.52	14.53	1.01	0.202				
	U	5	5	15.05	16.16	1.11	0.222				
	V	5	5	13.65	14.72	1.07	0.214				
	W	5	5	14.42	15.53	1.11	0.222				
	X	5	4	13.03	14.00	0.97	0.194				
500	Y	5	3	13.82	14.44	0.62	0.124	55.0	0.120	21.1	44.5
	Z	5	3	15.82	16.40	0.58	0.116				
	AA	5	3	14.06	14.84	0.78	0.156				
	BB	5	2	12.80	13.29	0.49	0.098				
	CC	5	3	13.71	14.47	0.76	0.152				
	DD	5	2	13.83	14.23	0.40	0.080				
	EE	5	3	14.37	14.98	0.61	0.122				
	FF	5	3	13.54	14.10	0.56	0.112				
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: 0.0177
PMSD: 8.2

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

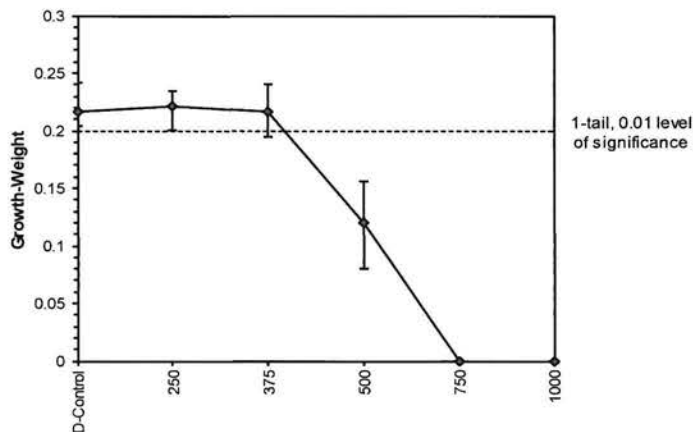
Upper PMSD bound determined by USEPA (90th percentile) = 37%.

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

Mysid Survival and Growth Test-7 Day Survival												
Start Date:	12/3/2024	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant							
End Date:	12/10/2024	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	0.8000				
500	0.6000	0.6000	0.6000	0.4000	0.6000	0.4000	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				
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	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	0.6847	0.8861	11.153	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												
Statistic							Critical		Skew		Kurt	
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)							0.63372		0.904		-2.4249 5.84709	
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												
Maximum Likelihood-Probit												
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	15.8775	3.06249	9.87504	21.88	0	0.24502	7.81472	0.97001	2.70444	0.06298	4	
Intercept	-37.94	8.23191	-54.074	-21.805								
TSCR												
Point	Probits	mg/L	95% Fiducial Limits									
EC01	2.674	361.343	298.176	396.465								
EC05	3.355	398.88	347.877	427.966								
EC10	3.718	420.459	376.957	446.62								
EC15	3.964	435.674	397.363	460.328								
EC20	4.158	448.158	413.826	472.138								
EC25	4.326	459.153	427.94	483.133								
EC40	4.747	488.07	462.193	515.847								
EC50	5.000	506.336	481.151	539.88								
EC60	5.253	525.285	498.837	567.354								
EC75	5.674	558.366	526.466	619.923								
EC80	5.842	572.065	537.141	642.958								
EC85	6.036	588.458	549.531	671.29								
EC90	6.282	609.752	565.158	709.182								
EC95	6.645	642.739	588.611	770.004								
EC99	7.326	709.507	634.212	900.007								

Dose (mg/L)	Response
1	1.0
250	1.0
375	1.0
500	0.55
750	0.0
1000	0.0

Dose (mg/L)	7 Day Survival
D-Control	1.0
250	1.0
375	1.0
500	0.55
750	0.0
1000	0.0



AbKCICR Test Number: **258**

Daily Chemistry:

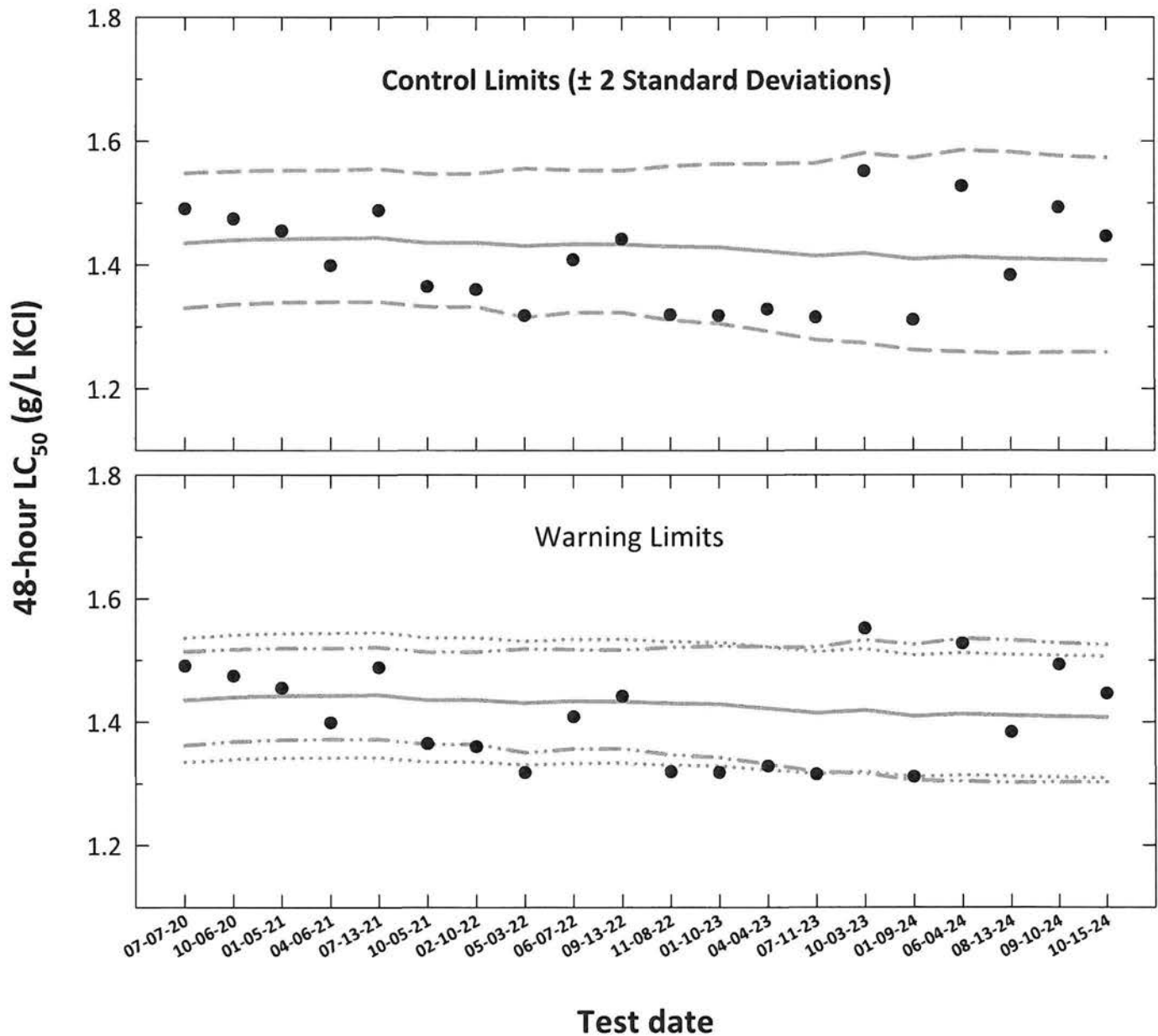
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst					
CONTROL, Salt SW	pH (S.U.)	<i>K. Jp</i>	<i>EM</i>	<i>EM</i>	<i>EM</i>	<i>EM</i>	<i>EM</i>
	DO (mg/L)	<i>7.70</i>	<i>7.75</i>	<i>7.79</i>	<i>7.73</i>	<i>7.77</i>	<i>7.77</i>
	Salinity (ppt)	<i>25.0</i>	<i>25.1</i>	<i>25.0</i>	<i>25.2</i>	<i>25.0</i>	<i>25.2</i>
	Alkalinity (mg CaCO ₃ /L)	<i>120</i>				<i>120</i>	
	Temperature (°C)	<i>25.6</i>	<i>26.2</i>	<i>25.1</i>	<i>26.2</i>	<i>25.1</i>	<i>26.3</i>
250 mg KCl/L	pH (S.U.)	<i>7.74</i>	<i>7.78</i>	<i>7.79</i>	<i>7.75</i>	<i>7.78</i>	<i>7.78</i>
	DO (mg/L)	<i>7.0</i>	<i>7.7</i>	<i>7.9</i>	<i>7.5</i>	<i>7.7</i>	<i>7.5</i>
	Salinity (ppt)	<i>25.3</i>	<i>25.4</i>	<i>25.1</i>	<i>25.3</i>	<i>25.1</i>	<i>25.3</i>
	Temperature (°C)	<i>25.5</i>	<i>26.0</i>	<i>25.1</i>	<i>26.2</i>	<i>25.1</i>	<i>26.2</i>
375 mg KCl/L	pH (S.U.)	<i>7.75</i>	<i>7.80</i>	<i>7.82</i>	<i>7.78</i>	<i>7.82</i>	<i>7.78</i>
	DO (mg/L)	<i>7.0</i>	<i>7.6</i>	<i>7.9</i>	<i>7.5</i>	<i>7.7</i>	<i>7.4</i>
	Salinity (ppt)	<i>25.4</i>	<i>25.6</i>	<i>25.4</i>	<i>25.5</i>	<i>25.2</i>	<i>25.4</i>
	Temperature (°C)	<i>25.6</i>	<i>26.2</i>	<i>26.2</i>	<i>26.2</i>	<i>25.2</i>	<i>26.2</i>
500 mg KCl/L	pH (S.U.)	<i>7.76</i>	<i>7.81</i>	<i>7.82</i>	<i>7.78</i>	<i>7.82</i>	<i>7.81</i>
	DO (mg/L)	<i>7.0</i>	<i>7.7</i>	<i>7.9</i>	<i>7.5</i>	<i>7.8</i>	<i>7.4</i>
	Salinity (ppt)	<i>25.4</i>	<i>25.8</i>	<i>25.4</i>	<i>25.6</i>	<i>25.4</i>	<i>25.5</i>
	Temperature (°C)	<i>25.6</i>	<i>26.3</i>	<i>25.1</i>	<i>26.2</i>	<i>25.1</i>	<i>26.2</i>
750 mg KCl/L	pH (S.U.)	<i>7.77</i>	<i>7.82</i>				
	DO (mg/L)	<i>7.0</i>	<i>7.0</i>				
	Salinity (ppt)	<i>25.8</i>	<i>26.0</i>				
	Temperature (°C)	<i>25.7</i>	<i>26.1</i>				
1000 mg KCl/L	pH (S.U.)	<i>7.70</i>	<i>7.80</i>				
	DO (mg/L)	<i>7.0</i>	<i>7.9</i>				
	Salinity (ppt)	<i>25.9</i>	<i>26.1</i>				
	Temperature (°C)	<i>25.7</i>	<i>26.3</i>				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: **258**

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.)	7.76	7.64	7.72	7.91	7.62	7.88	7.84	7.62
	DO (mg/L)	7.7	7.1	7.0	6.5	7.0	7.3	7.9	7.3
	Salinity (ppt)	24.8	25.3	25.3	25.4	25.0	24.6	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	120-140		120				110-140	
	Temperature (°C)	25.0	26.3	25.2	26.0	25.1	26.3	25.2	26.1
250 mg KCl/L	pH (S.U.)	7.77	7.60	7.74	7.91	7.70	7.83	7.87	7.67
	DO (mg/L)	7.8	7.2	7.0	6.2	7.0	7.4	7.9	7.3
	Salinity (ppt)	25.1	25.3	25.2	25.2	25.6	25.4	25.3	25.4
	Temperature (°C)	25.0	26.1	25.2	26.1	25.1	26.1	25.1	26.3
375 mg KCl/L	pH (S.U.)	7.84	7.66	7.79	7.53	7.70	7.86	7.89	7.68
	DO (mg/L)	7.8	7.1	7.9	6.3	7.9	7.4	7.9	7.3
	Salinity (ppt)	25.1	25.4	25.3	25.5	25.6	25.6	25.5	25.5
	Temperature (°C)	25.1	26.1	25.2	26.1	25.1	26.1	25.1	26.3
500 mg KCl/L	pH (S.U.)	7.86	7.62	7.82	7.57	7.72	7.87	7.91	7.69
	DO (mg/L)	7.8	6.0	6.0	6.3	7.9	7.4	7.9	7.3
	Salinity (ppt)	25.1	25.0	25.3	25.0	25.6	26.0	25.6	25.8
	Temperature (°C)	25.1	26.2	25.2	26.2	25.2	26.1	25.2	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)						7.7		
	DO (mg/L)						2.04-2.4		
	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 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 Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	07-07-20	1.4906	0.1734	0.1569	0.0165	1.4351	1.3302	1.5483	1.3620	1.5139
2	10-06-20	1.4741	0.1685	0.1583	0.0162	1.4397	1.3362	1.5513	1.3678	1.5172
3	01-05-21	1.4546	0.1627	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189
4	04-06-21	1.3988	0.1458	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187
5	07-13-21	1.4877	0.1725	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207
6	10-05-21	1.3650	0.1351	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132
7	02-10-22	1.3599	0.1335	0.1570	0.0163	1.4356	1.3320	1.5473	1.3635	1.5134
8	05-03-22	1.3180	0.1199	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182
9	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169
10	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163
11	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205
12	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227
13	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213
14	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209
15	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331
16	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256
17	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351
18	08-13-24	1.3832	0.1409	0.1493	0.0250	1.4102	1.2569	1.5823	1.3015	1.5322
19	09-10-24	1.4930	0.1740	0.1488	0.0244	1.4087	1.2589	1.5763	1.3024	1.5277
20	10-15-24	1.4461	0.1602	0.1484	0.0242	1.4075	1.2593	1.5731	1.3022	1.5251

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

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

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

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

CV = Coefficient of variation.



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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 96

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 #:

2319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.72	7.75	7.73
	Dissolved oxygen (mg/L)	7.0	7.6	7.7
	*Salinity (ppt)	24.9	25.0	25.2
	*Alkalinity (mg/L CaCO ₃)	76		
	*Temperature (°C)	24.7	25.0	25.1
1000 mg/L	pH (S.U.)	7.74	7.74	7.70
	Dissolved oxygen (mg/L)	7.7	7.7	7.7
	*Salinity (ppt)	25.4	25.5	25.6
	*Temperature (°C)	24.6	25.0	24.8
1250 mg/L	pH (S.U.)	7.74	7.74	7.70
	Dissolved oxygen (mg/L)	7.7	7.7	7.6
	*Salinity (ppt)	25.2	25.6	25.8
	*Temperature (°C)	24.7	25.2	24.8
1500 mg/L	pH (S.U.)	7.75	7.74	7.70
	Dissolved oxygen (mg/L)	7.9	7.6	7.6
	*Salinity (ppt)	25.8	25.9	26.0
	*Temperature (°C)	24.7	25.2	25.0
1750 mg/L	pH (S.U.)	7.73	7.74	7.68
	Dissolved oxygen (mg/L)	7.9	7.6	7.6
	*Salinity (ppt)	26.0	26.1	26.2
	*Temperature (°C)	24.5	25.0	25.0
2000 mg/L	pH (S.U.)	7.72	7.72	
	Dissolved oxygen (mg/L)	7.9	7.5	
	*Salinity (ppt)	26.2	26.3	
	*Temperature (°C)	24.7	25.0	

*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	1306646f

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 # 916

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-15-24	1215	JL	1427	JL	1B	ORANGE	10-13-24A
24	10-16-24			1430	JL			
48 Termination	10-17-24			1428	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):	10-05-24
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	10-04-24 1200 10-05-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.42 Temperature (°C) 24.9

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	9 ^u	9 ^u	7 ^u	8 ^u	2 ^u	4 ^u	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	8 ^u	9	5 ^u	5 ^u	0 ^u	0 ^u	0	0
Mean Survival	100%		100%		85%		50%		0%		0%	

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

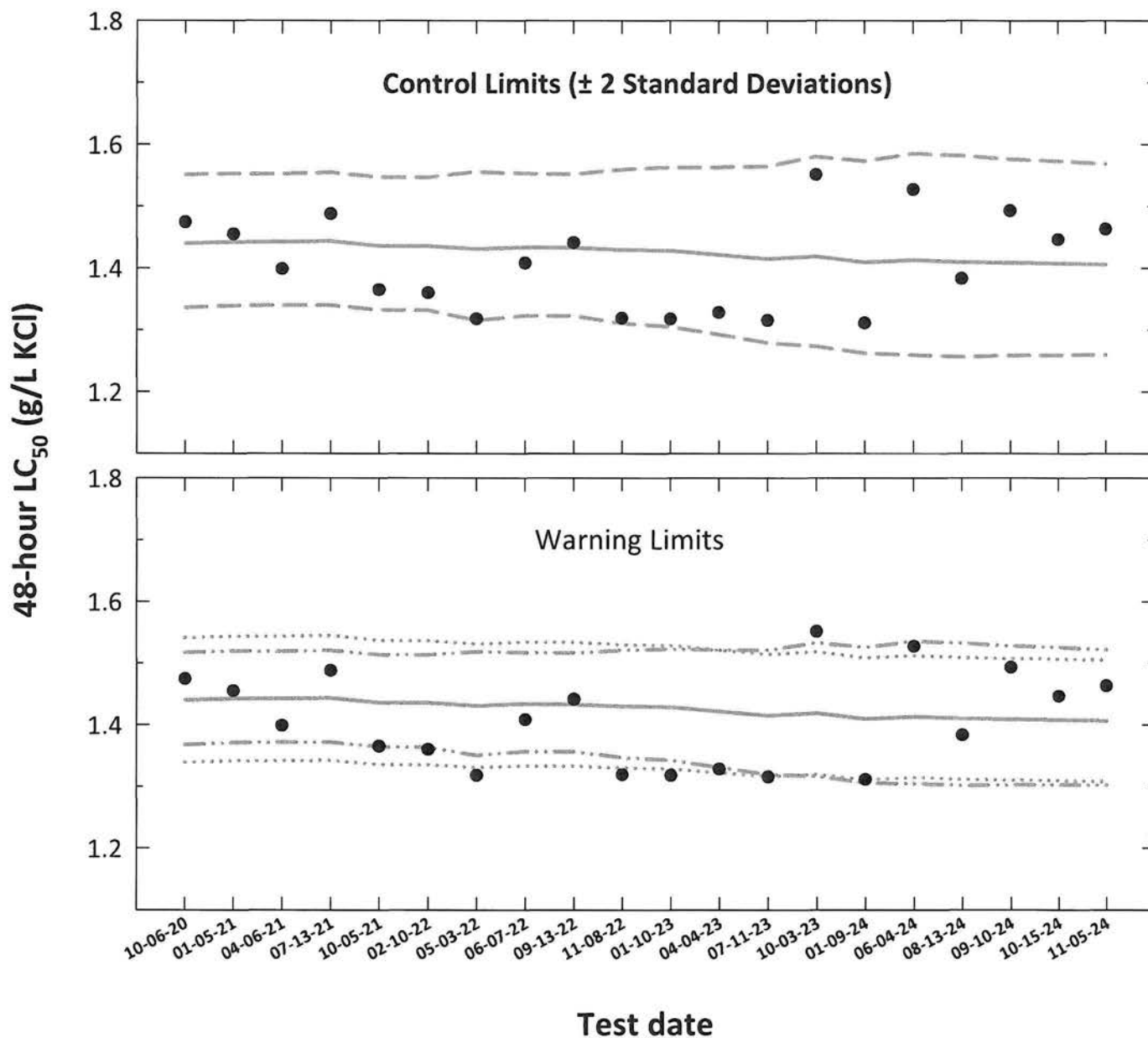
Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1573.7
Upper 95% confidence limit (mg KCl/L)	1515.5
48-hour LC ₅₀ (mg KCl/L)	1446.1

Comments:

Start Date:	10/15/2024	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant							
End Date:	10/17/2024	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	0.9000	0.9000										
1500	0.7000	0.8000										
1750	0.2000	0.4000										
2000	0.0000	0.0000										
Transform: Arcsin Square Root												
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total 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	0.2250	0	20
1250	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.064	2.850	0.2250	2	20
*1500	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	4.596	2.850	0.2250	5	20
*1750	0.3000	0.3000	0.5742	0.4636	0.6847	27.225	2	10.613	2.850	0.2250	14	20
2000	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								Statistic		Critical	Skew	Kurt
Equality of variance cannot be confirmed												
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test			1250	1500	1369.31		0.1152	0.11815	0.24414	0.00623	5.8E-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	16.1081	2.82857	10.5641	21.6521	0	3.47479	7.81472	0.32405	3.20139	0.06208	4	
Intercept	-46.568	9.0698	-64.345	-28.792								
TSCR												
Point	Probits	mg/L	95% Fiducial Limits									
EC01	2.674	1140.18	942.886	1257.38								
EC05	3.355	1256.84	1090	1356.7								
EC10	3.718	1323.84	1176.24	1414.43								
EC15	3.964	1371.04	1237.27	1455.93								
EC20	4.158	1409.76	1287.14	1490.79								
EC25	4.326	1443.85	1330.65	1522.36								
EC40	4.747	1533.44	1441.06	1611.44								
EC50	5.000	1589.99	1505.84	1674.18								
EC60	5.253	1648.62	1567.73	1745.79								
EC75	5.674	1750.92	1663.78	1885.74								
EC80	5.842	1793.26	1700.18	1948.16								
EC85	6.036	1843.9	1741.93	2025.47								
EC90	6.282	1909.65	1793.99	2129.42								
EC95	6.645	2011.44	1871.28	2296.74								
EC99	7.326	2217.25	2020.06	2653.81								
Dose-Response Plot												

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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	10-06-20	1.4741	0.1685	0.1583	0.0162	1.4397	1.3362	1.5513	1.3678	1.5172	1.3390	1.5405
2	01-05-21	1.4546	0.1627	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189	1.3411	1.5430
3	04-06-21	1.3988	0.1458	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187	1.3415	1.5434
4	07-13-21	1.4877	0.1725	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207	1.3424	1.5444
5	10-05-21	1.3650	0.1351	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132	1.3353	1.5363
6	02-10-22	1.3599	0.1335	0.1570	0.0163	1.4356	1.3320	1.5473	1.3635	1.5134	1.3351	1.5361
7	05-03-22	1.3180	0.1199	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182	1.3305	1.5308
8	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169	1.3332	1.5339
9	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163	1.3328	1.5335
10	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205	1.3296	1.5298
11	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227	1.3284	1.5283
12	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213	1.3221	1.5211
13	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209	1.3158	1.5139
14	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331	1.3197	1.5184
15	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256	1.3107	1.5081
16	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351	1.3142	1.5120
17	08-13-24	1.3832	0.1409	0.1493	0.0250	1.4102	1.2569	1.5823	1.3015	1.5322	1.3115	1.5089
18	09-10-24	1.4930	0.1740	0.1488	0.0244	1.4087	1.2589	1.5763	1.3024	1.5277	1.3101	1.5073
19	10-15-24	1.4461	0.1602	0.1484	0.0242	1.4075	1.2593	1.5731	1.3022	1.5251	1.3090	1.5060
20	11-05-24	1.4632	0.1653	0.1480	0.0238	1.4062	1.2603	1.5689	1.3024	1.5219	1.3077	1.5046

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 # 97

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 #: 7319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.89	7.50	7.79
	Dissolved oxygen (mg/L)	7.7	7.7	7.5
	*Salinity (ppt)	25.1	25.4	25.2
	*Alkalinity (mg/L CaCO ₃)	9.4		
	*Temperature (°C)	24.9	25.0	25.2
1000 mg/L	pH (S.U.)	7.81	7.50	7.78
	Dissolved oxygen (mg/L)	8.0	7.7	7.5
	*Salinity (ppt)	25.6	26.1	25.8
	*Temperature (°C)	24.8	25.2	25.2
1250 mg/L	pH (S.U.)	7.82	7.58	7.76
	Dissolved oxygen (mg/L)	7.9	7.7	7.4
	*Salinity (ppt)	25.8	26.0	26.4
	*Temperature (°C)	25.0	25.2	25.1
1500 mg/L	pH (S.U.)	7.82	7.50	7.78
	Dissolved oxygen (mg/L)	7.9	7.7	7.4
	*Salinity (ppt)	25.8	26.2	25.6
	*Temperature (°C)	25.0	25.1	25.1
1750 mg/L	pH (S.U.)	7.82	7.57	7.78
	Dissolved oxygen (mg/L)	8.0	7.8	7.3
	*Salinity (ppt)	26.0	26.4	25.7
	*Temperature (°C)	25.0	25.3	25.3
2000 mg/L	pH (S.U.)	7.82	7.57	
	Dissolved oxygen (mg/L)	8.0	7.8	
	*Salinity (ppt)	26.1	26.5	
	*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-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	13066468

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 # 97

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-05-24	* 1130	JK	1338	JK	6C	LIGHT GREEN	10-30-24B
24	11-06-24			1335	JK			
48 Termination	11-07-24			1334	JK			

*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):	10-26-24
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	10-25-24 1200 TO 10-26-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.59 Temperature (°C) 24.8

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	10	5 ^{sd} 5	7 ^{sd} 7	1 ^{qd} 1	1 ^{qd} 1	0 ^{ud} 0	0 ^{ud} 0
48 Termination	10	10	10	10	9 ^{id} 9	9 ^{id} 9	4 ^{id} 4	5 ^{sd} 5	0 ^{id} 0	1 ^{id} 1	0 ^{id} 0	0 ^{id} 0
Mean Survival	100%		100%		90%		45%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1390.2
Upper 95% confidence limit (mg KCl/L)	1533.3
48-hour LC ₅₀ (mg KCl/L)	1463.2

Comments:



Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	1.0000
1500	0.5000	0.7000
1750	0.1000	0.1000
2000	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
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1854		0	20
1250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1854		0	20
*1500	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	8.049	2.850	0.1854		8	20
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	16.756	2.850	0.1854		18	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		0.08888	0.09116	0.47099	0.00423	4.5E-05	4, 5

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	25.9707	5.38335	15.4194	36.5221	0	0.39555	7.81472	0.94116	3.18925	0.0385
Intercept	-77.827	17.1955	-111.53	-44.124						

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1257.97	1075.4 1350.53
EC05	3.355	1336.33	1187.23 1413.82
EC10	3.718	1380.07	1250.42 1450.05
EC15	3.964	1410.39	1294.17 1475.89
EC20	4.158	1434.96	1329.36 1497.52
EC25	4.326	1456.39	1359.66 1517.05
EC40	4.747	1511.79	1434.91 1571.99
EC50	5.000	1546.14	1477.94 1610.59
EC60	5.253	1581.26	1518.16 1654.6
EC75	5.674	1641.42	1578.35 1740.4
EC80	5.842	1665.92	1600.37 1778.48
EC85	6.036	1694.94	1625.11 1825.38
EC90	6.282	1732.18	1655.27 1887.89
EC95	6.645	1788.88	1698.85 1987.01
EC99	7.326	1900.3	1779.65 2192.17

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	0.9000
1500	0.4000	0.5000
1750	0.0000	0.1000
2000	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
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726		0	20
1250	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.690	2.850	0.1726		2	20
*1500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	11.175	2.850	0.1726		11	20
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.343	2.850	0.1726		19	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		0.08088	0.08295	0.52423	0.00367	2.4E-05	4, 5
Treatments vs D-Control										

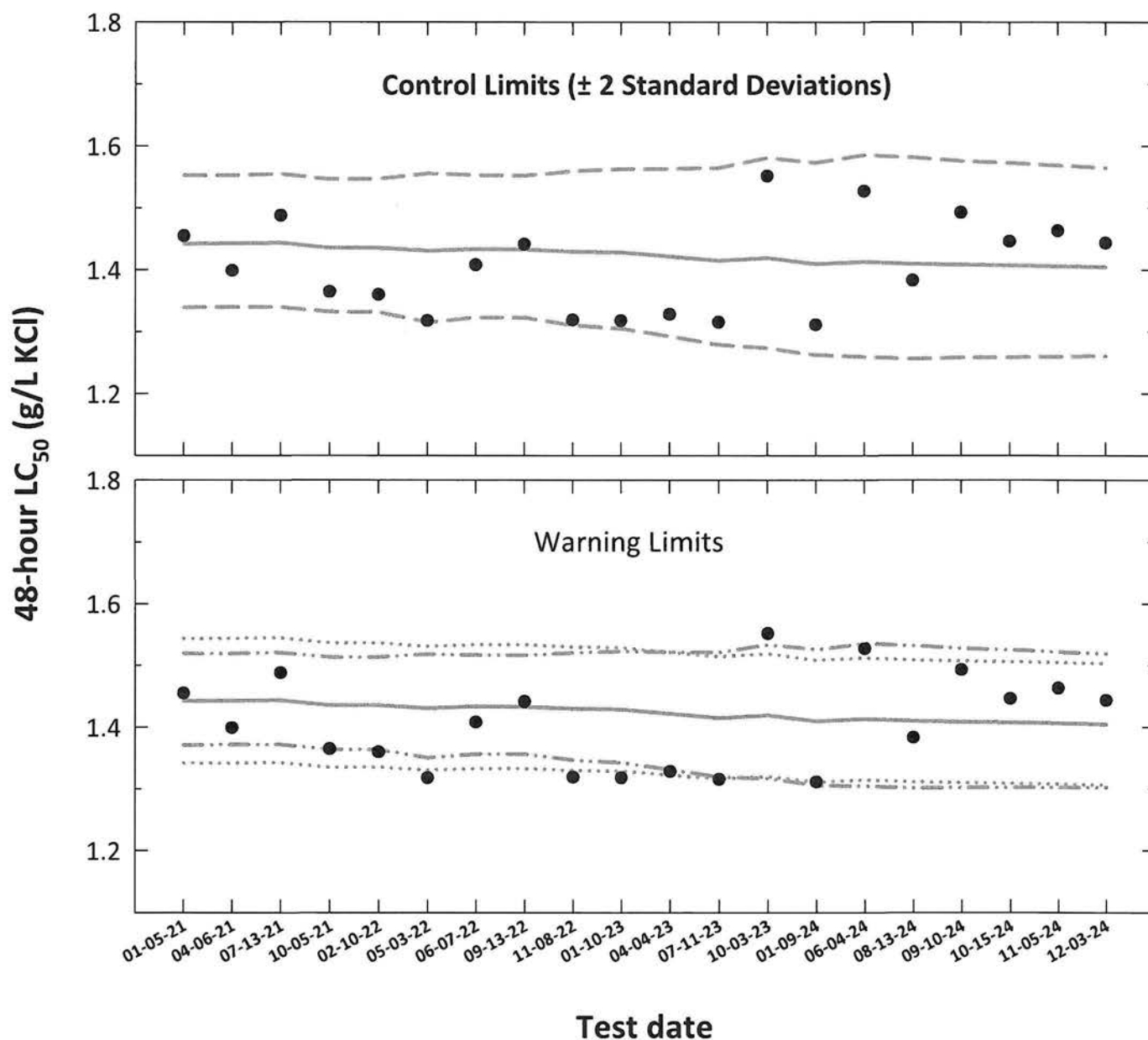
Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	20.195	3.71139	12.9207 27.4693	0	0.27292	7.81472	0.96504	3.1653	0.04952	3
Intercept	-58.923	11.7733	-81.999 -35.848							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1122.3	949.268 1220.58
EC05	3.355	1212.99	1068.31 1296.61
EC10	3.718	1264.29	1136.62 1340.4
EC15	3.964	1300.12	1184.36 1371.71
EC20	4.158	1329.32	1223.04 1397.89
EC25	4.326	1354.89	1256.56 1421.51
EC40	4.747	1421.54	1340.94 1487.48
EC50	5.000	1463.2	1390.18 1533.27
EC60	5.253	1506.09	1437.15 1584.96
EC75	5.674	1580.17	1509.55 1685
EC80	5.842	1610.57	1536.66 1729.38
EC85	6.036	1646.74	1567.47 1784.18
EC90	6.282	1693.41	1605.47 1857.5
EC95	6.645	1765.03	1661.09 1974.61
EC99	7.326	1907.65	1766.02 2220.41

Menidia beryllina

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)				
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	01-05-21	1.4546	0.1627	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189
2	04-06-21	1.3988	0.1458	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187
3	07-13-21	1.4877	0.1725	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207
4	10-05-21	1.3650	0.1351	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132
5	02-10-22	1.3599	0.1335	0.1570	0.0163	1.4356	1.3320	1.5473	1.3635	1.5134
6	05-03-22	1.3180	0.1199	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182
7	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169
8	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163
9	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205
10	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227
11	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213
12	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209
13	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331
14	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256
15	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351
16	08-13-24	1.3832	0.1409	0.1493	0.0250	1.4102	1.2569	1.5823	1.3015	1.5322
17	09-10-24	1.4930	0.1740	0.1488	0.0244	1.4087	1.2589	1.5763	1.3024	1.5277
18	10-15-24	1.4461	0.1602	0.1484	0.0242	1.4075	1.2593	1.5731	1.3022	1.5251
19	11-05-24	1.4632	0.1653	0.1480	0.0238	1.4062	1.2603	1.5689	1.3024	1.5219
20	12-03-24	1.4436	0.1594	0.1476	0.0235	1.4047	1.2609	1.5649	1.3023	1.5188
									CT - S _{A,10}	CT + S _{A,10}
									1.3411	1.5430
									1.3415	1.5434
									1.3424	1.5444
									1.3353	1.5363
									1.3351	1.5361
									1.3305	1.5308
									1.3332	1.5339
									1.3328	1.5335
									1.3296	1.5298
									1.3284	1.5283
									1.3221	1.5211
									1.3158	1.5139
									1.3197	1.5184
									1.3107	1.5081
									1.3142	1.5120
									1.3115	1.5089
									1.3101	1.5073
									1.3090	1.5060
									1.3077	1.5046
									1.3064	1.5030

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

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 98

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 #: 2334

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.70	7.85	7.83
	Dissolved oxygen (mg/L)	7.0	7.0	7.3
	*Salinity (ppt)	25.2	25.4	25.4
	*Alkalinity (mg/L CaCO ₃)	120		
	*Temperature (°C)	25.4	25.2	25.0
1000 mg/L	pH (S.U.)	7.80	7.85	7.83
	Dissolved oxygen (mg/L)	7.7	7.7	7.4
	*Salinity (ppt)	25.5	25.6	25.5
	*Temperature (°C)	25.3	25.3	25.1
1250 mg/L	pH (S.U.)	7.80	7.84	7.80
	Dissolved oxygen (mg/L)	7.7	7.6	7.4
	*Salinity (ppt)	25.9	25.9	25.9
	*Temperature (°C)	25.4	25.1	24.9
1500 mg/L	pH (S.U.)	7.81	7.83	7.82
	Dissolved oxygen (mg/L)	7.7	7.6	7.4
	*Salinity (ppt)	26.1	26.0	26.0
	*Temperature (°C)	25.4	25.3	25.2
1750 mg/L	pH (S.U.)	7.82	7.81	
	Dissolved oxygen (mg/L)	7.7	7.6	
	*Salinity (ppt)	26.4	26.3	
	*Temperature (°C)	25.4	25.3	
2000 mg/L	pH (S.U.)	7.82	7.80	
	Dissolved oxygen (mg/L)	7.8	7.6	
	*Salinity (ppt)	26.4	26.5	
	*Temperature (°C)	25.4	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-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	13066465

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 # 98

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-03-24	1145	Y	1353	Y	6B	ORANGE	11-27-24A
24	12-04-24			1345	Y			
48 Termination	12-05-24			1345	Y			

*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):	11-23-24
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	11-22-24 1200 TO 11-23-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.13
	Temperature (°C) 24.2

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	10	4 ^{ud}	4 ^{ud}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9 ^{ud}	3 ^{ud}	4	0	0	0	0
Mean Survival	100%		100%		95%		35%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1379.6
Upper 95% confidence limit (mg KCl/L)	1504.3
48-hour LC ₅₀ (mg KCl/L)	1443.6

Comments:



Acute Silverside Test-24 Hr Survival

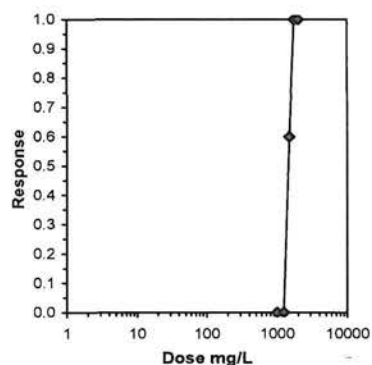
Start Date: 12/3/2024 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 12/5/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia berylina
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	1.0000
1500	0.4000	0.4000
1750	0.0000	0.0000
2000	0.0000	0.0000

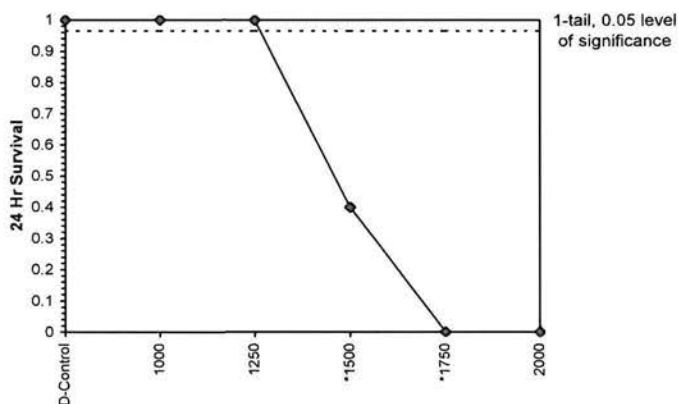
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
1250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*1500	0.4000	0.4000	0.6847	0.6847	0.6847	0.000	2	72.730	2.850	0.0285	12	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.00967	0.00991	0.65753	0.0001	1.7E-09	4, 5
Treatments vs D-Control										

Trim Level	EC50	95% CL	
0.0%	1464.62	1411.62	1519.62
5.0%	1463.2	1404.68	1524.16
10.0%	1461.79	1396.84	1529.76
20.0%	1459.06	1376.72	1546.34
Auto-0.0%	1464.62	1411.62	1519.62



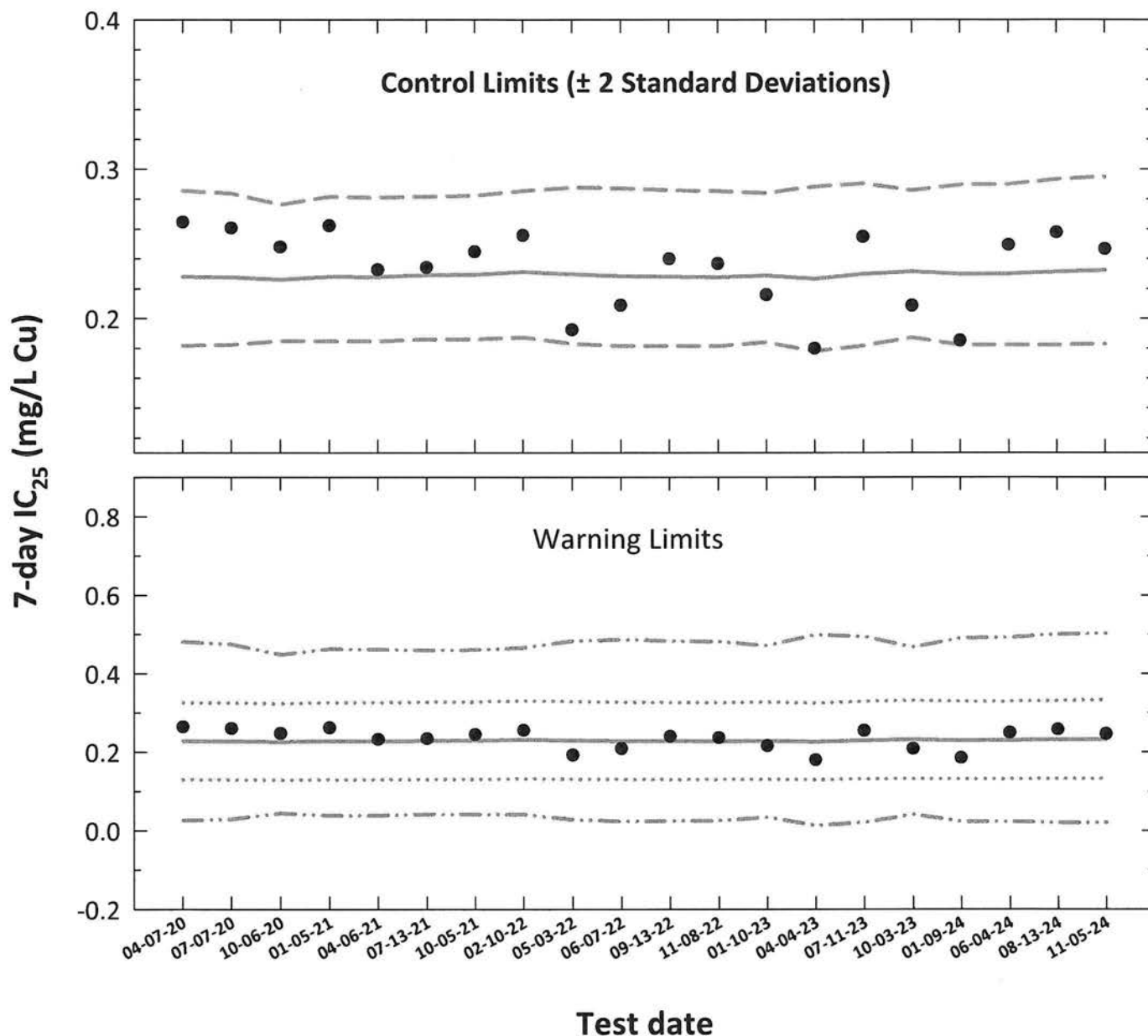
Dose-Response Plot



Menidia beryllina

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC_{25}** = 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 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)

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 Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.2312	0.1823	0.2932	0.0196	0.4996	0.1318	0.3306
20	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.2324	0.1831	0.2950	0.0201	0.5019	0.1325	0.3323

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the copper concentration that would cause a 25% reduction in *Menidia* 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.

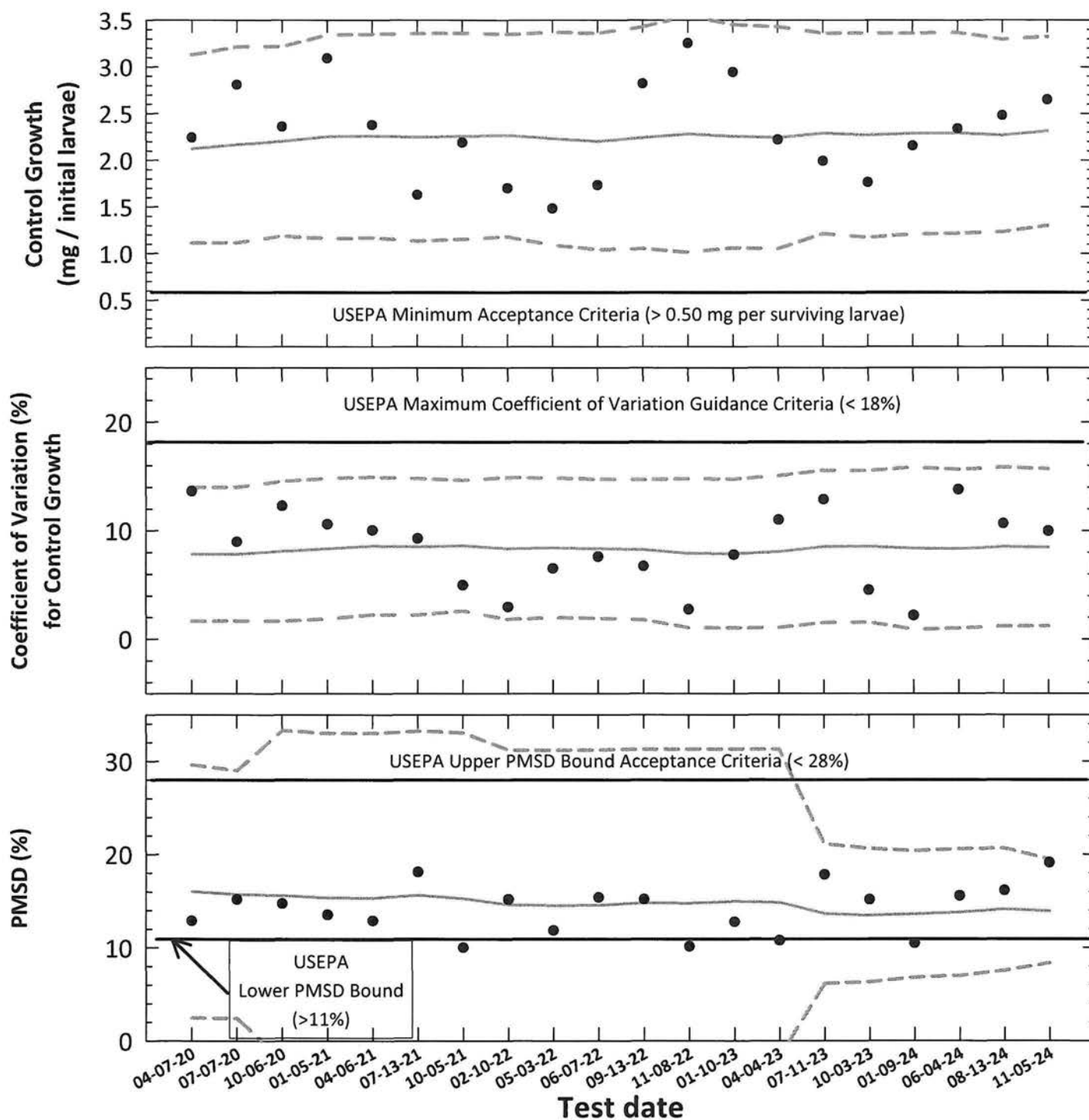
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.43).

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	04-07-20	100	2.244	13.6	0.2889	2.1227	1.114	7.8	1.7	16.1	2.5
2	07-07-20	100	2.809	9.0	0.4264	2.166	1.117	7.8	1.7	15.7	2.4
3	10-06-20	100	2.361	12.3	0.3484	2.205	1.188	8.1	1.7	15.6	-2.1
4	01-05-21	100	3.093	10.6	0.4186	2.253	1.163	8.3	1.9	15.4	-2.3
5	04-06-21	100	2.377	10.0	0.3062	2.258	1.167	8.6	2.2	15.3	-2.3
6	07-13-21	100	1.631	9.3	0.2956	2.247	1.136	8.5	2.2	15.7	-2.0
7	10-05-21	100	2.189	5.0	0.2192	2.258	1.153	8.6	2.6	15.3	-2.5
8	02-10-22	100	1.701	3.0	0.2583	2.266	1.180	8.4	1.8	14.6	-2.0
9	05-03-22	100	1.483	6.5	0.1756	2.232	1.092	8.4	2.0	14.5	-2.1
10	06-07-22	100	1.733	7.6	0.2665	2.201	1.041	8.3	1.9	14.6	-2.1
11	09-13-22	100	2.822	6.8	0.4290	2.243	1.057	8.3	1.8	14.8	-1.7
12	11-08-22	100	3.255	2.7	0.3294	2.280	1.014	7.9	1.0	14.8	-1.8
13	01-10-23	100	2.945	7.8	0.3755	2.257	1.062	7.9	1.0	15.0	-1.4
14	04-04-23	100	2.220	11.0	0.2389	2.243	1.053	8.1	1.1	14.9	-1.6
15	07-11-23	100	1.992	12.9	0.3549	2.287	1.213	8.5	1.5	13.6	6.2
16	10-03-23	100	1.763	4.5	0.2673	2.270	1.174	8.5	1.5	13.5	6.3
17	01-09-24	100	2.156	2.2	0.2257	2.287	1.211	8.4	0.9	13.6	6.8
18	06-04-24	100	2.339	13.8	0.3637	2.291	1.215	8.3	1.0	13.8	7.0
19	08-13-24	100	2.479	10.7	0.4001	2.266	1.235	8.5	1.2	14.1	7.6
20	11-05-24	100	2.648	10.0	0.5061	2.312	1.299	8.5	1.2	13.9	8.3

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: **147**

Dilution preparation information:						Comments:
Cu Stock INSS number:	INSS 1335					
Stock preparation:	100 mg Cu/L: Dissolve 0.1965 g CuSO ₄ in 500-mL deionized water					
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Test information:

Organism source:	Aquatic Indicators, Inc.	Randomizing template:	ORANGE
Age:	10-days old	Incubator number and shelf location:	6B
Batch:	AI Mb 10-26-24	Artemia CHM number:	CHM1294
Hatch dates and times:	10-25-24 1200 to 10-26-24 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = 7.59 Temperature (°C) = 24.8	Date / Time in oven:	11-12-24 1105
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	60 °C
		Date / Time out of oven:	11-13-24 1105
		*Final oven temperature:	60 °C
		Total drying time:	24-Hours

*60°C Oven, Thermometer SN: 14-98585

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	11-05-24	1130	JL	1500	JL	1300	JL	11-04-24 A 10-30-24 A 11-06-24 11-08-24 11-10-24 11-12-24	10-30-24 A
1	11-06-24	0500	JL	1130	JL	0900	JL		11-04-24 A
2	11-07-24	0500	JL	1350	JL	1300	JL		10-30-24 A
3	11-08-24	0500	JL	1100	JL	0900	JL		11-06-24
4	11-09-24	0500	JL	1300	JL	1215	JL		11-06-24
5	11-10-24	0500	JL	1100	JL	0900	JL		11-08-24
6	11-11-24	0500	JL	1100	JL	0903	JL		11-08-24
7	11-12-24					1105	JL		↓

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	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	0%	≤ 20%	7-day LC ₅₀ (%)	0.286
Average weight per initial larvae:	2.648		NOEC (%)	0.1
Average weight per surviving larvae:	2.648	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	0.141
			IC ₂₅ (%)	0.247

Species: Menidia beryllina

MbCuCR Test Number: 148

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) <u>11-01-24</u> Tray color code: <u>Next Navy</u> Analyst: <u>JO</u> Date: <u>11-01-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JO</u> Date: <u>11-14-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JO</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JO</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
2.648 2.846 -7.57 2.847 -7.57												

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

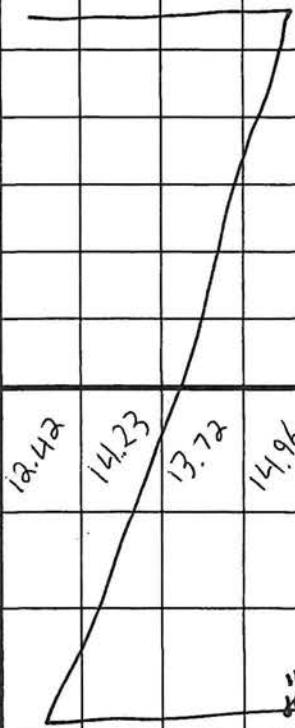
Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: Menidia beryllina

MbCuCR Test Number: 148

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	9 ^{id}	10	9 ^{id}	10					
6	10	10	10	10	9	8 ^{id}	9	10					
7	10	10	10	10	9	8	9	9 ^{id}					
*A = Pan weight (mg) Tray color code: <u>NOV4</u> Analyst: <u>JP</u> Date: <u>11-21-24</u>													
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>11-14-24</u>													
C = Larvae weight (mg) = B - A Analyst: <u>JP</u>													
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JP</u>													
Average weight per initial number of larvae (mg) Percent reduction from control (%)													
2.880 - 8.6% 2.491 5.97 0 100%													

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

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

Comments:

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

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

Test number: 148

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 (Mean weight per surviving number of larvae) (%)	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	12.48	37.71	25.23	2.523	2.648	10.0	2.523	100.0	2.648	10.0	Not applicable
	B	10	10	13.46	42.46	29.00	2.900			2.900				
	C	10	10	13.86	42.17	28.31	2.831			2.831				
	D	10	10	12.91	36.28	23.37	2.337			2.337				
0.025	E	10	10	12.89	43.40	30.51	3.051	2.846	8.7	3.051	100.0	2.846	8.7	-7.5
	F	10	10	14.48	41.42	26.94	2.694			2.694				
	G	10	10	11.94	42.58	30.64	3.064			3.064				
	H	10	10	12.21	37.97	25.76	2.576			2.576				
0.050	I	10	10	14.33	41.93	27.60	2.760	2.847	9.6	2.760	100.0	2.847	9.6	-7.5
	J	10	10	14.01	41.62	27.61	2.761			2.761				
	K	10	10	14.23	40.42	26.19	2.619			2.619				
	L	10	10	13.50	45.96	32.46	3.246			3.246				
0.100	M	10	10	11.58	44.29	32.71	3.271	2.880	14.9	3.271	100.0	2.880	14.9	-8.8
	N	10	10	13.66	42.06	28.40	2.840			2.840				
	O	10	10	13.43	36.38	22.95	2.295			2.295				
	P	10	10	12.81	43.94	31.13	3.113			3.113				
0.200	Q	10	9	14.21	40.67	26.46	2.940	2.855	10.9	2.646	87.5	2.491	9.0	5.9
	R	10	8	12.80	37.86	25.06	3.133			2.506				
	S	10	9	14.91	41.34	26.43	2.937			2.643				
	T	10	9	12.68	34.36	21.68	2.409			2.168				
0.500	U	10	0	0.00	0.00	0.00	0.000	0.000	0.0	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: 0.5061
PMSD: 19.1

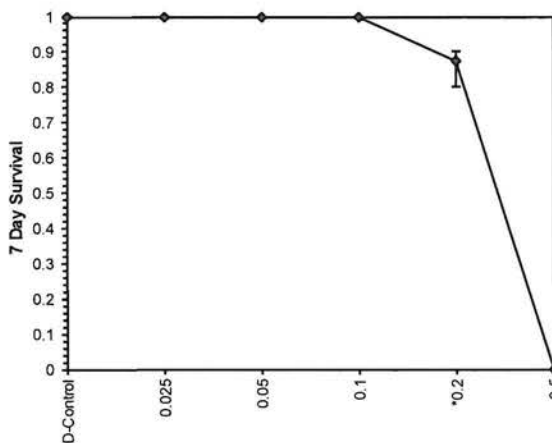
MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

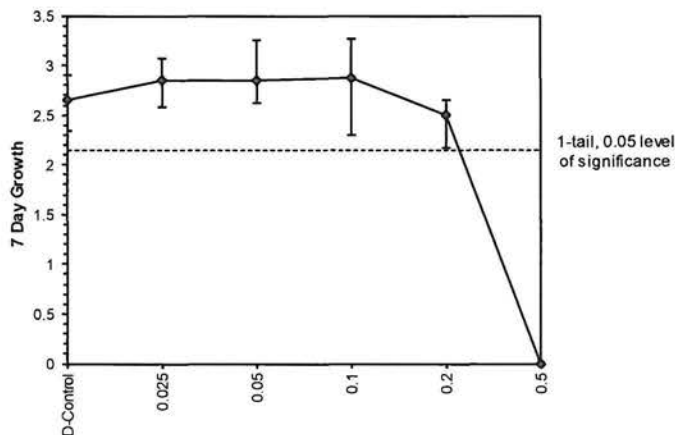
Lower PMSD bound determined by USEPA (10th percentile) = 11%.
Upper PMSD bound determined by USEPA (90th percentile) = 28%.
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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

Reviewed and
Approved by
Jim Sumner







Species: Menidia beryllina

MbCuCR Test Number: 148

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	
Concentration	Parameter	IL	NO BM	NO BM	BM	BM	IL
CONTROL, SaltSW	pH (S.U.)	7.87	7.88	8.05	7.85	7.98	7.72
	Dissolved oxygen (mg/L)	7.7	7.0	7.7	7.4	7.7	7.0
	Salinity (ppt)	25.1	25.1	25.0	25.3	24.9	23.5
	Alkalinity (mg CaCO ₃ /L)	94		* 130		130	
	Temperature (°C)	24.9	25.0	24.8	25.0	24.8	25.2
0.025 mg/L	pH (S.U.)	7.77	7.89	8.00	7.76	7.96	7.73
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.4	7.7	7.0
	Salinity (ppt)	25.1	25.3	25.0	25.5	25.0	25.6
	Temperature (°C)	24.8	25.2	24.9	25.1	24.9	25.4
0.05 mg/L	pH (S.U.)	7.78	7.88	8.01	7.75	7.96	7.69
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.4	7.7	7.1
	Salinity (ppt)	25.1	25.5	25.2	25.6	25.0	25.6
	Temperature (°C)	24.8	25.2	24.9	25.1	24.9	25.1
0.1 mg/L	pH (S.U.)	7.79	7.89	8.01	7.75	7.97	7.68
	Dissolved oxygen (mg/L)	7.9	7.7	8.0	7.5	7.7	7.1
	Salinity (ppt)	25.1	25.5	25.3	25.2	25.0	25.4
	Temperature (°C)	25.0	25.2	24.9	24.9	24.9	25.3
0.2 mg/L	pH (S.U.)	7.80	7.89	8.01	7.77	7.96	7.77
	Dissolved oxygen (mg/L)	7.9	7.7	8.0	7.5	7.7	7.1
	Salinity (ppt)	25.1	25.5	25.2	25.0	25.0	25.4
	Temperature (°C)	24.8	25.2	25.0	24.9	25.0	25.3
0.5 mg/L	pH (S.U.)	7.79	7.87	8.00	X		
	Dissolved oxygen (mg/L)	7.9	7.7	8.0			
	Salinity (ppt)	25.0	25.2	25.2			
	Alkalinity (mg CaCO ₃ /L)		105.41				
	Temperature (°C)	24.8	25.2	25.0			
		Initial	Final	Initial	Final	Initial	Final

* New batch of salt.

Species: *Menidia beryllina*

MbCuCR Test Number: 148

Concentration		Analyst	Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
			3		4		5		6	
			N	N	N	N	N	N	N	N
Parameter										
CONTROL, SaltSW	pH (S.U.)		7.62	7.69	7.68	7.79	7.74	7.87	7.75	7.67
	Dissolved oxygen (mg/L)		7.6	7.2	7.8	7.5	7.0	7.6	7.7	7.6
	Salinity (ppt)		25.0	25.4	24.8	25.1	25.2	25.2	25.2	25.2
	Alkalinity (mg CaCO ₃ /L)		110-115	110-115	120	110-115	130	110-115	110-115	110-115
	Temperature (°C)		25.0	25.2	25.0	25.2	24.7	25.0	25.0	25.3
0.025 mg/L	pH (S.U.)		7.96	7.67	7.95	7.81	8.04	7.85	7.96	7.67
	Dissolved oxygen (mg/L)		7.7	7.3	8.0	7.5	7.8	7.5	7.8	7.7
	Salinity (ppt)		25.3	25.6	25.0	25.4	25.1	25.3	25.2	25.6
	Temperature (°C)		25.1	25.0	25.0	25.0	24.7	24.9	24.8	25.0
0.05 mg/L	pH (S.U.)		7.95	7.67	7.97	7.78	8.04	7.85	7.96	7.67
	Dissolved oxygen (mg/L)		7.7	7.1	8.0	7.5	7.9	7.6	7.8	7.7
	Salinity (ppt)		25.3	25.4	24.9	25.4	25.0	25.4	25.2	25.6
	Temperature (°C)		25.1	25.3	25.1	25.0	24.7	24.9	24.8	25.0
0.1 mg/L	pH (S.U.)		7.95	7.69	7.97	7.80	8.04	7.86	7.96	7.67
	Dissolved oxygen (mg/L)		7.7	7.2	8.0	7.6	7.8	7.6	7.8	7.7
	Salinity (ppt)		25.3	25.6	24.8	25.2	25.0	25.5	25.2	25.9
	Temperature (°C)		25.1	25.3	25.1	25.0	24.7	25.1	24.8	25.0
0.2 mg/L	pH (S.U.)		7.96	7.73	7.97	7.82	8.04	7.86	7.96	7.70
	Dissolved oxygen (mg/L)		7.7	7.5	8.1	7.8	7.9	7.6	7.8	7.7
	Salinity (ppt)		25.2	25.7	24.7	25.4	24.9	25.4	25.2	25.6
	Temperature (°C)		25.2	25.1	25.1	24.8	24.8	24.8	24.7	25.2
0.5 mg/L	pH (S.U.)									
	Dissolved oxygen (mg/L)									
	Salinity (ppt)									
	Temperature (°C)									
Initial			Initial	Final	Initial	Final	Initial	Final	Initial	Final