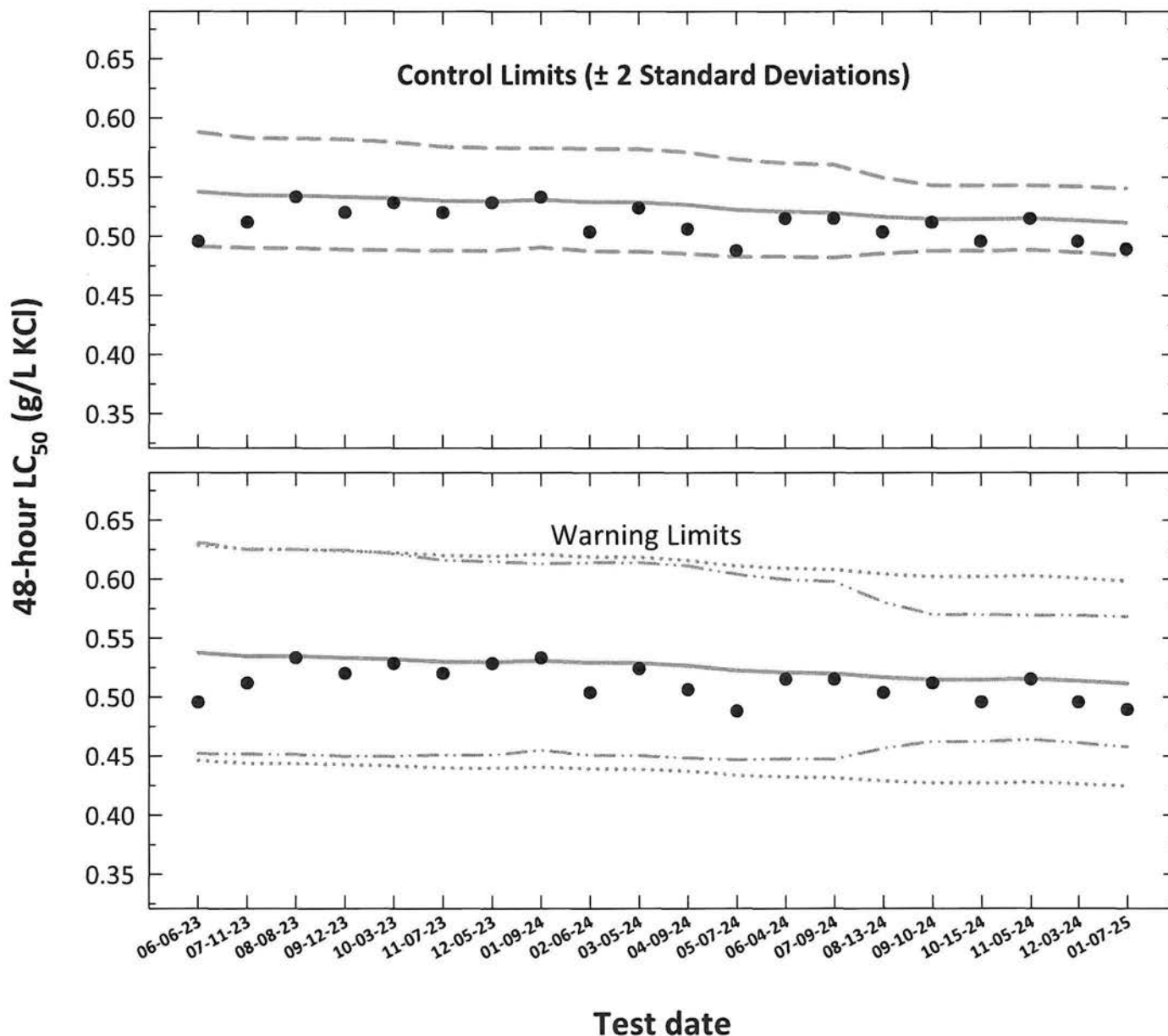


Americamysis (Mysidopsis) bahia

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	01-07-25	0.4892	-0.3105	-0.2912	0.0120	0.5114	0.4839 0.5405	0.4576 0.5683	0.4245 0.5984

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 286

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	5.0	7.5	10	15	20
mL Dilution water	995.0	992.5	990	985	980
Total volume (mL)	1000	1000	1000	1000	1000

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 #:

2348

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	8.17	8.00	7.90
	Dissolved oxygen (mg/L)	7.7	7.5	7.5
	*Salinity (ppt)	25.0	25.1	25.3
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	25.0	25.0	24.7
250 mg/L	pH (S.U.)	8.09	8.01	7.92
	Dissolved oxygen (mg/L)	7.7	7.5	7.5
	*Salinity (ppt)	25.1	25.3	25.4
	*Temperature (°C)	25.2	24.8	25.1
375 mg/L	pH (S.U.)	8.10	8.02	7.92
	Dissolved oxygen (mg/L)	7.7	7.6	7.4
	*Salinity (ppt)	25.2	25.4	25.5
	*Temperature (°C)	25.0	24.8	25.0
500 mg/L	pH (S.U.)	8.11	8.01	7.94
	Dissolved oxygen (mg/L)	7.7	7.6	7.5
	*Salinity (ppt)	25.2	25.4	25.6
	*Temperature (°C)	25.0	25.1	24.7
750 mg/L	pH (S.U.)	8.11	8.03	
	Dissolved oxygen (mg/L)	7.7	7.6	
	*Salinity (ppt)	25.5	25.8	
	*Temperature (°C)	25.1	25.1	
1000 mg/L	pH (S.U.)	8.12	7.96	7.8
	Dissolved oxygen (mg/L)	7.8	7.7	7.5
	*Salinity (ppt)	25.5	25.8	
	*Temperature (°C)	25.0	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	13066468

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 286

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-07-25	* 1145	jl	1345	jl	1F	PINK	01-02-25B
24	01-08-25			1400	jl			
48 Termination	01-09-25			1339	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):	01-06-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	01-05-25 1100 TO 01-06-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.90 Temperature (°C) 15.0

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	9 ^{1d}	7 ^{2d}	7 ^{2d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{1d}	9	5 ^{2d}	5 ^{2d}	0	0	0	0
Mean Survival	100%		100%		90%		50%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	449.4
Upper 95% confidence limit (mg KCl/L)	546.3
48-hour LC ₅₀ (mg KCl/L)	489.2

Comments:



Start Date:	1/7/2025	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	1/9/2025	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:					

Cone-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	0.9000
500	0.7000	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-CON/L	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	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	8.166	2.850	0.1469	6	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

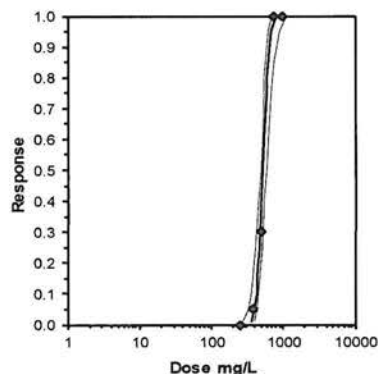
Equality of variance cannot be confirmed

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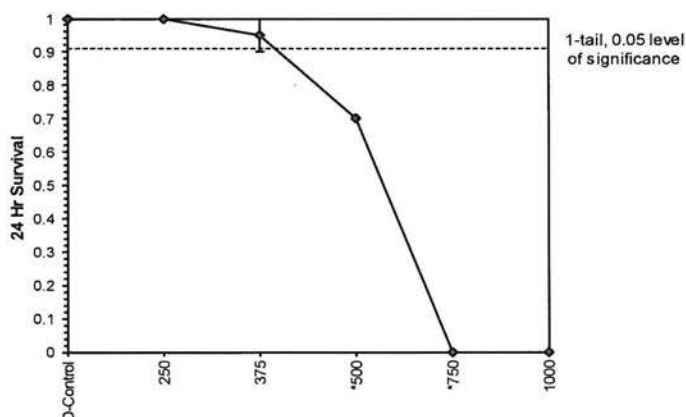
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	13.5631	2.95357	7.77407	19.352	0	1.60351	7.81472	0.65859	2.7229	0.07373	6
Intercept	-31.931	7.99322	-47.598	-16.264							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	355.945	268.013	403.777
EC05	3.355	399.603	325.274	441.49
EC10	3.718	425.025	359.567	464.411
EC15	3.964	443.085	383.912	481.556
EC20	4.158	457.984	403.707	496.522
EC25	4.326	471.165	420.796	510.585
EC40	4.747	506.086	462.887	552.84
EC50	5.000	528.328	486.642	584.178
EC60	5.253	551.547	508.984	620.484
EC75	5.674	592.425	543.79	691.734
EC80	5.84	609.475	557.134	723.678
EC85	6.036	629.969	572.547	763.518
EC90	6.282	656.737	591.895	817.679
EC95	6.645	698.518	620.809	906.515
EC99	7.326	784.195	676.896	1103.25



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 1/7/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 1/9/2025 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	0.9000	0.9000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*375	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	20
*500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	62.662	2.850	0.0285	10	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
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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
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Dunnett's Test 250 375 306.186 0.00967 0.00991 0.57759 0.0001 2.4E-09 4, 5

Treatments vs D-Control

Maximum Likelihood-Probit										
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Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
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Slope 12.2852 2.70352 6.9863 17.5841 0 0.5411 7.81472 0.90977 2.68948 0.0814 5

Intercept -28.041 7.23729 -42.226 -13.856

TSCR

Point	Probits	mg/L	95% Fiducial Limits
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EC01 2.674 316.317 228.545 363.918

EC05 3.355 359.415 284.002 400.827

EC10 3.718 384.741 318.008 423.159

EC15 3.964 402.829 342.542 439.798

EC20 4.158 417.809 362.75 454.279

EC25 4.326 431.104 380.387 467.877

EC40 4.747 466.512 424.438 509.067

EC50 5.000 489.198 449.414 540.273

EC60 5.253 512.988 472.755 577.159

EC75 5.674 555.121 508.762 651.101

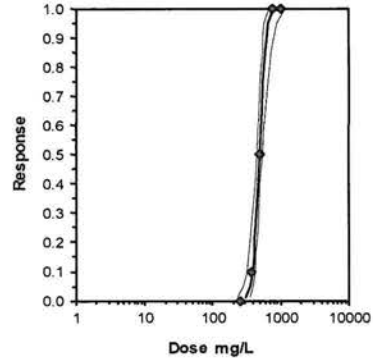
EC80 5.842 572.785 522.508 684.696

EC85 6.036 594.086 538.382 726.881

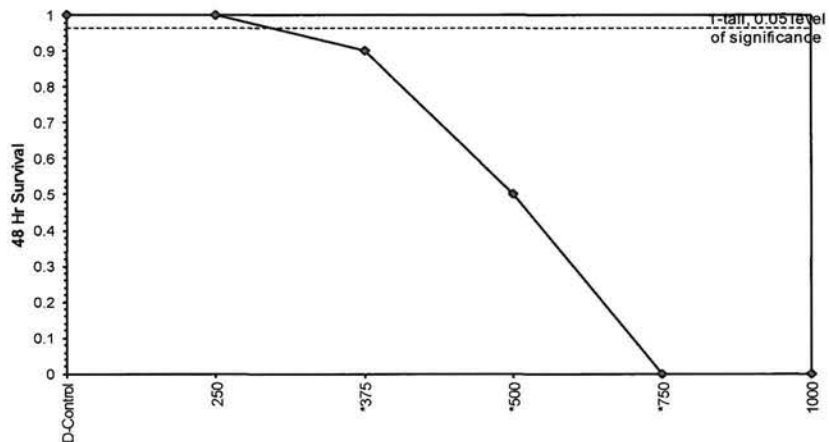
EC90 6.282 622.016 558.334 784.667

EC95 6.645 665.846 588.246 880.407

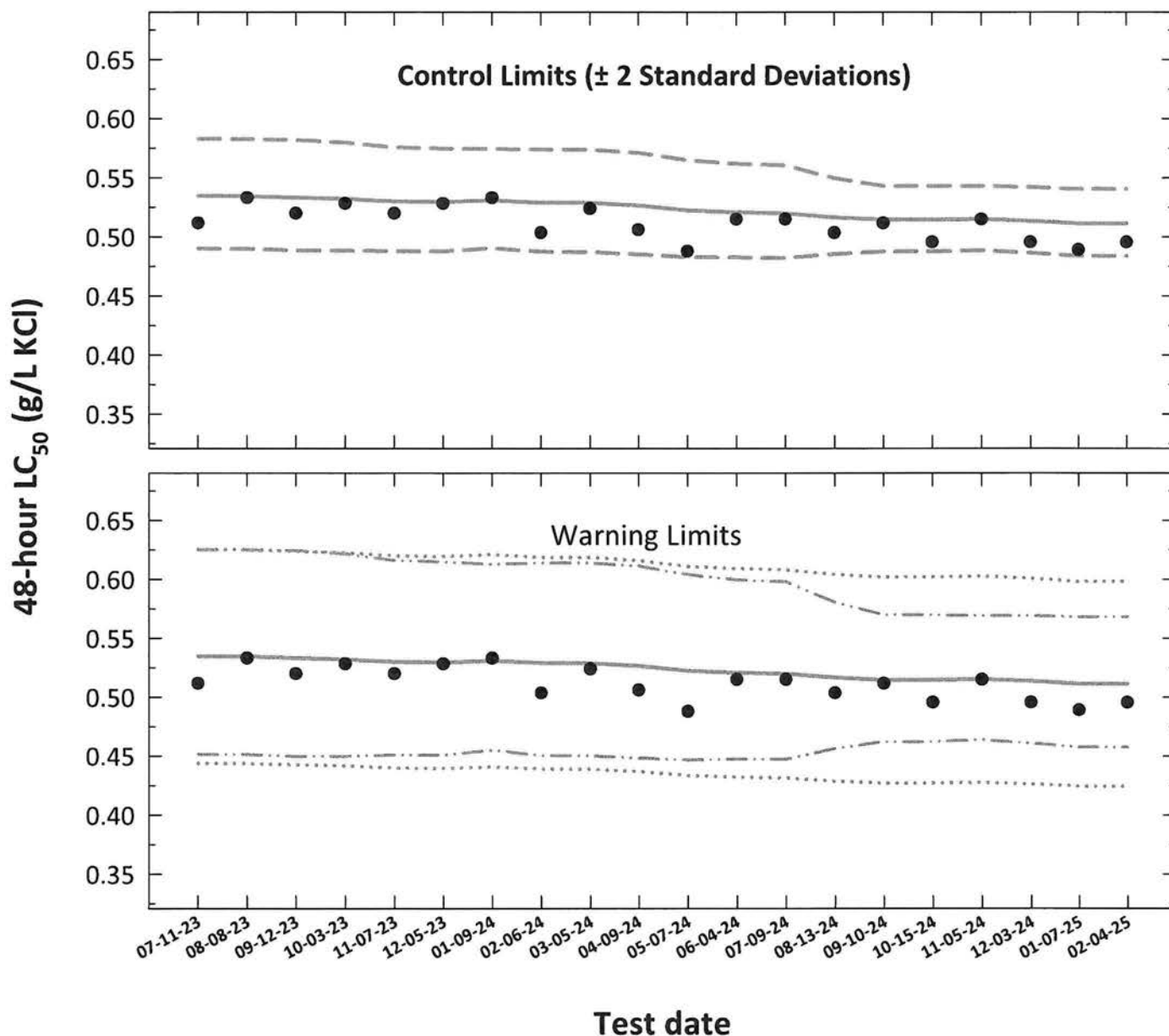
EC99 7.326 756.566 646.669 1096.14



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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252
2	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250
3	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244
4	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217
5	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161
6	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151
7	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550	0.6131
8	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875	0.5740	0.4505	0.6141
9	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872	0.5738	0.4502	0.6140
10	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853	0.5711	0.4483	0.6113
11	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829	0.5651	0.4467	0.6041
12	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4827	0.5620	0.4476	0.5998
13	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822	0.5606	0.4473	0.5982
14	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4856	0.5497	0.4565	0.5807
15	09-10-24	0.5117	-0.2910	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700
16	10-15-24	0.4957	-0.3048	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700
17	11-05-24	0.5149	-0.2882	-0.2880	0.0115	0.5153	0.4888	0.5432	0.4638	0.5695
18	12-03-24	0.4957	-0.3048	-0.2893	0.0118	0.5136	0.4866	0.5422	0.4609	0.5693
19	01-07-25	0.4892	-0.3105	-0.2912	0.0120	0.5114	0.4839	0.5405	0.4576	0.5683
20	02-04-25	0.4957	-0.3048	-0.2912	0.0120	0.5114	0.4839	0.5405	0.4576	0.5683
									CT - S _{A,10}	CT + S _{A,10}
									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
									0.4277	0.6029
									0.4263	0.6009
									0.4245	0.5984
									0.4245	0.5984

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 # 287

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	5.0	7.5	10	15	20
mL Dilution water	995.0	992.5	990	985	980
Total volume (mL)	1000	1000	1000	1000	1000

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 #: 7348

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>KL</i>	<i>20.8.1</i>	<i>20.8.1</i>
	pH (S.U.)	<i>7.95</i>	<i>7.88</i>	<i>7.85</i>
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.9</i>	<i>7.4</i>
	*Salinity (ppt)	<i>24.9</i>	<i>25.3</i>	<i>25.6</i>
	*Alkalinity (mg/L CaCO ₃)	<i>100</i>		
250 mg/L	*Temperature (°C)	<i>25.0</i>	<i>25.6</i>	<i>25.2</i>
	pH (S.U.)	<i>7.97</i>	<i>7.88</i>	<i>7.82</i>
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.9</i>	<i>7.4</i>
	*Salinity (ppt)	<i>24.9</i>	<i>25.4</i>	<i>25.7</i>
	*Temperature (°C)	<i>25.2</i>	<i>25.5</i>	<i>25.5</i>
375 mg/L	pH (S.U.)	<i>7.99</i>	<i>7.88</i>	<i>7.83</i>
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.8</i>	<i>7.4</i>
	*Salinity (ppt)	<i>24.9</i>	<i>25.7</i>	<i>26.2</i>
	*Temperature (°C)	<i>25.2</i>	<i>25.5</i>	<i>25.5</i>
	pH (S.U.)	<i>8.00</i>	<i>7.89</i>	<i>7.85</i>
500 mg/L	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.8</i>	<i>7.3</i>
	*Salinity (ppt)	<i>25.0</i>	<i>25.6</i>	<i>25.9</i>
	*Temperature (°C)	<i>25.0</i>	<i>25.7</i>	<i>25.6</i>
	pH (S.U.)	<i>8.01</i>	<i>7.89</i>	
	Dissolved oxygen (mg/L)	<i>7.9</i>	<i>7.8</i>	
750 mg/L	*Salinity (ppt)	<i>25.0</i>	<i>26.0</i>	
	*Temperature (°C)	<i>25.0</i>	<i>25.4</i>	
	pH (S.U.)	<i>8.02</i>	<i>7.89</i>	
	Dissolved oxygen (mg/L)	<i>8.0</i>	<i>7.9</i>	
	*Salinity (ppt)	<i>25.3</i>	<i>25.9</i>	
1000 mg/L	*Temperature (°C)	<i>25.1</i>	<i>25.5</i>	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	<i>130661685</i>

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 # 287

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-04-15	* 1045	JK	1245	JK	6D	LIBAT GREEN	01-28-15 B
24	02-05-15			1300	JK			
48 Termination	02-06-15			1250	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 Ab):	02-03-15
Age (1 to 5 days old):	1.2 DAYS
Date organisms were born:	02-02-15 1200 TD 02-05-15 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.88 Temperature (°C) 24.5

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^{id}	9 ^{id}	0 ^{ud}	0 ^{ud}	0 ^{ud}	0 ^{ud}
48 Termination	10	10	10	10	10	9 ^{id}	6 ^{sd}	4 ^{sd}	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: 2/4/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 2/6/2025 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:

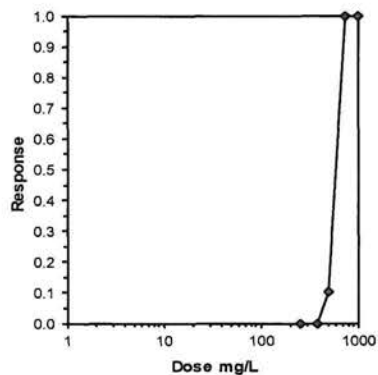
Comments:

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

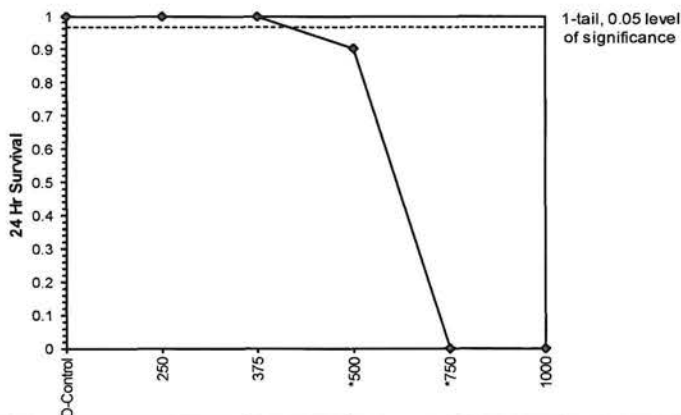
Transform: Arcsin Square Root								1-Tailed	Number	Total		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.59802	0.0001	2.2E-09	4, 5
Treatments vs D-Control										

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



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 2/4/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 2/6/2025 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	0.9000
500	0.6000	0.4000
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	0.2335	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.995	2.850	0.2335	1	20
*500	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	7.649	2.850	0.2335	10	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.299	2.850	0.2335	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

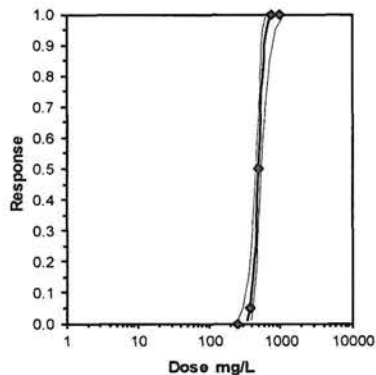
Auxiliary Tests

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

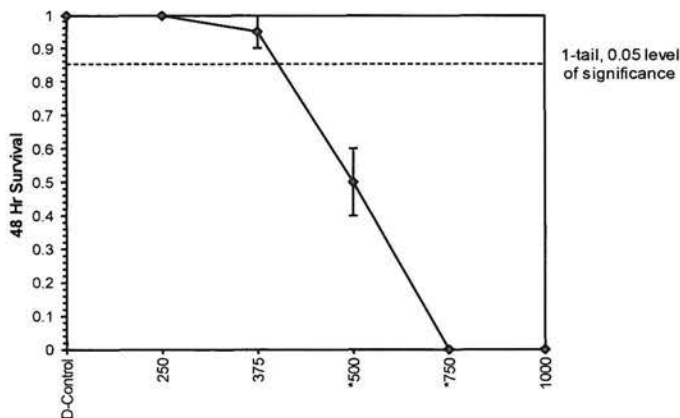
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	Chv	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.12113	0.12423	0.60026	0.00671	7.7E-05	4, 5

Treatments vs D-Control

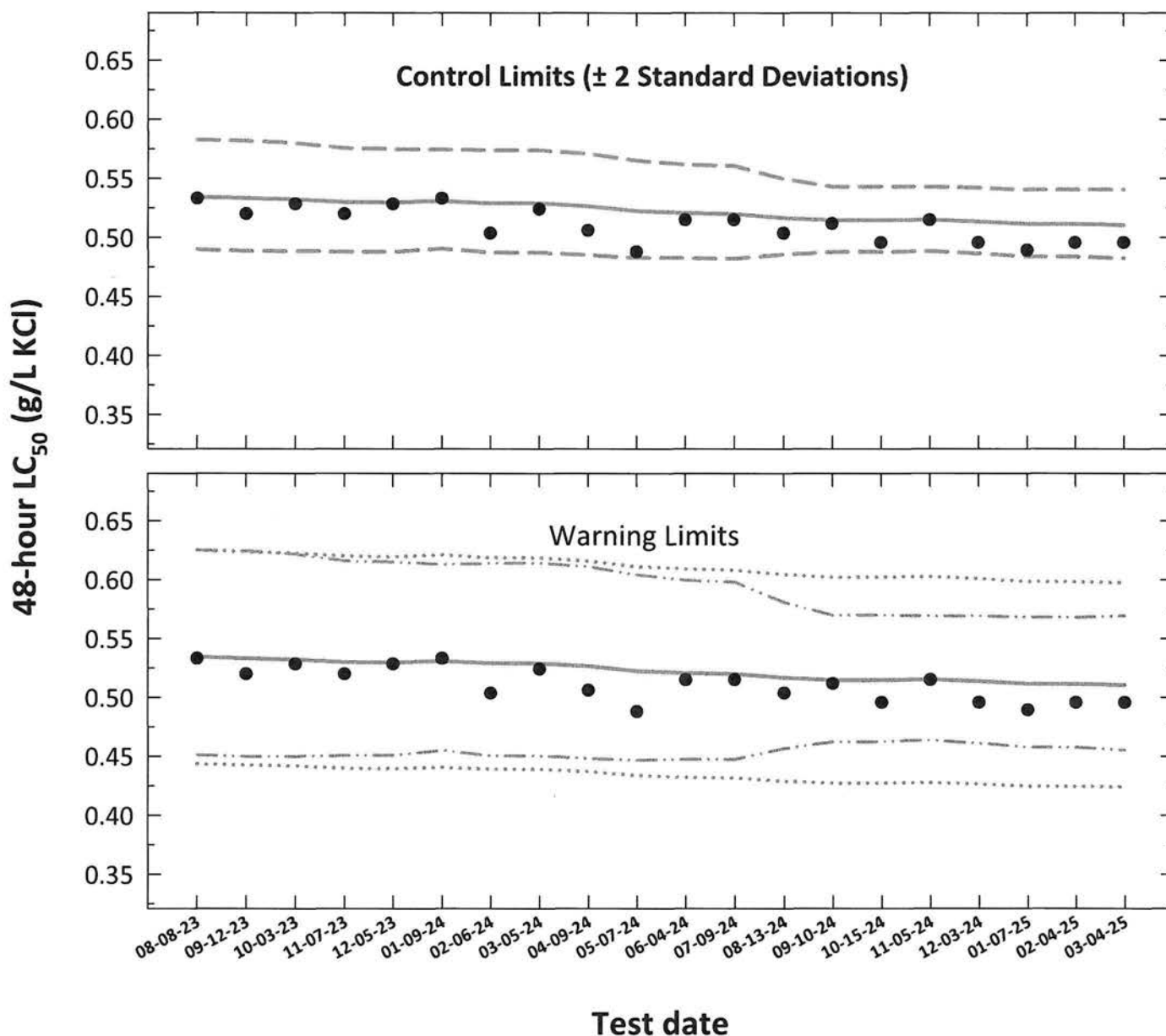
Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.3605	3.51976	7.4618	21.2592	0	0.17806	7.81472	0.98105	2.69519	0.06964	4
Intercept	-33.704	9.43318	-52.193	-15.215							
TSCR											
Point	Probits	mg/L	95% Fiducial 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 LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10} = 10^{th}$ 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		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	01-07-25	0.4892	-0.3105	-0.2912	0.0120	0.5114	0.4839	0.5405	0.4576	0.5683	0.4245	0.5984
19	02-04-25	0.4957	-0.3048	-0.2912	0.0120	0.5114	0.4839	0.5405	0.4576	0.5683	0.4245	0.5984
20	03-04-25	0.4957	-0.3048	-0.2919	0.0124	0.5106	0.4823	0.5406	0.4551	0.5693	0.4238	0.5974

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

Page 1 c

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 288

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	5.0	7.5	10	15	20
mL Dilution water	995.0	992.5	990	985	980
Total volume (mL)	1000	1000	1000	1000	1000

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 #:

2357

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	8.08	8.01	7.94
	Dissolved oxygen (mg/L)	8.1	7.7	7.7
	*Salinity (ppt)	25.0	25.4	25.5
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	25.2	24.7	24.6
250 mg/L	pH (S.U.)	8.05	8.01	7.93
	Dissolved oxygen (mg/L)	8.2	7.7	7.6
	*Salinity (ppt)	25.1	25.5	25.7
	*Temperature (°C)	25.0	24.9	24.3
375 mg/L	pH (S.U.)	8.06	8.01	7.89
	Dissolved oxygen (mg/L)	8.2	7.7	7.6
	*Salinity (ppt)	25.2	25.7	25.8
	*Temperature (°C)	25.2	24.6	24.8
500 mg/L	pH (S.U.)	8.06	8.01	7.91
	Dissolved oxygen (mg/L)	8.2	7.7	7.6
	*Salinity (ppt)	25.3	25.7	26.0
	*Temperature (°C)	25.2	24.6	24.7
750 mg/L	pH (S.U.)	8.07	8.00	
	Dissolved oxygen (mg/L)	8.2	7.6	
	*Salinity (ppt)	25.41	25.8	
	*Temperature (°C)	25.2	24.5	
1000 mg/L	pH (S.U.)	8.07	8.01	
	Dissolved oxygen (mg/L)	8.2	7.6	
	*Salinity (ppt)	25.5	26.0	
	*Temperature (°C)	25.2	24.7	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13064685

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 # 288

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-04-25	* 1100	JL	1300	JL	1E	PINK	0216-3A
24	03-05-25			1250	JL			
48 Termination	03-06-25			1255	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	03-63-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	03-02-25 1200 TO 03-03-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01 Temperature (°C) 24.5

Survival Data (number of living organisms):

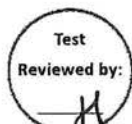
Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	8 ^{2d}	9 ^{4d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9	5 ^{3d}	5 ^{4d}	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: 3/4/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/6/2025 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	0.8000	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	0.1279	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*500	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	5.213	2.850	0.1279	3	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	27.929	2.850	0.1279	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

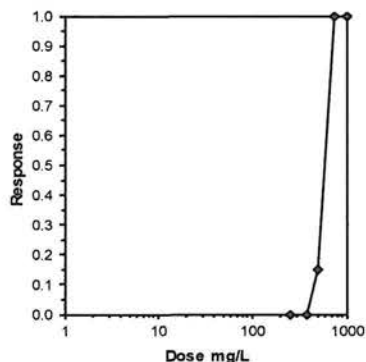
Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.05495	0.05636	0.5915	0.00201	4.1E-06	4, 5

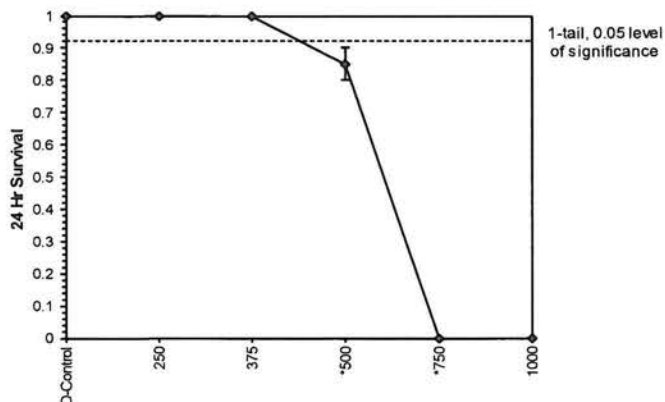
Treatments vs D-Control

Trimmed Spearman-Kärber

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



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 3/4/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/6/2025 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	0.9000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	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)

Dunnett's Test

Treatments vs D-Control

Maximum Likelihood-Probit

Parameter Value SE 95% Fiducial Limits

Slope 14.3605 3.51976 7.4618 21.2592

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

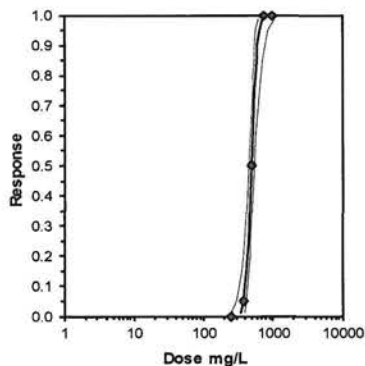
EC80 5.842 567.283 520.655 685.187

EC85 6.036 585.283 533.773 724.894

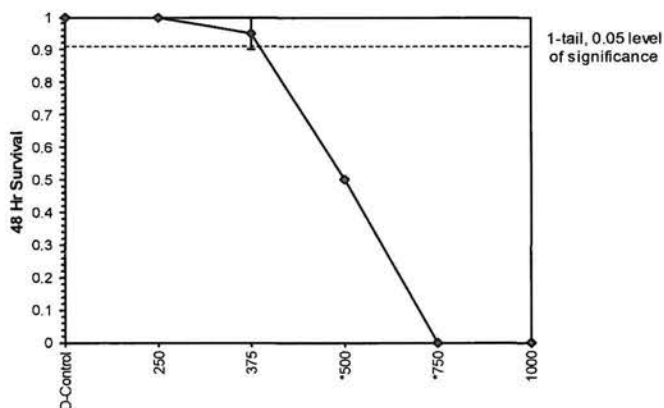
EC90 6.282 608.744 550.147 778.99

EC95 6.645 645.258 574.488 867.975

EC99 7.326 719.763 621.393 1066.13



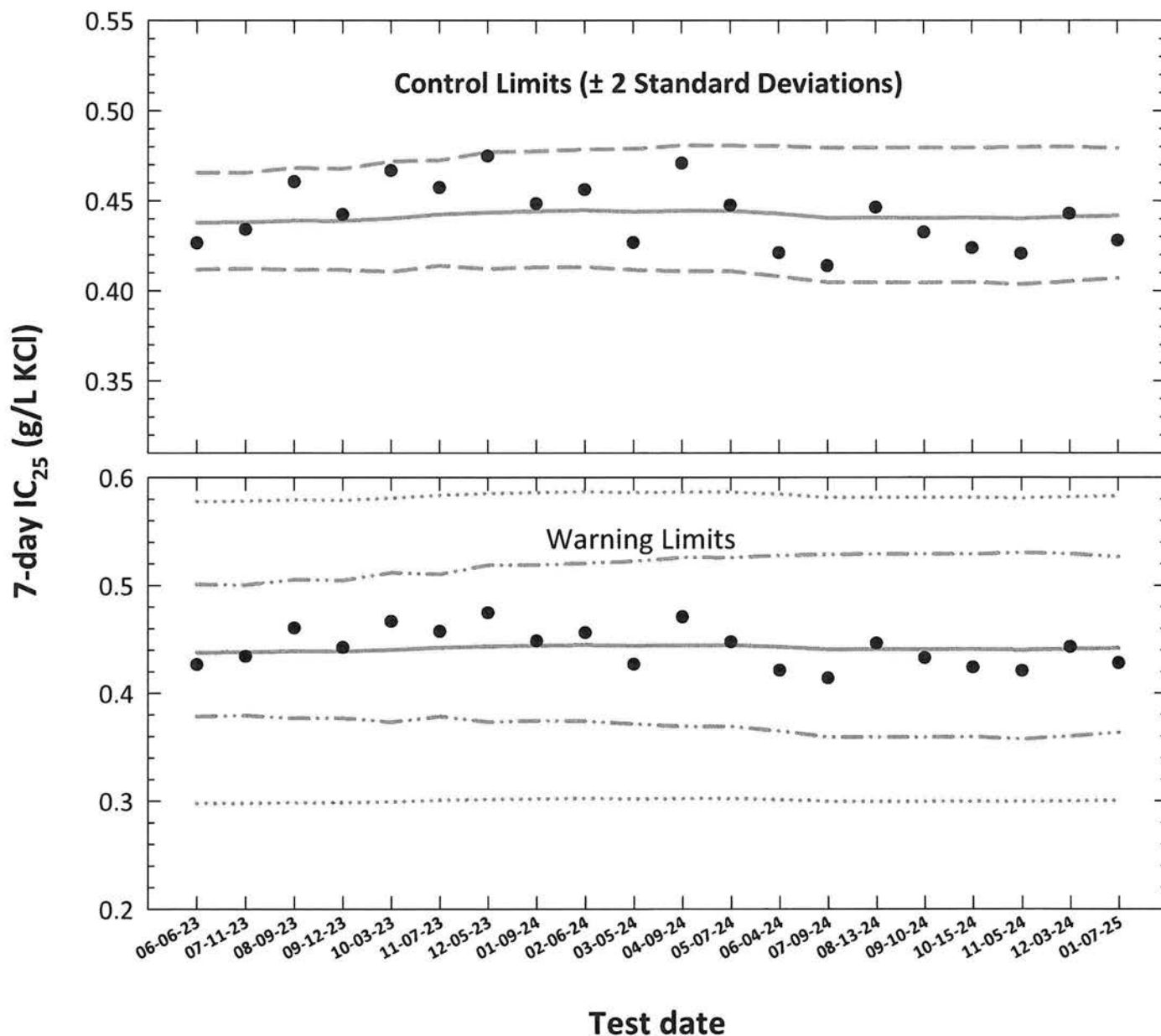
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053	0.4800	0.3600	0.5294	0.2999	0.5822
20	01-07-25	0.4280	-0.3686	-0.3549	0.0177	0.4417	0.4071	0.4792	0.3634	0.5266	0.3003	0.5830

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(c)).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

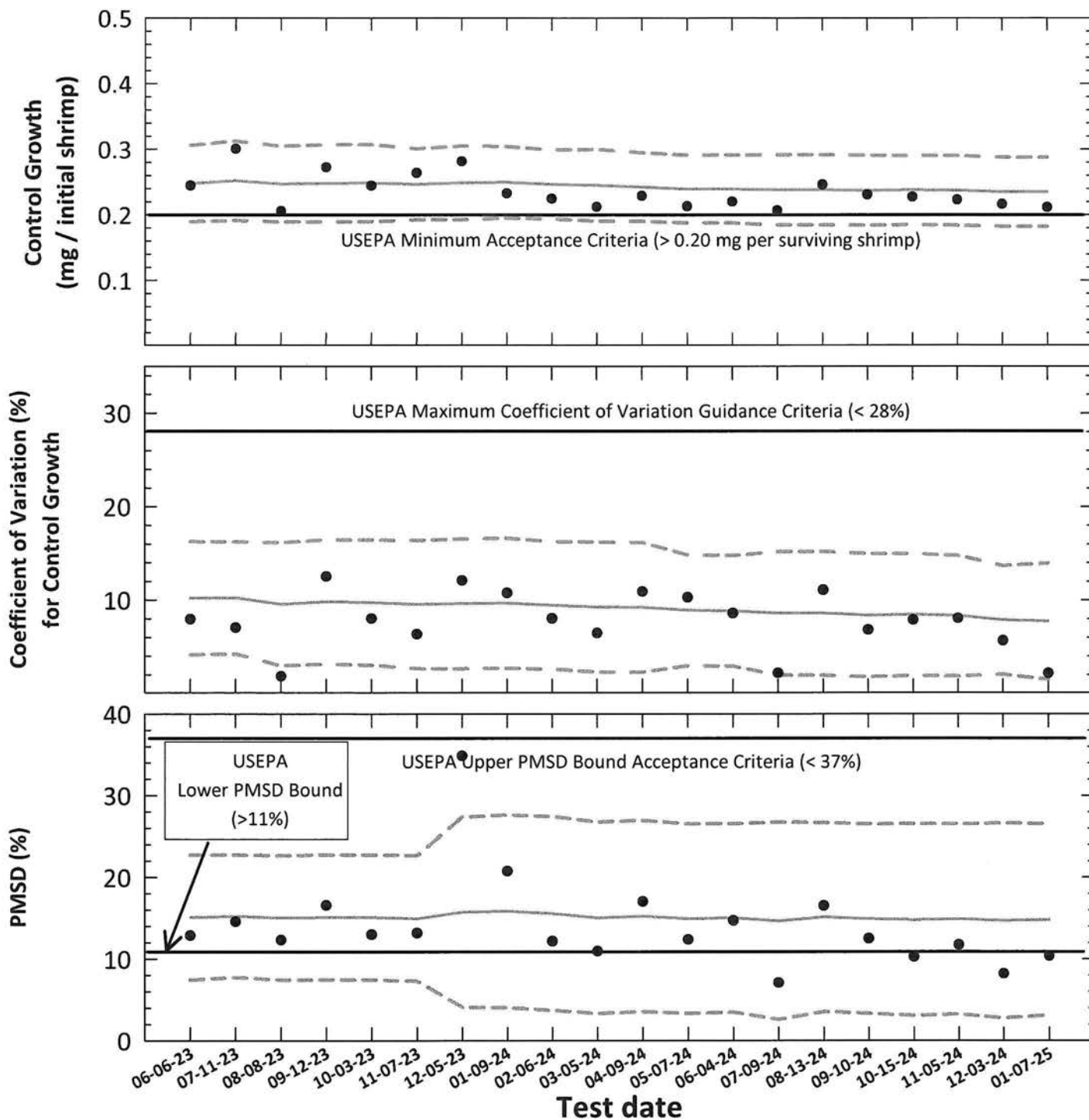
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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

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

CV = Coefficient of variation.

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



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

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

Test number	Test date	ToxCal Determination				Control Growth			Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial shrimp)	CV (%)													
1	06-06-23	100	0.245	8.0	0.0314	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7	15.1	7.4	22.7
2	07-11-23	100	0.300	7.0	0.0437	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7	15.2	7.7	22.7
3	08-08-23	100	0.206	1.8	0.0253	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6	15.0	7.4	22.6
4	09-12-23	100	0.273	12.5	0.0451	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7	15.1	7.4	22.7
5	10-03-23	100	0.244	8.0	0.0317	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7	15.1	7.4	22.7
6	11-07-23	100	0.264	6.3	0.0347	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6	14.9	7.3	22.6
7	12-05-23	100	0.282	12.1	0.0981	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4	15.7	4.1	27.4
8	01-09-24	100	0.233	10.8	0.0482	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6	15.8	4.0	27.6
9	02-06-24	100	0.225	8.0	0.0273	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4	15.5	3.7	27.4
10	03-05-24	100	0.212	6.5	0.0232	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8	15.0	3.3	26.8
11	04-09-24	100	0.229	10.9	0.0389	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9	15.2	3.5	26.9
12	05-07-24	100	0.213	10.3	0.0263	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5	14.9	3.3	26.5
13	06-04-24	100	0.220	8.6	0.0323	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6	15.0	3.5	26.6
14	07-09-24	100	0.207	2.2	0.0146	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7	14.6	2.6	26.7
15	08-13-24	100	0.246	11.1	0.0406	0.238	0.185	0.292	8.6	1.9	15.2	15.1	3.5	26.7	15.1	3.5	26.7
16	09-10-24	100	0.231	6.8	0.0288	0.238	0.184	0.291	8.4	1.8	15.0	14.9	3.3	26.5	14.9	3.3	26.5
17	10-15-24	100	0.228	7.9	0.0233	0.238	0.185	0.291	8.5	1.9	15.0	14.8	3.1	26.5	14.8	3.1	26.5
18	11-05-24	100	0.223	8.0	0.0261	0.237	0.184	0.291	8.3	1.9	14.8	14.9	3.2	26.5	14.9	3.2	26.5
19	12-03-24	100	0.216	5.7	0.0177	0.235	0.182	0.288	7.9	2.0	13.7	14.7	2.7	26.6	14.7	2.7	26.6
20	01-07-25	100	0.212	2.2	0.0219	0.235	0.183	0.288	7.7	1.5	14.0	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: **259**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2348					
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)	10	15	20	30	40	
Diluent volume (mL)	1990	1985	1980	1970	1960	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:			Test information:		
Organism age:	7-days old		Randomizing template:	Guel	
Date and times organisms were born between:	12-31-24 1200 to 01-01-25 1130		Incubator number and shelf location:	SB	
Organism source:	AI Batch Ab: 01-01-25		Artemia CHM number:	CHM1294	
			Drying information for weight determination:		
Transfer bowl information:	pH =	7.90	S.U.	Date / Time in oven:	01-14-25 1225
	Temperature =	25.0	°C	*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL		Date / Time out of oven:	01-15-25 1225	
			*Final oven temperature:	60 °C	
			Total drying time:	24.00025	

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	01-07-25	1145	JH	1430	JH	1300	JH	01-02-25 B
1	01-08-25	0500	JH	1100	JH	0900	JH	↓
2	01-09-25	0500	JH	1215	JH	0900	JH	01-06-25
3	01-10-25	0500	JH	1100	JH	0815	JH	↓
4	01-11-25	0600	JH	1300	JH	1145	JH	01-10-25
5	01-12-25	0600	JH	1200	JH	0912	JH	↓
6	01-13-25	0500	JH	1230	JH	0846	JH	↓
7	01-14-25					1100	JH	

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:	02.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	506.1
Average weight per initial shrimp:	0.212		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.212	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	428.0

AbKCICR Test Number: **259**

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: Rubyl Analyst: XL Date: 12-17-24	13.75	13.18	13.48	11.96	13.23	14.27	12.49	12.52	12.02	14.73	14.04	14.44	12.52	14.45	14.30	11.53
*B = Pan + Shrimp weight (mg) Analyst: H Date: 01-16-25	14.79	14.22	14.56	13.00	14.26	15.36	13.55	13.60	13.11	15.82	15.16	15.50	13.61	15.65	15.42	12.66
C = Shrimp weight (mg) = B - A Hand calculated Analyst: H	1.04	1.04	1.08	1.04	1.03	1.09	1.06	1.08	1.09	1.09	1.12	1.06	1.09	1.20	1.12	1.13
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: H	0.208	0.208	0.216	0.208	0.206	0.218	0.212	0.216	0.218	0.218	0.224	0.212	0.218	0.240	0.224	0.226
Average weight per initial number of shrimp (mg) 0.212								Average weight per initial number of shrimp (mg) 0.213				Percent reduction from control (%) -5.27				

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

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

Comments:

AbKCICR Test Number: **259**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	4 ^{4d}	4 ^{4d}	4 ^{1d}	5	4 ^{4d}	4 ^{1d}	4 ^{1d}	4 ^{4d}
4	S	S	S	S	S	S	S	S	4	3 ^{1d}	3 ^{1d}	4 ^{1d}	4	4	4	3 ^{1d}
5	S	S	S	S	S	S	S	S	3 ^{1d}	3	2 ^{1d}	4	2 ^{2d}	2 ^{2d}	3 ^{1d}	2 ^{1d}
6	S	S	S	S	S	S	S	S	3	3	2	2 ^{2d}	2	2	2 ^{1d}	2
7	S	S	S	S	S	S	S	S	3	3	2	2	2	2	2	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Rub Analyst: XL Date: 12-17-24	14.51	13.48	12.95	13.01	14.26	13.45	12.91	12.62	13.78	13.54	14.69	14.74	12.53	13.72	12.74	13.50
*B = Pan + Shrimp weight (mg) Analyst: J Date: 01-16-25	15.61	14.49	14.00	14.02	15.48	14.66	14.01	13.60	14.40	14.15	15.34	15.10	12.86	14.08	13.16	13.91
C = Shrimp weight (mg) = B - A Hand calculated Analyst: J	1.10	1.01	1.05	1.01	1.22	1.21	1.10	0.98	0.62	0.61	0.65	0.36	0.33	0.36	0.42	0.41
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: J	0.220	0.202	0.210	0.202	0.244	0.242	0.220	0.196	0.124	0.122	0.090	0.072	0.066	0.072	0.084	0.082
Average weight per initial number of shrimp (mg) 0.217				Percent reduction from control (%) -2.67				Average weight per initial number of shrimp (mg) 0.089				Percent reduction from control (%) 57.97				

*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:

AbKClCR Test Number: 259

Survival and Growth Data

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

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

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

Comments:

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

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

Test number: 259

Test dates: January 07-14, 2025

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	13.75	14.79	1.04	0.208	100.0	0.212	2.2	Not applicable
	B	5	5	13.18	14.22	1.04	0.208				
	C	5	5	13.48	14.56	1.08	0.216				
	D	5	5	11.96	13.00	1.04	0.208				
	E	5	5	13.23	14.26	1.03	0.206				
	F	5	5	14.27	15.36	1.09	0.218				
	G	5	5	12.49	13.55	1.06	0.212				
	H	5	5	12.52	13.60	1.08	0.216				
250	I	5	5	12.02	13.11	1.09	0.218	100.0	0.223	3.8	-5.2
	J	5	5	14.73	15.82	1.09	0.218				
	K	5	5	14.04	15.16	1.12	0.224				
	L	5	5	14.44	15.50	1.06	0.212				
	M	5	5	12.52	13.61	1.09	0.218				
	N	5	5	14.45	15.65	1.20	0.240				
	O	5	5	14.30	15.42	1.12	0.224				
	P	5	5	11.53	12.66	1.13	0.226				
375	Q	5	5	14.51	15.61	1.10	0.220	100.0	0.217	8.4	-2.6
	R	5	5	13.48	14.49	1.01	0.202				
	S	5	5	12.95	14.00	1.05	0.210				
	T	5	5	13.01	14.02	1.01	0.202				
	U	5	5	14.26	15.48	1.22	0.244				
	V	5	5	13.45	14.66	1.21	0.242				
	W	5	5	12.91	14.01	1.10	0.220				
	X	5	5	12.62	13.60	0.98	0.196				
500	Y	5	3	13.78	14.40	0.62	0.124	45.0	0.089	25.1	57.9
	Z	5	3	13.54	14.15	0.61	0.122				
	AA	5	2	14.89	15.34	0.45	0.090				
	BB	5	2	14.74	15.10	0.36	0.072				
	CC	5	2	12.53	12.86	0.33	0.066				
	DD	5	2	13.72	14.08	0.36	0.072				
	EE	5	2	12.74	13.16	0.42	0.084				
	FF	5	2	13.50	13.91	0.41	0.082				
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: 0.0219
PMSD: 10.4

MSD =
PMSD =

Minimum Significant Difference
Percent Minimum Significant Difference

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

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

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

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

Mysid Survival and Growth Test-7 Day Survival

Start Date:	1/7/2025	Test ID:	AbKICICR	Sample ID:	REF-Ref Toxicant
End Date:	1/14/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americanmysis bahia
Comments:					

[illegible]

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.4500	0.4500	0.7351	0.6847	0.8861	12.681	8	36.00	48.00	22	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

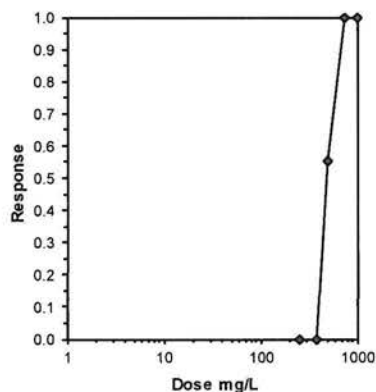
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.55437	0.904	2.42457	7.66092
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)

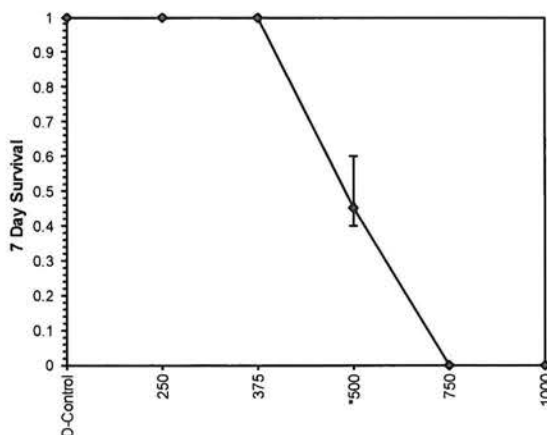
Hypothesis Test (T, F, and S)	1992	1993	SN
Steel's Many-One Rank Test	375	500	433.013

Treatments vs D-Control

Trim Level	EC50	95% CL	
0.0%	506.10	479.24	534.46
5.0%	503.74	474.23	535.07
10.0%	501.39	468.85	536.20
20.0%	496.78	456.13	541.04
Auto-0.0%	506.10	479.24	534.46



Dose-Response Plot



AbKCICR Test Number: **259**

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.)	80 EM	80 EM	80 EM	80 EM	80 EM	80 EM
	DO (mg/L)	8.17	7.98	8.04	7.99	8.05	7.93
	Salinity (ppt)	25.0	25.2	25.0	25.2	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	110		110	110	110	
	Temperature (°C)	25.2	25.9	25.2	26.2	25.1	26.3
250 mg KCl/L	pH (S.U.)	8.09	7.98	8.02	7.94	8.03	7.92
	DO (mg/L)	7.7	7.6	7.7	7.7	7.8	7.2
	Salinity (ppt)	25.1	25.3	24.9	25.2	25.1	25.2
	Temperature (°C)	25.2	26.1	25.2	26.0	25.2	26.0
375 mg KCl/L	pH (S.U.)	8.10	7.96	8.02	7.97	8.03	7.94
	DO (mg/L)	7.7	7.4	7.8	7.7	7.9	7.2
	Salinity (ppt)	25.2	25.4	25.3	25.9	25.7	25.9
	Temperature (°C)	25.5	26.2	25.3	26.0	25.2	26.0
500 mg KCl/L	pH (S.U.)	8.11	7.94	8.03	7.98	8.04	7.92
	DO (mg/L)	7.7	7.6	7.9	7.7	7.9	7.2
	Salinity (ppt)	25.2	25.5	25.5	26.1	25.7	26.0
	Temperature (°C)	25.6	26.2	25.2	26.3	25.2	26.2
750 mg KCl/L	pH (S.U.)	8.11	7.94				
	DO (mg/L)	7.7	7.7				
	Salinity (ppt)	25.4	25.7				
	Temperature (°C)	25.3	26.2				
1000 mg KCl/L	pH (S.U.)	8.12	7.94				
	DO (mg/L)	7.8	7.8				
	Salinity (ppt)	25.6	25.9				
	Temperature (°C)	25.6	26.2				
		Initial	Final	Initial	Final	Initial	Final

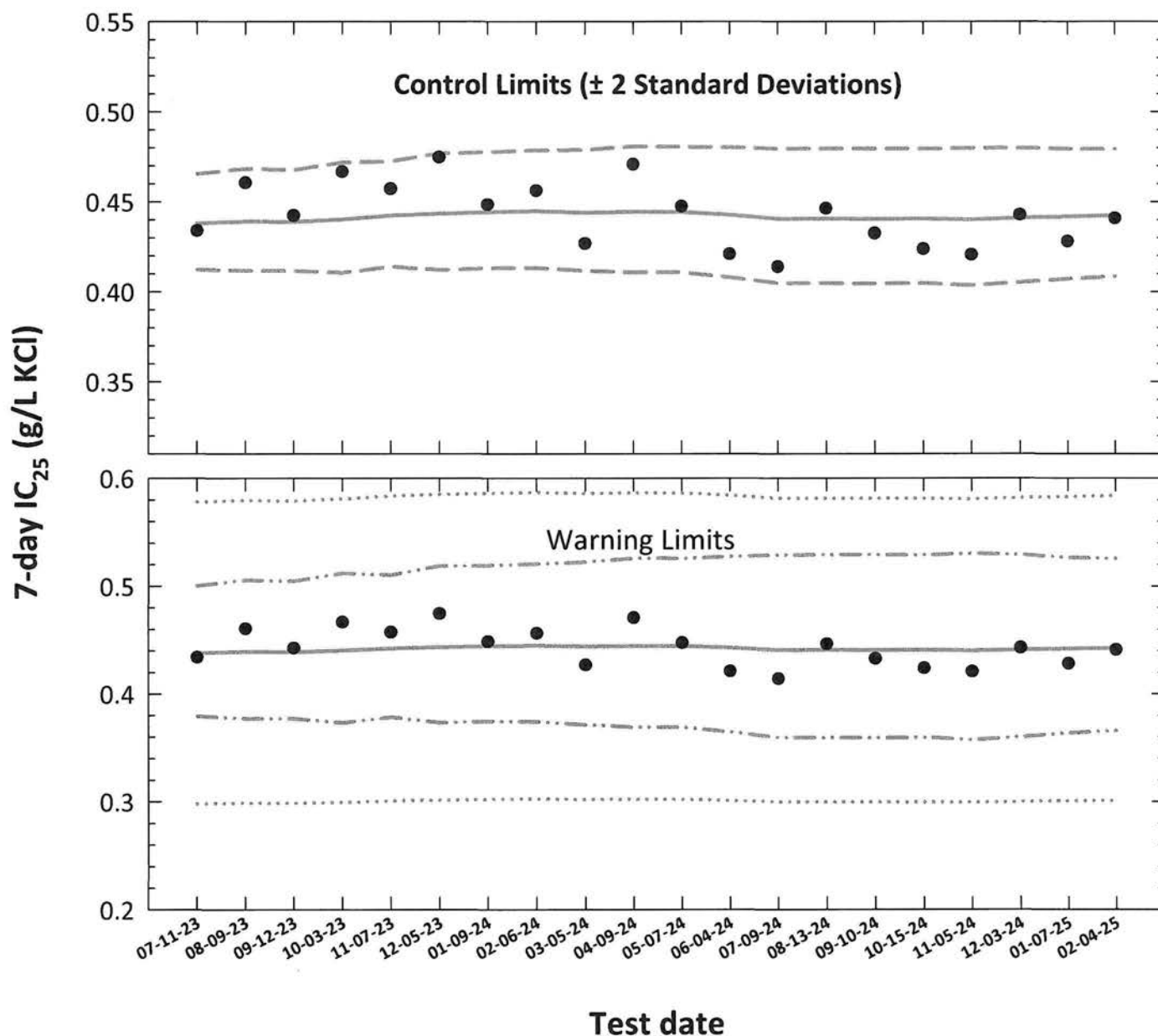
AbKCICR Test Number: **259**

		Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
Conc.	Parameter	3		4		5		6	
Analyst		Em	N	N	N	N	N	N	Em
CONTROL, Salt SW	pH (S.U.)	8.05	7.87	8.09	7.89	8.08	7.90	7.96	7.86
	DO (mg/L)	0.0	7.4	7.7	6.8	7.6	7.2	8.1	7.5
	Salinity (ppt)	25.1	25.2	25.1	25.4	25.1	25.2	25.3	25.5
	Alkalinity (mg CaCO ₃ /L)	non-sal		100				non-sal	
	Temperature (°C)	25.3	26.1	25.2	26.3	25.1	26.2	25.2	26.3
250 mg KCl/L	pH (S.U.)	8.04	7.88	8.10	7.89	8.08	7.92	7.96	7.92
	DO (mg/L)	0.0	7.4	7.7	6.9	7.6	7.2	8.1	7.5
	Salinity (ppt)	25.2	25.3	25.2	25.4	25.3	25.4	25.2	25.5
	Temperature (°C)	25.2	26.0	25.3	26.0	25.2	26.3	25.2	26.0
375 mg KCl/L	pH (S.U.)	8.04	7.92	8.09	7.92	8.08	7.88	7.98	7.89
	DO (mg/L)	0.0	7.4	7.7	6.9	7.5	7.2	8.1	7.5
	Salinity (ppt)	25.3	25.6	25.3	25.8	25.3	25.6	25.3	25.7
	Temperature (°C)	25.2	26.3	25.5	26.0	25.2	26.3	25.2	25.9
500 mg KCl/L	pH (S.U.)	8.05	7.94	8.10	7.92	8.09	7.88	7.99	7.93
	DO (mg/L)	0.0	7.4	7.7	6.8	7.5	7.3	8.1	7.6
	Salinity (ppt)	25.3	25.7	25.4	25.9	25.3	25.5	25.4	25.8
	Temperature (°C)	25.2	26.1	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.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

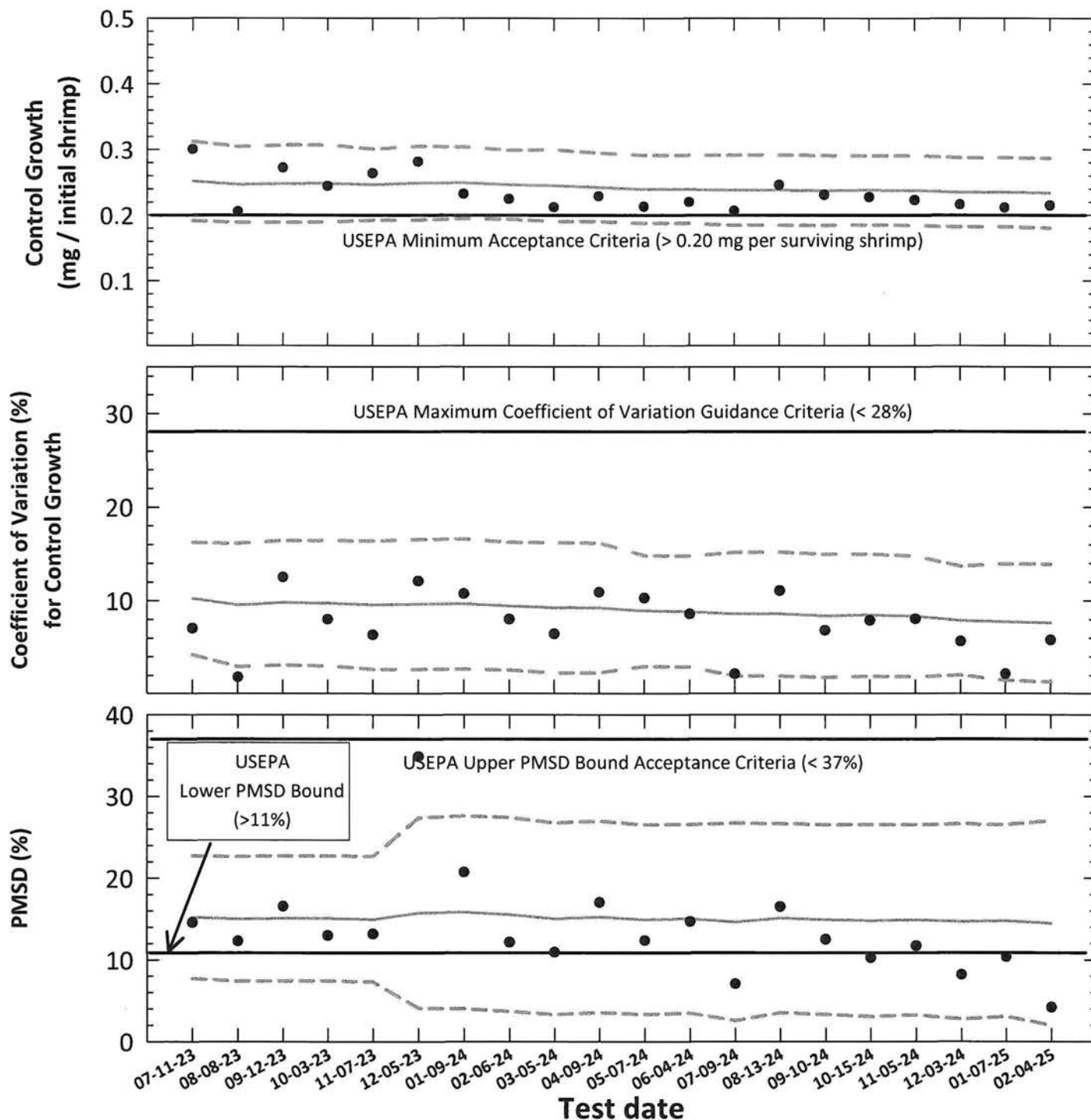


- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits**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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053	0.4800	0.3600	0.5294	0.2999	0.5822
19	01-07-25	0.4280	-0.3686	-0.3549	0.0177	0.4417	0.4071	0.4792	0.3634	0.5266	0.3003	0.5830
20	02-04-25	0.4408	-0.3558	-0.3542	0.0173	0.4424	0.4085	0.4792	0.3657	0.5255	0.3008	0.5840

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 (%)	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 (%)	MSD										
1	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
2	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
3	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
4	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
5	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
6	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
7	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
8	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
9	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
10	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
11	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
12	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
13	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	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	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
16	10-15-24	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
17	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.9	3.2	26.5
18	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.7	2.7	26.6
19	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.8	3.1	26.5
20	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	14.6	2.3	26.8

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: **260**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2318					
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)	10	15	20	30	40	
Diluent volume (mL)	1990	1985	1980	1970	1960	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	GREY
Date and times organisms were born between:	01-28-25 1200 to 01-29-25 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 01-29-25	Artemia CHM number:	CHM1294
Transfer bowl information:	pH = 7.28 S.U. Temperature = 25.0 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	02-11-25 1148
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	02-12-25 1148
		*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	02-04-25	1045	<i>JK</i>	1430	<i>JK</i>	1330	<i>JK</i>	01-28-25 B
1	02-05-25	0500	<i>JK</i>	1500	<i>JK</i>	0900	<i>JK</i>	↓
2	02-06-25	0500	<i>JK</i>	1300	<i>JK</i>	0900	<i>JK</i>	02-05-25
3	02-07-25	0500	<i>JK</i>	1100	<i>JK</i>	0900	<i>JK</i>	↓
4	02-08-25	0500	<i>JK</i>	1200	<i>JK</i>	1152	<i>JK</i>	02-06-25 B
5	02-09-25	0500	<i>JK</i>	1100	<i>JK</i>	0825	<i>JK</i>	↓
6	02-10-25	0500	<i>JK</i>	1200	<i>JK</i>	0811	<i>JK</i>	↓
7	02-11-25					1130	<i>JK</i>	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	498.4
Average weight per initial shrimp:	0.215		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.215	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	440.8

AbKCICR Test Number: **260**

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	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>gray</u> Analyst: <u>KL</u> Date: <u>01-23-25</u>	13.71	11.78	13.30	14.32	12.66	13.46	12.64	11.18	12.21	13.50	11.65	13.95	12.60	10.12	12.79	15.57
*B = Pan + Shrimp weight (mg) Analyst: <u>KL</u> Date: <u>01-23-25</u>	14.76	12.86	14.46	15.50	13.67	14.50	13.66	12.24	13.30	14.61	12.71	15.01	13.63	11.16	13.80	16.59
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>KL</u>	1.05	1.08	1.16	1.18	1.01	1.04	1.02	1.06	1.09	1.11	1.06	1.06	1.03	1.04	1.01	1.02
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>KL</u>	0.210	0.216	0.232	0.236	0.202	0.208	0.204	0.212	0.218	0.222	0.212	0.212	0.206	0.208	0.202	0.204
Average weight per initial number of shrimp (mg)								Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.215								0.211				2.17				

*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: 260

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	4 nd	5	5	5	4 nd	5	5
2	5	5	5	5	5	5	5	5	5	4	5	4 nd	4 nd	4	4 nd	4 nd
3	5	5	5	5	5	5	5	5	5	4	5	4	1 st	4	3 rd	3 rd
4	5	5	5	5	5	5	5	5	5	4	4 nd	4	1	4	3	3
5	5	5	5	5	5	5	5	5	5	4	4	4	1	4	3	3
6	5	4 th	5	5	5	5	5	5	5	4	4	4	1	4	2 nd	3
7	5	4	5	5	5	5	5	5	3 rd	4	2 nd	2 nd	1	3 rd	2	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>10400136</u> Analyst: <u>XL</u> Date: <u>01-23-25</u>	11.94	13.34	13.85	13.26	14.16	11.18	13.77	15.13	15.47	11.34	14.41	11.84	14.26	12.73	13.71	14.01
*B = Pan + Shrimp weight (mg) Analyst: <u>K</u> Date: <u>01-23-25</u>	13.02	14.34	14.89	14.29	15.20	12.20	14.84	16.21	16.21	12.31	14.94	12.35	14.58	13.23	14.25	14.63
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.08	1.00	1.04	1.03	1.04	1.02	1.07	1.08	0.73	0.97	0.53	0.51	0.32	0.50	0.54	0.62
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.216	0.200	0.208	0.206	0.208	0.204	0.214	0.216	0.146	0.194	0.106	0.102	0.064	0.100	0.108	0.124
Average weight per initial number of shrimp (mg)	0.209								0.118							
Percent reduction from control (%)	2.87								45.17							

*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: 260

Survival and Growth Data

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

*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: 260
Test dates: February 04-11, 2025

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	13.71	14.76	1.05	0.210	100.0	0.215	5.8	Not applicable
	B	5	5	11.78	12.86	1.08	0.216				
	C	5	5	13.30	14.46	1.16	0.232				
	D	5	5	14.32	15.50	1.18	0.236				
	E	5	5	12.66	13.67	1.01	0.202				
	F	5	5	13.46	14.50	1.04	0.208				
	G	5	5	12.64	13.66	1.02	0.204				
	H	5	5	11.18	12.24	1.06	0.212				
250	I	5	5	12.21	13.30	1.09	0.218	100.0	0.211	3.3	2.1
	J	5	5	13.50	14.61	1.11	0.222				
	K	5	5	11.65	12.71	1.06	0.212				
	L	5	5	13.95	15.01	1.06	0.212				
	M	5	5	12.60	13.63	1.03	0.206				
	N	5	5	10.12	11.16	1.04	0.208				
	O	5	5	12.79	13.80	1.01	0.202				
	P	5	5	15.57	16.59	1.02	0.204				
375	Q	5	5	11.94	13.02	1.08	0.216	97.5	0.209	2.8	2.8
	R	5	4	13.34	14.34	1.00	0.200				
	S	5	5	13.85	14.89	1.04	0.208				
	T	5	5	13.26	14.29	1.03	0.206				
	U	5	5	14.16	15.20	1.04	0.208				
	V	5	5	11.18	12.20	1.02	0.204				
	W	5	5	13.77	14.84	1.07	0.214				
	X	5	5	15.13	16.21	1.08	0.216				
500	Y	5	3	15.47	16.20	0.73	0.146	50.0	0.118	32.6	45.1
	Z	5	4	11.34	12.31	0.97	0.194				
	AA	5	2	14.41	14.94	0.53	0.106				
	BB	5	2	11.84	12.35	0.51	0.102				
	CC	5	1	14.26	14.58	0.32	0.064				
	DD	5	3	12.73	13.23	0.50	0.100				
	EE	5	2	13.71	14.25	0.54	0.108				
	FF	5	3	14.01	14.63	0.62	0.124				
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: 0.0125
PMSD: 5.8

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

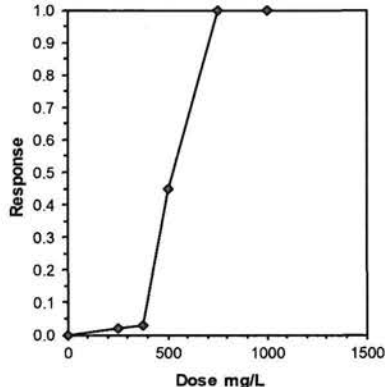
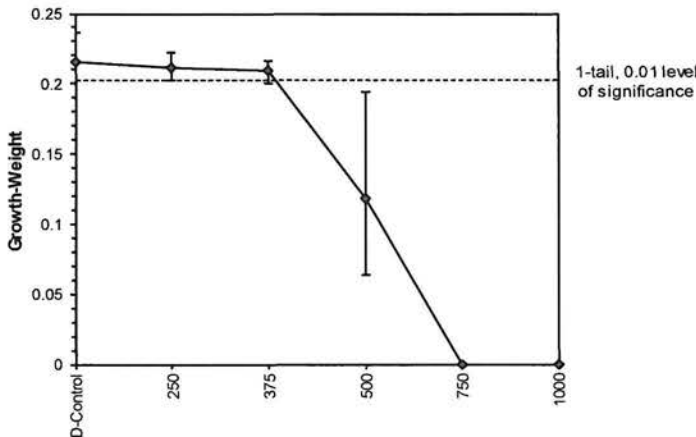
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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.

Dose (mg/kg)	7 Day Survival (Mean)	7 Day Survival (Lower Bound)	7 Day Survival (Upper Bound)
Control	1.0	1.0	1.0
250	1.0	1.0	1.0
375	0.98	0.80	1.0
*500	0.50	0.20	0.80
750	0.0	0.0	0.0
1000	0.0	0.0	0.0

Mysid Survival and Growth Test-Growth-Weight												
Start Date:	2/4/2025		Test ID:	AbKCICR		Sample ID:	REF-Ref Toxicant					
End Date:	2/11/2025		Lab ID:	ETS-Envir. Testing Sol.		Sample Type:	KCL-Potassium chloride					
Sample Date:			Protocol:	SWCHR-EPA-821-R-02-014		Test Species:	AB-Americanysis bahia					
Comments:												
Conc-mg/L	1	2	3	4	5	6	7	8				
D-Control	0.2100	0.2160	0.2320	0.2360	0.2020	0.2080	0.2040	0.2120				
250	0.2180	0.2220	0.2120	0.2120	0.2060	0.2080	0.2020	0.2040				
375	0.2160	0.2000	0.2080	0.2060	0.2080	0.2040	0.2140	0.2160				
500	0.1480	0.1940	0.1060	0.1020	0.0640	0.1000	0.1080	0.1240				
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: Untransformed												
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Isotonic Mean	Isotonic N-Mean
D-Control	0.2150	1.0000	0.2150	0.2020	0.2360	5.841	8				0.2150	1.0000
250	0.2105	0.9791	0.2105	0.2020	0.2220	3.281	8	1.007	2.799	0.0125	0.2105	0.9791
375	0.2090	0.9721	0.2090	0.2000	0.2160	2.802	8	1.342	2.799	0.0125	0.2090	0.9721
500	0.1183	0.5500	0.1183	0.0640	0.1940	32.677	8				0.1183	0.5500
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000
Auxiliary Tests												
Statistic						Critical		Skew		Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)						0.94574		0.884		0.83984		
Bartlett's Test indicates equal variances (p = 0.11)						4.48742		9.21035				
Hypothesis Test (1-tail, 0.01)			NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test			375	>375			0.01251	0.05818	7.8E-05	8E-05	0.39321	2, 21
Treatments vs D-Control												
Linear Interpolation (200 Resamples)												
Point	mg/L	SD	95%CL		Skew							
IC05	381.54	46.97	199.60	391.63	-3.0782							
IC10	396.35	6.35	384.63	408.95	0.1264							
IC15	411.16	7.71	398.89	427.19	0.5062							
IC20	425.96	9.50	412.23	445.45	0.7115							
IC25	440.77	11.50	425.21	464.40	0.8135							
IC40	485.19	17.44	461.92	525.17	0.6184							
IC50	522.73	22.01	484.68	562.64	0.0525							
												
Dose-Response Plot												
												

AbKCICR Test Number: **260**

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>W</i>	<i>JPB</i>	<i>JPB</i>	<i>JPB</i>	<i>JPB</i>	<i>W</i>
	DO (mg/L)	7.95	7.81	7.86	7.81	7.40	7.74
	Salinity (ppt)	7.9	7.5	7.0	7.4	7.0	6.5
	Alkalinity (mg CaCO ₃ /L)	24.9	25.3	25.1	25.0	25.0	25.3
	Temperature (°C)	100				110	
250 mg KCl/L	pH (S.U.)	25.7	26.2	26.1	26.0	25.2	25.9
	DO (mg/L)	7.97	7.82	7.85	7.85	7.92	7.76
	Salinity (ppt)	7.9	7.5	7.0	7.4	7.7	6.4
	Temperature (°C)	24.9	25.6	25.4	25.5	25.4	25.3
375 mg KCl/L	pH (S.U.)	26.5	26.0	26.2	26.1	26.1	26.0
	DO (mg/L)	7.99	7.83	7.85	7.85	7.92	7.73
	Salinity (ppt)	7.9	7.6	7.0	7.5	7.0	6.4
	Temperature (°C)	24.9	25.7	25.4	25.7	25.6	25.3
500 mg KCl/L	pH (S.U.)	25.9	26.0	26.2	26.3	26.2	26.0
	DO (mg/L)	0.00	7.81	7.86	7.87	7.93	7.02
	Salinity (ppt)	7.9	7.6	7.0	7.6	7.7	7.1
	Temperature (°C)	25.0	26.0	25.5	26.0	25.7	26.0
750 mg KCl/L	pH (S.U.)	25.9	26.3	26.2	26.0	25.2	26.2
	DO (mg/L)	0.01	7.80				
	Salinity (ppt)	7.9	7.6				
	Temperature (°C)	25.0	25.7				
1000 mg KCl/L	pH (S.U.)	26.0	26.3				
	DO (mg/L)	0.02	7.85				
	Salinity (ppt)	0.0	7.4				
	Temperature (°C)	25.3	25.4				
		Initial	Final	Initial	Final	Initial	Final

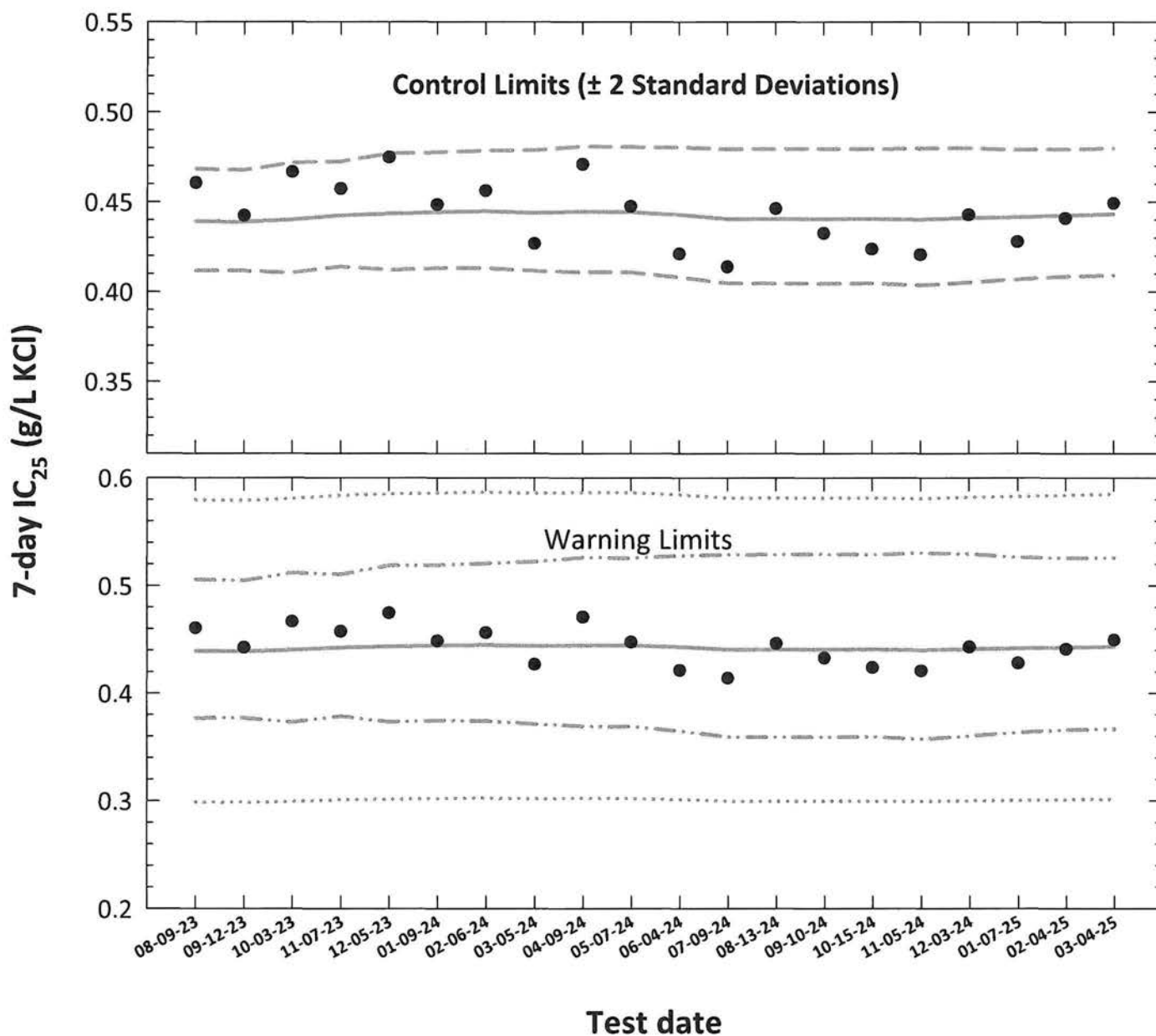
AbKCICR Test Number: 260

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	N	N	N	N	N	N	N	N
CONTROL, Salt SW	pH (S.U.)	8.22	7.73	8.09	7.79	8.05	7.93	7.96	7.76
	DO (mg/L)	0.1	5.9	0.0	6.7	7.0	7.6	8.0	6.7
	Salinity (ppt)	24.0	25.0	24.9	25.1	24.4	24.9	24.9	25.0
	Alkalinity (mg CaCO ₃ /L)	12.5 N		9.6				12.5 N	
	Temperature (°C)	25.1	25.1	25.2	26.0	25.2	26.1	25.2	26.0
250 mg KCl/L	pH (S.U.)	7.98	7.74	8.03	7.75	7.97	7.94	8.03	7.75
	DO (mg/L)	0.1	0.2	0.1	0.7	7.8	7.5	8.0	6.4
	Salinity (ppt)	25.2	25.3	25.2	25.4	25.1	25.3	25.1	25.1
	Temperature (°C)	26.1	26.1	25.3	26.2	25.3	26.2	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.97	7.72	8.03	7.77	7.98	7.94	8.03	7.74
	DO (mg/L)	0.1	0.2	0.1	0.8	8.0	7.5	8.0	6.3
	Salinity (ppt)	25.2	25.5	25.4	25.0	25.1	25.4	25.3	25.5
	Temperature (°C)	25.1	26.0	25.2	26.2	25.3	26.0	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.98	7.72	8.03	7.78	7.98	7.93	8.03	7.78
	DO (mg/L)	0.1	0.3	0.1	7.0	8.0	7.5	8.0	6.1
	Salinity (ppt)	25.2	25.5	25.5	25.9	25.2	25.5	25.4	25.6
	Temperature (°C)	25.1	26.0	25.2	26.3	25.3	26.2	25.2	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.

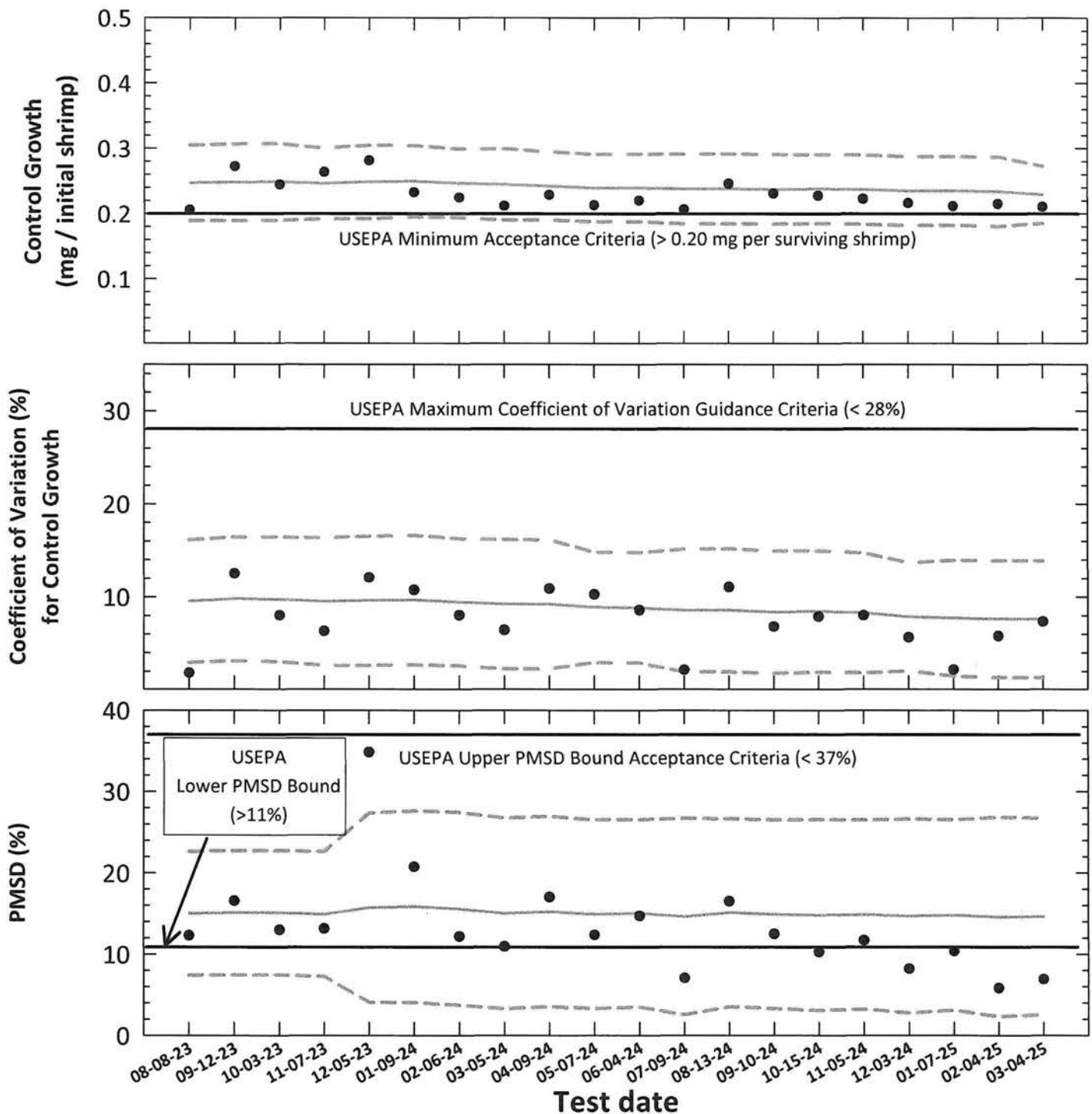


- **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- - - **Control Limits** (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 CT - 2S CT + 2S
1	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116 0.4683
2	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116 0.4677
3	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106 0.4719
4	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140 0.4725
5	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123 0.4769
6	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131 0.4775
7	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133 0.4785
8	03-05-24	0.4268	-0.3698	-0.3527	0.0164	0.4440	0.4117 0.4788
9	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109 0.4808
10	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109 0.4805
11	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082 0.4804
12	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047 0.4794
13	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048 0.4796
14	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046 0.4796
15	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048 0.4795
16	11-05-24	0.4206	-0.3761	-0.3565	0.0188	0.4401	0.4036 0.4798
17	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053 0.4800
18	01-07-25	0.4280	-0.3686	-0.3549	0.0177	0.4417	0.4071 0.4792
19	02-04-25	0.4408	-0.3558	-0.3542	0.0173	0.4424	0.4085 0.4792
20	03-04-25	0.4493	-0.3474	-0.3534	0.0173	0.4432	0.4093 0.4799
							Warning Limits
							CT - 2CV CT + 2CV
							Warning Limits
							CT - $S_{A,75}$ CT + $S_{A,75}$
							75th Percentile CV
							Warning Limits
							CT - $S_{A,75}$ CT + $S_{A,75}$
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							Warning Limits
							CT - <

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 (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	
			Mean (mg/initial shrimp)	CV (%)											
1	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
2	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
3	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
4	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
5	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	10-15-24	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
16	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.9	3.2	26.5
17	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.7	2.7	26.6
18	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.8	3.1	26.5
19	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	14.6	2.3	26.8
20	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	14.6	2.5	26.7

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: 261

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2357					
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)	10	15	20	30	40	
Diluent volume (mL)	1990	1985	1980	1970	1960	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	PALE GREEN
Date and times organisms were born between:	02-25-25 1200 to 02-26-25 1130	Incubator number and shelf location:	5C
Organism source:	AI Batch Ab: 02-26-25	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 8.02 S.U. Temperature = 25.0 °C		Date / Time in oven:	03-11-25 1025
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	03-12-25 1025
		*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	03-04-25	1100	jl	1420	jl	1150	jl	02-26-25A
1	03-05-25	0500	jl	1400	jl	0822	jl	↓
2	03-06-25	0500	jl	1315	jl	0910	jl	03-04-25
3	03-07-25	0500	jl	1100	jl	0812	jl	↓
4	03-08-25	0530	jl	1210	jl	0930	jl	03-06-25
5	03-09-25	0530	jl	1130	jl	0830	jl	↓
6	03-10-25	0500	jl	1100	jl	0922	jl	↓
7	03-11-25					0955	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:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	533.1
Average weight per initial shrimp:	0.211		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.211	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	449.3

AbKCICR Test Number: 261**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>Forest Green</u> Analyst: <u>XL</u> Date: <u>02-19-25</u>	14.53	12.67	15.23	12.26	13.42	13.67	14.09	12.24	14.13	14.91	11.95	14.85	12.84	12.38	12.75	13.82
*B = Pan + Shrimp weight (mg) Analyst: <u>fl</u> Date: <u>02-19-25</u>	15.55	13.69	16.28	13.50	14.48	14.69	15.09	13.26	15.20	15.98	13.00	15.89	13.91	13.42	13.84	14.90
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>fl</u>	1.02	1.02	1.05	1.24	1.06	1.02	1.00	1.02	1.07	1.07	1.05	1.04	1.07	1.04	1.09	1.08
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>fl</u>	0.204	0.204	0.210	0.248	0.212	0.204	0.200	0.204	0.214	0.214	0.210	0.208	0.214	0.208	0.216	0.216
Average weight per initial number of shrimp (mg) 0.211												Average weight per initial number of shrimp (mg) 0.213				Percent reduction from control (%) - 0.97.

*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: **261**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	4 ¹⁰	5	4 ¹⁰	4 ¹⁰	5	5	4 ¹⁰	5
4	5	5	5	5	5	5	5	5	4	5	4	4	5	5	4	5
5	5	5	5	5	5	5	5	5	4	4 ¹⁴	4	4	5	5	4	5
6	5	5	5	5	5	5	5	5	4	4	4	3 ¹⁰	4 ¹⁰	5	4	5
7	5	5	5	5	5	5	5	5	3 ¹⁴	3 ¹⁴	2 ²⁴	3	4	3 ²⁴	3 ²⁴	3 ²⁴
# females with eggs in brood sac																
# females with developing ova in oviducts																
# Immature females																
# males																0.34
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>XL</u> Date: <u>02-19-15</u>	12.83	14.42	13.33	13.51	13.68	13.37	12.50	13.78	13.24	12.94	12.56	14.85	12.12	15.36	14.03	12.23
*B = Pan + Shrimp weight (mg) Analyst: <u>KL</u> Date: <u>02-13-15</u>	13.94	15.51	14.53	14.54	14.69	14.40	13.59	14.80	13.88	13.58	12.96	15.58	12.93	15.91	14.54	12.91
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>KL</u>	1.11	1.09	1.00	1.03	1.03	1.03	1.09	1.02	0.64	0.64	0.40	0.73	0.81	0.55	0.51	0.69
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>KL</u>	0.222	0.218	0.200	0.206	0.206	0.206	0.218	0.204	0.128	0.128	0.080	0.146	0.162	0.110	0.102	0.138
Average weight per initial number of shrimp (mg)	0.210								0.124							
Percent reduction from control (%)	0.37.								41.07.							

*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: **261**

Survival and Growth Data

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

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

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

Comments:



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

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

Test number: 261

Test dates: March 04-11, 2025

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	14.53	15.55	1.02	0.204	100.0	0.211	7.4	Not applicable
	B	5	5	12.67	13.69	1.02	0.204				
	C	5	5	15.23	16.28	1.05	0.210				
	D	5	5	12.26	13.50	1.24	0.248				
	E	5	5	13.42	14.48	1.06	0.212				
	F	5	5	13.67	14.69	1.02	0.204				
	G	5	5	14.09	15.09	1.00	0.200				
	H	5	5	12.24	13.26	1.02	0.204				
250	I	5	5	14.13	15.20	1.07	0.214	100.0	0.213	1.7	-0.9
	J	5	5	14.91	15.98	1.07	0.214				
	K	5	5	11.95	13.00	1.05	0.210				
	L	5	5	14.85	15.89	1.04	0.208				
	M	5	5	12.84	13.91	1.07	0.214				
	N	5	5	12.38	13.42	1.04	0.208				
	O	5	5	12.75	13.84	1.09	0.218				
	P	5	5	13.82	14.90	1.08	0.216				
375	Q	5	5	12.83	13.94	1.11	0.222	100.0	0.210	3.8	0.3
	R	5	5	14.42	15.51	1.09	0.218				
	S	5	5	13.53	14.53	1.00	0.200				
	T	5	5	13.51	14.54	1.03	0.206				
	U	5	5	13.66	14.69	1.03	0.206				
	V	5	5	13.37	14.40	1.03	0.206				
	W	5	5	12.50	13.59	1.09	0.218				
	X	5	5	13.78	14.80	1.02	0.204				
500	Y	5	3	13.24	13.88	0.64	0.128	60.0	0.124	21.0	41.0
	Z	5	3	12.94	13.58	0.64	0.128				
	AA	5	2	12.56	12.96	0.40	0.080				
	BB	5	3	14.85	15.58	0.73	0.146				
	CC	5	4	12.12	12.93	0.81	0.162				
	DD	5	3	15.36	15.91	0.55	0.110				
	EE	5	3	14.03	14.54	0.51	0.102				
	FF	5	3	12.23	12.92	0.69	0.138				
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: 0.0146
PMSD: 6.9

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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

Start Date:	3/4/2025	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/11/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americanmysis bahia
Comments:					

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

Kurt

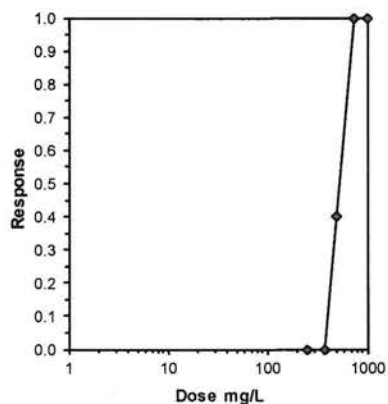
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0.0020

TU

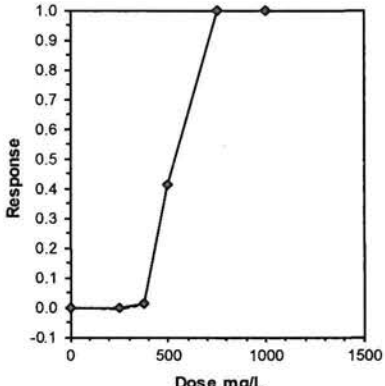
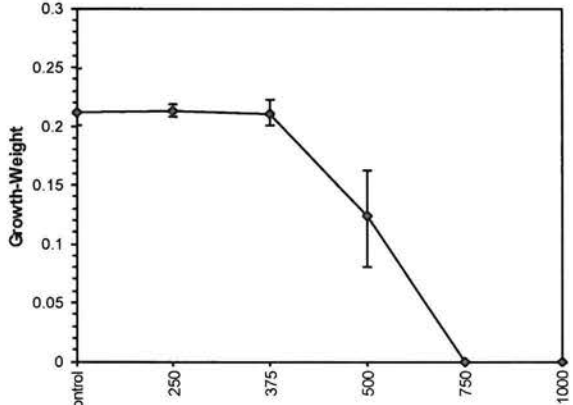
33.013

Trim Level	EC50	95% CL		
0.0%	533.10	505.23	562.51	
5.0%	533.38	502.42	566.25	
10.0%	533.65	498.69	571.07	
20.0%	534.18	486.77	586.22	
Auto-0.0%	533.10	505.23	562.51	



A line graph showing 7 Day Survival (Y-axis, 0 to 1) versus Dose (mg/kg) (X-axis, 0 to 1000). The graph displays two data series: 100% survival (solid line) and 50% survival (dashed line). The 100% survival group shows a sharp decline in survival starting at 375 mg/kg, reaching 0 survival at 750 mg/kg. The 50% survival group shows a more gradual decline, reaching 0 survival at 1000 mg/kg. Error bars are shown for the 50% survival group at 500 mg/kg.

Dose (mg/kg)	100% Survival (Solid Line)	50% Survival (Dashed Line)
0 (Q-Control)	1.0	1.0
250	1.0	1.0
375	1.0	1.0
500	0.6	0.6
750	0.0	0.6
1000	0.0	0.0

Mysid Survival and Growth Test-Growth-Weight											
Start Date: 3/4/2025		Test ID: AbKCICR		Sample ID:		REF-Ref Toxicant					
End Date: 3/11/2025		Lab ID: ETS-Envir. Testing Sol.		Sample Type:		KCL-Potassium chloride					
Sample Date:		Protocol: SWCHR-EPA-821-R-02-014		Test Species:		AB-Americanysis bahia					
Comments:											
Conc-mg/L	1	2	3	4	5	6	7	8			
D-Control	0.2040	0.2040	0.2100	0.2480	0.2120	0.2040	0.2000	0.2040			
250	0.2140	0.2140	0.2100	0.2080	0.2140	0.2080	0.2180	0.2160			
375	0.2220	0.2180	0.2000	0.2060	0.2020	0.2060	0.2180	0.2040			
500	0.1280	0.1280	0.0800	0.1460	0.1620	0.1100	0.1020	0.1380			
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: Untransformed											
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Isotonic Mean	N-Mean
D-Control	0.2108	1.0000	0.2108	0.2000	0.2480	7.367	8			0.2118	1.0000
250	0.2128	1.0095	0.2128	0.2080	0.2180	1.736	8	86.50	43.00	0.2118	1.0000
375	0.2095	0.9941	0.2095	0.2000	0.2220	4.042	8	73.00	43.00	0.2095	0.9894
500	0.1243	0.5896	0.1243	0.0800	0.1620	21.013	8			0.1243	0.5868
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8			0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8			0.0000	0.0000
Auxiliary Tests											
							Statistic	Critical	Skew	Kurt	
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)							0.77078	0.884	2.43399	7.98571	
Bartlett's Test indicates unequal variances (p = 3.74E-03)							11.1751	9.21035			
Hypothesis Test (1-tail, 0.01)											
			NOEC	LOEC	ChV	TU					
Steel's Many-One Rank Test			375	>375							
Treatments vs D-Control											
Linear Interpolation (200 Resamples)											
Point	mg/L	SD	95% CL		Skew						
IC05	387.23	21.57	322.84	393.18	-5.9451						
IC10	402.75	5.86	388.14	411.36	-0.8931						
IC15	418.27	6.42	403.61	429.54	-0.4858						
IC20	433.80	7.31	418.94	447.73	-0.1642						
IC25	449.32	8.42	434.32	465.91	0.0440						
IC40	495.89	12.66	475.91	521.89	0.2555						
IC50	536.97	14.69	507.51	559.90	-0.4422						
											
Dose-Response Plot											
											

AbKCICR Test Number: 261**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	EM	EM	EM	EM	EM	EM
CONTROL, Salt SW	pH (S.U.)	8.08	7.96	7.98	7.96	8.09	7.95
	DO (mg/L)	8.1	7.7	8.0	7.5	8.0	7.3
	Salinity (ppt)	25.0	25.7	25.0	25.1	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)	110				100	
	Temperature (°C)	25.2	25.9	25.2	25.8	25.1	26.1
250 mg KCl/L	pH (S.U.)	8.07	7.97	7.99	7.97	8.08	7.97
	DO (mg/L)	8.2	7.7	8.0	7.7	8.1	7.2
	Salinity (ppt)	25.1	25.8	25.1	25.2	25.1	25.3
	Temperature (°C)	25.2	25.7	25.2	26.0	25.2	26.0
375 mg KCl/L	pH (S.U.)	8.07	7.99	7.99	7.98	8.08	7.97
	DO (mg/L)	8.2	7.7	8.0	7.6	8.1	7.0
	Salinity (ppt)	25.1	26.0	25.2	25.4	25.2	25.8
	Temperature (°C)	25.3	25.7	25.1	26.0	25.1	26.0
500 mg KCl/L	pH (S.U.)	8.07	7.98	8.00	7.95	8.08	7.95
	DO (mg/L)	8.2	7.7	7.9	7.6	8.1	6.9
	Salinity (ppt)	25.5	26.0	25.5	25.7	25.3	25.8
	Temperature (°C)	25.3	26.0	25.1	25.8	25.1	26.2
750 mg KCl/L	pH (S.U.)	8.08	7.96				
	DO (mg/L)	8.2	7.4				
	Salinity (ppt)	25.6	26.4				
	Temperature (°C)	25.3	25.2				
1000 mg KCl/L	pH (S.U.)	8.07	7.98				
	DO (mg/L)	8.2	7.6				
	Salinity (ppt)	25.7	26.5				
	Temperature (°C)	25.3	25.2				
		Initial	Final	Initial	Final	Initial	Final

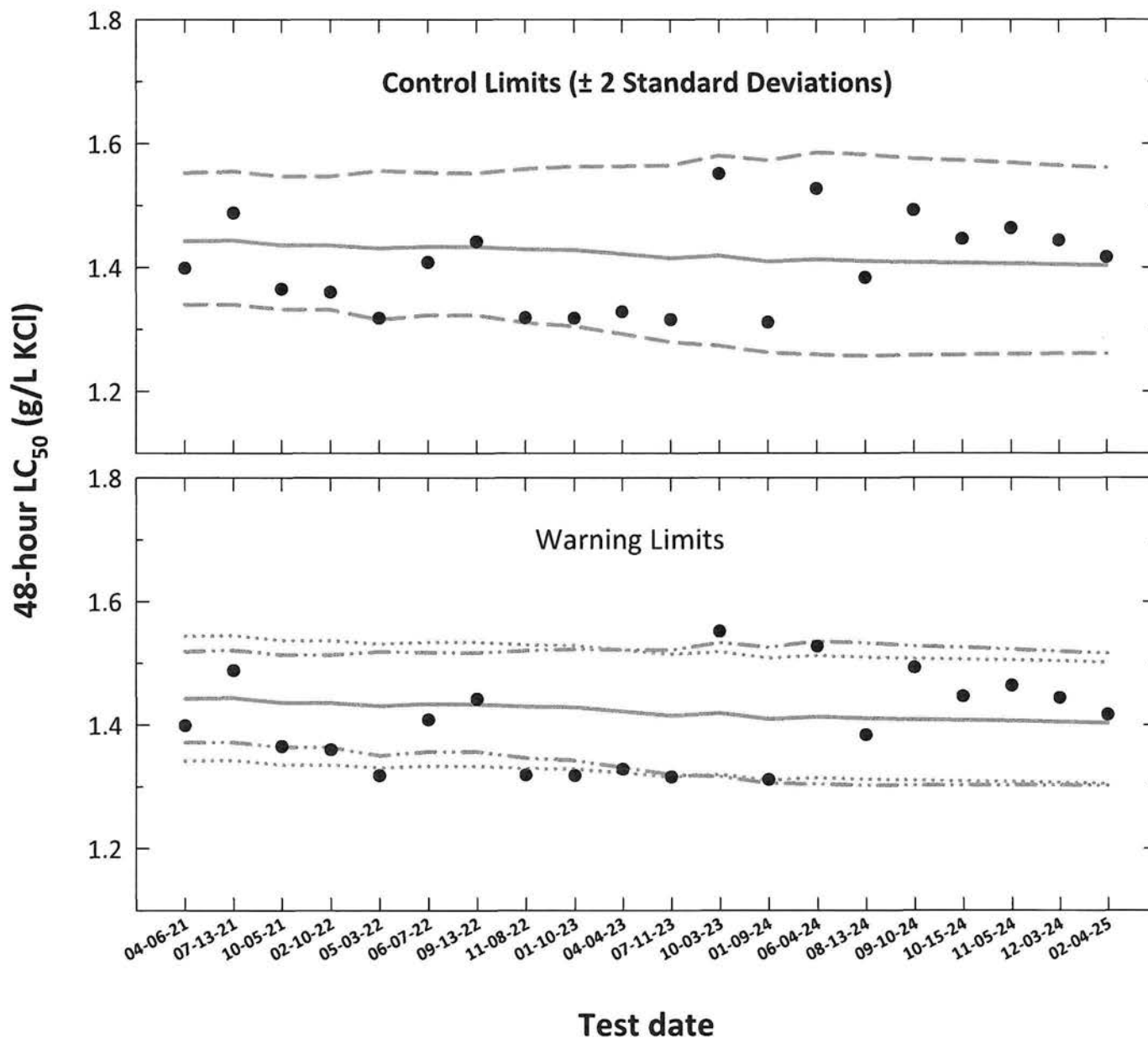
AbKCICR Test Number: **261**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	<i>JK</i>	<i>JK</i>	<i>JK</i>	<i>JK</i>	<i>JK</i>	<i>JK</i>	<i>JK</i>	<i>JK</i>
	DO (mg/L)	7.91	7.76	7.97	7.72	8.01	7.79	7.95	7.65
	Salinity (ppt)	7.8	6.5	7.9	6.4	7.8	7.7	8.2	5.3
	Alkalinity (mg CaCO ₃ /L)	24.0	24.0	14.7	24.9	25.0	25.0	25.3	25.3
	Temperature (°C)	—	—	9.6	—	—	—	—	—
250 mg KCl/L	pH (S.U.)	25.2	26.2	25.3	25.9	25.3	26.2	25.1	25.9
	DO (mg/L)	7.96	7.79	7.99	7.83	8.00	7.87	7.94	7.75
	Salinity (ppt)	7.9	6.5	7.9	6.4	7.9	7.7	8.2	5.5
	Temperature (°C)	25.0	25.0	24.9	25.0	25.2	25.4	25.3	25.4
375 mg KCl/L	pH (S.U.)	26.2	26.0	25.1	25.8	25.3	26.2	25.3	26.1
	DO (mg/L)	7.98	7.86	8.00	7.82	8.01	7.91	7.95	7.76
	Salinity (ppt)	8.0	6.5	7.9	6.6	7.8	7.8	8.1	5.6
	Temperature (°C)	25.0	25.2	25.1	25.5	25.2	25.4	25.4	25.0
500 mg KCl/L	pH (S.U.)	25.2	25.9	25.3	26.0	25.2	26.4	25.1	26.1
	DO (mg/L)	8.00	7.80	8.00	7.85	8.02	7.90	7.96	7.78
	Salinity (ppt)	8.0	6.6	8.0	6.9	8.0	7.8	8.1	5.9
	Temperature (°C)	25.0	25.3	25.3	25.6	25.2	25.6	25.5	25.0
750 mg KCl/L	pH (S.U.)	25.1	25.9	25.2	26.0	25.3	26.1	25.1	26.2
	DO (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	—	—	—	—	—	—	—	—
	Temperature (°C)	—	—	—	—	—	—	—	—
1000 mg KCl/L	pH (S.U.)	—	—	—	—	—	—	—	—
	DO (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	—	—	—	—	—	—	—	—
	Temperature (°C)	—	—	—	—	—	—	—	—
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Menidia beryllina

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	12-03-24	1.4436	0.1594	0.1476	0.0235	1.4047	1.2609 1.5649	1.3023 1.5188	1.3064 1.5030
20	02-04-25	1.4168	0.1513	0.1470	0.0232	1.4029	1.2607 1.5611	1.3015 1.5156	1.3047 1.5011

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

Page 1 of

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 99

Dilution Preparation:

Test concentrations (mg/L KCl)	1000	1250	1500	1750	2000
mL Stock solution	20	25	30	35	40
mL Dilution water	980	975	970	965	960
Total volume (mL)	1000	1000	1000	1000	1000

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 #: 2348

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.95	7.90	7.88
	Dissolved oxygen (mg/L)	7.9	7.7	7.4
	*Salinity (ppt)	24.9	25.6	26.0
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	25.0	25.2	25.2
1000 mg/L	pH (S.U.)	8.01	7.90	7.88
	Dissolved oxygen (mg/L)	7.9	7.7	7.6
	*Salinity (ppt)	25.1	26.0	26.4
	*Temperature (°C)	25.2	25.4	25.0
1250 mg/L	pH (S.U.)	8.02	7.89	7.87
	Dissolved oxygen (mg/L)	7.8	7.7	7.4
	*Salinity (ppt)	25.4	26.2	26.7
	*Temperature (°C)	25.1	25.6	25.2
1500 mg/L	pH (S.U.)	8.03	7.89	7.88
	Dissolved oxygen (mg/L)	7.9	7.7	7.6
	*Salinity (ppt)	25.5	26.2	26.8
	*Temperature (°C)	25.1	25.2	25.4
1750 mg/L	pH (S.U.)	8.03	7.88	7.87
	Dissolved oxygen (mg/L)	7.9	7.7	7.5
	*Salinity (ppt)	25.7	26.5	27.0
	*Temperature (°C)	25.0	25.5	25.2
2000 mg/L	pH (S.U.)	8.03	7.88	
	Dissolved oxygen (mg/L)	7.9	7.7	
	*Salinity (ppt)	25.8	26.5	
	*Temperature (°C)	25.0	25.5	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ 8-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664645

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 # 99

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-04-25	1045	JK	1252	JK	6D	LIGHT GREEN	01-28-25B
24	02-05-25			1300	JK			
48 Termination	02-06-25			1255	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):	01-25-25
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	01-24-25 1200 TO 01-25-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.54 Temperature (°C) 24.5

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	8 ^u	5 ^{sd}	4 ^{sd}	2 ^{sd}	2 ^{sd}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^u	8	2 ^{sd}	3 ^u	1 ^u	1 ^u	0	0
Mean Survival	100%		100%		85%		25%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1339.6
Upper 95% confidence limit (mg KCl/L)	1490.2
48-hour LC ₅₀ (mg KCl/L)	1416.8

Comments:



Acute Silverside Test-24 Hr Survival

Start Date:	2/4/2025	Test ID:	MBKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	0.8000
1500	0.5000	0.4000
1750	0.2000	0.2000
2000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20
1250	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.501	2.850	0.2894	2	20
*1500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	6.668	2.850	0.2894	11	20
*1750	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	2	9.341	2.850	0.2894	16	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

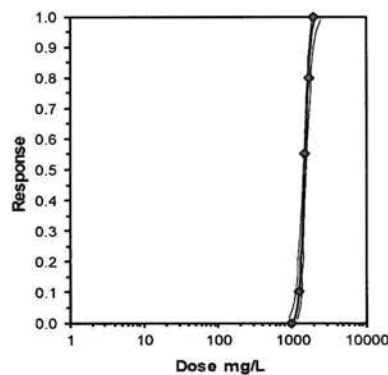
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.16274	0.16691	0.37441	0.01031	6.9E-04	4, 5

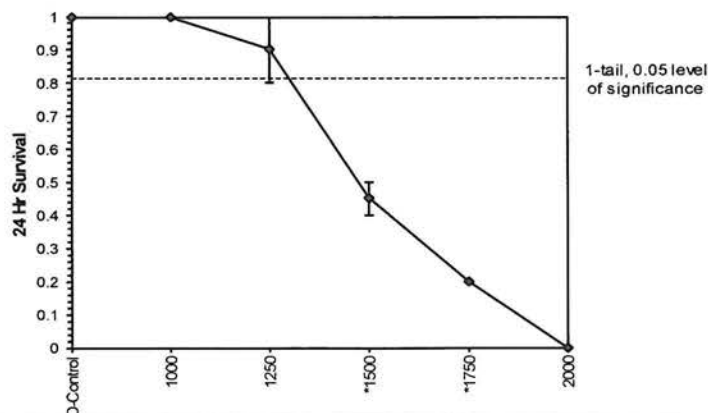
Treatments vs D-Control

				Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.2137	2.77272	10.7792 21.6483	0	1.31812	7.81472	0.72484	3.17557	0.06168	4
Intercept	-46.488	8.82922	-63.793 -29.183							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1076.69	893.657	1187.55
EC05	3.355	1186.11	1030.05	1281.35
EC10	3.718	1248.91	1109.86	1335.8
EC15	3.964	1293.15	1166.32	1374.85
EC20	4.158	1329.43	1212.47	1407.58
EC25	4.326	1361.36	1252.78	1437.13
EC40	4.747	1445.26	1355.53	1519.79
EC50	5.000	1498.21	1416.39	1577.32
EC60	5.253	1553.09	1475.06	1642.47
EC75	5.674	1648.82	1566.8	1769.37
EC80	5.842	1688.42	1601.6	1826.01
EC85	6.036	1735.78	1641.48	1896.24
EC90	6.282	1797.27	1691.11	1990.77
EC95	6.645	1892.43	1764.61	2143.03
EC99	7.326	2084.74	1954.71	2467.89



Dose-Response Plot



Acute Silverside Test-48 Hr Survival

Start Date: 2/4/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 2/6/2025 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:

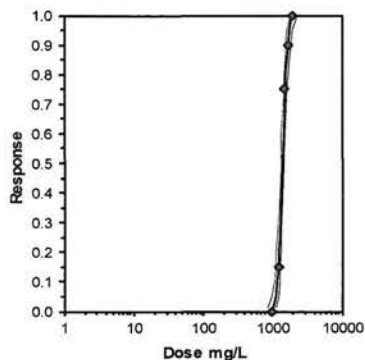
Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	0.8000
1500	0.2000	0.3000
1750	0.1000	0.1000
2000	0.0000	0.0000

Transform: Arcsin Square Root											1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1652	0	20	
*1250	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	4.036	2.850	0.1652	3	20	
*1500	0.2500	0.2500	0.5216	0.4636	0.5796	15.723	2	15.363	2.850	0.1652	15	20	
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	18.812	2.850	0.1652	18	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

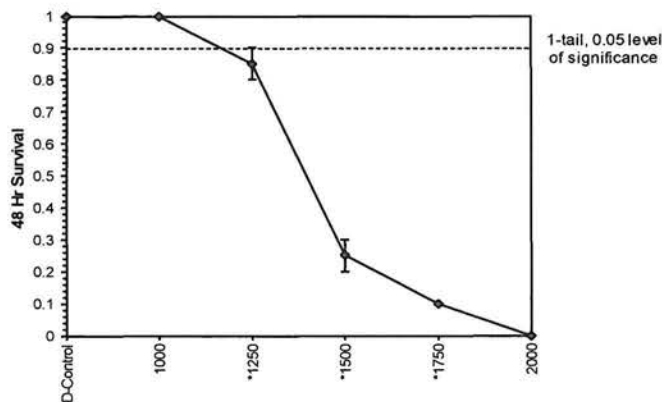
Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.07633	0.07828	0.52765	0.00336	1.9E-05	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	17.5161	3.08785	11.4639 23.5683	0	1.66094	7.81472	0.64565	3.1513	0.05709	3
Intercept	-50.198	9.75968	-69.327 -31.069							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1043.48	869.388 1146.49
EC05	3.355	1141.28	993.444 1229.71
EC10	3.718	1197.11	1065.53 1277.87
EC15	3.964	1236.31	1116.31 1312.35
EC20	4.158	1268.38	1157.71 1341.2
EC25	4.326	1296.55	1193.78 1367.22
EC40	4.747	1370.36	1285.44 1439.86
EC50	5.000	1416.76	1339.57 1490.23
EC60	5.253	1464.74	1391.68 1547.14
EC75	5.674	1548.12	1472.81 1657.78
EC80	5.842	1582.51	1503.41 1707.13
EC85	6.036	1623.56	1538.34 1768.28
EC90	6.282	1676.72	1581.61 1850.49
EC95	6.645	1758.74	1645.32 1982.63
EC99	7.326	1923.58	1766.61 2263.15



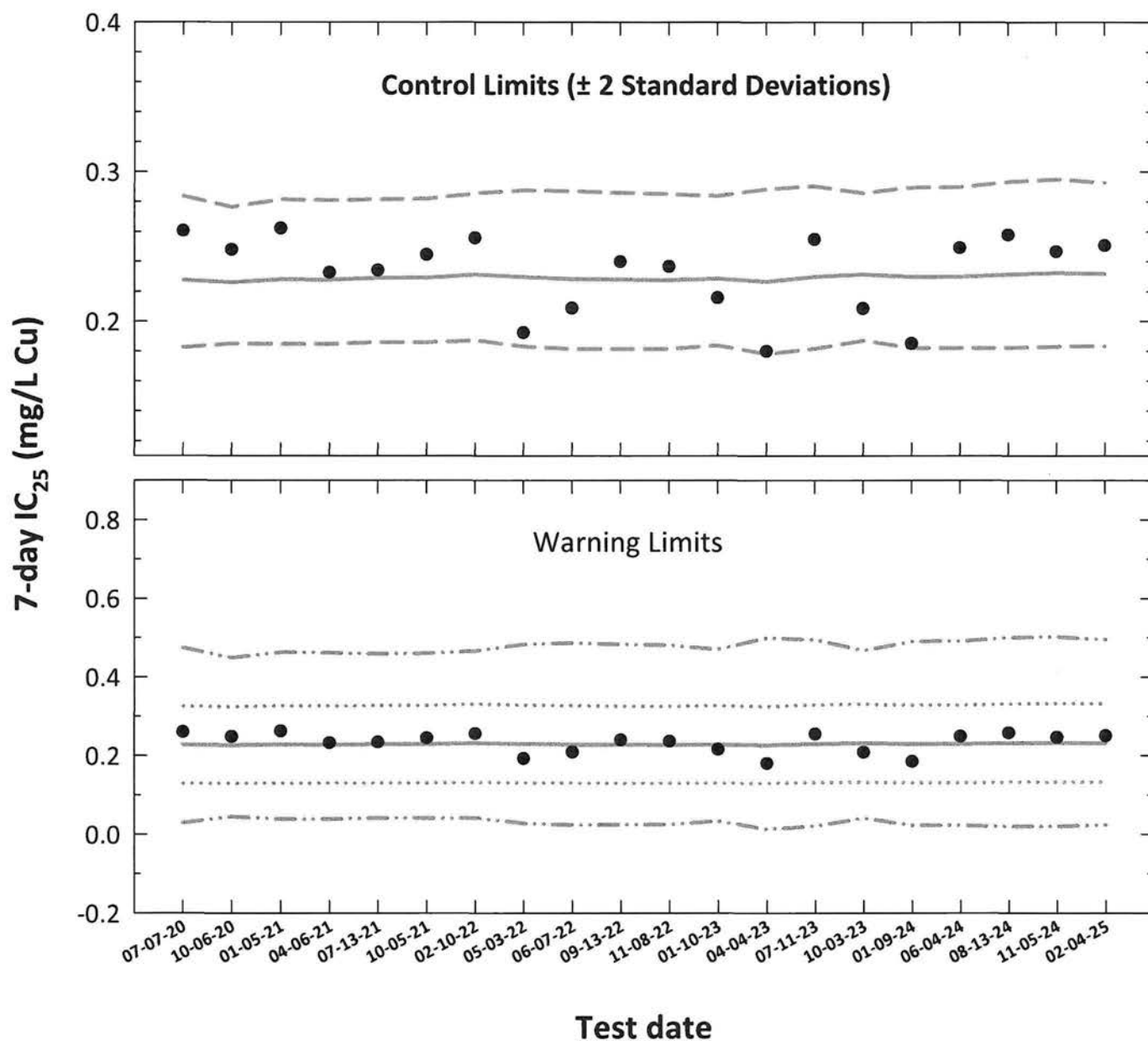
Dose-Response Plot



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		CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
			7-day IC ₂₅	CT		CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	07-07-20	0.2606	-0.5840	-0.6429	0.0479	0.1825	0.2838	0.0295	0.4745	0.1297	0.3254
2	10-06-20	0.2479	-0.6057	-0.6457	0.0436	0.1850	0.2763	0.0443	0.4483	0.1289	0.3233
3	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.1848	0.2816	0.0382	0.4625	0.1300	0.3262
4	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.1847	0.2810	0.0385	0.4612	0.1298	0.3258
5	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.1862	0.2817	0.0420	0.4590	0.1305	0.3274
6	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.1861	0.2822	0.0414	0.4604	0.1306	0.3277
7	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.1874	0.2855	0.0414	0.4656	0.1318	0.3307
8	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.1832	0.2877	0.0276	0.4827	0.1309	0.3283
9	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.1815	0.2872	0.0233	0.4861	0.1301	0.3265
10	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.1816	0.2861	0.0246	0.4831	0.1299	0.3259
11	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.1816	0.2853	0.0254	0.4811	0.1297	0.3255
12	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.1841	0.2841	0.0337	0.4708	0.1304	0.3270
13	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.1781	0.2884	0.0125	0.4991	0.1292	0.3241
14	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.1818	0.2905	0.0208	0.4939	0.1310	0.3286
15	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.1873	0.2859	0.0408	0.4669	0.1319	0.3309
16	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.1824	0.2896	0.0233	0.4900	0.1310	0.3286
17	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.1823	0.2901	0.0227	0.4914	0.1311	0.3288
18	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.1823	0.2932	0.0196	0.4996	0.1318	0.3306
19	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.1831	0.2950	0.0201	0.5019	0.1325	0.3323
20	02-04-25	0.2507	-0.6008	-0.6349	0.0507	0.1835	0.2928	0.0234	0.4950	0.1321	0.3314

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

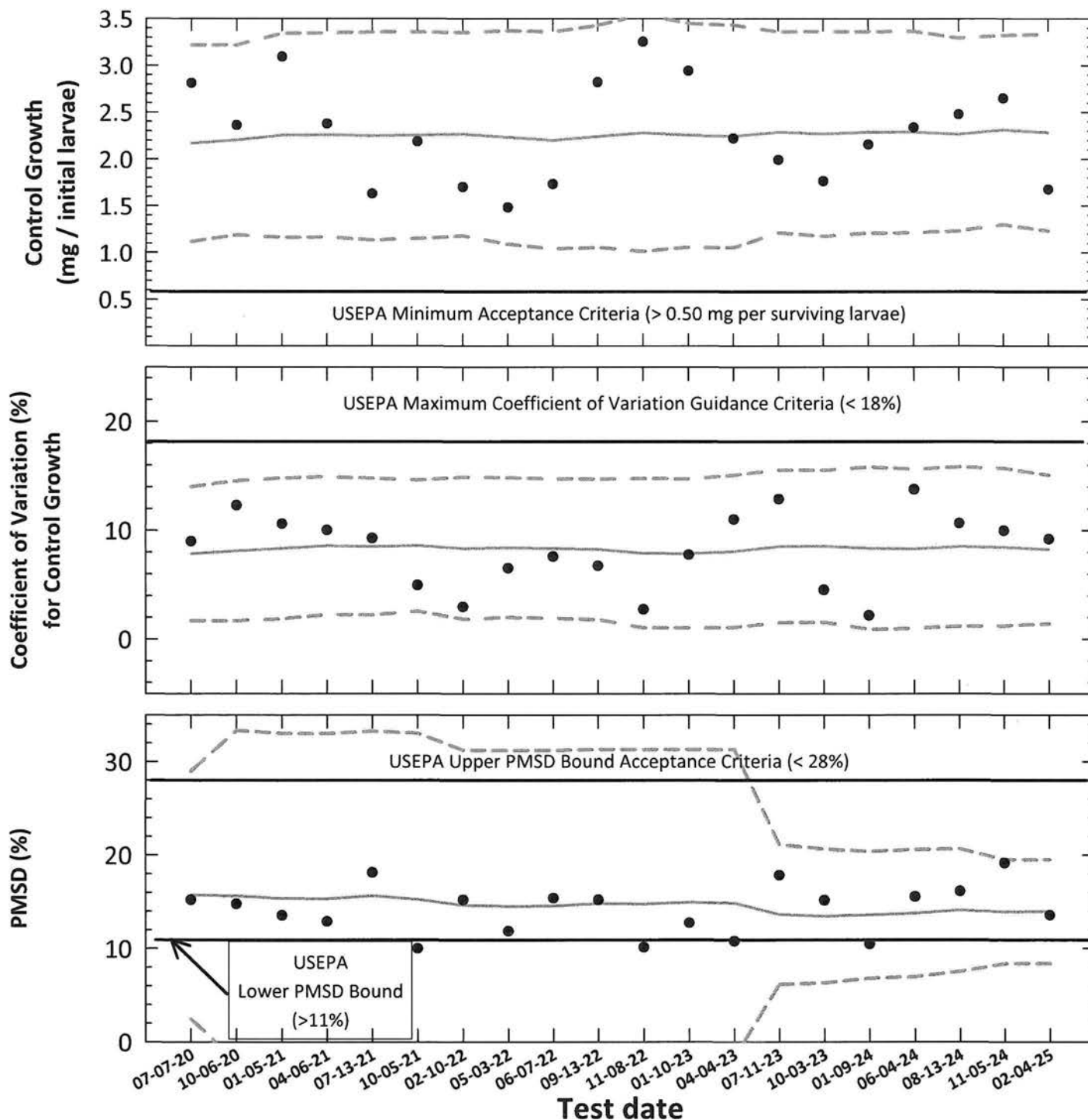
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Menidia beryllina

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



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

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

Test number	Test date	ToxCat Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		(mg/initial larvae)			(%)			(%)		
			Mean (mg/initial larvae)	CV (%)	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
1	07-07-20	100	2.809	9.0	0.4264	15.2	2.1660	1.117	3.215	7.8	1.7	14.0	15.7	2.4	29.0
2	10-06-20	100	2.361	12.3	0.3484	14.8	2.205	1.188	3.221	8.1	1.7	14.6	15.6	-2.1	33.3
3	01-05-21	100	3.093	10.6	0.4186	13.5	2.253	1.163	3.343	8.3	1.9	14.8	15.4	-2.3	33.0
4	04-06-21	100	2.377	10.0	0.3062	12.9	2.258	1.167	3.349	8.6	2.2	14.9	15.3	-2.3	33.0
5	07-13-21	100	1.631	9.3	0.2956	18.1	2.247	1.136	3.359	8.5	2.2	14.8	15.7	-2.0	33.3
6	10-05-21	100	2.189	5.0	0.2192	10.0	2.258	1.153	3.363	8.6	2.6	14.7	15.3	-2.5	33.1
7	02-10-22	100	1.701	3.0	0.2583	15.2	2.266	1.180	3.351	8.4	1.8	14.9	14.6	-2.0	31.2
8	05-03-22	100	1.483	6.5	0.1756	11.8	2.232	1.092	3.372	8.4	2.0	14.9	14.5	-2.1	31.2
9	06-07-22	100	1.733	7.6	0.2665	15.4	2.201	1.041	3.361	8.3	1.9	14.8	14.6	-2.1	31.2
10	09-13-22	100	2.822	6.8	0.4290	15.2	2.243	1.057	3.430	8.3	1.8	14.7	14.8	-1.7	31.3
11	11-08-22	100	3.255	2.7	0.3294	10.1	2.280	1.014	3.545	7.9	1.0	14.8	14.8	-1.8	31.3
12	01-10-23	100	2.945	7.8	0.3755	12.8	2.257	1.062	3.453	7.9	1.0	14.8	15.0	-1.4	31.3
13	04-04-23	100	2.220	11.0	0.2389	10.8	2.243	1.053	3.433	8.1	1.1	15.1	14.9	-1.6	31.3
14	07-11-23	100	1.992	12.9	0.3549	17.8	2.287	1.213	3.360	8.5	1.5	15.6	13.6	6.2	21.1
15	10-03-23	100	1.763	4.5	0.2673	15.2	2.270	1.174	3.366	8.5	1.5	15.5	13.5	6.3	20.6
16	01-09-24	100	2.156	2.2	0.2257	10.5	2.287	1.211	3.364	8.4	0.9	15.8	13.6	6.8	20.4
17	06-04-24	100	2.339	13.8	0.3637	15.5	2.291	1.215	3.368	8.3	1.0	15.6	13.8	7.0	20.6
18	08-13-24	100	2.479	10.7	0.4001	16.1	2.266	1.235	3.297	8.5	1.2	15.9	14.1	7.6	20.7
19	11-05-24	100	2.648	10.0	0.5061	19.1	2.312	1.299	3.324	8.5	1.2	15.7	13.9	8.3	19.5
20	02-04-25	100	1.675	9.2	0.2268	13.5	2.283	1.232	3.335	8.2	1.4	15.1	13.9	8.4	19.5

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: **149**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2359				
Stock preparation:		100 mg Cu/L: Dissolve 0.1965 g CuSO ₄ in 500-mL deionized water				
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Organism source:	Aquatic Indicators, Inc.
Age:	10-days old
Batch:	AI Mb 01-25-25
Hatch dates and times:	01-24-25 1200 to 01-25-25 1130
Transfer vessel information:	pH (S.U.) = 7.54 Temperature (°C) = 24.5
Average transfer volume (mL):	< 0.25 mL

Test information:

Randomizing template:	ORANGE
Incubator number and shelf location:	6B
Artemia CHM number:	CHM1294
Drying information for weight determination:	
Date / Time in oven:	02-11-25 1148
*Initial oven temperature:	60°C
Date / Time out of oven:	02-12-25 1148
*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	02-04-25	1045	H	1430	H	1315	H	NA	01-28-25 B
1	02-05-25	0500	H	1500	H	0845	H	↓	↓
2	02-06-25	0500	H	1300	H	0845	H	↓	02-03-25
3	02-07-25	0500	H	1100	H	0840	H	↓	↓
4	02-08-25	0500	H	1200	H	1142	H	↓	02-06-25 B
5	02-09-25	0500	H	1100	H	0810	H	↓	↓
6	02-10-25	0500	H	1200	H	0800	H	↓	↓
7	02-11-25		H		H	1115	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	130664685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (%)	0.326
Average weight per initial larvae:	1.675		NOEC (%)	0.2
Average weight per surviving larvae:	1.675	≥ 0.25mg/larvae	LOEC (%)	0.5
			ChV (%)	0.316
			IC ₂₅ (%)	6.251

Species: Menidia beryllina

MbCuCR Test Number: 149

Survival and Growth Data

Day	CONTROL				0.025 mg/L				0.05 mg/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>Lime Green</u> Analyst: <u>XL</u> Date: <u>01-23-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>02-13-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>XL</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>XL</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
1.675 1.625 3.07 1.597 4.72												

*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: 149

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	6 ^{4d}	4 ^{6d}	4 ^{6d}	5 ^{5d}
2	10	10	10	10	10	9 ^{1k}	10	10	6	4	4	5
3	10	10	10	10	10	9	10	10	5 ^{1d}	4	4	5
4	10	10	10	10	10	9	10	10	3 ^{2d}	2 ^{2d}	3 ^{1d}	3 ^{2d}
5	10	10	10	10	10	9	10	10	0 ^{2d}	0 ^{2d}	2 ^{1d}	1 ^{2d}
6	10	10	10	10	10	9	10	10	0	0	2	1
7	10	10	10	10	10	9	9 ^{1d}	10	0	0	2	1
*A = Pan weight (mg) Tray color code: <u>lime green</u> Analyst: <u>XL</u> Date: <u>01-23-29</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>02-13-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>XL</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>XL</u>												
Average weight per initial number of larvae (mg)	1.737				1.492				0.097			
Percent reduction from control (%)	-3.77				10.97				94.27			

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

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

Comments:

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: 149

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	Not for Compliance Assessment, Internal Laboratory QC			Weight / Initial number of larvae (mg)	Mean survival (%)	Coefficient of variation (%)	Percent reduction from control (%)
							Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)				
Control	A	10	10	13.96	29.66	15.70	1.570			1.570			
	B	10	10	13.56	31.89	18.33	1.833			1.833			
	C	10	10	15.06	32.84	17.78	1.778			1.778	100.0	9.2	Not applicable
	D	10	10	14.01	29.19	15.18	1.518			1.518			
0.025	E	10	10	13.50	29.83	16.33	1.633			1.633			
	F	10	10	13.54	30.06	16.52	1.652			1.652			
	G	10	10	13.91	30.80	16.89	1.689			1.689	100.0	4.3	3.0
	H	10	10	13.46	28.72	15.26	1.526			1.526			
0.050	I	10	10	14.25	32.66	18.41	1.841			1.841			
	J	10	10	13.22	27.48	14.26	1.426			1.426			
	K	10	10	12.68	27.21	14.53	1.453			1.453	100.0	12.2	4.7
	L	10	10	14.55	31.22	16.67	1.667			1.667			
0.100	M	10	10	15.33	32.10	16.77	1.677			1.677			
	N	10	10	14.43	32.04	17.61	1.761			1.761	100.0	3.8	-3.7
	O	10	10	12.53	29.45	16.92	1.692			1.692			
	P	10	10	14.63	32.81	18.18	1.818			1.818			
0.200	Q	10	10	13.42	27.39	13.97	1.397			1.397			
	R	10	9	10.28	27.37	17.09	1.899			1.709	95.0	9.8	10.9
	S	10	9	11.56	26.00	14.44	1.604			1.444			
	T	10	10	10.78	24.95	14.17	1.417			1.417			
0.500	U	10	0	0.00	0.00	0.00	0.000			0.000			
	V	10	0	0.00	0.00	0.00	0.000			0.000			
	W	10	2	14.71	17.07	2.36	1.180			0.236	7.5	0.0	94.2
	X	10	1	13.29	14.81	1.52	1.520			0.152			

Dunnett's MSD value: 0.2268
PMSD: 13.5

MSD =
PMSD =

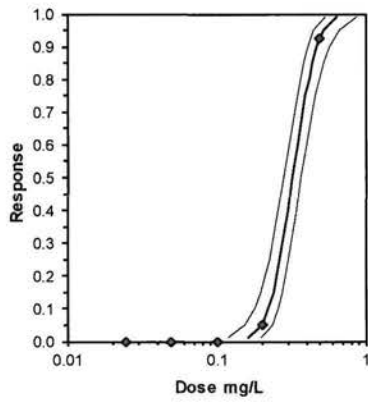
Minimum Significant Difference
Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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.

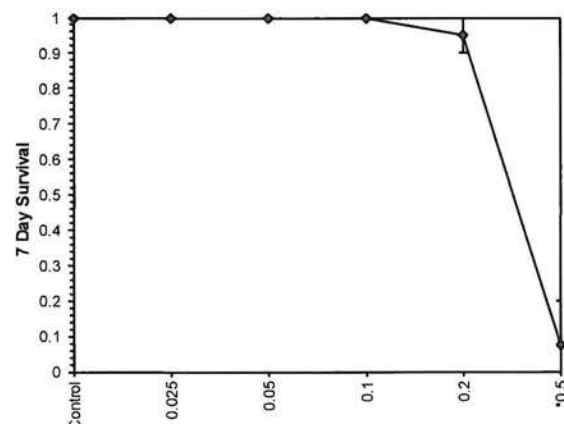
USEPA. 2001a, 2001b. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2-Appendix. EPA-821-R-01-004 and EPA-821-R-01-005. US Environmental Protection Agency, Cincinnati, OH.

Generated and
Reviewed by
[Signature]

Larval Fish Growth and Survival Test-7 Day Survival													
Start Date:	2/4/2025	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant								
End Date:	2/11/2025	Lab ID:	ETS-Envr. Testing Sol.	Sample Type:	CUSO4								
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina								
Comments:													
Conc-mg/L	1	2	3	4									
D-Control	1.0000	1.0000	1.0000	1.0000									
0.025	1.0000	1.0000	1.0000	1.0000									
0.05	1.0000	1.0000	1.0000	1.0000									
0.1	1.0000	1.0000	1.0000	1.0000									
0.2	1.0000	0.9000	0.9000	1.0000									
0.5	0.0000	0.0000	0.2000	0.1000									
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	1.4120	1.4120	1.4120	0.000	4			0	40		
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40		
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40		
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40		
0.2	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40		
*0.5	0.0750	0.0750	0.2757	0.1588	0.4636	53.294	4	10.00	10.00	37	40		
Auxiliary Tests								Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)								0.78293	0.884	0.66942	3.01534		
Equality of variance cannot be confirmed													
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU							
Steel's Many-One Rank Test			0.2	0.5	0.31623								
Treatments vs D-Control													
Maximum Likelihood-Probit													
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	7.75469	1.11464	5.56998	9.93939	0	0.00139	7.81472	0.99999	-0.4867	0.12895	3		
Intercept	8.7743	0.57399	7.64929	9.89932									
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	0.16342	0.1188	0.19865									
EC05	3.355	0.20007	0.15564	0.23533									
EC10	3.718	0.22286	0.17923	0.25834									
EC15	3.964	0.23968	0.19678	0.27561									
EC20	4.158	0.25395	0.21167	0.29053									
EC25	4.326	0.26688	0.22508	0.30431									
EC40	4.747	0.30242	0.26129	0.34397									
EC50	5.000	0.32605	0.28448	0.37202									
EC60	5.253	0.35153	0.30851	0.40394									
EC75	5.674	0.39835	0.3501	0.46706									
EC80	5.842	0.41862	0.36718	0.49603									
EC85	6.036	0.44355	0.38755	0.53289									
EC90	6.282	0.47703	0.41398	0.58431									
EC95	6.645	0.53137	0.45511	0.67188									
EC99	7.326	0.65055	0.54003	0.87884									



Dose-Response Plot



Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	2/4/2025	Test ID:	McCuCR	Sample ID:	REF-Ref Toxicant
End Date:	2/11/2025	Lab ID:	ETS-Envr. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

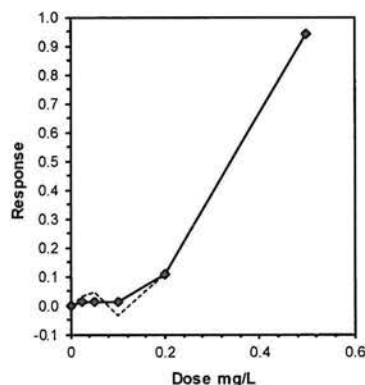
Conc-mg/L	1	2	3	4
D-Control	1.5700	1.8330	1.7780	1.5180
0.025	1.6330	1.6520	1.6890	1.5260
0.05	1.8410	1.4260	1.4530	1.6670
0.1	1.6770	1.7610	1.6920	1.8180
0.2	1.3970	1.7090	1.4440	1.4170
0.5	0.0000	0.0000	0.2360	0.1520

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed			Isotonic	
			Mean	Min	Max	CV%			Critical	MSD	Mean	N-Mean	
D-Control	1.6748	1.0000	1.6748	1.5180	1.8330	9.202	4					1.6748	1.0000
0.025	1.6250	0.9703	1.6250	1.5260	1.6890	4.306	4	0.518	2.360	0.2268		1.6529	0.9870
0.05	1.5968	0.9534	1.5968	1.4260	1.8410	12.230	4	0.812	2.360	0.2268		1.6529	0.9870
0.1	1.7370	1.0372	1.7370	1.6770	1.8180	3.755	4	-0.648	2.360	0.2268		1.6529	0.9870
0.2	1.4918	0.8907	1.4918	1.3970	1.7090	9.794	4	1.904	2.360	0.2268		1.4918	0.8907
0.5	0.0970	0.0579	0.0970	0.0000	0.2360	120.761	4					0.0970	0.0579

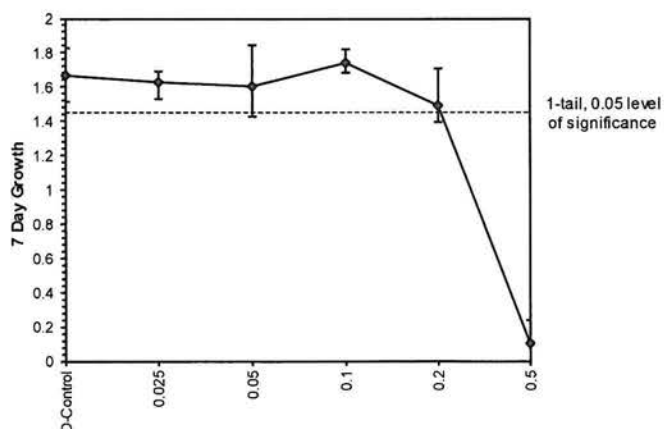
Auxiliary Tests					Statistic		Critical	Skew	Kurt					
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)					0.95233		0.868	0.51314	-0.5367					
Bartlett's Test indicates equal variances ($p = 0.35$)					4.43787		13.2767							
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					0.2	>0.2			0.22684	0.13544	0.03357	0.01848	0.17809	4, 15
Treatments vs D-Control														

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95%CL(Exp)	Skew
IC05	0.1384	0.0579	0.0000	0.2541
IC10	0.1904	0.0388	0.0000	0.2496
IC15	0.2147	0.0212	0.1310	0.2617
IC20	0.2327	0.0161	0.1853	0.2775
IC25	0.2507	0.0147	0.2071	0.2934
IC40	0.3047	0.0123	0.2678	0.3408
IC50	0.3408	0.0110	0.3077	0.3735



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 149

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	
		IN	EM	EM	EM	EM	IN
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	7.45	7.87	7.86	7.87	7.90	7.77
	Dissolved oxygen (mg/L)	7.9	7.5	7.9	7.4	7.0	6.0
	Salinity (ppt)	24.9	25.4	25.0	25.2	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	100				110	
	Temperature (°C)	25.0	25.2	24.7	25.2	24.6	24.8
0.025 mg/L	pH (S.U.)	8.04	7.87	7.94	7.86	7.98	7.79
	Dissolved oxygen (mg/L)	7.9	7.5	7.0	7.4	7.7	6.9
	Salinity (ppt)	24.7	25.5	25.0	25.6	25.3	25.3
	Temperature (°C)	25.2	25.1	24.7	25.0	24.6	24.8
0.05 mg/L	pH (S.U.)	8.04	7.86	7.94	7.86	7.98	7.70
	Dissolved oxygen (mg/L)	8.0	7.5	7.8	7.4	7.7	6.9
	Salinity (ppt)	24.8	25.5	25.1	25.5	25.0	25.4
	Temperature (°C)	25.2	25.2	24.7	24.9	24.7	24.7
0.1 mg/L	pH (S.U.)	8.04	7.87	7.94	7.87	7.98	7.75
	Dissolved oxygen (mg/L)	8.0	7.6	7.7	7.4	7.0	6.9
	Salinity (ppt)	24.7	25.4	25.1	25.6	25.0	25.3
	Temperature (°C)	25.0	25.2	24.7	25.3	24.6	24.9
0.2 mg/L	pH (S.U.)	8.05	7.88	7.93	7.86	7.98	7.74
	Dissolved oxygen (mg/L)	8.0	7.6	7.0	7.4	7.0	6.7
	Salinity (ppt)	24.7	25.2	25.1	25.6	25.0	25.3
	Temperature (°C)	25.0	25.0	24.8	25.3	24.6	24.9
0.5 mg/L	pH (S.U.)	8.04	7.85	7.93	7.88	7.97	7.00
	Dissolved oxygen (mg/L)	8.0	7.6	7.0	7.4	7.7	6.7
	Salinity (ppt)	24.7	25.6	25.1	25.4	24.9	25.1
	Alkalinity (mg CaCO ₃ /L)	24 NA					
	Temperature (°C) 020420	25.1	25.0	24.7	25.1	24.6	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: Menidia beryllina

MbCuCR Test Number: 149

		Day							
		(Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
Analyst		KL	N	N	N	KL	N	N	N
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	8.22	7.02	8.01	7.83	8.05	7.91	7.96	7.76
	Dissolved oxygen (mg/L)	8.1	6.6	8.1	7.2	7.8	7.5	8.0	6.9
	Salinity (ppt)	24.0	25.3	24.9	25.2	24.6	24.9	24.9	24.9
	Alkalinity (mg CaCO ₃ /L)	no results		96				no results	
	Temperature (°C)	24.9	24.7	24.8	24.6	24.7	24.6	24.7	24.9
0.025 mg/L	pH (S.U.)	8.01	7.78	8.07	7.85	8.04	7.91	7.97	7.69
	Dissolved oxygen (mg/L)	8.1	6.6	8.1	7.2	7.4	7.6	8.0	6.8
	Salinity (ppt)	25.0	25.3	25.1	25.3	25.1	25.2	24.9	25.0
	Temperature (°C)	24.9	24.6	24.9	24.8	24.7	24.7	24.8	24.7
0.05 mg/L	pH (S.U.)	7.99	7.74	8.08	7.82	8.05	7.91	7.98	7.72
	Dissolved oxygen (mg/L)	8.1	6.6	8.1	7.1	7.9	7.6	8.0	6.8
	Salinity (ppt)	24.9	25.3	25.1	25.2	25.1	25.4	24.6	25.0
	Temperature (°C)	25.0	24.8	24.9	24.5	24.8	24.7	24.8	24.7
0.1 mg/L	pH (S.U.)	7.99	7.74	8.08	7.84	8.05	7.91	7.99	7.73
	Dissolved oxygen (mg/L)	8.1	6.6	8.1	7.2	8.0	7.6	8.0	6.9
	Salinity (ppt)	25.1	25.3	25.1	25.3	25.1	25.3	24.7	25.0
	Temperature (°C)	25.0	24.9	24.9	24.8	24.9	24.7	24.9	24.8
0.2 mg/L	pH (S.U.)	7.99	7.72	8.09	7.82	8.05	7.91	7.99	7.73
	Dissolved oxygen (mg/L)	8.1	6.4	8.2	7.1	8.0	7.5	8.0	6.9
	Salinity (ppt)	25.0	25.3	25.1	25.3	25.0	25.4	24.7	25.0
	Temperature (°C)	25.0	24.9	25.0	24.7	24.9	24.7	24.9	24.8
0.5 mg/L	pH (S.U.)	7.99	7.81	8.09	7.96	8.05	7.91	8.00	7.84
	Dissolved oxygen (mg/L)	8.1	6.9	8.2	7.3	8.1	7.5	8.0	6.9
	Salinity (ppt)	24.9	25.2	25.0	25.3	25.0	25.4	25.1	25.2
	Temperature (°C)	25.0	25.0	25.0	24.7	24.9	24.6	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final