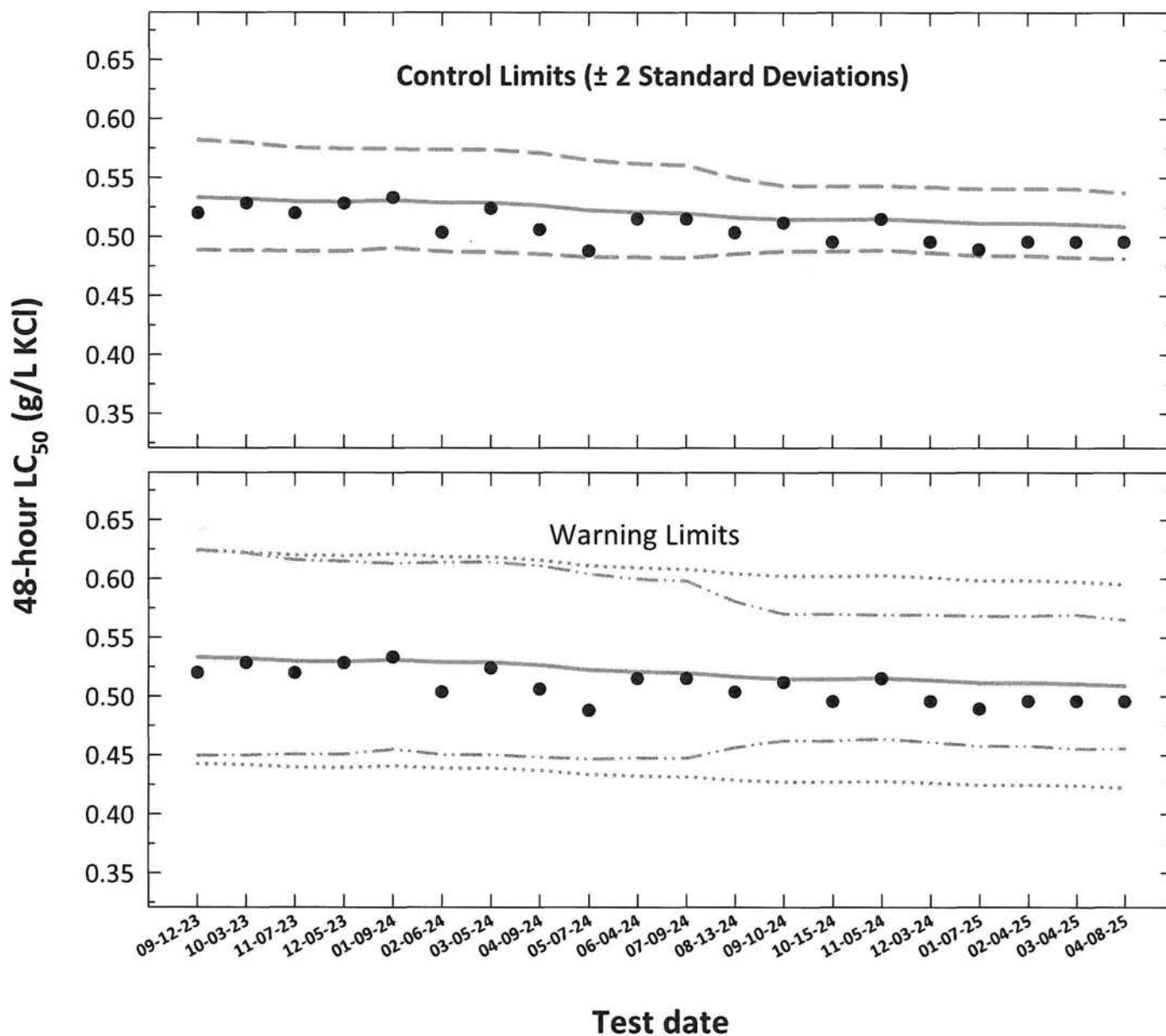


Americamysis (Mysidopsis) bahia
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.



- **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 Warning Limits		10th Percentile CV Warning Limits
1	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}
2	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4887	0.5819	0.4497	0.6244	0.4426
3	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4884	0.5798	0.4499	0.6217	0.4417
4	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4881	0.5757	0.4508	0.6161	0.4400
5	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4878	0.5748	0.4507	0.6151	0.4395
6	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4906	0.5746	0.4550	0.6131	0.4407
7	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4875	0.5740	0.4505	0.6141	0.4391
8	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4872	0.5738	0.4502	0.6140	0.4389
9	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4853	0.5711	0.4483	0.6113	0.4370
10	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4829	0.5651	0.4467	0.6041	0.4335
11	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4827	0.5620	0.4476	0.5998	0.4323
12	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4822	0.5606	0.4473	0.5982	0.4315
13	09-10-24	0.5117	-0.2910	-0.2884	0.0117	0.5147	0.4856	0.5497	0.4565	0.5807	0.4288
14	10-15-24	0.4957	-0.3048	-0.2884	0.0117	0.5147	0.4877	0.5431	0.4623	0.5700	0.4272
15	11-05-24	0.5149	-0.2882	-0.2880	0.0115	0.5153	0.4877	0.5431	0.4623	0.5700	0.4272
16	12-03-24	0.4957	-0.3048	-0.2893	0.0118	0.5136	0.4888	0.5432	0.4638	0.5695	0.4277
17	01-07-25	0.4892	-0.3105	-0.2912	0.0120	0.5114	0.4866	0.5422	0.4609	0.5693	0.4263
18	02-04-25	0.4957	-0.3048	-0.2912	0.0120	0.5114	0.4839	0.5405	0.4576	0.5683	0.4245
19	03-04-25	0.4957	-0.3048	-0.2919	0.0124	0.5106	0.4839	0.5405	0.4576	0.5683	0.4245
20	04-08-25	0.4957	-0.3048	-0.2935	0.0119	0.5088	0.4823	0.5406	0.4551	0.5693	0.4238
							0.4817	0.5374	0.4555	0.5650	0.4223

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 # 289

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	En JP	JP En	En
Control, SaltSW	pH (S.U.)	8.23	7.93	7.95
	Dissolved oxygen (mg/L)	0.3	7.0	0.0
	*Salinity (ppt)	25.0	25.6	26.0
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	25.1	25.2	25.3
250 mg/L	pH (S.U.)	8.08	7.96	7.95
	Dissolved oxygen (mg/L)	0.4	7.9	0.0
	*Salinity (ppt)	25.0	25.4	25.8
	*Temperature (°C)	25.3	25.2	25.0
375 mg/L	pH (S.U.)	8.08	7.96	7.94
	Dissolved oxygen (mg/L)	0.4	7.9	0.0
	*Salinity (ppt)	25.2	25.7	26.0
	*Temperature (°C)	25.3	25.3	25.0
500 mg/L	pH (S.U.)	8.08	7.97	7.93
	Dissolved oxygen (mg/L)	0.4	7.9	0.0
	*Salinity (ppt)	25.1	25.6	25.0
	*Temperature (°C)	25.0	25.0	25.2
750 mg/L	pH (S.U.)	8.09	7.95	
	Dissolved oxygen (mg/L)	0.4	7.9	
	*Salinity (ppt)	25.5	26.0	
	*Temperature (°C)	25.2	25.1	
1000 mg/L	pH (S.U.)	8.09	7.96	
	Dissolved oxygen (mg/L)	0.3	7.0	
	*Salinity (ppt)	25.5	25.9	
	*Temperature (°C)	25.0	25.1	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664615

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 # 289

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-08-25	* 1045	H	1320	H	LD	Yellow	04-0258
24	04-09-25			1324	H			
48 Termination	04-10-25			1317	H			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	04-01-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	04-06-25 1200 TO 04-07-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.89 Temperature (°C) 24.6

Survival Data (number of living organisms):

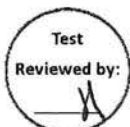
Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	11	10	10	9 ^{id}	9 ^{id}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	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: 4/8/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/10/2025 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:

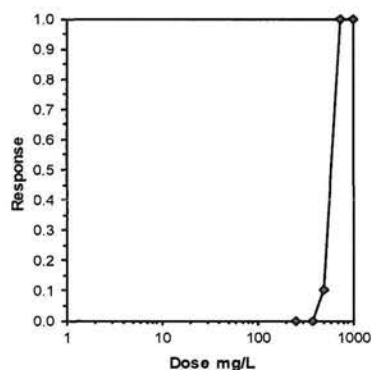
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.9000	0.9000
750	0.0000	0.0000
1000	0.0000	0.0000

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

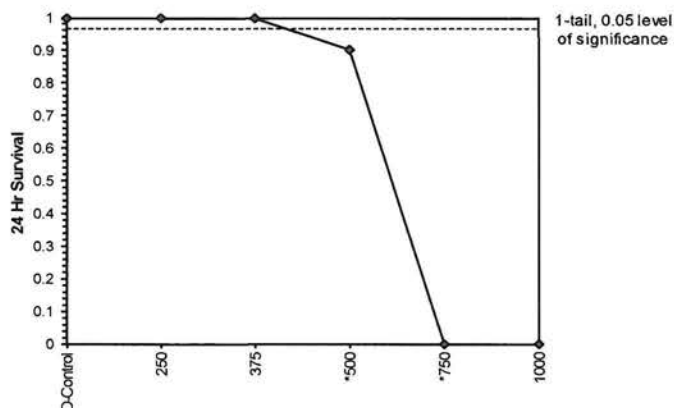
Auxiliary Tests					Statistic		Critical	Skew	Kurt		
Normality of the data set cannot be confirmed											
Equality of variance cannot be confirmed											
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		375	500	433.013		0.00967	0.00991	0.59802	0.0001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

Trimmed Spearman-Kärber



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 4/8/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/10/2025 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:

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

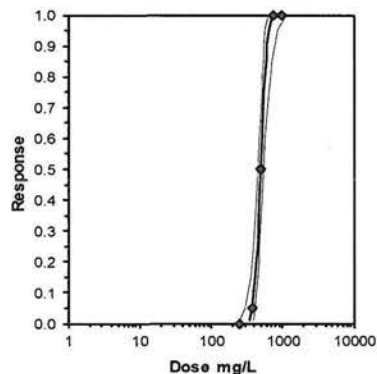
Auxiliary Tests

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

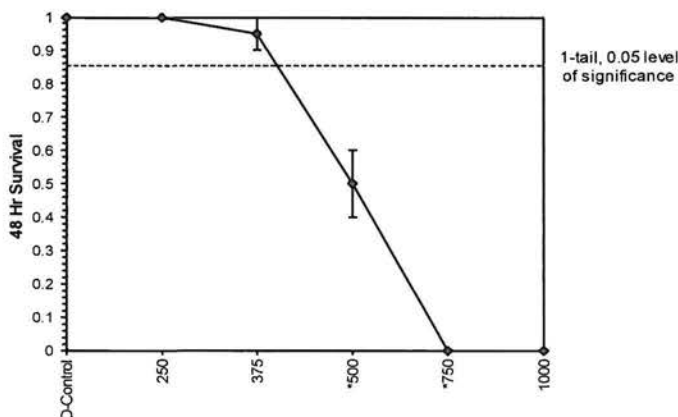
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.12113	0.12423	0.60026	0.00671	7.7E-05	4, 5
Treatments vs D-Control										

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							

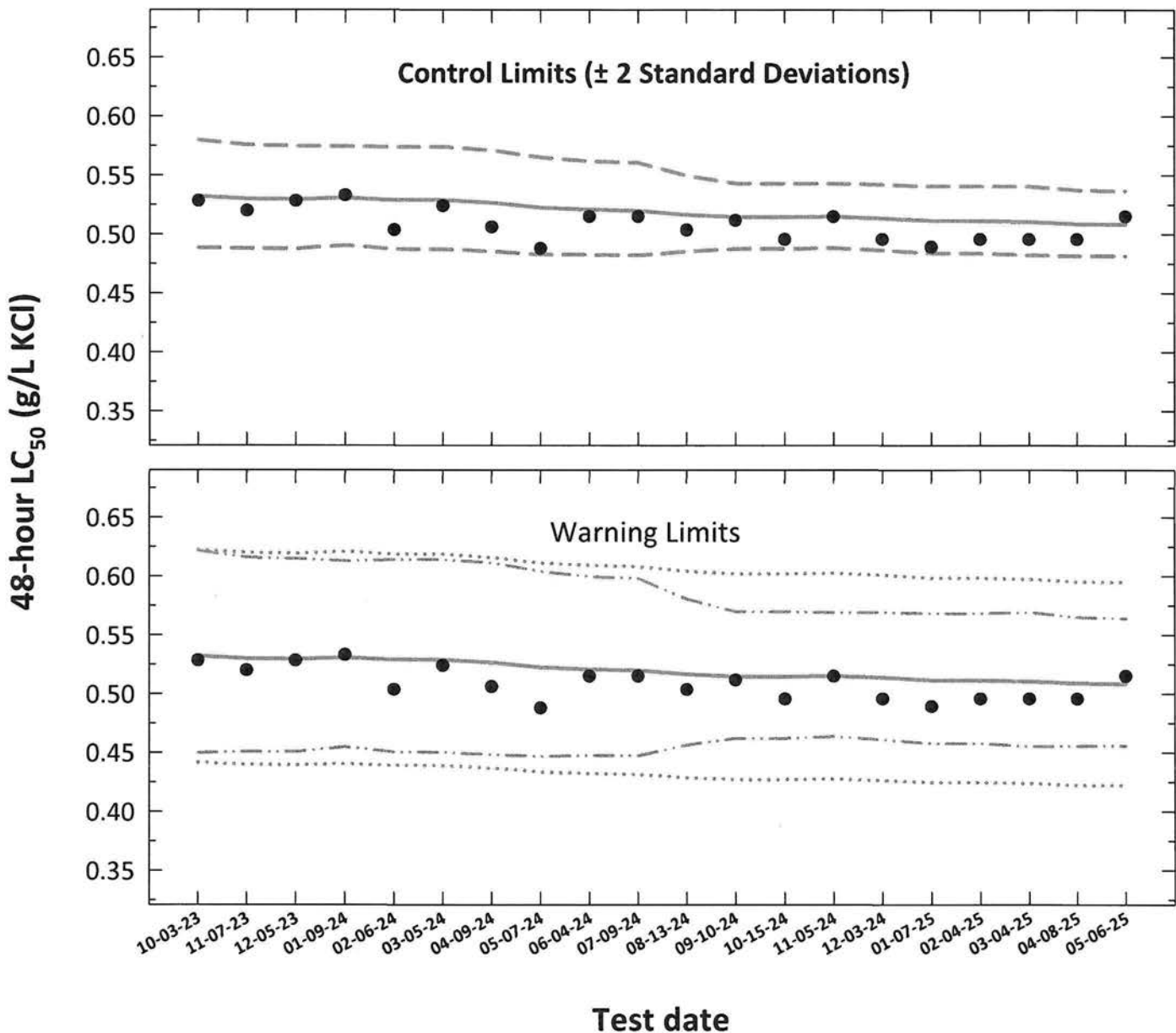
Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	341.348	245.982 387.152
EC05	3.355	380.762	301.411 419.772
EC10	3.718	403.601	334.964 439.493
EC15	3.964	419.779	358.932 454.273
EC20	4.158	433.099	378.48 467.26
EC25	4.326	444.862	395.35 479.6
EC40	4.747	475.939	436.335 517.953
EC50	5.000	495.671	458.62 547.68
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,
 $S_{A.10} = 10^{\text{th}}$ percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	04-08-25	0.4957	-0.3048	-0.2935	0.0119	0.5088	0.4817	0.5374	0.4555	0.5650	0.4223	0.5952
20	05-06-25	0.5149	-0.2882	-0.2937	0.0117	0.5085	0.4817	0.5368	0.4559	0.5641	0.4221	0.5950

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 # 290

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 #: 2376

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>U P</i>	<i>JP En</i>	<i>JP En</i>
	pH (S.U.)	<i>8.00</i>	<i>7.85</i>	<i>7.81</i>
	Dissolved oxygen (mg/L)	<i>8.3</i>	<i>7.8</i>	<i>7.9</i>
	*Salinity (ppt)	<i>25.0</i>	<i>25.5</i>	<i>26.0</i>
	*Alkalinity (mg/L CaCO ₃)	<i>92</i>		
250 mg/L	*Temperature (°C)	<i>25.9</i>	<i>25.4</i>	<i>24.6</i>
	pH (S.U.)	<i>8.00</i>	<i>7.86</i>	<i>7.79</i>
	Dissolved oxygen (mg/L)	<i>8.2</i>	<i>7.8</i>	<i>7.9</i>
	*Salinity (ppt)	<i>25.3</i>	<i>25.6</i>	<i>25.9</i>
	*Temperature (°C)	<i>25.7</i>	<i>25.2</i>	<i>24.2</i>
375 mg/L	pH (S.U.)	<i>8.01</i>	<i>7.87</i>	<i>7.77</i>
	Dissolved oxygen (mg/L)	<i>8.3</i>	<i>7.8</i>	<i>7.9</i>
	*Salinity (ppt)	<i>25.3</i>	<i>25.6</i>	<i>26.0</i>
	*Temperature (°C)	<i>25.7</i>	<i>25.2</i>	<i>24.8</i>
500 mg/L	pH (S.U.)	<i>8.00</i>	<i>7.87</i>	<i>7.82</i>
	Dissolved oxygen (mg/L)	<i>8.3</i>	<i>7.7</i>	<i>7.8</i>
	*Salinity (ppt)	<i>25.3</i>	<i>25.6</i>	<i>26.0</i>
	*Temperature (°C)	<i>25.6</i>	<i>25.3</i>	<i>24.8</i>
750 mg/L	pH (S.U.)	<i>8.00</i>	<i>7.87</i>	
	Dissolved oxygen (mg/L)	<i>8.3</i>	<i>7.7</i>	
	*Salinity (ppt)	<i>25.5</i>	<i>25.8</i>	
	*Temperature (°C)	<i>25.6</i>	<i>25.3</i>	
1000 mg/L	pH (S.U.)	<i>8.00</i>	<i>7.88</i>	
	Dissolved oxygen (mg/L)	<i>8.3</i>	<i>7.7</i>	
	*Salinity (ppt)	<i>25.6</i>	<i>26.0</i>	
	*Temperature (°C)	<i>25.6</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>13066468</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 # 290

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-06-25	* 1130	JL	1330	JL	6D	Yellow	04-30-25B
24	05-07-25			1335	JL			
48 Termination	05-08-25			1325	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):	AINB 05-06-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	05-04-25 1200 TO 05-05-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.05 Temperature (°C) 24.7

Survival Data (number of living organisms):

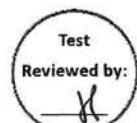
Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	9 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
48 Termination	10	10	10	10	10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

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

Statistics:

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

Comments:



Start Date:	5/6/2025	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	5/8/2025	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:					

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

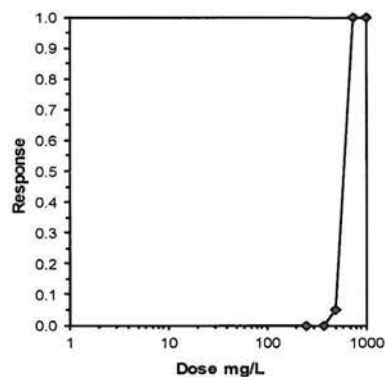
Statistic	Critical	Skew	Kurt
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Equality of variance cannot be confirmed

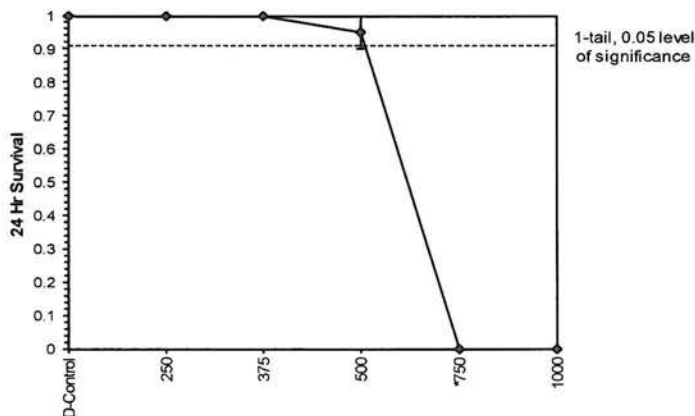
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.06555	0.06723	0.61047	0.00266	7.5E-06	4, 5

Treatments vs D-Control

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



Dose-Response Plot



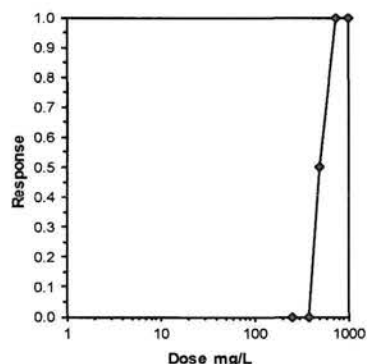
Start Date:	5/6/2025	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	5/8/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanmysis bahia
Comments:					

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

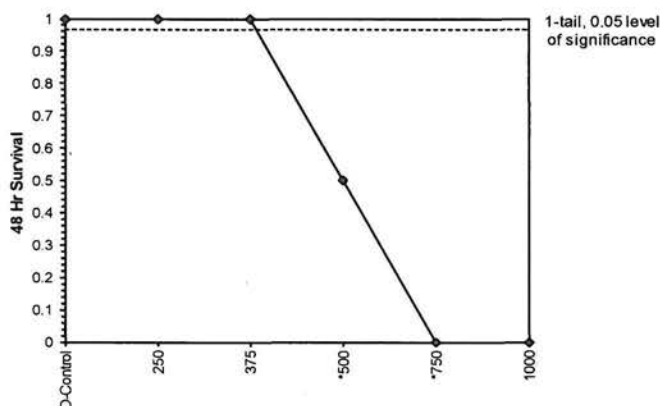
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120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

Auxiliary Tests					Statistic		Critical	Skew	Kurt	
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.62824	0.0001	2.0E-09	4,5
Treatments vs D-Control										

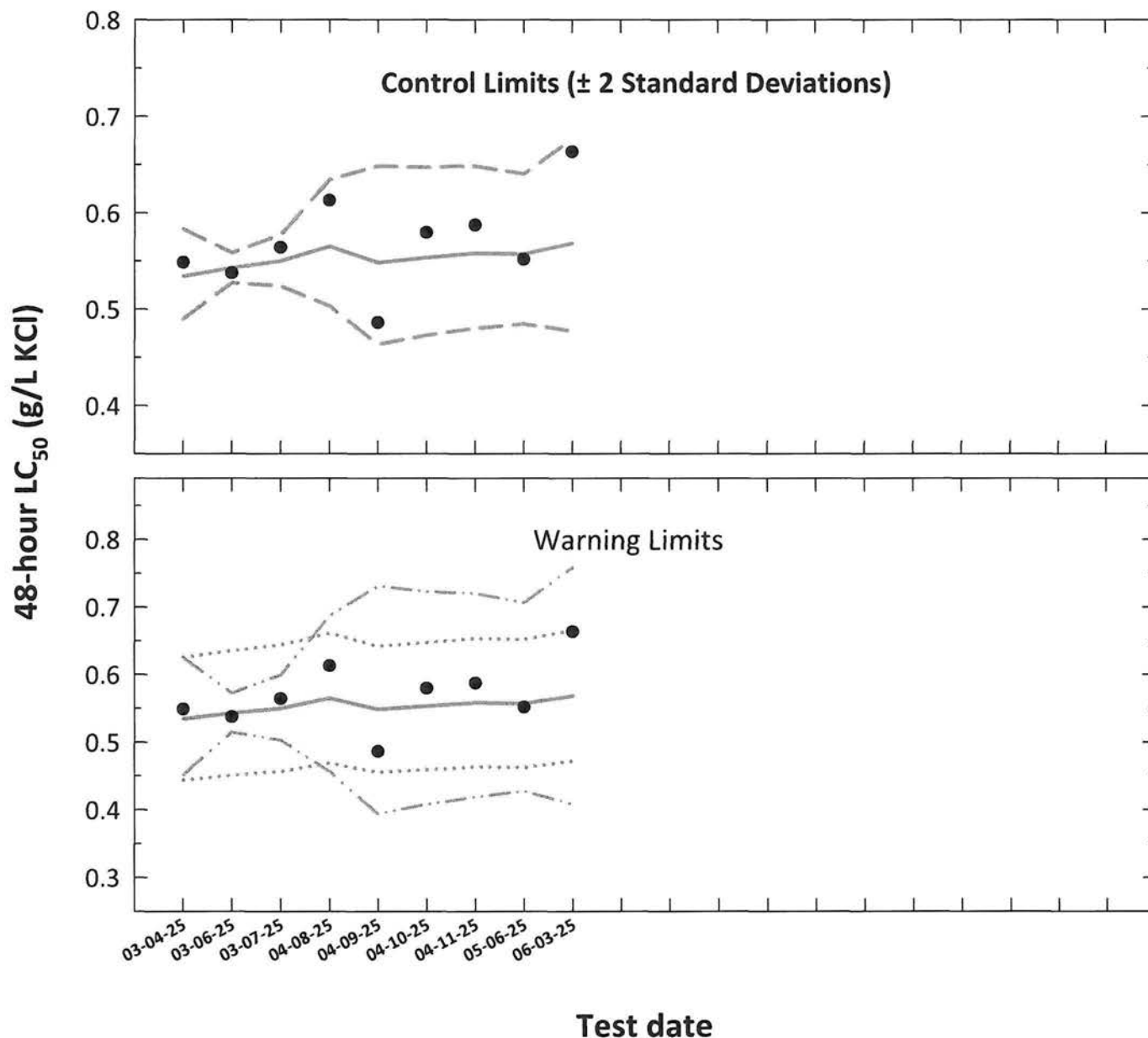
Trim Level	EC50	95% CL
0.0%	514.94	476.54 556.43
5.0%	513.43	471.14 559.51
10.0%	511.92	464.96 563.62
20.0%	508.91	448.82 577.05
Auto-0.0%	514.94	476.54 556.43



Dose-Response Plot



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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	03-04-25	0.5485	-0.2608			0.5430	0.5275	0.5588	0.5145	0.5722	0.4506	0.6353
2	03-06-25	0.5374	-0.2697	-0.2652	0.0063	0.5499	0.5239	0.5772	0.5026	0.5995	0.4564	0.6434
3	03-07-25	0.5640	-0.2487	-0.2597	0.0105	0.5650	0.5034	0.6342	0.4559	0.6874	0.4689	0.6610
4	04-08-25	0.6129	-0.2126	-0.2480	0.0251	0.5482	0.4635	0.6484	0.3937	0.7310	0.4550	0.6414
5	04-09-25	0.4860	-0.3134	-0.2610	0.0364	0.5533	0.4730	0.6473	0.4082	0.7231	0.4593	0.6474
6	04-10-25	0.5796	-0.2369	-0.2570	0.0341	0.5581	0.4803	0.6484	0.4187	0.7200	0.4632	0.6529
7	04-11-25	0.5872	-0.2312	-0.2533	0.0326	0.5573	0.4849	0.6405	0.4273	0.7066	0.4625	0.6520
8	05-06-25	0.5517	-0.2583	-0.2539	0.0302	0.5681	0.4772	0.6763	0.4082	0.7586	0.4716	0.6647
9	06-03-25	0.6631	-0.1784	-0.2455	0.0379							
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

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 # 1

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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.)	8.08	7.98	7.77
	Dissolved oxygen (mg/L)	8.1	7.7	7.6
	*Salinity (ppt)	25.7	25.4	25.2
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	24.4	24.7	24.4
150 mg/L	pH (S.U.)	8.07	7.98	7.77
	Dissolved oxygen (mg/L)	8.1	7.7	7.6
	*Salinity (ppt)	25.2	25.5	25.2
	*Temperature (°C)	24.6	24.5	24.4
300 mg/L	pH (S.U.)	8.07	7.96	7.76
	Dissolved oxygen (mg/L)	8.2	7.7	7.6
	*Salinity (ppt)	25.8	25.8	25.6
	*Temperature (°C)	24.4	24.5	24.5
450 mg/L	pH (S.U.)	8.07	7.99	7.81
	Dissolved oxygen (mg/L)	8.2	7.7	7.6
	*Salinity (ppt)	25.4	25.8	25.8
	*Temperature (°C)	24.5	24.3	24.3
600 mg/L	pH (S.U.)	8.08	7.98	7.84
	Dissolved oxygen (mg/L)	8.2	7.6	7.6
	*Salinity (ppt)	25.4	26.3	26.1
	*Temperature (°C)	24.2	24.5	24.3
750 mg/L	pH (S.U.)	8.07	8.00	
	Dissolved oxygen (mg/L)	8.2	7.6	
	*Salinity (ppt)	25.9	26.3	
	*Temperature (°C)	24.3	24.5	

*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+ 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	1306646

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 # 1

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-04-25	*1100	JP	1415	JP	6B	orange	02-26-25A
24	07-05-25			1430	JP			
48 Termination	03-06-25			1435	JP			

*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):	03-03-25
Age (1 to 5 days old):	1 to 2 days old
Date organisms were born:	03-02-25 1200 17 03-03-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01 Temperature (°C): 24.0°C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 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	8 ^{2d}	10	10	10	9 ^{1d}	9 ^{1d}	8 ^{2d}	5 ^{5d}	5 ^{5d}	0 ^{10d}	0 ^{10d}
48 Termination	10	8	10	10	10	9	9	7 ^{1d}	3 ^{2d}	4 ^{1d}	0	0
Mean Survival	90%.		100%.		95%.		90%.		35%.		0%.	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	492.93
Upper 95% confidence limit (mg KCl/L)	592.96
48-hour LC ₅₀ (mg KCl/L)	548.52

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: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	0.8000
150	1.0000	1.0000
300	1.0000	0.9000
450	0.9000	0.8000
600	0.5000	0.5000
750	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	Number Resp	Total Number
			Mean	Min	Max	CV%				
D-Control	0.9000	1.0000	1.2596	1.1071	1.4120	17.115	2	2	20	
150	1.0000	1.1111	1.4120	1.4120	1.4120	0.000	2	0	20	
300	0.9500	1.0556	1.3305	1.2490	1.4120	8.661	2	1	20	
450	0.8500	0.9444	1.1781	1.1071	1.2490	8.517	2	3	20	
600	0.5000	0.5556	0.7854	0.7854	0.7854	0.000	2	10	20	
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20	

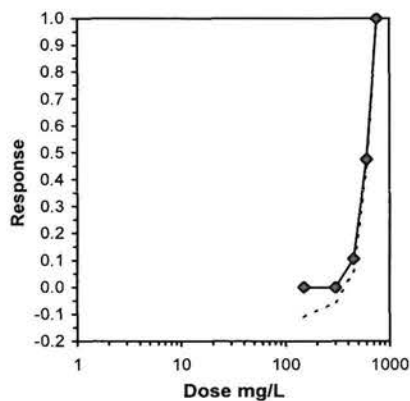
Auxiliary Tests

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

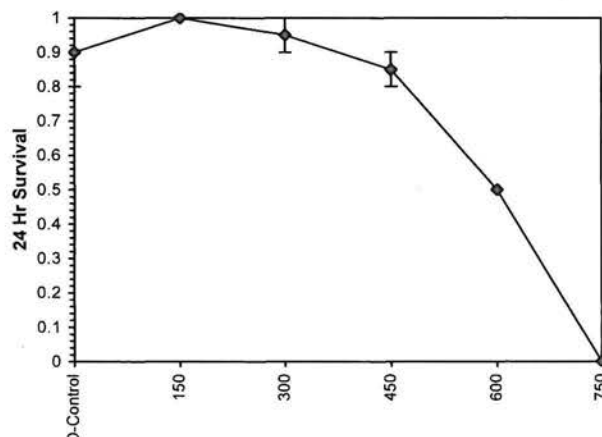
Statistic Critical Skew Kurt

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	573.09	532.07	617.27
5.0%	582.47	538.75	629.75
10.0%	588.09	545.29	634.26
20.0%	593.37	539.94	652.08
Auto-0.0%	573.09	532.07	617.27



Dose-Response Plot





Acute Mysid Test-48 Hr Survival

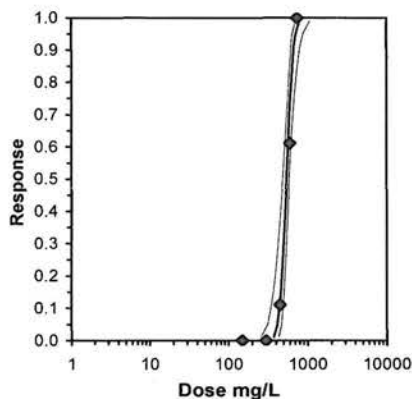
Start Date: 3/4/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/6/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	0.8000
150	1.0000	1.0000
300	1.0000	0.9000
450	0.9000	0.7000
600	0.3000	0.4000
750	0.0000	0.0000

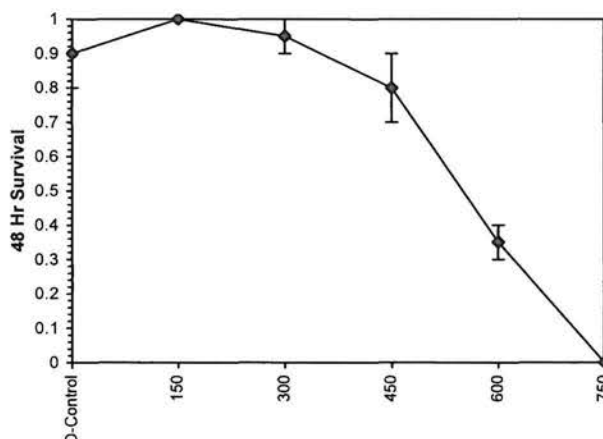
Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	0.9000	1.0000	1.2596	1.1071	1.4120	17.115	2	2	20
150	1.0000	1.1111	1.4120	1.4120	1.4120	0.000	2	0	20
300	0.9500	1.0556	1.3305	1.2490	1.4120	8.661	2	1	20
450	0.8000	0.8889	1.1201	0.9912	1.2490	16.280	2	4	20
600	0.3500	0.3889	0.6322	0.5796	0.6847	11.753	2	13	20
750	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				

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	13.9	3.22558	7.57791	20.2222	0.1	2.40387	7.81472	0.49291	2.73919	0.07194
Intercept	-33.075	8.90534	-50.529	-15.62						
TSCR	0.05276	0.0288	-0.0037	0.10921						
Point	Probits	mg/L	95% Fiducial Limits							
EC01	2.674	373.103	254.572	434.477						
EC05	3.355	417.692	311.715	471.685						
EC10	3.718	443.602	346.788	493.461						
EC15	3.964	461.985	372.325	509.17						
EC20	4.158	477.137	393.66	522.402						
EC25	4.326	490.531	412.639	534.412						
EC40	4.747	525.974	462.504	568.501						
EC50	5.000	548.518	492.93	592.956						
EC60	5.253	572.028	522.482	621.866						
EC75	5.674	613.36	567.418	682.756						
EC80	5.842	630.578	583.677	711.731						
EC85	6.036	651.26	601.756	748.875						
EC90	6.282	678.248	623.588	800.572						
EC95	6.645	720.32	654.992	887.093						
EC99	7.326	806.406	713.748	1082.16						



Dose-Response Plot



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 #

2Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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 #:

2348Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	<u>BM</u>	<u>BM</u>	<u>HP BM</u>
	pH (S.U.)	<u>7.98</u>	<u>7.86</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.7</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.0</u>	<u>25.0</u>
	*Alkalinity (mg/L CaCO ₃)	<u>110</u>		
Control, SaltSW	*Temperature (°C)	<u>24.6</u>	<u>24.5</u>	<u>24.2</u>
	pH (S.U.)	<u>8.07</u>	<u>7.83</u>	<u>7.89</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.7</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.0</u>	<u>25.4</u>
	*Temperature (°C)	<u>24.7</u>	<u>24.5</u>	<u>24.2</u>
150 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.83</u>	<u>7.89</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.7</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.0</u>	<u>25.4</u>
	*Temperature (°C)	<u>24.7</u>	<u>24.5</u>	<u>24.2</u>
300 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.83</u>	<u>7.89</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.6</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.4</u>	<u>25.2</u>	<u>25.5</u>
	*Temperature (°C)	<u>24.7</u>	<u>24.6</u>	<u>24.1</u>
450 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.91</u>	<u>7.92</u>
	Dissolved oxygen (mg/L)	<u>8.1</u>	<u>7.6</u>	<u>7.6</u>
	*Salinity (ppt)	<u>25.5</u>	<u>25.8</u>	<u>25.8</u>
	*Temperature (°C)	<u>25.0</u>	<u>24.5</u>	<u>24.1</u>
600 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.91</u>	<u>7.89</u>
	Dissolved oxygen (mg/L)	<u>8.1</u>	<u>7.7</u>	<u>7.6</u>
	*Salinity (ppt)	<u>25.6</u>	<u>25.8</u>	<u>25.6</u>
	*Temperature (°C)	<u>25.1</u>	<u>24.3</u>	<u>24.1</u>
750 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.89</u>	
	Dissolved oxygen (mg/L)	<u>8.1</u>	<u>7.6</u>	
	*Salinity (ppt)	<u>25.6</u>	<u>25.8</u>	
	*Temperature (°C)	<u>24.9</u>	<u>24.6</u>	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13066461

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC #

2

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-05-25	* 0500	JP	0930	JP	6C	yellow	02-26-250
24	03-06-25			0945	JP			
48 Termination	03-07-25			* 1200	JP			

*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):	03-03-25
Age (1 to 5 days old):	2 to 3 days old
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.): 7.93
	Temperature (°C) 24.8

* INVALID TEST. TEST TERMINATION EXCEEDED + 2 HOURS FROM INITIATION TIME. JP

NOT INCLUDED IN CONTROL CHART

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 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	9 ^{1d}	10	10	10	10	10	9 ^{1d}	10	8 ^{2d}	5 ^{5d}	0 ^{10d}	0 ^{10d}
48 Termination	9	10	10	10	10	10	9	9 ^{1d}	4 ^{4d}	* 10 ⁰⁷⁻²⁵	0	0
Mean Survival	95%		100%		100%		90%		40%		0%	

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

* spilled

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	496.04
Upper 95% confidence limit (mg KCl/L)	574.43
48-hour LC ₅₀ (mg KCl/L)	537.54

Comments:

501.20
604.35
556.96

JP 04-20-25





Acute Mysid Test-24 Hr Survival

Start Date: 3/5/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/7/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

Conc-mg/L	1	2
D-Control	0.9000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	1.0000
600	0.8000	0.5000
750	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	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2				1	20
150	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-0.651	2.850	0.3567	0	20
300	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-0.651	2.850	0.3567	0	20
450	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2	0.000	2.850	0.3567	1	20
*600	0.6500	0.6842	0.9463	0.7854	1.1071	24.043	2	3.070	2.850	0.3567	7	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

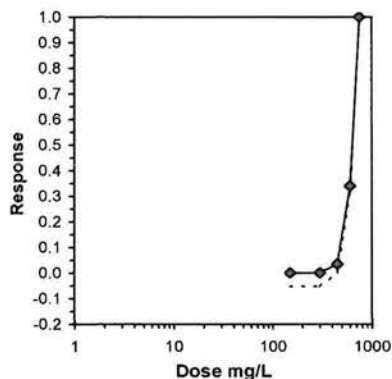
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

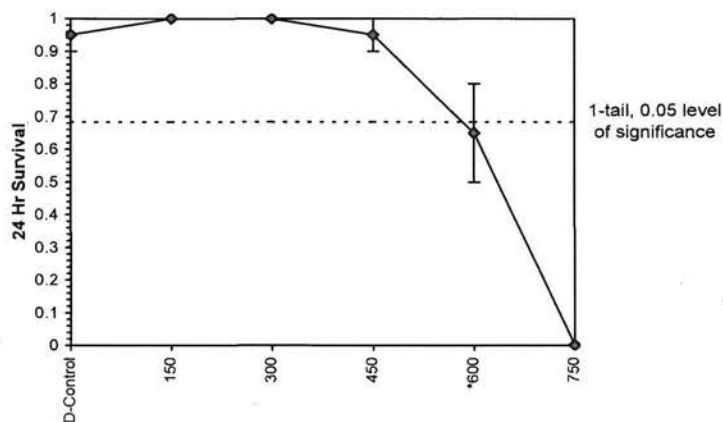
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.25937	0.27494	0.07557	0.01566	0.05735	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	608.00	572.07 646.18
5.0%	615.97	579.44 654.80
10.0%	619.97	578.48 664.44
20.0%	627.37	566.74 694.49
Auto-0.0%	608.00	572.07 646.18



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

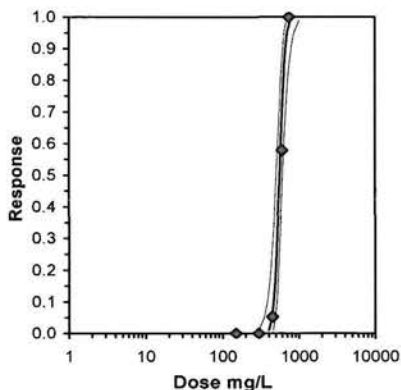
Start Date: 3/5/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/7/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

Conc-mg/L	1	2
D-Control	0.9000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	0.9000
600	0.4000	
750	0.0000	0.0000

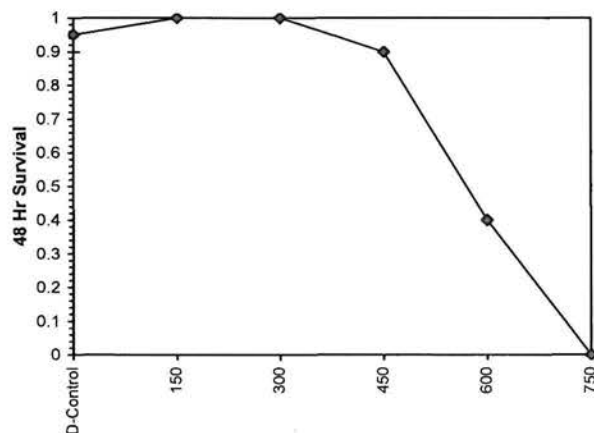
Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2	1	20
150	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.9000	0.9474	1.2490	1.2490	1.2490	0.000	2	2	20
600	0.4000	0.4211	0.6847	0.6847	0.6847	0.000	1	6	10
750	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				

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	16.3449	3.6849	9.12251 23.5673	0.05	1.68073	7.81472	0.64123	2.74738	0.06118	4	
Intercept	-39.906	10.1544	-59.808 -20.003								
TSCR	0.01797	0.0171	-0.0155 0.05149								
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	402.763	299.098 457.625								
EC05	3.355	443.347	352.903 492.369								
EC10	3.718	466.628	384.718 512.911								
EC15	3.964	483.023	407.302 527.885								
EC20	4.158	496.463	425.784 540.614								
EC25	4.326	508.29	441.921 552.261								
EC40	4.747	539.359	482.97 585.604								
EC50	5.000	558.956	507.199 609.351								
EC60	5.253	579.266	530.48 636.647								
EC75	5.674	614.673	566.473 690.913								
EC80	5.842	629.317	579.881 715.609								
EC85	6.036	646.827	595.026 746.622								
EC90	6.282	669.553	613.541 788.974								
EC95	6.645	704.712	640.352 858.476								
EC99	7.326	775.723	690.314 1010.93								



Dose-Response Plot





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 # 3

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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
Control, SaltSW	Analyst	<i>En</i>	<i>JP En</i>	<i>JP IL</i>
	pH (S.U.)	8.09	7.98	7.02
	Dissolved oxygen (mg/L)	8.0	7.7	7.1
	*Salinity (ppt)	25.0	25.0	24.9
	*Alkalinity (mg/L CaCO ₃)	110		
150 mg/L	*Temperature (°C)	25.7	24.1	25.2
	pH (S.U.)	8.06	8.00	7.02
	Dissolved oxygen (mg/L)	8.1	7.7	6.9
	*Salinity (ppt)	25.0	25.0	25.2
	*Temperature (°C)	25.6	24.1	25.4
300 mg/L	pH (S.U.)	8.05	8.00	7.05
	Dissolved oxygen (mg/L)	8.1	7.7	7.0
	*Salinity (ppt)	25.0	25.1	25.2
	*Temperature (°C)	25.7	24.2	25.3
450 mg/L	pH (S.U.)	8.06	7.99	7.05
	Dissolved oxygen (mg/L)	8.1	7.7	7.0
	*Salinity (ppt)	25.0	25.3	25.3
	*Temperature (°C)	25.8	24.1	25.2
600 mg/L	pH (S.U.)	8.06	7.97	7.04
	Dissolved oxygen (mg/L)	8.1	7.7	7.0
	*Salinity (ppt)	25.1	25.2	25.3
	*Temperature (°C)	25.7	24.2	25.5
750 mg/L	pH (S.U.)	8.06	8.00	7.09
	Dissolved oxygen (mg/L)	8.0	7.6	7.1
	*Salinity (ppt)	25.2	25.3	25.4
	*Temperature (°C)	25.8	24.1	25.2

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	132664

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 # 3

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-06-25	* 0500	JP	0404	JP	66	yellow	02-26-25B
24	03-07-25			0923	JP			
48 Termination	03-08-25			0900	JP			

*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):	03-03-25
Age (1 to 5 days old):	3 to 4 days old
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.): 7.79
	Temperature (°C) 24.1°C

Survival Data (number of living organisms):

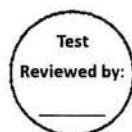
Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	7 ^{sd}	4 ^{sd}	1 ^{sd}	0
48 Termination	10	10	10	10	10	10	1 ^{sd}	3 ^{sd}	5 ^{sd}	4	1	0
Mean Survival	100%		100%		100%		80%		30%		5%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	490.43
Upper 95% confidence limit (mg KCl/L)	582.44
48-hour LC ₅₀ (mg KCl/L)	537.44

Comments:





Acute Mysid Test-24 Hr Survival

Start Date: 3/6/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/8/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanmysis bahia
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	1.0000
600	0.7000	0.4000
750	0.1000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
600	0.5500	0.5500	0.8379	0.6847	0.9912	25.859	2	9	20
750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19	20

Auxiliary Tests

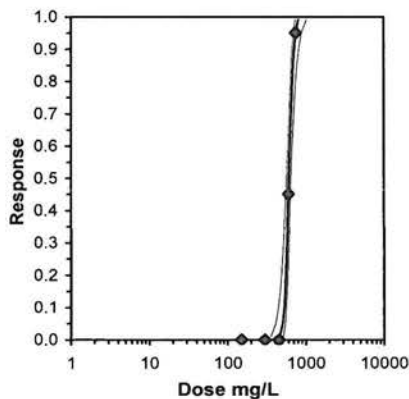
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Statistic Critical Skew Kurt

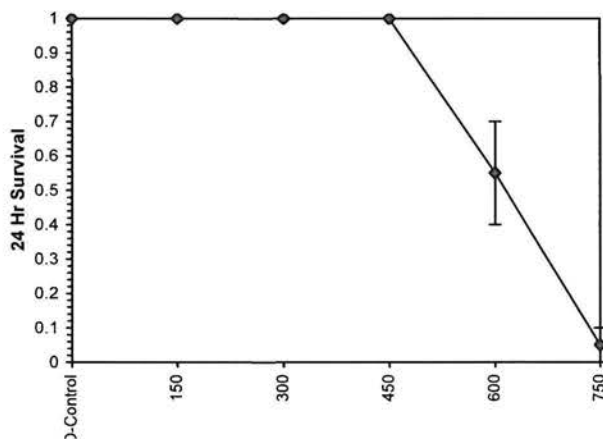
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	19.5637	4.72456	10.3035 28.8238	0	0.14507	7.81472	0.98593	2.78699	0.05112	3
Intercept	-49.524	13.2077	-75.411 -23.637							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	465.669	354.224 517.058
EC05	3.355	504.559	410.973 548.009
EC10	3.718	526.601	444.328 565.928
EC15	3.964	542.015	467.963 578.825
EC20	4.158	554.586	487.293 589.706
EC25	4.326	565.603	504.156 599.625
EC40	4.747	594.345	546.791 628.214
EC50	5.000	612.334	571.394 649.188
EC60	5.253	630.868	594.165 674.183
EC75	5.674	662.926	627.211 725.697
EC80	5.842	676.096	638.973 749.386
EC85	6.036	691.777	652.046 779.069
EC90	6.282	712.025	667.854 819.344
EC95	6.645	743.131	690.595 884.684
EC99	7.326	805.193	732.827 1025.17



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 3/6/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/8/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

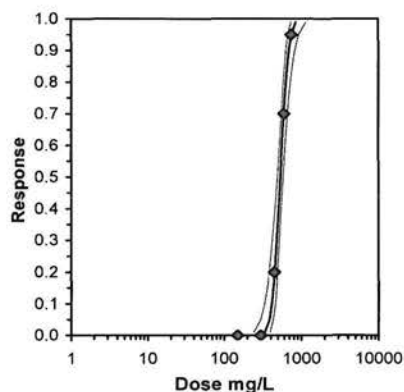
Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	0.7000
600	0.2000	0.4000
750	0.1000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.8000	0.8000	1.1201	0.9912	1.2490	16.280	2	4	20
600	0.3000	0.3000	0.5742	0.4636	0.6847	27.225	2	14	20
750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19	20

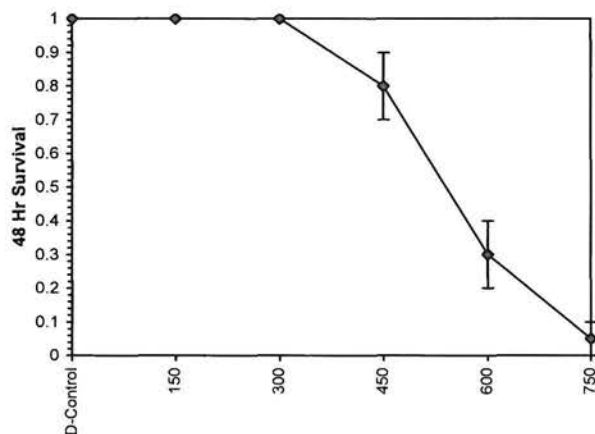
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	11.3432	2.243	6.94689 15.7395	0	0.05549	7.81472	0.99658	2.73033	0.08816
Intercept	-25.971	6.15794	-38.04 -13.901						
TSCR									

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	335.152	240.009 391.67
EC05	3.355	384.877	299.28 434.979
EC10	3.718	414.334	336.099 460.743
EC15	3.964	435.471	363.057 479.523
EC20	4.158	453.037	385.644 495.471
EC25	4.326	468.671	405.756 510.054
EC40	4.747	510.5	458.482 552
EC50	5.000	537.44	490.431 582.437
EC60	5.253	565.803	521.403 618.328
EC75	5.674	616.3	569.592 692.164
EC80	5.842	637.568	587.779 726.5
EC85	6.036	663.286	608.61 770.071
EC90	6.282	697.124	634.599 830.288
EC95	6.645	750.479	673.36 930.808
EC99	7.326	861.823	749.048 1158.77



Dose-Response Plot





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 # 4

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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
Control, SaltSW	Analyst	<u>JP</u>	<u>N</u>	<u>N</u>
	pH (S.U.)	<u>7.91</u>	<u>7.78</u>	<u>7.53</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.0</u>	<u>5.8</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.1</u>	<u>24.9</u>
	*Alkalinity (mg/L CaCO ₃)	<u>100</u>		
	*Temperature (°C)	<u>25.8</u>	<u>24.6</u>	<u>24.8</u>
150 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.78</u>	<u>7.62</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.0</u>	<u>5.8</u>
	*Salinity (ppt)	<u>24.9</u>	<u>24.8</u>	<u>25.0</u>
	*Temperature (°C)	<u>24.7</u>	<u>24.5</u>	<u>24.7</u>
300 mg/L	pH (S.U.)	<u>8.08</u>	<u>7.74</u>	<u>7.62</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>6.9</u>	<u>6.0</u>
	*Salinity (ppt)	<u>24.9</u>	<u>25.0</u>	<u>25.3</u>
	*Temperature (°C)	<u>24.4</u>	<u>24.6</u>	<u>24.7</u>
450 mg/L	pH (S.U.)	<u>8.08</u>	<u>7.79</u>	<u>7.63</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>6.8</u>	<u>6.0</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.3</u>	<u>25.4</u>
	*Temperature (°C)	<u>25.8</u>	<u>24.3</u>	<u>24.9</u>
600 mg/L	pH (S.U.)	<u>8.08</u>	<u>7.80</u>	<u>7.58</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.0</u>	<u>5.8</u>
	*Salinity (ppt)	<u>25.1</u>	<u>25.5</u>	<u>25.7</u>
	*Temperature (°C)	<u>24.3</u>	<u>24.2</u>	<u>24.6</u>
750 mg/L	pH (S.U.)	<u>8.07</u>	<u>7.86</u>	<u>7.70</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.1</u>	<u>6.1</u>
	*Salinity (ppt)	<u>25.7</u>	<u>25.4</u>	<u>25.6</u>
	*Temperature (°C)	<u>25.6</u>	<u>24.5</u>	<u>24.6</u>

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	<u>170634</u>

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC #

4

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-07-25	* 0500	JP	0938	JP	6B	orange	03-21-25
24	03-08-25			0930	X			
48 Termination	03-09-25			0930	XL			

*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):	03-03-25
Age (1 to 5 days old):	41 to 5 days old
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.): 7.95 Temperature (°C) 74.1 to 74.5 (03-07-25)

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	4d 6	5d 5	10d 0	9d 1
48 Termination	10	10	10	10	10	10	10	9d	4d 4	2d 3	0	0d
Mean Survival	100%		100%		100%		95%		35%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	525.13 512.13 (03-08-25)
Upper 95% confidence limit (mg KCl/L)	600.52
48-hour LC ₅₀ (mg KCl/L)	563.99

Comments:





Acute Mysid Test-24 Hr Survival

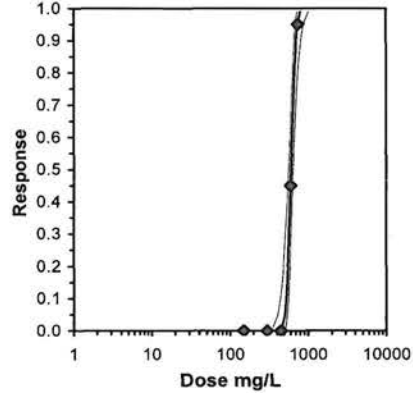
Start Date: 3/7/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/8/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	1.0000
600	0.6000	0.5000
750	0.0000	0.1000

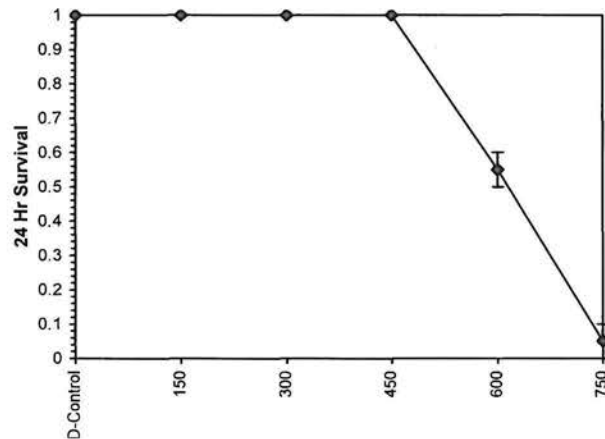
Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
600	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9	20
750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	19.5637	4.72456	10.3035 28.8238	0	0.14507	7.81472	0.98593	2.78699	0.05112
Intercept	-49.524	13.2077	-75.411 -23.637						
TSCR									
Point	Probits	mg/L	95% Fiducial Limits						
EC01	2.674	465.669	354.224 517.058						
EC05	3.355	504.559	410.973 548.009						
EC10	3.718	526.601	444.328 565.928						
EC15	3.964	542.015	467.963 578.825						
EC20	4.158	554.586	487.293 589.706						
EC25	4.326	565.603	504.156 599.625						
EC40	4.747	594.345	546.791 628.214						
EC50	5.000	612.334	571.394 649.188						
EC60	5.253	630.868	594.165 674.183						
EC75	5.674	662.926	627.211 725.697						
EC80	5.842	676.096	638.973 749.386						
EC85	6.036	691.777	652.046 779.069						
EC90	6.282	712.025	667.854 819.344						
EC95	6.645	743.131	690.595 884.684						
EC99	7.326	805.193	732.827 1025.17						



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 3/7/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/8/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

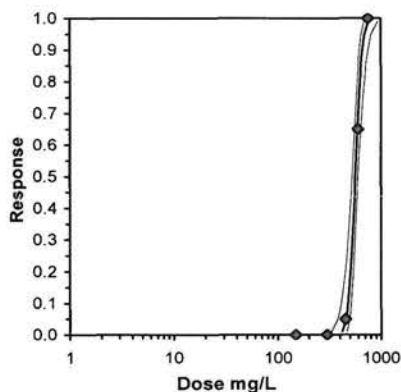
Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	0.9000
600	0.4000	0.3000
750	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1	20
600	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	13	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20

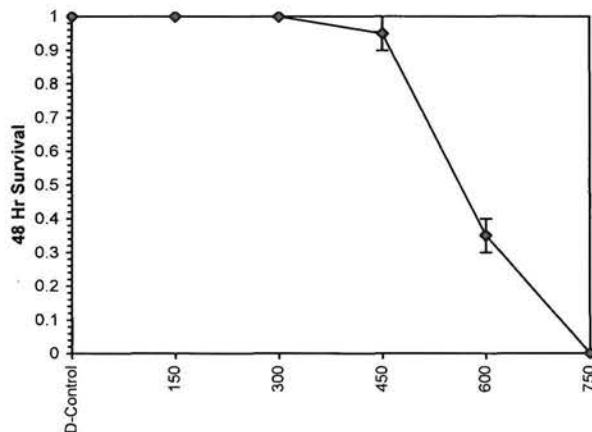
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.0334	3.78729	10.6104 25.4565	0	0.45137	7.81472	0.92944	2.75127	0.05545	4
Intercept	-44.615	10.4575	-65.112 -24.118							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	419.054	330.969 465.979
EC05	3.355	457.152	382.028 497.804
EC10	3.718	478.858	411.851 516.328
EC15	3.964	494.082	432.898 529.677
EC20	4.158	506.526	450.064 540.923
EC25	4.326	517.452	465.018 551.134
EC40	4.747	546.039	502.908 580.06
EC50	5.000	563.991	525.129 600.518
EC60	5.253	582.534	546.31 623.996
EC75	5.674	614.716	578.684 670.525
EC80	5.842	627.975	590.667 691.562
EC85	6.036	643.791	604.191 717.815
EC90	6.282	664.259	620.743 753.364
EC95	6.645	695.798	644.787 810.985
EC99	7.326	759.056	689.82 934.745



Dose-Response Plot



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 #

5

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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
Control, SaltSW	Analyst	EM JO	JPEN	JPEN
	pH (S.U.)	8.23	7.92	7.79
	Dissolved oxygen (mg/L)	0.3	0.0	0.1
	*Salinity (ppt)	25.0	25.5	25.8
	*Alkalinity (mg/L CaCO ₃)	100		
150 mg/L	*Temperature (°C)	24.2	25.4	24.7
	pH (S.U.)	8.09	7.90	7.80
	Dissolved oxygen (mg/L)	0.3	0.0	0.0
	*Salinity (ppt)	24.9	25.4	25.6
	*Temperature (°C)	24.7	24.7	25.1
300 mg/L	pH (S.U.)	8.10	7.89	7.78
	Dissolved oxygen (mg/L)	0.3	0.0	7.9
	*Salinity (ppt)	25.2	25.8	25.7
	*Temperature (°C)	24.1	25.6	25.2
	pH (S.U.)	8.10	7.93	7.79
450 mg/L	Dissolved oxygen (mg/L)	0.3	7.9	7.0
	*Salinity (ppt)	25.2	25.7	25.8
	*Temperature (°C)	24.5	24.5	24.1
	pH (S.U.)	8.10	7.95	7.83
	Dissolved oxygen (mg/L)	0.3	7.0	7.7
600 mg/L	*Salinity (ppt)	25.3	25.9	26.0
	*Temperature (°C)	24.2	24.9	24.5
	pH (S.U.)	8.11	7.93	7.82
	Dissolved oxygen (mg/L)	0.3	7.0	7.6
	*Salinity (ppt)	25.4	25.9	26.1
750 mg/L	*Temperature (°C)	24.3	24.7	24.3

*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	1306-990

130664

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 # 5

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-08-25	* 1045	JP	1521	JP	6E	1.9h 5112	04-02-25B
24	04-09-25			1532	JP			
48 Termination	04-10-25			1528	JP			

*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 (All Batch Ab):	04-07-25
Age (1 to 5 days old):	1-2 days old
Date organisms were born:	04-06-25 1200 to 04-07-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.89 Temperature (°C): 24.2°C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	8 ^{2d}	10	9 ^{1d}	0 ^{10d}	3 ^{7d}
48 Termination	10	10	10	10	10	10	10	7 ^{1d}	10	7 ^{2d}	0	0 ^{3d}
Mean Survival	100 %		100 %		100 %		85 %		85 %		0 %	

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

Statistics:

Method	Spearman-Kärber
Lower 95% confidence limit (mg KCl/L)	572.18
Upper 95% confidence limit (mg KCl/L)	656.52
48-hour LC ₅₀ (mg KCl/L)	612.90

Comments:





Acute Mysid Test-24 Hr Survival

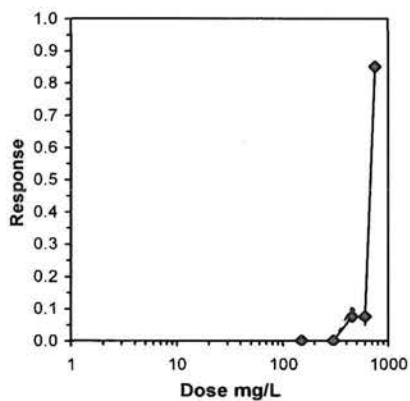
Start Date: 4/8/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 4/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	0.8000
600	1.0000	0.9000
750	0.0000	0.3000

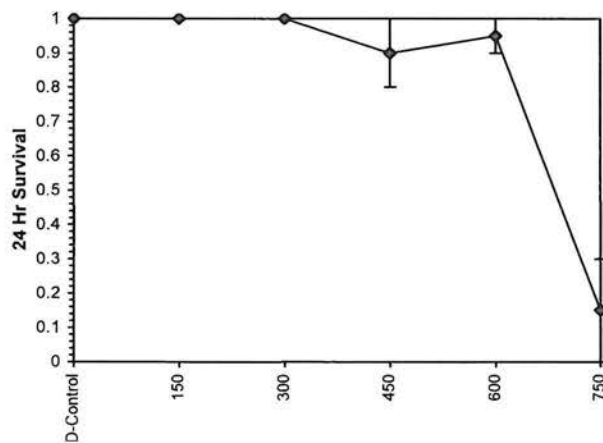
Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	2	20
600	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1	20
750	0.1500	0.1500	0.3692	0.1588	0.5796	80.603	2	17	20

Auxiliary Tests Statistic Critical Skew Kurt
 Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	678.10	658.39	698.41
Auto-15.0%	678.10	658.39	698.41



Dose-Response Plot





Acute Mysid Test-48 Hr Survival

Start Date: 4/8/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 4/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	0.7000
600	1.0000	0.7000
750	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.8500	0.8500	1.2016	0.9912	1.4120	24.767	2	3	20
600	0.8500	0.8500	1.2016	0.9912	1.4120	24.767	2	3	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20

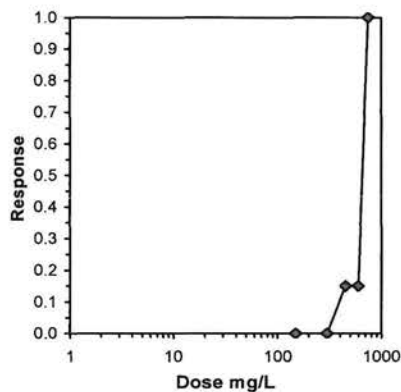
Auxiliary Tests

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

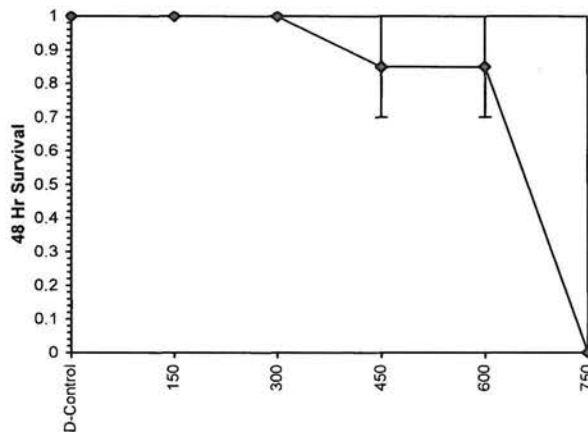
Statistic Critical Skew Kurt

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	612.90	572.18 656.52
5.0%	628.47	584.13 676.18
10.0%	643.56	598.84 691.63
20.0%	657.74	641.72 674.16
Auto-0.0%	612.90	572.18 656.52



Dose-Response Plot



Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 6

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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
Control, SaltSW	Analyst	APM	APM	APM
	pH (S.U.)	7.89	7.85	7.70
	Dissolved oxygen (mg/L)	9.0	7.7	7.0
	*Salinity (ppt)	25.0	25.2	25.3
	*Alkalinity (mg/L CaCO ₃)	96		
	*Temperature (°C)	24.6	24.3	24.5
150 mg/L	pH (S.U.)	8.04	7.84	7.77
	Dissolved oxygen (mg/L)	9.0	7.0	7.7
	*Salinity (ppt)	25.3	25.7	25.9
	*Temperature (°C)	24.1	24.8	24.4
300 mg/L	pH (S.U.)	8.04	7.83	7.76
	Dissolved oxygen (mg/L)	8.0	7.7	7.7
	*Salinity (ppt)	25.4	25.7	25.8
	*Temperature (°C)	24.6	24.5	24.1
450 mg/L	pH (S.U.)	8.04	7.85	7.79
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	*Salinity (ppt)	25.5	25.7	25.8
	*Temperature (°C)	24.1	24.5	24.3
600 mg/L	pH (S.U.)	8.04	7.88	7.84
	Dissolved oxygen (mg/L)	8.0	7.0	7.7
	*Salinity (ppt)	25.5	25.8	25.9
	*Temperature (°C)	24.7	24.2	24.4
750 mg/L	pH (S.U.)	8.04	7.90	
	Dissolved oxygen (mg/L)	8.0	7.0	
	*Salinity (ppt)	25.6	26.0	
	*Temperature (°C)	24.4	24.4	

*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	1306647

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 # 6

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-29-25	* 0500	JP	0827	JP	6E	1: 9h+ Blue	04-07-25 B
24	04-10-25			0833	JP			
48 Termination	04-11-25			0841	JP			

*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):	04-07-25
Age (1 to 5 days old):	2 to 3 days old
Date organisms were born:	04-06-25 1200 04-07-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.69 Temperature (°C) 25.4

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	7 ^{3d}	9 ^{1d}	8 ^{2d}	5 ^{5d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9 ^{1d}	7	8 ^{1d}	1 ^{7d}	2 ^{3d}	0	0
Mean Survival	100%		100%		95%		75%		15%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	439.15
Upper 95% confidence limit (mg KCl/L)	529.40
48-hour LC ₅₀ (mg KCl/L)	485.97

Comments:



Acute Mysid Test-24 Hr Survival

Start Date: 4/9/2025	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 4/11/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americamysis bahia

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.7000	0.9000
600	0.8000	0.5000
750	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.8000	0.8000	1.1201	0.9912	1.2490	16.280	2	4	20
600	0.6500	0.6500	0.9463	0.7854	1.1071	24.043	2	7	20
750	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

Statistic

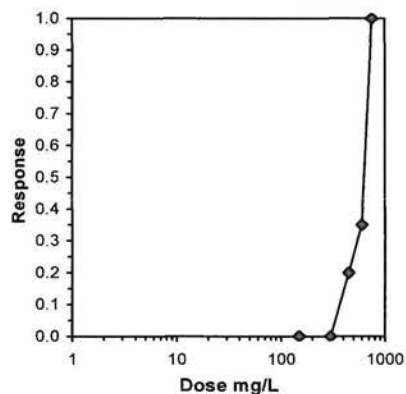
Critical

Skew

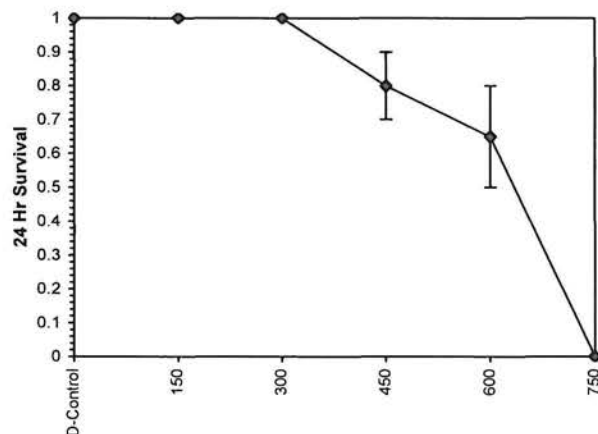
Kurt

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%	572.37	527.03 621.62
5.0%	583.08	533.04 637.82
10.0%	593.62	540.12 652.41
20.0%	613.33	556.61 675.83
Auto-0.0%	572.37	527.03 621.62



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 4/9/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

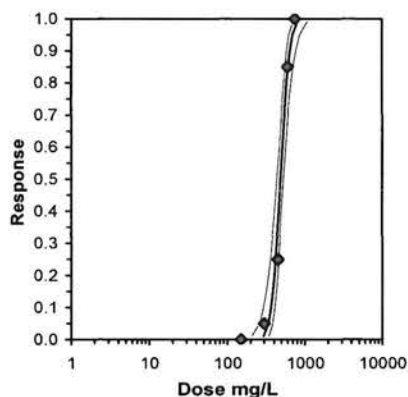
Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	0.9000
450	0.7000	0.8000
600	0.1000	0.2000
750	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1	20
450	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5	20
600	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	17	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20

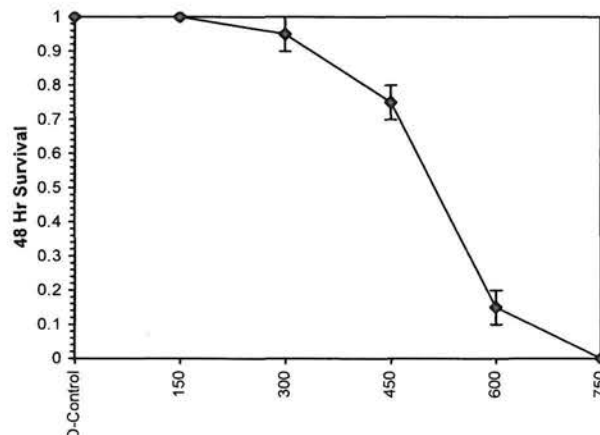
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma
Slope	10.5292	1.98918	6.63045 14.428	0	3.58698	7.81472	0.30966	2.68661	0.09497
Intercept	-23.288	5.39049	-33.853 -12.723						
TSCR									

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	292.19	206.949 345.485
EC05	3.355	339.148	260.775 387.296
EC10	3.718	367.192	294.496 412.29
EC15	3.964	387.412	319.326 430.531
EC20	4.158	404.274	340.231 446.015
EC25	4.326	419.323	358.935 460.148
EC40	4.747	459.776	408.524 500.498
EC50	5.000	485.967	439.145 529.395
EC60	5.253	513.651	469.395 563.143
EC75	5.674	563.204	517.473 632.375
EC80	5.842	584.17	535.815 664.719
EC85	6.036	609.594	556.883 705.948
EC90	6.282	643.162	583.221 763.24
EC95	6.645	696.345	622.583 859.549
EC99	7.326	808.256	699.785 1080.24



Dose-Response Plot



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 #

7

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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	<u>AP EM</u>	<u>AP EM</u>	<u>BSC</u>
Control, SaltSW	pH (S.U.)	<u>7.97</u>	<u>7.80</u>	<u>7.91</u>
	Dissolved oxygen (mg/L)	<u>0.2</u>	<u>7.0</u>	<u>7.9</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.2</u>	<u>25.5</u>
	*Alkalinity (mg/L CaCO ₃)	<u>96</u>		
	*Temperature (°C)	<u>24.6</u>	<u>24.2</u>	<u>25.3</u>
150 mg/L	pH (S.U.)	<u>8.02</u>	<u>7.79</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>0.0</u>	<u>7.0</u>	<u>7.8</u>
	*Salinity (ppt)	<u>25.2</u>	<u>25.4</u>	<u>25.0</u>
	*Temperature (°C)	<u>24.4</u>	<u>24.1</u>	<u>25.1</u>
300 mg/L	pH (S.U.)	<u>8.03</u>	<u>7.79</u>	<u>7.91</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.7</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.3</u>	<u>25.5</u>	<u>25.7</u>
	*Temperature (°C)	<u>24.1</u>	<u>24.5</u>	<u>25.1</u>
450 mg/L	pH (S.U.)	<u>8.03</u>	<u>7.84</u>	<u>7.92</u>
	Dissolved oxygen (mg/L)	<u>0.0</u>	<u>7.7</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.3</u>	<u>25.5</u>	<u>25.9</u>
	*Temperature (°C)	<u>24.1</u>	<u>24.2</u>	<u>25.2</u>
600 mg/L	pH (S.U.)	<u>8.03</u>	<u>7.86</u>	<u>7.90</u>
	Dissolved oxygen (mg/L)	<u>0.0</u>	<u>7.7</u>	<u>7.10</u>
	*Salinity (ppt)	<u>25.5</u>	<u>25.7</u>	<u>25.9</u>
	*Temperature (°C)	<u>24.2</u>	<u>24.1</u>	<u>25.2</u>
750 mg/L	pH (S.U.)	<u>8.02</u>	<u>7.85</u>	<u>(7.82)</u>
	Dissolved oxygen (mg/L)	<u>0.0</u>	<u>7.7</u>	<u>(6.9)</u>
	*Salinity (ppt)	<u>25.5</u>	<u>25.9</u>	<u>25.5</u>
	*Temperature (°C)	<u>24.3</u>	<u>24.2</u>	<u>25.2</u>

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	<u>130664</u>

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 7

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-10-25	* 2500	JP	0859	JP	6 D	light + green	040825
24	04-11-25			0909	JP			
48 Termination	04-12-25			0900	JP			

*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):	04-01-25
Age (1 to 5 days old):	3 to 4 days old
Date organisms were born:	04-06-25 1200 to 04-07-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.55 Temperature (°C): 24.8°C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 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 ^{2d}	10	10	9 ^{1d}	8 ^{2d}	0 ^{10d}	10 ^{1d}
48 Termination	10	10	10	10	10	8	9 ^u	10	5 ^u	6 ^{2d}	0	0 ^{1d}
Mean Survival	100%		100%		90%		95%		50%		5%	

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

Statistics:

Method	Trimmed Spearman-Kärber
Lower 95% confidence limit (mg KCl/L)	551.42
Upper 95% confidence limit (mg KCl/L)	636.96
48-hour LC ₅₀ (mg KCl/L)	579.59

Comments:



Acute Mysid Test-24 Hr Survival

Start Date: 4/10/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/12/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
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
150	1.0000	1.0000
300	1.0000	0.8000
450	1.0000	1.0000
600	0.9000	0.8000
750	0.0000	0.1000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	2	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
600	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3	20
750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19	20

Auxiliary Tests

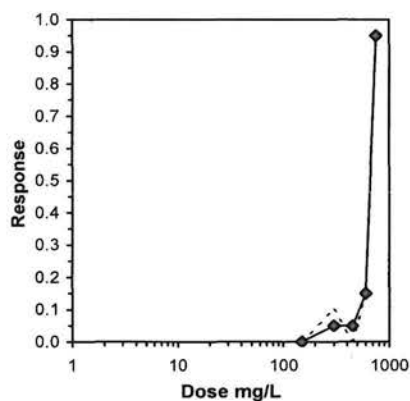
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

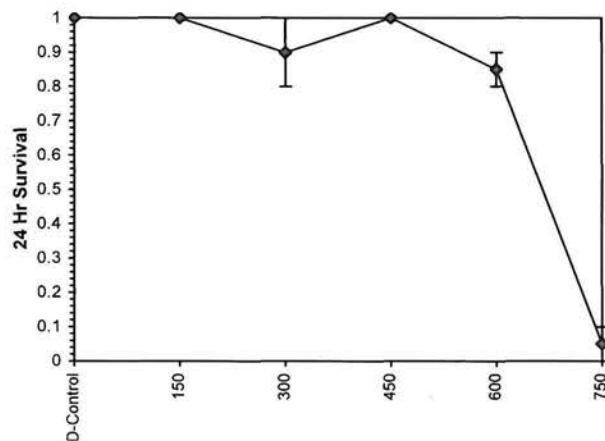
Statistic Critical Skew Kurt

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL
0.0%		
5.0%		
10.0%	658.85	620.82 699.21
20.0%	661.53	643.43 680.13
Auto-5.0%	652.05	620.61 685.08



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 4/10/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/12/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
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
150	1.0000	1.0000
300	1.0000	0.8000
450	0.9000	1.0000
600	0.4000	0.6000
750	0.0000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	Number Resp	Total Number
			Mean	Min	Max	CV%				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2		0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2		0	20
300	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2		2	20
450	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2		1	20
600	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2		10	20
750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2		19	20

Auxiliary Tests

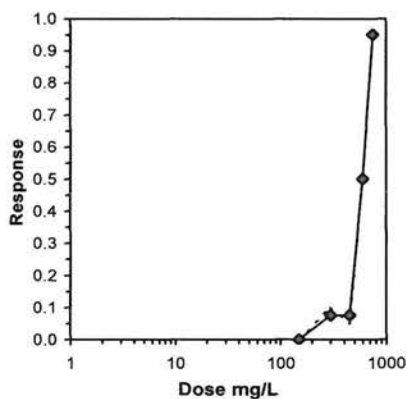
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

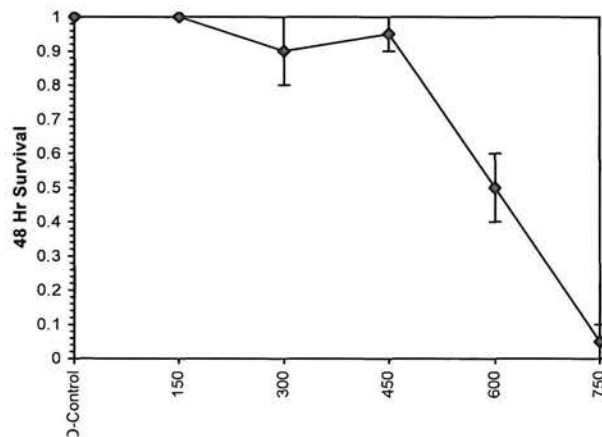
Statistic Critical Skew Kurt

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	589.24	547.01	634.72
20.0%	591.91	537.42	651.92
Auto-5.0%	579.59	527.40	636.96



Dose-Response Plot



Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 8

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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 no 04-10-25
2376

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.99	7.93	7.93
	Dissolved oxygen (mg/L)	7.3	7.6	7.8
	*Salinity (ppt)	24.8	25.0	25.1
	*Alkalinity (mg/L CaCO ₃)	90		
	*Temperature (°C)	25.1	25.2	25.2
150 mg/L	pH (S.U.)	8.03	7.92	7.88
	Dissolved oxygen (mg/L)	7.9	7.6	7.8
	*Salinity (ppt)	25.0	25.1	25.2
	*Temperature (°C)	25.8	25.0	24.9
300 mg/L	pH (S.U.)	8.03	7.93	7.88
	Dissolved oxygen (mg/L)	8.0	7.7	7.8
	*Salinity (ppt)	25.3	25.3	25.5
	*Temperature (°C)	24.5	25.0	24.9
450 mg/L	pH (S.U.)	8.04	7.93	7.92
	Dissolved oxygen (mg/L)	8.1	7.8	7.8
	*Salinity (ppt)	25.3	25.3	25.4
	*Temperature (°C)	25.3	25.0	25.1
600 mg/L	pH (S.U.)	8.04	7.93	7.91
	Dissolved oxygen (mg/L)	8.1	7.8	7.8
	*Salinity (ppt)	25.3	25.4	25.0
	*Temperature (°C)	24.3	25.0	25.1
750 mg/L	pH (S.U.)	8.04	7.93	
	Dissolved oxygen (mg/L)	8.1	7.7	
	*Salinity (ppt)	25.3	25.6	
	*Temperature (°C)	25.3	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664

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 # 8

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-11-25	* 0700	JP	0955	JP	6 F	light plate	04-09-25
24	04-12-25			0950	JP			
48 Termination	04-13-25			0950	JP			

*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):	04-07-25
Age (1 to 5 days old):	4 to 5 days old
Date organisms were born:	04-06-25 1200 to 04-07-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.64 Temperature (°C) 25.0 °C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^u	10	7 ^u	6 ^u	0 ^u	0 ^u
48 Termination	10	10	10	10	10	10	8 ^u	10	7	5 ^u	0	0
Mean Survival	100%		100%		100%		90%		60%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	543.91
Upper 95% confidence limit (mg KCl/L)	543.97 ^{no du} 630.08
48-hour LC ₅₀ (mg KCl/L)	587.23

Comments:





Acute Mysid Test-24 Hr Survival

Start Date: 4/11/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 4/13/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
 Comments:

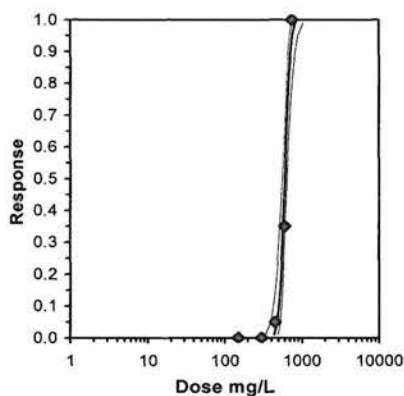
Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	1.0000
600	0.7000	0.6000
750	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1	20
600	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	7	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20

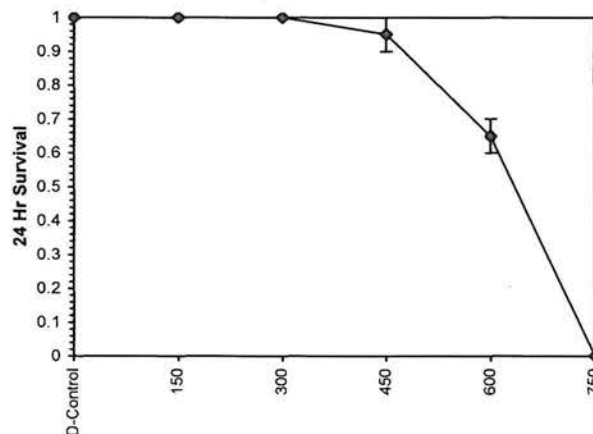
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.0394	3.72878	9.73103 24.3478	0	4.23386	7.81472	0.23729	2.78107	0.05869	5
Intercept	-42.388	10.4041	-62.78 -21.996							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	441.107	339.103 493.6
EC05	3.355	483.659	396.779 528.665
EC10	3.718	507.996	430.869 549.086
EC15	3.964	525.105	455.098 563.815
EC20	4.158	539.112	474.958 576.246
EC25	4.326	551.426	492.319 587.565
EC40	4.747	583.718	536.436 619.954
EC50	5.000	604.048	562.203 643.317
EC60	5.253	625.086	586.49 670.652
EC75	5.674	661.691	622.935 725.923
EC80	5.842	676.806	636.276 751.151
EC85	6.036	694.86	651.296 782.736
EC90	6.282	718.261	669.676 825.631
EC95	6.645	754.403	696.432 895.421
EC99	7.326	827.178	746.813 1046.45



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 4/11/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 4/13/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
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
150	1.0000	1.0000
300	1.0000	1.0000
450	0.8000	1.0000
600	0.7000	0.5000
750	0.0000	0.0000

Transform: Arcsin Square Root								Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
450	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	2	20
600	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	8	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20	20

Auxiliary Tests

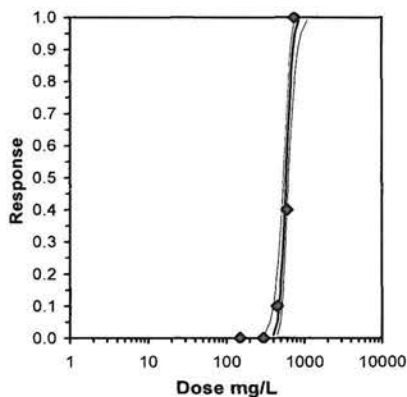
Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

Statistic Critical Skew Kurt

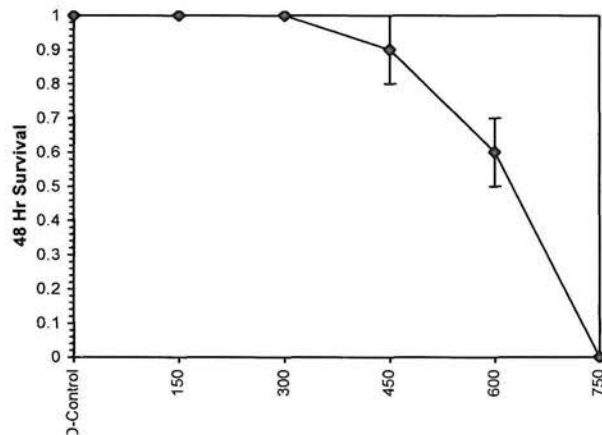
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1711	2.87113	8.54364 19.7985	0	4.30851	7.81472	0.23002	2.76881	0.07057	5
Intercept	-34.237	7.97304	-49.864 -18.61							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	402.389	306.032 456.421
EC05	3.355	449.507	365.965 496.455
EC10	3.718	476.841	401.959 520.01
EC15	3.964	496.216	427.772 537.096
EC20	4.158	512.174	449.061 551.57
EC25	4.326	526.274	467.762 564.787
EC40	4.747	563.547	515.654 602.721
EC50	5.000	587.23	543.914 630.077
EC60	5.253	611.908	570.872 661.965
EC75	5.674	655.246	612.268 726.1
EC80	5.842	673.284	627.768 755.341
EC85	6.036	694.937	645.436 792.009
EC90	6.282	723.174	667.338 841.993
EC95	6.645	767.148	699.69 923.894
EC99	7.326	856.979	761.78 1103.79



Dose-Response Plot



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 # 9

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997.0	994.0	991.0	988	985
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 #: 2376

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	8.00	7.75	7.66
	Dissolved oxygen (mg/L)	0.3	7.9	7.8
	*Salinity (ppt)	25.0	25.3	25.7
	*Alkalinity (mg/L CaCO ₃)	92		
	*Temperature (°C)	25.8	24.6	25.0
150 mg/L	pH (S.U.)	8.07	7.76	7.69
	Dissolved oxygen (mg/L)	0.3	7.9	7.9
	*Salinity (ppt)	25.1	25.5	25.9
	*Temperature (°C)	25.5	24.6	25.5
300 mg/L	pH (S.U.)	8.07	7.76	7.62
	Dissolved oxygen (mg/L)	0.3	7.8	7.8
	*Salinity (ppt)	25.1	25.5	25.8
	*Temperature (°C)	25.5	24.6	25.2
450 mg/L	pH (S.U.)	8.07	7.79	7.66
	Dissolved oxygen (mg/L)	0.3	7.8	7.8
	*Salinity (ppt)	25.4	25.6	25.9
	*Temperature (°C)	25.7	24.8	25.0
600 mg/L	pH (S.U.)	8.07	7.82	7.74
	Dissolved oxygen (mg/L)	0.3	7.7	7.8
	*Salinity (ppt)	25.4	25.8	26.1
	*Temperature (°C)	25.8	24.6	24.9
750 mg/L	pH (S.U.)	8.12	7.80	7.74
	Dissolved oxygen (mg/L)	0.3	7.7	7.8
	*Salinity (ppt)	25.6	25.8	26.2
	*Temperature (°C)	25.6	24.6	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+ 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	1306647

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 9

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-06-25	* 1130	JP	1340	JP	6E	light blue	04-3025B
24	05-07-25			1340	JP			
48 Termination	05-08-25			1330	JP			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	05-05-25
Age (1 to 5 days old):	1-2 days old
Date organisms were born:	05-04-25 1200 05-05-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.05
	Temperature (°C): 24.7 °C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 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 ^{1d}	8 ^{2d}	9 ^{1d} 10	10 ⁰⁵⁻⁰⁷⁻²⁵	2 ^{2d}	1 ^{9d}
48 Termination	10	10	10	10	10	10	9	8	3 ^{2d}	5 ^{5d}	0 ^{2d}	0 ^{1d}
Mean Survival	100%		100%		100%		85%		40%		0%	

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

Statistics:

Method	probit
Lower 95% confidence limit (mg KCl/L)	Spearman-Kärker 481.11 ⁰⁵⁻²⁰⁻²⁵
Upper 95% confidence limit (mg KCl/L)	593.31 ⁰⁵⁻²⁰⁻²⁵
48-hour LC ₅₀ (mg KCl/L)	551.74

05-20-25

Comments:





Statistical Analyses

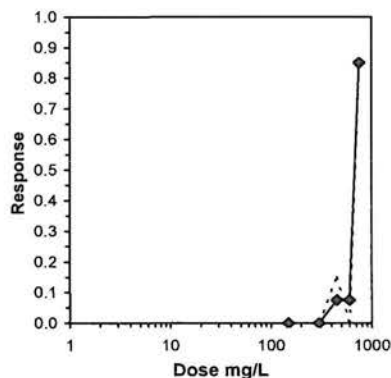
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Start Date: 5/6/2025	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant	
End Date: 5/8/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia	
Comments:			

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	0.8000
600	1.0000	1.0000
750	0.2000	0.1000

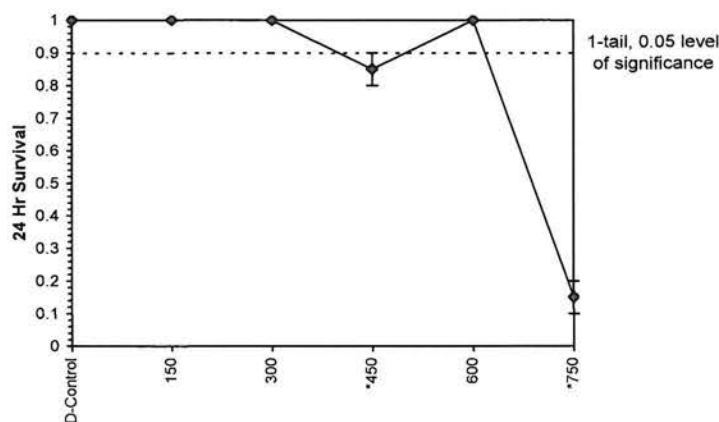
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2					0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1639		0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1639		0	20
*450	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	4.038	2.830	0.1639		3	20
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1639		0	20
*750	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	17.596	2.830	0.1639		17	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	300	450	367.423		0.07558	0.07752	0.33278	0.00336	1.1E-05	5, 6
Treatments vs D-Control										

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	678.10	658.39	698.41
Auto-15.0%	678.10	658.39	698.41



Dose-Response Plot



Statistical Analyses

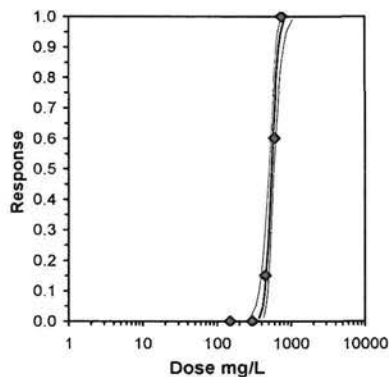
Acute Mysid Test-48 Hr Survival			
Start Date: 5/6/2025	Test ID: AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date: 5/8/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type:	DMR-Discharge Monitoring Report
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanysis bahia
Comments:			

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	0.8000
600	0.3000	0.5000
750	0.0000	0.0000

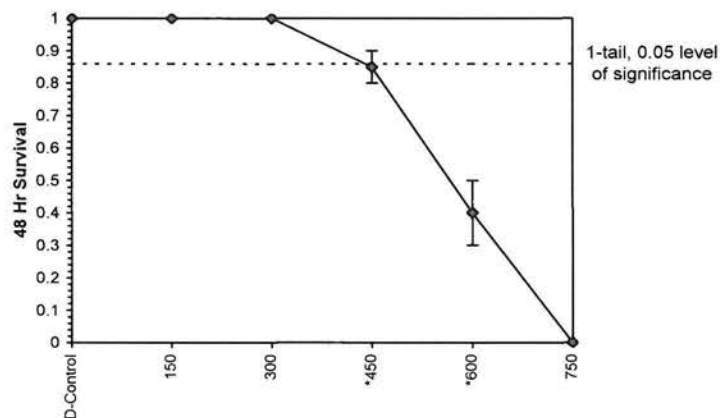
Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2253	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2253	0	20
*450	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	2.960	2.850	0.2253	3	20
*600	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	9.230	2.850	0.2253	12	20
750	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		300	450	367.423		0.11538	0.11834	0.20063	0.00625	9.3E-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	13.5089	2.68391	8.2484	18.7693	0	1.70659	7.81472	0.63547	2.74174	0.07403	5
Intercept	-32.038	7.38893	-46.52	-17.555							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	371.132	279.845	423.578							
EC05	3.355	416.847	336.819	462.79							
EC10	3.718	443.476	371.223	485.9							
EC15	3.964	462.397	395.986	502.665							
EC20	4.158	478.009	416.473	516.856							
EC25	4.326	491.822	434.524	529.797							
EC40	4.747	528.425	481.06	566.785							
EC50	5.000	551.744	508.801	593.311							
EC60	5.253	576.092	535.49	624.154							
EC75	5.674	618.967	576.809	686.289							
EC80	5.842	636.853	592.334	714.725							
EC85	6.036	658.355	610.047	750.482							
EC90	6.282	686.444	632.019	799.373							
EC95	6.645	730.296	664.505	879.797							
EC99	7.326	820.251	726.994	1057.5							



Dose-Response Plot





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 # 10

Dilution Preparation:

Test concentrations (mg/L KCl)	150	300	450	600	750
mL Stock solution	3.0	6.0	9.0	12	15
mL Dilution water	997	994	991	988	985
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 #: 2376

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL 2P	RL	XL
Control, SaltSW	pH (S.U.)	7.87	7.76	7.86
	Dissolved oxygen (mg/L)	8.7	7.9	8.0
	*Salinity (ppt)	25.0	25.3	25.4
	*Alkalinity (mg/L CaCO ₃)	95		
	*Temperature (°C)	25.8	24.7	24.6
150 mg/L	pH (S.U.)	7.91	7.76	7.84
	Dissolved oxygen (mg/L)	8.6	7.9	8.0
	*Salinity (ppt)	25.7	25.7	25.7
	*Temperature (°C)	25.6	24.8	24.2
300 mg/L	pH (S.U.)	7.92	7.77	7.85
	Dissolved oxygen (mg/L)	8.6	7.9	8.0
	*Salinity (ppt)	25.5	25.7	25.7
	*Temperature (°C)	25.8	24.5	24.5
450 mg/L	pH (S.U.)	7.90	7.78	7.85
	Dissolved oxygen (mg/L)	8.6	7.9	7.9
	*Salinity (ppt)	25.6	25.9	25.9
	*Temperature (°C)	25.6	24.8	24.5
600 mg/L	pH (S.U.)	7.91	7.79	7.88
	Dissolved oxygen (mg/L)	8.6	7.9	7.9
	*Salinity (ppt)	25.8	26.0	26.0
	*Temperature (°C)	25.4	24.8	24.5
750 mg/L	pH (S.U.)	7.93	7.79	7.87
	Dissolved oxygen (mg/L)	8.6	7.9	7.9
	*Salinity (ppt)	25.9	26.2	26.2
	*Temperature (°C)	25.8	24.6	24.3

*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	130664

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 10

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	06-03-25	* 1105	JP	1336	JP	15	Yellow	05-28-25A
24	06-04-25			1405	JP			
48 Termination	06-05-25			1336	JP			

*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):	06-02-25
Age (1 to 5 days old):	1 to 2 days old
Date organisms were born:	06-01-25 1200 to 06-02-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.89 Temperature (°C) 24.6°C

Survival Data (number of living organisms):

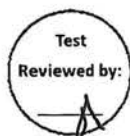
Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^d	10	10	10	8 ^{zc}	1 ^{9d}	1 ^{9d}
48 Termination	10	10	10	10	10	9	10	10	10	8	0 ^{9d}	1
Mean Survival	100%		100%		95%		100%		90%		5%	

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

Statistics:

Method	Trimmed SK
Lower 95% confidence limit (mg KCl/L)	635.53 639.93 3/06-11-25
Upper 95% confidence limit (mg KCl/L)	691.53
48-hour LC ₅₀ (mg KCl/L)	663.13

Comments:



Statistical Analyses

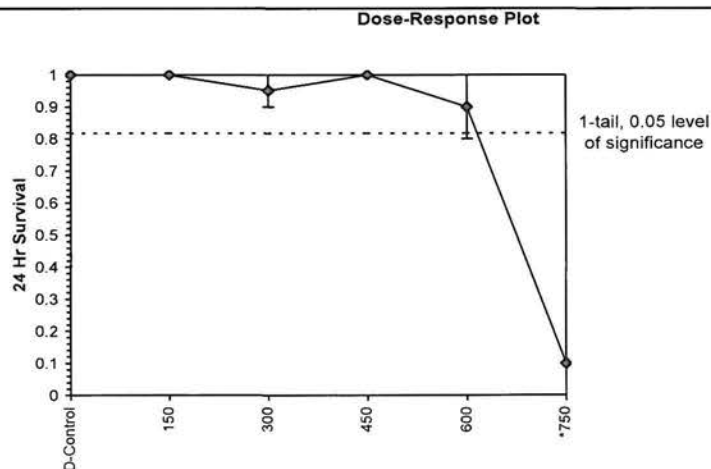
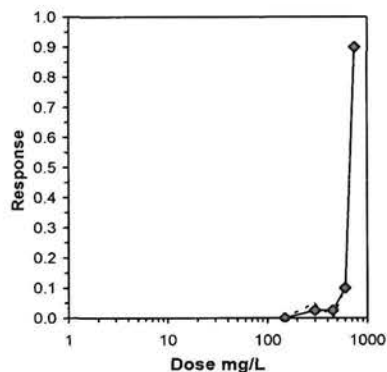
Acute Mysid Test-24 Hr Survival			
Start Date: 6/3/2025	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant	
End Date: 6/5/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia	
Comments:			

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	0.9000
450	1.0000	1.0000
600	1.0000	0.8000
750	0.1000	0.1000

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
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2824	0	20
300	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.817	2.830	0.2824	1	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2824	0	20
600	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.528	2.830	0.2824	2	20
*750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	10.925	2.830	0.2824	18	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	600	750	670.82		0.15735	0.16138	0.37052	0.00996	1.9E-04	5, 6
Treatments vs D-Control										

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	670.82	653.30	688.81
20.0%	670.82	653.30	688.81
Auto-10.0%	670.82	625.77	719.12



Statistical Analyses

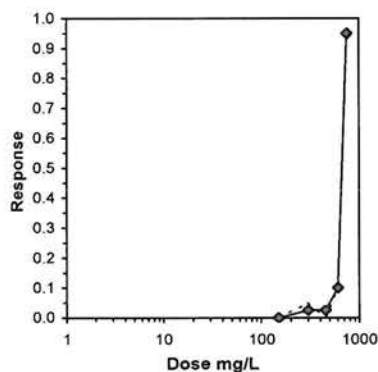
Acute Mysid Test-48 Hr Survival			
Start Date: 6/3/2025	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant	
End Date: 6/5/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia	
Comments:			

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	0.9000
450	1.0000	1.0000
600	1.0000	0.8000
750	0.0000	0.1000

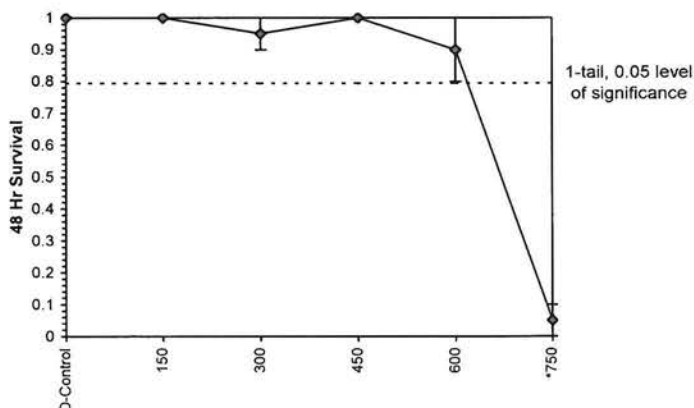
Transform: Arcsin Square Root									1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.3122	0	20
300	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.739	2.830	0.3122	1	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.3122	0	20
600	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.382	2.830	0.3122	2	20
*750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	10.621	2.830	0.3122	19	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	600	750	670.82		0.18092	0.18556	0.42942	0.01217	2.3E-04	5, 6
Treatments vs D-Control										

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	666.43	651.80	681.39
20.0%	666.43	651.80	681.39
Auto-5.0%	663.13	635.53	691.93



Dose-Response Plot





Environmental Testing Solutions, Inc.

Surveillance and Corrective Action Report (SCAR)

SURVEILLANCE NUMBER: SCAR-250618
SURVEILLANCE: Reference Toxicant Testing
DATE OF INSPECTION: 06-18-25
TYPE OF INSPECTION: Internal
EVALUATORS: Jim Sumner, Jaydon Perez
LABORATORY PERSONNEL: Jim Sumner, Jaydon Perez

I. DEVIATION / DECREPANCY:

Errors in the *A. bahia* and *P. promelas* chronic PMSD calculation spreadsheets were identified.

- Calculations of upper and lower PMSD bounds were not correct.
- These spreadsheets are used to create PMSD control charts.

Error did not impact the validity of reference tests performed but identify a larger issue with how data is calculated in the laboratory.

II. ROOT CAUSE INVESTIGATION:

Verbal instructions are provided for using spreadsheets used to validate results and calculate upper and lower control limits. These spreadsheets have formulas for log transforming data used to calculate central tendencies/standard deviations and calculating control limits using anti-log values.

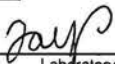
Spreadsheets are not locked to prevent an analyst from accidentally changing or deleting formulas. Individual spreadsheets do not have instructions for completing the data entry.

III. EXECUTION:

Spreadsheets containing formulas are locked to prevent calculations from being altered.

Step by step instructions are included on the top of each spreadsheet tab.

IV. VERIFICATION:

Signature: 
Laboratory Analyst

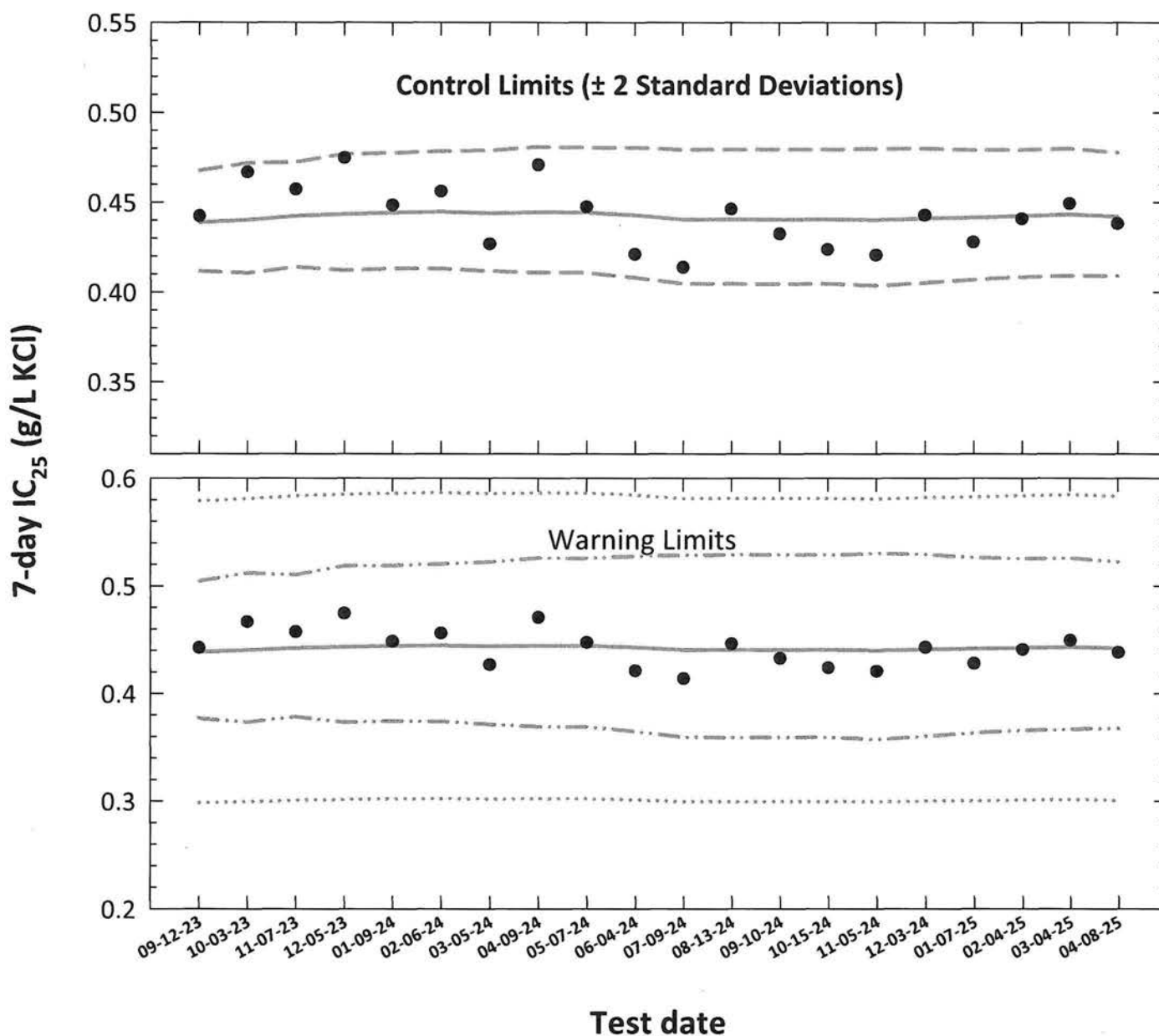
Date: 06-23-25

V. CLOSURE:

Signature: 
QA Officer

Date: 06-23-25

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	03-04-25	0.4493	-0.3474	-0.3534	0.0173	0.4432	0.4093	0.4799	0.3667	0.5260	0.3014	0.5850
20	04-08-25	0.4382	-0.3583	-0.3545	0.0169	0.4421	0.4091	0.4777	0.3674	0.5228	0.3006	0.5835

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

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

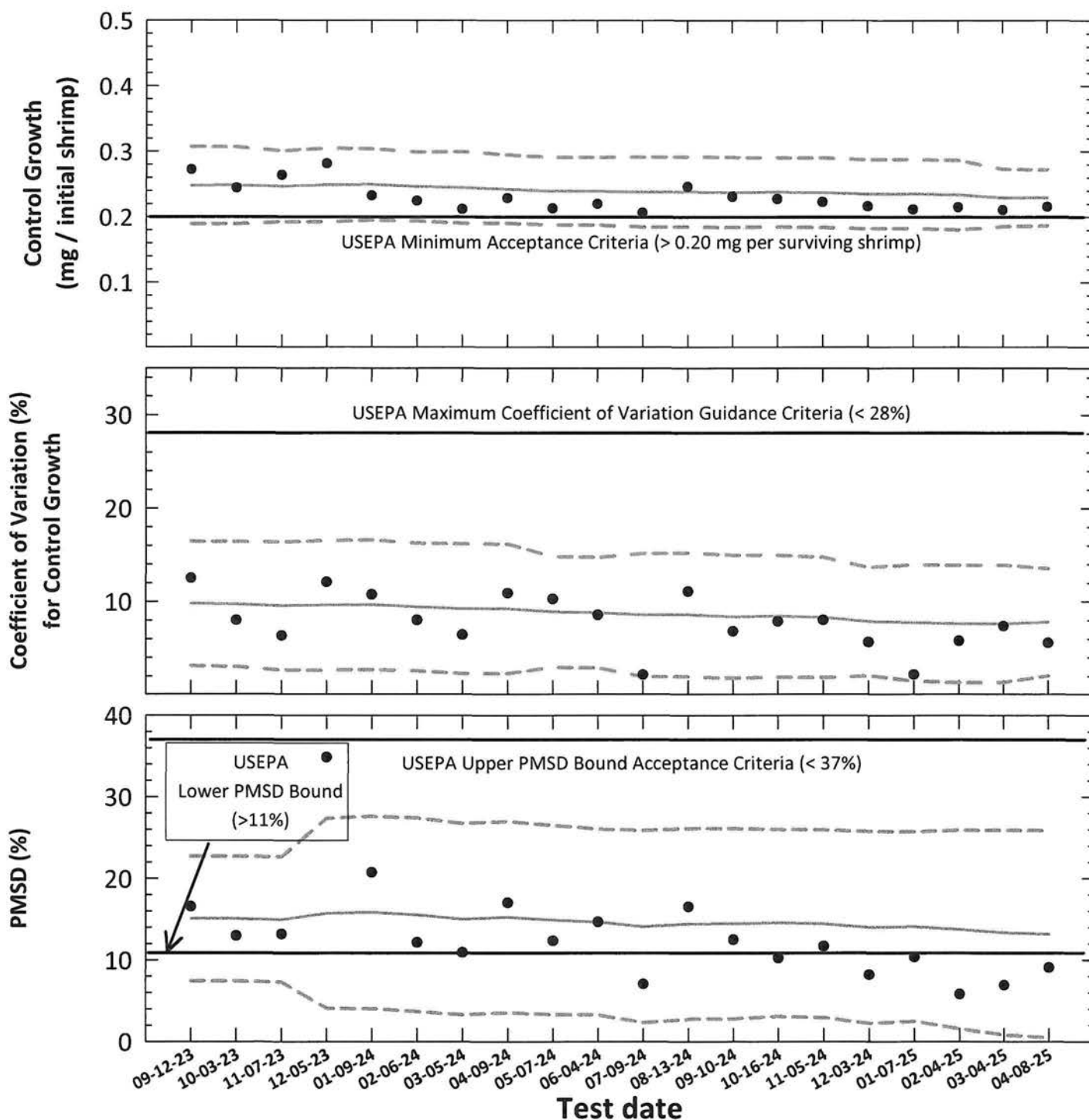
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

CV = Coefficient of variation.

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		CT	Control Growth		CT	Control Growth CV		CT	Test PMSD	
			Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)		95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S		95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S		95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3	26.1
11	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3	25.9
12	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.4	2.7	26.1
13	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8	26.1
14	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1	26.0
15	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9	26.0
16	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	1.9	13.7	14.0	2.2	25.8
17	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5	25.7
18	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5	25.9
19	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8	25.9
20	04-08-25	100	0.216	5.6	0.0196	9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5	25.9

Note: Control Survival = USEPA minimum test acceptability criteria $\geq$ 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria $\geq$ 0.20 mg/surviving shrimp.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
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: **262**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 7357					
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:	04-01-25 1200 to 04-02-25 1130		Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 04-02-25		Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 7.82 S.U. Temperature = 25.0 °C		Drying information for weight determination:	
Average transfer volume:	< 0.25 mL		Date / Time in oven:	04-15-25 1345
			*Initial oven temperature:	60°C
			Date / Time out of oven:	04-16-25 1345
			*Final oven temperature:	60°C
			Total drying time:	24-HOURS

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-98585

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	04-08-25	1045	<i>h</i>	1530	<i>h</i>	1400	<i>h</i>	04-02-25 B
1	04-09-25	0500	<i>h</i>	1510	<i>h</i>	0944	<i>h</i>	↓
2	04-10-25	0500	<i>h</i>	1400	<i>h</i>	1230	<i>h</i>	04-09-25
3	04-11-25	0500	<i>h</i>	1215	<i>h</i>	0942	<i>h</i>	↓
4	04-12-25	0530	<i>h</i>	1230	<i>h</i>	1215	<i>h</i>	04-11-25 A
5	04-13-25	0500	<i>h</i>	1100	<i>h</i>	0925	<i>h</i>	04-11-25 B
6	04-14-25	0500	<i>h</i>	1100	<i>h</i>	0940	<i>h</i>	↓
7	04-15-25					1226	<i>h</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)	503.6
Average weight per initial shrimp:	0.216		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.216	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	438.2

AbKCICR Test Number: **262**

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	6	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: Analyst: <u>TLV</u> Date: <u>03-20-25</u>	15.01	12.64	13.27	12.60	14.30	13.52	13.16	13.97	13.90	13.50	14.66	12.98	14.94	14.55	13.27	11.87
*B = Pan + Shrimp weight (mg) Analyst: <u>TLV</u> Date: <u>04-17-25</u>	16.64	13.64	14.33	13.78	15.40	14.67	14.23	15.02	15.08	14.64	15.71	14.21	16.14	15.60	14.33	13.05
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>TLV</u>	1.03	1.00	1.06	1.18	1.10	1.15	1.07	1.05	1.18	1.14	1.05	1.23	1.20	1.05	1.06	1.16
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>TLV</u>	0.206	0.200	0.212	0.236	0.220	0.230	0.214	0.210	0.236	0.228	0.210	0.246	0.240	0.210	0.212	0.232
Average weight per initial number of shrimp (mg) 0.216								Average weight per initial number of shrimp (mg) 0.227				Percent reduction from control (%) -5.07.				

*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: **262**

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	6	410	5	410	410	5	5	410
3	5	5	5	5	5	5	5	5	5	4	322	312	312	232	6	310
4	5	410	5	5	5	5	5	5	5	4	3	3	3	2	5	3
5	5	4	5	5	5	5	5	5	5	4	3	3	3	2	5	3
6	5	4	5	5	5	5	5	410	5	4	3	210	3	2	5	3
7	5	4	5	5	5	5	5	4	3	2	3	2	3	2	4	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Analyst: <u>WV</u> Date: <u>03-20-25</u>	13.63	15.49	14.76	15.07	14.19	12.57	14.60	14.68	14.36	13.50	15.39	12.99	13.16	13.81	14.61	12.94
*B = Pan + Shrimp weight (mg) Analyst: <u>WV</u> Date: <u>04-17-25</u>	14.64	16.50	15.94	16.14	15.40	13.62	15.72	15.83	15.13	14.08	16.05	13.55	13.70	14.16	15.33	13.26
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>WV</u>	1.01	1.01	1.18	1.07	1.21	1.05	1.12	1.15	0.77	0.58	0.66	0.56	0.54	0.35	0.72	0.35
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>WV</u>	0.202	0.202	0.236	0.214	0.242	0.210	0.244	0.230	0.154	0.116	0.132	0.112	0.108	0.070	0.144	0.070
Average weight per initial number of shrimp (mg) 0.220				Percent reduction from control (%) - 1.97				Average weight per initial number of shrimp (mg) 0.113				Percent reduction from control (%) 47.67				

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

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

Comments:

AbKCICR Test Number: **262**

Survival and Growth Data

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

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

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

Comments:

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

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

Test number: 262
Test dates: April 08-15, 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	15.61	16.64	1.03	0.206	100.0	0.216	5.6	Not applicable
	B	5	5	12.64	13.64	1.00	0.200				
	C	5	5	13.27	14.33	1.06	0.212				
	D	5	5	12.60	13.78	1.18	0.236				
	E	5	5	14.30	15.40	1.10	0.220				
	F	5	5	13.52	14.67	1.15	0.230				
	G	5	5	13.16	14.23	1.07	0.214				
	H	5	5	13.97	15.02	1.05	0.210				
250	I	5	5	13.90	15.08	1.18	0.236	100.0	0.227	6.3	-5.0
	J	5	5	13.50	14.64	1.14	0.228				
	K	5	5	14.66	15.71	1.05	0.210				
	L	5	5	12.98	14.21	1.23	0.246				
	M	5	5	14.94	16.14	1.20	0.240				
	N	5	5	14.55	15.60	1.05	0.210				
	O	5	5	13.27	14.33	1.06	0.212				
	P	5	5	11.87	13.03	1.16	0.232				
375	Q	5	5	13.63	14.64	1.01	0.202	95.0	0.220	7.0	-1.9
	R	5	4	15.49	16.50	1.01	0.202				
	S	5	5	14.76	15.94	1.18	0.236				
	T	5	5	15.07	16.14	1.07	0.214				
	U	5	5	14.19	15.40	1.21	0.242				
	V	5	5	12.57	13.62	1.05	0.210				
	W	5	5	14.60	15.72	1.12	0.224				
	X	5	4	14.68	15.83	1.15	0.230				
500	Y	5	3	14.36	15.13	0.77	0.154	55.0	0.113	27.4	47.6
	Z	5	2	13.50	14.08	0.58	0.116				
	AA	5	3	15.39	16.05	0.66	0.132				
	BB	5	2	12.99	13.55	0.56	0.112				
	CC	5	3	13.16	13.70	0.54	0.108				
	DD	5	2	13.81	14.16	0.35	0.070				
	EE	5	4	14.61	15.33	0.72	0.144				
	FF	5	3	12.94	13.29	0.35	0.070				
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: 0.0196
PMSD: 9.1

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

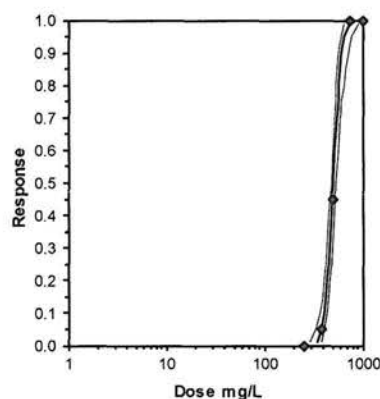
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance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

Start Date:	4/8/2025	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/15/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

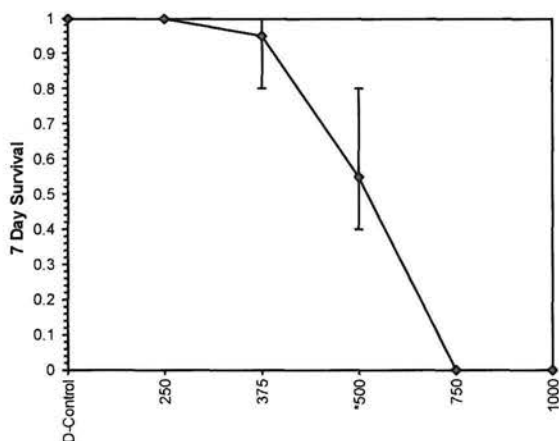
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.5500	0.5500	0.8382	0.6847	1.1071	17.590	8	36.00	48.00	18	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

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

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	343.437	286.779	378.558
EC05	3.355	384.19	337.136	414.034
EC10	3.718	407.857	366.794	435.125
EC15	3.964	424.643	387.722	450.587
EC20	4.158	438.476	404.714	463.826
EC25	4.326	450.701	419.402	476.041
EC40	4.747	483.041	455.873	511.553
EC50	5.000	503.603	476.82	536.983
EC60	5.253	525.04	496.899	565.756
EC75	5.674	582.714	529.032	620.689
EC80	5.842	578.403	541.615	644.817
EC85	6.036	597.244	556.303	674.561
EC90	6.282	621.825	574.935	714.457
EC95	6.645	660.131	603.097	778.757
EC99	7.326	738.465	658.446	917.13



Dose-Response Plot



Mysid Survival and Growth Test-Growth-Weight

Start Date:	4/8/2025	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/15/2025	Lab ID:	ETS-Envr. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

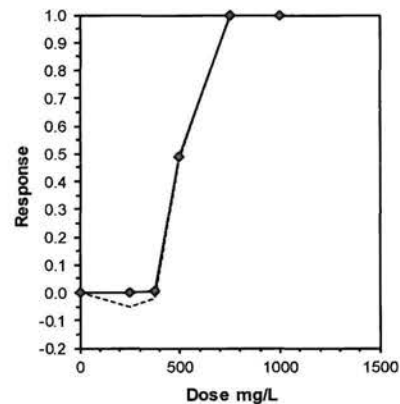
[illegible]

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
D-Control	0.2160	1.0000	0.2160	0.2000	0.2360	5.599	8				0.2214	1.0000
250	0.2268	1.0498	0.2268	0.2100	0.2460	6.324	8	-1.538	2.799	0.0196	0.2214	1.0000
375	0.2200	1.0185	0.2200	0.2020	0.2420	6.957	8	-0.572	2.799	0.0196	0.2200	0.9938
500	0.1133	0.5243	0.1133	0.0700	0.1540	27.390	8				0.1133	0.5116
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

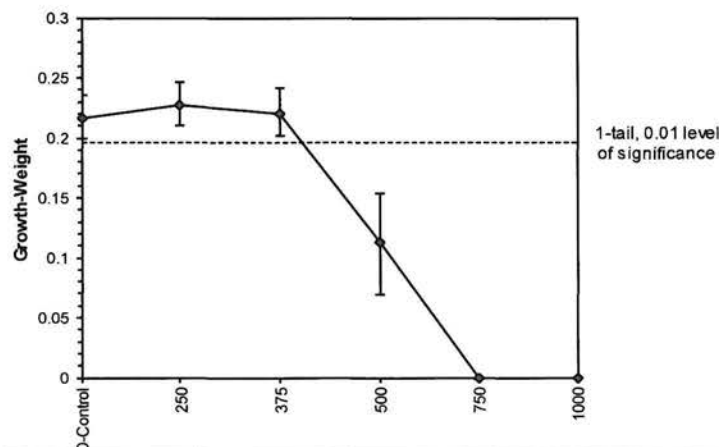
Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.92825	0.884	0.12224	-1.3276		
Bartlett's Test indicates equal variances (p = 0.83)					0.37502	9.21035				
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	>375			0.01956	0.09056	0.00024	0.0002	0.31856	2, 21
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95%CL		Skew
IC05	386.35	7.20	366.86	390.43	-3.7770
IC10	399.31	4.68	387.88	405.85	-0.5729
IC15	412.27	5.21	400.04	421.28	-0.1139
IC20	425.23	5.96	413.10	436.74	0.1807
IC25	438.20	6.86	425.87	452.52	0.3304
IC40	477.08	10.12	460.44	499.37	0.4458
IC50	505.66	16.10	481.82	540.97	0.4469



Dose-Response Plot



AbKCICR Test Number: **262**

Daily Chemistry:

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

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst	Analyst	Analyst	Analyst	Analyst	Analyst
CONTROL, Salt SW	pH (S.U.)	8.23	7.85	7.89	7.89	7.97	7.71
	DO (mg/L)	0.3	7.9	0.0	0.0	0.2	6.5
	Salinity (ppt)	25.0	25.5	25.0	25.3	24.7	25.4
	Alkalinity (mg CaCO ₃ /L)	100				25.0	
	Temperature (°C)	25.2	25.6	25.3	25.9	25.2	25.9
250 mg KCl/L	pH (S.U.)	8.08	7.89	7.94	7.93	8.01	7.73
	DO (mg/L)	0.3	7.9	0.1	0.0	0.1	6.6
	Salinity (ppt)	25.2	26.1	25.3	25.4	25.2	25.4
	Temperature (°C)	25.2	25.7	25.3	25.7	25.2	25.7
375 mg KCl/L	pH (S.U.)	8.08	7.91	7.95	7.94	8.03	7.75
	DO (mg/L)	0.3	7.9	0.1	0.0	0.1	6.7
	Salinity (ppt)	25.4	25.9	25.5	25.9	25.4	25.9
	Temperature (°C)	25.2	25.9	25.2	25.7	25.3	25.9
500 mg KCl/L	pH (S.U.)	8.08	7.89	7.96	7.97	8.03	7.01
	DO (mg/L)	0.3	7.9	0.0	0.0	0.1	7.0
	Salinity (ppt)	25.3	25.6	25.4	25.0	25.6	25.9
	Temperature (°C)	25.3	25.9	25.3	25.9	25.3	25.9
750 mg KCl/L	pH (S.U.)	8.09	7.90				
	DO (mg/L)	0.3	7.9				
	Salinity (ppt)	25.4	25.7				
	Temperature (°C)	25.3	25.7				
1000 mg KCl/L	pH (S.U.)	8.09	7.92				
	DO (mg/L)	0.3	7.9				
	Salinity (ppt)	25.6	26.0				
	Temperature (°C)	25.1	25.7				
		Initial	Final	Initial	Final	Initial	Final

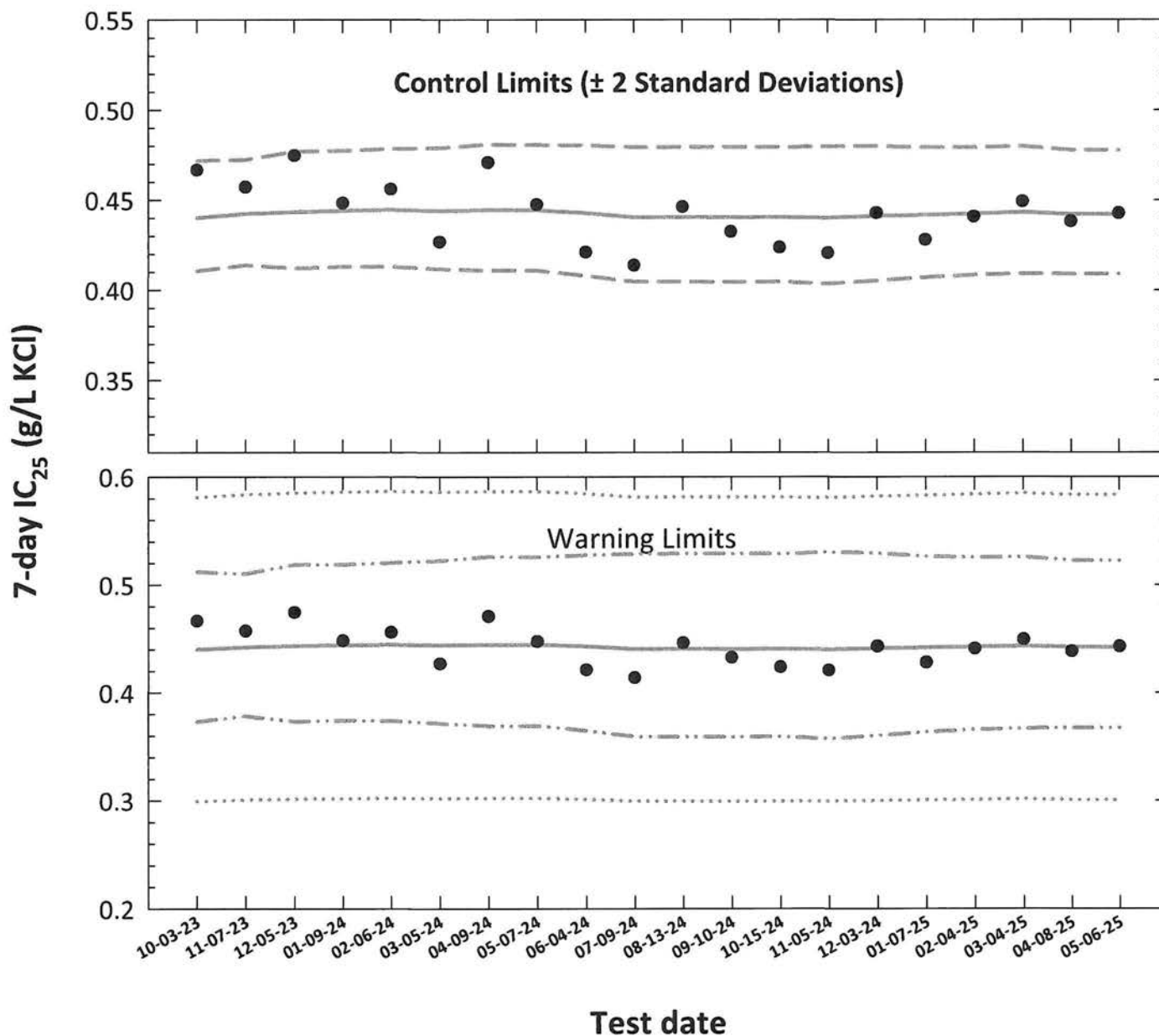
AbKCICR Test Number: 262

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	N	BSL	BSL	BSL	BSL	BSL	N	N
	DO (mg/L)	7.99	7.89	8.24	7.93	8.07	7.81	7.93	7.65
	Salinity (ppt)	7.0	7.8	8.1	7.8	8.0	7.5	8.0	5.0
	Alkalinity (mg CaCO ₃ /L)	24.0	25.2	24.9	25.2	24.0	25.2	25.0	25.3
	Temperature (°C)	—	8.15-15.11	9.5	—	9.0	—	8.15-15.11	—
250 mg KCl/L	pH (S.U.)	7.3	7.9	7.3	7.0	7.4	7.1	7.3	7.0
	DO (mg/L)	7.97	7.90	8.05	7.94	8.07	7.82	7.92	7.73
	Salinity (ppt)	7.9	7.8	8.1	7.8	8.1	7.6	7.9	6.5
	Temperature (°C)	25.1	25.4	25.1	25.0	25.0	25.7	25.1	25.7
375 mg KCl/L	pH (S.U.)	7.2	7.8	7.2	7.8	7.5	7.8	7.2	7.7
	DO (mg/L)	7.97	7.91	8.07	7.90	8.07	7.79	7.93	7.76
	Salinity (ppt)	7.9	7.8	8.1	7.8	8.1	7.6	7.9	6.6
	Temperature (°C)	25.2	25.5	25.3	25.9	25.1	25.7	25.2	26.0
500 mg KCl/L	pH (S.U.)	7.2	7.9	7.2	7.7	7.5	7.9	7.2	7.7
	DO (mg/L)	7.97	7.93	8.07	7.93	8.07	7.86	7.93	7.79
	Salinity (ppt)	7.0	7.8	8.1	7.8	8.1	7.6	7.9	6.5
	Temperature (°C)	25.4	25.7	25.5	25.0	25.2	25.5	25.4	25.7
750 mg KCl/L	pH (S.U.)	7.2	7.9	7.3	7.9	7.5	7.9	7.2	7.0
	DO (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	—	—	—	—	—	—	—	—
	Temperature (°C)	—	—	—	—	—	—	—	—
1000 mg KCl/L	pH (S.U.)	—	—	—	—	—	—	—	—
	DO (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	—	—	—	—	—	—	—	—
	Temperature (°C)	—	—	—	—	—	—	—	—
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			7-day IC_{25}	CT	S	CT	Control Limits		75th Percentile CV Warning Limits
							CT - 2S	CT + 2S	
1	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.2993
2	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3008
3	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3015
4	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3020
5	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3024
6	03-05-24	0.4268	-0.3698	-0.3527	0.0164	0.4440	0.4117	0.4788	0.3019
7	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109	0.4808	0.3022
8	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109	0.4805	0.3022
9	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082	0.4804	0.3011
10	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047	0.4794	0.2995
11	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048	0.4796	0.2996
12	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046	0.4796	0.2995
13	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048	0.4795	0.2996
14	11-05-24	0.4206	-0.3761	-0.3565	0.0188	0.4401	0.4036	0.4798	0.2993
15	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053	0.4800	0.2999
16	01-07-25	0.4280	-0.3686	-0.3549	0.0177	0.4417	0.4071	0.4792	0.3003
17	02-04-25	0.4408	-0.3558	-0.3542	0.0173	0.4424	0.4085	0.4792	0.3008
18	03-04-25	0.4493	-0.3474	-0.3534	0.0173	0.4432	0.4093	0.4799	0.3014
19	04-08-25	0.4382	-0.3583	-0.3545	0.0169	0.4421	0.4091	0.4777	0.3006
20	05-06-25	0.4428	-0.3538	-0.3545	0.0169	0.4421	0.4091	0.4778	0.3006

Note: 7-day IC_{25} = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
CT = Central tendency of the IC_{25} values.

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

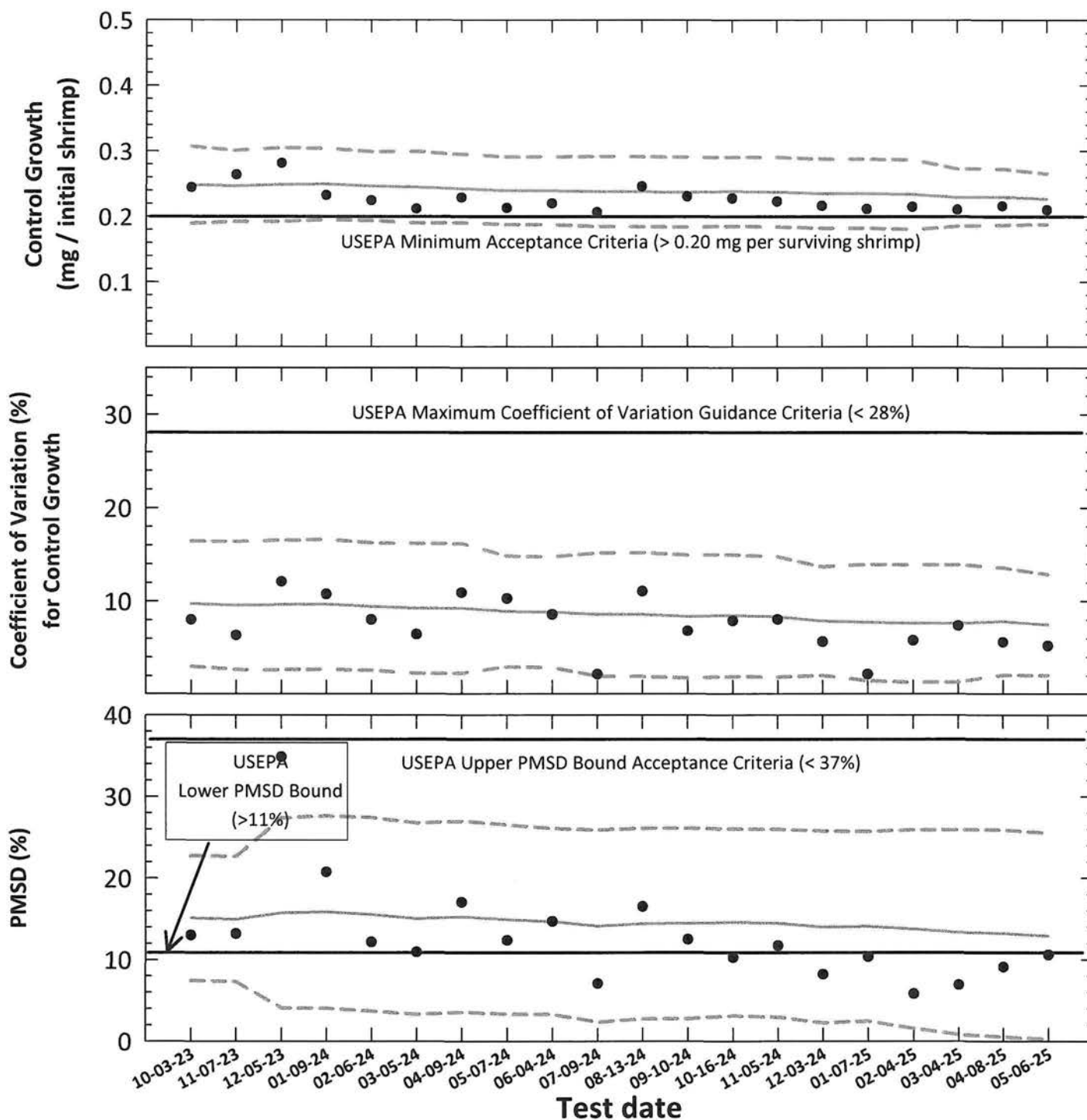
Control Limits = Mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

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

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

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 CV (%)	Control Growth		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			
			Mean (mg/initial shrimp)	CV (%)									MSD	PMSD (%)	
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3	26.1
10	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3	25.9
11	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.4	2.7	26.1
12	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8	26.1
13	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1	26.0
14	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9	26.0
15	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.0	2.2	25.8
16	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5	25.7
17	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5	25.9
18	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8	25.9
19	04-08-25	100	0.216	5.6	0.0196	9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5	25.9
20	05-06-25	100	0.210	5.2	0.0222	10.6	0.227	0.188	0.265	7.5	2.0	12.9	12.9	0.3	25.5

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 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: **263**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2376					
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:	04-29-25 1200 to 04-30-25 1130		Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 04-30-25		Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 7.92 Temperature = 25.0 °C	S.U.	Drying information for weight determination:	
			Date / Time in oven:	05-13-25 1145
Average transfer volume:	< 0.25 mL		*Initial oven temperature:	60 °C
			Date / Time out of oven:	05-14-25 1145
			*Final oven temperature:	60 °C
			Total drying time:	24 HOURS

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	05-06-25	1130	JK	1400	JK	1310	JK	04-30-25 B
1	05-07-25	0500	JK	1420	JK	0950	JK	05-05-25
2	05-08-25	0500	JK	1330	JK	1220	JK	05-06-25
3	05-09-25	0500	JK	1100	JK	0855	JK	05-07-25
4	05-10-25	0600	JK	1200	JK	1158	JK	05-08-25
5	05-11-25	0600	JK	1200	JK	0942	JK	↓
6	05-12-25	0500	JK	1100	JK	0900	JK	05-09-25
7	05-13-25					1110	JK	

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	15066468 5

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	01.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	494.6
Average weight per initial shrimp:	0.210		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.210	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	442.8

AbKCICR Test Number: **263**

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>SL</u> Date: <u>04-22-25</u>	10.64	10.38	10.27	10.32	10.41	10.14	10.55	10.34	10.23	10.38	10.25	10.30	10.70	10.24	10.58	10.25
*B = Pan + Shrimp weight (mg) Analyst: <u>SL</u> Date: <u>05-15-25</u>	11.67	11.42	11.31	11.50	11.45	11.16	11.55	11.39	11.30	11.58	11.36	11.38	11.74	11.27	11.61	11.26
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>SL</u>	1.03	1.04	1.04	1.18	1.04	1.02	1.00	1.05	1.07	1.20	1.11	1.08	1.04	1.03	1.03	1.04
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>SL</u>	0.206	0.208	0.208	0.236	0.208	0.204	0.200	0.210	0.214	0.240	0.222	0.216	0.208	0.206	0.206	0.209
Average weight per initial number of shrimp (mg) 0.210								Average weight per initial number of shrimp (mg) 0.215								Percent reduction from control (%) - 2.47.

*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: **263**

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 ^{1d}	4 ^{1d}	5	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}
2	5	5	5	5	5	5	5	5	5	4	4	5	3 ^{1d}	3 ^{1d}	3 ^{1d}	3 ^{1d}
3	5	5	5	5	5	5	5	5	2 ^{3d}	3 ^{1d}	1 ^{3d}	5	3	3	3	3
4	5	5	5	5	5	5	5	5	2	3	1	4 ^{1d}	3	3	2	3
5	5	5	5	5	5	5	5	5	2	3	1	3 ^{1d}	3	3	1 ^{1d}	3
6	5	5	5	4 ^{1d}	5	5	5	5	2	3	1	3	3	3	1	3
7	5	5	5	4	5	5	5	5	2	3	1	3	3	3	1	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# Immature females																
# males																18.05.15.1
*A = Pan weight (mg) Tray color code: Analyst: <u>Light pink</u> Date: <u>04-22-25</u>	10.32	10.27	10.05	10.25	10.00	10.54	10.71	10.28	10.18	9.96	10.09	10.19	10.49	10.02	10.24	10.4
*B = Pan + Shrimp weight (mg) Analyst: <u>JK</u> Date: <u>05-15-26</u>	11.38	11.43	11.25	11.17	11.24	11.56	11.71	11.29	10.74	10.66	10.31	10.91	11.09	10.75	10.53	10.94
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JK</u>	1.06	1.16	1.20	0.92	1.24	1.02	1.00	1.01	0.56	0.90	0.22	0.72	0.60	0.73	0.29	0.54
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JK</u>	0.212	0.232	0.240	0.184	0.248	0.204	0.200	0.202	0.112	0.180	0.044	0.144	0.120	0.146	0.058	0.116
Average weight per initial number of shrimp (mg)					Percent reduction from control (%)					Average weight per initial number of shrimp (mg)					Percent reduction from control (%)	
0.215					- 2.57.					0.115					45.27.	

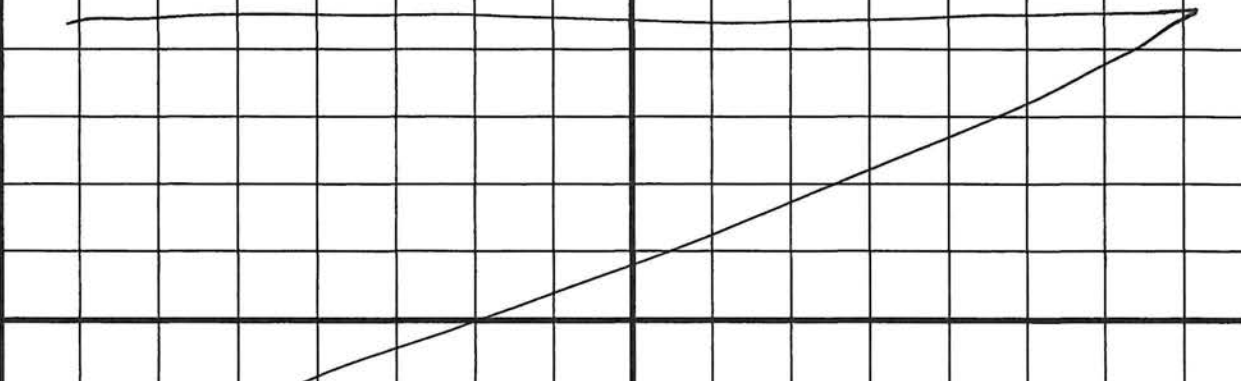
*Weight measurements performed using Cahn 28 Automatic Electrobalance, SN 41520.

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: **263**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: _____																
Analyst: _____																
Date: _____																
*B = Pan + Shrimp weight (mg)																
Analyst: _____																
Date: _____																
C = Shrimp weight (mg) = B - A																
Hand calculated																
Analyst: _____																
Weight per initial number of shrimp (mg) = C / Initial number of shrimp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hand calculated																
Analyst: _____																
Average weight per initial number of shrimp (mg)	0								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: 263

Test dates: May 06-13, 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	10.64	11.67	1.03	0.206	100.0	0.210	5.2	Not applicable
	B	5	5	10.38	11.42	1.04	0.208				
	C	5	5	10.27	11.31	1.04	0.208				
	D	5	5	10.32	11.50	1.18	0.236				
	E	5	5	10.41	11.45	1.04	0.208				
	F	5	5	10.14	11.16	1.02	0.204				
	G	5	5	10.55	11.55	1.00	0.200				
	H	5	5	10.34	11.39	1.05	0.210				
250	I	5	5	10.23	11.30	1.07	0.214	100.0	0.215	5.4	-2.4
	J	5	5	10.38	11.58	1.20	0.240				
	K	5	5	10.25	11.36	1.11	0.222				
	L	5	5	10.30	11.38	1.08	0.216				
	M	5	5	10.70	11.74	1.04	0.208				
	N	5	5	10.24	11.27	1.03	0.206				
	O	5	5	10.58	11.61	1.03	0.206				
	P	5	5	10.25	11.29	1.04	0.208				
375	Q	5	5	10.32	11.38	1.06	0.212	97.5	0.215	10.4	-2.5
	R	5	5	10.27	11.43	1.16	0.232				
	S	5	5	10.05	11.25	1.20	0.240				
	T	5	4	10.25	11.17	0.92	0.184				
	U	5	5	16.00	17.24	1.24	0.248				
	V	5	5	10.54	11.56	1.02	0.204				
	W	5	5	10.71	11.71	1.00	0.200				
	X	5	5	10.28	11.29	1.01	0.202				
500	Y	5	2	10.18	10.74	0.56	0.112	47.5	0.115	39.4	45.2
	Z	5	3	9.96	10.86	0.90	0.180				
	AA	5	1	10.09	10.31	0.22	0.044				
	BB	5	3	10.19	10.91	0.72	0.144				
	CC	5	3	10.49	11.09	0.60	0.120				
	DD	5	3	16.02	16.75	0.73	0.146				
	EE	5	1	10.24	10.53	0.29	0.058				
	FF	5	3	10.41	10.99	0.58	0.116				
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: 0.0222
 PMSD: 10.6

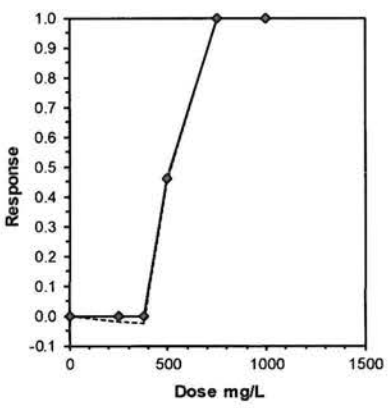
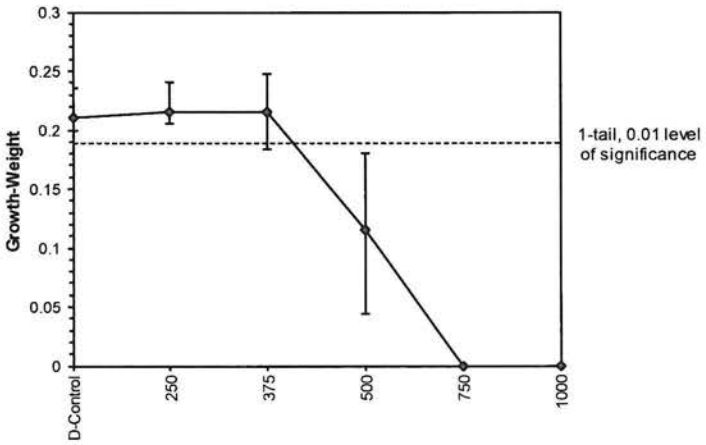
MSD =
 PMSD =

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:	5/6/2025		Test ID:		AbKCICR		Sample ID:		REF-Ref Toxicant		
End Date:	5/13/2025		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	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
375	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000			
500	0.4000	0.6000	0.2000	0.6000	0.6000	0.6000	0.2000	0.6000			
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
Transform: Arcsin Square Root											
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.4750	0.4750	0.7553	0.4636	0.8861	25.547	8	36.00	48.00	21	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
Auxiliary Tests								Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)								0.72331	0.904	-1.6376	3.66412
Equality of variance cannot be confirmed											
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU					
Steel's Many-One Rank Test			375	500	433.013						
Treatments vs D-Control											
Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.6709	3.35252	10.1	23.2418	0	0.06921	7.81472	0.99526	2.69424	0.05998	3
Intercept	-39.915	8.99074	-57.537	-22.294							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	358.671	293.812	393.338							
EC05	3.355	394.072	341.769	422.569							
EC10	3.718	414.351	369.842	439.761							
EC15	3.964	428.619	389.578	452.329							
EC20	4.158	440.309	405.537	463.115							
EC25	4.326	450.591	419.252	473.133							
EC40	4.747	477.578	452.589	503.004							
EC50	5.000	494.586	470.903	525.205							
EC60	5.253	512.199	487.759	550.857							
EC75	5.674	542.876	513.648	600.34							
EC80	5.842	555.554	523.547	622.079							
EC85	6.036	570.705	534.988	648.825							
EC90	6.282	590.358	549.364	684.584							
EC95	6.645	620.738	570.857	741.928							
EC99	7.326	682.005	612.446	864.208							

Dose-Response Plot

Mysid Survival and Growth Test-Growth-Weight												
Start Date:	5/6/2025		Test ID:	AbKCICR		Sample ID:	REF-Ref Toxicant					
End Date:	5/13/2025		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	0.2060	0.2080	0.2080	0.2360	0.2080	0.2040	0.2000	0.2100				
250	0.2140	0.2400	0.2220	0.2160	0.2080	0.2060	0.2060	0.2080				
375	0.2120	0.2320	0.2400	0.1840	0.2480	0.2040	0.2000	0.2020				
500	0.1120	0.1800	0.0440	0.1440	0.1200	0.1460	0.0580	0.1160				
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	0.2100	1.0000	0.2100	0.2000	0.2360	5.216	8				0.2134	1.0000
250	0.2150	1.0238	0.2150	0.2060	0.2400	5.378	8	-0.632	2.799	0.0222	0.2134	1.0000
375	0.2153	1.0250	0.2153	0.1840	0.2480	10.370	8	-0.663	2.799	0.0222	0.2134	1.0000
500	0.1150	0.5476	0.1150	0.0440	0.1800	39.374	8	36.000	42.000		0.1150	0.5389
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)							0.90382		0.884		0.64714	
Bartlett's Test indicates equal variances (p = 0.11)							4.46173		9.21035			
Hypothesis Test (1-tail, 0.01)			NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test			375	>375			0.02215	0.10549	7E-05	0.00025	0.7586	2, 21
Treatments vs D-Control												
Linear Interpolation (200 Resamples)												
Point	mg/L	SD	95% CL		Skew							
IC05	388.55	8.89	352.88	393.82	-4.0130							
IC10	402.11	6.09	385.96	412.65	-0.1085							
IC15	415.66	7.79	400.01	431.47	0.5674							
IC20	429.21	9.73	411.56	450.29	0.8603							
IC25	442.77	11.79	424.35	469.12	0.9875							
IC40	483.43	17.71	458.19	525.44	0.7397							
IC50	518.03	24.17	480.40	562.86	0.2446							
												
Dose-Response Plot												
												

AbKCICR Test Number: **263**

Daily Chemistry:

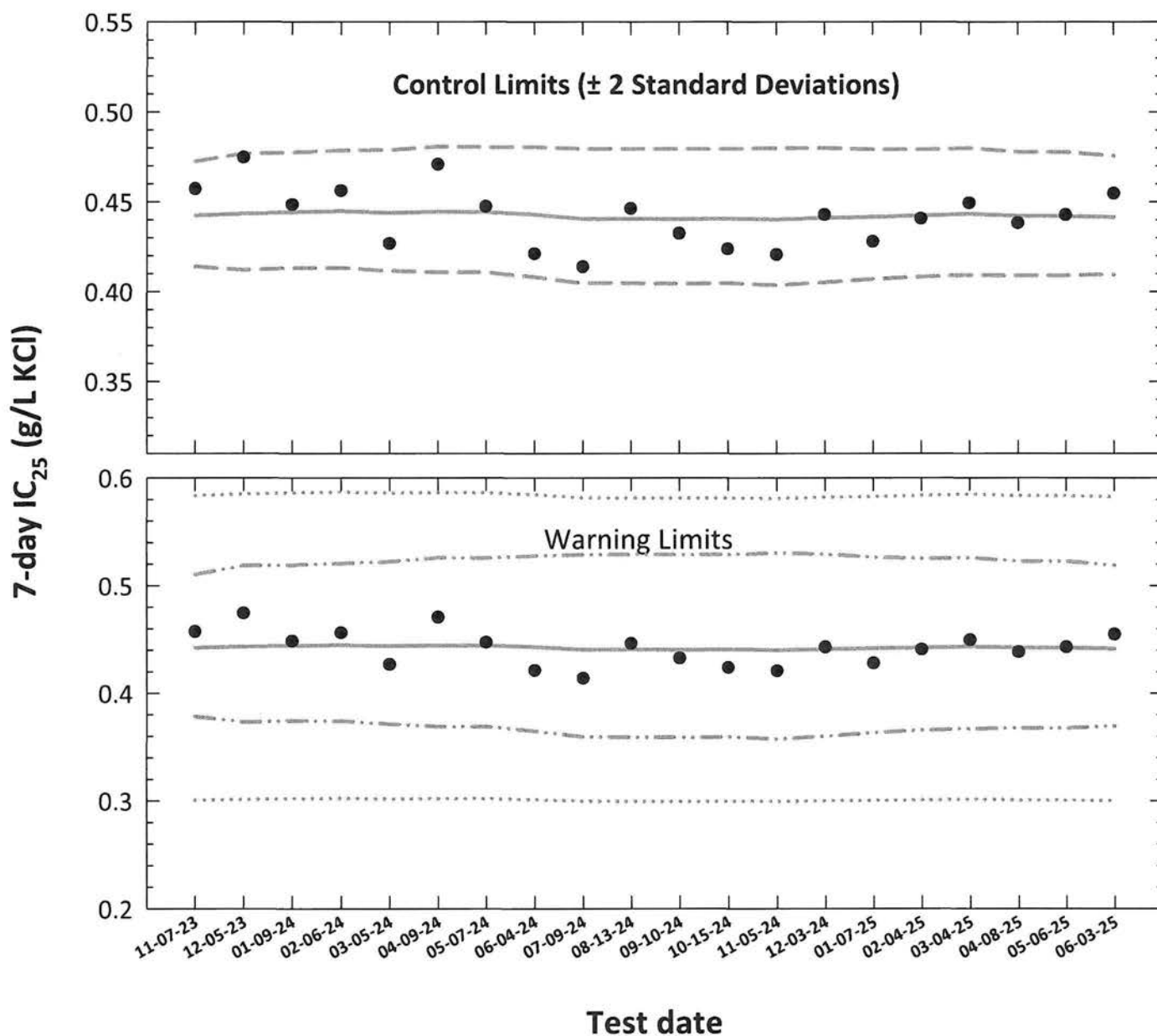
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

		Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
Conc.	Parameter	0		1		2	
Analyst		NP	JP	JP	JP	JP	XL
CONTROL, Salt SW	pH (S.U.)	8.00	7.88	7.91	7.79	7.99	7.78
	DO (mg/L)	0.3	7.8	8.0	7.8	8.0	7.9
	Salinity (ppt)	25.0	25.5	25.0	25.1	25.0	25.3 ← 25.3 05-04-23
	Alkalinity (mg CaCO ₃ /L)	92		89		77	
	Temperature (°C)	25.2	25.8	25.2	26.1	25.0	26.3
250 mg KCl/L	pH (S.U.)	8.00	7.88	7.93	7.84	7.90	7.84
	DO (mg/L)	0.2	7.8	7.9	7.8	8.0	8.0
	Salinity (ppt)	25.0	25.6	25.3	25.6	25.4	25.5
	Temperature (°C)	25.1	26.0	25.2	26.0	25.2	26.0
375 mg KCl/L	pH (S.U.)	8.01	7.88	7.93	7.83	7.90	7.84
	DO (mg/L)	0.3	7.9	7.9	7.8	8.0	8.0
	Salinity (ppt)	25.3	25.8	25.6	25.9	25.9	26.0
	Temperature (°C)	25.1	26.2	25.2	26.0	25.2	26.2
500 mg KCl/L	pH (S.U.)	8.00	7.89	7.93	7.84	7.90	7.83
	DO (mg/L)	0.3	7.9	7.9	7.8	8.0	7.9
	Salinity (ppt)	25.3	25.8	25.6	26.1	25.8	26.1
	Temperature (°C)	25.6	26.0	25.2	26.2	25.1	26.1
750 mg KCl/L	pH (S.U.)	8.00	7.86				
	DO (mg/L)	0.3	7.9				
	Salinity (ppt)	25.6	25.8				
	Temperature (°C)	25.6	26.0				
1000 mg KCl/L	pH (S.U.)	8.00	7.89				
	DO (mg/L)	0.3	7.9				
	Salinity (ppt)	25.7	25.9				
	Temperature (°C)	25.6	25.7				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 263

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst	XL	BSL	BSL	BSL	BSL	XL	XL
CONTROL, Salt SW	pH (S.U.)	7.88	7.82	8.04	7.87	7.02	7.88	7.99	7.91
	DO (mg/L)	8.1	7.7	8.1	7.7	8.1	7.8	8.1	6.2
	Salinity (ppt)	25.0	25.2	24.9	25.3	25.0	25.3	24.4	24.4
	Alkalinity (mg CaCO ₃ /L)	91		89		0.114-15 M		91	
	Temperature (°C)	25.2	25.9	25.3	26.0	25.2	26.0	25.2	26.0
250 mg KCl/L	pH (S.U.)	7.84	7.82	7.92	7.81	8.02	7.88	8.00	7.61
	DO (mg/L)	8.1	7.7	8.1	7.7	8.1	7.7	8.1	6.8
	Salinity (ppt)	25.2	25.4	25.2	25.4	25.1	25.3	25.1	25.2
	Temperature (°C)	25.1	25.9	25.2	26.2	25.2	26.2	25.1	26.0
375 mg KCl/L	pH (S.U.)	7.84	7.84	7.92	7.79	8.02	7.89	8.02	7.65
	DO (mg/L)	8.1	7.8	8.1	7.7	8.1	7.8	8.1	6.5
	Salinity (ppt)	25.4	25.7	25.5	25.7	25.2	25.4	25.2	25.5
	Temperature (°C)	25.1	26.1	25.2	26.2	25.3	26.1	25.1	26.0
500 mg KCl/L	pH (S.U.)	7.85	7.84	7.92	7.83	8.02	7.91	8.02	7.74
	DO (mg/L)	8.1	7.8	8.1	7.7	8.1	7.8	8.1	6.9
	Salinity (ppt)	25.4	25.8	25.5	25.2	25.2	25.6	25.3	25.7
	Temperature (°C)	25.1	25.8	25.2	26.2	25.3	26.1	25.1	26.0
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105	0.3008	0.5838
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	03-04-25	0.4493	-0.3474	-0.3534	0.0173	0.4432	0.4093	0.4799	0.3667	0.5260	0.3014	0.5850
18	04-08-25	0.4382	-0.3583	-0.3545	0.0169	0.4421	0.4091	0.4777	0.3674	0.5228	0.3006	0.5835
19	05-06-25	0.4428	-0.3538	-0.3545	0.0169	0.4421	0.4091	0.4778	0.3674	0.5228	0.3006	0.5836
20	06-03-25	0.4548	-0.3422	-0.3550	0.0162	0.4415	0.4098	0.4757	0.3696	0.5190	0.3002	0.5828

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

CT = Central tendency of the IC_{25} values.

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

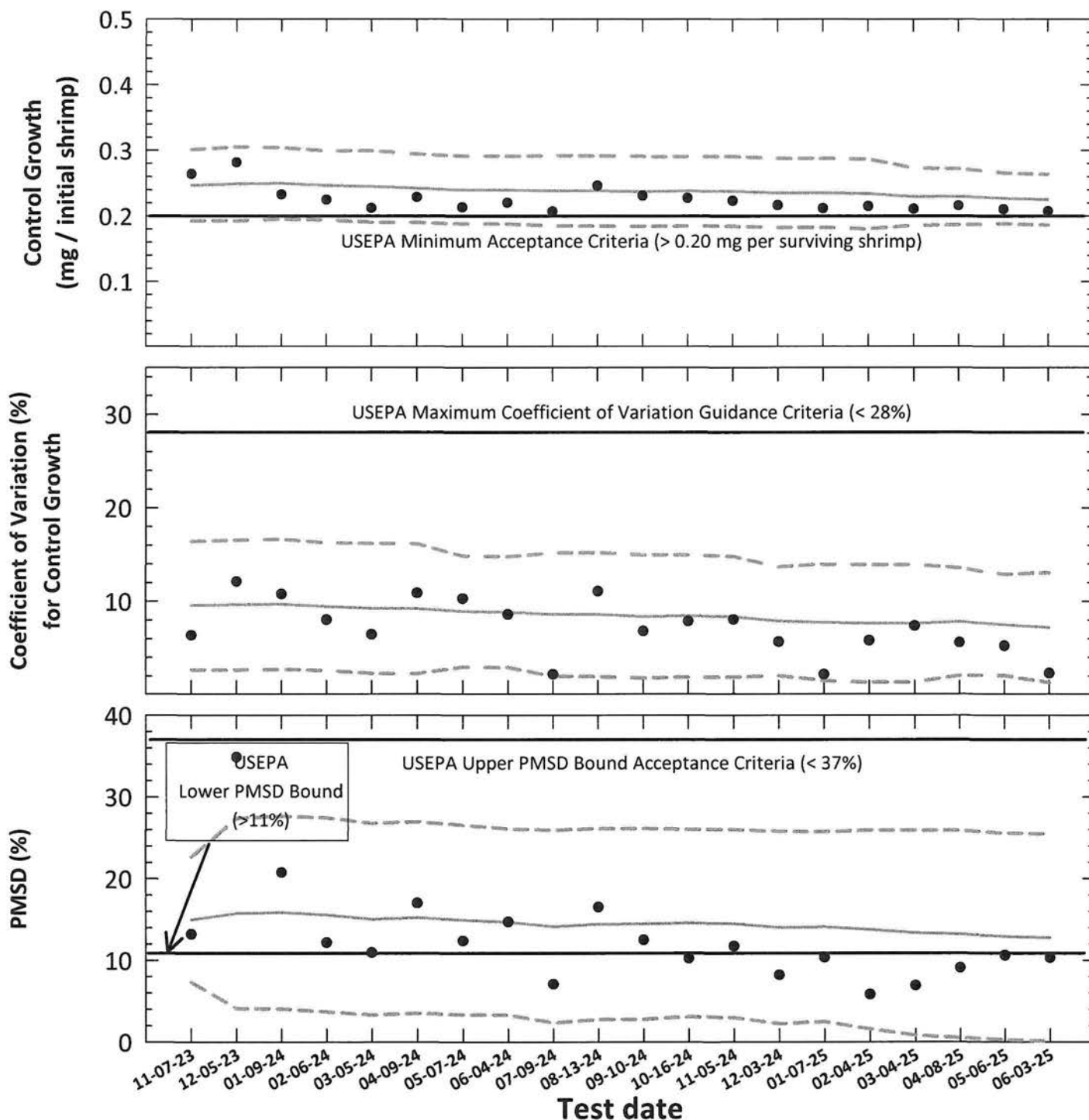
Control Limits = Mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

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

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

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	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
			Mean (mg/initial shrimp)	CV (%)										
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3
9	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3
10	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.7
11	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8
12	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1
13	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9
14	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.0	2.2
15	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5
16	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5
17	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8
18	04-08-25	100	0.216	5.6	0.0196	9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5
19	05-06-25	100	0.210	5.2	0.0222	10.6	0.227	0.188	0.265	7.5	2.0	12.9	12.9	0.3
20	06-03-25	100	0.207	2.3	0.0213	10.3	0.225	0.186	0.264	7.2	1.3	13.1	12.8	0.1

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

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

AbKCICR Test Number: 264

AbKCICR Test Number:

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2376				
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:	05-27-25 1200 to 05-28-25 1130		Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 05-28-25		Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 7.91 S.U. Temperature = 25.0 °C		Drying information for weight determination:	
Average transfer volume:	< 0.25 mL		Date / Time in oven:	06-10-25 1130
			*Initial oven temperature:	60 °C
			Date / Time out of oven:	06-11-25 1130
			*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	06-03-25	1105	H	1345	H	1210	H	05-28-25 A
1	06-04-25	0500	H	1730	H	0900	H	↓
2	06-05-25	0500	H	1300	H	0900	H	05-28-25 B
3	06-06-25	0600	H	1200	H	0855	H	↓
4	06-07-25	0600	H	1200	H	1100	H	06-04-25
5	06-08-25	0600	H	1130	H	0850	H	↓
6	06-09-25	0500	H	1100	H	0800	H	↓
7	06-10-25					1110	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 (mg/L KCl)	522.8
Average weight per initial shrimp:	0.207		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.207	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	456.4330
			IC ₂₅ (mg/L KCl)	456.911

AbKCICR Test Number: **264****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>Forest Green</u> Analyst: <u>Y</u> Date: <u>05-23-25</u>	13.65	13.52	12.73	14.14	14.00	12.98	13.54	12.91	14.02	11.56	15.13	13.56	13.08	15.51	14.16	13.7
*B = Pan + Shrimp weight (mg) Analyst: <u>Y</u> Date: <u>06-13-25</u>	14.71	14.54	13.77	15.14	15.01	14.02	14.60	13.97	15.11	12.70	16.21	14.59	14.12	16.59	15.26	14.85
C = Shrimp weight (mg) = B - A Hand calculated: <u>Y</u> Analyst: <u>Y</u>	1.06	1.02	1.04	1.00	1.01	1.04	1.06	1.06	1.09	1.14	1.08	1.03	1.04	1.08	1.10	1.06
Weight per Initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>Y</u> Analyst: <u>Y</u>	0.212	0.204	0.208	0.200	0.202	0.208	0.212	0.212	0.218	0.228	0.216	0.206	0.208	0.216	0.220	0.210
Average weight per initial number of shrimp (mg)	0.207								0.216				Percent reduction from control (%)			
													-4.0%			

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

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

Comments:



Environmental Testing Solutions, Inc.

AbKCICR Test Number: 264**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	5	5	5	4 ^{1d}	5	4 ^{1d}	5	4 ^{1d}
4	5	5	5	5	5	5	5	5	5	5	5	4	4 ^{1d}	3 ^{1d}	5	4
5	5	5	5	5	5	5	5	4 ^{1d}	5	3 ^{2d}	4 ^{1d}	4	3 ^{1d}	3	5	4
6	5	5	5	5	5	5	5	4	5	3	4	4	3	3	5	4
7	5	5	5	5	5	5	5	4	3 ^{2d}	3	3 ^{1d}	4	3	3	4 ^{1d}	3 ^{1d}
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Analyst: <u>Robert Golden</u> Date: <u>06-23-25</u>	12.13	13.22	15.16	13.92	12.83	12.66	11.73	10.40	14.20	14.53	13.16	13.02	14.53	12.78	14.97	12.60
*B = Pan + Shrimp weight (mg) Analyst: <u>XL</u> Date: <u>06-12-25</u>	13.14	14.25	16.20	14.97	13.88	13.66	12.80	11.15	14.80	15.26	13.86	13.84	15.21	13.38	15.72	13.12
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.01	1.03	1.24	1.05	1.05	1.00	1.07	0.75	0.60	0.73	0.70	0.82	0.68	0.60	0.75	0.52
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.202	0.206	0.208	0.210	0.210	0.200	0.214	0.150	0.120	0.146	0.140	0.164	0.136	0.120	0.150	0.104
Average weight per initial number of shrimp (mg)	0.200								0.135							
Percent reduction from control (%)	3.5 %								34.9 %							

*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: **264**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd	0 sd
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: _____																
Analyst: _____ Date: _____																
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B - A																
Hand calculated Analyst: _____																
Weight per initial number of shrimp (mg) = C / Initial number of shrimp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hand calculated Analyst: _____																
Average weight per initial number of shrimp (mg)	0								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: 264
Test dates: June 03-10, 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	13.65	14.71	1.06	0.212	100.0	0.207	2.3	Not applicable
	B	5	5	13.52	14.54	1.02	0.204				
	C	5	5	12.73	13.77	1.04	0.208				
	D	5	5	14.14	15.14	1.00	0.200				
	E	5	5	14.00	15.01	1.01	0.202				
	F	5	5	12.98	14.02	1.04	0.208				
	G	5	5	13.54	14.60	1.06	0.212				
	H	5	5	12.91	13.97	1.06	0.212				
250	I	5	5	14.02	15.11	1.09	0.218	100.0	0.216	3.2	-4.0
	J	5	5	11.56	12.70	1.14	0.228				
	K	5	5	15.13	16.21	1.08	0.216				
	L	5	5	13.56	14.59	1.03	0.206				
	M	5	5	13.08	14.12	1.04	0.208				
	N	5	5	15.51	16.59	1.08	0.216				
	O	5	5	14.16	15.26	1.10	0.220				
	P	5	5	13.79	14.85	1.06	0.212				
375	Q	5	5	12.13	13.14	1.01	0.202	97.5	0.200	10.4	3.5
	R	5	5	13.22	14.25	1.03	0.206				
	S	5	5	15.16	16.20	1.04	0.208				
	T	5	5	13.92	14.97	1.05	0.210				
	U	5	5	12.83	13.88	1.05	0.210				
	V	5	5	12.66	13.66	1.00	0.200				
	W	5	5	11.73	12.80	1.07	0.214				
	X	5	4	10.40	11.15	0.75	0.150				
500	Y	5	3	14.20	14.80	0.60	0.120	65.0	0.135	14.3	34.9
	Z	5	3	14.53	15.26	0.73	0.146				
	AA	5	3	13.16	13.86	0.70	0.140				
	BB	5	4	13.02	13.84	0.82	0.164				
	CC	5	3	14.53	15.21	0.68	0.136				
	DD	5	3	12.78	13.38	0.60	0.120				
	EE	5	4	14.97	15.72	0.75	0.150				
	FF	5	3	12.60	13.12	0.52	0.104				
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: 0.0213
PMSD: 10.3

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

Mysid Survival and Growth Test-7 Day Survival

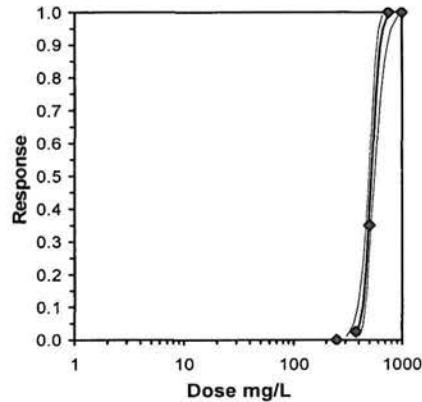
Start Date: 6/3/2025 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 6/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanysis bahia
Comments:

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

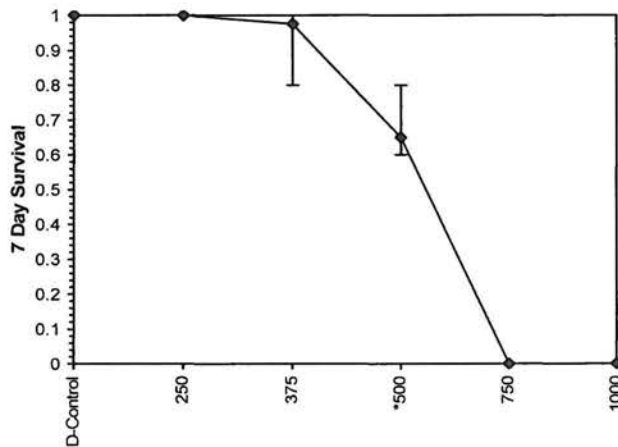
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.6500	0.6500	0.9413	0.8861	1.1071	10.871	8	36.00	48.00	14	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)					0.77803	0.904	-0.1046	5.2967
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					375	500	433.013	
Treatments vs D-Control								

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.3979	2.77332	9.96216	20.8336	0	0.93438	7.81472	0.81712	2.71835	0.06494	5
Intercept	-36.857	7.48591	-51.529	-22.184							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	369.204	309.198	404.519							
EC05	3.355	408.814	360.01	438.511							
EC10	3.718	431.638	389.612	458.742							
EC15	3.964	447.753	410.32	473.641							
EC20	4.158	460.989	426.992	486.473							
EC25	4.326	472.656	441.278	498.385							
EC40	4.747	503.379	476.148	533.364							
EC50	5.000	522.816	495.797	558.539							
EC60	5.253	543.003	514.447	586.96							
EC75	5.674	578.3	544.071	640.888							
EC80	5.842	592.935	555.617	664.448							
EC85	6.036	610.463	569.061	693.4							
EC90	6.282	633.254	586.062	732.098							
EC95	6.645	668.609	611.646	794.18							
EC99	7.326	740.34	661.558	926.768							



Dose-Response Plot



Mysid Survival and Growth Test-Growth-Weight

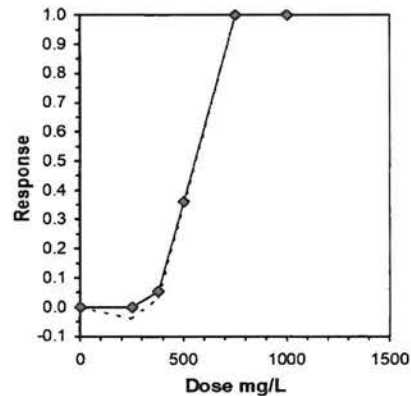
Start Date: 6/3/2025 Test ID: AbKQCR Sample ID: REF-Ref Toxicant
End Date: 6/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2120	0.2040	0.2080	0.2000	0.2020	0.2080	0.2120	0.2120
250	0.2180	0.2280	0.2160	0.2060	0.2080	0.2160	0.2200	0.2120
375	0.2020	0.2060	0.2080	0.2100	0.2100	0.2000	0.2140	0.1500
500	0.1200	0.1460	0.1400	0.1640	0.1360	0.1200	0.1500	0.1040
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

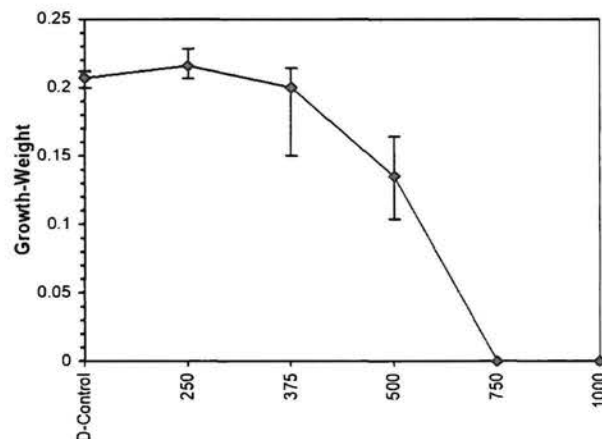
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
D-Control	0.2073	1.0000	0.2073	0.2000	0.2120	2.303	8			0.2114	1.0000
250	0.2155	1.0398	0.2155	0.2060	0.2280	3.244	8	89.50	43.00	0.2114	1.0000
375	0.2000	0.9650	0.2000	0.1500	0.2140	10.351	8	63.00	43.00	0.2000	0.9462
500	0.1350	0.6514	0.1350	0.1040	0.1640	14.342	8			0.1350	0.6387
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 ($p \leq 0.01$)				0.70403	0.884	-3.0053	12.0483
Bartlett's Test indicates unequal variances ($p = 5.63E-04$)				14.9662	9.21035		
Hypothesis Test (1-tail, 0.01)				NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test				375	>375		
Treatments vs D-Control							

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	366.14	29.82	298.50	390.93	-0.7036
IC10	393.77	16.07	346.99	408.58	-1.7127
IC15	414.10	11.61	385.01	429.01	-1.2621
IC20	434.42	10.04	408.63	450.84	-0.7693
IC25	454.75	9.69	433.34	472.86	-0.2725
IC40	515.14	11.05	493.86	532.94	-0.3595
IC50	554.28	9.54	534.89	569.11	-0.5629



Dose-Response Plot



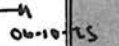
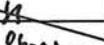
AbKCICR Test Number: **264**

Daily Chemistry:

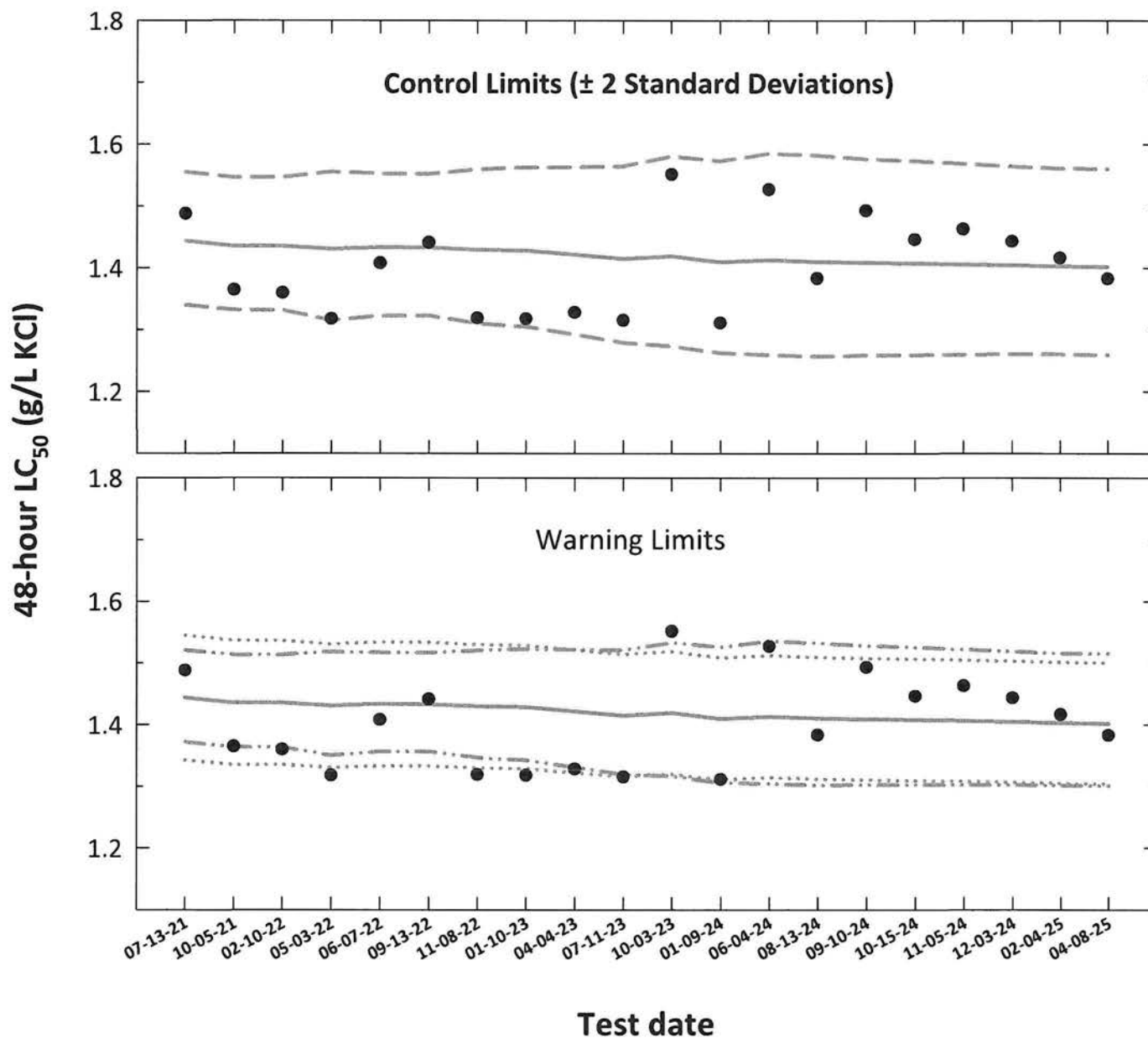
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

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.)	Em K2 40	N	N	XL	XL	N
	DO (mg/L)	7.87	7.75	7.76	7.80	7.88	7.74
	Salinity (ppt)	8.7	8.0	8.0	7.9	8.2	7.6
	Alkalinity (mg CaCO ₃ /L)	25.0	25.5	25.0	25.3	25.0	25.1
	Temperature (°C)	95			89		
250 mg KCl/L	pH (S.U.)	25.6	26.3	25.2	26.2	25.1	26.0
	DO (mg/L)	7.88	7.75	7.76	7.84	7.90	7.74
	Salinity (ppt)	8.7	8.0	8.0	7.9	8.2	7.7
	Temperature (°C)	25.3	25.6	25.5	25.6	25.4	25.7
375 mg KCl/L	pH (S.U.)	25.8	26.5	25.1	26.2	26.1	26.2
	DO (mg/L)	7.89	7.75	7.77	7.83	7.92	7.74
	Salinity (ppt)	8.6	8.0	8.0	7.9	8.2	7.7
	Temperature (°C)	25.7	25.8	25.6	25.7	25.6	26.1
500 mg KCl/L	pH (S.U.)	26.0	26.1	25.1	26.1	25.2	26.2
	DO (mg/L)	7.89	7.75	7.78	7.83	7.92	7.77
	Salinity (ppt)	8.6	8.0	8.0	7.8	8.1	7.8
	Temperature (°C)	25.2	26.0	25.7	25.9	25.7	26.3
750 mg KCl/L	pH (S.U.)	26.0	26.0	25.1	26.4	25.1	26.4
	DO (mg/L)	7.89	7.72				
	Salinity (ppt)	8.6	2.9				
	Temperature (°C)	25.7	26.1				
1000 mg KCl/L	pH (S.U.)	26.0	26.2				
	DO (mg/L)	7.91	7.72				
	Salinity (ppt)	8.6	7.0				
	Temperature (°C)	26.0	26.2				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 264

		Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
Conc.	Parameter	3		4		5		6	
Analyst			BSL	BSL	BSL	BSL	XL	XL	
CONTROL, Salt SW	pH (S.U.)	7.81	7.83	8.00	7.82	8.02	7.80	7.91	7.43
	DO (mg/L)	8.0	7.8	8.2	7.4	8.3	7.7	8.0	5.3
	Salinity (ppt)	25.0	25.0	25.1	25.1	25.2	25.2	25.1	26.0
	Alkalinity (mg CaCO ₃ /L)	 06-04-25		94				 06-04-25	
	Temperature (°C)	25.1	26.2	25.2	26.1	25.1	26.3	25.2	26.1
250 mg KCl/L	pH (S.U.)	7.83	7.83	8.00	7.80	8.02	7.81	7.92	7.54
	DO (mg/L)	8.0	7.8	8.3	7.6	8.2	7.7	8.0	5.4
	Salinity (ppt)	25.4	25.4	25.3	25.3	25.3	25.6	25.4	25.7
	Temperature (°C)	25.2	26.3	25.1	26.2	26.2	26.0	25.3	26.2
375 mg KCl/L	pH (S.U.)	7.83	7.88	8.00	7.83	8.03	7.81	7.92	7.58
	DO (mg/L)	8.1	7.8	8.2	7.6	8.2	7.7	8.0	5.7
	Salinity (ppt)	25.4	25.6	25.4	25.6	25.4	25.7	25.6	25.9
	Temperature (°C)	25.2	26.3	25.2	26.2	26.2	26.2	25.3	26.2
500 mg KCl/L	pH (S.U.)	7.83	7.89	8.00	7.89	8.03	7.80	7.91	7.57
	DO (mg/L)	8.1	7.8	8.1	7.7	8.1	7.6	8.0	5.4
	Salinity (ppt)	25.4	25.7	25.6	25.8	25.5	25.8	25.7	26.0
	Temperature (°C)	25.2	26.4	25.2	26.2	25.1	26.2	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.)						 06-04-25		
	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 $\pm$ 2 standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ $\pm$ 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm$ S_{A.10} converted to anti-logarithmic values,
S_{A.10} = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV		
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	Warning Limits	CT - S _{A,10}	CT + S _{A,10}
1	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	
2	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	
3	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	
4	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	
5	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	
6	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	
7	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	
8	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	
9	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	
10	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	
11	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	
12	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	
13	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	
14	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	
15	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	
16	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	
17	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	
18	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	
19	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	
20	04-08-25	1.3832	0.1409	0.1468	0.0232	1.4021	1.2597	1.5605	1.3006	1.5150	1.3039	1.5002	

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

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 100

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 #: 2357

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	Em JP	JP Em	Em
	pH (S.U.)	8.23	7.99	7.97
	Dissolved oxygen (mg/L)	0.3	7.9	0.1
	*Salinity (ppt)	25.5	25.1	26.0
	*Alkalinity (mg/L CaCO ₃)	100		
1000 mg/L	*Temperature (°C)	25.0	25.3	25.1
	pH (S.U.)	8.08	7.99	7.98
	Dissolved oxygen (mg/L)	0.4	7.9	0.1
	*Salinity (ppt)	25.3	25.9	26.5
	*Temperature (°C)	25.2	25.1	25.0
1250 mg/L	pH (S.U.)	8.08	7.98	7.96
	Dissolved oxygen (mg/L)	0.4	7.9	0.0
	*Salinity (ppt)	25.7	26.2	26.5
	*Temperature (°C)	25.2	25.1	25.0
1500 mg/L	pH (S.U.)	8.09	7.99	7.97
	Dissolved oxygen (mg/L)	0.4	7.9	0.1
	*Salinity (ppt)	25.7	26.1	26.9
	*Temperature (°C)	25.2	25.1	25.2
1750 mg/L	pH (S.U.)	8.09	7.98	7.97
	Dissolved oxygen (mg/L)	0.4	7.9	0.0
	*Salinity (ppt)	26.3	26.9	27.0
	*Temperature (°C)	25.2	25.2	25.2
2000 mg/L	pH (S.U.)	8.09	7.96	
	Dissolved oxygen (mg/L)	0.4	7.9	
	*Salinity (ppt)	26.1	26.5	
	*Temperature (°C)	25.0	25.1	
				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	19000164

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 # 100

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-08-25	* 1045	H	1330	H	6D	Yellow	04-02-28
24	04-09-25			1328	H			
48 Termination	04-10-25			1325	H			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Mb):	03-30-25
Age (9 to 14 days old):	
Date organisms were born:	03-19-25 1200 TO 03-30-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.73 Temperature (°C) 24.7

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	8 ^{2d}	7 ^{2d}	6 ^{4d}	4 ^{6d}	2 ^{5d}	1 ^{9d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	6 ^{2d}	7	5 ^{1d}	3 ^{1d}	0 ^{2d}	1	0	0
Mean Survival	100%		100%		65%		40%		5%		0%	

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

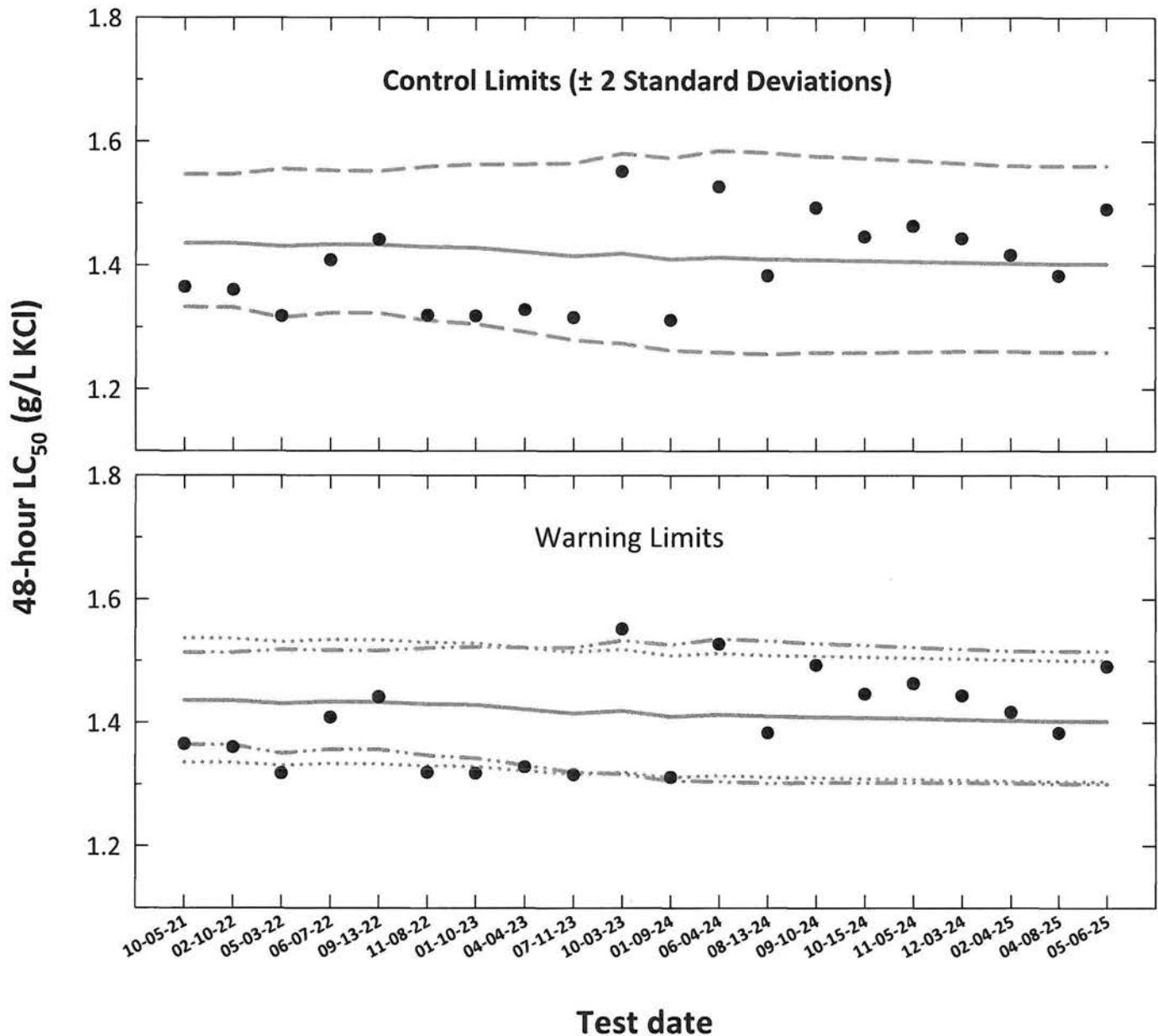
Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	1300.9
Upper 95% confidence limit (mg KCl/L)	1461.5
48-hour LC ₅₀ (mg KCl/L)	1383.2

Comments:



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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	04-08-25	1.3832	0.1409	0.1468	0.0232	1.4021	1.2597	1.5605	1.3006	1.5150	1.3039	1.5002
20	05-06-25	1.4906	0.1734	0.1468	0.0233	1.4022	1.2596	1.5610	1.3005	1.5154	1.3040	1.5004

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

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Menidia beryllina*

EPA-821-R-02-012, Method 2006.0

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 101

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 #: 7376

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>U JP</i>	<i>JP En</i>	<i>JP En</i>
	pH (S.U.)	<i>8.00</i>	<i>7.89</i>	<i>7.85</i>
	Dissolved oxygen (mg/L)	<i>0.3</i>	<i>7.8</i>	<i>7.8</i>
	*Salinity (ppt)	<i>25.0</i>	<i>25.3</i>	<i>25.9</i>
	*Alkalinity (mg/L CaCO ₃)	<i>42</i>		
	*Temperature (°C)	<i>25.6</i>	<i>25.3</i>	<i>24.5</i>
1000 mg/L	pH (S.U.)	<i>0.04</i>	<i>7.88</i>	<i>7.84</i>
	Dissolved oxygen (mg/L)	<i>0.3</i>	<i>7.8</i>	<i>7.8</i>
	*Salinity (ppt)	<i>25.3</i>	<i>25.7</i>	<i>25.9</i>
	*Temperature (°C)	<i>25.8</i>	<i>25.5</i>	<i>24.3</i>
1250 mg/L	pH (S.U.)	<i>0.04</i>	<i>7.88</i>	<i>7.83</i>
	Dissolved oxygen (mg/L)	<i>0.3</i>	<i>7.8</i>	<i>7.9</i>
	*Salinity (ppt)	<i>25.8</i>	<i>26.3</i>	<i>26.5</i>
	*Temperature (°C)	<i>25.6</i>	<i>25.5</i>	<i>24.7</i>
1500 mg/L	pH (S.U.)	<i>0.05</i>	<i>7.87</i>	<i>7.84</i>
	Dissolved oxygen (mg/L)	<i>0.3</i>	<i>7.8</i>	<i>7.9</i>
	*Salinity (ppt)	<i>26.2</i>	<i>26.2</i>	<i>26.5</i>
	*Temperature (°C)	<i>25.6</i>	<i>25.6</i>	<i>25.0</i>
1750 mg/L	pH (S.U.)	<i>0.05</i>	<i>7.86</i>	<i>7.86</i>
	Dissolved oxygen (mg/L)	<i>0.3</i>	<i>7.8</i>	<i>7.8</i>
	*Salinity (ppt)	<i>26.2</i>	<i>26.7</i>	<i>26.9</i>
	*Temperature (°C)	<i>25.3</i>	<i>25.2</i>	<i>24.7</i>
2000 mg/L	pH (S.U.)	<i>0.05</i>	<i>7.87</i>	
	Dissolved oxygen (mg/L)	<i>0.3</i>	<i>7.8</i>	
	*Salinity (ppt)	<i>26.2</i>	<i>26.5</i>	
	*Temperature (°C)	<i>25.4</i>	<i>25.2</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>1306468</i>

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 # 101

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-06-25	* 1130	yl	1340	yl	6 D	Yellow	04-30-58
24	05-07-25			1345	h			
48 Termination	05-08-25			1322	zp			

*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):	AC Mb 04-26-25
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	04-25-25 1200 TO 04-26-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.29
	Temperature (°C) 24.7

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^d	6 ^u	5 ^s	1 ^u	1 ^u	0 ^u	0 ^u
48 Termination	10	10	10	10	10	9	5 ^u	5	0 ^u	1	0	0
Mean Survival	100%		100%		95%		50%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1420.6
Upper 95% confidence limit (mg KCl/L)	1557.7
48-hour LC ₅₀ (mg KCl/L)	1490.6

Comments:

Acute Silverside Test-24 Hr Survival													
Start Date:	5/6/2025	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	5/8/2025	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	0.9000											
1500	0.6000	0.5000											
1750	0.1000	0.1000											
2000	0.0000	0.0000											
Transform: Arcsin Square Root													
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20	
1250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.345	2.850	0.1726	1	20	
*1500	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20	
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	17.998	2.850	0.1726	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)													
Dunnett's Test	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df			
Treatments vs D-Control	1250	1500	1369.31		0.08088	0.08295	0.45815	0.00367	3.4E-05	4, 5			
Maximum Likelihood-Probit													
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter			
Slope	20.8349	3.83725	13.3139 28.3559	0	0.18423	7.81472	0.98009	3.18016	0.048	3			
Intercept	-61.258	12.2281	-85.225 -37.291										
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	1170.85	995.133 1270.1										
EC05	3.355	1262.44	1116.03 1346.68										
EC10	3.718	1314.15	1185.21 1390.74										
EC15	3.964	1350.24	1233.48 1422.2										
EC20	4.158	1379.63	1272.55 1448.51										
EC25	4.326	1405.35	1306.38 1472.23										
EC40	4.747	1472.3	1391.37 1538.46										
EC50	5.000	1514.11	1440.87 1584.41										
EC60	5.253	1557.1	1488.01 1636.25										
EC75	5.674	1631.28	1560.54 1736.46										
EC80	5.842	1661.69	1587.66 1780.86										
EC85	6.036	1697.86	1618.47 1835.63										
EC90	6.282	1744.48	1656.44 1908.83										
EC95	6.645	1815.95	1711.99 2025.55										
EC99	7.326	1958	1816.62 2269.86										

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

Response

1 10 100 1000 10000

Dose mg/L

1-tail, 0.05 level of significance

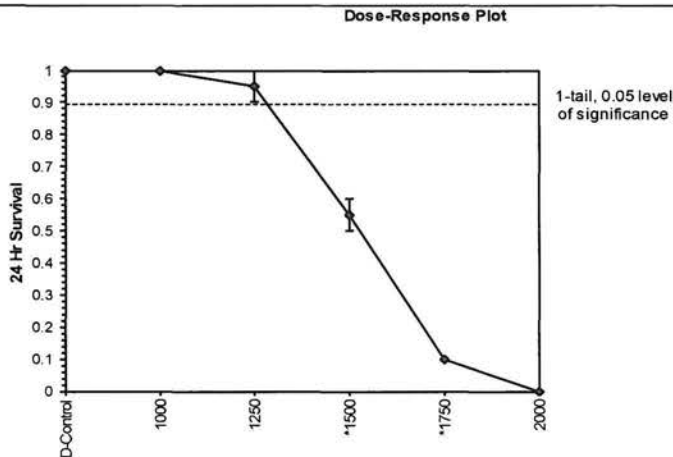
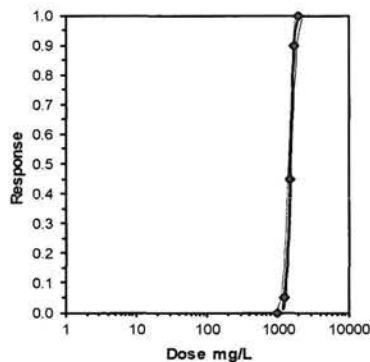
1
0.9
0.8
0.7
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0.5
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0.3
0.2
0.1
0

24 Hr Survival

D-Control 1000 1250 *1500 *1750 2000

Dose mg/L

1-tail, 0.05 level of significance



Acute Silverside Test-48 Hr Survival													
Start Date:	5/6/2025	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	5/8/2025	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	0.9000											
1500	0.5000	0.5000											
1750	0.0000	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	0.2077	0	20	
1250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.118	2.850	0.2077	1	20	
*1500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	8.598	2.850	0.2077	10	20	
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	16.077	2.850	0.2077	19	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	
Auxiliary Tests								Statistic		Critical		Skew Kurt	
Normality of the data set cannot be confirmed													
Equality of variance cannot be confirmed													
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df	
Dunnett's Test			1250	1500	1369.31		0.10341	0.10606	0.53276	0.00531	5.8E-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	22.7855	4.3743	14.2119	31.3592	0	0.14458	7.81472	0.986	3.17336	0.04389	3		
Intercept	-67.307	13.9063	-94.563	-40.05									
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	1178.33	1005.61	1272.5									
EC05	3.355	1262.34	1119.49	1342									
EC10	3.718	1309.54	1184.25	1381.91									
EC15	3.964	1342.39	1229.26	1410.41									
EC20	4.158	1369.07	1265.57	1434.24									
EC25	4.326	1392.39	1296.92	1455.74									
EC40	4.747	1452.93	1375.29	1515.88									
EC50	5.000	1490.61	1420.63	1557.7									
EC60	5.253	1529.26	1463.56	1604.95									
EC75	5.674	1595.75	1529.1	1696.28									
EC80	5.842	1622.93	1553.44	1736.71									
EC85	6.036	1655.2	1581.01	1786.52									
EC90	6.282	1696.71	1614.84	1853									
EC95	6.645	1760.16	1664.09	1958.74									
EC99	7.326	1885.65	1756.25	2178.97									

Dose-Response Plot

48 Hr Survival

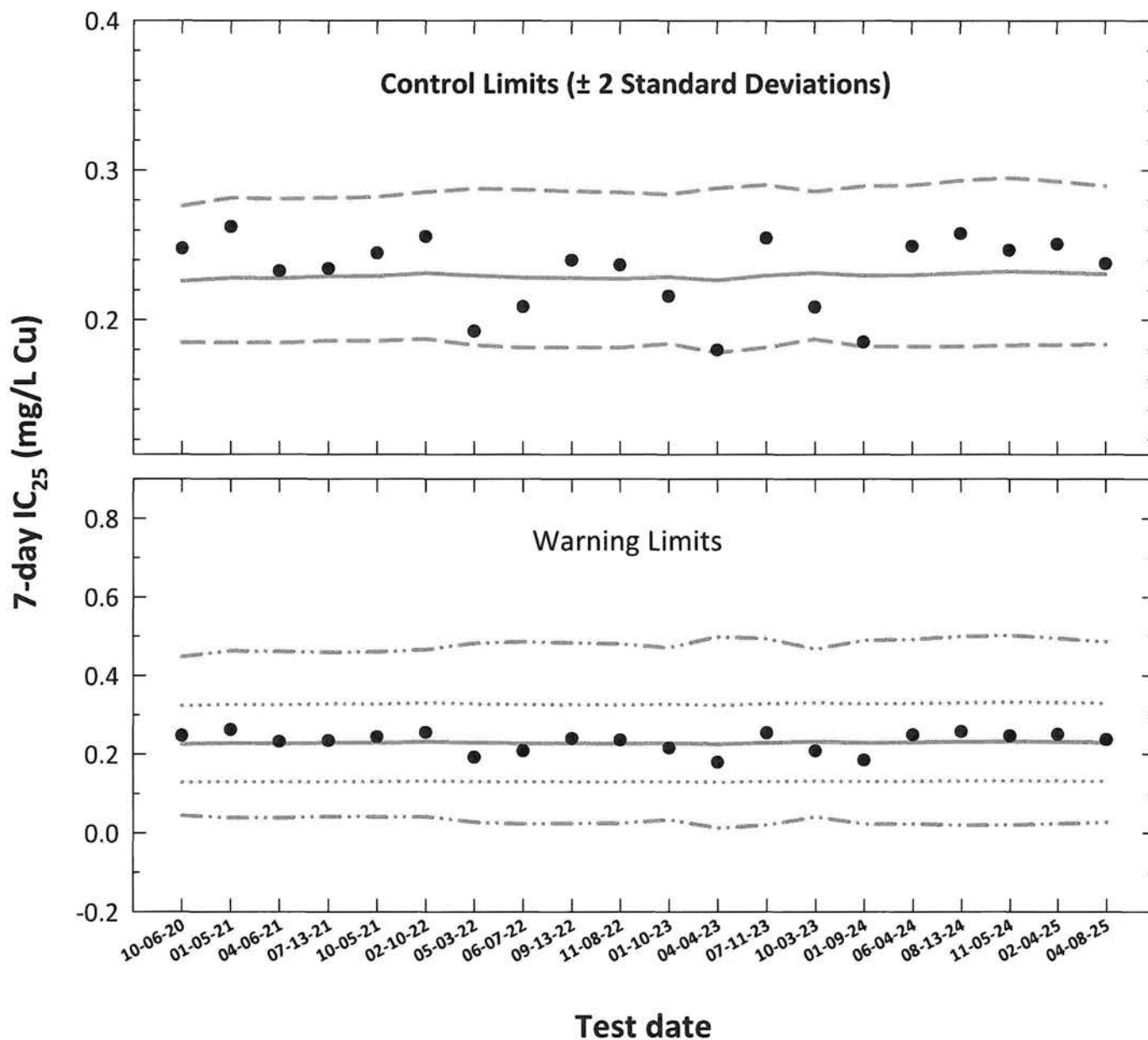
Dose mg/L

1-tail, 0.05 level of significance

Menidia beryllina

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion		Anti-logarithmic Values (mg/L Cu)								
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits		
								CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	10-06-20	0.2479	-0.6057	-0.6457	0.0436	0.2261	0.1850	0.2763		0.0443	0.4483	0.1289	0.3233
2	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.2281	0.1848	0.2816		0.0382	0.4625	0.1300	0.3262
3	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.2278	0.1847	0.2810		0.0385	0.4612	0.1298	0.3258
4	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.2290	0.1862	0.2817		0.0420	0.4590	0.1305	0.3274
5	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.2292	0.1861	0.2822		0.0414	0.4604	0.1306	0.3277
6	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.2313	0.1874	0.2855		0.0414	0.4656	0.1318	0.3307
7	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.2296	0.1832	0.2877		0.0276	0.4827	0.1309	0.3283
8	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.2283	0.1815	0.2872		0.0233	0.4861	0.1301	0.3265
9	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.2279	0.1816	0.2861		0.0246	0.4831	0.1299	0.3259
10	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.2276	0.1816	0.2853		0.0254	0.4811	0.1297	0.3255
11	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.2287	0.1841	0.2841		0.0337	0.4708	0.1304	0.3270
12	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.2266	0.1781	0.2884		0.0125	0.4991	0.1292	0.3241
13	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.2298	0.1818	0.2905		0.0208	0.4939	0.1310	0.3286
14	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.2314	0.1873	0.2859		0.0408	0.4669	0.1319	0.3309
15	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.2298	0.1824	0.2896		0.0233	0.4900	0.1310	0.3286
16	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.2300	0.1823	0.2901		0.0227	0.4914	0.1311	0.3288
17	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.2312	0.1823	0.2932		0.0196	0.4996	0.1318	0.3306
18	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.2324	0.1831	0.2950		0.0201	0.5019	0.1325	0.3323
19	02-04-25	0.2507	-0.6008	-0.6349	0.0507	0.2318	0.1835	0.2928		0.0234	0.4950	0.1321	0.3314
20	04-08-25	0.2377	-0.6240	-0.6369	0.0494	0.2307	0.1838	0.2896		0.0273	0.4861	0.1315	0.3299

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

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

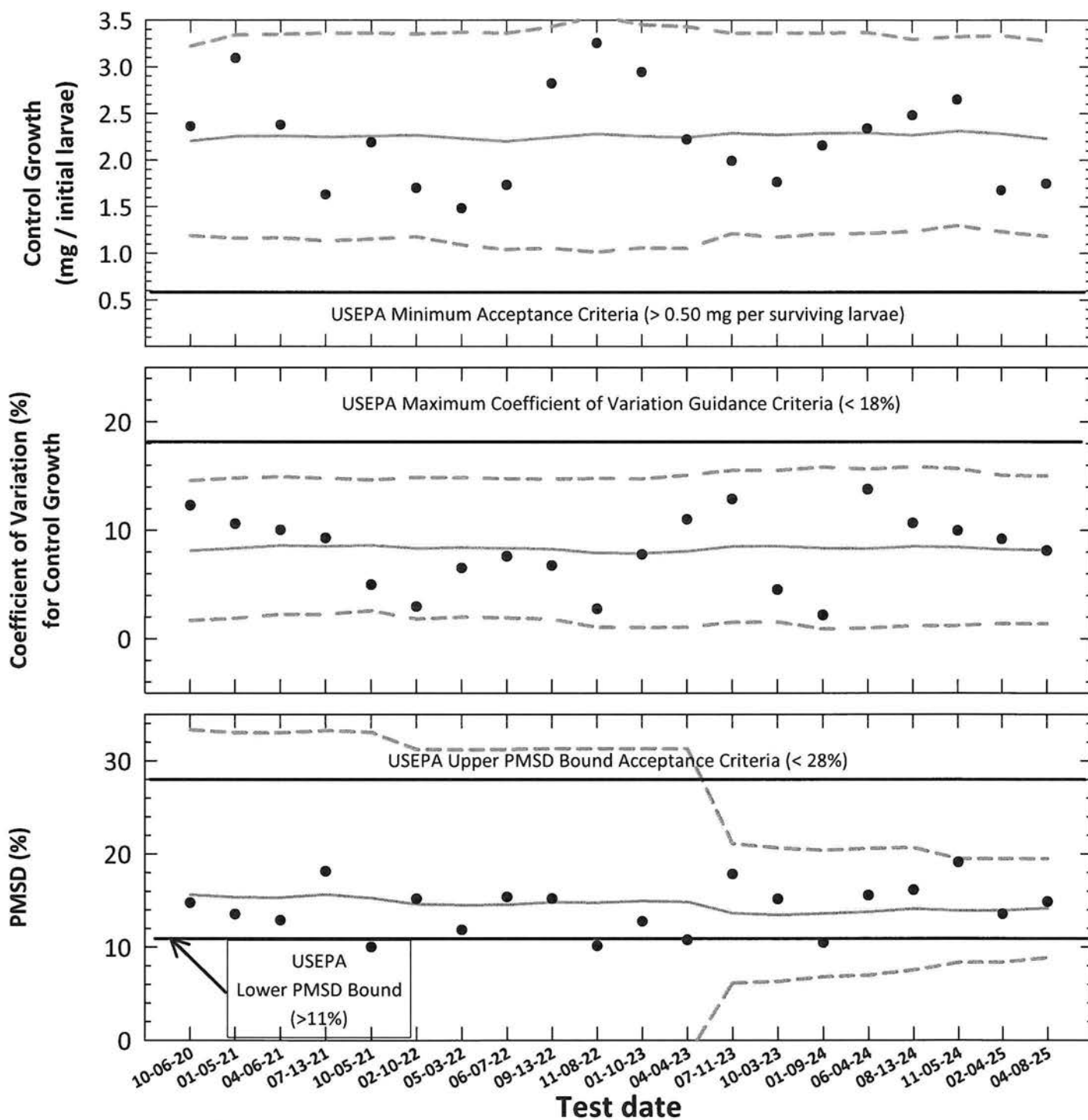
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.43).

CV = Coefficient of variation.

Menidia beryllina

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD	
		Control Survival (%)	Control Growth		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 larvae)	CV (%)							
1	10-06-20	100	2.361	12.3	14.8	2.2045	1.188 3.221	8.1	1.7 14.6	15.6	-2.1 33.3
2	01-05-21	100	3.093	10.6	0.4186	2.253	1.163 3.343	8.3	1.9 14.8	15.4	-2.3 33.0
3	04-06-21	100	2.377	10.0	0.3062	2.258	1.167 3.349	8.6	2.2 14.9	15.3	-2.3 33.0
4	07-13-21	100	1.631	9.3	0.2956	2.247	1.136 3.359	8.5	2.2 14.8	15.7	-2.0 33.3
5	10-05-21	100	2.189	5.0	0.2192	2.258	1.153 3.363	8.6	2.6 14.7	15.3	-2.5 33.1
6	02-10-22	100	1.701	3.0	0.2583	2.266	1.180 3.351	8.4	1.8 14.9	14.6	-2.0 31.2
7	05-03-22	100	1.483	6.5	0.1756	2.232	1.092 3.372	8.4	2.0 14.9	14.5	-2.1 31.2
8	06-07-22	100	1.733	7.6	0.2665	2.201	1.041 3.361	8.3	1.9 14.8	14.6	-2.1 31.2
9	09-13-22	100	2.822	6.8	0.4290	2.243	1.057 3.430	8.3	1.8 14.7	14.8	-1.7 31.3
10	11-08-22	100	3.255	2.7	0.3294	2.280	1.014 3.545	7.9	1.0 14.8	14.8	-1.8 31.3
11	01-10-23	100	2.945	7.8	0.3755	2.257	1.062 3.453	7.9	1.0 14.8	15.0	-1.4 31.3
12	04-04-23	100	2.220	11.0	0.2389	2.243	1.053 3.433	8.1	1.1 15.1	14.9	-1.6 31.3
13	07-11-23	100	1.992	12.9	0.3549	2.287	1.213 3.360	8.5	1.5 15.6	13.6	6.2 21.1
14	10-03-23	100	1.763	4.5	0.2673	2.270	1.174 3.366	8.5	1.5 15.5	13.5	6.3 20.6
15	01-09-24	100	2.156	2.2	0.2257	2.287	1.211 3.364	8.4	0.9 15.8	13.6	6.8 20.4
16	06-04-24	100	2.339	13.8	0.3637	2.291	1.215 3.368	8.3	1.0 15.6	13.8	7.0 20.6
17	08-13-24	100	2.479	10.7	0.4001	2.266	1.235 3.297	8.5	1.2 15.9	14.1	7.6 20.7
18	11-05-24	100	2.648	10.0	0.5061	2.312	1.299 3.324	8.5	1.2 15.7	13.9	8.3 19.5
19	02-04-25	100	1.675	9.2	0.2268	2.283	1.232 3.335	8.2	1.4 15.1	13.9	8.4 19.5
20	04-08-25	100	1.747	8.1	0.2598	2.230	1.183 3.278	8.2	1.4 15.0	14.2	8.9 19.5

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.50 mg/surviving larvae.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 18%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
Upper PMSD bound 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: **150**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2377				
Stock preparation:		100 mg Cu/L: Dissolve 0.1965 g CuSO ₄ in 500-mL deionized water				
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Test information:

Organism source:	Aquatic Indicators, Inc.	Randomizing template:	ORANGE
Age:	10-days old	Incubator number and shelf location:	6B
Batch:	AI Mb 03-30-25	Artemia CHM number:	CHM1385
Hatch dates and times:	03-29-25 1200 to 03-30-25 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = 7.73 Temperature (°C) = 24.7	Date / Time in oven:	04-15-25 1217
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	60°C
		Date / Time out of oven:	04-16-25 1217
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	04-08-25	0500	H	1530	H	1330	H	NA	04-01-25B
1	04-09-25	0500	H	1510	H	0934	H		↓
2	04-10-25	0500	H	1400 1500	H	1220	H		04-09-25
3	04-11-25	0500	H	1215	H	0930	H		↓
4	04-12-25	0530	H	1230	H	1205	H		04-11-25A
5	04-13-25	0500	H	1100	H	0914	H		04-11-25B
6	04-14-25	0500	H	1100	H	0930	H		↓
7	04-15-25					1204	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:	0%	≤ 20%	7-day LC ₅₀ (%)	0.33
Average weight per initial larvae:	1.747		NOEC (%)	0.10
Average weight per surviving larvae:	1.747	≥ 0.25mg/larvae	LOEC (%)	0.20
			ChV (%)	0.14
			IC ₂₅ (%)	0.238

Species: Menidia beryllina

MbCuCR Test Number: 150

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>plum</u> Analyst: <u>XL</u> Date: <u>03-20-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>04-17-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JS</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JS</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
1.747 1.942 -11.27. 1.844 -5.97.												

*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:

Species: Menidia beryllina

MbCuCR Test Number: 150

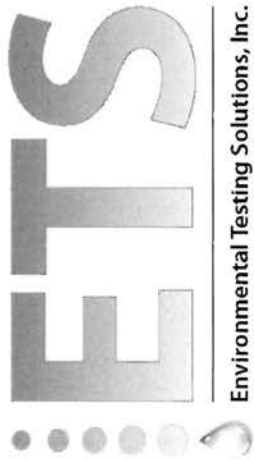
Survival and Growth Data

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	8 ^{2d}	8 ^{2d}	8 ^{2d}	9 ^{1d}
2	10	10	10	10	10	10	10	10	7 ^{1d}	6 ^{2d}	6 ^{2d}	6 ^{3d}
3	10	10	10	10	10	10	10	10	7	6	6	6
4	10	10	10	10	10	10	10	10	6 ^{1d}	5 ^{1d}	6	5 ^{1d}
5	10	10	10	10	9 ^{1d}	10	10	10	5 ^{1d}	3 ^{2d}	4 ^{2d}	4 ^{1d}
6	10	10	10	10	9	9 ^{1d}	10	8 ^{2d}	1 ^{4d}	2 ^{1d}	2 ^{1d}	3 ^{1d}
7	10	10	10	10	9	9	9 ^{1d}	8	1	2	2	2 ^{1d}
*A = Pan weight (mg) Tray color code: <u>plum</u> Analyst: <u>XL</u> Date: <u>03-20-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>04-17-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JS</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JS</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
1.926 -10.27 1.558 10.87 0.299 82.97												

*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:



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

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

Environmental Testing Solutions, Inc.

Test number: 150

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan * Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Not for Compliance Assessment: Internal Laboratory QC	Weight / Initial number of larvae (mg)	Mean survival (%)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.01	32.70	18.69	1.869	1.747	8.1	1.869	1.747	100.0	8.1	Not applicable
	B	10	10	14.19	32.21	18.02	1.802			1.802				
	C	10	10	15.12	32.86	17.74	1.774			1.774				
	D	10	10	13.84	29.26	15.42	1.542			1.542				
0.025	E	10	10	12.89	30.17	17.28	1.728	1.942	9.7	1.728	1.942	100.0	9.7	-11.2
	F	10	10	11.91	31.45	19.54	1.954			1.954				
	G	10	10	12.54	31.58	19.04	1.904			1.904				
	H	10	10	13.57	35.40	21.83	2.183			2.183				
0.050	I	10	10	14.22	33.46	19.24	1.924	1.849	4.4	1.924	1.849	100.0	4.4	-5.9
	J	10	10	14.70	33.10	18.40	1.840			1.840				
	K	10	10	12.10	31.03	18.93	1.893			1.893				
	L	10	10	13.70	31.09	17.39	1.739			1.739				
0.100	M	10	10	12.57	32.13	19.56	1.956	1.926	10.5	1.956	1.926	100.0	10.5	-10.2
	N	10	10	14.54	32.36	17.42	1.742			1.742				
	O	10	10	14.02	36.00	21.98	2.198			2.198				
	P	10	10	14.40	32.46	18.06	1.806			1.806				
0.200	Q	10	9	15.44	30.99	15.55	1.728	1.778	3.6	1.555	1.778	87.5	8.3	10.8
	R	10	9	11.73	28.22	16.49	1.832			1.649				
	S	10	9	15.06	31.57	16.51	1.834			1.651				
	T	10	8	12.11	25.96	13.75	1.719			1.375				
0.500	U	10	1	13.52	15.30	1.78	1.780	1.718	29.5	0.178	1.718	17.5	0.0	82.9
	V	10	2	11.80	14.96	3.16	1.580			0.316				
	W	10	2	13.69	18.42	4.73	2.365			0.473				
	X	10	2	12.80	15.09	2.29	1.145			0.229				

Dunnett's MSD value: 0.2598
PMDS: 14.9

MSD = Minimum Significant Difference
PMDS = Percent Minimum Significant Difference
PMDS 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.

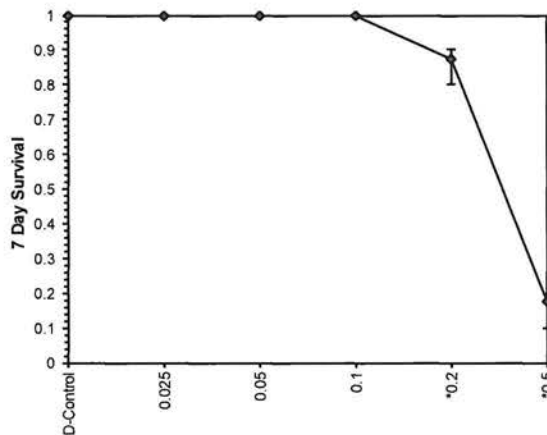
Lower PMSD bound determined by USEPA (10th percentile) = 11%.

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-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.





Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/8/2025	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	4/15/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

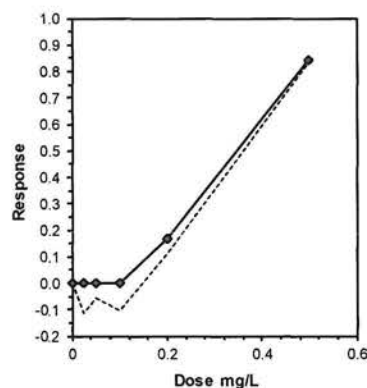
Conc-mg/L	1	2	3	4
D-Control	1.8690	1.8020	1.7740	1.5420
0.025	1.7280	1.9540	1.9040	2.1830
0.05	1.9240	1.8400	1.8930	1.7390
0.1	1.9560	1.7420	2.1980	1.8060
0.2	1.5550	1.6490	1.6510	1.3750
0.5	0.1780	0.3160	0.4730	0.2290

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean
D-Control	1.7468	1.0000	1.7468	1.5420	1.8690	8.141	4				1.8659	1.0000
0.025	1.9423	1.1119	1.9423	1.7280	2.1830	9.654	4	-1.723	2.290	0.2598	1.8659	1.0000
0.05	1.8490	1.0585	1.8490	1.7390	1.9240	4.387	4	-0.901	2.290	0.2598	1.8659	1.0000
0.1	1.9255	1.1023	1.9255	1.7420	2.1980	10.522	4	-1.575	2.290	0.2598	1.8659	1.0000
0.2	1.5575	0.8917	1.5575	1.3750	1.6510	8.324	4				1.5575	0.8347
0.5	0.2990	0.1712	0.2990	0.1780	0.4730	43.223	4				0.2990	0.1602

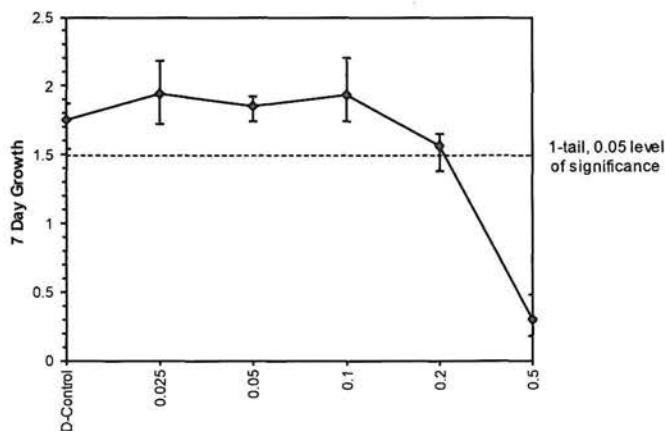
Auxiliary Tests					Statistic		Critical	Skew	Kurt					
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95167		0.844	0.23321	-0.306					
Bartlett's Test indicates equal variances (p = 0.54)					2.18247		11.3449							
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					0.1	>0.1			0.25985	0.14876	0.03182	0.02575	0.33973	3, 12
Treatments vs D-Control														

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1303	0.0154	0.0528	0.1563	-2.9549
IC10	0.1605	0.0148	0.1118	0.2126	0.2032
IC15	0.1908	0.0152	0.1416	0.2346	0.0045
IC20	0.2154	0.0134	0.1677	0.2536	-0.1917
IC25	0.2377	0.0127	0.1929	0.2730	-0.1961
IC40	0.3044	0.0105	0.2670	0.3340	0.0724
IC50	0.3489	0.0099	0.3198	0.3769	0.2561



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 150

Daily Chemistry:

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

Concentration		Day					
		(Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
Analyst		Em N	Em N	Em N	Em N	Em N	N
Parameter	Parameter						
CONTROL, SaltSW	pH (S.U.)	8.23	7.96	7.89	7.86	7.97	7.02
	Dissolved oxygen (mg/L)	0.3	0.0	0.0	0.0	0.2	7.0
	Salinity (ppt)	25.0	25.1	25.0	25.0	24.7	24.25 25.2
	Alkalinity (mg CaCO ₃ /L)	100				(250) 90	
	Temperature (°C)	24.9	24.9	24.8	24.8	24.7	24.7
0.025 mg/L	pH (S.U.)	8.10	7.94	8.07	7.87	7.95	7.03
	Dissolved oxygen (mg/L)	0.3	0.0	0.0	0.0	0.2	7.1
	Salinity (ppt)	25.0	25.2	25.2	25.5	25.2	25.5
	Temperature (°C)	25.0	25.1	24.8	24.5	24.7	24.9
0.05 mg/L	pH (S.U.)	8.10	7.96	8.05	7.86	7.94	7.03
	Dissolved oxygen (mg/L)	0.3	0.0	0.0	0.0	0.2	7.1
	Salinity (ppt)	24.9	25.4	25.3	25.5	25.3	25.5
	Temperature (°C)	25.0	24.9	24.9	24.7	24.6	24.9
0.1 mg/L	pH (S.U.)	8.10	7.98	8.05	7.88	7.95	7.29
	Dissolved oxygen (mg/L)	0.4	0.0	0.0	0.0	0.2	7.0
	Salinity (ppt)	25.1	25.7	25.3	25.6	25.2	25.5
	Temperature (°C)	25.0	25.0	24.9	24.7	24.6	24.6
0.2 mg/L	pH (S.U.)	8.10	7.96	8.05	7.88	7.96	7.01
	Dissolved oxygen (mg/L)	0.4	0.0	0.0	0.0	0.2	7.1
	Salinity (ppt)	25.0	25.6	25.2	25.5	25.1	25.5
	Temperature (°C)	24.8	25.0	24.9	24.6	24.6	24.6
0.5 mg/L	pH (S.U.)	8.11	7.96	8.06	7.88	7.97	7.04
	Dissolved oxygen (mg/L)	0.4	0.0	0.0	0.0	0.2	7.2
	Salinity (ppt)	25.0	25.6	25.1	25.4	25.1	25.4
	Alkalinity (mg CaCO ₃ /L)						
	Temperature (°C)	25.0	25.0	24.9	24.6	24.6	24.8
Initial		Final	Initial	Final	Initial	Final	

Species: Menidia beryllina

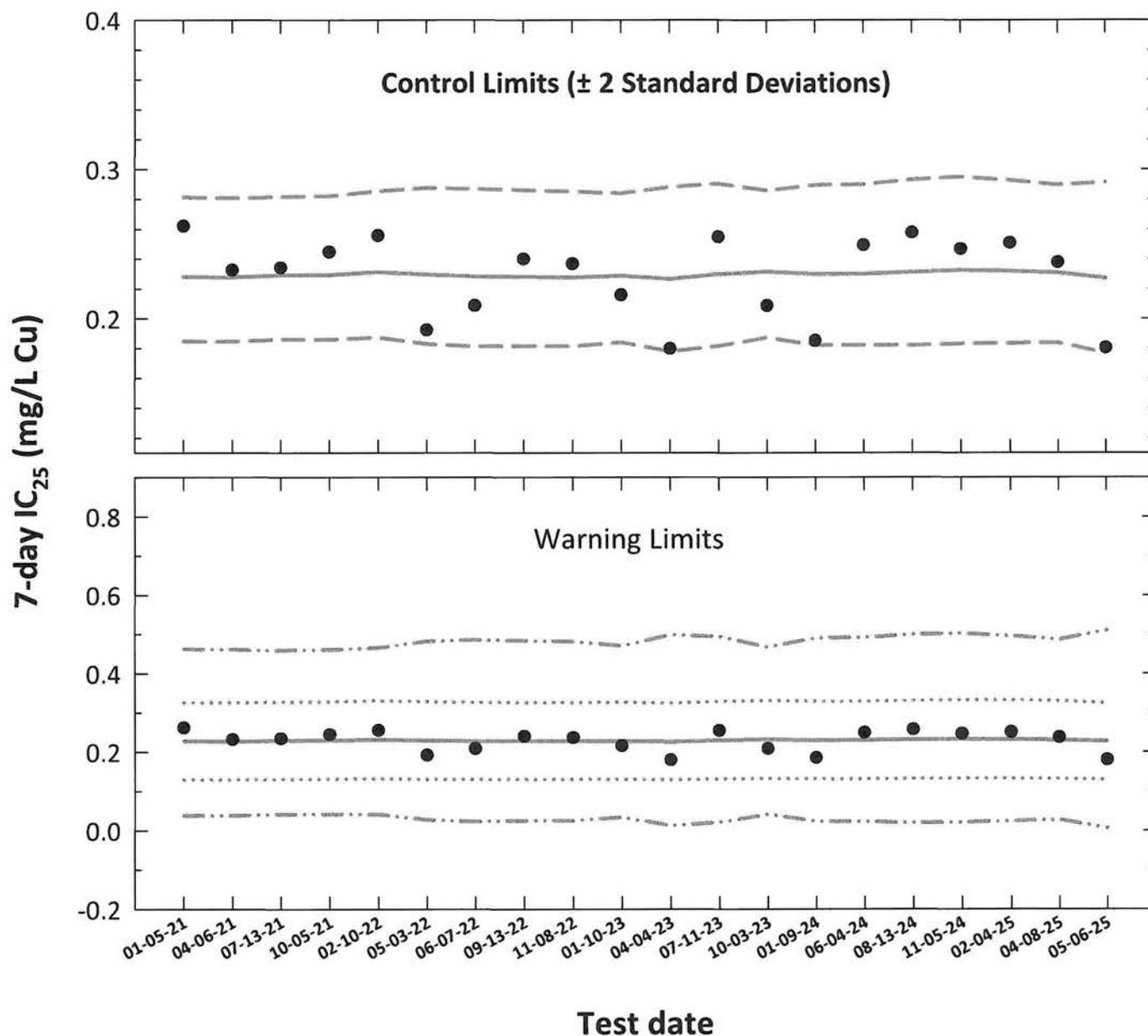
MbCuCR Test Number: 150

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
		Analyst							
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	N	BSL	BSL	BSL	BSL	EM	EM	N
	Dissolved oxygen (mg/L)	7.99	7.89	8.24	7.93	8.07	7.88	7.93	7.74
	Salinity (ppt)	24.0	25.6	24.9	25.3	24.8	25.2	25.0	25.3
	Alkalinity (mg CaCO ₃ /L)			95		90			
	Temperature (°C)	24.7	24.8	24.7	24.6	24.7	24.3	24.6	24.5
0.025 mg/L	pH (S.U.)	8.03	7.87	8.08	7.91	8.05	7.90	8.00	7.76
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.8	7.9	7.7	8.0	6.4
	Salinity (ppt)	25.2	25.4	25.2	25.4	25.1	25.4	25.2	25.3
	Temperature (°C)	24.8	24.5	24.7	24.6	24.6	24.6	24.6	24.1
0.05 mg/L	pH (S.U.)	8.04	7.88	8.08	7.92	8.07	7.91	7.99	7.77
	Dissolved oxygen (mg/L)	8.0	7.7	8.1	7.8	7.9	7.7	8.0	6.6
	Salinity (ppt)	25.2	25.4	25.2	25.6	25.0	25.4	25.1	25.3
	Temperature (°C)	24.9	24.5	24.8	24.5	24.6	24.6	24.6	24.5
0.1 mg/L	pH (S.U.)	8.03	7.87	8.08	7.92	8.07	7.88	7.99	7.77
	Dissolved oxygen (mg/L)	8.0	7.7	8.1	7.8	8.0	7.6	8.0	6.5
	Salinity (ppt)	25.1	25.4	25.2	25.5	25.0	25.4	25.1	25.4
	Temperature (°C)	24.9	24.7	24.9	24.6	24.7	24.5	24.7	24.5
0.2 mg/L	pH (S.U.)	8.03	7.90	8.08	7.93	8.08	7.90	7.99	7.70
	Dissolved oxygen (mg/L)	8.0	7.8	8.1	7.8	8.0	7.6	8.0	6.6
	Salinity (ppt)	25.1	25.6	25.1	25.3	25.0	25.4	25.2	25.3
	Temperature (°C)	24.9	24.7	24.9	24.5	24.9	24.5	24.7	24.5
0.5 mg/L	pH (S.U.)	8.03	7.86	8.08	7.90	8.07	7.89	7.99	7.80
	Dissolved oxygen (mg/L)	8.0	7.8	8.1	7.6	7.9	7.6	8.0	6.6
	Salinity (ppt)	25.0	25.5	25.1	25.7	24.9	25.2	25.0	25.2
	Temperature (°C)	24.9	24.9	24.9	24.5	24.9	24.7	24.6	24.4
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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	Anti-logarithmic Values (mg/L Cu)		
			7-day IC ₂₅	CT	S	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A.75} CT + S _{A.75}
1	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.1848 0.2816	0.0382 0.4625	0.1300 0.3262
2	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.1847 0.2810	0.0385 0.4612	0.1298 0.3258
3	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.1862 0.2817	0.0420 0.4590	0.1305 0.3274
4	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.1861 0.2822	0.0414 0.4604	0.1306 0.3277
5	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.1874 0.2855	0.0414 0.4656	0.1318 0.3307
6	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.1832 0.2877	0.0276 0.4827	0.1309 0.3283
7	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.1815 0.2872	0.0233 0.4861	0.1301 0.3265
8	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.1816 0.2861	0.0246 0.4831	0.1299 0.3259
9	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.1816 0.2853	0.0254 0.4811	0.1297 0.3255
10	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.1841 0.2841	0.0337 0.4708	0.1304 0.3270
11	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.1781 0.2884	0.0125 0.4991	0.1292 0.3241
12	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.1818 0.2905	0.0208 0.4939	0.1310 0.3286
13	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.1873 0.2859	0.0408 0.4669	0.1319 0.3309
14	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.1824 0.2896	0.0233 0.4900	0.1310 0.3286
15	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.1823 0.2901	0.0227 0.4914	0.1311 0.3288
16	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.1823 0.2932	0.0196 0.4996	0.1318 0.3306
17	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.1831 0.2950	0.0201 0.5019	0.1325 0.3323
18	02-04-25	0.2507	-0.6008	-0.6349	0.0507	0.1835 0.2928	0.0234 0.4950	0.1321 0.3314
19	04-08-25	0.2377	-0.6240	-0.6369	0.0494	0.1838 0.2896	0.0273 0.4861	0.1315 0.3299
20	05-06-25	0.1805	-0.7435	-0.6438	0.0542	0.1769 0.2915	0.0062 0.5105	0.1294 0.3247

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

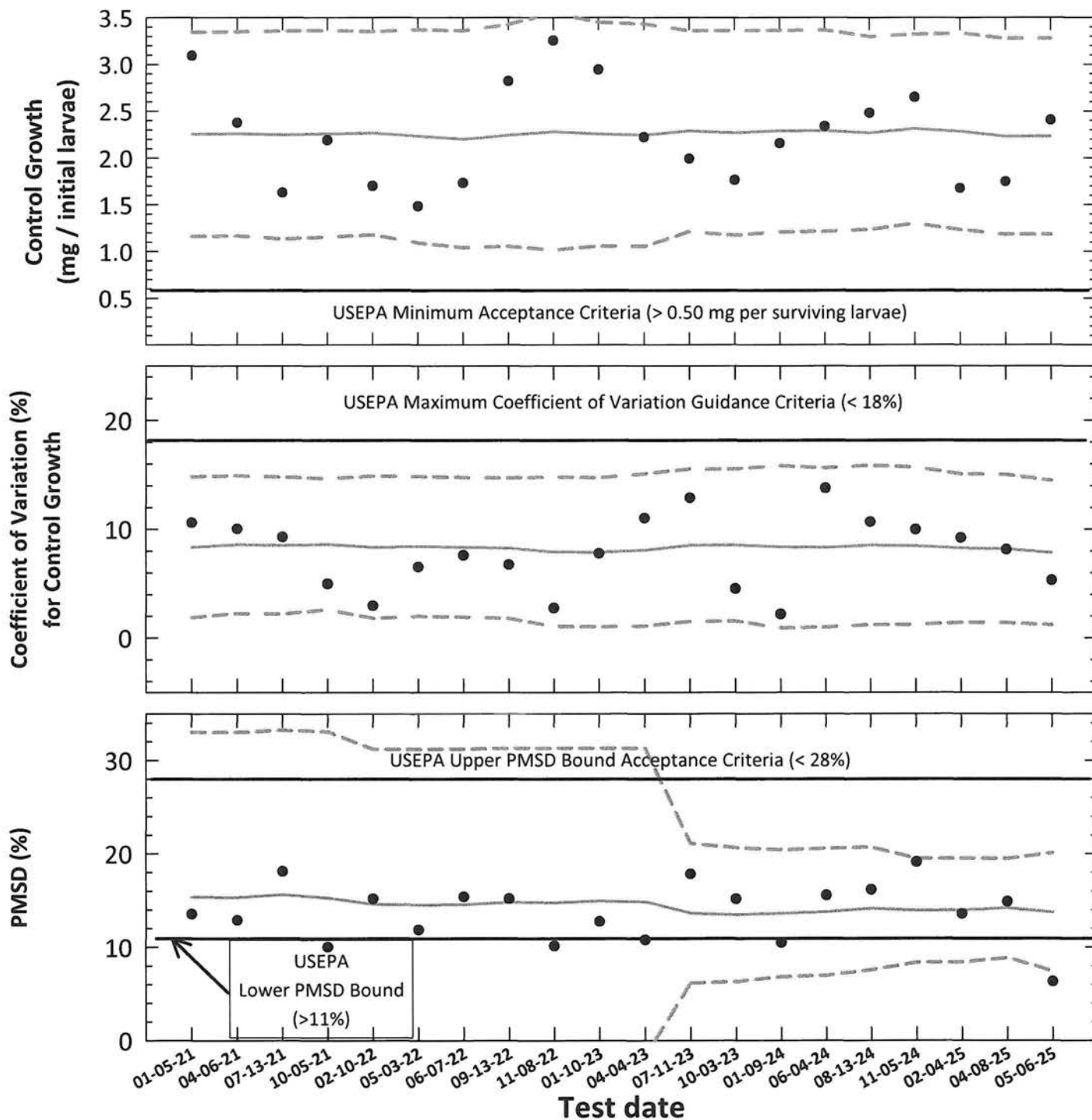
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A.75} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Menidia beryllina

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



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

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

Test number	Test date	ToxCal Determination				Control Growth		Control Growth		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
			Mean (mg/initial larvae)	CV (%)									
1	01-05-21	100	3.093	10.6	0.4186	2.2532	1.163	3.343	8.3	1.9	14.8	15.4	-2.3
2	04-06-21	100	2.377	10.0	0.3062	2.258	1.167	3.349	8.6	2.2	14.9	15.3	-2.3
3	07-13-21	100	1.631	9.3	0.2956	2.247	1.136	3.359	8.5	2.2	14.8	15.7	-2.0
4	10-05-21	100	2.189	5.0	0.2192	2.258	1.153	3.363	8.6	2.6	14.7	15.3	-2.5
5	02-10-22	100	1.701	3.0	0.2583	2.266	1.180	3.351	8.4	1.8	14.9	14.6	-2.0
6	05-03-22	100	1.483	6.5	0.1756	2.232	1.092	3.372	8.4	2.0	14.9	14.5	-2.1
7	06-07-22	100	1.733	7.6	0.2665	2.201	1.041	3.361	8.3	1.9	14.8	14.6	-2.1
8	09-13-22	100	2.822	6.8	0.4290	2.243	1.057	3.430	8.3	1.8	14.7	14.8	-1.7
9	11-08-22	100	3.255	2.7	0.3294	2.280	1.014	3.545	7.9	1.0	14.8	14.8	-1.8
10	01-10-23	100	2.945	7.8	0.3755	2.257	1.062	3.453	7.9	1.0	14.8	15.0	-1.4
11	04-04-23	100	2.220	11.0	0.2389	2.243	1.053	3.433	8.1	1.1	15.1	14.9	-1.6
12	07-11-23	100	1.992	12.9	0.3549	2.287	1.213	3.360	8.5	1.5	15.6	13.6	6.2
13	10-03-23	100	1.763	4.5	0.2673	2.270	1.174	3.366	8.5	1.5	15.5	13.5	6.3
14	01-09-24	100	2.156	2.2	0.2257	2.287	1.211	3.364	8.4	0.9	15.8	13.6	6.8
15	06-04-24	100	2.339	13.8	0.3637	2.291	1.215	3.368	8.3	1.0	15.6	13.8	7.0
16	08-13-24	100	2.479	10.7	0.4001	2.266	1.235	3.297	8.5	1.2	15.9	14.1	7.6
17	11-05-24	100	2.648	10.0	0.5061	2.312	1.299	3.324	8.5	1.2	15.7	13.9	8.3
18	02-04-25	100	1.675	9.2	0.2268	2.283	1.232	3.335	8.2	1.4	15.1	13.9	8.4
19	04-08-25	100	1.747	8.1	0.2598	2.230	1.183	3.278	8.2	1.4	15.0	14.2	8.9
20	05-06-25	100	2.406	5.3	0.1521	2.233	1.184	3.281	7.9	1.2	14.5	13.7	7.4

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: 151

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS <u>2387</u>				
Stock preparation:		100 mg Cu/L: Dissolve 0.1965 g CuSO ₄ in 500-mL deionized water				
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Organism source:	Aquatic Indicators, Inc.	Randomizing template:	<u>ORANGE</u>
Age:	10-days old	Incubator number and shelf location:	<u>6B</u>
Batch:	AI Mb 04-26-25	Artemia CHM number:	CHM1385
Hatch dates and times:	04-25-25 1200 to 04-26-25 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = <u>7.29</u> Temperature (°C) = <u>24.7</u>	Date / Time in oven:	<u>05-13-25 1155</u>
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>05-14-25 1155</u>
		*Final oven temperature:	<u>60 °C</u>
		Total drying time:	<u>24-HOURS</u>

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	05-06-25	1130	H	1400	H	1352	H	NA	04-30-25 B
1	05-07-25	0500	H	1420	H	0925	H		05-06-25
2	05-08-25	0500	H	1330	H	1210	H		05-06-25
3	05-09-25	0500	H	1100	H	0845	H		05-06-25
4	05-10-25	0600	H	1200	H	1150	H		05-06-25
5	05-11-25	0600	H	1200	H	0933	H		05-06-25
6	05-12-25	0500	H	1100	H	0830	H		05-06-25
7	05-13-25					1152	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:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (%)	<u>0.310</u>
Average weight per initial larvae:	<u>2.406</u>		NOEC (%)	<u>0.025</u>
Average weight per surviving larvae:	<u>2.406</u>	≥ 0.25mg/larvae	LOEC (%)	<u>0.050</u>
* INTERPRETED DOSE RESPONSE. LOWEST VALUES USED FOR HYPOTHESIS TESTS - MOST CONSERVATIVE.			ChV (%)	<u>0.035</u>
			IC ₂₅ (%)	<u>0.181</u>

Species: Menidia beryllina

MbCuCR Test Number: 151

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>Dark Blue</u> Analyst: <u>XL</u> Date: <u>04-22-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>05-15-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JA</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JA</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
2.406 2.313 3.97 2.101 12.77												

*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: 151

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	8 ^{2d}	7 ^{3d}	8 ^{2d}	7 ^{3d}
2	10	10	10	10	10	10	10	10	7 ^{1d}	7	6 ^{2d}	6 ^{1d}
3	10	10	10	10	10	10	10	10	6 ^{1d}	5 ^{1d}	6	6
4	10	10	10	10	9 ^{1d}	10	10	9 ^{1d}	3 ^{3d}	3 ^{2d}	4 ^{2d}	2 ^{4d}
5	10	10	10	10	9	10	10	9	3	3	4	2
6	10	10	10	10	9	8 ^{1d}	10	9	1 ^{2d}	3	2 ^{2d}	1 ^{1d}
7	10	10	10	10	8 ^{1d}	8	9 ^{1d}	9	1	1 ^{2d}	2	1
*A = Pan weight (mg) Tray color code: <u>Dark Blue</u> Analyst: <u>XL</u> Date: <u>04-22-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>05-15-25</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 (%)												
2.287 4.97 1.710 28.97 0.227 90.67												

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

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

Comments:

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: 151

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / surviving number of larvae (mg)	Mean weight / surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / initial number of larvae (mg)	Mean survival (%)	Mean weight / initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	9.80	34.88	25.08	2.508	2.406	5.3	2.508		2.406		
	B	10	10	10.81	33.06	22.25	2.225			2.225	100.0	2.406	5.3	Not applicable
	C	10	10	10.84	34.90	24.06	2.406			2.406				
	D	10	10	10.97	35.81	24.84	2.484			2.484				
0.025	E	10	10	10.21	32.11	21.90	2.190			2.190				
	F	10	10	9.78	33.48	23.70	2.370	2.313	4.1	2.370	100.0	2.313	4.1	3.9
	G	10	10	10.17	34.21	24.04	2.404			2.404				
	H	10	10	10.33	33.20	22.87	2.287			2.287				
0.050	I	10	10	10.59	31.25	20.66	2.066			2.066				
	J	10	10	10.67	32.00	21.33	2.133	2.101	1.9	2.133	100.0	2.101	1.9	12.7
	K	10	10	10.35	31.74	21.39	2.139			2.139				
	L	10	10	10.41	31.06	20.65	2.065			2.065				
0.100	M	10	10	10.51	32.28	21.77	2.177			2.177				
	N	10	10	10.28	32.86	22.58	2.258	2.287	3.9	2.258	100.0	2.287	3.9	4.9
	O	10	10	10.09	33.94	23.85	2.385			2.385				
	P	10	10	15.81	39.10	23.29	2.329			2.329				
0.200	Q	10	8	10.33	25.68	15.35	1.919			1.535				
	R	10	8	10.03	23.70	13.67	1.709	2.001	11.8	1.367	85.0	1.710	18.0	28.9
	S	10	9	10.33	30.43	20.10	2.233			2.010				
	T	10	9	10.34	29.62	19.28	2.142			1.928				
0.500	U	10	1	9.95	10.79	0.84	0.840			0.084				
	V	10	1	10.74	13.03	2.29	2.290	1.770	36.0	0.229	12.5	0.227	0.0	90.6
	W	10	2	10.50	14.46	3.96	1.980			0.396				
	X	10	1	10.75	12.72	1.97	1.970			0.197				

Dunnett's MSD value: 0.1521
PMSD: 6.3

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.025	1.0000	1.0000	1.0000	1.0000
0.05	1.0000	1.0000	1.0000	1.0000
0.1	1.0000	1.0000	1.0000	1.0000
0.2	0.8000	0.8000	0.9000	0.9000
0.5	0.1000	0.1000	0.2000	0.1000

Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.2	0.8500	0.8500	1.1781	1.1071	1.2490	6.954	4	10.00	10.00	6	40
*0.5	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77127	0.884	0.84747	2.20368
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.6295	0.81389	4.03428	7.22473	0	0.14073	7.81472	0.98654	-0.5084	0.17764	3
Intercept	7.86214	0.46087	6.95883	8.76545							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	0.11977	0.0805	0.15188
EC05	3.355	0.15827	0.11726	0.19115
EC10	3.718	0.18363	0.14272	0.21695
EC15	3.964	0.20299	0.16253	0.23693
EC20	4.158	0.21983	0.17983	0.25464
EC25	4.326	0.23538	0.19576	0.27142
EC40	4.747	0.27963	0.23993	0.32208
EC50	5.000	0.31016	0.26883	0.36012
EC60	5.253	0.34402	0.29914	0.40542
EC75	5.674	0.40869	0.35269	0.50012
EC80	5.842	0.43761	0.37521	0.54544
EC85	6.036	0.4739	0.40256	0.60458
EC90	6.282	0.52388	0.43888	0.68964
EC95	6.645	0.60781	0.49727	0.84083
EC99	7.326	0.8032	0.62478	1.22697

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	5/6/2025	Test ID:	MBCuCR	Sample ID:	REF-Ref Toxicant
End Date:	5/13/2025	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	2.5080	2.2250	2.4060	2.4840
0.025	2.1900	2.3700	2.4040	2.2870
0.05	2.0660	2.1330	2.1390	2.0650
0.1	2.1770	2.2580	2.3850	2.3290
0.2	1.5350	1.3670	2.0100	1.9280
0.5	0.0840	0.2290	0.3960	0.1970

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean	
D-Control	2.4058	1.0000	2.4058	2.2250	2.5080	5.326	4					2.4058	1.0000
0.025	2.3128	0.9613	2.3128	2.1900	2.4040	4.127	4	1.400	2.290	0.1521		2.3128	0.9613
*0.05	2.1008	0.8732	2.1008	2.0650	2.1390	1.941	4	4.592	2.290	0.1521		2.1940	0.9120
0.1	2.2873	0.9507	2.2873	2.1770	2.3850	3.936	4	1.784	2.290	0.1521		2.1940	0.9120
0.2	1.7100	0.7108	1.7100	1.3670	2.0100	18.050	4					1.7100	0.7108
0.5	0.2265	0.0941	0.2265	0.0840	0.3960	56.948	4					0.2265	0.0941

Auxiliary Tests

Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)

Bartlett's Test indicates equal variances ($p = 0.41$)

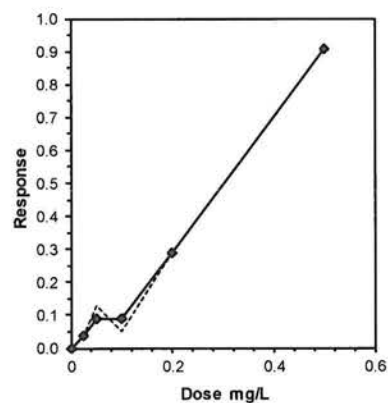
Hypothesis Test (1-tail, 0.05)

Statistic	Critical	Skew	Kurt
0.92765	0.844	-0.7236	-0.1677
2.90025	11.3449		

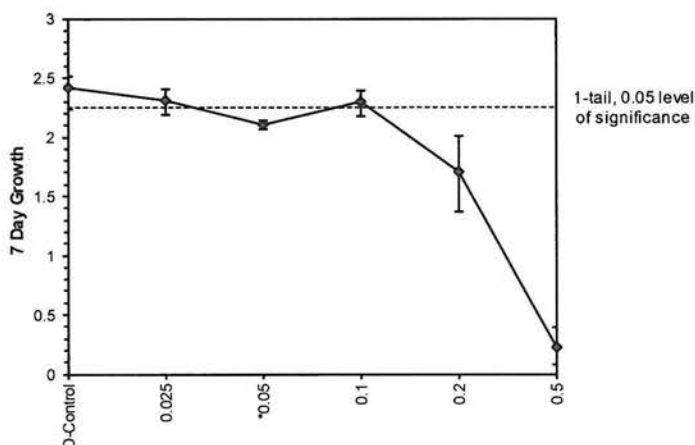
[illegible]

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95%CL(Exp)	Skew	
IC05	0.0307	0.0183	0.0039	0.1440	2.3606
IC10	0.1060	0.0353	0.0000	0.1427	-0.4689
IC15	0.1308	0.0166	0.0984	0.1922	1.0766
IC20	0.1557	0.0208	0.1162	0.2425	0.8741
IC25	0.1805	0.0224	0.1300	0.2596	0.4099
IC40	0.2539	0.0235	0.1742	0.3178	-0.4224
IC50	0.3026	0.0198	0.2307	0.3552	-0.5433



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 151

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.

		Day					
		(Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
Analyst		JP VL	JP EM	JP EM	JP EM	JP EM	XL
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	8.00	7.85	7.91	7.80	7.99	7.81
	Dissolved oxygen (mg/L)	8.3	7.8	8.0	7.8	8.0	8.0
	Salinity (ppt)	25.0	25.8	25.0	25.1	25.0	25.3
	Alkalinity (mg CaCO ₃ /L)	92		89		77	
	Temperature (°C)	24.8	25.2	24.7	24.5	24.7	24.7
0.025 mg/L	pH (S.U.)	8.04	7.85	7.95	7.81	7.88	7.82
	Dissolved oxygen (mg/L)	8.3	7.8	8.0	7.9	8.0	8.0
	Salinity (ppt)	25.0	25.8	25.4	25.6	25.2	25.4
	Temperature (°C)	24.9	25.2	24.8	24.3	24.6	24.9
0.05 mg/L	pH (S.U.)	8.04	7.85	7.95	7.83	7.88	7.83
	Dissolved oxygen (mg/L)	8.3	7.8	8.0	7.9	8.0	8.0
	Salinity (ppt)	25.2	25.6	25.4	25.6	25.2	25.8
	Temperature (°C)	24.8	25.0	24.8	24.3	24.6	24.6
0.1 mg/L	pH (S.U.)	8.04	7.85	7.95	7.83	7.88	7.81
	Dissolved oxygen (mg/L)	8.3	7.8	8.0	7.9	8.0	8.0
	Salinity (ppt)	25.5	25.8	25.4	25.8	25.2	25.4
	Temperature (°C)	24.3	25.0	24.8	24.6	24.7	24.6
0.2 mg/L	pH (S.U.)	8.05	7.86	7.95	7.83	7.88	7.80
	Dissolved oxygen (mg/L)	8.3	7.8	8.0	7.9	8.0	8.0
	Salinity (ppt)	25.2	25.8	25.4	25.7	25.0	25.3
	Temperature (°C)	24.3	25.1	24.9	24.2	24.6	24.9
0.5 mg/L	pH (S.U.)	8.05	7.84	7.95	7.83	7.87	7.83
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	7.9	8.0	25.3
	Salinity (ppt)	25.1	25.4	25.3	25.6	25.0	
	Alkalinity (mg CaCO ₃ /L)	→ 85	→ 85				
	Temperature (°C)	24.4	25.2	24.9	24.2	24.6	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: *Menidia beryllina*

MbCuCR Test Number: 151

Analyst		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
		XL	BSL	BSL	BSL	BSL	XL	XL	IV
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.88	7.87	8.04	7.88	8.02	7.88	7.99	7.66
	Dissolved oxygen (mg/L)	8.1	7.9	8.1	7.9	8.1	7.9	8.1	7.0
	Salinity (ppt)	25.0	25.2	24.9	25.1	25.0	25.3	24.4	24.9
	Alkalinity (mg CaCO ₃ /L)	91		89			91		
	Temperature (°C)	24.7	24.9	24.8	24.7	24.7	24.5	24.8	24.5
0.025 mg/L	pH (S.U.)	7.90	7.84	8.01	7.86	8.05	7.84	8.03	7.65
	Dissolved oxygen (mg/L)	8.3	8.0	8.1	7.9	8.0	7.9	8.2	6.7
	Salinity (ppt)	25.1	25.3	25.0	25.2	24.9	25.2	25.0	25.3
	Temperature (°C)	24.6	25.0	24.7	24.7	24.7	24.7	24.9	24.5
0.05 mg/L	pH (S.U.)	7.89	7.82	8.01	7.86	8.06	7.87	8.03	7.65
	Dissolved oxygen (mg/L)	8.3	8.0 (7.9)	8.0	7.9	8.0	7.9	8.1	6.9
	Salinity (ppt)	25.0	25.3	25.0	25.4	24.9	25.3	25.0	25.3
	Temperature (°C)	24.6	25.1	24.7	24.6	24.7	24.7	24.9	24.6
0.1 mg/L	pH (S.U.)	7.89	7.82	8.01	7.84	8.06	7.85	8.04	7.66
	Dissolved oxygen (mg/L)	8.3	7.9	8.1	7.8	8.1	7.9	8.1	7.0
	Salinity (ppt)	25.0	25.4	25.0	25.2	24.9	25.3	25.0	25.3
	Temperature (°C)	24.6	25.1	24.7	24.6	24.8	24.4	25.0	24.6
0.2 mg/L	pH (S.U.)	7.90	7.82	8.01	7.87	8.06	7.87	8.04	7.66
	Dissolved oxygen (mg/L)	8.2	7.9	8.1	7.8	8.1	7.8	8.1	7.0
	Salinity (ppt)	25.0	25.4	25.0	25.3	24.9	25.3	25.0	25.3
	Temperature (°C)	24.7	25.2	25.0	24.8	24.8	24.6	25.0	24.7
0.5 mg/L	pH (S.U.)	7.89	7.80	8.01	7.83	8.06	7.85	8.04	7.67
	Dissolved oxygen (mg/L)	8.2	7.8	8.1	7.8	8.1	7.7	8.1	6.7
	Salinity (ppt)	25.1	25.2	25.0	25.4	24.9	25.1	25.0	25.3
	Temperature (°C)	24.6	25.2	25.0	24.6	24.8	24.6	25.0	24.5
		Initial	Final	Initial	Final	Initial	Final	Initial	Final