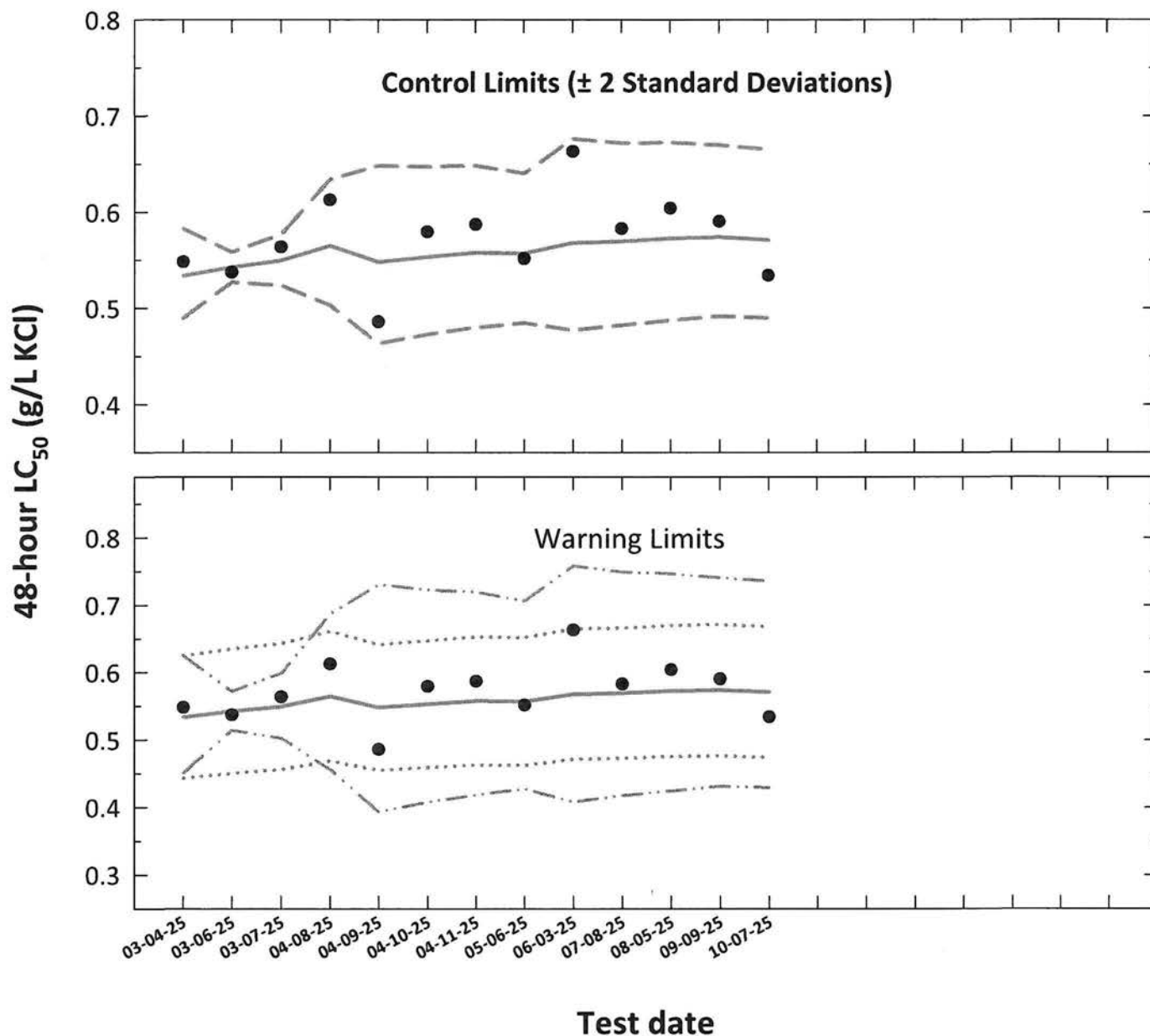


Americamysis (Mysidopsis) bahia
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)					
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	03-04-25	0.5485	-0.2608	-0.2652	0.0063	0.5430	0.5275	0.5588	0.5145	0.5722
2	03-06-25	0.5374	-0.2697	-0.2597	0.0105	0.5499	0.5239	0.5772	0.5026	0.5995
3	03-07-25	0.5640	-0.2487	-0.2480	0.0251	0.5650	0.5034	0.6342	0.4559	0.6874
4	04-08-25	0.6129	-0.2126	-0.2610	0.0364	0.5482	0.4635	0.6484	0.3937	0.7310
5	04-09-25	0.4860	-0.3134	-0.2570	0.0341	0.5533	0.4730	0.6473	0.4082	0.7231
6	04-10-25	0.5796	-0.2369	-0.2533	0.0326	0.5581	0.4803	0.6484	0.4187	0.7200
7	04-11-25	0.5872	-0.2312	-0.2539	0.0302	0.5573	0.4849	0.6405	0.4273	0.7066
8	05-06-25	0.5517	-0.2583	-0.2455	0.0379	0.5681	0.4772	0.6763	0.4082	0.7586
9	06-03-25	0.6631	-0.1784	-0.2444	0.0359	0.5696	0.4829	0.6719	0.4174	0.7492
10	07-08-25	0.5828	-0.2345	-0.2421	0.0349	0.5726	0.4877	0.6724	0.4242	0.7469
11	08-05-25	0.6040	-0.2189	-0.2410	0.0335	0.5741	0.4921	0.6698	0.4312	0.7408
12	09-09-25	0.5904	-0.2289	-0.2434	0.0332	0.5709	0.4899	0.6653	0.4291	0.7362
13	10-07-25	0.5341	-0.2724							
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 # 14

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 #: 2412

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.93	7.94	8.01
	Dissolved oxygen (mg/L)	8.4	7.8	8.0
	*Salinity (ppt)	25.0	25.5	25.5
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	25.6	24.7	25.0
150 mg/L	pH (S.U.)	7.99	7.93	7.94
	Dissolved oxygen (mg/L)	8.4	7.8	8.0
	*Salinity (ppt)	25.0	25.5	26.1
	*Temperature (°C)	25.6	25.0	24.6
300 mg/L	pH (S.U.)	7.99	7.93	7.95
	Dissolved oxygen (mg/L)	8.4	7.8	8.1
	*Salinity (ppt)	25.1	25.8	26.1
	*Temperature (°C)	25.5	25.2	24.8
450 mg/L	pH (S.U.)	7.99	7.95	7.96
	Dissolved oxygen (mg/L)	8.4	7.9	8.1
	*Salinity (ppt)	25.2	25.6	26.1
	*Temperature (°C)	25.6	25.2	24.8
600 mg/L	pH (S.U.)	7.99	7.94	7.96
	Dissolved oxygen (mg/L)	8.6	7.9	8.0
	*Salinity (ppt)	25.2	25.7	26.1
	*Temperature (°C)	25.3	25.0	25.0
750 mg/L	pH (S.U.)	8.00	7.96	
	Dissolved oxygen (mg/L)	8.4	7.8	
	*Salinity (ppt)	25.3	25.8	
	*Temperature (°C)	25.5	24.9	

*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 # 14

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-07-25	* 1200	JP	1410	JP	6D	Light + Green	09-29-25A
24	10-08-25			1423	JP			
48 Termination	10-09-25			1355	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):	10-06-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	10-05-25 1200 10-06-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.21 Temperature (°C): 24.9°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	10	10	10	10
48 Termination	10	10	10	10	10	10	10	10	10	10	10	10
Mean Survival	100%		100%		100%		90%		20%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	497.6
Upper 95% confidence limit (mg KCl/L)	570.3
48-hour LC ₅₀ (mg KCl/L)	534.1

Comments:

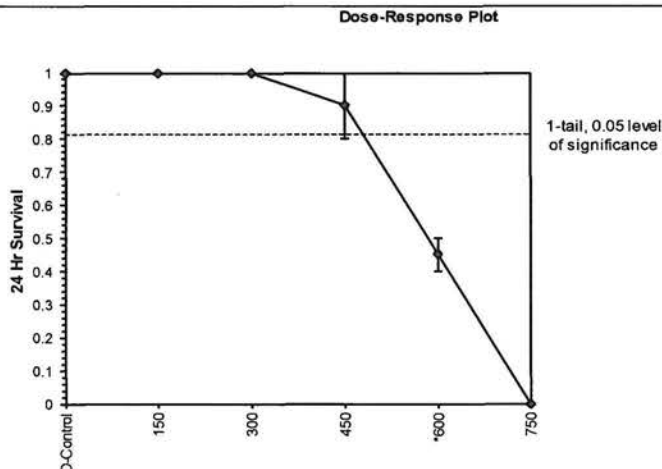
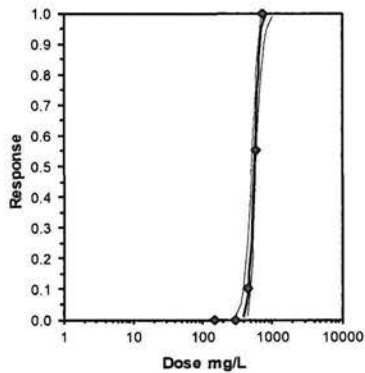


Statistical Analyses

Acute Mysid Test-24 Hr Survival													
Start Date:	10/7/2025	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	10/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride								
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanysis bahia								
Comments:													
Conc-mg/L	1	2											
D-Control	1.0000	1.0000											
150	1.0000	1.0000											
300	1.0000	1.0000											
450	1.0000	0.8000											
600	0.4000	0.5000											
750	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	
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20	
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20	
450	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.501	2.850	0.2894	2	20	
*600	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	6.668	2.850	0.2894	11	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	450	600	519.615		0.16274	0.16691	0.17196	0.01031	0.00427	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	14.7748	2.94669	8.99929 20.5503	0	1.78082	7.81472	0.61912	2.75373	0.06768	5			
Intercept	-35.686	8.14294	-51.646 -19.726										
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	394.711	304.451 445.762										
EC05	3.355	438.94	360.712 483.447										
EC10	3.718	464.509	394.255 505.569										
EC15	3.964	482.597	418.213 521.586										
EC20	4.158	497.473	437.921 535.125										
EC25	4.326	510.601	455.203 547.457										
EC40	4.747	545.238	499.426 582.606										
EC50	5.000	567.196	525.597 607.677										
EC60	5.253	590.039	550.692 636.645										
EC75	5.674	630.064	589.452 694.46										
EC80	5.842	646.69	603.986 720.731										
EC85	6.036	666.625	620.538 753.632										
EC90	6.282	692.583	641.017 798.405										
EC95	6.645	732.928	671.176 871.576										
EC99	7.326	815.055	728.789 1031.41										

Dose (mg/L)	Response
1	0.0
10	0.0
100	0.0
200	0.05
300	0.1
400	0.55
500	0.95
600	1.0
700	1.0
800	1.0
900	1.0
1000	1.0

Dose (mg/L)	24 Hr Survival
Control	1.0
150	1.0
300	1.0
450	0.9
600	0.45
750	0.0

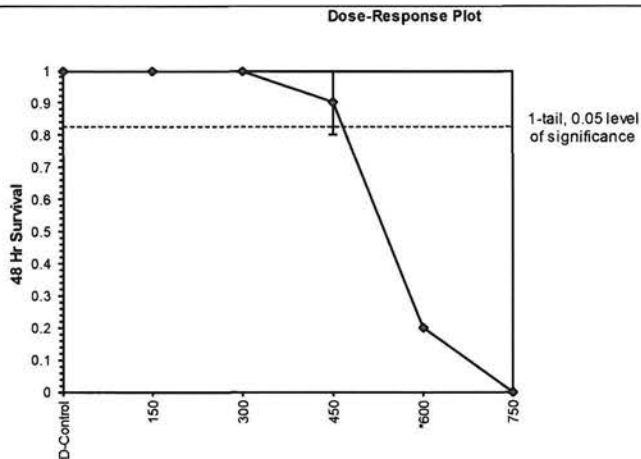
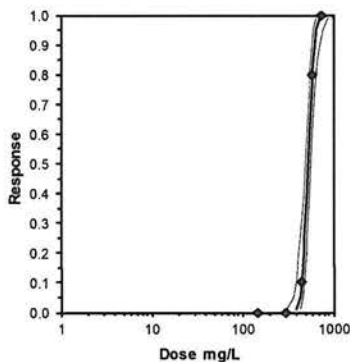


Statistical Analyses

Acute Mysid Test-48 Hr Survival													
Start Date:	10/7/2025	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	10/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride								
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-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	0.8000											
600	0.2000	0.2000											
750	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	
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2748	0	20	
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2748	0	20	
450	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.581	2.850	0.2748	2	20	
*600	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	2	9.837	2.850	0.2748	16	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			450	600	519.615		0.15147	0.15536	0.34014	0.00929	6.8E-04	4, 5	
Treatments vs D-Control													
Maximum Likelihood-Probit													
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	17.6438	3.58798	10.6113	24.6762	0	0.12478	7.81472	0.98871	2.72762	0.05668	3		
Intercept	-43.126	9.81137	-62.356	-23.895									
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	394.251	314.812	437.929									
EC05	3.355	430.921	363.2	468.976									
EC10	3.718	451.844	391.392	487.129									
EC15	3.964	466.532	411.242	500.254									
EC20	4.158	478.545	427.397	511.342									
EC25	4.326	489.097	441.437	521.431									
EC40	4.747	516.731	476.878	550.063									
EC50	5.000	534.101	497.607	570.264									
EC60	5.253	552.055	517.409	593.294									
EC75	5.674	583.246	547.974	638.408									
EC80	5.842	596.107	559.414	658.641									
EC85	6.036	611.456	572.404	683.807									
EC90	6.282	631.332	588.395	717.794									
EC95	6.645	661.986	611.755	772.771									
EC99	7.326	723.559	655.754	890.693									

Dose (mg/L)	Response
1	1.0
150	1.0
300	1.0
450	0.9
600	0.2
750	0.0

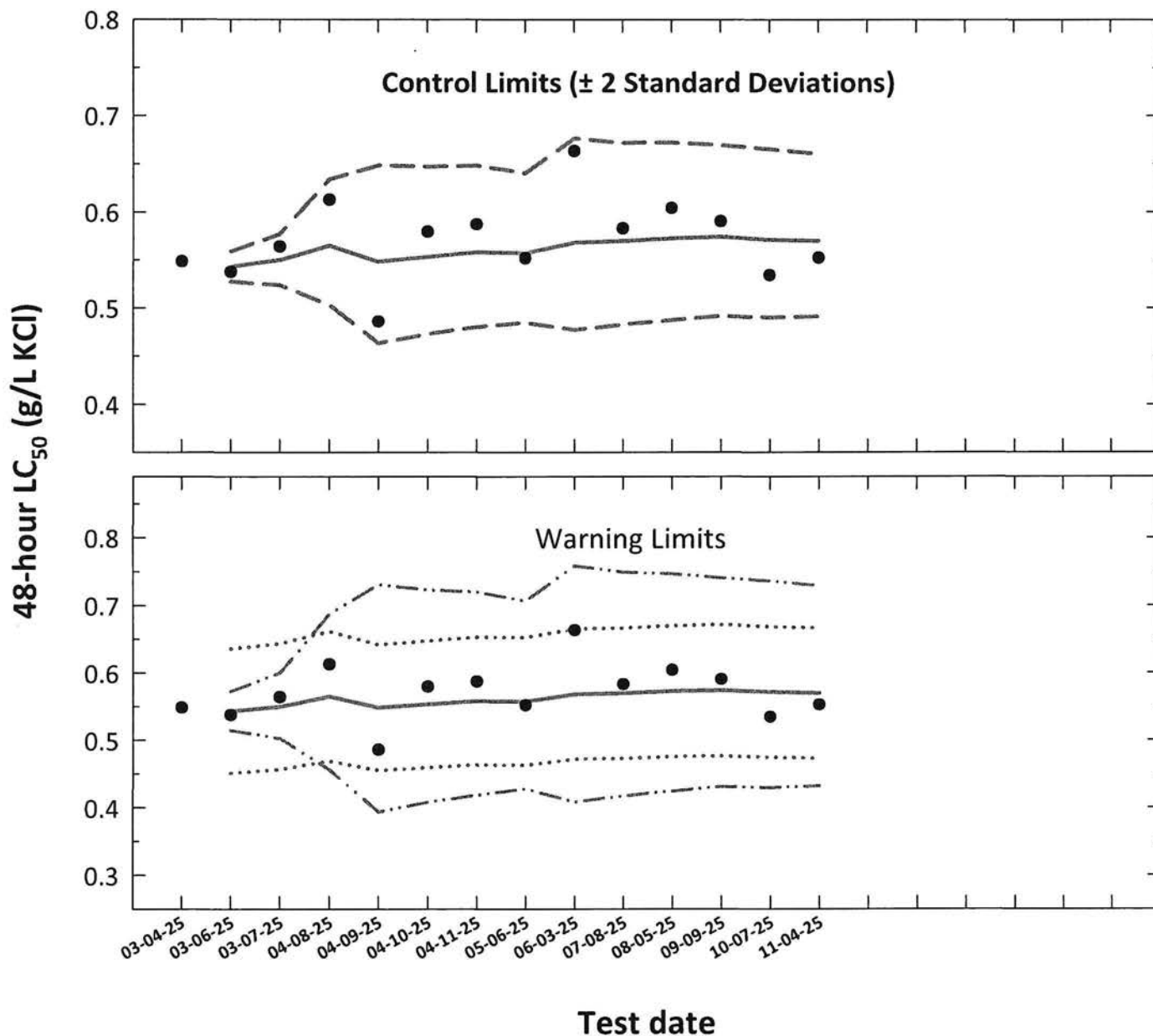
Dose (mg/L)	Survival
D-Control	1.0
150	1.0
300	1.0
450	0.9
600	0.2
750	0.0



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S		Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV		10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}	
1	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	0.5696	0.4829	0.6719	0.4174	0.7492	0.4728	0.6664
10	07-08-25	0.5828	-0.2345	-0.2444	0.0359	0.5726	0.4877	0.6724	0.4242	0.7469	0.4753	0.6700
11	08-05-25	0.6040	-0.2189	-0.2421	0.0349	0.5741	0.4921	0.6698	0.4312	0.7408	0.4765	0.6717
12	09-09-25	0.5904	-0.2289	-0.2410	0.0335	0.5709	0.4899	0.6653	0.4291	0.7362	0.4739	0.6680
13	10-07-25	0.5341	-0.2724	-0.2434	0.0332	0.5696	0.4912	0.6604	0.4320	0.7291	0.4727	0.6664
14	11-04-25	0.5523	-0.2578	-0.2445	0.0321							
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 # 15

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 #: 2437

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	8	XL	XL
	Dissolved oxygen (mg/L)	7.98	7.90	7.86
	*Salinity (ppt)	25.0	25.4	25.3
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	24.5	25.0	25.3
150 mg/L	pH (S.U.)	7.99	7.90	7.86
	Dissolved oxygen (mg/L)	8.5	8.0	8.0
	*Salinity (ppt)	25.1	26.1	25.9
	*Temperature (°C)	24.5	25.4	25.5
300 mg/L	pH (S.U.)	8.00	7.90	7.87
	Dissolved oxygen (mg/L)	8.5	8.0	8.0
	*Salinity (ppt)	25.5	25.6	25.6
	*Temperature (°C)	24.6	25.2	25.1
450 mg/L	pH (S.U.)	8.00	7.90	7.87
	Dissolved oxygen (mg/L)	8.5	7.9	8.0
	*Salinity (ppt)	25.2	25.21	25.6
	*Temperature (°C)	24.5	25.2	25.1
600 mg/L	pH (S.U.)	8.00	7.91	7.87
	Dissolved oxygen (mg/L)	8.5	7.9	7.9
	*Salinity (ppt)	25.6	25.9	26.3
	*Temperature (°C)	24.6	25.3	25.1
750 mg/L	pH (S.U.)	8.00	7.91	7.87
	Dissolved oxygen (mg/L)	8.4	7.9	7.9
	*Salinity (ppt)	25.3	26.0	26.4
	*Temperature (°C)	24.6	25.4	25.2

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numb
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	13066

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 # 15

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-01-15	1042	JK	1329	JP	6B	orange	10-29-250 11-10-25
24	11-05-15			1349	JP			
48 Termination	11-06-15			1341	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):	11-03-15
Age (1 to 5 days old):	1.2 DAYS
Date organisms were born:	11-01-15 1200 TO 11-03-15 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.98 Temperature (°C) 24.5°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 ¹²	8 ^{2d}	8 ^{2d}	7 ^{3d}	3 ^{7d}	2 ^{8d}
48 Termination	10	10	10	10	10	9 ¹²	9	8	5 ^{3d}	6 ^{1d}	0 ^{3d}	0 ^{2d}
Mean Survival	100%		100%		95%		85%		55%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	501.9
Upper 95% confidence limit (mg KCl/L)	607.8
48-hour LC ₅₀ (mg KCl/L)	552.3

Comments:





Statistical Analyses

Acute Mysid Test-24 Hr Survival			
Start Date:	11/4/2025	Test ID:	AbKCIAC
End Date:	11/6/2025	Lab ID:	ETS-Envir. Testing Sol.
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012
Comments:		Sample ID:	REF-Ref Toxicant
		Sample Type:	DMR-Discharge Monitoring Report
		Test Species:	AB-Americanysis bahia

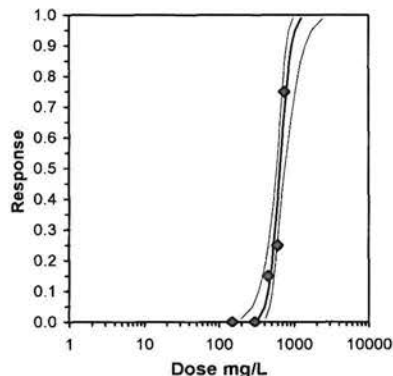
Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	0.9000	0.8000
600	0.8000	0.7000
750	0.3000	0.2000

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	0.1772	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1772	0	20
*450	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.736	2.830	0.1772	3	20
*600	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5.795	2.830	0.1772	5	20
*750	0.2500	0.2500	0.5216	0.4636	0.5796	15.723	2	14.220	2.830	0.1772	15	20

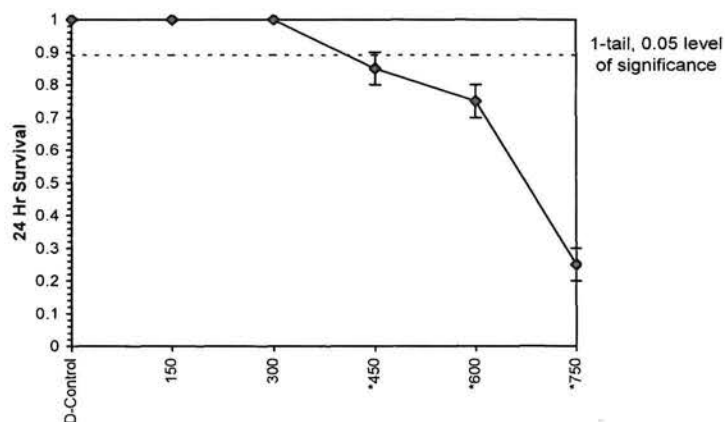
Auxiliary Tests					Statistic		Critical	Skew	Kurt		
Normality of the data set cannot be confirmed											
Equality of variance cannot be confirmed											
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		300	450	367.423		0.08369	0.08584	0.24422	0.00392	4.3E-05	5, 6
Treatments vs D-Control											

Maximum Likelihood-Probit										Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
										Slope	8.06006	1.93992	4.25783 11.8623	0	2.62936	7.81472	0.45237	2.81608	0.12407	3
										Intercept	-17.698	5.40059	-28.283 -7.1126							
										TSCR										

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	336.864	195.933 413.858
EC05	3.355	409.266	280.978 476.205
EC10	3.718	454.026	339.283 515.058
EC15	3.964	486.958	384.14 544.705
EC20	4.158	514.828	422.683 571.232
EC25	4.326	540.005	457.257 597.048
EC40	4.747	609.043	544.415 683.365
EC50	5.000	654.757	591.989 757.055
EC60	5.253	703.903	635.243 849.885
EC75	5.674	793.896	702.62 1047.11
EC80	5.842	832.72	729.098 1140.96
EC85	6.036	880.378	760.3 1262.54
EC90	6.282	944.236	800.451 1435.92
EC95	6.645	1047.5	862.467 1740.5
EC99	7.326	1272.64	989.166 2504.12



Dose-Response Plot



Statistical Analyses

Acute Mysid Test-48 Hr Survival

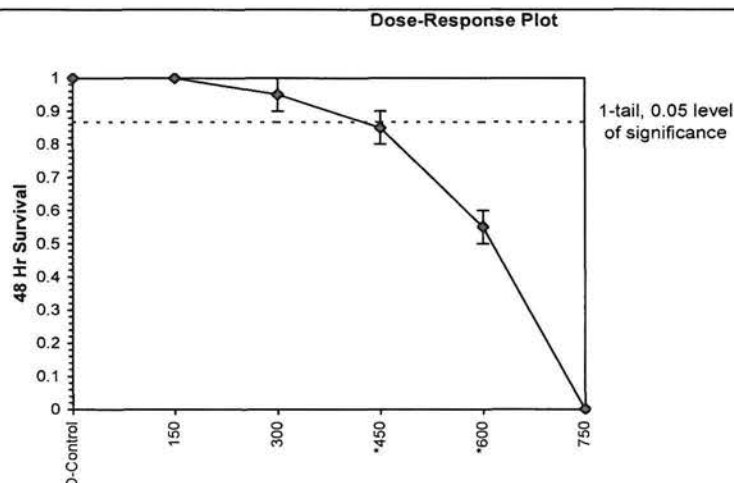
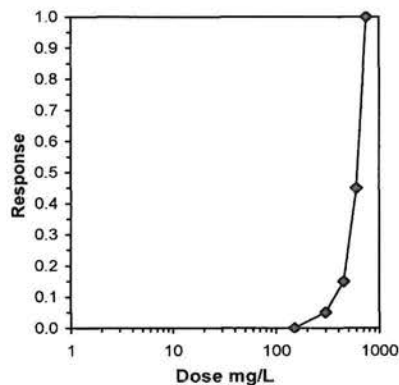
Start Date: 11/4/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/6/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	0.9000
450	0.9000	0.8000
600	0.5000	0.6000
750	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
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2148	0	20
300	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.081	2.850	0.2148	1	20
*450	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.103	2.850	0.2148	3	20
*600	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	7.644	2.850	0.2148	9	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		0.10822	0.111	0.11722	0.00568	0.00262	4, 5
Treatments vs D-Control											

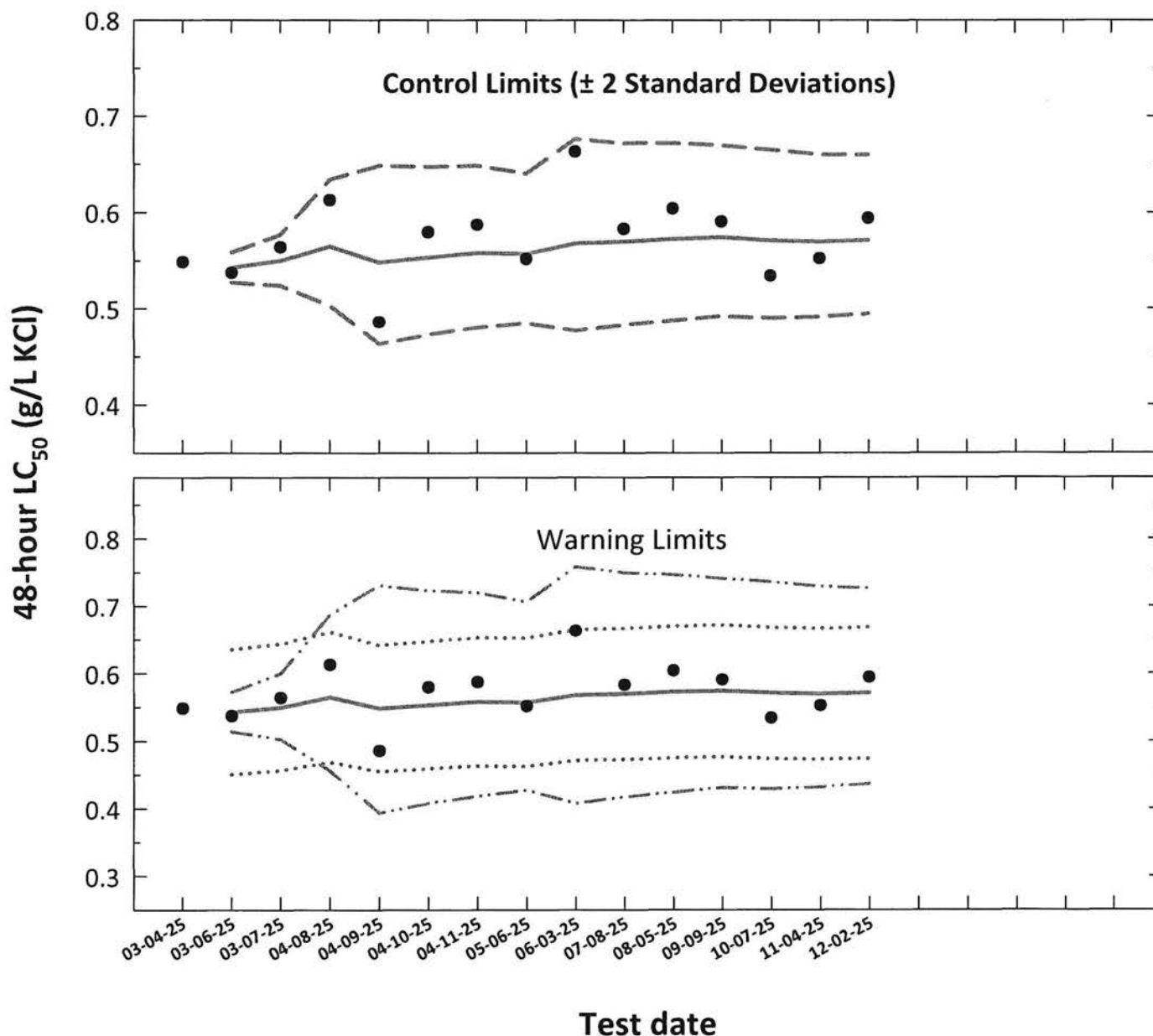
Trim Level	EC50	95% CL
0.0%	552.31	501.91 607.78
5.0%	572.97	523.31 627.36
10.0%	584.07	532.84 640.23
20.0%	594.90	541.24 653.89
Auto-0.0%	552.31	501.91 607.78



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)								
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV		
								CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	03-04-25	0.5485	-0.2608	-0.2652	0.0063	0.5430	0.5275	0.5588		0.5145	0.5722	0.4506	0.6353
2	03-06-25	0.5374	-0.2697	-0.2597	0.0105	0.5499	0.5239	0.5772		0.5026	0.5995	0.4564	0.6434
3	03-07-25	0.5640	-0.2487	-0.2480	0.0251	0.5650	0.5034	0.6342		0.4559	0.6874	0.4689	0.6610
4	04-08-25	0.6129	-0.2126	-0.2610	0.0364	0.5482	0.4635	0.6484		0.3937	0.7310	0.4550	0.6414
5	04-09-25	0.4860	-0.3134	-0.2570	0.0341	0.5533	0.4730	0.6473		0.4082	0.7231	0.4593	0.6474
6	04-10-25	0.5796	-0.2369	-0.2533	0.0326	0.5581	0.4803	0.6484		0.4187	0.7200	0.4632	0.6529
7	04-11-25	0.5872	-0.2312	-0.2539	0.0302	0.5573	0.4849	0.6405		0.4273	0.7066	0.4625	0.6520
8	05-06-25	0.5517	-0.2583	-0.2455	0.0379	0.5681	0.4772	0.6763		0.4082	0.7586	0.4716	0.6647
9	06-03-25	0.6631	-0.1784	-0.2444	0.0359	0.5696	0.4829	0.6719		0.4174	0.7492	0.4728	0.6664
10	07-08-25	0.5828	-0.2345	-0.2421	0.0349	0.5726	0.4877	0.6724		0.4242	0.7469	0.4753	0.6700
11	08-05-25	0.6040	-0.2189	-0.2410	0.0335	0.5741	0.4921	0.6698		0.4312	0.7408	0.4765	0.6717
12	09-09-25	0.5904	-0.2289	-0.2434	0.0332	0.5709	0.4899	0.6653		0.4291	0.7362	0.4739	0.6680
13	10-07-25	0.5341	-0.2724	-0.2445	0.0321	0.5696	0.4912	0.6604		0.4320	0.7291	0.4727	0.6664
14	11-04-25	0.5523	-0.2578	-0.2432	0.0313	0.5712	0.4944	0.6598		0.4368	0.7264	0.4741	0.6683
15	12-02-25	0.5941	-0.2261										
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 ToxCal).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 16

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 #: 2437

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	8.06	7.96	7.89
	Dissolved oxygen (mg/L)	8.4	8.1	8.2
	*Salinity (ppt)	25.0	25.2	25.2
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	24.8	24.7	25.0
150 mg/L	pH (S.U.)	8.05	7.94	7.90
	Dissolved oxygen (mg/L)	8.4	8.0	8.2
	*Salinity (ppt)	25.1	25.3	25.2
	*Temperature (°C)	25.0	24.4	24.7
300 mg/L	pH (S.U.)	8.04	7.95	7.90
	Dissolved oxygen (mg/L)	8.4	8.0	8.2
	*Salinity (ppt)	25.2	25.4	25.5
	*Temperature (°C)	25.0	24.5	24.6
450 mg/L	pH (S.U.)	8.06	7.96	7.90
	Dissolved oxygen (mg/L)	8.4	8.0	8.2
	*Salinity (ppt)	25.3	25.7	25.6
	*Temperature (°C)	25.1	24.5	25.0
600 mg/L	pH (S.U.)	8.05	7.96	7.91
	Dissolved oxygen (mg/L)	8.4	8.0	8.2
	*Salinity (ppt)	25.4	25.9	25.7
	*Temperature (°C)	25.1	24.6	24.7
750 mg/L	pH (S.U.)	8.05	7.95	
	Dissolved oxygen (mg/L)	8.3	7.9	
	*Salinity (ppt)	25.5	26.0	
	*Temperature (°C)	25.2	24.6	

*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	30664685

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 # 16

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-02-25	* 1120	JP	1423	JP	1B	orange	11-24-25A
24	12-03-25			1433	JP			
48 Termination	12-04-25			1420	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):	12-01-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	11-30-25 1200 TO 12-01-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.05 Temperature (°C): 25.7 °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	9 ^{1d}	10	10	10	7 ^{3d}	8 ^{2d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9	9 ^{1d}	10	10	7	7 ^{1d}	0	0
Mean Survival	100%		100%		90%		100%		70%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	547.3
Upper 95% confidence limit (mg KCl/L)	645.0
48-hour LC ₅₀ (mg KCl/L)	594.1

Comments:



Statistical Analyses

Acute Mysid Test-24 Hr Survival

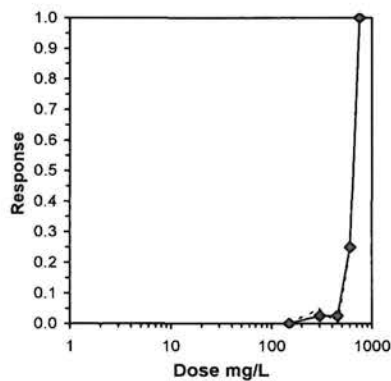
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End Date: 12/4/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

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

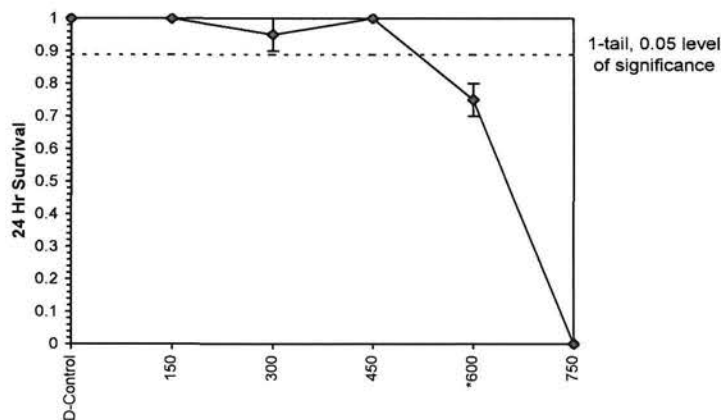
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1803	0	20
300	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.288	2.850	0.1803	1	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1803	0	20
*600	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5.736	2.850	0.1803	5	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		450	600	519.615		0.08562	0.08782	0.04941	0.004	0.00838	4, 5
Treatments vs D-Control											

Trimmed Spearman-Kärber				
Trim Level	EC50	95% CL		
0.0%	615.39	575.45	658.10	
5.0%	632.39	597.99	668.77	
10.0%	637.47	597.09	680.59	
20.0%	645.01	579.00	718.54	
Auto-0.0%	615.39	575.45	658.10	



Dose-Response Plot



Statistical Analyses

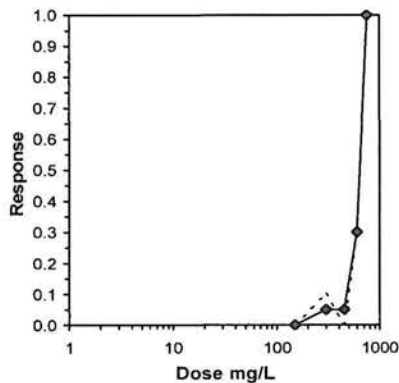
Acute Mysid Test-48 Hr Survival					
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End Date:	12/4/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	DMR-Discharge Monitoring Report
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanysis bahia
Comments:					

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

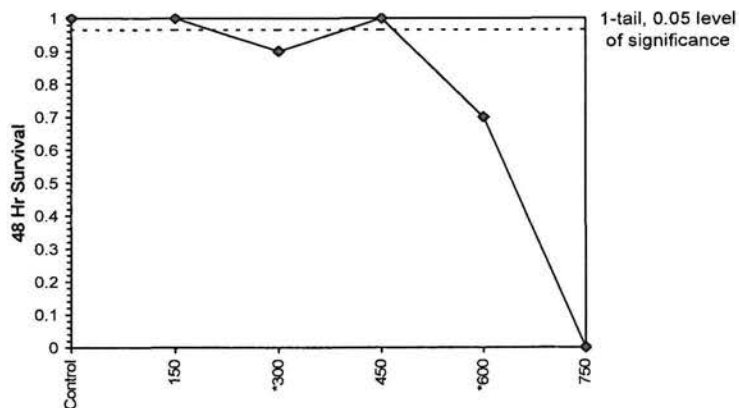
Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*300	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*600	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	42.086	2.850	0.0285	6	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				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	150	300	212.132		0.00967	0.00991	0.06776	0.0001	5.1E-07	4, 5
Treatments vs D-Control										

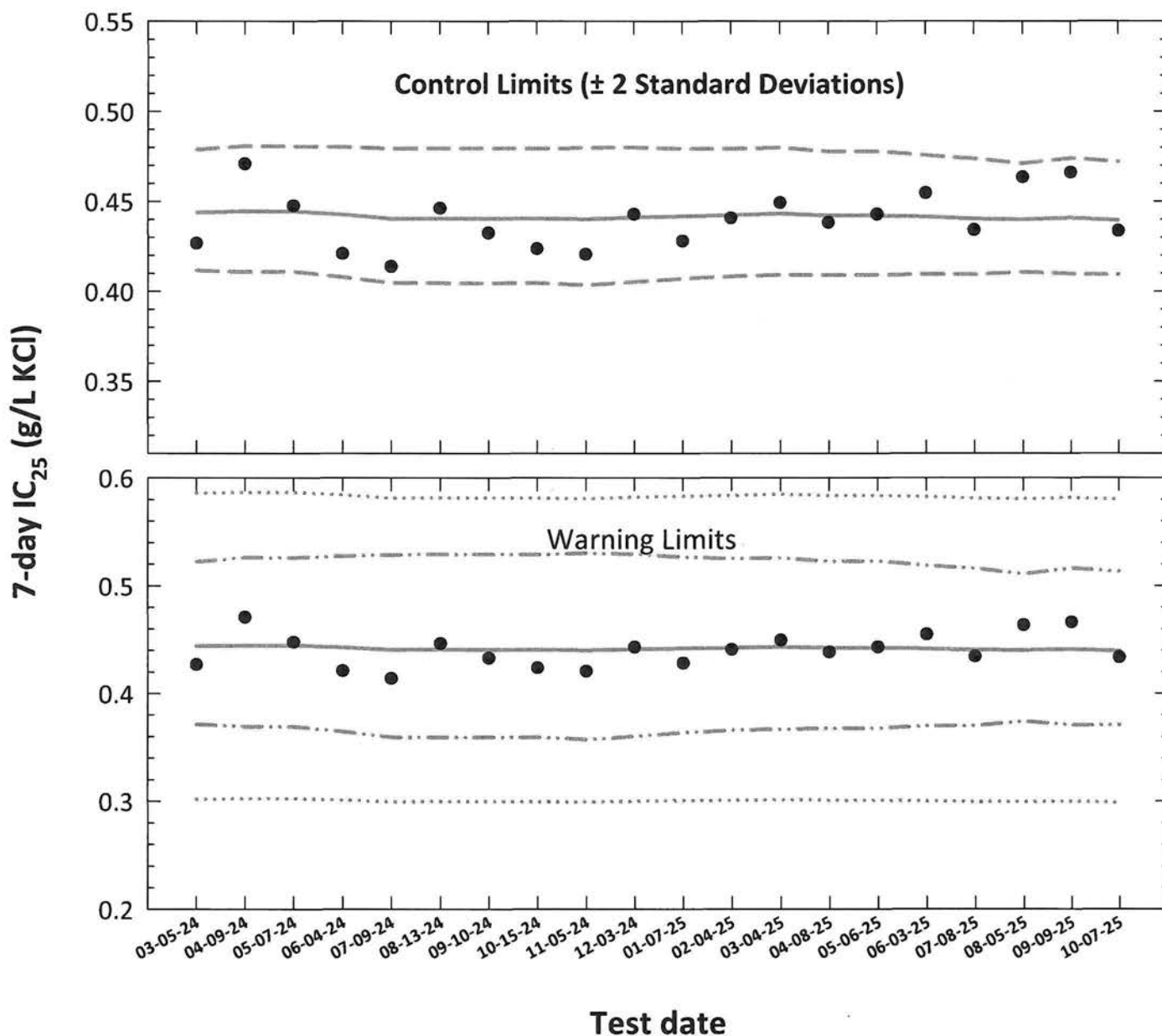
Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	594.12	547.27	644.98
5.0%	621.29	585.06	659.76
10.0%	626.34	585.59	669.91
20.0%	635.08	573.56	703.20
Auto-0.0%	594.12	547.27	644.98



Dose-Response Plot



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



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

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-08-25	0.4342	-0.3624	-0.3562	0.0159	0.4404	0.4093	0.4738	0.3699	0.5162	0.2995	0.5813
18	08-05-25	0.4634	-0.3340	-0.3567	0.0149	0.4398	0.4107	0.4710	0.3736	0.5108	0.2991	0.5806
19	09-09-25	0.4660	-0.3316	-0.3559	0.0158	0.4407	0.4097	0.4740	0.3705	0.5162	0.2997	0.5817
20	10-07-25	0.4336	-0.3629	-0.3570	0.0155	0.4396	0.4093	0.4721	0.3708	0.5135	0.2989	0.5802

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.

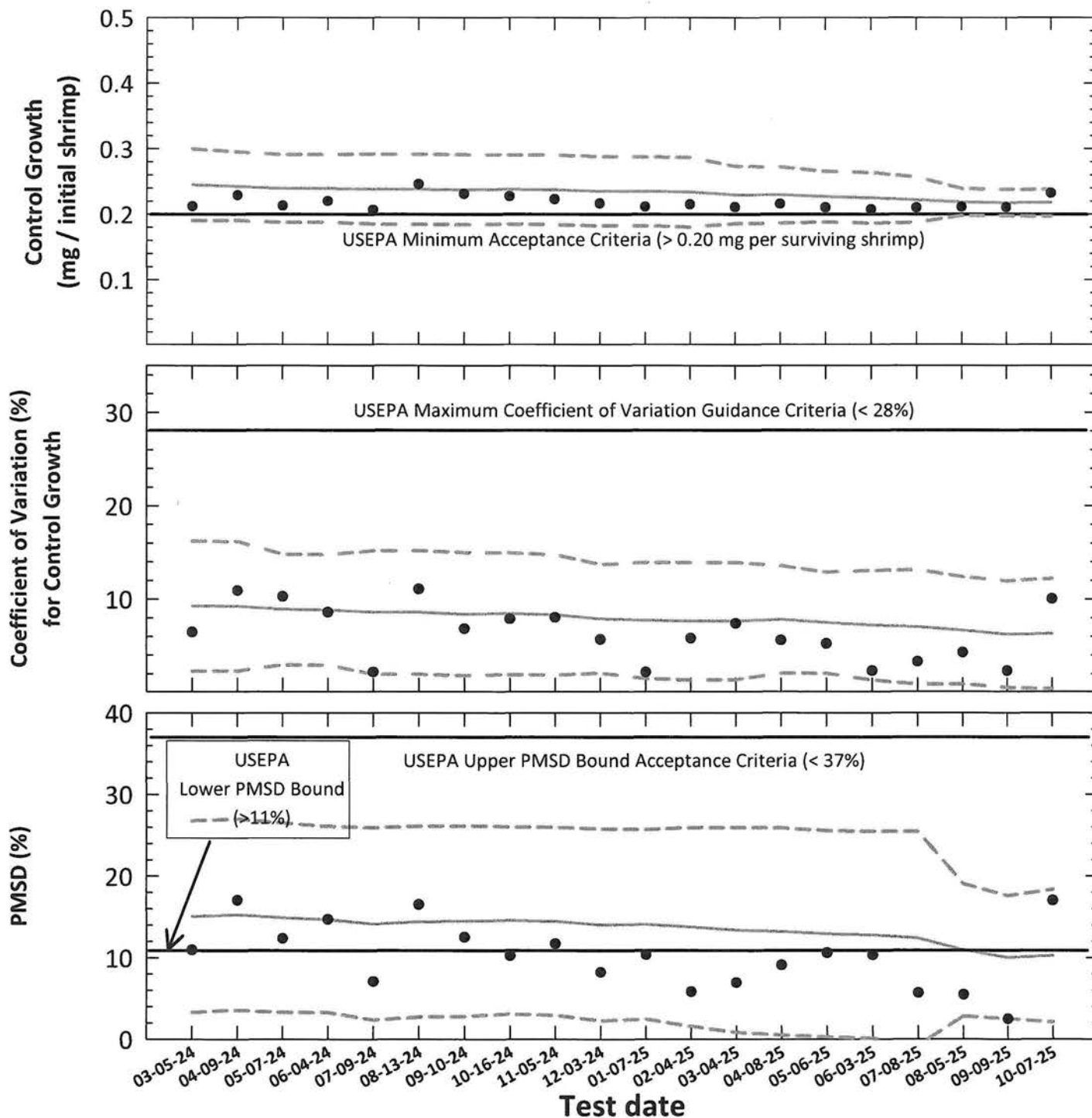
Control Limits = Mean logarithmic $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

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 CV		Test PMSD	
		Control Survival (%)	Control Growth		PMSD (%)	MSD	CT	95% Confidence Interval CT - 25 CT + 25 (mg/initial shrimp)	CT	95% Confidence Interval CT - 25 CT + 25 (%)	CT	95% Confidence Interval CT - 25 CT + 25 (%)
			Mean (mg/initial shrimp)	CV (%)								
1	03-05-24	100	0.212	6.5	10.9	0.0232	0.245	0.190	9.3	2.3	15.0	3.3
2	04-09-24	100	0.229	10.9	17.0	0.0389	0.243	0.190	9.2	2.3	15.2	3.5
3	05-07-24	100	0.213	10.3	12.3	0.0263	0.239	0.188	8.9	2.9	14.9	3.3
4	06-04-24	100	0.220	8.6	14.7	0.0323	0.239	0.188	8.8	2.9	14.7	3.3
5	07-09-24	100	0.207	2.2	7.1	0.0146	0.238	0.185	8.6	1.9	14.1	2.3
6	08-13-24	100	0.246	11.1	16.5	0.0406	0.238	0.185	8.6	1.9	14.4	2.7
7	09-10-24	100	0.231	6.8	12.5	0.0288	0.238	0.184	8.4	1.8	14.4	2.8
8	10-16-24	100	0.228	7.9	10.2	0.0233	0.238	0.185	8.5	1.9	14.6	3.1
9	11-05-24	100	0.223	8.0	11.7	0.0261	0.237	0.184	8.3	1.9	14.4	2.9
10	12-03-24	100	0.216	5.7	8.2	0.0177	0.235	0.182	7.9	2.0	14.0	2.2
11	01-07-25	100	0.212	2.2	10.4	0.0219	0.235	0.183	7.7	1.5	14.1	2.5
12	02-04-25	100	0.215	5.8	5.8	0.0125	0.234	0.181	7.6	1.3	13.7	1.5
13	03-04-25	100	0.211	7.4	6.9	0.0146	0.229	0.185	7.6	1.3	13.3	0.8
14	04-08-25	100	0.216	5.6	9.1	0.0196	0.230	0.187	7.8	2.1	13.2	0.5
15	05-06-25	100	0.210	5.2	10.6	0.0222	0.227	0.188	7.5	2.0	12.9	0.3
16	06-03-25	100	0.207	2.3	10.3	0.0213	0.225	0.186	7.2	1.3	12.8	0.1
17	07-08-25	100	0.210	3.3	5.7	0.0119	0.222	0.188	7.0	0.9	12.4	-0.7
18	08-05-25	100	0.211	4.3	5.5	0.0115	0.219	0.198	6.6	0.9	10.9	2.8
19	09-09-25	100	0.210	2.3	2.5	0.0052	0.218	0.198	6.2	0.5	10.0	2.5
20	10-07-25	100	0.232	10.0	17.0	0.0395	0.218	0.197	6.3	0.4	10.2	2.1

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.

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 (90th percentile) < 37%.

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: **268**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2412					
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:	09-30-25 1200 to 10-01-25 1130		Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 10-01-25		Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 8.05 S.U.		Drying information for weight determination:	
	Temperature = 25.0 °C		Date / Time in oven:	10-14-25 1145
Average transfer volume:	< 0.25 mL		*Initial oven temperature:	60°C
			Date / Time out of oven:	10-15-25 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	10-07-25	1200	H	1500	H	1230	H	09-29-25 A
1	10-08-25	0500	H	1400	H	0900	H	↓
2	10-09-25	0500	H	1330	H	1130	H	10-06-25
3	10-10-25	0300	H	1100	H	0755	H	↓
4	10-11-25	0600	H	1200	H	1110	H	10-10-25
5	10-12-25	0600	H	1200	H	0850	H	↓
6	10-13-25	0500	H	1100	H	0850	H	↓
7	10-14-25					1030	H	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	533.1
Average weight per initial shrimp:	0.232		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.232	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	433.6

AbKCICR Test Number: **268**

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>Lavender</u> Analyst: <u>VL</u> Date: <u>09-19-25</u>	13.27	14.28	14.58	13.64	10.30	12.20	12.86	13.96	13.01	14.14	12.96	15.46	10.86	10.56	10.07	11.00
*B = Pan + Shrimp weight (mg) Analyst: <u>H</u> Date: <u>10-16-25</u>	14.38	15.28	15.72	14.45	11.52	13.48	12.87	15.18	14.52	15.34	14.33	16.65	11.89	11.85	11.26	12.7
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>H</u>	1.11	1.00	1.14	1.31	1.22	1.28	1.01	1.22	1.51	1.20	1.37	1.39	1.03	1.29	1.19	1.2
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>H</u>	0.222	0.200	0.228	0.262	0.244	0.256	0.202	0.244	0.302	0.240	0.274	0.278	0.206	0.256	0.238	0.254
Average weight per initial number of shrimp (mg) 0.232												Average weight per initial number of shrimp (mg) 0.256				Percent reduction from control (%) - 10.37.

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

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

Comments:

AbKCICR Test Number: **268**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	S	S	S	4 ^{1d}
3	5	5	S	S	S	S	S	S	4	4	4	4	5	S	3 ^{2d}	3 ^{1d}
4	5	S	S	S	S	S	S	S	4	4	4	4	4 ^{1d}	5	3	3
5	S	S	S	S	S	S	S	S	3 ^{1d}	3 ^{1d}	3 ^{1d}	4	4	4 ^{1d}	3	3
6	S	S	S	S	S	S	S	S	3	3	3	4	4	4	3	3
7	S	S	S	S	S	S	S	S	3	3	3	2 ^{2d}	4	3 ^{1d}	3	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males	110 ^{1d}															
*A = Pan weight (mg) Tray color code: <u>WATER</u> Analyst: <u>XL</u> Date: <u>09-19-25</u>	13.75	12.68	13.06	13.63	14.31	12.10	10.92	14.52	11.58	12.80	14.41	12.63	12.41	10.24	9.97	10.18
*B = Pan + Shrimp weight (mg) Analyst: <u>XL</u> Date: <u>10-16-25</u>	15.01	13.86	14.26	15.06	15.34	13.10	11.87	15.63	12.01	13.69	15.22	13.06	12.36	11.03	10.67	10.4
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>XL</u>	1.26	1.20	1.20	1.43	1.03	1.00	0.95	1.11	0.43	0.89	0.81	0.43	0.95	0.79	0.70	0.22
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>XL</u>	0.252	0.240	0.240	0.286	0.206	0.200	0.190	0.222	0.086	0.178	0.162	0.086	0.190	0.168	0.140	0.046
	Average weight per initial number of shrimp (mg) 0.230				Percent reduction from control (%) 1.27.				Average weight per initial number of shrimp (mg) 0.131				Percent reduction from control (%) 43.77.			

*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:

AbKCICR Test Number: **268**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# Immature females																
# males																
*A = Pan weight (mg) Tray color code: _____ Analyst: _____ Date: _____																
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____																
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average weight per initial number of shrimp (mg)	0				100%				0				100%			
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: 26

Test dates: October 07-14, 202

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.27	14.38	1.11	0.222	100.0	0.232	10.0	Not applicable
	B	5	5	14.28	15.28	1.00	0.200				
	C	5	5	14.58	15.72	1.14	0.228				
	D	5	5	13.64	14.95	1.31	0.262				
	E	5	5	10.30	11.52	1.22	0.244				
	F	5	5	12.20	13.48	1.28	0.256				
	G	5	5	12.86	13.87	1.01	0.202				
	H	5	5	13.96	15.18	1.22	0.244				
250	I	5	5	13.01	14.52	1.51	0.302	100.0	0.256	11.4	-10.3
	J	5	5	14.14	15.34	1.20	0.240				
	K	5	5	12.96	14.33	1.37	0.274				
	L	5	5	15.46	16.85	1.39	0.278				
	M	5	5	10.86	11.89	1.03	0.206				
	N	5	5	10.56	11.85	1.29	0.258				
	O	5	5	10.07	11.26	1.19	0.238				
	P	5	5	11.00	12.27	1.27	0.254				
375	Q	5	5	13.75	15.01	1.26	0.252	100.0	0.230	13.7	1.2
	R	5	5	12.68	13.88	1.20	0.240				
	S	5	5	13.06	14.26	1.20	0.240				
	T	5	5	13.63	15.06	1.43	0.286				
	U	5	5	14.31	15.34	1.03	0.206				
	V	5	5	12.10	13.10	1.00	0.200				
	W	5	5	10.92	11.87	0.95	0.190				
	X	5	5	14.52	15.63	1.11	0.222				
500	Y	5	3	11.58	12.01	0.43	0.086	60.0	0.131	39.6	43.7
	Z	5	3	12.80	13.69	0.89	0.178				
	AA	5	3	14.41	15.22	0.81	0.162				
	BB	5	2	12.63	13.06	0.43	0.086				
	CC	5	4	12.41	13.36	0.95	0.190				
	DD	5	3	10.24	11.03	0.79	0.158				
	EE	5	3	9.97	10.67	0.70	0.140				
	FF	5	3	10.18	10.41	0.23	0.046				
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: 0.0395
PMSD: 17.0

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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

Start Date:	10/7/2025	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/14/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americanysis bahia
Comments:					

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

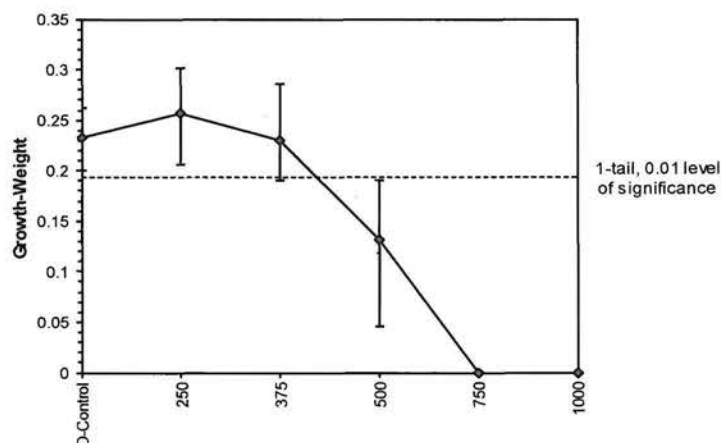
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000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

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.3625	0.904	0.44003	15.5766
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				

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

Dose-Response Plot

7 Day Survival



AbKCICR Test Number: **268**

Daily Chemistry:

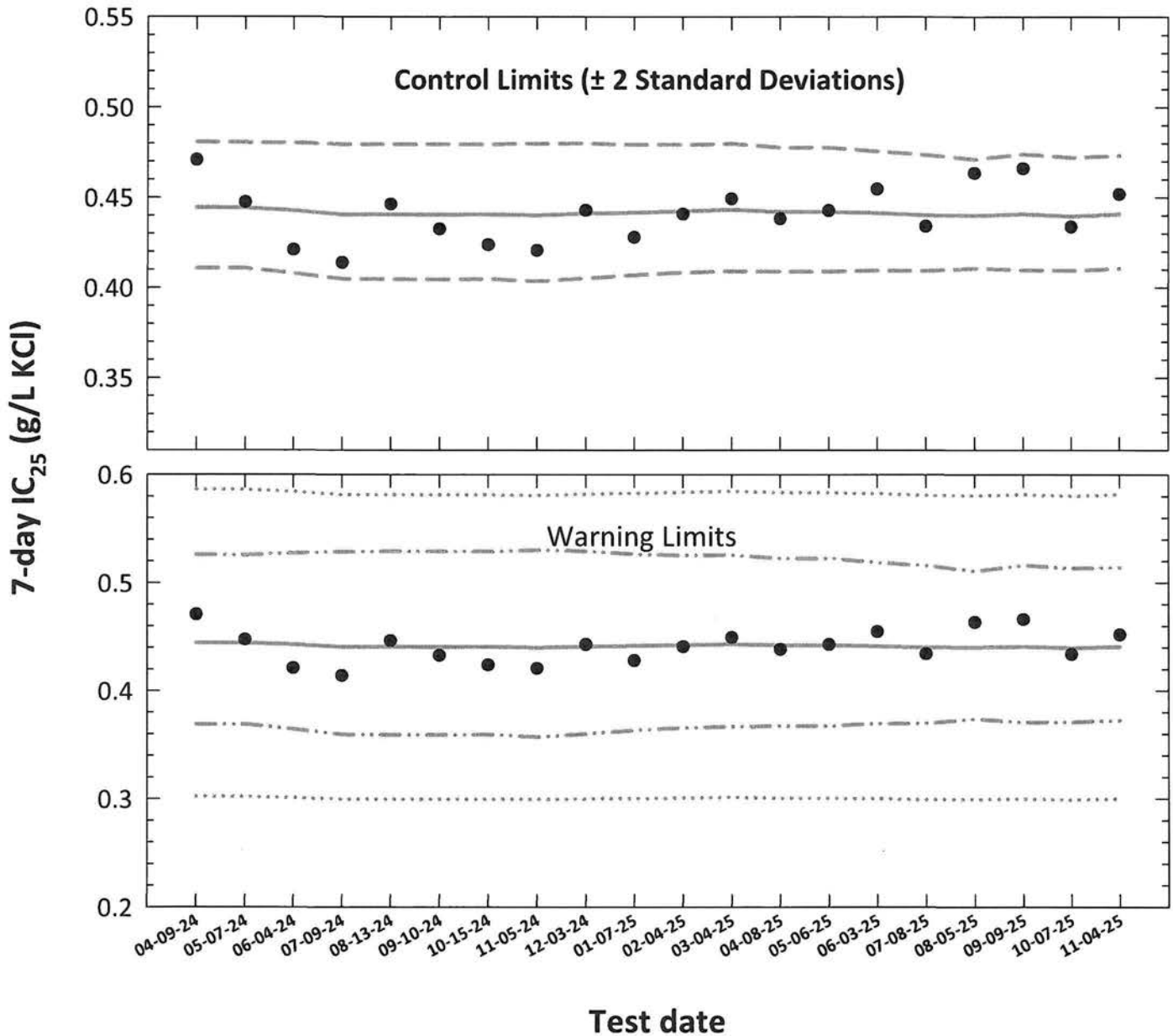
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst	XL	XL	XL	XL	XL
CONTROL, Salt SW	pH (S.U.)		7.93	7.89	7.91	7.80	8.05
	DO (mg/L)		8.6	7.8	8.2	7.4	8.3
	Salinity (ppt)		25.0	25.0	25.0	25.1	25.0
	Alkalinity (mg CaCO ₃ /L)		110				100
	Temperature (°C)		25.2	25.9	25.2	25.5	25.2
250 mg KCl/L	pH (S.U.)		7.97	7.91	7.94	7.88	8.00
	DO (mg/L)		8.6	7.9	8.1	7.7	8.2
	Salinity (ppt)		25.0	25.4	25.0	25.1	25.2
	Temperature (°C)		25.3	25.5	25.2	25.9	25.2
375 mg KCl/L	pH (S.U.)		7.98	7.91	7.94	7.86	8.00
	DO (mg/L)		8.6	7.9	8.1	7.7	8.2
	Salinity (ppt)		25.1	25.5	25.4	25.3	25.2
	Temperature (°C)		25.3	25.6	25.1	25.6	25.2
500 mg KCl/L	pH (S.U.)		7.98	7.91	7.94	7.89	8.00
	DO (mg/L)		8.6	7.9	8.1	7.7	8.2
	Salinity (ppt)		25.2	25.5	25.5	25.4	25.3
	Temperature (°C)		25.2	25.6	25.1	25.7	25.2
750 mg KCl/L	pH (S.U.)		7.98	7.90			
	DO (mg/L)		8.6	7.9			
	Salinity (ppt)		25.5	26.3			
	Temperature (°C)		25.2	25.8			
1000 mg KCl/L	pH (S.U.)		7.98	7.90			
	DO (mg/L)		8.6	7.7			
	Salinity (ppt)		25.6	26.1			
	Temperature (°C)		25.2	25.5			
			Initial	Final	Initial	Final	Initial
							Final

AbKCICR Test Number: **268**

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.)	XL	BSL	BSL	BSL	BSL	XL	XL	✓
	DO (mg/L)	7.90	7.86	8.05	7.89	8.06	7.84	7.98	7.52
	Salinity (ppt)	8.3	7.8	8.3	7.8	8.2	7.7	8.2	5.3
	Alkalinity (mg CaCO ₃ /L)	25.0	24.9	25.0	25.1	25.0	24.9	24.9	25.0
	Temperature (°C)	—	—	100	—	—	—	—	—
250 mg KCl/L	pH (S.U.)	25.2	25.9	25.2	26.2	25.2	26.0	25.2	26.1
	DO (mg/L)	7.89	7.89	8.04	7.90	8.06	7.84	7.94	7.50
	Salinity (ppt)	8.3	7.8	8.3	7.8	8.2	7.8	8.1	6.1
	Temperature (°C)	25.1	25.1	25.2	25.3	25.3	25.3	25.4	25.4
375 mg KCl/L	pH (S.U.)	25.1	25.9	25.1	26.0	25.3	26.2	25.2	26.0
	DO (mg/L)	7.90	7.86	8.04	7.87	8.06	7.83	7.95	7.61
	Salinity (ppt)	8.2	7.7	8.3	7.7	8.2	7.8	8.1	5.9
	Temperature (°C)	25.1	25.3	25.4	25.5	25.3	25.5	25.6	25.7
500 mg KCl/L	pH (S.U.)	25.1	25.9	25.3	25.9	25.2	26.2	25.4	26.2
	DO (mg/L)	7.90	7.94	8.04	7.88	8.07	7.83	7.95	7.63
	Salinity (ppt)	8.2	7.8	8.3	7.7	8.2	7.8	8.1	7.9
	Temperature (°C)	25.2	25.4	25.4	25.6	25.4	25.6	25.6	25.7
750 mg KCl/L	pH (S.U.)	25.4	26.0	25.2	25.9	25.2	26.1	25.4	26.2
	DO (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	—	—	—	—	—	—	—	—
	Temperature (°C)	—	—	—	—	—	—	—	—
1000 mg KCl/L	pH (S.U.)	—	—	—	—	—	—	—	—
	DO (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	—	—	—	—	—	—	—	—
	Temperature (°C)	—	—	—	—	—	—	—	—
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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		CT	Anti-logarithmic Values (g/L KCl)		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
			7-day IC_{25}	CT	S	CT	Control Limits CT - 2S CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-08-25	0.4342	-0.3624	-0.3562	0.0159	0.4404	0.4093 0.4738	0.3699	0.5162	0.2995	0.5813
17	08-05-25	0.4634	-0.3340	-0.3567	0.0149	0.4398	0.4107 0.4710	0.3736	0.5108	0.2991	0.5806
18	09-09-25	0.4660	-0.3316	-0.3559	0.0158	0.4407	0.4097 0.4740	0.3705	0.5162	0.2997	0.5817
19	10-07-25	0.4336	-0.3629	-0.3570	0.0155	0.4396	0.4093 0.4721	0.3708	0.5135	0.2989	0.5802
20	11-04-25	0.4518	-0.3451	-0.3557	0.0154	0.4408	0.4107 0.4732	0.3724	0.5143	0.2998	0.5819

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.

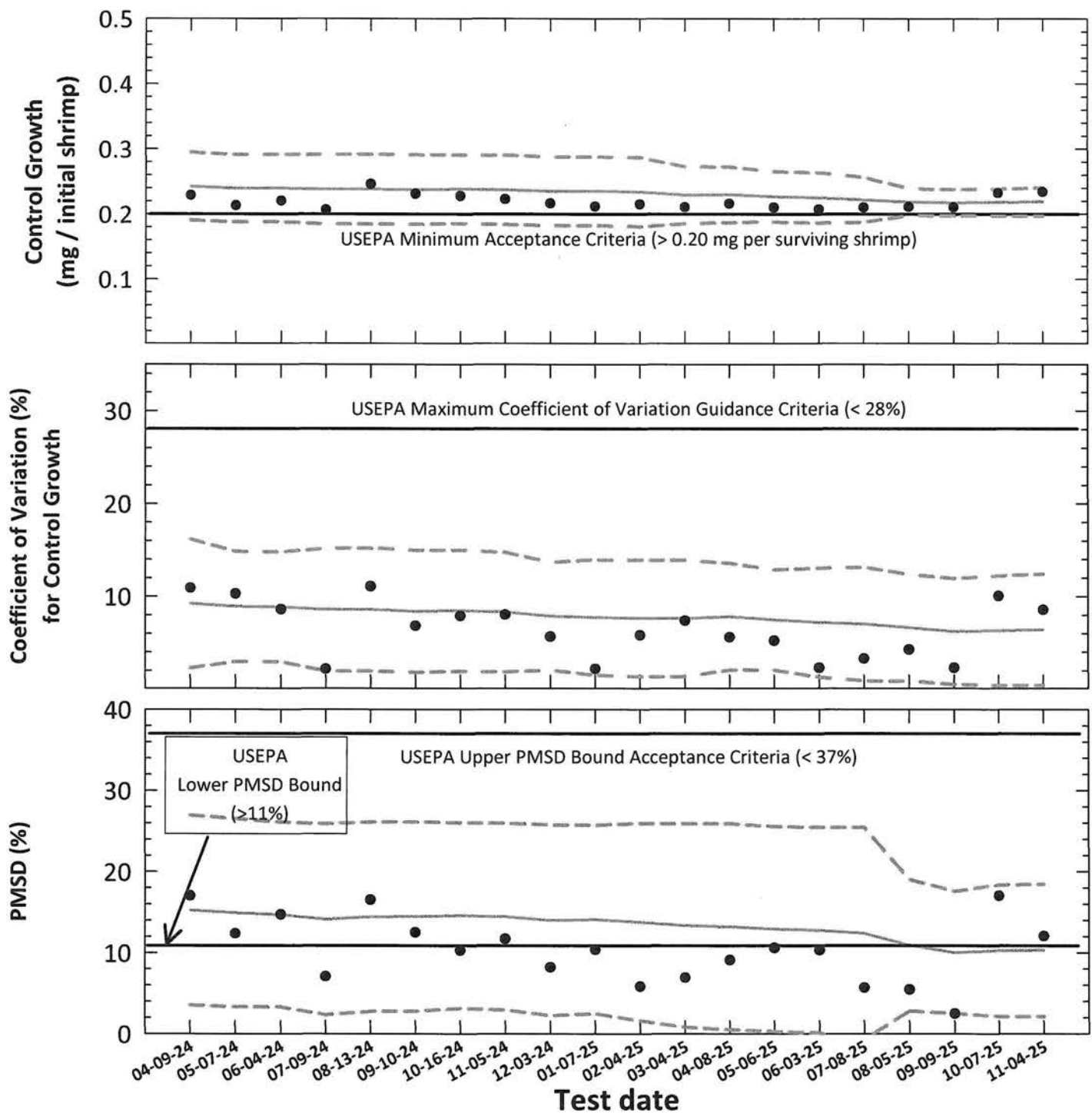
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75} = 0.32$).

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 CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
			Mean (mg/initial shrimp)	CV (%)											
1	04-09-24	100	0.229	10.9		17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9
2	05-07-24	100	0.213	10.3		12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5
3	06-04-24	100	0.220	8.6		14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3	26.1
4	07-09-24	100	0.207	2.2		7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3	25.9
5	08-13-24	100	0.246	11.1		16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.4	2.7	26.1
6	09-10-24	100	0.231	6.8		12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8	26.1
7	10-16-24	100	0.228	7.9		10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1	26.0
8	11-05-24	100	0.223	8.0		11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9	26.0
9	12-03-24	100	0.216	5.7		8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.0	2.2	25.8
10	01-07-25	100	0.212	2.2		10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5	25.7
11	02-04-25	100	0.215	5.8		5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5	25.9
12	03-04-25	100	0.211	7.4		6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8	25.9
13	04-08-25	100	0.216	5.6		9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5	25.9
14	05-06-25	100	0.210	5.2		10.6	0.227	0.188	0.265	7.5	2.0	12.9	12.9	0.3	25.5
15	06-03-25	100	0.207	2.3		10.3	0.225	0.186	0.264	7.2	1.3	13.1	12.8	0.1	25.4
16	07-08-25	100	0.210	3.3		5.7	0.222	0.188	0.257	7.0	0.9	13.2	12.4	-0.7	25.5
17	08-05-25	100	0.211	4.3		5.5	0.219	0.198	0.239	6.6	0.9	12.4	10.9	2.8	19.0
18	09-09-25	100	0.210	2.3		2.5	0.218	0.198	0.237	6.2	0.5	11.9	10.0	2.5	17.5
19	10-07-25	100	0.232	10.0		17.0	0.218	0.197	0.239	6.3	0.4	12.2	10.2	2.1	18.4
20	11-04-25	100	0.234	8.6		12.1	0.219	0.197	0.241	6.4	0.4	12.4	10.3	2.1	18.4

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.20 mg/surviving shrimp.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 37%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.

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

AbKCICR Test Number: 269

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2437					
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:	10-28-25 1200 to 10-29-25 1130	Incubator number and shelf location:	5 B
Organism source:	AI Batch Ab: 10-29-25	Artemia CHM number:	CHM1385
Transfer bowl information:		Drying information for weight determination:	
pH = 8.09 S.U. Temperature = 25.0 °C		Date / Time in oven:	11-11-25 1015
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	11-11-25 1015
		*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	11-04-25	1042	H	1200	H	1150	H	10-29-25 B
1	11-05-25	0900	H	1230	H	0900	H	↓
2	11-06-25	0905	H	1120	H	0900	H	11-03-25
3	11-07-25	0900	H	1100	H	0815	H	↓
4	11-08-25	0600	H	1200	H	0900	H	11-06-25
5	11-09-25	0600	H	1100	H	0842	H	↓
6	11-10-25	0600	H	1200	H	0840	H	↓
7	11-11-25					0950	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	13066465

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	537.7
Average weight per initial shrimp:	0.234		NOEC (mg/L KCl)	315
Average weight per surviving shrimp:	0.234	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	451.8

AbKCICR Test Number: **269**

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>XL</u> Date: <u>10/22/15</u>	9.79	10.11	10.15	10.28	12.62	12.70	13.89	12.31	13.73	12.25	15.18	15.32	12.54	10.48	13.45	15.05
*B = Pan + Shrimp weight (mg) Analyst: <u>11-13-21</u> Date: <u>11</u>	10.95	11.27	11.27	11.29	13.99	13.88	15.10	13.46	14.91	13.67	16.63	16.71	13.79	11.71	15.11	16.2
C = Shrimp weight (mg) = B - A Hand calculated: <u>11</u> Analyst: <u>11</u>	1.16	1.16	1.12	1.01	1.37	1.18	1.21	1.15	1.18	1.42	1.45	1.39	1.25	1.23	1.26	1.2
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>11</u> Analyst: <u>11</u>	0.232	0.232	0.224	0.202	0.274	0.236	0.242	0.230	0.236	0.284	0.290	0.278	0.250	0.246	0.252	0.24
Average weight per initial number of shrimp (mg) 0.234												Average weight per initial number of shrimp (mg) 0.260				Percent reduction from control (%) -10.9 7.

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

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

Comments:

AbKCICR Test Number: **269**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	4 nd	4 nd	S	S
3	5	S	5	5	S	S	S	S	S	S	S	S	4	4	S	S
4	S	S	S	S	S	S	S	S	S	4 nd	4 nd	S	4	4	S	S
5	S	S	S	S	S	S	S	S	3 rd	4	4	S	3 rd	4	4 nd	3 rd
6	S	S	S	S	S	S	S	S	3	4	4	3 rd	3	4	4	3
7	S	S	S	S	S	S	S	S	3	4	3 rd	2 nd	3	3 rd	4	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# Immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>XL</u> Date: <u>10-22-25</u>	14.88	15.46	14.15	13.48	13.59	10.42	10.33	14.02	12.55	14.43	12.46	13.12	10.01	9.93	13.20	14.91
*B = Pan + Shrimp weight (mg) Analyst: <u>jl</u> Date: <u>11-13-25</u>	16.22	16.63	15.45	14.96	14.86	11.51	11.46	15.14	13.33	15.13	13.03	13.67	10.67	10.79	14.09	15.6
C = Shrimp weight (mg) = B - A Hand calculated: <u>jl</u> Analyst: <u>jl</u>	1.34	1.17	1.30	1.28	1.27	1.09	1.13	1.12	0.78	0.70	0.57	0.55	0.66	0.86	0.89	0.69
Weight per Initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>jl</u> Analyst: <u>jl</u>	0.266	0.234	0.260	0.256	0.254	0.218	0.226	0.224	0.156	0.140	0.114	0.110	0.132	0.172	0.178	0.190
Average weight per initial number of shrimp (mg) 0.243				Percent reduction from control (%) -3.67				Average weight per initial number of shrimp (mg) 0.149				Percent reduction from control (%) 36.37				

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

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

Comments:

AbKCICR Test Number: **269**

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: _____ 26

Test dates: _____ November 04-11, 201

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	9.79	10.95	1.16	0.232	100.0	0.234	8.6	Not applicable
	B	5	5	10.11	11.27	1.16	0.232				
	C	5	5	10.15	11.27	1.12	0.224				
	D	5	5	10.28	11.29	1.01	0.202				
	E	5	5	12.62	13.99	1.37	0.274				
	F	5	5	12.70	13.88	1.18	0.236				
	G	5	5	13.89	15.10	1.21	0.242				
	H	5	5	12.31	13.46	1.15	0.230				
250	I	5	5	13.73	14.91	1.18	0.236	100.0	0.260	8.2	-10.9
	J	5	5	12.25	13.67	1.42	0.284				
	K	5	5	15.18	16.63	1.45	0.290				
	L	5	5	15.32	16.71	1.39	0.278				
	M	5	5	12.54	13.79	1.25	0.250				
	N	5	5	10.48	11.71	1.23	0.246				
	O	5	5	13.85	15.11	1.26	0.252				
	P	5	5	15.05	16.25	1.20	0.240				
375	Q	5	5	14.88	16.22	1.34	0.268	100.0	0.243	7.9	-3.6
	R	5	5	15.46	16.63	1.17	0.234				
	S	5	5	14.15	15.45	1.30	0.260				
	T	5	5	13.68	14.96	1.28	0.256				
	U	5	5	13.59	14.86	1.27	0.254				
	V	5	5	10.42	11.51	1.09	0.218				
	W	5	5	10.33	11.46	1.13	0.226				
	X	5	5	14.02	15.14	1.12	0.224				
500	Y	5	3	12.55	13.33	0.78	0.156	62.5	0.149	20.0	36.3
	Z	5	4	14.43	15.13	0.70	0.140				
	AA	5	3	12.46	13.03	0.57	0.114				
	BB	5	2	13.12	13.67	0.55	0.110				
	CC	5	3	10.01	10.67	0.66	0.132				
	DD	5	3	9.93	10.79	0.86	0.172				
	EE	5	4	13.20	14.09	0.89	0.178				
	FF	5	3	14.91	15.86	0.95	0.190				
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: 0.0282
PMSD: 12.1

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.

Mysid Survival and Growth Test-Growth-Weight													
Start Date:	11/4/2025		Test ID:	AbKCICR		Sample ID:	REF-Ref Toxicant						
End Date:	11/11/2025		Lab ID:	ETS-Envr. 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	0.2320	0.2320	0.2240	0.2020	0.2740	0.2360	0.2420	0.2300					
250	0.2360	0.2840	0.2900	0.2780	0.2500	0.2460	0.2520	0.2400					
375	0.2680	0.2340	0.2600	0.2560	0.2540	0.2180	0.2260	0.2240					
500	0.1560	0.1400	0.1140	0.1100	0.1320	0.1720	0.1780	0.1900					
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	N-Mean	
D-Control	0.2340	1.0000	0.2340	0.2020	0.2740	8.571	8				0.2468	1.0000	
250	0.2595	1.1090	0.2595	0.2360	0.2900	8.154	8	-2.534	2.799	0.0282	0.2468	1.0000	
375	0.2425	1.0363	0.2425	0.2180	0.2680	7.883	8	-0.845	2.799	0.0282	0.2425	0.9828	
500	0.1490	0.6368	0.1490	0.1100	0.1900	20.000	8				0.1490	0.6039	
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)							0.96849		0.884		0.37152		-0.7182
Bartlett's Test indicates equal variances (p = 0.97)							0.068		9.21035				
Hypothesis Test (1-tail, 0.01)			NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df	
Dunnett's Test			375	>375			0.02817	0.12036	0.00135	0.00041	0.05549	2, 21	
Treatments vs D-Control													
Linear Interpolation (200 Resamples)													
Point	mg/L	SD	95% CL		Skew								
IC05	385.81	16.91	333.74	393.33	-2.0629								
IC10	402.31	8.05	384.45	412.54	-0.8621								
IC15	418.80	7.96	401.03	431.31	-0.4338								
IC20	435.29	8.45	417.25	452.32	-0.0857								
IC25	451.79	9.33	432.44	471.81	0.2059								
IC40	501.59	12.99	480.35	528.65	0.2473								
IC50	542.99	13.43	513.21	565.54	-0.3681								

AbKCICR Test Number: **269**

Daily Chemistry:

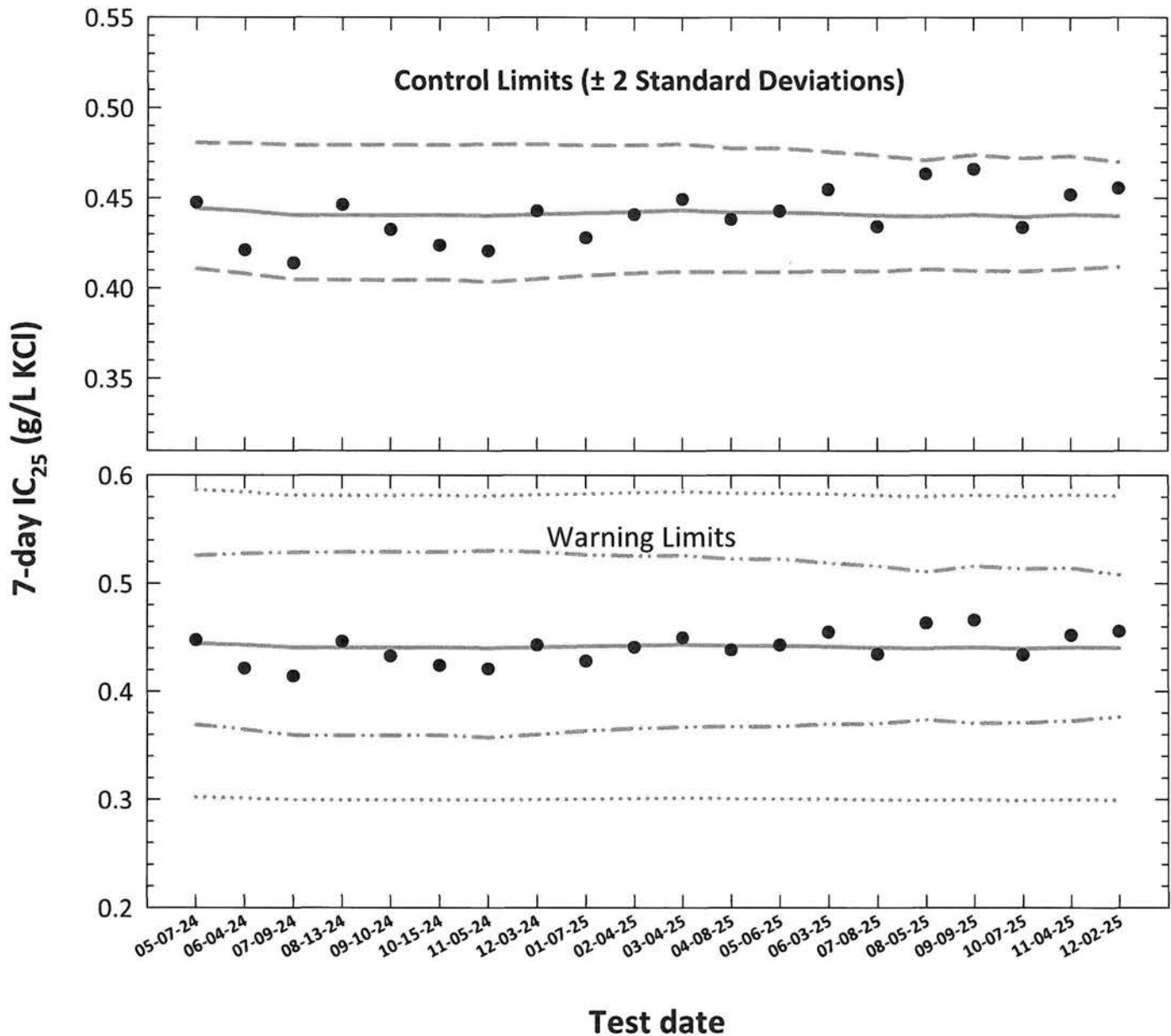
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

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 XL	XL	XL	XL	XL	XL
	DO (mg/L)	7.98	7.89	7.93	7.81	7.93	7.86
	Salinity (ppt)	8.5	8.0	8.2	7.8	8.2	7.9
	Alkalinity (mg CaCO ₃ /L)	25.0	25.1	25.0	25.0	25.0	25.1
	Temperature (°C)	100				98	
250 mg KCl/L	pH (S.U.)	25.2	26.0	25.2	26.3	25.2	26.1
	DO (mg/L)	8.00	7.90	7.93	7.87	7.93	7.86
	Salinity (ppt)	8.5	8.0	8.1	7.9	8.2	7.9
	Temperature (°C)	25.0	25.3	25.0	25.3	25.1	25.2
375 mg KCl/L	pH (S.U.)	25.4	26.2	25.1	26.0	25.2	26.3
	DO (mg/L)	8.00	7.90	7.93	7.86	7.93	7.86
	Salinity (ppt)	8.5	7.9	8.1	7.9	8.1	7.9
	Temperature (°C)	25.1	25.4	25.1	25.6	25.4	25.5
500 mg KCl/L	pH (S.U.)	25.4	26.0	25.1	26.3	25.3	26.2
	DO (mg/L)	8.01	7.90	7.92	7.85	7.91	7.86
	Salinity (ppt)	8.5	7.9	8.1	7.9	8.2	7.8
	Temperature (°C)	25.2	25.7	25.2	25.6	25.5	25.7
750 mg KCl/L	pH (S.U.)	25.4	26.0	25.4	26.1	25.3	26.2
	DO (mg/L)	8.01	7.90				
	Salinity (ppt)	8.4	7.9				
	Temperature (°C)	25.3	26.0				
1000 mg KCl/L	pH (S.U.)	25.3	25.9				
	DO (mg/L)	8.01	7.90				
	Salinity (ppt)	8.4	7.9				
	Temperature (°C)	25.4	26.1				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: **269**

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.)		7.92	7.79	7.96	7.81	7.99	7.82	7.99
	DO (mg/L)		8.1	8.1	8.2	7.9	8.2	7.9	8.2
	Salinity (ppt)		25.0	25.2	25.0	25.1	25.0	25.2	25.0
	Alkalinity (mg CaCO ₃ /L)		11.15 M	9.5				11.15 M	
	Temperature (°C)		25.2	26.0	25.2	26.4	25.1	26.2	25.2
250 mg KCl/L	pH (S.U.)		7.92	7.81	7.95	7.80	7.77	7.78	7.91
	DO (mg/L)		8.0	8.1	8.3	7.7	8.2	7.8	8.1
	Salinity (ppt)		25.2	25.3	25.2	25.3	25.1	25.4	25.3
	Temperature (°C)		25.2	26.3	25.1	26.2	25.3	26.2	25.3
375 mg KCl/L	pH (S.U.)		7.92	7.81	7.95	7.79	7.97	7.78	7.95
	DO (mg/L)		8.0	8.1	8.3	7.7	8.2	7.7	8.1
	Salinity (ppt)		25.1	25.4	25.4	25.5	25.2	25.5	25.6
	Temperature (°C)		25.3	26.2	25.1	26.4	25.1	26.3	25.3
500 mg KCl/L	pH (S.U.)		7.91	7.83	7.95	7.77	7.97	7.79	7.95
	DO (mg/L)		8.0	8.0	8.3	7.7	8.2	7.8	8.2
	Salinity (ppt)		25.3	25.6	25.5	25.6	25.3	25.7	25.6
	Temperature (°C)		25.2	26.2	25.3	26.5	25.1	26.3	25.3
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
			Initial	Final	Initial	Final	Initial	Final	Initial
									Final

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



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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)		
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S
1	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109 0.4805
2	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082 0.4804
3	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047 0.4794
4	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048 0.4796
5	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046 0.4796
6	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048 0.4795
7	11-05-24	0.4206	-0.3761	-0.3565	0.0188	0.4401	0.4036 0.4798
8	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053 0.4800
9	01-07-25	0.4280	-0.3686	-0.3549	0.0177	0.4417	0.4071 0.4792
10	02-04-25	0.4408	-0.3558	-0.3542	0.0173	0.4424	0.4085 0.4792
11	03-04-25	0.4493	-0.3474	-0.3534	0.0173	0.4432	0.4093 0.4799
12	04-08-25	0.4382	-0.3583	-0.3545	0.0169	0.4421	0.4091 0.4777
13	05-06-25	0.4428	-0.3538	-0.3545	0.0169	0.4421	0.4091 0.4778
14	06-03-25	0.4548	-0.3422	-0.3550	0.0162	0.4415	0.4098 0.4757
15	07-08-25	0.4342	-0.3624	-0.3562	0.0159	0.4404	0.4093 0.4738
16	08-05-25	0.4634	-0.3340	-0.3567	0.0149	0.4398	0.4107 0.4710
17	09-09-25	0.4660	-0.3316	-0.3559	0.0158	0.4407	0.4097 0.4740
18	10-07-25	0.4336	-0.3629	-0.3570	0.0155	0.4396	0.4093 0.4721
19	11-04-25	0.4518	-0.3451	-0.3557	0.0154	0.4408	0.4107 0.4732
20	12-02-25	0.4557	-0.3414	-0.3564	0.0143	0.4401	0.4121 0.4701
						Laboratory Calculated CV Warning Limits	
						CT - 2CV	CT + 2CV
						0.3690	0.5258
						0.3645	0.5278
						0.3593	0.5288
						0.3593	0.5291
						0.3590	0.5292
						0.3594	0.5289
						0.3572	0.5304
						0.3600	0.5294
						0.3634	0.5266
						0.3657	0.5255
						0.3667	0.5260
						0.3674	0.5228
						0.3674	0.5228
						0.3696	0.5190
						0.3699	0.5162
						0.3736	0.5108
						0.3705	0.5162
						0.3708	0.5135
						0.3724	0.5143
						0.3764	0.5082
						75th Percentile CV Warning Limits	
						CT - S _{A,75}	CT + S _{A,75}
						0.3022	0.5865
						0.3011	0.5845
						0.2995	0.5814
						0.2996	0.5816
						0.2995	0.5814
						0.2996	0.5815
						0.2993	0.5809
						0.2999	0.5822
						0.3003	0.5830
						0.3008	0.5840
						0.3014	0.5850
						0.3006	0.5835
						0.3006	0.5836
						0.3002	0.5828
						0.2995	0.5813
						0.2991	0.5806
						0.2997	0.5817
						0.2989	0.5802
						0.2998	0.5819
						0.2993	0.5810

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

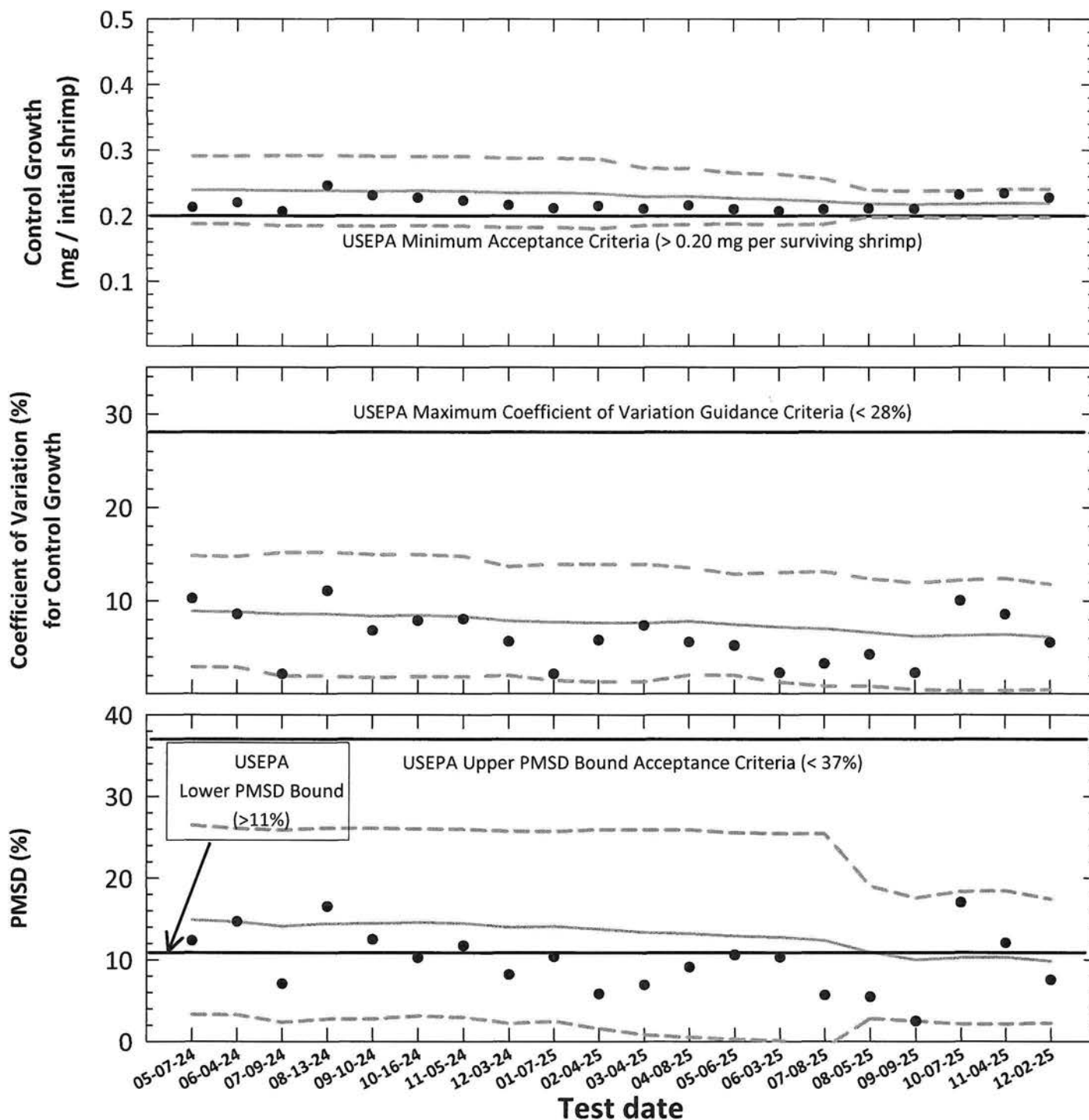
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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

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

CV = Coefficient of variation.

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



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

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

Test number	Test date	ToxCat Determination				Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial shrimp)	CV (%)										
1	05-07-24	100	0.213	10.3	0.0263	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5
2	06-04-24	100	0.220	8.6	0.0323	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3	26.1
3	07-09-24	100	0.207	2.2	0.0146	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3	25.9
4	08-13-24	100	0.246	11.1	0.0406	0.238	0.185	0.292	8.6	1.9	15.2	14.4	2.7	26.1
5	09-10-24	100	0.231	6.8	0.0288	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8	26.1
6	10-16-24	100	0.228	7.9	0.0233	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1	26.0
7	11-05-24	100	0.223	8.0	0.0261	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9	26.0
8	12-03-24	100	0.216	5.7	0.0177	0.235	0.182	0.288	7.9	2.0	13.7	14.0	2.2	25.8
9	01-07-25	100	0.212	2.2	0.0219	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5	25.7
10	02-04-25	100	0.215	5.8	0.0125	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5	25.9
11	03-04-25	100	0.211	7.4	0.0146	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8	25.9
12	04-08-25	100	0.216	5.6	0.0196	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5	25.9
13	05-06-25	100	0.210	5.2	0.0222	0.227	0.188	0.265	7.5	2.0	12.9	12.9	0.3	25.5
14	06-03-25	100	0.207	2.3	0.0213	0.225	0.186	0.264	7.2	1.3	13.1	12.8	0.1	25.4
15	07-08-25	100	0.210	3.3	0.0119	0.222	0.188	0.257	7.0	0.9	13.2	12.4	-0.7	25.5
16	08-05-25	100	0.211	4.3	0.0115	0.219	0.198	0.239	6.6	0.9	12.4	10.9	2.8	19.0
17	09-09-25	100	0.210	2.3	0.0052	0.218	0.198	0.237	6.2	0.5	11.9	10.0	2.5	17.5
18	10-07-25	100	0.232	10.0	0.0395	0.218	0.197	0.239	6.3	0.4	12.2	10.2	2.1	18.4
19	11-04-25	100	0.234	8.6	0.0282	0.219	0.197	0.241	6.4	0.4	12.4	10.3	2.1	18.4
20	12-02-25	100	0.228	5.6	0.0171	0.219	0.197	0.241	6.2	0.5	11.8	9.8	2.2	17.4

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

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

CV = Coefficient of variation for control growth.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

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

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

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

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

Species: *Americamysis (Mysidopsis) bahia*

AbKCICR Test Number: **270**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2437					
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:	6NEX
Date and times organisms were born between:	11-26-25 1200 to 11-27-25 1130		Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 11-27-25		Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 7.94 S.U.		Drying information for weight determination:	
	Temperature = 25.0 °C		Date / Time in oven:	12-09-25 1015
Average transfer volume:	< 0.25 mL		*Initial oven temperature:	60 °C
			Date / Time out of oven:	12-10-25 1015
			*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	12-02-25	1120	<i>h</i>	1430	<i>h</i>	1145	<i>h</i>	11-24-25 A
1	12-03-25	0500	<i>h</i>	1100	<i>h</i>	0130	<i>h</i>	↓
2	12-04-25	0500	<i>h</i>	1340	<i>h</i>	0745	<i>h</i>	11-24-25 B
3	12-05-25	0500	<i>h</i>	1100	<i>h</i>	0130	<i>h</i>	↓
4	12-06-25	0630	<i>h</i>	1230	<i>h</i>	0900	<i>h</i>	12-02-25
5	12-07-25	0600	<i>h</i>	1200	<i>h</i>	0930	<i>h</i>	↓
6	12-08-25	0500	<i>h</i>	1100	<i>h</i>	0845	<i>h</i>	↓
7	12-09-25					0945	<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)	547.1
Average weight per initial shrimp:	0.228		NOEC (mg/L KCl)	250
Average weight per surviving shrimp:	0.228	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	375
			ChV (mg/L KCl)	306.2
			IC ₂₅ (mg/L KCl)	455.7

375
500
433
12-10-25 *h*

AbKCICR Test Number: **270**

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>Light Pink</u> Analyst: <u>fen</u> Date: <u>11-18-25</u>	13.56	13.78	15.06	13.72	10.35	12.41	13.41	15.44	11.66	14.39	14.51	15.03	13.59	13.36	14.09	11.71
*B = Pan + Shrimp weight (mg) Analyst: <u>M</u> Date: <u>12-15-25</u>	14.71	14.79	16.19	14.88	11.61	13.57	14.64	16.54	12.77	15.61	15.61	16.04	14.75	14.47	15.13	12.81
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>M</u>	1.15	1.01	1.13	1.16	1.16	1.16	1.23	1.10	1.11	1.22	1.10	1.01	1.16	1.11	1.04	1.10
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>M</u>	0.230	0.202	0.226	0.232	0.232	0.232	0.246	0.220	0.222	0.244	0.220	0.202	0.232	0.222	0.208	0.220
Average weight per initial number of shrimp (mg) 0.228								Average weight per initial number of shrimp (mg) 0.221				Percent reduction from control (%) 2.77				

*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:

AbKCICR Test Number: **270**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	4 ^{1d}	4 ^{1d}	S	S	4 ^{1d}	S	S
4	S	S	S	S	S	S	S	S	S	4	4	S	S	4	S	S
5	S	S	S	S	S	S	S	S	S	4	4	S	S	4	S	4 ^{1d}
6	S	S	S	S	S	S	S	S	S	4	4	S	S	4	S	4
7	S	S	S	S	S	S	S	S	3 ^{2d}	4	3 ^{1d}	3 ^{2d}	4 ^{1d}	4	3 ^{2d}	5 ^{1d}
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																11-12-23
*A = Pan weight (mg) Tray color code: <u>Light Pink</u> Analyst: <u>ELM</u> Date: <u>11-18-25</u>	14.28	11.78	10.17	13.86	13.50	10.29	13.09	11.63	12.21	9.98	13.52	12.68	10.35	14.79	13.62	9.62
*B = Pan + Shrimp weight (mg) Analyst: <u>ELM</u> Date: <u>11-18-25</u>	15.36	12.93	17.20	14.88	14.52	11.30	14.07	12.72	12.90	10.63	14.27	13.41	11.18	15.52	14.47	10.57
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>ELM</u>	1.08	1.15	1.03	1.02	1.02	1.01	0.98	1.09	0.69	0.65	0.75	0.73	0.83	0.73	0.85	0.74
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>ELM</u>	0.216	0.230	0.206	0.204	0.204	0.202	0.196	0.218	0.138	0.130	0.150	0.146	0.166	0.146	0.170	0.148
Average weight per initial number of shrimp (mg) 0.210				Percent reduction from control (%) 7.97				Average weight per initial number of shrimp (mg) 0.149				Percent reduction from control (%) 34.47				

*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: **270**

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	11.03.15															
*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: _____	11.03.15															
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 (%) 1001-				Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 1001.			

*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: 27

Test dates: December 02-09, 202

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.56	14.71	1.15	0.230	100.0	0.228	5.6	Not applicable
	B	5	5	13.78	14.79	1.01	0.202				
	C	5	5	15.06	16.19	1.13	0.226				
	D	5	5	13.72	14.88	1.16	0.232				
	E	5	5	10.35	11.51	1.16	0.232				
	F	5	5	12.41	13.57	1.16	0.232				
	G	5	5	13.41	14.64	1.23	0.246				
	H	5	5	15.44	16.54	1.10	0.220				
250	I	5	5	11.66	12.77	1.11	0.222	100.0	0.221	5.9	2.7
	J	5	5	14.39	15.61	1.22	0.244				
	K	5	5	14.51	15.61	1.10	0.220				
	L	5	5	15.03	16.04	1.01	0.202				
	M	5	5	13.59	14.75	1.16	0.232				
	N	5	5	13.36	14.47	1.11	0.222				
	O	5	5	14.09	15.13	1.04	0.208				
	P	5	5	11.71	12.81	1.10	0.220				
375	Q	5	5	14.28	15.36	1.08	0.216	100.0	0.210	5.2	7.9
	R	5	5	11.78	12.93	1.15	0.230				
	S	5	5	16.17	17.20	1.03	0.206				
	T	5	5	13.86	14.88	1.02	0.204				
	U	5	5	13.50	14.52	1.02	0.204				
	V	5	5	10.29	11.30	1.01	0.202				
	W	5	5	13.09	14.07	0.98	0.196				
	X	5	5	11.63	12.72	1.09	0.218				
500	Y	5	3	12.21	12.90	0.69	0.138	67.5	0.149	8.9	34.4
	Z	5	4	9.98	10.63	0.65	0.130				
	AA	5	3	13.52	14.27	0.75	0.150				
	BB	5	3	12.68	13.41	0.73	0.146				
	CC	5	4	10.35	11.18	0.83	0.166				
	DD	5	4	14.79	15.52	0.73	0.146				
	EE	5	3	13.62	14.47	0.85	0.170				
	FF	5	3	9.63	10.37	0.74	0.148				
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:

0.0171

PMSD:

7.5

MSD =

PMSD =

Minimum Significant Difference

Percent Minimum Significant Difference

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

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

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

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

Mysid Survival and Growth Test-7 Day Survival

Start Date: 12/2/2025 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 12/9/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanmysis bahia
Comments:

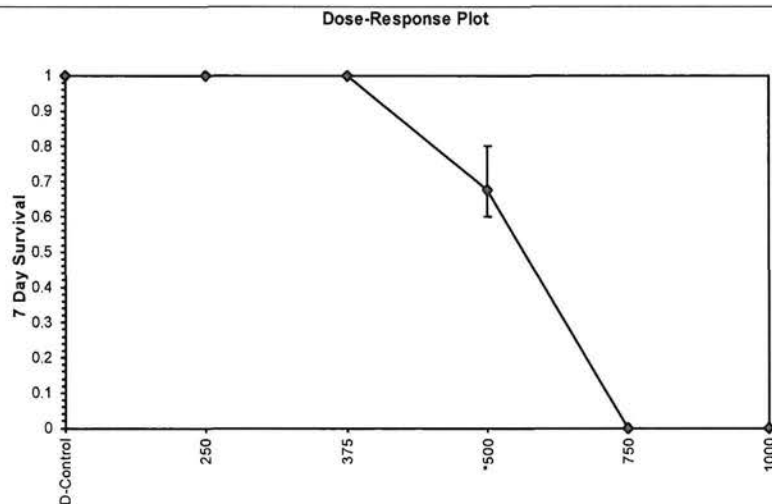
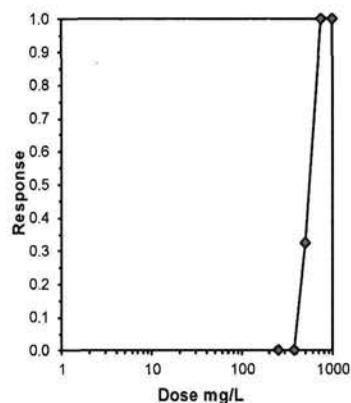
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.8000	0.6000	0.6000	0.8000	0.8000	0.6000	0.6000
750	0.0000	0.0000	0.0000	0.0000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

Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.6750	0.6750	0.9690	0.8861	1.1071	11.808	8	36.00	48.00	13	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)
Steel's Many-One Rank Test
Treatments vs D-Control

Trim Level	EC50	95% CL
0.0%	547.14	519.76 575.96
5.0%	548.82	518.20 581.25
10.0%	550.45	515.36 587.92
20.0%	553.37	503.42 608.28
Auto-0.0%	547.14	519.76 575.96



Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2300	0.2020	0.2260	0.2320	0.2320	0.2320	0.2460	0.2200
250	0.2220	0.2440	0.2200	0.2020	0.2320	0.2220	0.2080	0.2200
375	0.2160	0.2300	0.2060	0.2040	0.2040	0.2020	0.1960	0.2180
500	0.1380	0.1300	0.1500	0.1460	0.1660	0.1460	0.1700	0.1480
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed			Isotonic	
			Mean	Min	Max	CV%			Critical	MSD	Mean	N-Mean	
D-Control	0.2275	1.0000	0.2275	0.2020	0.2460	5.555	8					0.2275	1.0000
250	0.2213	0.9725	0.2213	0.2020	0.2440	5.877	8	1.021	2.799	0.0171		0.2213	0.9725
*375	0.2095	0.9209	0.2095	0.1960	0.2300	5.247	8	2.941	2.799	0.0171		0.2095	0.9209
500	0.1493	0.6560	0.1493	0.1300	0.1700	8.887	8					0.1493	0.6560
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 ($p > 0.01$)					0.9778	0.884	-0.0174	0.23055		
Bartlett's Test indicates equal variances ($p = 0.90$)					0.20541	9.21035				
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	250	375	306.186		0.01713	0.0753	0.00067	0.00015	0.02434	2, 21
Treatments vs D-Control										

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	304.52	53.04	178.80	379.56	-0.6454
IC10	384.85	16.12	338.98	401.47	-2.2858
IC15	408.45	8.85	391.09	424.54	-0.1378
IC20	432.05	8.53	416.01	448.06	-0.0972
IC25	455.65	8.87	439.97	472.29	0.0590
IC40	521.36	7.70	505.22	535.21	-0.2159
IC50	559.46	6.41	546.02	571.01	-0.2091

Dose-Response Plot

Growth-Weight Plot

AbKCICR Test Number: 270

Daily Chemistry:

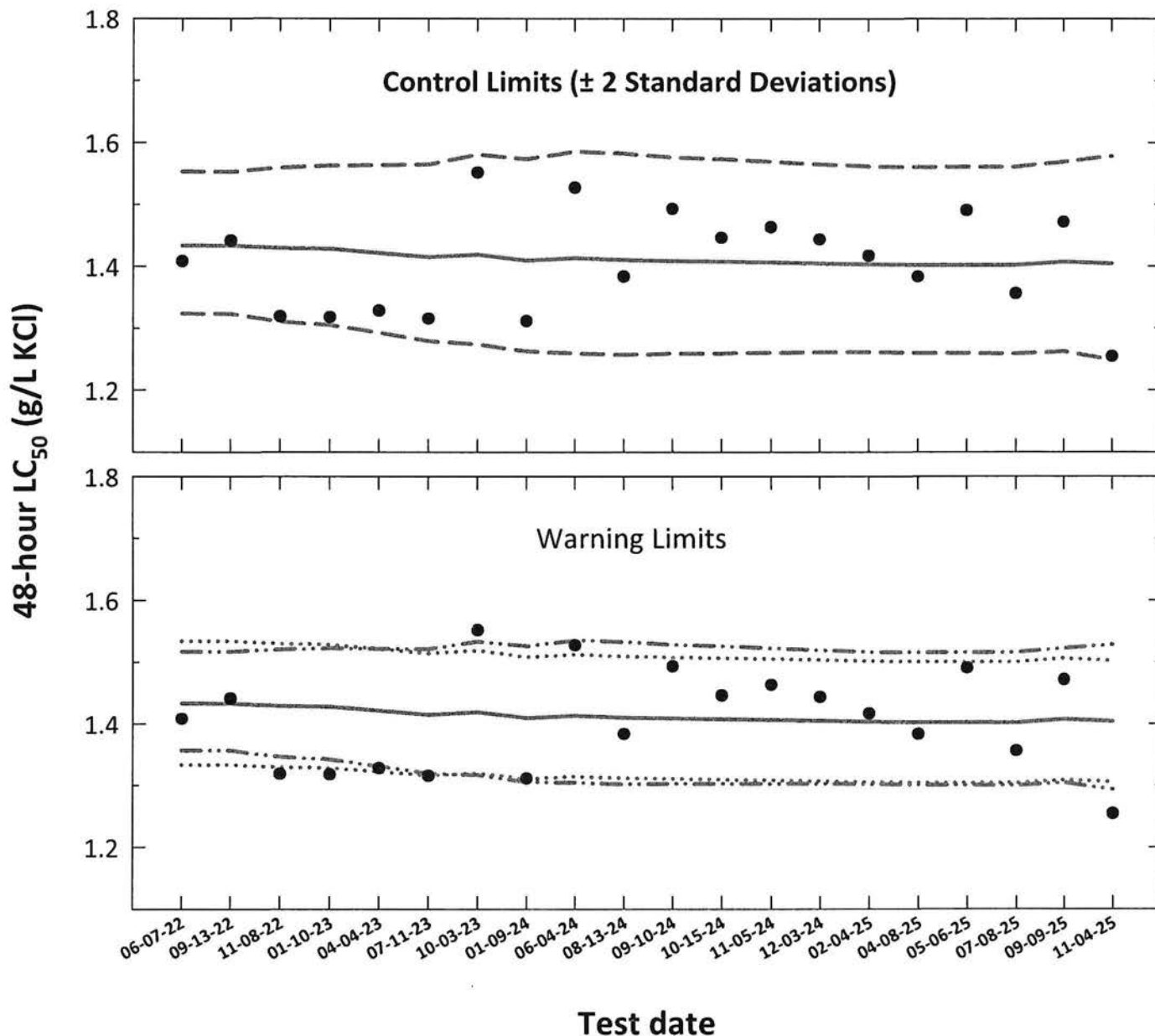
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

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 XL	XL	XL	XL	XL	XL
	DO (mg/L)	8.06	7.94	8.00	7.87	7.97	7.88
	Salinity (ppt)	8.4	8.0	8.1	8.3	8.5	7.8
	Alkalinity (mg CaCO ₃ /L)	25.0	25.6	25.0	25.1	25.0	25.1
	Temperature (°C)	100			98		
250 mg KCl/L	pH (S.U.)	26.6	26.1	25.1	26.3	25.2	26.3
	DO (mg/L)	8.03	7.95	7.99	7.88	7.96	7.89
	Salinity (ppt)	8.4	8.0	8.1	8.3	8.5	7.8
	Temperature (°C)	25.0	25.6	25.3	25.6	25.4	24.9
375 mg KCl/L	pH (S.U.)	25.9	26.4	25.2	26.1	25.2	26.1
	DO (mg/L)	8.03	7.95	8.00	7.87	7.97	7.89
	Salinity (ppt)	8.4	8.0	8.2	8.2	8.5	7.8
	Temperature (°C)	25.3	25.8	25.5	26.0	25.6	25.5
500 mg KCl/L	pH (S.U.)	25.9	26.0	25.2	26.1	25.2	26.2
	DO (mg/L)	8.04	7.95	8.01	7.87	7.97	7.89
	Salinity (ppt)	8.4	8.0	8.2	8.2	8.5	7.8
	Temperature (°C)	25.3	26.0	25.6	26.2	25.7	26.2
750 mg KCl/L	pH (S.U.)	26.0	26.2	25.1	26.4	25.2	26.2
	DO (mg/L)	8.04	7.94				
	Salinity (ppt)	8.4	8.0				
	Temperature (°C)	25.5	26.2				
1000 mg KCl/L	pH (S.U.)	25.8	26.2				
	DO (mg/L)	8.04	7.94				
	Salinity (ppt)	8.4	8.0				
	Temperature (°C)	25.6	26.5				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 270

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		XL	BSC	BSL	BSC	BSL	XL	XL	BSL ^{XL}
CONTROL, Salt SW	Analyst								
	pH (S.U.)	7.98	7.93	8.10	7.96	8.11	7.98	8.07	7.92
	DO (mg/L)	8.1	8.2	8.3	7.5	8.1	8.0	8.2	8.0
	Salinity (ppt)	25.2	25.1	25.1	25.1	25.1	25.2	25.0	25.3
	Alkalinity (mg CaCO ₃ /L)	12.0		12.0		12.0		12.0	
250 mg KCl/L	Temperature (°C)	25.1	26.3	25.1	26.3	25.2	26.3	25.3	26.2
	pH (S.U.)	7.97	7.93	8.03	7.89	8.12	7.95	8.06	7.93
	DO (mg/L)	8.1	8.2	8.4	7.6	8.1	7.9	8.2	8.0
	Salinity (ppt)	25.2	25.3	25.2	25.2	25.3	25.4	25.3	25.4
375 mg KCl/L	Temperature (°C)	25.2	26.0	25.2	26.1	25.2	26.0	25.2	26.3
	pH (S.U.)	7.97	7.92	8.03	7.88	8.13	7.95	8.07	7.91
	DO (mg/L)	8.0	8.1	8.4	7.6	8.1	7.9	8.1	8.0
	Salinity (ppt)	25.2	25.4	25.2	25.4	25.3	25.5	25.5	25.8
500 mg KCl/L	Temperature (°C)	25.2	26.0	25.2	26.2	25.3	26.2	25.2	26.3
	pH (S.U.)	7.97	7.90	8.03	7.86	8.13	7.97	8.07	7.96
	DO (mg/L)	8.0	8.1	8.4	7.5	8.1	7.8	8.1	7.9
	Salinity (ppt)	25.4	25.6	25.2	25.0	25.4	25.5	25.5	26.0
750 mg KCl/L	Temperature (°C)	25.2	26.3	25.2	26.2	25.1	26.2	25.2	26.3
	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
1000 mg KCl/L	Temperature (°C)								
	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)					
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169
2	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163
3	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205
4	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227
5	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213
6	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209
7	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331
8	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256
9	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351
10	08-13-24	1.3832	0.1409	0.1493	0.0250	1.4102	1.2569	1.5823	1.3015	1.5322
11	09-10-24	1.4930	0.1740	0.1488	0.0244	1.4087	1.2589	1.5763	1.3024	1.5277
12	10-15-24	1.4461	0.1602	0.1484	0.0242	1.4075	1.2593	1.5731	1.3022	1.5251
13	11-05-24	1.4632	0.1653	0.1480	0.0238	1.4062	1.2603	1.5689	1.3024	1.5219
14	12-03-24	1.4436	0.1594	0.1476	0.0235	1.4047	1.2609	1.5649	1.3023	1.5188
15	02-04-25	1.4168	0.1513	0.1470	0.0232	1.4029	1.2607	1.5611	1.3015	1.5156
16	04-08-25	1.3832	0.1409	0.1468	0.0232	1.4021	1.2597	1.5605	1.3006	1.5150
17	05-06-25	1.4906	0.1734	0.1468	0.0233	1.4022	1.2596	1.5610	1.3005	1.5154
18	07-08-25	1.3559	0.1322	0.1467	0.0234	1.4017	1.2587	1.5611	1.2997	1.5154
19	09-09-25	1.4716	0.1678	0.1484	0.0236	1.4073	1.2623	1.5690	1.3042	1.5222
20	11-04-25	1.2543	0.0984	0.1473	0.0254	1.4038	1.2488	1.5780	1.2934	1.5279

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 # 104

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 #: 2437

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.98	7.88	7.80
	Dissolved oxygen (mg/L)	8.5	8.0	7.9
	*Salinity (ppt)	25.0	25.3	25.3
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	24.4	25.2	25.2
1000 mg/L	pH (S.U.)	7.99	7.87	7.79
	Dissolved oxygen (mg/L)	8.5	8.0	7.9
	*Salinity (ppt)	25.1	25.8	26.0
	*Temperature (°C)	24.5	25.0	25.4
1250 mg/L	pH (S.U.)	8.00	7.87	7.80
	Dissolved oxygen (mg/L)	8.5	8.0	7.9
	*Salinity (ppt)	26.0	26.1	26.3
	*Temperature (°C)	24.1	25.0	25.4
1500 mg/L	pH (S.U.)	8.00	7.87	7.81
	Dissolved oxygen (mg/L)	8.5	7.9	7.9
	*Salinity (ppt)	25.9	26.0	26.2
	*Temperature (°C)	24.3	25.2	25.1
1750 mg/L	pH (S.U.)	8.00	7.86	
	Dissolved oxygen (mg/L)	8.5	7.9	
	*Salinity (ppt)	26.4	27.0	
	*Temperature (°C)	24.5	25.2	
2000 mg/L	pH (S.U.)	8.00	7.84	
	Dissolved oxygen (mg/L)	8.4	7.9	
	*Salinity (ppt)	26.5	26.8	
	*Temperature (°C)	24.5	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	130664

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 # 104

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-04-25	* 1042	JP	1400	JP	6 B	orange	10-29-25B
24	11-05-25			1403	JP			
48 Termination	11-06-25			1455	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 Mb):	10-25-25
Age (9 to 14 days old):	10 DMS
Date organisms were born:	10-24-25 1200 TO 10-25-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.35 Temperature (°C) 25.7 °C

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	9 ^{1d}	7 ^{3d}	10	0 ^{10d}	3 ^{7d}	0 ^{10d}	0 ^{12d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	8 ^{1d}	4 ^{7d}	9 ^{1d}	0	1 ^{1d}	0	0	0	0
Mean Survival	100 %		90 %		65 %		5 %		0 %		0 %	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	1179.6
Upper 95% confidence limit (mg KCl/L)	1325.1
48-hour LC ₅₀ (mg KCl/L)	1254.3

Comments:



Acute Silverside Test-24 Hr Survival

Start Date: 11/4/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/6/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	0.9000
1250	0.7000	1.0000
1500	0.0000	0.3000
1750	0.0000	0.0000
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	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.418	2.850	0.5562	1	20
1250	0.8500	0.8500	1.2016	0.9912	1.4120	24.767	2	1.078	2.850	0.5562	3	20
*1500	0.1500	0.1500	0.3692	0.1588	0.5796	80.603	2	5.344	2.850	0.5562	17	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	6.422	2.850	0.5562	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

Normality of the data set cannot be confirmed

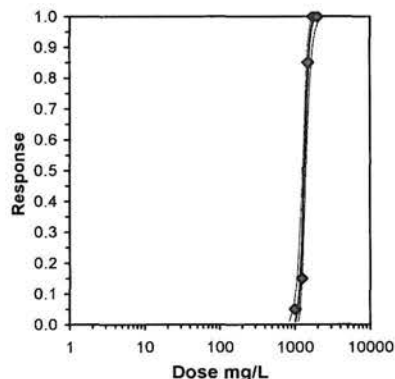
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	Chv	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.40477	0.41515	0.68473	0.03808	0.00359	4, 5

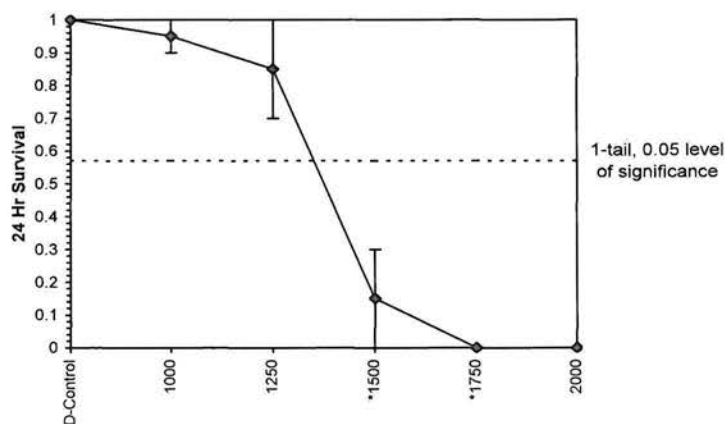
Treatments vs D-Control

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.7028	3.47938	11.8832	25.5224	0	6.37418	7.81472	0.09476	3.12802	0.05347	4
Intercept	-53.503	10.9145	-74.895	-32.11							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1008.41	837.406 1105.68
EC05	3.355	1096.67	952.294 1179.9
EC10	3.718	1146.83	1018.78 1222.77
EC15	3.964	1181.97	1065.48 1253.46
EC20	4.158	1210.66	1103.47 1279.15
EC25	4.326	1235.83	1136.51 1302.33
EC40	4.747	1301.6	1220.14 1367.18
EC50	5.000	1342.84	1269.25 1412.33
EC60	5.253	1385.38	1316.24 1463.51
EC75	5.674	1459.1	1388.73 1563.34
EC80	5.842	1489.44	1415.85 1607.93
EC85	6.036	1525.59	1446.66 1663.2
EC90	6.282	1572.33	1484.66 1737.47
EC95	6.645	1644.26	1540.29 1856.72
EC99	7.326	1788.17	1645.46 2109.18



Dose-Response Plot



Acute Silverside Test-48 Hr Survival

Start Date: 11/4/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/6/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
Comments:

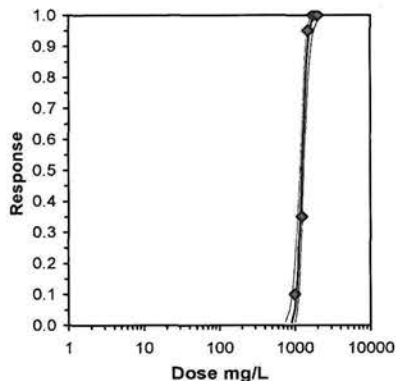
Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	0.8000
1250	0.4000	0.9000
1500	0.0000	0.1000
1750	0.0000	0.0000
2000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							1-Tailed		Number		Total
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	0.728	2.850	0.5964	2	20
1250	0.6500	0.6500	0.9669	0.6847	1.2490	41.271	2	2.127	2.850	0.5964	7	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	5.599	2.850	0.5964	19	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	5.988	2.850	0.5964	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

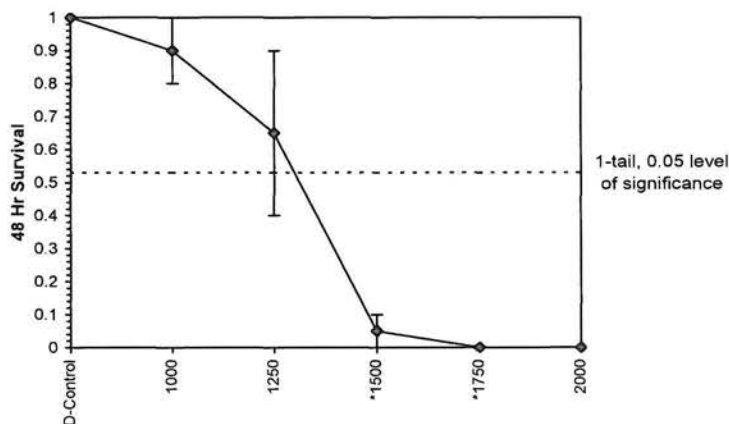
Auxiliary Tests												Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed															
Equality of variance cannot be confirmed															
Hypothesis Test (1-tail, 0.05)												NOEC	LOEC	ChV	TU
Dunnett's Test												1250	1500	1369.31	0.44484
Treatments vs D-Control												0.45625	0.66891	0.0438	0.00521
												4, 5			

Maximum Likelihood-Probit												Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope												0	3.30338	7.81472	0.34717	3.09841	0.0597	3
Intercept												-46.902	9.39439	-65.315	-28.489			

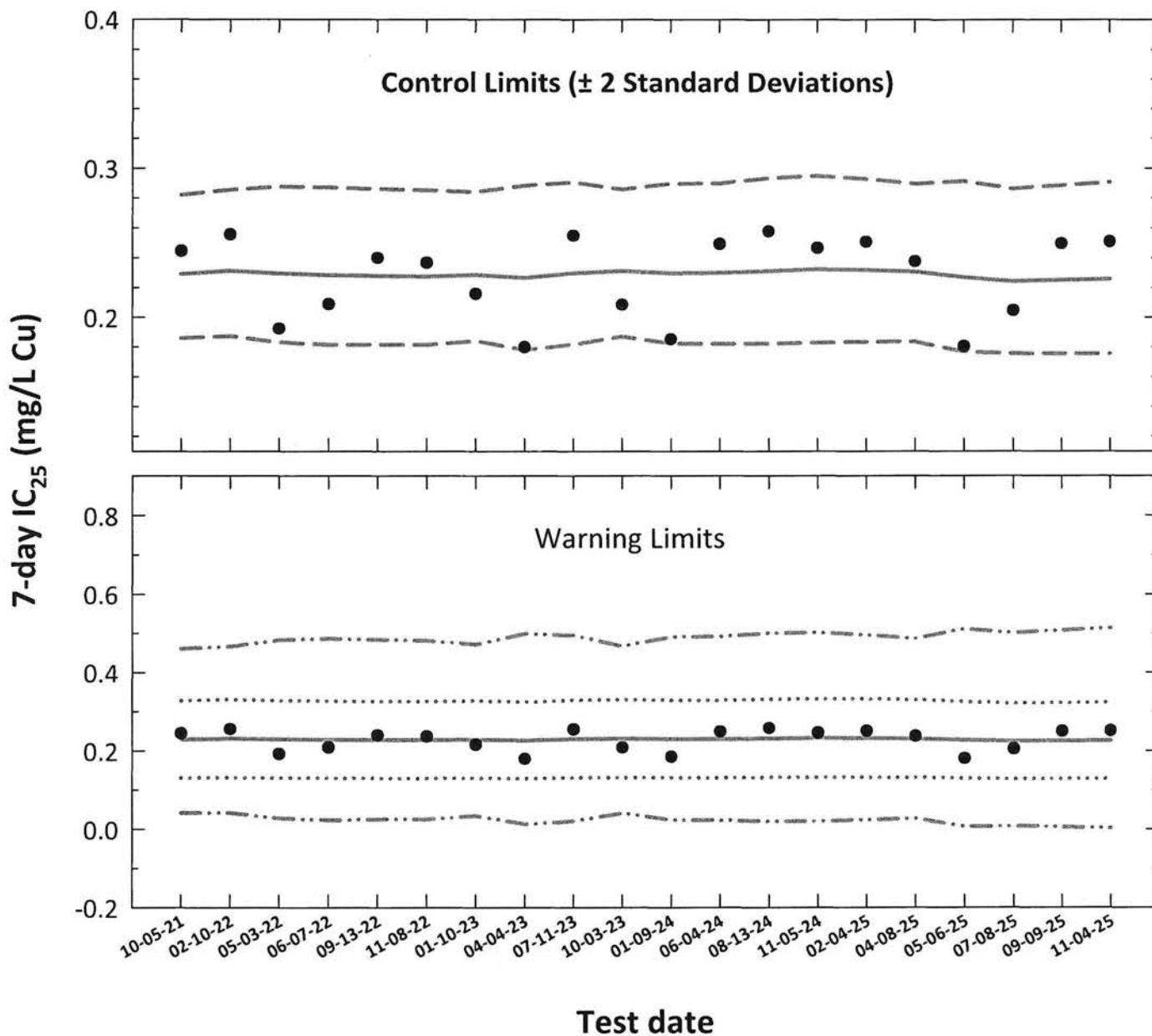
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	911.026	746.691	1007.82
EC05	3.355	1000.49	859.855	1084.15
EC10	3.718	1051.72	925.984	1128.45
EC15	3.964	1087.76	972.714	1160.24
EC20	4.158	1117.29	1010.9	1186.88
EC25	4.326	1143.25	1044.23	1210.94
EC40	4.747	1211.39	1129.19	1278.25
EC50	5.000	1254.32	1179.55	1325.07
EC60	5.253	1298.77	1228.12	1378.1
EC75	5.674	1376.17	1303.84	1481.71
EC80	5.842	1408.15	1332.39	1528.13
EC85	6.036	1446.37	1364.95	1585.82
EC90	6.282	1495.93	1405.26	1663.65
EC95	6.645	1572.53	1464.57	1789.29
EC99	7.326	1726.96	1577.47	2057.87



Dose-Response Plot



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



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

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

Test number	Test date	7-day IC_{25} ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion		Anti-logarithmic Values (mg/L Cu)				
			7-day IC_{25}	CT	S	CT	Control Limits		75th Percentile CV Warning Limits
							CT - 2S	CT + 2S	
1	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.2292	0.1861	0.2822	0.1306
2	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.2313	0.1874	0.2855	0.1318
3	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.2296	0.1832	0.2877	0.1309
4	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.2283	0.1815	0.2872	0.1301
5	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.2279	0.1816	0.2861	0.1299
6	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.2276	0.1816	0.2853	0.1297
7	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.2287	0.1841	0.2841	0.1304
8	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.2266	0.1781	0.2884	0.1292
9	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.2298	0.1818	0.2905	0.1310
10	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.2314	0.1873	0.2859	0.1319
11	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.2298	0.1824	0.2896	0.1310
12	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.2300	0.1823	0.2901	0.1311
13	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.2312	0.1823	0.2932	0.1318
14	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.2324	0.1831	0.2950	0.1325
15	02-04-25	0.2507	-0.6008	-0.6349	0.0507	0.2318	0.1835	0.2928	0.1321
16	04-08-25	0.2377	-0.6240	-0.6369	0.0494	0.2307	0.1838	0.2896	0.1315
17	05-06-25	0.1805	-0.7435	-0.6438	0.0542	0.2271	0.1769	0.2915	0.1294
18	07-08-25	0.2047	-0.6889	-0.6492	0.0530	0.2243	0.1757	0.2863	0.1278
19	09-09-25	0.2496	-0.6028	-0.6477	0.0539	0.2251	0.1756	0.2885	0.1283
20	11-04-25	0.2509	-0.6005	-0.6462	0.0548	0.2259	0.1755	0.2907	0.1287

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

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

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

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

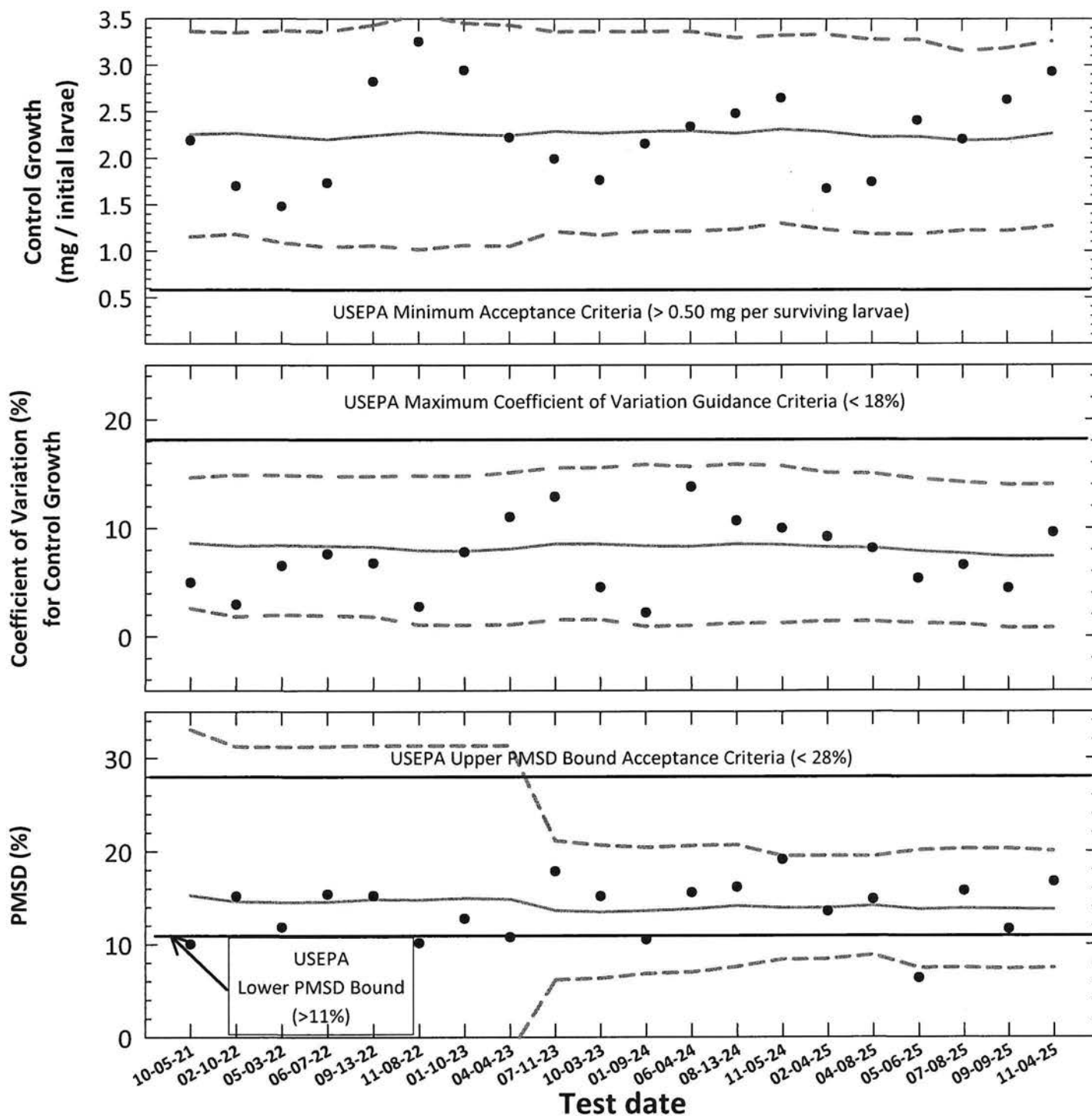
Warning Limits = Mean logarithmic $IC_{25} \pm 2CV$ or $S_{A,75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Menidia beryllina

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



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

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

Test number	Test date	ToxCal Determination				Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial larvae)	CV (%)										
1	10-05-21	100	2.189	5.0	0.2192	2.2578	1.153	3.363	8.6	2.6	14.7	15.3	-2.5	33.1
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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	20.1
18	07-08-25	100	2.201	6.6	0.3464	2.188	1.221	3.155	7.6	1.1	14.2	13.8	7.4	20.3
19	09-09-25	100	2.628	4.4	0.3054	2.201	1.217	3.185	7.4	0.8	14.0	13.8	7.3	20.3
20	11-04-25	100	2.930	9.6	0.4894	2.266	1.268	3.263	7.4	0.8	14.0	13.7	7.4	20.0

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

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

CV = Coefficient of variation for control growth.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

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

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

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

Species: Menidia beryllina

MbCuCR Test Number: **154**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2445				
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:	GREEN
Age:	10-days old	Incubator number and shelf location:	7F
Batch:	AI Mb 10-25-25	Artemia CHM number:	CHM1385
Hatch dates and times:	10-24-25 1200 to 10-25-25 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = 7.35 Temperature (°C) = 24.8	Date / Time in oven:	11-11-25 0925
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	60°C
		Date / Time out of oven:	11-12-25 0925
		*Final oven temperature:	60°C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	11-04-25	1042	H	1200	H	1125	H	NA	11-08-25B
1	11-05-25	0500	H	1230	H	0540	H		
2	11-06-25	0505	H	1120	H	0530	H		11-03-25
3	11-07-25	0500	H	1100	H	0500	H		
4	11-08-25	0600	H	1200	H	0540	H		11-06-25
5	11-09-25	0600	H	1100	H	0532	H		
6	11-10-25	0600	H	1200	H	0530	H		
7	11-11-25					0925	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	1306646f5

Control information:		Acceptance criteria	Summary of test endpoints: (mg/L)	
% Mortality:	0%	≤ 20%	7-day LC ₅₀ (%)	0.286
Average weight per initial larvae:	2.930		NOEC (%)	0.1
Average weight per surviving larvae:	2.930	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	0.141
			IC ₂₅ (%)	0.251

Species: Menidia beryllina

MbCuCR Test Number: 154

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>p14m</u> Analyst: <u>JP</u> Date: <u>10-23-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>11-16-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JP</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JP</u>												
Average weight per initial number of larvae (mg)	Percent reduction from control (%)											
	2.930				2.835	3.27	2.934	-0.37				

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

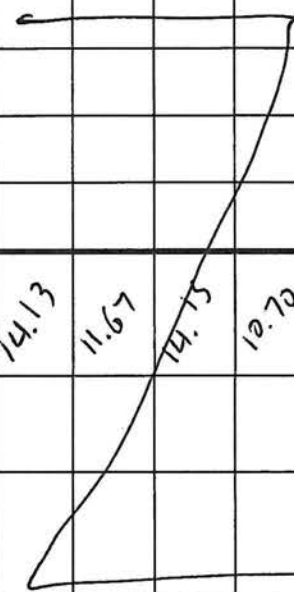
Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: Menidia beryllina

MbCuCR Test Number: 154

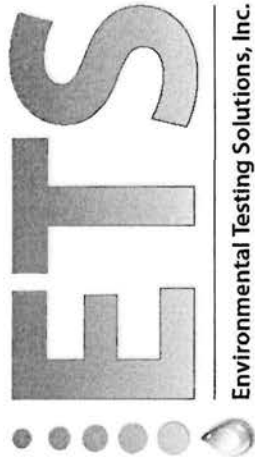
Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	0 ^{10d}	1 ^{9d}	0 ^{10d}	0 ^{10d}
2	10	10	10	10	10	10	10	10	0	1	0	0
3	10	10	10	10	10	10	10	10	0	0 ^d	0	0
4	10	10	10	10	10	10	10	10				
5	10	10	10	10	8 ^{2d}	10	10	10				
6	10	10	10	10	8	9 ^{1d}	10	9 ^{1d}				
7	10	10	10	10	8	9	9 ^{1d}	9				
*A = Pan weight (mg) Tray color code: <u>plum</u> Analyst: <u>JP</u> Date: <u>10-23-25</u>	14.92	9.82	12.17	14.12	14.34	13.49	12.16	12.69	14.13	11.67	14.75	10.70
*B = Pan + Larvae weight (mg) Analyst: <u>JS</u> Date: <u>11-16-25</u>	44.71	38.51	33.99	46.91	37.83	38.19	42.16	40.33				
C = Larvae weight (mg) = B - A Analyst: <u>JP</u>	29.79	28.69	21.82	32.79	23.49	24.70	30.00	27.64				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JP</u>	2.979	2.869	2.182	3.279	2.349	2.470	3.00	2.764	0	0	0	0
Average weight per initial number of larvae (mg)	2.827				2.646				0			
Percent reduction from control (%)	3.57.				9.77.				1007.			

*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: 154

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Not for Compliance Assessment: Internal Laboratory QC	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	15.32	41.03	25.71	Weight / Surviving number of larvae (mg)	2.571				
	B	10	10	14.35	45.35	31.00	Mean weight/ Surviving number of larvae (mg)	3.100				
	C	10	10	13.76	45.74	31.98	Coefficient of variation (Mean weight per surviving number of larvae) (%)	3.198	100.0	2.930	9.6	Not applicable
	D	10	10	13.78	42.27	28.49		2.849				
0.025	E	10	10	14.80	44.44	29.64		2.964				
	F	10	10	13.14	41.91	28.77		2.877				
	G	10	10	13.97	42.27	28.30		2.830				
	H	10	10	14.58	41.28	26.70		2.670	100.0	2.835	4.4	3.2
0.050	I	10	10	14.13	43.80	29.67		2.967				
	J	10	10	14.78	45.73	30.95		3.095				
	K	10	10	14.68	45.67	30.99		3.099	100.0	2.939	8.1	-0.3
	L	10	10	12.88	38.83	25.95		2.595				
0.100	M	10	10	14.92	44.71	29.79		2.979				
	N	10	10	9.82	38.51	28.69		2.869	100.0	2.827	16.4	3.5
	O	10	10	12.17	33.99	21.82		2.182				
	P	10	10	14.12	46.91	32.79		3.279				
0.200	Q	10	8	14.34	37.83	23.49		2.936				
	R	10	9	13.49	38.19	24.70		2.744	87.5	3.021	8.2	9.7
	S	10	9	12.16	42.16	30.00		3.333				
	T	10	9	12.69	40.33	27.64		3.071				
0.500	U	10	0	0.00	0.00	0.00		0.000				
	V	10	0	0.00	0.00	0.00		0.000	0.0	0.000	0.0	100.0
	W	10	0	0.00	0.00	0.00		0.000				
	X	10	0	0.00	0.00	0.00		0.000				

Dunnett's MSD value: 0.4894
 PMSD: 16.7
 MSD = Minimum Significant Difference
 PMSD = Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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

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



Larval Fish Growth and Survival Test-7 Day Survival

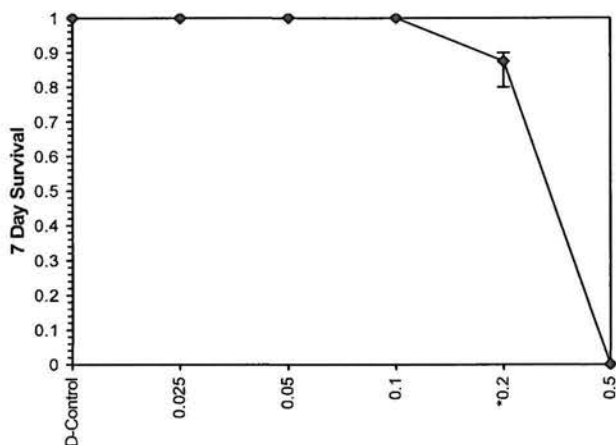
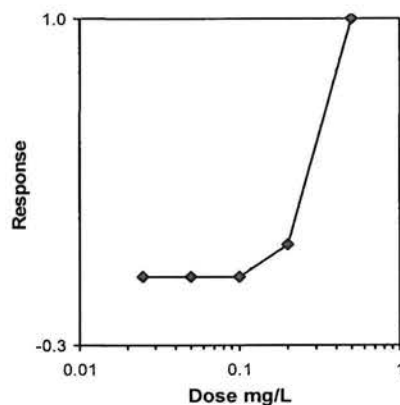
Start Date: 11/4/2025 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
End Date: 11/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUSO4
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	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	0.9000	0.9000	0.9000
0.5	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	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	0.8750	0.8750	1.2136	1.1071	1.2490	5.846	4	10.00	10.00	5	40
0.5	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.5089	0.868	-2.7962	11.6732
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Trim Level	EC50	95% CL	
0.0%	0.2860	0.2629	0.3111
5.0%	0.2921	0.2643	0.3227
10.0%	0.2957	0.2587	0.3379
20.0%	0.2962	0.2782	0.3153
Auto-0.0%	0.2860	0.2629	0.3111



Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 11/4/2025 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
End Date: 11/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUS04
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina
Comments:

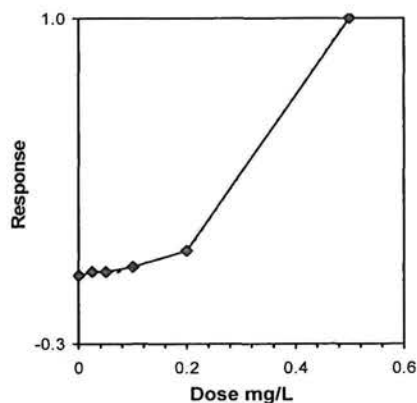
Conc-mg/L	1	2	3	4
D-Control	2.5710	3.1000	3.1980	2.8490
0.025	2.9640	2.8770	2.8300	2.6700
0.05	2.9670	3.0950	3.0990	2.5950
0.1	2.9790	2.8690	2.1820	3.2790
0.2	2.3490	2.4700	3.0000	2.7640
0.5	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	2.9295	1.0000	2.9295	2.5710	3.1980	9.578	4					2.9295	1.0000
0.025	2.8353	0.9678	2.8353	2.6700	2.9640	4.351	4	0.441	2.290	0.4894		2.8871	0.9855
0.05	2.9390	1.0032	2.9390	2.5950	3.0990	8.077	4	-0.044	2.290	0.4894		2.8871	0.9855
0.1	2.8273	0.9651	2.8273	2.1820	3.2790	16.403	4	0.478	2.290	0.4894		2.8273	0.9651
0.2	2.6458	0.9031	2.6458	2.3490	3.0000	11.093	4					2.6458	0.9031
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4					0.0000	0.0000

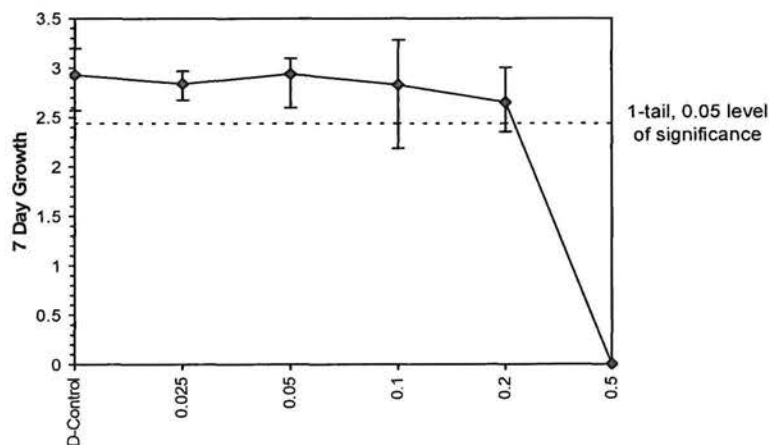
Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)					0.93008	0.844	-0.8882	1.03837
Bartlett's Test indicates equal variances ($p = 0.25$)					4.12864	11.3449		
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Dunnnett's Test					0.1	>0.1		
Treatments vs D-Control								
					MSDu	MSDp	MSB	MSE
					0.48938	0.16705	0.01425	0.09134
					F-Prob	df		
					0.9238	3, 12		

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	0.1244	0.0589	0.0000	0.2604
IC10	0.2010	0.0479	0.0000	0.2388
IC15	0.2177	0.0342	0.0182	0.2534
IC20	0.2343	0.0159	0.1742	0.2679
IC25	0.2509	0.0135	0.1967	0.2824
IC40	0.3007	0.0108	0.2573	0.3259
IC50	0.3339	0.0090	0.2978	0.3549



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 154

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		Analyst	Day (Analyst identified for each day, performed pH and D.O. measurements only.)					
			0		1		2	
			XL	XL	XL	XL	XL	XL
CONTROL, SaltSW	pH (S.U.)		7.98	7.88	7.93	7.82	7.93	7.85
	Dissolved oxygen (mg/L)		8.5	8.0	8.2	8.0	8.2	7.8
	Salinity (ppt)		25.0	25.3	25.0	25.5	25.0	25.6
	Alkalinity (mg CaCO ₃ /L)		100				98	
	Temperature (°C)		24.8	24.9	24.6	24.6	24.8	24.9
0.025 mg/L	pH (S.U.)		8.02	7.89	7.99	7.83	7.97	7.85
	Dissolved oxygen (mg/L)		8.5	7.9	8.1	7.9	8.0	7.8
	Salinity (ppt)		24.9	25.4	24.9	25.6	25.0	25.7
	Temperature (°C)		24.9	25.0	24.4	24.5	24.8	25.1
0.05 mg/L	pH (S.U.)		8.02	7.89	7.98	7.84	7.96	7.85
	Dissolved oxygen (mg/L)		8.5	7.9	8.1	7.9	8.0	7.8
	Salinity (ppt)		25.1	25.4	25.0	25.4	25.1	25.3
	Temperature (°C)		24.9	24.7	24.4	24.7	24.7	25.1
0.1 mg/L	pH (S.U.)		8.03	7.89	7.98	7.83	7.96	7.84
	Dissolved oxygen (mg/L)		8.5	7.9	8.1	7.8	8.1	7.8
	Salinity (ppt)		25.5	25.6	25.0	25.4	25.0	25.3
	Temperature (°C)		24.9	24.7	24.5	24.7	24.7	24.8
0.2 mg/L	pH (S.U.)		8.03	7.88	7.97	7.83	7.95	7.83
	Dissolved oxygen (mg/L)		8.5	7.9	8.1	7.8	8.1	7.7
	Salinity (ppt)		25.2	25.4	25.0	25.5	25.0	25.3
	Temperature (°C)		25.0	24.9	24.5	24.7	24.7	24.8
0.5 mg/L	pH (S.U.)		8.03	7.88	7.98	7.87	7.95	7.86
	Dissolved oxygen (mg/L)		8.5	7.9	8.1	7.8	8.2	7.6
	Salinity (ppt)		25.6	25.6	24.9	25.6	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)		NA					
	Temperature (°C)		25.0	24.8	24.5	24.8	24.7	24.8
Initial				Final	Initial	Final	Initial	Final

Species: Menidia beryllina

MbCuCR Test Number: 154

Analyst		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
		XL	BS	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.92	7.80	8.09 (7.10) 8.2 (7.10)	7.81	7.99	7.84	7.94	7.70
	Dissolved oxygen (mg/L)	8.1	8.1	8.2	7.9	8.2	7.5	8.2	6.4
	Salinity (ppt)	25.0	25.2	25.0	25.0	25.0	25.8	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)		9.5						
	Temperature (°C)	24.7	24.7	24.7	24.6	25.0	25.1	24.8	24.6
0.025 mg/L	pH (S.U.)	7.96	7.83	7.98	7.82	8.01	7.82	8.04	7.73
	Dissolved oxygen (mg/L)	8.0	8.2	8.3	7.9	8.1	7.6	8.0	6.6
	Salinity (ppt)	25.1	25.0	25.0	25.0	25.0	25.8	25.1	25.2
	Temperature (°C)	24.6	24.7	24.8	24.8	25.1	24.9	24.9	24.6
0.05 mg/L	pH (S.U.)	7.95	7.81	7.97	7.81	8.02	7.81	8.04	7.72
	Dissolved oxygen (mg/L)	8.0	8.0	8.3	7.9	8.3	7.6	8.0	6.6
	Salinity (ppt)	25.0	25.6	25.0	25.9	25.0	25.6	25.3	25.9
	Temperature (°C)	24.6	25.0	24.8	24.8	24.9	24.9	24.9	24.5
0.1 mg/L	pH (S.U.)	7.95	7.80	7.98	7.81	8.01	7.80	8.04	7.72
	Dissolved oxygen (mg/L)	8.0	8.0	8.4	7.8	8.2	7.5	8.0	6.6
	Salinity (ppt)	25.0	25.3	25.0	25.5	25.0	25.8	25.3	25.9
	Temperature (°C)	24.7	25.0	24.8	24.8	24.9	25.0	24.9	24.7
0.2 mg/L	pH (S.U.)	7.95	7.90	7.98	7.76	8.01	7.80	8.04	7.73
	Dissolved oxygen (mg/L)	8.0	8.0	8.4	7.8	8.3	7.5	8.0	6.5
	Salinity (ppt)	25.1	25.5	25.0	25.4	25.0	25.4	25.3	25.7
	Temperature (°C)	24.7	25.0	24.7	24.8	24.9	25.0	24.9	24.7
0.5 mg/L	pH (S.U.)	7.96							
	Dissolved oxygen (mg/L)	8.0							
	Salinity (ppt)	24.9							
	Temperature (°C)	24.9							
		Initial	Final	Initial	Final	Initial	Final	Initial	Final