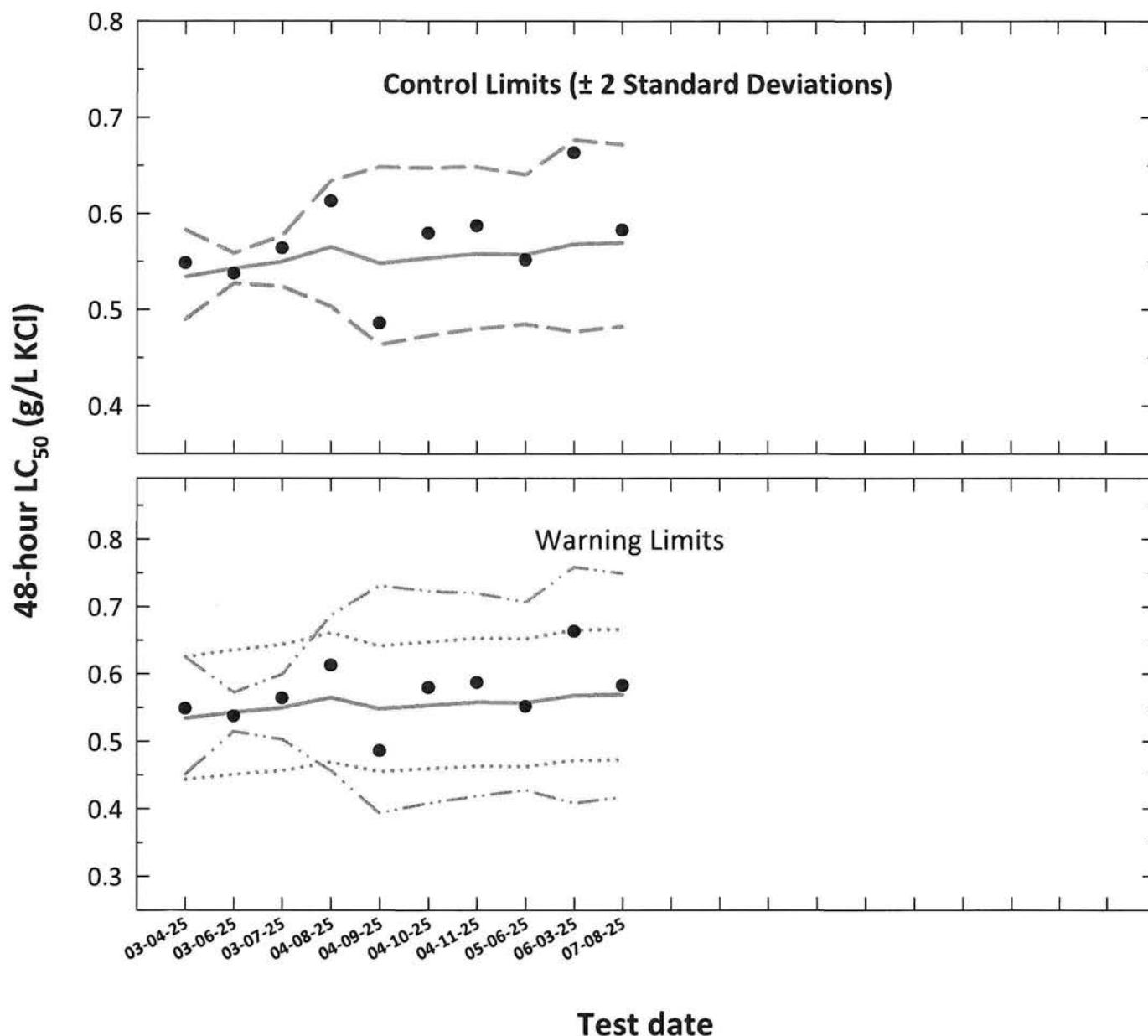


Americamysis (Mysidopsis) bahia Acute Reference Toxicant Control Chart Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}
1	03-04-25	0.5485	-0.2608			0.5430			
2	03-06-25	0.5374	-0.2697	-0.2652	0.0063	0.5430	0.5275	0.5145	0.4506
3	03-07-25	0.5640	-0.2487	-0.2597	0.0105	0.5499	0.5239	0.5026	0.4564
4	04-08-25	0.6129	-0.2126	-0.2480	0.0251	0.5650	0.5034	0.4559	0.4689
5	04-09-25	0.4860	-0.3134	-0.2610	0.0364	0.5482	0.4635	0.3937	0.4550
6	04-10-25	0.5796	-0.2369	-0.2570	0.0341	0.5533	0.4730	0.4082	0.4593
7	04-11-25	0.5872	-0.2312	-0.2533	0.0326	0.5581	0.4803	0.4187	0.4632
8	05-06-25	0.5517	-0.2583	-0.2539	0.0302	0.5573	0.4849	0.4273	0.4625
9	06-03-25	0.6631	-0.1784	-0.2455	0.0379	0.5681	0.4772	0.4082	0.4716
10	07-08-25	0.5828	-0.2345	-0.2444	0.0359	0.5696	0.4829	0.4174	0.4728
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

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

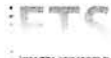
S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 11

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 #: 2394

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, SaltSW	pH (S.U.)	7.97	7.96	7.84
	Dissolved oxygen (mg/L)	8.4	8.2	7.8
	*Salinity (ppt)	25.0	25.6	25.5
	*Alkalinity (mg/L CaCO ₃)	8.40 ¹⁰⁰		
	*Temperature (°C)	24.0	24.3	24.3
150 mg/L	pH (S.U.)	7.95	7.90	7.84
	Dissolved oxygen (mg/L)	8.5	8.0	7.8
	*Salinity (ppt)	25.3	25.7	25.6
	*Temperature (°C)	24.1	24.2	24.4
300 mg/L	pH (S.U.)	7.95	7.90	7.84
	Dissolved oxygen (mg/L)	8.5	8.0	7.8
	*Salinity (ppt)	25.4	26.0	26.0
	*Temperature (°C)	24.2	24.2	24.4
450 mg/L	pH (S.U.)	7.95	7.90	7.86
	Dissolved oxygen (mg/L)	8.5	8.0	7.8
	*Salinity (ppt)	25.5	26.0	26.1
	*Temperature (°C)	24.1	24.2	24.6
600 mg/L	pH (S.U.)	7.95	7.90	7.86
	Dissolved oxygen (mg/L)	8.5	8.0	7.8
	*Salinity (ppt)	25.5	26.1	26.1
	*Temperature (°C)	24.1	24.6	24.6
750 mg/L	pH (S.U.)	7.94	7.91	
	Dissolved oxygen (mg/L)	8.5	8.0	
	*Salinity (ppt)	25.6	26.3	
	*Temperature (°C)	24.0	24.3	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066470

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 # 11

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-08-25	* 1100	JP	1420	JP	1C	Yellow	07-07-25A
24	07-09-25			1428	JP			
48 Termination	07-11-25			1430	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):	07-07-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	07-06-25 1200 07-07-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.09 Temperature (°C) 25.1°C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ¹²	10	10	10	9 ¹²	10	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9	8 ²²	10	9 ¹²	6 ³²	10	0	0
Mean Survival	100%		100%		95%		95%		80%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	528.2
Upper 95% confidence limit (mg KCl/L)	643.1
48-hour LC ₅₀ (mg KCl/L)	582.8

Comments:





Statistical Analyses

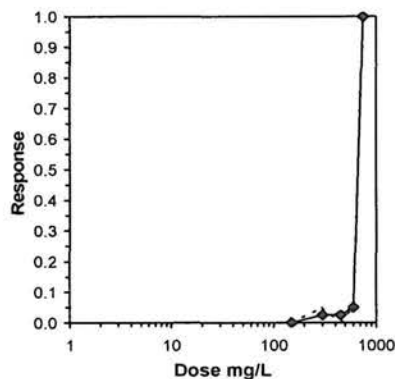
Acute Mysid Test-24 Hr Survival			
Start Date:	7/8/2025	Test ID:	AbKCIAC
End Date:	7/10/2025	Lab ID:	ETS-Envir. Testing Sol.
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012
Comments:		Sample ID:	REF-Ref Toxicant
		Sample Type:	DMR-Discharge Monitoring Report
		Test Species:	AB-Americanysis bahia

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

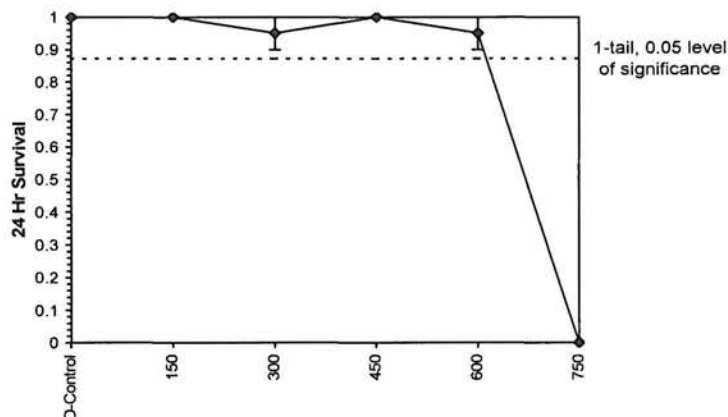
Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2077	0	20
300	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.118	2.850	0.2077	1	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2077	0	20
600	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.118	2.850	0.2077	1	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
Dunnett's Test												600	750	670.82	
Treatments vs D-Control															
												MSDu	MSDp	MSB	MSE
												0.10341	0.10606	0.00398	0.00531
												F-Prob			df
												0.59833			4, 5

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	647.64	614.99	682.03
5.0%	666.89	658.91	674.98
10.0%	666.89	658.91	674.98
20.0%	666.89	658.91	674.98
Auto-0.0%	647.64	614.99	682.03



Dose-Response Plot



Statistical Analyses

Acute Mysid Test-48 Hr Survival

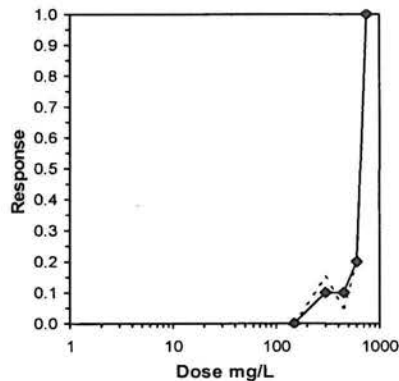
Start Date: 7/8/2025 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 7/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: DMR-Discharge Monitoring Report
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanmysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	0.9000	0.8000
450	1.0000	0.9000
600	0.6000	1.0000
750	0.0000	0.0000

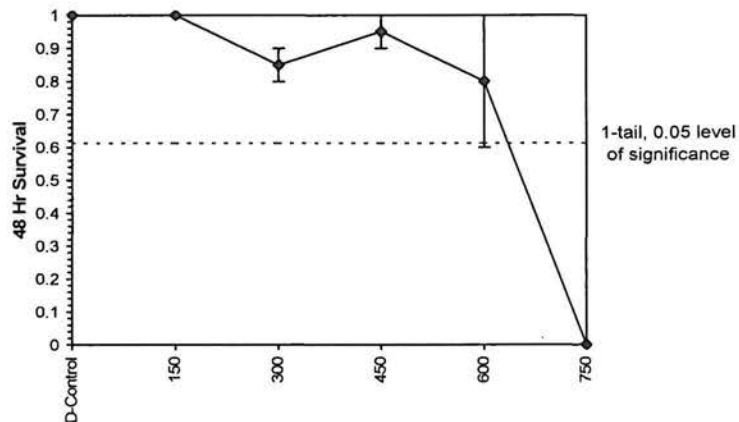
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2					0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.5125		0	20
300	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	1.301	2.850	0.5125		3	20
450	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.453	2.850	0.5125		1	20
600	0.8000	0.8000	1.1490	0.8861	1.4120	32.366	2	1.463	2.850	0.5125		4	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	600	750	670.82		0.36182	0.3711	0.0318	0.03233	0.49219	4, 5
Treatments vs D-Control										

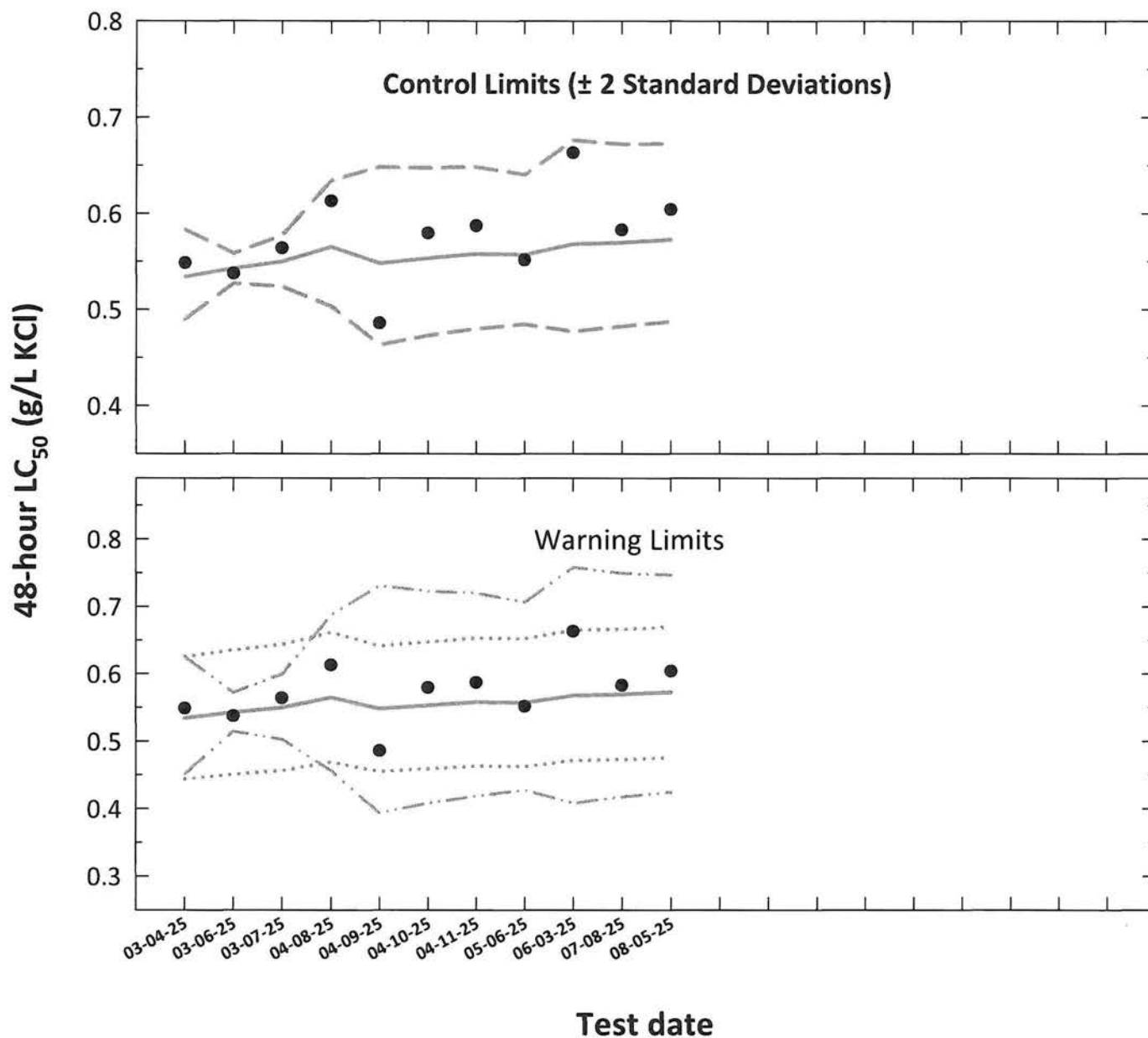
Trim Level	EC50	95% CL	
0.0%	582.79	528.18	643.05
5.0%	613.99	555.58	678.54
10.0%	641.86	603.49	682.67
20.0%	652.37	632.34	673.03
Auto-0.0%	582.79	528.18	643.05



Dose-Response Plot



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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			48-hour LC ₅₀	CT	S	CT	Control Limits 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	-0.2652	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	0.4753 0.6700
11	08-05-25	0.6040	-0.2189						
12									
13									
14									
15									
16									
17									
18									
19									
20									

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

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 12

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 #: 2394

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	8.03	7.90	7.87
	Dissolved oxygen (mg/L)	8.4	8.1	7.9
	*Salinity (ppt)	25.2	25.4	25.8
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	24.5	25.2	25.4
150 mg/L	pH (S.U.)	8.06	7.90	7.85
	Dissolved oxygen (mg/L)	8.4	8.1	7.9
	*Salinity (ppt)	25.4	25.5	25.8
	*Temperature (°C)	24.8	24.7	25.2
300 mg/L	pH (S.U.)	8.05	7.91	7.85
	Dissolved oxygen (mg/L)	8.4	8.1	7.9
	*Salinity (ppt)	25.1	25.7	25.9
	*Temperature (°C)	24.8	25.1	25.5
450 mg/L	pH (S.U.)	8.06	7.91	7.85
	Dissolved oxygen (mg/L)	8.4	8.0	7.8
	*Salinity (ppt)	25.2	25.6	26.0
	*Temperature (°C)	24.9	25.0	25.5
600 mg/L	pH (S.U.)	8.06	7.92	7.85
	Dissolved oxygen (mg/L)	8.3	8.0	7.6
	*Salinity (ppt)	25.3	25.7	26.1
	*Temperature (°C)	24.9	25.3	25.3
750 mg/L	pH (S.U.)	8.05	7.93	
	Dissolved oxygen (mg/L)	8.3	7.9	
	*Salinity (ppt)	25.6	25.8	
	*Temperature (°C)	25.0	25.0	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306647

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 12

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	08-05-25	* 1045	JP	1347	JP	1 D	White	07-31-25A
24	08-06-25			1346	JP			
48 Termination	08-07-25			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):	08-04-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	08-03-25 1200 08-04-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.92 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 ^d	10	9 ^d	6 ^u	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	8 ^u 9 ^u 08-07-29	10	8 ^{1d}	5 ^{1d}	0	0
Mean Survival	100%		100%		100%		45%		(85%) 75% 91% 08-07-29			

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	562.2
Upper 95% confidence limit (mg KCl/L)	643.3
48-hour LC ₅₀ (mg KCl/L)	604.0

Comments:



Statistical Analyses

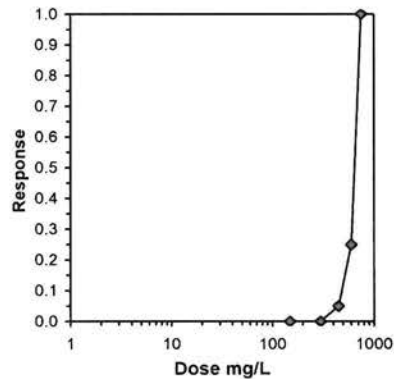
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Start Date: 8/5/2025	Test ID: AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date: 8/7/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type:	DMR-Discharge Monitoring Report
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanmysis bahia
Comments:			

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

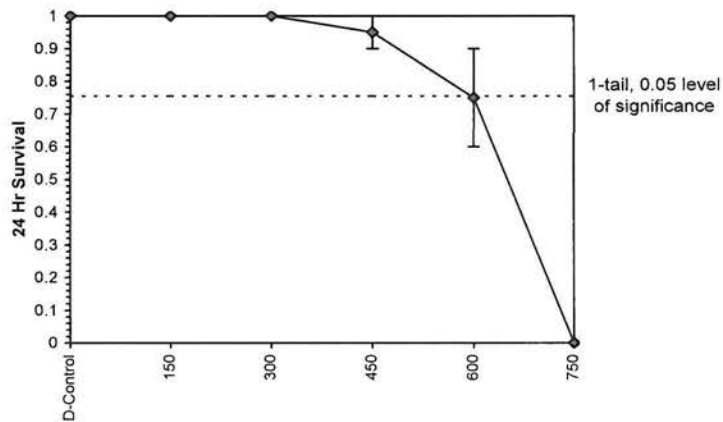
Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.3586	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.3586	0	20
450	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.648	2.850	0.3586	1	20
600	0.7500	0.7500	1.0676	0.8861	1.2490	24.041	2	2.738	2.850	0.3586	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		600	750	670.82		0.21962	0.22525	0.0445	0.01583	0.14352	4, 5
Treatments vs D-Control											

Trim Level	EC50	95% CL
0.0%	618.51	582.56 656.69
5.0%	630.15	595.22 667.12
10.0%	636.04	596.44 678.27
20.0%	644.80	579.95 716.90
Auto-0.0%	618.51	582.56 656.69



Dose-Response Plot





Statistical Analyses

Acute Mysid Test-48 Hr Survival			
Start Date: 8/5/2025	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant	
End Date: 8/7/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia	
Comments:			

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

Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	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	0.3251	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.3251	0	20
450	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.714	2.850	0.3251	1	20
*600	0.6500	0.6500	0.9463	0.7854	1.1071	24.043	2	4.084	2.850	0.3251	7	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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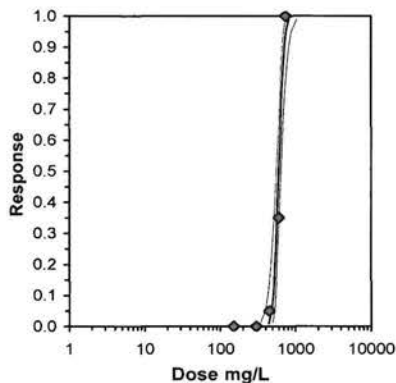
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

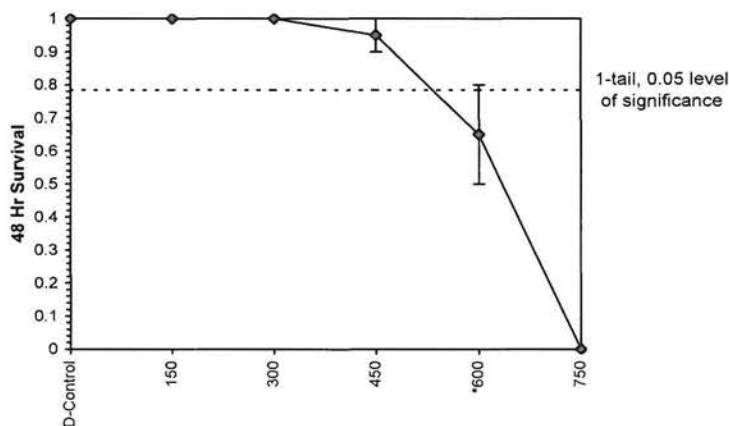
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.19139	0.1963	0.08183	0.01301	0.03452	4, 5
Treatments vs D-Control										

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

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



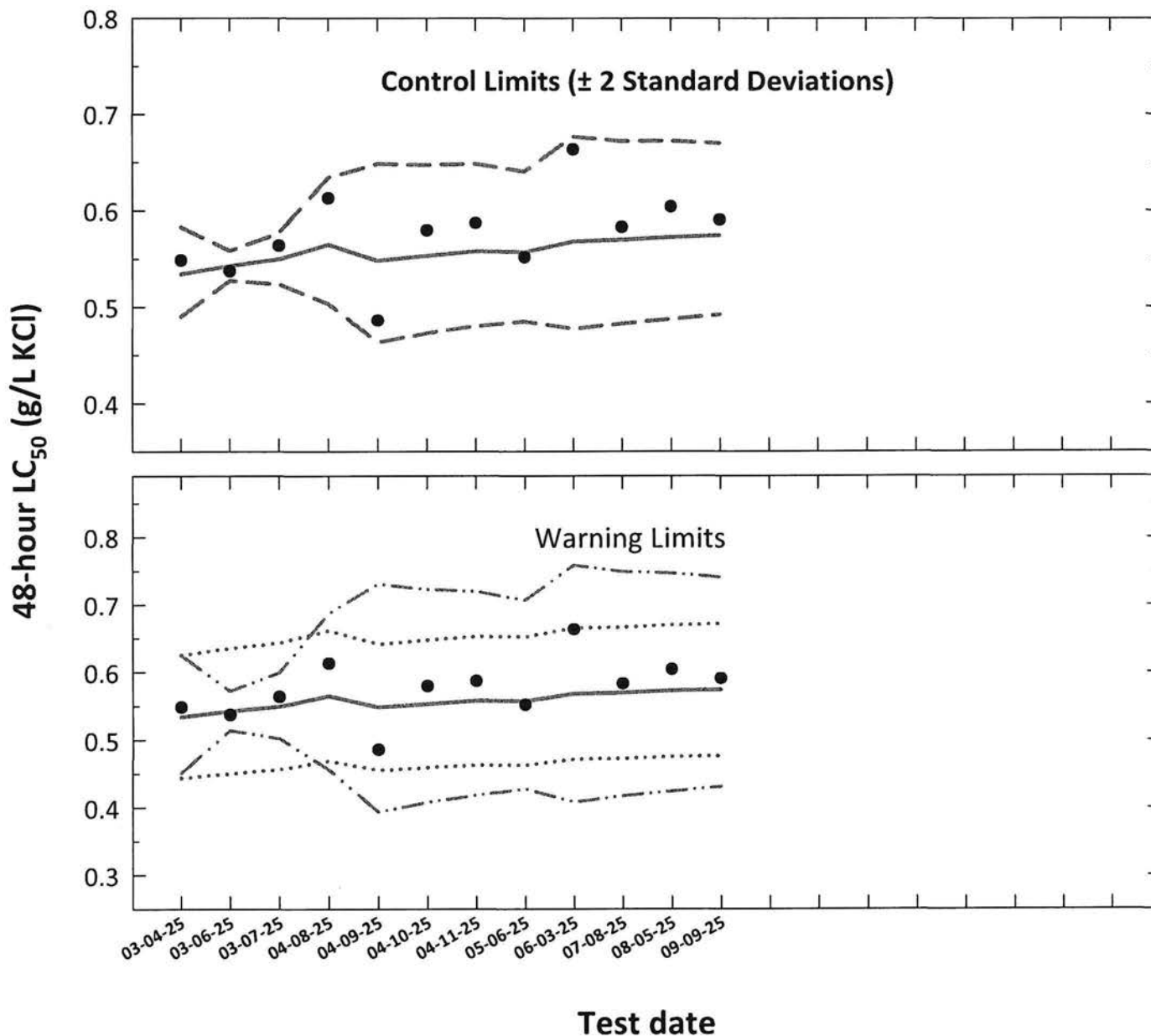
Dose-Response Plot



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		CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
			48-hour LC ₅₀	CT		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	0.5430	0.5275	0.5588	0.5145	0.5722	0.4506	0.6353
2	03-06-25	0.5374	-0.2697	-0.2652	0.5499	0.5239	0.5772	0.5026	0.5995	0.4564	0.6434
3	03-07-25	0.5640	-0.2487	-0.2597	0.5650	0.5034	0.6342	0.4559	0.6874	0.4689	0.6610
4	04-08-25	0.6129	-0.2126	-0.2480	0.5482	0.4635	0.6484	0.3937	0.7310	0.4550	0.6414
5	04-09-25	0.4860	-0.3134	-0.2610	0.5533	0.4730	0.6473	0.4082	0.7231	0.4593	0.6474
6	04-10-25	0.5796	-0.2369	-0.2570	0.5581	0.4803	0.6484	0.4187	0.7200	0.4632	0.6529
7	04-11-25	0.5872	-0.2312	-0.2533	0.5573	0.4849	0.6405	0.4273	0.7066	0.4625	0.6520
8	05-06-25	0.5517	-0.2583	-0.2539	0.5681	0.4772	0.6763	0.4082	0.7586	0.4716	0.6647
9	06-03-25	0.6631	-0.1784	-0.2455	0.5696	0.4829	0.6719	0.4174	0.7492	0.4728	0.6664
10	07-08-25	0.5828	-0.2345	-0.2444	0.5726	0.4877	0.6724	0.4242	0.7469	0.4753	0.6700
11	08-05-25	0.6040	-0.2189	-0.2421	0.5741	0.4921	0.6698	0.4312	0.7408	0.4765	0.6717
12	09-09-25	0.5904	-0.2289	-0.2410							
13											
14											
15											
16											
17											
18											
19											
20											

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 13

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	XL	XL	XL
Control, SaltSW	pH (S.U.)	8.03	7.95	7.93
	Dissolved oxygen (mg/L)	8.2	7.9	7.8
	*Salinity (ppt)	24.9	25.0	25.1
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	25.1	24.7	25.0
150 mg/L	pH (S.U.)	8.01	7.96	7.91
	Dissolved oxygen (mg/L)	8.3	8.0	7.8
	*Salinity (ppt)	26.0	25.3	25.8
	*Temperature (°C)	25.0	24.6	24.5
300 mg/L	pH (S.U.)	8.01	7.97	7.90
	Dissolved oxygen (mg/L)	8.3	8.0	7.8
	*Salinity (ppt)	24.9	25.21	25.9
	*Temperature (°C)	25.0	24.9	24.8
450 mg/L	pH (S.U.)	8.01	7.97	7.89
	Dissolved oxygen (mg/L)	8.3	8.0	7.8
	*Salinity (ppt)	24.9	25.5	25.9
	*Temperature (°C)	24.9	24.9	24.6
600 mg/L	pH (S.U.)	8.01	7.97	7.89
	Dissolved oxygen (mg/L)	8.3	8.0	7.8
	*Salinity (ppt)	24.9	25.7	26.0
	*Temperature (°C)	25.0	24.8	24.9
750 mg/L	pH (S.U.)	8.00	7.95	7.89
	Dissolved oxygen (mg/L)	8.3	8.0	7.8
	*Salinity (ppt)	24.0	25.7	26.2
	*Temperature (°C)	25.1	24.5	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	13066461

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant TestAbKCIAC # 13

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-09-25	* 1045	JP	1315	JP	1D	WHITE	09-04-25B
24	09-10-25			1324	JP			
48 Termination	09-11-25			1324	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):	09-08-25
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	09-07-25 1200 TO 09-08-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.87 Temperature (°C) 24.2 °C

Survival Data (number of living organisms):

Hours	Control		150 mg/L		300 mg/L		450 mg/L		600 mg/L		750 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	9 ^{1d}	6 ^{4d}	2 ^{8d}	1 ^{9d}
48 Termination	10	10	10	10	10	10	10	10	6 ^{7d}	4 ^{2d}	0 ^{2d}	0 ^{1d}
Mean Survival	100%		100%		100%		100%		50%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	557.6
Upper 95% confidence limit (mg KCl/L)	625.1
48-hour LC ₅₀ (mg KCl/L)	590.41

Comments:





Statistical Analyses

Acute Mysid Test-24 Hr Survival			
Start Date: 9/9/2025	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant	
End Date: 9/11/2025	Lab ID: ETS-Envir. Testing Sol.	Sample Type: DMR-Discharge Monitoring Report	
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia	

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	1.0000
600	0.9000	0.6000
750	0.2000	0.1000

Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.3184	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.3184	0	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.3184	0	20
*600	0.7500	0.7500	1.0676	0.8861	1.2490	24.041	2	3.062	2.830	0.3184	5	20
*750	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	9.060	2.830	0.3184	17	20

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

Equality of variance cannot be confirmed

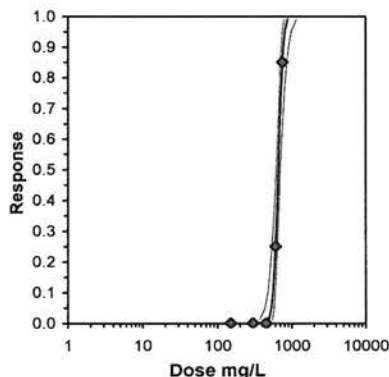
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.18592	0.19069	0.33907	0.01266	4.9E-04	5, 6

Treatments vs D-Control

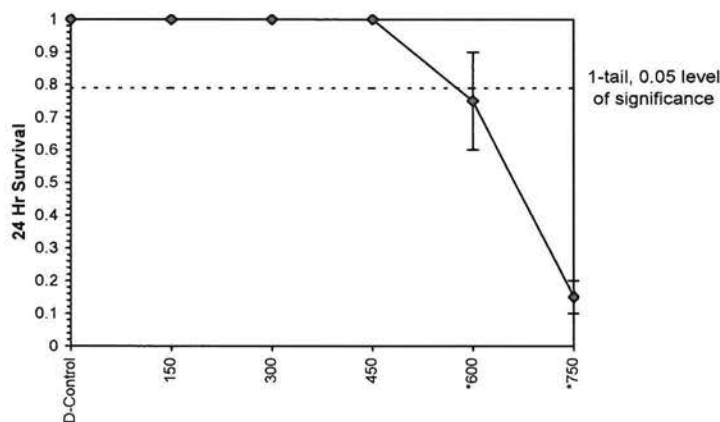
Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.0422	4.32841	9.55853 26.5259	0	0.03905	7.81472	0.99797	2.81675	0.05543	3
Intercept	-45.82	12.1998	-69.732 -21.909							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	487.317	369.308 542.359
EC05	3.355	531.599	433.412 577.781
EC10	3.718	556.827	471.344 598.455
EC15	3.964	574.521	498.278 613.454
EC20	4.158	588.984	520.298 626.22
EC25	4.326	601.682	539.463 637.974
EC40	4.747	634.905	587.369 672.651
EC50	5.000	655.769	614.363 698.765
EC60	5.253	677.318	638.891 730.105
EC75	5.674	714.718	674.449 793.96
EC80	5.842	730.127	687.314 822.96
EC85	6.036	748.507	701.793 859.112
EC90	6.282	772.292	719.537 908.009
EC95	6.645	808.943	745.43 987.282
EC99	7.326	882.45	794.258 1158.44



Dose-Response Plot



Statistical Analyses

Acute Mysid Test-48 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
150	1.0000	1.0000
300	1.0000	1.0000
450	1.0000	1.0000
600	0.6000	0.4000
750	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
150	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1815	0	20
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1815	0	20
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1815	0	20
*600	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	9.841	2.850	0.1815	10	20
750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

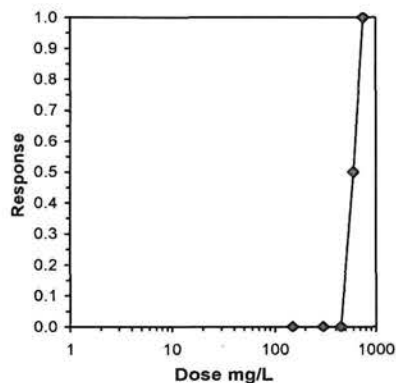
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

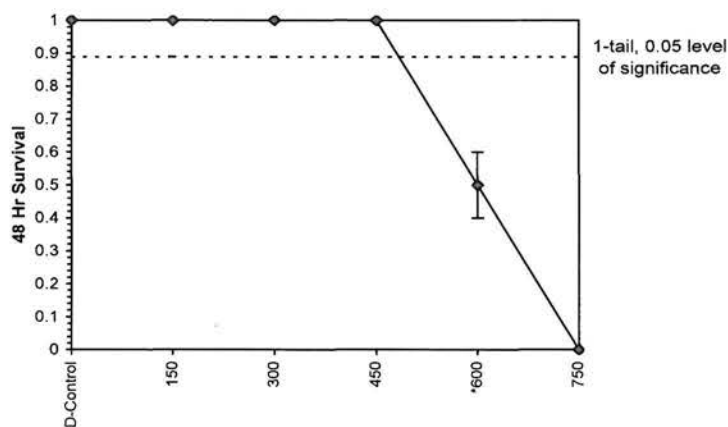
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.08637	0.08859	0.15706	0.00405	5.9E-04	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber

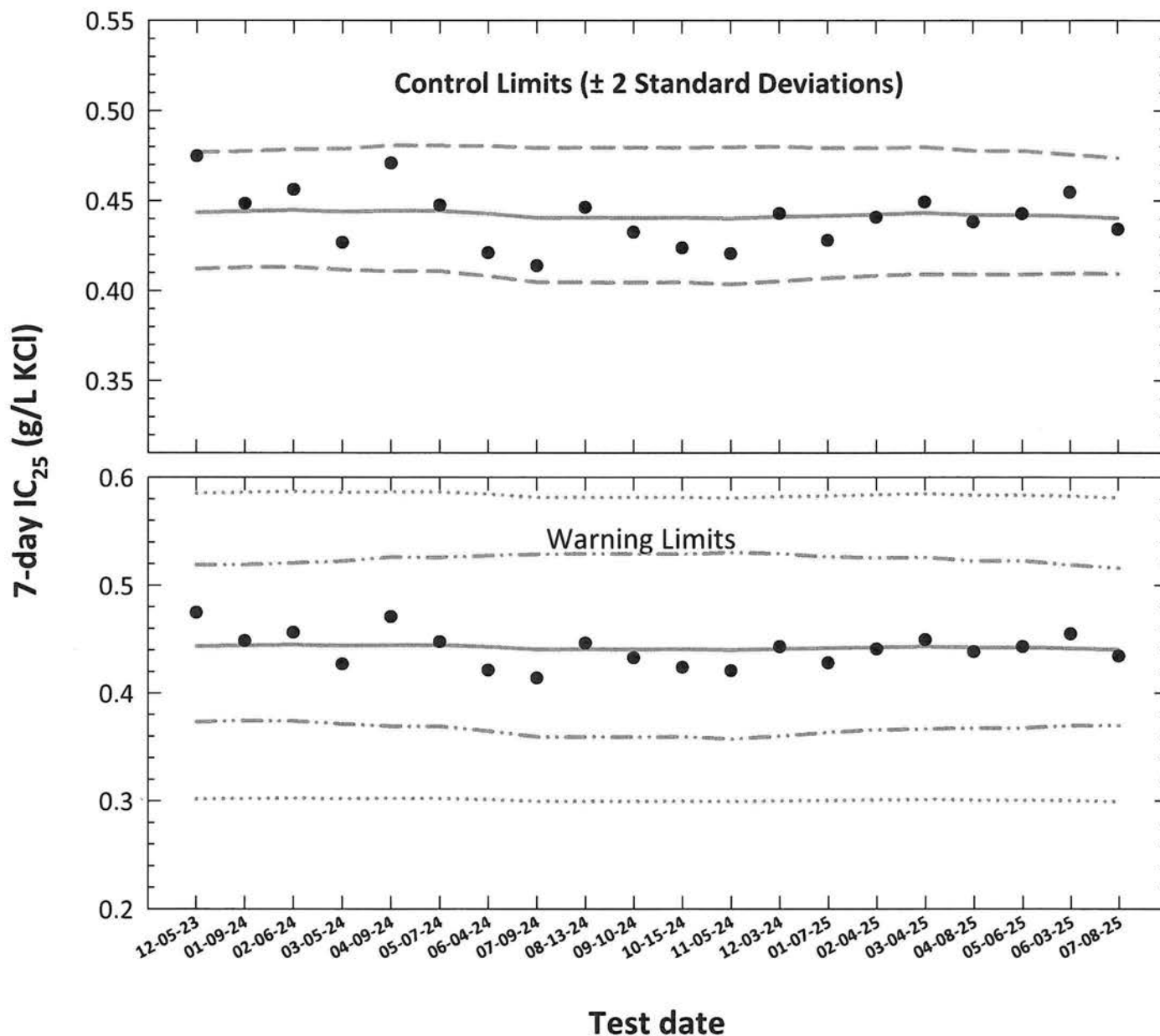
Trim Level	EC50	95% CL	
0.0%	590.40	557.62	625.10
5.0%	591.35	554.95	630.14
10.0%	592.31	551.30	636.37
20.0%	594.22	539.23	654.82
Auto-0.0%	590.40	557.62	625.10



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	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3732	0.5190	0.3015	0.5853
2	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3744	0.5192	0.3020	0.5863
3	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3740	0.5208	0.3024	0.5870
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

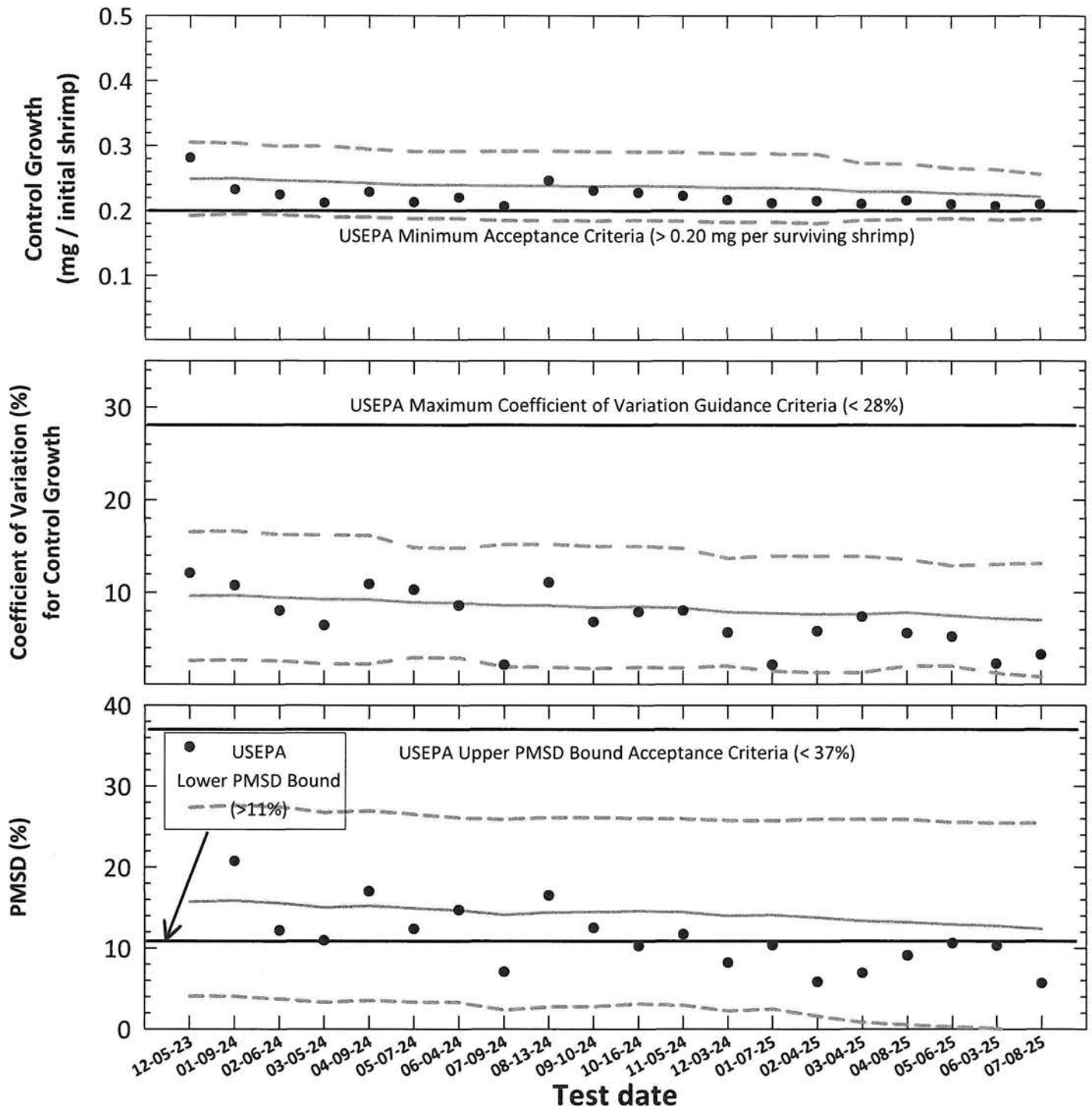
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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

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

CV = Coefficient of variation.

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



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency (mean Control Growth, CV or PMSD)**
- - - **95% Confidence Interval (mean Control Growth, CV or PMSD ± 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		Test		Control Growth (mg/initial shrimp)		Control Growth CV (%)		Test PMSD (%)	
		Control Survival (%)	Control Growth		CV (%)	Mean (mg/initial shrimp)	MSD	PMSD (%)	CT	95% Confidence Interval CT - 25	95% Confidence Interval CT + 25	CT	95% Confidence Interval CT - 25	95% Confidence Interval CT + 25	CT
1	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	27.4	4.1
2	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	27.6	4.0
3	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	27.4	3.7
4	03-05-24	100	0.212	6.5	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	26.8	3.3
5	04-09-24	100	0.229	10.9	0.0389	17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	26.9	3.5
6	05-07-24	100	0.213	10.3	0.0263	12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	26.5	3.3
7	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	26.1	3.3
8	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	25.9	2.3
9	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.4	26.1	2.7
10	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	26.1	2.8
11	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	26.0	3.1
12	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	26.0	2.9
13	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.0	25.8	2.2
14	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	25.7	2.5
15	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	25.9	1.5
16	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	25.9	0.8
17	04-08-25	100	0.216	5.6	0.0196	9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	25.9	0.5
18	05-06-25	100	0.210	5.2	0.0222	10.6	0.227	0.188	0.265	7.5	2.0	12.9	12.9	25.5	0.3
19	06-03-25	100	0.207	2.3	0.0213	10.3	0.225	0.186	0.264	7.2	1.3	13.1	12.8	25.4	0.1
20	07-08-25	100	0.210	3.3	0.0119	5.7	0.222	0.188	0.257	7.0	0.9	13.2	12.4	25.5	-0.7

Note:
Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.20 mg/surviving shrimp.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
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: 265

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2394					
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:	624
Date and times organisms were born between:	07-01-25 1200 to 07-02-25 1130	Incubator number and shelf location:	5A
Organism source:	AI Batch Ab: 07-02-25	Artemia CHM number:	CHM1385
Transfer bowl information:		Drying information for weight determination:	
pH = 8.63 S.U. Temperature = 25.0 °C		Date / Time in oven:	07-15-25 1200
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	07-16-25 1200
		*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	07-08-25	1100	X	1440	H	1225	H	07-07-25A
1	07-09-25	0500	H	1215	H	1040	H	07-07-25B
2	07-10-25	0500	H	1215	H	0900	H	07-09-25
3	07-11-25	0500	H	1100	H	0820	H	07-10-25A
4	07-12-25	0600	H	1200	H	0940	H	07-11-25
5	07-13-25	0600	H	1200	H	0910	H	07-12-25A
6	07-14-25	0500	H	1100	H	0830	H	07-12-25B
7	07-15-25					1042	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	13064665

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	508.3
Average weight per initial shrimp:	0.210		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.210	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	434.2

AbKCICR Test Number: **265**

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>BM</u> Date: <u>06-13-25</u>	12.34	14.24	13.59	13.82	12.78	14.22	13.47	11.02	12.01	13.01	14.04	14.30	12.90	13.65	14.31	12.71
*B = Pan + Shrimp weight (mg) Analyst: <u>BM</u> Date: <u>07-07-25</u>	13.41	15.28	14.66	14.93	13.64	15.22	14.50	12.04	13.09	14.11	15.09	15.43	13.95	14.71	15.32	13.81
C = Shrimp weight (mg) = B - A Hand calculated: <u>BM</u> Analyst: <u>BM</u>	1.07	1.04	1.07	1.11	1.06	1.00	1.03	1.02	1.08	1.10	1.05	1.13	1.05	1.06	1.01	1.09
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>BM</u> Analyst: <u>BM</u>	0.214	0.208	0.214	0.222	0.212	0.200	0.206	0.204	0.216	0.220	0.210	0.226	0.210	0.212	0.202	0.218
Average weight per initial number of shrimp (mg)								Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.210								0.214				-2.07.				

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

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

Comments:

AbKCICR Test Number: **265**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	4 ^{1d}	3 ^{2d}	3 ^{2d}	4 ^{1d}
3	S	S	S	S	S	4 ^{1d}	4 ^{1d}	S	4	4	4	4	4	2 ^{1d}	3	4
4	S	S	S	S	S	4	4	S	4	4	4	4	4	2	3	4
5	S	S	S	S	S	4	4	S	4	4	4	4	4	2	3	4
6	S	S	S	4 ^{1d}	S	4	4	S	4	4	4	4	4	2	3	4
7	S	S	S	4	S	4	4	S	3 ^{1d}	3 ^{1d}	4	2 ^{2d}	4	2	3	3 ^{1d}
# 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>EM</u> Date: <u>06-13-25</u>	14.48	14.35	14.63	12.23	14.43	10.28	10.20	10.55	10.21	13.01	13.86	13.80	15.40	14.00	13.90	10.35
*B = Pan + Shrimp weight (mg) Analyst: <u>EM</u> Date: <u>06-13-25</u>	15.55	15.46	15.71	13.41	15.50	11.30	11.21	11.61	10.74	13.52	14.38	14.44	16.18	14.33	14.38	10.88
C = Shrimp weight (mg) = B - A Hand calculated <u>EM</u> Analyst: <u>EM</u>	1.07	1.11	1.08	1.18	1.07	1.02	1.01	1.06	0.53	0.51	0.52	0.64	0.78	0.33	0.48	0.53
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated <u>EM</u> Analyst: <u>EM</u>	0.214	0.222	0.216	0.236	0.214	0.204	0.202	0.212	0.106	0.102	0.104	0.068	0.156	0.066	0.096	0.106
	Average weight per initial number of shrimp (mg) 0.215				Percent reduction from control (%) - 2.47				Average weight per initial number of shrimp (mg) 0.101				Percent reduction from control (%) 52.17			

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

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

Comments:

AbKCICR Test Number: **265**

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: 261

Test dates: July 08-15, 2021

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	12.34	13.41	1.07	0.214	100.0	0.210	3.3	Not applicable
	B	5	5	14.24	15.28	1.04	0.208				
	C	5	5	13.59	14.66	1.07	0.214				
	D	5	5	13.82	14.93	1.11	0.222				
	E	5	5	12.78	13.84	1.06	0.212				
	F	5	5	14.22	15.22	1.00	0.200				
	G	5	5	13.47	14.50	1.03	0.206				
	H	5	5	11.02	12.04	1.02	0.204				
250	I	5	5	12.01	13.09	1.08	0.216	100.0	0.214	3.4	-2.0
	J	5	5	13.01	14.11	1.10	0.220				
	K	5	5	14.04	15.09	1.05	0.210				
	L	5	5	14.30	15.43	1.13	0.226				
	M	5	5	12.90	13.95	1.05	0.210				
	N	5	5	13.65	14.71	1.06	0.212				
	O	5	5	14.31	15.32	1.01	0.202				
	P	5	5	12.71	13.80	1.09	0.218				
375	Q	5	5	14.48	15.55	1.07	0.214	92.5	0.215	4.9	-2.4
	R	5	5	14.35	15.46	1.11	0.222				
	S	5	5	14.63	15.71	1.08	0.216				
	T	5	4	12.23	13.41	1.18	0.236				
	U	5	5	14.43	15.50	1.07	0.214				
	V	5	4	10.28	11.30	1.02	0.204				
	W	5	4	10.20	11.21	1.01	0.202				
	X	5	5	10.55	11.61	1.06	0.212				
500	Y	5	3	10.21	10.74	0.53	0.106	60.0	0.101	27.7	52.1
	Z	5	3	13.01	13.52	0.51	0.102				
	AA	5	4	13.86	14.38	0.52	0.104				
	BB	5	2	13.80	14.14	0.34	0.068				
	CC	5	4	15.40	16.18	0.78	0.156				
	DD	5	2	14.00	14.33	0.33	0.066				
	EE	5	3	13.90	14.38	0.48	0.096				
	FF	5	3	10.35	10.88	0.53	0.106				
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:

0.0119

PMSD:

5.7

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.

1-tail, 0.01 level of significance

Dose (mg/kg)	Growth-Weight (100% Control)	Growth-Weight (50% Control)
0 (Control)	0.21	0.21
250	0.21	0.21
375	0.21	0.21
500	0.10	0.21
750	0.00	0.21
1000	0.00	0.00

AbKCICR Test Number: **265**

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.

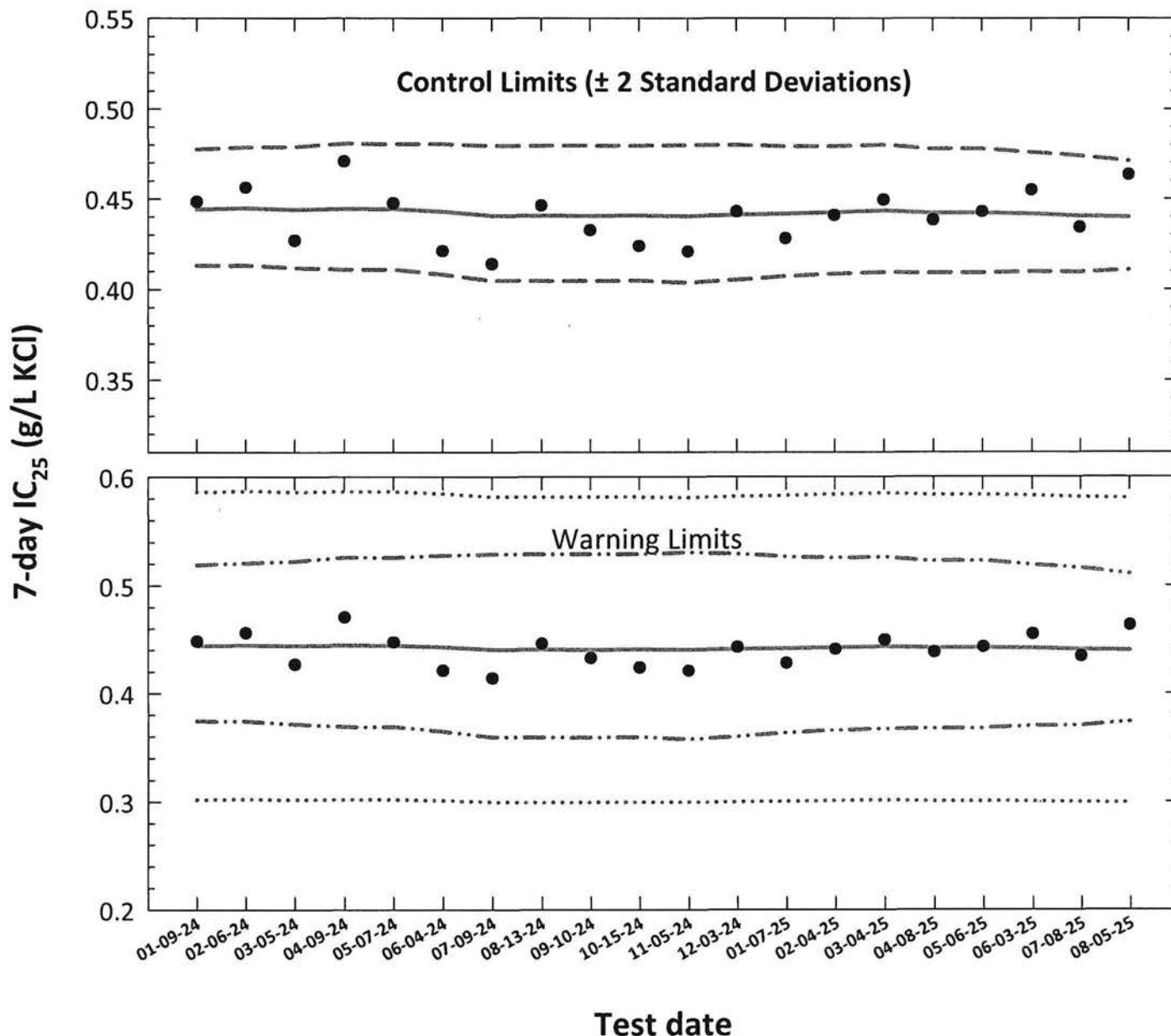
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst					
		XL <i>EM</i>	<i>PM</i>	<i>EM</i> XL	XL <i>joia</i>	XL	<i>KL</i>
CONTROL, Salt SW	pH (S.U.)	7.97	7.86	7.96	7.96 7.85	7.92	7.62
	DO (mg/L)	8.4	7.9	8.2	7.7	8.1	6.6
	Salinity (ppt)	25.0	25.2	25.0	25.2	26.0	25.9
	Alkalinity (mg CaCO ₃ /L)	100		100		82	
	Temperature (°C)	25.7	26.0	25.2	26.3	25.2	26.4
250 mg KCl/L	pH (S.U.)	7.91	7.87	7.92	7.85	7.94	7.72
	DO (mg/L)	8.4	7.9	8.2	7.8	8.1	6.7
	Salinity (ppt)	25.4	26.1	25.6	25.5	26.1	25.4
	Temperature (°C)	25.6	25.8	25.2	26.2	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.91	7.87	7.93	7.84	7.94	7.75
	DO (mg/L)	8.4	7.9	8.2	7.8	8.1	7.0
	Salinity (ppt)	25.4	26.3	25.8	25.7	25.3	25.9
	Temperature (°C)	25.6	25.8	25.2	26.5	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.92	7.88	7.94	7.85	7.94	7.80
	DO (mg/L)	8.4	7.9	8.2	7.8	8.1	7.2
	Salinity (ppt)	25.5	26.0	25.8	25.8	25.5	26.1
	Temperature (°C)	25.6	25.7	25.2	26.5	25.2	26.3
750 mg KCl/L	pH (S.U.)	7.92	7.91				
	DO (mg/L)	8.4	8.0				
	Salinity (ppt)	25.7	26.2				
	Temperature (°C)	25.8	26.1				
1000 mg KCl/L	pH (S.U.)	7.92	7.90				
	DO (mg/L)	8.4	8.0				
	Salinity (ppt)	25.9	26.6				
	Temperature (°C)	25.7	26.0				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: **265**

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.)	✓	BSL	BSL	BSL	BSL	XL	XL	✓
	DO (mg/L)	0.06	7.80	8.02	7.90	8.28	7.92	7.96	7.77
	Salinity (ppt)	0.2	7.9	8.2	7.9	8.4	7.9	8.1	5.5
	Alkalinity (mg CaCO ₃ /L)	25.0	25.1	25.0	25.3	24.9	25.1	25.1	25.2
	Temperature (°C)	76		95		95		100	
250 mg KCl/L	pH (S.U.)	25.4	26.3	25.2	26.4	25.3	26.4	25.2	26.3
	DO (mg/L)	7.07	7.84	*8.02 (7.12)	7.86	8.05	7.82	7.93	7.76
	Salinity (ppt)	8.1	7.9	8.2	8.0	8.3	7.8	8.1	5.6
	Temperature (°C)	25.2	25.4	25.2	25.4	25.2	25.3	25.1	25.4
375 mg KCl/L	pH (S.U.)	25.5	26.4	25.2	26.1	25.3	26.2	25.4	26.2
	DO (mg/L)	7.89	7.81	*8.03 (7.92)	7.79	8.05	7.83	7.93	7.69
	Salinity (ppt)	8.1	7.8	8.3	7.8	8.3	7.8	8.1	5.4
	Temperature (°C)	25.2	25.0	25.4	25.4	25.2	25.5	25.4	25.6
500 mg KCl/L	pH (S.U.)	25.5	26.5	25.4	26.2	25.2	26.6	25.4	26.2
	DO (mg/L)	7.89	7.91	7.94	7.83	8.05	7.83	7.93	7.70
	Salinity (ppt)	0.2	7.9	8.3	7.8	8.4	7.8	8.1	5.4
	Temperature (°C)	25.2	25.5	25.4	25.4	25.2	25.5	25.5	25.6
750 mg KCl/L	pH (S.U.)	25.5	26.2	25.4	26.2	25.2	26.4	25.3	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

* BSL 07-12-25 wrong on wrong page.

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	01-09-24	0.4484	-0.3483	-0.3525	0.0157	0.4441	0.4131	0.4775	0.3744	0.5192	0.3020	0.5863
2	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3740	0.5208	0.3024	0.5870
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

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

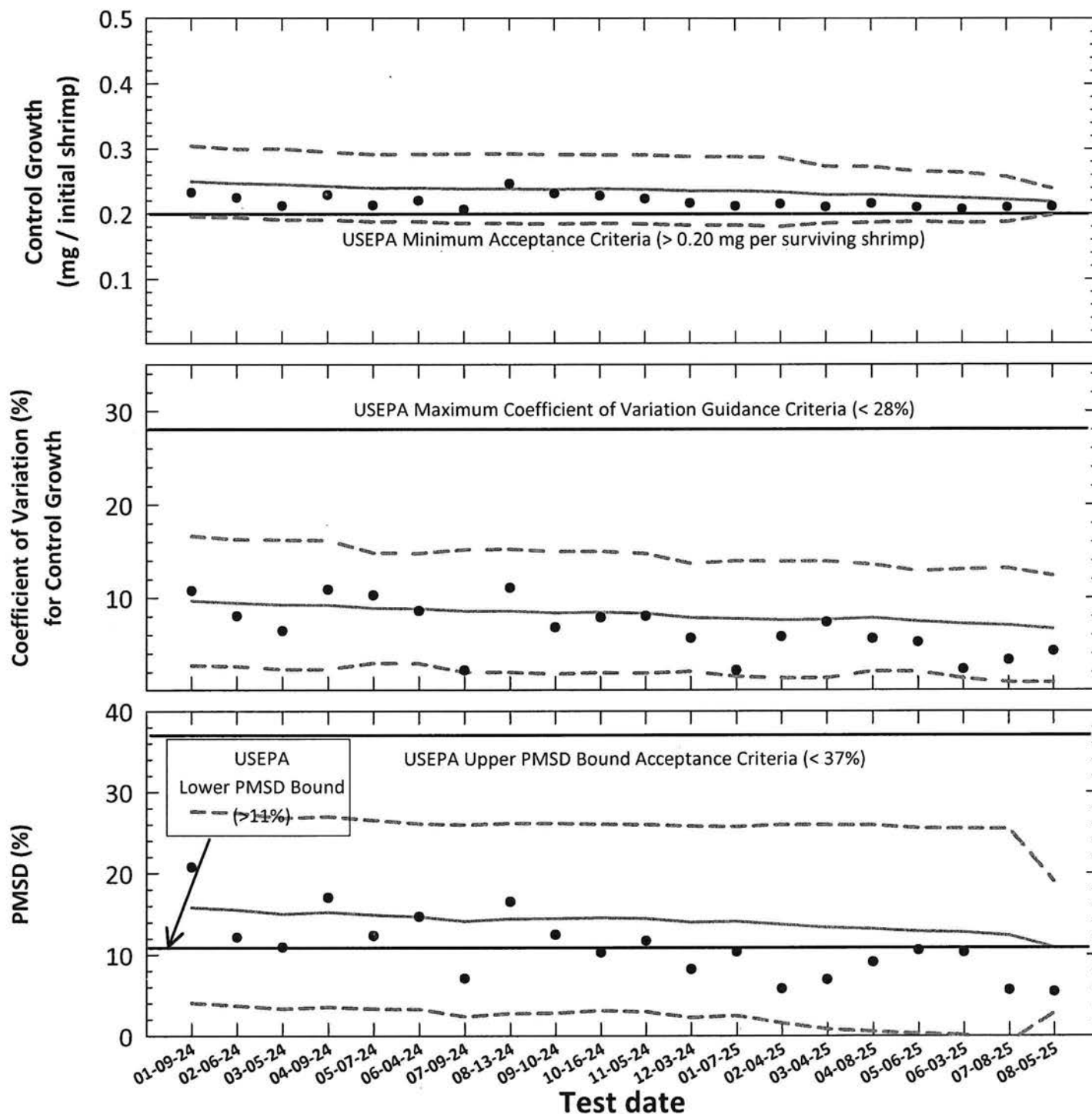
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

CV = Coefficient of variation.

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
			Mean (mg/initial shrimp)	CV (%)											
1	01-09-24	100	0.233	10.8	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6
2	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4
3	03-05-24	100	0.212	6.5	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8
4	04-09-24	100	0.229	10.9	0.0389	17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9
5	05-07-24	100	0.213	10.3	0.0263	12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5
6	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3	26.1
7	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3	25.9
8	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.4	2.7	26.1
9	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8	26.1
10	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1	26.0
11	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9	26.0
12	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.0	2.2	25.8
13	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5	25.7
14	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5	25.9
15	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8	25.9
16	04-08-25	100	0.216	5.6	0.0196	9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5	25.9
17	05-06-25	100	0.210	5.2	0.0222	10.6	0.227	0.188	0.265	7.5	2.0	12.9	12.9	0.3	25.5
18	06-03-25	100	0.207	2.3	0.0213	10.3	0.225	0.186	0.264	7.2	1.3	13.1	12.8	0.1	25.4
19	07-08-25	100	0.210	3.3	0.0119	5.7	0.222	0.188	0.257	7.0	0.9	13.2	12.4	-0.7	25.5
20	08-05-25	100	0.211	4.3	0.0115	5.5	0.219	0.198	0.239	6.6	0.9	12.4	10.9	2.8	19.0

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*

AbKICR Test Number: **266**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2394					
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:	07-29-25 1200 to 07-30-25 1130		Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 07-30-25		Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 8.17 S.U. Temperature = 25.0 °C		Drying information for weight determination:	
Average transfer volume:	< 0.25 mL		Date / Time in oven:	08-12-25 1010
			*Initial oven temperature:	60°C
			Date / Time out of oven:	08-13-25 1010
			*Final oven temperature:	60°C
			Total drying time:	24 Hours

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-98585

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	08-05-25	1045	K	1430	K	1130	K	07-31-25 B
1	08-06-25	0500	K	1100	K	0925	K	↓
2	08-07-25	0500	K	1130	K	0930	K	07-31-25 B
3	08-08-25	0600	K	1100	K	0950	K	↓
4	08-09-25	0600	K	1100	K	1050	K	08-05-25
5	08-10-25	0600	K	1100	K	0900	K	↓
6	08-11-25	0500	K	1100	K	0815	K	↓
7	08-12-25					0930	K	

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	13064085

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07	≤ 20%	7-day LC ₅₀ (mg/L KCl)	512.5
Average weight per initial shrimp:	0.211		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.211	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	463.4

AbKCICR Test Number: **266**

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>BP</u> Date: <u>07-21-25</u>	13.94	13.01	10.21	10.25	10.02	10.74	10.59	13.06	14.16	14.10	14.24	14.67	13.38	11.97	13.56	14.00
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>08-14-25</u>	15.07	14.05	11.24	11.37	11.06	11.74	11.64	14.10	15.19	15.17	15.25	15.68	14.46	13.06	14.61	15.17
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JP</u>	1.13	1.04	1.03	1.12	1.04	1.00	1.05	1.04	1.03	1.07	1.01	1.01	1.08	1.09	1.05	1.05
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JP</u>	0.226	0.208	0.206	0.224	0.208	0.200	0.210	0.208	0.206	0.214	0.202	0.202	0.202	0.218	0.210	0.211
Average weight per initial number of shrimp (mg) 0.211								Average weight per initial number of shrimp (mg) 0.211				Percent reduction from control (%) 0.4%				

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

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

Comments:

AbKCICR Test Number: **266**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	410	5	5	5	5	5	5	410	5	5	5	5	410	5	5
5	5	4	5	5	5	5	5	5	4	324	414	414	414	4	414	414
6	5	4	5	5	410	410	5	410	4	3	4	310	4	4	4	4
7	5	4	5	5	4	4	5	4	4	3	4	3	314	314	314	314
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males	2 of 12-5 H															
*A = Pan weight (mg) Tray color code: <u>Light Pink</u> Analyst: <u>EB</u> Date: <u>07-24-75</u>	13.87	14.03	12.81	12.24	14.42	12.55	14.56	13.76	11.81	12.25	14.50	14.00	13.95	14.83	13.52	13.48
*B = Pan + Shrimp weight (mg) Analyst: <u>SI</u> Date: <u>08-14-75</u>	14.97	15.06	13.80	13.26	15.50	13.60	13.66	14.75	12.56	12.95	15.16	14.63	14.77	15.29	14.26	14.27
C = Shrimp weight (mg) = B - A Hand calculated <u>AP</u> Analyst: <u>AP</u>	1.10	1.03	0.99	1.02	1.08	1.05	1.10	0.99	0.75	0.70	0.66	0.63	0.82	0.46	0.74	0.74
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated <u>AP</u> Analyst: <u>AP</u>	0.220	0.206	0.198	0.204	0.216	0.210	0.220	0.198	0.150	0.140	0.132	0.126	0.164	0.092	0.148	0.145
Average weight per initial number of shrimp (mg) 0.209				Percent reduction from control (%) 1.1%				Average weight per initial number of shrimp (mg) 0.138				Percent reduction from control (%) 34.9%				

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

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

Comments:

AbKCICR Test Number: **266**

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: _____ August 05-12, 202

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.94	15.07	1.13	0.226	100.0	0.211	4.3	Not applicable
	B	5	5	13.01	14.05	1.04	0.208				
	C	5	5	10.21	11.24	1.03	0.206				
	D	5	5	10.25	11.37	1.12	0.224				
	E	5	5	10.02	11.06	1.04	0.208				
	F	5	5	10.74	11.74	1.00	0.200				
	G	5	5	10.59	11.64	1.05	0.210				
	H	5	5	13.06	14.10	1.04	0.208				
250	I	5	5	14.16	15.19	1.03	0.206	100.0	0.211	3.1	0.4
	J	5	5	14.10	15.17	1.07	0.214				
	K	5	5	14.24	15.25	1.01	0.202				
	L	5	5	14.67	15.68	1.01	0.202				
	M	5	5	13.38	14.46	1.08	0.216				
	N	5	5	11.97	13.06	1.09	0.218				
	O	5	5	13.56	14.61	1.05	0.210				
	P	5	5	14.09	15.17	1.08	0.216				
375	Q	5	5	13.87	14.97	1.10	0.220	90.0	0.209	4.3	1.1
	R	5	4	14.03	15.06	1.03	0.206				
	S	5	5	12.81	13.80	0.99	0.198				
	T	5	5	12.24	13.26	1.02	0.204				
	U	5	4	14.42	15.50	1.08	0.216				
	V	5	4	12.55	13.60	1.05	0.210				
	W	5	5	14.56	15.66	1.10	0.220				
	X	5	4	13.76	14.75	0.99	0.198				
500	Y	5	4	11.81	12.56	0.75	0.150	65.0	0.138	15.8	34.9
	Z	5	3	12.25	12.95	0.70	0.140				
	AA	5	4	14.50	15.16	0.66	0.132				
	BB	5	3	14.00	14.63	0.63	0.126				
	CC	5	3	13.95	14.77	0.82	0.164				
	DD	5	3	14.83	15.29	0.46	0.092				
	EE	5	3	13.52	14.26	0.74	0.148				
	FF	5	3	13.48	14.22	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: 0.0115
PMSD: 5.4

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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

Mysid Survival and Growth Test-7 Day Survival

Start Date: 8/5/2025 Test ID: AbKICIR Sample ID: REF-Ref Toxicant
End Date: 8/12/2025 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:

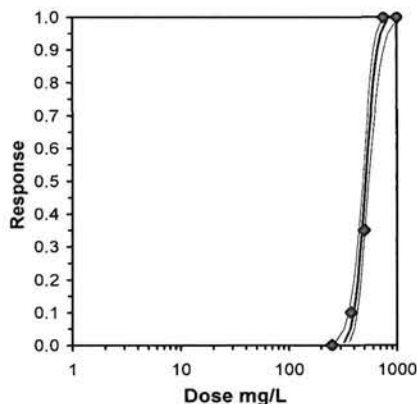
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	0.8000	1.0000	1.0000	0.8000	0.8000	1.0000	0.8000
500	0.8000	0.6000	0.8000	0.6000	0.6000	0.6000	0.6000	0.6000
750	0.0000	0.0000	0.0000	0.0000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

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9000	0.9000	1.2262	1.1071	1.3453	10.381	8	52.00	48.00	4	40
*500	0.6500	0.6500	0.9413	0.8861	1.1071	10.871	8	36.00	48.00	14	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

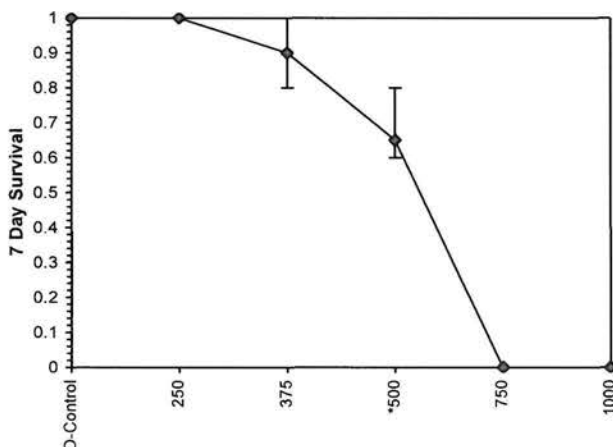
Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)				0.85337	0.904	0.59644	0.11307
Equality of variance cannot be confirmed							
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test				375	500	433.013	
Treatments vs D-Control							

Maximum Likelihood-Probit									
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu
Slope	11.6925	1.67467	8.41012	14.9748	0	4.16426	7.81472	0.24426	2.70995
Intercept	-26.686	4.51706	-35.539	-17.833					
TSCR									

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	324.33	270.808	361.173
EC05	3.355	370.913	324.549	403.309
EC10	3.718	398.422	356.75	428.562
EC15	3.964	418.126	379.761	447.077
EC20	4.158	434.479	398.665	462.868
EC25	4.326	449.017	415.207	477.347
EC40	4.747	487.844	457.418	518.782
EC50	5.000	512.801	482.594	547.972
EC60	5.253	539.034	507.374	580.837
EC75	5.674	585.645	548.047	643.819
EC80	5.842	605.241	564.223	671.683
EC85	6.036	628.912	583.245	706.216
EC90	6.282	660.014	607.569	752.847
EC95	6.645	708.965	644.711	828.699
EC99	7.326	810.792	718.935	994.521



Dose-Response Plot



Mysid Survival and Growth Test-Growth-Weight

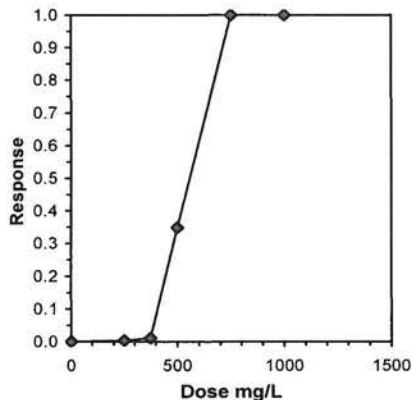
Start Date: 8/5/2025 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
End Date: 8/12/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2260	0.2080	0.2060	0.2240	0.2080	0.2000	0.2100	0.2080
250	0.2060	0.2140	0.2020	0.2020	0.2160	0.2180	0.2100	0.2160
375	0.2200	0.2060	0.1980	0.2040	0.2160	0.2100	0.2200	0.1980
500	0.1500	0.1400	0.1320	0.1260	0.1640	0.0920	0.1480	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

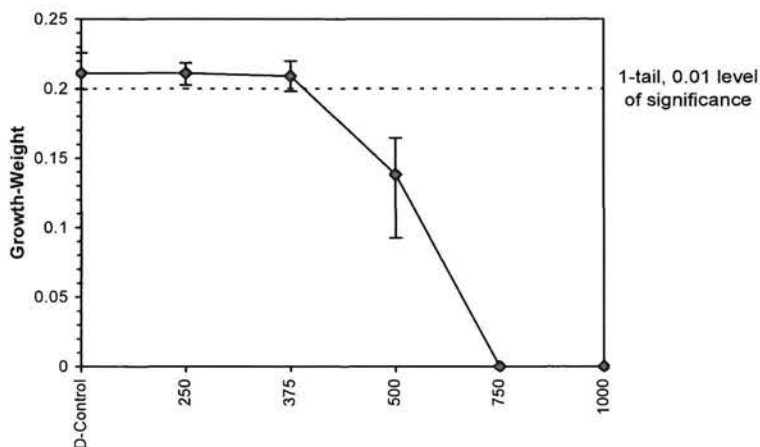
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	Critical			MSD	Mean	N-Mean	
D-Control	0.2113	1.0000	0.2113	0.2000	0.2260	4.262	8	0.182	2.799	0.0115	0.2113	1.0000	
250	0.2105	0.9964	0.2105	0.2020	0.2180	3.079	8				0.2105	0.9964	
375	0.2090	0.9893	0.2090	0.1980	0.2200	4.310	8	0.545	2.799	0.0115	0.2090	0.9893	
500	0.1375	0.6509	0.1375	0.0920	0.1640	15.834	8				0.1375	0.6509	
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)					0.94429		0.884		0.31734		-0.9714			
Bartlett's Test indicates equal variances (p = 0.65)					0.87187		9.21035							
Hypothesis Test (1-tail, 0.01)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					375	>375			0.01154	0.05465	1.1E-05	6.8E-05	0.85802	2, 21
Treatments vs D-Control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL		Skew
IC05	389.53	8.23	372.09	394.55	-4.1665
IC10	408.00	5.59	394.83	415.70	-0.6279
IC15	426.46	6.39	413.88	437.11	-0.1819
IC20	444.93	7.64	429.66	458.92	0.0625
IC25	463.40	9.16	445.05	480.48	0.1750
IC40	519.55	11.61	492.61	537.88	-0.3826
IC50	557.95	10.04	533.24	573.24	-0.5887



Dose-Response Plot



AbKCICR Test Number: **266**

Daily Chemistry:

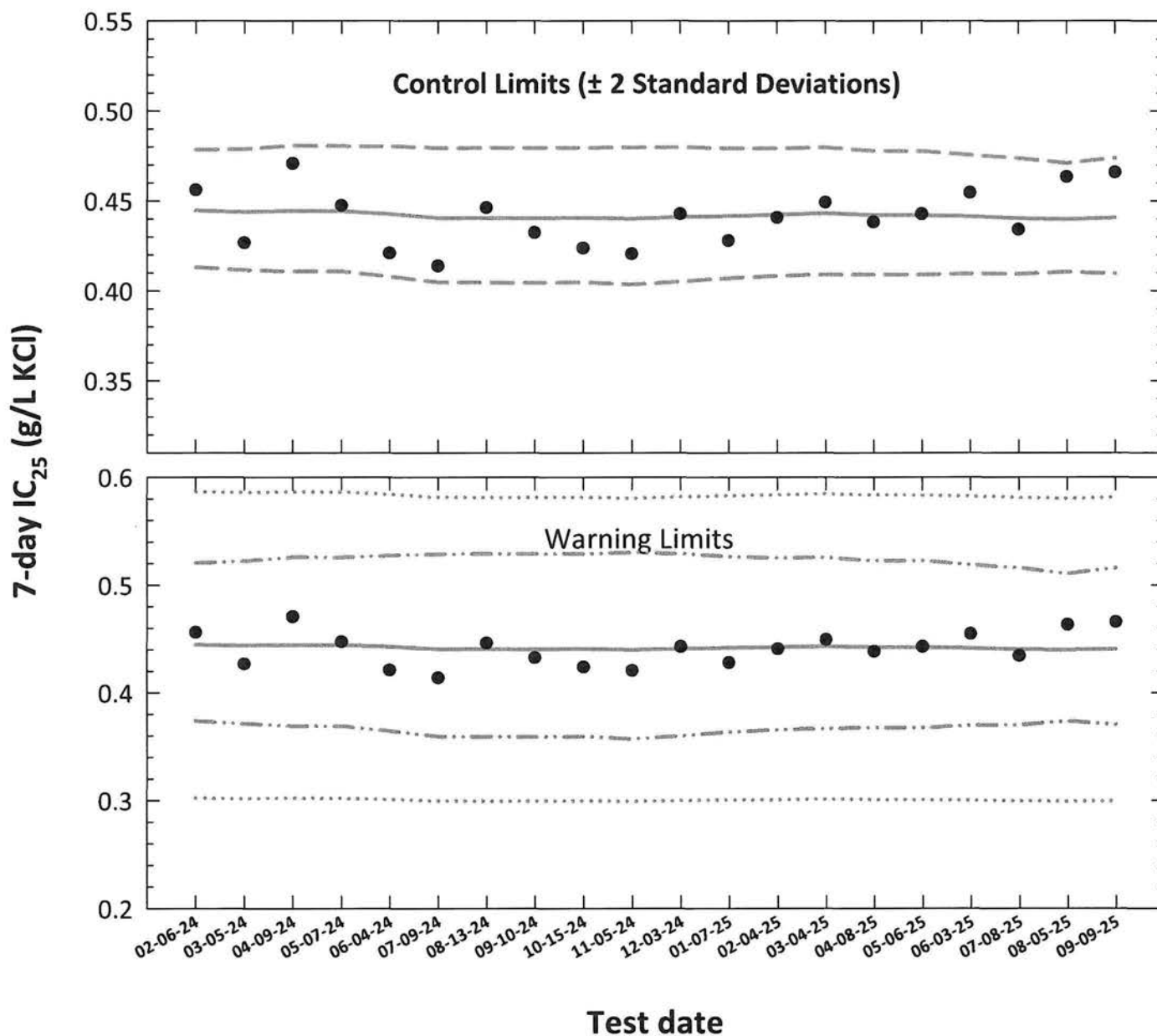
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

		Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
Conc.	Parameter	0		1		2	
Analyst		W	XL	XL	XL	XL	XL
CONTROL, Salt SW	pH (S.U.)	8.03	7.85	7.89	7.82	7.87	7.81
	DO (mg/L)	0.4	7.9	8.3	7.9	8.2	7.8
	Salinity (ppt)	25.2	25.2	25.0	25.4	25.2 ^{25.0}	25.0
	Alkalinity (mg CaCO ₃ /L)	110			08-11-25 H	110	
	Temperature (°C)	25.2	25.2	25.0	26.2	25.2	26.3
250 mg KCl/L	pH (S.U.)	8.04	7.86	7.90	7.83	7.88	7.84
	DO (mg/L)	0.4	8.0	8.2	7.9	8.1	7.8
	Salinity (ppt)	25.2	25.6	25.2	25.7	25.2	25.3
	Temperature (°C)	25.3	25.6	25.1	26.4	25.1	26.0
375 mg KCl/L	pH (S.U.)	8.04	7.86	7.90	7.83	7.88	7.86
	DO (mg/L)	0.4	8.0	8.2	7.8	8.1	7.8
	Salinity (ppt)	25.2	25.7	25.4	25.9	25.4	25.7
	Temperature (°C)	25.3	25.7	25.1	26.2	25.1	26.0
500 mg KCl/L	pH (S.U.)	8.04	7.88	7.91	7.81	7.87	7.84
	DO (mg/L)	0.4	8.0	8.2	7.8	8.1	7.6
	Salinity (ppt)	25.4	25.9	25.5	25.9	25.5	25.7
	Temperature (°C)	25.3	25.7	25.1	26.2	25.1	26.2
750 mg KCl/L	pH (S.U.)	8.04	7.82				
	DO (mg/L)	0.4	7.9				
	Salinity (ppt)	25.4	25.7				
	Temperature (°C)	25.3	25.5				
1000 mg KCl/L	pH (S.U.)	8.04	7.83				
	DO (mg/L)	0.4	7.8				
	Salinity (ppt)	25.4	26.0				
	Temperature (°C)	25.3	25.7				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: **266**

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.)		7.93	7.95	8.07	7.97	8.08	7.84	7.99
	DO (mg/L)		8.1	7.9	8.2	7.7	8.1	7.7	8.0
	Salinity (ppt)		25.0	25.2	25.2	25.2	25.4	25.3	25.5
	Alkalinity (mg CaCO ₃ /L)		110	110					
	Temperature (°C)		25.2	26.1	26.2	26.4	26.2	26.1	26.2
250 mg KCl/L	pH (S.U.)		7.92	7.95	8.04	7.91	8.07	7.84	7.97
	DO (mg/L)		8.1	7.9	8.2	7.6	8.1	7.4	8.0
	Salinity (ppt)		25.3	25.5	25.3	25.4	25.5	25.4	25.5
	Temperature (°C)		25.3	26.2	25.2	26.6	25.4	26.5	25.4
375 mg KCl/L	pH (S.U.)		7.92	7.97	8.07	7.94	8.07	7.87	7.97
	DO (mg/L)		8.1	7.9	8.2	7.7	8.1	7.7	8.0
	Salinity (ppt)		25.3	25.6	25.3	25.4	25.5	25.4	25.6
	Temperature (°C)		25.2	26.2	25.3	26.3	25.4	26.6	25.4
500 mg KCl/L	pH (S.U.)		7.92	7.99	8.06	7.90	8.07	7.85	7.97
	DO (mg/L)		8.0	7.9	8.2	7.7	8.1	7.7	8.0
	Salinity (ppt)		25.4	25.7	25.4	25.5	25.5	25.7	25.6
	Temperature (°C)		25.2	26.0	25.3	26.3	25.4	26.6	25.4
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		75th Percentile CV Warning Limits
							CT - 2S	CT + 2S	
1	02-06-24	0.4561	-0.3409	-0.3519	0.0159	0.4447	0.4133	0.4785	0.3024
2	03-05-24	0.4268	-0.3698	-0.3527	0.0164	0.4440	0.4117	0.4788	0.3019
3	04-09-24	0.4708	-0.3272	-0.3521	0.0170	0.4445	0.4109	0.4808	0.3022
4	05-07-24	0.4475	-0.3492	-0.3523	0.0170	0.4443	0.4109	0.4805	0.3022
5	06-04-24	0.4211	-0.3756	-0.3538	0.0177	0.4428	0.4082	0.4804	0.3011
6	07-09-24	0.4139	-0.3832	-0.3561	0.0184	0.4405	0.4047	0.4794	0.2995
7	08-13-24	0.4462	-0.3505	-0.3560	0.0184	0.4406	0.4048	0.4796	0.2996
8	09-10-24	0.4325	-0.3640	-0.3561	0.0185	0.4405	0.4046	0.4796	0.2995
9	10-15-24	0.4238	-0.3729	-0.3560	0.0184	0.4406	0.4048	0.4795	0.2996
10	11-05-24	0.4206	-0.3761	-0.3565	0.0188	0.4401	0.4036	0.4798	0.2993
11	12-03-24	0.4428	-0.3538	-0.3555	0.0184	0.4411	0.4053	0.4800	0.2999
12	01-07-25	0.4280	-0.3686	-0.3549	0.0177	0.4417	0.4071	0.4792	0.3003
13	02-04-25	0.4408	-0.3558	-0.3542	0.0173	0.4424	0.4085	0.4792	0.3008
14	03-04-25	0.4493	-0.3474	-0.3534	0.0173	0.4432	0.4093	0.4799	0.3014
15	04-08-25	0.4382	-0.3583	-0.3545	0.0169	0.4421	0.4091	0.4777	0.3006
16	05-06-25	0.4428	-0.3538	-0.3545	0.0169	0.4421	0.4091	0.4778	0.3006
17	06-03-25	0.4548	-0.3422	-0.3550	0.0162	0.4415	0.4098	0.4757	0.3002
18	07-08-25	0.4342	-0.3624	-0.3562	0.0159	0.4404	0.4093	0.4738	0.2995
19	08-05-25	0.4634	-0.3340	-0.3567	0.0149	0.4398	0.4107	0.4710	0.2991
20	09-09-25	0.4660	-0.3316	-0.3559	0.0158	0.4407	0.4097	0.4740	0.2997

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

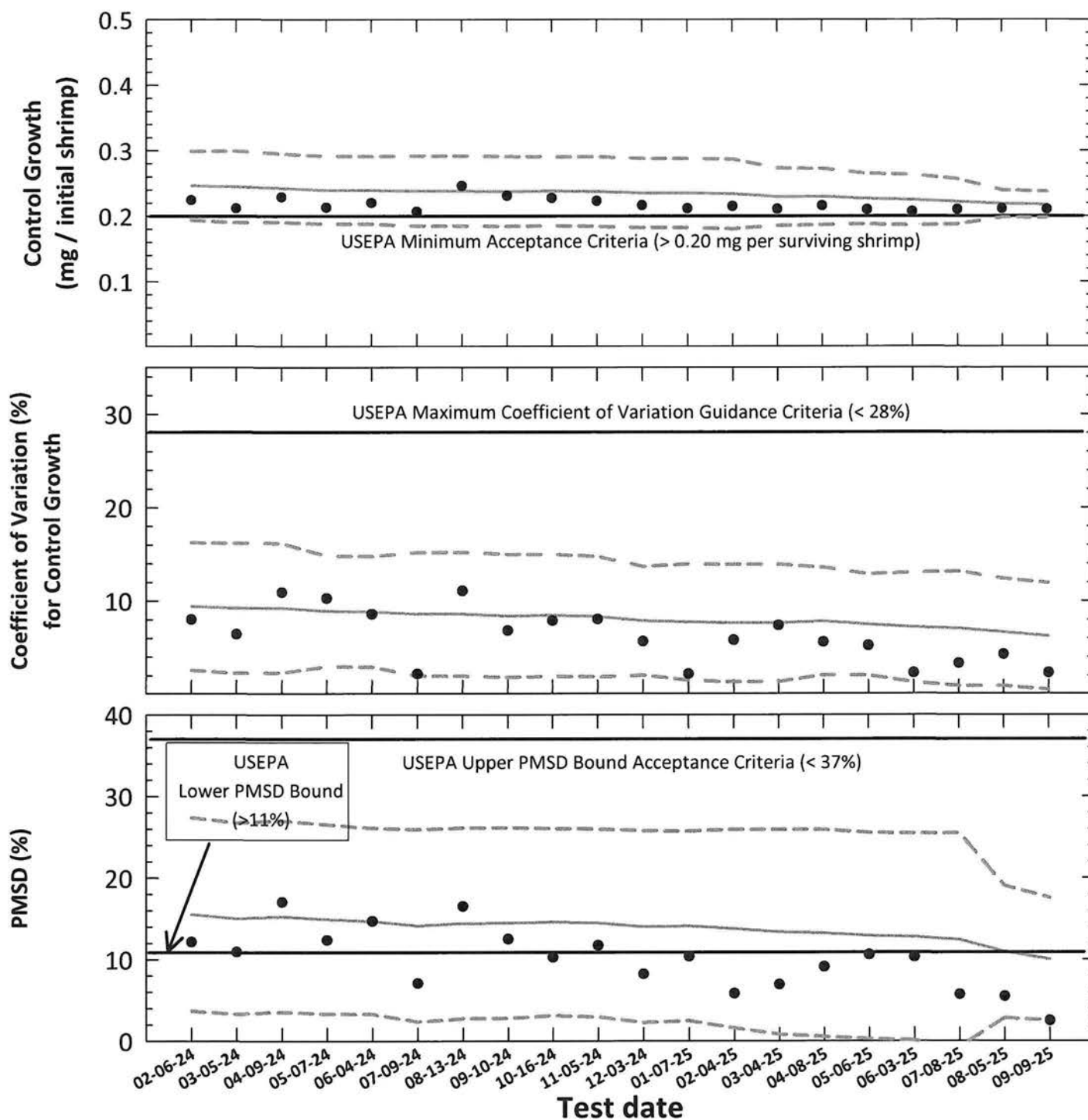
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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

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

CV = Coefficient of variation.

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



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

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD				
		Control Survival (%)	Control Growth		Test	PMSD (%)	CV (%)	MSD	CT	Control Growth (mg/Initial shrimp)		CT	Control Growth CV (%)		CT	Test PMSD (%)	
			Mean (mg/Initial shrimp)							95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S		95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S			
1	02-06-24	100	0.225	8.0	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4		
2	03-05-24	100	0.212	6.5	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8		
3	04-09-24	100	0.229	10.9	0.0389	17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9		
4	05-07-24	100	0.213	10.3	0.0263	12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5		
5	06-04-24	100	0.220	8.6	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	14.7	3.3	26.1		
6	07-09-24	100	0.207	2.2	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.1	2.3	25.9		
7	08-13-24	100	0.246	11.1	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	14.4	2.7	26.1		
8	09-10-24	100	0.231	6.8	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.4	2.8	26.1		
9	10-16-24	100	0.228	7.9	0.0233	10.2	0.238	0.185	0.291	8.5	1.9	15.0	14.6	3.1	26.0		
10	11-05-24	100	0.223	8.0	0.0261	11.7	0.237	0.184	0.291	8.3	1.9	14.8	14.4	2.9	26.0		
11	12-03-24	100	0.216	5.7	0.0177	8.2	0.235	0.182	0.288	7.9	2.0	13.7	14.0	2.2	25.8		
12	01-07-25	100	0.212	2.2	0.0219	10.4	0.235	0.183	0.288	7.7	1.5	14.0	14.1	2.5	25.7		
13	02-04-25	100	0.215	5.8	0.0125	5.8	0.234	0.181	0.287	7.6	1.3	13.9	13.7	1.5	25.9		
14	03-04-25	100	0.211	7.4	0.0146	6.9	0.229	0.185	0.273	7.6	1.3	13.9	13.3	0.8	25.9		
15	04-08-25	100	0.216	5.6	0.0196	9.1	0.230	0.187	0.273	7.8	2.1	13.6	13.2	0.5	25.9		
16	05-06-25	100	0.210	5.2	0.0222	10.6	0.227	0.188	0.265	7.5	2.0	12.9	12.9	0.3	25.5		
17	06-03-25	100	0.207	2.3	0.0213	10.3	0.225	0.186	0.264	7.2	1.3	13.1	12.8	0.1	25.4		
18	07-08-25	100	0.210	3.3	0.0119	5.7	0.222	0.188	0.257	7.0	0.9	13.2	12.4	-0.7	25.5		
19	08-05-25	100	0.211	4.3	0.0115	5.5	0.219	0.198	0.239	6.6	0.9	12.4	10.9	2.8	19.0		
20	09-09-25	100	0.210	2.3	0.0052	2.5	0.218	0.198	0.237	6.2	0.5	11.9	10.0	2.5	17.5		

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

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

AbKCICR Test Number: 267

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:	6124
Date and times organisms were born between:	09-02-25 1200 to 09-03-25 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 09-03-25	Artemia CHM number:	CHM1385
Transfer bowl information:	pH = 7.81 S.U. Temperature = 24.5°C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	09-16-25 1050
		*Initial oven temperature:	60°C
		Date / Time out of oven:	09-17-25 1050
		*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	09-09-25	1045	H	1445	H	1200	H	09-09-25 B
1	09-10-25	0500	H	1315	H	1010	H	↓
2	09-11-25	0500	H	1330	H	1000	H	09-09-25
3	09-12-25	0500	H	1100	H	0800	H	09-10-25
4	09-13-25	0600	H	1130	H	0930	H	09-11-25
5	09-14-25	0600	H	1130	H	0900	H	09-12-25
6	09-15-25	0500	H	1100	H	0800	H	09-13-25
7	09-16-25					1013	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	13066464 S

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	540.2
Average weight per initial shrimp:	0.210		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.210	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	466.0

09-15-25 H

AbKCICR Test Number: **267**

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>km</u> Date: <u>08-20-25</u>	14.29	13.38	12.84	10.64	10.05	14.24	10.36	13.79	13.84	14.06	10.74	14.20	10.44	14.16	12.76	11.14
*B = Pan + Shrimp weight (mg) Analyst: <u>dl</u> Date: <u>08-14-25</u>	15.33	14.41	13.90	11.72	11.14	15.29	11.40	14.81	14.90	15.11	11.81	15.27	11.49	15.20	13.82	12.2
C = Shrimp weight (mg) = B - A Hand calculated: <u>dl</u> Analyst: <u>dl</u>	1.04	1.03	1.06	1.08	1.09	1.05	1.04	1.02	1.06	1.05	1.07	1.07	1.05	1.04	1.06	1.06
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>dl</u> Analyst: <u>dl</u>	0.208	0.206	0.212	0.216	0.218	0.210	0.208	0.204	0.212	0.210	0.214	0.214	0.210	0.208	0.212	0.212
Average weight per initial number of shrimp (mg)								Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
6.210								0.212				- 0.67.				

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

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

Comments:

AbKCICR Test Number: 267

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	4 ^d	S	4 ^{id}	4 ^{id}	4 ^{id}	S	4 ^{id}	4 ^{id}	4 ^{id}	S
3	S	S	S	S	S	S	4 ^d	S	4	4	4	S	4	4	4	S
4	S	S	S	S	S	S	4	S	4	4	4	4 ^{id}	4	4	4	4 ^{id}
5	S	S	S	S	S	S	4	S	4	4	4	4	4	4	4	4
6	S	S	S	S	S	S	4	S	4	4	4	4	4	4	4	4
7	S	S	S	S	S	S	4 ^{id}	S	4	3 ^{id}	4	4	3 ^{id}	4	4	4
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>Laverde</u> Analyst: <u>guy</u> Date: <u>08-20-25</u>	13.16	10.27	10.31	13.25	13.58	10.72	15.47	10.22	12.82	14.97	14.54	14.52	10.23	11.02	13.21	12.99
*B = Pan + Shrimp weight (mg) Analyst: <u>guy</u> Date: <u>08-18-25</u>	14.22	11.30	11.39	14.31	14.64	11.80	16.51	11.30	13.61	15.76	15.26	15.20	10.82	11.57	13.73	13.90
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>guy</u>	1.06	1.03	1.08	1.06	1.06	1.08	1.04	1.08	0.79	0.79	0.72	0.68	0.59	0.55	0.52	0.91
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>guy</u>	0.212	0.206	0.216	0.212	0.212	0.216	0.208	0.216	0.158	0.158	0.144	0.136	0.118	0.110	0.104	0.182
Average weight per initial number of shrimp (mg)					Percent reduction from control (%)					Average weight per initial number of shrimp (mg)					Percent reduction from control (%)	
0.212					-1.07					0.139					34.07	

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

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

Comments:

AbKCICR Test Number: **267**

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 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol			
2																			
3																			
4																			
5																			
6																			
7																			
# females with eggs in brood sac																			
# females with developing ova in oviducts																			
# immature females																			
# males																			
*A = Pan weight (mg) Tray color code: _____ Analyst: _____ Date: _____																			
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																			
C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____																			
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Average weight per initial number of shrimp (mg)		0		Percent reduction from control (%)				100%				Average weight per initial number of shrimp (mg)		0		Percent reduction from control (%)		100%	

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

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

Comments:

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

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

Test number: 267
Test dates: September 09-16, 2025

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.29	15.33	1.04	0.208	100.0	0.210	2.3	Not applicable
	B	5	5	13.38	14.41	1.03	0.206				
	C	5	5	12.84	13.90	1.06	0.212				
	D	5	5	10.64	11.72	1.08	0.216				
	E	5	5	10.05	11.14	1.09	0.218				
	F	5	5	14.24	15.29	1.05	0.210				
	G	5	5	10.36	11.40	1.04	0.208				
	H	5	5	13.79	14.81	1.02	0.204				
250	I	5	5	13.84	14.90	1.06	0.212	100.0	0.212	1.0	-0.6
	J	5	5	14.06	15.11	1.05	0.210				
	K	5	5	10.74	11.81	1.07	0.214				
	L	5	5	14.20	15.27	1.07	0.214				
	M	5	5	10.44	11.49	1.05	0.210				
	N	5	5	14.16	15.20	1.04	0.208				
	O	5	5	12.76	13.82	1.06	0.212				
	P	5	5	11.14	12.20	1.06	0.212				
375	Q	5	5	13.16	14.22	1.06	0.212	97.5	0.212	1.8	-1.0
	R	5	5	10.27	11.30	1.03	0.206				
	S	5	5	10.31	11.39	1.08	0.216				
	T	5	5	13.25	14.31	1.06	0.212				
	U	5	5	13.58	14.64	1.06	0.212				
	V	5	5	10.72	11.80	1.08	0.216				
	W	5	4	15.47	16.51	1.04	0.208				
	X	5	5	10.22	11.30	1.08	0.216				
500	Y	5	4	12.82	13.61	0.79	0.158	75.0	0.139	19.5	34.0
	Z	5	3	14.97	15.76	0.79	0.158				
	AA	5	4	14.54	15.26	0.72	0.144				
	BB	5	4	14.52	15.20	0.68	0.136				
	CC	5	3	10.23	10.82	0.59	0.118				
	DD	5	4	11.02	11.57	0.55	0.110				
	EE	5	4	13.21	13.73	0.52	0.104				
	FF	5	4	12.99	13.90	0.91	0.182				
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: 0.0052
PMSD: 2.5

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.

1-tail, 0.01 level of significance

Dose (mg/kg)	Growth-Weight (100% Control)	Growth-Weight (50% Control)
Control	0.21	0.21
250	0.21	0.21
375	0.21	0.21
500	0.14	0.21
750	0.00	0.21
1000	0.00	0.00

AbKCICR Test Number: **267**

Daily Chemistry:

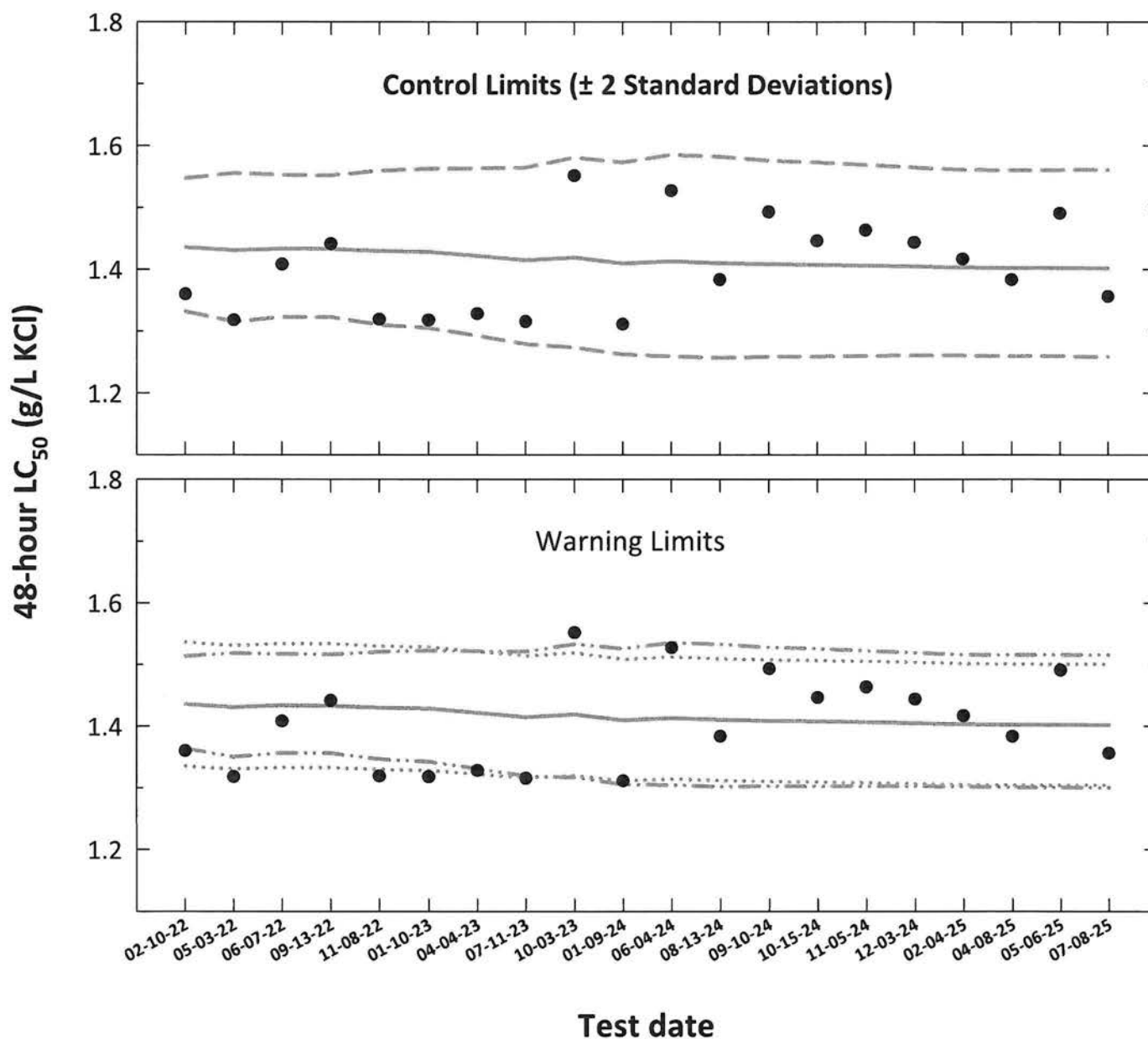
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

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.)		8.03	7.95	7.97	7.84	8.00	7.88
	DO (mg/L)		8.2	7.9	8.2	7.7	8.1	7.8
	Salinity (ppt)		24.9	25.2	25.0	25.2	25.0	24.2
	Alkalinity (mg CaCO ₃ /L)		110		115.15	100		
	Temperature (°C)		26.3	26.3	25.2	26.3	25.1	26.4
250 mg KCl/L	pH (S.U.)		7.98	7.95	7.99	7.91	7.99	7.87
	DO (mg/L)		8.2	7.9	8.1	7.7	8.2	7.8
	Salinity (ppt)		25.1	25.4	25.3	25.5	25.2	25.5
	Temperature (°C)		26.2	26.6	25.1	26.2	25.2	25.9
375 mg KCl/L	pH (S.U.)		7.98	7.94	7.99	7.88	7.99	7.85
	DO (mg/L)		8.2	7.9	8.1	7.7	8.2	7.8
	Salinity (ppt)		25.0	25.5	25.5	25.7	25.6	25.0
	Temperature (°C)		26.2	26.4	25.1	26.4	25.1	25.9
500 mg KCl/L	pH (S.U.)		7.98	7.94	7.99	7.88	7.99	7.88
	DO (mg/L)		8.2	7.8	8.1	7.7	8.2	7.8
	Salinity (ppt)		25.1	25.6	25.5	25.9	25.7	25.9
	Temperature (°C)		26.2	26.4	25.3	26.5	25.1	26.2
750 mg KCl/L	pH (S.U.)		7.99	7.92				
	DO (mg/L)		8.3	7.8				
	Salinity (ppt)		25.3	26.4				
	Temperature (°C)		26.4	26.3				
1000 mg KCl/L	pH (S.U.)		7.99	7.93				
	DO (mg/L)		8.3	7.8				
	Salinity (ppt)		25.4	26.3				
	Temperature (°C)		26.4	26.3				
			Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 267

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSC	BSC	BSC	BSC	XL	XL	XL
CONTROL, Salt SW	pH (S.U.)	7.94	7.87	7.94	7.85	8.03	7.77	8.06	7.88
	DO (mg/L)	8.0	7.9	8.2	7.7	8.3	7.7	8.0	6.0
	Salinity (ppt)	250	250	24.9	25.1	24.8	25.0	24.9	25.3
	Alkalinity (mg CaCO ₃ /L)	99		86		78		110	
	Temperature (°C)	25.2	26.4	25.1	26.4	25.3	26.3	25.2	26.4
250 mg KCl/L	pH (S.U.)	7.96	7.90	8.03	7.87	8.01	7.83	7.91	7.74
	DO (mg/L)	8.1	7.9	8.1	7.8	8.3	7.8	8.1	6.1
	Salinity (ppt)	25.2	25.2	25.2	25.3	25.1	25.2	25.2	25.3
	Temperature (°C)	25.1	26.1	25.1	26.2	25.2	26.2	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.96	7.86	8.03	7.95	8.01	7.84	7.91	7.68
	DO (mg/L)	8.1	7.9	8.1	7.9	8.3	7.8	8.1	6.1
	Salinity (ppt)	25.3	25.4	25.4	25.5	25.1	25.3	25.4	25.6
	Temperature (°C)	25.1	26.0	25.1	26.4	25.2	26.2	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.96	7.92	8.03	7.90	8.00	7.83	7.91	7.68
	DO (mg/L)	8.1	7.9	8.2	7.7	8.3	7.8	8.1	6.6
	Salinity (ppt)	25.3	25.5	25.5	25.6	25.2	25.4	25.4	25.7
	Temperature (°C)	25.2	26.3	25.1	26.4	25.2	26.3	25.2	26.5
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
1	02-10-22	1.3599	0.1335	0.1570	0.0163	1.4356	1.3320	1.5473	1.3635	1.5134	1.3351	1.5361
2	05-03-22	1.3180	0.1199	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182	1.3305	1.5308
3	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169	1.3332	1.5339
4	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163	1.3328	1.5335
5	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205	1.3296	1.5298
6	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227	1.3284	1.5283
7	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213	1.3221	1.5211
8	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209	1.3158	1.5139
9	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331	1.3197	1.5184
10	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256	1.3107	1.5081
11	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351	1.3142	1.5120
12	08-13-24	1.3832	0.1409	0.1493	0.0250	1.4102	1.2569	1.5823	1.3015	1.5322	1.3115	1.5089
13	09-10-24	1.4930	0.1740	0.1488	0.0244	1.4087	1.2589	1.5763	1.3024	1.5277	1.3101	1.5073
14	10-15-24	1.4461	0.1602	0.1484	0.0242	1.4075	1.2593	1.5731	1.3022	1.5251	1.3090	1.5060
15	11-05-24	1.4632	0.1653	0.1480	0.0238	1.4062	1.2603	1.5689	1.3024	1.5219	1.3077	1.5046
16	12-03-24	1.4436	0.1594	0.1476	0.0235	1.4047	1.2609	1.5649	1.3023	1.5188	1.3064	1.5030
17	02-04-25	1.4168	0.1513	0.1470	0.0232	1.4029	1.2607	1.5611	1.3015	1.5156	1.3047	1.5011
18	04-08-25	1.3832	0.1409	0.1468	0.0232	1.4021	1.2597	1.5605	1.3006	1.5150	1.3039	1.5002
19	05-06-25	1.4906	0.1734	0.1468	0.0233	1.4022	1.2596	1.5610	1.3005	1.5154	1.3040	1.5004
20	07-08-25	1.3559	0.1322	0.1467	0.0234	1.4017	1.2587	1.5611	1.2997	1.5154	1.3036	1.4999

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 # 102

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 #: 2394

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	X2 EN	X1	X1
Control, SaltSW	pH (S.U.)	7.97	7.91	7.89
	Dissolved oxygen (mg/L)	8.4	8.1	7.9
	*Salinity (ppt)	25.0	25.4	25.3
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	24.1	24.2	24.3
1000 mg/L	pH (S.U.)	7.94	7.92	7.91
	Dissolved oxygen (mg/L)	8.5	8.1	7.9
	*Salinity (ppt)	25.5 28.5	25.7	25.6
	*Temperature (°C)	24.1	24.4	24.3
1250 mg/L	pH (S.U.)	7.94	7.90	7.90
	Dissolved oxygen (mg/L)	8.5	8.1	7.9
	*Salinity (ppt)	25.8 27.1	25.9	25.7
	*Temperature (°C)	24.0	24.4	24.6
1500 mg/L	pH (S.U.)	7.94	7.90	7.90
	Dissolved oxygen (mg/L)	8.5	8.1	7.9
	*Salinity (ppt)	25.9 27.2	26.0	26.0
	*Temperature (°C)	24.1	24.4	24.6
1750 mg/L	pH (S.U.)	7.94	7.91	7.89
	Dissolved oxygen (mg/L)	8.5	8.0	7.8
	*Salinity (ppt)	26.1 26.8	26.3	26.2
	*Temperature (°C)	24.1	24.5	24.6
2000 mg/L	pH (S.U.)	7.94	7.85	7.87
	Dissolved oxygen (mg/L)	8.5	7.9	7.8
	*Salinity (ppt)	26.4 26.8	26.6	26.5
	*Temperature (°C)	24.6	24.5	24.5

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306470

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 # 102

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-06-25	* 1100	JP	1343	JP	1F	Pink	07-07-25A
24	07-09-25			1330	JP			
48 Termination	07-10-25			1323	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):	06-28-25
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	06-27-25 1200 TO 06-28-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.59
	Temperature (°C) 24.5°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	10	9 ^{1d}	10	5 ^{5d}	4 ^{6d}	1 ^{9d}	1 ^{9d}	0 ^{10d}	1 ^{9d}
48 Termination	10	10	10	10	8 ^{1d}	7 ^{3d}	3 ^{2d}	0 ^{4d}	0 ^{1d}	1	0	6 ^{1d}
Mean Survival	100%		100%		75%		15%		5%		0%	

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

Statistics:

Method	Mbbit
Lower 95% confidence limit (mg KCl/L)	1281.7
Upper 95% confidence limit (mg KCl/L)	1426.1
48-hour LC ₅₀ (mg KCl/L)	1355.9

Comments:



Acute Silverside Test-24 Hr Survival

Start Date: 7/8/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 7/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina

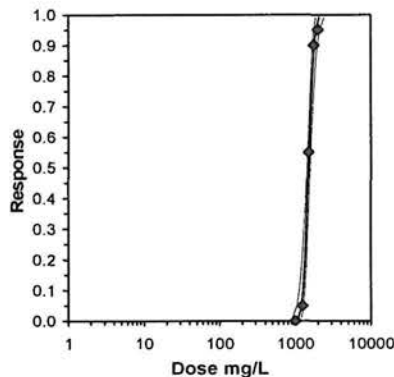
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	1.0000
1500	0.5000	0.4000
1750	0.1000	0.1000
2000	0.0000	0.1000

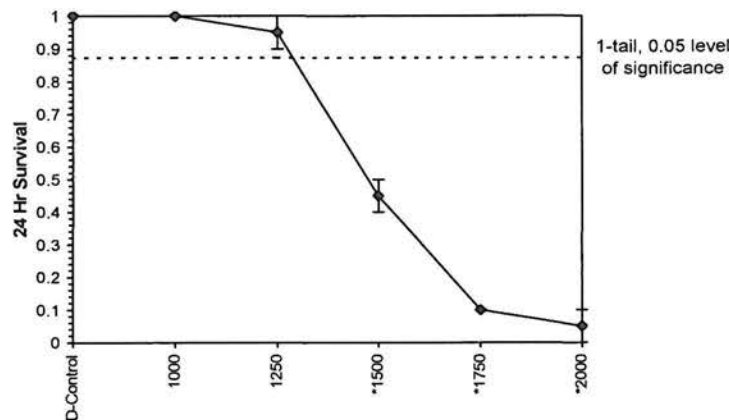
Transform: Arcsin Square Root								1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2055	0	20
1250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.122	2.830	0.2055	1	20
*1500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	9.324	2.830	0.2055	11	20
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	15.017	2.830	0.2055	18	20
*2000	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	16.139	2.830	0.2055	19	20

Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Normality of the data set cannot be confirmed											
Equality of variance cannot be confirmed											
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		1250	1500	1369.31		0.10191	0.10452	0.60242	0.00527	7.3E-06	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	17.0575	2.95442	11.2669	22.8482	0	2.01626	7.81472	0.56904	3.17752	0.05863	3
Intercept	-49.201	9.41217	-67.649	-30.753							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	1099.35	917.662	1208							
EC05	3.355	1205.28	1051.15	1298.36							
EC10	3.718	1265.87	1128.88	1350.7							
EC15	3.964	1308.45	1183.69	1388.2							
EC20	4.158	1343.32	1228.41	1419.6							
EC25	4.326	1373.97	1267.38	1447.94							
EC40	4.747	1454.34	1366.44	1527.14							
EC50	5.000	1504.94	1424.9	1582.19							
EC60	5.253	1557.3	1481.13	1644.46							
EC75	5.674	1648.4	1568.78	1765.49							
EC80	5.842	1686.01	1601.94	1819.41							
EC85	6.036	1730.93	1639.87	1886.19							
EC90	6.282	1789.16	1686.98	1975.91							
EC95	6.645	1879.1	1756.57	2120.11							
EC99	7.326	2060.16	1889.63	2426.39							



Dose-Response Plot



Acute Silverside Test-48 Hr Survival

Start Date: 7/8/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 7/10/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.8000	0.7000
1500	0.3000	0.0000
1750	0.0000	0.1000
2000	0.0000	0.0000

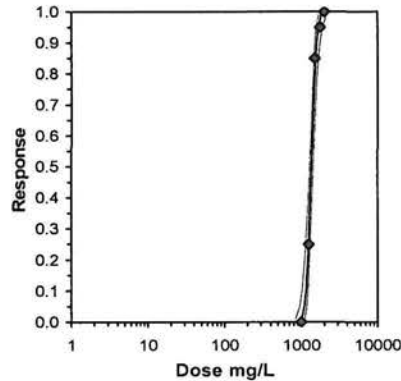
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.4200	0	20
1250	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	2.463	2.850	0.4200	5	20
*1500	0.1500	0.1500	0.3692	0.1588	0.5796	80.603	2	7.077	2.850	0.4200	17	20
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	7.952	2.850	0.4200	19	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

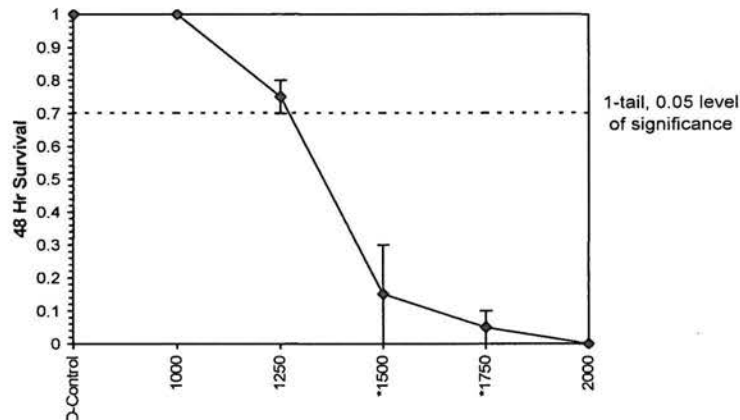
Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.4772	3.40221	11.8088	25.1455	0	1.47537	7.81472	0.68797	3.13223	0.05412	4
Intercept	-52.875	10.6871	-73.821	-31.928							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	1014.67	843.204	1112.96							
EC05	3.355	1104.6	959.684	1188.76							
EC10	3.718	1155.76	1027.14	1232.56							
EC15	3.964	1191.61	1074.55	1263.91							
EC20	4.158	1220.9	1113.13	1290.16							
EC25	4.326	1246.59	1146.69	1313.83							
EC40	4.747	1313.76	1231.7	1380.04							
EC50	5.000	1355.9	1281.68	1426.09							
EC60	5.253	1399.39	1329.55	1478.26							
EC75	5.674	1474.79	1403.55	1579.94							
EC80	5.842	1505.83	1431.28	1625.34							
EC85	6.036	1542.84	1462.81	1681.61							
EC90	6.282	1590.69	1501.72	1757.23							
EC95	6.645	1664.36	1558.76	1878.67							
EC99	7.326	1811.89	1666.72	2135.9							

The graph displays a sigmoidal dose-response curve. The x-axis represents the dose in mg/L on a logarithmic scale, ranging from 1 to 10,000. The y-axis represents the response, ranging from 0.0 to 1.0. The curve shows that the response remains near zero for doses up to approximately 100 mg/L, then rises sharply between 100 and 1,000 mg/L, and finally plateaus at a response of 1.0 for doses above 1,000 mg/L. Several data points are plotted along the curve, corresponding to the EC values listed in the table.

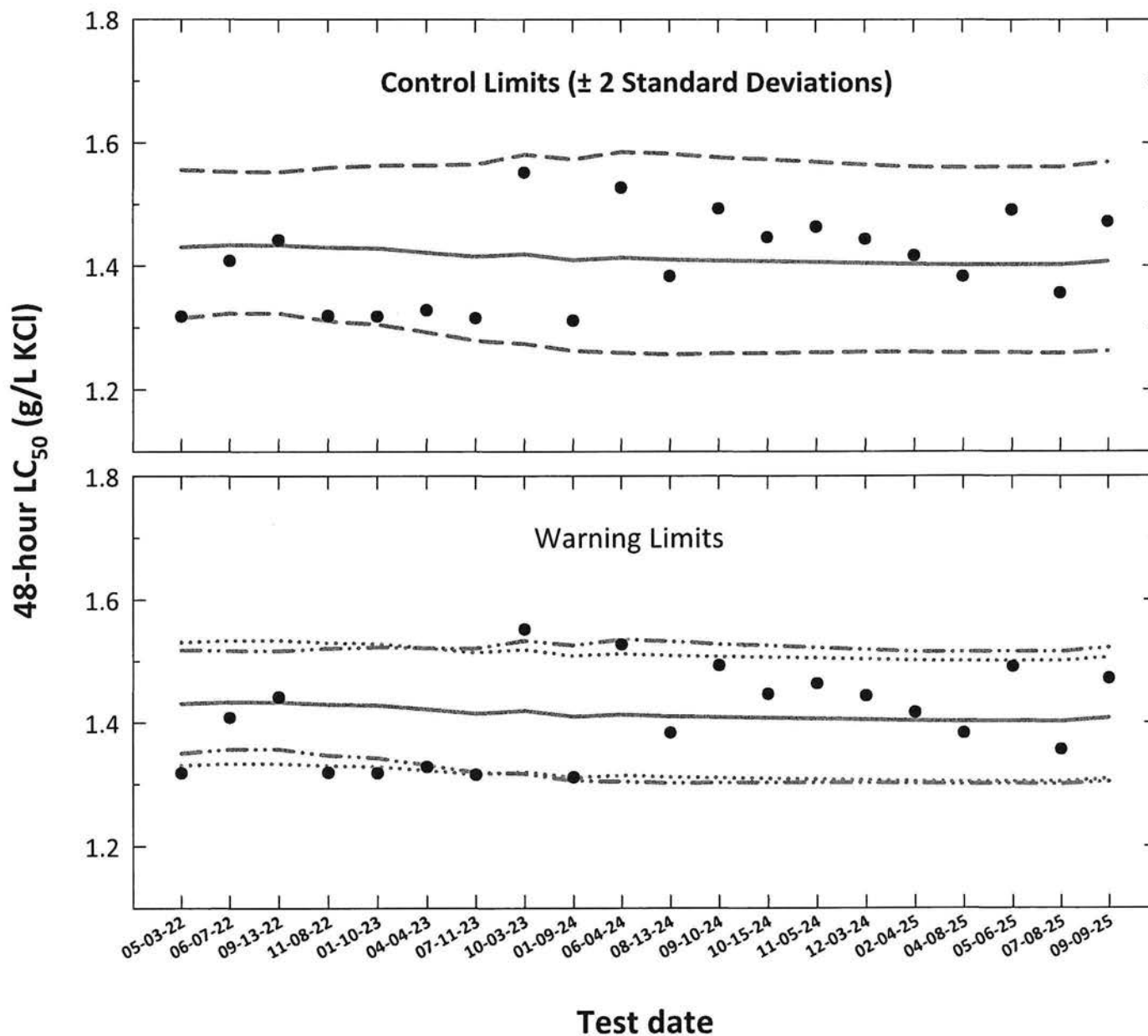
Dose (mg/L)	Response
100	0.00
1000	0.25
1500	0.85
2000	0.95
2500	0.98



Dose-Response Plot



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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCat Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
1	05-03-22	1.3180	0.1199	0.1555	0.0182	1.4306	1.3154	1.5559	1.3501	1.5182	1.3305	1.5308
2	06-07-22	1.4081	0.1486	0.1564	0.0174	1.4336	1.3233	1.5530	1.3566	1.5169	1.3332	1.5339
3	09-13-22	1.4415	0.1588	0.1563	0.0174	1.4331	1.3231	1.5524	1.3563	1.5163	1.3328	1.5335
4	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205	1.3296	1.5298
5	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227	1.3284	1.5283
6	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213	1.3221	1.5211
7	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209	1.3158	1.5139
8	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331	1.3197	1.5184
9	01-09-24	1.3113	0.1177	0.1490	0.0239	1.4094	1.2627	1.5732	1.3053	1.5256	1.3107	1.5081
10	06-04-24	1.5271	0.1839	0.1502	0.0250	1.4131	1.2594	1.5854	1.3043	1.5351	1.3142	1.5120
11	08-13-24	1.3832	0.1409	0.1493	0.0250	1.4102	1.2569	1.5823	1.3015	1.5322	1.3115	1.5089
12	09-10-24	1.4930	0.1740	0.1488	0.0244	1.4087	1.2589	1.5763	1.3024	1.5277	1.3101	1.5073
13	10-15-24	1.4461	0.1602	0.1484	0.0242	1.4075	1.2593	1.5731	1.3022	1.5251	1.3090	1.5060
14	11-05-24	1.4632	0.1653	0.1480	0.0238	1.4062	1.2603	1.5689	1.3024	1.5219	1.3077	1.5046
15	12-03-24	1.4436	0.1594	0.1476	0.0235	1.4047	1.2609	1.5649	1.3023	1.5188	1.3064	1.5030
16	02-04-25	1.4168	0.1513	0.1470	0.0232	1.4029	1.2607	1.5611	1.3015	1.5156	1.3047	1.5011
17	04-08-25	1.3832	0.1409	0.1468	0.0232	1.4021	1.2597	1.5605	1.3006	1.5150	1.3039	1.5002
18	05-06-25	1.4906	0.1734	0.1468	0.0233	1.4022	1.2596	1.5610	1.3005	1.5154	1.3040	1.5004
19	07-08-25	1.3559	0.1322	0.1467	0.0234	1.4017	1.2587	1.5611	1.2997	1.5154	1.3036	1.4999
20	09-09-25	1.4716	0.1678	0.1484	0.0236	1.4073	1.2623	1.5690	1.3042	1.5222	1.3088	1.5058

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 103

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 #:

2412

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, SaltSW	pH (S.U.)	8.03	7.84	7.90
	Dissolved oxygen (mg/L)	8.2	8.1	7.9
	*Salinity (ppt)	24.9	25.0	26.2
	*Alkalinity (mg/L CaCO ₃)	110		
	*Temperature (°C)	25.2	25.0	24.9
1000 mg/L	pH (S.U.)	8.00	7.84	7.84
	Dissolved oxygen (mg/L)	8.3	8.0	7.9
	*Salinity (ppt)	25.4	25.7	26.4
	*Temperature (°C)	25.0	24.7	25.1
1250 mg/L	pH (S.U.)	8.00	7.85	7.84
	Dissolved oxygen (mg/L)	8.3	8.0	7.9
	*Salinity (ppt)	25.6	26.0	26.6
	*Temperature (°C)	25.3	24.9	24.6
1500 mg/L	pH (S.U.)	8.01	7.84	7.88
	Dissolved oxygen (mg/L)	8.3	7.9	7.9
	*Salinity (ppt)	25.7	26.2	27.4
	*Temperature (°C)	25.2	24.9	24.6
1750 mg/L	pH (S.U.)	8.01	7.83	7.88
	Dissolved oxygen (mg/L)	8.3	7.8	7.9
	*Salinity (ppt)	26.0	26.5	26.9
	*Temperature (°C)	25.4	25.0	25.1
2000 mg/L	pH (S.U.)	8.00	7.78	
	Dissolved oxygen (mg/L)	8.3	7.6	
	*Salinity (ppt)	26.2	26.6	
	*Temperature (°C)	25.4	24.7	

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

Chemical analyses:

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

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 # 103

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-09-25	* 1045	M	1342	JP	1E	PINK	09-04-25B
24	09-10-25			1344	JP			
48 Termination	09-11-25			1330	JP			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Mb):	08-31-25
Age (9 to 14 days old):	9-10 DAYS
Date organisms were born:	08-30-25 1200 TO 08-31-25 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.76
	Temperature (°C): 25.0 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	10	10	10	6 ^d	4 ^d	3 ^d	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{1d}	8 ^{2d}	5 ^{1d}	4	3	0	0	0
Mean Survival	100 %		100 %		85 %		45 %		15 %		0 %	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	1389.6
Upper 95% confidence limit (mg KCl/L)	1550.5
48-hour LC ₅₀ (mg KCl/L)	1471.6

Comments:



Acute Silverside Test-24 Hr Survival

Start Date: 9/9/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 9/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
Comments:

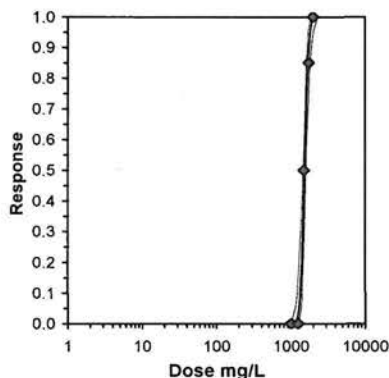
Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	1.0000
1500	0.6000	0.4000
1750	0.3000	0.0000
2000	0.0000	0.0000

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
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.4205	0	20
1250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.4205	0	20
*1500	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	4.247	2.850	0.4205	10	20
*1750	0.1500	0.1500	0.3692	0.1588	0.5796	80.603	2	7.068	2.850	0.4205	17	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

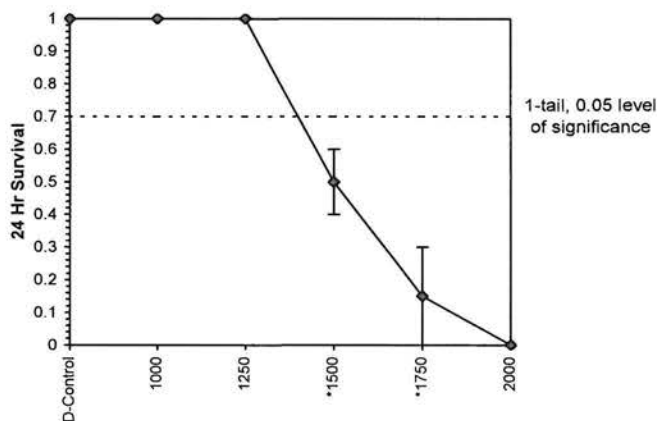
Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.27465	0.28169	0.46135	0.02177	0.00246	4, 5
Treatments vs D-Control										

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	22.3012	4.22887	14.0127 30.5898	0	1.72458	7.81472	0.63148	3.18582	0.04484	5
Intercept	-66.048	13.4978	-92.503 -39.592							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1206.44	1029.27 1303.9
EC05	3.355	1294.39	1147.69 1376.78
EC10	3.718	1343.87	1215.14 1418.62
EC15	3.964	1378.31	1262.07 1448.48
EC20	4.158	1406.32	1299.97 1473.45
EC25	4.326	1430.79	1332.72 1495.95
EC40	4.747	1494.38	1414.72 1558.87
EC50	5.000	1533.99	1462.24 1602.6
EC60	5.253	1574.64	1507.27 1652.01
EC75	5.674	1644.62	1576.1 1747.6
EC80	5.842	1673.25	1601.7 1789.93
EC85	6.036	1707.24	1630.7 1842.11
EC90	6.282	1751	1666.33 1911.76
EC95	6.645	1817.93	1718.27 2022.58
EC99	7.326	1950.46	1815.65 2253.6



Dose-Response Plot



Acute Silverside Test-48 Hr Survival

Start Date: 9/9/2025 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
End Date: 9/11/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	0.8000
1500	0.5000	0.4000
1750	0.3000	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	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.4104	0	20
1250	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	1.624	2.850	0.4104	3	20
*1500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	4.701	2.850	0.4104	11	20
*1750	0.1500	0.1500	0.3692	0.1588	0.5796	80.603	2	7.241	2.850	0.4104	17	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

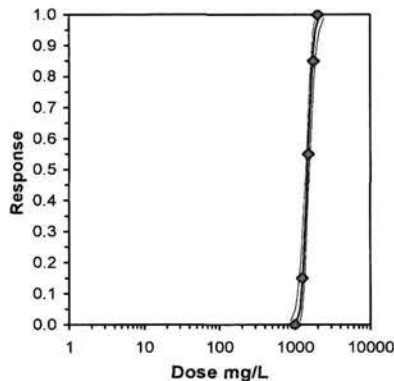
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

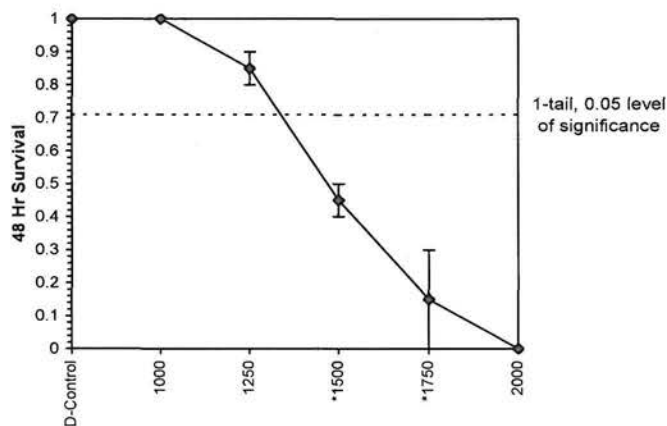
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	1250	1500	1369.31		0.26549	0.2723	0.41853	0.02074	0.00276	4, 5
Treatments vs D-Control										

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.9054	2.70053	10.6124	21.1985	0	0.72601	7.81472	0.86707	3.16779	0.06287	4
Intercept	-45.385	8.5806	-62.203	-28.567							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1050.82	870.068 1161.08
EC05	3.355	1159.78	1005.12 1254.77
EC10	3.718	1222.41	1084.29 1309.21
EC15	3.964	1266.57	1140.36 1348.26
EC20	4.158	1302.8	1186.24 1380.98
EC25	4.326	1334.7	1226.35 1410.51
EC40	4.747	1418.61	1328.8 1493.09
EC50	5.000	1471.6	1389.64 1550.48
EC60	5.253	1526.58	1448.44 1615.44
EC75	5.674	1622.55	1540.6 1742.01
EC80	5.842	1662.28	1575.61 1798.57
EC85	6.036	1709.83	1615.74 1868.75
EC90	6.282	1771.59	1665.7 1963.3
EC95	6.645	1867.26	1739.73 2115.79
EC99	7.326	2060.88	1881.98 2441.77



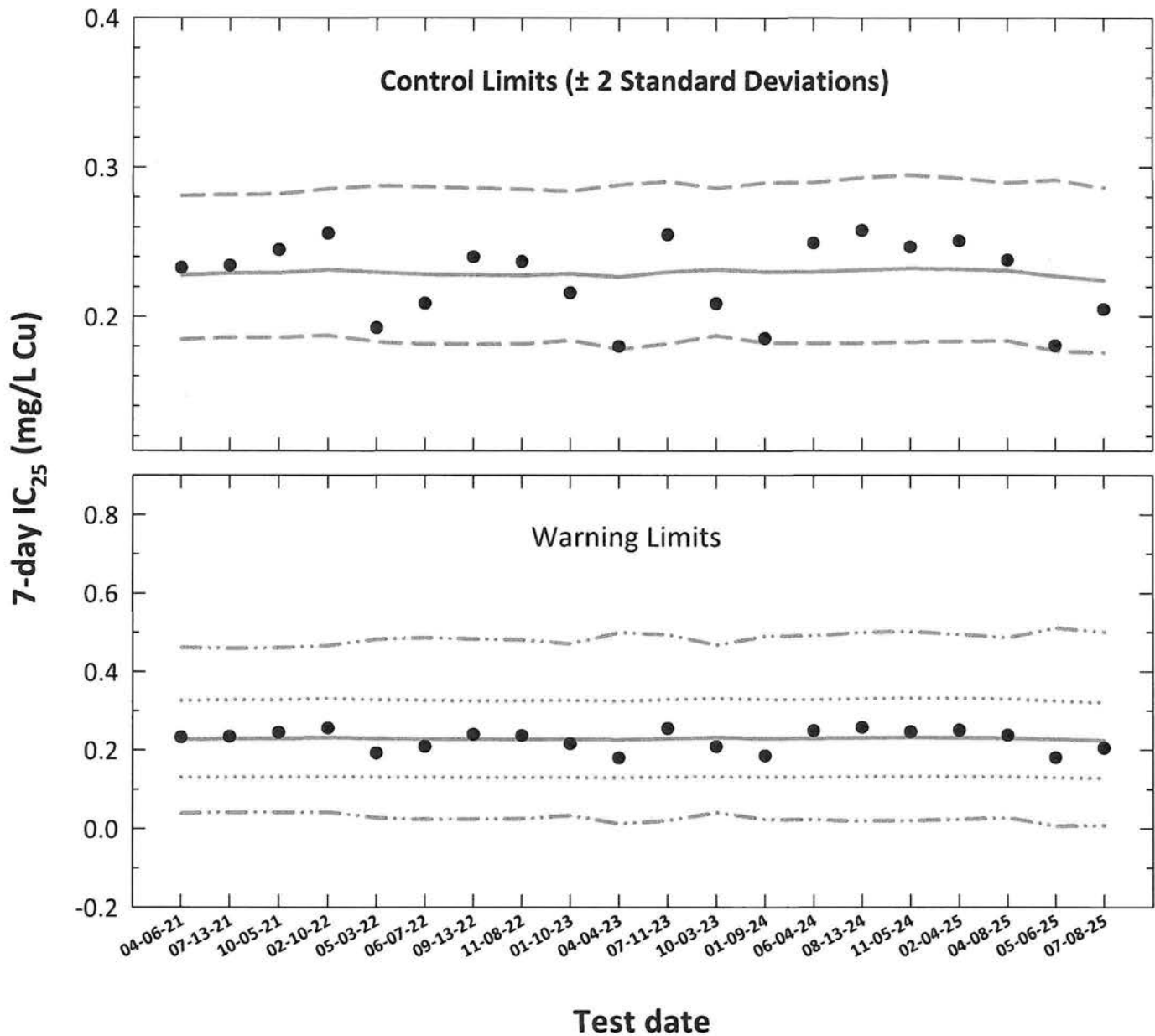
Dose-Response Plot



Menidia beryllina

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion		Anti-logarithmic Values (mg/L Cu)							
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
1	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.2278	0.1847	0.2810	0.0385	0.4612	0.1298	0.3258
2	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.2290	0.1862	0.2817	0.0420	0.4590	0.1305	0.3274
3	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.2292	0.1861	0.2822	0.0414	0.4604	0.1306	0.3277
4	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.2313	0.1874	0.2855	0.0414	0.4656	0.1318	0.3307
5	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.2296	0.1832	0.2877	0.0276	0.4827	0.1309	0.3283
6	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.2283	0.1815	0.2872	0.0233	0.4861	0.1301	0.3265
7	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.2279	0.1816	0.2861	0.0246	0.4831	0.1299	0.3259
8	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.2276	0.1816	0.2853	0.0254	0.4811	0.1297	0.3255
9	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.2287	0.1841	0.2841	0.0337	0.4708	0.1304	0.3270
10	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.2266	0.1781	0.2884	0.0125	0.4991	0.1292	0.3241
11	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.2298	0.1818	0.2905	0.0208	0.4939	0.1310	0.3286
12	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.2314	0.1873	0.2859	0.0408	0.4669	0.1319	0.3309
13	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.2298	0.1824	0.2896	0.0233	0.4900	0.1310	0.3286
14	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.2300	0.1823	0.2901	0.0227	0.4914	0.1311	0.3288
15	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.2312	0.1823	0.2932	0.0196	0.4996	0.1318	0.3306
16	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.2324	0.1831	0.2950	0.0201	0.5019	0.1325	0.3323
17	02-04-25	0.2507	-0.6008	-0.6349	0.0507	0.2318	0.1835	0.2928	0.0234	0.4950	0.1321	0.3314
18	04-08-25	0.2377	-0.6240	-0.6369	0.0494	0.2307	0.1838	0.2896	0.0273	0.4861	0.1315	0.3299
19	05-06-25	0.1805	-0.7435	-0.6438	0.0542	0.2271	0.1769	0.2915	0.0062	0.5105	0.1294	0.3247
20	07-08-25	0.2047	-0.6889	-0.6492	0.0530	0.2243	0.1757	0.2863	0.0077	0.5007	0.1278	0.3207

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

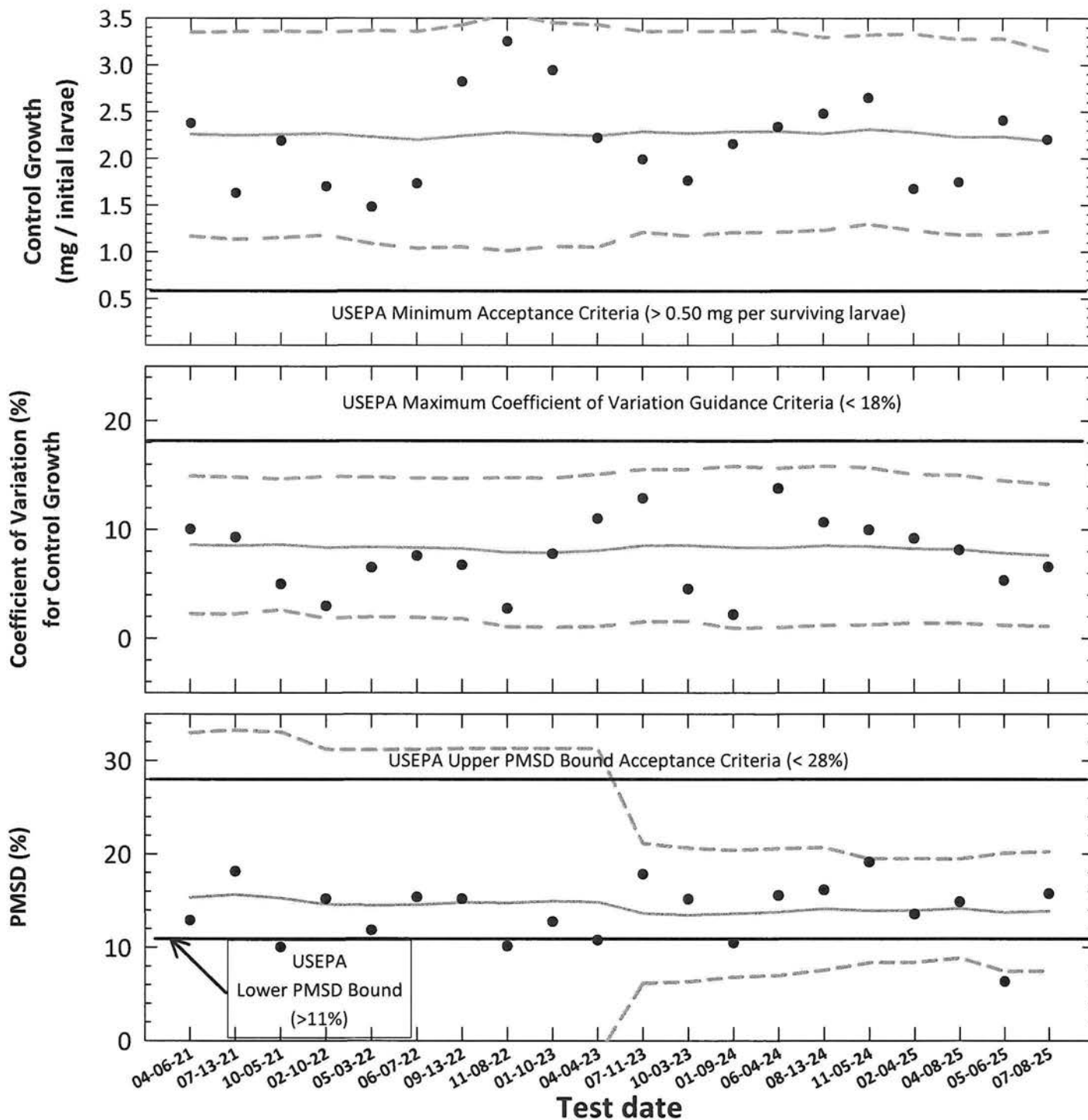
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Menidia beryllina

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



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

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		CT	(mg/initial larvae)		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S			
1	04-06-21	100	2.377	10.0	0.3062	12.9	2.2579	1.167	3.349	8.6	2.2	14.9	15.3	-2.3	33.0
2	07-13-21	100	1.631	9.3	0.2956	18.1	2.247	1.136	3.359	8.5	2.2	14.8	15.7	-2.0	33.3
3	10-05-21	100	2.189	5.0	0.2192	10.0	2.258	1.153	3.363	8.6	2.6	14.7	15.3	-2.5	33.1
4	02-10-22	100	1.701	3.0	0.2583	15.2	2.266	1.180	3.351	8.4	1.8	14.9	14.6	-2.0	31.2
5	05-03-22	100	1.483	6.5	0.1756	11.8	2.232	1.092	3.372	8.4	2.0	14.8	14.5	-2.1	31.2
6	06-07-22	100	1.733	7.6	0.2665	15.4	2.201	1.041	3.361	8.3	1.9	14.8	14.6	-2.1	31.2
7	09-13-22	100	2.822	6.8	0.4290	15.2	2.243	1.057	3.430	8.3	1.8	14.7	14.8	-1.7	31.3
8	11-08-22	100	3.255	2.7	0.3294	10.1	2.280	1.014	3.545	7.9	1.0	14.8	14.8	-1.8	31.3
9	01-10-23	100	2.945	7.8	0.3755	12.8	2.257	1.062	3.453	7.9	1.0	14.8	15.0	-1.4	31.3
10	04-04-23	100	2.220	11.0	0.2389	10.8	2.243	1.053	3.433	8.1	1.1	15.1	14.9	-1.6	31.3
11	07-11-23	100	1.992	12.9	0.3549	17.8	2.287	1.213	3.360	8.5	1.5	15.6	13.6	6.2	21.1
12	10-03-23	100	1.763	4.5	0.2673	15.2	2.270	1.174	3.366	8.5	1.5	15.5	13.5	6.3	20.6
13	01-09-24	100	2.156	2.2	0.2257	10.5	2.287	1.211	3.364	8.4	0.9	15.8	13.6	6.8	20.4
14	06-04-24	100	2.339	13.8	0.3637	15.5	2.291	1.215	3.368	8.3	1.0	15.6	13.8	7.0	20.6
15	08-13-24	100	2.479	10.7	0.4001	16.1	2.266	1.235	3.297	8.5	1.2	15.9	14.1	7.6	20.7
16	11-05-24	100	2.648	10.0	0.5061	19.1	2.312	1.299	3.324	8.5	1.2	15.7	13.9	8.3	19.5
17	02-04-25	100	1.675	9.2	0.2268	13.5	2.283	1.232	3.335	8.2	1.4	15.1	13.9	8.4	19.5
18	04-08-25	100	1.747	8.1	0.2598	14.9	2.230	1.183	3.278	8.2	1.4	15.0	14.2	8.9	19.5
19	05-06-25	100	2.406	5.3	0.1521	6.3	2.233	1.184	3.281	7.9	1.2	14.5	13.7	7.4	20.1
20	07-08-25	100	2.201	6.6	0.3464	15.7	2.188	1.221	3.155	7.6	1.1	14.2	13.8	7.4	20.3

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

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

CV = Coefficient of variation for control growth.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) $< 28\%$.

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

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

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

Species: *Menidia beryllina*

MbCuCR Test Number: **152**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2406				
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:	ORANGE
Age:	10-days old	Incubator number and shelf location:	6B
Batch:	AI Mb 06-28-25	Artemia CHM number:	CHM1385
Hatch dates and times:	06-27-25 1200 to 06-28-25 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = 7.59 Temperature (°C) = 24.6	Date / Time in oven:	07-05-25 1200
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	60°C
		Date / Time out of oven:	07-16-25 1200
		*Final oven temperature:	60°C
		Total drying time:	24-Hours

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	07-08-25	1100	H	1440	H	1215	H	NA	07-08-25 A
1	07-09-25	0500	H	1215	H	0835	H		07-09-25 B
2	07-10-25	0500	H	1215	H	0500	H		07-09-25
3	07-11-25	0500	H	1100	H	0752	H		07-10-25 A
4	07-12-25	0600	H	1200	H	0940	H		07-11-25
5	07-13-25	0600	H	1200	H	0850	H		07-12-25 A
6	07-14-25	0500	H	1100	H	0815	H		07-12-25 B
7	07-15-25					1015	H		

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (%)	0.272
Average weight per initial larvae:	2.201		NOEC (%)	0.1
Average weight per surviving larvae:	2.201	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	0.141
			IC ₂₅ (%)	0.205

Species: Menidia beryllina

MbCuCR Test Number: 152

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>Ruby</u> Analyst: <u>XC</u> Date: <u>07-17-25</u>		10.40	10.50	10.37	10.77	12.38	10.65	10.90	10.21	13.09	10.30	12.04	10.25
*B = Pan + Larvae weight (mg) Analyst: <u>XC</u> Date: <u>07-18-25</u>		31.45	31.40	34.41	32.93	35.98	31.97	30.40	30.08	32.48	34.18	31.15	30.33
C = Larvae weight (mg) = B - A Analyst: <u>JA</u>		21.05	20.90	24.04	22.06	23.60	21.32	19.50	19.87	19.39	25.88	19.11	20.08
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JA</u>		2.105	2.090	2.404	2.206	2.360	2.132	1.950	1.987	1.939	2.588	1.911	2.008
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	2.201				2.107		4.37.		2.112		4.17.	

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

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

Comments:

Species: Menidia beryllina

MbCuCR Test Number: 152

Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	8 rd	8 rd	7 ^{sd}	6 ^{td}
2	10	9 ^{td}	10	10	10	10	9 ^{td}	10	7 ^{td}	7 ^{td}	7	4 ^{td}
3	10	9	10	10	10	10	9	10	6 ^{td}	5 ^{sd}	7	4
4	10	9	10	10	10	10	9	10	4 ^{td}	5	5 ^{sd}	4
5	10	9	10	10	10	10	9	10	4	5	5	4
6	10	9	10	10	8 rd	9 ^{td}	9	10	2 rd	4 ^{td}	4 ^{td}	3 ^{td}
7	10	9	10	10	8	8 ^{td}	8 ^{td}	9 ^{td}	1 ^{td}	0 ^{td}	0 ^{td}	1
*A = Pan weight (mg) Tray color code: <u>Ruby</u> Analyst: <u>XL</u> Date: <u>06-17-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>XL</u> Date: <u>07-18-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JA</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JA</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
2.096 4.87 1.676 23.97 0.044 98.07												

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

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

Comments:

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

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

Test number: 152

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	Not for Compliance Assessment, Internal Laboratory QC				Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
				A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)					
Control	A	10	10	10.40	31.45	21.05	2.105					
	B	10	10	10.50	31.40	20.90	2.090					
	C	10	10	10.37	34.41	24.04	2.404		100.0	2.201	6.6	Not applicable
	D	10	10	10.77	32.83	22.06	2.206					
0.025	E	10	10	12.38	35.98	23.60	2.360					
	F	10	10	10.65	31.97	21.32	2.132					
	G	10	10	10.90	30.40	19.50	1.950		100.0	2.107	8.8	4.3
	H	10	10	10.21	30.08	19.87	1.987					
0.050	I	10	10	13.09	32.48	19.39	1.939					
	J	10	10	10.30	36.18	25.88	2.588					
	K	10	10	12.04	31.15	19.11	1.911		100.0	2.112	15.2	4.1
	L	10	10	10.25	30.33	20.08	2.008					
0.100	M	10	10	10.35	32.75	22.40	2.240					
	N	10	9	10.31	32.38	22.07	2.452					
	O	10	10	10.39	29.40	19.01	1.901			2.157	11.2	4.8
	P	10	10	10.31	30.65	20.34	2.034					
0.200	Q	10	8	10.31	23.05	12.74	1.593					
	R	10	8	10.78	31.48	20.70	2.588					
	S	10	8	14.29	26.44	12.15	1.519		82.5	2.021	26.9	23.9
	T	10	9	15.68	37.14	21.46	2.384					
0.500	U	10	1	11.75	12.30	0.55	0.550					
	V	10	0	0.00	0.00	0.00	0.000			0.880	53.0	98.0
	W	10	0	0.00	0.00	0.00	0.000					
	X	10	1	13.63	14.84	1.21	1.210					

Dunnett's MSD value: $\frac{0.3464}{15.7}$ MSD = Minimum Significant Difference
 PMSD: 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.

Entered and
Reviewed by
Initials
8

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 7/8/2025 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
End Date: 7/15/2025 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUSO4
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	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	0.9000	1.0000	1.0000
0.2	0.8000	0.8000	0.8000	0.9000
0.5	0.1000	0.0000	0.0000	0.1000

Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	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	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*0.2	0.8250	0.8250	1.1426	1.1071	1.2490	6.209	4	10.00	10.00	7	40
*0.5	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

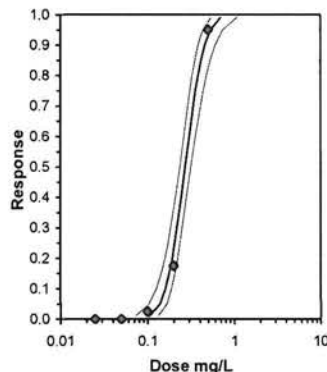
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9082	0.884	-0.1889	0.82506

Equality of variance cannot be confirmed

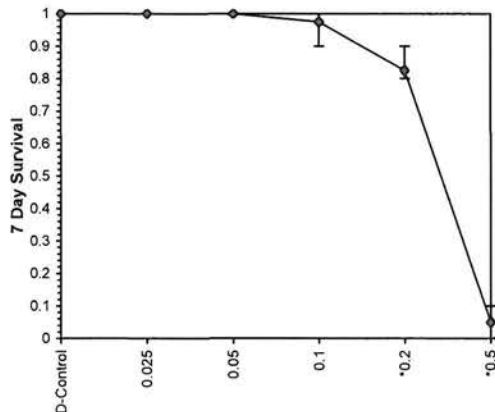
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.63449	0.80741	4.05197 7.21701	0	2.5947	7.81472	0.45842	-0.5654	0.17748	3
Intercept	8.18577	0.50703	7.19199 9.17956							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	0.10513	0.0716 0.13248
EC05	3.355	0.13889	0.10408 0.16684
EC10	3.718	0.16112	0.12652 0.18946
EC15	3.964	0.17809	0.14393 0.20701
EC20	4.158	0.19285	0.1591 0.22261
EC25	4.326	0.20648	0.17303 0.2374
EC40	4.747	0.24526	0.21149 0.28223
EC50	5.000	0.27202	0.23655 0.31594
EC60	5.253	0.30169	0.26281 0.35605
EC75	5.674	0.35835	0.30922 0.43971
EC80	5.842	0.38368	0.32878 0.47966
EC85	6.036	0.41547	0.35257 0.53171
EC90	6.282	0.45924	0.38419 0.6065
EC95	6.645	0.53275	0.43511 0.73923
EC99	7.326	0.70383	0.54645 1.07756



Dose-Response Plot



Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	7/8/2025	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	7/15/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

Conc.-mg/L	1	2	3	4
D-Control	2.1050	2.0900	2.4040	2.2060
0.025	2.3600	2.1320	1.9500	1.9870
0.05	1.9390	2.5880	1.9110	2.0080
0.1	2.2400	2.2070	1.9010	2.0340
0.2	1.2740	2.0700	1.2150	2.1460
0.5	0.0550	0.0000	0.0000	0.1210

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean
D-Control	2.2013	1.0000	2.2013	2.0900	2.4040	6.571	4				2.2013	1.0000
0.025	2.1073	0.9573	2.1073	1.9500	2.3600	8.822	4	0.621	2.290	0.3464	2.1094	0.9583
0.05	2.1115	0.9592	2.1115	1.9110	2.5880	15.168	4	0.593	2.290	0.3464	2.1094	0.9583
0.1	2.0955	0.9520	2.0955	1.9010	2.2400	7.542	4	0.699	2.290	0.3464	2.0955	0.9520
0.2	1.6763	0.7615	1.6763	1.2150	2.1460	29.834	4				1.6763	0.7615
0.5	0.0440	0.0200	0.0440	0.0000	0.1210	130.703	4				0.0440	0.0200

Auxiliary Tests

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

Statistic

0.88435

Critical

0.844

Skew

1.16726

Kurt

.97797

Bartlett's Test indicates equal variances ($p = 0.52$)

2.26915

11.3449

1000000

1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 2679, 2680, 26

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
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MSDu	MSD
------	-----

MSB

F-Prob

df

Dunnnett's Test

ROLES	ROLES
0.1	>0.1

MOBA	MOB
0.34638	0.157

0.0095

0.88924

3.12

Treatments vs D-Control

0.1 0.1

0.34030 0.157

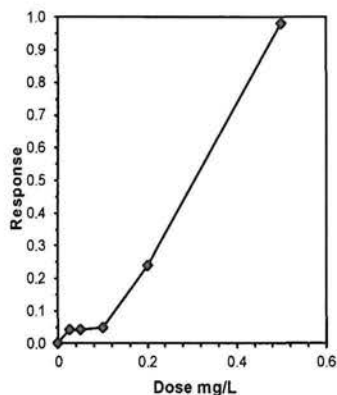
0.0055

0.00324

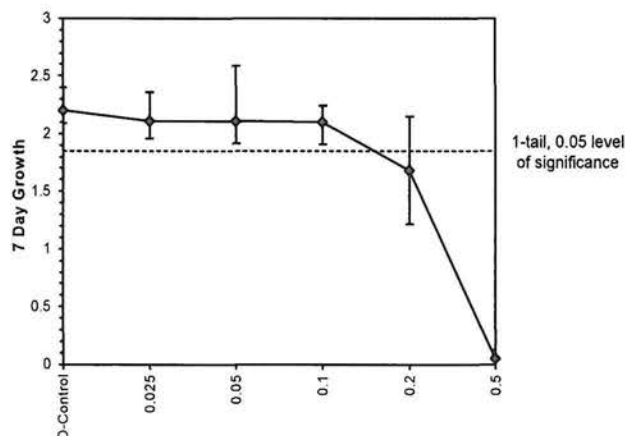
3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1010	0.0437	0.0000	0.2188	0.6570
IC10	0.1273	0.0394	0.0000	0.2651	0.1130
IC15	0.1535	0.0357	0.0908	0.2752	0.4942
IC20	0.1798	0.0342	0.1083	0.2854	0.1743
IC25	0.2047	0.0334	0.1185	0.2966	-0.0407
IC40	0.2653	0.0314	0.1489	0.3391	-0.4653
IC50	0.3058	0.0268	0.1985	0.3675	-0.6158



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 152

Daily Chemistry:

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

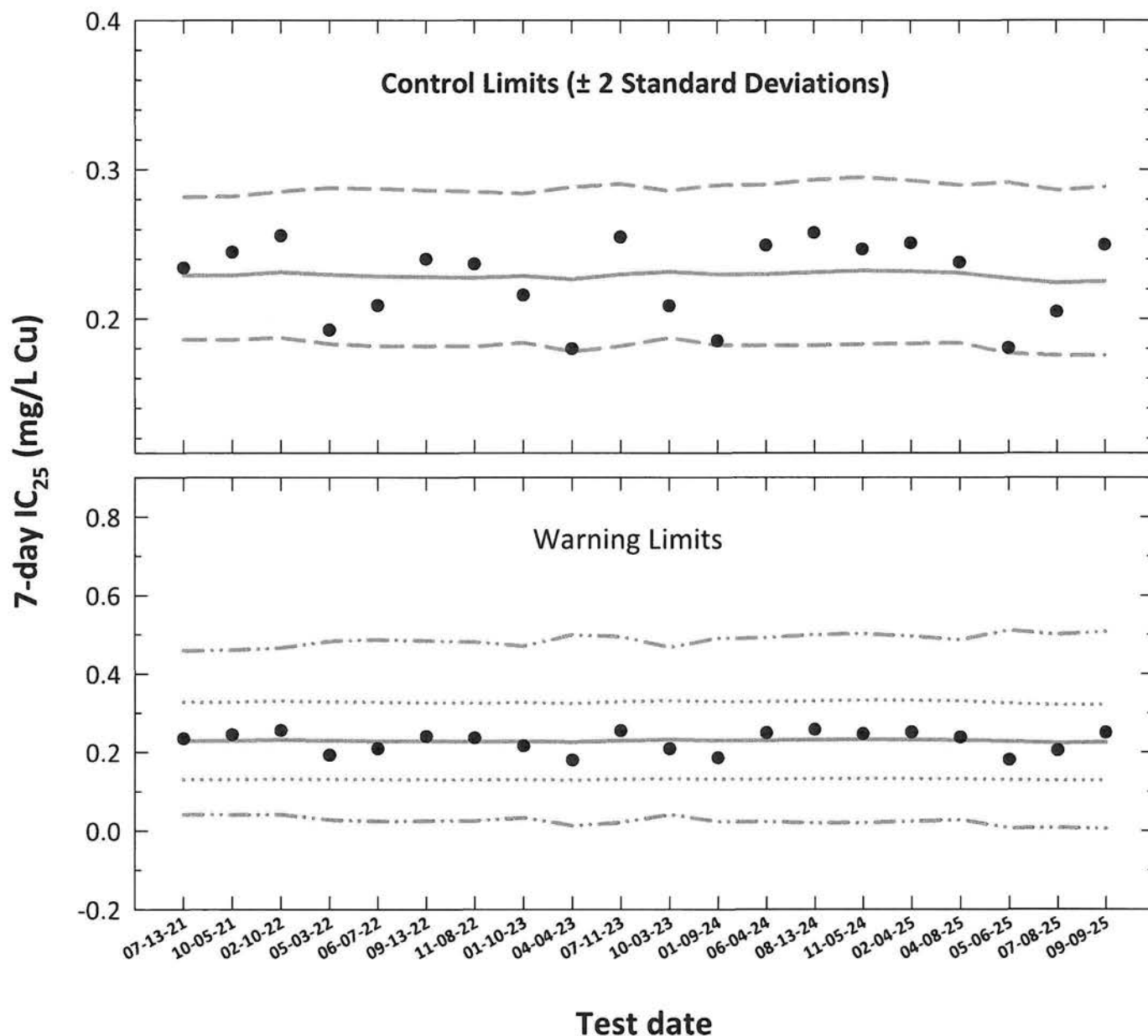
Analyst		Day (Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
		XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	7.97	7.79	7.96	7.85	7.92	7.67
	Dissolved oxygen (mg/L)	8.4	7.8	8.2	7.9	8.1	7.2
	Salinity (ppt)	25.0	25.7	25.0	25.0	25.0	25.3
	Alkalinity (mg CaCO ₃ /L)	100		100		82	
	Temperature (°C)	24.8	24.2	24.5	24.7	24.6	24.8
0.025 mg/L	pH (S.U.)	7.94	7.82	7.89	7.79	7.91	7.62
	Dissolved oxygen (mg/L)	8.4	7.9	8.2	7.7	8.1	7.0
	Salinity (ppt)	25.3	25.8	25.5	25.4	25.1	25.3
	Temperature (°C)	24.7	24.6	24.5	24.8	24.5	24.7
0.05 mg/L	pH (S.U.)	7.94	7.82	7.89	7.79	7.92	7.62
	Dissolved oxygen (mg/L)	8.4	7.9	8.2	7.7	8.1	6.9
	Salinity (ppt)	25.3	25.8	25.6	25.4	25.1	25.3
	Temperature (°C)	24.7	24.7	24.5	24.7	24.5	24.6
0.1 mg/L	pH (S.U.)	7.94	7.83	7.89	7.79	7.92	7.65
	Dissolved oxygen (mg/L)	8.4	7.9	8.1	7.6	8.1	6.9
	Salinity (ppt)	25.3	25.9	25.5	25.6	25.0	25.4
	Temperature (°C)	24.7	24.5	24.5	24.7	24.5	24.5
0.2 mg/L	pH (S.U.)	7.94	7.84	7.90	7.80	7.93	7.66
	Dissolved oxygen (mg/L)	8.4	7.9	8.1	7.7	8.1	7.0
	Salinity (ppt)	25.2	25.7	25.5	25.4	25.0	25.4
	Temperature (°C)	24.7	24.5	24.6	24.9	25.0	24.8
0.5 mg/L	pH (S.U.)	7.94	7.83	7.90	7.80	7.93	7.67
	Dissolved oxygen (mg/L)	8.4	7.9	8.1	7.6	8.1	7.0
	Salinity (ppt)	25.3	25.7	25.3	25.4	24.8	25.3
	Alkalinity (mg CaCO ₃ /L)						
	Temperature (°C)	24.7	24.6	24.6	24.6	25.0	24.7
		Initial	Final	Initial	Final	Initial	Final

Species: Menidia beryllina

MbCuCR Test Number: 152

Analyst		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
		KL	BSL	BSL	BSL	BSL	XL	XL	KL
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	8.00	7.80	8.02	7.87	8.28	7.84	7.90	7.69
	Dissolved oxygen (mg/L)	0.2	8.0	8.2	7.9	8.4	7.9	8.1	6.3
	Salinity (ppt)	25.0	24.9	25.0	25.0	24.9	24.9	25.1	25.1
	Alkalinity (mg CaCO ₃ /L)	76		95		95		100	
	Temperature (°C)	24.8	24.6	24.7	24.7	24.7	24.4	24.6	24.3
0.025 mg/L	pH (S.U.)	7.07	7.78	8.02	7.85	8.07	7.84	7.94	7.70
	Dissolved oxygen (mg/L)	0.3	7.8	8.2	7.9	8.4	7.9	8.1	6.2
	Salinity (ppt)	25.1	25.1	25.1	25.0	25.2	25.2	25.0	25.2
	Temperature (°C)	24.6	24.5	24.8	24.6	24.7	24.6	24.7	24.5
0.05 mg/L	pH (S.U.)	7.05	7.76	8.03	7.85	8.07	7.84	7.94	7.70
	Dissolved oxygen (mg/L)	0.3	7.8	8.2	7.7	8.4	7.9	8.1	5.7
	Salinity (ppt)	25.0	25.1	25.0	25.1	25.2	25.3	25.0	25.2
	Temperature (°C)	24.6	24.9	24.8	24.6	24.7	24.6	24.7	24.5
0.1 mg/L	pH (S.U.)	7.05	7.79	8.04	7.86	8.08	7.85	7.94	7.70
	Dissolved oxygen (mg/L)	0.3	7.8	8.2	7.7	8.4	7.8	8.1	5.8
	Salinity (ppt)	24.9	25.1	24.9	25.2	25.2	25.3	25.0	25.2
	Temperature (°C)	24.6	24.9	24.9	24.5	24.7	24.3	24.7	24.6
0.2 mg/L	pH (S.U.)	7.06	7.83	8.04	7.85	8.08	7.84	7.94	7.72
	Dissolved oxygen (mg/L)	0.3	7.9	8.2	7.7	8.4	7.8	8.1	5.7
	Salinity (ppt)	25.0	25.3	24.9	25.0	25.0	25.3	25.0	25.1
	Temperature (°C)	24.5	24.7	24.9	24.9	24.8	24.7	24.6	24.6
0.5 mg/L	pH (S.U.)	7.05	7.77	8.04	7.86	8.08	7.85	7.94	7.00
	Dissolved oxygen (mg/L)	0.3	7.6	8.3	7.7	8.4	7.8	8.1	5.0
	Salinity (ppt)	24.9	25.1	24.9	25.0	25.0	25.2	24.0	25.0
	Temperature (°C)	24.8	24.7	24.7	24.6	24.8	24.6	24.6	24.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion			Anti-logarithmic Values (mg/L Cu)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.2290	0.1862	0.2817	0.0420	0.4590	0.1305	0.3274
2	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.2292	0.1861	0.2822	0.0414	0.4604	0.1306	0.3277
3	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.2313	0.1874	0.2855	0.0414	0.4656	0.1318	0.3307
4	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.2296	0.1832	0.2877	0.0276	0.4827	0.1309	0.3283
5	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.2283	0.1815	0.2872	0.0233	0.4861	0.1301	0.3265
6	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.2279	0.1816	0.2861	0.0246	0.4831	0.1299	0.3259
7	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.2276	0.1816	0.2853	0.0254	0.4811	0.1297	0.3255
8	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.2287	0.1841	0.2841	0.0337	0.4708	0.1304	0.3270
9	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.2266	0.1781	0.2884	0.0125	0.4991	0.1292	0.3241
10	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.2298	0.1818	0.2905	0.0208	0.4939	0.1310	0.3286
11	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.2314	0.1873	0.2859	0.0408	0.4669	0.1319	0.3309
12	01-09-24	0.1852	-0.7324	-0.6387	0.0502	0.2298	0.1824	0.2896	0.0233	0.4900	0.1310	0.3286
13	06-04-24	0.2493	-0.6033	-0.6383	0.0504	0.2300	0.1823	0.2901	0.0227	0.4914	0.1311	0.3288
14	08-13-24	0.2577	-0.5889	-0.6360	0.0516	0.2312	0.1823	0.2932	0.0196	0.4996	0.1318	0.3306
15	11-05-24	0.2466	-0.6080	-0.6338	0.0518	0.2324	0.1831	0.2950	0.0201	0.5019	0.1325	0.3323
16	02-04-25	0.2507	-0.6008	-0.6349	0.0507	0.2318	0.1835	0.2928	0.0234	0.4950	0.1321	0.3314
17	04-08-25	0.2377	-0.6240	-0.6369	0.0494	0.2307	0.1838	0.2896	0.0273	0.4861	0.1315	0.3299
18	05-06-25	0.1805	-0.7435	-0.6438	0.0542	0.2271	0.1769	0.2915	0.0062	0.5105	0.1294	0.3247
19	07-08-25	0.2047	-0.6889	-0.6492	0.0530	0.2243	0.1757	0.2863	0.0077	0.5007	0.1278	0.3207
20	09-09-25	0.2496	-0.6028	-0.6477	0.0539	0.2251	0.1756	0.2885	0.0052	0.5069	0.1283	0.3219

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 ToxCal).
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.

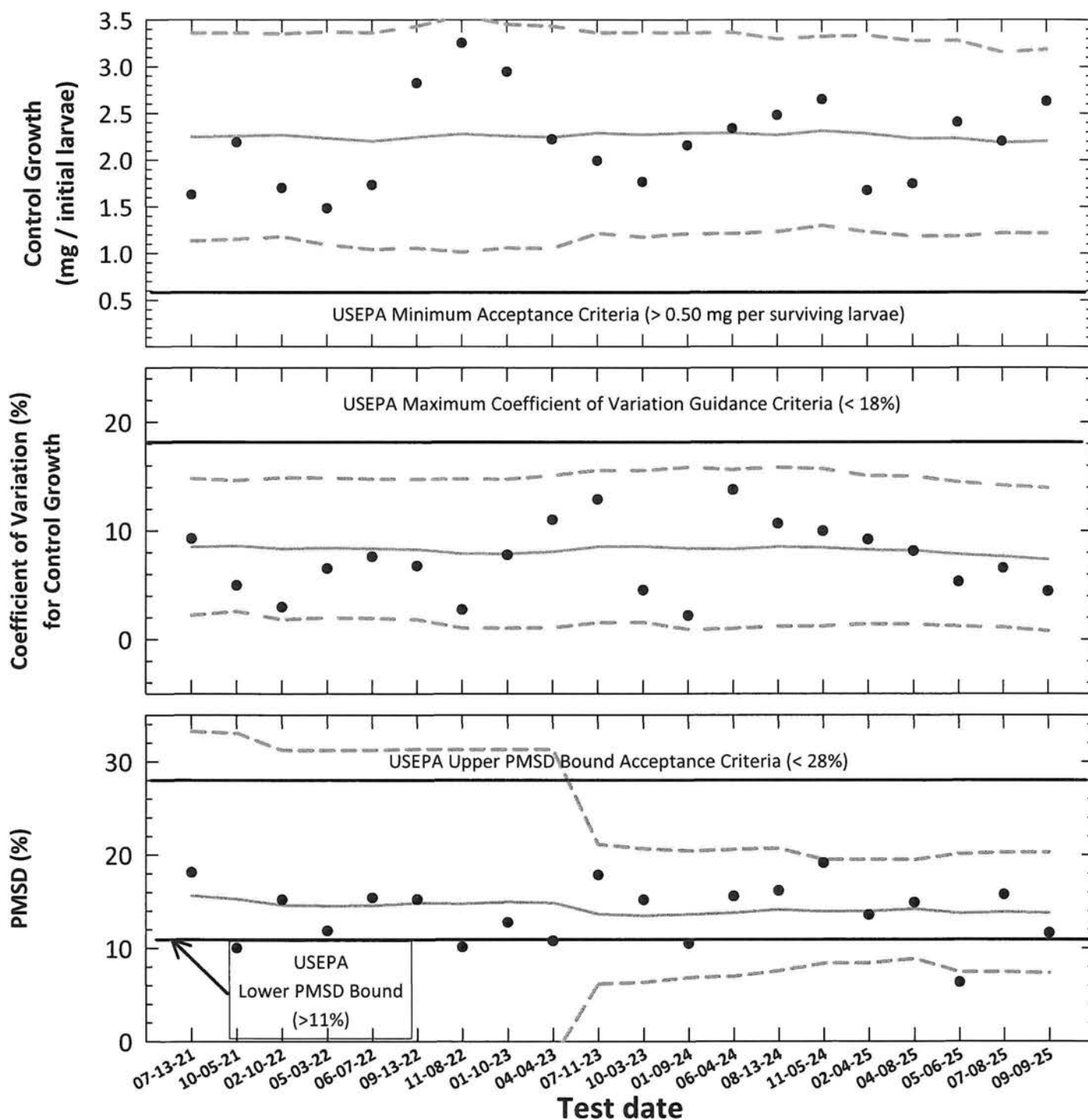
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75} = 0.43$).

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	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S
			Mean (mg/initial larvae)	CV (%)								
1	07-13-21	100	1.631	9.3	MSD	1.136	2.2474	3.359	8.5	2.2	15.7	-2.0
2	10-05-21	100	2.189	5.0	0.2956	1.153	2.258	3.363	8.6	2.6	15.3	-2.5
3	02-10-22	100	1.701	3.0	0.2192	1.180	2.266	3.351	8.4	1.8	14.6	-2.0
4	05-03-22	100	1.483	6.5	0.2583	1.092	2.232	3.372	8.4	2.0	14.5	-2.1
5	06-07-22	100	1.733	7.6	0.1756	1.041	2.201	3.361	8.3	1.9	14.6	-2.1
6	09-13-22	100	2.822	6.8	0.2665	1.057	2.243	3.430	8.3	1.8	14.8	-1.7
7	11-08-22	100	3.255	2.7	0.4290	1.014	2.280	3.545	7.9	1.0	14.8	-1.8
8	01-10-23	100	2.945	7.8	0.3294	1.062	2.257	3.453	7.9	1.0	15.0	-1.4
9	04-04-23	100	2.220	11.0	0.3755	1.053	2.243	3.433	8.1	1.1	14.9	-1.6
10	07-11-23	100	1.992	12.9	0.2389	1.213	2.287	3.360	8.5	1.5	13.6	6.2
11	10-03-23	100	1.763	4.5	0.3549	1.174	2.270	3.366	8.5	1.5	13.5	6.3
12	01-09-24	100	2.156	2.2	0.2673	1.211	2.287	3.364	8.4	0.9	13.6	6.8
13	06-04-24	100	2.339	13.8	0.2257	1.215	2.291	3.368	8.3	1.0	13.8	7.0
14	08-13-24	100	2.479	10.7	0.3637	1.235	2.266	3.297	8.5	1.2	14.1	7.6
15	11-05-24	100	2.648	10.0	0.4001	1.299	2.312	3.324	8.5	1.2	13.9	8.3
16	02-04-25	100	1.675	9.2	0.5061	1.232	2.283	3.335	8.2	1.4	15.1	8.4
17	04-08-25	100	1.747	8.1	0.2268	1.183	2.230	3.278	8.2	1.4	14.2	8.9
18	05-06-25	100	2.406	5.3	0.2598	1.184	2.233	3.281	7.9	1.2	13.7	7.4
19	07-08-25	100	2.201	6.6	0.1521	1.221	2.188	3.155	7.6	1.1	13.8	7.4
20	09-09-25	100	2.628	4.4	0.3464	1.217	2.201	3.185	7.4	0.8	13.8	7.3

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

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

CV = Coefficient of variation for control growth.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 28%.

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

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

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

Species: *Menidia beryllina*

MbCuCR Test Number: **153**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2425				
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:	ORANGE
Age:	10-days old	Incubator number and shelf location:	1 B
Batch:	AI Mb 08-31-25	Artemia CHM number:	CHM1385
Hatch dates and times:	08-30-25 1200 to 08-31-25 1130	Drying information for weight determination:	
		Date / Time in oven:	09-16-25 1050
Transfer vessel information:	pH (S.U.) = 7.76 Temperature (°C) = 24.5	*Initial oven temperature:	60°C
		Date / Time out of oven:	09-17-25 1050
Average transfer volume (mL):	< 0.25 mL	*Final oven temperature:	60°C
		Total drying time:	24 hours

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	09-09-25	1045	K	1445	K	1150	K	NA	09-04-25 B
1	09-10-25	0500	K	1315	K	0840	K	↓	↓
2	09-11-25	0500	K	1330	K	0845	K		09-04-25
3	09-12-25	0500	K	1100	K	0750	K		09-10-25
4	09-13-25	0600	K	1130	K	0940	K		09-11-25
5	09-14-25	0600	K	1130	K	0840	K		09-12-25
6	09-15-25	0500	K	1100	K	0851	K		09-13-25
7	09-16-25					1000	K		

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (%)	0.285
Average weight per initial larvae:	2.62g		NOEC (%)	0.1
Average weight per surviving larvae:	2.62g	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	0.141
			IC ₂₅ (%)	0.256

Species: Menidia beryllina

MbCuCR Test Number: 153

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>orange</u> Analyst: <u>EM</u> Date: <u>08-19-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>09-16-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JK</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JK</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
2.628 2.526 3.97 2.620 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: 153

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	9 ^{1d}	10	10	10	1 ^{2d}	1 ^{2d}	2 ^{8d}	0 ^{10d}
2	10	10	10	10	9	10	10	10	1	1	1 ^{1d}	0
3	10	10	10	10	9	10	10	10	1	1	1	0
4	10	10	10	10	9	10	10	10	1	1	1	0
5	10	10	10	10	9	10	10	10	1	1	1	0
6	10	10	10	10	9	9 ^{1d}	10	8 ^{2d}	1	1	1	0
7	10	10	10	10	9	9	8 ^{2d}	8	1	1	0 ^{1d}	0
*A = Pan weight (mg) Tray color code: <u>Orange</u> Analyst: <u>EM</u> Date: <u>08-19-25</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JL</u> Date: <u>09-18-25</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
2.548 3.17 2.535 11.17 0.133 94.97												

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

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

Comments:

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

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

Test number: 153

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pin weight (mg)	B = Pin + Larvae weight (mg)	Larvae weight (mg) = B - A	Not for Compliance Assessment, Internal Laboratory QC				Test number:			
							Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (from weight-surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	10.41	35.33	24.92	2.492			2.492				
	B	10	10	14.49	41.63	27.14	2.714			2.714				
	C	10	10	13.91	39.62	25.71	2.571			2.571	100.0	2.628	4.4	Not applicable
	D	10	10	13.25	40.60	27.35	2.735			2.735				
0.025	E	10	10	10.05	35.21	25.16	2.516			2.516				
	F	10	10	14.17	42.44	28.27	2.827			2.827				
	G	10	10	10.46	34.94	24.48	2.448			2.448	100.0	2.526	8.6	3.9
	H	10	10	10.32	33.44	23.12	2.312			2.312				
0.050	I	10	10	13.67	38.52	24.85	2.485			2.485				
	J	10	10	13.45	39.79	26.34	2.634			2.634				
	K	10	10	14.66	39.66	25.00	2.500			2.500	100.0	2.620	6.6	0.3
	L	10	10	12.39	41.00	28.61	2.861			2.861				
0.100	M	10	10	14.39	39.85	25.46	2.546			2.546				
	N	10	10	10.38	39.05	28.67	2.867			2.867				
	O	10	10	12.73	36.69	23.96	2.396			2.396	100.0	2.548	8.8	3.1
	P	10	10	11.06	34.88	23.82	2.382			2.382				
0.200	Q	10	9	12.86	36.84	23.98	2.664			2.398				
	R	10	9	10.35	32.45	22.10	2.456			2.210	85.0	2.335	3.7	11.1
	S	10	8	13.96	37.74	23.78	2.973			2.378				
	T	10	8	16.02	39.56	23.54	2.943			2.354				
0.500	U	10	1	11.98	15.20	3.22	3.220			0.322				
	V	10	1	13.64	15.75	2.11	2.110			0.211	5.0	0.133	120.4	94.9
	W	10	0	0.00	0.00	0.00	0.000			0.000				
	X	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.3054
PMSD: 11.6

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:	9/9/2025		Test ID:	MbCuCR		Sample ID:	REF-Ref Toxicant				
End Date:	9/16/2025		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	0.9000	0.9000	0.8000	0.8000							
0.5	0.1000	0.1000	0.0000	0.0000							
Transform: Arcsin Square Root											
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.2	0.8500	0.8500	1.1781	1.1071	1.2490	6.954	4	10.00	10.00	6	40
*0.5	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40
Auxiliary Tests								Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)								0.77628	0.884	-2E-15	0.36938
Equality of variance cannot be confirmed											
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU					
Steel's Many-One Rank Test			0.1	0.2	0.14142						
Treatments vs D-Control											
Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.80038	0.99933	4.84169	8.75907	0	0.04343	7.81472	0.99762	-0.5446	0.14705	3
Intercept	8.70318	0.60204	7.52318	9.88319							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	0.12982	0.09312	0.15861							
EC05	3.355	0.16352	0.12705	0.19229							
EC10	3.718	0.18492	0.14933	0.21394							
EC15	3.964	0.20093	0.16611	0.2305							
EC20	4.158	0.21463	0.18041	0.24506							
EC25	4.326	0.22712	0.19332	0.25874							
EC40	4.747	0.26193	0.22809	0.29929							
EC50	5.000	0.28539	0.25022	0.32895							
EC60	5.253	0.31096	0.27309	0.36341							
EC75	5.674	0.35862	0.31277	0.43305							
EC80	5.842	0.37949	0.32922	0.46546							
EC85	6.036	0.40537	0.34899	0.50702							
EC90	6.282	0.44045	0.37494	0.56558							
EC95	6.645	0.49811	0.41591	0.66675							
EC99	7.326	0.62739	0.50263	0.9126							

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	9/9/2025	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	9/16/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUS04
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

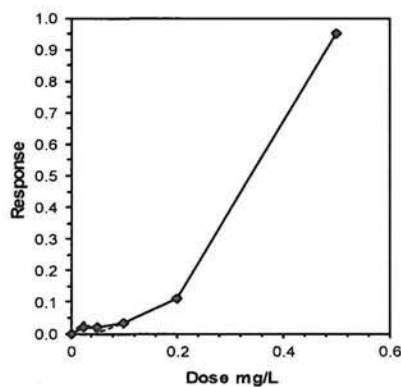
Conc-mg/L	1	2	3	4
D-Control	2.4920	2.7140	2.5710	2.7350
0.025	2.5160	2.8270	2.4480	2.3120
0.05	2.4850	2.6340	2.5000	2.8610
0.1	2.5460	2.8670	2.3950	2.3820
0.2	2.3980	2.2100	2.3780	2.3540
0.5	0.3220	0.2110	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean
D-Control	2.6280	1.0000	2.6280	2.4920	2.7350	4.426	4				2.6280	1.0000
0.025	2.5258	0.9611	2.5258	2.3120	2.8270	8.631	4	0.767	2.290	0.3054	2.5729	0.9790
0.05	2.6200	0.9970	2.6200	2.4850	2.8610	6.644	4	0.060	2.290	0.3054	2.5729	0.9790
0.1	2.5475	0.9694	2.5475	2.3820	2.8670	8.857	4	0.604	2.290	0.3054	2.5475	0.9694
0.2	2.3350	0.8885	2.3350	2.2100	2.3980	3.651	4				2.3350	0.8885
0.5	0.1333	0.0507	0.1333	0.0000	0.3220	120.374	4				0.1333	0.0507

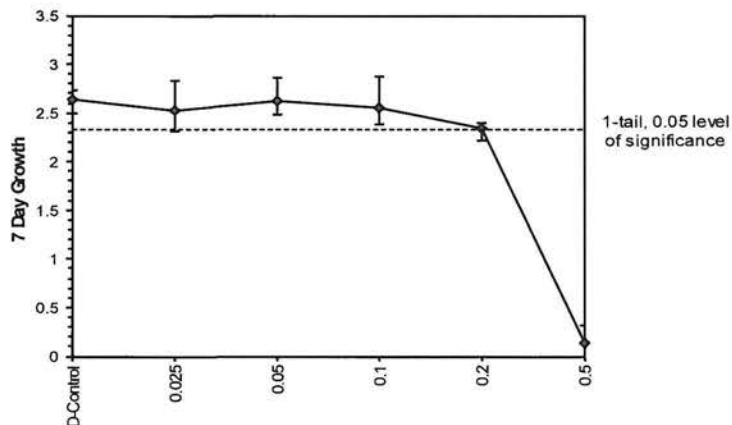
Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.90177	0.844	0.80339	-0.4448		
Bartlett's Test indicates equal variances (p = 0.74)					1.25971	11.3449				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.1	>0.1			0.30538	0.1162	0.01054	0.03557	0.82742	3, 12
Treatments vs D-Control										

Treatments to 2 Control	
	Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1240	0.0426	0.0000	0.1787	-0.4028
IC10	0.1858	0.0296	0.0458	0.2218	-0.9471
IC15	0.2138	0.0072	0.1900	0.2320	-0.7459
IC20	0.2317	0.0064	0.2106	0.2493	-0.1235
IC25	0.2496	0.0062	0.2295	0.2657	-0.1293
IC40	0.3033	0.0059	0.2846	0.3187	-0.1211
IC50	0.3391	0.0060	0.3208	0.3562	-0.0713



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 153

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
		XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	8.03	7.90	7.97	7.79	8.00	7.82
	Dissolved oxygen (mg/L)	8.2	8.0	8.2	7.6	8.1	7.7
	Salinity (ppt)	24.9	25.1	25.0	25.4	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	110			115.5	100	
	Temperature (°C)	24.7	24.9	24.6	24.7	24.6	24.8
0.025 mg/L	pH (S.U.)	8.00	7.89	7.98	7.83	7.98	7.80
	Dissolved oxygen (mg/L)	8.3	7.9	8.2	7.6	8.1	7.4
	Salinity (ppt)	25.0	25.5	25.3	25.6	25.3	25.4
	Temperature (°C)	24.9	24.5	24.6	24.9	24.5	25.0
0.05 mg/L	pH (S.U.)	8.00	7.89	7.98	7.87	7.98	7.80
	Dissolved oxygen (mg/L)	8.3	7.9	8.1	7.6	8.1	7.6
	Salinity (ppt)	24.9	25.4	25.3	25.6	25.4	25.5
	Temperature (°C)	25.0	24.8	24.7	25.0	24.5	24.9
0.1 mg/L	pH (S.U.)	8.00	7.90	7.98	7.81	7.98	7.81
	Dissolved oxygen (mg/L)	8.3	7.9	8.1	7.6	8.1	7.7
	Salinity (ppt)	24.9	25.3	25.2	25.5	25.4	25.6
	Temperature (°C)	24.8	24.7	24.6	24.5	24.6	24.9
0.2 mg/L	pH (S.U.)	8.00	7.89	7.98	7.81	7.98	7.83
	Dissolved oxygen (mg/L)	8.3	7.9	8.1	7.5	8.2	7.7
	Salinity (ppt)	24.9	25.2	25.1	25.5	25.2	25.5
	Temperature (°C)	24.8	24.7	24.6	24.6	24.6	25.1
0.5 mg/L	pH (S.U.)	8.00	7.88	7.98	7.85	7.98	7.80
	Dissolved oxygen (mg/L)	8.3	7.9	8.1	7.5	8.2	7.7
	Salinity (ppt)	24.8	25.7	25.2	25.4	25.3	25.4
	Alkalinity (mg CaCO ₃ /L)		110.5				
	Temperature (°C)	24.8	24.8	24.6	24.6	24.6	24.8
		Initial	Final	Initial	Final	Initial	Final

Species: Menidia beryllina

MbCuCR Test Number: 153

		Day							
		(Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
Analyst		XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.94	7.87	7.94	7.80	8.03	7.75	8.06	7.62
	Dissolved oxygen (mg/L)	8.0	8.0	8.2	7.8	8.3	7.9	8.0	6.2
	Salinity (ppt)	25.0	25.0	24.9	24.9	24.0	25.0	24.9	25.3
	Alkalinity (mg CaCO ₃ /L)	99		86		78		110	
	Temperature (°C)	24.7	24.7	24.6	25.0	24.5	24.8	24.7	25.2
0.025 mg/L	pH (S.U.)	7.93	7.90	7.94	7.86	8.00	7.78	7.85	7.53
	Dissolved oxygen (mg/L)	8.0	7.9	8.2	7.8	8.3	7.9	8.0	6.1
	Salinity (ppt)	25.1	25.2	25.1	25.3	25.1	25.3	25.1	25.2
	Temperature (°C)	24.6	24.5	24.6	24.6	24.5	24.7	24.7	25.2
0.05 mg/L	pH (S.U.)	7.93	7.87	7.95	7.84	8.00	7.78	7.85	7.49
	Dissolved oxygen (mg/L)	8.0	7.9	8.2	7.8	8.3	7.9	8.0	6.1
	Salinity (ppt)	24.9	25.3	25.0	25.3	25.0	25.1	25.1	25.2
	Temperature (°C)	24.6	24.7	24.5	24.6	24.7	24.6	24.6	25.0
0.1 mg/L	pH (S.U.)	7.93	7.89	7.96	7.84	8.00	7.78	7.85	7.51
	Dissolved oxygen (mg/L)	8.0	7.9	8.2	7.8	8.3	7.9	8.1	6.6
	Salinity (ppt)	24.9	25.2	25.0	25.1	25.0	25.1	25.1	25.4
	Temperature (°C)	24.6	24.6	24.5	24.7	24.7	24.8	24.7	25.0
0.2 mg/L	pH (S.U.)	7.93	7.88	7.96	7.86	8.00	7.78	7.85	7.51
	Dissolved oxygen (mg/L)	8.0	7.9	8.2	7.8	8.4	7.9	8.1	6.1
	Salinity (ppt)	25.0	25.2	25.0	25.1	24.9	25.0	25.0	25.3
	Temperature (°C)	24.6	24.6	24.5	24.7	24.6	24.8	24.6	25.3
0.5 mg/L	pH (S.U.)	7.93	7.91	7.97	7.95	8.00	7.79	7.86	7.55
	Dissolved oxygen (mg/L)	8.0	7.8	8.2	7.8	8.3	7.8	8.1	6.6
	Salinity (ppt)	24.9	25.1	25.0	25.0	24.0	25.2	25.0	25.1
	Temperature (°C)	24.6	24.6	24.5	24.9	24.6	24.6	24.6	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final