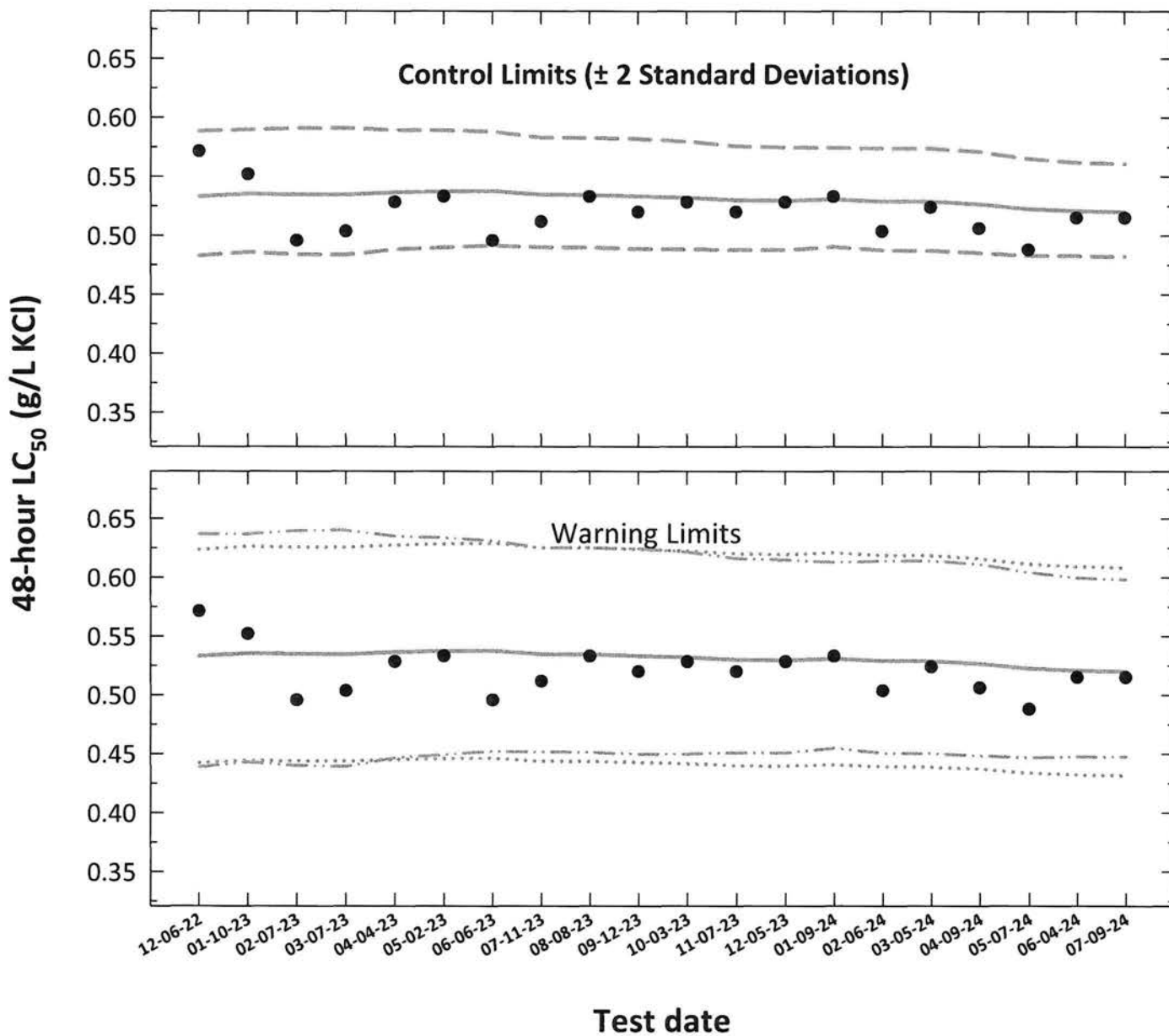


Americamysis (Mysidopsis) bahia

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	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829 0.5884	0.4390 0.6368	0.4424 0.6236
2	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860 0.5897	0.4431 0.6369	0.4443 0.6263
3	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840 0.5909	0.4399 0.6396	0.4439 0.6257
4	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836 0.5911	0.4392 0.6402	0.4438 0.6255
5	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882 0.5892	0.4466 0.6350	0.4452 0.6275
6	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900 0.5891	0.4493 0.6338	0.4459 0.6286
7	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916 0.5880	0.4521 0.6312	0.4462 0.6290
8	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902 0.5830	0.4515 0.6252	0.4437 0.6255
9	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900 0.5828	0.4514 0.6250	0.4436 0.6253
10	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887 0.5819	0.4497 0.6244	0.4426 0.6239
11	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884 0.5798	0.4499 0.6217	0.4417 0.6226
12	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881 0.5757	0.4508 0.6161	0.4400 0.6202
13	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878 0.5748	0.4507 0.6151	0.4395 0.6195
14	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906 0.5746	0.4550 0.6131	0.4407 0.6212
15	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875 0.5740	0.4505 0.6141	0.4391 0.6189
16	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872 0.5738	0.4502 0.6140	0.4389 0.6187
17	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853 0.5711	0.4483 0.6113	0.4370 0.6160
18	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829 0.5651	0.4467 0.6041	0.4335 0.6111
19	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4827 0.5620	0.4476 0.5998	0.4323 0.6094
20	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822 0.5606	0.4473 0.5982	0.4315 0.6083

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 280

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	2.5	3.75	5.0	7.5	10.0
mL Dilution water	497.5	496.25	495.0	492.5	490.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 7286

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Concentration	kd	kd	kd
	Analyst			
	pH (S.U.)	7.91	7.84	7.74
	Dissolved oxygen (mg/L)	7.9	7.4	7.5
	*Salinity (ppt)	24.8	25.0	25.1
250 mg/L	*Alkalinity (mg/L CaCO ₃)	150		
	*Temperature (°C)	25.0	25.2	25.2
	pH (S.U.)	7.89	7.85	7.75
	Dissolved oxygen (mg/L)	7.4	7.7	7.4
375 mg/L	*Salinity (ppt)	24.9	25.0	25.2
	*Temperature (°C)	24.9	25.0	25.0
	pH (S.U.)	7.91	7.85	7.77
	Dissolved oxygen (mg/L)	7.4	7.7	7.4
500 mg/L	*Salinity (ppt)	24.9	25.1	25.2
	*Temperature (°C)	25.1	25.1	25.0
	pH (S.U.)	7.92	7.88	7.79
	Dissolved oxygen (mg/L)	7.4	7.7	7.4
750 mg/L	*Salinity (ppt)	25.0	25.1	25.4
	*Temperature (°C)	25.1	25.1	25.0
	pH (S.U.)	7.93	7.87	
	Dissolved oxygen (mg/L)	8.0	7.9	
1000 mg/L	*Salinity (ppt)	25.2	25.4	
	*Temperature (°C)	25.0	25.1	
	pH (S.U.)	7.93	7.87	
	Dissolved oxygen (mg/L)	8.0	7.9	
	*Salinity (ppt)	25.4	25.5	
	*Temperature (°C)	25.0	25.1	
	pH (S.U.)			
	Dissolved oxygen (mg/L)			

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

Chemical analyses:

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 280

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-09-24	* 1120	JL	1320	JL	1D	WHITE	07-01-24B
24	07-10-24			1318	JL			
48 Termination	07-11-24			1322	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	07-08-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	07-07-24 1200 TO 07-08-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.84
	Temperature (°C) 25.1

Survival Data (number of living organisms):

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

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

Statistics:

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

Comments:

Acute Mysid Test-24 Hr Survival

Start Date: 7/9/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 7/11/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

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

Transform: Arcsin Square Root								1-Tailed		Number	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Total
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0
*500	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	42.086	2.850	0.0285	6
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20

Auxiliary Tests

Normality of the data set cannot be confirmed

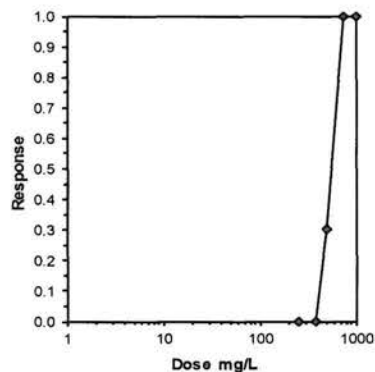
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.5936	0.0001	2.3E-09	4, 5

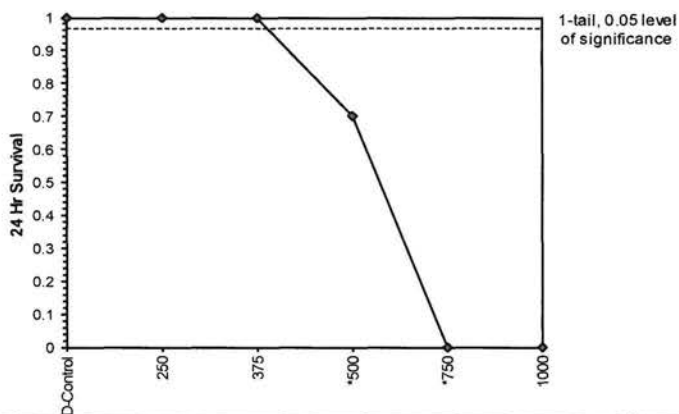
Treatments vs D-Control

Trimmed Spearman-Kärber

Trim Level	EC50	95%CL
0.0%	551.90	514.06 592.53
5.0%	554.06	511.67 599.96
10.0%	556.11	507.32 609.59
20.0%	559.64	489.18 640.25
Auto-0.0%	551.90	514.06 592.53



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 7/9/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 7/11/2024 Lab ID: ETS-Envr. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

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

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

Auxiliary Tests

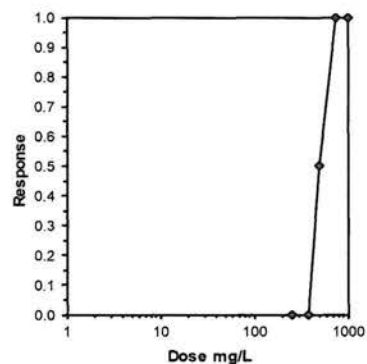
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

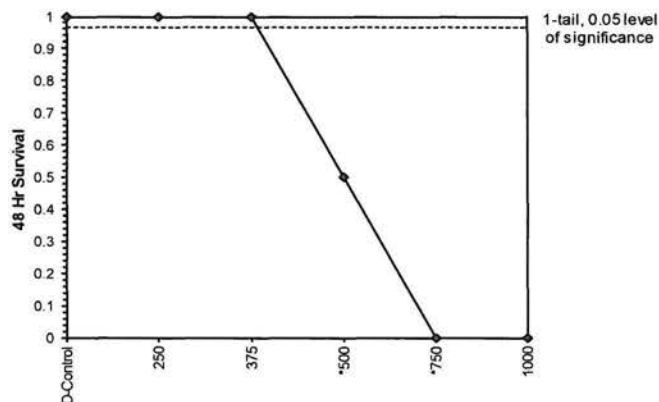
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.62824	0.0001	2.0E-09	4, 5
Treatments vs D-Control										

Trimmed Spearman-Kärber

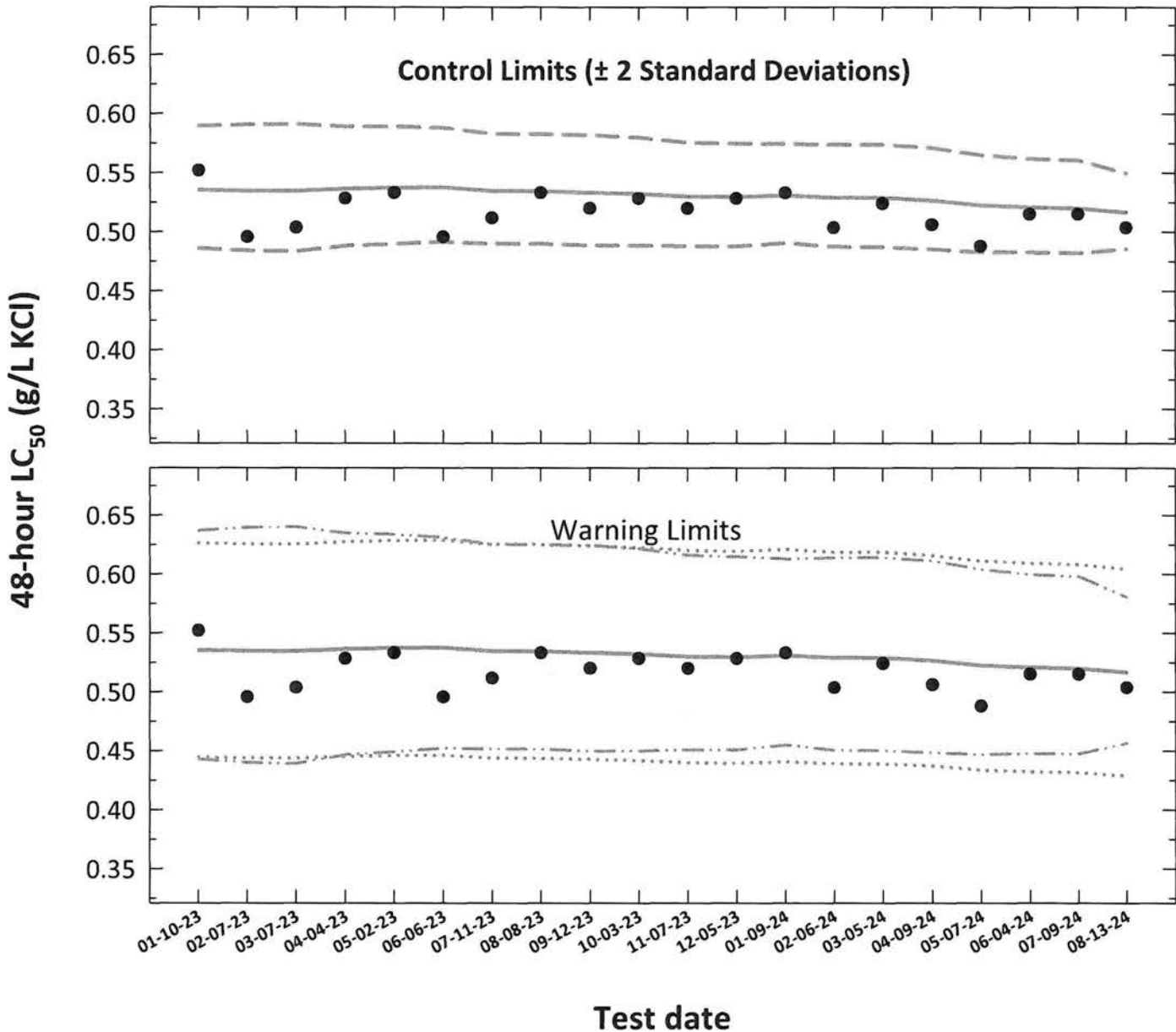
Trim Level	EC50	95% CL	
0.0%	514.94	476.54	556.43
5.0%	513.43	471.14	559.51
10.0%	511.92	464.96	563.62
20.0%	508.91	448.82	577.05
Auto-0.0%	514.94	476.54	556.43



Dose-Response Plot



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



- **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A,10}$ converted to anti-logarithmic values,
 $S_{A,10}$ = 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	Anti-logarithmic Values (g/L KCl)		10th Percentile CV Warning Limits	
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	CT - S _{A,10} CT + S _{A,10}
1	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431
2	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399
3	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392
4	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466
5	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493
6	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521
7	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515
8	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514
9	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497
10	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499
11	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508
12	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507
13	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906	0.5746	0.4550
14	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875	0.5740	0.4505
15	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872	0.5738	0.4502
16	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853	0.5711	0.4483
17	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829	0.5651	0.4467
18	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4827	0.5620	0.4476
19	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822	0.5606	0.4473
20	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4856	0.5497	0.4565

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 281

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	2.5	3.75	5.0	7.5	10.0
mL Dilution water	497.5	496.25	495.0	492.5	490.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2298

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.60	7.71	7.55
	Dissolved oxygen (mg/L)	7.9	7.7	7.5
	*Salinity (ppt)	25.0	25.3	25.3
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	25.0	24.5	24.9
250 mg/L	pH (S.U.)	7.61	7.72	7.58
	Dissolved oxygen (mg/L)	7.9	7.6	7.4
	*Salinity (ppt)	25.2	25.4	25.4
	*Temperature (°C)	25.1	24.8	24.7
375 mg/L	pH (S.U.)	7.62	7.73	7.57
	Dissolved oxygen (mg/L)	7.9	7.6	7.6
	*Salinity (ppt)	25.3	25.7	25.7
	*Temperature (°C)	25.1	24.4	24.9
500 mg/L	pH (S.U.)	7.62	7.73	7.57
	Dissolved oxygen (mg/L)	7.9	7.6	7.7
	*Salinity (ppt)	25.6	25.9	25.8
	*Temperature (°C)	25.1	24.6	24.9
750 mg/L	pH (S.U.)	7.62	7.72	
	Dissolved oxygen (mg/L)	8.0	7.7	
	*Salinity (ppt)	25.5	26.0	
	*Temperature (°C)	25.1	24.2	
1000 mg/L	pH (S.U.)	7.63	7.73	
	Dissolved oxygen (mg/L)	8.0	7.7	
	*Salinity (ppt)	25.7	26.1	
	*Temperature (°C)	25.0	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	13066461

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 281

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-13-24 08-03-24	1030	J	1230	J	1C	Yellow	08-04-24B
24	08-14-24			1232	J			
48 Termination	08-15-24			1227	J			

*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):	08-12-24
Age (1 to 5 days old):	1-2 days
Date organisms were born:	08-11-24 1200 TO 08-12-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.71 Temperature (°C): 21.8

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	8 ^u	7 ^u	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{1u}	10	5 ^{3u}	6 ^{1u}	0	0	0	0
Mean Survival	100%		100%		45%		55%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	465.3
Upper 95% confidence limit (mg KCl/L)	557.0
48-hour LC ₅₀ (mg KCl/L)	503.6

Comments:

Acute Mysid Test-24 Hr Survival

Start Date: 8/13/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 8/15/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americamysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.8000	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

Transform: Arcsin Square Root								1-Tailed		Number		Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045	0	20
*500	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	9.893	2.850	0.1045	5	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	34.167	2.850	0.1045	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

Normality of the data set cannot be confirmed

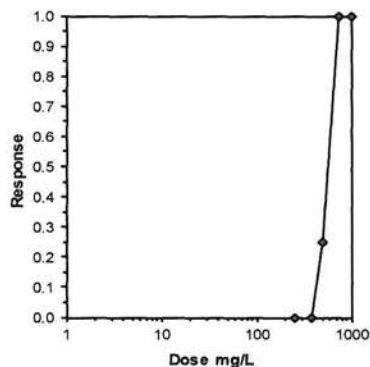
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.04275	0.04384	0.58996	0.00135	1.5E-06	4, 5

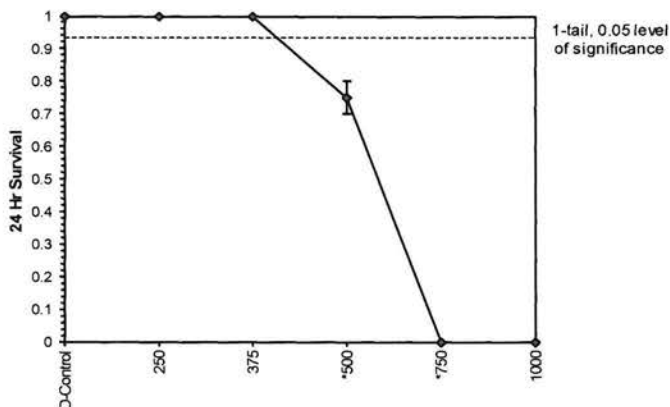
Treatments vs D-Control

Trimmed Spearman-Kärber

Trim Level	EC50	95%CL
0.0%	561.55	525.10 600.53
5.0%	564.65	523.53 609.00
10.0%	567.47	519.36 620.03
20.0%	571.63	498.49 655.51
Auto-0.0%	561.55	525.10 600.53



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 8/13/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 8/15/2024 Lab ID: ETS-Envr. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanmysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	1.0000
500	0.5000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.345	2.850	0.1726	1	20
*500	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20.688	2.850	0.1726	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

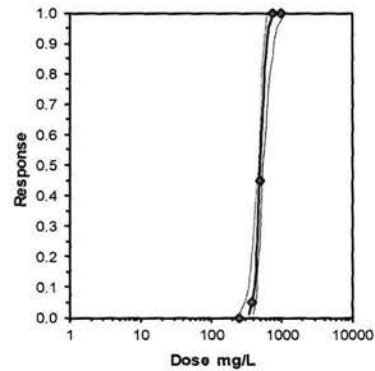
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

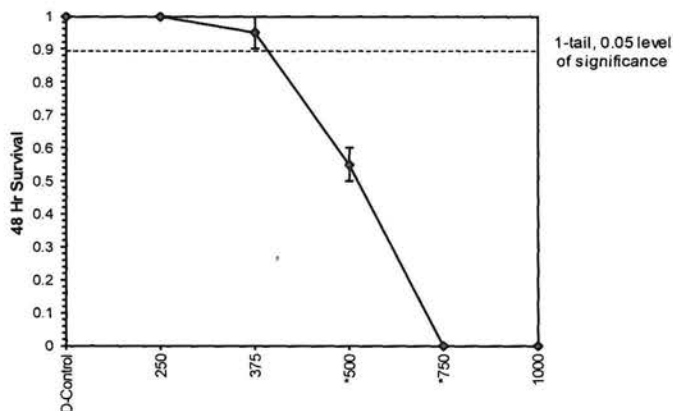
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.08088	0.08295	0.58948	0.00367	1.8E-05	4, 5
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
					Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.9938	3.31443	7.4975	20.4901	0	0.34385	7.81472	0.95158	2.70209	0.07146	4
Intercept	-32.812	8.90228	-50.261	-15.364							

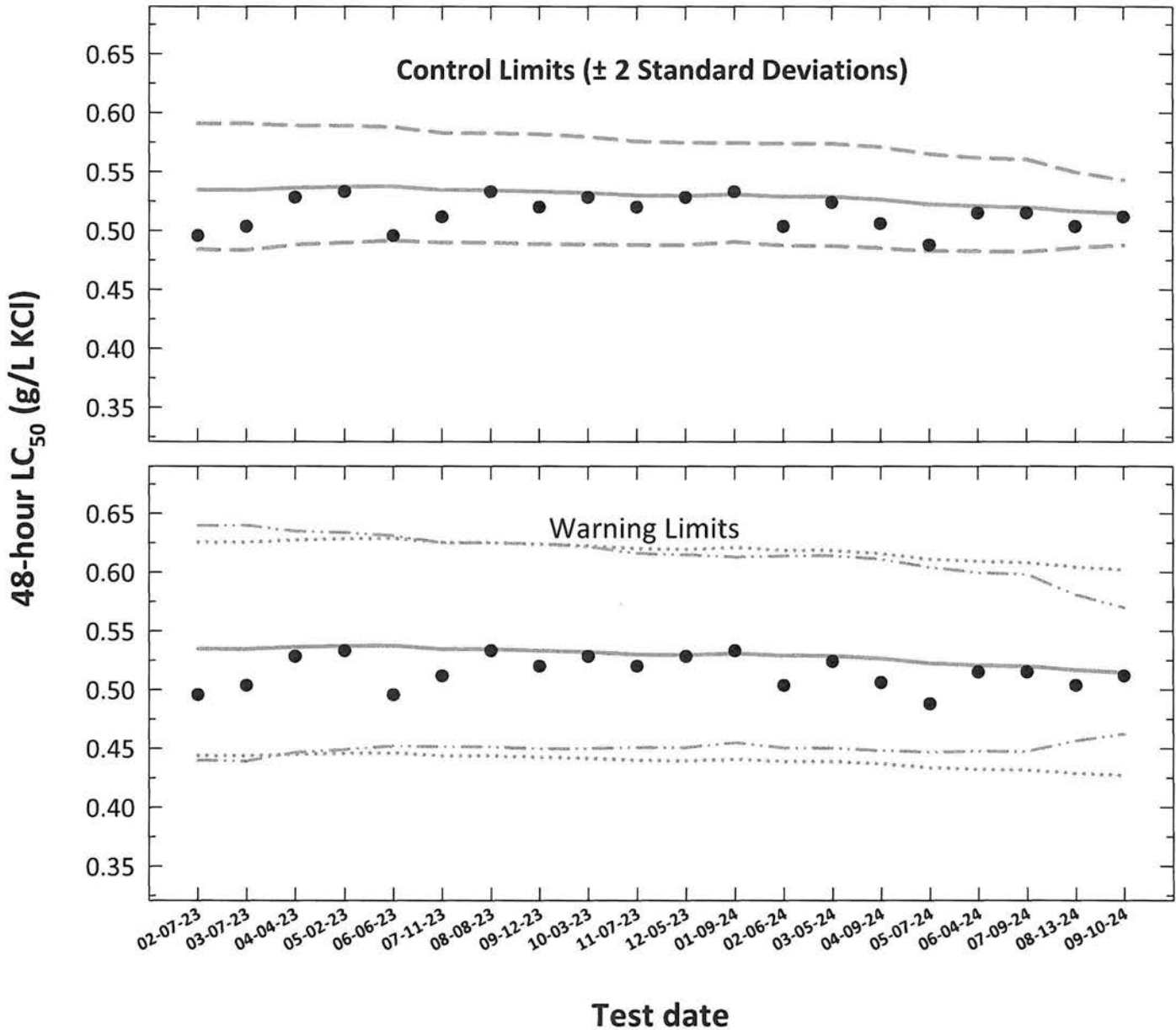
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	343.437	250.566	389.808
EC05	3.355	384.19	306.605	423.985
EC10	3.718	407.857	340.45	444.696
EC15	3.964	424.643	364.587	460.226
EC20	4.158	438.476	384.252	473.857
EC25	4.326	450.701	401.218	486.778
EC40	4.747	483.041	442.565	526.574
EC50	5.000	503.603	465.286	557.021
EC60	5.253	525.04	486.136	592.91
EC75	5.674	562.714	517.856	664.144
EC80	5.842	578.403	529.87	696.226
EC85	6.036	597.244	543.691	736.307
EC90	6.282	621.825	560.982	790.894
EC95	6.645	660.131	586.743	880.657
EC99	7.326	738.465	636.526	1080.43



Dose-Response Plot



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



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			48-hour LC ₅₀	CT	S	CT	Control Limits 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	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840 0.5909	0.4399 0.6396	0.4439 0.6257
2	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836 0.5911	0.4392 0.6402	0.4438 0.6255
3	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882 0.5892	0.4466 0.6350	0.4452 0.6275
4	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900 0.5891	0.4493 0.6338	0.4459 0.6286
5	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916 0.5880	0.4521 0.6312	0.4462 0.6290
6	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902 0.5830	0.4515 0.6252	0.4437 0.6255
7	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900 0.5828	0.4514 0.6250	0.4436 0.6253
8	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887 0.5819	0.4497 0.6244	0.4426 0.6239
9	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884 0.5798	0.4499 0.6217	0.4417 0.6226
10	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881 0.5757	0.4508 0.6161	0.4400 0.6202
11	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878 0.5748	0.4507 0.6151	0.4395 0.6195
12	01-09-24	0.5331	-0.2732	-0.2749	0.0172	0.5310	0.4906 0.5746	0.4550 0.6131	0.4407 0.6212
13	02-06-24	0.5036	-0.2979	-0.2766	0.0177	0.5290	0.4875 0.5740	0.4505 0.6141	0.4391 0.6189
14	03-05-24	0.5239	-0.2807	-0.2767	0.0178	0.5288	0.4872 0.5738	0.4502 0.6140	0.4389 0.6187
15	04-09-24	0.5061	-0.2958	-0.2786	0.0177	0.5265	0.4853 0.5711	0.4483 0.6113	0.4370 0.6160
16	05-07-24	0.4879	-0.3117	-0.2820	0.0171	0.5223	0.4829 0.5651	0.4467 0.6041	0.4335 0.6111
17	06-04-24	0.5149	-0.2882	-0.2833	0.0165	0.5208	0.4827 0.5620	0.4476 0.5998	0.4323 0.6094
18	07-09-24	0.5149	-0.2882	-0.2841	0.0164	0.5199	0.4822 0.5606	0.4473 0.5982	0.4315 0.6083
19	08-13-24	0.5036	-0.2979	-0.2868	0.0135	0.5166	0.4856 0.5497	0.4565 0.5807	0.4288 0.6045
20	09-10-24	0.5117	-0.2910	-0.2884	0.0117	0.5147	0.4877 0.5431	0.4623 0.5700	0.4272 0.6022

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 # 282

Dilution Preparation:

Test concentrations (mg/L KCl)	250	375	500	750	1000
mL Stock solution	2.5	3.75	5.0	7.5	10.0
mL Dilution water	497.5	496.25	495.0	492.5	490.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2319

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>JK</i>	<i>JP</i>	<i>JP</i>
	pH (S.U.)	7.63	7.45	7.73
	Dissolved oxygen (mg/L)	7.7	7.4	7.4
	*Salinity (ppt)	25.0	25.1	25.6
	*Alkalinity (mg/L CaCO ₃)	95		
	*Temperature (°C)	25.3	25.3	25.3
250 mg/L	pH (S.U.)	7.62	7.64	7.70
	Dissolved oxygen (mg/L)	7.7	7.4	7.4
	*Salinity (ppt)	25.2	25.5	25.7
	*Temperature (°C)	25.2	25.2	25.2
375 mg/L	pH (S.U.)	7.62	7.64	7.72
	Dissolved oxygen (mg/L)	7.7	7.5	7.5
	*Salinity (ppt)	25.3	25.6	25.9
	*Temperature (°C)	25.2	25.2	25.4
500 mg/L	pH (S.U.)	7.63	7.64	7.69
	Dissolved oxygen (mg/L)	7.7	7.5	7.5
	*Salinity (ppt)	25.4	25.7	26.0
	*Temperature (°C)	25.2	25.0	25.2
750 mg/L	pH (S.U.)	7.64	7.64	
	Dissolved oxygen (mg/L)	7.7	7.6	
	*Salinity (ppt)	25.7	25.8	
	*Temperature (°C)	25.2	25.2	
1000 mg/L	pH (S.U.)	7.65	7.64	
	Dissolved oxygen (mg/L)	7.8	7.7	
	*Salinity (ppt)	25.8	26.0	
	*Temperature (°C)	25.2	25.4	

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

Chemical analyses:

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

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 282

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-10-24	1050	J	1250	J	1F	PINK	09-0424A
24	09-11-24			1300	J			
48 Termination	09-12-24			1250	J			

*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):	0909-24
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	09-08-24 1200 TO 09-09-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.59
	Temperature (°C) 24.5

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^{1d}	8 ^{2d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{1d}	10	6 ^{3d}	6 ^{2d}	0	0	0	0
Mean Survival	100%		100%		95%		60%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	472.2
Upper 95% confidence limit (mg KCl/L)	566.1
48-hour LC ₅₀ (mg KCl/L)	511.7

Comments:

Start Date:	9/10/2024	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	9/12/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americanysis bahia
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.9000	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*500	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	5.213	2.850	0.1279	3	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	27.929	2.850	0.1279	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.05495	0.05636	0.5915	0.00201	4.1E-06	4, 5
Treatments vs D-Control										

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

Response

Dose mg/L

Dose-Response Plot

24 Hr Survival

Dose mg/L

1-tail, 0.05 level of significance

Acute Mysid Test-48 Hr Survival

Start Date: 9/10/2024 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 9/12/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia
Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	1.0000
500	0.6000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*500	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	10.205	2.850	0.1469	8	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

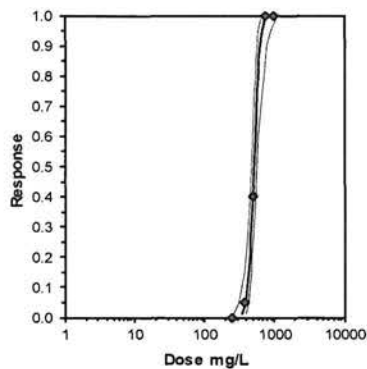
Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

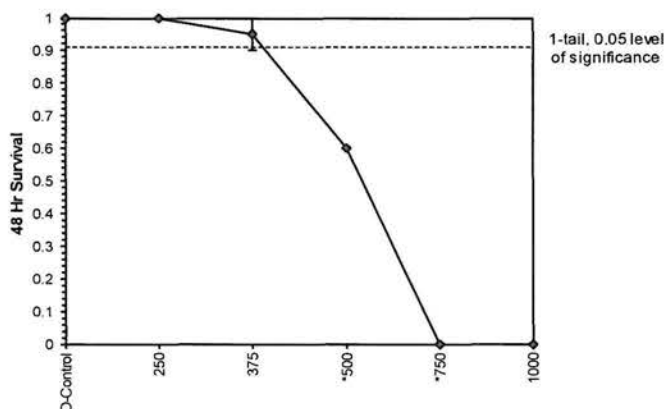
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.06555	0.06723	0.58072	0.00266	8.5E-06	4, 5
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						Mu	Sigma	Iter
					Control	Chi-Sq	Critical	P-value					
Slope	13.7501	3.15512	7.5661	19.9342	0	0.60941	7.81472	0.89428			2.709	0.07273	5
Intercept	-32.249	8.49454	-48.898	-15.6									

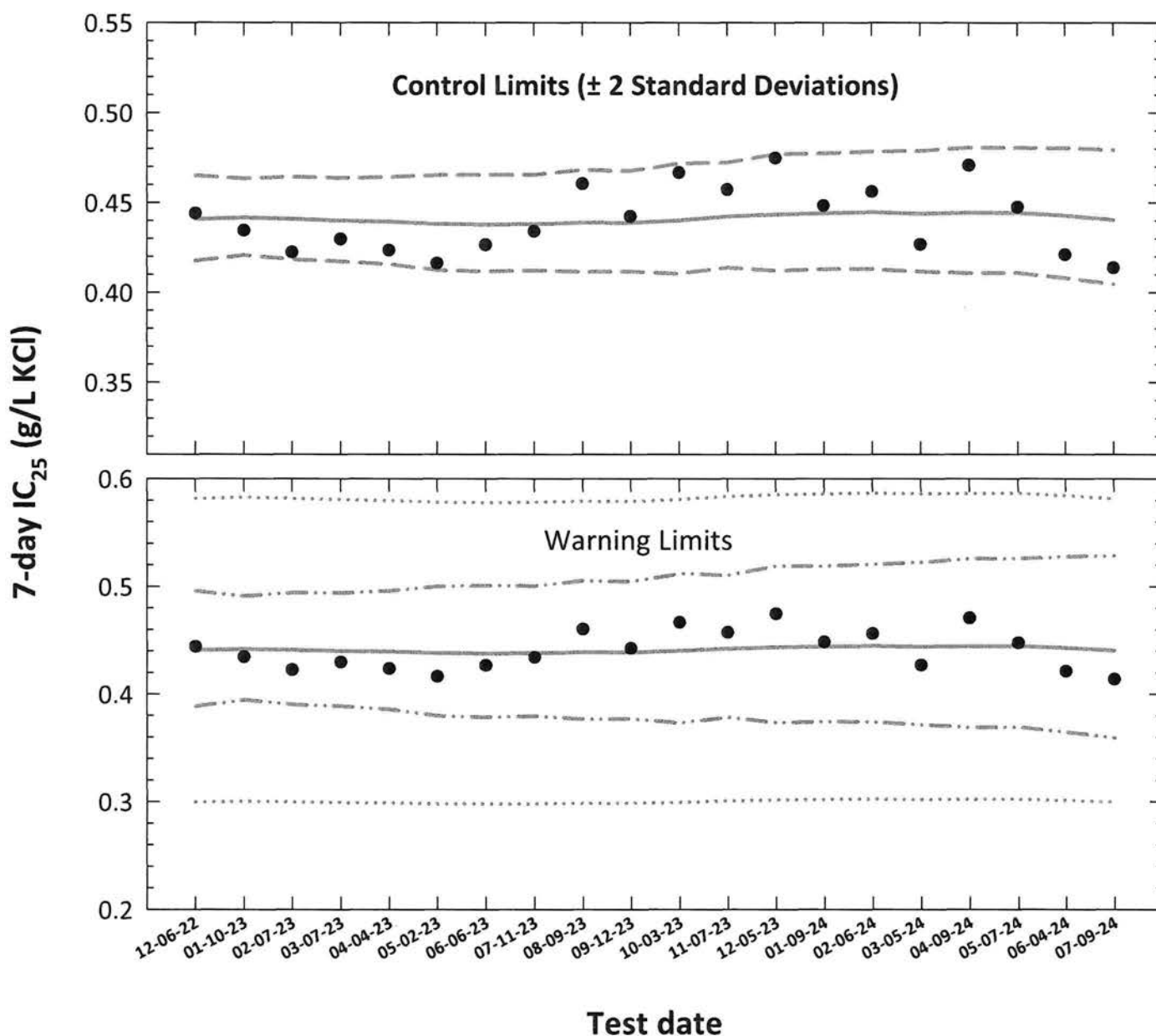
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	346.589	255.817	393.471
EC05	3.355	388.488	312.339	429.018
EC10	3.718	412.857	346.387	450.592
EC15	3.964	430.156	370.63	466.763
EC20	4.158	444.421	390.366	480.935
EC25	4.326	457.035	407.395	494.329
EC40	4.747	490.431	449.047	535.215
EC50	5.000	511.685	472.168	566.132
EC60	5.253	533.86	493.594	602.334
EC75	5.674	572.87	526.511	673.89
EC80	5.842	589.129	539.044	706.067
EC85	6.036	608.666	553.489	746.246
EC90	6.282	634.169	571.589	800.941
EC95	6.645	673.949	598.598	890.822
EC99	7.326	755.422	650.897	1090.62



Dose-Response Plot



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



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

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)			
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4408	0.4177 0.4651	0.3884 0.4960	0.2997 0.5818
2	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208 0.4635	0.3944 0.4911	0.3003 0.5829
3	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185 0.4644	0.3901 0.4943	0.2998 0.5820
4	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174 0.4638	0.3886 0.4941	0.2992 0.5807
5	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158 0.4643	0.3857 0.4961	0.2988 0.5800
6	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126 0.4654	0.3798 0.5003	0.2980 0.5785
7	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118 0.4655	0.3784 0.5011	0.2977 0.5780
8	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124 0.4655	0.3794 0.5006	0.2979 0.5783
9	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116 0.4683	0.3766 0.5056	0.2985 0.5795
10	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116 0.4677	0.3769 0.5047	0.2983 0.5791
11	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106 0.4719	0.3730 0.5122	0.2993 0.5810
12	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140 0.4725	0.3784 0.5105	0.3008 0.5838
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

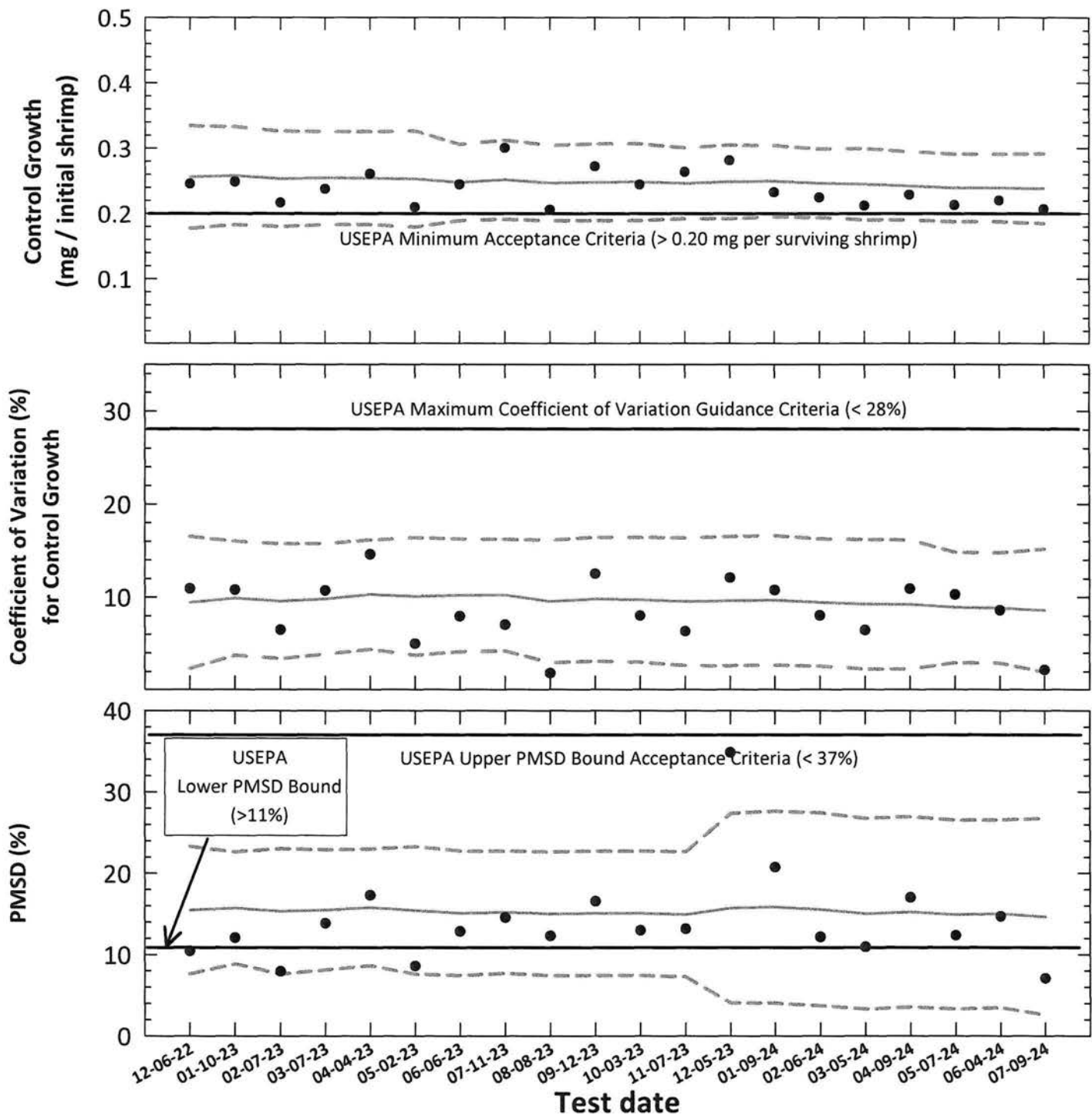
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	12-06-22	100	0.246	10.9	0.0256	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3	
2	01-10-23	100	0.249	10.8	0.0300	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6	
3	02-07-23	100	0.217	6.5	0.0172	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0	
4	03-07-23	100	0.238	10.7	0.0329	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9	
5	04-04-23	100	0.261	14.6	0.0450	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0	
6	05-02-23	100	0.210	5.0	0.0180	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3	
7	06-06-23	100	0.245	8.0	0.0314	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7	
8	07-11-23	100	0.300	7.0	0.0437	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7	
9	08-08-23	100	0.206	1.8	0.0253	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6	
10	09-12-23	100	0.273	12.5	0.0451	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7	
11	10-03-23	100	0.244	8.0	0.0317	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7	
12	11-07-23	100	0.264	6.3	0.0347	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6	
13	12-05-23	100	0.282	12.1	0.0981	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4	
14	01-09-24	100	0.233	10.8	0.0482	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6	
15	02-06-24	100	0.225	8.0	0.0273	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4	
16	03-05-24	100	0.212	6.5	0.0232	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8	
17	04-09-24	100	0.229	10.9	0.0389	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9	
18	05-07-24	100	0.213	10.3	0.0263	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5	
19	06-04-24	100	0.220	8.6	0.0323	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6	
20	07-09-24	100	0.207	2.2	0.0146	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7	

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: **253**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2288					
Stock preparation:	50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water					
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	Grey
Date and times organisms were born between:	07-02-24 1200 to 07-03-24 1130	Incubator number and shelf location:	01-10-24 2054 SB
Organism source:	AI Batch Ab: 07-03-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 7.84 S.U. Temperature = 25.1 °C		Date / Time in oven:	07-16-24 1245
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	07-17-24 1245
		*Final oven temperature:	60 °C
		Total drying time:	24-Hours

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-98585

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	07-09-24	1120	JL	1410	JL	1330	JL	07-01-24 B
1	07-10-24	0500	JL	1100	JL	0830	JL	↓
2	07-11-24	0510	JL	1215	JL	0830	JL	07-08-24
3	07-12-24	0530	JL	1200	JL	0930	JL	↓
4	07-13-24	0600	JL	1200	JL	0933	JL	07-11-24
5	07-14-24	0600	JL	1200	JL	0920	JL	↓
6	07-15-24	0500	JL	1100	JL	0845	JL	↓
7	07-16-24					1130	JL	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13064665

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	0%	≤ 20%	7-day LC ₅₀ (mg/L KCl)	443.4
Average weight per initial shrimp:	0.201		NOEC (mg/L KCl)	250
Average weight per surviving shrimp:	0.201	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	375
			ChV (mg/L KCl)	306.2
			IC ₂₅ (mg/L KCl)	413.9

AbKCICR Test Number: **253**

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>mage/10</u> Analyst: <u>JP</u> Date: <u>06-17-24</u>	14.04	14.28	13.19	13.85	13.21	13.57	12.28	14.88	15.68	14.28	13.77	11.60	14.39	14.08	15.47	13.69
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>06-17-24</u>	15.10	15.31	14.14	14.85	14.24	14.62	13.32	15.93	16.73	15.38	14.87	12.68	15.43	15.34	16.58	14.70
C = Shrimp weight (mg) = B - A Hand calculated: <u>JP</u> Analyst: <u>JP</u>	1.06	1.03	1.00	1.00	1.03	1.05	1.04	1.05	1.05	1.10	1.10	1.08	1.04	1.26	1.11	1.01
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>JP</u> Analyst: <u>JP</u>	0.212	0.206	0.200	0.200	0.206	0.210	0.208	0.210	0.210	0.220	0.220	0.216	0.208	0.252	0.222	0.202
Average weight per initial number of shrimp (mg)										Average weight per initial number of shrimp (mg)					Percent reduction from control (%)	
0.207										0.219					-5.97	

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

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

Comments:

AbKCICR Test Number: 253

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	4"
3	S	S	S	S	S	S	S	S	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4
4	S	4 ^{id}	4 ^{id}	4 ^{id}	S	4 ^{id}	S	4 ^{id}	4	3 ^{id}	4	4	4	4	22 ^d	3 ^{id}
5	S	4	4	4	S	4	S	4	4	3	4	4	4	4	2	3
6	S	4	4	4	S	4	S	4	3 ^{id}	3	3 ^{id}	3 ^{id}	2 ^{id}	3 ^{id}	2	3
7	S	4	4	4	S	4	S	4	3	3	3	3	2	3	2	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>magenta</u> Analyst: <u>JP</u> Date: <u>06-17-24</u>	14.50	14.70	13.26	14.40	15.25	14.23	13.07	11.56	14.44	14.61	13.30	14.48	14.08	14.09	14.13	14.05
*B = Pan + Shrimp weight (mg) Analyst: <u>JP</u> Date: <u>07-18-24</u>	15.50	15.49	14.27	15.39	16.76	15.28	14.08	12.58	14.92	15.01	13.55	14.86	14.27	14.56	14.38	14.60
C = Shrimp weight (mg) = B - A Hand calculated: <u>JP</u> Analyst: <u>JP</u>	1.00	0.79	1.01	0.99	1.01	1.05	1.01	1.02	0.48	0.40	0.25	0.38	0.27	0.47	0.25	0.55
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated: <u>JP</u> Analyst: <u>JP</u>	0.200	0.158	0.202	0.198	0.202	0.210	0.202	0.204	0.096	0.080	0.050	0.076	0.054	0.094	0.050	0.110
Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.147				4.67				0.076				30.37				

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

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

Comments:

AbKCICR Test Number: **253**

Survival and Growth Data

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

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

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

Comments:

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

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

Test number: 253

Test dates: July 09-16, 2024

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.04	15.10	1.06	0.212	100.0	0.207	2.2	Not applicable
	B	5	5	14.28	15.31	1.03	0.206				
	C	5	5	13.19	14.19	1.00	0.200				
	D	5	5	13.85	14.85	1.00	0.200				
	E	5	5	13.21	14.24	1.03	0.206				
	F	5	5	13.57	14.62	1.05	0.210				
	G	5	5	12.28	13.32	1.04	0.208				
	H	5	5	14.88	15.93	1.05	0.210				
250	I	5	5	15.68	16.73	1.05	0.210	100.0	0.219	6.9	-5.9
	J	5	5	14.28	15.38	1.10	0.220				
	K	5	5	13.77	14.87	1.10	0.220				
	L	5	5	11.60	12.68	1.08	0.216				
	M	5	5	14.39	15.43	1.04	0.208				
	N	5	5	14.08	15.34	1.26	0.252				
	O	5	5	15.47	16.58	1.11	0.222				
	P	5	5	13.69	14.70	1.01	0.202				
375	Q	5	5	14.50	15.50	1.00	0.200	87.5	0.197	8.2	4.6
	R	5	4	14.70	15.49	0.79	0.158				
	S	5	4	13.26	14.27	1.01	0.202				
	T	5	4	14.40	15.39	0.99	0.198				
	U	5	5	15.25	16.26	1.01	0.202				
	V	5	4	14.23	15.28	1.05	0.210				
	W	5	5	13.07	14.08	1.01	0.202				
	X	5	4	11.56	12.58	1.02	0.204				
500	Y	5	3	14.44	14.92	0.48	0.096	55.0	0.076	30.3	63.1
	Z	5	3	14.61	15.01	0.40	0.080				
	AA	5	3	13.30	13.55	0.25	0.050				
	BB	5	3	14.48	14.86	0.38	0.076				
	CC	5	2	14.00	14.27	0.27	0.054				
	DD	5	3	14.09	14.56	0.47	0.094				
	EE	5	2	14.13	14.38	0.25	0.050				
	FF	5	3	14.05	14.60	0.55	0.110				
750	GG	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	HH	5	0	0.00	0.00	0.00	0.000				
	II	5	0	0.00	0.00	0.00	0.000				
	JJ	5	0	0.00	0.00	0.00	0.000				
	KK	5	0	0.00	0.00	0.00	0.000				
	LL	5	0	0.00	0.00	0.00	0.000				
	MM	5	0	0.00	0.00	0.00	0.000				
	NN	5	0	0.00	0.00	0.00	0.000				
1000	OO	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	PP	5	0	0.00	0.00	0.00	0.000				
	QQ	5	0	0.00	0.00	0.00	0.000				
	RR	5	0	0.00	0.00	0.00	0.000				
	SS	5	0	0.00	0.00	0.00	0.000				
	TT	5	0	0.00	0.00	0.00	0.000				
	UU	5	0	0.00	0.00	0.00	0.000				
	VV	5	0	0.00	0.00	0.00	0.000				

Dunnett's MSD value: 0.0146
PMSD: 7.1

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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

Dose (mg/L)	Response
0	0.0
100	-0.05
250	0.05
500	0.65
750	1.0
1000	1.0

Dose-Response Plot

AbKCICR Test Number: **253**

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.

Sheet.

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst		KD	KD N	KD N	KD N
CONTROL, Salt SW	pH (S.U.)	7.91	7.72	7.72	7.69	7.78	7.68
	DO (mg/L)	7.9	7.8	7.8	7.6	7.7	7.4
	Salinity (ppt)	24.6	24.8	24.7	25.0	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	150		15.24 N		150	
	Temperature (°C)	25.8	26.1	25.1	26.0	25.2	26.0
250 mg KCl/L	pH (S.U.)	7.89	7.73	7.72	7.70	7.76	7.63
	DO (mg/L)	7.9	7.8	7.8	7.5	7.6	7.4
	Salinity (ppt)	24.9	25.3	25.0	25.2	25.1	25.2
	Temperature (°C)	25.7	26.0	25.2	26.3	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.91	7.75	7.73	7.72	7.78	7.70
	DO (mg/L)	7.9	7.8	7.7	7.5	7.6	7.4
	Salinity (ppt)	24.9	25.5	25.1	25.4	25.1	25.5
	Temperature (°C)	25.7	26.0	25.2	26.1	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.92	7.75	7.74	7.79	7.79	7.74
	DO (mg/L)	7.9	7.8	7.7	7.4	7.5	7.3
	Salinity (ppt)	25.0	25.5	25.0	25.5	25.3	25.9
	Temperature (°C)	25.9	25.9	25.2	26.1	25.2	26.0
750 mg KCl/L	pH (S.U.)	7.93	7.77				
	DO (mg/L)	8.0	7.8				
	Salinity (ppt)	25.2	25.8				
	Temperature (°C)	25.9	25.9				
1000 mg KCl/L	pH (S.U.)	7.93	7.78				
	DO (mg/L)	8.0	7.9				
	Salinity (ppt)	25.4	25.9				
	Temperature (°C)	25.9	26.1				
		Initial	Final	Initial	Final	Initial	Final

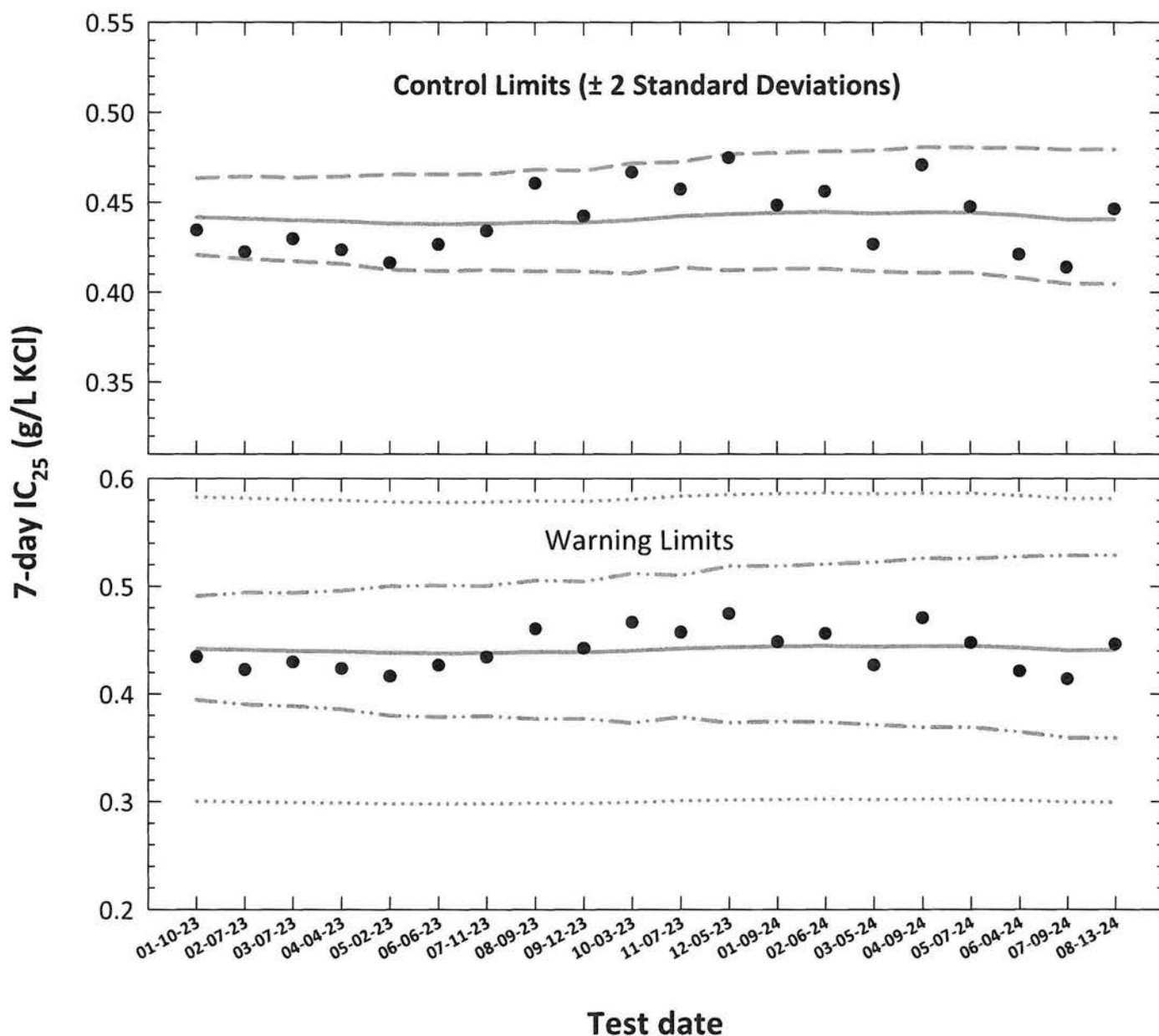
AbKCICR Test Number: 253

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3	4	5	6				
	Analyst	<i>V</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>VD R</i>	<i>VD R</i>	<i>R</i>
CONTROL, Salt SW	pH (S.U.)	<i>7.79</i>	<i>7.86</i>	<i>7.87</i>	<i>7.72</i>	<i>7.81</i>	<i>7.75</i>	<i>7.78</i>	<i>7.52</i>
	DO (mg/L)	<i>7.8</i>	<i>8.0</i>	<i>7.8</i>	<i>7.7</i>	<i>7.8</i>	<i>6.3</i>	<i>7.7</i>	<i>6.9</i>
	Salinity (ppt)	<i>25.0</i>	<i>25.0</i>	<i>25.0</i>	<i>25.2</i>	<i>24.8</i>	<i>24.9</i>	<i>24.9</i>	<i>24.9</i>
	Alkalinity (mg CaCO ₃ /L)	<i>—</i>	<i>150</i>	<i>150</i>	<i>—</i>	<i>—</i>	<i>—</i>	<i>—</i>	<i>—</i>
	Temperature (°C)	<i>25.2</i>	<i>26.1</i>	<i>25.2</i>	<i>25.6</i>	<i>25.2</i>	<i>26.1</i>	<i>25.2</i>	<i>26.0</i>
250 mg KCl/L	pH (S.U.)	<i>7.79</i>	<i>7.82</i>	<i>7.87</i>	<i>7.71</i>	<i>7.81</i>	<i>7.76</i>	<i>7.82</i>	<i>7.52</i>
	DO (mg/L)	<i>7.8</i>	<i>8.0</i>	<i>8.0</i>	<i>7.8</i>	<i>7.8</i>	<i>6.8</i>	<i>7.7</i>	<i>6.8</i>
	Salinity (ppt)	<i>25.0</i>	<i>25.1</i>	<i>25.2</i>	<i>25.2</i>	<i>25.3</i>	<i>25.3</i>	<i>25.3</i>	<i>25.1</i>
	Temperature (°C)	<i>25.2</i>	<i>26.3</i>	<i>25.2</i>	<i>25.9</i>	<i>25.2</i>	<i>26.0</i>	<i>25.1</i>	<i>26.0</i>
375 mg KCl/L	pH (S.U.)	<i>7.79</i>	<i>7.86</i>	<i>7.90</i>	<i>7.72</i>	<i>7.81</i>	<i>7.81</i>	<i>7.83</i>	<i>7.54</i>
	DO (mg/L)	<i>7.9</i>	<i>8.0</i>	<i>8.0</i>	<i>7.7</i>	<i>7.8</i>	<i>6.8</i>	<i>7.8</i>	<i>6.8</i>
	Salinity (ppt)	<i>25.0</i>	<i>25.4</i>	<i>25.2</i>	<i>25.4</i>	<i>25.3</i>	<i>25.5</i>	<i>25.5</i>	<i>25.4</i>
	Temperature (°C)	<i>25.3</i>	<i>26.0</i>	<i>25.2</i>	<i>25.9</i>	<i>25.3</i>	<i>26.0</i>	<i>25.1</i>	<i>26.1</i>
500 mg KCl/L	pH (S.U.)	<i>7.80</i>	<i>7.78</i>	<i>7.93</i>	<i>7.71</i>	<i>7.82</i>	<i>7.78</i>	<i>7.84</i>	<i>7.55</i>
	DO (mg/L)	<i>8.0</i>	<i>8.0</i>	<i>8.0</i>	<i>7.6</i>	<i>7.7</i>	<i>7.0</i>	<i>7.8</i>	<i>6.8</i>
	Salinity (ppt)	<i>25.0</i>	<i>25.5</i>	<i>25.3</i>	<i>25.6</i>	<i>25.3</i>	<i>25.6</i>	<i>25.6</i>	<i>25.6</i>
	Temperature (°C)	<i>25.2</i>	<i>26.0</i>	<i>25.2</i>	<i>25.9</i>	<i>25.1</i>	<i>26.2</i>	<i>25.3</i>	<i>26.0</i>
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)				
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208 0.4635	0.3944 0.4911	0.3003 0.5829
2	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185 0.4644	0.3901 0.4943	0.2998 0.5820
3	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174 0.4638	0.3886 0.4941	0.2992 0.5807
4	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158 0.4643	0.3857 0.4961	0.2988 0.5800
5	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126 0.4654	0.3798 0.5003	0.2980 0.5785
6	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118 0.4655	0.3784 0.5011	0.2977 0.5780
7	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124 0.4655	0.3794 0.5006	0.2979 0.5783
8	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116 0.4683	0.3766 0.5056	0.2985 0.5795
9	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116 0.4677	0.3769 0.5047	0.2983 0.5791
10	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106 0.4719	0.3730 0.5122	0.2993 0.5810
11	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140 0.4725	0.3784 0.5105	0.3008 0.5838
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

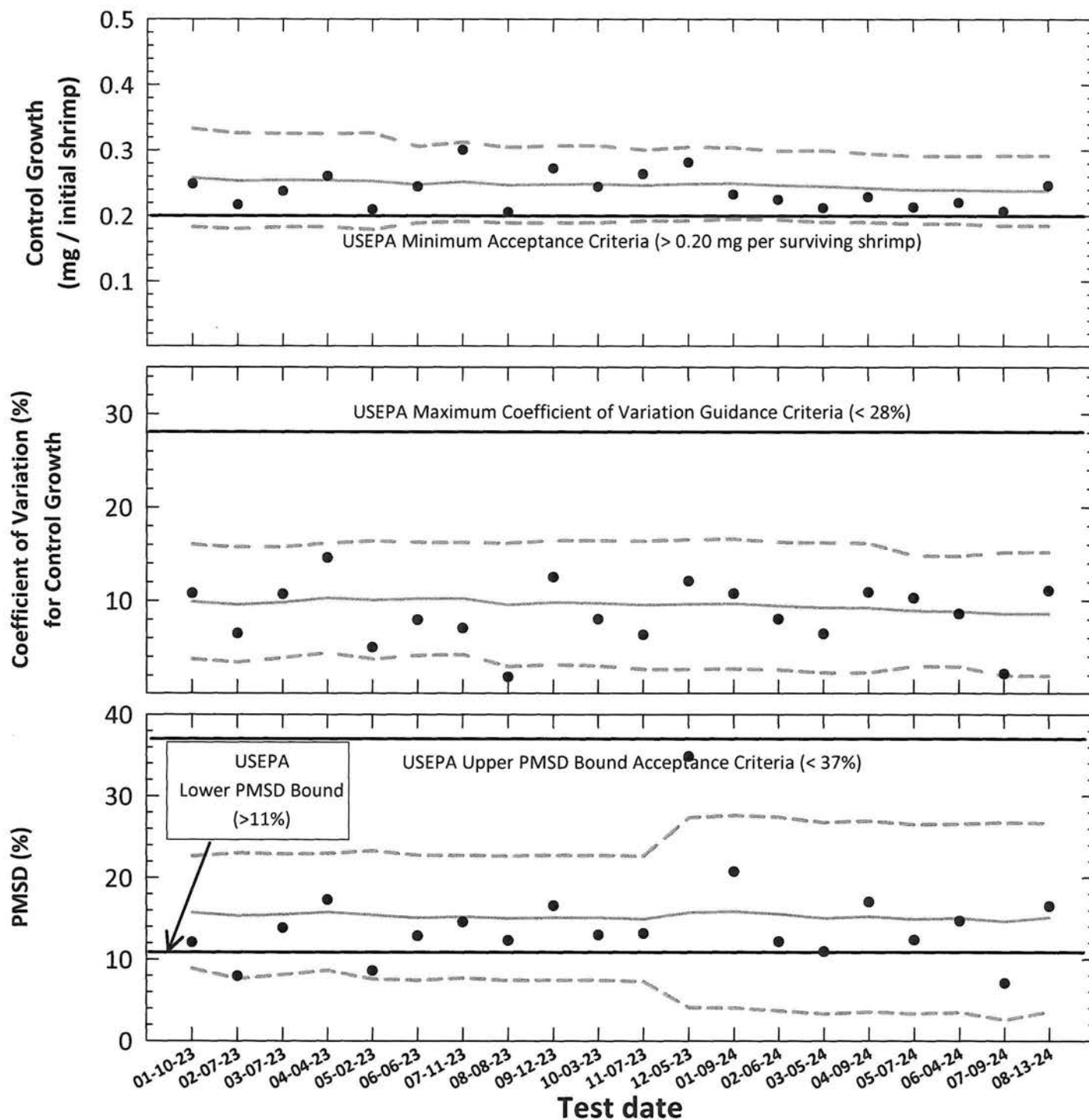
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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 ± 2 Standard Deviations)**

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

Test number	Test date	ToxCal Determination				Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval	CT + 2S	CT	95% Confidence Interval	CT + 2S	CT	95% Confidence Interval	CT + 2S
			Mean (mg/initial shrimp)	CV (%)										
1	01-10-23	100	0.249	10.8	0.0300	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6
2	02-07-23	100	0.217	6.5	0.0172	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0
3	03-07-23	100	0.238	10.7	0.0329	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9
4	04-04-23	100	0.261	14.6	0.0450	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
5	05-02-23	100	0.210	5.0	0.0180	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
6	06-06-23	100	0.245	8.0	0.0314	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
7	07-11-23	100	0.300	7.0	0.0437	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
8	08-08-23	100	0.206	1.8	0.0253	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
9	09-12-23	100	0.273	12.5	0.0451	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
10	10-03-23	100	0.244	8.0	0.0317	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
11	11-07-23	100	0.264	6.3	0.0347	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
12	12-05-23	100	0.282	12.1	0.0581	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4
13	01-09-24	100	0.233	10.8	0.0482	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6
14	02-06-24	100	0.225	8.0	0.0273	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4
15	03-05-24	100	0.212	6.5	0.0232	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8
16	04-09-24	100	0.229	10.9	0.0389	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9
17	05-07-24	100	0.213	10.3	0.0263	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5
18	06-04-24	100	0.220	8.6	0.0323	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6
19	07-09-24	100	0.207	2.2	0.0146	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7
20	08-13-24	100	0.246	11.1	0.0406	0.238	0.185	0.292	8.6	1.9	15.2	15.1	3.5	26.7

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: 254

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2298					
Stock preparation:	50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water					
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	Gray
Date and times organisms were born between:	08-06-24 1200 to 08-07-24 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 08-07-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 7.71 S.U. Temperature = 25.0 °C		Date / Time in oven:	08-10-24 1000
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	08-11-24 1000
		*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	08-13-24	1030	JH	1400	JH	1100	JH	08-09-24 B
1	08-14-24	0600	JH	1320	JH	0900	JH	↓
2	08-15-24	0600	JH	1210	JH	0900	JH	08-12-24
3	08-16-24	0600	JH	1130	JH	0900	JH	↓
4	08-17-24	0710	JH	1200	JH	0920	JH	08-14-24
5	08-18-24	0700	JH	1200	JH	0910	JH	08-15-24
6	08-19-24	0700	JH	1200	JH	0910	JH	↓
7	08-20-24		JH		JH	0900	JH	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	537.7
Average weight per initial shrimp:	0.246		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.246	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	446.2

AbKCICR Test Number: **254**

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>4540356</u> Analyst: <u>Ben</u> Date: <u>07-24-24</u>	15.03	15.44	14.52	13.07	13.58	13.10	13.25	12.58	12.76	15.15	15.39	13.54	12.68	13.13	13.85	10.96
*B = Pan + Shrimp weight (mg) Analyst: <u>11</u> Date: <u>07-24-24</u>	16.42	16.86	15.59	14.18	14.92	14.27	14.37	13.80	14.15	16.41	16.57	15.04	13.86	14.37	15.35	12.62
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JA</u>	1.39	1.42	1.07	1.11	1.34	1.17	1.12	1.22	1.39	1.26	1.18	1.50	1.18	1.24	1.50	1.66
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JA</u>	0.276	0.284	0.214	0.222	0.268	0.234	0.224	0.244	0.278	0.252	0.236	0.300	0.236	0.248	0.300	0.332
Average weight per initial number of shrimp (mg) 0.246												Average weight per initial number of shrimp (mg) 0.273				Percent reduction from control (%) -10.97

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

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

Comments:

AbKCICR Test Number: **254**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	4	S	S	S	4	S	S
4	S	S	S	S	S	S	S	S	S	4	4 ^d	S	S	4	4 ^d	S
5	S	S	S	S	S	S	S	S	3 ^{2d}	4	4	4 ^{1d}	S	4	4	S
6	S	S	S	S	S	S	S	S	3	4	4	4	3 ^{2d}	4	3 ^{1d}	4 ¹
7	S	S	S	S	S	S	S	S	3	4	4	3 ^{1d}	3	2 ^{2d}	3	3 ^{1d}
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																101
*A = Pan weight (mg) Tray color code: Analyst: <u>Julia Oise</u> Date: <u>07-24-24</u>	13.82	15.16	12.77	13.93	14.79	13.25	13.18	13.33	14.49	13.31	13.92	13.42	12.71	11.58	12.37	11.94
*B = Pan + Shrimp weight (mg) Analyst: <u>JK</u> Date: <u>08-21-24</u>	15.02	16.39	13.88	15.05	16.26	14.90	14.33	14.49	15.34	14.42	14.85	14.19	13.63	12.07	12.89	12.69
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JK</u>	1.20	1.23	1.11	1.12	1.47	1.25	1.15	1.16	0.85	1.11	0.93	0.77	0.92	0.49	0.52	0.75
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JK</u>	0.240	0.246	0.222	0.224	0.294	0.250	0.230	0.232	0.170	0.222	0.186	0.154	0.184	0.098	0.104	0.150
Average weight per initial number of shrimp (mg)	0.242								0.159							
Percent reduction from control (%)	1.57								35.67							

*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: **254**

Survival and Growth Data

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

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

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

Comments:

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

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

Test number: 2
Test dates: August 13-20, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	15.03	16.42	1.39	0.278	100.0	0.246	11.1	Not applicable
	B	5	5	15.44	16.86	1.42	0.284				
	C	5	5	14.52	15.59	1.07	0.214				
	D	5	5	13.07	14.18	1.11	0.222				
	E	5	5	13.58	14.92	1.34	0.268				
	F	5	5	13.10	14.27	1.17	0.234				
	G	5	5	13.25	14.37	1.12	0.224				
	H	5	5	12.58	13.80	1.22	0.244				
250	I	5	5	12.76	14.15	1.39	0.278	100.0	0.273	13.0	-10.9
	J	5	5	15.15	16.41	1.26	0.252				
	K	5	5	15.39	16.57	1.18	0.236				
	L	5	5	13.54	15.04	1.50	0.300				
	M	5	5	12.68	13.86	1.18	0.236				
	N	5	5	13.13	14.37	1.24	0.248				
	O	5	5	13.85	15.35	1.50	0.300				
	P	5	5	10.96	12.62	1.66	0.332				
375	Q	5	5	13.82	15.02	1.20	0.240	100.0	0.242	9.6	1.5
	R	5	5	15.16	16.39	1.23	0.246				
	S	5	5	12.77	13.88	1.11	0.222				
	T	5	5	13.93	15.05	1.12	0.224				
	U	5	5	14.79	16.26	1.47	0.294				
	V	5	5	13.25	14.50	1.25	0.250				
	W	5	5	13.18	14.33	1.15	0.230				
	X	5	5	13.33	14.49	1.16	0.232				
500	Y	5	3	14.49	15.34	0.85	0.170	62.5	0.159	26.4	35.6
	Z	5	4	13.31	14.42	1.11	0.222				
	AA	5	4	13.92	14.85	0.93	0.186				
	BB	5	3	13.42	14.19	0.77	0.154				
	CC	5	3	12.71	13.63	0.92	0.184				
	DD	5	2	11.58	12.07	0.49	0.098				
	EE	5	3	12.37	12.89	0.52	0.104				
	FF	5	3	11.94	12.69	0.75	0.150				
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: 0.0406
PMSD: 16.5

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%.

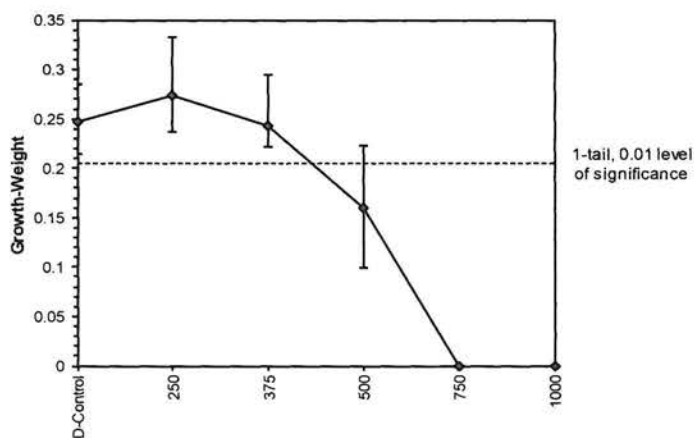
Upper PMSD bound determined by USEPA (90th percentile) = 37%.

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:	8/13/2024	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant						
End Date:	8/20/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride						
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americanysis bahia						
Comments:											
Conc-mg/L	1	2	3	4	5	6	7	8			
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
375	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
500	0.6000	0.8000	0.8000	0.6000	0.6000	0.4000	0.6000	0.6000			
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
Transform: Arcsin Square Root											
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.6250	0.6250	0.9162	0.6847	1.1071	14.934	8	36.00	48.00	15	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)							0.53774		0.904		0.17453 8.81285
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											
Trimmed Spearman-Kärber											
Trim Level	EC50	95% CL									
0.0%	537.74	509.95	567.04								
5.0%	538.48	507.54	571.30								
10.0%	539.21	504.12	576.74								
20.0%	540.60	492.31	593.63								
Auto-0.0%	537.74	509.95	567.04								

Dose (mg/L)	Response
0	0.0
375	0.0
500	0.38
750	1.0
1000	1.0

Dose (mg/L)	7 Day Survival
D-Control	1.0
250	1.0
375	1.0
500	0.62
750	0.0
1000	0.0



AbKCICR Test Number: 254

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	JP	SM N	PN N	N	N	JP N
CONTROL, Salt SW	pH (S.U.)	7.60	7.63	7.50	7.45	7.44	7.37
	DO (mg/L)	7.9	7.4	7.9	7.6	8.0	7.4
	Salinity (ppt)	25.0	25.2	25.0	25.3	24.9	25.1
	Alkalinity (mg CaCO ₃ /L)	100		no test		86	
	Temperature (°C)	25.2	26.0	25.1	25.8	25.2	26.0
250 mg KCl/L	pH (S.U.)	7.61	7.66	7.67	7.49	7.42	7.40
	DO (mg/L)	7.9	7.4	7.9	7.6	8.0	7.4
	Salinity (ppt)	25.2	25.4	25.3	25.5	25.4	25.5
	Temperature (°C)	25.4	26.2	25.1	25.5	25.1	26.2
375 mg KCl/L	pH (S.U.)	7.62	7.66	7.69	7.48	7.68	7.47
	DO (mg/L)	7.9	7.4	7.8	7.7	8.0	7.4
	Salinity (ppt)	25.6	25.6	25.4	25.7	25.5	25.9
	Temperature (°C)	25.4	25.8	25.1	25.9	25.1	26.2
500 mg KCl/L	pH (S.U.)	7.62	7.67	7.70	7.50	7.71	7.46
	DO (mg/L)	7.9	7.5	7.0	7.7	8.1	7.4
	Salinity (ppt)	25.6	25.6	25.4	25.9	25.6	26.4
	Temperature (°C)	25.3	25.7	25.1	25.7	25.1	26.4
750 mg KCl/L	pH (S.U.)	7.62	7.67				
	DO (mg/L)	8.0	7.5				
	Salinity (ppt)	25.6	25.7				
	Temperature (°C)	25.4	25.9				
1000 mg KCl/L	pH (S.U.)	7.63	7.67				
	DO (mg/L)	8.0	7.6				
	Salinity (ppt)	25.9	25.9				
	Temperature (°C)	25.4	26.1				
		Initial	Final	Initial	Final	Initial	Final

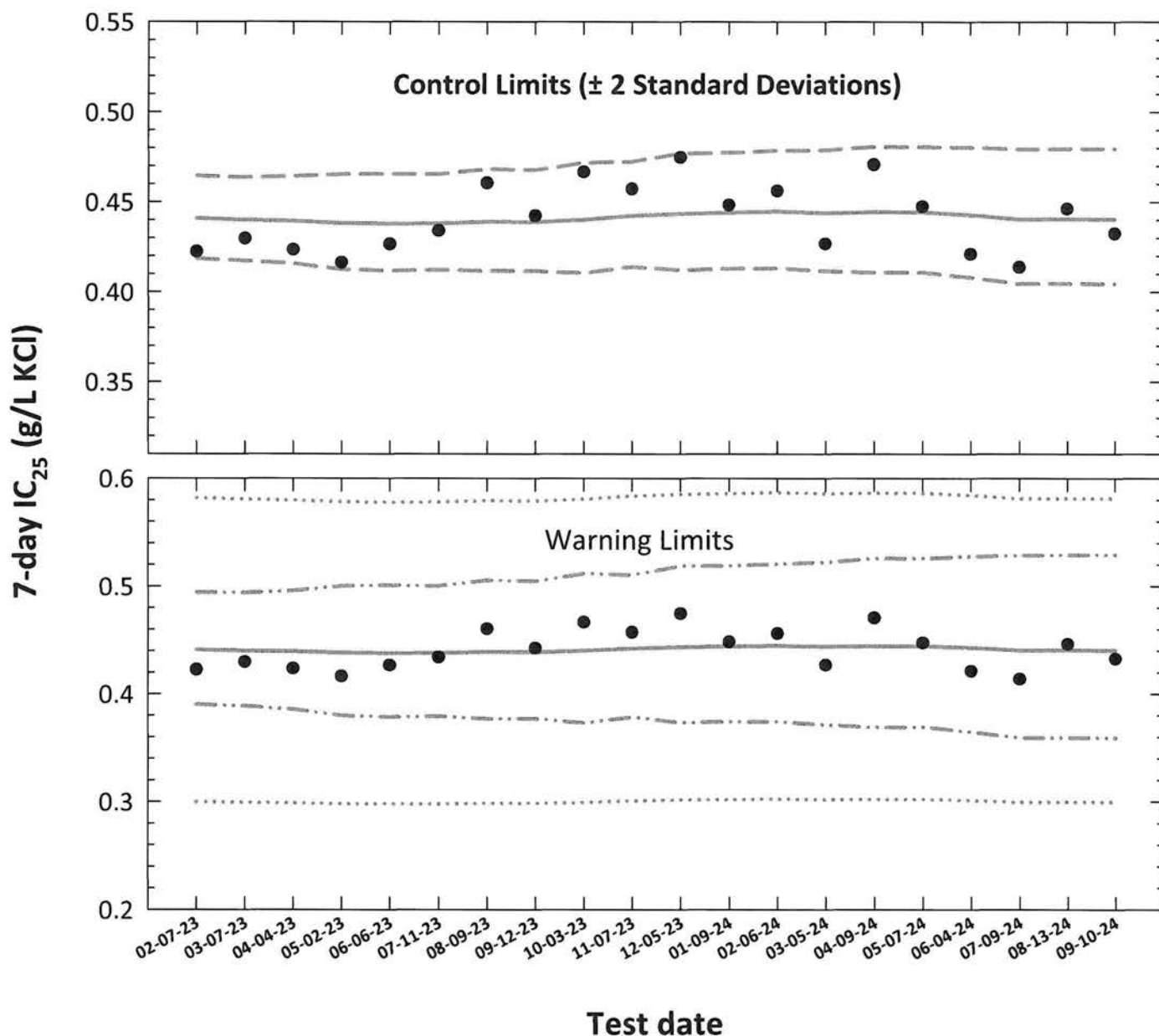
AbKCICR Test Number: **254**

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.)	JP ✓	BSL	BSL	✓	✓	JP ✓	JP ✓	✓
	DO (mg/L)	7.42	7.47	7.72	7.56	8.03	7.66	7.78	7.66
	Salinity (ppt)	7.9	6.9	7.7	5.7	7.8	7.4	7.7	6.4
	Alkalinity (mg CaCO ₃ /L)	25.0	25.1	25.3	25.2	25.2	25.1	25.0	25.1
	Temperature (°C)	—	7.4	7.9	—	9.2	—	—	—
250 mg KCl/L	pH (S.U.)	7.47	7.55	7.73	7.58	8.07	7.71	7.83	7.70
	DO (mg/L)	0.0	6.9	7.8	5.5	7.7	7.4	7.7	6.4
	Salinity (ppt)	25.3	25.3	25.3	25.2	25.2	25.5	24.9 (25.5)	25.2
	Temperature (°C)	25.1	26.0	25.2	26.3	25.2	26.3	25.1	25.8
375 mg KCl/L	pH (S.U.)	7.47	7.54	7.75	7.67	8.07	7.72	7.85	7.65
	DO (mg/L)	0.0	7.0	7.8	6.5	7.7	7.3	7.7	6.6
	Salinity (ppt)	25.4	25.4	25.4	25.3	25.1	25.5	25.1 (25.5)	25.4
	Temperature (°C)	25.2	25.7	25.0	26.0	25.1	26.2	25.1	25.8
500 mg KCl/L	pH (S.U.)	7.44	7.57	7.74	7.68	8.07	7.72	7.87	7.74
	DO (mg/L)	0.0	7.0	7.8	6.4	7.7	7.3	7.7	6.7
	Salinity (ppt)	25.4	25.6	25.6	25.4	25.2	25.7	25.4	25.5
	Temperature (°C)	25.2	25.7	25.0	26.0	25.1	26.2	25.1	25.8
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		CT	Anti-logarithmic Values (g/L KCl)			75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	
1	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185 0.4644	0.3901 0.4943	0.2998 0.5820
2	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174 0.4638	0.3886 0.4941	0.2992 0.5807
3	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158 0.4643	0.3857 0.4961	0.2988 0.5800
4	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126 0.4654	0.3798 0.5003	0.2980 0.5785
5	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118 0.4655	0.3784 0.5011	0.2977 0.5780
6	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124 0.4655	0.3794 0.5006	0.2979 0.5783
7	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116 0.4683	0.3766 0.5056	0.2985 0.5795
8	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116 0.4677	0.3769 0.5047	0.2983 0.5791
9	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106 0.4719	0.3730 0.5122	0.2993 0.5810
10	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140 0.4725	0.3784 0.5105	0.3008 0.5838
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

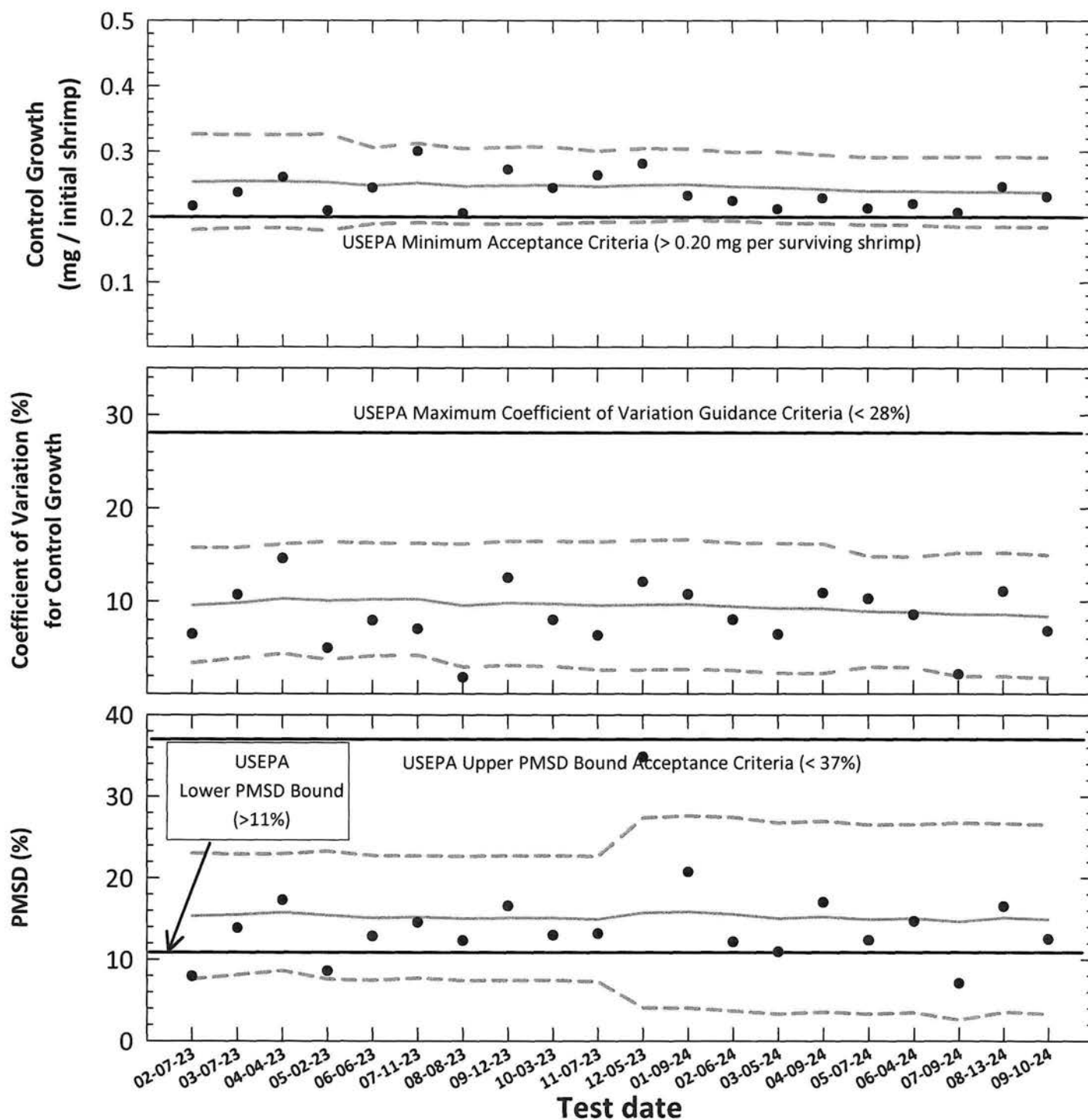
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

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		CV (%)	Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	MSD		PMSD (%)	CT - 2S		CT + 2S	CT - 2S		CT + 2S	CT - 2S		CT + 2S	
1	02-07-23	100	0.217	0.0172	7.9	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0		
2	03-07-23	100	0.238	0.0329	13.9	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9		
3	04-04-23	100	0.261	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0		
4	05-02-23	100	0.210	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3		
5	06-06-23	100	0.245	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7		
6	07-11-23	100	0.300	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7		
7	08-08-23	100	0.206	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6		
8	09-12-23	100	0.273	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7		
9	10-03-23	100	0.244	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7		
10	11-07-23	100	0.264	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6		
11	12-05-23	100	0.282	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4		
12	01-09-24	100	0.233	0.0482	20.7	0.250	0.195	0.304	9.7	2.7	16.6	15.8	4.0	27.6		
13	02-06-24	100	0.225	0.0273	12.2	0.247	0.194	0.299	9.4	2.6	16.3	15.5	3.7	27.4		
14	03-05-24	100	0.212	0.0232	10.9	0.245	0.190	0.300	9.3	2.3	16.2	15.0	3.3	26.8		
15	04-09-24	100	0.229	0.0389	17.0	0.243	0.190	0.295	9.2	2.3	16.2	15.2	3.5	26.9		
16	05-07-24	100	0.213	0.0263	12.3	0.239	0.188	0.291	8.9	2.9	14.8	14.9	3.3	26.5		
17	06-04-24	100	0.220	0.0323	14.7	0.239	0.188	0.291	8.8	2.9	14.8	15.0	3.5	26.6		
18	07-09-24	100	0.207	0.0146	7.1	0.238	0.185	0.292	8.6	1.9	15.2	14.6	2.6	26.7		
19	08-13-24	100	0.246	0.0406	16.5	0.238	0.185	0.292	8.6	1.9	15.2	15.1	3.5	26.7		
20	09-10-24	100	0.231	0.0288	12.5	0.238	0.184	0.291	8.4	1.8	15.0	14.9	3.3	26.5		

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.20 mg/surviving shrimp.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) < 37%.
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: 255

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 7319					
Stock preparation:	50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water					
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		Test information:	
Organism age:	7-days old	Randomizing template:	624
Date and times organisms were born between:	09-03-24 1200 to 09-04-24 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 09-04-24	Artemia CHM number:	CHM1294
Transfer bowl information:		Drying information for weight determination:	
pH = 7.65 S.U. Temperature = 25.0 °C		Date / Time in oven:	09-17-24 1030
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	09-16-24 1030
		*Final oven temperature:	60 °C
		Total drying time:	74-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	09-10-24	1050	H	1430	H	1210	H	09-04-24 A
1	09-11-24	0500	H	1100	H	1022	H	↓
2	09-12-24	0500	H	1200	H	1010	H	09-04-24 B
3	09-13-24	0500	H	1100	H	0850	H	↓
4	09-14-24	0600	H	1200	H	0937	H	09-12-24
5	09-15-24	0600	H	1200	H	0930	H	↓
6	09-16-24	0500	H	1100	H	0846	H	↓
7	09-17-24					1010	H	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066465

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07	≤ 20%	7-day LC ₅₀ (mg/L KCl)	523.9
Average weight per initial shrimp:	0.231		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.231	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	432.5

AbKCICR Test Number: **255**

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>JHC</u> Date: <u>08-22-24</u>	14.12	13.83	12.69	14.81	13.78	13.74	14.29	12.67	15.65	14.06	13.83	13.70	14.98	13.08	13.33	14.04
*B = Pan + Shrimp weight (mg) Analyst: <u>W</u> Date: <u>09-04-24</u>	15.26	14.93	13.74	15.94	14.97	14.89	15.44	13.99	16.66	15.26	15.22	14.94	16.01	14.12	14.56	15.26
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JH</u>	1.14	1.10	1.05	1.13	1.19	1.15	1.15	1.32	1.01	1.20	1.59	1.24	1.03	1.04	1.23	1.22
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JH</u>	0.228	0.220	0.210	0.226	0.238	0.230	0.230	0.264	0.202	0.240	0.278	0.248	0.206	0.208	0.246	0.244
Average weight per initial number of shrimp (mg)								Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.231								0.234				-1.47				

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

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

Comments:

AbKCICR Test Number: **255**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	4 ^{1d}	3 ^{2d}	4 ^{1d}	1 ^{4d}	3 ^{2d}
3	S	S	S	S	S	S	S	S	4 ^{1d}	4 ^{1d}	5	3 ^{1d}	3	4	1	3
4	S	S	S	S	S	S	S	S	3 ^{1d}	3 ^{1d}	3 ^{2d}	3	3	4	1	3
5	S	S	S	S	S	S	S	S	3	3	3	3	2 ^{1d}	4	1	3
6	S	S	S	S	S	S	S	S	3	3	3	3	2	4	1	3
7	S	S	S	S	S	S	S	S	3	3	3	3	2	4	1	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																1
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>JHC</u> Date: <u>08-22-24</u>	14.69	14.08	13.91	15.50	12.63	14.31	12.41	13.41	12.94	13.04	13.16	14.00	14.78	13.84	13.93	14.79
*B = Pan + Shrimp weight (mg) Analyst: <u>Y</u> Date: <u>09-19-24</u>	15.89	15.10	14.99	16.74	13.73	15.57	13.58	14.46	13.46	13.52	13.85	14.99	15.20	14.45	14.19	15.26
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JA</u>	1.20	1.02	1.08	1.24	1.10	1.26	1.17	1.05	0.52	0.48	0.69	0.99	0.42	0.61	0.26	0.48
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JA</u>	0.240	0.204	0.216	0.248	0.220	0.252	0.234	0.210	0.104	0.096	0.138	0.198	0.084	0.122	0.052	0.096
Average weight per initial number of shrimp (mg)	0.228								0.111							
Percent reduction from control (%)	1.27								51.87							

*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: 255

Survival and Growth Data

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

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

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

Comments:

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

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

Test number: 2
Test dates: September 10-17, 200

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	14.12	15.26	1.14	0.228	100.0	0.231	6.8	Not applicable
	B	5	5	13.83	14.93	1.10	0.220				
	C	5	5	12.69	13.74	1.05	0.210				
	D	5	5	14.81	15.94	1.13	0.226				
	E	5	5	13.78	14.97	1.19	0.238				
	F	5	5	13.74	14.89	1.15	0.230				
	G	5	5	14.29	15.44	1.15	0.230				
	H	5	5	12.67	13.99	1.32	0.264				
250	I	5	5	15.65	16.66	1.01	0.202	100.0	0.234	11.3	-1.4
	J	5	5	14.06	15.26	1.20	0.240				
	K	5	5	13.83	15.22	1.39	0.278				
	L	5	5	13.70	14.94	1.24	0.248				
	M	5	5	14.98	16.01	1.03	0.206				
	N	5	5	13.08	14.12	1.04	0.208				
	O	5	5	13.33	14.56	1.23	0.246				
	P	5	5	14.04	15.26	1.22	0.244				
375	Q	5	5	14.69	15.89	1.20	0.240	100.0	0.228	7.9	1.2
	R	5	5	14.08	15.10	1.02	0.204				
	S	5	5	13.91	14.99	1.08	0.216				
	T	5	5	15.50	16.74	1.24	0.248				
	U	5	5	12.63	13.73	1.10	0.220				
	V	5	5	14.31	15.57	1.26	0.252				
	W	5	5	12.41	13.58	1.17	0.234				
	X	5	5	13.41	14.46	1.05	0.210				
500	Y	5	3	12.94	13.46	0.52	0.104	55.0	0.111	38.9	51.8
	Z	5	3	13.04	13.52	0.48	0.096				
	AA	5	3	13.16	13.85	0.69	0.138				
	BB	5	3	14.00	14.99	0.99	0.198				
	CC	5	2	14.78	15.20	0.42	0.084				
	DD	5	4	13.84	14.45	0.61	0.122				
	EE	5	1	13.93	14.19	0.26	0.052				
	FF	5	3	14.78	15.26	0.48	0.096				
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: 0.0288
PMSD: 12.5

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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

AbKCICR Test Number: **255**

Daily Chemistry:

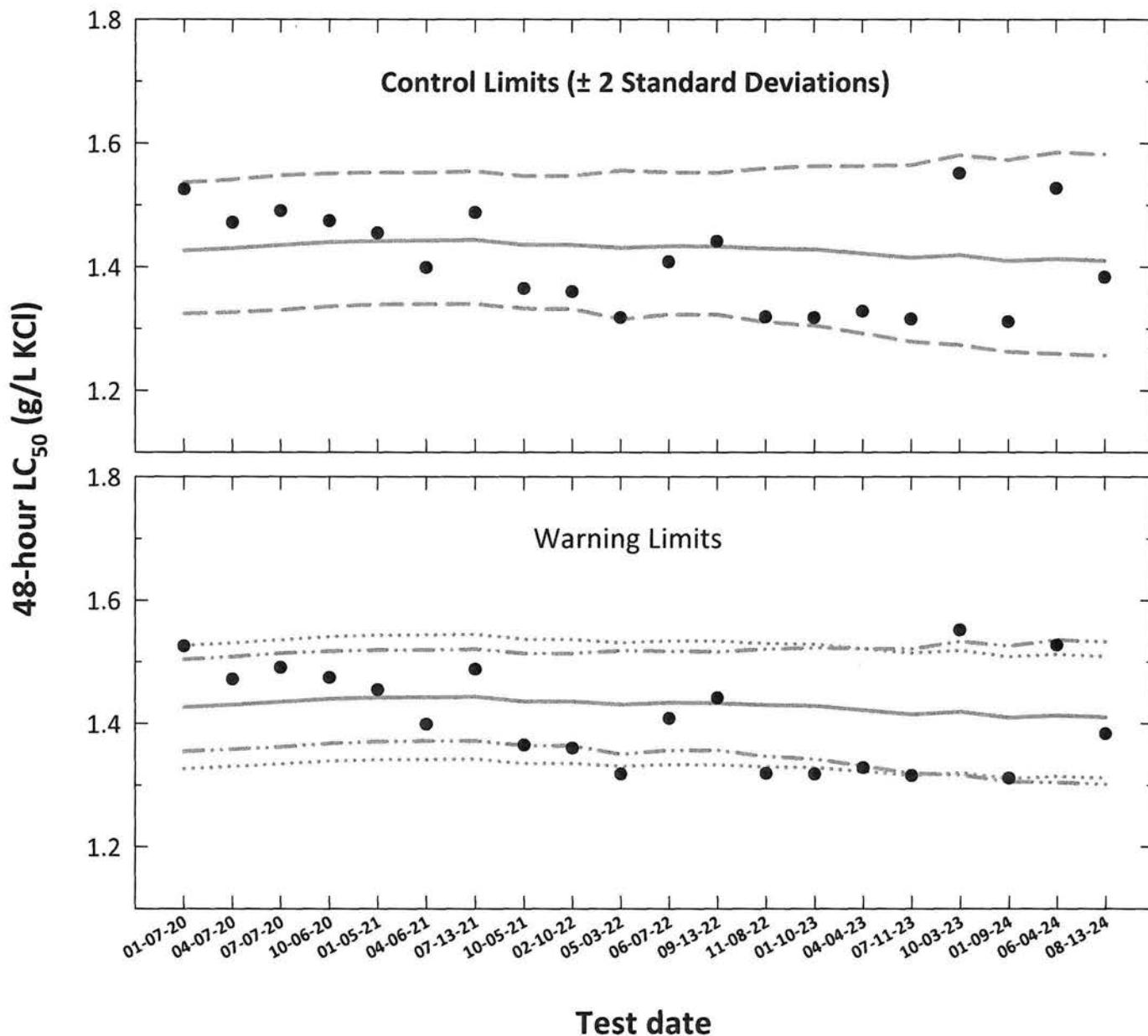
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	N		N		N	
CONTROL, Salt SW	pH (S.U.)	7.43	7.62	7.45	7.56	7.66	7.44
	DO (mg/L)	7.7	7.4	7.7	7.6	7.7	6.5
	Salinity (ppt)	25.0	25.7	25.0	25.4	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)	95				100	
	Temperature (°C)	25.9	25.9	25.2	26.0	26.2	26.2
250 mg KCl/L	pH (S.U.)	7.62	7.61	7.46	7.55	7.71	7.43
	DO (mg/L)	7.7	7.4	7.7	7.3	7.7	6.4
	Salinity (ppt)	25.2	25.8	25.5	25.8	25.6	25.5
	Temperature (°C)	25.7	26.2	25.3	26.2	25.3	26.0
375 mg KCl/L	pH (S.U.)	7.62	7.61	7.46	7.65	7.72	7.45
	DO (mg/L)	7.7	7.4	7.7	7.3	7.7	6.4
	Salinity (ppt)	25.3	26.1	25.5	26.1	25.6	25.3
	Temperature (°C)	25.9	26.2	25.1	26.2	25.3	26.0
500 mg KCl/L	pH (S.U.)	7.63	7.61	7.49	7.65	7.75	7.49
	DO (mg/L)	7.7	7.4	7.7	7.3	7.7	6.9
	Salinity (ppt)	25.4	26.2	25.6	26.4	25.7	25.9
	Temperature (°C)	26.0	26.0	25.1	26.0	25.2	26.3
750 mg KCl/L	pH (S.U.)	7.64	7.61				
	DO (mg/L)	7.7	7.5				
	Salinity (ppt)	25.7	26.3				
	Temperature (°C)	26.0	26.0				
1000 mg KCl/L	pH (S.U.)	7.65	7.59				
	DO (mg/L)	7.8	7.4				
	Salinity (ppt)	25.8	26.1				
	Temperature (°C)	26.0	26.2				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 255

		Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
Conc.	Parameter	3		4		5		6	
Analyst		W	BSC	BSC	BSC	BSC	BW	BW	BW
CONTROL, Salt SW	pH (S.U.)	7.50	7.59	7.68	7.51	7.71	7.73	7.83	7.60
	DO (mg/L)	7.6	7.5	7.8	7.4	8.0	7.5	7.9	7.4
	Salinity (ppt)	24.8	24.9	24.8	24.9	24.0	24.0	25.1	25.3
	Alkalinity (mg CaCO ₃ /L)	100						100	
	Temperature (°C)	25.1	26.3	25.2	26.2	25.2	26.3	25.2	26.3
250 mg KCl/L	pH (S.U.)	7.60	7.69	7.77	7.54	7.70	7.75	7.81	7.56
	DO (mg/L)	7.6	7.5	7.8	7.5	8.0	7.4	7.9	7.3
	Salinity (ppt)	25.0	25.2	25.1	25.1	25.1	25.1	25.1	25.3
	Temperature (°C)	25.3	26.1	25.2	26.0	25.2	26.1	25.2	26.0
375 mg KCl/L	pH (S.U.)	7.61	7.72	7.78	7.63	7.70	7.80	7.83	7.60
	DO (mg/L)	7.6	7.5	7.8	7.4	8.0	7.4	7.9	7.3
	Salinity (ppt)	25.0	25.4	25.1	25.3	25.1	25.3	25.1	25.5
	Temperature (°C)	25.3	26.1	25.2	26.0	25.3	26.1	25.2	26.0
500 mg KCl/L	pH (S.U.)	7.61	7.71	7.79	7.70	7.73	7.81	7.85	7.61
	DO (mg/L)	7.6	7.5	7.8	7.4	8.0	7.3	7.9	7.3
	Salinity (ppt)	25.0	25.5	25.1	25.4	25.2	25.4	25.2	25.5
	Temperature (°C)	25.1	26.2	25.2	26.0	25.3	26.2	25.2	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	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		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	01-07-20	1.5253	0.1834	0.1542	0.0162	1.4264	1.3242	1.5365	1.3547	1.5036	1.3266	1.5262
2	04-07-20	1.4716	0.1678	0.1554	0.0163	1.4302	1.3269	1.5414	1.3580	1.5079	1.3300	1.5303
3	07-07-20	1.4906	0.1734	0.1569	0.0165	1.4351	1.3302	1.5483	1.3620	1.5139	1.3347	1.5356
4	10-06-20	1.4741	0.1685	0.1583	0.0162	1.4397	1.3362	1.5513	1.3678	1.5172	1.3390	1.5405
5	01-05-21	1.4546	0.1627	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189	1.3411	1.5430
6	04-06-21	1.3988	0.1458	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187	1.3415	1.5434
7	07-13-21	1.4877	0.1725	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207	1.3424	1.5444
8	10-05-21	1.3650	0.1351	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132	1.3353	1.5363
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

S = Standard deviation of the LC₅₀ values.

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

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

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

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 # 94

Dilution Preparation:

Test concentrations (mg/L KCl)	1000	1250	1500	1750	2000
mL Stock solution	10.0	12.5	15.0	17.5	20.0
mL Dilution water	490.0	487.50	485.0	482.5	480.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2298

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.60	7.74	7.41
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	*Salinity (ppt)	25.0	25.3	25.2
	*Alkalinity (mg/L CaCO ₃)	100		
	*Temperature (°C)	24.9	24.4	24.8
1000 mg/L	pH (S.U.)	7.60	7.72	7.58
	Dissolved oxygen (mg/L)	8.0	7.6	7.7
	*Salinity (ppt)	25.6	25.7	25.6
	*Temperature (°C)	25.0	24.9	24.6
1250 mg/L	pH (S.U.)	7.62	7.73	7.42
	Dissolved oxygen (mg/L)	8.0	7.5	7.6
	*Salinity (ppt)	25.8	26.0	25.9
	*Temperature (°C)	25.0	24.6	24.6
1500 mg/L	pH (S.U.)	7.65	7.72	7.45
	Dissolved oxygen (mg/L)	8.0	7.4	7.6
	*Salinity (ppt)	26.2	26.3	26.3
	*Temperature (°C)	25.0	24.3	24.9
1750 mg/L	pH (S.U.)	7.65	7.69	7.41
	Dissolved oxygen (mg/L)	8.0	7.4	7.6
	*Salinity (ppt)	26.1	26.4	26.4
	*Temperature (°C)	25.1	24.7	24.5
2000 mg/L	pH (S.U.)	7.66	7.70	
	Dissolved oxygen (mg/L)	8.0	7.5	
	*Salinity (ppt)	26.2	26.4	
	*Temperature (°C)	25.0	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	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 # 94

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	08-13-24	1030	J	1240	J	1C	Yellow	08-09-24B
24	08-14-24			1238	J			
48 Termination	08-15-24			1235	J			

*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):	08-03-24
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	08-02-24 1200 TO 08-03-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.38 Temperature (°C) 24.8

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ^{1d}	8 ^{2d}	3 ^{7d}	2 ^{8d}	1 ^{9d}	2 ^{8d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9	8	2 ^{1d}	1 ^{1d}	1	0 ^{2d}	0	0
Mean Survival	100%		100%		85%		15%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1312.5
Upper 95% confidence limit (mg KCl/L)	1450.8
48-hour LC ₅₀ (mg KCl/L)	1383.2

Comments:



Acute Silverside Test-24 Hr Survival													
Start Date:	8/13/2024	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	8/15/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride								
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina								
Comments:													
Conc-mg/L	1	2											
D-Control	1.0000	1.0000											
1000	1.0000	1.0000											
1250	0.9000	0.8000											
1500	0.3000	0.2000											
1750	0.1000	0.2000											
2000	0.0000	0.0000											
Transform: Arcsin Square Root													
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2089	0	20	
*1250	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.191	2.850	0.2089	3	20	
*1500	0.2500	0.2500	0.5216	0.4636	0.5796	15.723	2	12.148	2.850	0.2089	15	20	
*1750	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	13.907	2.850	0.2089	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	1000	1250	1118.03	0.1042	0.10687	0.48374	0.00537	7.6E-05	4, 5				
Treatments vs D-Control													
Maximum Likelihood-Probit													
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter			
Slope	16.1221	2.74859	10.7349 21.5093	0	3.03416	7.81472	0.38639	3.15471	0.06203	4			
Intercept	-45.86	8.69957	-62.912 -28.809										
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	1024.26	848.155 1131.45										
EC05	3.355	1128.97	978.136 1221.46										
EC10	3.718	1189.1	1054.24 1273.72										
EC15	3.964	1231.46	1108.09 1311.2										
EC20	4.158	1266.2	1152.15 1342.58										
EC25	4.326	1296.79	1190.65 1370.89										
EC40	4.747	1377.19	1289 1449.92										
EC50	5.000	1427.93	1347.47 1504.72										
EC60	5.253	1480.54	1404.04 1566.66										
EC75	5.674	1572.33	1492.75 1687.25										
EC80	5.842	1610.31	1526.41 1741.14										
EC85	6.036	1655.75	1564.96 1808.03										
EC90	6.282	1714.74	1612.9 1898.16										
EC95	6.645	1806.06	1683.82 2043.51										
EC99	7.326	1990.69	1819.79 2354.07										

Response

Dose mg/L

1-tail, 0.05 level of significance

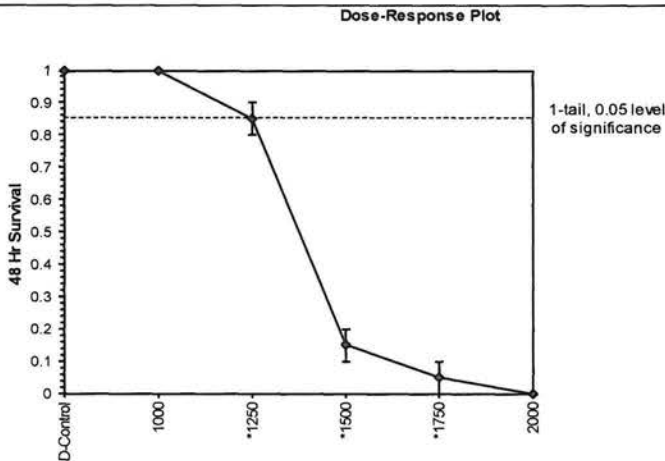
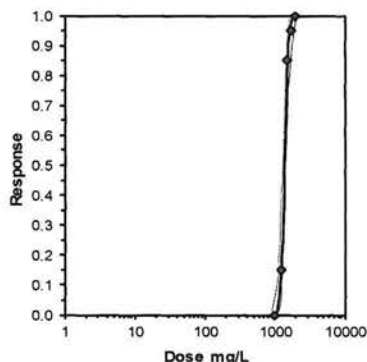
24 Hr Survival

Dose mg/L

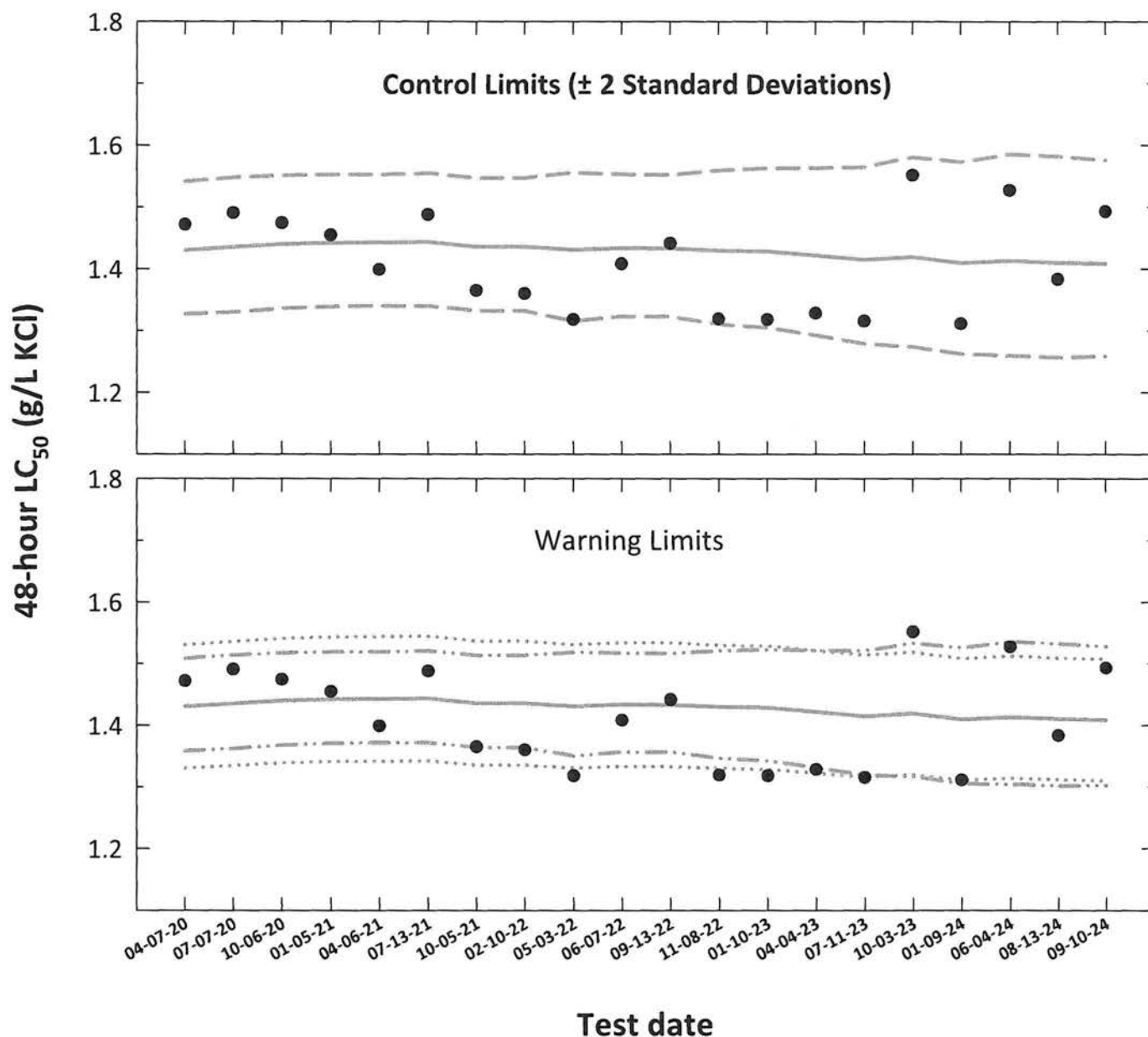
1-tail, 0.05 level of significance

Acute Silverside Test-48 Hr Survival													
Start Date:	8/13/2024	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	8/15/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride								
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina								
Comments:													
Conc-mg/L	1	2											
D-Control	1.0000	1.0000											
1000	1.0000	1.0000											
1250	0.9000	0.8000											
1500	0.2000	0.1000											
1750	0.1000	0.0000											
2000	0.0000	0.0000											
Transform: Arcsin Square Root													
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2330	0	20	
*1250	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	2.861	2.850	0.2330	3	20	
*1500	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	12.469	2.850	0.2330	17	20	
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	14.334	2.850	0.2330	19	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	
Auxiliary Tests													
								Statistic	Critical	Skew	Kurt		
Normality of the data set cannot be confirmed													
Equality of variance cannot be confirmed													
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df	
Dunnett's Test			1000	1250	1118.03		0.12079	0.12388	0.64531	0.00668	6.4E-05	4, 5	
Treatments vs D-Control													
Maximum Likelihood-Probit													
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	20.7256	3.99919	12.8872	28.564	0	2.23037	7.81472	0.52599	3.1409	0.04825	3		
Intercept	-60.097	12.5882	-84.77	-35.424									
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	1068.2	895.879	1162.7									
EC05	3.355	1152.22	1008.52	1232.47									
EC10	3.718	1199.68	1073.15	1272.65									
EC15	3.964	1232.8	1118.31	1301.39									
EC20	4.158	1259.77	1154.89	1325.46									
EC25	4.326	1283.38	1186.58	1347.2									
EC40	4.747	1344.85	1266.19	1408.16									
EC50	5.000	1383.24	1312.46	1450.75									
EC60	5.253	1422.73	1356.36	1499.1									
EC75	5.674	1490.88	1423.44	1593.27									
EC80	5.842	1518.82	1448.38	1635.19									
EC85	6.036	1552.05	1476.62	1687.01									
EC90	6.282	1594.9	1511.33	1756.42									
EC95	6.645	1660.59	1561.97	1867.35									
EC99	7.326	1791.2	1657.09	2100.35									

Dose (mg/L)	48 Hr Survival	Significance
D-Control	1.00	
1000	1.00	
*1250	0.85	*
*1500	0.15	*
*1750	0.05	*
2000	0.00	



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



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

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		CT	Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT		S	Control Limits CT - 2S CT + 2S		Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV		10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}	
1	04-07-20	1.4716	0.1678	0.1554	0.0163	1.4302	1.3269	1.5414	1.3580	1.5079	1.3300	1.5303
2	07-07-20	1.4906	0.1734	0.1569	0.0165	1.4351	1.3302	1.5483	1.3620	1.5139	1.3347	1.5356
3	10-06-20	1.4741	0.1685	0.1583	0.0162	1.4397	1.3362	1.5513	1.3678	1.5172	1.3390	1.5405
4	01-05-21	1.4546	0.1627	0.1590	0.0161	1.4420	1.3392	1.5528	1.3707	1.5189	1.3411	1.5430
5	04-06-21	1.3988	0.1458	0.1591	0.0160	1.4424	1.3402	1.5525	1.3715	1.5187	1.3415	1.5434
6	07-13-21	1.4877	0.1725	0.1594	0.0162	1.4434	1.3399	1.5549	1.3717	1.5207	1.3424	1.5444
7	10-05-21	1.3650	0.1351	0.1571	0.0162	1.4358	1.3326	1.5470	1.3639	1.5132	1.3353	1.5363
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 95

Dilution Preparation:

Test concentrations (mg/L KCl)	1000	1250	1500	1750	2000
mL Stock solution	10.0	12.5	15.0	17.5	20.0
mL Dilution water	490.0	487.50	485.0	482.5	480.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 7319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, SaltSW	pH (S.U.)	7.63	7.67	7.77
	Dissolved oxygen (mg/L)	7.7	7.5	7.4
	*Salinity (ppt)	25.0	25.4	25.7
	*Alkalinity (mg/L CaCO ₃)	95		
	*Temperature (°C)	25.1	25.0	25.2
1000 mg/L	pH (S.U.)	7.66	7.65	7.75
	Dissolved oxygen (mg/L)	7.7	7.6	7.5
	*Salinity (ppt)	25.7	25.9	26.2
	*Temperature (°C)	25.2	25.2	25.0
1250 mg/L	pH (S.U.)	7.66	7.65	7.76
	Dissolved oxygen (mg/L)	7.7	7.5	7.4
	*Salinity (ppt)	25.7	26.2	26.4
	*Temperature (°C)	25.2	25.2	25.2
1500 mg/L	pH (S.U.)	7.67	7.64	7.75
	Dissolved oxygen (mg/L)	7.8	7.6	7.4
	*Salinity (ppt)	26.0	26.2	26.7
	*Temperature (°C)	25.2	25.2	25.0
1750 mg/L	pH (S.U.)	7.67	7.65	7.76
	Dissolved oxygen (mg/L)	7.9	7.6	7.6
	*Salinity (ppt)	25.8	26.3	26.7
	*Temperature (°C)	25.2	25.0	25.1
2000 mg/L	pH (S.U.)	7.68	7.62	7.75
	Dissolved oxygen (mg/L)	7.9	7.7	7.4
	*Salinity (ppt)	26.1	26.5	27.1
	*Temperature (°C)	25.0	25.3	25.1

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Salinity	1.0 ppt	SM 2520 B-2021	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2021	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 95

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09.10.24	* 1050	N	1258	N	1F	PINK	09.04.24A
24	09.11.24			1300	N			
48 Termination	09.12.24			1250	N			

*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):	08-31-24
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	08-31-24 1200 TO 08-31-24 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.41 Temperature (°C) 24.5

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ^u	10	9 ^u	7 ^u	2 ^{sd}	2 ^{sd}	0 ^{10d}	1 ^u
48 Termination	10	10	10	10	8 ^u	9 ^u	7 ^u	5 ^u	1 ^u	1 ^u	0	0 ^u
Mean Survival	100%		100%		85%		60%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1413.0
Upper 95% confidence limit (mg KCl/L)	1570.0
48-hour LC ₅₀ (mg KCl/L)	1493.0

Comments:

Test
Reviewed by:
N

Acute Silverside Test-24 Hr Survival

Start Date:	9/10/2024	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	9/12/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	1.0000
1500	0.9000	0.7000
1750	0.2000	0.2000
2000	0.0000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2826	0	20
1250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.816	2.830	0.2826	1	20
*1500	0.8000	0.8000	1.1201	0.9912	1.2490	16.280	2	2.924	2.830	0.2826	4	20
*1750	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	2	9.499	2.830	0.2826	16	20
*2000	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.736	2.830	0.2826	19	20

Auxiliary Tests

Normality of the data set cannot be confirmed

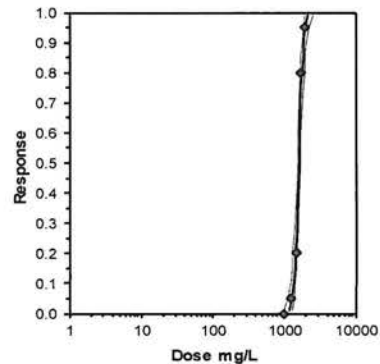
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.15746	0.16149	0.53119	0.00997	6.8E-05	5, 6

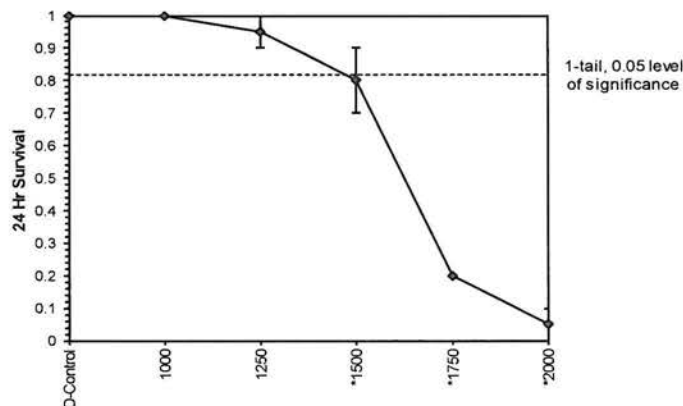
Treatments vs D-Control

Parameter	Maximum Likelihood-Probit								Mu	Sigma	Iter
	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value			
Slope	18.0466	3.2436	11.6891	24.404	0	1.73708	7.81472	0.62872	3.2069	0.05541	3
Intercept	-52.873	10.4184	-73.293	-32.453							

TSCR				
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1196.71	1002.88	1308.9
EC05	3.355	1305.43	1143.14	1400.51
EC10	3.718	1367.36	1224.45	1453.48
EC15	3.964	1410.8	1281.61	1491.45
EC20	4.158	1446.31	1328.11	1523.28
EC25	4.326	1477.48	1368.52	1552.06
EC40	4.747	1559.05	1470.48	1633
EC50	5.000	1610.27	1529.93	1689.77
EC60	5.253	1663.17	1586.52	1754.33
EC75	5.674	1754.98	1673.77	1879.94
EC80	5.842	1792.81	1706.62	1935.76
EC85	6.036	1837.93	1744.15	2004.72
EC90	6.282	1896.32	1790.72	2097.12
EC95	6.645	1986.29	1859.46	2245.07
EC99	7.326	2166.74	1990.63	2557.73



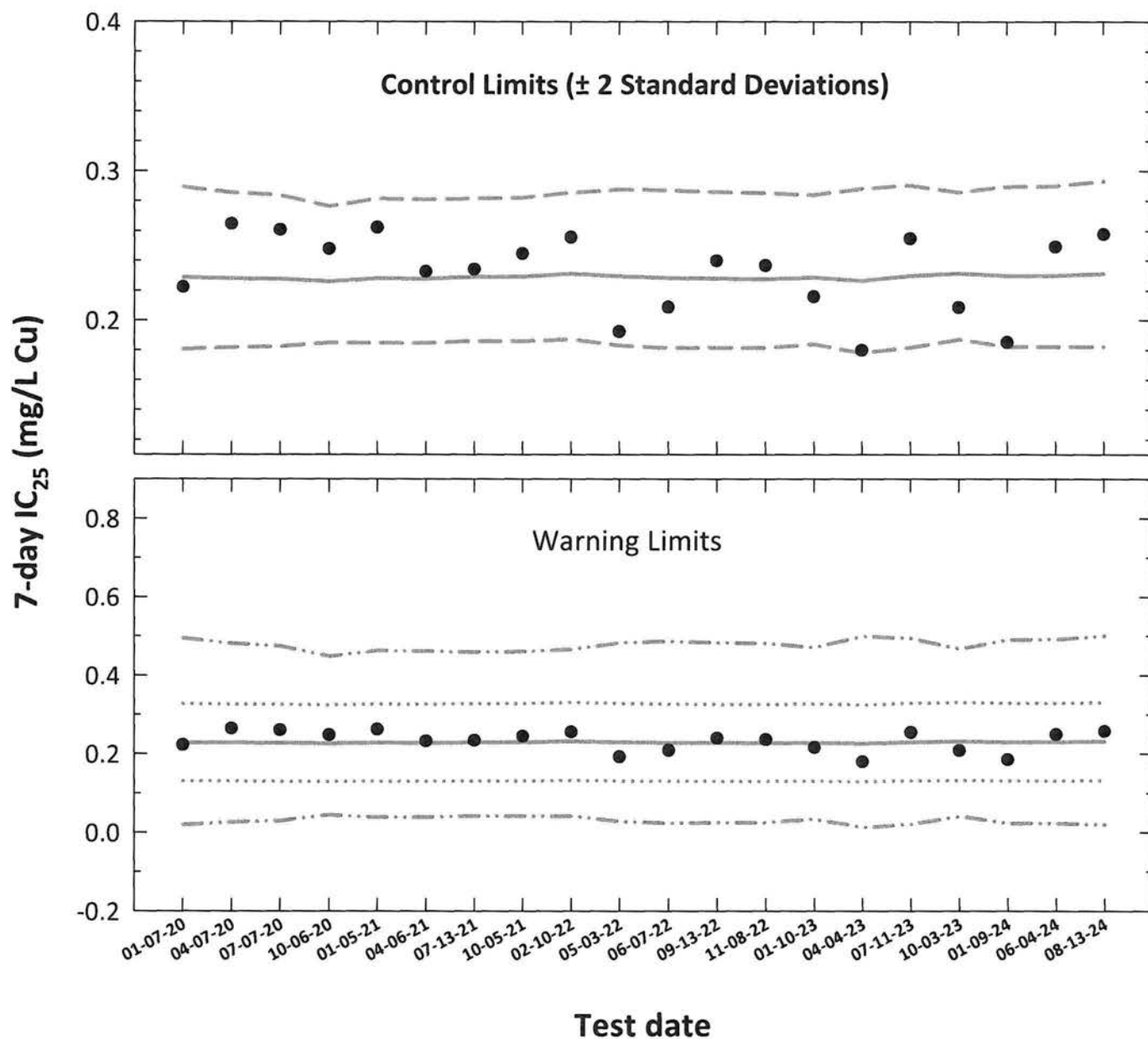
Dose-Response Plot



Acute Silverside Test-48 Hr Survival													
Start Date:	9/10/2024	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant								
End Date:	9/12/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride								
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina								
Comments:													
Conc-mg/L	1	2											
D-Control	1.0000	1.0000											
1000	1.0000	1.0000											
1250	0.8000	0.9000											
1500	0.7000	0.5000											
1750	0.1000	0.1000											
2000	0.0000	0.0000											
Transform: Arcsin Square Root													
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number	
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2253	0	20	
*1250	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	2.960	2.850	0.2253	3	20	
*1500	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	6.626	2.850	0.2253	8	20	
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	13.794	2.850	0.2253	18	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	
Auxiliary Tests													
Statistic Critical Skew Kurt													
Normality of the data set cannot be confirmed													
Equality of variance cannot be confirmed													
Hypothesis Test (1-tail, 0.05)													
	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df			
Dunnett's Test	1000	1250	1118.03		0.11538	0.11834	0.41737	0.00625	1.6E-04	4, 5			
Treatments vs D-Control													
Maximum Likelihood-Probit													
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter			
Slope	16.9676	2.9321	11.2207 22.7145	0	2.16136	7.81472	0.5396	3.17405	0.05894	3			
Intercept	-48.856	9.33187	-67.147 -30.566										
TSCR													
Point	Probits	mg/L	95% Fiducial Limits										
EC01	2.674	1088.79	908.197 1197.02										
EC05	3.355	1194.28	1040.9 1287.1										
EC10	3.718	1254.64	1118.2 1339.29										
EC15	3.964	1297.08	1172.73 1376.68										
EC20	4.158	1331.82	1217.23 1407.98										
EC25	4.326	1362.37	1256.02 1436.22										
EC40	4.747	1442.5	1354.69 1515.14										
EC50	5.000	1492.96	1412.99 1569.96										
EC60	5.253	1545.18	1469.11 1631.94										
EC75	5.674	1636.06	1556.66 1752.41										
EC80	5.842	1673.59	1589.79 1806.11										
EC85	6.036	1718.43	1627.69 1872.62										
EC90	6.282	1776.55	1674.77 1962.02										
EC95	6.645	1866.33	1744.31 2105.75										
EC99	7.326	2047.17	1877.3 2411.22										

1-tail, 0.05 level of significance

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



- **7-day IC₂₅** = 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 IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ $\pm S_{A.75}$ 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		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	01-07-20	0.2222	-0.6533	-0.6408	0.0511	0.2286	0.1807	0.2893	0.0189	0.4941	0.1303	0.3270
2	04-07-20	0.2646	-0.5774	-0.6422	0.0490	0.2279	0.1819	0.2856	0.0261	0.4809	0.1299	0.3260
3	07-07-20	0.2606	-0.5840	-0.6429	0.0479	0.2276	0.1825	0.2838	0.0295	0.4745	0.1297	0.3254
4	10-06-20	0.2479	-0.6057	-0.6457	0.0436	0.2261	0.1850	0.2763	0.0443	0.4483	0.1289	0.3233
5	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.2281	0.1848	0.2816	0.0382	0.4625	0.1300	0.3262
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

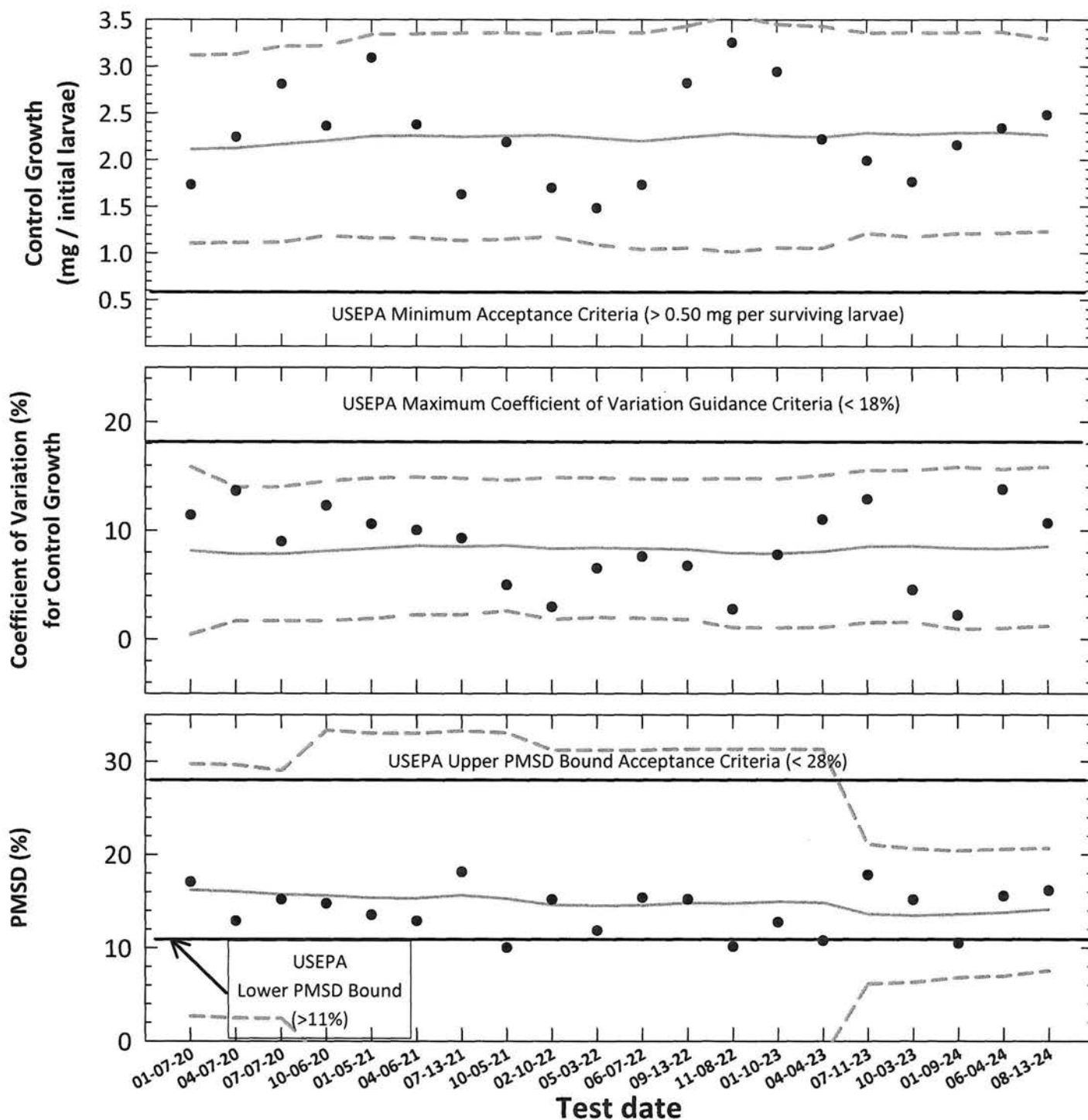
Warning Limits = Mean logarithmic $IC_{25} \pm 2CV$ or $S_{A,75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Menidia beryllina

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



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

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	01-07-20	100	1.732	11.4	0.2954	2.1123	1.105	3.120	8.1	0.4	15.9	16.2	2.7	29.7	
2	04-07-20	100	2.244	13.6	0.2889	2.123	1.114	3.131	7.8	1.7	14.0	16.1	2.5	29.6	
3	07-07-20	100	2.809	9.0	0.4264	2.166	1.117	3.215	7.8	1.7	14.0	15.7	2.4	29.0	
4	10-06-20	100	2.361	12.3	0.3484	2.205	1.188	3.221	8.1	1.7	14.6	15.6	-2.1	33.3	
5	01-05-21	100	3.093	10.6	0.4186	2.253	1.163	3.343	8.3	1.9	14.8	15.4	-2.3	33.0	
6	04-06-21	100	2.377	10.0	0.3062	2.258	1.167	3.349	8.6	2.2	14.9	15.3	-2.3	33.0	
7	07-13-21	100	1.631	9.3	0.2956	2.247	1.136	3.359	8.5	2.2	14.8	15.7	-2.0	33.3	
8	10-05-21	100	2.189	5.0	0.2192	2.258	1.153	3.363	8.6	2.6	14.7	15.3	-2.5	33.1	
9	02-10-22	100	1.701	3.0	0.2583	2.266	1.180	3.351	8.4	1.8	14.9	14.6	-2.0	31.2	
10	05-03-22	100	1.483	6.5	0.1756	2.232	1.092	3.372	8.4	2.0	14.9	14.5	-2.1	31.2	
11	06-07-22	100	1.733	7.6	0.2665	2.201	1.041	3.361	8.3	1.9	14.8	14.6	-2.1	31.2	
12	09-13-22	100	2.822	6.8	0.4290	2.243	1.057	3.430	8.3	1.8	14.7	14.8	-1.7	31.3	
13	11-08-22	100	3.255	2.7	0.3294	2.280	1.014	3.545	7.9	1.0	14.8	14.8	-1.8	31.3	
14	01-10-23	100	2.945	7.8	0.3755	2.257	1.062	3.453	7.9	1.0	14.8	15.0	-1.4	31.3	
15	04-04-23	100	2.220	11.0	0.2389	2.243	1.053	3.433	8.1	1.1	15.1	14.9	-1.6	31.3	
16	07-11-23	100	1.992	12.9	0.3549	2.287	1.213	3.360	8.5	1.5	15.6	13.6	6.2	21.1	
17	10-03-23	100	1.763	4.5	0.2673	2.270	1.174	3.366	8.5	1.5	15.5	13.5	6.3	20.6	
18	01-09-24	100	2.156	2.2	0.2257	2.287	1.211	3.364	8.4	0.9	15.8	13.6	6.8	20.4	
19	06-04-24	100	2.339	13.8	0.3637	2.291	1.215	3.368	8.3	1.0	15.6	13.8	7.0	20.6	
20	08-13-24	100	2.479	10.7	0.4001	2.266	1.235	3.297	8.5	1.2	15.9	14.1	7.6	20.7	

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

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

CV = Coefficient of variation for control growth.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

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

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

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

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

Species: Menidia beryllina

MbCuCR Test Number: **147**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2311				
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:	1B
Batch:	AI Mb 08-03-24	Artemia CHM number:	CHM1294
Hatch dates and times:	08-02-24 1200 to 08-03-24 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = 7.38 Temperature (°C) = 24.8	Date / Time in oven:	08-20-24 1000
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	60 °C
		Date / Time out of oven:	08-21-24 1000
		*Final oven temperature:	60 °C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	08-13-24	1030	JL	1400	JL	1200	JL	NA	08-09-24 B
1	08-14-24	0600	JL	1320	JL	0800	JL	↓	↓
2	08-15-24	0600	JL	1210	JL	0800	JL	↓	08-12-24
3	08-16-24	0600	JL	1130	JL	0800	JL	↓	↓
4	08-17-24	0700	JL	1200	JL	0910	JL	↓	08-14-24
5	08-18-24	0700	JL	1200	JL	0900	JL	↓	08-15-24
6	08-19-24	0700	JL	1200	JL	0900	JL	↓	↓
7	08-20-24					1000	JL		

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (%)	0.286
Average weight per initial larvae:	2.479		NOEC (%)	0.1
Average weight per surviving larvae:	2.479	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	6.141
			IC ₂₅ (%)	0.258

Species: Menidia beryllina

MbCuCR Test Number: 147

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>Gold</u> Analyst: <u>En</u> Date: <u>07-23-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JK</u> Date: <u>08-21-24</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 (%)												
2.479 2.806 -13.27. 2.574 -3.87.												

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: Menidia beryllina

MbCuCR Test Number: 147

Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L																		
	M	N	O	P	Q	R	S	T	U	V	W	X															
0	10	10	10	10	10	10	10	10	10	10	10	10															
1	10	10	10	10	10	10	10	10	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}															
2	10	10	10	10	10	10	10	10																			
3	10	10	10	10	10	10	10	10																			
4	10	10	10	10	10	10	10	10																			
5	10	10	10	10	9 ^{1d}	10	9 ^{1d}	10																			
6	10	10	10	10	9	9 ^{1d}	9	10																			
7	10	10	10	10	8 ^{1d}	9	9	9 ^{1d}																			
*A = Pan weight (mg) Tray color code: <u>Gold</u> Analyst: <u>JS</u> Date: <u>07-23-24</u>													14.45				12.10	14.78	14.52	15.21	13.69	12.48	11.60	12.25	13.07	12.74	15.67
*B = Pan + Larvae weight (mg) Analyst: <u>JS</u> Date: <u>08-21-24</u>													43.09				37.66	38.46	35.71	33.80	34.60	42.85	39.88				
C = Larvae weight (mg) = B - A Analyst: <u>JS</u>													28.64				25.56	23.68	21.19	18.59	20.91	30.37	28.28				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JS</u>													2.864				2.556	2.368	2.119	1.859	2.091	3.037	2.828	0			
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		2.477		0.17		0.1001 2.454					1.07		0		100%										

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

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

Comments:

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

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

Test number: 147

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Not for Compliance Assessment, Internal Laboratory QC	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / initial number of larvae (mg)	Mean survival (%)	Mean weight/ initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	11.61	36.58	24.97	2.497	2.497	10.7	2.498	100.0	2.479	10.7	Not applicable
	B	10	10	13.75	38.73	24.98	2.498	2.498		2.138				
	C	10	10	11.98	33.36	21.38	2.138	2.138		2.784				
	D	10	10	14.57	42.41	27.84	2.784	2.784		2.725				
0.025	E	10	10	12.59	39.84	27.25	2.725	2.725	6.9	3.087	100.0	2.806	6.9	-13.2
	F	10	10	12.31	43.18	30.87	3.087	3.087		2.765				
	G	10	10	13.42	41.07	27.65	2.765	2.765		2.648				
	H	10	10	11.76	38.24	26.48	2.648	2.648		2.855				
0.050	I	10	10	14.23	42.78	28.55	2.855	2.855	7.6	2.544	100.0	2.574	7.6	-3.8
	J	10	10	12.99	38.43	25.44	2.544	2.544		2.409				
	K	10	10	11.72	35.81	24.09	2.409	2.409		2.488				
	L	10	10	11.38	36.26	24.88	2.488	2.488		2.864				
0.100	M	10	10	14.45	43.09	28.64	2.864	2.864	12.7	2.556	100.0	2.477	12.7	0.1
	N	10	10	12.10	37.66	25.56	2.556	2.556		2.368				
	O	10	10	14.78	38.46	23.68	2.368	2.368		2.119				
	P	10	10	14.52	35.71	21.19	2.119	2.119		1.859				
0.200	Q	10	8	15.21	33.80	18.59	2.324	2.324	19.6	2.091	87.5	2.454	23.1	1.0
	R	10	9	13.69	34.60	20.91	2.323	2.323		3.037				
	S	10	9	12.48	42.85	30.37	3.374	3.374		2.828				
	T	10	9	11.60	39.88	28.28	3.142	3.142		0.000				
0.500	U	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	V	10	0	0.00	0.00	0.00	0.000	0.000		0.000				
	W	10	0	0.00	0.00	0.00	0.000	0.000		0.000				
	X	10	0	0.00	0.00	0.00	0.000	0.000		0.000				

Dunnett's MSD value: 0.4001
PMSD: 16.1

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	8/13/2024	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	8/20/2024	Lab ID:	ETS-Envr. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.025	1.0000	1.0000	1.0000	1.0000
0.05	1.0000	1.0000	1.0000	1.0000
0.1	1.0000	1.0000	1.0000	1.0000
0.2	0.8000	0.9000	0.9000	0.9000
0.5	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.2	0.8750	0.8750	1.2136	1.1071	1.2490	5.846	4	10.00	10.00	5	40
0.5	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

Auxiliary Tests

Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)

Equality of variance cannot be confirmed

Statistic	Critical	Skew	Kurt
0.5089	0.868	-2.7962	11.6732

Hypothesis Test (1-tail, 0.05)

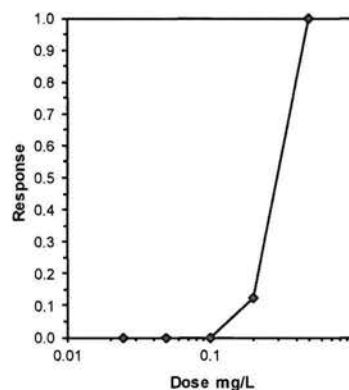
NOEC	LOEC	ChV	TU
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Steel's Many-One Rank Test

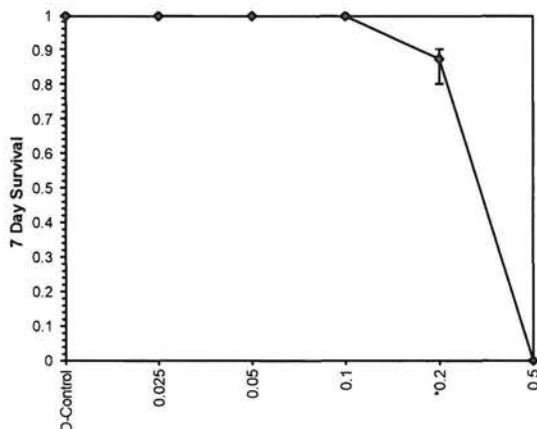
0.1	0.2	0.14142
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Trimmed Spearman-Kärber

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



Dose-Response Plot



Larval Fish Growth and Survival Test-7 Day Growth

Start Date: 8/13/2024 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
End Date: 8/20/2024 Lab ID: ETS-Envr. Testing Sol. Sample Type: CUSO4
Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina
Comments:

Conc-mg/L	1	2	3	4
D-Control	2.4970	2.4980	2.1380	2.7840
0.025	2.7250	3.0870	2.7650	2.6480
0.05	2.8550	2.5440	2.4090	2.4880
0.1	2.8640	2.5560	2.3680	2.1190
0.2	1.8590	2.0910	3.0370	2.8280
0.5	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	2.4793	1.0000	2.4793	2.1380	2.7840	10.671	4				2.6428	1.0000
0.025	2.8063	1.1319	2.8063	2.6480	3.0870	6.890	4	-1.872	2.290	0.4001	2.6428	1.0000
0.05	2.5740	1.0382	2.5740	2.4090	2.8550	7.589	4	-0.542	2.290	0.4001	2.5740	0.9740
0.1	2.4768	0.9990	2.4768	2.1190	2.8640	12.684	4	0.014	2.290	0.4001	2.4768	0.9372
0.2	2.4538	0.9897	2.4538	1.8590	3.0370	23.121	4				2.4538	0.9285
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests

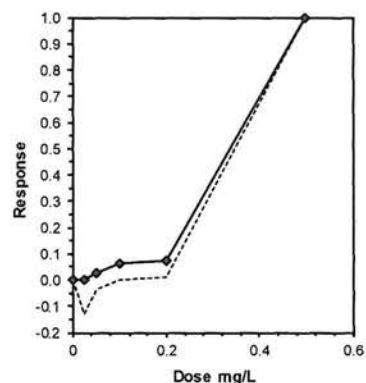
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)
Bartlett's Test indicates equal variances ($p = 0.82$)

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.1	>0.1			0.40012	0.16139	0.09596	0.06106	0.24752	3, 12

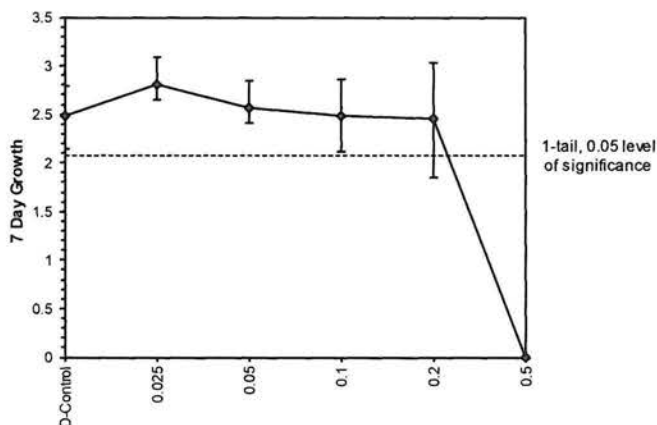
Treatments vs D-Control

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	0.0826	0.0689	0.0118	0.2944
IC10	0.2092	0.0558	0.0000	0.2425
IC15	0.2254	0.0385	0.0242	0.2568
IC20	0.2415	0.0267	0.1128	0.2711
IC25	0.2577	0.0234	0.1455	0.2854
IC40	0.3061	0.0185	0.2199	0.3283
IC50	0.3384	0.0154	0.2666	0.3569



Dose-Response Plot



Species: Menidia beryllina

MbCuCR Test Number: 147

Daily Chemistry:

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

Concentration		Parameter	Day (Analyst identified for each day, performed pH and D.O. measurements only.)					
			0		1		2	
			Analyst	Analyst	Analyst	Analyst	Analyst	Analyst
			JD	PH	PH N	N	N	PH N
CONTROL, SaltSW	pH (S.U.)		7.66	7.71	7.50	7.49	7.64	7.50
	Dissolved oxygen (mg/L)		7.9	7.7	7.0	7.5	8.0	7.6
	Salinity (ppt)		25.0	25.2	25.0	25.2	24.9	25.0 (25.3) 5-16-24
	Alkalinity (mg CaCO ₃ /L)		100				86	
	Temperature (°C)		24.9	24.6	24.5	24.9	24.6	24.8
0.025 mg/L	pH (S.U.)		7.65	7.71	7.78	7.52	7.73	7.47
	Dissolved oxygen (mg/L)		7.9	7.7	7.0	7.6	8.1	7.5
	Salinity (ppt)		25.2	25.4	25.2	25.4	25.2	25.3
	Temperature (°C)		25.0	24.5	24.4	24.7	24.6	24.7
0.05 mg/L	pH (S.U.)		7.66	7.70	7.78	7.52	7.73	7.49
	Dissolved oxygen (mg/L)		7.9	7.7	7.0	7.6	8.1	7.6
	Salinity (ppt)		25.4	25.5	25.2	25.4	25.2	25.4 (25.6) 5-16-24
	Temperature (°C)		24.9	24.4	24.4	24.9	24.9	24.9
0.1 mg/L	pH (S.U.)		7.66	7.71	7.79	7.53	7.73	7.49
	Dissolved oxygen (mg/L)		7.9	7.0	7.0	7.6	8.1	7.6
	Salinity (ppt)		25.2	25.3	25.1	25.3	25.2	25.2
	Temperature (°C)		24.9	24.7	24.5	24.8	24.6	25.0
0.2 mg/L	pH (S.U.)		7.67	7.70	7.79	7.53	7.74	7.49
	Dissolved oxygen (mg/L)		8.0	7.7	7.0	7.5	8.2	7.7
	Salinity (ppt)		25.4	25.5	25.1	25.3	25.2	25.4
	Temperature (°C)		25.1	24.6	24.4	25.0	24.9	25.0
0.5 mg/L	pH (S.U.)		7.67	7.66	7.78			
	Dissolved oxygen (mg/L)		8.0	7.7	7.0			
	Salinity (ppt)		25.3	25.2	25.0			
	Alkalinity (mg CaCO ₃ /L)		NA					
	Temperature (°C)		24.9	24.5	24.4			
			Initial	Final	Initial	Final	Initial	Final

Species: Menidia beryllina

MbCuCR Test Number: 147

		Day							
		(Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
Analyst		JP	BSL	BSL	N	N	JP	JP	N
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.42	7.57	7.72	7.00	6.03	7.78	7.78	7.33
	Dissolved oxygen (mg/L)	7.9	7.5	7.7	6.3	7.0	7.0	7.7	6.9
	Salinity (ppt)	25.0	25.4	25.3	25.4	25.2	25.3	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)	—	—	7.9	—	92	—	—	—
	Temperature (°C)	24.6	24.9	24.8	24.9	24.6	24.8	24.6	25.3
0.025 mg/L	pH (S.U.)	7.59	7.56	7.80	7.03	0.08	7.76	7.90	7.09
	Dissolved oxygen (mg/L)	0.0	7.5	7.8	6.0	7.6	6.9	7.8	7.1
	Salinity (ppt)	25.0	25.4	25.1	25.5	25.0	25.2	25.3	25.4
	Temperature (°C)	24.5	24.7	24.9	24.7	24.6	24.9	24.5	25.0
0.05 mg/L	pH (S.U.)	7.59	7.59	7.80	7.03	0.08	7.76	7.90	7.05
	Dissolved oxygen (mg/L)	0.0	7.6	7.9	6.5	7.6	7.1	7.8	7.1
	Salinity (ppt)	25.0	25.4	25.1	25.5	24.9	25.2	25.3	25.4
	Temperature (°C)	24.5	24.7	24.9	24.7	24.6	24.9	24.5	25.3
0.1 mg/L	pH (S.U.)	7.59	7.55	7.80	7.05	0.08	7.74	7.90	7.09
	Dissolved oxygen (mg/L)	0.0	7.6	7.9	6.0	7.7	7.1	7.8	7.1
	Salinity (ppt)	25.0	25.1	25.1	25.6	24.0	25.0	25.3	25.4
	Temperature (°C)	24.5	24.8	24.9	24.8	24.6	25.0	24.6	25.2
0.2 mg/L	pH (S.U.)	7.60	7.59	7.80	7.03	0.09	7.77	7.90	7.02
	Dissolved oxygen (mg/L)	0.0	7.5	7.9	6.6	7.0	7.0	7.8	7.2
	Salinity (ppt)	24.9	25.1	25.1	25.6	24.0	25.1	25.3	25.4
	Temperature (°C)	24.5	24.5	24.9	25.0	24.7	24.8	24.4	25.2
0.5 mg/L	pH (S.U.)	—	—	—	—	—	—	—	—
	Dissolved oxygen (mg/L)	—	—	—	—	—	—	—	—
	Salinity (ppt)	25.16	24	—	—	—	—	—	—
	Temperature (°C)	24.4	—	—	—	—	—	—	—
Initial			Final	Initial	Final	Initial	Final	Initial	Final