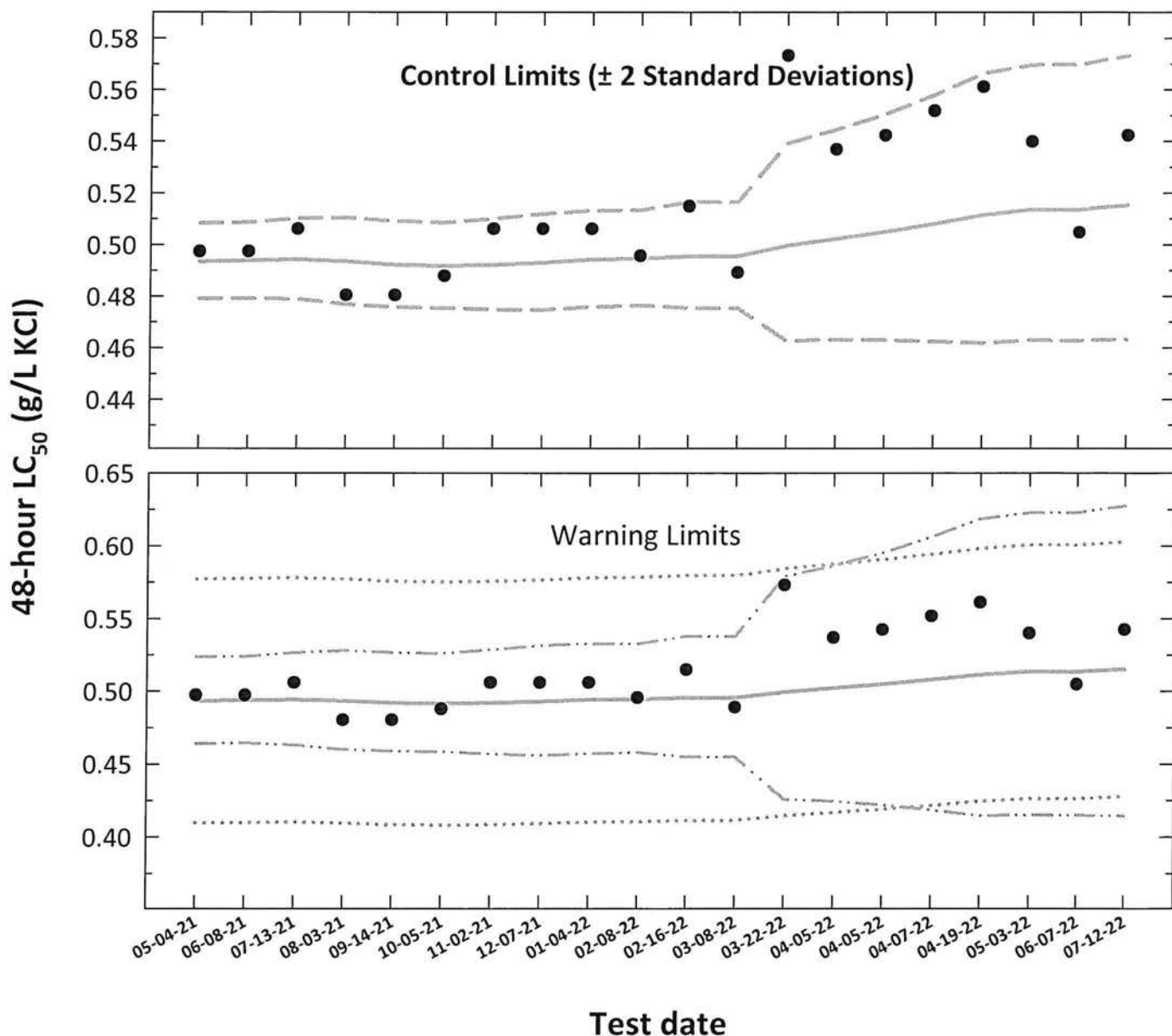


Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	05-04-21	0.4974	-0.3033	-0.3068	0.0064	0.4934	0.4790	0.5083	0.4642	0.5236	0.4096	0.5773
2	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
3	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
4	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
5	09-14-21	0.4804	-0.3184	-0.3079	0.0074	0.4922	0.4758	0.5091	0.4589	0.5266	0.4085	0.5759
6	10-05-21	0.4879	-0.3117	-0.3083	0.0073	0.4917	0.4754	0.5086	0.4586	0.5260	0.4081	0.5753
7	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
8	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
9	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
10	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
11	02-16-22	0.5149	-0.2882	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
12	03-08-22	0.4892	-0.3105	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
13	03-22-22	0.5733	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
14	04-05-22	0.5369	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
15	04-05-22	0.5424	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
16	04-07-22	0.5519	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
17	04-19-22	0.5612	-0.2509	-0.2911	0.0221	0.5115	0.4620	0.5664	0.4146	0.6188	0.4246	0.5985
18	05-03-22	0.5399	-0.2677	-0.2894	0.0225	0.5136	0.4630	0.5698	0.4151	0.6229	0.4263	0.6009
19	06-07-22	0.5048	-0.2969	-0.2894	0.0225	0.5135	0.4629	0.5697	0.4149	0.6229	0.4262	0.6009
20	07-12-22	0.5424	-0.2657	-0.2879	0.0231	0.5153	0.4633	0.5732	0.4144	0.6276	0.4277	0.6029

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 # 255

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 #: 2101

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	U	U	U
Control, SaltSW	pH (S.U.)	7.01	7.70	7.77
	Dissolved oxygen (mg/L)	0.0	0.0	7.7
	*Salinity (ppt)	24.9	25.0	25.2
	*Alkalinity (mg/L CaCO ₃)	99		
	*Temperature (°C)	25.0	25.2	25.6
250 mg/L	pH (S.U.)	7.00	7.74	7.77
	Dissolved oxygen (mg/L)	7.9	0.0	7.7
	*Salinity (ppt)	25.3	25.3	25.4
	*Temperature (°C)	25.1	25.0	25.2
375 mg/L	pH (S.U.)	7.07	7.75	7.77
	Dissolved oxygen (mg/L)	7.0	0.0	7.0
	*Salinity (ppt)	25.3	25.4	25.4
	*Temperature (°C)	25.1	25.2	25.2
500 mg/L	pH (S.U.)	7.00	7.75	7.77
	Dissolved oxygen (mg/L)	7.0	0.0	7.0
	*Salinity (ppt)	25.4	25.6	25.7
	*Temperature (°C)	25.0	25.2	25.2
750 mg/L	pH (S.U.)	7.00	7.75	
	Dissolved oxygen (mg/L)	7.9	0.0	
	*Salinity (ppt)	25.7	25.8	
	*Temperature (°C)	25.2	25.1	
1000 mg/L	pH (S.U.)	7.00	7.75	
	Dissolved oxygen (mg/L)	0.0	0.0	
	*Salinity (ppt)	25.7	25.9	
	*Temperature (°C)	25.0	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	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 # 255

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-12-22	* 1022	J	1310	J	6E	UHT BWE	07-07-21A
24	07-13-22			1308	K			
48 Termination	07-14-22			1311	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):	07-11-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	07-10-22 1200 TO 07-11-22
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.84
	Temperature (°C) 25.1

Survival Data (number of living organisms):

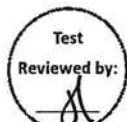
Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^d	8 ^{ud}	0 ^{100d}	0 ^{100d}	0 ^{100d}	0 ^{100d}
48 Termination	10	10	10	10	10	10	6 ^{3d}	7 ^{1d}	0	0	0	0
Mean Survival	100%		100%		100%		65%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	503.8
Upper 95% confidence limit (mg KCl/L)	584.0
48-hour LC ₅₀ (mg KCl/L)	542.4

Comments:



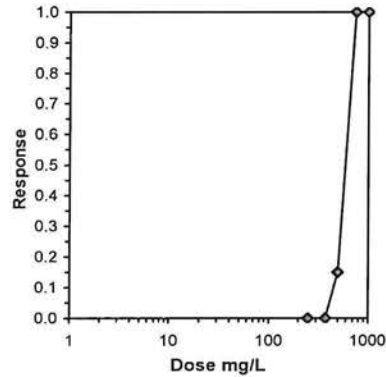
Acute Mysid Test-24 Hr Survival

Start Date: 7/12/2022	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 7/14/2022	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

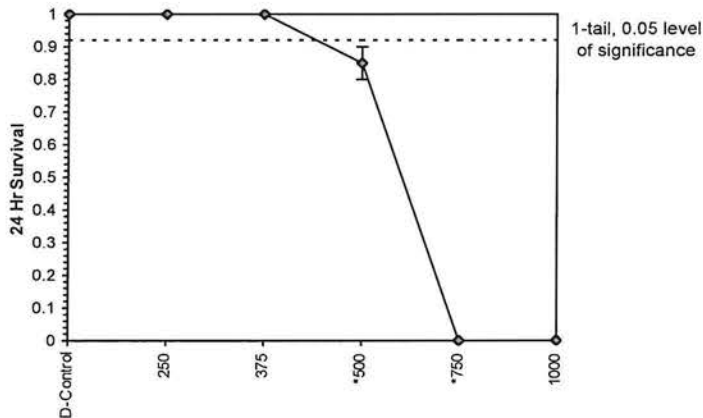
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.9000	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							1-Tailed			Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
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
Dunnett's Test	375	500	433.013	0.05495
Treatments vs D-Control				
Trimmed Spearman-Kärber				
Trim Level	EC50	95% CL		
0.0%	581.35	550.05	614.43	
5.0%	586.14	549.69	625.01	
10.0%	589.52	543.45	639.50	
20.0%	590.85	564.96	617.93	
Auto-0.0%	581.35	550.05	614.43	



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 7/12/2022	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 7/14/2022	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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.6000	0.7000
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	0.0947	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0947	0	20
*500	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	14.247	2.850	0.0947	7	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	37.715	2.850	0.0947	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

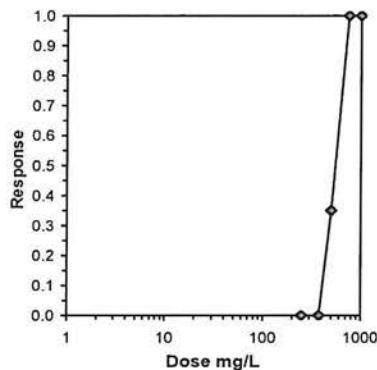
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)

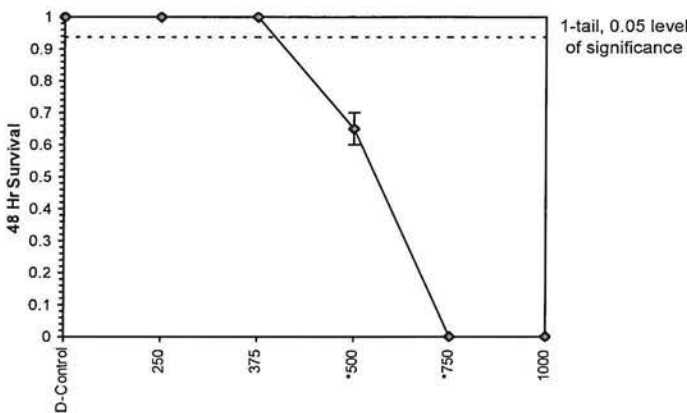
	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.03789	0.03886	0.59923	0.0011	8.8E-07	4, 5

Treatments vs D-Control Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	542.42	503.77	584.04
5.0%	543.63	500.55	590.41
10.0%	544.81	495.80	598.66
20.0%	547.01	478.84	624.87
Auto-0.0%	542.42	503.77	584.04



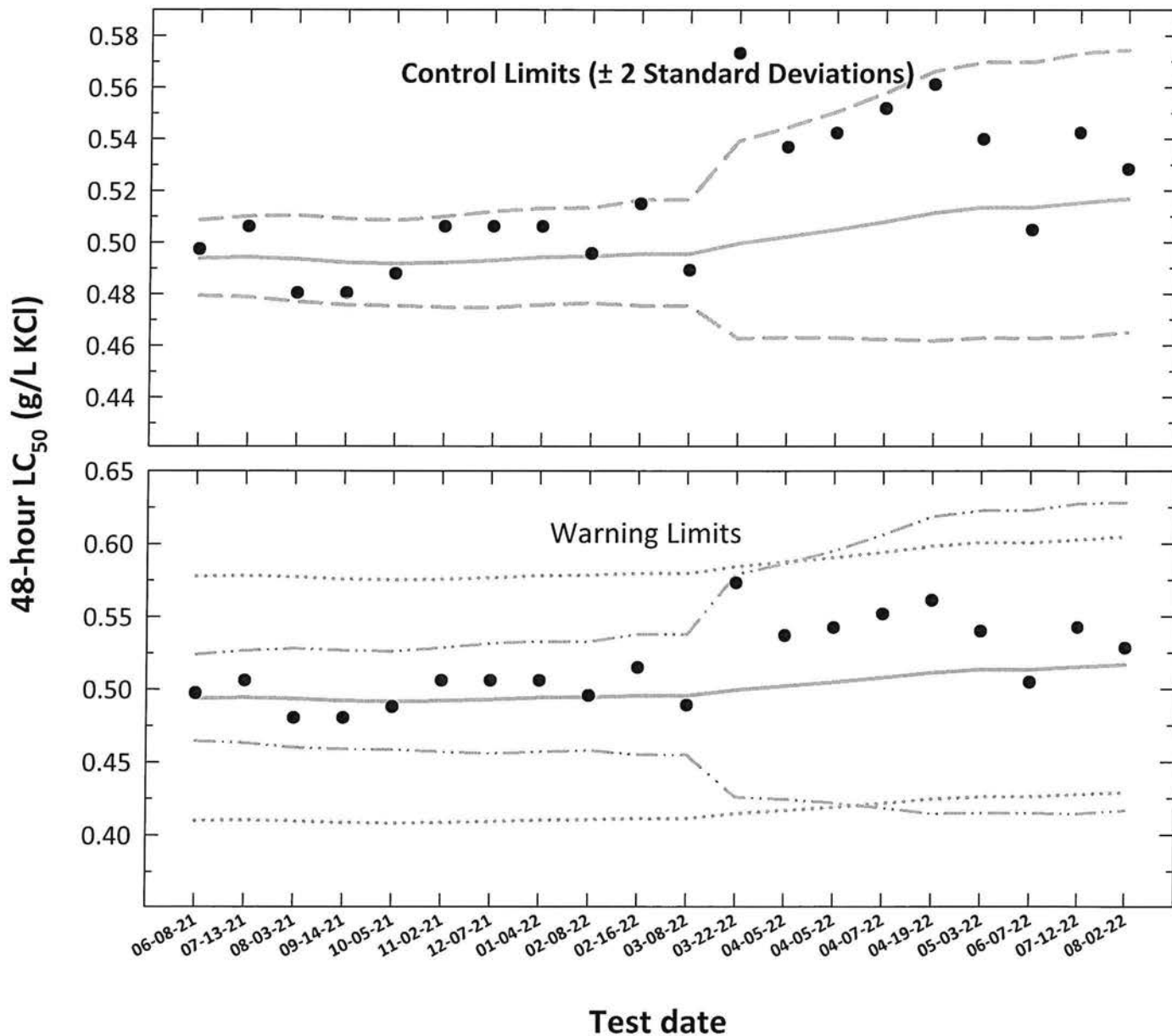
Dose-Response Plot



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 \pm 2 standard deviations converted to anti-logarithmic values)
- · - · - Laboratory Warning Limits (mean logarithmic LC₅₀ \pm 2 coefficient of variations converted to anti-logarithmic values)
- · · · · USEPA Warning Limits (mean logarithmic LC₅₀ \pm S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV Warning Limits	
			CT - 2S	CT + 2S	CT - 2CV		CT + 2CV	CT - S _{A,10}	CT + S _{A,10}			
1	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
2	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
3	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
4	09-14-21	0.4804	-0.3184	-0.3079	0.0074	0.4922	0.4758	0.5091	0.4589	0.5266	0.4085	0.5759
5	10-05-21	0.4879	-0.3117	-0.3083	0.0073	0.4917	0.4754	0.5086	0.4586	0.5260	0.4081	0.5753
6	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
7	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
8	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
9	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
10	02-16-22	0.5149	-0.2882	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
11	03-08-22	0.4892	-0.3105	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
12	03-22-22	0.5733	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
13	04-05-22	0.5369	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
14	04-05-22	0.5424	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
15	04-07-22	0.5519	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
16	04-19-22	0.5612	-0.2509	-0.2911	0.0221	0.5115	0.4620	0.5664	0.4146	0.6188	0.4246	0.5985
17	05-03-22	0.5399	-0.2677	-0.2894	0.0225	0.5136	0.4630	0.5698	0.4151	0.6229	0.4263	0.6009
18	06-07-22	0.5048	-0.2969	-0.2894	0.0225	0.5135	0.4629	0.5697	0.4149	0.6229	0.4262	0.6009
19	07-12-22	0.5424	-0.2657	-0.2879	0.0231	0.5153	0.4633	0.5732	0.4144	0.6276	0.4277	0.6029
20	08-02-22	0.5283	-0.2771	-0.2866	0.0229	0.5169	0.4651	0.5744	0.4167	0.6282	0.4290	0.6048

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 # 756

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 #: 2118

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<u>JK</u>	<u>JK</u>	<u>EC</u>
	pH (S.U.)	<u>7.73</u>	<u>7.78</u>	<u>7.72</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>0.0</u>	<u>7.5</u>
	*Salinity (ppt)	<u>24.9</u>	<u>25.2</u>	<u>25.3</u>
	*Alkalinity (mg/L CaCO ₃)	<u>91</u>		
	*Temperature (°C)	<u>25.6</u>	<u>25.2</u>	<u>25.7</u>
250 mg/L	pH (S.U.)	<u>7.77</u>	<u>7.70</u>	<u>7.09</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>0.0</u>	<u>7.5</u>
	*Salinity (ppt)	<u>25.1</u>	<u>25.3</u>	<u>25.5</u>
	*Temperature (°C)	<u>25.6</u>	<u>25.4</u>	<u>25.6</u>
375 mg/L	pH (S.U.)	<u>7.81</u>	<u>7.75</u>	<u>7.09</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>0.1</u>	<u>7.4</u>
	*Salinity (ppt)	<u>25.1</u>	<u>25.3</u>	<u>25.5</u>
	*Temperature (°C)	<u>25.5</u>	<u>25.4</u>	<u>25.6</u>
500 mg/L	pH (S.U.)	<u>7.83</u>	<u>7.75</u>	<u>7.09</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>0.1</u>	<u>7.4</u>
	*Salinity (ppt)	<u>25.2</u>	<u>25.4</u>	<u>25.6</u>
	*Temperature (°C)	<u>25.5</u>	<u>25.4</u>	<u>25.6</u>
750 mg/L	pH (S.U.)	<u>7.83</u>	<u>7.75</u>	
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>0.1</u>	
	*Salinity (ppt)	<u>25.3</u>	<u>25.6</u>	
	*Temperature (°C)	<u>25.6</u>	<u>25.5</u>	
1000 mg/L	pH (S.U.)	<u>7.84</u>	<u>7.74</u>	
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>0.1</u>	
	*Salinity (ppt)	<u>25.4</u>	<u>25.8</u>	
	*Temperature (°C)	<u>25.5</u>	<u>25.5</u>	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	<u>30664685</u>

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 256

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	08-02-22*	1015	J	1215	J	SF	PURPLE	08-29-22A
24	08-03-22			1212	J			
48 Termination	08-04-22			1710	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):	AIAb 08-01-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	07-31-22 1200 TO 08-01-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86
	Temperature (°C) 25.0

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^{1d}	9 ^{1d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9 ^{1d}	7 ^{2d}	7 ^{2d}	0	0	0	0
Mean Survival	100%		100%		95%		70%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	486.6
Upper 95% confidence limit (mg KCl/L)	584.2
48-hour LC ₅₀ (mg KCl/L)	528.3

Comments:



Acute Mysid Test-24 Hr Survival

Start Date: 8/2/2022	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 8/4/2022	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	0.9000
750	0.0000	0.0000
1000	0.0000	0.0000

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

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

Equality of variance cannot be confirmed

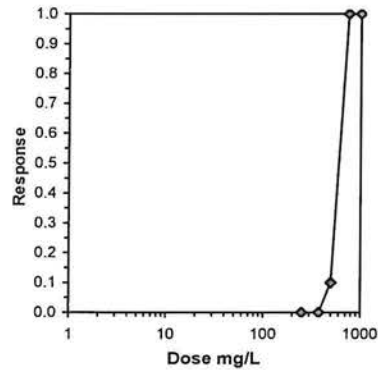
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
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Dunnett's Test	375	500	433.013		0.00967	0.00991	0.59802	0.0001	2.2E-09	4, 5
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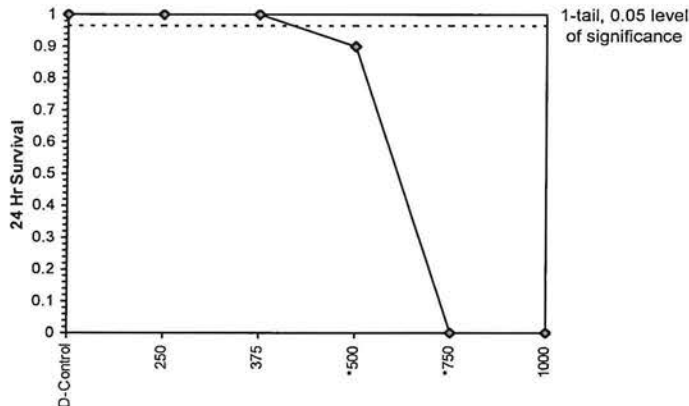
Treatments vs D-Control

Trimmed Spearman-Kärber

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



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 8/2/2022	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 8/4/2022	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

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

Conc-mg/L	Transform: Arcsin Square Root							1-Tailed			Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
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	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	8.166	2.850	0.1469	6	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

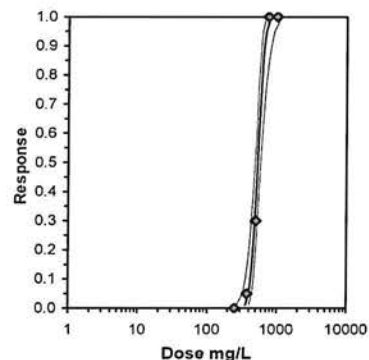
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	375	500	433.013		0.06555	0.06723	0.56898	0.00266	8.9E-06	4, 5

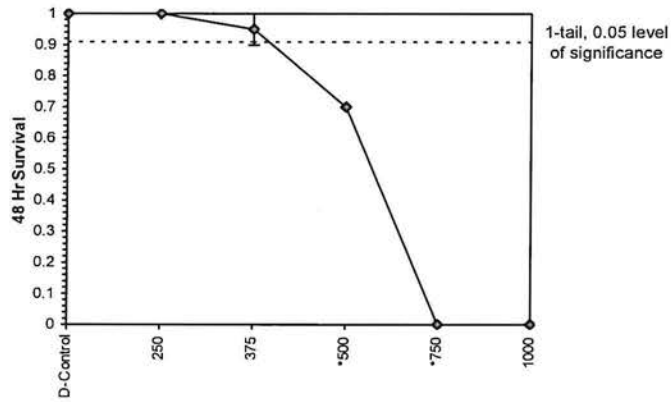
Maximum Likelihood-Probit

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

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	355.945	268.013	403.777
EC05	3.355	399.603	325.274	441.497
EC10	3.718	425.025	359.567	464.411
EC15	3.964	443.085	383.912	481.556
EC20	4.158	457.984	403.707	496.522
EC25	4.326	471.165	420.796	510.585
EC40	4.747	506.086	462.887	552.84
EC50	5.000	528.328	486.642	584.178
EC60	5.253	551.547	508.984	620.484
EC75	5.674	592.425	543.79	691.734
EC80	5.842	609.475	557.134	723.678
EC85	6.036	629.969	572.547	763.518
EC90	6.282	656.737	591.895	817.679
EC95	6.645	698.518	620.809	906.515
EC99	7.326	784.195	676.896	1103.29



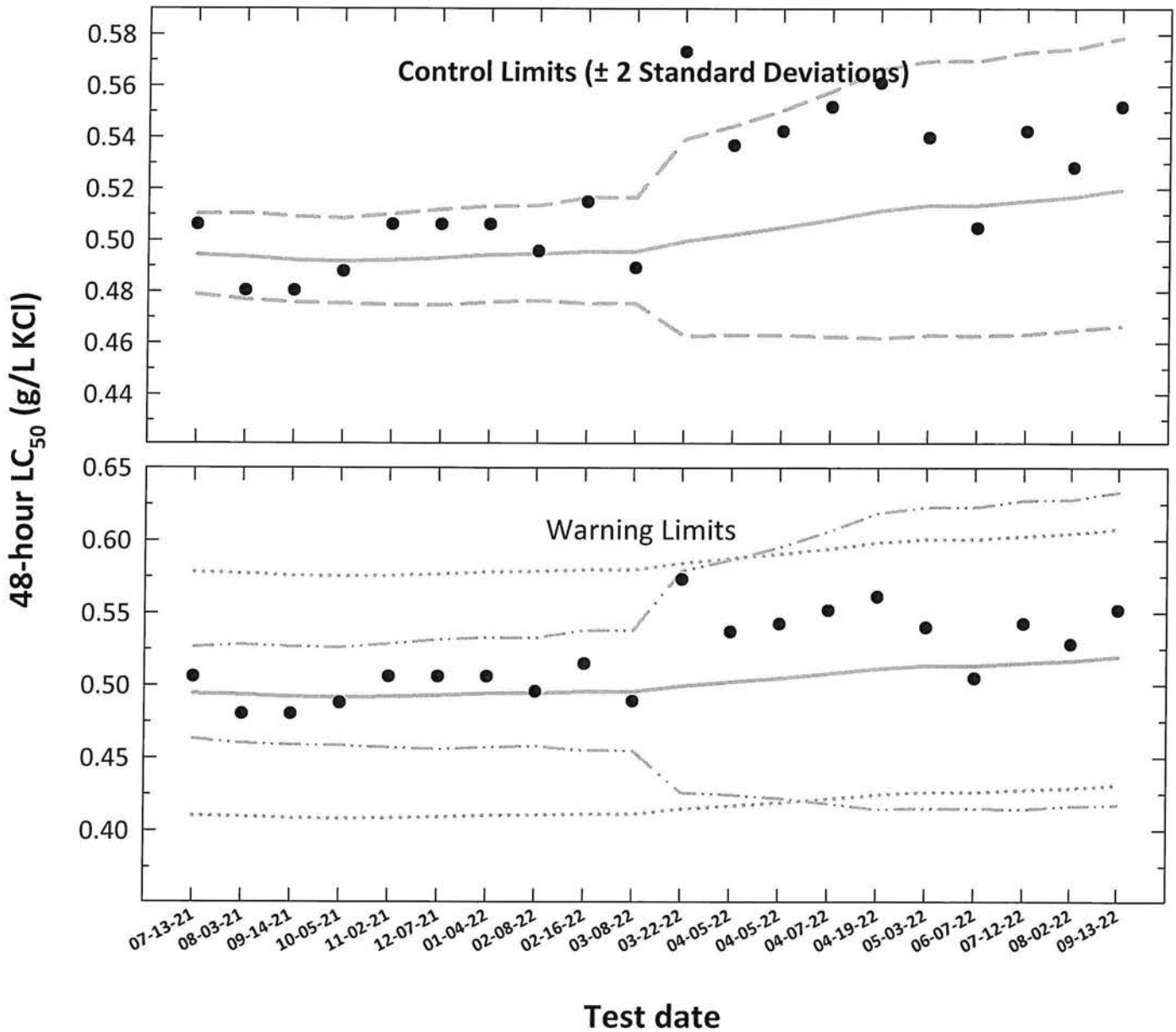
Dose-Response Plot



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 \pm 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic LC₅₀ \pm 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ \pm S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
2	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
3	09-14-21	0.4804	-0.3184	-0.3079	0.0074	0.4922	0.4758	0.5091	0.4589	0.5266	0.4085	0.5759
4	10-05-21	0.4879	-0.3117	-0.3083	0.0073	0.4917	0.4754	0.5086	0.4586	0.5260	0.4081	0.5753
5	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
6	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
7	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
8	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
9	02-16-22	0.5149	-0.2882	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
10	03-08-22	0.4892	-0.3105	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
11	03-22-22	0.5733	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
12	04-05-22	0.5369	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
13	04-05-22	0.5424	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
14	04-07-22	0.5519	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
15	04-19-22	0.5612	-0.2509	-0.2911	0.0221	0.5115	0.4620	0.5664	0.4146	0.6188	0.4246	0.5985
16	05-03-22	0.5399	-0.2677	-0.2894	0.0225	0.5136	0.4630	0.5698	0.4151	0.6229	0.4263	0.6009
17	06-07-22	0.5048	-0.2969	-0.2894	0.0225	0.5135	0.4629	0.5697	0.4149	0.6229	0.4262	0.6009
18	07-12-22	0.5424	-0.2657	-0.2879	0.0231	0.5153	0.4633	0.5732	0.4144	0.6276	0.4277	0.6029
19	08-02-22	0.5283	-0.2771	-0.2866	0.0229	0.5169	0.4651	0.5744	0.4167	0.6282	0.4290	0.6048
20	09-13-22	0.5519	-0.2581	-0.2843	0.0234	0.5196	0.4665	0.5787	0.4174	0.6334	0.4313	0.6079

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 # 257

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 #: 2128

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	X	EC	EC
	pH (S.U.)	7.86	7.82	7.82
	Dissolved oxygen (mg/L)	7.8	7.9	7.8
	*Salinity (ppt)	24.5	24.8	25.0
	*Alkalinity (mg/L CaCO ₃)	86		
	*Temperature (°C)	24.8	24.9	25.1
250 mg/L	pH (S.U.)	7.91	7.82	7.82
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	*Salinity (ppt)	25.1	25.2	25.4
	*Temperature (°C)	25.0	25.1	25.0
375 mg/L	pH (S.U.)	7.92	7.87	7.82
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	*Salinity (ppt)	25.0	25.2	25.5
	*Temperature (°C)	25.0	25.0	25.0
500 mg/L	pH (S.U.)	7.93	7.88	7.82
	Dissolved oxygen (mg/L)	7.9	7.8	7.9
	*Salinity (ppt)	25.2	25.3	25.7
	*Temperature (°C)	25.0	25.1	25.2
750 mg/L	pH (S.U.)	7.94	7.89	
	Dissolved oxygen (mg/L)	7.9	7.9	
	*Salinity (ppt)	25.1	25.5	
	*Temperature (°C)	25.0	25.1	
1000 mg/L	pH (S.U.)	7.95	7.88	
	Dissolved oxygen (mg/L)	7.9	7.9	
	*Salinity (ppt)	25.4	25.6	
	*Temperature (°C)	25.0	25.1	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1316465

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 # 257

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-13-22*	1030	J	1230	J	6F	ORANGE	09-08-22 B
24	09-14-22			1235	J			
48 Termination	09-15-22			1232	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):	09-12-22
Age (1 to 5 days old):	
Date organisms were born: (time organisms were born between is not provided by supplier)	09-11-22 1200 TO 09-17-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.93 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	10	9 ^{ud}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	7 ^{3d}	7 ^{2d}	0	0	0	0
Mean Survival	100%		100%		100%		70%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	581.9
Upper 95% confidence limit (mg KCl/L)	622.5
48-hour LC ₅₀ (mg KCl/L)	601.9

Comments:

Test Reviewed by: J

Acute Mysid Test-48 Hr Survival

Start Date: 9/13/2022 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 9/15/2022 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	0.7000	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

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

Auxiliary Tests **Statistic** **Critical** **Skew** **Kurt**

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

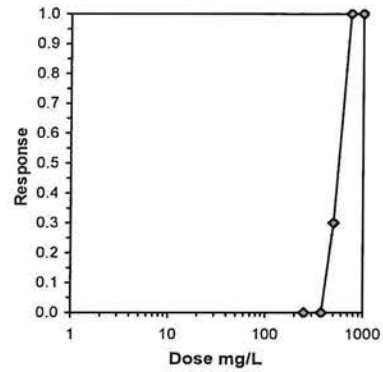
Hypothesis Test (1-tail, 0.05) **NOEC** **LOEC** **ChV** **TU** **MSDu** **MSDp** **MSB** **MSE** **F-Prob** **df**

Dunnett's Test 375 500 433.013 0.00967 0.00991 0.5936 0.0001 2.3E-09 4, 5

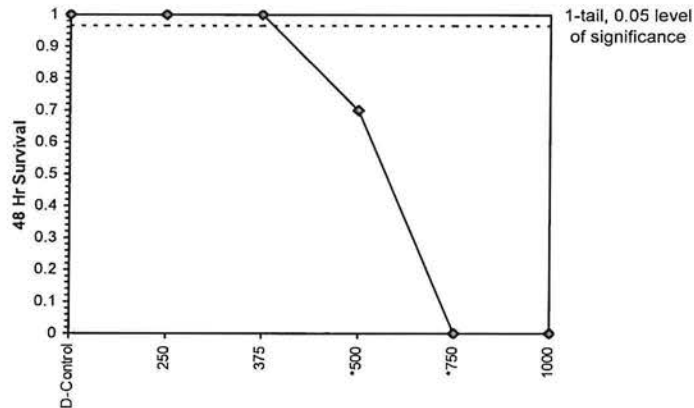
Treatments vs D-Control

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-24 Hr Survival

Start Date: 9/13/2022	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 9/15/2022	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

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

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

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

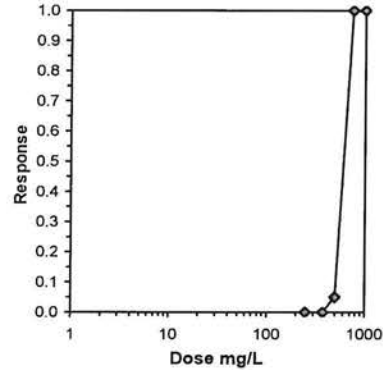
Equality of variance cannot be confirmed

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

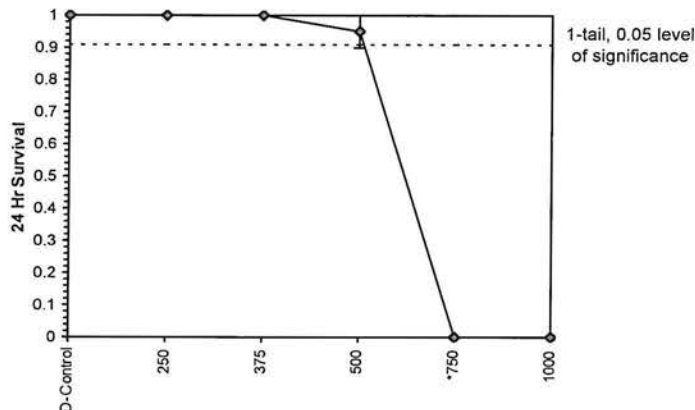
Treatments vs D-Control

Trimmed Spearman-Kärber

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



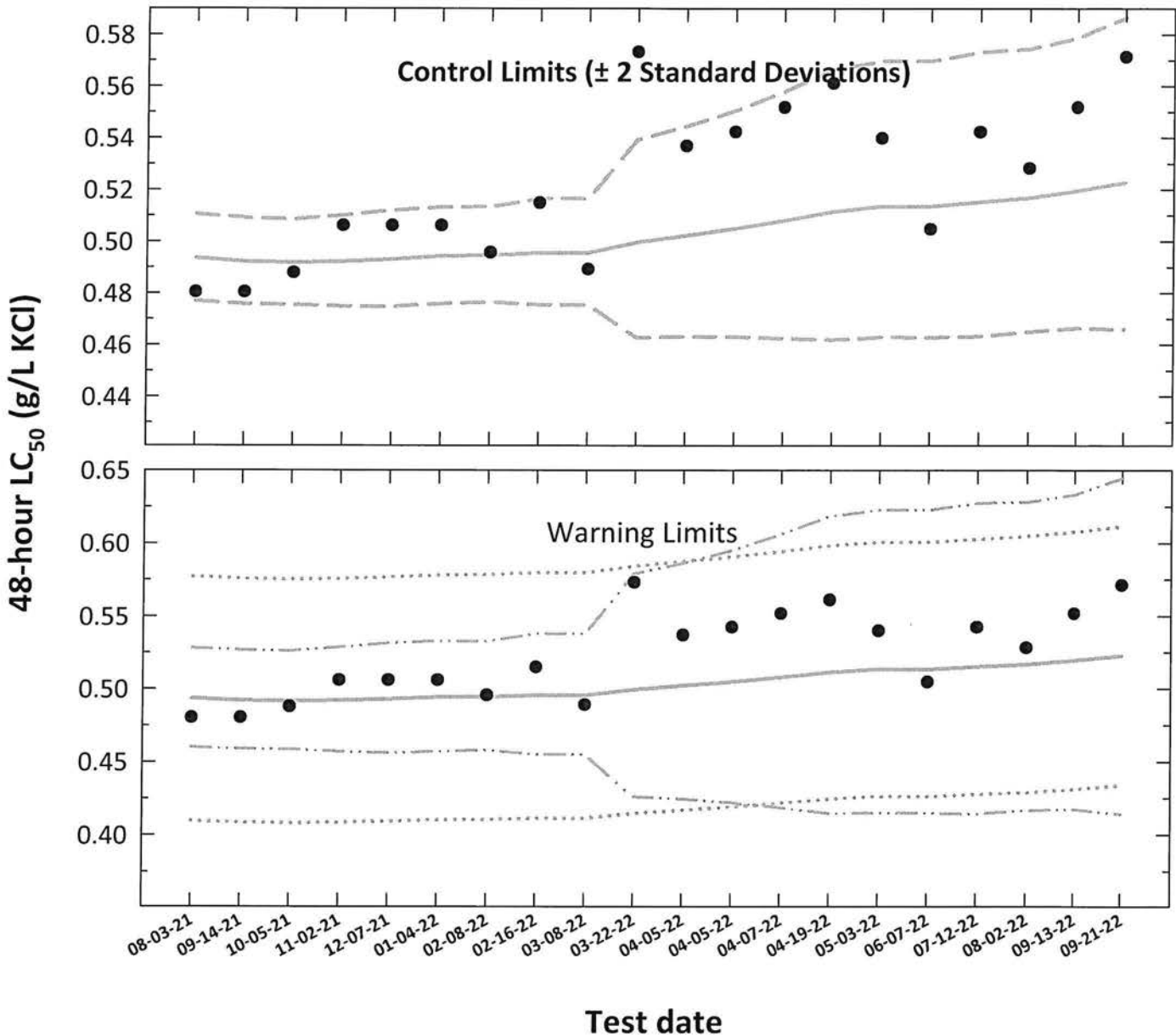
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
2	09-14-21	0.4804	-0.3184	-0.3079	0.0074	0.4922	0.4758	0.5091	0.4589	0.5266	0.4085	0.5759
3	10-05-21	0.4879	-0.3117	-0.3083	0.0073	0.4917	0.4754	0.5086	0.4586	0.5260	0.4081	0.5753
4	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
5	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
6	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
7	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
8	02-16-22	0.5149	-0.2882	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
9	03-08-22	0.4892	-0.3105	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
10	03-22-22	0.5733	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
11	04-05-22	0.5369	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
12	04-05-22	0.5424	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
13	04-07-22	0.5519	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
14	04-19-22	0.5612	-0.2509	-0.2911	0.0221	0.5115	0.4620	0.5664	0.4146	0.6188	0.4246	0.5985
15	05-03-22	0.5399	-0.2677	-0.2894	0.0225	0.5136	0.4630	0.5698	0.4151	0.6229	0.4263	0.6009
16	06-07-22	0.5048	-0.2969	-0.2894	0.0225	0.5135	0.4629	0.5697	0.4149	0.6229	0.4262	0.6009
17	07-12-22	0.5424	-0.2657	-0.2879	0.0231	0.5153	0.4633	0.5732	0.4144	0.6276	0.4277	0.6029
18	08-02-22	0.5283	-0.2771	-0.2866	0.0229	0.5169	0.4651	0.5744	0.4167	0.6282	0.4290	0.6048
19	09-13-22	0.5519	-0.2581	-0.2843	0.0234	0.5196	0.4665	0.5787	0.4174	0.6334	0.4313	0.6079
20	09-21-22	0.5714	-0.2431	-0.2817	0.0250	0.5227	0.4660	0.5864	0.4141	0.6446	0.4339	0.6116

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 # 758

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 #: 2128

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	SCN	JK	SC
Control, SaltSW	pH (S.U.)	7.76	7.35	7.72
	Dissolved oxygen (mg/L)	7.7	7.5	7.5
	*Salinity (ppt)	24.9	25.3	25.4
	*Alkalinity (mg/L CaCO ₃)	83		
	*Temperature (°C)	25.0	25.2	25.2
250 mg/L	pH (S.U.)	7.77	7.37	7.69
	Dissolved oxygen (mg/L)	7.7	7.2	7.5
	*Salinity (ppt)	25.0	25.4	25.6
	*Temperature (°C)	24.9	25.0	25.0
375 mg/L	pH (S.U.)	7.79	7.38	7.70
	Dissolved oxygen (mg/L)	7.7	7.2	7.4
	*Salinity (ppt)	25.3	25.5	25.5
	*Temperature (°C)	24.9	25.0	25.0
500 mg/L	pH (S.U.)	7.80	7.49	7.76
	Dissolved oxygen (mg/L)	7.7	7.1	7.4
	*Salinity (ppt)	25.4	25.7	25.8
	*Temperature (°C)	25.1	25.0	25.0
750 mg/L	pH (S.U.)	7.80	7.54	
	Dissolved oxygen (mg/L)	7.7	7.1	
	*Salinity (ppt)	25.6	25.8	
	*Temperature (°C)	24.8	25.1	
1000 mg/L	pH (S.U.)	7.81	7.54	
	Dissolved oxygen (mg/L)	7.8	7.0	
	*Salinity (ppt)	25.7	25.9	
	*Temperature (°C)	25.1	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-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 258

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-21-22	0520	J	0730	J	7F	GREEN	09-11-22B
24	09-22-22			0732	J			
48 Termination	09-23-22			0725	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):	09-19-22
Age (1 to 5 days old):	2 to 3 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	09-18-22 1200 TO 09-19-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.80
	Temperature (°C) 24.9

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	10	8 ^{2d}	8 ^{2d}	0	0	0	0
Mean Survival	100%		100%		100%		80%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	537.0
Upper 95% confidence limit (mg KCl/L)	607.9
48-hour LC ₅₀ (mg KCl/L)	571.4

Comments:

Test Reviewed by: J

Acute Mysid Test-24 Hr Survival

Start Date: 9/21/2022	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 9/23/2022	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	1.0000	1.0000
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	0.0285	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	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests Statistic Critical Skew Kurt

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

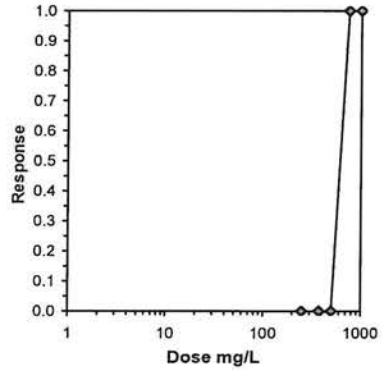
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.00967	0.00991	0.62824	0.0001	2.0E-09	4, 5

Treatments vs D-Control

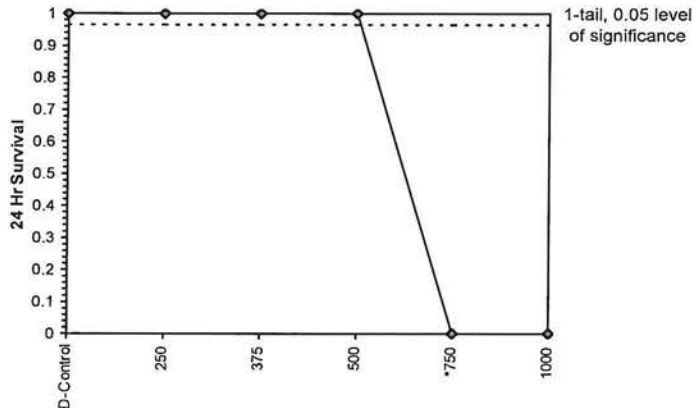
Graphical Method

Trim Level	EC50
0.0%	612.37

612.37



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 9/21/2022 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 9/23/2022 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	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
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	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	2	30.487	2.850	0.0285	4	20	
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20	
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

Auxiliary Tests Statistic Critical Skew Kurt

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

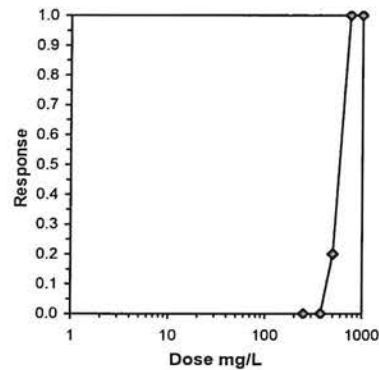
Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU MSDu MSDp MSB MSE F-Prob df

Dunnett's Test 375 500 433.013 0.00967 0.00991 0.589 0.0001 2.3E-09 4, 5

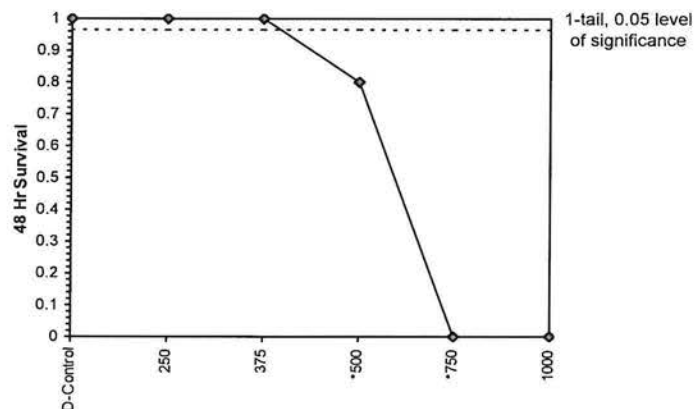
Treatments vs D-Control

Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	571.36	537.02	607.91
5.0%	575.37	536.19	617.41
10.0%	578.73	531.66	629.96
20.0%	582.11	550.04	616.05
Auto-0.0%	571.36	537.02	607.91



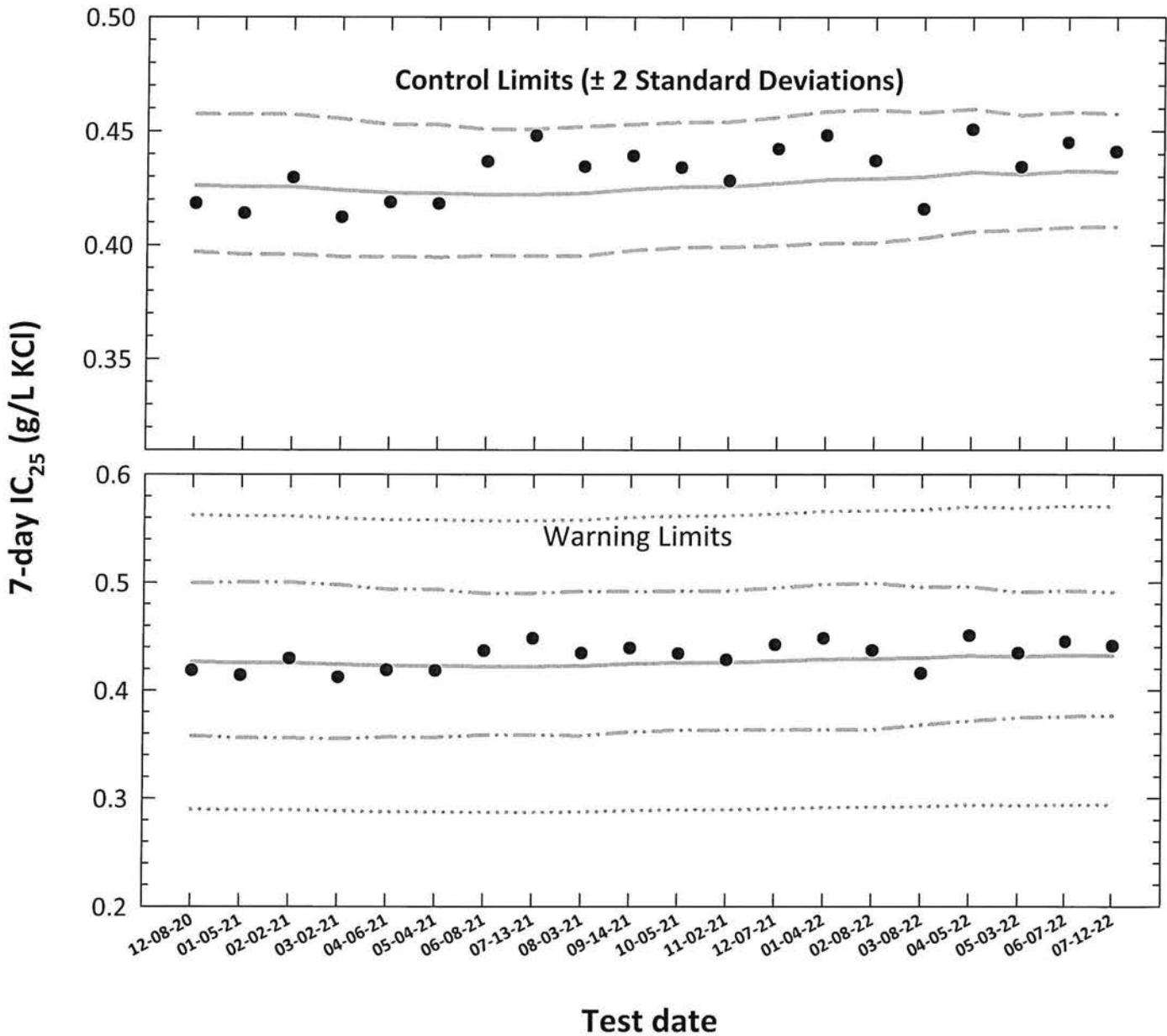
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 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 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)



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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		CT - S _{A,75}
1	12-08-20	0.4183	-0.3786	-0.3704	0.0154	0.4262	0.3970	0.4575	0.3577	0.4996	0.2898	0.5625
2	01-05-21	0.4140	-0.3830	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
3	02-02-21	0.4296	-0.3669	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
4	03-02-21	0.4122	-0.3849	-0.3725	0.0155	0.4241	0.3949	0.4554	0.3553	0.4980	0.2884	0.5598
5	04-06-21	0.4188	-0.3780	-0.3737	0.0149	0.4229	0.3949	0.4529	0.3567	0.4939	0.2876	0.5582
6	05-04-21	0.4182	-0.3787	-0.3740	0.0149	0.4227	0.3946	0.4527	0.3562	0.4938	0.2874	0.5579
7	06-08-21	0.4366	-0.3599	-0.3745	0.0142	0.4222	0.3954	0.4508	0.3587	0.4900	0.2871	0.5573
8	07-13-21	0.4480	-0.3487	-0.3745	0.0143	0.4222	0.3953	0.4509	0.3585	0.4903	0.2871	0.5573
9	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.4227	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
10	09-14-21	0.4392	-0.3574	-0.3721	0.0142	0.4245	0.3977	0.4531	0.3614	0.4918	0.2887	0.5603
11	10-05-21	0.4340	-0.3625	-0.3710	0.0140	0.4256	0.3990	0.4539	0.3631	0.4922	0.2894	0.5617
12	11-02-21	0.4283	-0.3683	-0.3708	0.0140	0.4258	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
13	12-07-21	0.4422	-0.3544	-0.3695	0.0143	0.4271	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
14	01-04-22	0.4482	-0.3485	-0.3678	0.0146	0.4288	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
15	02-08-22	0.4370	-0.3595	-0.3673	0.0147	0.4293	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
16	03-08-22	0.4158	-0.3811	-0.3667	0.0139	0.4298	0.4032	0.4582	0.3679	0.4959	0.2923	0.5674
17	04-05-22	0.4507	-0.3461	-0.3645	0.0135	0.4320	0.4059	0.4597	0.3716	0.4962	0.2938	0.5702
18	05-03-22	0.4343	-0.3622	-0.3654	0.0126	0.4311	0.4067	0.4569	0.3745	0.4911	0.2931	0.5690
19	06-07-22	0.4450	-0.3516	-0.3641	0.0127	0.4324	0.4078	0.4584	0.3756	0.4925	0.2940	0.5707
20	07-12-22	0.4410	-0.3556	-0.3644	0.0124	0.4321	0.4081	0.4576	0.3764	0.4911	0.2938	0.5704

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.

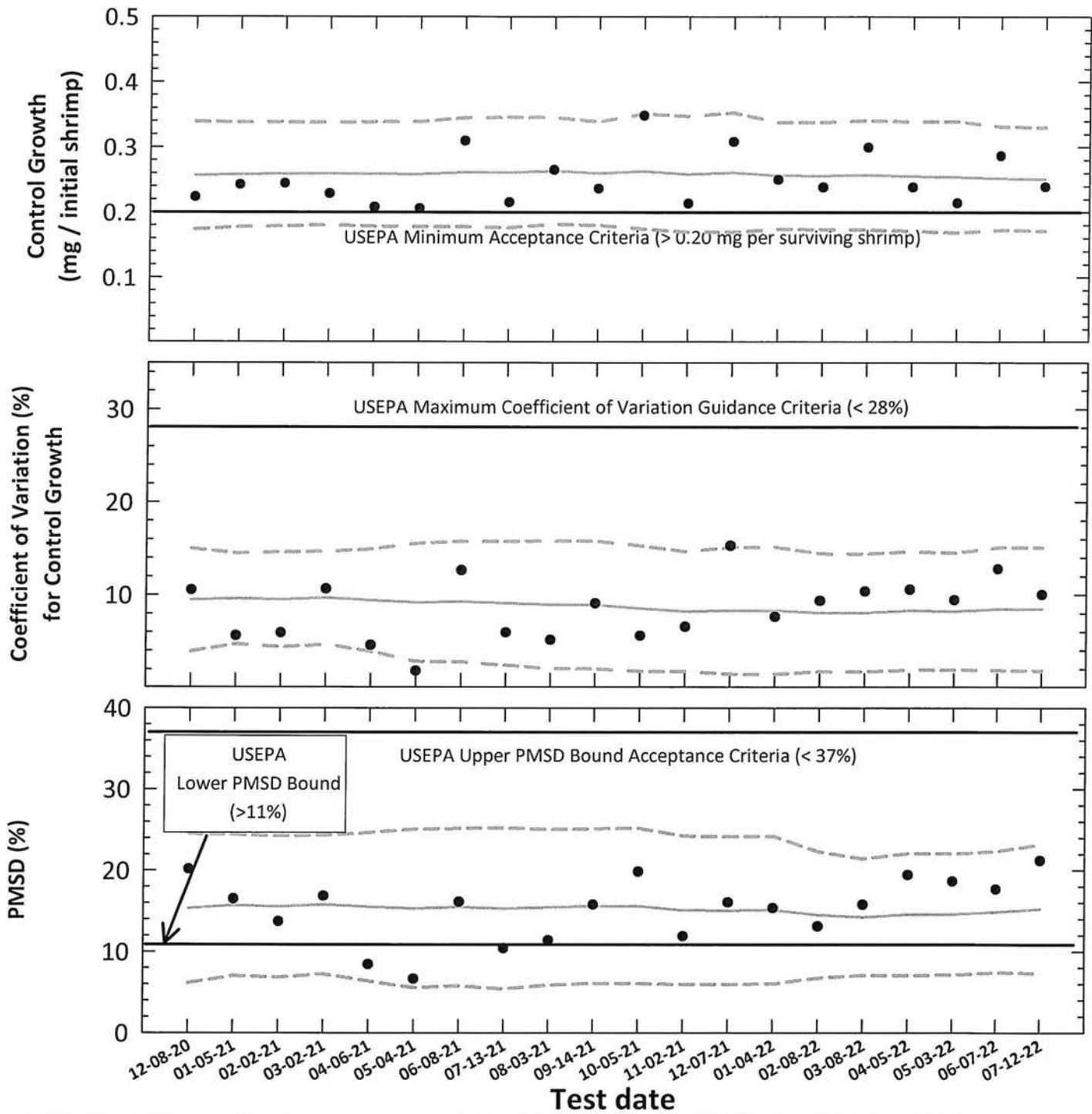
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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)

Entered and Reviewed by
 Jim Sumner

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 (%)	MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	Mean (mg/initial shrimp)					CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	12-08-20	100	0.223	0.0450	10.5	20.2	0.256	0.174	0.339	9.5	3.9	15.0	15.3	6.1	24.5	
2	01-05-21	100	0.243	0.0400	5.6	16.5	0.258	0.178	0.338	9.6	4.7	14.5	15.7	7.0	24.4	
3	02-02-21	100	0.244	0.0335	5.9	13.7	0.259	0.179	0.338	9.5	4.4	14.6	15.5	6.8	24.2	
4	03-02-21	100	0.229	0.0385	10.6	16.8	0.259	0.181	0.338	9.7	4.7	14.7	15.8	7.3	24.3	
5	04-06-21	100	0.208	0.0175	4.6	8.4	0.259	0.179	0.339	9.4	3.9	14.9	15.5	6.4	24.6	
6	05-04-21	100	0.206	0.0137	1.8	6.7	0.258	0.178	0.339	9.2	2.8	15.6	15.3	5.5	25.0	
7	06-08-21	100	0.310	0.0499	12.7	16.1	0.261	0.178	0.345	9.3	2.8	15.8	15.5	5.8	25.1	
8	07-13-21	100	0.215	0.0224	5.9	10.4	0.261	0.176	0.346	9.1	2.4	15.8	15.3	5.4	25.2	
9	08-03-21	100	0.265	0.0301	5.2	11.4	0.263	0.181	0.345	8.9	2.0	15.8	15.4	5.9	25.0	
10	09-14-21	100	0.236	0.0373	9.1	15.8	0.260	0.180	0.339	8.9	2.0	15.8	15.6	6.1	25.1	
11	10-05-21	100	0.348	0.0691	5.6	19.8	0.263	0.174	0.351	8.5	1.8	15.3	15.6	6.1	25.2	
12	11-02-21	100	0.214	0.0254	6.6	11.9	0.258	0.169	0.347	8.2	1.7	14.7	15.1	6.0	24.2	
13	12-07-21	100	0.308	0.0495	15.3	16.1	0.261	0.169	0.353	8.3	1.5	15.1	15.0	5.9	24.1	
14	01-04-22	100	0.250	0.0384	7.6	15.4	0.256	0.174	0.338	8.3	1.5	15.1	15.1	6.0	24.2	
15	02-08-22	100	0.238	0.0312	9.4	13.1	0.256	0.174	0.338	8.3	1.7	14.4	14.5	6.7	22.3	
16	03-08-22	100	0.299	0.0472	10.4	15.8	0.257	0.173	0.341	8.1	1.7	14.4	14.2	7.1	21.4	
17	04-05-22	100	0.238	0.0463	10.6	19.4	0.255	0.171	0.339	8.3	1.9	14.7	14.6	7.1	22.1	
18	05-03-22	100	0.214	0.0399	9.4	18.6	0.254	0.169	0.340	8.2	1.9	14.5	14.6	7.1	22.0	
19	06-07-22	100	0.287	0.0506	12.8	17.7	0.252	0.173	0.331	8.5	1.8	15.1	14.9	7.4	22.3	
20	07-12-22	100	0.239	0.0507	10.0	21.2	0.251	0.171	0.330	8.5	1.8	15.1	15.2	7.3	23.2	

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



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

Species: *Americamysis (Mysidopsis) bahia*

AbKCICR Test Number: 229

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2101				
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:	PURPLE
Date and times organisms were born between:	07-05-22 1200 to 07-06-22 1130	Incubator number and shelf location:	SF
Organism source:	AI Batch Ab: 07-06-22	Artemia CHM number:	CHM1149
Transfer bowl information:	pH = 7.84 S.U. Temperature = 25.1 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	07-19-22 1211
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	07-20-22 1255
		*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	07-12-22	1022	JL	1250	JL	1152	JL	07-07-22 A
1	07-13-22	0500	JL	1100	JL	1006	JL	↓
2	07-14-22	0500	JL	1200	JL	1110	JL	07-07-22 B
3	07-15-22	0500	JL	1100	JL	0952	JL	↓
4	07-16-22	0600	JL	1300	JL	1130	JL	07-14-22 (07-14-22)
5	07-17-22	0500	JL	1100	JL	1003	JL	↓
6	07-18-22	0500	JL	1100	JL	1003	JL	↓
7	07-19-22					1040	JL	↓

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	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)	500.4
Average weight per initial shrimp:	0.239		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.239	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	441.0

AbKCICR Test Number: 229

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>JR</u> Date: <u>06-29-22</u>	14.37	13.20	14.77	12.64	13.62	15.57	13.70	14.09	13.11	15.44	13.96	14.69	12.25	15.25	15.12	13.09
*B = Pan + Shrimp weight (mg) Analyst: <u>EC</u> Date: <u>07-25-22</u>	15.65	14.41	15.89	13.64	14.96	16.66	15.08	15.33	14.29	16.56	15.10	15.69	13.32	16.66	16.33	14.02
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>K</u>	1.28	1.21	1.12	1.00	1.24	1.09	1.38	1.24	1.18	1.12	1.14	1.00	1.07	1.41	1.21	0.93
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.256	0.242	0.224	0.200	0.248	0.218	0.276	0.248	0.236	0.224	0.228	0.200	0.214	0.282	0.242	0.186
Average weight per initial number of shrimp (mg)	0.239								0.227							
Percent reduction from control (%)	5.27															

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

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

Comments:

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	4 ^{1d}	S	4 ^{1d}	4 ^{1d}	3 ^{2d}	3 ^{2d}	4 ^{1d}
4	S	S	S	S	S	S	S	S	4 ^{1d}	3 ^{1d}	3 ^{2d}	3 ^{1d}	3 ^{1d}	3	3	3 ^{1d}
5	S	S	S	4 ^{1d}	S	S	S	S	3 ^{1d}	3	3	2	3	3	2 ^{1d}	3
6	S	S	S	4	S	4 ^{1d}	S	S	3	3	3	2	3	3	2	3
7	S	S	S	4	S	4	S	4 ^{1d}	3	3	3	2	3	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>Light Pink</u> Analyst: <u>JR</u> Date: <u>06-29-22</u>	13.69	12.12	15.30	14.65	15.28	13.57	15.35	14.88	13.16	16.32	14.47	15.46	15.14	15.65	15.25	13.89
*B = Pan + Shrimp weight (mg) Analyst: <u>EC</u> Date: <u>07-25-22</u>	15.80	13.36	16.73	15.43	16.62	14.93	16.90	15.86	13.80	17.16	15.20	16.00	15.70	16.24	15.72	14.49
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JR</u>	1.32	1.24	1.43	0.78	1.34	1.36	1.55	0.98	0.65	0.84	0.73	0.54	0.64	0.59	0.47	0.60
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JR</u>	0.264	0.248	0.286	0.156	0.268	0.272	0.310	0.196	0.130	0.168	0.146	0.108	0.128	0.118	0.094	0.120
Average weight per initial number of shrimp (mg) 0.250				Percent reduction from control (%) -4.67.				Average weight per initial number of shrimp (mg) 0.127				Percent reduction from control (%) 47.17.				

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

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

Comments:

AbKCICR Test Number: 229

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				Percent reduction from control (%) 100%				Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 100%			

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

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

Comments:

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

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

Test number: 229

Test dates: July 12-19, 2022

Concentration (mg/L KCD)	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	14.37	15.65	1.28	0.256	100.0	0.239	10.0	Not applicable
	B	5	5	13.20	14.41	1.21	0.242				
	C	5	5	14.77	15.89	1.12	0.224				
	D	5	5	12.64	13.64	1.00	0.200				
	E	5	5	13.62	14.86	1.24	0.248				
	F	5	5	15.57	16.66	1.09	0.218				
	G	5	5	13.70	15.08	1.38	0.276				
	H	5	5	14.09	15.33	1.24	0.248				
250	I	5	5	13.11	14.29	1.18	0.236	100.0	0.227	12.8	5.2
	J	5	5	15.44	16.56	1.12	0.224				
	K	5	5	13.96	15.10	1.14	0.228				
	L	5	5	14.69	15.69	1.00	0.200				
	M	5	5	12.25	13.32	1.07	0.214				
	N	5	5	15.25	16.66	1.41	0.282				
	O	5	5	15.12	16.33	1.21	0.242				
	P	5	5	13.09	14.02	0.93	0.186				
375	Q	5	5	14.48	15.80	1.32	0.264	92.5	0.250	20.1	-4.6
	R	5	5	12.12	13.36	1.24	0.248				
	S	5	5	15.30	16.73	1.43	0.286				
	T	5	4	14.65	15.43	0.78	0.156				
	U	5	5	15.28	16.62	1.34	0.268				
	V	5	4	13.57	14.93	1.36	0.272				
	W	5	5	15.35	16.90	1.55	0.310				
	X	5	4	14.88	15.86	0.98	0.196				
500	Y	5	3	13.16	13.81	0.65	0.130	55.0	0.127	18.0	47.1
	Z	5	3	16.32	17.16	0.84	0.168				
	AA	5	3	14.47	15.20	0.73	0.146				
	BB	5	2	15.46	16.00	0.54	0.108				
	CC	5	3	15.14	15.78	0.64	0.128				
	DD	5	3	15.65	16.24	0.59	0.118				
	EE	5	2	15.25	15.72	0.47	0.094				
	FF	5	3	13.89	14.49	0.60	0.120				
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: 0.0507
PMSD: 21.2

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.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date: 7/12/2022	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant	
End Date: 7/19/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanysis bahia	

Comments:

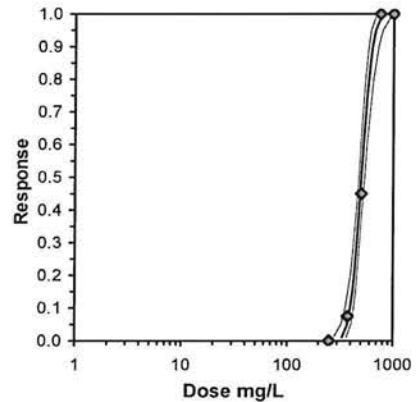
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	1.0000	0.8000	1.0000	0.8000	1.0000	0.8000
500	0.6000	0.6000	0.6000	0.4000	0.6000	0.6000	0.4000	0.6000
750	0.0000	0.0000	0.0000	0.0000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

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9250	0.9250	1.2560	1.1071	1.3453	9.813	8	56.00	48.00	3	40
*500	0.5500	0.5500	0.8357	0.6847	0.8861	11.153	8	36.00	48.00	18	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

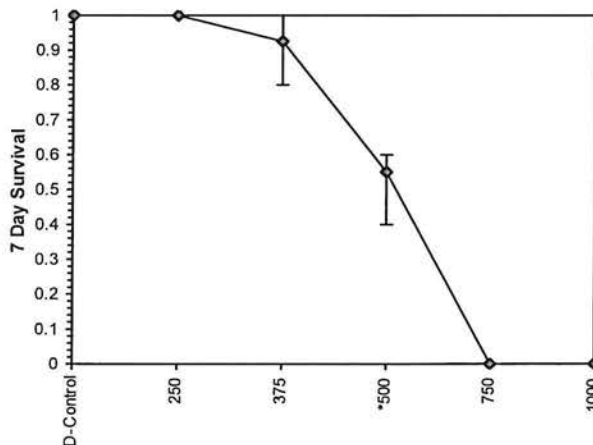
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77838	0.904	-1.0823	0.55012
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			Lower	Upper							
Slope	12.8464	2.01629	8.89446	16.7983	0	1.21634	7.81472	0.74909	2.6993	0.07784	5
Intercept	-29.676	5.413	-40.286	-19.067							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	329.767	274.848	365.409
EC05	3.355	372.612	326.157	403.306
EC10	3.718	397.683	356.637	425.901
EC15	3.964	415.545	378.283	442.455
EC20	4.158	430.311	395.96	456.599
EC25	4.326	443.397	411.331	469.606
EC40	4.747	478.162	449.988	507.159
EC50	5.000	500.376	472.566	533.885
EC60	5.253	523.622	494.446	564.1
EC75	5.674	564.678	529.818	621.968
EC80	5.842	581.85	543.756	647.487
EC85	6.036	602.526	560.076	679.028
EC90	6.282	629.588	580.85	721.474
EC95	6.645	671.949	612.389	790.187
EC99	7.326	759.252	674.838	939.177



Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date: 7/12/2022 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 7/19/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanysis bahia
 Comments:

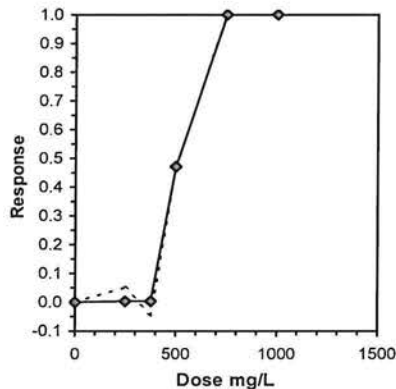
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2560	0.2420	0.2240	0.2000	0.2480	0.2180	0.2760	0.2480
250	0.2360	0.2240	0.2280	0.2000	0.2140	0.2820	0.2420	0.1860
375	0.2640	0.2480	0.2860	0.1560	0.2680	0.2720	0.3100	0.1960
500	0.1300	0.1680	0.1460	0.1080	0.1280	0.1180	0.0940	0.1200
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2390	1.0000	0.2390	0.2000	0.2760	10.022	8				0.2390	1.0000
250	0.2265	0.9477	0.2265	0.1860	0.2820	12.837	8	0.690	2.799	0.0507	0.2383	0.9969
375	0.2500	1.0460	0.2500	0.1560	0.3100	20.089	8	-0.607	2.799	0.0507	0.2383	0.9969
500	0.1265	0.5293	0.1265	0.0940	0.1680	18.002	8				0.1265	0.5293
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000

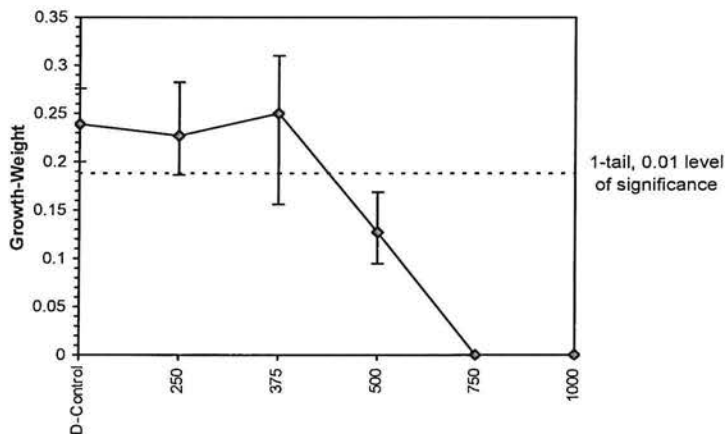
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95849	0.884	-0.7221	1.23009						
Bartlett's Test indicates equal variances ($p = 0.13$)	4.06222	9.21035								
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	>375			0.05072	0.21221	0.00111	0.00131	0.44495	2, 21

Treatments vs D-Control Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	387.53	87.00	127.72	389.83	-1.5504
IC10	400.89	31.78	266.13	404.65	-4.4419
IC15	414.26	11.12	386.39	419.48	-3.9510
IC20	427.63	8.51	402.66	434.31	-1.3808
IC25	441.00	8.01	420.03	449.13	-0.8574
IC40	481.10	8.55	464.35	497.24	0.4158
IC50	513.83	13.47	491.47	538.82	0.3375



Dose-Response Plot



AbKCICR Test Number: 229

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		u	u	u	u	u	u
CONTROL, Salt SW	pH (S.U.)	7.04	7.72	7.79	7.77	7.06	7.70
	DO (mg/L)	0.0	7.9	7.9	7.7	7.6	7.3
	Salinity (ppt)	24.9	25.1	25.1	25.2	25.1	25.5
	Alkalinity (mg CaCO ₃ /L)	99				100	
	Temperature (°C)	25.7	25.8	25.4	25.9	25.3	25.9
250 mg KCl/L	pH (S.U.)	7.00	7.72	7.79	7.77	7.06	7.71
	DO (mg/L)	7.9	7.9	7.0	7.0	7.6	7.1
	Salinity (ppt)	25.3	25.3	25.3	25.5	25.2	25.4
	Temperature (°C)	25.6	25.7	25.3	25.8	25.4	25.7
	375 mg KCl/L	pH (S.U.)	7.07	7.75	7.79	7.78	7.06
DO (mg/L)		7.0	7.0	7.0	7.0	7.7	7.1
Salinity (ppt)		25.3	25.4	25.3	25.5	25.4	25.7
Temperature (°C)		25.6	25.7	25.3	25.8	25.4	25.8
500 mg KCl/L		pH (S.U.)	7.06	7.75	7.00	7.78	7.07
	DO (mg/L)	7.0	7.0	7.0	7.0	7.7	7.4
	Salinity (ppt)	25.4	25.6	25.5	25.5	25.5	20.0
	Temperature (°C)	25.6	25.6	25.3	25.8	25.4	25.8
	750 mg KCl/L	pH (S.U.)	7.06	7.74			
DO (mg/L)		7.9	7.9				
Salinity (ppt)		25.7	25.8				
Temperature (°C)		25.7	25.9				
1000 mg KCl/L		pH (S.U.)	7.06	7.73			
	DO (mg/L)	0.0	7.9				
	Salinity (ppt)	25.7	25.8				
	Temperature (°C)	25.7	25.8				
			Initial	Final	Initial	Final	Initial

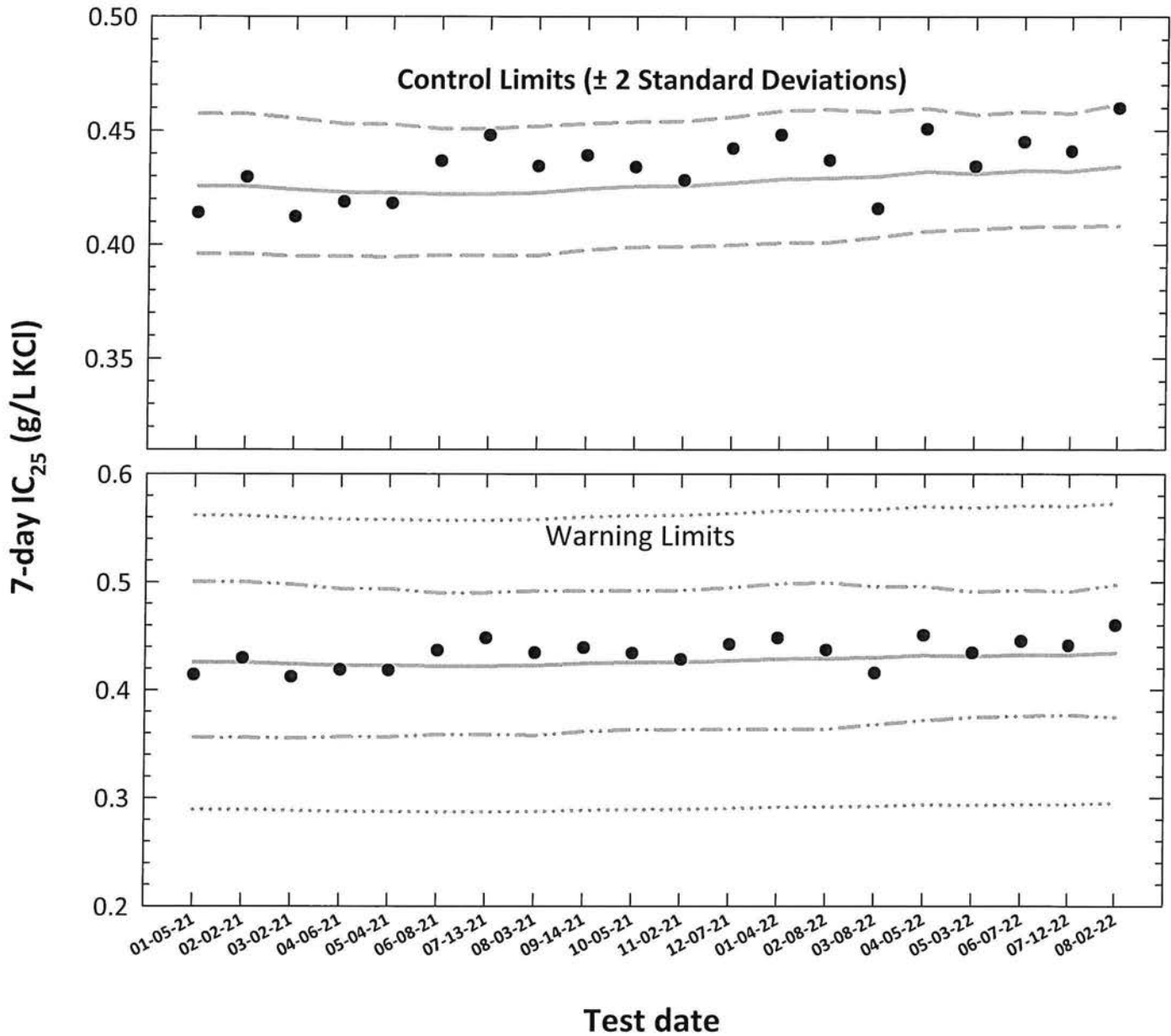
AbKCICR Test Number: 229

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	U	BSC	BSC	BSC	BSC	TC	TC	U
CONTROL, Salt SW	pH (S.U.)	7.05	7.77	7.94	7.69	8.00	7.82	7.75	7.69
	DO (mg/L)	7.6	7.4	7.7	7.1	7.6	7.6	7.6	7.0
	Salinity (ppt)	25.4	25.4	24.0	25.4	24.6	24.9	25.0	25.5
	Alkalinity (mg CaCO ₃ /L)	---	89						
	Temperature (°C)	25.3	25.9	25.4	26.1	25.5	26.0	25.7	25.8
250 mg KCl/L	pH (S.U.)	7.05	7.82	7.95	7.76	7.98	7.81	7.80	7.70
	DO (mg/L)	7.5	7.3	7.7	7.1	7.4	7.6	7.7	6.9
	Salinity (ppt)	25.5	25.6	25.5	25.7	25.1	25.2	25.1	25.3
	Temperature (°C)	25.4	26.0	25.5	26.0	25.7	26.2	25.7	26.0
375 mg KCl/L	pH (S.U.)	7.05	7.81	7.94	7.79	7.94	7.83	7.84	7.70
	DO (mg/L)	7.5	7.5	7.7	7.2	7.7	7.6	7.6	6.9
	Salinity (ppt)	25.4	25.7	25.6	25.8	25.1	25.2	25.3	25.5
	Temperature (°C)	25.4	25.8	25.5	26.0	25.5	26.1	25.6	25.9
500 mg KCl/L	pH (S.U.)	7.05	7.85	7.94	7.79	7.94	7.81	7.85	7.71
	DO (mg/L)	7.6	7.5	7.7	7.3	7.7	7.5	7.7	7.0
	Salinity (ppt)	25.5	25.9	25.7	25.9	25.1	25.4	25.4	25.6
	Temperature (°C)	25.4	25.8	25.4	26.0	25.5	26.0	25.6	25.9
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 IC₂₅ = 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 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)

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	01-05-21	0.4140	-0.3830	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
2	02-02-21	0.4296	-0.3669	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
3	03-02-21	0.4122	-0.3849	-0.3725	0.0155	0.4241	0.3949	0.4554	0.3553	0.4980	0.2884	0.5598
4	04-06-21	0.4188	-0.3780	-0.3737	0.0149	0.4229	0.3949	0.4529	0.3567	0.4939	0.2876	0.5582
5	05-04-21	0.4182	-0.3787	-0.3740	0.0149	0.4227	0.3946	0.4527	0.3562	0.4938	0.2874	0.5579
6	06-08-21	0.4366	-0.3599	-0.3745	0.0142	0.4222	0.3954	0.4508	0.3587	0.4900	0.2871	0.5573
7	07-13-21	0.4480	-0.3487	-0.3745	0.0143	0.4222	0.3953	0.4509	0.3585	0.4903	0.2871	0.5573
8	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.4227	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
9	09-14-21	0.4392	-0.3574	-0.3721	0.0142	0.4245	0.3977	0.4531	0.3614	0.4918	0.2887	0.5603
10	10-05-21	0.4340	-0.3625	-0.3710	0.0140	0.4256	0.3990	0.4539	0.3631	0.4922	0.2894	0.5617
11	11-02-21	0.4283	-0.3683	-0.3708	0.0140	0.4258	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
12	12-07-21	0.4422	-0.3544	-0.3695	0.0143	0.4271	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
13	01-04-22	0.4482	-0.3485	-0.3678	0.0146	0.4288	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
14	02-08-22	0.4370	-0.3595	-0.3673	0.0147	0.4293	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
15	03-08-22	0.4158	-0.3811	-0.3667	0.0139	0.4298	0.4032	0.4582	0.3679	0.4959	0.2923	0.5674
16	04-05-22	0.4507	-0.3461	-0.3645	0.0135	0.4320	0.4059	0.4597	0.3716	0.4962	0.2938	0.5702
17	05-03-22	0.4343	-0.3622	-0.3654	0.0126	0.4311	0.4067	0.4569	0.3745	0.4911	0.2931	0.5690
18	06-07-22	0.4450	-0.3516	-0.3641	0.0127	0.4324	0.4078	0.4584	0.3756	0.4925	0.2940	0.5707
19	07-12-22	0.4410	-0.3556	-0.3644	0.0124	0.4321	0.4081	0.4576	0.3764	0.4911	0.2938	0.5704
20	08-02-22	0.4599	-0.3373	-0.3623	0.0134	0.4342	0.4083	0.4617	0.3745	0.4976	0.2952	0.5731

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.

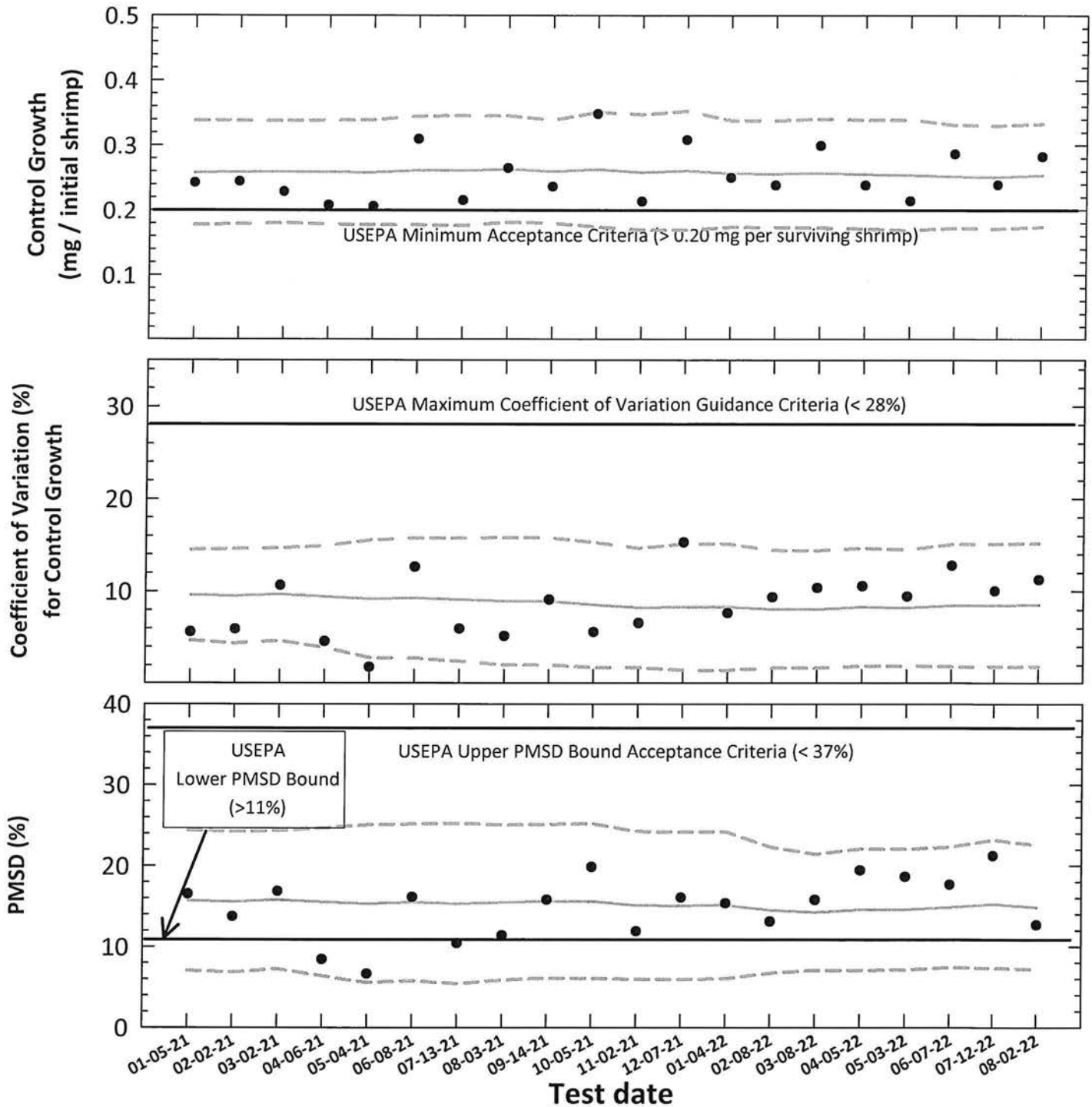
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

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)**

Entered and Reviewed by
Jim Sumner

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		MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	CV (%)				CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	01-05-21	100	0.243	5.6	0.0400	16.5	0.258	0.178	0.338	9.6	4.7	14.5	15.7	7.0	24.4
2	02-02-21	100	0.244	5.9	0.0335	13.7	0.259	0.179	0.338	9.5	4.4	14.6	15.5	6.8	24.2
3	03-02-21	100	0.229	10.6	0.0385	16.8	0.259	0.181	0.338	9.7	4.7	14.7	15.8	7.3	24.3
4	04-06-21	100	0.208	4.6	0.0175	8.4	0.259	0.179	0.339	9.4	3.9	14.9	15.5	6.4	24.6
5	05-04-21	100	0.206	1.8	0.0137	6.7	0.258	0.178	0.339	9.2	2.8	15.6	15.3	5.5	25.0
6	06-08-21	100	0.310	12.7	0.0499	16.1	0.261	0.178	0.345	9.3	2.8	15.8	15.5	5.8	25.1
7	07-13-21	100	0.215	5.9	0.0224	10.4	0.261	0.176	0.346	9.1	2.4	15.8	15.3	5.4	25.2
8	08-03-21	100	0.265	5.2	0.0301	11.4	0.263	0.181	0.345	8.9	2.0	15.8	15.4	5.9	25.0
9	09-14-21	100	0.236	9.1	0.0373	15.8	0.260	0.180	0.339	8.9	2.0	15.8	15.6	6.1	25.1
10	10-05-21	100	0.348	5.6	0.0691	19.8	0.263	0.174	0.351	8.5	1.8	15.3	15.6	6.1	25.2
11	11-02-21	100	0.214	6.6	0.0254	11.9	0.258	0.169	0.347	8.2	1.7	14.7	15.1	6.0	24.2
12	12-07-21	100	0.308	15.3	0.0495	16.1	0.261	0.169	0.353	8.3	1.5	15.1	15.0	5.9	24.1
13	01-04-22	100	0.250	7.6	0.0384	15.4	0.256	0.174	0.338	8.3	1.5	15.1	15.1	6.0	24.2
14	02-08-22	100	0.238	9.4	0.0312	13.1	0.256	0.174	0.338	8.1	1.7	14.4	14.5	6.7	22.3
15	03-08-22	100	0.299	10.4	0.0472	15.8	0.257	0.173	0.341	8.1	1.7	14.4	14.2	7.1	21.4
16	04-05-22	100	0.238	10.6	0.0463	19.4	0.255	0.171	0.339	8.3	1.9	14.7	14.6	7.1	22.1
17	05-03-22	100	0.214	9.4	0.0399	18.6	0.254	0.169	0.340	8.2	1.9	14.5	14.6	7.1	22.0
18	06-07-22	100	0.287	12.8	0.0506	17.7	0.252	0.173	0.331	8.5	1.8	15.1	14.9	7.4	22.3
19	07-12-22	100	0.239	10.0	0.0507	21.2	0.251	0.171	0.330	8.5	1.8	15.1	15.2	7.3	23.2
20	08-02-22	100	0.282	11.3	0.0358	12.7	0.254	0.174	0.333	8.5	1.8	15.2	14.9	7.2	22.5

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



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

AbKCICR Test Number: 230

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2118					
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:	07-26-22 1200 to 07-27-22 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 07-27-22	Artemia CHM number:	CHM1149
Transfer bowl information:	pH = 7.75 S.U. Temperature = 25.0 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	08-09-22 1010
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	08-10-22 1010
		*Final oven temperature:	60 °C
		Total drying time:	24-Hours

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-98585

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	08-02-22	1015	J	1145	J	1120	J	07-29-22 A
1	08-03-22	0500	J	1100	J	0925	J	↓
2	08-04-22	0500	J	1200	J	1120	J	07-29-22 B
3	08-05-22	0500	J	1100	J	0925	J	↓
4	08-06-22	0610	J	1210	J	1000	J	08-03-22
5	08-07-22	0600	J	1200	J	0920	J	↓
6	08-08-22	0600	J	1200	J	0920	J	↓
7	08-09-22					0927	J	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	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:	0.282		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.282	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	459.9

AbKCICR Test Number: 230

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>Gray</u> Analyst: <u>JK</u> Date: <u>07-26-22</u>	15.78	13.17	15.91	14.48	12.92	11.54	14.32	15.87	13.71	13.18	15.86	12.92	13.62	14.26	16.34	13.56	
*B = Pan + Shrimp weight (mg) Analyst: <u>EC</u> Date: <u>08-12-22</u>	17.03	14.05	17.59	16.01	14.33	12.92	15.71	17.04	14.93	14.40	16.99	14.34	14.99	15.45	17.67	14.75	
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JA</u>	1.25	1.48	1.68	1.53	1.41	1.38	1.39	1.17	1.22	1.22	1.13	1.42	1.37	1.19	1.33	1.19	
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JA</u>	0.250	0.296	0.336	0.306	0.282	0.276	0.278	0.234	0.244	0.244	0.226	0.284	0.274	0.238	0.266	0.238	
Average weight per initial number of shrimp (mg)	0.282								0.252								Percent reduction from control (%) 10.87.

*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: 230

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 ^{id}	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	4	S	S	S	S
4	S	S	S	S	S	S	S	S	4 ^{id}	4 ^{id}	4 ^{id}	4	S	4 ^{id}	3 ^{id}	4 ^{id}
5	S	S	S	S	S	S	S	S	3 ^{id}	4	3 ^{id}	4	4 ^{id}	3 ^{id}	3	4
6	S	S	S	S	S	S	S	S	3	4	3	3 ^{id}	3 ^{id}	3	3	4
7	S	S	S	S	S	S	S	S	3	4	3	3	2 ^{id}	3	3	4
# females with eggs in brood sac	/															
# females with developing ova in oviducts	/															
# immature females	/															
# males	/															
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>UJP</u> Date: <u>07-26-22</u>	12.69	13.95	13.66	14.36	13.18	15.66	14.23	12.53	16.15	12.84	14.65	15.50	13.27	14.17	14.32	15.58
*B = Pan + Shrimp weight (mg) Analyst: <u>EC</u> Date: <u>08-12-22</u>	14.18	15.54	14.93	15.82	14.01	17.05	15.68	14.17	17.17	13.82	15.52	16.30	13.88	15.13	15.19	16.81
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>A</u>	1.41	1.59	1.27	1.46	1.43	1.39	1.45	1.64	1.02	0.98	0.87	0.80	0.61	0.96	0.87	1.23
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>A</u>	0.282	0.318	0.254	0.292	0.286	0.278	0.290	0.328	0.204	0.196	0.174	0.160	0.22	0.192	0.174	0.246
Average weight per initial number of shrimp (mg)	0.291								0.184							
Percent reduction from control (%)	-3.17								35.07							

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

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

Comments:

AbKCICR Test Number: 230

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																
	*A = Pan weight (mg) Tray color code: _____ Analyst: _____ Date: _____															
	*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____															
	C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____															
	Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____															
	Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				Average weight per initial number of shrimp (mg)				Percent reduction from control (%)			
	0				100%				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:



**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: 230

Test dates: August 02-09, 2022

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	15.78	17.03	1.25	0.250	100.0	0.282	11.3	Not applicable
	B	5	5	13.17	14.65	1.48	0.296				
	C	5	5	15.91	17.59	1.68	0.336				
	D	5	5	14.48	16.01	1.53	0.306				
	E	5	5	12.92	14.33	1.41	0.282				
	F	5	5	11.54	12.92	1.38	0.276				
	G	5	5	14.32	15.71	1.39	0.278				
	H	5	5	15.87	17.04	1.17	0.234				
250	I	5	5	13.71	14.93	1.22	0.244	100.0	0.252	8.1	10.8
	J	5	5	13.18	14.40	1.22	0.244				
	K	5	5	15.86	16.99	1.13	0.226				
	L	5	5	12.92	14.34	1.42	0.284				
	M	5	5	13.62	14.99	1.37	0.274				
	N	5	5	14.26	15.45	1.19	0.238				
	O	5	5	16.34	17.67	1.33	0.266				
	P	5	5	13.56	14.75	1.19	0.238				
375	Q	5	5	12.69	14.10	1.41	0.282	100.0	0.291	7.9	-3.1
	R	5	5	13.95	15.54	1.59	0.318				
	S	5	5	13.66	14.93	1.27	0.254				
	T	5	5	14.36	15.82	1.46	0.292				
	U	5	5	13.18	14.61	1.43	0.286				
	V	5	5	15.66	17.05	1.39	0.278				
	W	5	5	14.23	15.68	1.45	0.290				
	X	5	5	12.53	14.17	1.64	0.328				
500	Y	5	3	16.15	17.17	1.02	0.204	62.5	0.184	19.6	35.0
	Z	5	4	12.84	13.82	0.98	0.196				
	AA	5	3	14.65	15.52	0.87	0.174				
	BB	5	3	15.50	16.30	0.80	0.160				
	CC	5	2	13.27	13.88	0.61	0.122				
	DD	5	3	14.17	15.13	0.96	0.192				
	EE	5	3	14.32	15.19	0.87	0.174				
	FF	5	4	15.58	16.81	1.23	0.246				
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: 0.0358
 PMSD: 12.7

MSD = Minimum Significant Difference
 PMSD = Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
 Lower PMSD bound determined by USEPA (10th percentile) = 11%.
 Upper PMSD bound determined by USEPA (90th percentile) = 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.

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



Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date: 8/2/2022	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 8/9/2022	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

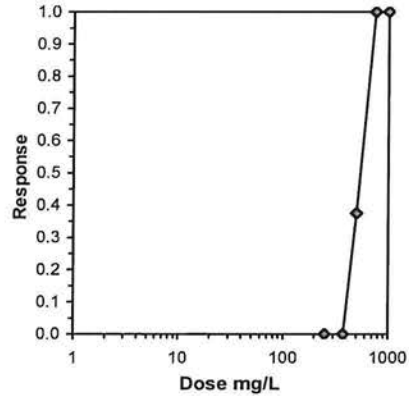
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.8000	0.6000	0.6000	0.4000	0.6000	0.6000	0.8000
750	0.0000	0.0000	0.0000	0.0000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

Conc-mg/L	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%				
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	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	40
*500	0.6250	0.6250	0.9162	0.6847	1.1071	14.934	8	36.00	48.00	15
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40

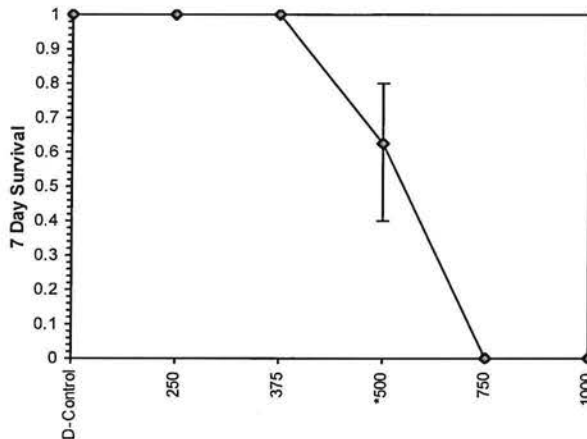
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.53774	0.904	0.17453	8.81285

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

Trim Level	Trimmed Spearman-Kärber		
	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-Response Plot



Entered and Reviewed by
 Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date: 8/2/2022	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 8/9/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanmysid bahia

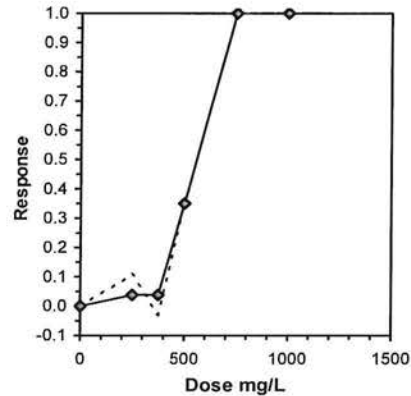
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2500	0.2960	0.3360	0.3060	0.2820	0.2760	0.2780	0.2340
250	0.2440	0.2440	0.2260	0.2840	0.2740	0.2380	0.2660	0.2380
375	0.2820	0.3180	0.2540	0.2920	0.2860	0.2780	0.2900	0.3280
500	0.2040	0.1960	0.1740	0.1600	0.1220	0.1920	0.1740	0.2460
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	t-Stat		Critical	MSD	Mean	N-Mean	
D-Control	0.2823	1.0000	0.2823	0.2340	0.3360	11.258	8				0.2823	1.0000	
250	0.2518	0.8919	0.2518	0.2260	0.2840	8.084	8	2.387	2.799	0.0358	0.2714	0.9615	
375	0.2910	1.0310	0.2910	0.2540	0.3280	7.947	8	-0.685	2.799	0.0358	0.2714	0.9615	
500	0.1835	0.6501	0.1835	0.1220	0.2460	19.616	8				0.1835	0.6501	
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000	
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000	

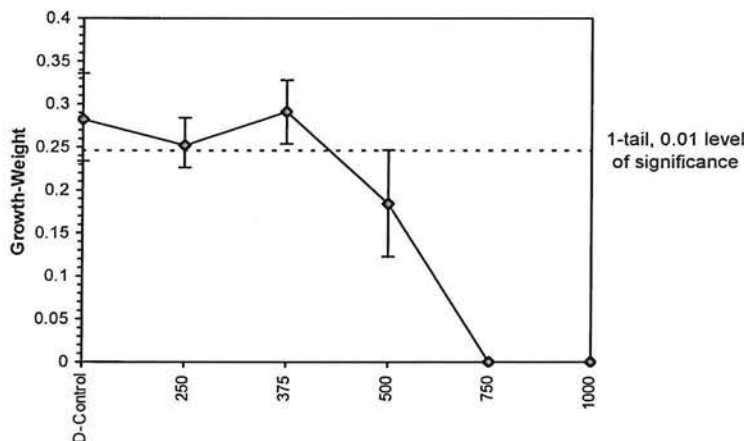
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97585	0.884	0.21498	-0.0339
Bartlett's Test indicates equal variances (p = 0.49)	1.43857	9.21035		

Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	>375			0.03575	0.12668	0.0034	0.00065	0.01462	2, 21

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	379.61	110.64	106.83	396.18	-0.6226
IC10	399.68	47.55	213.67	418.28	-3.2863
IC15	419.75	13.89	389.79	441.02	-0.2942
IC20	439.83	14.50	410.54	466.22	0.0250
IC25	459.90	15.76	429.08	495.02	0.2299
IC40	519.28	16.58	482.50	547.37	-0.2437
IC50	557.73	14.85	521.40	581.15	-0.5969



Dose-Response Plot



Entered and Reviewed by
Jim Sumner

AbKCICR Test Number: 230

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	EC	N	N	EC	EC	N
CONTROL, Salt SW	pH (S.U.)	7.73	7.58	7.57	7.60	7.69	7.50
	DO (mg/L)	7.8	7.6	7.6	7.7	7.7	6.2
	Salinity (ppt)	24.9	25.2	24.9	25.4	24.9	25.2
	Alkalinity (mg CaCO ₃ /L)	91			08-01-22	87	
	Temperature (°C)	25.7	25.8	25.6	25.8	25.3	25.8
250 mg KCl/L	pH (S.U.)	7.77	7.58	7.57	7.60	7.73	7.52
	DO (mg/L)	7.8	7.6	7.6	7.7	7.8	6.4
	Salinity (ppt)	25.1	25.4	25.2	25.5	25.3	25.4
	Temperature (°C)	25.6	26.0	25.6	25.8	25.3	25.9
375 mg KCl/L	pH (S.U.)	7.81	7.58	7.58	7.60	7.74	7.55
	DO (mg/L)	7.8	7.6	7.7	7.7	7.8	7.0
	Salinity (ppt)	25.1	25.4	25.2	25.7	25.5	25.6
	Temperature (°C)	25.6	26.0	25.5	25.6	25.3	25.7
500 mg KCl/L	pH (S.U.)	7.83	7.59	7.60	7.65	7.74	7.57
	DO (mg/L)	7.8	7.7	7.8	7.7	7.8	7.3
	Salinity (ppt)	25.2	25.5	25.3	25.8	25.6	26.1
	Temperature (°C)	25.8	25.9	25.5	25.8	25.3	25.8
750 mg KCl/L	pH (S.U.)	7.83	7.60				
	DO (mg/L)	7.8	7.9				
	Salinity (ppt)	25.3	25.8				
	Temperature (°C)	25.8	25.9				
1000 mg KCl/L	pH (S.U.)	7.84	7.60				
	DO (mg/L)	7.8	7.9				
	Salinity (ppt)	25.4	25.9				
	Temperature (°C)	25.8	26.0				
		Initial	Final	Initial	Final	Initial	Final

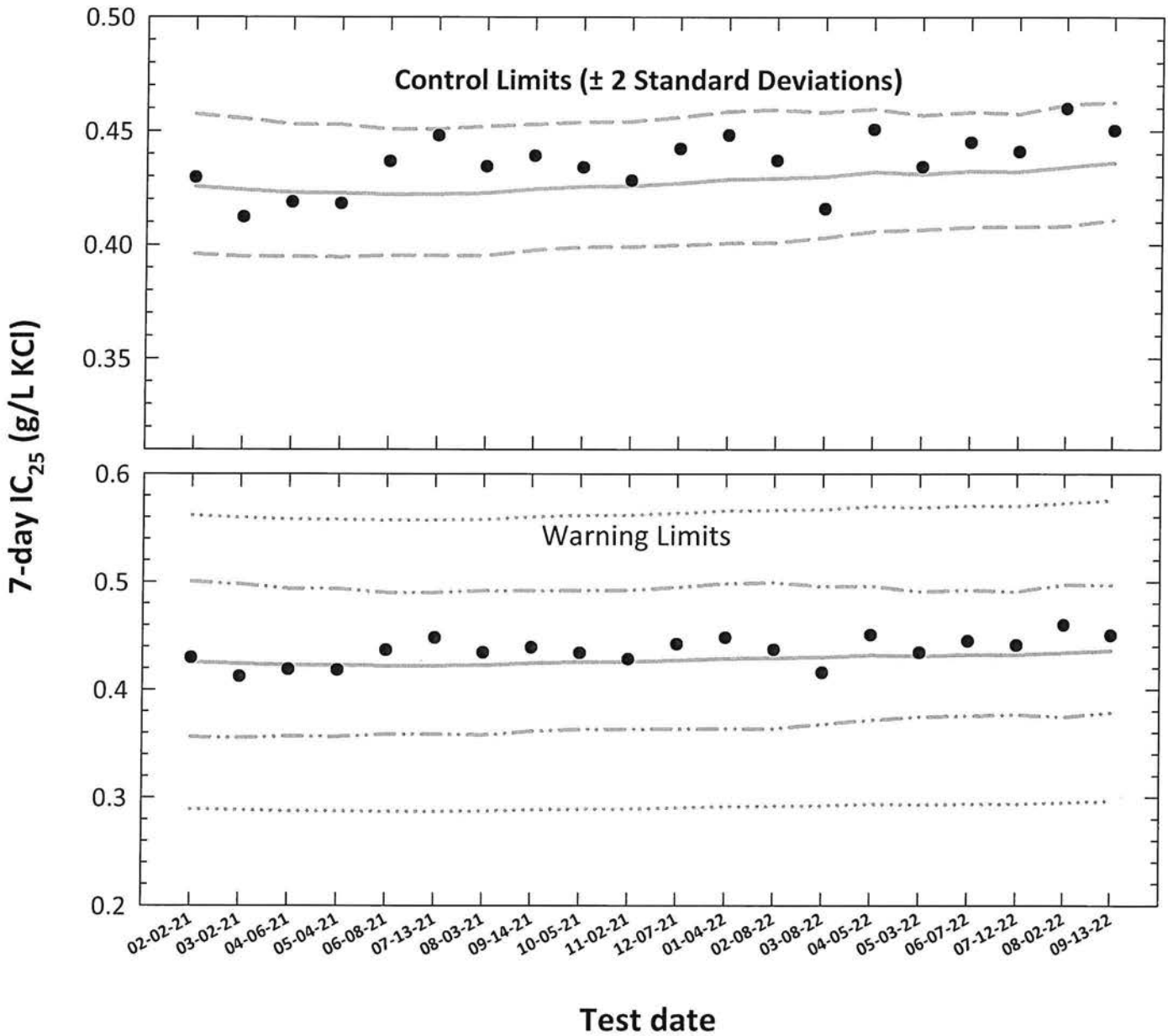
AbKCICR Test Number: 230

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	N	BSL	BSL	N	N	EC N	EC N	EC
	DO (mg/L)	7.71	7.68	7.80	7.61	7.80	7.74	7.83	7.70
	Salinity (ppt)	7.8	7.4	7.8	6.4	7.7	7.6	7.9	7.3
	Alkalinity (mg CaCO ₃ /L)	24.9	25.2	25.1	25.1	25.2	25.1	25.1	24.7
	Temperature (°C)	—	8.11.12.8	9.4	—	—	—	—	8.08.11.12.11
250 mg KCl/L	pH (S.U.)	75.4	75.9	75.3	75.8	75.4	75.9	75.4	76.1
	DO (mg/L)	7.71	7.64	7.78	7.57	7.81	7.73	7.87	7.75
	Salinity (ppt)	7.8	7.4	7.8	6.7	7.6	7.6	7.9	7.4
	Temperature (°C)	25.2	25.3	25.5	25.3	25.4	25.2	25.0	25.2
375 mg KCl/L	pH (S.U.)	75.4	75.9	75.4	76.0	75.2	76.0	75.4	76.1
	DO (mg/L)	7.72	7.63	7.78	7.50	7.82	7.71	7.87	7.77
	Salinity (ppt)	7.8	7.5	7.8	6.7	7.6	7.7	7.8	7.4
	Temperature (°C)	25.3	25.6	25.6	25.7	25.3	25.3	25.3	25.6
500 mg KCl/L	pH (S.U.)	75.3	76.0	75.4	75.8	75.4	76.0	75.3	76.0
	DO (mg/L)	7.74	7.63	7.79	7.50	7.83	7.74	7.88	7.76
	Salinity (ppt)	7.8	7.5	7.8	6.7	7.6	7.7	7.8	7.3
	Temperature (°C)	25.4	26.0	25.6	25.5	25.4	25.5	25.5	25.7
750 mg KCl/L	pH (S.U.)	75.3	76.0	75.4	75.8	75.4	75.8	75.3	76.1
	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 IC₂₅** = 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 IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ \pm 2 standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic IC₂₅ \pm 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)

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	02-02-21	0.4296	-0.3669	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
2	03-02-21	0.4122	-0.3849	-0.3725	0.0155	0.4241	0.3949	0.4554	0.3553	0.4980	0.2884	0.5598
3	04-06-21	0.4188	-0.3780	-0.3737	0.0149	0.4229	0.3949	0.4529	0.3567	0.4939	0.2876	0.5582
4	05-04-21	0.4182	-0.3787	-0.3740	0.0149	0.4227	0.3946	0.4527	0.3562	0.4938	0.2874	0.5579
5	06-08-21	0.4366	-0.3599	-0.3745	0.0142	0.4222	0.3954	0.4508	0.3587	0.4900	0.2871	0.5573
6	07-13-21	0.4480	-0.3487	-0.3745	0.0143	0.4222	0.3953	0.4509	0.3585	0.4903	0.2871	0.5573
7	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.4227	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
8	09-14-21	0.4392	-0.3574	-0.3721	0.0142	0.4245	0.3977	0.4531	0.3614	0.4918	0.2887	0.5603
9	10-05-21	0.4340	-0.3625	-0.3710	0.0140	0.4256	0.3990	0.4539	0.3631	0.4922	0.2894	0.5617
10	11-02-21	0.4283	-0.3683	-0.3708	0.0140	0.4258	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
11	12-07-21	0.4422	-0.3544	-0.3695	0.0143	0.4271	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
12	01-04-22	0.4482	-0.3485	-0.3678	0.0146	0.4288	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
13	02-08-22	0.4370	-0.3595	-0.3673	0.0147	0.4293	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
14	03-08-22	0.4158	-0.3811	-0.3667	0.0139	0.4298	0.4032	0.4582	0.3679	0.4959	0.2923	0.5674
15	04-05-22	0.4507	-0.3461	-0.3645	0.0135	0.4320	0.4059	0.4597	0.3716	0.4962	0.2938	0.5702
16	05-03-22	0.4343	-0.3622	-0.3654	0.0126	0.4311	0.4067	0.4569	0.3745	0.4911	0.2931	0.5690
17	06-07-22	0.4450	-0.3516	-0.3641	0.0127	0.4324	0.4078	0.4584	0.3756	0.4925	0.2940	0.5707
18	07-12-22	0.4410	-0.3556	-0.3644	0.0124	0.4321	0.4081	0.4576	0.3764	0.4911	0.2938	0.5704
19	08-02-22	0.4599	-0.3373	-0.3623	0.0134	0.4342	0.4083	0.4617	0.3745	0.4976	0.2952	0.5731
20	09-13-22	0.4502	-0.3466	-0.3605	0.0129	0.4360	0.4109	0.4626	0.3785	0.4970	0.2965	0.5755

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.

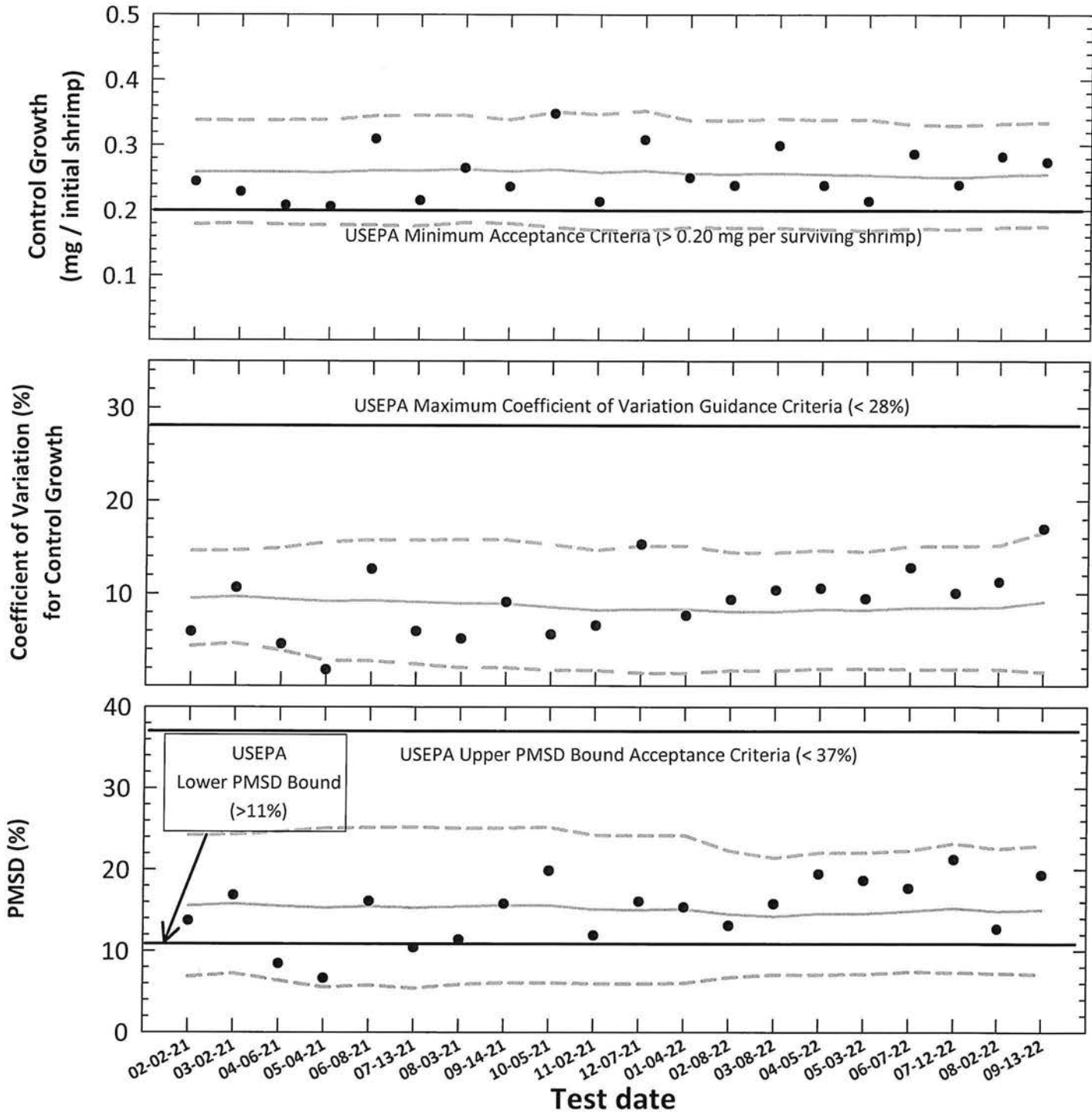
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

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)

Entered and Reviewed by
Jim Sumner

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		MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S			
			Mean (mg/initial shrimp)	CV (%)											
1	02-02-21	100	0.244	5.9	0.0335	13.7	0.259	0.179	0.338	9.5	4.4	14.6	15.5	6.8	24.2
2	03-02-21	100	0.229	10.6	0.0385	16.8	0.259	0.181	0.338	9.7	4.7	14.7	15.8	7.3	24.3
3	04-06-21	100	0.208	4.6	0.0175	8.4	0.259	0.179	0.339	9.4	3.9	14.9	15.5	6.4	24.6
4	05-04-21	100	0.206	1.8	0.0137	6.7	0.258	0.178	0.339	9.2	2.8	15.6	15.3	5.5	25.0
5	06-08-21	100	0.310	12.7	0.0499	16.1	0.261	0.178	0.345	9.3	2.8	15.8	15.5	5.8	25.1
6	07-13-21	100	0.215	5.9	0.0224	10.4	0.261	0.176	0.346	9.1	2.4	15.8	15.3	5.4	25.2
7	08-03-21	100	0.265	5.2	0.0301	11.4	0.263	0.181	0.345	8.9	2.0	15.8	15.4	5.9	25.0
8	09-14-21	100	0.236	9.1	0.0373	15.8	0.260	0.180	0.339	8.9	2.0	15.8	15.6	6.1	25.1
9	10-05-21	100	0.348	5.6	0.0691	19.8	0.263	0.174	0.351	8.5	1.8	15.3	15.6	6.1	25.2
10	11-02-21	100	0.214	6.6	0.0254	11.9	0.258	0.169	0.347	8.2	1.7	14.7	15.1	6.0	24.2
11	12-07-21	100	0.308	15.3	0.0495	16.1	0.261	0.169	0.353	8.3	1.5	15.1	15.0	5.9	24.1
12	01-04-22	100	0.250	7.6	0.0384	15.4	0.256	0.174	0.338	8.3	1.5	15.1	15.1	6.0	24.2
13	02-08-22	100	0.238	9.4	0.0312	13.1	0.256	0.174	0.338	8.1	1.7	14.4	14.5	6.7	22.3
14	03-08-22	100	0.299	10.4	0.0472	15.8	0.257	0.173	0.341	8.1	1.7	14.4	14.2	7.1	21.4
15	04-05-22	100	0.238	10.6	0.0463	19.4	0.255	0.171	0.339	8.3	1.9	14.7	14.6	7.1	22.1
16	05-03-22	100	0.214	9.4	0.0399	18.6	0.254	0.169	0.340	8.2	1.9	14.5	14.6	7.1	22.0
17	06-07-22	100	0.287	12.8	0.0506	17.7	0.252	0.173	0.331	8.5	1.8	15.1	14.9	7.4	22.3
18	07-12-22	100	0.239	10.0	0.0507	21.2	0.251	0.171	0.330	8.5	1.8	15.1	15.2	7.3	23.2
19	08-02-22	100	0.282	11.3	0.0358	12.7	0.254	0.174	0.333	8.5	1.8	15.2	14.9	7.2	22.5
20	09-13-22	100	0.274	17.0	0.0529	19.3	0.255	0.175	0.335	9.1	1.6	16.6	15.0	7.1	22.9

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



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

Species: *Americamysis (Mysidopsis) bahia*

AbKCICR Test Number: **231**

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2128				
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:	PALE GREEN
Date and times organisms were born between:	09-06-22 1200 to 09-07-22 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 09-07-22	Artemia CHM number:	CHM1149
Transfer bowl information:		Drying information for weight determination:	
pH = 7.82 S.U. Temperature = 25.0 °C		Date / Time in oven:	09-20-22 1150
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	09-21-22 1150
		*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	09-13-22	1030	JL	1200	JL	1120	JL	09-08-22B
1	09-14-22	0500	JL	1100	JL	0950	JL	↓
2	09-15-22	0800	JL	1400	JL	1200	JL	09-14-22
3	09-16-22	0500	JL	1100	JL	0950	JL	↓
4	09-17-22	0600	JL	1215	JL	1200	JL	09-15-22
5	09-18-22	0600	JL	1200	JL	1040	JL	↓
6	09-19-22	0500	JL	1100	JL	0948	JL	09-17-22 A
7	09-20-22					1056	JL	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13064685

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	0%	≤ 20%	7-day LC ₅₀ (mg/L KCl)	561.6
Average weight per initial shrimp:	0.274		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.274	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	450.2

AbKCICR Test Number: 231

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>JAVANER</u> Analyst: <u>EC</u> Date: <u>07-17-22</u>	13.47	12.79	15.70	10.15	12.03	14.05	14.81	13.77	13.82	14.53	14.32	15.14	13.55	13.80	15.49	14.07
*B = Pan + Shrimp weight (mg) Analyst: <u>EC</u> Date: <u>09-23-22</u>	14.09	13.89	17.31	17.82	13.94	16.15	16.12	14.77	14.93	15.96	16.05	16.40	15.07	15.15	16.80	15.33
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.52	1.10	1.55	1.67	1.31	1.50	1.31	1.00	1.11	1.42	1.73	1.26	1.52	1.29	1.31	1.26
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.304	0.220	0.310	0.334	0.262	0.300	0.262	0.200	0.222	0.284	0.346	0.252	0.304	0.258	0.274	0.252
Average weight per initial number of shrimp (mg)	0.274								0.274				0.07			
Percent reduction from control (%)	0.07															

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

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

Comments:

AbKCICR Test Number: 231

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	4 ^{1st}	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5
5	5	5	5	5	5	5	5	5	4 ^{1st}	4 ^{1st}	4	5	3 ^{2nd}	5	4 ^{1st}	4 ^{1st}
6	5	5	5	5	5	5	5	5	3 ^{1st}	4	4	5	3	3 ^{2nd}	4	4
7	5	5	5	5	5	5	5	5	3	4	4	5	3	3	4	4
# females with eggs in brood sac	/															
# females with developing ova in oviducts	/															
# immature females	/															
# males	/															
*A = Pan weight (mg) Tray color code: <u>lavender</u> Analyst: <u>EC</u> Date: <u>08-17-22</u>	14.62	12.98	15.09	15.76	14.05	13.91	15.20	14.58	15.03	13.19	15.26	15.84	14.21	14.80	14.37	15.05
*B = Pan + Shrimp weight (mg) Analyst: <u>EC</u> Date: <u>09-23-22</u>	16.06	14.12	16.19	17.17	15.41	15.21	16.35	15.82	15.72	14.15	15.99	16.73	15.00	15.72	15.41	16.64
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.44	1.14	1.10	1.41	1.36	1.30	1.15	1.24	0.69	0.96	0.73	0.89	0.79	0.86	1.04	0.99
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.288	0.228	0.220	0.282	0.272	0.260	0.230	0.248	0.138	0.192	0.146	0.178	0.158	0.172	0.208	0.198
Average weight per initial number of shrimp (mg)	0.254								0.174							
Percent reduction from control (%)	7.57								36.67							

*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: **231**

Survival and Growth Data

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

*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: _____ 231
Test dates: _____ September 13-20, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.47	14.99	1.52	0.304	100.0	0.274	17.0	Not applicable
	B	5	5	12.79	13.89	1.10	0.220				
	C	5	5	15.76	17.31	1.55	0.310				
	D	5	5	16.15	17.82	1.67	0.334				
	E	5	5	12.63	13.94	1.31	0.262				
	F	5	5	14.65	16.15	1.50	0.300				
	G	5	5	14.81	16.12	1.31	0.262				
	H	5	5	13.77	14.77	1.00	0.200				
250	I	5	5	13.82	14.93	1.11	0.222	100.0	0.274	13.9	0.0
	J	5	5	14.53	15.95	1.42	0.284				
	K	5	5	14.32	16.05	1.73	0.346				
	L	5	5	15.14	16.40	1.26	0.252				
	M	5	5	13.55	15.07	1.52	0.304				
	N	5	5	13.86	15.15	1.29	0.258				
	O	5	5	15.49	16.86	1.37	0.274				
	P	5	5	14.07	15.33	1.26	0.252				
375	Q	5	5	14.62	16.06	1.44	0.288	100.0	0.254	10.3	7.5
	R	5	5	12.98	14.12	1.14	0.228				
	S	5	5	15.09	16.19	1.10	0.220				
	T	5	5	15.76	17.17	1.41	0.282				
	U	5	5	14.05	15.41	1.36	0.272				
	V	5	5	13.91	15.21	1.30	0.260				
	W	5	5	15.20	16.35	1.15	0.230				
	X	5	5	14.58	15.82	1.24	0.248				
500	Y	5	3	15.03	15.72	0.69	0.138	75.0	0.174	14.4	36.6
	Z	5	4	13.19	14.15	0.96	0.192				
	AA	5	4	15.26	15.99	0.73	0.146				
	BB	5	5	15.84	16.73	0.89	0.178				
	CC	5	3	14.21	15.00	0.79	0.158				
	DD	5	3	14.86	15.72	0.86	0.172				
	EE	5	4	14.37	15.41	1.04	0.208				
	FF	5	4	15.65	16.64	0.99	0.198				
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: 0.0529
PMSD: 19.3

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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

Mysid Survival and Growth Test-7 Day Survival

Start Date: 9/13/2022 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 9/20/2022 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:

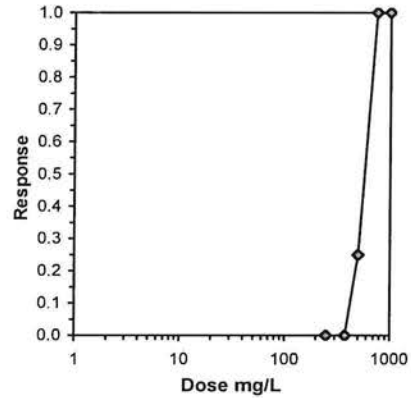
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.8000	0.8000	1.0000	0.6000	0.6000	0.8000	0.8000
750	0.0000	0.0000	0.0000	0.0000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

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.7500	0.7500	1.0540	0.8861	1.3453	15.245	8	40.00	48.00	10	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

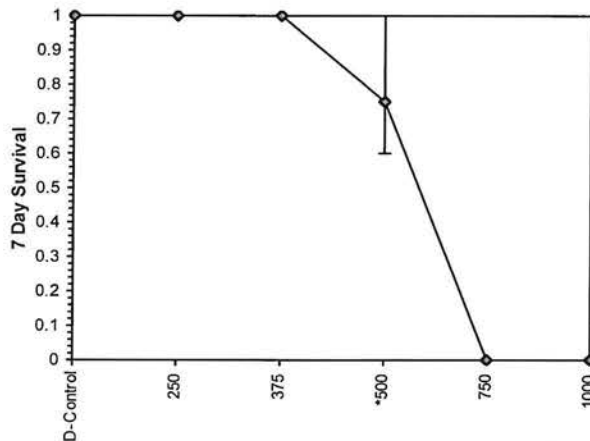
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.61046	0.904	0.85808	7.76198

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

Trim Level	Trimmed Spearman-Kärber		
	EC50	95% CL	
0.0%	561.55	535.52	588.84
5.0%	564.65	535.25	595.66
10.0%	567.47	533.01	604.15
20.0%	571.63	518.88	629.74
Auto-0.0%	561.55	535.52	588.84



Dose-Response Plot



Entered and
 Reviewed by
 Jim Sumner
JS

Mysid Survival and Growth Test-Growth-Weight

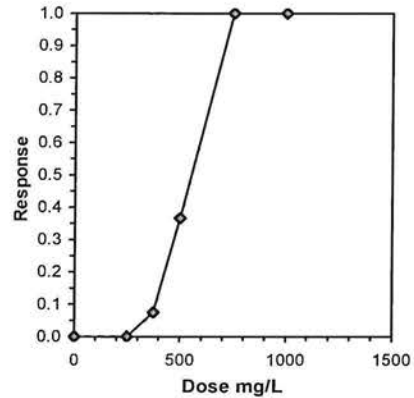
Start Date: 9/13/2022	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 9/20/2022	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanmysis bahia
Comments:		

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.3040	0.2200	0.3100	0.3340	0.2620	0.3000	0.2620	0.2000
250	0.2220	0.2840	0.3460	0.2520	0.3040	0.2580	0.2740	0.2520
375	0.2880	0.2280	0.2200	0.2820	0.2720	0.2600	0.2300	0.2480
500	0.1380	0.1920	0.1460	0.1780	0.1580	0.1720	0.2080	0.1980
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

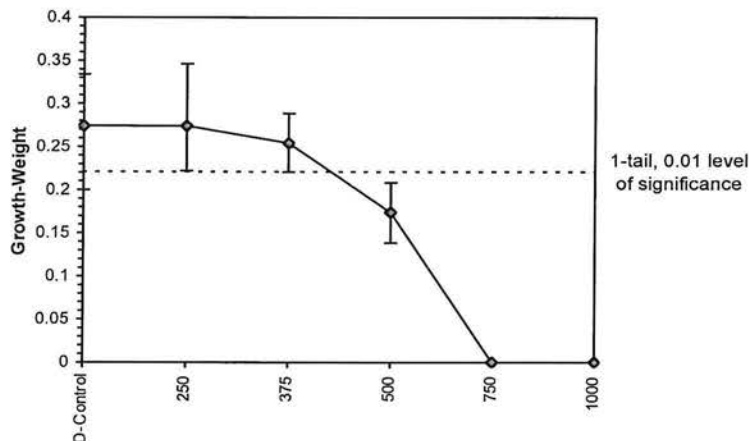
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	0.2740	1.0000	0.2740	0.2000	0.3340	16.980	8				0.2740	1.0000	
250	0.2740	1.0000	0.2740	0.2220	0.3460	13.871	8	0.000	2.799	0.0529	0.2740	1.0000	
375	0.2535	0.9252	0.2535	0.2200	0.2880	10.267	8	1.085	2.799	0.0529	0.2535	0.9252	
500	0.1738	0.6341	0.1738	0.1380	0.2080	14.448	8				0.1738	0.6341	
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000	
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8				0.0000	0.0000	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.98183	0.884	-0.0203	-0.3112						
Bartlett's Test indicates equal variances ($p = 0.35$)	2.10654	9.21035								
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	>375			0.05289	0.19304	0.00112	0.00143	0.46935	2, 21

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	333.54	82.85	115.81	392.54	-1.0354
IC10	385.82	47.89	231.61	412.82	-1.8254
IC15	407.29	21.73	339.18	434.81	-1.2206
IC20	428.76	17.93	389.55	457.63	-0.1049
IC25	450.24	17.69	418.75	481.67	0.1733
IC40	513.45	15.87	483.86	539.32	-0.0219
IC50	552.88	13.58	525.07	574.43	-0.1000



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKCICR Test Number: 231

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		EC	EC N	EC N	EC N	EC N	EC N
CONTROL, Salt SW	pH (S.U.)	7.80	7.77	7.78	7.76	7.82	7.77
	DO (mg/L)	7.8	7.0	0.0	7.0	7.9	7.0
	Salinity (ppt)	24.5	25.1	24.8	25.2	24.8	25.2
	Alkalinity (mg CaCO ₃ /L)	86				86	
	Temperature (°C)	25.6	25.8	25.4	25.9	25.5	25.6
250 mg KCl/L	pH (S.U.)	7.91	7.82	7.87	7.79	7.90	7.79
	DO (mg/L)	7.8	7.0	0.0	7.0	7.9	7.0
	Salinity (ppt)	25.1	25.4	25.0	25.4	25.1	25.4
	Temperature (°C)	25.7	25.9	25.5	26.0	25.3	26.6
375 mg KCl/L	pH (S.U.)	7.92	7.83	7.87	7.78	7.90	7.79
	DO (mg/L)	7.8	7.0	0.1	7.9	7.0	7.0
	Salinity (ppt)	25.0	25.5	25.1	25.5	25.2	25.0
	Temperature (°C)	25.7	25.9	25.5	26.0	25.4	26.0
500 mg KCl/L	pH (S.U.)	7.93	7.84	7.88	7.79	7.90	7.77
	DO (mg/L)	7.9	7.0	0.1	7.9	7.0	7.0
	Salinity (ppt)	25.2	25.7	25.3	25.7	25.4	25.0
	Temperature (°C)	25.7	25.7	25.5	26.2	25.4	25.9
750 mg KCl/L	pH (S.U.)	7.94	7.80				
	DO (mg/L)	7.9	7.9				
	Salinity (ppt)	25.1	25.7				
	Temperature (°C)	25.7	25.7				
1000 mg KCl/L	pH (S.U.)	7.95	7.80				
	DO (mg/L)	7.9	7.9				
	Salinity (ppt)	25.4	25.9				
	Temperature (°C)	25.7	25.9				
		Initial	Final	Initial	Final	Initial	Final

* REANALYZED +
CONFIRMED D.O.
JL

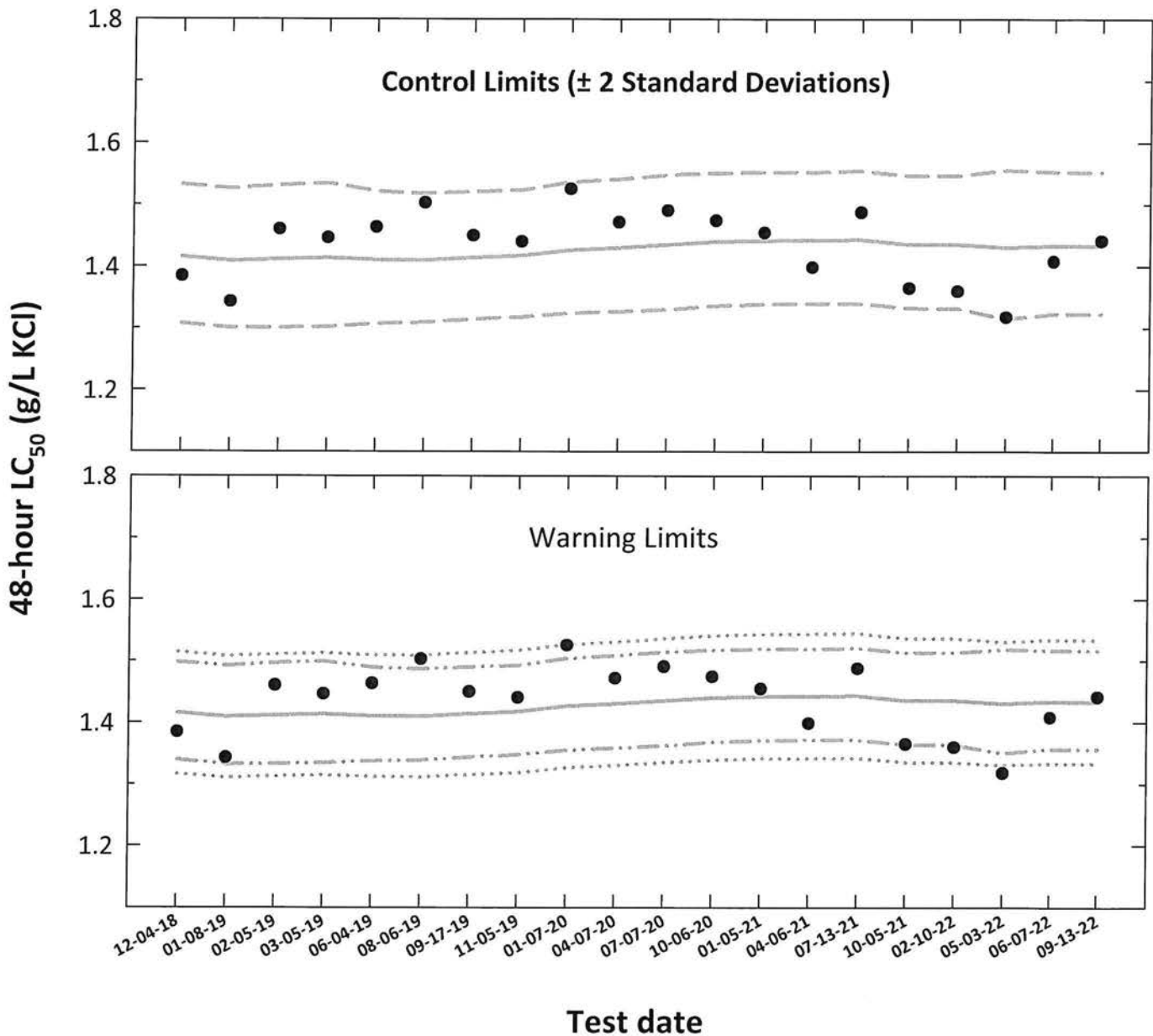
AbKCICR Test Number: 231

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
Analyst	EC	BSL	BSL	BSL	BSL	EC _{AP22}	EC _{AP22}	EC	
CONTROL, Salt SW	pH (S.U.)	7.92	7.64	7.86	7.80	8.17	7.78 (7.85)	7.87 (7.90)	7.64
	DO (mg/L)	0.0	7.7	7.7	7.8	7.7	7.0	7.8	7.7
	Salinity (ppt)	24.7	25.4	24.0	25.3	24.0	25.2	24.0	25.2
	Alkalinity (mg CaCO ₃ /L)		09.14.22	100	85		09.14.22	100	86
	Temperature (°C)	25.8	26.1	25.4	25.8	25.8	26.0	25.7	26.1
250 mg KCl/L	pH (S.U.)	7.87	7.73	7.83	7.85	7.91	7.89	7.92	7.70
	DO (mg/L)	0.0	7.7	7.7	7.8	7.9	7.2	7.9	7.5
	Salinity (ppt)	25.2	25.3	25.1	25.4	25.1	25.3	25.1	25.3
	Temperature (°C)	25.7	26.0	25.3	26.0	25.9	26.1	25.6	26.2
375 mg KCl/L	pH (S.U.)	7.87	7.70	7.82	7.77	7.92	7.87	7.90	7.71
	DO (mg/L)	0.0	7.6	7.6	7.7	7.8	7.2	7.9	7.6
	Salinity (ppt)	25.1	25.4	25.2	25.2	25.1	25.4	25.2	25.5
	Temperature (°C)	25.7	26.2	25.3	26.2	25.7	26.1	25.6	26.2
500 mg KCl/L	pH (S.U.)	7.88	7.76	7.83	7.80	7.91	7.85	7.94	7.69
	DO (mg/L)	0.0	7.6	7.6	7.6	7.8	7.1	7.9	7.6
	Salinity (ppt)	25.2	25.5	25.2	25.6	25.1	25.6	25.3	25.6
	Temperature (°C)	25.7	26.1	25.3	26.0	25.7	26.0	25.6	26.3
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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 ± S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

Menidia beryllina

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	12-04-18	1.3844	0.1412	0.1509	0.0172	1.4155	1.3076	1.5322	1.3393	1.4980	1.3164	1.5146
2	01-08-19	1.3428	0.1280	0.1488	0.0174	1.4088	1.3005	1.5261	1.3319	1.4920	1.3102	1.5074
3	02-05-19	1.4601	0.1644	0.1496	0.0177	1.4113	1.3007	1.5312	1.3330	1.4962	1.3125	1.5101
4	03-05-19	1.4461	0.1602	0.1503	0.0179	1.4134	1.3019	1.5345	1.3345	1.4991	1.3145	1.5124
5	06-04-19	1.4632	0.1653	0.1494	0.0165	1.4105	1.3074	1.5216	1.3374	1.4893	1.3117	1.5092
6	08-06-19	1.5030	0.1769	0.1491	0.0160	1.4097	1.3095	1.5176	1.3386	1.4862	1.3110	1.5084
7	09-17-19	1.4497	0.1613	0.1504	0.0159	1.4139	1.3143	1.5212	1.3434	1.4898	1.3150	1.5129
8	11-05-19	1.4402	0.1584	0.1514	0.0157	1.4172	1.3182	1.5235	1.3474	1.4922	1.3180	1.5164
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 86

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 #: 2128

Chemical Analyses:

Concentration		Hours		
		0	24	48
Control, SaltSW	Analyst	EC	EC	EC
	pH (S.U.)	7.80	7.78	7.84
	Dissolved oxygen (mg/L)	7.8	7.9	8.0
	*Salinity (ppt)	24.5	25.1	25.2
	*Alkalinity (mg/L CaCO ₃)	86		
*Temperature (°C)	25.0	25.1	25.0	
1000 mg/L	pH (S.U.)	7.90	7.80	7.82
	Dissolved oxygen (mg/L)	7.8	7.8	8.0
	*Salinity (ppt)	25.2	25.5	25.5
	*Temperature (°C)	25.1	24.9	24.9
1250 mg/L	pH (S.U.)	7.97	7.85	7.83
	Dissolved oxygen (mg/L)	7.8	7.8	8.0
	*Salinity (ppt)	25.5	25.6	25.7
	*Temperature (°C)	25.0	25.0	25.2
1500 mg/L	pH (S.U.)	7.97	7.85	7.82
	Dissolved oxygen (mg/L)	7.8	7.8	8.0
	*Salinity (ppt)	25.6	25.8	26.0
	*Temperature (°C)	25.0	25.0	25.2
1750 mg/L	pH (S.U.)	7.97	7.83	7.80
	Dissolved oxygen (mg/L)	7.8	7.9	8.0
	*Salinity (ppt)	25.6	25.9	26.4
	*Temperature (°C)	25.0	25.0	25.1
2000 mg/L	pH (S.U.)	7.97	7.81	
	Dissolved oxygen (mg/L)	7.8	7.9	
	*Salinity (ppt)	25.9	26.2	
	*Temperature (°C)	25.0	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664605

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 # 86

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-13-22	1530	JL	1240	JL	6F	ORANGE	09-01-22B
24	09-14-22			1240	JL			
48 Termination	09-15-22			1240	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 #):	Mb 09-04-22
Age (9 to 14 days old):	10 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	09-03-22 1100 TD 09-04-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.66 Temperature (°C) 25.1

Survival Data (number of living organisms):

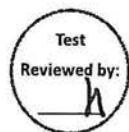
Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^{id}	5 ^{sd}	5 ^{sd}	1 ^{sd}	1 ^{sd}	0 ^{sd}	0 ^{sd}
48 Termination	10	10	10	10	10	8 ^{id}	3 ^{sd}	4 ^{id}	1	0 ^{id}	0	0
Mean Survival	100%		100%		90%		35%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1369.6
Upper 95% confidence limit (mg KCl/L)	1510.5
48-hour LC ₅₀ (mg KCl/L)	1441.5

Comments:



Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date: 9/13/2022 Test ID: MbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 9/15/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: MB-Menidia beryllina
 Comments:

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

Conc-mg/L	Transform: Arcsin Square Root							1-Tailed			Number		Total
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	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	0.1469	0	20	
1250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20	
*1500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	12.159	2.850	0.1469	10	20	
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	21.156	2.850	0.1469	18	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

Auxiliary Tests

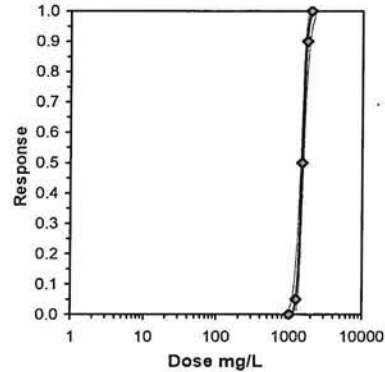
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.06555	0.06723	0.47057	0.00266	1.4E-05	4, 5

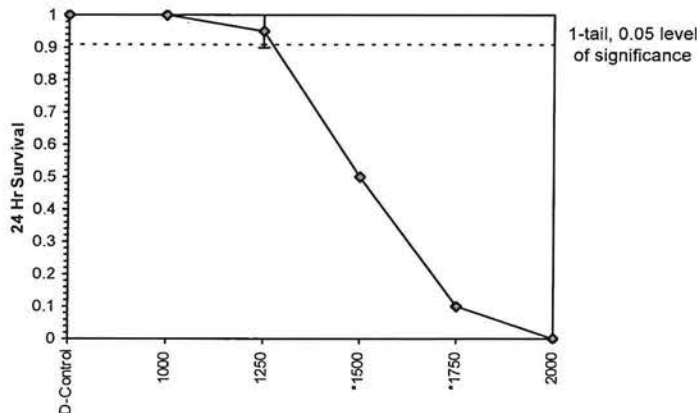
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	20.6442	3.79257	13.2107	28.0776	0	0.15971	7.81472	0.98381	3.17695	0.04844	3
Intercept	-60.585	12.0739	-84.25	-36.921							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1159.47	984.55	1258.56
EC05	3.355	1251.04	1105.15	1335.21
EC10	3.718	1302.78	1174.21	1379.32
EC15	3.964	1338.89	1222.41	1410.83
EC20	4.158	1368.3	1261.44	1437.18
EC25	4.326	1394.04	1295.24	1460.94
EC40	4.747	1461.09	1380.2	1527.27
EC50	5.000	1502.96	1429.72	1573.29
EC60	5.253	1546.04	1476.91	1625.2
EC75	5.674	1620.39	1549.57	1725.55
EC80	5.842	1650.88	1576.76	1770.02
EC85	6.036	1687.15	1607.66	1824.88
EC90	6.282	1733.91	1645.75	1898.23
EC95	6.645	1805.61	1701.49	2015.23
EC99	7.326	1948.21	1806.54	2260.29



Dose-Response Plot



Entered and
 Analyzed by
 Jm Sumner

Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date:	9/13/2022	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	9/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	0.8000
1500	0.3000	0.4000
1750	0.1000	0.0000
2000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.3256	0	20	
1250	0.9000	0.9000	1.2596	1.1071	1.4120	17.115	2	1.334	2.850	0.3256	2	20	
*1500	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	6.825	2.850	0.3256	13	20	
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	10.255	2.850	0.3256	19	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

Auxiliary Tests

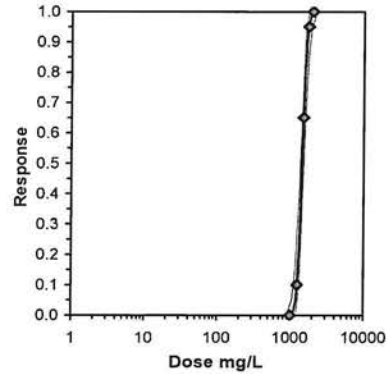
Normality of the data set cannot be confirmed
 Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	1250	1500	1369.31		0.19186	0.19678	0.5595	0.01305	4.6E-04	4, 5

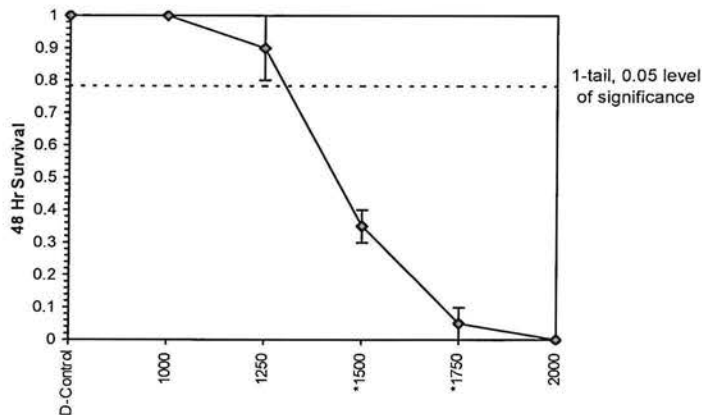
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	20.5696	3.83628	13.0505	28.0887	0	0.09204	7.81472	0.99278	3.15881	0.04862	3
Intercept	-59.975	12.1437	-83.777	-36.174							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1110.99	939.119	1207.53
EC05	3.355	1199.06	1055.61	1281.14
EC10	3.718	1248.83	1122.38	1323.53
EC15	3.964	1283.57	1169.01	1353.83
EC20	4.158	1311.87	1206.76	1379.19
EC25	4.326	1336.65	1239.46	1402.06
EC40	4.747	1401.17	1321.67	1466.03
EC50	5.000	1441.48	1369.55	1510.49
EC60	5.253	1482.94	1415.14	1560.73
EC75	5.674	1554.53	1485.24	1658.05
EC80	5.842	1583.89	1511.43	1701.23
EC85	6.036	1618.81	1541.17	1754.54
EC90	6.282	1663.84	1577.8	1825.87
EC95	6.645	1732.9	1631.35	1939.75
EC99	7.326	1870.27	1732.2	2178.6



Dose-Response Plot

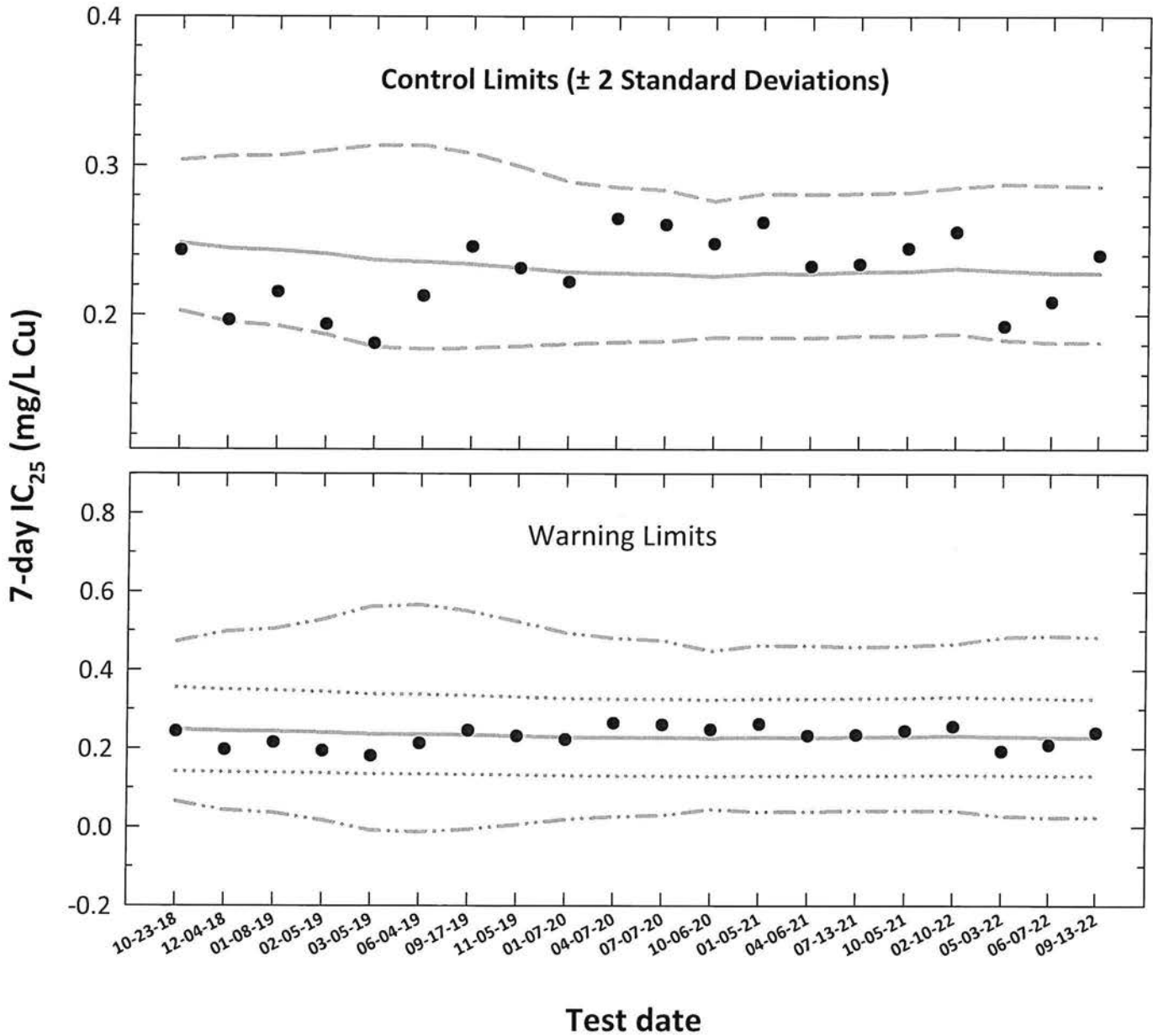


Checked and
 Approved by
 Jan Thomas
JT

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	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	10-23-18	0.2434	-0.6137	-0.6052	0.0439	0.2027	0.3039	0.0650	0.4725	0.1415	0.3549
2	12-04-18	0.1966	-0.7064	-0.6115	0.0489	0.1953	0.3064	0.0429	0.4973	0.1394	0.3498
3	01-08-19	0.2154	-0.6668	-0.6140	0.0505	0.1928	0.3068	0.0359	0.5048	0.1386	0.3478
4	02-05-19	0.1937	-0.7129	-0.6182	0.0550	0.1869	0.3104	0.0170	0.5294	0.1373	0.3445
5	03-05-19	0.1810	-0.7423	-0.6257	0.0612	0.1786	0.3138	-0.0088	0.5623	0.1349	0.3385
6	06-04-19	0.2128	-0.6720	-0.6275	0.0620	0.1772	0.3137	-0.0128	0.5665	0.1344	0.3371
7	09-17-19	0.2458	-0.6094	-0.6304	0.0597	0.1779	0.3083	-0.0061	0.5505	0.1335	0.3349
8	11-05-19	0.2315	-0.6354	-0.6352	0.0557	0.1792	0.2994	0.0054	0.5240	0.1320	0.3312
9	01-07-20	0.2222	-0.6533	-0.6408	0.0511	0.1807	0.2893	0.0189	0.4941	0.1303	0.3270
10	04-07-20	0.2646	-0.5774	-0.6422	0.0490	0.1819	0.2856	0.0261	0.4809	0.1299	0.3260
11	07-07-20	0.2606	-0.5840	-0.6429	0.0479	0.1825	0.2838	0.0295	0.4745	0.1297	0.3254
12	10-06-20	0.2479	-0.6057	-0.6457	0.0436	0.1850	0.2763	0.0443	0.4483	0.1289	0.3233
13	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.1848	0.2816	0.0382	0.4625	0.1300	0.3262
14	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.1847	0.2810	0.0385	0.4612	0.1298	0.3258
15	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.1862	0.2817	0.0420	0.4590	0.1305	0.3274
16	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.1861	0.2822	0.0414	0.4604	0.1306	0.3277
17	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.1874	0.2855	0.0414	0.4656	0.1318	0.3307
18	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.1832	0.2877	0.0276	0.4827	0.1309	0.3283
19	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.1815	0.2872	0.0233	0.4861	0.1301	0.3265
20	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.1816	0.2861	0.0246	0.4831	0.1299	0.3259

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

S = Standard deviation of the IC₂₅ values.

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

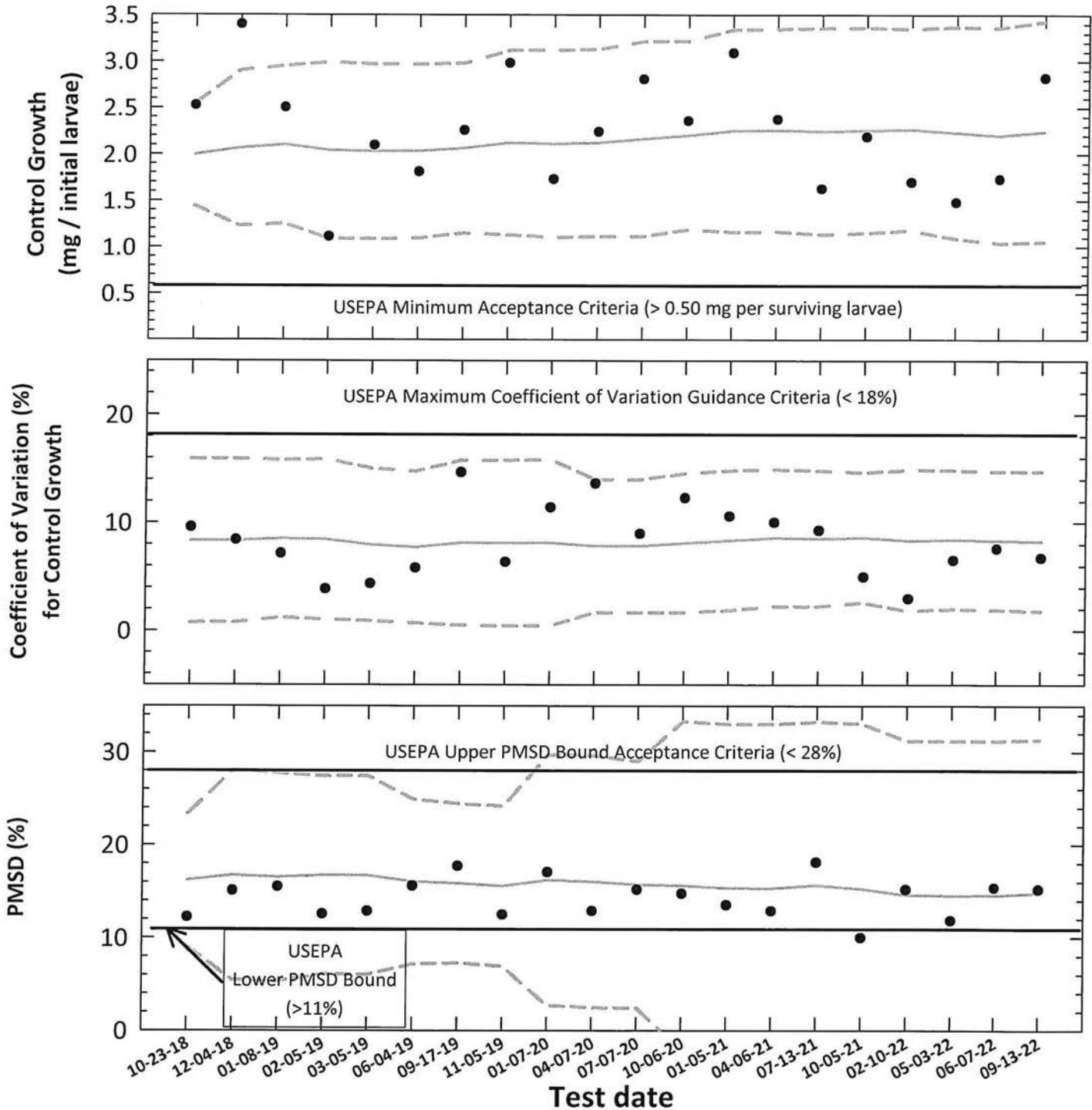
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Menidia beryllina

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



- **Control Growth, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**
PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
- **Central Tendency (mean Control Growth, CV or PMSD)**
- - - **95% Confidence Interval (mean Control Growth, CV or PMSD ± 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		CT	95% Confidence Interval		CT	95% Confidence Interval				
			Mean (mg/initial larvae)	CV (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S			
1	10-23-18	100	2.526	9.6	1.9964	1.443	2.550	8.3	0.7	15.9	16.2	9.0	23.4
2	12-04-18	100	3.399	8.4	2.067	1.230	2.903	8.3	0.8	15.9	16.7	5.4	28.1
3	01-08-19	100	2.504	7.2	2.102	1.253	2.950	8.5	1.2	15.8	16.5	5.3	27.7
4	02-05-19	100	1.114	3.9	2.042	1.092	2.992	8.5	1.0	15.9	16.7	6.0	27.4
5	03-05-19	100	2.098	4.3	2.030	1.090	2.969	8.0	0.9	15.0	16.7	6.0	27.4
6	06-04-19	100	1.812	5.8	2.032	1.096	2.969	7.7	0.7	14.8	16.0	7.1	24.9
7	09-17-19	100	2.259	14.7	2.067	1.151	2.982	8.1	0.5	15.8	15.8	7.3	24.4
8	11-05-19	100	2.983	6.4	2.125	1.132	3.119	8.1	0.4	15.8	15.5	6.9	24.2
9	01-07-20	100	1.732	11.4	2.112	1.105	3.120	8.1	0.4	15.9	16.2	2.7	29.7
10	04-07-20	100	2.244	13.6	2.123	1.114	3.131	7.8	1.7	14.0	16.1	2.5	29.6
11	07-07-20	100	2.809	9.0	2.166	1.117	3.215	7.8	1.7	14.0	15.7	2.4	29.0
12	10-06-20	100	2.361	12.3	0.4264	1.188	3.221	8.1	1.7	14.6	15.6	-2.1	33.3
13	01-05-21	100	3.093	10.6	0.4186	1.163	3.343	8.3	1.9	14.8	15.4	-2.3	33.0
14	04-06-21	100	2.377	10.0	0.3062	1.167	3.349	8.6	2.2	14.9	15.3	-2.3	33.0
15	07-13-21	100	1.631	9.3	0.2956	1.136	3.359	8.5	2.2	14.8	15.7	-2.0	33.3
16	10-05-21	100	2.189	5.0	0.2192	1.153	3.363	8.6	2.6	14.7	15.3	-2.5	33.1
17	02-10-22	100	1.701	3.0	0.2583	1.180	3.351	8.4	1.8	14.9	14.6	-2.0	31.2
18	05-03-22	100	1.483	6.5	0.1756	1.092	3.372	8.4	2.0	14.9	14.5	-2.1	31.2
19	06-07-22	100	1.733	7.6	0.2665	1.041	3.361	8.3	1.9	14.8	14.6	-2.1	31.2
20	09-13-22	100	2.822	6.8	0.4290	1.057	3.430	8.3	1.8	14.7	14.8	-1.7	31.3

Note: Control Survival = USEPA minimum test acceptability criteria \geq 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria \geq 0.50 mg/surviving larvae.
CV = Coefficient of variation for control growth.
MSD = USEPA maximum CV guidance criteria (90th percentile) < 18%
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
Upper PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



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

MbCuCR Test Number: 139

Dilution preparation information:						Comments:
Cu Stock INSS number:	INSS <u>2138</u>					
Stock preparation:	100 mg Cu/L: Dissolve 0.1965 g CuSO ₄ in 500-mL deionized water					
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Test information:

Organism source:	Aquatic Indicators, Inc.	Randomizing template:	<u>ORANGE</u>
Age:	10-days old	Incubator number and shelf location:	<u>6B</u>
Batch:	AI Mb 09-04-22	Artemia CHM number:	CHM1149
Hatch dates and times:	09-03-22 1200 to 09-04-22 1130	Drying information for weight determination:	
		Date / Time in oven:	<u>09-20-22 1150</u> ^{09-20-22 1150}
Transfer vessel information:	pH (S.U.) = <u>7.66</u> Temperature (°C) = <u>25.1</u>	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>09-21-22 1150</u>
Average transfer volume (mL):	< 0.25 mL	*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>2.4 Hours</u>

*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	09-13-22	1030	JL	1200	JL	1148	JL	09-13-22	09-08-22B
1	09-14-22	0500	JL	1100	JL	1025	JL		↓
2	09-15-22	0600	JL	1400	JL	1220	JL		09-14-22
3	09-16-22	0500	JL	1100	JL	1025	JL		↓
4	09-17-22	0600	JL	1215	JL	1450	JL		09-15-22
5	09-18-22	0600	JL	1200	JL	1029	JL		↓
6	09-19-22	0500	JL	1100	JL	1020	JL		09-17-22A
7	09-20-22					1147	JL		

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (%)	<u>0.292</u>
Average weight per initial larvae:	<u>2.822</u>	<u>0.5 mg/larvae</u>	NOEC (%)	<u>0.1</u>
Average weight per surviving larvae:	<u>2.822</u>	≥ 0.25 mg/larvae	LOEC (%)	<u>0.2</u>
			ChV (%)	<u>0.14</u>
			IC ₂₅ (%)	<u>0.240</u>

Species: Menidia beryllina

MbCuCR Test Number: 139

Survival and Growth Data

Day	CONTROL				0.025 mg/L				0.05 mg/L				
	A	B	C	D	E	F	G	H	I	J	K	L	
0	10	10	10	10	10	10	10	10	10	10	10	10	
1	10	10	10	10	10	10	10	10	10	10	10	10	
2	10	10	10	10	10	10	10	10	10	10	10	10	
3	10	10	10	10	10	10	10	10	10	10	10	10	
4	10	10	10	10	10	10	10	10	10	10	10	10	
5	10	10	10	10	10	10	10	10	10	10	10	10	
6	10	10	10	10	10	10	10	10	10	10	10	10	
7	10	10	10	10	10	10	10	10	10	10	10	10	
*A = Pan weight (mg) Tray color code: <u>orange</u> Analyst: <u>EC</u> Date: <u>08-17-22</u>		13.00	14.08	13.17	14.44	14.23	14.02	13.00	15.24	11.54	10.02	15.14	13.23
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>09-22-22</u>		42.67	44.58	38.07	42.92	39.37	45.75	30.43	42.78	44.11	44.90	39.55	40.84
C = Larvae weight (mg) = B - A Analyst: <u>J</u>		29.01	29.90	25.50	28.48	25.14	31.13	23.37	27.54	32.57	28.88	24.41	27.61
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>K</u>		2.901	2.990	2.550	2.848	2.514	3.113	2.337	2.754	3.257	2.888	2.441	2.761
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	2.822				2.680		5.17.		2.837		-0.57.	

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

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

Comments:

Species: Menidia beryllina

MbCuCR Test Number: 139

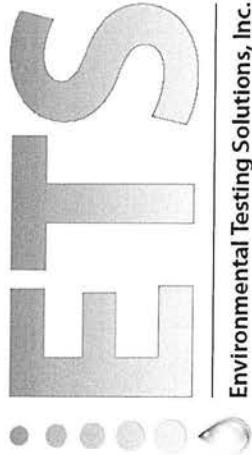
Survival and Growth Data

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 ^{dead}	0 ^{dead}	0 ^{dead}	0 ^{dead}			
2	10	10	10	10	9 ^d	10	10	10							
3	10	10	10	10	9	10	10								
4	10	10	10	10	9	10	9 ^d								
5	10	10	10	10	9	10	9								
6	10	10	10	10	9	10	9								
7	10	10	10	10	9	9 ^d	9								
*A = Pan weight (mg) Tray color code: <u>Orange</u> Analyst: <u>EC</u> Date: <u>08-17-22</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>09-22-22</u>															
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		2.863		-1.47		2.441		13.57		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

Environmental Testing Solutions, Inc.

Test number: 139

Concentration (mg/L Co)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Not for Compliance Assessment, Internal Laboratory QC			Weight / initial number of larvae (mg)	Mean survival (%)	Mean weight/ initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
							Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)					
Control	A	10	10	13.66	42.67	29.01	2.901				2.901			
	B	10	10	14.68	44.58	29.90	2.990			100.0	2.822	6.8	Not applicable	
	C	10	10	13.17	38.67	25.50	2.550							
	D	10	10	14.44	42.92	28.48	2.848							
0.025	E	10	10	14.23	39.37	25.14	2.514							
	F	10	10	14.62	45.75	31.13	3.113			100.0	2.680	12.5	5.1	
	G	10	10	13.06	36.43	23.37	2.337							
	H	10	10	15.24	42.78	27.54	2.754							
0.050	I	10	10	11.54	44.11	32.57	3.257							
	J	10	10	16.02	44.90	28.88	2.888			100.0	2.837	11.9	-0.5	
	K	10	10	15.14	39.55	24.41	2.441							
	L	10	10	13.23	40.84	27.61	2.761							
0.100	M	10	10	12.72	40.83	28.11	2.811							
	N	10	10	15.34	45.31	29.97	2.997			100.0	2.863	4.7	-1.4	
	O	10	10	14.06	43.49	29.43	2.943							
	P	10	10	13.73	40.74	27.01	2.701							
0.200	Q	10	9	14.01	37.09	23.08	2.564							
	R	10	9	14.92	36.69	21.77	2.419			90.0	2.441	9.9	13.5	
	S	10	9	13.99	41.10	27.11	3.012							
	T	10	9	12.23	37.92	25.69	2.854							
0.500	U	10	0	0.00	0.00	0.00	0.000							
	V	10	0	0.00	0.00	0.00	0.000			0.0	0.000	0.0	100.0	
	W	10	0	0.00	0.00	0.00	0.000							
	X	10	0	0.00	0.00	0.00	0.000							

Dunnett's MSD value: $\frac{0.4290}{15.2}$ MSD = Minimum Significant Difference
 PMSD: PMSD = Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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

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



Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 9/13/2022 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
 End Date: 9/20/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUSO4
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina

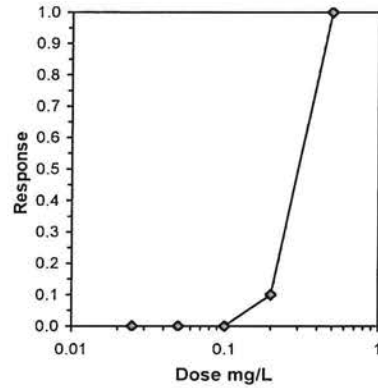
Comments:

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

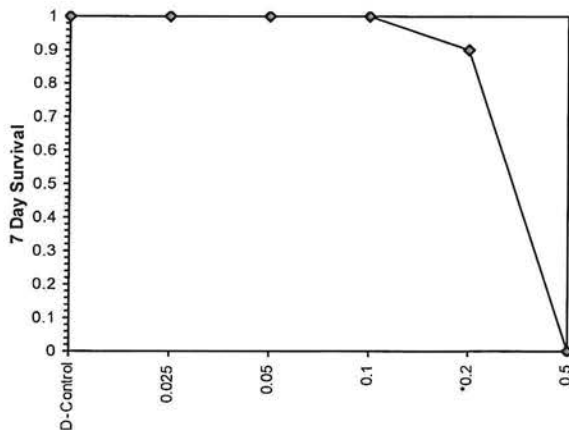
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	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	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	4	10.00	10.00	4	40
0.5	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

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

Trim Level	EC50	95% CL	
0.0%	0.2918	0.2703	0.3149
5.0%	0.2981	0.2714	0.3274
10.0%	0.3005	0.2848	0.3171
20.0%	0.3005	0.2848	0.3171
Auto-0.0%	0.2918	0.2703	0.3149



Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

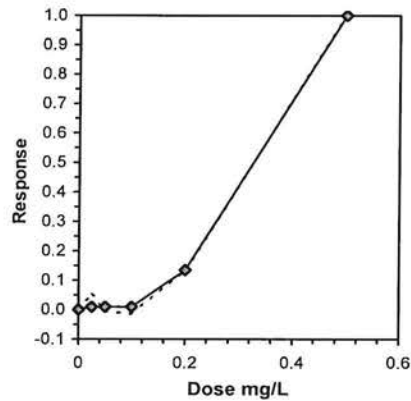
Start Date: 9/13/2022 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
 End Date: 9/20/2022 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUSO4
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina

Conc-mg/L	1	2	3	4
D-Control	2.9010	2.9900	2.5500	2.8480
0.025	2.5140	3.1130	2.3370	2.7540
0.05	3.2570	2.8880	2.4410	2.7610
0.1	2.8110	2.9970	2.9430	2.7010
0.2	2.3080	2.1770	2.7110	2.5690
0.5	0.0000	0.0000	0.0000	0.0000

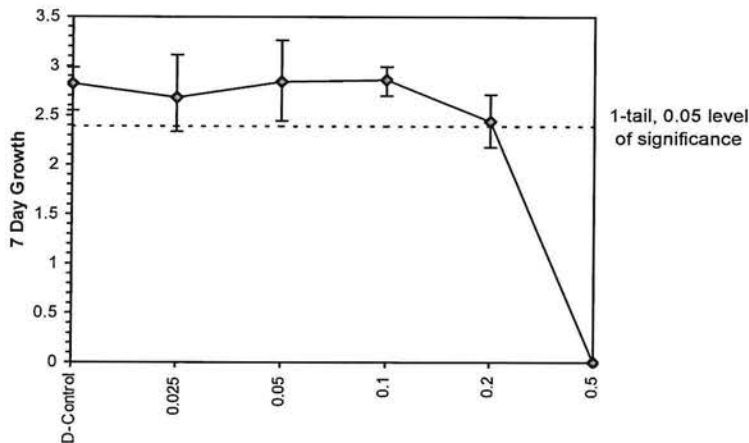
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	2.8223	1.0000	2.8223	2.5500	2.9900	6.758	4				2.8223	1.0000
0.025	2.6795	0.9494	2.6795	2.3370	3.1130	12.530	4	0.762	2.290	0.4290	2.7931	0.9897
0.05	2.8368	1.0051	2.8368	2.4410	3.2570	11.895	4	-0.077	2.290	0.4290	2.7931	0.9897
0.1	2.8630	1.0144	2.8630	2.7010	2.9970	4.656	4	-0.218	2.290	0.4290	2.7931	0.9897
0.2	2.4413	0.8650	2.4413	2.1770	2.7110	9.940	4				2.4413	0.8650
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95699	0.844	0.18715	-0.1373
Bartlett's Test indicates equal variances ($p = 0.42$)	2.82691	11.3449		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	0.1	>0.1		
Treatments vs D-Control	0.42898	0.152	0.02711	0.07018
	MSE	F-Prob	df	
	0.07018	0.76489	3, 12	

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL(Exp)	Skew	
IC05	0.1318	0.0439	0.0000	0.1963	-1.0246
IC10	0.1719	0.0279	0.0897	0.2357	-0.8848
IC15	0.2052	0.0200	0.1258	0.2413	-0.4927
IC20	0.2225	0.0149	0.1589	0.2565	-0.4220
IC25	0.2399	0.0132	0.1865	0.2717	-0.1719
IC40	0.2919	0.0106	0.2492	0.3174	-0.1685
IC50	0.3266	0.0088	0.2910	0.3478	-0.1685



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Menidia beryllina

MbCuCR Test Number: 139

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		EC	EC N	EC W	EC H	EC N	EC	
CONTROL, SaltSW	pH (S.U.)	7.810	7.85	7.78	7.81	7.82	7.80	
	Dissolved oxygen (mg/L)	7.8	7.0	8.0	7.0	7.0	7.7	
	Salinity (ppt)	24.5	24.7	24.8	25.2	24.8	25.0	
	Alkalinity (mg CaCO ₃ /L)	86			100	100		
	Temperature (°C)	24.8	24.7	24.8	25.1	24.9	24.8	
0.025 mg/L	pH (S.U.)	7.95	7.83	7.80	7.75	7.97	7.80	
	Dissolved oxygen (mg/L)	7.7	7.0	8.0	7.0	7.0	7.7	
	Salinity (ppt)	24.7	24.9	24.8	25.2	25.0	25.1	
	Temperature (°C)	24.9	24.9	24.7	25.0	25.0	24.6	
0.05 mg/L	pH (S.U.)	7.95	7.83	7.85	7.75	7.93	7.79	
	Dissolved oxygen (mg/L)	7.7	7.0	8.0	7.0	7.0	7.7	
	Salinity (ppt)	24.6	24.8	24.7	25.1	24.8	25.1	
	Temperature (°C)	24.9	24.9	24.7	25.0	25.0	24.6	
0.1 mg/L	pH (S.U.)	7.90	7.84	7.80	7.70	7.92	7.80	
	Dissolved oxygen (mg/L)	7.8	7.0	8.0	7.0	7.9	7.7	
	Salinity (ppt)	24.0	24.8	24.7	25.2	24.7	25.2	
	Temperature (°C)	24.9	24.8	24.7	25.0	25.0	24.7	
0.2 mg/L	pH (S.U.)	7.97	7.83	7.80	7.70	7.92	7.70	
	Dissolved oxygen (mg/L)	8.0	7.0	8.1	7.0	7.9	7.7	
	Salinity (ppt)	24.6	24.7	24.7	25.2	24.7	25.2	
	Temperature (°C)	24.9	24.9	24.7	24.8	24.9	24.7	
0.5 mg/L	pH (S.U.)	7.97	7.84	7.80				
	Dissolved oxygen (mg/L)	8.0	7.0	8.1				
	Salinity (ppt)	24.5	24.7	24.6				
	Alkalinity (mg CaCO ₃ /L)							
	Temperature (°C)	24.9	24.6	24.8				
		Initial	Final	Initial	Final	Initial	Final	

Species: Menidia beryllina

MbCuCR Test Number: 139

Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH and D.O. measurements only.)							
			3		4		5		6	
Analyst		EC	W	BSL	BSL	BSL	BSL	EC	EC	EC
CONTROL, SaltSW	pH (S.U.)	7.92	7.77	7.86	7.84	8.17	7.87	7.90	7.73	
	Dissolved oxygen (mg/L)	0.0	7.9	7.7	7.9	7.7	7.1	7.8	7.6	
	Salinity (ppt)	24.7	25.0	24.8	25.1	24.8	25.0	24.0	25.2	
	Alkalinity (mg CaCO ₃ /L)	86	86	86		86	86	86		
	Temperature (°C)	24.8	24.6	24.8	24.7	24.8	24.8	24.9	24.9	
0.025 mg/L	pH (S.U.)	7.93	7.76	7.92	7.84	7.97	7.80	7.90	7.75	
	Dissolved oxygen (mg/L)	0.0	7.9	7.9	7.8	7.7	7.2	7.8	7.7	
	Salinity (ppt)	24.9	24.8	25.1	25.0	25.0	25.1	25.0	25.2	
	Temperature (°C)	24.9	24.9	24.8	24.9	24.7	25.0	24.8	24.7	
	0.05 mg/L	pH (S.U.)	7.93	7.75	7.92	7.83	8.00	7.85	7.90	7.75
Dissolved oxygen (mg/L)		7.9	8.0	8.0	7.8	7.7	7.2	7.8	7.7	
Salinity (ppt)		24.9	25.1	25.0	25.1	24.9	25.2	25.0	25.3	
Temperature (°C)		24.9	25.0	24.9	24.9	24.7	24.9	24.8	24.7	
0.1 mg/L		pH (S.U.)	7.93	7.75	7.92	7.82	8.00	7.84	7.90	7.75
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.7	7.8	7.3	7.9	7.7	
	Salinity (ppt)	24.8	25.0	25.0	25.1	24.9	25.1	25.0	25.2	
	Temperature (°C)	24.9	24.7	24.9	24.8	24.7	24.7	24.8	24.9	
	0.2 mg/L	pH (S.U.)	7.93	7.75	7.92	7.83	7.98	7.86	7.97	7.75
Dissolved oxygen (mg/L)		7.9	8.0	8.0	7.7	7.9	7.3	7.9	7.8	
Salinity (ppt)		24.8	25.3	25.0	25.4	24.8	25.1	25.0	25.2	
Temperature (°C)		24.9	24.7	25.0	24.8	24.7	24.7	24.8	24.8	
0.5 mg/L		pH (S.U.)								
	Dissolved oxygen (mg/L)									
	Salinity (ppt)									
	Temperature (°C)									
			Initial	Final	Initial	Final	Initial	Final	Initial	Final