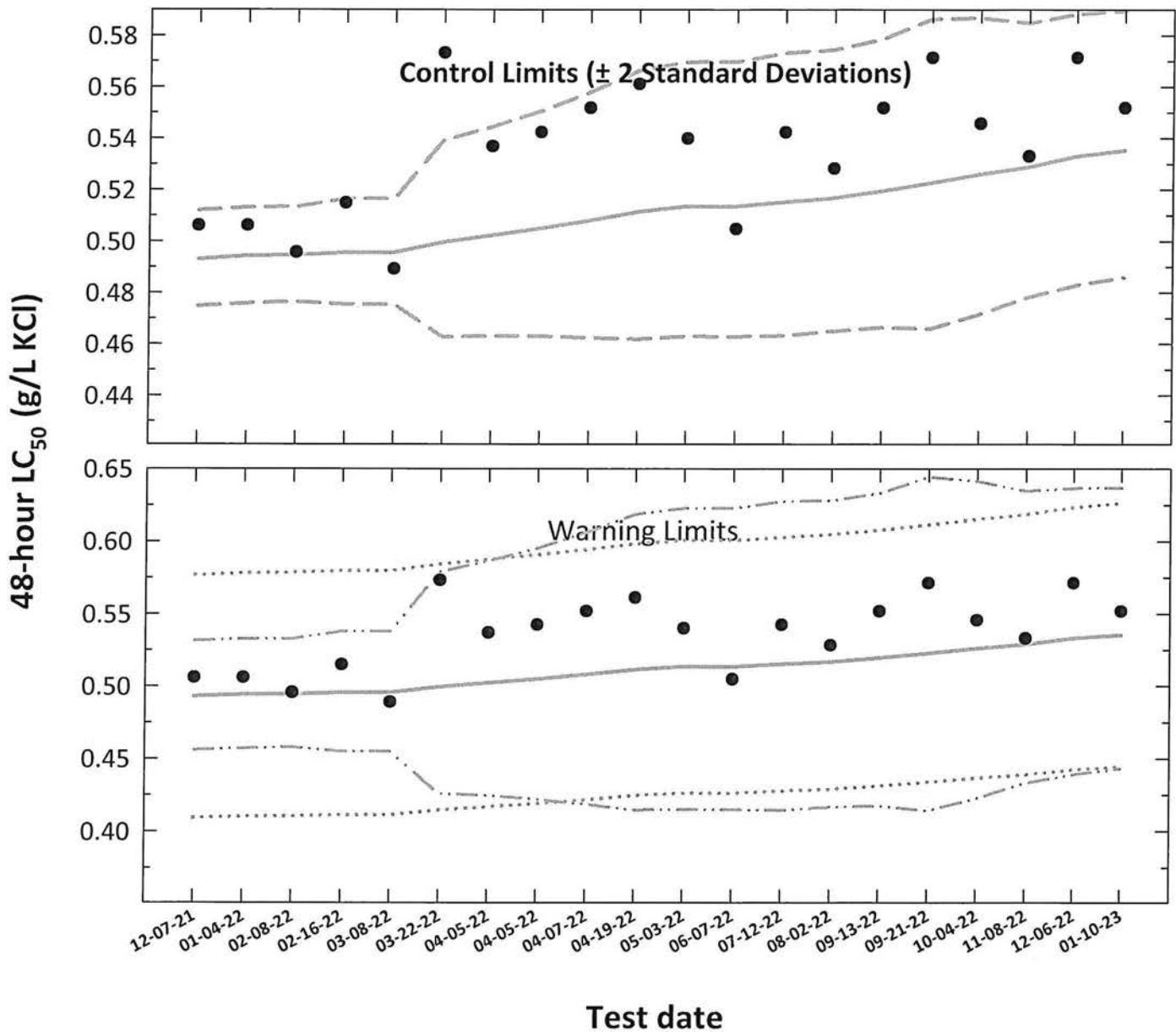


Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour LC₅₀** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ ± 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415	0.4367	0.6155
18	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347	0.4389	0.6187
19	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368	0.4424	0.6236
20	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369	0.4443	0.6263

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 # 262

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 #: 2166

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Concentration			
	Analyst	JL	EW	N
	pH (S.U.)	8.08	7.90	7.92
	Dissolved oxygen (mg/L)	7.7	7.7	7.2
	*Salinity (ppt)	25.0	25.2	25.0
250 mg/L	pH (S.U.)	8.04	8.03	7.92
	Dissolved oxygen (mg/L)	7.8	7.8	7.0
	*Salinity (ppt)	25.0	25.2	25.7
	*Temperature (°C)	25.0	25.0	25.2
	375 mg/L	pH (S.U.)	8.04	8.04
Dissolved oxygen (mg/L)		7.7	7.0	7.1
*Salinity (ppt)		25.1	25.3	26.0
*Temperature (°C)		25.0	25.0	25.2
500 mg/L		pH (S.U.)	8.05	8.05
	Dissolved oxygen (mg/L)	7.7	7.0	7.4
	*Salinity (ppt)	25.3	25.4	25.0
	*Temperature (°C)	25.0	25.2	25.1
	750 mg/L	pH (S.U.)	8.06	8.05
Dissolved oxygen (mg/L)		7.8	7.9	
*Salinity (ppt)		25.5	25.6	
*Temperature (°C)		25.0	25.2	
1000 mg/L		pH (S.U.)	8.07	8.04
	Dissolved oxygen (mg/L)	0.1023 2 [#] 7.9	7.0	
	*Salinity (ppt)	25.0	25.7	
	*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	13066469

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 # 262

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltsW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-10-23	* 1020	JL	1230	JL	6D	LIGHT GREEN	01-09-23A
24	01-11-23			1230	JL			
48 Termination	01-12-23			1230	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	01-09-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	01-08-23 1200 TU 01-09-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.79 Temperature (°C) 24.3

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	7 ^{3d}	7 ^{3d}	0	0	0	0
Mean Survival	100%		100%		100%		70%		0%		0%	

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

Statistics:

Method	(SK) GRAPHICAL	Comments:
Lower 95% confidence limit (mg KCl/L)	514.1	
Upper 95% confidence limit (mg KCl/L)	592.5	
48-hour LC ₅₀ (mg KCl/L)	551.9	



Acute Mysid Test-24 Hr Survival

Start Date: 1/10/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/12/2023	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	1.0000	1.0000
750	0.0000	0.0000
1000	0.0000	0.0000

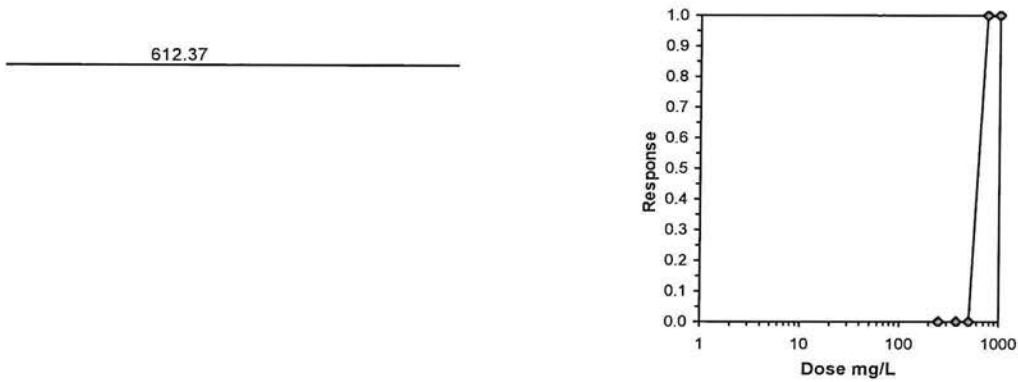
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20	
375	1.0000	1.0000	1.4120	1.4120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

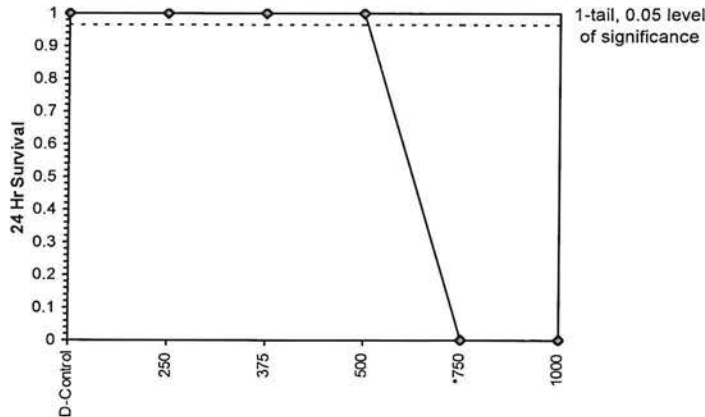
Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed			
Equality of variance cannot be confirmed			
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV
Dunnnett's Test	500	750	612.372
Treatments vs D-Control	MSDu	MSDp	MSB
	0.00967	0.00991	0.62824
	MSE	F-Prob	df
	0.0001	2.0E-09	4, 5

Trim Level	EC50
0.0%	612.37

Graphical Method



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 1/10/2023 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 1/12/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

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

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	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

Normality of the data set cannot be confirmed

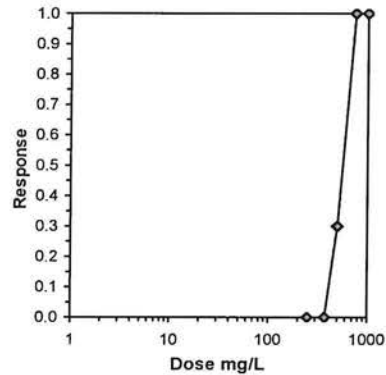
Equality of variance cannot be confirmed

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

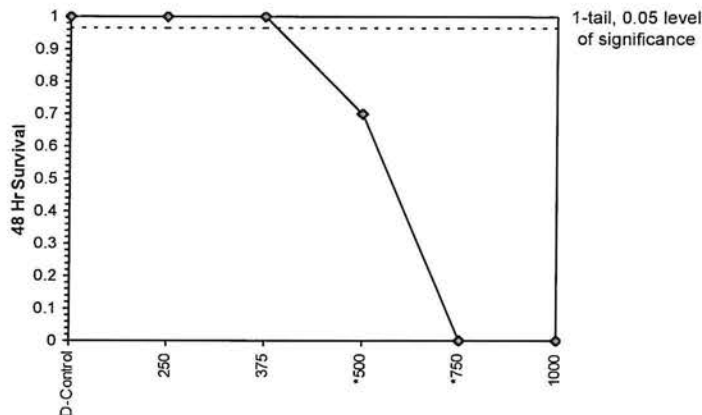
Treatments vs D-Control

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



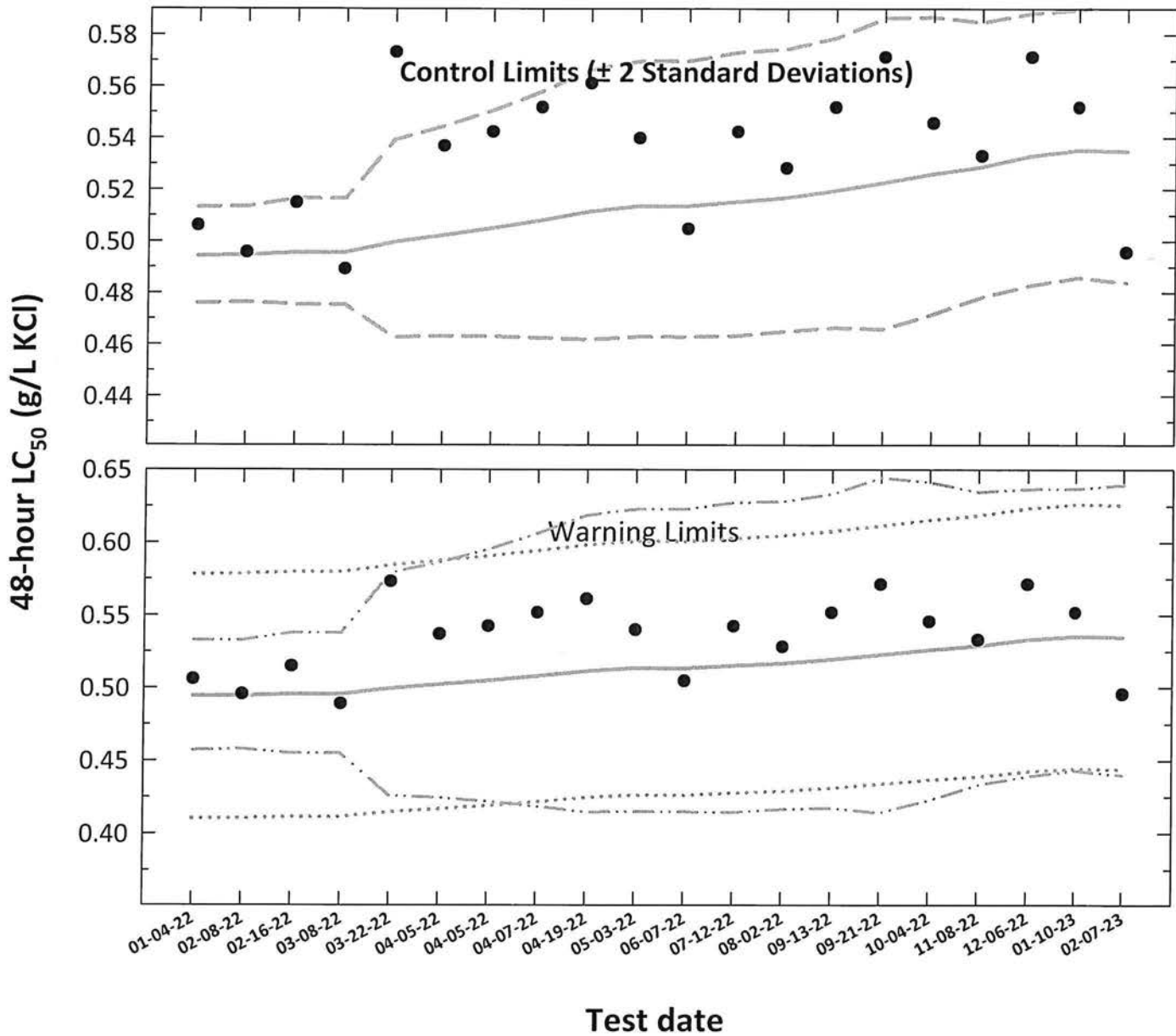
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	Control Limits		Warning Limits		10th Percentile CV		
						CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}	
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415	0.4367	0.6155
17	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347	0.4389	0.6187
18	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368	0.4424	0.6236
19	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369	0.4443	0.6263
20	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399	0.6396	0.4439	0.6257

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 # 263

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 #: 2166

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Concentration			
	Analyst	u	u	u
	pH (S.U.)	7.00	7.04	7.00
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	*Salinity (ppt)	25.0	25.1	25.7
250 mg/L	pH (S.U.)	7.97	7.02	7.77
	Dissolved oxygen (mg/L)	8.0	7.8	7.9
	*Salinity (ppt)	25.2	25.2	25.6
	*Temperature (°C)	25.1	25.0	25.4
	375 mg/L	pH (S.U.)	7.99	7.03
Dissolved oxygen (mg/L)		8.0	7.8	7.9
*Salinity (ppt)		25.2	25.3	25.8
*Temperature (°C)		25.1	25.0	25.4
500 mg/L		pH (S.U.)	8.01	7.02
	Dissolved oxygen (mg/L)	8.0	7.8	7.9
	*Salinity (ppt)	25.3	25.2	25.7
	*Temperature (°C)	25.1	25.2	25.5
	750 mg/L	pH (S.U.)	8.00	7.04
Dissolved oxygen (mg/L)		8.1	7.9	
*Salinity (ppt)		25.4	25.6	
*Temperature (°C)		25.0	25.2	
1000 mg/L		pH (S.U.)	8.00	7.03
	Dissolved oxygen (mg/L)	8.1	8.0	
	*Salinity (ppt)	25.6	25.4	
	*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 # 263

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-07-23*	1040	JL	1240	JL	1C	Yellow	02-01-23 A
24	02-08-23			1242	JL			
48 Termination	02-09-23			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 Ab):	02-06-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	02-05-23 1200 TD 02-06-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.96
	Temperature (°C) 24.8

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9 ^{1d}	5 ^{sd}	5 ^{sd}	0	0	0	0
Mean Survival	100%		100%		95%		50%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	458.6
Upper 95% confidence limit (mg KCl/L)	547.7
48-hour LC ₅₀ (mg KCl/L)	495.7

Comments:



Acute Mysid Test-24 Hr Survival

Start Date: 2/7/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 2/9/2023	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

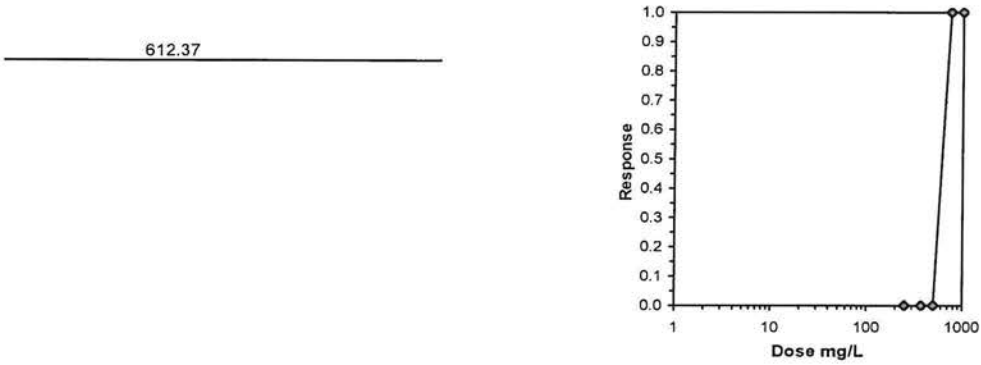
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	1.0000	1.0000
750	0.0000	0.0000
1000	0.0000	0.0000

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	

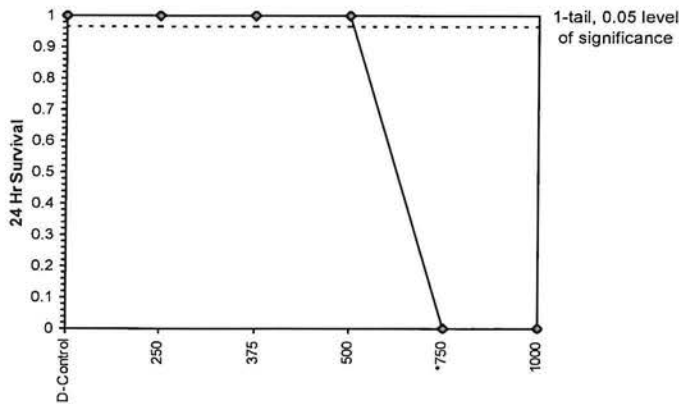
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.00967	0.00991	0.62824	0.0001	2.0E-09	4, 5

Trim Level	EC50
0.0%	612.37

Graphical Method



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 2/7/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 2/9/2023	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	0.9000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	12.159	2.850	0.1469	10	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	0.60026	0.00266	7.8E-06	4, 5

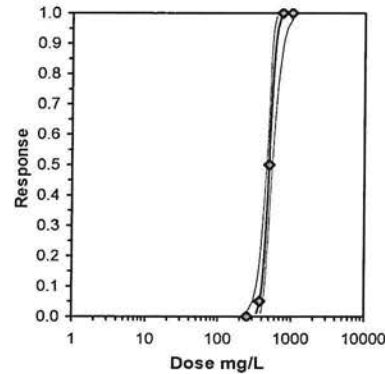
Treatments vs D-Control

Maximum Likelihood-Probit

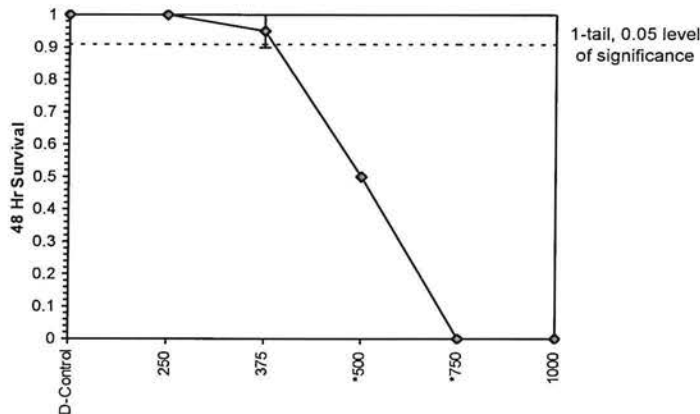
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.3605	3.51976	7.4618 21.2592	0	0.17806	7.81472	0.98105	2.69519	0.06964	4
Intercept	-33.704	9.43318	-52.193 -15.215							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	341.348	245.982 387.152
EC05	3.355	380.762	301.411 419.772
EC10	3.718	403.601	334.964 439.493
EC15	3.964	419.779	358.932 454.273
EC20	4.158	433.099	378.48 467.26
EC25	4.326	444.862	395.35 479.6
EC40	4.747	475.939	436.335 517.953
EC50	5.000	495.671	458.62 547.68
EC60	5.253	516.22	478.836 582.992
EC75	5.674	552.283	509.223 653.418
EC80	5.842	567.283	520.655 685.187
EC85	6.036	585.283	533.773 724.894
EC90	6.282	608.744	550.147 778.99
EC95	6.645	645.258	574.488 867.975
EC99	7.326	719.763	621.393 1066.13



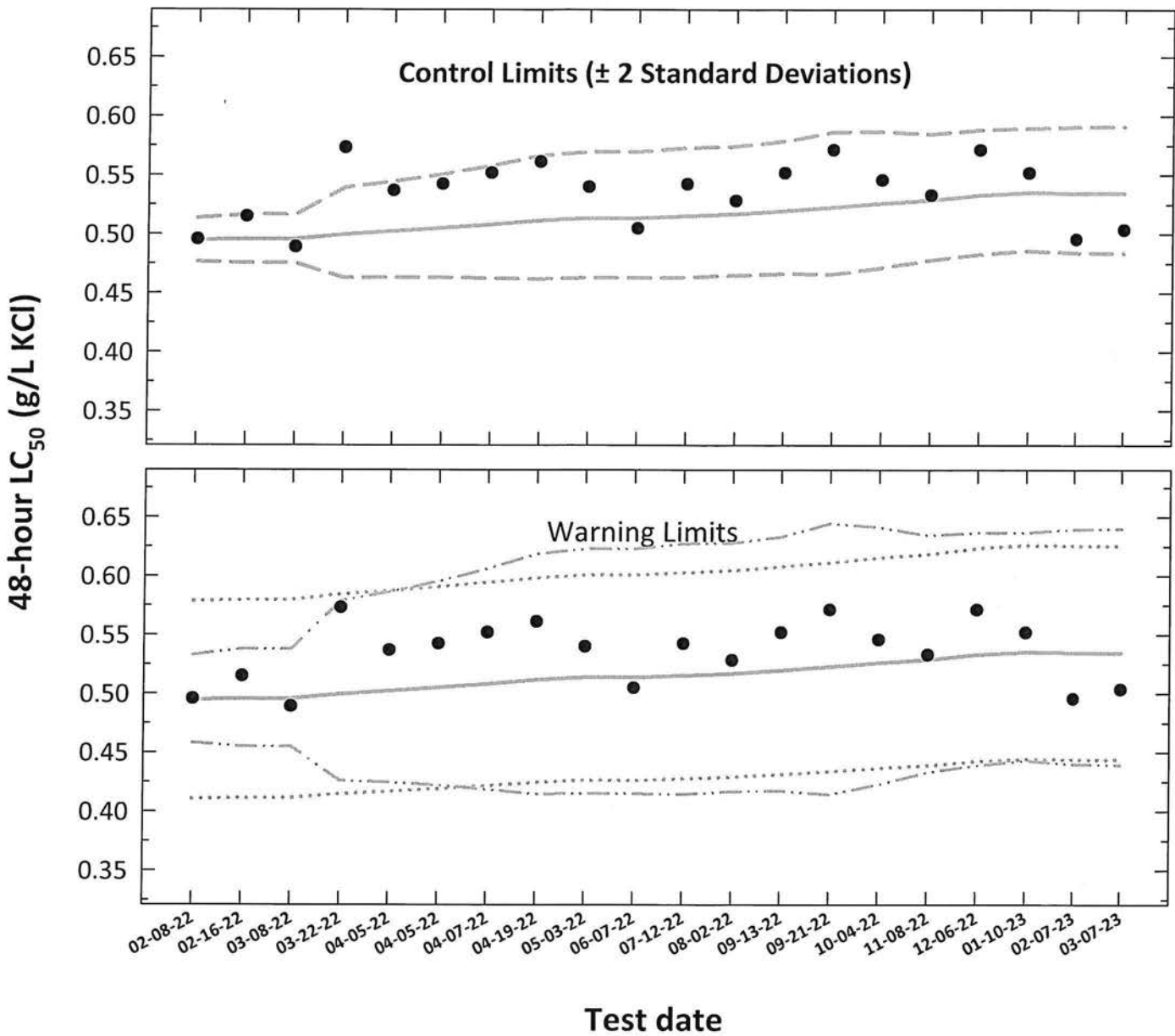
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **48-hour 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)

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	10-04-22	0.5458	-0.2630	-0.2789	0.0237	0.5261	0.4716	0.5868	0.4226	0.6415	0.4367	0.6155
16	11-08-22	0.5331	-0.2732	-0.2767	0.0218	0.5288	0.4782	0.5848	0.4331	0.6347	0.4389	0.6187
17	12-06-22	0.5714	-0.2431	-0.2733	0.0214	0.5330	0.4829	0.5884	0.4390	0.6368	0.4424	0.6236
18	01-10-23	0.5519	-0.2581	-0.2714	0.0210	0.5353	0.4860	0.5897	0.4431	0.6369	0.4443	0.6263
19	02-07-23	0.4957	-0.3048	-0.2718	0.0217	0.5348	0.4840	0.5909	0.4399	0.6396	0.4439	0.6257
20	03-07-23	0.5036	-0.2979	-0.2719	0.0218	0.5347	0.4836	0.5911	0.4392	0.6402	0.4438	0.6255

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 # 264

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 #: 2166

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	<u>KL</u>	<u>BLK</u>	<u>KL</u>
Control, SaltSW	pH (S.U.)	<u>7.97</u>	<u>7.92</u>	<u>7.75</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.7</u>	<u>7.5</u>
	*Salinity (ppt)	<u>25.0</u>	<u>25.0</u>	<u>25.5</u>
	*Alkalinity (mg/L CaCO ₃)	<u>100</u>		
	*Temperature (°C)	<u>24.9</u>	<u>24.8</u>	<u>25.3</u>
250 mg/L	pH (S.U.)	<u>7.96</u>	<u>7.92</u>	<u>7.74</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.7</u>	<u>7.5</u>
	*Salinity (ppt)	<u>25.4</u>	<u>25.4</u>	<u>25.7</u>
	*Temperature (°C)	<u>24.7</u>	<u>24.7</u>	<u>25.2</u>
	375 mg/L	pH (S.U.)	<u>7.96</u>	<u>7.94</u>
Dissolved oxygen (mg/L)		<u>8.0</u>	<u>7.8</u>	<u>7.4</u>
*Salinity (ppt)		<u>25.5</u>	<u>25.5</u>	<u>25.9</u>
*Temperature (°C)		<u>24.7</u>	<u>24.7</u>	<u>25.4</u>
500 mg/L		pH (S.U.)	<u>7.98</u>	<u>7.89</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.8</u>	<u>7.3</u>
	*Salinity (ppt)	<u>25.5</u>	<u>25.5</u>	<u>26.0</u>
	*Temperature (°C)	<u>24.7</u>	<u>25.0</u>	<u>25.4</u>
	750 mg/L	pH (S.U.)	<u>7.98</u>	<u>7.89</u>
Dissolved oxygen (mg/L)		<u>8.0</u>	<u>7.9</u>	
*Salinity (ppt)		<u>26.3</u>	<u>26.0</u>	
*Temperature (°C)		<u>24.9</u>	<u>24.9</u>	
1000 mg/L		pH (S.U.)	<u>7.99</u>	<u>7.86</u>
	Dissolved oxygen (mg/L)	<u>8.1</u>	<u>7.9</u>	
	*Salinity (ppt)	<u>26.3</u>	<u>26.2</u>	
	*Temperature (°C)	<u>24.8</u>	<u>24.9</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>130664665</u>

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Americamysis bahia

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 264

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-07-23	* 1020	JL	1220	JL	SF	PURAE	0228-13A
24	03-08-23			1220	JL			
48 Termination	03-09-23			1218	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	03-06-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	03-05-23 1200 TO 03-06-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.19
	Temperature (°C) 24.6

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	7 ^{sd}	7 ^{sd}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9 ^{id}	5 ^{sd}	6 ^{id}	0	0	0	0
Mean Survival	100%		100%		95%		55%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	465.3
Upper 95% confidence limit (mg KCl/L)	557.0
48-hour LC ₅₀ (mg KCl/L)	505.6

Comments:

Test Reviewed by:


Acute Mysid Test-24 Hr Survival

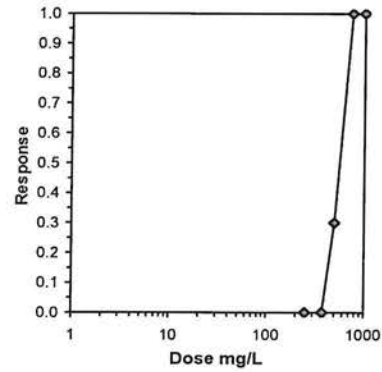
Start Date: 3/7/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 3/9/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americamysis bahia

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

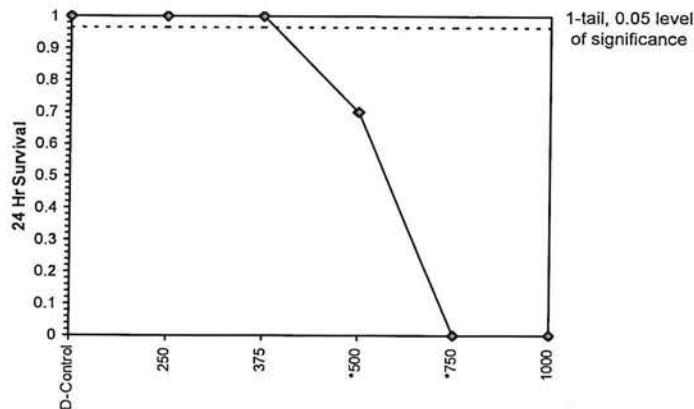
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20	
375	1.0000	1.0000	1.4120	1.4120	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
Dunnett's Test	375	500	433.013	0.00967
Treatments vs D-Control				0.00991
				0.5936
				0.0001
				2.3E-09
				4, 5

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



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 3/7/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 3/9/2023	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	0.9000
500	0.5000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

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

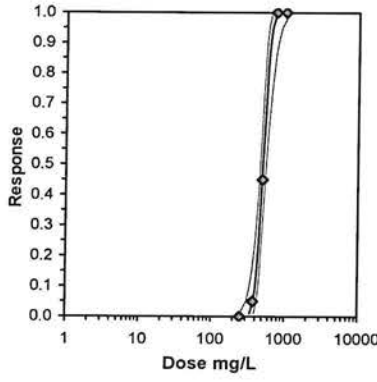
Auxiliary Tests

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

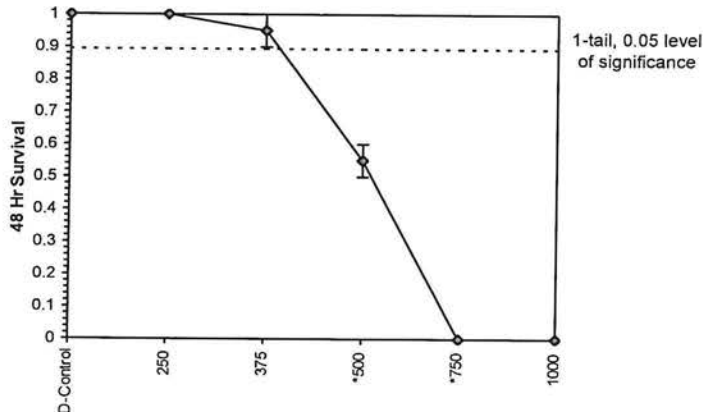
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.08088	0.08295	0.58948	0.00367	1.8E-05	4, 5

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

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



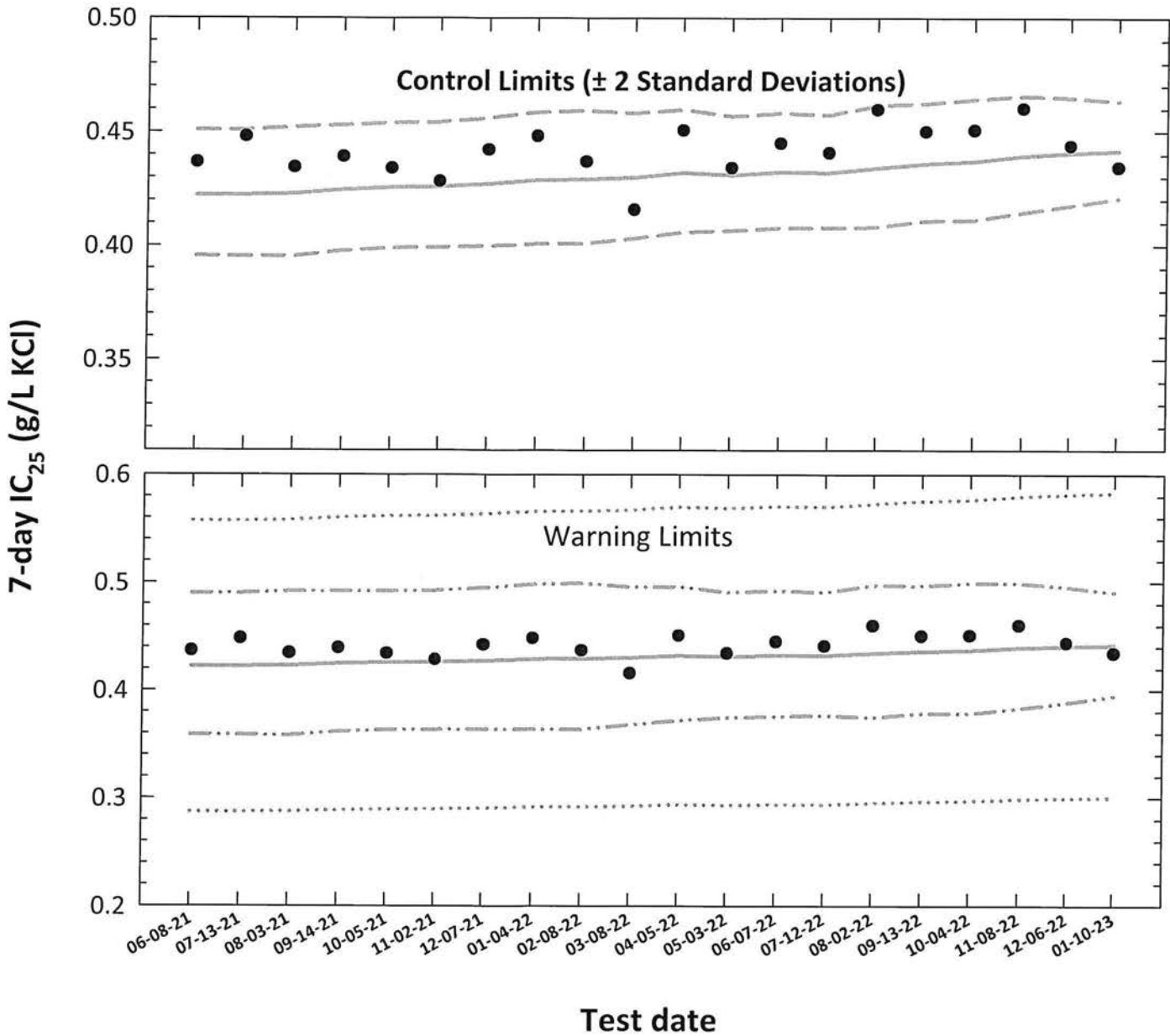
Dose-Response Plot



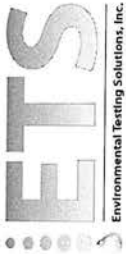
Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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



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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}			
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	10-04-22	0.4509	-0.3459	-0.3595	0.0132	0.4371	0.4113	0.4644	0.3782	0.4995	0.2972	0.5769
18	11-08-22	0.4604	-0.3368	-0.3571	0.0127	0.4395	0.4146	0.4658	0.3829	0.4995	0.2988	0.5801
19	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4408	0.4177	0.4651	0.3884	0.4960	0.2997	0.5818
20	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208	0.4635	0.3944	0.4911	0.3003	0.5829

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).

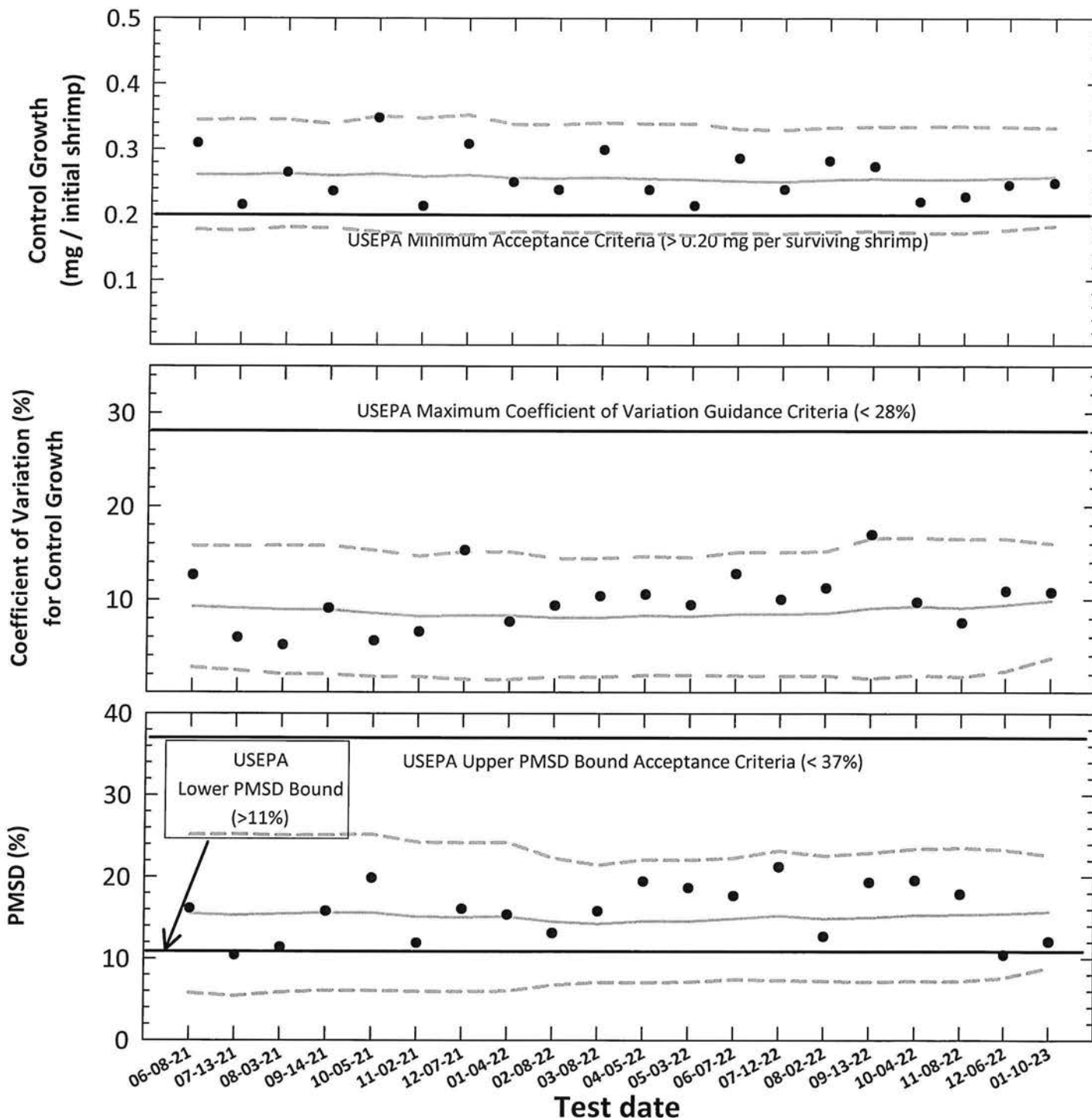
CV = Coefficient of variation.



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		CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S				
			Mean (mg/initial shrimp)	CV (%)								MSD	PMSD (%)		
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	10-04-22	100	0.220	9.7	0.0430	19.5	0.254	0.173	0.335	9.3	1.9	16.7	15.3	7.2	23.4
18	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	0.335	9.1	1.7	16.5	15.4	7.2	23.5
19	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3
20	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6

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.
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
USEPA maximum CV guidance criteria (90th percentile) < 28%
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 37%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.

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

Species: *Americamysis (Mysidopsis) bahia*

AbKICR Test Number: 235

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2166					
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:	01-03-23 1200 to 01-04-23 1130	Incubator number and shelf location:	SF
Organism source:	AI Batch Ab: 01-04-23	Artemia CHM number:	CHM1222
Transfer bowl information:	pH = 7.72 S.U. Temperature = 25.0 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	01-17-23 1115
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	01-18-23 1115
		*Final oven temperature:	60 °C
		Total drying time:	24-HOURS

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	01-10-23	1020	JL	1145	JL	1115	JL	01-09-23 A
1	01-11-23	0500	JL	1100	JL	0915	JL	↓
2	01-12-23	0500	JL	1130	JL	1100	JL	01-11-23
3	01-13-23	0500	JL	1115	JL	0925	JL	↓
4	01-14-23	0600	JL	1200	JL	1138	JL	01-12-23
5	01-15-23	0550	JL	1150	JL	0920	JL	01-13-23
6	01-16-23	0500	JL	1100	JL	0915	JL	↓
7	01-17-23					0938	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	1306646 & S

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07	≤ 20%	7-day LC ₅₀ (mg/L KCl)	536.7
Average weight per initial shrimp:	0.249		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.249	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	434.4

AbKCICR Test Number: 235

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>Forest Green</u> Analyst: <u>EC</u> Date: <u>12-29-22</u>	13.80	14.21	14.10	14.54	13.11	15.45	14.19	14.00	14.80	14.89	15.74	12.73	14.11	14.27	14.30	13.44
*B = Pan + Shrimp weight (mg) Analyst: <u>jl</u> Date: <u>01-23-23</u>	15.32	15.41	15.24	15.83	14.16	16.73	15.43	15.94	15.92	15.91	16.88	13.94	15.19	15.36	15.65	14.49
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>jl</u>	1.46	1.20	1.08	1.29	1.05	1.28	1.24	1.34	1.12	1.02	1.14	1.21	1.08	1.09	1.29	1.05
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>jl</u>	0.292	0.240	0.216	0.258	0.210	0.256	0.248	0.268	0.224	0.204	0.228	0.242	0.216	0.218	0.258	0.210
Average weight per initial number of shrimp (mg)	0.249								0.225							
Percent reduction from control (%)	9.57															

*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:

AbKCICR Test Number: 235

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	4 ^{id}	5	5	5	5	5	5	5	5	5	5	4 ^{id}	5	4 ^{id}
4	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	4
5	5	5	4	5	5	5	5	5	5	5	5	5	5	4	5	4
6	5	5	4	5	5	5	5	5	4 ^{id}	5	5	5	5	4	5	4
7	5	5	4	5	5	5	5	5	3 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	3 ^{id}	3 ^{id}	4 ^{id}	4
# females with eggs in brood sac	/															
# females with developing ova in oviducts	/															
# immature females	/															
# males	/															
*A = Pan weight (mg) Tray color code: <u>Forces + Green</u> Analyst: <u>SC</u> Date: <u>12-29-22</u>	14.27	14.27	13.10	14.17	14.80	14.38	15.73	15.00	14.29	13.31	12.93	15.29	14.85	12.58	14.03	13.87
*B = Pan + Shrimp weight (mg) Analyst: <u>J</u> Date: <u>01-23-23</u>	15.31	15.30	14.20	15.19	15.94	15.67	16.81	16.73	14.98	13.99	13.72	16.05	15.56	13.18	15.53	14.70
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.04	1.03	1.04	1.02	1.14	1.29	1.08	1.17	0.69	0.68	0.79	0.76	0.71	0.60	0.90	0.83
Weight per Initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.208	0.206	0.208	0.204	0.228	0.258	0.216	0.234	0.138	0.136	0.158	0.152	0.142	0.120	0.180	0.166
Average weight per initial number of shrimp (mg)	0.220															
Percent reduction from control (%)	11.47															
Average weight per initial number of shrimp (mg)									0.149							
Percent reduction from control (%)									40.07							

*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: 235

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	01-11-13															
*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: _____	01-11-13															
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average weight per initial number of shrimp (mg)	0								0							
Percent reduction from control (%)	100%								100%							

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

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

Comments:



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

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

Test number: 235

Test dates: January 10-17, 2023

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	13.86	15.32	1.46	0.292	100.0	0.249	10.8	Not applicable
	B	5	5	14.21	15.41	1.20	0.240				
	C	5	5	14.16	15.24	1.08	0.216				
	D	5	5	14.54	15.83	1.29	0.258				
	E	5	5	13.11	14.16	1.05	0.210				
	F	5	5	15.45	16.73	1.28	0.256				
	G	5	5	14.19	15.43	1.24	0.248				
	H	5	5	14.60	15.94	1.34	0.268				
250	I	5	5	14.80	15.92	1.12	0.224	100.0	0.225	7.9	9.5
	J	5	5	14.89	15.91	1.02	0.204				
	K	5	5	15.74	16.88	1.14	0.228				
	L	5	5	12.73	13.94	1.21	0.242				
	M	5	5	14.11	15.19	1.08	0.216				
	N	5	5	14.27	15.36	1.09	0.218				
	O	5	5	14.36	15.65	1.29	0.258				
	P	5	5	13.44	14.49	1.05	0.210				
375	Q	5	5	14.27	15.31	1.04	0.208	97.5	0.220	8.5	11.4
	R	5	5	14.27	15.30	1.03	0.206				
	S	5	4	13.16	14.20	1.04	0.208				
	T	5	5	14.17	15.19	1.02	0.204				
	U	5	5	14.80	15.94	1.14	0.228				
	V	5	5	14.38	15.67	1.29	0.258				
	W	5	5	15.73	16.81	1.08	0.216				
	X	5	5	15.60	16.77	1.17	0.234				
500	Y	5	3	14.29	14.98	0.69	0.138	72.5	0.149	12.7	40.0
	Z	5	4	13.31	13.99	0.68	0.136				
	AA	5	4	12.93	13.72	0.79	0.158				
	BB	5	4	15.29	16.05	0.76	0.152				
	CC	5	3	14.85	15.56	0.71	0.142				
	DD	5	3	12.58	13.18	0.60	0.120				
	EE	5	4	14.63	15.53	0.90	0.180				
	FF	5	4	13.87	14.70	0.83	0.166				
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: 0.0300
PMSD: 12.1

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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



Mysid Survival and Growth Test-7 Day Survival

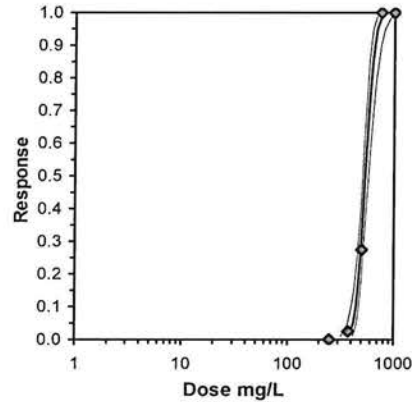
Start Date:	1/10/2023	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/17/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americanysis bahia
Comments:					

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	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.8000	0.8000	0.8000	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

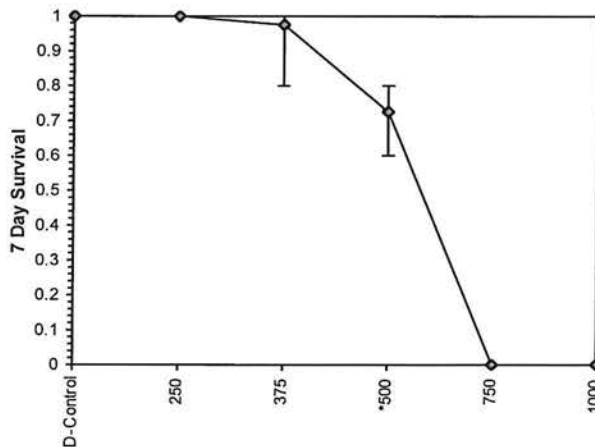
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number	
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8		0	40	
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.7250	0.7250	1.0242	0.8861	1.1071	11.171	8	36.00	48.00	11	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)	0.75505	0.904	-1.5579	2.75863
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		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	15.4089	2.66011	10.1951	20.6227	0	2.19372	7.81472	0.53319	2.72894	0.0649	6
Intercept	-37.05	7.20657	-51.175	-22.925							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	378.413	320.227	413.799							
EC05	3.355	418.98	371.378	449.078							
EC10	3.718	442.355	401.043	470.11							
EC15	3.964	458.858	421.739	485.596							
EC20	4.158	472.413	438.381	498.909							
EC25	4.326	484.36	452.643	511.228							
EC40	4.747	515.821	487.619	547.041							
EC50	5.000	535.723	507.546	572.479							
EC60	5.253	556.394	526.634	600.98							
EC75	5.674	592.534	557.198	654.772							
EC80	5.842	607.519	569.154	678.214							
EC85	6.036	625.464	583.089	706.995							
EC90	6.282	648.799	600.726	745.425							
EC95	6.645	684.995	627.28	806.994							
EC99	7.326	758.429	679.108	938.175							



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Mysid Survival and Growth Test-Growth-Weight

Start Date:	1/10/2023	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/17/2023	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:					

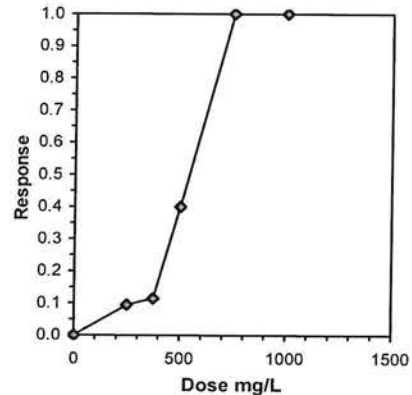
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2920	0.2400	0.2160	0.2580	0.2100	0.2560	0.2480	0.2680
250	0.2240	0.2040	0.2280	0.2420	0.2160	0.2180	0.2580	0.2100
375	0.2080	0.2060	0.2080	0.2040	0.2280	0.2580	0.2160	0.2340
500	0.1380	0.1360	0.1580	0.1520	0.1420	0.1200	0.1800	0.1660
750	0.0000	0.0000	0.0000	0.0000	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	0.2485	1.0000	0.2485	0.2100	0.2920	10.787	8				0.2485	1.0000	
250	0.2250	0.9054	0.2250	0.2040	0.2580	7.850	8	2.190	2.799	0.0300	0.2250	0.9054	
375	0.2203	0.8863	0.2203	0.2040	0.2580	8.511	8	2.632	2.799	0.0300	0.2203	0.8863	
500	0.1490	0.5996	0.1490	0.1200	0.1800	12.734	8				0.1490	0.5996	
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	

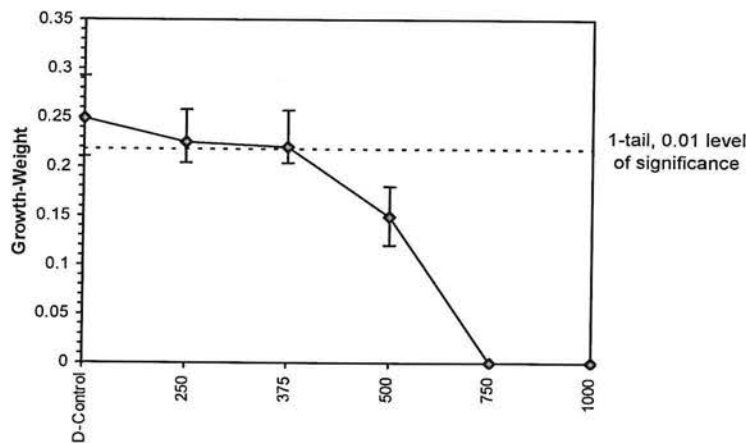
Auxiliary Tests		Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)		0.97081	0.884	0.39641	0.0138						
Bartlett's Test indicates equal variances (p = 0.49)		1.4204	9.21035								
Hypothesis Test (1-tail, 0.01)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		375	>375			0.03003	0.12085	0.00183	0.00046	0.03439	2, 21
Treatments vs D-Control											

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL		Skew
IC05*	132.18	77.98	79.97	376.13	1.5606
IC10	285.53	81.54	159.94	395.58	0.1392
IC15	390.83	42.01	239.91	413.94	-2.2164
IC20	412.63	14.69	381.83	436.83	0.0092
IC25	434.43	13.93	407.79	459.99	0.1381
IC40	499.82	13.74	474.06	526.38	0.0091
IC50	541.53	11.96	518.69	563.65	-0.1147

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Entered and Reviewed by
Jim Sumner

AbKCICR Test Number: 235

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	EC	EC	EC	EC
CONTROL, Salt SW	pH (S.U.)	8.08	7.92	7.98	7.94	8.12	7.87
	DO (mg/L)	7.7	7.7	7.7	7.0	7.6	7.7
	Salinity (ppt)	25.0	25.2	24.7	25.1	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	120		—	01-15-23	110	
	Temperature (°C)	25.4	26.1	25.3	26.2	25.2	26.3
250 mg KCl/L	pH (S.U.)	8.04	8.01	8.00	7.96	8.11	7.79
	DO (mg/L)	7.8	7.6	7.7	7.0	7.5	7.1
	Salinity (ppt)	25.0	25.5	25.0	25.5	25.1	25.4
	Temperature (°C)	25.3	26.2	25.3	26.2	25.2	26.2
375 mg KCl/L	pH (S.U.)	8.04	8.01	8.05	7.96	8.09	7.91
	DO (mg/L)	7.7	7.6	7.7	7.2	7.6	7.6
	Salinity (ppt)	25.1	25.6	25.3	25.8	25.4	25.9
	Temperature (°C)	25.3	26.2	25.3	26.1	25.2	26.2
500 mg KCl/L	pH (S.U.)	8.05	8.01	8.05	7.99	8.10	7.85
	DO (mg/L)	7.7	7.7	7.8	7.4	7.7	7.6
	Salinity (ppt)	25.3	25.7	25.4	25.9	25.6	26.0
	Temperature (°C)	25.3	26.2	25.3	26.2	25.2	26.4
750 mg KCl/L	pH (S.U.)	8.00	8.00				
	DO (mg/L)	7.8	7.7				
	Salinity (ppt)	25.5	25.9				
	Temperature (°C)	25.5	26.2				
1000 mg KCl/L	pH (S.U.)	8.07	8.00				
	DO (mg/L)	7.9	7.8				
	Salinity (ppt)	25.6	26.0				
	Temperature (°C)	25.5	26.0				
		Initial	Final	Initial	Final	Initial	Final

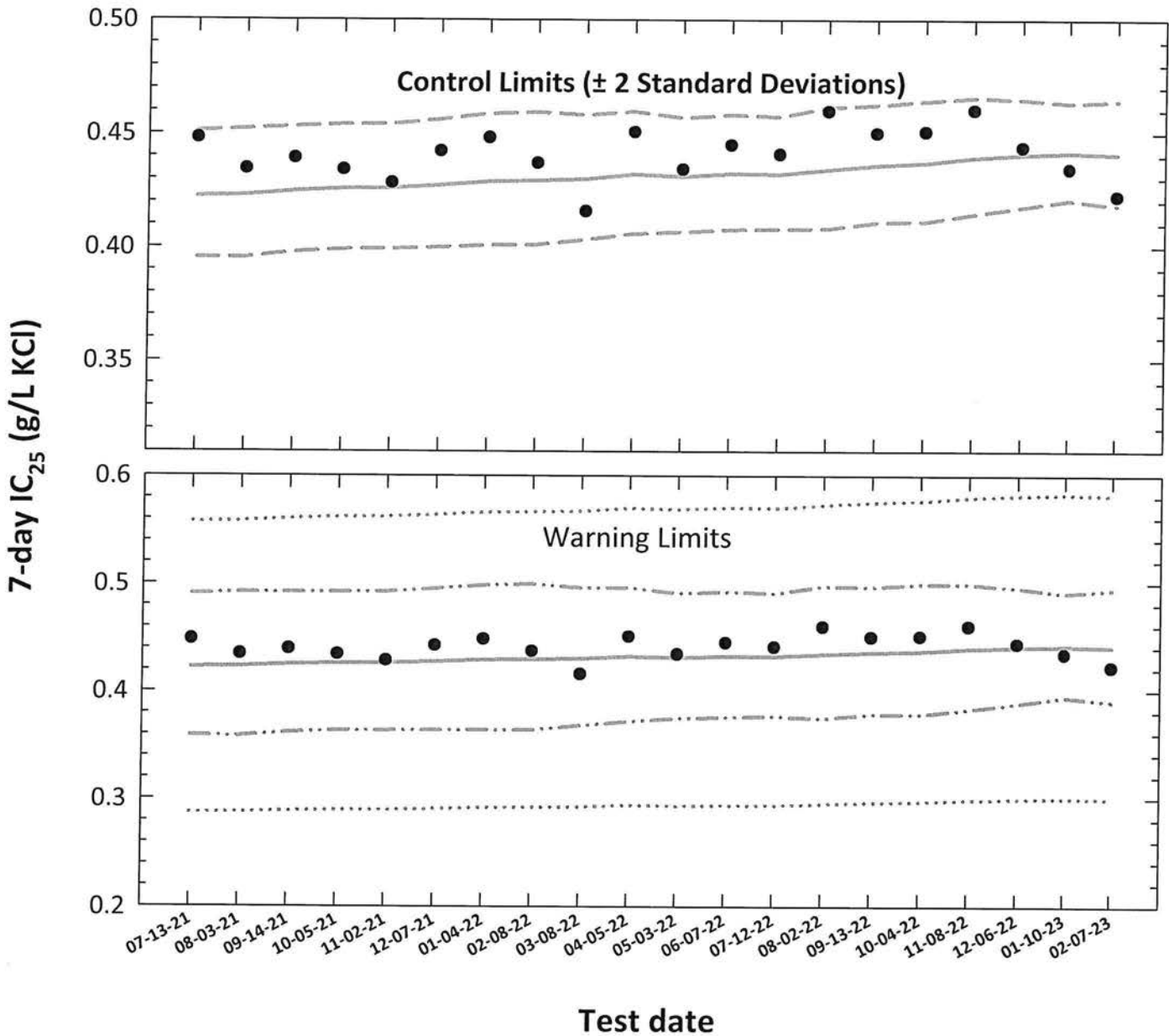
AbKCICR Test Number: 235

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	W	BSC	BSC	W	W	EC	EC	W
CONTROL, Salt SW	pH (S.U.)	8.03	7.80	8.08	7.75	8.05	7.86	8.03	7.70
	DO (mg/L)	7.9	8.0	7.8	7.2	7.8	7.2	7.9	6.6
	Salinity (ppt)	24.8	25.3	25.1	25.4	24.9	25.2	25.2	25.4
	Alkalinity (mg CaCO ₃ /L)	—	100	100	—	94	—	—	—
	Temperature (°C)	25.4	26.4	25.3	26.2	25.2	26.1	25.3	26.4
250 mg KCl/L	pH (S.U.)	8.03	7.95	8.10	7.82	8.03	7.94	8.03	7.75
	DO (mg/L)	7.8	7.7	7.7	7.1	7.7	7.1	7.9	6.5
	Salinity (ppt)	25.2	25.5	25.2	25.4	25.2	25.4	25.0	25.3
	Temperature (°C)	25.3	26.2	25.2	26.4	25.4	26.3	25.4	26.0
375 mg KCl/L	pH (S.U.)	8.03	7.92	8.11	7.83	8.04	7.91	8.04	7.77
	DO (mg/L)	7.8	7.7	7.8	7.3	7.7	7.0	7.9	6.7
	Salinity (ppt)	25.2	25.7	25.4	25.7	25.1	25.5	25.2	25.6
	Temperature (°C)	25.3	26.2	25.2	26.4	25.4	26.5	25.4	26.2
500 mg KCl/L	pH (S.U.)	8.06	7.92	8.08	7.85	8.04	7.94	8.04	7.79
	DO (mg/L)	7.9	7.7	7.8	7.2	7.7	7.1	7.9	7.0
	Salinity (ppt)	25.2	25.7	25.6	25.8	25.2	25.4	25.4	25.7
	Temperature (°C)	25.3	26.3	25.2	26.3	25.4	26.2	25.4	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

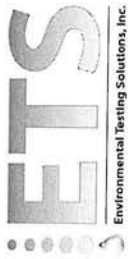
Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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



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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV			
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	75th Percentile CV Warning Limits	
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	10-04-22	0.4509	-0.3459	-0.3595	0.0132	0.4371	0.4113	0.4644	0.3782	0.4995	0.2972	0.5769
17	11-08-22	0.4604	-0.3368	-0.3571	0.0127	0.4395	0.4146	0.4658	0.3829	0.4995	0.2988	0.5801
18	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4408	0.4177	0.4651	0.3884	0.4960	0.2997	0.5818
19	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4416	0.4208	0.4635	0.3944	0.4911	0.3003	0.5829
20	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4409	0.4185	0.4644	0.3901	0.4943	0.2998	0.5820

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).

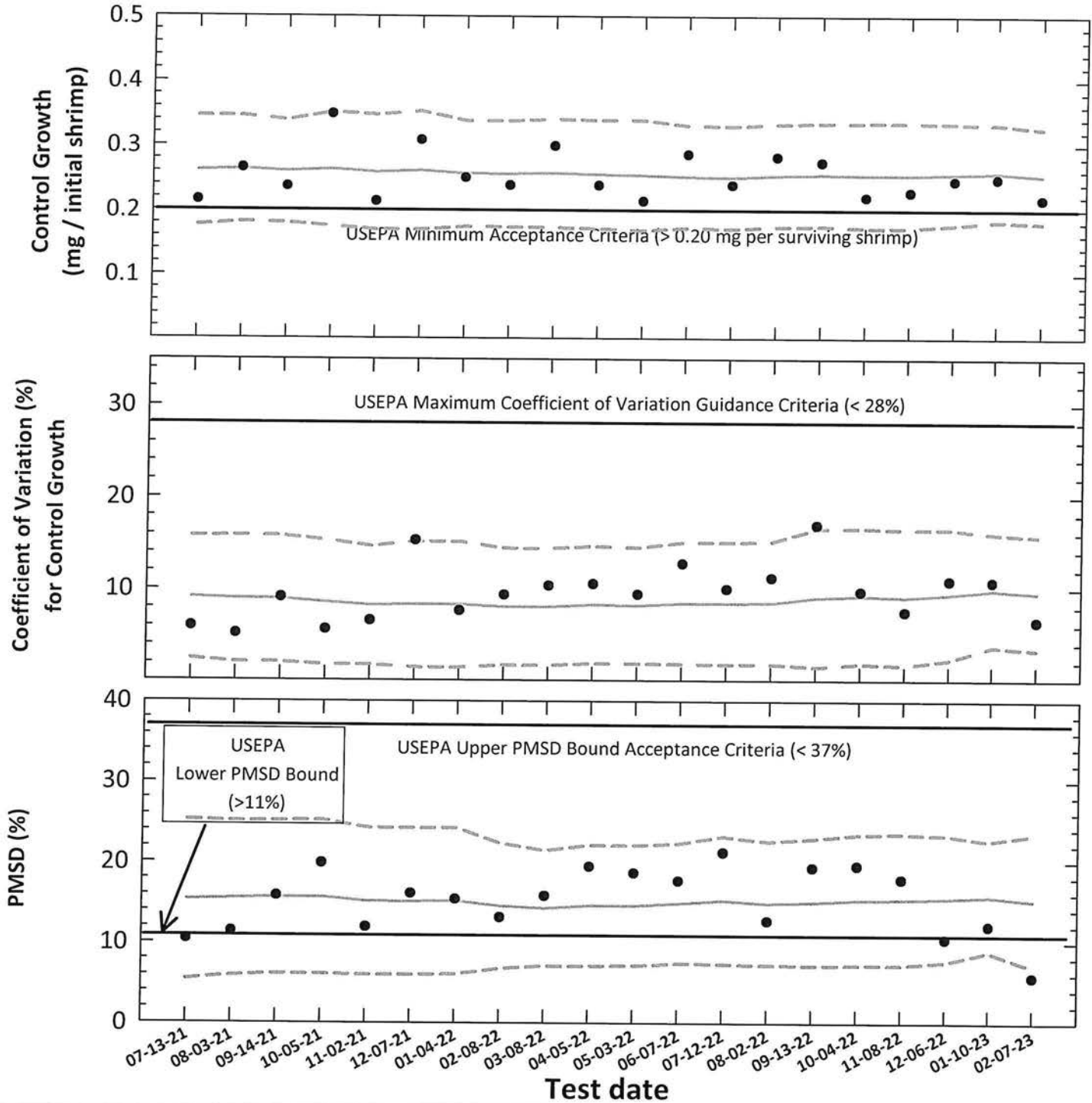
CV = Coefficient of variation.



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 - 2S	CT	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
			Mean (mg/initial shrimp)	CV (%)									
1	07-13-21	100	0.215	5.9	0.0224	10.4	0.261	0.176	9.1	2.4	15.3	5.4	25.2
2	08-03-21	100	0.265	5.2	0.0301	11.4	0.263	0.181	8.9	2.0	15.4	5.9	25.0
3	09-14-21	100	0.236	9.1	0.0373	15.8	0.260	0.180	8.9	2.0	15.6	6.1	25.1
4	10-05-21	100	0.348	5.6	0.0691	19.8	0.263	0.174	8.5	1.8	15.6	6.1	25.2
5	11-02-21	100	0.214	6.6	0.0254	11.9	0.258	0.169	8.2	1.7	14.7	6.0	24.2
6	12-07-21	100	0.308	15.3	0.0495	16.1	0.261	0.169	8.3	1.5	15.1	5.9	24.1
7	01-04-22	100	0.250	7.6	0.0384	15.4	0.256	0.174	8.3	1.5	15.1	6.0	24.2
8	02-08-22	100	0.238	9.4	0.0312	13.1	0.256	0.174	8.1	1.7	14.4	6.7	22.3
9	03-08-22	100	0.299	10.4	0.0472	15.8	0.257	0.173	8.1	1.7	14.4	7.1	21.4
10	04-05-22	100	0.238	10.6	0.0463	19.4	0.255	0.171	8.3	1.9	14.7	7.1	22.1
11	05-03-22	100	0.214	9.4	0.0399	18.6	0.254	0.169	8.2	1.9	14.6	7.1	22.0
12	06-07-22	100	0.287	12.8	0.0506	17.7	0.252	0.173	8.5	1.8	14.9	7.4	23.2
13	07-12-22	100	0.239	10.0	0.0507	21.2	0.251	0.171	8.5	1.8	15.1	7.3	23.2
14	08-02-22	100	0.282	11.3	0.0358	12.7	0.254	0.174	8.5	1.8	15.2	7.2	22.5
15	09-13-22	100	0.274	17.0	0.0529	19.3	0.255	0.175	9.1	1.6	15.0	7.1	22.9
16	10-04-22	100	0.220	9.7	0.0430	19.5	0.254	0.173	9.3	1.9	15.3	7.2	23.4
17	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	9.1	1.7	15.4	7.2	23.5
18	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	9.4	2.3	16.5	7.6	23.3
19	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	9.9	3.7	16.0	8.8	22.6
20	02-07-23	100	0.217	6.5	0.0172	7.9	0.253	0.180	9.6	3.4	15.8	7.6	23.0

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

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

AbKICR Test Number: 236

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2166					
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:	01-31-23 1200 to 02-01-23 1130	Incubator number and shelf location:	SD
Organism source:	AI Batch Ab: 02-01-23	Artemia CHM number:	CHM1222
Transfer bowl information:	pH = 7.96 S.U. Temperature = 25.1 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	02-14-23 1030
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	02-15-23 1030
		*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	02-07-23	1040	JL	1215	JL	1200	JL	02-01-23 A
1	02-08-23	0500	JL	1100	JL	1000	JL	↓
2	02-09-23	0500	JL	1145	JL	1000	JL	02-01-23 B
3	02-10-23	0500	JL	1200	JL	1000	JL	↓
4	02-11-23	0600	JL	1200	JL	1015	JL	02-09-23
5	02-12-23	0500	JL	1100	JL	1000	JL	↓
6	02-13-23	0500	JL	1100	JL	1000	JL	↓
7	02-14-23					1000	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)	495.7
Average weight per initial shrimp:	0.217		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.217	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	422.4

AbKCICR Test Number: 236

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac	/															
# females with developing ova in oviducts	/															
# immature females	/															
# males	/															
*A = Pan weight (mg) Tray color code: <u>lavender</u> Analyst: <u>AL</u> Date: <u>02-04-23</u>	14.52	13.12	16.34	15.53	15.59	15.27	15.19	14.76	16.07	14.78	15.33	15.51	14.38	15.36	14.94	14.02
*B = Pan + Shrimp weight (mg) Analyst: <u>J</u> Date: <u>02-21-23</u>	15.71	14.23	17.42	16.66	16.72	16.26	16.20	15.78	17.22	15.98	16.36	16.71	15.48	16.39	16.02	15.10
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.19	1.11	1.08	1.13	1.13	0.99	1.01	1.02	1.15	1.20	1.03	1.20	1.10	1.03	1.08	1.08
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.238	0.222	0.216	0.226	0.226	0.198	0.202	0.204	0.230	0.240	0.206	0.240	0.220	0.206	0.216	0.216
Average weight per initial number of shrimp (mg)	0.217								0.222							
Percent reduction from control (%)	- 2.47.															

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

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

Comments:

AbKCICR Test Number: 236

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	4 ^{1st}	3 ^{2nd}	4 ^{1st}	3 ^{2nd}	4 ^{1st}	4 ^{1st}	4 ^{1st}	4 ^{1st}
2	S	S	S	S	S	S	S	S	4	3	4	3	4	4	4	4
3	S	S	S	S	S	S	S	S	4	3	4	3	4	4	4	4
4	S	S	S	S	S	S	S	S	4	3	4	3	4	4	4	4
5	S	S	S	S	S	S	S	S	4	3	3 ^{1st}	3	3 ^{1st}	4	3 ^{1st}	3 ^{1st}
6	S	S	S	S	S	S	S	S	3	2 ^{1st}	3	3	3	4	2 ^{1st}	2 ^{1st}
7	S	S	S	S	S	S	S	4 ^{1st}	3	2	3	3	3	2 ^{1st}	2	2
# 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>ALC</u> Date: <u>02-04-23</u>	13.38	15.34	14.46	15.25	14.47	14.72	13.06	15.15	16.08	14.63	15.49	13.60	14.26	14.74	13.19	14.61
*B = Pan + Shrimp weight (mg) Analyst: <u>JL</u> Date: <u>02-21-23</u>	14.42	16.36	15.53	16.27	15.58	15.81	14.19	16.19	16.81	14.93	16.00	14.05	14.54	15.11	13.55	15.00
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JL</u>	1.04	1.02	1.07	1.02	1.11	1.09	1.13	1.04	0.73	0.30	0.51	0.45	0.28	0.37	0.36	0.39
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JL</u>	0.208	0.204	0.214	0.204	0.222	0.218	0.226	0.208	0.146	0.060	0.102	0.090	0.056	0.074	0.072	0.078
Average weight per initial number of shrimp (mg) 0.213				Percent reduction from control (%) 1.67.				Average weight per initial number of shrimp (mg) 0.085				Percent reduction from control (%) 60.97.				

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

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

Comments:

AbKCICR Test Number: 236

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

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

Test number: 236

Test dates: February 07-14, 2023

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.52	15.71	1.19	0.238	100.0	0.217	6.5	Not applicable
	B	5	5	13.12	14.23	1.11	0.222				
	C	5	5	16.34	17.42	1.08	0.216				
	D	5	5	15.53	16.66	1.13	0.226				
	E	5	5	15.59	16.72	1.13	0.226				
	F	5	5	15.27	16.26	0.99	0.198				
	G	5	5	15.19	16.20	1.01	0.202				
	H	5	5	14.76	15.78	1.02	0.204				
250	I	5	5	16.07	17.22	1.15	0.230	100.0	0.222	6.1	-2.4
	J	5	5	14.78	15.98	1.20	0.240				
	K	5	5	15.33	16.36	1.03	0.206				
	L	5	5	15.51	16.71	1.20	0.240				
	M	5	5	14.38	15.48	1.10	0.220				
	N	5	5	15.36	16.39	1.03	0.206				
	O	5	5	14.94	16.02	1.08	0.216				
	P	5	5	14.02	15.10	1.08	0.216				
375	Q	5	5	13.38	14.42	1.04	0.208	97.5	0.213	3.9	1.6
	R	5	5	15.34	16.36	1.02	0.204				
	S	5	5	14.46	15.53	1.07	0.214				
	T	5	5	15.25	16.27	1.02	0.204				
	U	5	5	14.47	15.58	1.11	0.222				
	V	5	5	14.72	15.81	1.09	0.218				
	W	5	5	13.06	14.19	1.13	0.226				
	X	5	4	15.15	16.19	1.04	0.208				
500	Y	5	3	16.08	16.81	0.73	0.146	50.0	0.085	34.1	60.9
	Z	5	2	14.63	14.93	0.30	0.060				
	AA	5	3	15.49	16.00	0.51	0.102				
	BB	5	3	13.60	14.05	0.45	0.090				
	CC	5	3	14.26	14.54	0.28	0.056				
	DD	5	2	14.74	15.11	0.37	0.074				
	EE	5	2	13.19	13.55	0.36	0.072				
	FF	5	2	14.61	15.00	0.39	0.078				
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: 0.0172
PMSD: 7.9

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: 2/7/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 2/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americamysis bahia

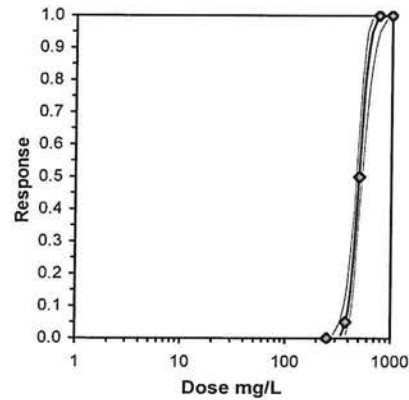
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	0.8000
500	0.6000	0.4000	0.6000	0.6000	0.6000	0.4000	0.4000	0.4000
750	0.0000	0.0000	0.0000	0.0000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

Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.5000	0.5000	0.7854	0.6847	0.8861	13.704	8	36.00	48.00	20	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

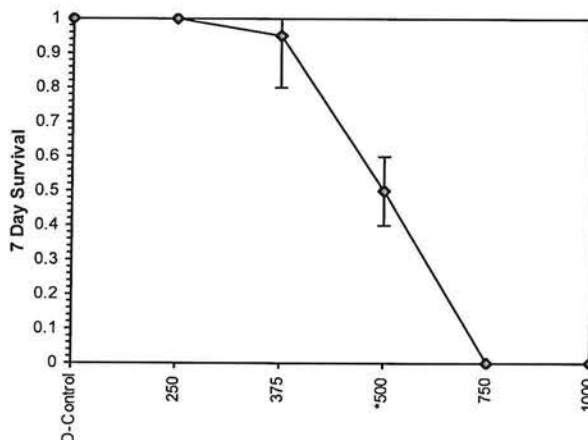
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84785	0.904	-0.8881	0.68294
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.3605	2.48884	9.48239	19.2387	0	0.35613	7.81472	0.94915	2.69519	0.06964	4
Intercept	-33.704	6.67026	-46.778	-20.631							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	341.348	283.758	376.145
EC05	3.355	380.762	333.191	410.113
EC10	3.718	403.601	362.298	430.257
EC15	3.964	419.779	382.838	445.01
EC20	4.158	433.099	399.511	457.642
EC25	4.326	444.862	413.914	469.308
EC40	4.747	475.939	449.557	503.369
EC50	5.000	495.671	469.872	527.932
EC60	5.253	516.22	489.203	555.847
EC75	5.674	552.283	519.905	609.289
EC80	5.842	567.283	531.875	632.777
EC85	6.036	585.283	545.822	661.728
EC90	6.282	608.744	563.486	700.549
EC95	6.645	645.258	590.134	763.077
EC99	7.326	719.763	642.372	897.48



Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

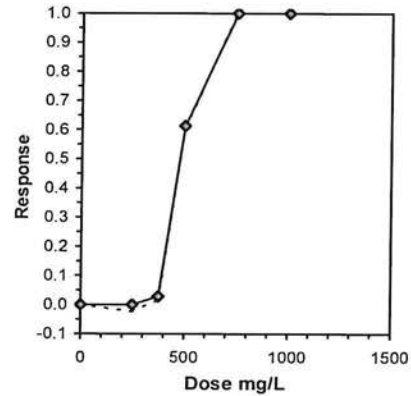
Start Date: 2/7/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 2/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanysis bahia
Comments:		

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2380	0.2220	0.2160	0.2260	0.2260	0.1980	0.2020	0.2040
250	0.2300	0.2400	0.2060	0.2400	0.2200	0.2060	0.2160	0.2160
375	0.2080	0.2040	0.2140	0.2040	0.2220	0.2180	0.2260	0.2080
500	0.1460	0.0600	0.1020	0.0900	0.0560	0.0740	0.0720	0.0780
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

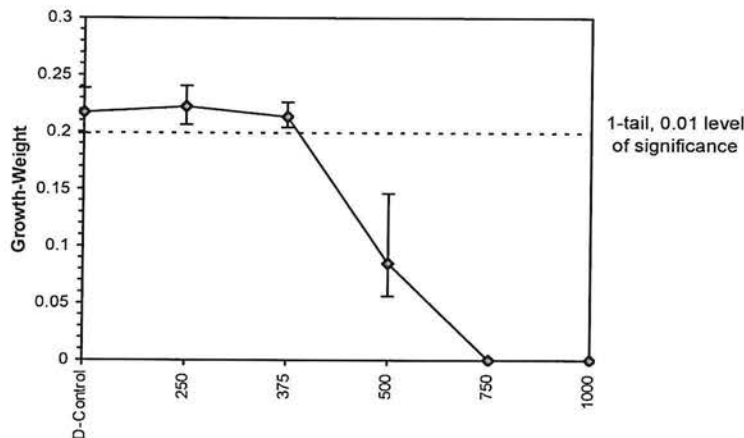
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
D-Control	0.2165	1.0000	0.2165	0.1980	0.2380	6.490	8				0.2191	1.0000	
250	0.2218	1.0242	0.2218	0.2060	0.2400	6.144	8	-0.855	2.799	0.0172	0.2191	1.0000	
375	0.2130	0.9838	0.2130	0.2040	0.2260	3.920	8	0.570	2.799	0.0172	0.2130	0.9720	
500	0.0848	0.3915	0.0848	0.0560	0.1460	34.080	8				0.0848	0.3868	
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 ($p > 0.01$)		0.95793	0.884	0.18615	-1.0013						
Bartlett's Test indicates equal variances ($p = 0.38$)		1.95365	9.21035								
Hypothesis Test (1-tail, 0.01)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		375	>375			0.01719	0.0794	0.00016	0.00015	0.37501	2, 21
Treatments vs D-Control											

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL		Skew
IC05	379.71	17.16	322.38	385.70	-5.0938
IC10	390.39	4.00	381.90	396.87	-0.3241
IC15	401.07	4.18	392.91	408.42	-0.1443
IC20	411.74	4.53	403.76	420.66	0.0582
IC25	422.42	5.02	414.22	433.08	0.2466
IC40	454.46	6.98	443.80	468.81	0.6111
IC50	475.82	8.69	462.68	492.72	0.9284



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

AbKCICR Test Number: 236

Daily Chemistry:

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

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	N	N	N	N	N	N
CONTROL, Salt SW	pH (S.U.)	7.08	7.79	7.70	7.67	7.79	7.73
	DO (mg/L)	0.0	7.7	7.0	7.5	8.0	7.5
	Salinity (ppt)	15.0	25.0	25.1	24.9	24.4	25.0 (25.2) 02-10
	Alkalinity (mg CaCO ₃ /L)	99		25.1 02-10	25.28	93	
	Temperature (°C)	25.1	26.2	25.3	26.2	25.1	25.9
250 mg KCl/L	pH (S.U.)	7.97	7.70	7.02	7.73	7.01	7.00
	DO (mg/L)	0.0	7.7	7.0	7.5	0.0	7.5
	Salinity (ppt)	25.2	25.2	25.0	25.0	24.8	25.0 (25.2) 02-10
	Temperature (°C)	25.2	26.2	25.4	26.2	25.0	26.1
	375 mg KCl/L	pH (S.U.)	7.99	7.79	7.00	7.74	7.01
DO (mg/L)		0.0	7.7	7.9	7.6	7.9	7.4
Salinity (ppt)		25.2	25.6	25.1	25.4	25.1	25.6
Temperature (°C)		25.2	26.1	25.4	26.0	25.2	26.2
500 mg KCl/L		pH (S.U.)	0.01	7.00	7.02	7.24	7.02
	DO (mg/L)	0.0	7.0	0.0	7.7	7.9	7.9
	Salinity (ppt)	25.3	25.5	25.1	25.3	25.1	25.6
	Temperature (°C)	25.2	26.3	25.4	26.0	25.2	26.0
	750 mg KCl/L	pH (S.U.)	0.00	7.79			
DO (mg/L)		0.1	7.9				
Salinity (ppt)		25.4	25.4				
Temperature (°C)		25.2	26.0				
1000 mg KCl/L		pH (S.U.)	0.00	7.00			
	DO (mg/L)	0.1	0.0				
	Salinity (ppt)	25.6	25.7				
	Temperature (°C)	25.2	26.0				
			Initial	Final	Initial	Final	Initial

AbKCICR Test Number: 236

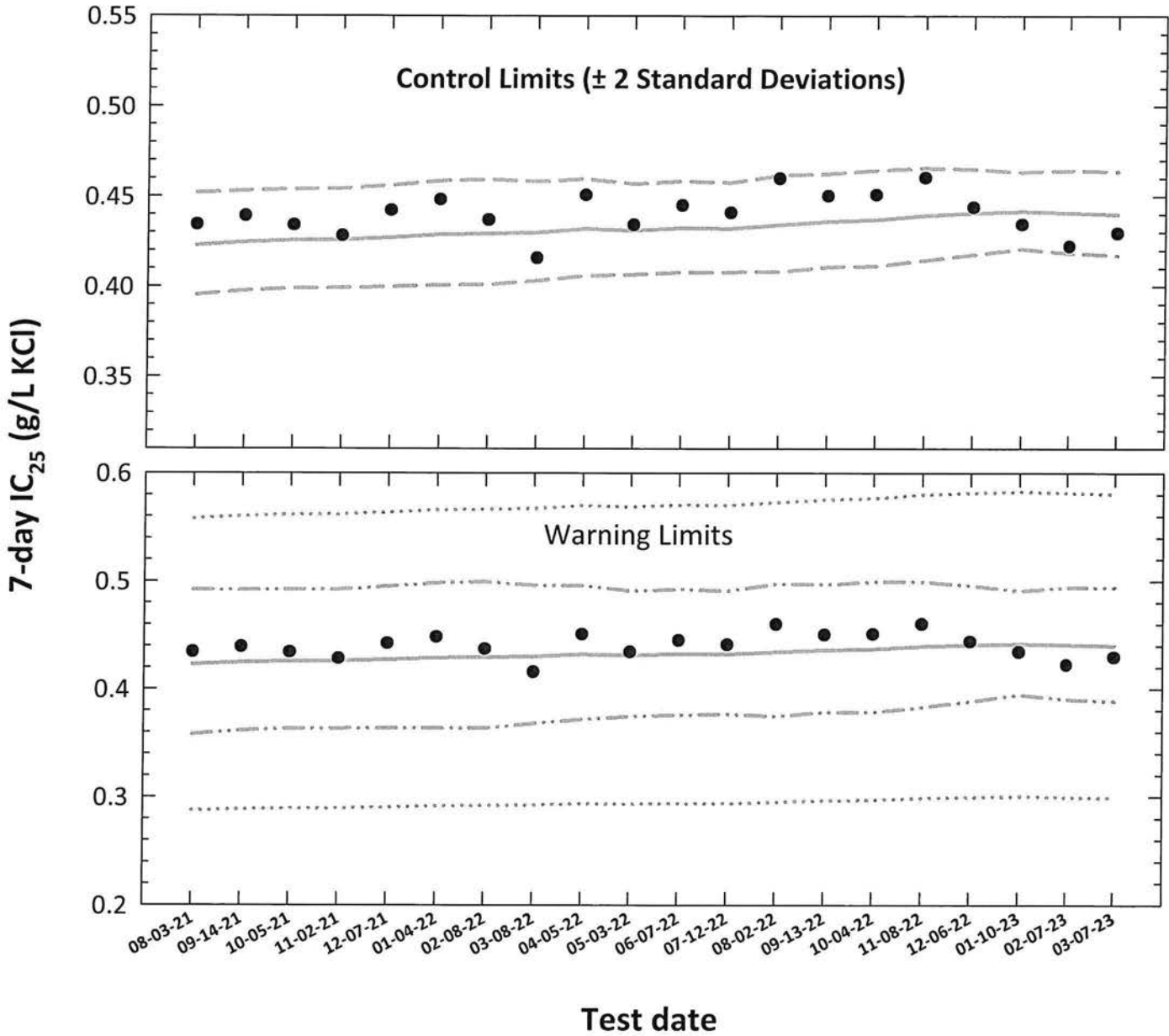
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
		Analyst							
CONTROL, Salt SW	pH (S.U.)	N	BSL	BSL	N	N	N	N	N
	DO (mg/L)	7.91	*7.87 (7.38)	8.01	7.60	7.94	7.71	7.91	7.57
	Salinity (ppt)	0.0	7.5	7.9	6.3	7.9	7.2	7.0	5.4
	Alkalinity (mg CaCO ₃ /L)	25.0	25.1	24.9	25.5	25.4	25.5	25.0	25.1
	Temperature (°C)	—	94	—	—	—	—	—	—
250 mg KCl/L	pH (S.U.)	25.3	26.2	25.2	26.3	25.4	26.1	25.3	26.1
	DO (mg/L)	7.90	7.82	7.98	7.71	7.92	7.72	7.91	7.60
	Salinity (ppt)	7.9	7.4	7.8	6.3	7.6	7.2	7.0	5.4
	Temperature (°C)	25.0	25.1	25.2	25.6	25.4	25.7	25.4	25.5
375 mg KCl/L	pH (S.U.)	25.5	26.2	25.4	26.0	25.5	26.3	25.4	26.1
	DO (mg/L)	7.90	7.84	7.97	7.71	7.92	7.73	7.91	7.60
	Salinity (ppt)	7.9	7.4	7.8	6.0	7.6	7.2	7.0	5.6
	Temperature (°C)	25.1	25.3	25.2	25.7	25.4	25.8	25.6	25.7
500 mg KCl/L	pH (S.U.)	25.5	26.2	25.4	25.9	25.3	26.1	25.2	26.2
	DO (mg/L)	7.90	7.87	7.98	7.70	7.92	7.81	7.93	7.01
	Salinity (ppt)	0.0	7.5	7.8	6.6	7.6	7.4	7.0	7.0
	Temperature (°C)	25.2	25.3	25.3	25.0	25.7	25.9	25.7	25.0
750 mg KCl/L	pH (S.U.)	25.5	26.0	25.4	26.2	25.3	26.1	25.2	26.2
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 02-11-23

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	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	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
2	09-14-21	0.4392	-0.3574	-0.3721	0.0142	0.3977	0.4531	0.3614	0.4918	0.2887	0.5603
3	10-05-21	0.4340	-0.3625	-0.3710	0.0140	0.3990	0.4539	0.3631	0.4922	0.2894	0.5617
4	11-02-21	0.4283	-0.3683	-0.3708	0.0140	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
5	12-07-21	0.4422	-0.3544	-0.3695	0.0143	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
6	01-04-22	0.4482	-0.3485	-0.3678	0.0146	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
7	02-08-22	0.4370	-0.3595	-0.3673	0.0147	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
8	03-08-22	0.4158	-0.3811	-0.3667	0.0139	0.4032	0.4582	0.3679	0.4959	0.2923	0.5674
9	04-05-22	0.4507	-0.3461	-0.3645	0.0135	0.4059	0.4597	0.3716	0.4962	0.2938	0.5702
10	05-03-22	0.4343	-0.3622	-0.3654	0.0126	0.4067	0.4569	0.3745	0.4911	0.2931	0.5690
11	06-07-22	0.4450	-0.3516	-0.3641	0.0127	0.4078	0.4584	0.3756	0.4925	0.2940	0.5707
12	07-12-22	0.4410	-0.3556	-0.3644	0.0124	0.4081	0.4576	0.3764	0.4911	0.2938	0.5704
13	08-02-22	0.4599	-0.3373	-0.3623	0.0134	0.4083	0.4617	0.3745	0.4976	0.2952	0.5731
14	09-13-22	0.4502	-0.3466	-0.3605	0.0129	0.4109	0.4626	0.3785	0.4970	0.2965	0.5755
15	10-04-22	0.4509	-0.3459	-0.3595	0.0132	0.4113	0.4644	0.3782	0.4995	0.2972	0.5769
16	11-08-22	0.4604	-0.3368	-0.3571	0.0127	0.4146	0.4658	0.3829	0.4995	0.2988	0.5801
17	12-06-22	0.4440	-0.3526	-0.3558	0.0117	0.4177	0.4651	0.3884	0.4960	0.2997	0.5818
18	01-10-23	0.4344	-0.3621	-0.3550	0.0105	0.4208	0.4635	0.3944	0.4911	0.3003	0.5829
19	02-07-23	0.4224	-0.3743	-0.3557	0.0113	0.4185	0.4644	0.3901	0.4943	0.2998	0.5820
20	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4174	0.4638	0.3886	0.4941	0.2992	0.5807

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.

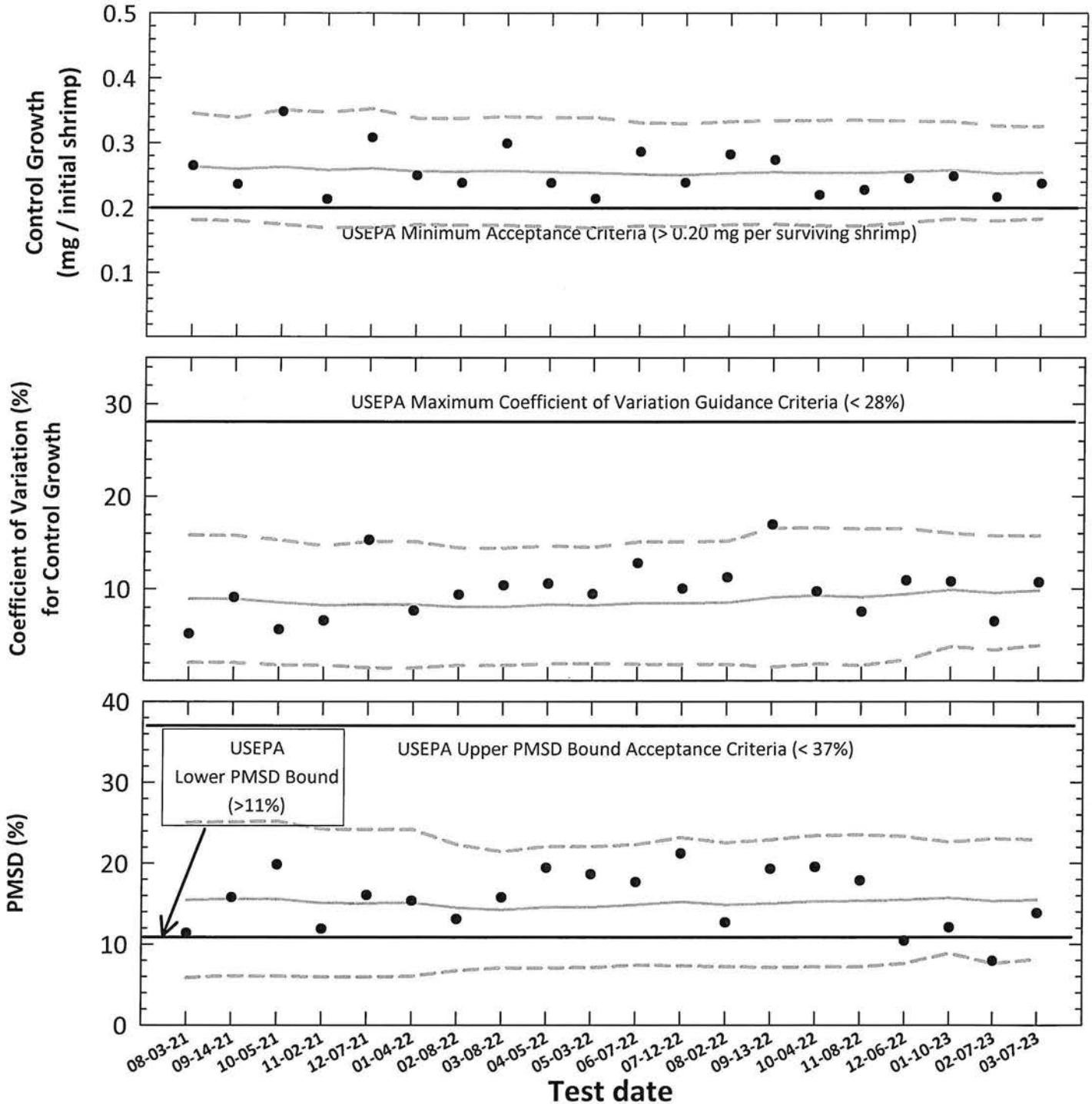
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

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		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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	10-04-22	100	0.220	9.7	0.0430	19.5	0.254	0.173	0.335	9.3	1.9	16.7	15.3	7.2	23.4
16	11-08-22	100	0.228	7.5	0.0407	17.9	0.254	0.173	0.335	9.1	1.7	16.5	15.4	7.2	23.5
17	12-06-22	100	0.246	10.9	0.0256	10.4	0.256	0.177	0.334	9.4	2.3	16.5	15.5	7.6	23.3
18	01-10-23	100	0.249	10.8	0.0300	12.1	0.258	0.183	0.333	9.9	3.7	16.0	15.7	8.8	22.6
19	02-07-23	100	0.217	6.5	0.0172	7.9	0.253	0.180	0.326	9.6	3.4	15.8	15.3	7.6	23.0
20	03-07-23	100	0.238	10.7	0.0329	13.9	0.254	0.183	0.326	9.8	3.9	15.8	15.5	8.1	22.9

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



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

AbKCICR Test Number: **237**

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2166					
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:	PURR
Date and times organisms were born between:	02-28-23 1200 to 03-01-23 1130	Incubator number and shelf location:	SE
Organism source:	AI Batch Ab: 03-01-23	Artemia CHM number:	CHM1222
Transfer bowl information:	pH = 8.17 S.U. Temperature = 25.0 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	03-14-23 1020
		*Initial oven temperature:	60°C
		Date / Time out of oven:	03-15-23 1020
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	03-07-23	1020	J	1225	J	1128	H	02-28-23 A
1	03-08-23	0500	H	1100	H	0930	H	↓
2	03-09-23	0500	H	1205	H	1150	H	02-28-23 B
3	03-10-23	0500	H	1100	H	0930	J	↓
4	03-11-23	0600	H	1200	H	1110	H	03-04-23
5	03-12-23	0600	H	1100	H	0940	H	↓
6	03-13-23	0500	H	1100	H	0930	H	↓
7	03-14-23					0940	H	

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)	491.8
Average weight per initial shrimp:	0.238		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.238	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	429.6

AbKCICR Test Number: 237

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	[Handwritten scribble]															
# females with developing ova in oviducts	[Handwritten scribble]															
# immature females	[Handwritten scribble]															
# males	[Handwritten scribble]															
*A = Pan weight (mg) Tray color code: <u>Lavender</u> Analyst: <u>BL</u> Date: <u>02-21-23</u>	14.21	12.62	15.18	13.52	12.36	14.68	16.21	12.43	15.85	15.28	15.05	15.24	15.17	14.91	14.24	12.62
*B = Pan + Shrimp weight (mg) Analyst: <u>K</u> Date: <u>02-21-23</u>	15.25	13.83	16.55	14.71	13.67	15.81	17.46	13.43	16.90	16.65	16.13	16.55	16.39	16.08	15.36	13.79
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.04	1.21	1.37	1.19	1.31	1.13	1.25	1.00	1.05	1.37	1.08	1.31	1.22	1.17	1.12	1.17
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.208	0.242	0.274	0.238	0.262	0.226	0.250	0.200	0.210	0.274	0.216	0.262	0.244	0.234	0.224	0.234
Average weight per initial number of shrimp (mg)	0.238								0.237							
Percent reduction from control (%)	0.17.															

*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: 237

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	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}	4 ^{id}
2	S	S	S	S	4 ^{id}	4 ^{id}	S	S	3 ^{id}	3 ^{id}	3 ^{id}	3 ^{id}	4	4	4	4
3	S	S	S	S	4	4	S	S	3	3	3	3	4	4	4	4
4	S	S	S	S	4	4	S	S	3	2 ^{id}	3	2 ^{id}	2 ^{id}	2 ^{id}	2 ^{id}	3 ^{id}
5	S	S	S	S	4	4	S	S	3	2	3	2	2	2	2	3
6	S	S	S	S	4	4	S	S	3	2	3	2	2	2	2	3
7	S	S	S	S	4	4	S	S	3	2	3	2	2	2	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>Lavender</u> Analyst: <u>BL</u> Date: <u>02-28-23</u> <u>02-21-23</u>	15.20	14.29	15.02	13.28	14.61	14.00	15.02	13.95	14.65	14.07	15.01	14.14	13.46	14.96	15.47	14.88
*B = Pan + Shrimp weight (mg) Analyst: <u>JK</u> Date: <u>03-21-23</u>	16.36	15.49	16.08	14.68	15.83	15.04	16.16	15.04	15.32	14.59	15.51	14.59	13.87	15.80	15.82	15.45
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JL</u>	1.16	1.20	1.06	1.40	1.22	1.04	1.14	1.09	0.67	0.52	0.50	0.45	0.41	0.84	0.35	0.57
Weight per Initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JL</u>	0.232	0.240	0.212	0.280	0.244	0.208	0.228	0.218	0.134	0.104	0.100	0.090	0.082	0.168	0.070	0.114
Average weight per initial number of shrimp (mg)	0.233								0.108							
Percent reduction from control (%)	2.07								54.67							

*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: **237**

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

03-08-13

03-11-13

*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: 237
Test dates: March 07-14, 2023

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.21	15.25	1.04	0.208	100.0	0.238	10.7	Not applicable
	B	5	5	12.62	13.83	1.21	0.242				
	C	5	5	15.18	16.55	1.37	0.274				
	D	5	5	13.52	14.71	1.19	0.238				
	E	5	5	12.36	13.67	1.31	0.262				
	F	5	5	14.68	15.81	1.13	0.226				
	G	5	5	16.21	17.46	1.25	0.250				
	H	5	5	12.43	13.43	1.00	0.200				
250	I	5	5	15.85	16.90	1.05	0.210	100.0	0.237	9.3	0.1
	J	5	5	15.28	16.65	1.37	0.274				
	K	5	5	15.05	16.13	1.08	0.216				
	L	5	5	15.24	16.55	1.31	0.262				
	M	5	5	15.17	16.39	1.22	0.244				
	N	5	5	14.91	16.08	1.17	0.234				
	O	5	5	14.24	15.36	1.12	0.224				
	P	5	5	12.62	13.79	1.17	0.234				
375	Q	5	5	15.20	16.36	1.16	0.232	95.0	0.233	9.9	2.0
	R	5	5	14.29	15.49	1.20	0.240				
	S	5	5	15.02	16.08	1.06	0.212				
	T	5	5	13.28	14.68	1.40	0.280				
	U	5	4	14.61	15.83	1.22	0.244				
	V	5	4	14.00	15.04	1.04	0.208				
	W	5	5	15.02	16.16	1.14	0.228				
	X	5	5	13.95	15.04	1.09	0.218				
500	Y	5	3	14.65	15.32	0.67	0.134	47.5	0.108	29.0	54.6
	Z	5	2	14.07	14.59	0.52	0.104				
	AA	5	3	15.01	15.51	0.50	0.100				
	BB	5	2	14.14	14.59	0.45	0.090				
	CC	5	2	13.46	13.87	0.41	0.082				
	DD	5	2	14.96	15.80	0.84	0.168				
	EE	5	2	15.47	15.82	0.35	0.070				
	FF	5	3	14.88	15.45	0.57	0.114				
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: 0.0329
PMSD: 13.9

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: 3/7/2023 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 3/14/2023 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:

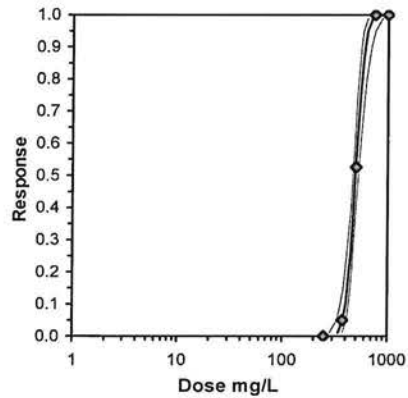
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	1.0000	1.0000	0.8000	0.8000	1.0000	1.0000
500	0.6000	0.4000	0.6000	0.4000	0.4000	0.4000	0.4000	0.6000
750	0.0000	0.0000	0.0000	0.0000	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				N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			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	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.4750	0.4750	0.7602	0.6847	0.8861	13.708	8	36.00	48.00	21	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

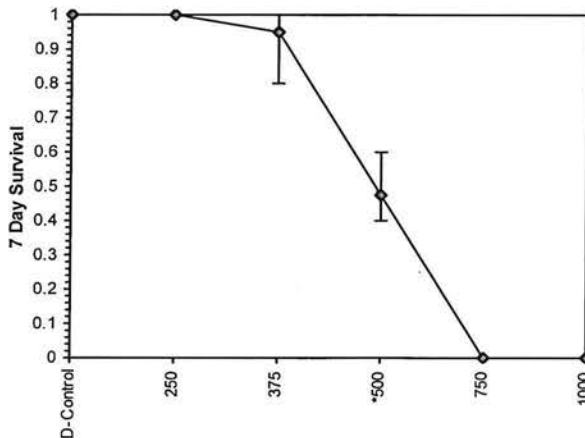
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.87524	0.904	-0.5788	1.07342
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	14.597	2.57122	9.55738	19.6365	0	0.24607	7.81472	0.96983	2.69176	0.06851	4
Intercept	-34.292	6.88422	-47.785	-20.798							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	340.713	282.722	375.305
EC05	3.355	379.381	331.592	408.461
EC10	3.718	401.758	360.356	428.091
EC15	3.964	417.597	380.652	442.456
EC20	4.158	430.629	397.126	454.752
EC25	4.326	442.133	411.354	466.107
EC40	4.747	472.503	446.518	499.314
EC50	5.000	491.768	466.49	523.335
EC60	5.253	511.819	485.423	550.696
EC75	5.674	546.976	515.366	603.153
EC80	5.842	561.588	527.01	626.214
EC85	6.036	579.114	540.563	654.636
EC90	6.282	601.944	557.71	692.736
EC95	6.645	637.449	583.548	754.074
EC99	7.326	709.794	634.118	885.79



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Mysid Survival and Growth Test-Growth-Weight

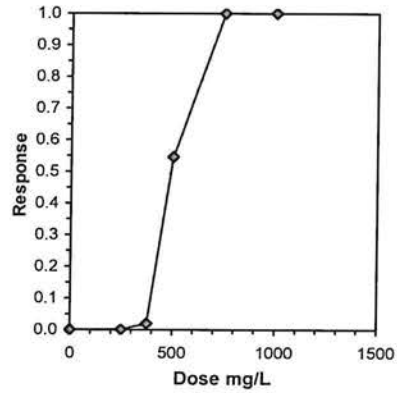
Start Date: 3/7/2023 Test ID: AbKCICR Sample ID: REF-Ref Toxicant
 End Date: 3/14/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: AB-Americanmysid bahia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2080	0.2420	0.2740	0.2380	0.2620	0.2260	0.2500	0.2000
250	0.2100	0.2740	0.2160	0.2620	0.2440	0.2340	0.2240	0.2340
375	0.2320	0.2400	0.2120	0.2800	0.2440	0.2080	0.2280	0.2180
500	0.1340	0.1040	0.1000	0.0900	0.0820	0.1680	0.0700	0.1140
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

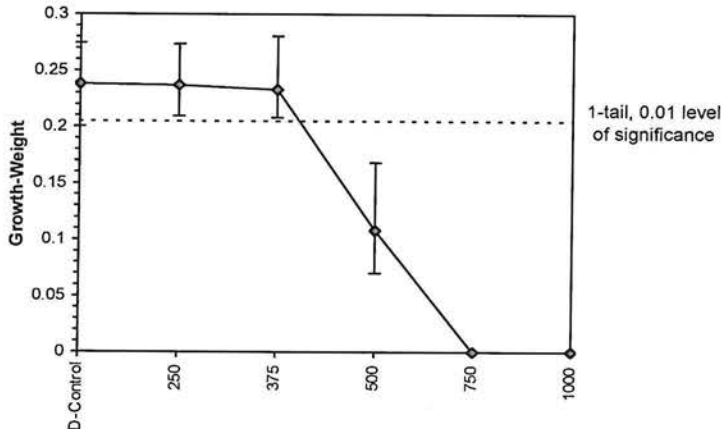
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.2375	1.0000	0.2375	0.2000	0.2740	10.697	8				0.2375	1.0000
250	0.2373	0.9989	0.2373	0.2100	0.2740	9.289	8	0.021	2.799	0.0329	0.2373	0.9989
375	0.2328	0.9800	0.2328	0.2080	0.2800	9.872	8	0.404	2.799	0.0329	0.2328	0.9800
500	0.1078	0.4537	0.1078	0.0700	0.1680	29.001	8				0.1078	0.4537
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)	0.96968	0.884	0.41057	-0.4424						
Bartlett's Test indicates equal variances (p = 0.93)	0.143	9.21035								
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	>375			0.03291	0.13855	5.7E-05	0.00055	0.90224	2, 21

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	382.13	66.28	146.63	387.59	-1.9151
IC10	394.00	23.71	315.87	400.72	-5.2006
IC15	405.88	7.31	384.78	414.02	-0.6116
IC20	417.75	7.17	398.73	427.03	-0.3941
IC25	429.63	7.25	411.79	440.04	-0.1373
IC40	465.25	8.63	448.34	480.72	0.4733
IC50	489.00	11.31	469.83	515.80	1.1063



Dose-Response Plot



Entered and
 Reviewed by
 Jim Sumner

AbKCICR Test Number: 237

Daily Chemistry:

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

Conc.	Parameter	Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
Analyst	N	BL N	BL N	N	N	BL N	
CONTROL, Salt SW	pH (S.U.)	7.97	7.92	7.96	7.82	8.02	7.81
	DO (mg/L)	0.0	7.9	7.8	7.5	7.7	7.7
	Salinity (ppt)	25.0	25.2	25.0	25.0	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	100			15-20 H	100	
	Temperature (°C)	25.7	26.1	25.1	26.3	25.3	26.4
250 mg KCl/L	pH (S.U.)	7.96	7.90	7.95	7.85	7.99	7.84
	DO (mg/L)	0.0	7.8	7.8	7.3	7.10	7.6
	Salinity (ppt)	25.4	26.0	25.4	25.6	25.4	25.6
	Temperature (°C)	25.5	26.2	25.2	26.2	25.3	26.2
375 mg KCl/L	pH (S.U.)	7.96	7.92	7.95	7.85	8.00	7.80
	DO (mg/L)	0.0	7.8	7.8	7.3	7.7	7.6
	Salinity (ppt)	25.5	26.0	25.5	25.9	25.9	26.2
	Temperature (°C)	25.5	26.2	25.2	26.4	25.3	26.2
500 mg KCl/L	pH (S.U.)	7.98	7.94	7.97	7.88	8.01	7.88
	DO (mg/L)	0.0	7.8	7.8	7.3	7.7	7.6
	Salinity (ppt)	25.5	26.2	25.6	26.3	25.9	26.3
	Temperature (°C)	25.6	26.2	25.2	26.4	25.4	26.3
750 mg KCl/L	pH (S.U.)	7.98	7.91				
	DO (mg/L)	0.0	7.9				
	Salinity (ppt)	26.3	26.2/26.6				
	Temperature (°C)	25.5	26.0				
1000 mg KCl/L	pH (S.U.)	7.99	7.90				
	DO (mg/L)	9.1	8.2				
	Salinity (ppt)	26.3	26.0				
	Temperature (°C)	25.6	26.0				
		Initial	Final	Initial	Final	Initial	Final

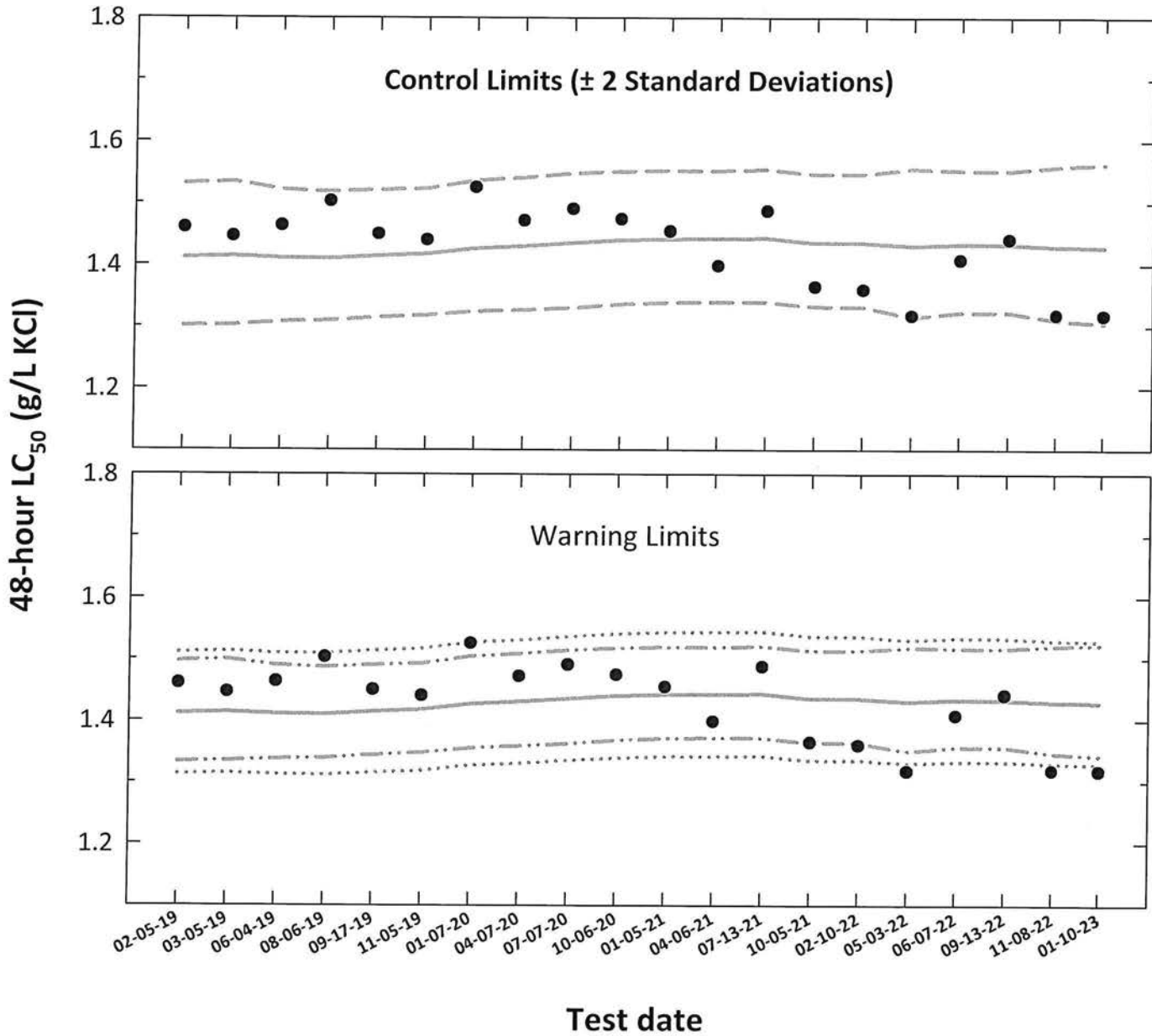
AbKCICR Test Number: 237

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
Analyst	BL	BL	BL	BL	BL	BL	BL	BL	
CONTROL, Salt SW	pH (S.U.)	7.94	7.83	8.05	7.73	7.99	7.81	7.99	7.75
	DO (mg/L)	0.0	7.8	7.8	7.7	7.8	4.6	7.8	7.0
	Salinity (ppt)	25.0	25.0	24.7	25.2	25.0	25.3	25.0	25.0
	Alkalinity (mg CaCO ₃ /L)	03-15-23 H		92				03-15-23 H	
	Temperature (°C)	25.2	26.2	25.2	26.3	25.3	26.2	26.1	26.2
250 mg KCl/L	pH (S.U.)	7.93	7.87	8.08	7.78	7.97	7.88	7.99	7.76
	DO (mg/L)	0.0	7.7	7.8	7.7	7.9	7.6	7.0	7.2
	Salinity (ppt)	25.2	25.2	25.3	25.5	25.4	25.6	25.4	25.5
	Temperature (°C)	25.3	26.0	25.1	26.3	25.3	26.3	26.2	26.0
375 mg KCl/L	pH (S.U.)	7.91	7.86	8.09	7.80	7.98	7.87	7.99	7.76
	DO (mg/L)	7.9	7.5	7.7	7.5	7.9	7.4	7.9	7.2
	Salinity (ppt)	25.3	25.2	25.5	25.9	25.5	25.8	25.8	25.9
	Temperature (°C)	25.3	26.0	25.1	26.2	25.3	26.5	26.2	26.0
500 mg KCl/L	pH (S.U.)	7.94	7.93	8.09	7.86	7.98	7.84	7.99	7.77
	DO (mg/L)	7.9	7.4	7.8	7.4	7.9	7.4	7.9	7.2
	Salinity (ppt)	25.4	25.1	25.6	26.0	25.5	26.0	25.8	26.1
	Temperature (°C)	25.2	26.2	25.3	26.2	25.4	26.2	26.2	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Menidia beryllina

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	11-08-22	1.3190	0.1202	0.1552	0.0189	1.4297	1.3106	1.5596	1.3464	1.5205	1.3296	1.5298
20	01-10-23	1.3180	0.1199	0.1548	0.0196	1.4284	1.3053	1.5630	1.3422	1.5227	1.3284	1.5283

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 88

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 #: 2166

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	EC	EC N	N
Control, SaltSW	pH (S.U.)	8.08	7.97	7.95
	Dissolved oxygen (mg/L)	7.7	7.9	7.7
	*Salinity (ppt)	25.0	24.7	25.5
	*Alkalinity (mg/L CaCO ₃)	120		
	*Temperature (°C)	24.9	25.1	25.0
1000 mg/L	pH (S.U.)	8.00	8.00	7.91
	Dissolved oxygen (mg/L)	7.8	7.9	7.6
	*Salinity (ppt)	25.5	25.2	26.5
	*Temperature (°C)	25.0	25.0	24.9
1250 mg/L	pH (S.U.)	8.09	7.99	7.89
	Dissolved oxygen (mg/L)	7.9	8.0	7.5
	*Salinity (ppt)	25.6	25.5	26.7
	*Temperature (°C)	25.0	25.2	25.0
1500 mg/L	pH (S.U.)	8.10	7.98	7.97
	Dissolved oxygen (mg/L)	7.9	8.0	7.5
	*Salinity (ppt)	25.8	25.7	26.7
	*Temperature (°C)	25.0	25.2	25.0
1750 mg/L	pH (S.U.)	8.10	7.99	7.97
	Dissolved oxygen (mg/L)	7.9	8.0	8.0
	*Salinity (ppt)	25.9	25.9	26.5
	*Temperature (°C)	25.0	25.0	25.1
2000 mg/L	pH (S.U.)	8.10	8.00	
	Dissolved oxygen (mg/L)	7.9	8.0	
	*Salinity (ppt)	26.0	26.0	
	*Temperature (°C)	24.9	25.0	

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

Chemical analyses:

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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 88

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-10-23	* 1020	JL	1220	JL	6D	LIGHT GREEN	01-09-23A
24	01-11-23			1225	JL			
48 Termination	01-12-23			1227	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 (All Batch Ad):	12-31-22
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	12-30-23 1700 TO 12-31-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.79 Temperature (°C) 24.3

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	sd 2	bd 4	sd 0	sd 1	sd 0	sd 0
48 Termination	10	10	10	10	4d 6	3d 7	sd 1	sd 2	0	0	0	0
Mean Survival	100%		100%		65%		15%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1247.8
Upper 95% confidence limit (mg KCl/L)	1384.0
48-hour LC ₅₀ (mg KCl/L)	1318.0

Comments:



Statistical Analyses

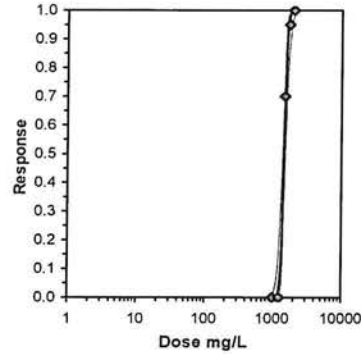
Acute Silverside Test-24 Hr Survival			
Start Date:	1/10/2023	Test ID:	MbKCIAC
End Date:	1/12/2023	Lab ID:	ETS-Envir. Testing Sol.
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012
Comments:		Sample ID:	REF-Ref Toxicant
		Sample Type:	KCL-Potassium chloride
		Test Species:	MB-Menidia beryllina

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	1.0000
1500	0.2000	0.4000
1750	0.0000	0.1000
2000	0.0000	0.0000

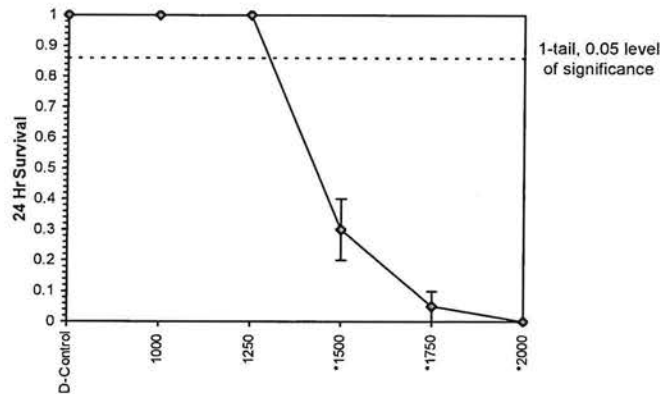
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2244	0	20	
1250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2244	0	20	
*1500	0.3000	0.3000	0.5742	0.4636	0.6847	27.225	2	10.567	2.830	0.2244	14	20	
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	14.779	2.830	0.2244	19	20	
*2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.807	2.830	0.2244	20	20	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.11476	0.11771	0.74849	0.00629	6.4E-06	5, 6
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	27.387	5.83968	15.9413	38.8328	0	2.53206	7.81472	0.46952	3.16613	0.03651	6
Intercept	-81.711	18.5172	-118	-45.417							
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	1205.55	1030.93	1292.03							
EC05	3.355	1276.64	1134.27	1349.22							
EC10	3.718	1316.24	1192.47	1381.98							
EC15	3.964	1343.65	1232.67	1405.37							
EC20	4.158	1365.84	1264.94	1424.96							
EC25	4.326	1385.17	1292.68	1442.68							
EC40	4.747	1435.09	1361.41	1492.57							
EC50	5.000	1465.99	1400.6	1527.66							
EC60	5.253	1497.55	1437.18	1567.64							
EC75	5.674	1551.52	1491.79	1645.56							
EC80	5.842	1573.48	1511.7	1680.14							
EC85	6.036	1599.46	1534.01	1722.73							
EC90	6.282	1632.77	1561.13	1779.49							
EC95	6.645	1683.41	1600.16	1869.46							
EC99	7.326	1782.68	1672.16	2055.43							



Dose-Response Plot



Retained and Approved by [Signature]

Statistical Analyses

Acute Silverside Test-48 Hr Survival					
Start Date:	1/10/2023	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	1/12/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.6000	0.7000
1500	0.1000	0.2000
1750	0.0000	0.0000
2000	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
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1591	0	20
*1250	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	8.478	2.850	0.1591	7	20
*1500	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	18.256	2.850	0.1591	17	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	22.445	2.850	0.1591	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

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

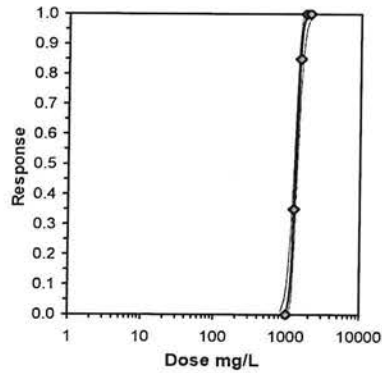
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.07271	0.07457	0.66283	0.00312	9.1E-06	4, 5

Maximum Likelihood-Probit

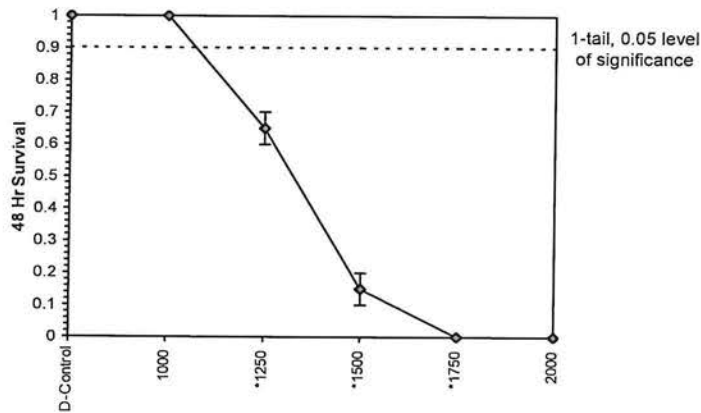
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	20.708	4.13036	12.6125 28.8035	0	0.48452	7.81472	0.92228	3.11993	0.04829	4
Intercept	-59.607	12.9176	-84.926 -34.289							

TSCR

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1017.62	844.477 1110.41
EC05	3.355	1097.73	953.149 1176.54
EC10	3.718	1142.98	1015.64 1214.63
EC15	3.964	1174.56	1059.37 1241.88
EC20	4.158	1200.28	1094.82 1264.72
EC25	4.326	1222.8	1125.56 1285.35
EC40	4.747	1281.42	1202.86 1343.35
EC50	5.000	1318.03	1247.78 1384.04
EC60	5.253	1355.69	1290.32 1430.45
EC75	5.674	1420.68	1354.93 1521.47
EC80	5.842	1447.33	1378.81 1562.18
EC85	6.036	1479.03	1405.78 1612.6
EC90	6.282	1519.89	1438.83 1680.27
EC95	6.645	1582.55	1486.91 1788.63
EC99	7.326	1707.13	1577 2016.83



Dose-Response Plot

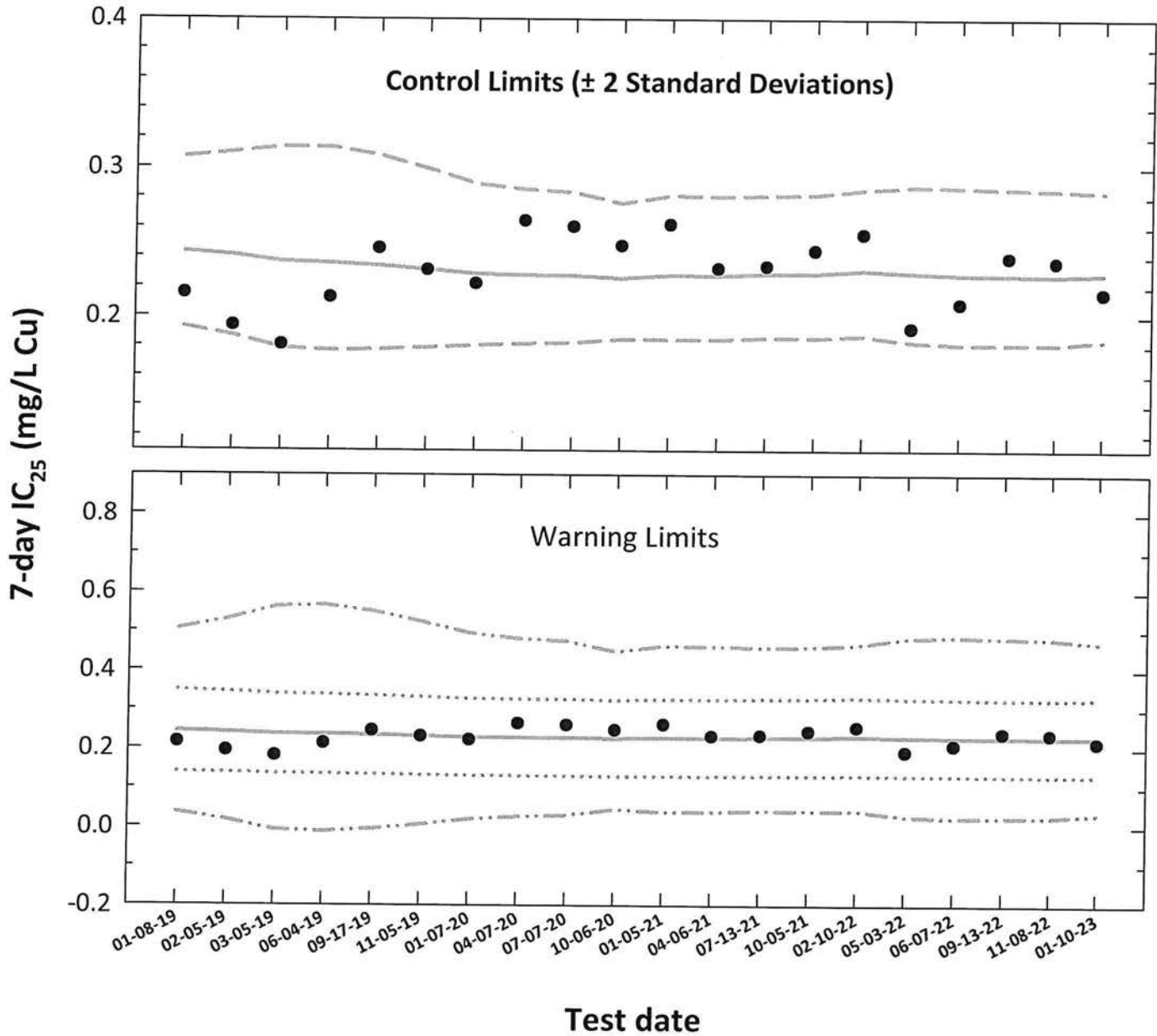


Checked and
 Approved by
 the Supervisor

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 ± S_{A,75} converted to anti-logarithmic values, S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion			Anti-logarithmic Values (mg/L Cu)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	01-08-19	0.2154	-0.6668	-0.6140	0.0505	0.2432	0.1928	0.3068	0.0359	0.5048	0.1386	0.3478
2	02-05-19	0.1937	-0.7129	-0.6182	0.0550	0.2409	0.1869	0.3104	0.0170	0.5294	0.1373	0.3445
3	03-05-19	0.1810	-0.7423	-0.6257	0.0612	0.2367	0.1786	0.3138	-0.0088	0.5623	0.1349	0.3385
4	06-04-19	0.2128	-0.6720	-0.6275	0.0620	0.2357	0.1772	0.3137	-0.0128	0.5665	0.1344	0.3371
5	09-17-19	0.2458	-0.6094	-0.6304	0.0597	0.2342	0.1779	0.3083	-0.0061	0.5505	0.1335	0.3349
6	11-05-19	0.2315	-0.6354	-0.6352	0.0557	0.2316	0.1792	0.2994	0.0054	0.5240	0.1320	0.3312
7	01-07-20	0.2222	-0.6533	-0.6408	0.0511	0.2286	0.1807	0.2893	0.0189	0.4941	0.1303	0.3270
8	04-07-20	0.2646	-0.5774	-0.6422	0.0490	0.2279	0.1819	0.2856	0.0261	0.4809	0.1299	0.3260
9	07-07-20	0.2606	-0.5840	-0.6429	0.0479	0.2276	0.1825	0.2838	0.0295	0.4745	0.1297	0.3254
10	10-06-20	0.2479	-0.6057	-0.6457	0.0436	0.2261	0.1850	0.2763	0.0443	0.4483	0.1289	0.3233
11	01-05-21	0.2621	-0.5815	-0.6419	0.0457	0.2281	0.1848	0.2816	0.0382	0.4625	0.1300	0.3262
12	04-06-21	0.2327	-0.6332	-0.6424	0.0456	0.2278	0.1847	0.2810	0.0385	0.4612	0.1298	0.3258
13	07-13-21	0.2342	-0.6304	-0.6402	0.0450	0.2290	0.1862	0.2817	0.0420	0.4590	0.1305	0.3274
14	10-05-21	0.2447	-0.6114	-0.6398	0.0452	0.2292	0.1861	0.2822	0.0414	0.4604	0.1306	0.3277
15	02-10-22	0.2557	-0.5923	-0.6359	0.0457	0.2313	0.1874	0.2855	0.0414	0.4656	0.1318	0.3307
16	05-03-22	0.1925	-0.7156	-0.6390	0.0490	0.2296	0.1832	0.2877	0.0276	0.4827	0.1309	0.3283
17	06-07-22	0.2088	-0.6803	-0.6415	0.0498	0.2283	0.1815	0.2872	0.0233	0.4861	0.1301	0.3265
18	09-13-22	0.2399	-0.6200	-0.6422	0.0494	0.2279	0.1816	0.2861	0.0246	0.4831	0.1299	0.3259
19	11-08-22	0.2368	-0.6256	-0.6428	0.0491	0.2276	0.1816	0.2853	0.0254	0.4811	0.1297	0.3255
20	01-10-23	0.2159	-0.6657	-0.6408	0.0471	0.2287	0.1841	0.2841	0.0337	0.4708	0.1304	0.3270

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.

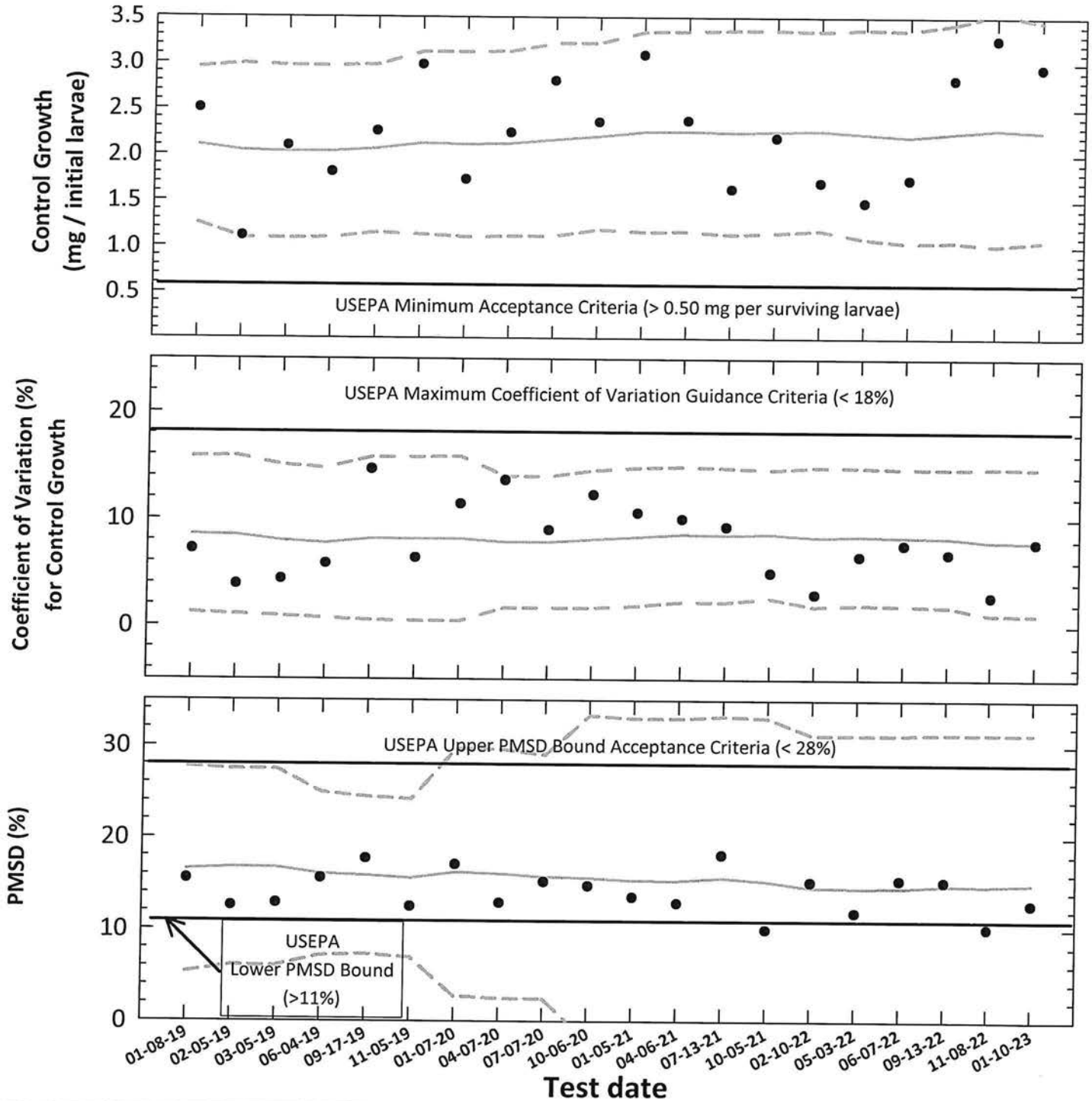
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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)**

Entered and Reviewed by
Jim Sumner

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

Test number	Test date	ToxCal Determination				Control Growth		Test		Control Growth (mg/initial larvae)		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	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial larvae)	CV (%)											
1	01-08-19	100	2.504	7.2	0.3880	15.5	2.1017	1.253	2.950	8.5	1.2	15.8	16.5	5.3	27.7
2	02-05-19	100	1.114	3.9	0.1400	12.6	2.042	1.092	2.992	8.5	1.0	15.9	16.7	6.0	27.4
3	03-05-19	100	2.098	4.3	0.2697	12.9	2.030	1.090	2.969	8.0	0.9	15.0	16.7	6.0	27.4
4	06-04-19	100	1.812	5.8	0.2823	15.6	2.032	1.096	2.969	7.7	0.7	14.8	16.0	7.1	24.9
5	09-17-19	100	2.259	14.7	0.4004	17.7	2.067	1.151	2.982	8.1	0.5	15.8	15.8	7.3	24.4
6	11-05-19	100	2.983	6.4	0.3722	12.5	2.125	1.132	3.119	8.1	0.4	15.8	15.5	6.9	24.2
7	01-07-20	100	1.732	11.4	0.2954	17.1	2.112	1.105	3.120	8.1	0.4	15.9	16.2	2.7	29.7
8	04-07-20	100	2.244	13.6	0.2889	12.9	2.123	1.114	3.131	7.8	1.7	14.0	16.1	2.5	29.6
9	07-07-20	100	2.809	9.0	0.4264	15.2	2.166	1.117	3.215	7.8	1.7	14.0	15.7	2.4	29.0
10	10-06-20	100	2.361	12.3	0.3484	14.8	2.205	1.188	3.221	8.1	1.7	14.6	15.6	-2.1	33.3
11	01-05-21	100	3.093	10.6	0.4186	13.5	2.253	1.163	3.343	8.3	1.9	14.8	15.4	-2.3	33.0
12	04-06-21	100	2.377	10.0	0.3062	12.9	2.258	1.167	3.349	8.6	2.2	14.9	15.3	-2.3	33.0
13	07-13-21	100	1.631	9.3	0.2956	18.1	2.247	1.136	3.359	8.5	2.2	14.8	15.7	-2.0	33.3
14	10-05-21	100	2.189	5.0	0.2192	10.0	2.258	1.153	3.363	8.6	2.6	14.7	15.3	-2.5	33.1
15	02-10-22	100	1.701	3.0	0.2583	15.2	2.266	1.180	3.351	8.4	1.8	14.9	14.6	-2.0	31.2
16	05-03-22	100	1.483	6.5	0.1756	11.8	2.232	1.092	3.372	8.4	2.0	14.9	14.5	-2.1	31.2
17	06-07-22	100	1.733	7.6	0.2665	15.4	2.201	1.041	3.361	8.3	1.9	14.8	14.6	-2.1	31.2
18	09-13-22	100	2.822	6.8	0.4290	15.2	2.243	1.057	3.430	8.3	1.8	14.7	14.8	-1.7	31.3
19	11-08-22	100	3.255	2.7	0.3294	10.1	2.280	1.014	3.545	7.9	1.0	14.8	14.8	-1.8	31.3
20	01-10-23	100	2.945	7.8	0.3755	12.8	2.257	1.062	3.453	7.9	1.0	14.8	15.0	-1.4	31.3

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.
USEPA maximum CV guidance criteria (90th percentile) < 18%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 28%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



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

MbCuCR Test Number: 141

Dilution preparation information:						Comments:
Cu Stock INSS number:	INSS <u>2165</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>CB</u>
Batch:	AI Mb 12-31-22	Artemia CHM number:	CHM1222
Hatch dates and times:	12-30-22 1200 to 12-31-22 1130	Drying information for weight determination:	
Transfer vessel information:	pH (S.U.) = <u>7.79</u> Temperature (°C) = <u>24.3</u>	Date / Time in oven:	<u>01-17-23 0915</u>
Average transfer volume (mL):	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>01-18-23 0925</u>
		*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24-Hours</u>

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	01-10-23	1020	JL	1145	JL	1100	JL		01-01-23 A
1	01-11-23	0500	JL	1100	JL	0900	JL	01-10-23	↓
2	01-12-23	0500	JL	1130	JL	1050	JL		01-11-23
3	01-13-23	0500	JL	1115	JL	0914	JL		↓
4	01-14-23	0600	JL	1200	JL	1128	JL		01-12-23
5	01-15-23	0550	JL	1150	JL	0910	JL		01-13-23
6	01-16-23	0500	JL	1100	JL	0900	JL		↓
7	01-17-23					0906	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: (<u>mg/L</u>)	
% Mortality:	<u>0%</u>	≤ 20%	7-day LC ₅₀ (%)	<u>0.298</u>
Average weight per initial larvae:	<u>2.945</u>		NOEC (%)	<u>0.1</u>
Average weight per surviving larvae:	<u>2.945</u>	≥ 0.25mg/larvae	LOEC (%)	<u>0.2</u>
		<u>0.5</u>	ChV (%)	<u>0.14</u>
			LC ₂₅ (%)	<u>0.216</u>

Species: Menidia beryllina

MbCuCR Test Number: 141

Survival and Growth Data

Day	CONTROL				0.025 mg/L				0.05 mg/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>Ruby</u> Analyst: <u>EC</u> Date: <u>12-29-22</u>	13.50	13.15	13.08	14.08	14.50	14.43	14.01	14.17	14.14	14.19	14.00	14.23
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>01-20-23</u>	41.35	41.72	41.66	41.92	41.95	45.45	41.01	47.80	45.25	41.10	41.20	39.72
C = Larvae weight (mg) = B - A Analyst: <u>J</u>	27.79	28.57	28.58	27.84	27.45	31.02	27.00	33.63	31.11	32.27	27.20	25.49
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>	2.779	2.857	2.858	2.784	2.745	3.102	2.700	3.363	3.111	3.227	2.720	2.549
Average weight per initial number of larvae (mg)	2.945				3.244				-10.27			
Percent reduction from control (%)									1.47			

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

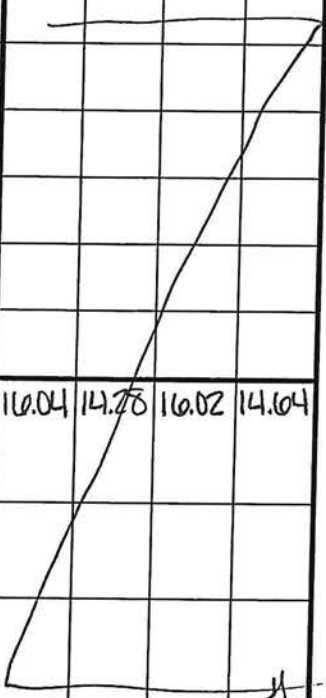
Comment codes: c = clear, d = dead, fg = fungus, k = killed, m = missing, sk = sick, sm = unusually small, lg = unusually large, d&r = decanted and returned, w = wounded.

Comments:

Species: Menidia beryllina

MbCuCR Test Number: 141

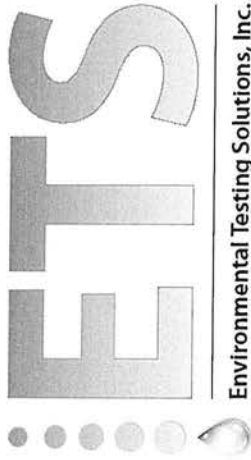
Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
2	10	10	10	10	10	10	8 ^{2d}	9 ^{1d}				
3	10	10	10	10	10	10	8	9				
4	10	10	10	10	10	10	8	9				
5	10	10	10	10	10	10	8	9				
6	10	10	10	10	10	10	8	9				
7	10	10	10	10	10	10	8	9				
*A = Pan weight (mg) Tray color code: <u>Ruby</u> Analyst: <u>EC</u> Date: <u>12-29-22</u>	14.87	14.58	14.54	15.48	15.23	14.47	14.40	12.48				
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>01-20-23</u>	47.19	45.39	44.25	49.77	39.96	39.33	35.96	39.34				
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	32.32	30.81	29.71	34.29	24.73	24.86	21.56	26.86				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	3.232	3.081	2.971	3.429	2.473	2.486	2.156	2.686	0	0	0	0
Average weight per initial number of larvae (mg)	3.178			-7.97			2.450		16.87		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:



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: 141

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Not for Compliance Assessment, Internal Laboratory QC			Weight / initial number of larvae (mg)	Mean survival (%)	Mean weight / initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
							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	13.56	41.35	27.79	2.779							
	B	10	10	13.15	41.72	28.57	2.857							
	C	10	10	13.08	41.66	28.58	2.858			100.0	2.945	7.8	Not applicable	
	D	10	10	14.08	46.92	32.84	3.284							
0.025	E	10	10	14.50	46.95	32.45	3.245							
	F	10	10	14.43	45.45	31.02	3.102							
	G	10	10	14.01	46.66	32.65	3.265			100.0	3.244	3.3	-10.2	
	H	10	10	14.17	47.80	33.63	3.363							
0.050	I	10	10	14.14	45.25	31.11	3.111							
	J	10	10	14.19	46.46	32.27	3.227							
	K	10	10	14.00	41.26	27.26	2.726			100.0	2.903	11.0	1.4	
	L	10	10	14.23	39.72	25.49	2.549							
0.100	M	10	10	14.87	47.19	32.32	3.232							
	N	10	10	14.58	45.39	30.81	3.081							
	O	10	10	14.54	44.25	29.71	2.971			100.0	3.178	6.2	-7.9	
	P	10	10	15.48	49.77	34.29	3.429							
0.200	Q	10	10	15.23	39.96	24.73	2.473							
	R	10	10	14.47	39.33	24.86	2.486							
	S	10	8	14.40	35.96	21.56	2.695			92.5	2.450	8.9	16.8	
	T	10	9	12.48	39.34	26.86	2.984							
0.500	U	10	0	0.00	0.00	0.00	0.000							
	V	10	0	0.00	0.00	0.00	0.000							
	W	10	0	0.00	0.00	0.00	0.000			0.0	0.000	0.0	100.0	
	X	10	0	0.00	0.00	0.00	0.000							

Dummett's MSD value: 0.3755
 MSD = 12.8
 PMSD = 12.8
 Minimum Significant Difference
 Percent Minimum Significant Difference
 PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

Lower PMSD bound determined by USEPA (10th percentile) = 11%.
 Upper PMSD bound determined by USEPA (90th percentile) = 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

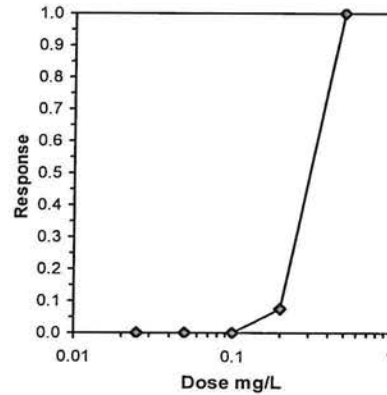
Start Date: 1/10/2023 Test ID: MbCuCR Sample ID: REF-Ref Toxicant
 End Date: 1/17/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: CUSO4
 Sample Date: Protocol: SWCHR-EPA-821-R-02-014 Test Species: MB-Menidia beryllina
 Comments:

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	1.0000	1.0000	0.8000	0.9000
0.5	0.0000	0.0000	0.0000	0.0000

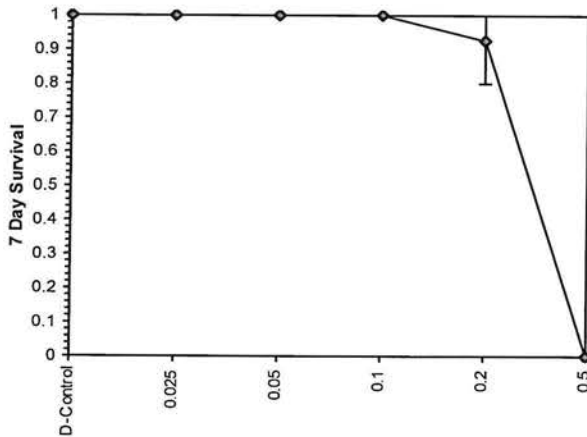
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number	
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4		0	40	
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	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	0.9250	0.9250	1.2951	1.1071	1.4120	11.347	4	14.00	10.00	3	40
0.5	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.59694	0.868	-1.0375	6.56057
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.2	0.5	0.31623	

Trim Level	Trimmed Spearman-Kärber		
	EC50	95% CL	
0.0%	0.2977	0.2784	0.3183
5.0%	0.3038	0.2781	0.3320
10.0%	0.3047	0.2914	0.3186
20.0%	0.3047	0.2914	0.3186
Auto-0.0%	0.2977	0.2784	0.3183



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

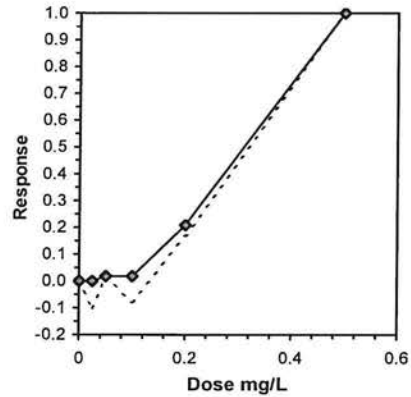
Start Date: 1/10/2023	Test ID: MbCuCR	Sample ID: REF-Ref Toxicant
End Date: 1/17/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: CUSO4
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: MB-Menidia beryllina

Conc-mg/L	1	2	3	4
D-Control	2.7790	2.8570	2.8580	3.2840
0.025	3.2450	3.1020	3.2650	3.3630
0.05	3.1110	3.2270	2.7260	2.5490
0.1	3.2320	3.0810	2.9710	3.4290
0.2	2.4730	2.4860	2.1560	2.6860
0.5	0.0000	0.0000	0.0000	0.0000

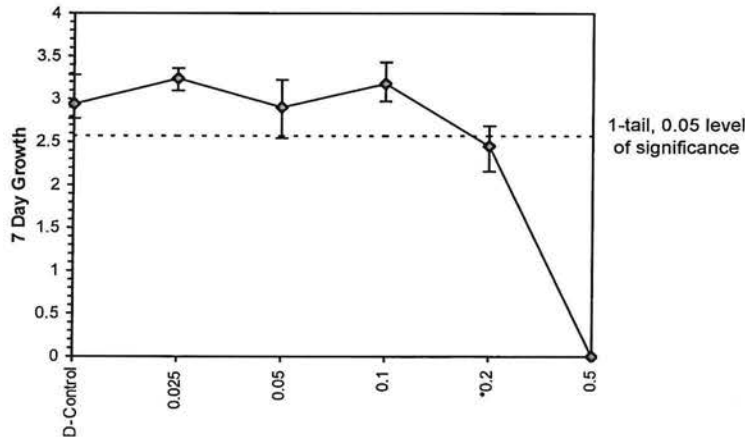
Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	2.9445	1.0000	2.9445	2.7790	3.2840	7.789	4				3.0941	1.0000
0.025	3.2438	1.1016	3.2438	3.1020	3.3630	3.319	4	-1.881	2.360	0.3755	3.0941	1.0000
0.05	2.9033	0.9860	2.9033	2.5490	3.2270	10.981	4	0.259	2.360	0.3755	3.0408	0.9827
0.1	3.1783	1.0794	3.1783	2.9710	3.4290	6.245	4	-1.469	2.360	0.3755	3.0408	0.9827
*0.2	2.4503	0.8321	2.4503	2.1560	2.6860	8.940	4	3.106	2.360	0.3755	2.4503	0.7919
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)		0.96725	0.868	0.13751	-0.7899						
Bartlett's Test indicates equal variances (p = 0.60)		2.76632	13.2767								
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		0.1	0.2	0.14142		0.37552	0.12753	0.39017	0.05064	0.00139	4, 15

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	0.1172	0.0287	0.0000	0.1379	-1.5557
IC10	0.1434	0.0117	0.1004	0.1776	-0.2188
IC15	0.1696	0.0133	0.1328	0.2186	0.3358
IC20	0.1958	0.0133	0.1530	0.2311	-0.2130
IC25	0.2159	0.0119	0.1736	0.2472	-0.4154
IC40	0.2727	0.0096	0.2380	0.2978	-0.4938
IC50	0.3106	0.0080	0.2817	0.3315	-0.4938



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Species: Menidia beryllina

MbCuCR Test Number: 141

Daily Chemistry:

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

Analyst		Day					
		(Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
Concentration	Parameter	<i>JL</i>	<i>EC N</i>	<i>EC N</i>	<i>N</i>	<i>N</i>	<i>N</i>
CONTROL, SaltSW	pH (S.U.)	8.08	7.98	7.98	7.95	8.12	7.09
	Dissolved oxygen (mg/L)	7.7	7.4	7.7	7.1	7.0	7.0
	Salinity (ppt)	25.0	25.2	24.7	25.2	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	120		101.15		110	
	Temperature (°C)	24.8	25.0	24.7	24.9	24.8	24.8
0.025 mg/L	pH (S.U.)	8.07	7.98	8.07	7.94	8.13	7.02
	Dissolved oxygen (mg/L)	7.7	7.4	7.5	7.1	8.0	7.0
	Salinity (ppt)	24.8	25.0	25.1	25.4	24.7	25.0
	Temperature (°C)	24.9	24.7	24.8	24.9	24.9	24.7
0.05 mg/L	pH (S.U.)	8.10	7.99	8.07	7.97	8.14	7.03
	Dissolved oxygen (mg/L)	7.7	7.5	7.6	7.2	8.0	7.7
	Salinity (ppt)	24.8	25.1	25.0	25.4	24.8	25.1
	Temperature (°C)	25.0	24.7	24.8	24.8	24.8	24.9
0.1 mg/L	pH (S.U.)	8.10	7.99	8.07	7.97	8.10	7.01
	Dissolved oxygen (mg/L)	7.8	7.5	7.5	7.1	8.0	7.6
	Salinity (ppt)	24.8	25.2	25.0	25.4	24.7	25.2
	Temperature (°C)	25.0	24.9	24.9	24.8	24.8	24.9
0.2 mg/L	pH (S.U.)	8.10	7.98	8.07	7.97	8.10	7.00
	Dissolved oxygen (mg/L)	7.8	7.5	7.6	7.2	8.0	7.4
	Salinity (ppt)	24.8	25.2	25.0	25.2	24.7	25.2
	Temperature (°C)	24.9	24.9	24.8	24.8	24.8	24.9
0.5 mg/L	pH (S.U.)	8.11	7.97	8.10			
	Dissolved oxygen (mg/L)	7.9	7.5	7.5			
	Salinity (ppt)	24.8	25.2	25.1			
	Alkalinity (mg CaCO ₃ /L)		101.15				101.15
	Temperature (°C)	24.9	24.8	24.8			
		Initial	Final	Initial	Final	Initial	Final

Species: Menidia beryllina

MbCuCR Test Number: 141

Concentration		Day							
		(Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
Parameter	Analyst	BSL	BSL	N	N	EC	EC	N	
CONTROL, SaltSW	pH (S.U.)	0.03	7.93	8.08	7.62	8.05	7.94	8.03	7.35
	Dissolved oxygen (mg/L)	7.9	8.0	7.8	7.6	7.0	7.2	7.9	6.0
	Salinity (ppt)	24.0	25.1	25.1	25.3	24.9	25.1	25.2	25.5
	Alkalinity (mg CaCO ₃ /L)	100		100		94		101-15-13	
	Temperature (°C)	24.8	25.1	24.7	25.0	24.8	25.1	24.8	24.8
0.025 mg/L	pH (S.U.)	0.05	7.92	8.13	7.77	8.04	7.90	8.07	7.74
	Dissolved oxygen (mg/L)	8.0	8.0	7.9	7.0	8.0	7.1	7.6	6.7
	Salinity (ppt)	24.9	25.2	25.1	25.3	25.1	25.2	25.2	25.4
	Temperature (°C)	24.9	24.9	24.7	25.2	24.9	25.2	24.9	25.0
	0.05 mg/L	pH (S.U.)	0.08	7.92	8.13	7.74	8.05	7.91	8.07
Dissolved oxygen (mg/L)		8.0	8.0	8.0	7.7	8.1	7.0	7.6	6.7
Salinity (ppt)		24.9	25.2	25.0	25.3	24.9	25.2	25.1	25.4
Temperature (°C)		24.9	24.9	24.8	24.9	24.9	25.0	24.9	25.0
0.1 mg/L		pH (S.U.)	0.08	7.89	8.12	7.70	8.05	7.89	8.07
	Dissolved oxygen (mg/L)	8.1	8.0	8.0	7.7	8.1	7.0	7.6	6.7
	Salinity (ppt)	24.9	25.3	25.1	25.3	25.1	25.2	25.1	25.4
	Temperature (°C)	24.9	25.0	24.7	24.9	24.9	25.0	25.0	24.9
	0.2 mg/L	pH (S.U.)	0.08	7.87	8.13	7.80	8.05	7.89	8.07
Dissolved oxygen (mg/L)		8.1	8.0	8.0	7.0	8.2	7.0	7.6	6.6
Salinity (ppt)		24.9	25.2	25.0	25.3	25.0	25.1	25.0	25.3
Temperature (°C)		24.9	24.8	24.8	25.1	24.9	25.3	25.0	24.9
0.5 mg/L		pH (S.U.)							
	Dissolved oxygen (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final