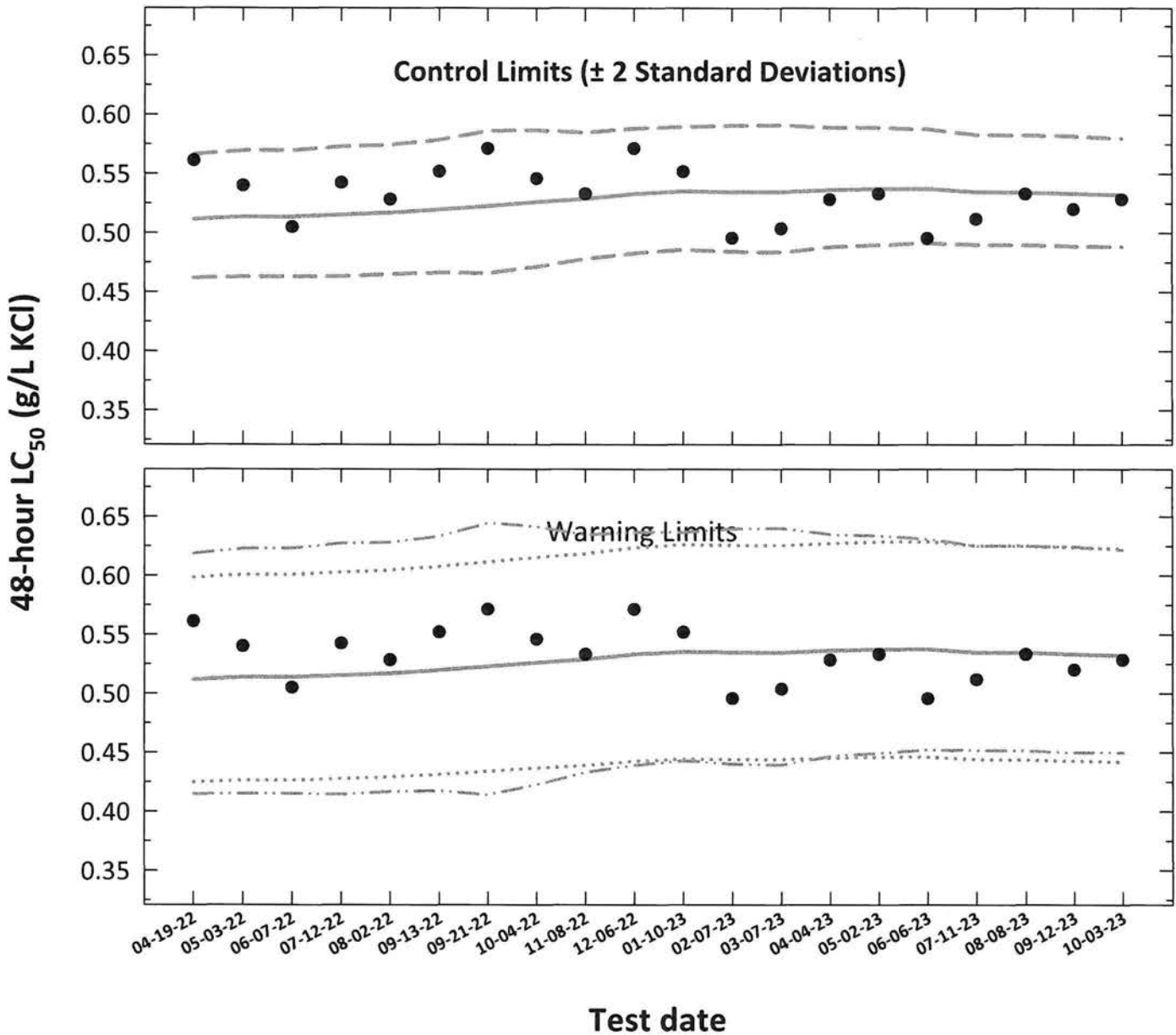


Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350	0.4452	0.6275
15	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338	0.4459	0.6286
16	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312	0.4462	0.6290
17	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252	0.4437	0.6255
18	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250	0.4436	0.6253
19	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244	0.4426	0.6239
20	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217	0.4417	0.6226

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 # 271

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 #: 227

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Concentration			
	Analyst	<i>N</i>	<i>N N</i>	<i>N J</i>
	pH (S.U.)	7.06	7.02	7.72
	Dissolved oxygen (mg/L)	7.7	7.0	7.7
	*Salinity (ppt)	24.9	25.5	25.5
250 mg/L	pH (S.U.)	7.01	7.02	7.71
	Dissolved oxygen (mg/L)	7.8	7.0	7.0
	*Salinity (ppt)	25.2	25.6	25.7
	*Temperature (°C)	25.0	25.0	25.1
	375 mg/L	pH (S.U.)	7.00	7.02
Dissolved oxygen (mg/L)		7.8	7.0	7.0
*Salinity (ppt)		25.2	25.6	26.0
*Temperature (°C)		25.0	25.3	24.8
500 mg/L		pH (S.U.)	7.01	7.03
	Dissolved oxygen (mg/L)	7.9	7.0	7.0
	*Salinity (ppt)	25.2	25.5	25.9
	*Temperature (°C)	25.0	25.2	24.8
	750 mg/L	pH (S.U.)	7.01	7.03
Dissolved oxygen (mg/L)		7.9	7.0	
*Salinity (ppt)		25.4	25.8	
*Temperature (°C)		25.2	25.2	
1000 mg/L		pH (S.U.)	7.01	7.03
	Dissolved oxygen (mg/L)	8.0	7.9	
	*Salinity (ppt)	25.9	25.8	
	*Temperature (°C)	25.0	25.4	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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 # 271

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-03-23	1015	JL	1225	JL	6D	LIGHT GREEN	09-27-23 10
24	10-04-23			1225	JL			
48 Termination	10-05-23			1221	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):	10-02-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	10-01-23 1200 TO 10-02-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.90
	Temperature (°C) 24.2

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	1 ^{1d} 9	3 ^{3d} 7	2 ^{2d} 7	0	0	0	0
Mean Survival	100%		100%		95%		70%		0%		0%	

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

Statistics:

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

Comments:

Test Reviewed by:

Acute Mysid Test-24 Hr Survival

Start Date: 10/3/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 10/5/2023	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	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

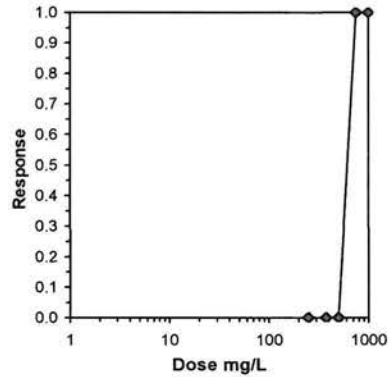
Hypothesis Test (1-tail, 0.05) NOEC LOEC ChV TU MSDu MSDp MSB MSE F-Prob df

Dunnett's Test 500 750 612.372 0.00967 0.00991 0.62824 0.0001 2.0E-09 4, 5

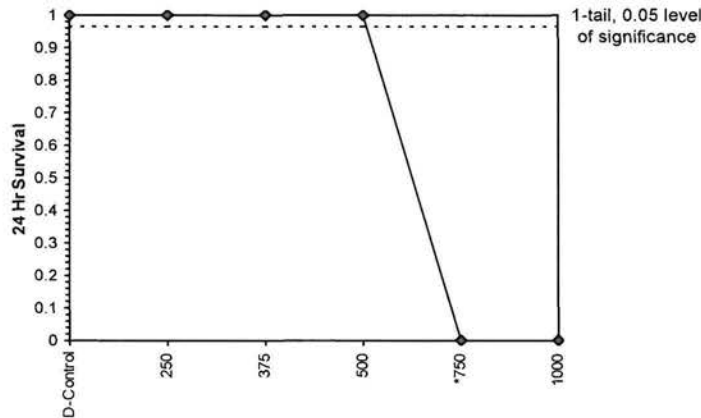
Treatments vs D-Control

Graphical Method

Trim Level	EC50
0.0%	612.37



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date:	10/3/2023	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	10/5/2023	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	0.9000
500	0.7000	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							1-Tailed			Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*500	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	8.166	2.850	0.1469	6	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

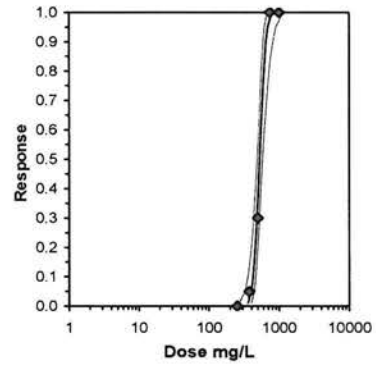
Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

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

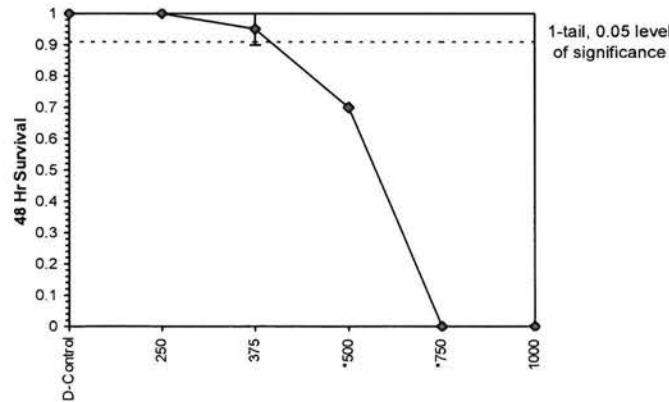
Maximum Likelihood-Probit

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

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



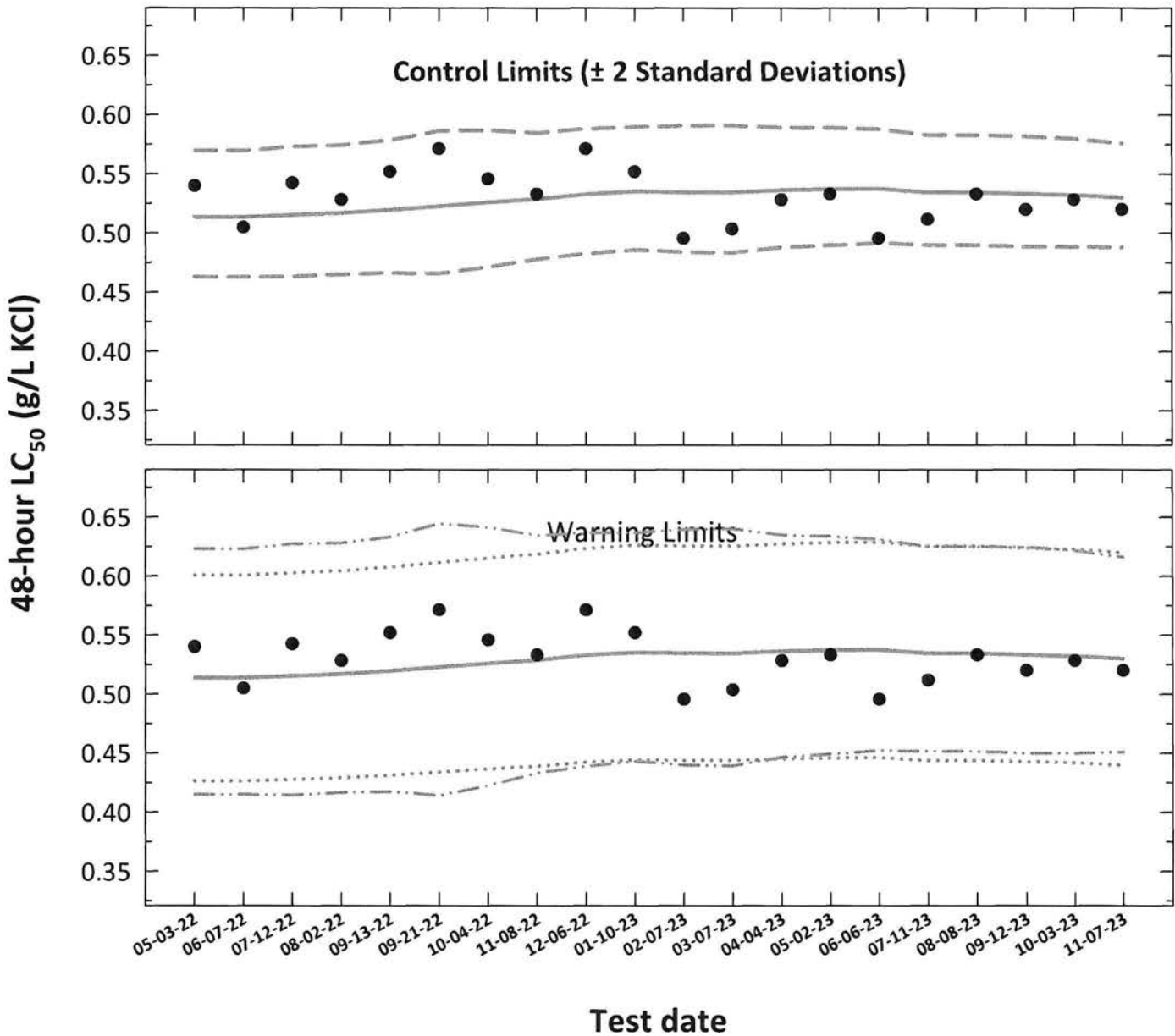
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350	0.4452	0.6275
14	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338	0.4459	0.6286
15	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312	0.4462	0.6290
16	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252	0.4437	0.6255
17	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250	0.4436	0.6253
18	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244	0.4426	0.6239
19	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217	0.4417	0.6226
20	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161	0.4400	0.6202

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 # 272

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 #: 2227

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<i>VGP</i>	<i>OL</i>	<i>PO</i>
	pH (S.U.)	7.97	8.00	7.90
	Dissolved oxygen (mg/L)	8.0	7.7	7.6
	*Salinity (ppt)	25.0	25.6	25.9
	*Alkalinity (mg/L CaCO ₃)	120		
	*Temperature (°C)	24.9	24.7	24.3
250 mg/L	pH (S.U.)	7.90	8.00	7.87
	Dissolved oxygen (mg/L)	8.0	7.7	7.5
	*Salinity (ppt)	25.0	25.5	25.7
	*Temperature (°C)	24.8	24.5	24.3
375 mg/L	pH (S.U.)	7.99	8.00	7.86
	Dissolved oxygen (mg/L)	8.0	7.7	7.6
	*Salinity (ppt)	25.2	25.7	26.5
	*Temperature (°C)	24.9	24.5	24.6
500 mg/L	pH (S.U.)	8.00	7.99	7.89
	Dissolved oxygen (mg/L)	8.0	7.6	7.6
	*Salinity (ppt)	25.2	25.6	25.9
	*Temperature (°C)	24.9	24.6	24.7
750 mg/L	pH (S.U.)	8.00	7.99	
	Dissolved oxygen (mg/L)	8.0	7.6	
	*Salinity (ppt)	25.3	25.7	
	*Temperature (°C)	25.0	24.6	
1000 mg/L	pH (S.U.)	8.00	7.96	
	Dissolved oxygen (mg/L)	8.1	7.7	
	*Salinity (ppt)	25.5	25.9	
	*Temperature (°C)	25.0	24.6	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 272

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	11-07-23	1005	J	1220	J	6C	Yellow	10-31-23A
24	11-08-23			1222	J			
48 Termination	11-09-23			1210	J			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	11-06-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	11-05-23 1200 TO 11-06-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.84 Temperature (°C) 25.0

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 ^d	9 ^d	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	10	9	7 ^{2d}	6 ^{3d}	0	0	0	0
Mean Survival	100%		100%		95%		65%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	479.3
Upper 95% confidence limit (mg KCl/L)	575.1
48-hour LC ₅₀ (mg KCl/L)	519.9

Comments:

Test Reviewed by:

Acute Mysid Test-24 Hr Survival

Start Date: 11/7/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 11/9/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: AB-Americanysis bahia

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

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

Auxiliary Tests Statistic Critical Skew Kurt

Normality of the data set cannot be confirmed

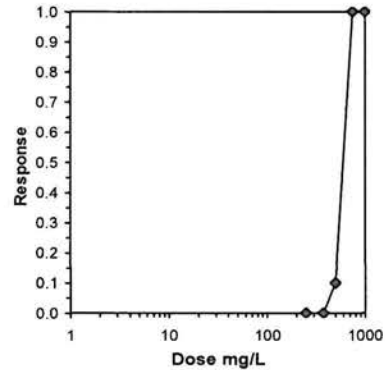
Equality of variance cannot be confirmed

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	0.59802	0.0001	2.2E-09	4, 5

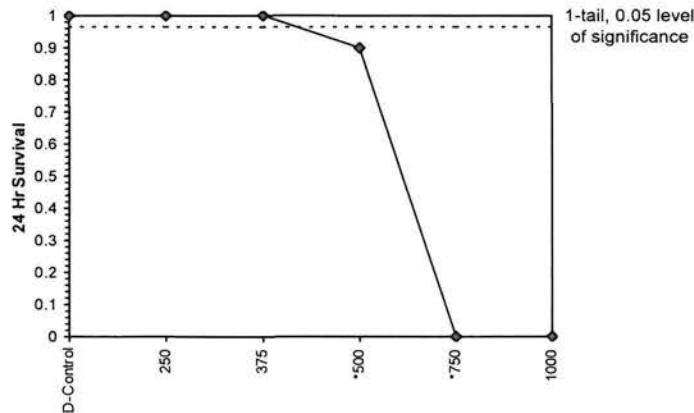
Treatments vs D-Control

Trimmed Spearman-Kärber

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



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 11/7/2023 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
End Date: 11/9/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: AB-Americanysis bahia

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	0.9000
500	0.7000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1748	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.329	2.850	0.1748	1	20
*500	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	7.720	2.850	0.1748	7	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20.438	2.850	0.1748	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)

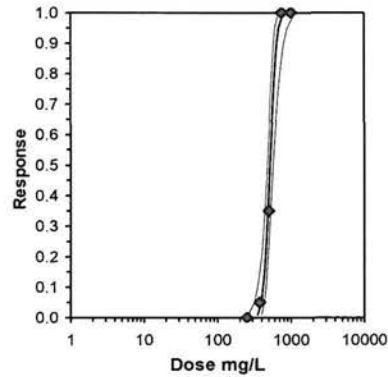
	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.08218	0.08429	0.57374	0.00376	2.1E-05	4, 5

Treatments vs D-Control

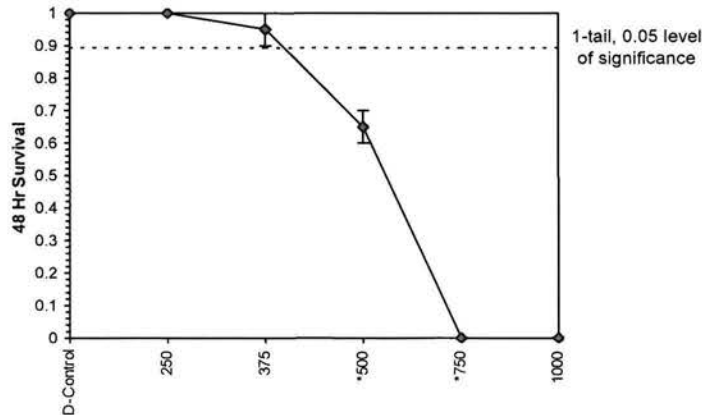
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.6104	3.03411	7.66352	19.5572	0	1.01009	7.81472	0.79881	2.71594	0.07347	5
Intercept	-31.965	8.18953	-48.016	-15.913							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	350.763	261.711	398.116
EC05	3.355	393.628	318.615	434.85
EC10	3.718	418.58	352.793	457.159
EC15	3.964	436.303	377.093	473.869
EC20	4.158	450.923	396.862	488.482
EC25	4.326	463.854	413.925	502.251
EC40	4.747	498.109	455.817	543.926
EC50	5.000	519.923	479.286	575.107
EC60	5.253	542.691	501.217	611.408
EC75	5.674	582.768	535.176	682.885
EC80	5.842	599.481	548.159	714.979
EC85	6.036	619.568	563.141	755.03
EC90	6.282	645.801	581.936	809.514
EC95	6.645	686.739	610.01	898.971
EC99	7.326	770.661	664.439	1097.49



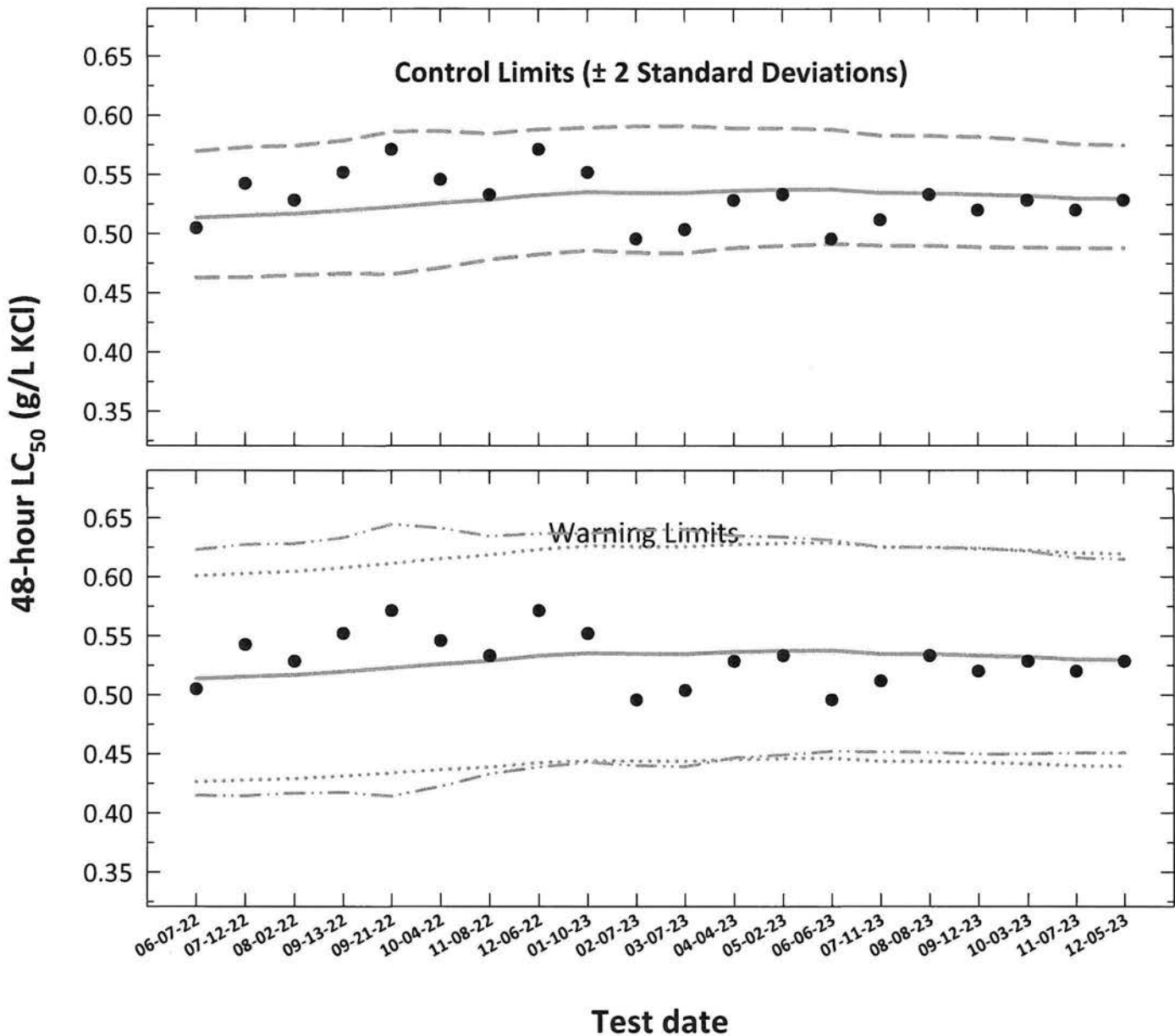
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	04-04-23	0.5283	-0.2771	-0.2705	0.0204	0.5364	0.4882	0.5892	0.4466	0.6350	0.4452	0.6275
13	05-02-23	0.5331	-0.2732	-0.2698	0.0200	0.5373	0.4900	0.5891	0.4493	0.6338	0.4459	0.6286
14	06-06-23	0.4957	-0.3048	-0.2695	0.0194	0.5376	0.4916	0.5880	0.4521	0.6312	0.4462	0.6290
15	07-11-23	0.5117	-0.2910	-0.2720	0.0188	0.5346	0.4902	0.5830	0.4515	0.6252	0.4437	0.6255
16	08-08-23	0.5331	-0.2732	-0.2721	0.0188	0.5344	0.4900	0.5828	0.4514	0.6250	0.4436	0.6253
17	09-12-23	0.5199	-0.2841	-0.2730	0.0189	0.5333	0.4887	0.5819	0.4497	0.6244	0.4426	0.6239
18	10-03-23	0.5283	-0.2771	-0.2740	0.0186	0.5321	0.4884	0.5798	0.4499	0.6217	0.4417	0.6226
19	11-07-23	0.5199	-0.2841	-0.2757	0.0179	0.5301	0.4881	0.5757	0.4508	0.6161	0.4400	0.6202
20	12-05-23	0.5283	-0.2771	-0.2761	0.0178	0.5295	0.4878	0.5748	0.4507	0.6151	0.4395	0.6195

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

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

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

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

CV = Coefficient of variation.



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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 273

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 #: 2249

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	JP JHC	DL	JP
	pH (S.U.)	7.92	8.00	7.79
	Dissolved oxygen (mg/L)	7.7	7.7	7.3
	*Salinity (ppt)	25.0	25.4	25.5
	*Alkalinity (mg/L CaCO ₃)	120		
	*Temperature (°C)	24.6	25.0	24.6
250 mg/L	pH (S.U.)	7.93	8.00	7.77
	Dissolved oxygen (mg/L)	7.7	7.7	7.3
	*Salinity (ppt)	25.0	25.4	25.7
	*Temperature (°C)	24.4	25.2	24.5
375 mg/L	pH (S.U.)	7.94	7.98	7.73
	Dissolved oxygen (mg/L)	7.7	7.8	7.2
	*Salinity (ppt)	25.3	25.6	25.7
	*Temperature (°C)	24.7	25.2	24.5
500 mg/L	pH (S.U.)	7.94	8.02	7.75
	Dissolved oxygen (mg/L)	7.7	7.8	7.4
	*Salinity (ppt)	25.5	25.6	25.8
	*Temperature (°C)	24.5	24.9	24.5
750 mg/L	pH (S.U.)	7.95	8.00	
	Dissolved oxygen (mg/L)	7.7	7.9	
	*Salinity (ppt)	25.6	25.8	
	*Temperature (°C)	24.5	24.9	
1000 mg/L	pH (S.U.)	7.94	8.02	
	Dissolved oxygen (mg/L)	7.6	7.9	
	*Salinity (ppt)	25.7	26.1	
	*Temperature (°C)	24.3	24.8	

*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	1306646

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 # 273

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	12-05-23	1210	JL	1410	JL	1C	Yellow	11-24-13A
24	12-06-23			1405	JL			
48 Termination	12-07-23			1407	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):	12-04-23
Age (1 to 5 days old):	1-2 DMS
Date organisms were born:	12-03-23 1200 TO 12-04-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86 Temperature (°C) 24.0°C

Survival Data (number of living organisms):

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

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

Statistics:

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

Comments:

Test Reviewed by: 

Acute Mysid Test-24 Hr Survival

Start Date: 12/5/2023	Test ID: AbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 12/7/2023	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

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

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

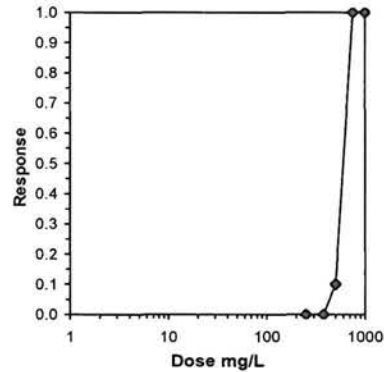
Auxiliary Tests Statistic Critical Skew Kurt

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

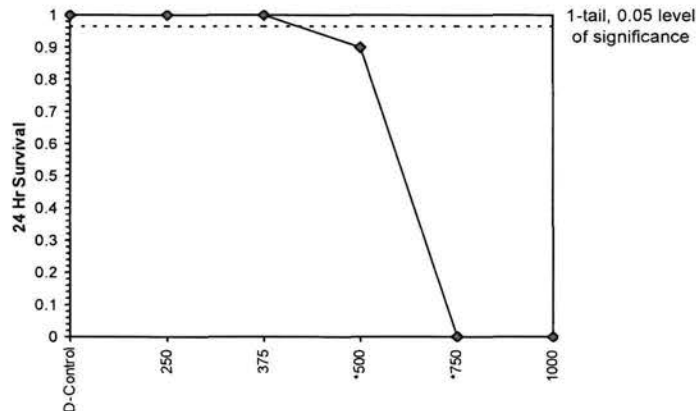
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.59802	0.0001	2.2E-09	4, 5

Treatments vs D-Control Trimmed Spearman-Kärber

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



Dose-Response Plot



Acute Mysid Test-48 Hr Survival

Start Date: 12/5/2023 Test ID: AbKCIAC Sample ID: REF-Ref Toxicant
 End Date: 12/7/2023 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	0.9000
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	0.1469	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*500	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	8.166	2.850	0.1469	6	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

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

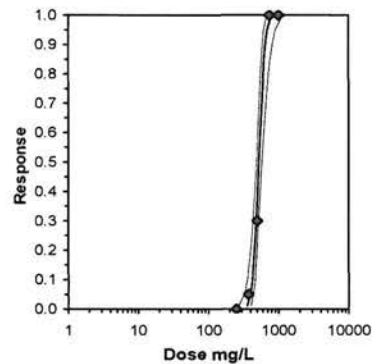
Treatments vs D-Control

Maximum Likelihood-Probit

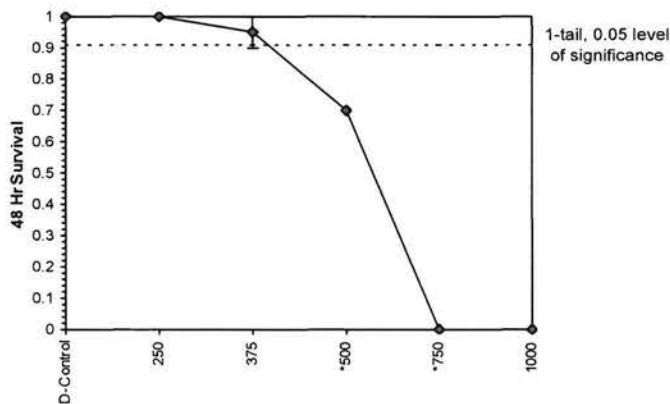
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.5631	2.95357	7.77407 19.352	0	1.60351	7.81472	0.65859	2.7229	0.07373	6
Intercept	-31.931	7.99322	-47.598 -16.264							

TSCR

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



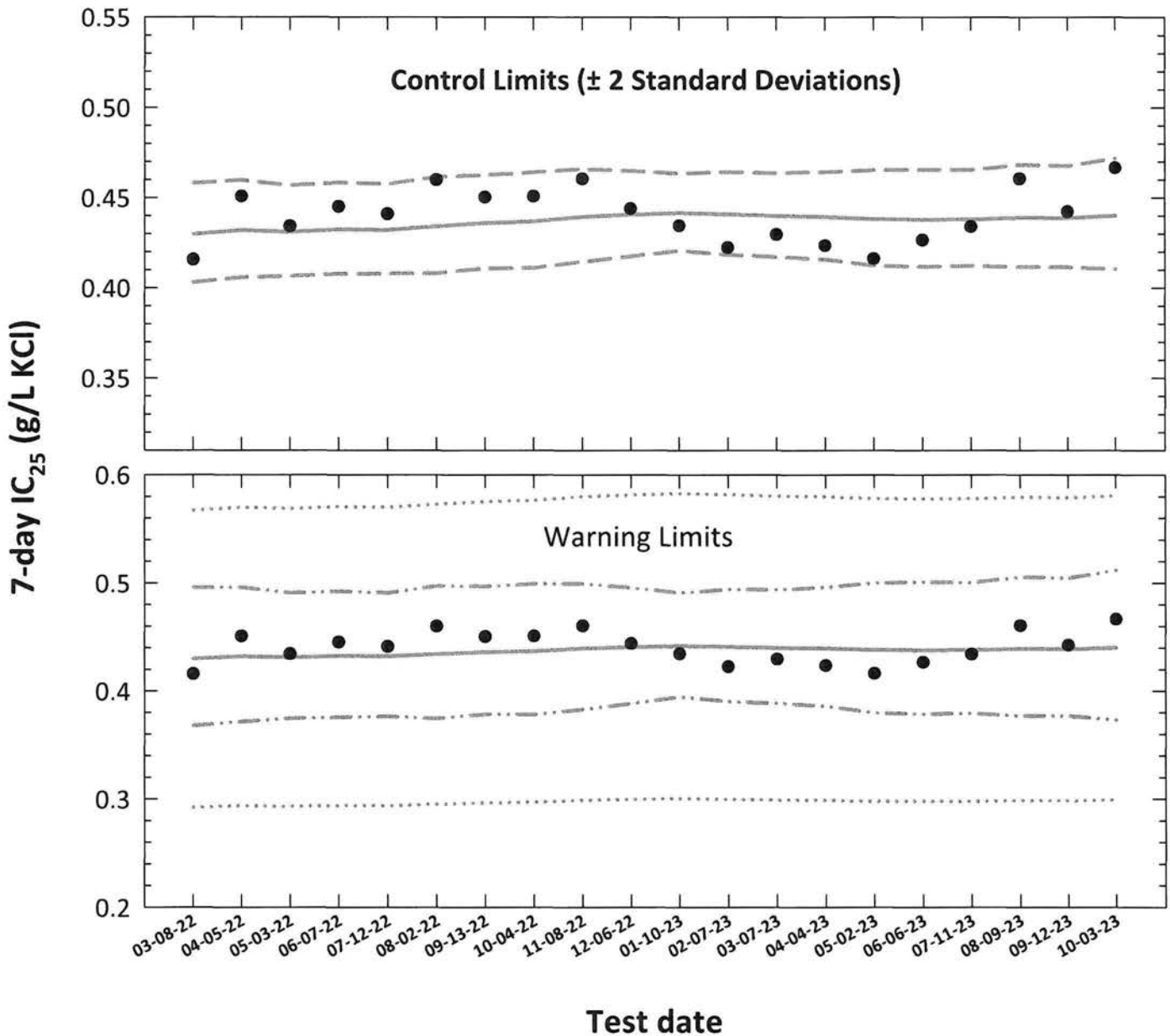
Dose-Response Plot



Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ $\pm S_{A,75}$ converted to anti-logarithmic values, $S_{A,75}$ = 75th percentile of CVs reported nationally by USEPA)

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

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

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

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

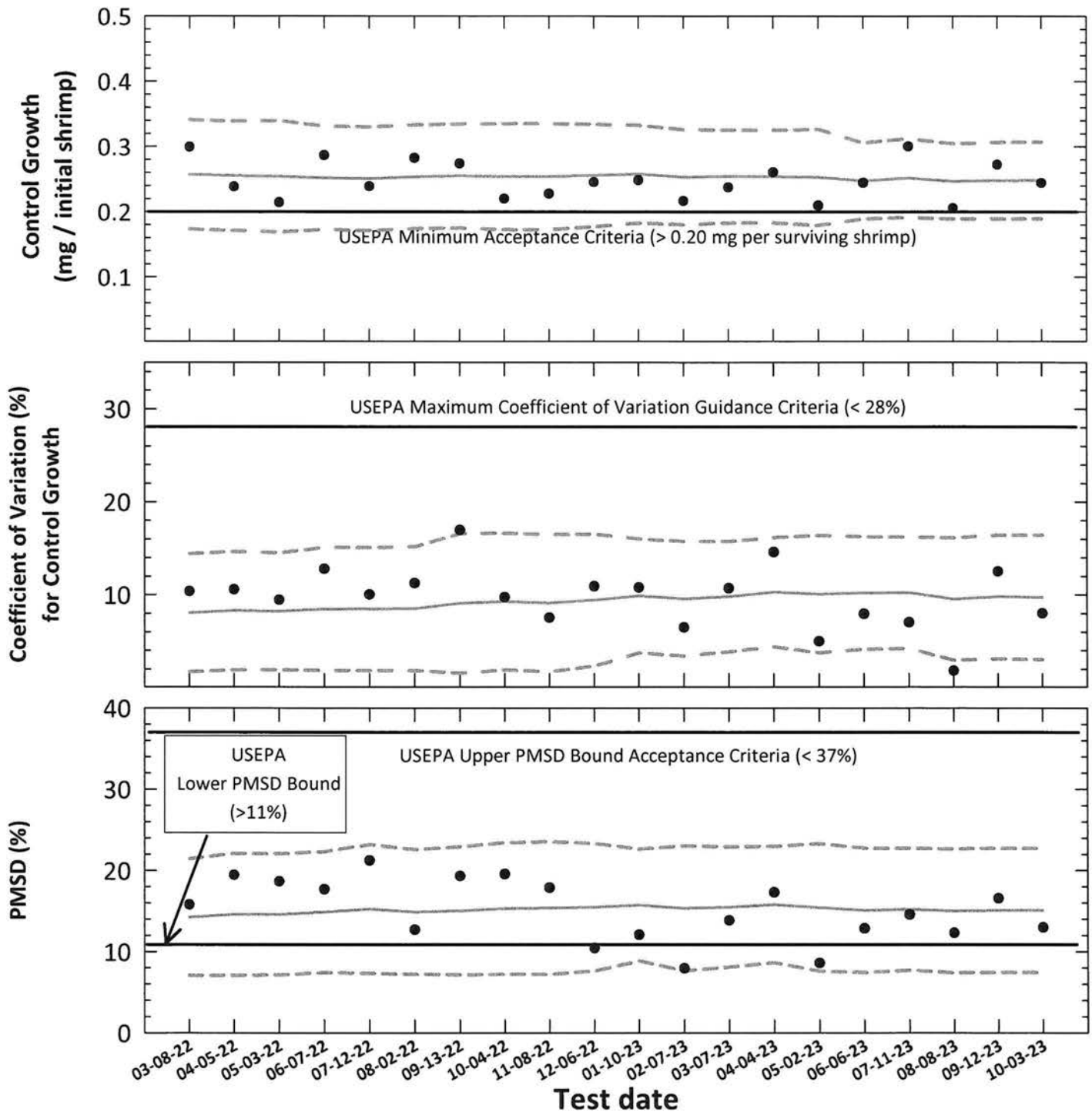
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

CV = Coefficient of variation.

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test		CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
			Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)									
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
15	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
16	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
17	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
18	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
19	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
20	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7

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) < 37%.
Upper PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



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

AbKCICR Test Number: **244**

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2227				
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:	09-26-23 1200 to 09-27-23 1130	Incubator number and shelf location:	SF
Organism source:	AI Batch Ab: 09-27-23	Artemia CHM number:	CHM1222
Transfer bowl information:		Drying information for weight determination:	
pH = 7.92 S.U. Temperature = 25.0 °C		Date / Time in oven:	10-10-23 1050
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	60 °C
		Date / Time out of oven:	10-10-23 1050
		*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	10-03-23	1015	JH	1240	JH	1145	JH	09-27-23 B
1	10-04-23	0500	JH	1100	JH	0945	JH	↓
2	10-05-23	0500	JH	1215	JH	1140	JH	10-05-23
3	10-06-23	0500	JH	1100	JH	0945	JH	10-04-23
4	10-07-23	0615	JH	1215	JH	1045	JH	10-05-23 A
5	10-08-23	0600	JH	1200	JH	0953	JH	10-05-23 B
6	10-09-23	0500	JH	1100	JH	0945	JH	10-07-23 A
7	10-10-23					0945	JH	

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)	531.4
Average weight per initial shrimp:	0.244		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.244	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	435.0
			IC ₂₅ (mg/L KCl)	466.7

AbKCICR Test Number: 244

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>Magenta</u> Analyst: <u>DL</u> Date: <u>09-25-23</u>	12.66	14.94	15.50	15.01	13.17	14.68	11.86	14.50	13.08	15.20	13.53	13.85	12.68	15.08	12.81	13.76
*B = Pan + Shrimp weight (mg) Analyst: <u>H</u> Date: <u>10-15-23</u>	15.78	15.95	16.69	16.28	14.57	15.95	13.01	15.76	14.39	16.45	14.85	14.94	13.96	16.27	14.02	15.01
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JL</u>	1.12	1.11	1.19	1.27	1.40	1.27	1.15	1.26	1.31	1.25	1.32	1.09	1.28	1.19	1.21	1.31
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>H</u>	0.224	0.222	0.238	0.254	0.280	0.254	0.230	0.252	0.262	0.250	0.264	0.218	0.256	0.238	0.242	0.267
	Average weight per initial number of shrimp (mg) <u>0.244</u>								Average weight per initial number of shrimp (mg) <u>0.249</u>				Percent reduction from control (%) <u>-1.97</u>			

*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: **244**

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	4 ^d	5	5	5	5	5	5	5								
3	5	5	5	5	5	5	5	5	3 ^{ld}	4 ^{ld}	5	5	5	5	5	5								
4	5	5	5	5	5	5	5	5	3	4	4 ^{ld}	5	5	5	5	4 ^{ld}								
5	5	5	5	5	5	5	5	5	3	4	4	4 ^{ld}	4 ^{ld}	4 ^{ld}	4 ^{ld}	4								
6	4 ^{ld}	5	5	5	5	5	5	5	3	4	3 ^{ld}	4	4	4	4	3 ^{ld}								
7	4	5	5	5	5	5	5	5	3	3 ^{ld}	3	4	4	4	4	3								
# females with eggs in brood sac																								
# females with developing ova in oviducts																								
# immature females																								
# males																								
*A = Pan weight (mg) Tray color code: <u>Magenta</u> Analyst: <u>BL</u> Date: <u>09-25-23</u>	12.29	13.38	13.86	15.07	15.11	12.64	12.80	13.36	DARK GREEN A-1								12.50	14.64	14.74	13.83	13.66	15.47	13.37	14.3
*B = Pan + Shrimp weight (mg) Analyst: <u>JL</u> Date: <u>10-15-23</u>	13.12	14.43	14.97	16.13	16.44	13.80	13.82	14.60									13.68	14.82	12.23	14.77	13.22	13.55	13.67	11.8
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JL</u>	0.83	1.05	1.11	1.06	1.33	1.16	1.02	1.24									0.89	0.87	0.97	0.95	0.86	0.72	1.10	0.5
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JL</u>	0.166	0.210	0.222	0.212	0.266	0.232	0.204	0.248									0.178	0.174	0.194	0.190	0.172	0.144	0.220	0.104
Average weight per initial number of shrimp (mg) 0.220				Percent reduction from control (%) 9.97				Average weight per initial number of shrimp (mg) 0.172				Percent reduction from control (%) 29.57												

*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: 244

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <u>megab2</u> Analyst: <u>DL</u> Date: <u>09-25-23</u>	15.76	14.36	14.16	14.64	13.68	14.82	12.23	14.77	13.22	13.55	13.67	11.89	14.03	14.01	14.25	14.31
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____															10.04	23
Weight per initial number of shrimp (mg) = C / initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average weight per initial number of shrimp (mg)	0				100%				0				100%			
Percent reduction from control (%)	0				100%				0				100%			

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

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

Comments:



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

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

Test number: _____

Test dates: October 03-10, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	12.66	13.78	1.12	0.224	100.0	0.244	8.0	Not applicable
	B	5	5	14.84	15.95	1.11	0.222				
	C	5	5	15.50	16.69	1.19	0.238				
	D	5	5	15.01	16.28	1.27	0.254				
	E	5	5	13.17	14.57	1.40	0.280				
	F	5	5	14.68	15.95	1.27	0.254				
	G	5	5	11.86	13.01	1.15	0.230				
	H	5	5	14.50	15.76	1.26	0.252				
250	I	5	5	13.08	14.39	1.31	0.262	100.0	0.249	6.3	-1.9
	J	5	5	15.20	16.45	1.25	0.250				
	K	5	5	13.53	14.85	1.32	0.264				
	L	5	5	13.85	14.94	1.09	0.218				
	M	5	5	12.68	13.96	1.28	0.256				
	N	5	5	15.08	16.27	1.19	0.238				
	O	5	5	12.81	14.02	1.21	0.242				
	P	5	5	13.70	15.01	1.31	0.262				
375	Q	5	4	12.29	13.12	0.83	0.166	97.5	0.220	13.7	9.9
	R	5	5	13.38	14.43	1.05	0.210				
	S	5	5	13.86	14.97	1.11	0.222				
	T	5	5	15.07	16.13	1.06	0.212				
	U	5	5	15.11	16.44	1.33	0.266				
	V	5	5	12.64	13.80	1.16	0.232				
	W	5	5	12.80	13.82	1.02	0.204				
	X	5	5	13.36	14.60	1.24	0.248				
500	Y	5	3	13.68	14.57	0.89	0.178	70.0	0.172	20.0	29.5
	Z	5	3	14.82	15.69	0.87	0.174				
	AA	5	3	12.23	13.20	0.97	0.194				
	BB	5	4	14.77	15.72	0.95	0.190				
	CC	5	4	13.22	14.08	0.86	0.172				
	DD	5	4	13.55	14.27	0.72	0.144				
	EE	5	4	13.67	14.77	1.10	0.220				
	FF	5	3	11.89	12.42	0.53	0.106				
750	GG	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	HH	5	0	0.00	0.00	0.00	0.000				
	II	5	0	0.00	0.00	0.00	0.000				
	JJ	5	0	0.00	0.00	0.00	0.000				
	KK	5	0	0.00	0.00	0.00	0.000				
	LL	5	0	0.00	0.00	0.00	0.000				
	MM	5	0	0.00	0.00	0.00	0.000				
	NN	5	0	0.00	0.00	0.00	0.000				
1000	OO	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	PP	5	0	0.00	0.00	0.00	0.000				
	QQ	5	0	0.00	0.00	0.00	0.000				
	RR	5	0	0.00	0.00	0.00	0.000				
	SS	5	0	0.00	0.00	0.00	0.000				
	TT	5	0	0.00	0.00	0.00	0.000				
	UU	5	0	0.00	0.00	0.00	0.000				
	VV	5	0	0.00	0.00	0.00	0.000				

Dunnett's MSD value: 0.0317
PMSD: 13.0

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

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



Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

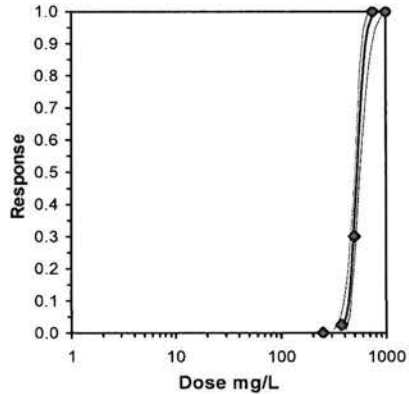
Start Date: 10/3/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 10/10/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americamysis bahia
Comments:		

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	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.6000	0.6000	0.8000	0.8000	0.8000	0.8000	0.6000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

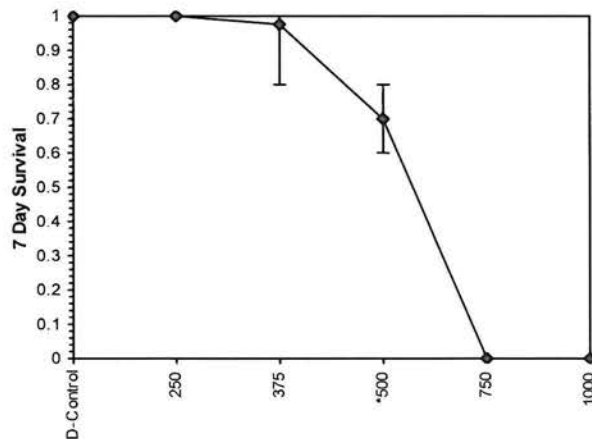
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.7000	0.7000	0.9966	0.8861	1.1071	11.857	8	36.00	48.00	12	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)	0.81737	0.904	-0.9304	2.03119
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.3719	2.68634	10.1067	20.6371	0	1.66025	7.81472	0.64581	2.72539	0.06505	5
Intercept	-36.894	7.26862	-51.141	-22.648							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	375.021	316.328	410.38							
EC05	3.355	415.326	367.392	445.294							
EC10	3.718	438.554	397.054	466.098							
EC15	3.964	454.956	417.768	481.418							
EC20	4.158	468.427	434.432	494.597							
EC25	4.326	480.303	448.714	506.803							
EC40	4.747	511.578	483.691	542.397							
EC50	5.000	531.365	503.556	567.782							
EC60	5.253	551.918	522.535	596.29							
EC75	5.674	587.856	552.855	650.187							
EC80	5.842	602.759	564.705	673.695							
EC85	6.036	620.607	578.512	702.568							
EC90	6.282	643.818	595.984	741.134							
EC95	6.645	679.825	622.289	802.953							
EC99	7.326	752.889	673.633	934.786							



Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

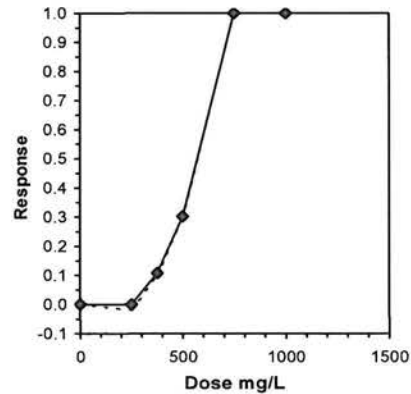
Start Date: 10/3/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 10/10/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americamysis bahia

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2240	0.2220	0.2380	0.2540	0.2800	0.2540	0.2300	0.2520
250	0.2620	0.2500	0.2640	0.2180	0.2560	0.2380	0.2420	0.2620
375	0.1660	0.2100	0.2220	0.2120	0.2660	0.2320	0.2040	0.2480
500	0.1780	0.1740	0.1940	0.1900	0.1720	0.1440	0.2200	0.1060
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

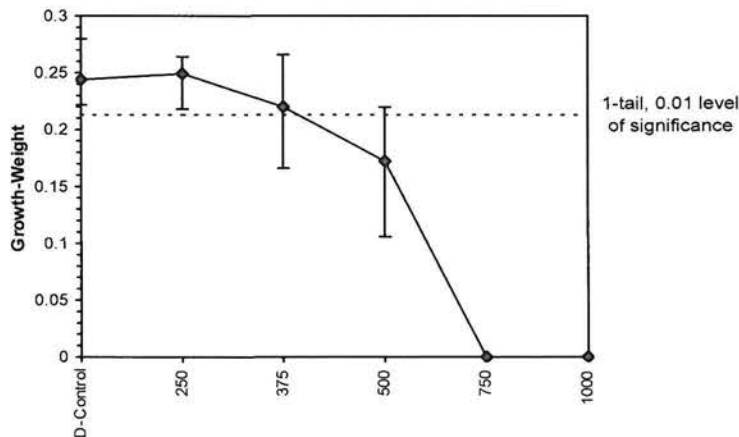
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2443	1.0000	0.2443	0.2220	0.2800	8.004	8				0.2466	1.0000
250	0.2490	1.0194	0.2490	0.2180	0.2640	6.339	8	-0.419	2.799	0.0317	0.2466	1.0000
375	0.2200	0.9007	0.2200	0.1660	0.2660	13.727	8	2.138	2.799	0.0317	0.2200	0.8920
500	0.1723	0.7052	0.1723	0.1060	0.2200	19.981	8				0.1723	0.6984
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)						0.97789	0.884	-0.1857	0.87912		
Bartlett's Test indicates equal variances (p = 0.23)						2.95971	9.21035				
Hypothesis Test (1-tail, 0.01)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		375	>375			0.03174	0.12994	0.00194	0.00051	0.04013	2, 21

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL	Skew	
IC05	307.89	36.70	239.07	387.44	-0.1304
IC10	365.79	32.27	297.78	412.76	-0.2856
IC15	402.14	28.04	338.31	446.10	-0.3668
IC20	434.42	27.15	379.16	484.65	-0.1105
IC25	466.70	25.30	417.18	511.20	-0.0519
IC40	535.23	15.51	492.46	558.96	-0.5888
IC50	571.03	12.98	535.86	590.80	-0.6151



Dose-Response Plot



Entered and
Reviewed by
Jan Sumner
JS

AbKCICR Test Number: 244

Daily Chemistry:

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

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
		Analyst					
CONTROL, Salt SW	pH (S.U.)	KL	KL 70	KL 70	KL 70	KL 70	KL
	DO (mg/L)	7.7	7.6	7.8	7.5	7.7	7.2
	Salinity (ppt)	24.9	25.0	25.0	25.0	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	86		10-11		110	
	Temperature (°C)	25.7	26.3	25.2	26.3	25.1	26.0
250 mg KCl/L	pH (S.U.)	7.01	7.01	7.08	7.74	7.91	7.71
	DO (mg/L)	7.0	7.0	7.0	7.2	7.7	7.0
	Salinity (ppt)	25.2	25.5	25.0	25.4	25.1	25.4
	Temperature (°C)	25.8	26.4	25.2	26.2	25.3	26.0
375 mg KCl/L	pH (S.U.)	7.00	7.01	7.08	7.74	7.08	7.107
	DO (mg/L)	7.8	7.0	7.9	7.4	7.0	7.0
	Salinity (ppt)	25.2	25.5	25.2	25.7	25.4	25.7
	Temperature (°C)	25.8	26.2	25.3	26.2	25.1	26.3
500 mg KCl/L	pH (S.U.)	7.01	7.01	7.08	7.74	7.07	7.165
	DO (mg/L)	7.9	7.9	7.9	7.7	8.0	7.0
	Salinity (ppt)	25.2	25.7	25.3	25.7	25.4	25.8
	Temperature (°C)	25.7	26.2	25.3	26.0	25.1	26.0
750 mg KCl/L	pH (S.U.)	7.01	7.01				
	DO (mg/L)	7.9	7.9				
	Salinity (ppt)	25.4	26.2				
	Temperature (°C)	25.7	26.2				
1000 mg KCl/L	pH (S.U.)	7.01	7.00				
	DO (mg/L)	8.0	8.0				
	Salinity (ppt)	25.9	26.5				
	Temperature (°C)	25.9	26.1				
		Initial	Final	Initial	Final	Initial	Final

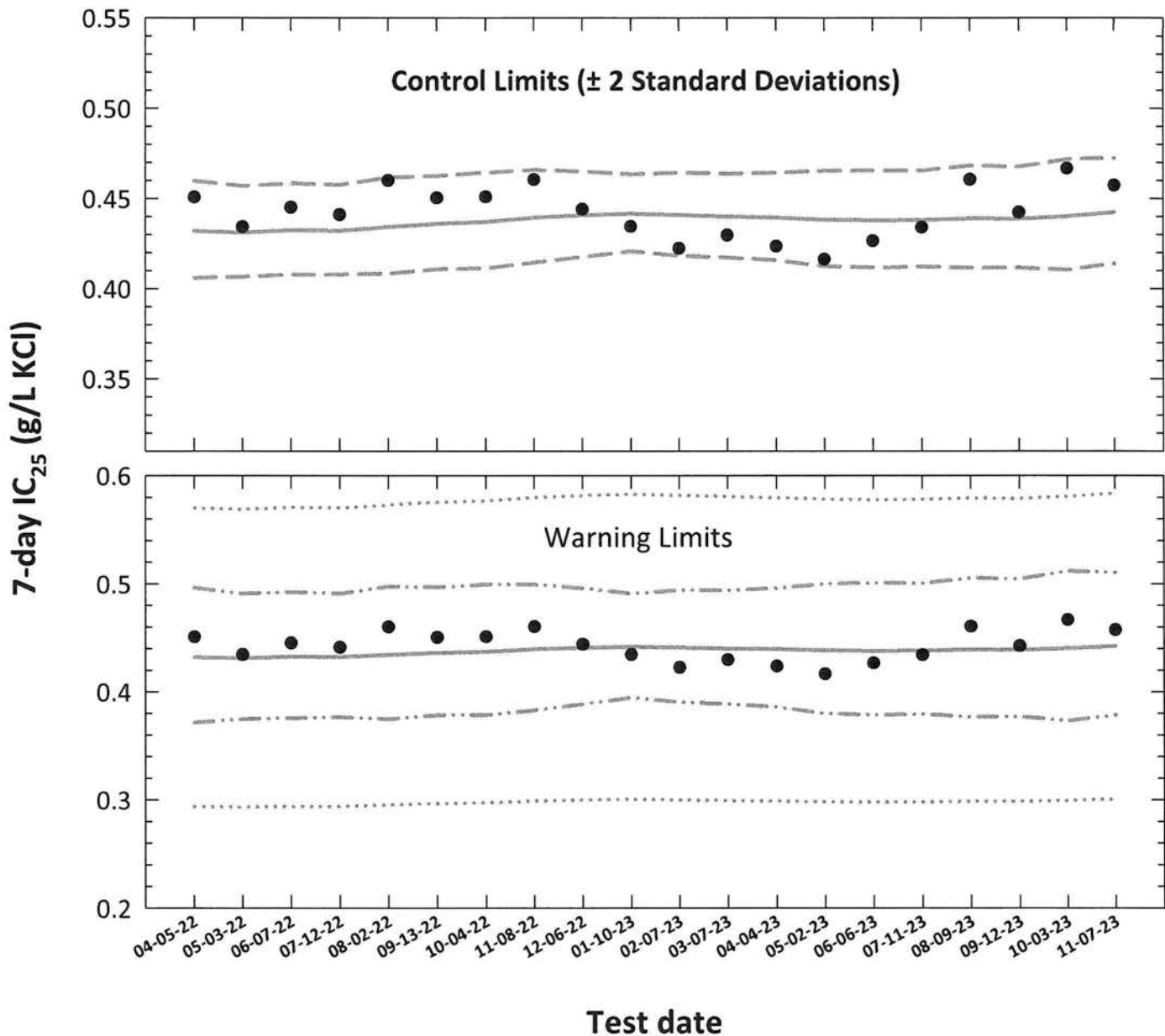
AbKCICR Test Number: 244

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
Analyst	✓	BL	BSL	BSL	BSL	BL	BL	✓	
CONTROL, Salt SW	pH (S.U.)	8.06	7.95	8.11	8.00	8.19	7.99	8.11	7.03
	DO (mg/L)	7.9	7.8	8.0	7.4	8.0	7.2	7.0	6.6
	Salinity (ppt)	25.1	25.5	25.2	25.4	24.0	25.2	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	130		140		130		130	
	Temperature (°C)	25.3	26.1	25.2	26.3	25.2	26.5	25.2	26.0
250 mg KCl/L	pH (S.U.)	7.96	7.87	8.03	7.87	8.19	8.01	8.13	7.07
	DO (mg/L)	7.0	7.9	8.0	7.5	8.0	7.2	7.0	6.7
	Salinity (ppt)	25.2	25.4	25.2	25.4	25.3	25.6	25.2	25.4
	Temperature (°C)	25.4	26.3	25.2	26.2	25.1	26.6	25.2	26.3
375 mg KCl/L	pH (S.U.)	7.86	7.87	8.02	7.93	8.19	8.01	8.15	7.06
	DO (mg/L)	7.0	7.9	8.0	7.5	8.0	7.4	7.0	7.0
	Salinity (ppt)	25.2	25.4	25.2	25.5	25.3	25.6	25.4	25.5
	Temperature (°C)	25.4	26.3	25.2	26.2	25.2	26.3	25.2	26.0
500 mg KCl/L	pH (S.U.)	7.96	7.88	8.02	7.91	8.19	8.02	8.15	7.07
	DO (mg/L)	7.9	7.9	8.0	7.5	8.0	7.4	7.9	7.0
	Salinity (ppt)	25.1	25.4	25.3	25.6	25.4	25.6	25.5	25.5
	Temperature (°C)	25.4	26.3	25.2	26.2	25.2	26.3	25.1	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		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174	0.4638	0.3886	0.4941	0.2992	0.5807
13	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158	0.4643	0.3857	0.4961	0.2988	0.5800
14	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126	0.4654	0.3798	0.5003	0.2980	0.5785
15	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118	0.4655	0.3784	0.5011	0.2977	0.5780
16	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124	0.4655	0.3794	0.5006	0.2979	0.5783
17	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116	0.4683	0.3766	0.5056	0.2985	0.5795
18	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116	0.4677	0.3769	0.5047	0.2983	0.5791
19	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.3730	0.5122	0.2993	0.5810
20	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105	0.3008	0.5838

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

S = Standard deviation of the IC₂₅ values.

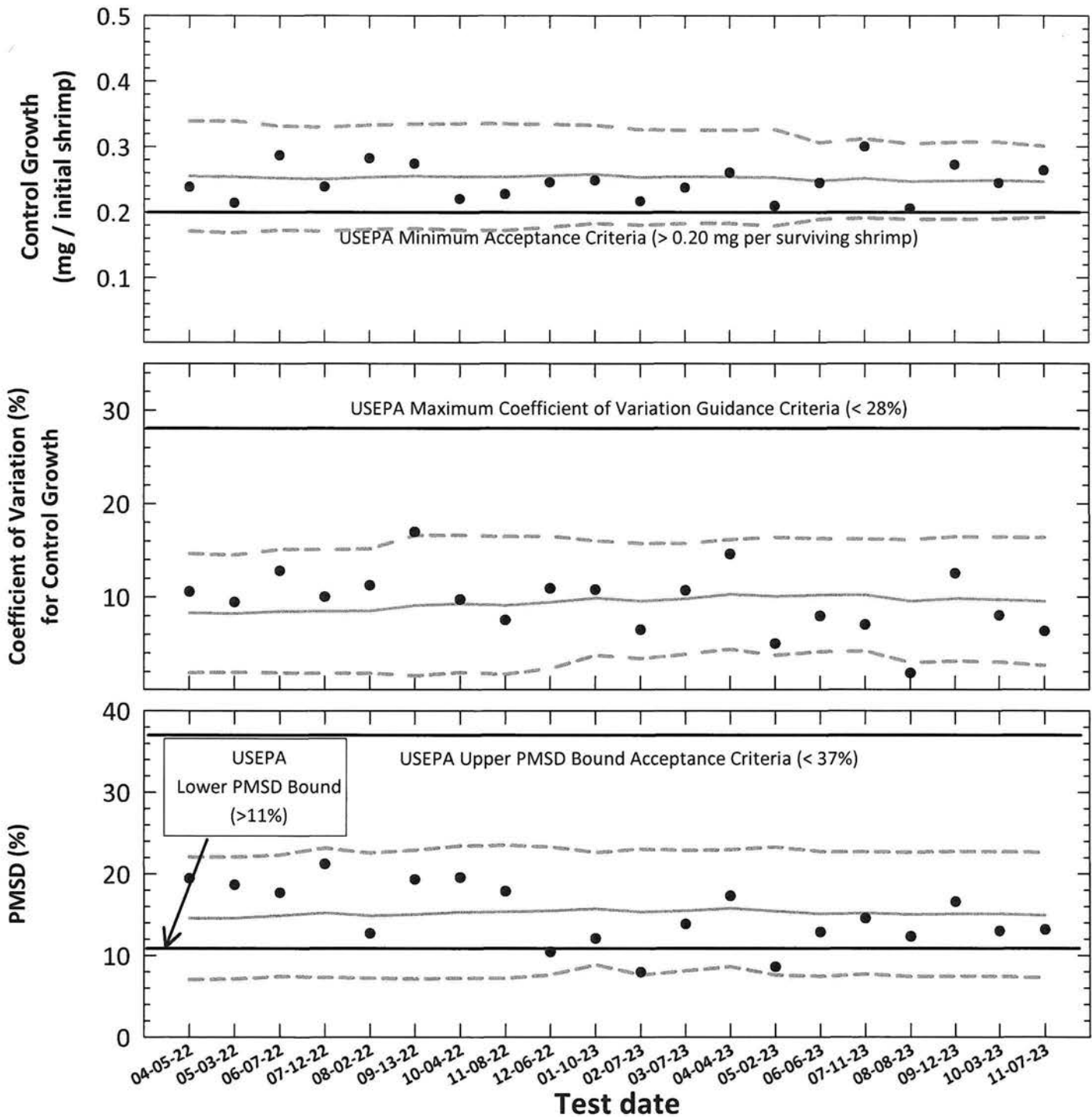
Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

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

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

CV = Coefficient of variation.

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



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

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

Test number	Test date	ToxCal Determination					Control Growth		Control Growth (mg/initial shrimp)		Control Growth CV (%)		Test PMSD (%)		
		Control Survival (%)	Control Growth		MSD	PMSD (%)	CT	95% Confidence Interval CT - 25	CT	95% Confidence Interval CT - 25	CT	95% Confidence Interval CT + 25	CT	95% Confidence Interval CT + 25	
			Mean (mg/initial shrimp)	CV (%)											
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
14	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
15	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
16	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
17	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
18	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
19	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
20	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6

Note:
Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.20 mg/surviving shrimp.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%.
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
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: 245

Dilution preparation information:						Comments:
KCl Stock INSS number:		INSS 2227				
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:	6/2/24
Date and times organisms were born between:	10-31-23 1200 to 11-01-23 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 11-01-23	Artemia CHM number:	CHM1222
Transfer bowl information:		Drying information for weight determination:	
pH = 7.65 S.U. Temperature = 25.0 °C		Date / Time in oven:	11-14-23 1030
Average transfer volume:		*Initial oven temperature:	60 °C
< 0.25 mL		Date / Time out of oven:	11-15-23 1030
		*Final oven temperature:	60 °C
		Total drying time:	21-HOURS

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	11-07-23	1005	J	1430	J	1200	J	10-31-23 A
1	11-08-23	0500	J	1100	J	1000	J	↓
2	11-09-23	0500	J	1230	J	1000	J	10-31-23 B
3	11-10-23	0500	J	1200	J	1000	J	↓
4	11-11-23	0600	J	1200	J	1010	J	11-10-23
5	11-12-23	0600	J	1200	J	1000	J	↓
6	11-13-23	0600	J	1200	J	1000	J	↓
7	11-14-23					1004	J	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07.	≤ 20%	7-day LC ₅₀ (mg/L KCl)	507.6
Average weight per initial shrimp:	0.261		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.261	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	457.3

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: Lavender Analyst: JP Date: 12-29-23	13.46	14.21	14.24	12.93	13.27	14.65	14.66	15.59	12.95	14.66	15.63	13.20	13.85	14.60	13.39	14.7				
*B = Pan + Shrimp weight (mg) Analyst: M Date: 1-16-25	14.66	15.49	15.54	14.17	14.70	16.03	15.96	17.00	14.27	16.12	16.88	14.40	15.42	15.67	14.72	16.0				
C = Shrimp weight (mg) = B - A Hand calculated Analyst: M	1.20	1.28	1.30	1.24	1.43	1.38	1.30	1.42	1.32	1.46	1.25	1.20	1.57	1.07	1.33	1.34				
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: M	0.240	0.256	0.260	0.248	0.286	0.276	0.260	0.284	0.264	0.292	0.250	0.240	0.314	0.214	0.266	0.260				
Average weight per initial number of shrimp (mg)	0.264								Average weight per initial number of shrimp (mg)				0.264				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: 245

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	4 ^{1d}	4 ^{1d}	4 ^{1d}	3 ^{2d}	4 ^{1d}	4 ^{1d}	5
3	5	5	4 ^{1d}	5	5	5	5	5	3 ^{2d}	4	2 ^{2d}	4	3	3 ^{1d}	3 ^{1d}	5
4	5	4 ^{1d}	4	5	5	5	5	5	3	4	2	4	3	3	3	5
5	5	4	4	5	5	5	5	5	3	4	2	4	3	3	3	5
6	5	4	4	5	5	5	5	5	3	3 ^{1d}	2	4	2 ^{1d}	3	3	3 ^{2d}
7	5	4	4	5	5	5	5	5	3	3	2	4	2	3	3	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>JP</u> Date: <u>10-20-23</u>	15.19	14.33	14.18	15.27	14.11	15.20	14.58	12.80	13.67	12.44	14.35	15.13	15.77	12.40	13.15	14.4
*B = Pan + Shrimp weight (mg) Analyst: <u>JL</u> Date: <u>10-16-23</u>	16.39	15.33	15.29	16.59	15.41	16.45	15.92	14.16	14.50	13.26	15.14	16.24	16.66	13.04	14.11	15.3
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>JL</u>	1.20	1.00	1.11	1.32	1.30	1.25	1.34	1.36	0.83	0.82	0.79	1.11	0.89	0.64	0.96	0.85
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>JL</u>	0.240	0.200	0.222	0.264	0.260	0.250	0.268	0.272	0.166	0.164	0.158	0.222	0.178	0.128	0.192	0.170
Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				Average weight per initial number of shrimp (mg)				Percent reduction from control (%)				
0.247				6.47				0.172				34.77				

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

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

Comments:

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}	0 ^{sd}
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: <i>lavender</i> Analyst: <i>AP</i> Date: <i>10-20-23</i>	<i>12.52</i>	<i>14.78</i>	<i>13.16</i>	<i>15.18</i>	<i>13.67</i>	<i>13.48</i>	<i>13.90</i>	<i>14.57</i>	<i>14.35</i>	<i>14.07</i>	<i>13.48</i>	<i>13.26</i>	<i>13.72</i>	<i>13.98</i>	<i>13.23</i>	<i>14.85</i>
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B - A Hand calculated Analyst: _____																<i>11-08-23</i>
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: _____	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average weight per initial number of shrimp (mg)	0								0							
Percent reduction from control (%)	100%								100%							

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

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

Comments:



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

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

Test number: 2
Test dates: November 07-14, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.46	14.66	1.20	0.240	100.0	0.264	6.3	Not applicable
	B	5	5	14.21	15.49	1.28	0.256				
	C	5	5	14.24	15.54	1.30	0.260				
	D	5	5	12.93	14.17	1.24	0.248				
	E	5	5	13.27	14.70	1.43	0.286				
	F	5	5	14.65	16.03	1.38	0.276				
	G	5	5	14.66	15.96	1.30	0.260				
	H	5	5	15.58	17.00	1.42	0.284				
250	I	5	5	12.95	14.27	1.32	0.264	100.0	0.264	11.6	0.1
	J	5	5	14.66	16.12	1.46	0.292				
	K	5	5	15.63	16.88	1.25	0.250				
	L	5	5	13.20	14.40	1.20	0.240				
	M	5	5	13.85	15.42	1.57	0.314				
	N	5	5	14.60	15.67	1.07	0.214				
	O	5	5	13.39	14.72	1.33	0.266				
	P	5	5	14.73	16.07	1.34	0.268				
375	Q	5	5	15.19	16.39	1.20	0.240	95.0	0.247	10.2	6.4
	R	5	4	14.33	15.33	1.00	0.200				
	S	5	4	14.18	15.29	1.11	0.222				
	T	5	5	15.27	16.59	1.32	0.264				
	U	5	5	14.11	15.41	1.30	0.260				
	V	5	5	15.20	16.45	1.25	0.250				
	W	5	5	14.58	15.92	1.34	0.268				
	X	5	5	12.80	14.16	1.36	0.272				
500	Y	5	3	13.67	14.50	0.83	0.166	57.5	0.172	15.8	34.7
	Z	5	3	12.44	13.26	0.82	0.164				
	AA	5	2	14.35	15.14	0.79	0.158				
	BB	5	4	15.13	16.24	1.11	0.222				
	CC	5	2	15.77	16.66	0.89	0.178				
	DD	5	3	12.40	13.04	0.64	0.128				
	EE	5	3	13.15	14.11	0.96	0.192				
	FF	5	3	14.46	15.31	0.85	0.170				
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: 0.0347
PMSD: 13.2

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (10th percentile) = 11%.
Upper PMSD bound determined by USEPA (90th percentile) = 37%.

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



Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date: 11/7/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant	
End Date: 11/14/2023	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	0.8000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.6000	0.4000	0.8000	0.4000	0.6000	0.6000	0.6000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

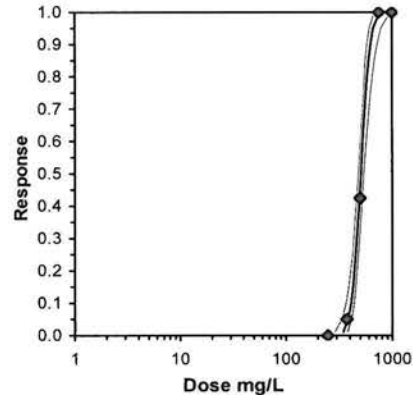
Conc-mg/L	Transform: Arcsin Square Root							Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.5750	0.5750	0.8634	0.6847	1.1071	15.530	8	36.00	48.00	17	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)	0.74299	0.904	-0.4284	3.18376
Equality of variance cannot be confirmed				

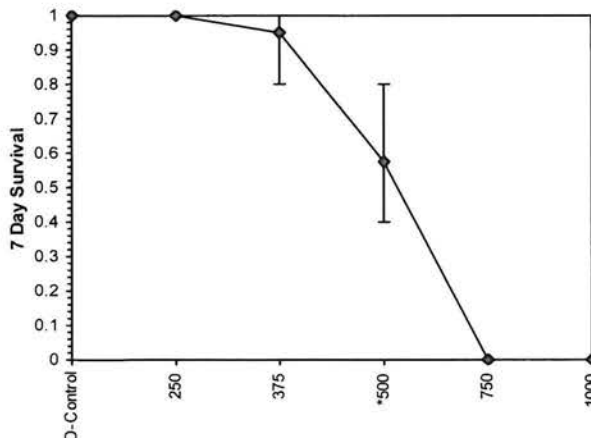
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	13.858	2.28416	9.38105	18.335	0	0.92485	7.81472	0.81943	2.70554	0.07216	5
Intercept	-32.493	6.1422	-44.532	-20.455							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	344.884	288.63	380.156
EC05	3.355	386.233	339.39	416.314
EC10	3.718	410.266	369.28	437.83
EC15	3.964	427.32	390.372	453.608
EC20	4.158	441.378	407.499	467.115
EC25	4.326	453.807	422.309	479.569
EC40	4.747	486.7	459.145	515.696
EC50	5.000	507.625	480.379	541.482
EC60	5.253	529.449	500.797	570.598
EC75	5.674	567.825	533.579	626.118
EC80	5.842	583.814	546.439	650.496
EC85	6.036	603.021	561.461	680.548
EC90	6.282	628.087	580.529	720.861
EC95	6.645	667.169	609.37	785.844
EC99	7.326	747.159	666.112	925.732



Dose-Response Plot



Entered and
 Reviewed by
 Jim Sumner

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

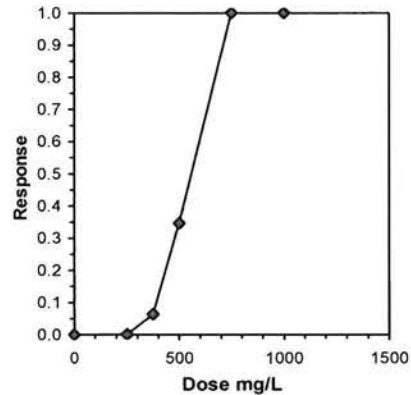
Start Date: 11/7/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant	
End Date: 11/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanysis bahia	

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2400	0.2560	0.2600	0.2480	0.2860	0.2760	0.2600	0.2840
250	0.2640	0.2920	0.2500	0.2400	0.3140	0.2140	0.2660	0.2680
375	0.2400	0.2000	0.2220	0.2640	0.2600	0.2500	0.2680	0.2720
500	0.1660	0.1640	0.1580	0.2220	0.1780	0.1280	0.1920	0.1700
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

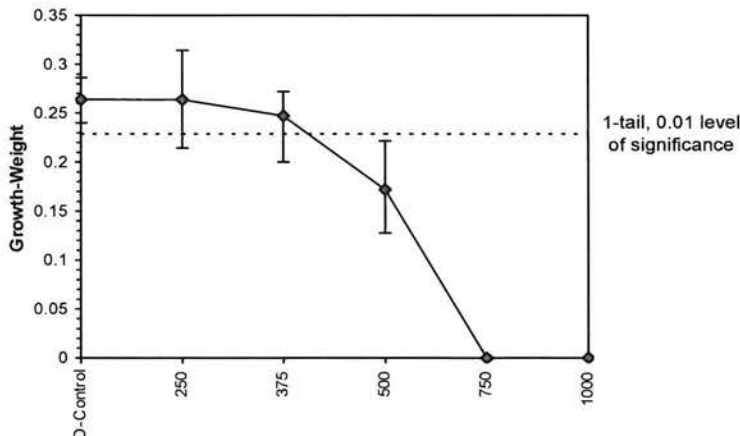
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	t-Stat		Critical	MSD	Mean	N-Mean	
D-Control	0.2638	1.0000	0.2638	0.2400	0.2860	6.337	8				0.2638	1.0000	
250	0.2635	0.9991	0.2635	0.2140	0.3140	11.630	8	0.020	2.799	0.0347	0.2635	0.9991	
375	0.2470	0.9365	0.2470	0.2000	0.2720	10.169	8	1.349	2.799	0.0347	0.2470	0.9365	
500	0.1723	0.6531	0.1723	0.1280	0.2220	15.780	8				0.1723	0.6531	
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)					0.97697	0.884	-0.2457	0.18233
Bartlett's Test indicates equal variances (p = 0.32)					2.28665	9.21035		
Hypothesis Test (1-tail, 0.01)					NOEC	LOEC	ChV	TU
Dunnett's Test					375	>375		
Treatments vs D-Control					0.03474	0.13172	0.00074	0.00062
					0.32223		2, 21	

Point	mg/L	SD	Linear Interpolation (200 Resamples)	
			95% CL	Skew
IC05	348.01	63.13	137.49	389.62
IC10	391.10	31.07	275.77	409.14
IC15	413.15	16.13	382.83	430.47
IC20	435.20	13.84	408.45	454.77
IC25	457.25	13.50	429.53	481.82
IC40	520.32	12.92	490.20	539.79
IC50	558.60	10.94	532.83	574.83



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS

AbKCICR Test Number: 245

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.

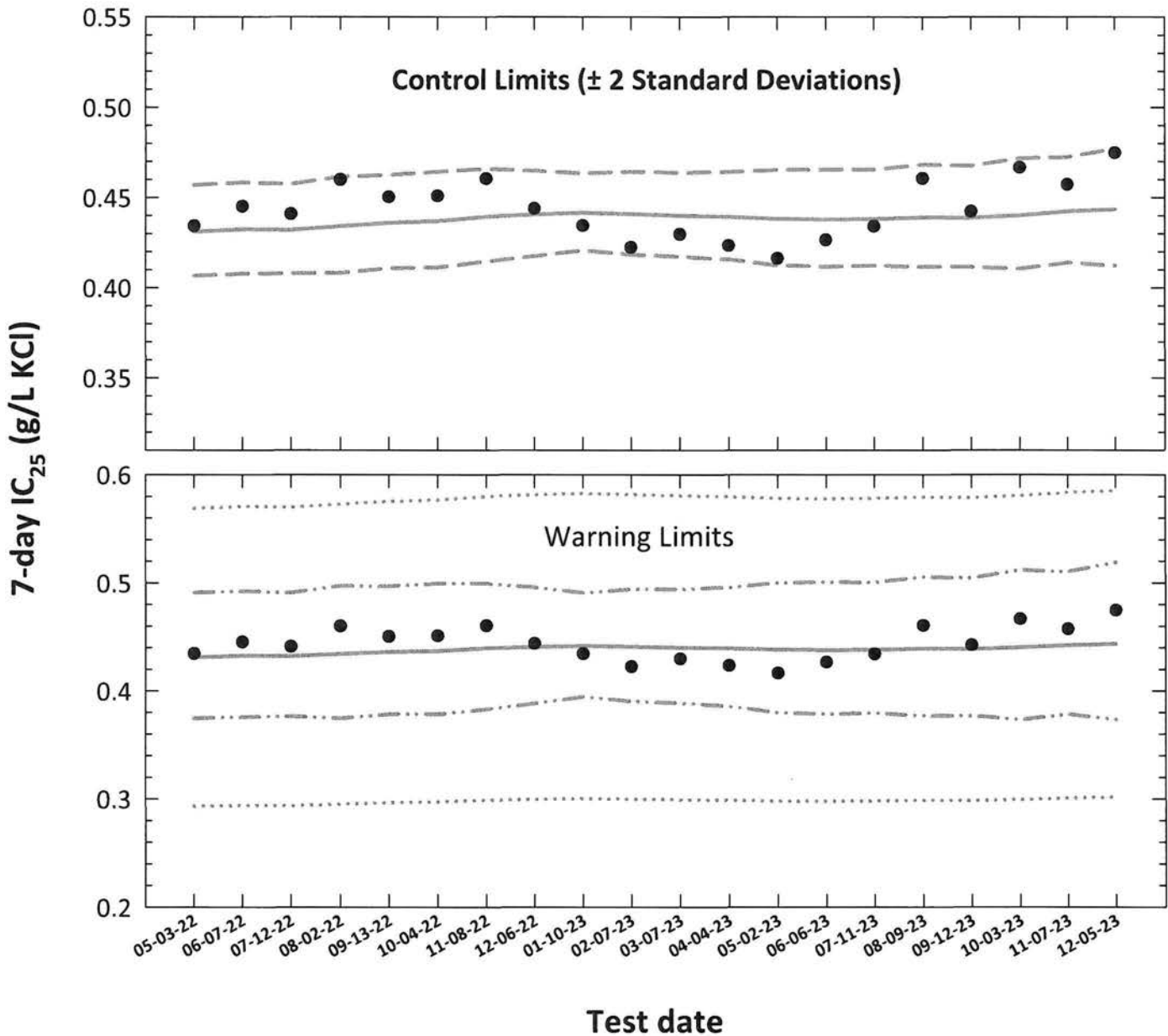
Conc.	Parameter	Day					
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)					
		0		1		2	
	Analyst	K. Ap	BL	DL	APBL	APBL	BL
CONTROL, Salt SW	pH (S.U.)	7.97	7.99	8.02	7.90	8.01	7.98
	DO (mg/L)	8.0	7.8	7.8	7.6	7.7	7.4
	Salinity (ppt)	25.0	25.2	24.9	25.2	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	120				120	
	Temperature (°C)	25.5	26.4	25.1	26.3	25.1	26.3
250 mg KCl/L	pH (S.U.)	7.98	8.02	8.03	7.96	8.01	8.01
	DO (mg/L)	8.0	7.8	7.8	7.6	7.7	7.4
	Salinity (ppt)	25.5	25.6	25.8 (25.8)	25.3	24.9	25.2
	Temperature (°C)	25.7	26.2	25.2	26.2	25.3	26.4
375 mg KCl/L	pH (S.U.)	7.99	7.99	8.04	7.94	8.01	8.00
	DO (mg/L)	8.0	7.8	7.9	7.6	7.7	7.3
	Salinity (ppt)	25.4	25.8	25.8 (25.8)	25.6	25.3	25.5
	Temperature (°C)	25.8	26.2	25.1	26.2	25.3	26.4
500 mg KCl/L	pH (S.U.)	8.00	8.01	8.04	7.95	8.02	7.99
	DO (mg/L)	8.0	7.8	8.0	7.6	7.7	7.5
	Salinity (ppt)	25.4	25.8	25.8 (25.8)	25.7	25.3	25.7
	Temperature (°C)	25.8	26.1	25.1	26.2	25.2	26.3
750 mg KCl/L	pH (S.U.)	8.00	7.99				
	DO (mg/L)	8.0	7.9				
	Salinity (ppt)	25.5	25.9	25.9 (25.9)			
	Temperature (°C)	25.7	26.5				
1000 mg KCl/L	pH (S.U.)	8.00	7.98				
	DO (mg/L)	8.1	7.7				
	Salinity (ppt)	25.7	26.4				
	Temperature (°C)	25.7	26.2				
		Initial	Final	Initial	Final	Initial	Final

AbKCICR Test Number: 245

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
Analyst	BL	BSL	BSL	BSL	BSL	AP BL	AP BL	SL	
CONTROL, Salt SW	pH (S.U.)	8.07	8.03	8.13	8.01	8.14	8.03	8.17	7.64
	DO (mg/L)	8.0	8.0	8.0	7.6	7.8	7.6	7.8	5.4
	Salinity (ppt)	24.0	25.1	25.2	25.3	25.0	25.4	25.3	25.1
	Alkalinity (mg CaCO ₃ /L)	110		140				110	
	Temperature (°C)	25.2	26.3	25.2	26.0	25.1	26.3	25.3	26.4
250 mg KCl/L	pH (S.U.)	8.07	7.98	8.08	7.94	8.14	8.04	8.15	7.73
	DO (mg/L)	8.0	8.0	8.0	7.4	7.8	7.5	7.9	5.5
	Salinity (ppt)	25.2	25.3	25.2	25.4	25.3	25.6	25.3	25.4
	Temperature (°C)	25.2	26.4	25.2	26.2	25.2	26.2	25.2	26.5
375 mg KCl/L	pH (S.U.)	8.07	7.99	8.08	7.94	8.15	8.05	8.17	7.73
	DO (mg/L)	8.0	8.0	8.0	7.4	7.8	7.5	7.9	5.6
	Salinity (ppt)	25.2	25.5	25.2	25.5	25.3	25.4	25.5	25.7
	Temperature (°C)	25.2	26.4	25.3	26.2	25.2	26.2	25.2	26.5
500 mg KCl/L	pH (S.U.)	8.07	7.97	8.08	7.97	8.15	8.05	8.18	7.83
	DO (mg/L)	8.0	8.0	8.0	7.4	7.8	7.5	7.9	5.5
	Salinity (ppt)	25.3	25.5	25.3	25.7	25.4	25.9	25.6	25.7
	Temperature (°C)	25.3	26.3	25.2	26.2	25.1	26.2	25.2	26.5
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Americamysis (Mysidopsis) bahia Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ \pm 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ \pm 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ \pm S_{A,75} converted to anti-logarithmic values, S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV			
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	Warning Limits	Warning Limits
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	03-07-23	0.4296	-0.3669	-0.3566	0.0114	0.4400	0.4174	0.4638	0.3886	0.4941	0.2992	0.5807
12	04-04-23	0.4235	-0.3732	-0.3571	0.0120	0.4394	0.4158	0.4643	0.3857	0.4961	0.2988	0.5800
13	05-02-23	0.4163	-0.3806	-0.3583	0.0131	0.4382	0.4126	0.4654	0.3798	0.5003	0.2980	0.5785
14	06-06-23	0.4265	-0.3700	-0.3587	0.0133	0.4378	0.4118	0.4655	0.3784	0.5011	0.2977	0.5780
15	07-11-23	0.4340	-0.3625	-0.3584	0.0132	0.4381	0.4124	0.4655	0.3794	0.5006	0.2979	0.5783
16	08-09-23	0.4605	-0.3368	-0.3575	0.0140	0.4390	0.4116	0.4683	0.3766	0.5056	0.2985	0.5795
17	09-12-23	0.4423	-0.3542	-0.3578	0.0139	0.4387	0.4116	0.4677	0.3769	0.5047	0.2983	0.5791
18	10-03-23	0.4667	-0.3310	-0.3564	0.0151	0.4402	0.4106	0.4719	0.3730	0.5122	0.2993	0.5810
19	11-07-23	0.4573	-0.3398	-0.3543	0.0143	0.4423	0.4140	0.4725	0.3784	0.5105	0.3008	0.5838
20	12-05-23	0.4747	-0.3236	-0.3532	0.0158	0.4434	0.4123	0.4769	0.3732	0.5190	0.3015	0.5853

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.

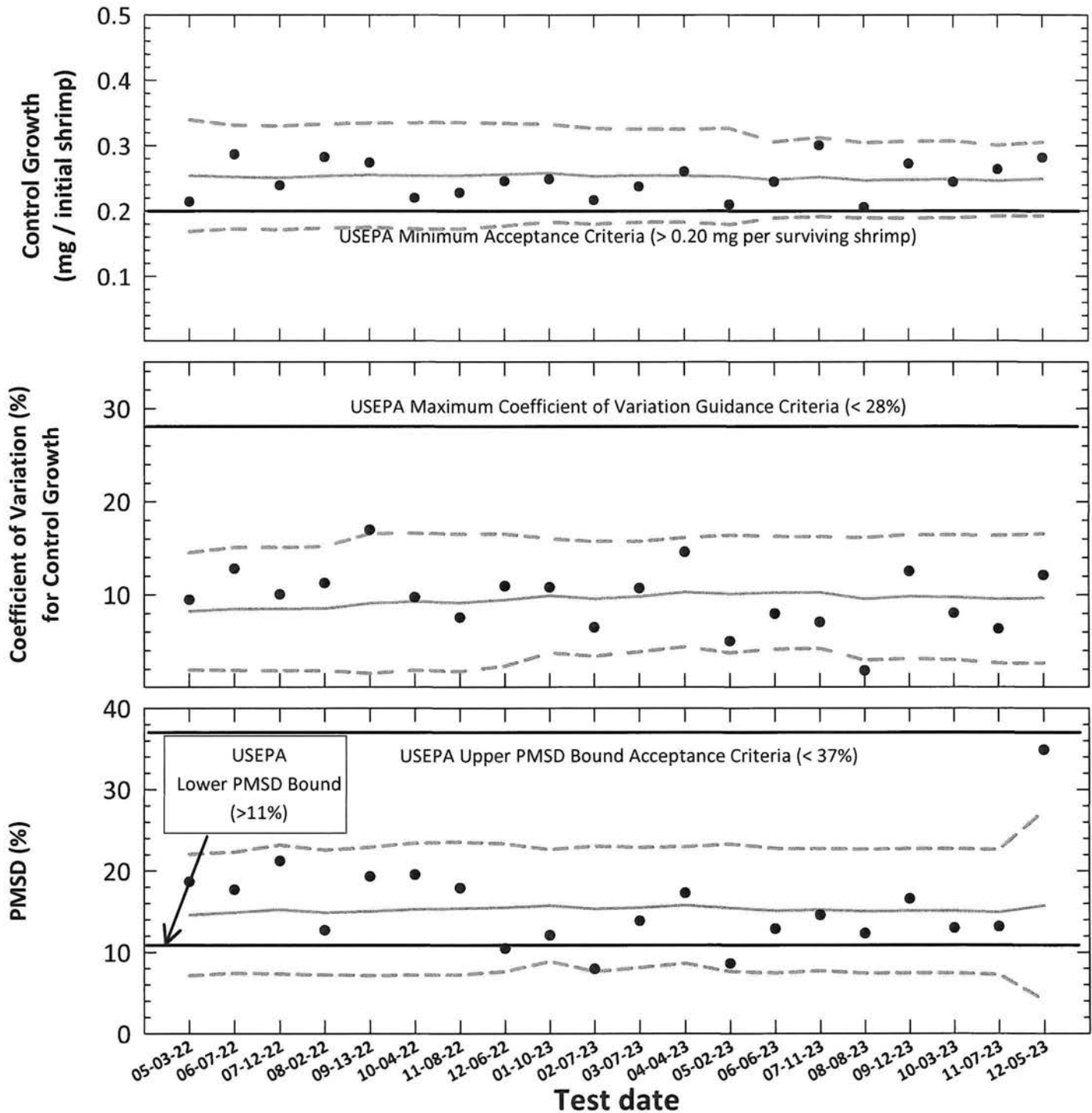
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.32).

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		Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	04-04-23	100	0.261	14.6	0.0450	17.3	0.254	0.183	0.325	10.3	4.4	16.2	15.8	8.6	23.0
13	05-02-23	100	0.210	5.0	0.0180	8.6	0.253	0.179	0.327	10.1	3.7	16.4	15.4	7.6	23.3
14	06-06-23	100	0.245	8.0	0.0314	12.8	0.248	0.189	0.306	10.2	4.1	16.3	15.1	7.4	22.7
15	07-11-23	100	0.300	7.0	0.0437	14.6	0.252	0.192	0.313	10.2	4.2	16.2	15.2	7.7	22.7
16	08-08-23	100	0.206	1.8	0.0253	12.3	0.247	0.189	0.305	9.6	2.9	16.2	15.0	7.4	22.6
17	09-12-23	100	0.273	12.5	0.0451	16.6	0.248	0.189	0.307	9.8	3.1	16.5	15.1	7.4	22.7
18	10-03-23	100	0.244	8.0	0.0317	13.0	0.248	0.190	0.307	9.7	3.0	16.4	15.1	7.4	22.7
19	11-07-23	100	0.264	6.3	0.0347	13.2	0.247	0.192	0.301	9.5	2.7	16.4	14.9	7.3	22.6
20	12-05-23	100	0.282	12.1	0.0981	34.8	0.249	0.192	0.305	9.6	2.6	16.6	15.7	4.1	27.4

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

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

CV = Coefficient of variation for control growth.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

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

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

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



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

AbKCICR Test Number: 246

Dilution preparation information:						Comments:
KCl Stock INSS number:	INSS 2249					
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:	Gret
Date and times organisms were born between:	11-28-23 1200 to 11-29-23 1130	Incubator number and shelf location:	5B
Organism source:	AI Batch Ab: 11-29-23	Artemia CHM number:	CHM1294
Transfer bowl information:	pH = 7.86 S.U. Temperature = 25.0 °C	Drying information for weight determination:	
Average transfer volume:	< 0.25 mL	Date / Time in oven:	12-12-23 1140
		*Initial oven temperature:	60 °C
		Date / Time out of oven:	12-13-23 1140
		*Final oven temperature:	60 °C
		Total drying time:	24 Hours

Daily feeding and renewal information:

*60°C Oven, Thermometer SN: 14-985B5

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	12-05-23	1210	JL	1415	JL	1300	JL	11-29-23 A
1	12-06-23	0500	JL	1115	JL	1100	JL	↓
2	12-07-23	0500	JL	1315	JL	1100	JL	11-29-23 B
3	12-08-23	0600	JL	1205	JL	1100	JL	↓
4	12-09-23	0600	JL	1235	JL	1100	JL	12-01-23
5	12-10-23	0500	JL	1150	JL	1100	JL	↓
6	12-11-23	0500	JL	1115	JL	1100	JL	↓
7	12-12-23					1112	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	1306646FS

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	07	≤ 20%	7-day LC ₅₀ (mg/L KCl)	528.3
Average weight per initial shrimp:	0.282		NOEC (mg/L KCl)	375
Average weight per surviving shrimp:	0.282	≥ 0.20 mg/shrimp	LOEC (mg/L KCl)	500
* HIGH PMSD - w/in BOUNDS			ChV (mg/L KCl)	433.0
			IC ₂₅ (mg/L KCl)	474.7

AbKCICR Test Number: 246

Survival and Growth Data

Day	CONTROL								250 mg KCl/L														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P							
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5							
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S							
# females with eggs in brood sac	_____																						
# females with developing ova in oviducts	_____																						
# immature females	_____																						
# males	_____																						
*A = Pan weight (mg) Tray color code: <u>Light Pink</u> Analyst: <u>BL</u> Date: <u>11-22-23</u>	13.70	13.84	14.32	12.88	14.93	13.65	15.02	13.75	16.08	15.63	12.16	12.58	11.59	13.61	14.20	14.7							
*B = Pan + Shrimp weight (mg) Analyst: <u>BL</u> Date: <u>12-14-23</u>	15.30	15.17	15.94	14.07	16.26	15.00	16.27	15.34	18.36	17.06	13.66	14.25	12.94	15.04	15.83	16.31							
C = Shrimp weight (mg) = B - A Hand calculated Analyst: <u>J</u>	1.60	1.33	1.62	1.19	1.33	1.35	1.25	1.59	2.28	1.43	1.50	1.67	1.35	1.43	1.63	1.65							
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>J</u>	0.320	0.266	0.324	0.238	0.266	0.270	0.250	0.318	0.456	0.286	0.300	0.334	0.270	0.286	0.326	0.331							
Average weight per initial number of shrimp (mg)				0.282				Average weight per initial number of shrimp (mg)				0.324				Percent reduction from control (%)				-14.97			

*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: 246

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	4 ^d	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5
4	5	5	5	4 ^{id}	5	5	5	5	3 ^{id}	5	5	5	4 ^{id}	5	5	5
5	5	5	5	4	5	5	5	5	3	4 ^{id}	4 ^{id}	3 ^{id}	4	5	5	4 ^{id}
6	5	5	5	4	5	5	5	5	3	4	4	3	4	3 ^{id}	5	4 ^{id}
7	5	5	5	4	5	5	5	4 ^{id}	3	3 ^{id}	4	3	4	3	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>Light Pink</u> Analyst: <u>DL</u> Date: <u>11-28-23</u>	14.69	11.39	13.71	12.42	12.64	13.05	14.55	13.49	12.56	14.07	14.06	13.07	13.58	15.08	15.25	14.02
*B = Pan + Shrimp weight (mg) Analyst: <u>BL</u> Date: <u>12-14-23</u>	16.62	13.69	15.59	13.20	14.34	14.34	15.87	14.61	13.48	15.09	15.37	13.83	14.76	16.07	16.25	15.21
C = Shrimp weight (mg). = B - A Hand calculated Analyst: <u>DL</u>	1.98	2.30	1.88	0.78	1.70	1.29	1.32	1.12	0.92	1.02	1.31	0.76	1.18	0.99	1.00	1.19
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: <u>DL</u>	0.396	0.460	0.376	0.156	0.340	0.258	0.264	0.224	0.184	0.204	0.262	0.152	0.236	0.198	0.200	0.238
Average weight per initial number of shrimp (mg)	0.309								0.209							
Percent reduction from control (%)	-9.97								25.77							

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

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

Comments:

AbKCICR Test Number: 246

Survival and Growth Data

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

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

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

Comments:



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

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

Test number: 2

Test dates: December 05-12, 20

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.70	15.30	1.60	0.320	100.0	0.282	12.1	Not applicable
	B	5	5	13.84	15.17	1.33	0.266				
	C	5	5	14.32	15.94	1.62	0.324				
	D	5	5	12.88	14.07	1.19	0.238				
	E	5	5	14.93	16.26	1.33	0.266				
	F	5	5	13.65	15.00	1.35	0.270				
	G	5	5	15.02	16.27	1.25	0.250				
	H	5	5	13.75	15.34	1.59	0.318				
250	I	5	5	16.08	18.36	2.28	0.456	100.0	0.324	18.1	-14.9
	J	5	5	15.63	17.06	1.43	0.286				
	K	5	5	12.16	13.66	1.50	0.300				
	L	5	5	12.58	14.25	1.67	0.334				
	M	5	5	11.59	12.94	1.35	0.270				
	N	5	5	13.61	15.04	1.43	0.286				
	O	5	5	14.20	15.83	1.63	0.326				
	P	5	5	14.71	16.36	1.65	0.330				
375	Q	5	5	14.69	16.67	1.98	0.396	95.0	0.309	32.6	-9.9
	R	5	5	11.39	13.69	2.30	0.460				
	S	5	5	13.71	15.59	1.88	0.376				
	T	5	4	12.42	13.20	0.78	0.156				
	U	5	5	12.64	14.34	1.70	0.340				
	V	5	5	13.05	14.34	1.29	0.258				
	W	5	5	14.55	15.87	1.32	0.264				
	X	5	4	13.49	14.61	1.12	0.224				
500	Y	5	3	12.56	13.48	0.92	0.184	70.0	0.209	16.6	25.7
	Z	5	3	14.07	15.09	1.02	0.204				
	AA	5	4	14.06	15.37	1.31	0.262				
	BB	5	3	13.07	13.83	0.76	0.152				
	CC	5	4	13.58	14.76	1.18	0.236				
	DD	5	3	15.08	16.07	0.99	0.198				
	EE	5	4	15.25	16.25	1.00	0.200				
	FF	5	4	14.02	15.21	1.19	0.238				
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: 0.0981
PMSD: 34.8

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: 12/5/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 12/12/2023	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
Comments:		

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

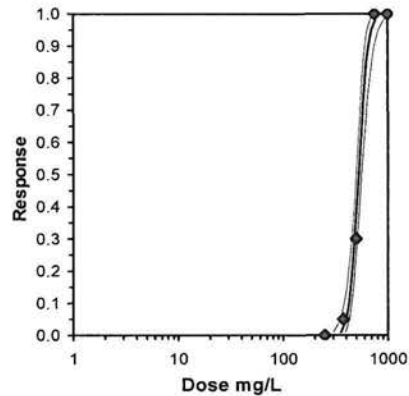
Conc-mg/L	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number	
	Mean	N-Mean	Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8		0	40	
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.7000	0.7000	0.9966	0.8861	1.1071	11.857	8	36.00	48.00	12	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)	0.85292	0.904	-0.7696	0.40706
Equality of variance cannot be confirmed				

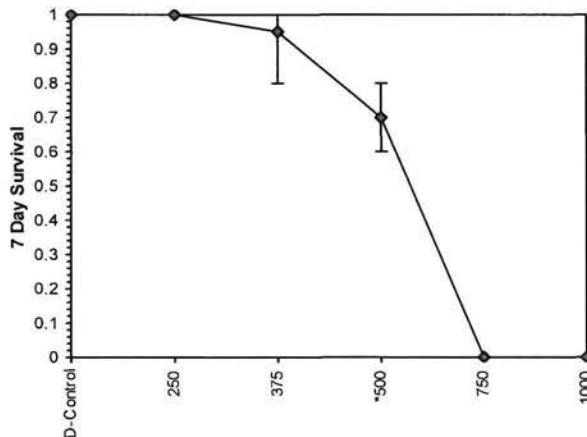
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	13.5631	2.08849	9.46963	17.6565	0	3.20703	7.81472	0.36079	2.7229	0.07373	6
Intercept	-31.931	5.65206	-43.009	-20.853							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	355.945	301.142	391.914
EC05	3.355	399.603	353.402	430.782
EC10	3.718	425.025	384.103	453.962
EC15	3.964	443.085	405.748	470.954
EC20	4.158	457.984	423.331	485.467
EC25	4.326	471.165	438.561	498.798
EC40	4.747	506.086	476.733	537.038
EC50	5.000	528.328	499.066	563.929
EC60	5.253	551.547	520.807	594.029
EC75	5.674	592.425	556.116	651.095
EC80	5.842	609.475	570.049	676.102
EC85	6.036	629.969	586.359	706.915
EC90	6.282	656.737	607.099	748.235
EC95	6.645	698.518	638.525	814.818
EC99	7.326	784.195	700.505	958.056



Dose-Response Plot



Entered and Reviewed by Jim Sumner
JS

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

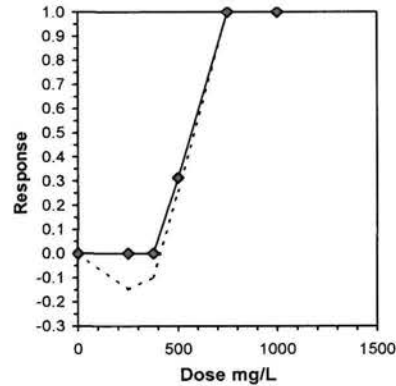
Start Date: 12/5/2023	Test ID: AbKCICR	Sample ID: REF-Ref Toxicant
End Date: 12/12/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: SWCHR-EPA-821-R-02-014	Test Species: AB-Americanysis bahia

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.3200	0.2660	0.3240	0.2380	0.2660	0.2700	0.2500	0.3180
250	0.4560	0.2860	0.3000	0.3340	0.2700	0.2860	0.3260	0.3300
375	0.3960	0.4600	0.3760	0.1560	0.3400	0.2580	0.2640	0.2240
500	0.1840	0.2040	0.2620	0.1520	0.2360	0.1980	0.2000	0.2380
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

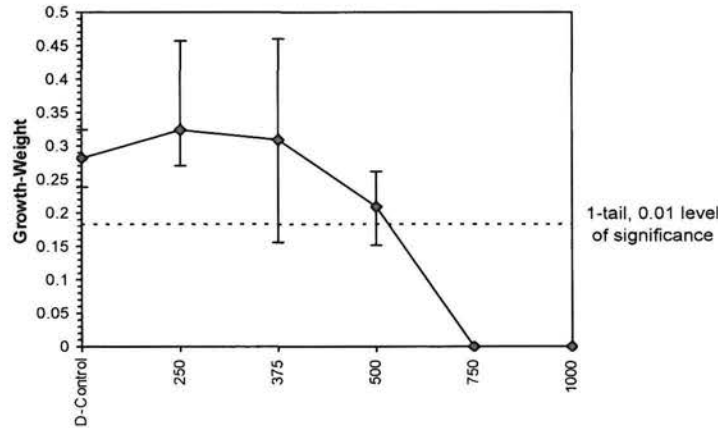
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2815	1.0000	0.2815	0.2380	0.3240	12.098	8				0.3048	1.0000
250	0.3235	1.1492	0.3235	0.2700	0.4560	18.078	8	-1.199	2.799	0.0981	0.3048	1.0000
375	0.3093	1.0986	0.3093	0.1560	0.4600	32.585	8	-0.792	2.799	0.0981	0.3048	1.0000
500	0.2093	0.7433	0.2093	0.1520	0.2620	16.633	8				0.2093	0.6866
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)					0.96141	0.884	0.32778	0.89458					
Bartlett's Test indicates equal variances (p = 0.03)					7.10137	9.21035							
Hypothesis Test (1-tail, 0.01)				NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test				375	>375			0.09806	0.34836	0.00365	0.00491	0.48775	2, 21
Treatments vs D-Control													

Point	Linear Interpolation (200 Resamples)				
	mg/L	SD	95% CL	Skew	
IC05	394.94	41.02	276.57	399.73	-0.9924
IC10	414.89	32.25	303.14	424.49	-1.6066
IC15	434.83	28.33	329.71	449.27	-1.7510
IC20	454.78	26.13	356.28	484.36	-1.7175
IC25	474.72	23.99	394.25	511.13	-1.4296
IC40	531.54	15.40	496.14	558.90	-0.4602
IC50	567.95	13.13	537.14	590.75	-0.5894



Dose-Response Plot



Entered and
Reviewed by
Jan Sumner
JS

AbKCICR Test Number: 246

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	AP	BL	BL	AP	AP	BL
CONTROL, Salt SW	pH (S.U.)	7.92	8.01	8.04	7.81	7.90	7.84
	DO (mg/L)	7.7	7.6	7.9	7.3	7.6	6.9
	Salinity (ppt)	25.0	25.2	24.9	25.3	25.0	25.2
	Alkalinity (mg CaCO ₃ /L)	120				100	
	Temperature (°C)	25.6	26.2	25.1	26.3	25.2	26.3
250 mg KCl/L	pH (S.U.)	7.93	8.02	8.06	7.85	7.97	7.90
	DO (mg/L)	7.7	7.6	7.9	7.2	7.6	6.9
	Salinity (ppt)	25.2	25.8	25.6	25.5	25.3	25.5
	Temperature (°C)	25.8	26.4	25.0	26.2	25.3	26.2
375 mg KCl/L	pH (S.U.)	7.94	8.04	8.07	7.89	8.00	7.92
	DO (mg/L)	7.7	7.6	7.9	7.3	7.6	7.0
	Salinity (ppt)	25.5	25.9	25.6	25.9	25.6	25.8
	Temperature (°C)	25.5	26.4	25.0	26.4	25.3	26.4
500 mg KCl/L	pH (S.U.)	7.94	8.04	8.08	7.89	8.01	7.89
	DO (mg/L)	7.7	7.7	8.0	7.3	7.7	7.0
	Salinity (ppt)	25.5	25.9	25.6	26.2	25.7	26.0
	Temperature (°C)	25.7	26.3	25.0	26.4	25.4	26.4
750 mg KCl/L	pH (S.U.)	7.95	8.03				
	DO (mg/L)	7.7	7.7				
	Salinity (ppt)	25.7	25.9				
	Temperature (°C)	25.7	26.5				
1000 mg KCl/L	pH (S.U.)	7.94	8.02				
	DO (mg/L)	7.6	7.6				
	Salinity (ppt)	25.8	26.2				
	Temperature (°C)	25.5	26.3				
		Initial	Final	Initial	Final	Initial	Final

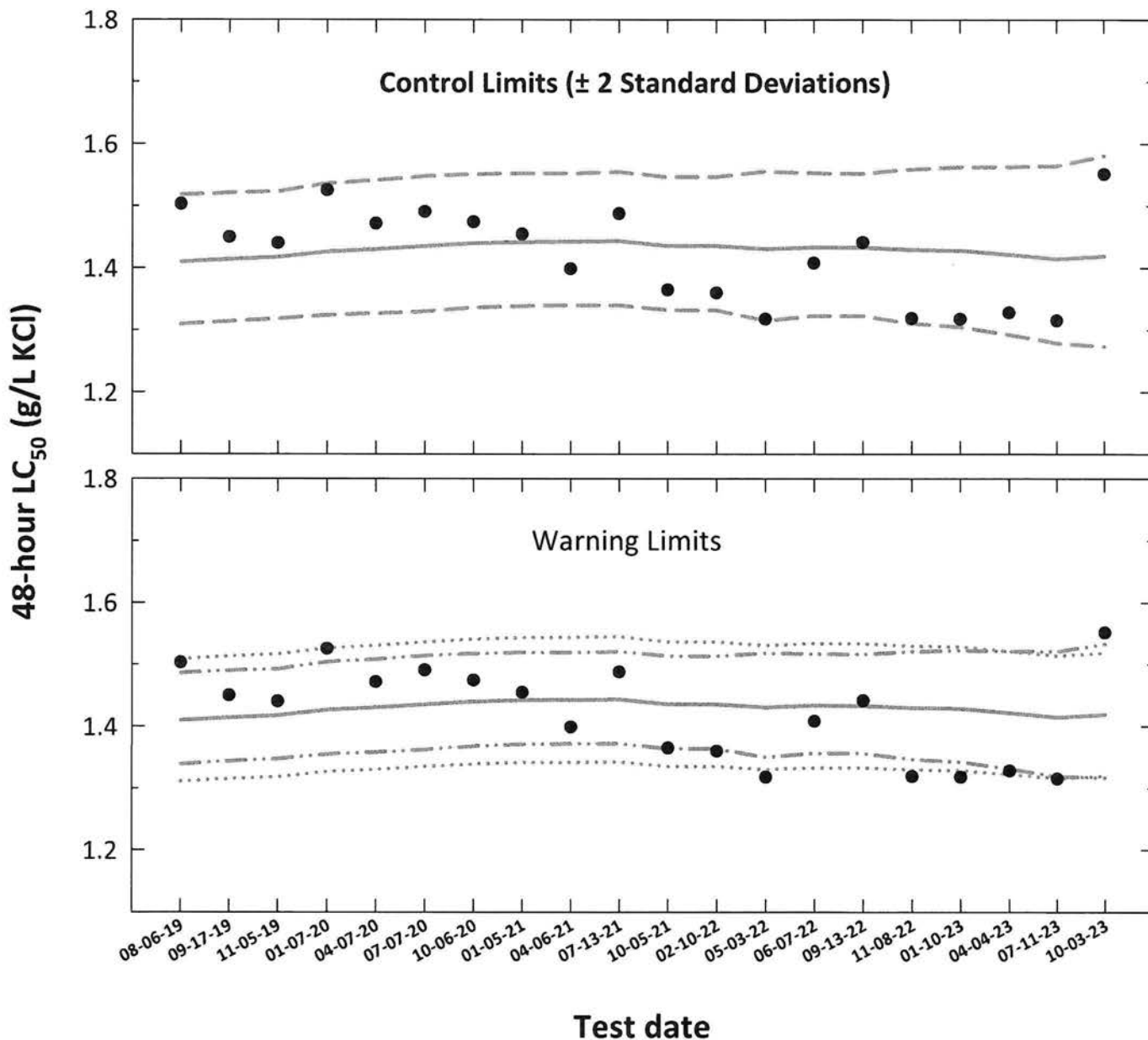
AbKCICR Test Number: 246

Conc.	Parameter	Day							
		(Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
Analyst	BL	PM/BSL	PM/BSL	PM/BSL	PM/BSL	APBL	APBL	IL	
CONTROL, Salt SW	pH (S.U.)	7.95	7.87	8.00	7.86	7.99	7.82	7.96	4.57
	DO (mg/L)	7.9	7.5	7.7	7.8	8.0	7.2	7.9	6.2
	Salinity (ppt)	25.0	25.2	24.8	25.2	25.1	25.4	25.0	25.1
	Alkalinity (mg CaCO ₃ /L)	---	---	110	---	---	---	---	---
	Temperature (°C)	25.3	26.2	25.1	26.5	25.2	26.3	25.2	26.4
250 mg KCl/L	pH (S.U.)	7.95	7.89	7.99	7.84	8.00	7.82	8.01	7.61
	DO (mg/L)	7.9	7.5	7.8	7.8	8.0	7.3	7.9	6.4
	Salinity (ppt)	25.2	25.3	25.2	25.5	25.1	25.4	24.9	25.7
	Temperature (°C)	25.2	26.4	25.2	26.2	25.3	26.5	25.1	26.4
375 mg KCl/L	pH (S.U.)	7.95	7.87	8.00	7.84	8.02	7.86	8.01	7.62
	DO (mg/L)	7.9	7.6	7.8	7.8	8.0	7.2	7.9	6.3
	Salinity (ppt)	25.2	25.5	25.5	25.2	25.1	25.5	25.4	26.0
	Temperature (°C)	25.2	26.4	25.2	26.2	25.3	26.2	25.1	26.2
500 mg KCl/L	pH (S.U.)	7.95	7.82	8.00	7.83	8.03	7.89	8.01	7.67
	DO (mg/L)	7.9	7.5	7.8	7.9	8.1	7.1	7.8	6.3
	Salinity (ppt)	25.3	25.7	25.5	25.0	25.1	25.6	25.5	26.1
	Temperature (°C)	25.2	26.3	25.2	26.3	25.3	26.2	25.1	26.4
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Menidia beryllina

Acute Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	04-04-23	1.3283	0.1233	0.1528	0.0206	1.4216	1.2927	1.5634	1.3309	1.5213	1.3221	1.5211
19	07-11-23	1.3153	0.1190	0.1507	0.0219	1.4149	1.2793	1.5649	1.3190	1.5209	1.3158	1.5139
20	10-03-23	1.5515	0.1908	0.1520	0.0235	1.4190	1.2738	1.5809	1.3167	1.5331	1.3197	1.5184

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: Menidia beryllina

EPA-821-R-02-012, Method 2006.0

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKCIAC # 91

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 #: 227

Chemical Analyses:

		Hours		
		0	24	48
Control, SaltSW	Analyst	<u>N</u>	<u>N P</u>	<u>V</u>
	pH (S.U.)	<u>7.06</u>	<u>7.04</u>	<u>7.73</u>
	Dissolved oxygen (mg/L)	<u>7.7</u>	<u>7.8</u>	<u>7.9</u>
	*Salinity (ppt)	<u>24.9</u>	<u>25.3</u>	<u>25.6</u>
	*Alkalinity (mg/L CaCO ₃)	<u>86</u>		
1000 mg/L	pH (S.U.)	<u>7.02</u>	<u>7.01</u>	<u>7.75</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.8</u>	<u>8.0</u>
	*Salinity (ppt)	<u>25.4</u>	<u>26.1</u>	<u>26.8</u>
	*Temperature (°C)	<u>25.0</u>	<u>25.0</u>	<u>24.7</u>
	1250 mg/L	pH (S.U.)	<u>7.03</u>	<u>7.01</u>
Dissolved oxygen (mg/L)		<u>7.9</u>	<u>7.8</u>	<u>8.0</u>
*Salinity (ppt)		<u>25.2</u>	<u>26.6</u>	<u>26.9</u>
*Temperature (°C)		<u>25.1</u>	<u>24.8</u>	<u>24.7</u>
1500 mg/L		pH (S.U.)	<u>7.03</u>	<u>7.01</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>7.8</u>	<u>8.1</u>
	*Salinity (ppt)	<u>25.6</u>	<u>26.2</u>	<u>26.6</u>
	*Temperature (°C)	<u>25.0</u>	<u>25.0</u>	<u>24.7</u>
	1750 mg/L	pH (S.U.)	<u>7.03</u>	<u>7.01</u>
Dissolved oxygen (mg/L)		<u>8.0</u>	<u>7.7</u>	<u>8.1</u>
*Salinity (ppt)		<u>25.3</u>	<u>26.3</u>	<u>26.9</u>
*Temperature (°C)		<u>25.2</u>	<u>24.7</u>	<u>24.5</u>
2000 mg/L		pH (S.U.)	<u>7.02</u>	<u>7.79</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>7.7</u>	<u>7.7</u>
	*Salinity (ppt)	<u>25.4</u>	<u>26.5</u>	<u>26.5</u>
	*Temperature (°C)	<u>25.0</u>	<u>24.9</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>1306646</u>

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 # 91

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	10-03-13	1015	JL	1235	JL	6D	LIGHT GREEN	09-27-13A
24	10-04-13			1230	JL			
48 Termination	10-05-13			1235	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 #):	AI Mb 01-13-13
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	01-12-13 1200 TO 01-13-13 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.73 Temperature (°C): 24.2

Survival Data (number of living organisms):

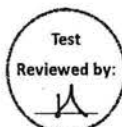
Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ^u	9 ^u	6 ^u	8 ^u	5 ^s	4 ^u	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9	8 ^u	6	7 ^u	3 ^u	3 ^u	0	0
Mean Survival	100%		100%		85%		65%		30%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1463.1
Upper 95% confidence limit (mg KCl/L)	1640.4
48-hour LC ₅₀ (mg KCl/L)	1551.5

Comments:



Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date: 10/3/2023	Test ID: MbKCIAC	Sample ID: REF-Ref Toxicant
End Date: 10/5/2023	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

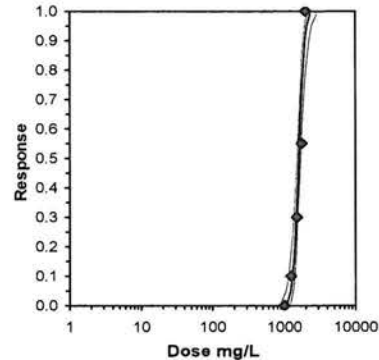
Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	0.9000
1500	0.6000	0.8000
1750	0.5000	0.4000
2000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%						
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2189	0	20
1250	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.122	2.850	0.2189	2	20
*1500	0.7000	0.7000	0.9966	0.8861	1.1071	15.685	2	5.408	2.850	0.2189	6	20
*1750	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	8.813	2.850	0.2189	11	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

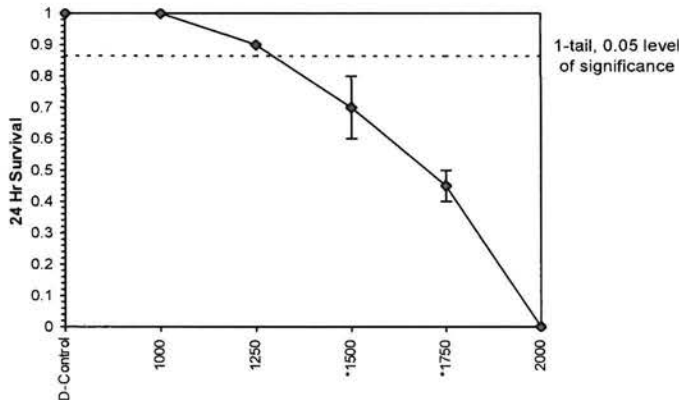
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	1250	1500	1369.31	0.11101
Treatments vs D-Control				0.11385
				0.17111
				0.0059
				0.00118
				4, 5

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	14.3656	2.5476	9.3723	19.3589	0	4.74564	7.81472	0.1914	3.20812	0.06961	5
Intercept	-41.087	8.17753	-57.115	-25.059							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1112.2	901.134	1238.15
EC05	3.355	1240.58	1061.29	1347.86
EC10	3.718	1314.96	1156.49	1412.1
EC15	3.964	1367.65	1224.37	1458.53
EC20	4.158	1411.03	1280.1	1497.72
EC25	4.326	1449.34	1328.86	1533.41
EC40	4.747	1550.55	1452.82	1635.38
EC50	5.000	1614.81	1525.3	1708.41
EC60	5.253	1681.74	1594.27	1792.68
EC75	5.674	1799.18	1701.39	1958.63
EC80	5.842	1848.03	1742.22	2032.91
EC85	6.036	1906.64	1789.3	2125.14
EC90	6.282	1983.04	1848.35	2249.58
EC95	6.645	2101.95	1936.67	2451.09
EC99	7.326	2344.56	2108.5	2886.41



Dose-Response Plot



Prepared and Reviewed by: *[Signature]*

Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date: 10/3/2023	Test ID: MbKCIAC	Sample ID: REF-Ref Toxicant	REF-Ref Toxicant
End Date: 10/5/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: MB-Menidia beryllina	MB-Menidia beryllina

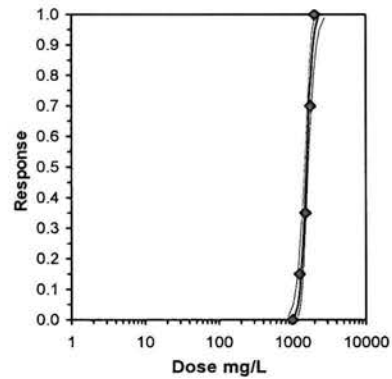
Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	0.8000
1500	0.6000	0.7000
1750	0.3000	0.3000
2000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1591	0	20	
*1250	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	4.189	2.850	0.1591	3	20	
*1500	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	8.478	2.850	0.1591	7	20	
*1750	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	2	14.908	2.850	0.1591	14	20	
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

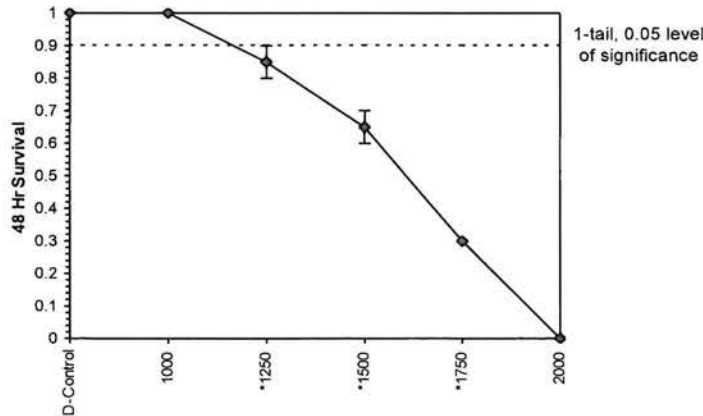
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.07271	0.07457	0.24877	0.00312	1.0E-04	4, 5

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1979	2.42337	9.44811	18.9477	0	3.08811	7.81472	0.37824	3.19076	0.07043	4
Intercept	-40.302	7.7467	-55.486	-25.119							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1063.92	865.579	1185.54
EC05	3.355	1188.25	1018.1	1292.8
EC10	3.718	1260.37	1108.75	1355.55
EC15	3.964	1311.48	1173.41	1400.81
EC20	4.158	1353.58	1226.58	1438.91
EC25	4.326	1390.77	1273.2	1473.47
EC40	4.747	1489.08	1392.49	1571.26
EC50	5.000	1551.53	1463.12	1640.38
EC60	5.253	1616.61	1531.05	1719.57
EC75	5.674	1730.88	1637.39	1875.25
EC80	5.842	1778.44	1677.98	1945.07
EC85	6.036	1835.52	1724.75	2031.87
EC90	6.282	1909.96	1783.37	2149.12
EC95	6.645	2025.88	1870.97	2339.18
EC99	7.326	2262.63	2041.29	2749.93



Dose-Response Plot

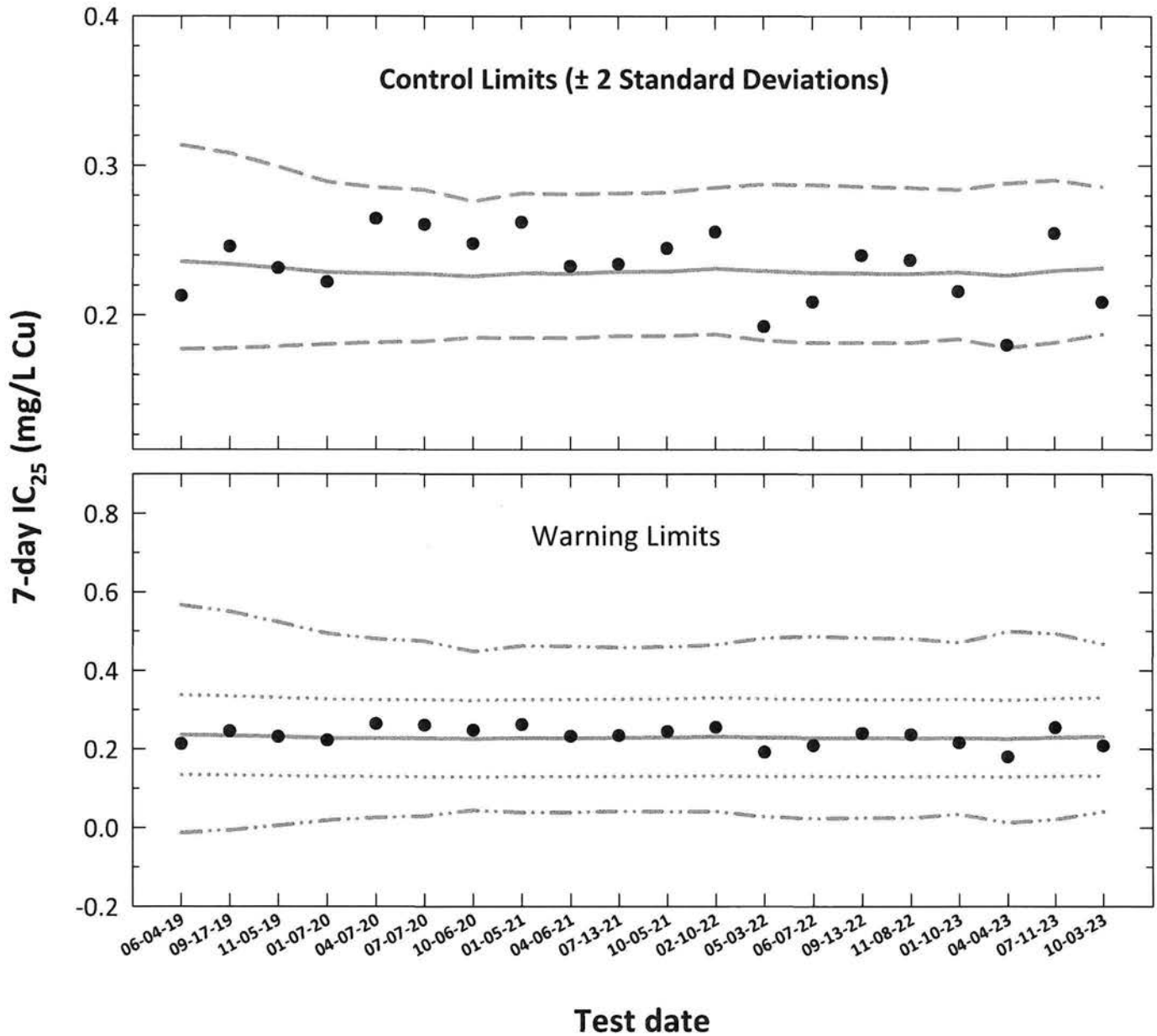


Checked and Approved by

Menidia beryllina

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the copper concentration which would cause a 25% reduction in *Menidia* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ $\pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (mg/L Cu)	Log ₁₀ Conversion			Anti-logarithmic Values (mg/L Cu)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	04-04-23	0.1799	-0.7450	-0.6447	0.0523	0.2266	0.1781	0.2884	0.0125	0.4991	0.1292	0.3241
19	07-11-23	0.2548	-0.5938	-0.6387	0.0509	0.2298	0.1818	0.2905	0.0208	0.4939	0.1310	0.3286
20	10-03-23	0.2086	-0.6807	-0.6356	0.0459	0.2314	0.1873	0.2859	0.0408	0.4669	0.1319	0.3309

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.

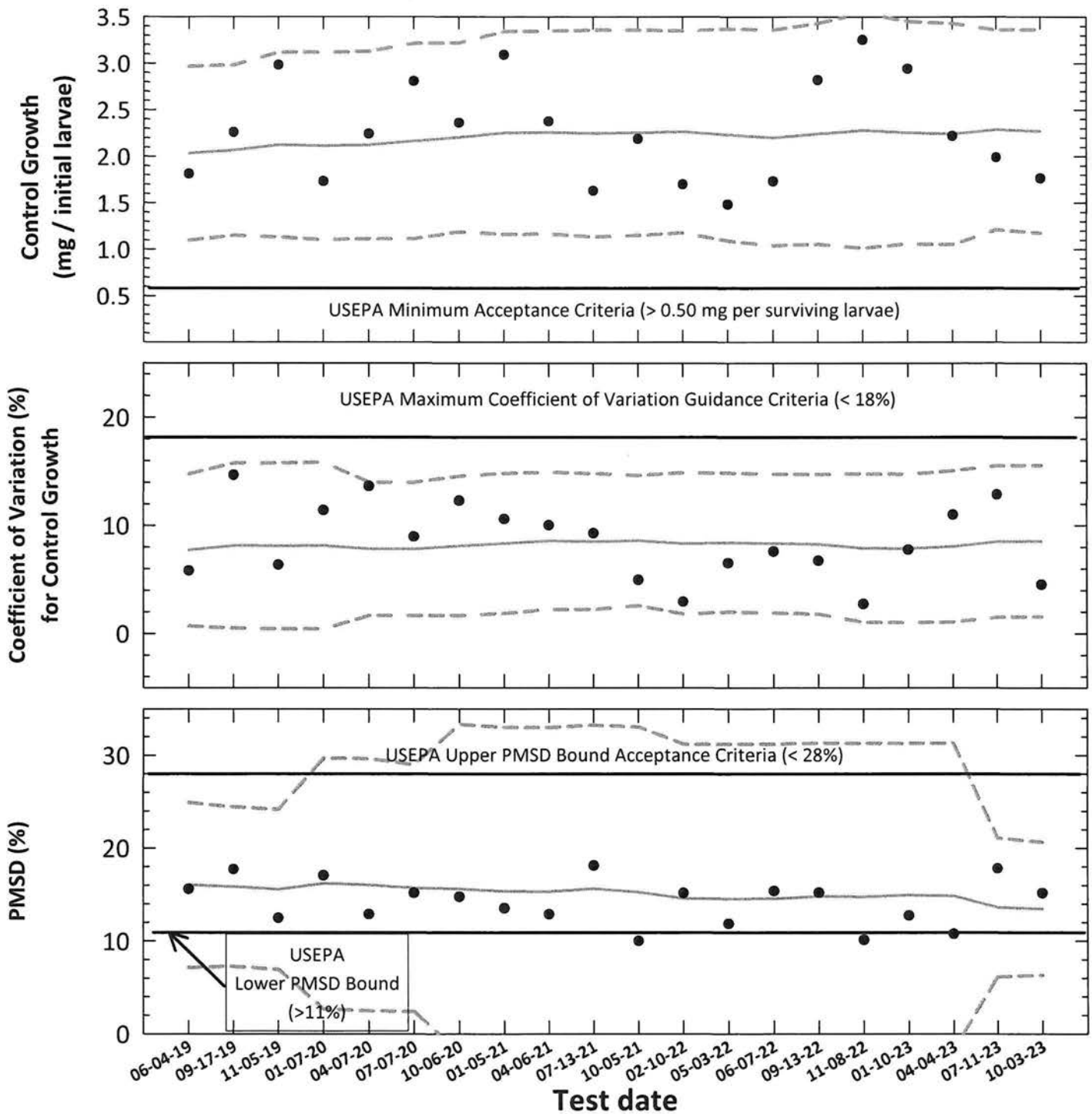
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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		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 larvae)	CV (%)								MSD	PMSD (%)		
1	06-04-19	100	1.812	5.8	0.2823	15.6	2.0324	1.096	2.969	7.7	0.7	14.8	16.0	7.1	24.9
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	04-04-23	100	2.220	11.0	0.2389	10.8	2.243	1.053	3.433	8.1	1.1	15.1	14.9	-1.6	31.3
19	07-11-23	100	1.992	12.9	0.3549	17.8	2.287	1.213	3.360	8.5	1.5	15.6	13.6	6.2	21.1
20	10-03-23	100	1.763	4.5	0.2673	15.2	2.270	1.174	3.366	8.5	1.5	15.5	13.5	6.3	20.6

Note: Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.50 mg/surviving larvae.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 18%
MSD = Minimum significant difference.
PMSD = Percent minimum significant difference.

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

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

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

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

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



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

Species: Menidia beryllina

MbCuCR Test Number: **144**

Dilution preparation information:						Comments:
Cu Stock INSS number:		INSS 2238				
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:	ORANGE
Age:	10-days old	Incubator number and shelf location:	LB
Batch:	Al Mb 09-23-23	Artemia CHM number:	CHM1222
Hatch dates and times:	09-22-23 1200 to	Drying information for weight determination:	
	09-23-23 1130	Date / Time in oven:	10-10-23 0935
Transfer vessel information:	pH (S.U.) = 7.75	*Initial oven temperature:	60°C
	Temperature (°C) = 24.2	Date / Time out of oven:	10-11-23 0935
Average transfer volume (mL):	< 0.25 mL	*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Sample number used	SaltSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst		
0	10-03-23	1115	JL	1240	JL	1135	JL	NA	09-23-23 B
1	10-04-23	0500	JL	1100	JL	0935	JL	↓	↓
2	10-05-23	0500	JL	1215	JL	1130	JL		10-03-23
3	10-06-23	0500	JL	1100	JL	0935	JL		10-04-23
4	10-07-23	0615	JL	1215	JL	1035	JL		10-05-23 A
5	10-08-23	0600	JL	1200	JL	0945	JL		10-05-23 B
6	10-09-23	0500	JL	1100	JL	0935	JL		10-05-23 A
7	10-10-23					0935	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 (%)	0.259
Average weight per initial larvae:	1.763		NOEC (%)	0.1
Average weight per surviving larvae:	1.763	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	0.14
			IC ₂₅ (%)	0.209

Species: Menidia beryllina

MbCuCR Test Number: 144

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>Turquoise</u> Analyst: <u>BL</u> Date: <u>09.27.23</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JL</u> Date: <u>10-13-23</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>												
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		1.763		1.859	-5.47	1.748	0.97			

*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:

Species: Menidia beryllina

MbCuCR Test Number: 144

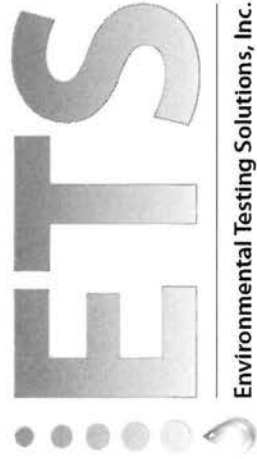
Survival and Growth Data

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 ^{100d}	0 ^{100d}	0 ^{100d}	0 ^{100d}			
2	10	10	10	10	8 ^{2d}	9 ^{1d}	9 ^{1d}	9 ^{1d}							
3	10	10	10	10	8	9	9	9							
4	10	10	10	10	8	9	9	9							
5	10	10	10	10	8	9	9	9							
6	10	10	10	10	8	9	9	9							
7	10	10	10	10	7 ^{1d}	8 ^{1d}	8	7 ^{2d}							
*A = Pan weight (mg) Tray color code: <u>Turquoise</u> Analyst: <u>DL</u> Date: <u>09-27-23</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>JP</u> Date: <u>10-13-23</u>															
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		1.827		-5.67		1.398		20.77		0		1007.	

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

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

Comments:



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

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

Environmental Testing Solutions, Inc.

Test number: 144

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)		B = Pan + Larvae weight (mg)		Not for Compliance Assessment, Internal Laboratory QC		Weight / Initial number of larvae (mg)	Mean survival (%)	Coefficient of variation (%)	Percent reduction from control (%)
				A	B	B - A	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	13.83	30.85	17.02	1.702	1.702	1.702	100.0	4.5	Not applicable	
	B	10	10	14.24	32.20	17.96	1.796	1.796	1.796	100.0	4.5	Not applicable	
	C	10	10	14.14	31.08	16.94	1.694	1.694	1.694	100.0	4.5	Not applicable	
	D	10	10	13.42	32.03	18.61	1.861	1.861	1.861	100.0	4.5	Not applicable	
0.025	E	10	10	15.06	34.66	19.60	1.960	1.960	1.960	100.0	9.1	-5.4	
	F	10	10	11.89	28.69	16.80	1.680	1.680	1.680	100.0	9.1	-5.4	
	G	10	10	12.44	32.82	20.38	2.038	2.038	2.038	100.0	9.1	-5.4	
	H	10	10	15.14	32.70	17.56	1.756	1.756	1.756	100.0	9.1	-5.4	
0.050	I	10	10	14.41	31.14	16.73	1.673	1.673	1.673	100.0	4.5	0.9	
	J	10	10	14.68	33.16	18.48	1.848	1.848	1.848	100.0	4.5	0.9	
	K	10	10	14.04	31.03	16.99	1.699	1.699	1.699	100.0	4.5	0.9	
	L	10	10	11.48	29.20	17.72	1.772	1.772	1.772	100.0	4.5	0.9	
0.100	M	10	10	13.68	29.98	16.30	1.630	1.630	1.630	100.0	14.3	-3.6	
	N	10	10	12.75	31.00	18.25	1.825	1.825	1.825	100.0	14.3	-3.6	
	O	10	10	14.73	36.69	21.96	2.196	2.196	2.196	100.0	14.3	-3.6	
	P	10	10	15.58	32.14	16.56	1.656	1.656	1.656	100.0	14.3	-3.6	
0.200	Q	10	7	14.78	25.96	11.18	1.597	1.118	1.118	75.0	10.9	20.7	
	R	10	8	13.93	29.74	15.81	1.976	1.581	1.581	75.0	10.9	20.7	
	S	10	8	13.83	28.36	14.53	1.816	1.453	1.453	75.0	10.9	20.7	
	T	10	7	13.60	28.01	14.41	2.059	1.441	1.441	75.0	10.9	20.7	
0.500	U	10	0	0.00	0.00	0.00	0.000	0.000	0.000	0.0	0.0	100.0	
	V	10	0	0.00	0.00	0.00	0.000	0.000	0.000	0.0	0.0	100.0	
	W	10	0	0.00	0.00	0.00	0.000	0.000	0.000	0.0	0.0	100.0	
	X	10	0	0.00	0.00	0.00	0.000	0.000	0.000	0.0	0.0	100.0	

Dummett's MSD value: 0.2673
 PMSD: 15.2

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

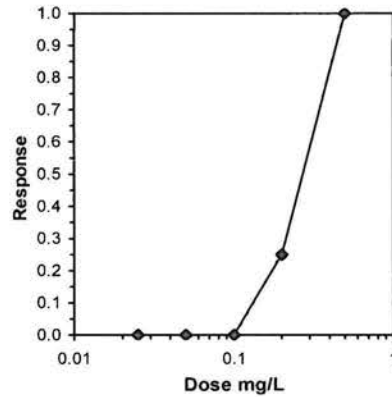
Start Date:	10/3/2023	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	10/10/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

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	0.7000	0.8000	0.8000	0.7000
0.5	0.0000	0.0000	0.0000	0.0000

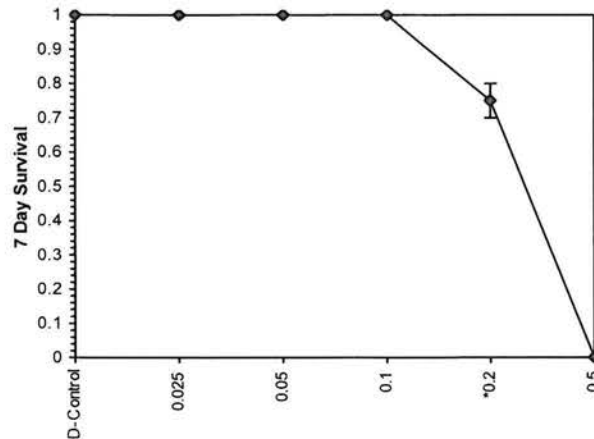
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number	
			Mean	Min	Max	CV%					N
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4		0	40	
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.2	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	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)	0.63123	0.868	5.4E-15	2.98039
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Trim Level	Trimmed Spearman-Kärber		
	EC50	95% CL	
0.0%	0.2586	0.2316	0.2887
5.0%	0.2622	0.2316	0.2969
10.0%	0.2656	0.2295	0.3073
20.0%	0.2706	0.2157	0.3395
Auto-0.0%	0.2586	0.2316	0.2887



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

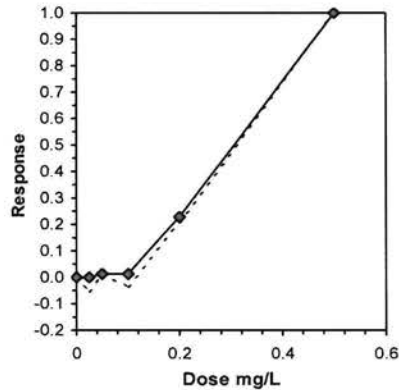
Start Date:	10/3/2023	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	10/10/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.7020	1.7960	1.6940	1.8610
0.025	1.9600	1.6800	2.0380	1.7560
0.05	1.6730	1.8480	1.6990	1.7720
0.1	1.6300	1.8250	2.1960	1.6560
0.2	1.1180	1.5810	1.4530	1.4410
0.5	0.0000	0.0000	0.0000	0.0000

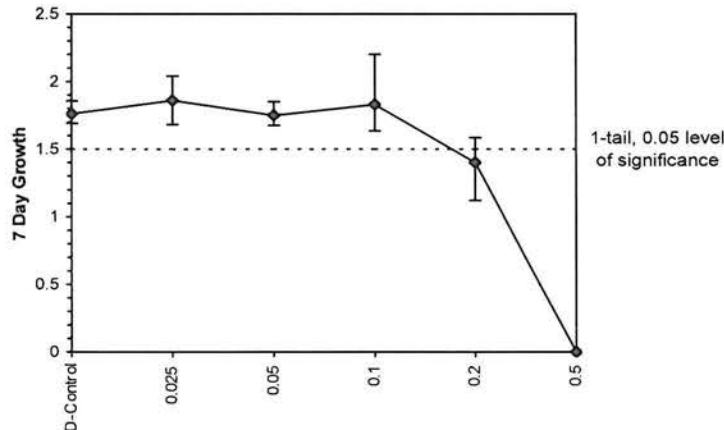
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	t-Stat		Critical	MSD	Mean	N-Mean	
D-Control	1.7633	1.0000	1.7633	1.6940	1.8610	4.534	4				1.8109	1.0000	
0.025	1.8585	1.0540	1.8585	1.6800	2.0380	9.051	4	-0.816	2.290	0.2673	1.8109	1.0000	
0.05	1.7480	0.9914	1.7480	1.6730	1.8480	4.505	4	0.131	2.290	0.2673	1.7874	0.9870	
0.1	1.8268	1.0360	1.8268	1.6300	2.1960	14.282	4	-0.544	2.290	0.2673	1.7874	0.9870	
0.2	1.3983	0.7930	1.3983	1.1180	1.5810	14.109	4				1.3983	0.7721	
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 ($p > 0.01$)	0.9358	0.844	0.9165	1.21643
Bartlett's Test indicates equal variances ($p = 0.16$)	5.2046	11.3449		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	0.1	>0.1		
Treatments vs D-Control	0.26725	0.15157	0.01092	0.02724
			0.75497	3, 12

Point	mg/L	SD	Linear Interpolation (200 Resamples)		
			95% CL(Exp)	Skew	
IC05	0.1172	0.0275	0.0000	0.1451	-1.2990
IC10	0.1405	0.0169	0.0674	0.1865	-0.2852
IC15	0.1638	0.0179	0.1055	0.2287	0.5777
IC20	0.1870	0.0185	0.1262	0.2425	0.2375
IC25	0.2086	0.0178	0.1513	0.2574	-0.0069
IC40	0.2669	0.0155	0.2091	0.3059	-0.3425
IC50	0.3057	0.0129	0.2576	0.3383	-0.3425



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Menidia beryllina

MbCuCR Test Number: 144

Daily Chemistry:

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

Concentration		Parameter	Day					
			(Analyst identified for each day, performed pH and D.O. measurements only.)					
			0		1		2	
Analyst		N	N	N	N	N	N	
CONTROL, SaltSW	pH (S.U.)	7.00	7.02	7.00	7.00	7.95	7.74	
	Dissolved oxygen (mg/L)	7.7	7.7	7.0	7.9	7.7	7.2	
	Salinity (ppt)	24.9	26.0	25.0	25.2	25.0	25.2	
	Alkalinity (mg CaCO ₃ /L)	86		10-11 23.8		110		
	Temperature (°C)	25.2	24.9	24.8	25.1	24.7	24.8	
0.025 mg/L	pH (S.U.)	7.06	7.02	7.05	7.75	7.99	7.75	
	Dissolved oxygen (mg/L)	7.0	7.7	7.0	7.9	8.1	7.2	
	Salinity (ppt)	25.0	25.5	24.9	25.2	24.9	25.4	
	Temperature (°C)	25.3	25.0	24.8	25.1	24.7	24.9	
0.05 mg/L	pH (S.U.)	7.04	7.02	7.06	7.75	8.00	7.75	
	Dissolved oxygen (mg/L)	7.0	7.6	7.9	7.0	8.1	7.2	
	Salinity (ppt)	25.0	25.6	25.0	25.3	25.3	25.4	
	Temperature (°C)	25.1	25.0	24.7	25.0	24.7	25.1	
0.1 mg/L	pH (S.U.)	7.04	7.01	7.06	7.74	8.00	7.70	
	Dissolved oxygen (mg/L)	7.9	7.0	8.0	7.0	8.0	7.2	
	Salinity (ppt)	25.0	25.2	25.0	25.3	25.3	25.6	
	Temperature (°C)	25.3	24.7	24.7	25.0	24.8	25.1	
0.2 mg/L	pH (S.U.)	7.06	7.00	7.07	7.73	8.00	7.70	
	Dissolved oxygen (mg/L)	8.6	7.0	8.0	7.0	8.1	7.4	
	Salinity (ppt)	24.9	25.2	25.0	25.3	25.3	25.6	
	Temperature (°C)	25.3	25.0	24.7	25.0	24.7	25.0	
0.5 mg/L	pH (S.U.)	7.06	7.70	7.07				
	Dissolved oxygen (mg/L)	8.1	7.9	8.0				
	Salinity (ppt)	24.9	25.3	24.4				
	Alkalinity (mg CaCO ₃ /L)		10-11 23.8				10-11 23.8	
	Temperature (°C)	25.0	24.7	24.7				
		Initial	Final	Initial	Final	Initial	Final	

Species: Menidia beryllina

MbCuCR Test Number: 144

Concentration		Parameter	Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
			3		4		5		6	
			Analyst							
CONTROL, SaltSW		pH (S.U.)	JK	BSC	BSC	BSU	BSC	BL	BL	JK
		Dissolved oxygen (mg/L)	8.06	8.02	8.11	8.03	8.19	8.05	8.11	7.70
		Salinity (ppt)	25.1	25.5	25.2	25.2	24.9	25.2	25.0	25.1
		Alkalinity (mg CaCO ₃ /L)	130	104	140	130	130	130	130	130
		Temperature (°C)	24.8	25.2	24.7	24.9	24.7	25.3	24.6	24.5
0.025 mg/L		pH (S.U.)	JK	BSC	BSC	BSU	BSC	BL	BL	JK
		Dissolved oxygen (mg/L)	7.93	7.93	8.12	8.01	8.19	8.04	8.16	7.70
		Salinity (ppt)	25.1	25.4	25.3	25.5	25.2	25.6	25.1	25.3
		Temperature (°C)	24.8	25.0	24.7	25.2	24.6	25.0	24.6	24.7
0.05 mg/L		pH (S.U.)	JK	BSC	BSC	BSU	BSC	BL	BL	JK
		Dissolved oxygen (mg/L)	7.95	7.92	8.12	8.00	8.19	8.04	8.17	7.75
		Salinity (ppt)	25.0	25.5	25.4	25.4	25.1	25.2	25.1	25.3
		Temperature (°C)	24.7	25.0	24.7	25.2	24.6	25.0	24.6	24.7
0.1 mg/L		pH (S.U.)	JK	BSC	BSC	BSU	BSC	BL	BL	JK
		Dissolved oxygen (mg/L)	7.94	7.91	8.13	8.00	8.20	8.03	8.17	7.79
		Salinity (ppt)	25.0	25.0	25.3	25.5	25.1	25.2	25.1	25.5
		Temperature (°C)	24.7	24.8	24.7	25.2	24.6	25.0	24.6	24.7
0.2 mg/L		pH (S.U.)	JK	BSC	BSC	BSU	BSC	BL	BL	JK
		Dissolved oxygen (mg/L)	7.96	7.92	8.13	8.07	8.19	8.05	8.16	7.80
		Salinity (ppt)	24.9	25.9	25.3	25.4	25.2	25.2	25.0	25.5
		Temperature (°C)	24.7	25.0	24.7	25.0	24.7	25.2	24.7	24.6
0.5 mg/L		pH (S.U.)								
		Dissolved oxygen (mg/L)								
		Salinity (ppt)								
		Temperature (°C)								
			Initial	Final	Initial	Final	Initial	Final	Initial	Final