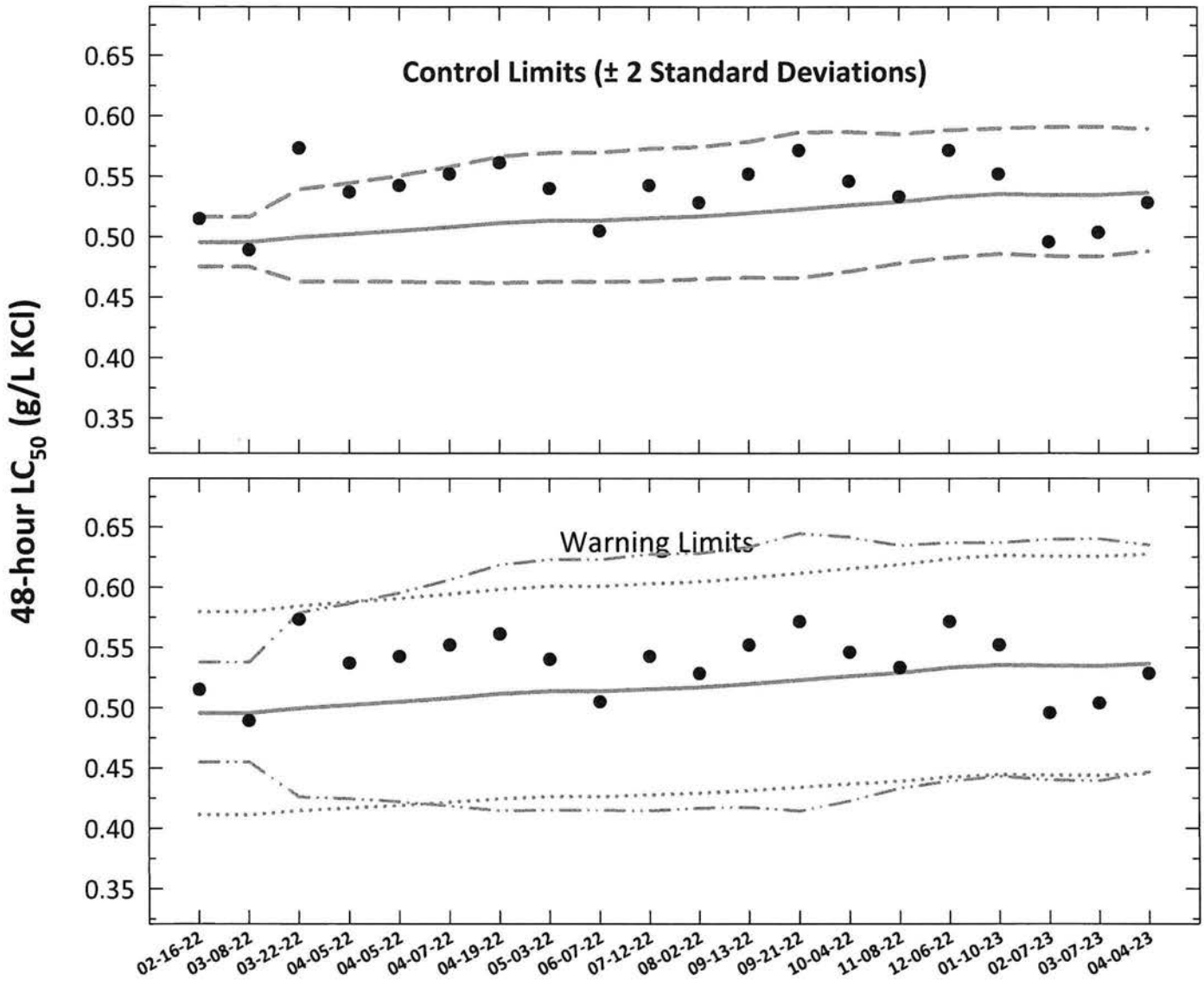
Americamysis (Mysidopsis) bahia
Acute Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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 Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**02-16-22**	**0.5149**	-0.2882	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
2	**03-08-22**	**0.4892**	-0.3105	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
3	**03-22-22**	**0.5733**	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
4	**04-05-22**	**0.5369**	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
5	**04-05-22**	**0.5424**	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
6	**04-07-22**	**0.5519**	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 265

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 #: 2176

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	[illegible]	BL [illegible]	[illegible]
Control, SaltSW	pH (S.U.)	7.87	7.87	7.85
	Dissolved oxygen (mg/L)	8.1	7.6	7.3
	*Salinity (ppt)	25.0	25.4	25.5
	*Alkalinity (mg/L $CaCO_3$)	100		
	*Temperature (°C)	25.1	25.2	25.0
250 mg/L	pH (S.U.)	7.87	7.87	7.80
	Dissolved oxygen (mg/L)	8.0	7.7	7.4
	*Salinity (ppt)	25.3	25.5	25.7
	*Temperature (°C)	25.2	25.5	25.0
375 mg/L	pH (S.U.)	7.88	7.86	7.77
	Dissolved oxygen (mg/L)	8.0	7.8	7.4
	*Salinity (ppt)	25.4	25.5	25.9
	*Temperature (°C)	25.2	25.3	25.1
500 mg/L	pH (S.U.)	7.90	7.88	7.83
	Dissolved oxygen (mg/L)	8.0	7.8	7.3
	*Salinity (ppt)	25.4	25.7	25.8
	*Temperature (°C)	25.2	25.4	25.0
750 mg/L	pH (S.U.)	7.91	7.84	
	Dissolved oxygen (mg/L)	8.1	7.9	
	*Salinity (ppt)	25.7	25.9	
	*Temperature (°C)	25.0	25.4	
1000 mg/L	pH (S.U.)	7.91	7.86	
	Dissolved oxygen (mg/L)	8.2	8.0	
	*Salinity (ppt)	25.9	26.0	
	*Temperature (°C)	25.2	25.5	

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

Chemical analyses:

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

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 265

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-04-23	* 1020	JL	1220	JL	6F	LIGHT BLUE	03-28-23B
24	04-05-23			1220	JL			
48 Termination	04-06-23			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):	04-03-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	04-02-23 1200 TO 04-03-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.04
	Temperature (°C) 24.8

Survival Data **(number of living organisms):**

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	2d &	3d 7	1od 0	1od 0	1od 0	1od 0
48 Termination	10	10	10	10	9 1d	10	8	6 1d	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:

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	4/4/2023	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/6/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

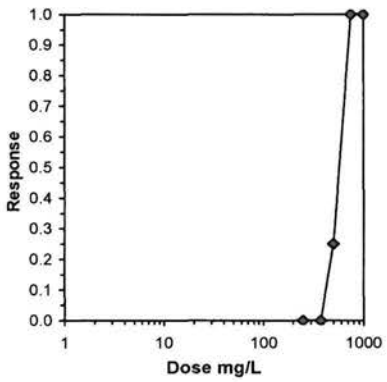
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

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

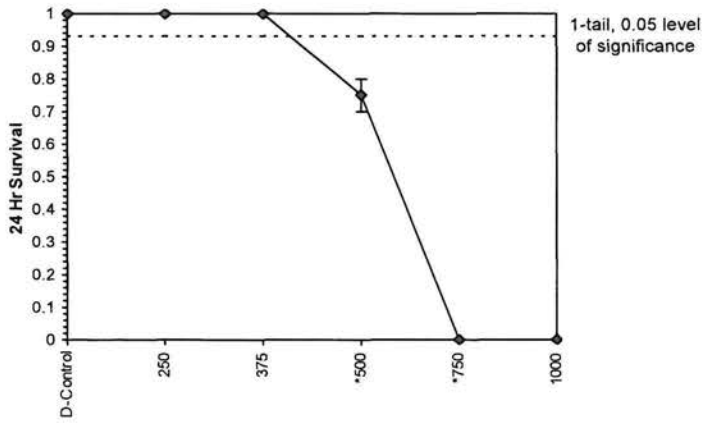
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.04275	0.04384	0.58996	0.00135	1.5E-06	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

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



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed By

Acute Mysid Test-48 Hr Survival

Start Date:	4/4/2023	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/6/2023	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	0.8000	0.6000
750	0.0000	0.0000
1000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2475	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.938	2.850	0.2475	1	20
*500	0.7000	0.7000	0.9966	0.8861	1.1071	15.685	2	4.783	2.850	0.2475	6	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	14.430	2.850	0.2475	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

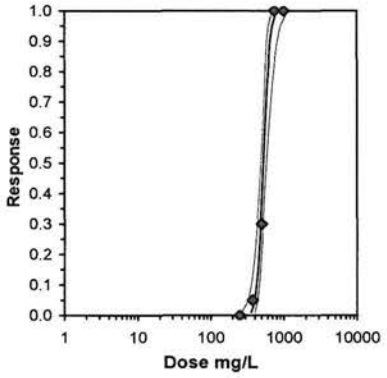
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.1312	0.13456	0.56861	0.00754	1.2E-04	4, 5
Treatments vs D-Control										

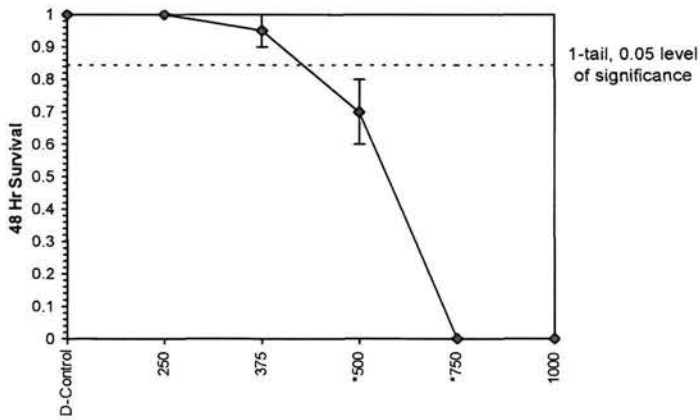
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	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



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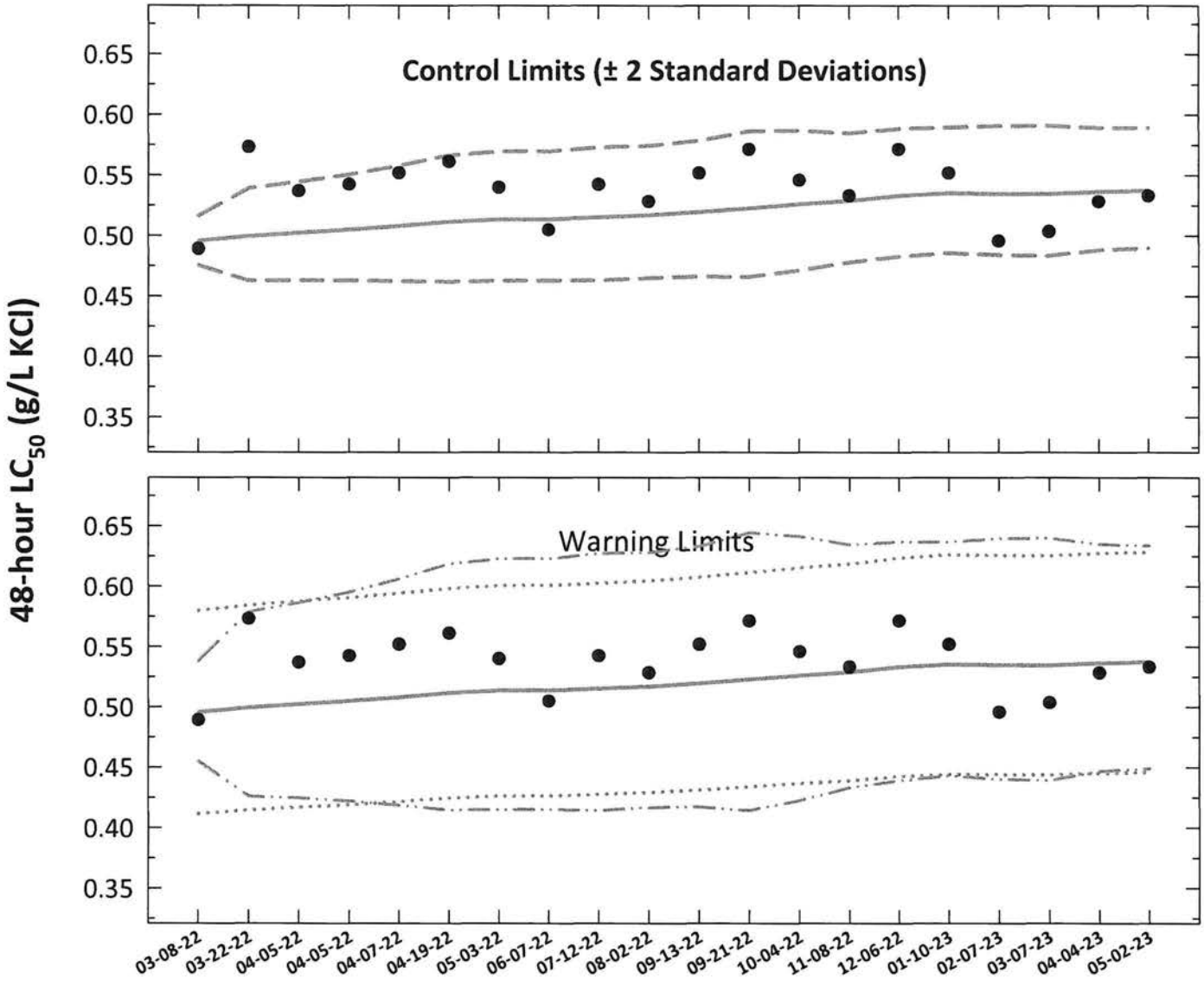

- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control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

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**03-08-22**	**0.4892**	-0.3105	-0.3049	0.0090	0.4956	0.4754	0.5165	0.4550	0.5378	0.4113	0.5798
2	**03-22-22**	**0.5733**	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
3	**04-05-22**	**0.5369**	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
4	**04-05-22**	**0.5424**	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
5	**04-07-22**	**0.5519**	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 266

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 #: 2176

Chemical Analyses:

		Hours		
		0 05-27	24	48
Concentration	Analyst	JP BL	JP BL	VL
Control, SaltSW	pH (S.U.)	7.87	7.86	7.72
	Dissolved oxygen (mg/L)	7.9	7.6	7.8
	*Salinity (ppt)	25.0	25.2	25.5
	*Alkalinity (mg/L $CaCO_3$)	97		
	*Temperature (°C)	25.2	25.7	25.5
250 mg/L	pH (S.U.)	7.89	7.88	7.67
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	*Salinity (ppt)	25.1	25.4	25.5
	*Temperature (°C)	25.4	25.6	25.5
375 mg/L	pH (S.U.)	7.90	7.86	7.62
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	*Salinity (ppt)	25.4	25.7	26.0
	*Temperature (°C)	25.4	25.6	25.6
500 mg/L	pH (S.U.)	7.90	7.87	7.60
	Dissolved oxygen (mg/L)	8.0	7.7	7.8
	*Salinity (ppt)	25.3	25.5	25.8
	*Temperature (°C)	25.4	25.6	25.6
750 mg/L	pH (S.U.)	7.90	7.87	
	Dissolved oxygen (mg/L)	8.0	7.7	
	*Salinity (ppt)	25.9	26.2	
	*Temperature (°C)	25.5	25.3	
1000 mg/L	pH (S.U.)	7.90	7.88	
	Dissolved oxygen (mg/L)	8.0	7.9	
	*Salinity (ppt)	25.6	25.9	
	*Temperature (°C)	25.5	25.6	

*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	80661685

ETC

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 266

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05-02-23	* 1106	JH	1310	JH	5F	PURPLE	04-26-BA
24	05-03-23			1311	JH			
48 Termination	05-04-23			1308	JH			

*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):	05-01-23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	04-30-23 1200 TO 05-01-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.83
	Temperature (°C) 24.5

Survival Data **(number of living organisms):**

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 1d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	6 4u	6 4u	0	0	0	0
Mean Survival	100%		100%		100%		60%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	494.1
Upper 95% confidence limit (mg KCl/L)	575.2
48-hour LC_{50} (mg KCl/L)	533.1

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-48 Hr Survival

Start Date:	5/2/2023	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/4/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

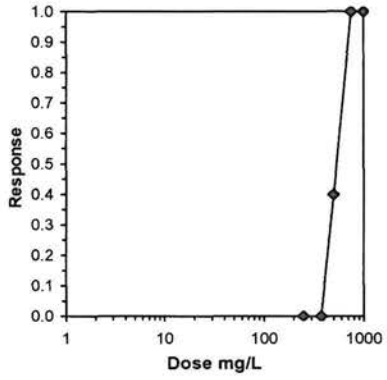
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	52.594	2.850	0.0285	8	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				

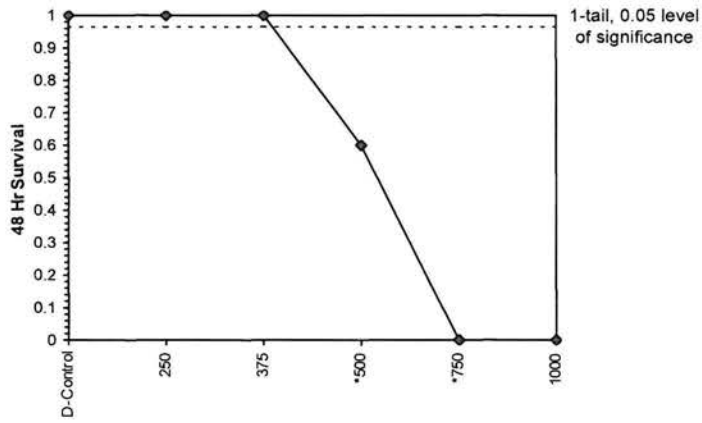
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	500	433.013		0.00967	0.00991	0.60706	0.0001	2.1E-09	4, 5

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	533.10	494.12	575.16
5.0%	533.38	490.12	580.45
10.0%	533.65	484.88	587.32
20.0%	534.18	468.38	609.23
Auto-0.0%	533.10	494.12	575.16



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-24 Hr Survival

Start Date:	5/2/2023	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/4/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

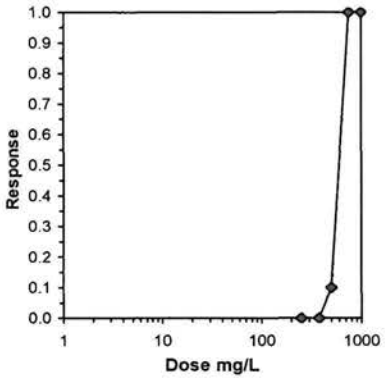
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

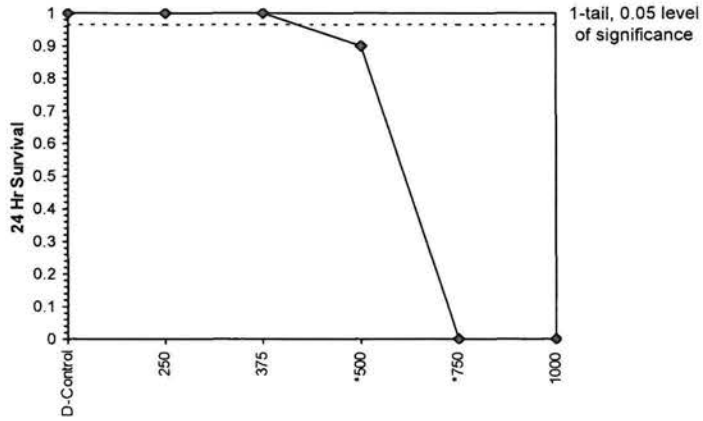
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.59802	0.0001	2.2E-09	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

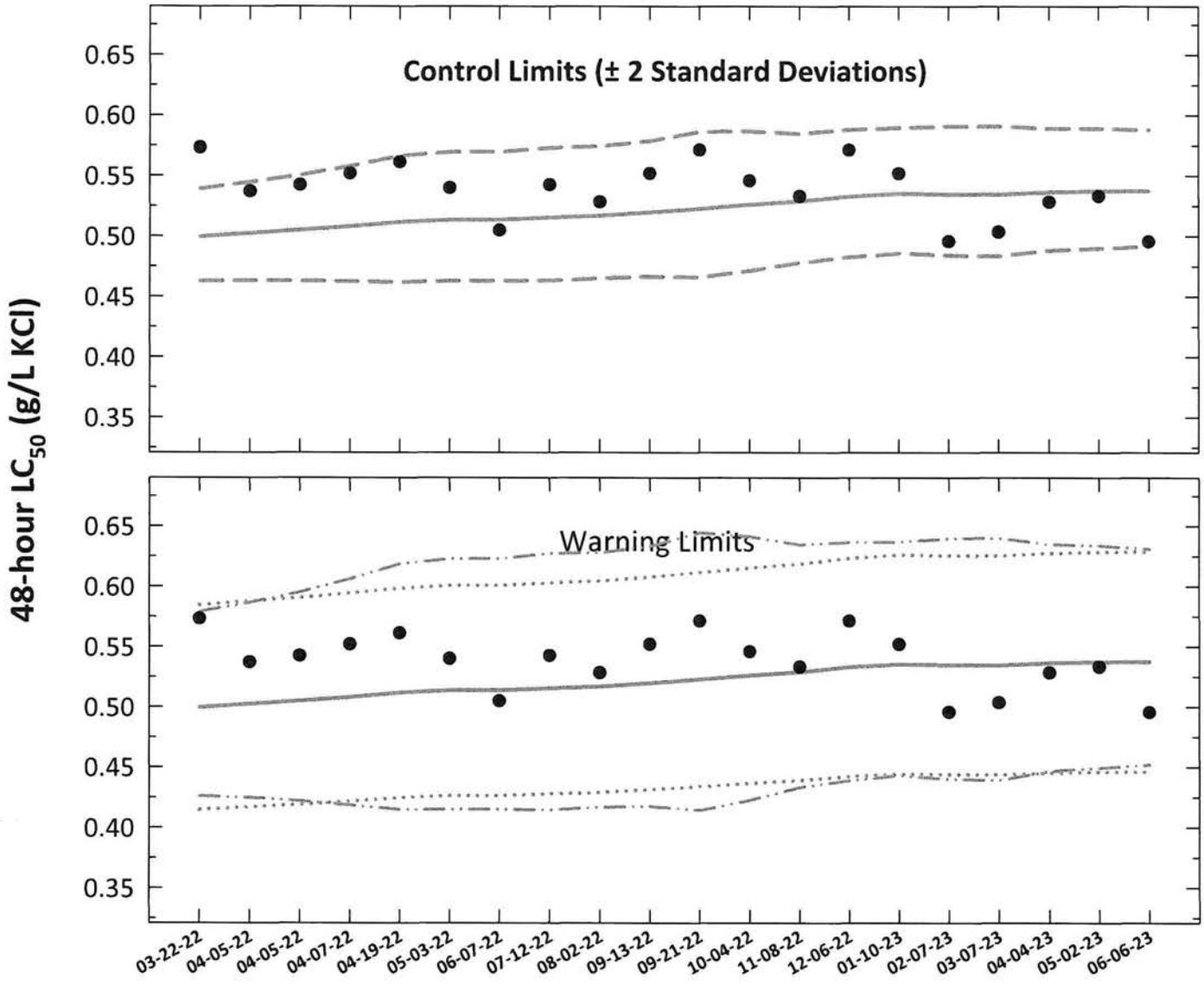
Trim Level	EC50	95% CL	
0.0%	591.51	564.64	619.67
5.0%	596.72	563.70	631.68
10.0%	598.73	578.96	619.18
20.0%	598.73	578.96	619.18
Auto-0.0%	591.51	564.64	619.67



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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 Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**03-22-22**	**0.5733**	-0.2417	-0.3014	0.0166	0.4996	0.4628	0.5393	0.4259	0.5791	0.4146	0.5845
2	**04-05-22**	**0.5369**	-0.2701	-0.2990	0.0176	0.5023	0.4633	0.5446	0.4246	0.5866	0.4169	0.5877
3	**04-05-22**	**0.5424**	-0.2657	-0.2967	0.0188	0.5050	0.4631	0.5506	0.4220	0.5954	0.4191	0.5908
4	**04-07-22**	**0.5519**	-0.2581	-0.2941	0.0204	0.5080	0.4625	0.5579	0.4185	0.6063	0.4216	0.5944
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 267

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 #: 2176

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JP	JP BLM	JP W
Control, SaltSW	pH (S.U.)	7.91	~~06.07.21~~ ~~7.86~~ 7.90	7.80
	Dissolved oxygen (mg/L)	7.6	7.0	7.3
	*Salinity (ppt)	25.0	25.8	25.5
	*Alkalinity (mg/L $CaCO_3$)	110		
	*Temperature (°C)	25.3	25.4	25.3
250 mg/L	pH (S.U.)	7.88	7.87	7.77
	Dissolved oxygen (mg/L)	7.5	7.6	7.1
	*Salinity (ppt)	25.1	25.5	26.0
	*Temperature (°C)	25.2	25.3	25.4
375 mg/L	pH (S.U.)	7.90	7.89	~~7.77~~
	Dissolved oxygen (mg/L)	7.5	7.6	7.1
	*Salinity (ppt)	25.2	25.7	26.1
	*Temperature (°C)	25.2	25.4	25.2
500 mg/L	pH (S.U.)	7.91	~~06.07.21~~ ~~7.8~~ 7.90	7.79
	Dissolved oxygen (mg/L)	7.5	7.7	7.1
	*Salinity (ppt)	25.3	25.2	26.0
	*Temperature (°C)	25.2	25.4	25.2
750 mg/L	pH (S.U.)	7.90	7.90	
	Dissolved oxygen (mg/L)	7.6	7.7	
	*Salinity (ppt)	25.3	26.2	
	*Temperature (°C)	25.3	25.6	
1000 mg/L	pH (S.U.)	7.91	7.89	
	Dissolved oxygen (mg/L)	7.6	7.7	
	*Salinity (ppt)	25.5	26.1	
	*Temperature (°C)	25.3	25.5	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	130664664

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 267

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-06-23	* 1015	JH	1215	JH	6B	ORANGE	05-30-23B
24	06-07-23			1210	JH			
48 Termination	06-08-23			1215	JH			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	AIAb 0605.23
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born:	06-04-23 1200 TO 06-05-23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.82
	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	10	10	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	9 9d	10	5 5d	5 5d	0	0	0	0
Mean Survival	100%		100%		95%		50%		0%		0%	

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

Statistics:

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

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-24 Hr Survival

Start Date:	6/6/2023	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	6/8/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

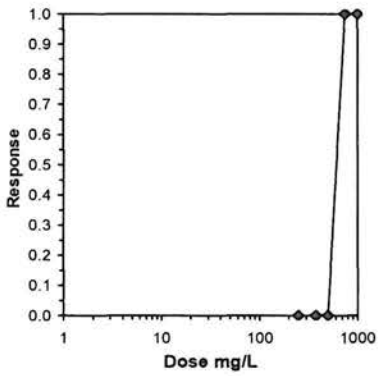
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	1.0000	1.0000
750	0.0000	0.0000
1000	0.0000	0.0000

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

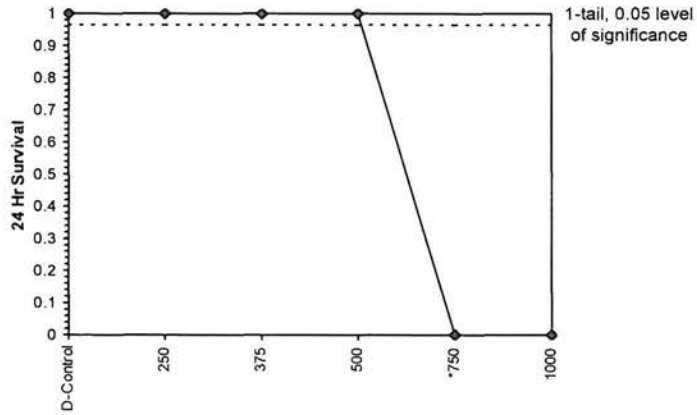
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	500	750	612.372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

Graphical Method

Trim Level	EC50
0.0%	612.37
	612.37



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-48 Hr Survival

Start Date:	6/6/2023	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/8/2023	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	0.9000	1.0000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	12.159	2.850	0.1469	10	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

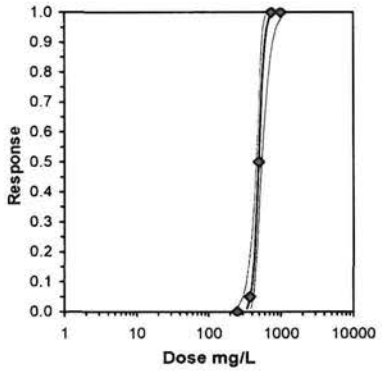
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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	0.60026	0.00266	7.8E-06	4, 5
Treatments vs D-Control										

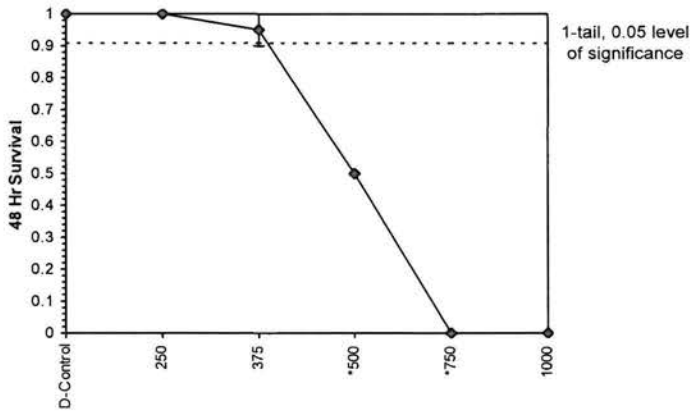
Maximum Likelihood-Probit

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

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

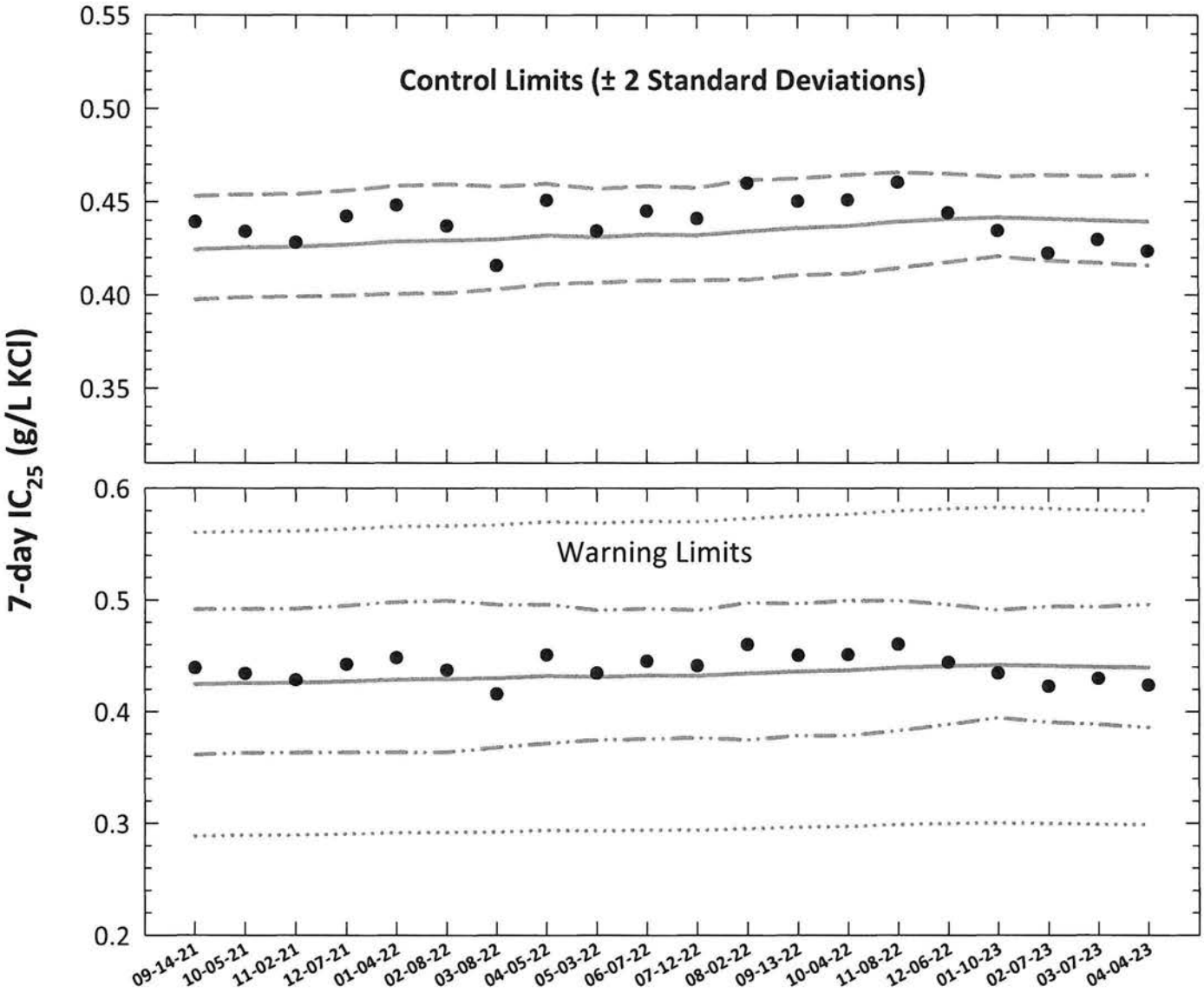


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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} ± 2 standard deviations converted to anti-logarithmic values)
- – · – · **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic IC_{25} ± $S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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 Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**09-14-21**	**0.4392**	-0.3574	-0.3721	0.0142	0.4245	0.3977	0.4531	0.3614	0.4918	0.2887	0.5603
2	**10-05-21**	**0.4340**	-0.3625	-0.3710	0.0140	0.4256	0.3990	0.4539	0.3631	0.4922	0.2894	0.5617
3	**11-02-21**	**0.4283**	-0.3683	-0.3708	0.0140	0.4258	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
4	**12-07-21**	**0.4422**	-0.3544	-0.3695	0.0143	0.4271	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
5	**01-04-22**	**0.4482**	-0.3485	-0.3678	0.0146	0.4288	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
6	**02-08-22**	**0.4370**	-0.3595	-0.3673	0.0147	0.4293	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note:

7-day IC_{25} = 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 IC_{25} values.

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

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

Warning Limits = Mean logarithmic IC_{25} ± 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.

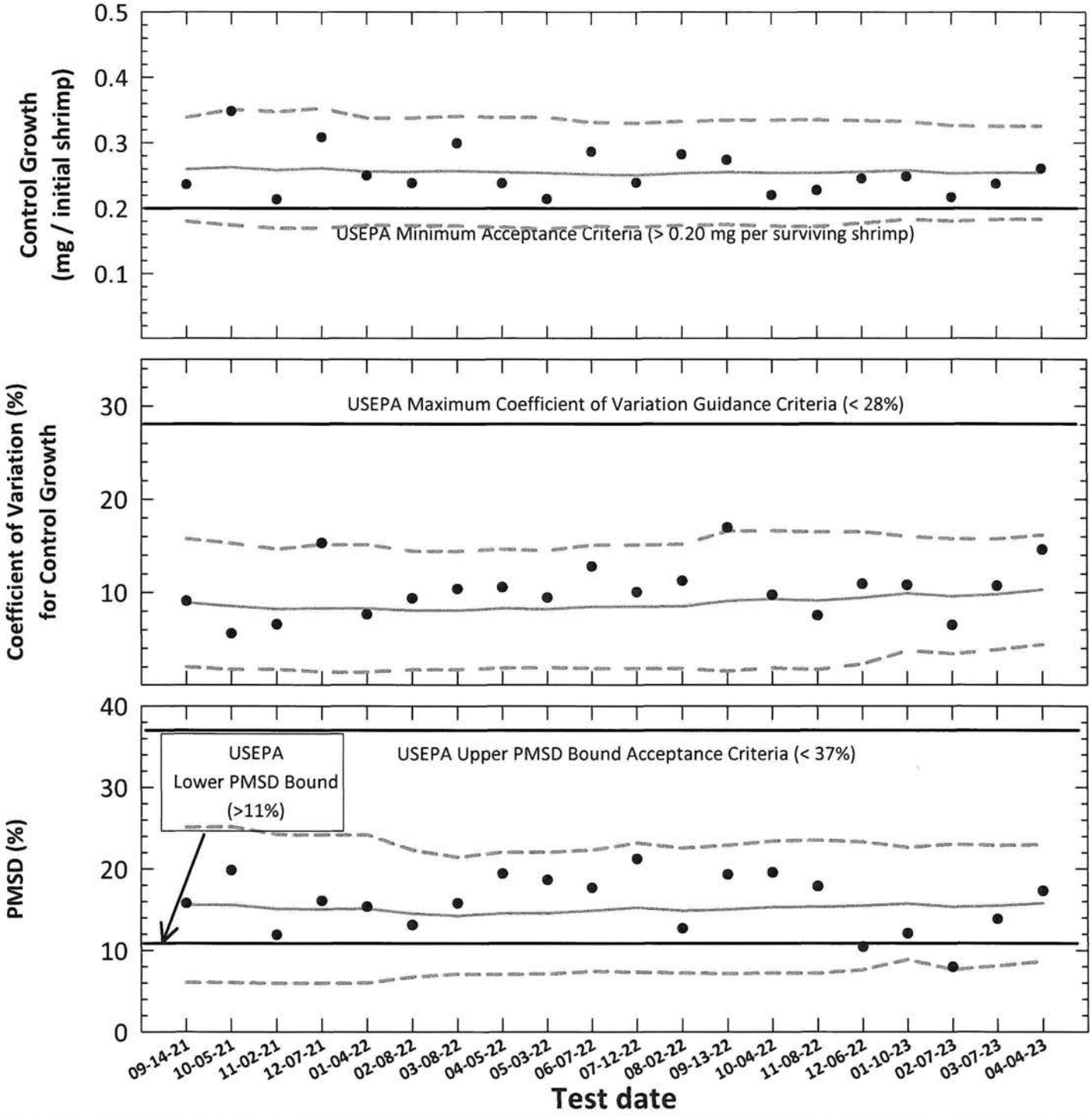
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control	Control Growth		Test										
		Survival	Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(mg/initial shrimp)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	09-14-21	100	0.236	9.1	0.0373	15.8	0.260	0.180	0.339	8.9	2.0	15.8	15.6	6.1	25.1
2	10-05-21	100	0.348	5.6	0.0691	19.8	0.263	0.174	0.351	8.5	1.8	15.3	15.6	6.1	25.2
3	11-02-21	100	0.214	6.6	0.0254	11.9	0.258	0.169	0.347	8.2	1.7	14.7	15.1	6.0	24.2
4	12-07-21	100	0.308	15.3	0.0495	16.1	0.261	0.169	0.353	8.3	1.5	15.1	15.0	5.9	24.1
5	01-04-22	100	0.250	7.6	0.0384	15.4	0.256	0.174	0.338	8.3	1.5	15.1	15.1	6.0	24.2
6	02-08-22	100	0.238	9.4	0.0312	13.1	0.256	0.174	0.338	8.1	1.7	14.4	14.5	6.7	22.3
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Americamysis (Mysidopsis) bahia*

AbKClCR Test Number: **238**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2176				
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:	03-28-23 1200 to 03-29-23 1130	Incubator number and shelf location:	SF
Organism source:	AI Batch Ab: 03-29-23	*Artemia* CHM number:	CHM1222
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.86 S.U. Temperature = 25.1 °C	Date / Time in oven:	04-11-23 1115
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	04-12-23 1115
		*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		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	04-04-23	1020	JK	1400	JK	1140	JK	03-28-23 B
1	04-05-23	0500	JK	1100	JK	0940	JK	↓
2	04-06-23	0500	JK	1240	JK	1216	JK	04-15-23
3	04-07-23	0500	JK	1100	JK	0940	JK	↓
4	04-08-23	0600	JK	1200	JK	0940	JK	04-07-23
5	04-09-23	0600	JK	1200	JK	0940	JK	↓
6	04-10-23	0500	JK	1100	JK	0940	JK	04-08-23
7	04-11-23					0957	JK	

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	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	523.9
Average weight per initial shrimp:	0.261		**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)	423.5

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **238**

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: forest green Analyst: BL Date: 03-23-23	13.93	15.82	12.05	13.92	14.92	15.24	12.62	14.66	14.60	15.16	15.01	13.09	14.41	15.59	14.71	14.5
*B = Pan + Shrimp weight (mg) Analyst: BL Date: 04·13·23	CONFIRMED WEIGHTS. JL															
	~~20.39~~	~~22.75~~	~~22.37~~	~~21.34~~	~~21.31~~	~~21.78~~	~~21.35~~	~~20.17~~	~~21.72~~	~~21.15~~	~~21.43~~	~~22.03~~	~~21.32~~	~~20.36~~	~~24.15~~	~~21.6~~
	15.27	16.78	13.52	15.30	16.30	16.70	14.00	15.71	15.69	16.53	16.46	14.43	15.57	16.93	16.03	15.8
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	1.34	0.96	1.47	1.38	1.38	1.46	1.38	1.05	1.09	1.37	1.45	1.34	1.16	1.34	1.32	1.3
Weight per Initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.268	0.192	0.294	0.276	0.276	0.292	0.276	0.210	0.218	0.274	0.290	0.268	0.232	0.268	0.264	0.26
	Average weight per initial number of shrimp (mg) 0.261								Average weight per initial number of shrimp (mg) 0.259				Percent reduction from control (%) 0.5 %			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: 238

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	4 id	4 id	4 id	S	4 id	4 id	4
2	S	S	S	S	S	S	S	S	4	3 id	4	3 id	3 2d	4	4	3 id
3	S	S	S	S	S	S	S	S	3 id	3	3 id	3	3	2	3 id	3
4	S	S	S	S	S	S	S	S	3	3	3	3	3	2	3	3
5	S	S	S	S	S	S	S	S	3	3	3	3	3	2	3	3
6	S	S	S	S	S	S	S	S	2 id	3	3	3	3	2	3	3
7	S	S	S	S	S	S	S	S	2	3	3	3	3	2	3	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																4 04-11
*A = Pan weight (mg) Tray color code: Sorest green Analyst: DL Date: 03-23-23	14.41	14.22	14.82	12.02	14.64	15.53	14.99	14.66	12.40	15.52	15.32	13.79	15.24	15.49	14.36	15.9
*B = Pan + Shrimp weight (mg) Analyst: DL Date: 04-13-23	~~21.14~~ 15.71	~~20.34~~ 15.37	~~21.86~~ 15.92	~~23.15~~ 13.31	~~19.98~~ 16.00	~~20.08~~ 16.71	~~21.79~~ 16.38	~~22.95~~ 15.54	04-13-23 DL 12.84	16.41	16.43	14.69	15.67	15.85	14.79	16.2
C = Shrimp weight (mg) = B – A Hand calculated Analyst: DL	1.30	1.15	1.10	1.29	1.36	1.18	1.39	0.88	0.44	0.89	1.11	0.90	0.43	0.36	0.43	0.31
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: DL	0.260	0.230	0.220	0.258	0.272	0.236	0.278	0.176	0.088	0.178	0.222	0.180 ~~0.086~~ 04-17-23	0.086 ~~0.172~~ 04-17-23	0.072	0.086	0.077
	Average weight per initial number of shrimp (mg) 0.241				Percent reduction from control (%) 7.47				Average weight per initial number of shrimp (mg) 0.123				Percent reduction from control (%) 52.67			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **238**

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															04-05-23	
*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:															04-05-23 JH	
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 100%				Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 100%			

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

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

Comments:

Environmental Testing Solutions, Inc.

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: April 04-11, 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 fro control (%)
Control	A	5	5	13.93	15.27	1.34	0.268	100.0	0.261	14.6	Not applicable
	B	5	5	15.82	16.78	0.96	0.192				
	C	5	5	12.05	13.52	1.47	0.294				
	D	5	5	13.92	15.30	1.38	0.276				
	E	5	5	14.92	16.30	1.38	0.276				
	F	5	5	15.24	16.70	1.46	0.292				
	G	5	5	12.62	14.00	1.38	0.276				
	H	5	5	14.66	15.71	1.05	0.210				
250	I	5	5	14.60	15.69	1.09	0.218	100.0	0.259	9.0	0.5
	J	5	5	15.16	16.53	1.37	0.274				
	K	5	5	15.01	16.46	1.45	0.290				
	L	5	5	13.09	14.43	1.34	0.268				
	M	5	5	14.41	15.57	1.16	0.232				
	N	5	5	15.59	16.93	1.34	0.268				
	O	5	5	14.71	16.03	1.32	0.264				
	P	5	5	14.52	15.82	1.30	0.260				
375	Q	5	5	14.41	15.71	1.30	0.260	100.0	0.241	13.8	7.4
	R	5	5	14.22	15.37	1.15	0.230				
	S	5	5	14.82	15.92	1.10	0.220				
	T	5	5	12.02	13.31	1.29	0.258				
	U	5	5	14.64	16.00	1.36	0.272				
	V	5	5	15.53	16.71	1.18	0.236				
	W	5	5	14.99	16.38	1.39	0.278				
	X	5	5	14.66	15.54	0.88	0.176				
500	Y	5	2	12.40	12.84	0.44	0.088	55.0	0.123	48.8	52.8
	Z	5	3	15.52	16.41	0.89	0.178				
	AA	5	3	15.32	16.43	1.11	0.222				
	BB	5	3	13.79	14.69	0.90	0.180				
	CC	5	3	15.24	15.67	0.43	0.086				
	DD	5	2	15.49	15.85	0.36	0.072				
	EE	5	3	14.36	14.79	0.43	0.086				
	FF	5	3	15.93	16.29	0.36	0.072				
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: 0.0450

PMSD: 17.3

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (10th percentile) = 11%.
Upper PMSD bound determined by USEPA (90th percentile) = 37%.
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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.

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	4/5/2023	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	4/12/2023	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	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.4000	0.6000	0.6000	0.6000	0.6000	0.4000	0.6000	0.6000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

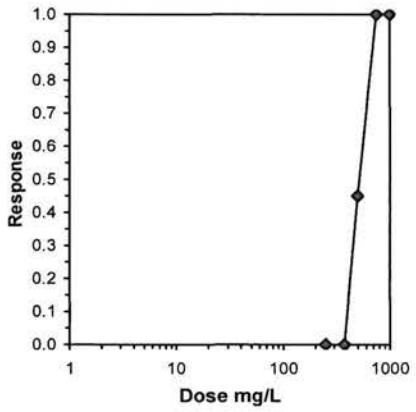
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00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

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.55437	0.904	-2.4246	7.66092
Equality of variance cannot be confirmed				

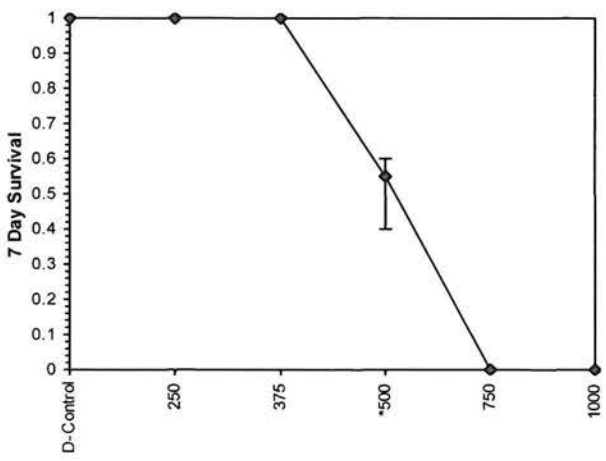
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	523.94	496.14	553.30
5.0%	523.31	492.54	556.00
10.0%	522.68	488.20	559.59
20.0%	521.43	475.93	571.27
Auto-0.0%	523.94	496.14	553.30



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	4/5/2023	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/12/2023	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	0.2680	0.1920	0.2940	0.2760	0.2760	0.2920	0.2760	0.2100
250	0.2180	0.2740	0.2900	0.2680	0.2320	0.2680	0.2640	0.2600
375	0.2600	0.2300	0.2200	0.2580	0.2720	0.2360	0.2780	0.1760
500	0.0880	0.1780	0.2220	0.1800	0.0860	0.0720	0.0860	0.0720
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

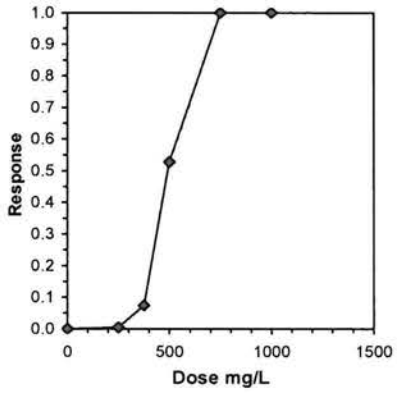
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	0.2605	1.0000	0.2605	0.1920	0.2940	14.606	8			0.2605	1.0000
250	0.2593	0.9952	0.2593	0.2180	0.2900	8.968	8	58.00	43.00	0.2593	0.9952
375	0.2413	0.9261	0.2413	0.1760	0.2780	13.829	8	55.00	43.00	0.2413	0.9261
500	0.1230	0.4722	0.1230	0.0720	0.2220	48.819	8			0.1230	0.4722
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 non-normal distribution (p <= 0.01)	0.88094	0.884	-1.023	0.1529
Bartlett's Test indicates equal variances (p = 0.46)	1.56499	9.21035		

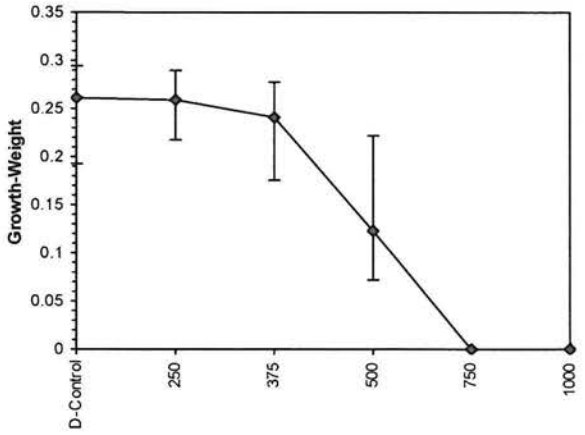
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	>375		
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	331.77	67.64	135.92	387.10	-0.9525
IC10	382.19	35.94	269.05	401.33	-1.5671
IC15	395.96	16.73	345.10	416.46	-1.3671
IC20	409.73	13.13	381.52	433.13	-0.1706
IC25	423.49	13.45	398.34	447.66	0.2998
IC40	464.80	18.81	441.63	516.33	0.9557
IC50	492.34	24.46	463.69	555.27	0.8304



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **238**

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.

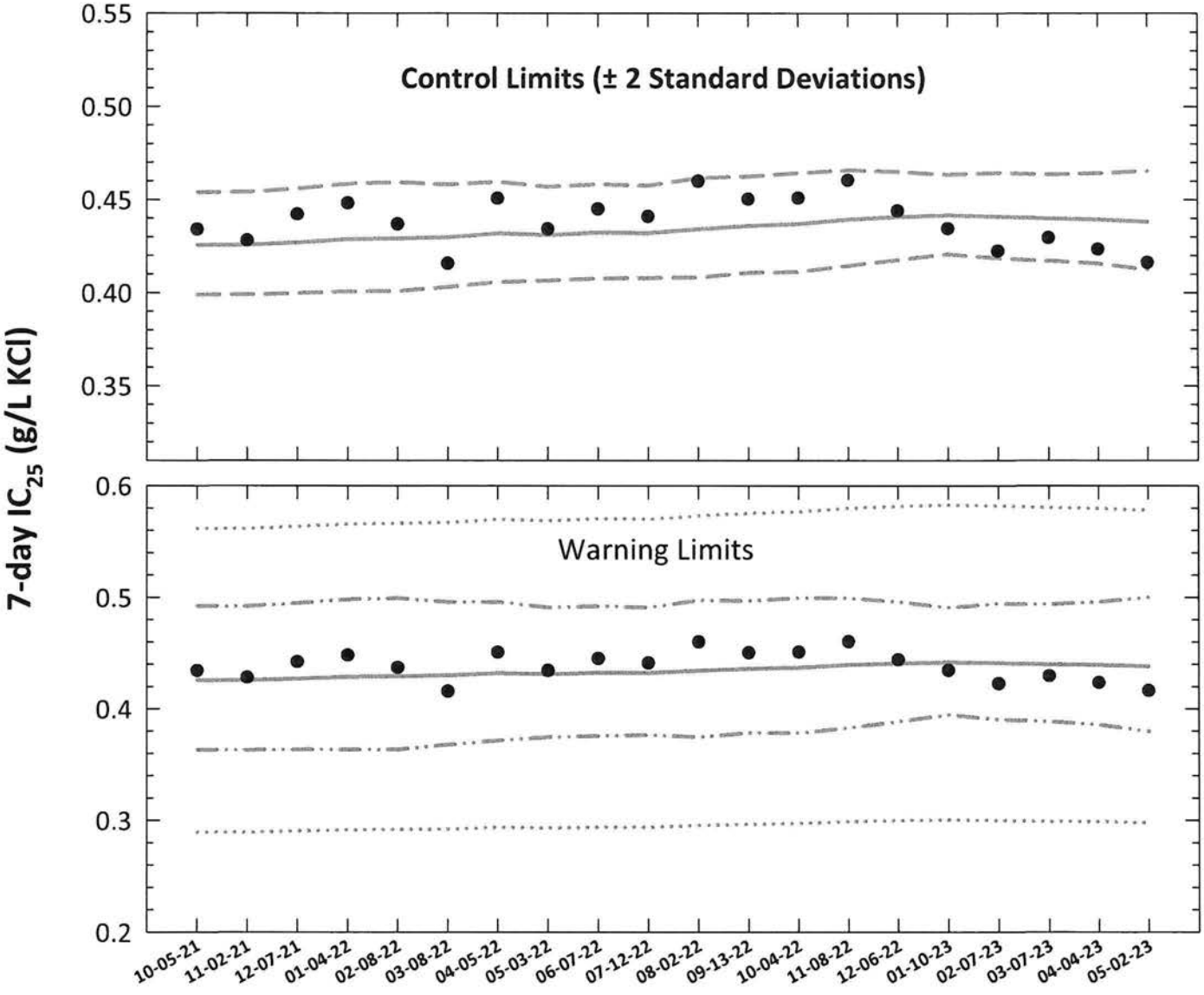
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	GP VL	GP BZ VL	BZ VL	GP VL	VL GP	GP VL
CONTROL, Salt SW	pH (S.U.)	7.87	7.85	7.97 04/05/23	7.75	8.00	7.76
	DO (mg/L)	8.1	8.0	~~7.7~~ 8.0	7.1	7.7	7.5
	Salinity (ppt)	25.0	25.6	25.0	25.0	25.0	25.3
	Alkalinity (mg $CaCO_3$/L)	100		→ 04/05/23		100	
	Temperature (°C)	25.6	25.7	25.3	25.9	25.2	26.1
250 mg KCl/L	pH (S.U.)	7.87	7.84	7.90	7.81	7.94	7.77
	DO (mg/L)	8.0	7.9	7.7	6.8	7.7	7.5
	Salinity (ppt)	25.0	25.7	25.4	25.5	25.2	25.7
	Temperature (°C)	25.7	25.9	25.2	25.8	25.2	26.0
375 mg KCl/L	pH (S.U.)	7.88	7.85	7.90	7.79	7.94	7.77
	DO (mg/L)	8.0	7.9	7.8	6.9	7.7	7.6
	Salinity (ppt)	25.5	25.9	25.4	25.8	25.5	26.0
	Temperature (°C)	25.7	25.9	25.2	25.8	25.1	26.0
500 mg KCl/L	pH (S.U.)	7.90	7.83	7.90	7.77	7.95	7.76
	DO (mg/L)	8.0	7.9	7.8	7.0	7.7	7.6
	Salinity (ppt)	25.5	26.0	25.4	26.0	25.5	26.2
	Temperature (°C)	25.7	25.6	25.2	25.8	25.1	26.2
750 mg KCl/L	pH (S.U.)	7.91	7.87				
	DO (mg/L)	8.1	8.0				
	Salinity (ppt)	25.6	26.1				
	Temperature (°C)	25.5	25.8				
1000 mg KCl/L	pH (S.U.)	7.91	7.84				
	DO (mg/L)	8.2	8.1			04-05-23	
	Salinity (ppt)	25.7	26.2				
	Temperature (°C)	25.5	25.8				
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

AbKClCR Test Number: 238

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 3		4		5		6	
	Analyst	AP M	BSL	BSL	BSL	BSL	AP BZ	BZ	M
CONTROL, Salt SW	pH (S.U.)	7.88	7.78	8.03	7.74	8.01	7.88	7.98	7.68
	DO (mg/L)	7.8	7.7	8.0	7.3	7.9	7.7	7.8	6.8
	Salinity (ppt)	25.0	25.0	14.8	25.0	25.1	25.0	25.0	25.0
	Alkalinity (mg $CaCO_3$/L)	→ 04-11-23 M		100		→ 04-11-23 M		100	
	Temperature (°C)	25.2	26.1	25.7	26.2	25.6	26.3	25.6	25.7
250 mg KCl/L	pH (S.U.)	7.97	7.82	8.02	7.75	7.99	7.90	7.98	7.78
	DO (mg/L)	7.8	7.7	8.0	7.3	7.9	7.8	8.1	6.6
	Salinity (ppt)	25.3	25.4	25.3	25.4	25.2	25.5	25.3	25.4
	Temperature (°C)	25.2	26.2	25.9	26.2	25.9	26.0	25.6	26.0
375 mg KCl/L	pH (S.U.)	7.97	7.83	8.02	7.79	7.99	7.88	7.98	7.69
	DO (mg/L)	7.6	7.7	8.0	7.3	7.7	7.8	8.1	6.8
	Salinity (ppt)	25.4	25.5	25.5	25.6	25.3	25.6	25.5	25.6
	Temperature (°C)	25.2	26.2	25.9	26.3	25.7	26.3	25.6	26.0
500 mg KCl/L	pH (S.U.)	7.97	7.87	8.02	7.83	8.00	7.91	7.98	7.86
	DO (mg/L)	7.8	7.7	8.0	7.5	7.9	8.0	8.1	7.0
	Salinity (ppt)	25.5	25.6	25.6	25.7	25.3	25.8	25.6	25.6
	Temperature (°C)	25.3	26.2	25.8	26.3	25.7	26.3	25.5	26.0
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)						04-07-23		
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



Test date

- ● **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} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic IC_{25} ± $S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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 Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**10-05-21**	**0.4340**	-0.3625	-0.3710	0.0140	0.4256	0.3990	0.4539	0.3631	0.4922	0.2894	0.5617
2	**11-02-21**	**0.4283**	-0.3683	-0.3708	0.0140	0.4258	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
3	**12-07-21**	**0.4422**	-0.3544	-0.3695	0.0143	0.4271	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
4	**01-04-22**	**0.4482**	-0.3485	-0.3678	0.0146	0.4288	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
5	**02-08-22**	**0.4370**	-0.3595	-0.3673	0.0147	0.4293	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note:

7-day IC_{25} = 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 IC_{25} values.

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

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

Warning Limits = Mean logarithmic IC_{25} ± 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.

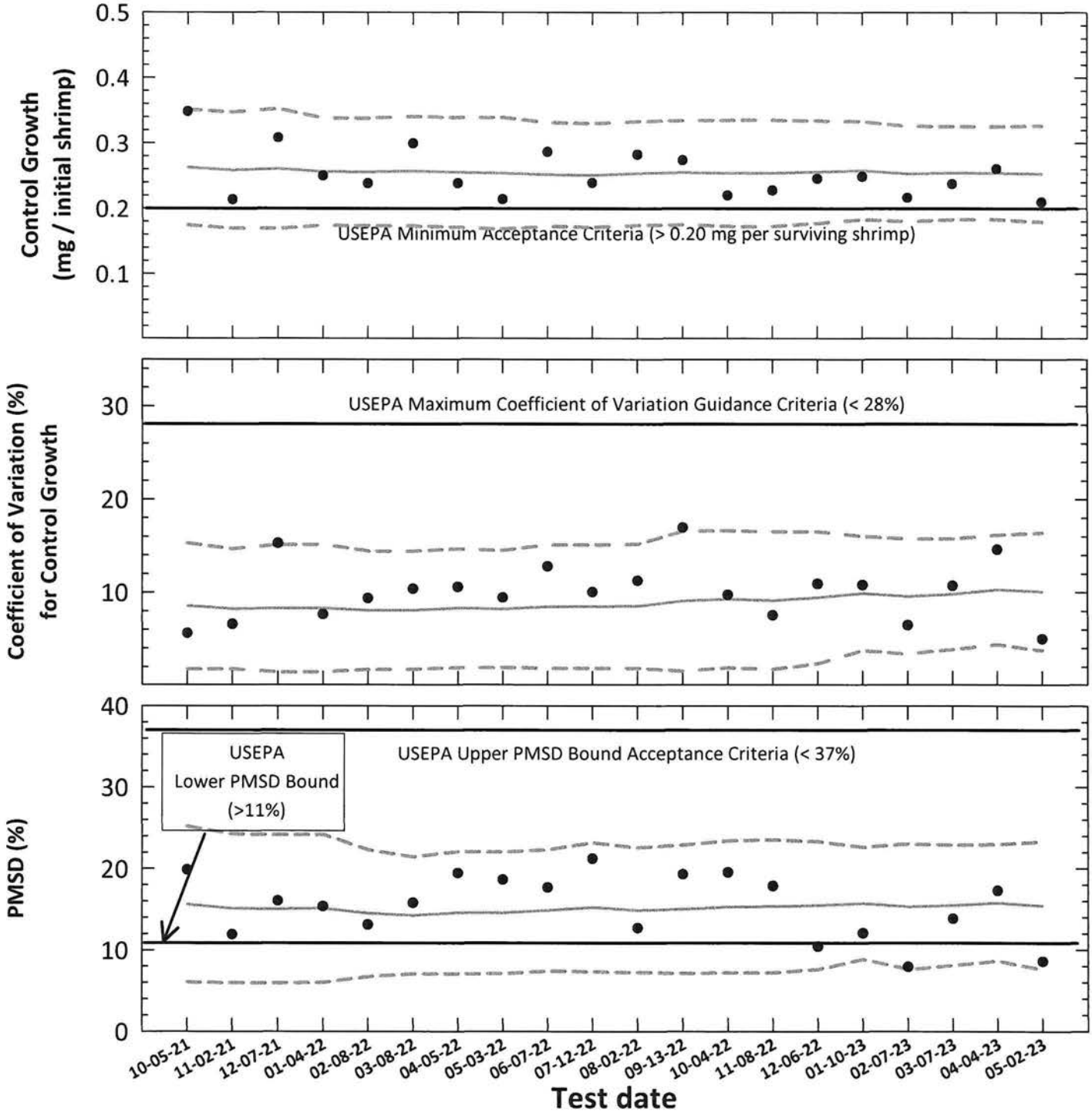
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
			Mean	CV	MSD	PMSD	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
		(%)	(mg/initial shrimp)	(%)		(%)									
1	10-05-21	100	0.348	5.6	0.0691	19.8	0.263	0.174	0.351	8.5	1.8	15.3	15.6	6.1	25.2
2	11-02-21	100	0.214	6.6	0.0254	11.9	0.258	0.169	0.347	8.2	1.7	14.7	15.1	6.0	24.2
3	12-07-21	100	0.308	15.3	0.0495	16.1	0.261	0.169	0.353	8.3	1.5	15.1	15.0	5.9	24.1
4	01-04-22	100	0.250	7.6	0.0384	15.4	0.256	0.174	0.338	8.3	1.5	15.1	15.1	6.0	24.2
5	02-08-22	100	0.238	9.4	0.0312	13.1	0.256	0.174	0.338	8.1	1.7	14.4	14.5	6.7	22.3
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Americamysis (Mysidopsis) bahia*

AbKClCR Test Number: **239**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2176				
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:	04-25-23 1200 to 04-26-23 1130	Incubator number and shelf location:	SE
Organism source:	AI Batch Ab: 04-26-23	*Artemia* CHM number:	CHM1222
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.82 S.U. Temperature = 25.0 °C	Date / Time in oven:	05-04-23 1100
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	05-10-23 1100
		*Final oven temperature:	60°C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	05-02-23	1106	JK	1230	JK	1225	JK	04-26-23 A
1	05-03-23	0500	JK	1100	JK	1026	JK	↓
2	05-04-23	0500	JK	1200	JK	1140	JK	04-26-23 B
3	05-05-23	0500	JK	1100	JK	1025	JK	↓
4	05-06-23	0610	JK	1210	JK	1141	JK	05-04-23
5	05-07-23	0600	JK	1200	JK	1025	JK	↓
6	05-08-23	0600	JK	1200	JK	1025	JK	↓
7	05-09-23					1038	JK	

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
% Mortality:	07.	≤ 20%
Average weight per initial shrimp:	0.210	
Average weight per surviving shrimp:	0.210	≥ 0.20 mg/shrimp

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	482.0
NOEC (mg/L KCl)	250
LOEC (mg/L KCl)	375
ChV (mg/L KCl)	306.2
IC_{25} (mg/L KCl)	416.3

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **239**

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: Ruby Analyst: BL Date: 04-21-23	14.62	14.88	13.73	15.07	12.87	BL 04-21-23 ~~12.87~~ 14.55	13.02	13.83	15.15	14.66	13.45	15.27	15.09	16.09	13.41	15.0
*B = Pan + Shrimp weight (mg) Analyst: JK Date: 05-15-23	15.64	15.90	14.79	16.08	13.92	15.57	14.05	15.00	16.28	15.93	14.50	16.31	16.15	17.14	14.51	16.8
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JK	1.02	1.02	1.06	1.01	1.05	1.02	1.03	1.17	1.13	1.27	1.05	1.04	1.06	1.05	1.10	1.0
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JK	0.204	0.204	0.212	0.202	0.210	0.204	0.206	0.234	0.226	0.254	0.210	0.208	0.212	0.210	0.220	0.20
	Average weight per initial number of shrimp (mg) 0.210								Average weight per initial number of shrimp (mg) 0.218				Percent reduction from control (%) -3.97			

*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:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **239**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	4 1d	4 1d	4 1d	S	S	4 1d	4 1d	4 1d
3	6	5	4 1d	5	4 1d	5	5	4 1d	4	4	3 1d	3 2d	3 2d	2 2d	3 1d	2 2d
4	4 1d	5	4	5	4	5	4 1d	4	3 1d	2 2d	3	3	2 1d	2	2 1d	2
5	4	5	4	5	4	5	4	4	3	2	3	3	2	2	2	2
6	4	5	4	5	4	5	4	4	3	2	3	3	2	2	2	2
7	4	5	4	5	4	5	4	4	3	2	3	3	2	2	2	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																5-04
*A = Pan weight (mg) Tray color code: Ruby Analyst: BL Date: 04-21-23	14.86	12.26	14.03	13.76	13.43	15.68	13.77	13.48	13.64	14.83	15.58	14.41	13.90	13.44	13.93	14.9
*B = Pan + Shrimp weight (mg) Analyst: JL Date: 05.15.23	15.78	13.42	14.90	15.26	14.34	16.31	14.81	14.31	14.32	15.19	16.23	14.79	14.25	13.78	14.35	15.21
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	0.92	1.16	0.87	1.50	0.91	0.63	1.04	0.83	0.68	0.36	0.65	0.38	0.35	0.34	0.42	0.2
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.184	0.232	0.174	0.300	0.182	0.126	0.208	0.166	0.136	0.072	0.130	0.076	0.070	0.068	0.084	0.05
	Average weight per initial number of shrimp (mg) 0.197				Percent reduction from control (%) 6.27				Average weight per initial number of shrimp (mg) 0.087				Percent reduction from control (%) 58.67			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **239**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males															05-03-23 JH	
*A = Pan weight (mg) Tray color code: Ruby Analyst: BL Date: 04-21-23	14.81	15.40	15.71	14.85	13.72	13.16	14.38	BL 04.21.23 ~~13.63~~ 15.77	12.48	12.70	13.90	15.59	15.70	15.02	14.69	12.5
*B = Pan + Shrimp weight (mg) Analyst: Date:																
C = Shrimp weight (mg) = B – A Hand calculated Analyst:															05-03-23 JH	
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 100%				Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 100%			

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

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

Comments:

Environmental Testing Solutions, Inc.

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: May 02-09, 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 fro control (%)
Control	A	5	5	14.62	15.64	1.02	0.204	100.0	0.210	5.0	Not applicable
	B	5	5	14.88	15.90	1.02	0.204				
	C	5	5	13.73	14.79	1.06	0.212				
	D	5	5	15.07	16.08	1.01	0.202				
	E	5	5	12.87	13.92	1.05	0.210				
	F	5	5	14.55	15.57	1.02	0.204				
	G	5	5	13.02	14.05	1.03	0.206				
	H	5	5	13.83	15.00	1.17	0.234				
250	I	5	5	15.15	16.28	1.13	0.226	100.0	0.218	7.5	-3.9
	J	5	5	14.66	15.93	1.27	0.254				
	K	5	5	13.45	14.50	1.05	0.210				
	L	5	5	15.27	16.31	1.04	0.208				
	M	5	5	15.09	16.15	1.06	0.212				
	N	5	5	16.09	17.14	1.05	0.210				
	O	5	5	13.41	14.51	1.10	0.220				
	P	5	5	15.06	16.07	1.01	0.202				
375	Q	5	4	14.86	15.78	0.92	0.184	87.5	0.197	26.4	6.2
	R	5	5	12.26	13.42	1.16	0.232				
	S	5	4	14.03	14.90	0.87	0.174				
	T	5	5	13.76	15.26	1.50	0.300				
	U	5	4	13.43	14.34	0.91	0.182				
	V	5	5	15.68	16.31	0.63	0.126				
	W	5	4	13.77	14.81	1.04	0.208				
	X	5	4	13.48	14.31	0.83	0.166				
500	Y	5	3	13.64	14.32	0.68	0.136	47.5	0.087	34.0	58.6
	Z	5	2	14.83	15.19	0.36	0.072				
	AA	5	3	15.58	16.23	0.65	0.130				
	BB	5	3	14.41	14.79	0.38	0.076				
	CC	5	2	13.90	14.25	0.35	0.070				
	DD	5	2	13.44	13.78	0.34	0.068				
	EE	5	2	13.93	14.35	0.42	0.084				
	FF	5	2	14.92	15.21	0.29	0.058				
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: 0.0180

PMSD: 8.6

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (10th percentile) = 11%.
Upper PMSD bound determined by USEPA (90th percentile) = 37%
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	5/2/2023	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2023	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	0.8000	1.0000	0.8000	1.0000	0.8000	0.8000
500	0.6000	0.4000	0.6000	0.6000	0.4000	0.4000	0.4000	0.4000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*375	0.8750	0.8750	1.1964	1.1071	1.3453	10.301	8	48.00	48.00	5	40
*500	0.4750	0.4750	0.7602	0.6847	0.8861	13.708	8	36.00	48.00	21	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.80385	0.904	0.77467	-0.2527
Equality of variance cannot be confirmed				

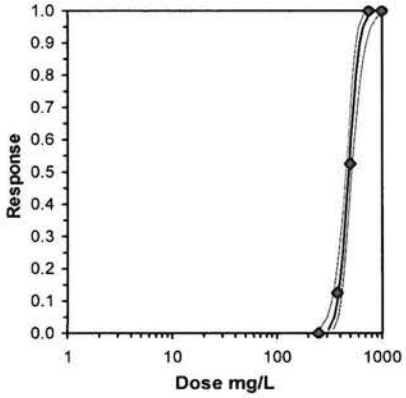
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	250	375	306.186	
Treatments vs D-Control				

Maximum Likelihood-Probit

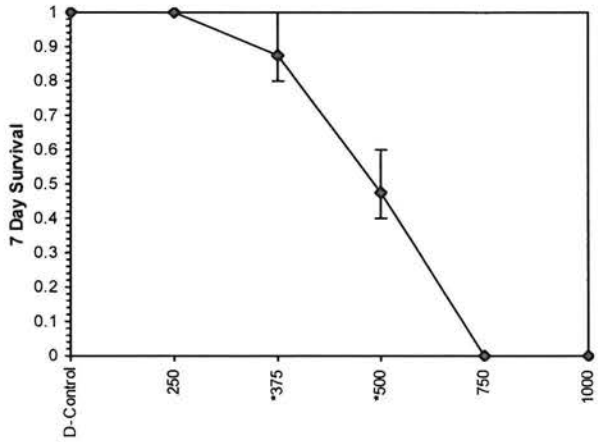
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.7608	1.80417	8.22459	15.2969	0	1.18185	7.81472	0.75736	2.68301	0.08503	5
Intercept	-26.554	4.82175	-36.005	-17.104							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	305.639	250.977	341.829
EC05	3.355	349.265	302.208	380.671
EC10	3.718	375.013	333.067	403.874
EC15	3.964	393.448	355.194	420.861
EC20	4.158	408.745	373.411	435.351
EC25	4.326	422.341	389.369	448.655
EC40	4.747	458.641	430.002	487.012
EC50	5.000	481.964	454.008	514.413
EC60	5.253	506.473	477.368	545.617
EC75	5.674	550.003	515.206	606.034
EC80	5.842	568.298	530.142	632.896
EC85	6.036	590.392	547.657	666.251
EC90	6.282	619.416	570.005	711.369
EC95	6.645	665.08	604.059	784.904
EC99	7.326	760.012	671.965	946.152



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	5/2/2023	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2023	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	0.2040	0.2040	0.2120	0.2020	0.2100	0.2040	0.2060	0.2340
250	0.2260	0.2540	0.2100	0.2080	0.2120	0.2100	0.2200	0.2020
375	0.1840	0.2320	0.1740	0.3000	0.1820	0.1260	0.2080	0.1660
500	0.1360	0.0720	0.1300	0.0760	0.0700	0.0680	0.0840	0.0580
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	0.2095	1.0000	0.2095	0.2020	0.2340	4.993	8			0.2136	1.0000
250	0.2178	1.0394	0.2178	0.2020	0.2540	7.533	8	81.50	45.00	0.2136	1.0000
375	0.1965	0.9379	0.1965	0.1260	0.3000	26.428	8			0.1965	0.9198
500	0.0868	0.4141	0.0868	0.0580	0.1360	34.016	8			0.0868	0.4061
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

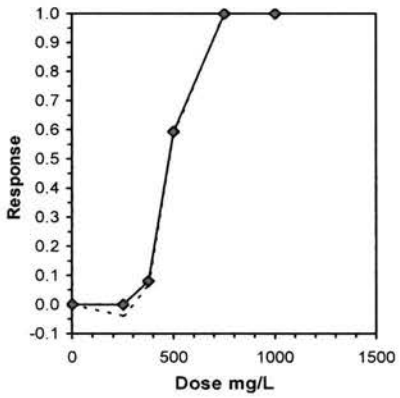
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.78957	0.844	1.81787	3.24239
F-Test indicates equal variances (p = 0.26)	2.45888	8.88531		

Hypothesis Test (1-tail, 0.01)

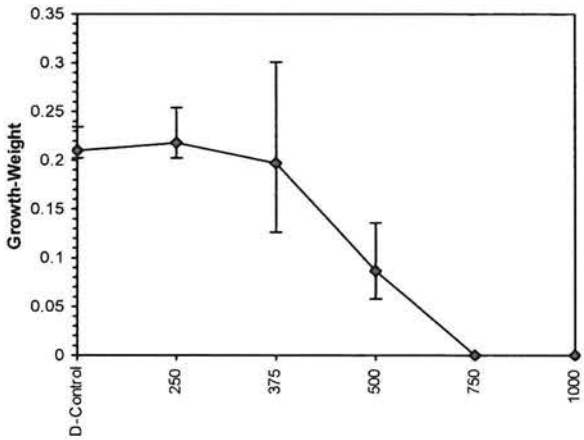
Wilcoxon Two-Sample Test indicates no significant differences
Treatments vs D-Control

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	327.97	42.47	270.92	385.95	0.2241
IC10	379.83	32.19	299.64	396.89	-0.4881
IC15	391.99	21.92	328.12	408.11	-0.9827
IC20	404.16	16.24	357.59	419.15	-0.9907
IC25	416.32	13.65	381.97	430.56	-0.7772
IC40	452.82	9.92	430.25	468.44	-0.3084
IC50	477.15	10.24	458.73	496.56	0.5993



Dose-Response Plot



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Environmental Testing Solutions, Inc.

AbKClCR Test Number: **239**

Daily Chemistry:

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

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	JP BL	JP BL N	JP BL N	N	N	N
CONTROL, Salt SW	pH (S.U.)	7.87	7.80	7.86	7.70	7.86	7.70
	DO (mg/L)	7.9	7.6	7.8	7.7	7.9	7.7
	Salinity (ppt)	25.0	24.9	25.0	25.5	25.2	25.4
	Alkalinity (mg $CaCO_3$/L)	97		N 05.03.23		110	
	Temperature (°C)	25.5	26.2	25.2	26.1	25.3	26.3
250 mg KCl/L	pH (S.U.)	7.89	7.81	7.87	7.69	7.85	7.69
	DO (mg/L)	7.8	7.6	7.8	7.7	8.0	7.7
	Salinity (ppt)	25.1	25.6	25.2	25.5	25.1	25.4
	Temperature (°C)	25.7	26.0	25.1	26.2	25.4	26.1
375 mg KCl/L	pH (S.U.)	7.90	7.82	7.89	7.70	7.86	7.69
	DO (mg/L)	7.9	7.6	7.8	7.8	8.0	7.7
	Salinity (ppt)	25.2	25.8	25.4	25.7	25.5	25.8
	Temperature (°C)	25.7	26.0	25.1	26.2	25.4	26.1
500 mg KCl/L	pH (S.U.)	7.90	7.81	7.90	7.71	7.87	7.76
	DO (mg/L)	8.0	7.6	7.8	7.8	8.2	7.8
	Salinity (ppt)	25.3	25.6	25.4	25.7	25.6	25.9
	Temperature (°C)	25.6	26.1	25.1	26.0	25.4	26.2
750 mg KCl/L	pH (S.U.)	7.90	7.86				
	DO (mg/L)	8.0	7.7				
	Salinity (ppt)	25.3	25.8				
	Temperature (°C)	25.6	26.2				
1000 mg KCl/L	pH (S.U.)	7.90	7.85				
	DO (mg/L)	8.0	7.7			N 05.03.23	
	Salinity (ppt)	25.6	26.1				
	Temperature (°C)	25.6	26.3				
		Initial	Final	Initial	Final	Initial	Final

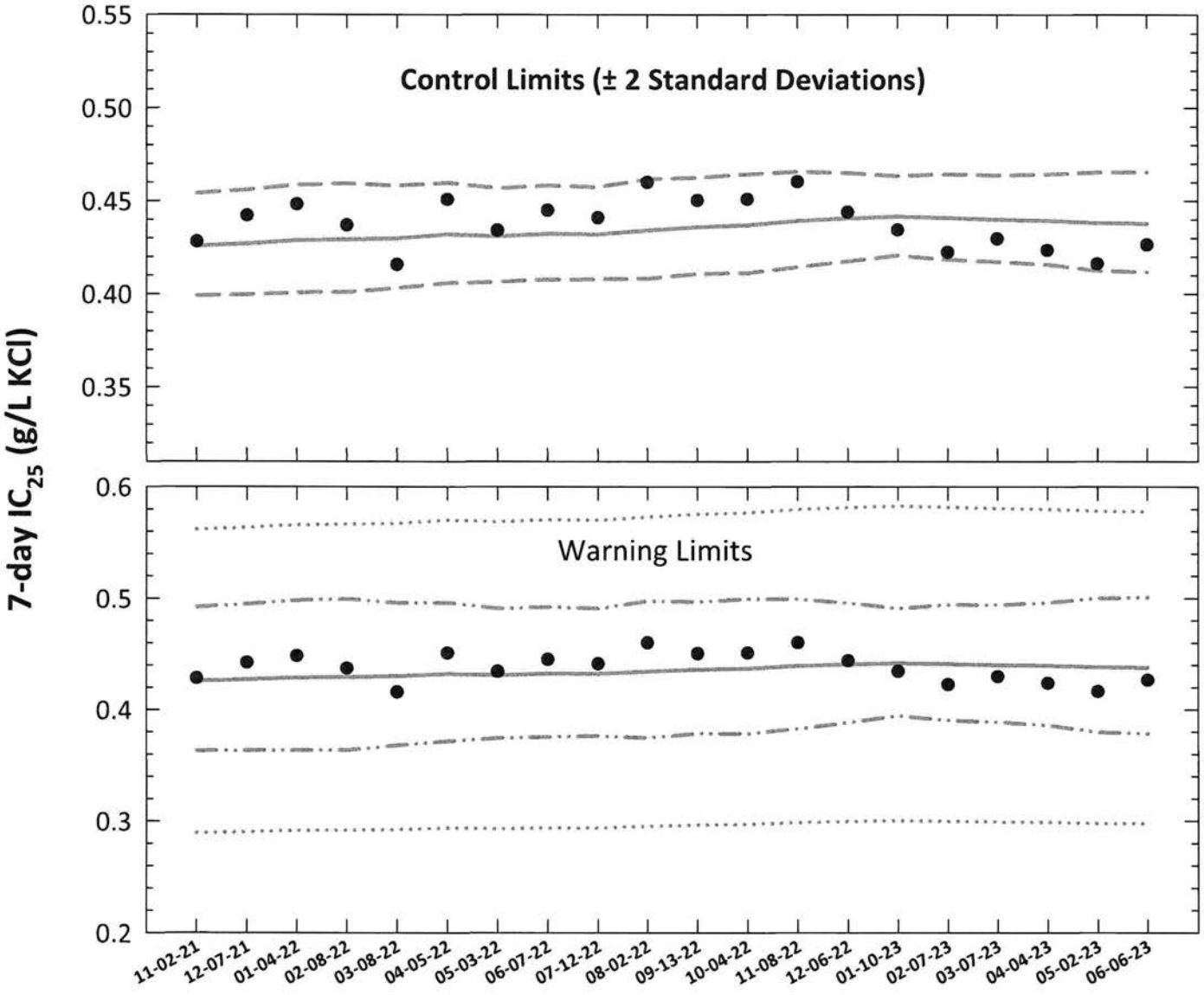
Environmental Testing Solutions, Inc.

AbKClCR Test Number: **239**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 3		4		5		6	
	Analyst	[illegible]	BSL	BSL	BSL	BSL	JPBL [illegible]	JPBL [illegible]	[illegible]
CONTROL, Salt SW	pH (S.U.)	7.98	7.71	7.91	7.68	7.89	7.73	7.88	7.33
	DO (mg/L)	7.6	7.5	8.0	7.7	7.7	7.0	7.7	5.2
	Salinity (ppt)	25.0	25.7	25.2	25.4	24.9	25.4	25.0	25.1
	Alkalinity (mg $CaCO_3$/L)			100					
	Temperature (°C)	25.5	26.3	25.3	25.2	25.2	25.7	25.2	26.3
250 mg KCl/L	pH (S.U.)	7.96	7.76	7.89	7.71	7.88	7.77	7.91	7.54
	DO (mg/L)	7.6	7.6	7.9	7.7	7.7	7.1	7.6	5.4
	Salinity (ppt)	25.5	25.6	25.5	25.7	25.7	25.9	25.4	25.8
	Temperature (°C)	25.6	26.2	25.4	25.3	25.2	25.6	25.2	26.4
375 mg KCl/L	pH (S.U.)	7.96	7.74	7.90	7.71	7.89	7.79	7.90	7.50
	DO (mg/L)	7.5	7.6	7.9	7.7	7.9	7.1	7.4	5.6
	Salinity (ppt)	25.5	25.7	25.6	25.8	25.7	26.1	26.1	26.2
	Temperature (°C)	25.4	26.2	25.4	25.3	25.2	26.0	25.2	26.2
500 mg KCl/L	pH (S.U.)	7.96	7.79	7.90	7.74	7.89	7.78	7.91	7.64
	DO (mg/L)	7.5	7.5	7.9	7.7	7.9	7.3	7.6	6.4
	Salinity (ppt)	25.5	25.7	25.7	25.9	25.8	26.0	26.1	26.2
	Temperature (°C)	25.4	26.2	25.3	25.4	25.2	26.0	25.2	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)						OS-03-[illegible]		
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

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} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic IC_{25} ± $S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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 Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**11-02-21**	**0.4283**	-0.3683	-0.3708	0.0140	0.4258	0.3992	0.4542	0.3633	0.4924	0.2895	0.5620
2	**12-07-21**	**0.4422**	-0.3544	-0.3695	0.0143	0.4271	0.3999	0.4561	0.3634	0.4950	0.2904	0.5637
3	**01-04-22**	**0.4482**	-0.3485	-0.3678	0.0146	0.4288	0.4008	0.4587	0.3636	0.4985	0.2916	0.5660
4	**02-08-22**	**0.4370**	-0.3595	-0.3673	0.0147	0.4293	0.4011	0.4594	0.3636	0.4995	0.2919	0.5666
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note:

7-day IC_{25} = 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 IC_{25} values.

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

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

Warning Limits = Mean logarithmic IC_{25} ± 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.

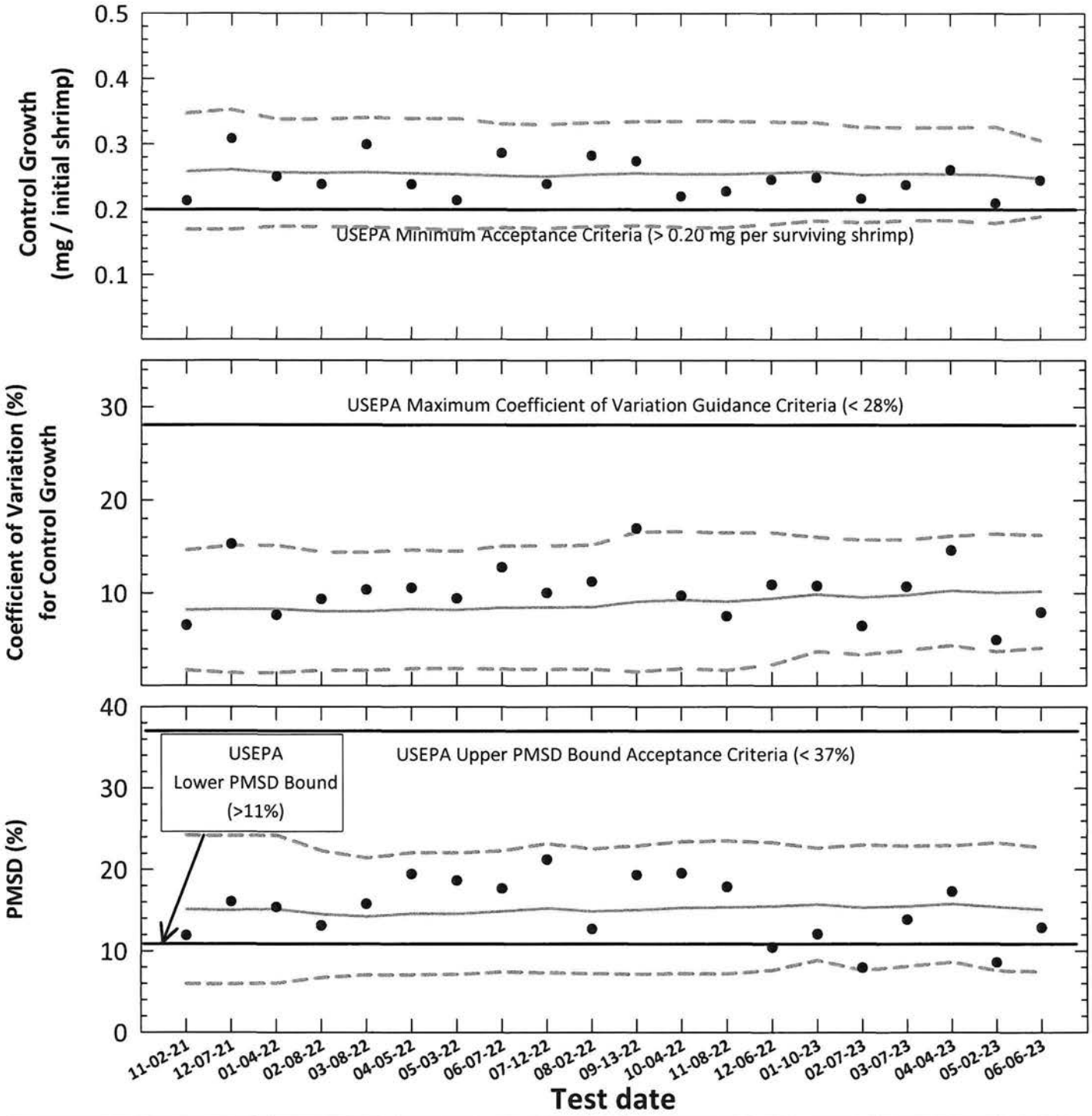
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		Survival (%)	Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	11-02-21	100	0.214	6.6	0.0254	11.9	0.258	0.169	0.347	8.2	1.7	14.7	15.1	6.0	24.2
2	12-07-21	100	0.308	15.3	0.0495	16.1	0.261	0.169	0.353	8.3	1.5	15.1	15.0	5.9	24.1
3	01-04-22	100	0.250	7.6	0.0384	15.4	0.256	0.174	0.338	8.3	1.5	15.1	15.1	6.0	24.2
4	02-08-22	100	0.238	9.4	0.0312	13.1	0.256	0.174	0.338	8.1	1.7	14.4	14.5	6.7	22.3
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Species: *Americamysis (Mysidopsis) bahia*

AbKClCR Test Number: **240**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2176				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	Grey
Date and times organisms were born between:	05-30-23 1200 to 05-31-23 1130	Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 05-31-23	*Artemia* CHM number:	CHM1222
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.82 S.U. Temperature = 25.0 ~~7.8~~ °C 06-06-23 JL	Date / Time in oven:	06-13-23 1100
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	06-14-23 1100
		*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		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	06-06-23	1015	JL	1230	JL	1130	JL	05-30-23 B
1	06-07-23	0500	JL	1100	JL	0930	JL	↓
2	06-08-23	0500	JL	1230	JL	1130	JL	06-05-23 ↓
3	06-09-23	0500	JL	1100	JL	0930	JL	↓
4	06-10-23	0610	JL	1210	JL	1110	JL	06-08-23
5	06-11-23	0600	JL	1200	JL	0950	JL	↓
6	06-12-23	0600	JL	1200	JL	0950	JL	
7	06-13-23					0952	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	130466465

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	499.6
Average weight per initial shrimp:	0.245		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.245	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	426.5

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **240**

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																06-14-23
*A = Pan weight (mg) Tray color code: Lavender Analyst: BL Date: 05-22-23	15.30	14.12	14.82	14.79	13.63	14.92	12.38	12.80	12.94	13.97	14.73	14.87	13.40	13.72	14.33	14.6
*B = Pan + Shrimp weight (mg) Analyst: JL Date: 06-18-23	16.63	15.34	15.92	16.05	14.81	16.26	13.46	14.07	14.24	15.18	16.04	16.04	14.60	15.03	15.55	15.8
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	1.33	1.22	1.10	1.26	1.18	1.34	1.08	1.27	1.30	1.21	1.31	1.17	1.20	1.31	1.22	1.25
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.266	0.244	0.220	0.252	0.236	0.268	0.216	0.254	0.260	0.242	0.262	0.234	0.240	0.262	0.244	0.25
	Average weight per initial number of shrimp (mg) 0.245								Average weight per initial number of shrimp (mg) 0.249				Percent reduction from control (%) -1.97.			

*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:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **240**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	4d	4d	S	4d	S	S	S	S
3	S	S	S	S	S	S	S	S	4	3d	4d	3d	3d	3d	3d	4d
4	S	S	S	S	S	S	S	S	4	3	4	3	3	3	3	4
5	S	S	S	S	4d	S	S	S	3d	3	4	3	3	3	1d	4
6	S	S	S	S	4	S	S	4d	3	3	4	3	3	3	1	4
7	S	S	S	S	4	S	S	4	2	3	2	3	3	3	1	4
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Lavender Analyst: BL Date: 05·22·23	14.11	14.16	13.37	13.89	14.55	15.07	13.57	15.50	12.89	13.90	14.51	14.99	12.53	15.21	15.95	15.0
*B = Pan + Shrimp weight (mg) Analyst: JL Date: 06·15·23	15.49	15.34	14.53	15.09	15.55	16.37	14.67	16.38	13.33	14.52	14.94	15.89	13.13	15.83	16.25	16.0
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	1.38	1.18	1.16	1.20	1.00	1.30	1.10	0.88	0.44	0.62	0.43	0.90	0.60	0.62	0.30	0.9
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.276	0.236	0.232	0.240	0.200	0.260	0.220	0.176	0.088	0.124	0.086	0.180	0.120	0.124	0.060	0.18
	Average weight per initial number of shrimp (mg) 0.230				Percent reduction from control (%) 5.97.				Average weight per initial number of shrimp (mg) 0.121				Percent reduction from control (%) 50.47.			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **240**

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:_____															06-07-23	
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				Percent reduction from control (%) 100%				Average weight per initial number of shrimp (mg) 0				Percent reduction from control (%) 100%			

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

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

Comments:

Environmental Testing Solutions, Inc.

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: June 06-13, 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 fro control (%)
Control	A	5	5	15.30	16.63	1.33	0.266	100.0	0.245	8.0	Not applicable
	B	5	5	14.12	15.34	1.22	0.244				
	C	5	5	14.82	15.92	1.10	0.220				
	D	5	5	14.79	16.05	1.26	0.252				
	E	5	5	13.63	14.81	1.18	0.236				
	F	5	5	14.92	16.26	1.34	0.268				
	G	5	5	12.38	13.46	1.08	0.216				
	H	5	5	12.80	14.07	1.27	0.254				
250	I	5	5	12.94	14.24	1.30	0.260	100.0	0.249	4.4	-1.9
	J	5	5	13.97	15.18	1.21	0.242				
	K	5	5	14.73	16.04	1.31	0.262				
	L	5	5	14.87	16.04	1.17	0.234				
	M	5	5	13.40	14.60	1.20	0.240				
	N	5	5	13.72	15.03	1.31	0.262				
	O	5	5	14.33	15.55	1.22	0.244				
	P	5	5	14.64	15.89	1.25	0.250				
375	Q	5	5	14.11	15.49	1.38	0.276	95.0	0.230	13.8	5.9
	R	5	5	14.16	15.34	1.18	0.236				
	S	5	5	13.37	14.53	1.16	0.232				
	T	5	5	13.89	15.09	1.20	0.240				
	U	5	4	14.55	15.55	1.00	0.200				
	V	5	5	15.07	16.37	1.30	0.260				
	W	5	5	13.57	14.67	1.10	0.220				
	X	5	4	15.50	16.38	0.88	0.176				
500	Y	5	2	12.89	13.33	0.44	0.088	52.5	0.121	36.9	50.4
	Z	5	3	13.90	14.52	0.62	0.124				
	AA	5	2	14.51	14.94	0.43	0.086				
	BB	5	3	14.99	15.89	0.90	0.180				
	CC	5	3	12.53	13.13	0.60	0.120				
	DD	5	3	15.21	15.83	0.62	0.124				
	EE	5	1	15.95	16.25	0.30	0.060				
	FF	5	4	15.06	16.00	0.94	0.188				
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: 0.0314

PMSD: 12.8

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.

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.

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	6/6/2023	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/13/2023	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	1.0000	0.8000	1.0000	1.0000	0.8000
500	0.4000	0.6000	0.4000	0.6000	0.6000	0.6000	0.2000	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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.5250	0.5250	0.8106	0.4636	1.1071	23.904	8	36.00	48.00	19	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

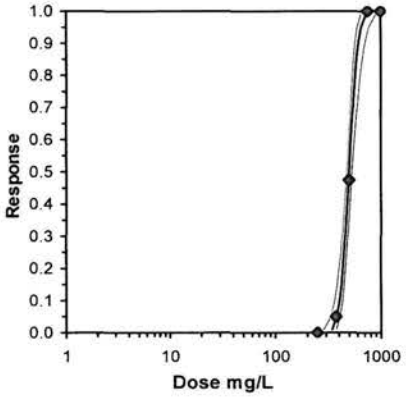
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.80535	0.904	-0.8122	4.44899
Equality of variance cannot be confirmed				

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

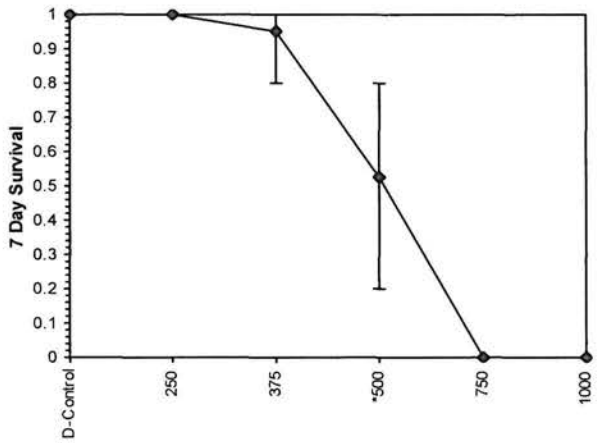
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1605	2.41274	9.43149	18.8894	0	0.50117	7.81472	0.91864	2.69864	0.07062	4
Intercept	-33.214	6.47316	-45.901	-20.527							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	342.257	285.126	377.227
EC05	3.355	382.366	335.049	411.97
EC10	3.718	405.635	364.451	432.602
EC15	3.964	422.129	385.2	447.721
EC20	4.158	435.715	402.044	460.668
EC25	4.326	447.719	416.599	472.621
EC40	4.747	479.453	452.675	507.448
EC50	5.000	499.617	473.316	532.478
EC60	5.253	520.629	493.03	560.859
EC75	5.674	557.531	524.464	615.118
EC80	5.842	572.89	536.748	638.958
EC85	6.036	591.329	551.076	668.345
EC90	6.282	615.374	569.237	707.758
EC95	6.645	652.823	596.661	771.264
EC99	7.326	729.326	650.498	907.863



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	6/6/2023	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/13/2023	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	0.2660	0.2440	0.2200	0.2520	0.2360	0.2680	0.2160	0.2540
250	0.2600	0.2420	0.2620	0.2340	0.2400	0.2620	0.2440	0.2500
375	0.2760	0.2360	0.2320	0.2400	0.2000	0.2600	0.2200	0.1760
500	0.0880	0.1240	0.0860	0.1800	0.1200	0.1240	0.0600	0.1880
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

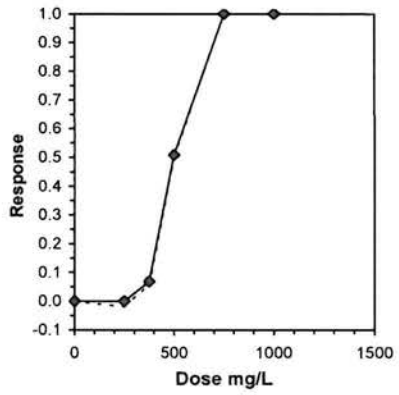
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2445	1.0000	0.2445	0.2160	0.2680	7.952	8				0.2469	1.0000
250	0.2493	1.0194	0.2493	0.2340	0.2620	4.394	8	-0.424	2.799	0.0314	0.2469	1.0000
375	0.2300	0.9407	0.2300	0.1760	0.2760	13.820	8	1.293	2.799	0.0314	0.2300	0.9316
500	0.1213	0.4959	0.1213	0.0600	0.1880	36.915	8				0.1213	0.4911
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)	0.97945	0.884	-0.3552	0.93533
Bartlett's Test indicates equal variances (p = 0.03)	6.71656	9.21035		

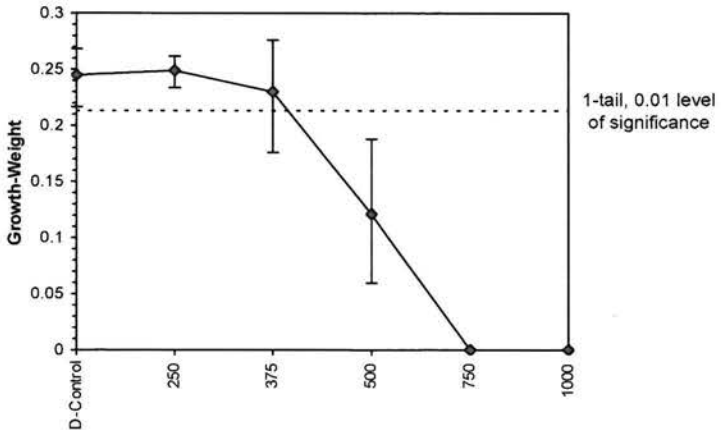
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	>375			0.03137	0.12832	0.0008	0.0005	0.2255	2, 21

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	341.44	38.93	271.88	386.86	-0.4548
IC10	383.98	20.46	329.09	399.77	-1.2201
IC15	398.17	11.21	376.37	414.78	-0.6060
IC20	412.36	10.22	391.66	430.17	-0.0531
IC25	426.54	10.10	406.87	445.41	0.2653
IC40	469.11	12.59	449.99	495.88	0.7540
IC50	497.49	17.75	474.35	537.52	0.7352



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **240**

Daily Chemistry:

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

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	JP BL	JP BLK	JP BLK	JP K	JP K	K
CONTROL, Salt SW	pH (S.U.)	7.91	7.82	7.90	7.75	7.95	7.75
	DO (mg/L)	7.6	7.6	7.9	6.9	7.0	6.4
	Salinity (ppt)	25.0	25.0	25.0	24.4	25.0	25.1
	Alkalinity (mg $CaCO_3$/L)	110		→ 06 13:23		130	
	Temperature (°C)	25.2	26.3	25.2	26.5	25.2	25.8
250 mg KCl/L	pH (S.U.)	7.88	7.85	7.88	7.82	7.94	7.70
	DO (mg/L)	7.5	7.6	7.9	7.0	7.0	6.4
	Salinity (ppt)	25.1	25.5	25.1	25.4	25.2	25.6
	Temperature (°C)	25.4	26.5	25.2	26.6	25.2	26.2
375 mg KCl/L	pH (S.U.)	7.90	7.87	7.89	7.82	7.94	7.72
	DO (mg/L)	7.5	7.5	7.9	7.2	7.0	6.4
	Salinity (ppt)	25.2	25.5	25.3	25.6	25.5	25.7
	Temperature (°C)	25.4	26.5	25.3	26.2	25.2	26.3
500 mg KCl/L	pH (S.U.)	7.91	7.87	7.89	7.83	7.94	7.73
	DO (mg/L)	7.5	7.5	7.9	7.3	7.9	7.1
	Salinity (ppt)	25.3	25.5	25.4	25.8	25.5	26.1
	Temperature (°C)	25.4	26.4	25.3	26.6	25.2	26.3
750 mg KCl/L	pH (S.U.)	7.90	7.86				
	DO (mg/L)	7.6	7.7				
	Salinity (ppt)	25.3	25.7				
	Temperature (°C)	25.4	26.2				
1000 mg KCl/L	pH (S.U.)	7.91	7.87				
	DO (mg/L)	7.6	7.6			06.07-25	
	Salinity (ppt)	25.5	26.0				
	Temperature (°C)	25.3	26.4				
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

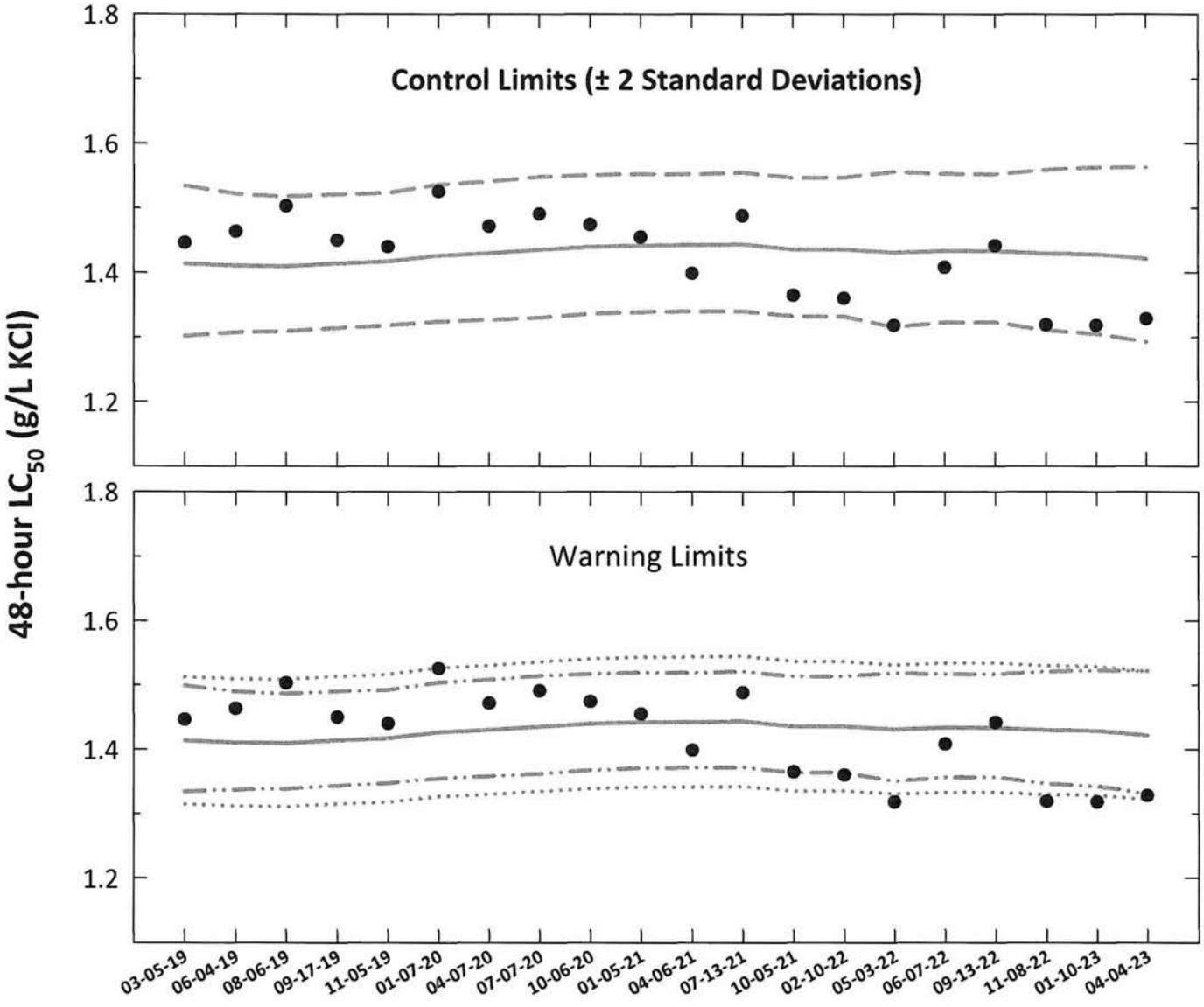
AbKClCR Test Number: **240**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 3		4		5		6	
	Analyst	N	BSL	BSL	BSL	BSL	NBLN	NBLN	N
CONTROL, Salt SW	pH (S.U.)	8.01	7.90	8.04	7.84	~~7.90~~ (7.99)	7.85	8.04	7.66
	DO (mg/L)	7.9	7.8	7.9	7.4	8.0	7.4	8.0	6.3
	Salinity (ppt)	25.3	25.1	25.2	25.4	24.8	25.1	25.0	25.0
	Alkalinity (mg $CaCO_3$/L)	→ 06.13.23		120		→		→ 06.13.23	
	Temperature (°C)	25.2	25.9	25.1	26.3	25.1	26.4	25.1	26.4
250 mg KCl/L	pH (S.U.)	7.90	7.92	8.01	7.79	~~7.93~~ (7.98)	7.84	8.04	7.73
	DO (mg/L)	7.7	7.8	7.9	7.5	8.0	7.7	8.0	6.2
	Salinity (ppt)	25.2	25.4	25.2	25.4	25.0	25.9	25.5	25.7
	Temperature (°C)	25.3	26.3	25.3	26.5	25.2	26.3	25.2	26.1
375 mg KCl/L	pH (S.U.)	7.90	7.90	8.02	7.82	~~7.92~~ (7.99)	7.88	8.04	7.75
	DO (mg/L)	7.7	7.8	7.9	7.5	8.0	7.2	8.0	6.8
	Salinity (ppt)	25.3	25.3	25.3	25.5	25.0	26.0	26.0	25.9
	Temperature (°C)	25.5	26.2	25.2	26.3	25.1	26.3	25.2	26.1
500 mg KCl/L	pH (S.U.)	7.99	7.90	8.02	7.88	7.99	7.93	8.03	7.80
	DO (mg/L)	7.8	7.8	7.9	7.4	8.0	7.4	8.0	7.2
	Salinity (ppt)	25.4	25.4	25.0	25.7	25.0	26.1	26.0	26.0
	Temperature (°C)	25.5	26.4	25.2	26.3	25.1	26.5	25.2	26.3
750 mg KCl/L	pH (S.U.)	→							
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)					06.07.23			
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 06.11.23

ETS
Environmental Testing Solutions, Inc.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**03-05-19**	**1.4461**	0.1602	0.1503	0.0179	1.4134	1.3019	1.5345	1.3345	1.4991	1.3145	1.5124
2	**06-04-19**	**1.4632**	0.1653	0.1494	0.0165	1.4105	1.3074	1.5216	1.3374	1.4893	1.3117	1.5092
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Luettmer

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Menidia beryllina*

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

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKClAC # 89

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 #: 2176

Chemical Analyses:

Concentration	Analyst	Hours 0	Hours 24	Hours 48
	Analyst	JL	BL JL	AP JL
Control, SaltSW	pH (S.U.)	7.87	7.87	7.81
	Dissolved oxygen (mg/L)	8.1	7.8	7.3
	*Salinity (ppt)	25.0	25.2	25.3
	*Alkalinity (mg/L $CaCO_3$)	100		
	*Temperature (°C)	25.1	24.9	25.0
1000 mg/L	pH (S.U.)	7.97	7.91	7.83
	Dissolved oxygen (mg/L)	7.9	7.7	7.4
	*Salinity (ppt)	25.8	25.7	26.3
	*Temperature (°C)	24.9	24.8	25.1
1250 mg/L	pH (S.U.)	7.97	7.90	7.85
	Dissolved oxygen (mg/L)	7.9	7.7	7.4
	*Salinity (ppt)	25.8	25.9	26.3
	*Temperature (°C)	24.9	25.0	25.1
1500 mg/L	pH (S.U.)	7.97	7.88	7.86
	Dissolved oxygen (mg/L)	7.9	7.8	7.3
	*Salinity (ppt)	26.0	26.0	26.4 (04-06-24)
	*Temperature (°C)	24.9	25.0	25.1
1750 mg/L	pH (S.U.)	7.98	7.86	7.82
	Dissolved oxygen (mg/L)	8.0	7.9	7.4
	*Salinity (ppt)	26.2	26.3	26.7
	*Temperature (°C)	25.0	25.0	25.1
2000 mg/L	pH (S.U.)	7.98	7.86	JL 04-05-23
	Dissolved oxygen (mg/L)	8.0	8.0	
	*Salinity (ppt)	26.2	26.4	
	*Temperature (°C)	25.0	24.9	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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 LC_{50} Whole Effluent Toxicity Test, Species: Menidia beryllina
EPA-821-R-02-012, Method 2006.0

Menidia beryllina Potassium Chloride Acute Reference Toxicant Test

MbKClAC # 89

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04.04.23	* 1020	JK	1230	JK	6 F	LIGHT BLUE	03.28.23 B
24	04.05.23			1225	JK			
48 Termination	04.06.23			1232	JK			

*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): Mb	03.25.23
Age (9 to 14 days old):	10 DAYS
Date organisms were born:	03.24.23 1200 TO 03.25.23 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.62
	Temperature (°C) 24.8

Survival Data (number of living organisms):

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	7 3d	7 3d	3 7d	2 8d	1 9d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	6 1d	6 1d	2 1d	2	0 1d	1 1d	0	0
Mean Survival	100%		100%		60%		20%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1303.4
Upper 95% confidence limit (mg KCl/L)	1461.0
48-hour LC_{50} (mg KCl/L)	1377.9

Comments:

Test Reviewed by: [signature]

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date:	4/4/2023	Test ID:	MbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/6/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.7000	0.7000
1500	0.3000	0.2000
1750	0.1000	0.2000
2000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1652	0	20
*1250	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	7.262	2.850	0.1652	6	20
*1500	0.2500	0.2500	0.5216	0.4636	0.5796	15.723	2	15.363	2.850	0.1652	15	20
*1750	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	17.588	2.850	0.1652	17	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

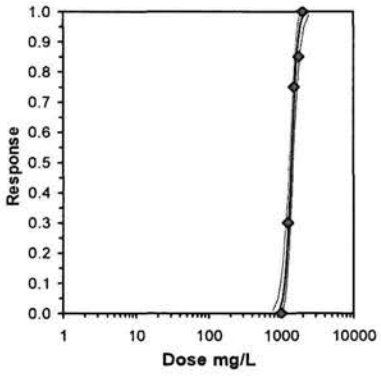
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.07633	0.07828	0.4613	0.00336	2.7E-05	4, 5
Treatments vs D-Control										

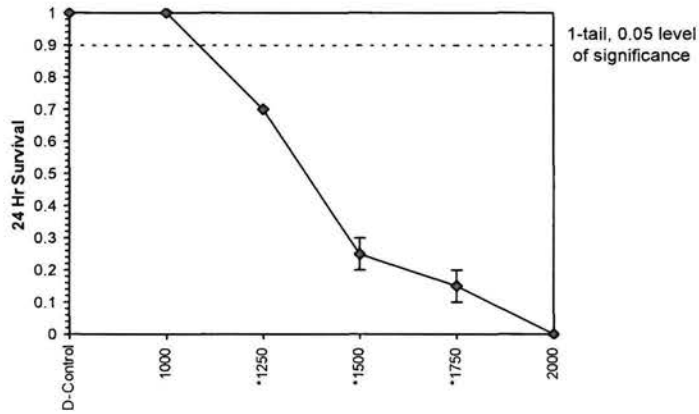
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.9755	2.28914	9.48873	18.4622	0	2.68354	7.81472	0.44303	3.14185	0.07155	4
Intercept	-38.909	7.22207	-53.064	-24.754							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	944.908	769.037	1056.28
EC05	3.355	1057.19	903.87	1154.4
EC10	3.718	1122.4	984.022	1211.78
EC15	3.964	1168.66	1041.27	1253.07
EC20	4.158	1206.78	1088.43	1287.72
EC25	4.326	1240.47	1129.88	1319.02
EC40	4.747	1329.6	1236.87	1406.56
EC50	5.000	1386.27	1301.25	1467.38
EC60	5.253	1445.36	1364.11	1536.31
EC75	5.674	1549.21	1463.76	1671.28
EC80	5.842	1592.47	1501.92	1731.97
EC85	6.036	1644.41	1545.82	1807.63
EC90	6.282	1712.18	1600.73	1910.12
EC95	6.645	1817.8	1682.56	2076.71
EC99	7.326	2033.8	1841.25	2437.62



Dose-Response Plot



ETS
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Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date:	4/4/2023	Test ID:	MbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/6/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.6000	0.6000
1500	0.2000	0.2000
1750	0.0000	0.1000
2000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
*1250	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	10.205	2.850	0.1469	8	20
*1500	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	2	18.402	2.850	0.1469	16	20
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	22.737	2.850	0.1469	19	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

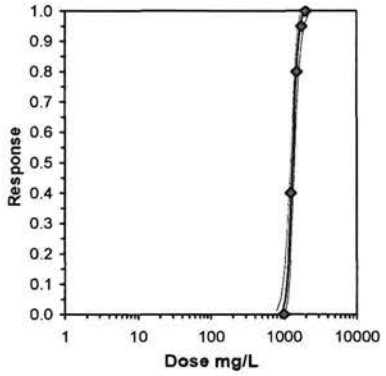
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.06555	0.06723	0.57434	0.00266	8.7E-06	4, 5
Treatments vs D-Control										

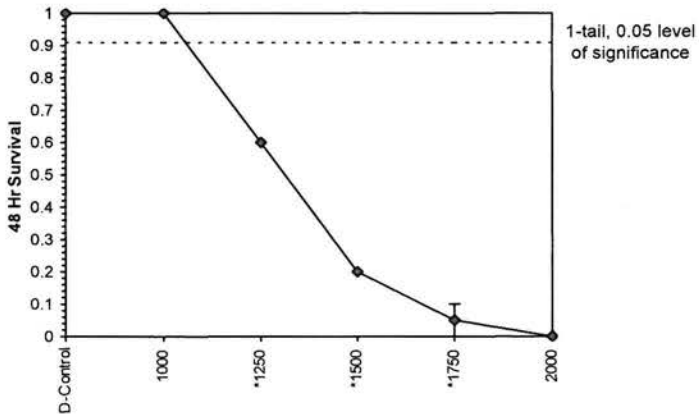
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.8961	2.73578	10.534	21.2583	0	1.2432	7.81472	0.74266	3.12329	0.06291	4
Intercept	-44.648	8.57659	-61.458	-27.838							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	948.29	779.807	1050.78
EC05	3.355	1046.68	901.692	1135.51
EC10	3.718	1103.23	973.189	1184.76
EC15	3.964	1143.11	1023.84	1220.1
EC20	4.158	1175.83	1065.32	1249.7
EC25	4.326	1204.64	1101.59	1276.41
EC40	4.747	1280.41	1194.43	1350.96
EC50	5.000	1328.27	1249.76	1402.63
EC60	5.253	1377.92	1303.42	1461.01
EC75	5.674	1464.6	1387.7	1574.83
EC80	5.842	1500.49	1419.69	1625.79
EC85	6.036	1543.43	1456.29	1689.15
EC90	6.282	1599.22	1501.76	1774.65
EC95	6.645	1685.63	1568.97	1912.84
EC99	7.326	1860.52	1697.69	2208.96



Dose-Response Plot



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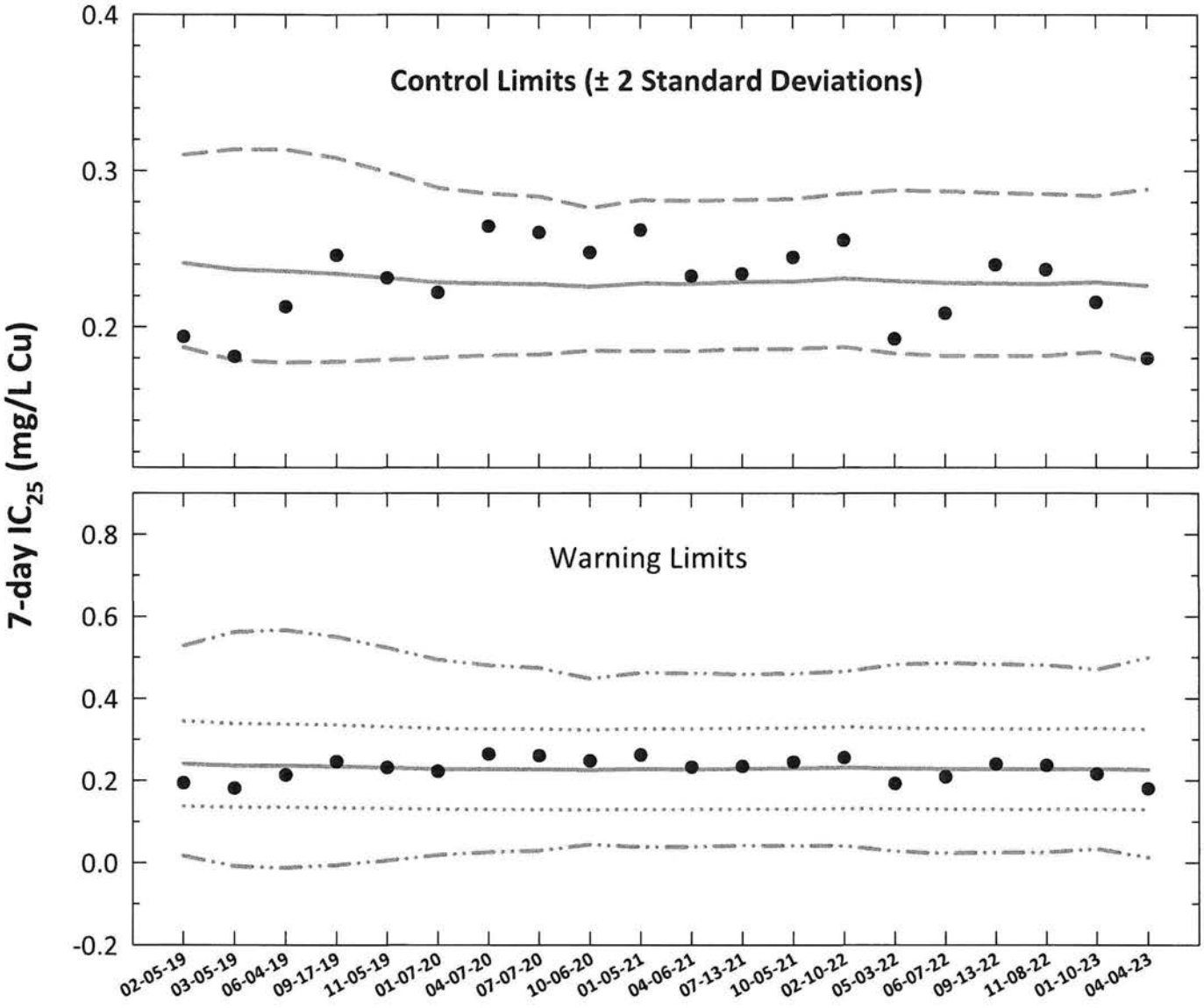
Menidia beryllina
Chronic Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Menidia beryllina

Chronic Reference Toxicant Control Chart

Source: Aquatic Indicators, Inc.

Test number	Test date	7-day IC_{25} ToxCal Determination (mg/L Cu)	Log_{10} Conversion			Anti-logarithmic Values (mg/L Cu)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**02-05-19**	**0.1937**	-0.7129	-0.6182	0.0550	0.2409	0.1869	0.3104	0.0170	0.5294	0.1373	0.3445
2	**03-05-19**	**0.1810**	-0.7423	-0.6257	0.0612	0.2367	0.1786	0.3138	-0.0088	0.5623	0.1349	0.3385
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**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

Note:

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

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 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.

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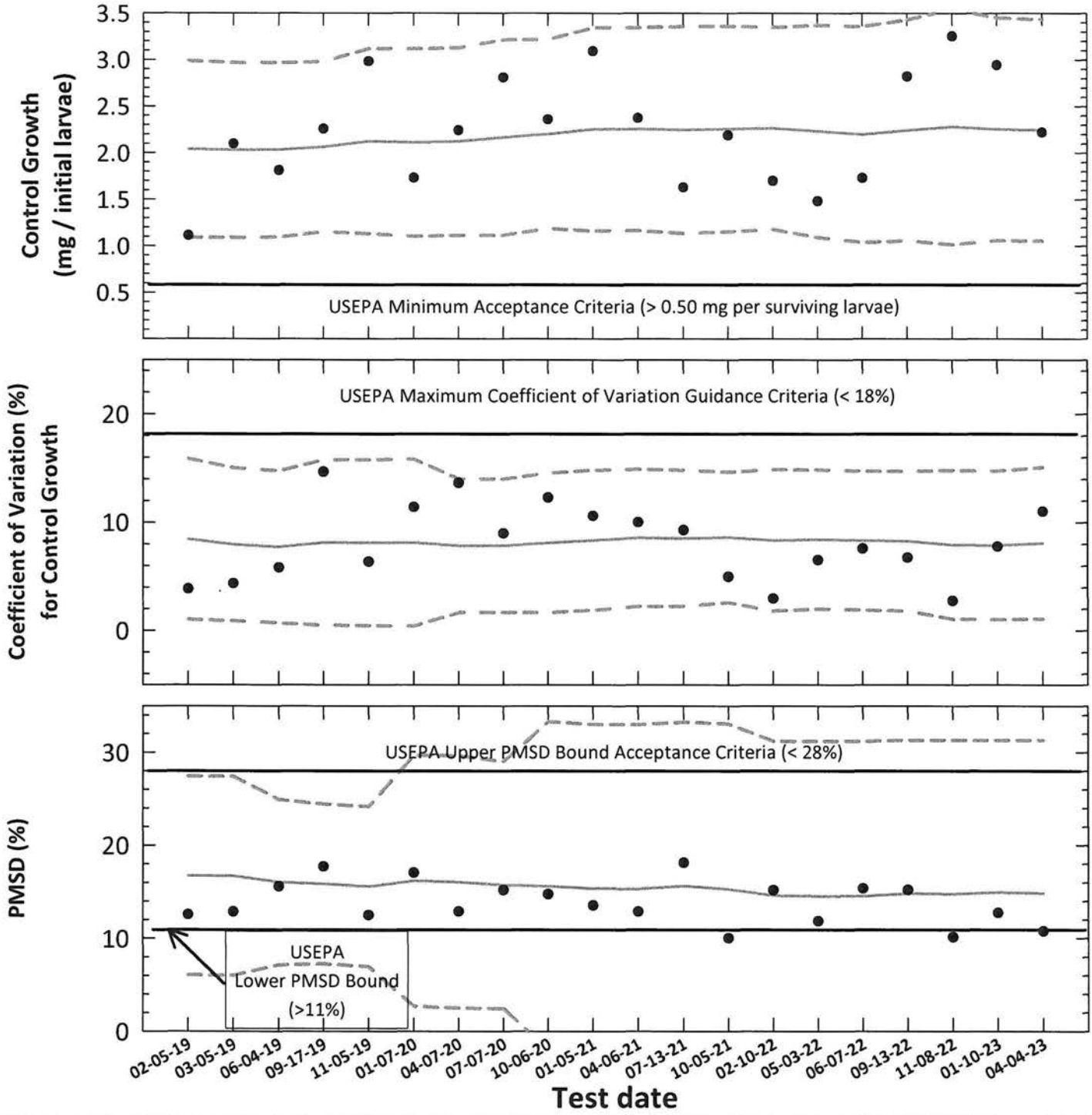
ETS
Environmental Testing Solutions, Inc.

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)

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ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	02-05-19	100	1.114	3.9	0.1400	12.6	2.0421	1.092	2.992	8.5	1.0	15.9	16.7	6.0	27.4
2	03-05-19	100	2.098	4.3	0.2697	12.9	2.030	1.090	2.969	8.0	0.9	15.0	16.7	6.0	27.4
3	06-04-19	100	1.812	5.8	0.2823	15.6	2.032	1.096	2.969	7.7	0.7	14.8	16.0	7.1	24.9
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

Entered and Reviewed by Jim Summey

Environmental Testing Solutions, Inc.

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

Species: *Menidia beryllina*

MbCuCR Test Number: **142**

Dilution preparation information:						***Comments:***
Cu Stock INSS number:		INSS 2187				
Stock preparation:		100 mg Cu/L: Dissolve 0.1965 g $CuSO_4$ in 500-mL deionized water				
Dilution prep (mg/L)	0.025	0.050	0.100	0.200	0.500	
Stock volume (mL)	0.5	1.0	2.0	4.0	10.0	
Diluent volume (mL)	1999.5	1999.0	1998.0	1996.0	1990.0	
Total volume (mL)	2000	2000	2000	2000	2000	

Test organism information:

Organism source:	Aquatic Indicators, Inc.
Age:	10-days old
Batch:	AI Mb 03-25-22
Hatch dates and times:	03-24-22 1200 to 03-25-22 1130
Transfer vessel information:	pH (S.U.) = 7.62 Temperature (°C) = 24.8
Average transfer volume (mL):	< 0.25 mL

Test information:

Randomizing template:	ORANGE
Incubator number and shelf location:	6B
Artemia CHM number:	CHM1222
Drying information for weight determination:	
Date / Time in oven:	04-11-23 1115
*Initial oven temperature:	60°C
Date / Time out of oven:	04-12-23 1115
*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	04-04-23	1020	JH	1400	JH	1130	JH		03-28-23 B
1	04-05-23	0500	JH	1100	JH	0930	JH		↓
2	04-06-23	0500	JH	1240	JH	1206	JH		04-05-23
3	04-07-23	0500	JH	1100	JH	0930	JH		↓
4	04-08-23	0600	JH	1200	JH	1100	JH		04-07-23
5	04-09-23	0600	JH	1200	JH	0930	JH		↓
6	04-10-23	0500	JH	1100	JH	0930	JH		04-08-23
7	04-11-23					0930	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	13066468S

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07	≤ 20%	7-day LC_{50} (%)	0.234
Average weight per initial larvae:	2.220		NOEC (%)	0.1
Average weight per surviving larvae:	2.220	≥ 0.25mg/larvae	LOEC (%)	0.2
			ChV (%)	0.14
			IC_{25} (%)	0.180

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **142**

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: yellow Analyst: BL Date: 03-22-23	13.89	12.90	14.84	13.93	14.28	14.59	14.67	13.05	14.91	13.13	13.14	13.39
*B = Pan + Larvae weight (mg) Analyst: BL Date: 04·13·23	38.98	33.48	~~38.17~~ 04·13·23 38.17	~~33.74~~ 04·13·23 33.74	37.18	37.00	38.25	36.18	36.49	36.93	37.10	35.65
C = Larvae weight (mg) = B – A Analyst: JH	25.09	20.58	23.33	19.81	22.90	22.41	23.58	23.13	21.58	23.80	23.96	22.26
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	2.509	2.058	2.333	1.981	2.290	2.241	2.358	2.313	2.158	2.380	2.396	2.226
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	2.220				2.301		-3.67		2.290		-3.17	

*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:

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: 142

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	6 4d	6 4d	6 4d	7 3d	0 10d	0 10d	0 10d	0 10d
2	10	10	10	10	6	6	6	7				
3	10	10	10	10	6	6	6 → 5 04-07-23	7				
4	10	10	10	10	6	6	6	7				
5	10	10	10	10	6	6	6	7				
6	10	10	10	10	6	6	6	7				
7	10	10	10	10	6	6	6	7				
*A = Pan weight (mg) Tray color code: yellow Analyst: BL Date: 03-22-23	14.35	12.73	14.14	13.86	14.25	14.31	14.34	14.42	15.11	14.81	14.87	14.02
*B = Pan + Larvae weight (mg) Analyst: BL Date: 04-13-23	35.30	33.69	37.25	36.13	30.78	28.94	29.99	30.88				
C = Larvae weight (mg) = B – A Analyst: JL	20.95	20.96	23.11	22.27	16.53	14.63	15.65	16.46				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	2.095	2.096	2.311	2.227	1.653	1.463	1.565	1.646	0	0	0	0
Average weight per initial number of larvae (mg)	2.182				1.582				0			
Percent reduction from control (%)			1.7%				28.8%				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:

Environmental Testing Solutions, Inc.

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

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

Test number: 142

							Not for Compliance Assessment, Internal Laboratory QC							
Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	13.89	38.98	25.09	2.509	2.220	11.0	2.509	100.0	2.220	11.0	Not applicable
	B	10	10	12.90	33.48	20.58	2.058			2.058				
	C	10	10	14.84	38.17	23.33	2.333			2.333				
	D	10	10	13.93	33.74	19.81	1.981			1.981				
0.025	E	10	10	14.28	37.18	22.90	2.290	2.301	2.1	2.290	100.0	2.301	2.1	-3.6
	F	10	10	14.59	37.00	22.41	2.241			2.241				
	G	10	10	14.67	38.25	23.58	2.358			2.358				
	H	10	10	13.05	36.18	23.13	2.313			2.313				
0.050	I	10	10	14.91	36.49	21.58	2.158	2.290	5.1	2.158	100.0	2.290	5.1	-3.1
	J	10	10	13.13	36.93	23.80	2.380			2.380				
	K	10	10	13.14	37.10	23.96	2.396			2.396				
	L	10	10	13.39	35.65	22.26	2.226			2.226				
0.100	M	10	10	14.35	35.30	20.95	2.095	2.182	4.9	2.095	100.0	2.182	4.9	1.7
	N	10	10	12.73	33.69	20.96	2.096			2.096				
	O	10	10	14.14	37.25	23.11	2.311			2.311				
	P	10	10	13.86	36.13	22.27	2.227			2.227				
0.200	Q	10	6	14.25	30.78	16.53	2.755	2.538	7.1	1.653	62.5	1.582	5.6	28.8
	R	10	6	14.31	28.94	14.63	2.438			1.463				
	S	10	6	14.34	29.99	15.65	2.608			1.565				
	T	10	7	14.42	30.88	16.46	2.351			1.646				
0.500	U	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	V	10	0	0.00	0.00	0.00	0.000			0.000				
	W	10	0	0.00	0.00	0.00	0.000			0.000				
	X	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.2389
PMSD: 10.8

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) = 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.

Entered and Reviewed by Jim Sumner

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	4/5/2023 04-04-23	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	4/12/2023 04-11-23	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	0.6000	0.6000	0.6000	0.7000
0.5	0.0000	0.0000	0.0000	0.0000

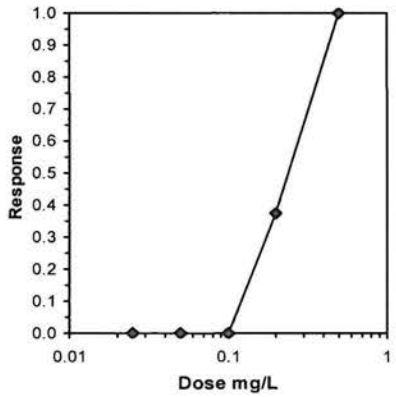
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*0.2	0.6250	0.6250	0.9123	0.8861	0.9912	5.759	4	10.00	10.00	15	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)	0.5089	0.868	2.79623	11.6732
Equality of variance cannot be confirmed				

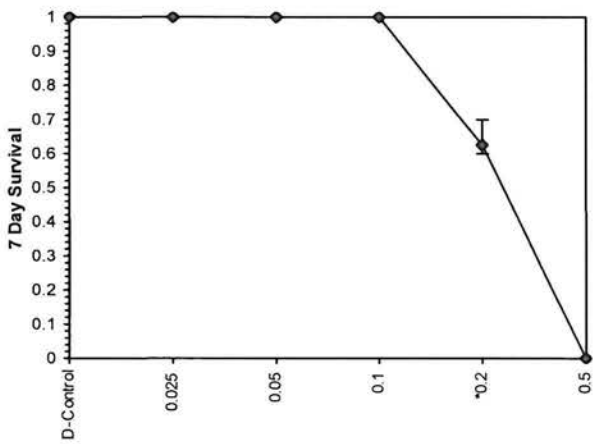
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	0.2339	0.2067	0.2645
5.0%	0.2349	0.2047	0.2695
10.0%	0.2359	0.2018	0.2759
20.0%	0.2379	0.1912	0.2960
Auto-0.0%	0.2339	0.2067	0.2645



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/5/2023 04-04-23	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	4/12/2023 04-11-23	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

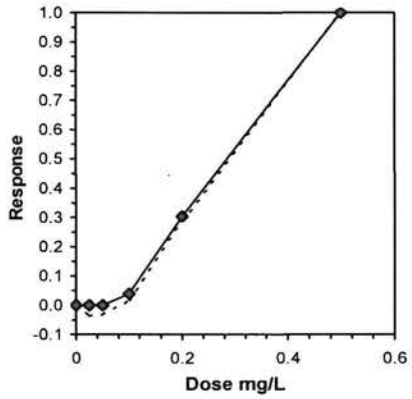
Conc-mg/L	1	2	3	4
D-Control	2.5090	2.0580	2.3330	1.9810
0.025	2.2900	2.2410	2.3580	2.3130
0.05	2.1580	2.3800	2.3960	2.2260
0.1	2.0950	2.0960	2.3110	2.2270
0.2	1.6530	1.4630	1.5650	1.6460
0.5	0.0000	0.0000	0.0000	0.0000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	2.2203	1.0000	2.2203	1.9810	2.5090	11.022	4				2.2703	1.0000
0.025	2.3005	1.0361	2.3005	2.2410	2.3580	2.117	4	-0.769	2.290	0.2389	2.2703	1.0000
0.05	2.2900	1.0314	2.2900	2.1580	2.3960	5.096	4	-0.669	2.290	0.2389	2.2703	1.0000
0.1	2.1823	0.9829	2.1823	2.0950	2.3110	4.852	4	0.364	2.290	0.2389	2.1823	0.9612
0.2	1.5818	0.7124	1.5818	1.4630	1.6530	5.606	4				1.5818	0.6967
0.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

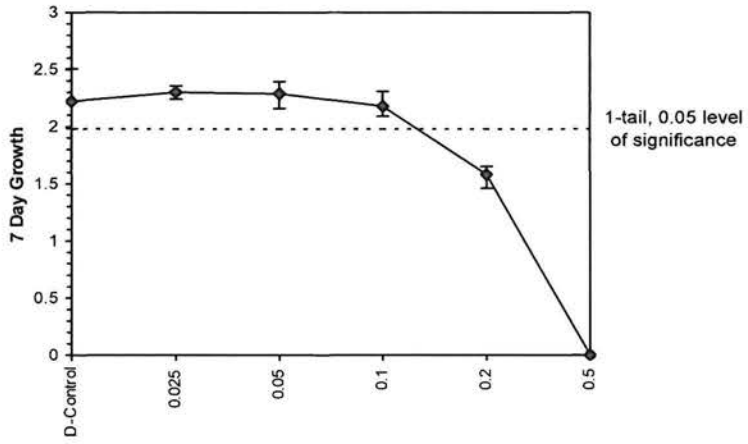
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.98086		0.844		0.25849	0.23601
Bartlett's Test indicates equal variances (p = 0.10)					6.15955		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	0.1	>0.1			0.23893	0.10761	0.01282	0.02177	0.63398	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1042	0.0202	0.0031	0.1255	-1.6100
IC10	0.1232	0.0099	0.0868	0.1422	-0.8918
IC15	0.1421	0.0088	0.1075	0.1624	-0.4734
IC20	0.1610	0.0085	0.1298	0.1828	-0.3896
IC25	0.1799	0.0086	0.1499	0.2037	-0.2576
IC40	0.2417	0.0089	0.2067	0.2642	-0.4771
IC50	0.2847	0.0074	0.2556	0.3035	-0.4771



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **142**

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)					
		0		1		2	
	Analyst	VL	BL VL	BL VL	QP VL	VL QP	QP VL
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	7.87	7.83	7.97	7.85	8.00	7.84
	Dissolved oxygen (mg/L)	8.1	7.9	8.0	7.8	7.7	7.8
	Salinity (ppt)	25.0	25.7	25.0	25.0	25.0	25.3
	Alkalinity (mg $CaCO_3$/L)	100		→	04-22-25 M	100	
	Temperature (°C)	24.9	25.0	24.8	24.9	24.8	24.7
0.025 mg/L	pH (S.U.)	7.92	7.84	7.90	7.77	8.01	7.81
	Dissolved oxygen (mg/L)	8.1	7.9	8.1	7.3	7.7	7.7
	Salinity (ppt)	25.2	25.7	25.2	25.5	25.2	25.7
	Temperature (°C)	25.0	24.9	24.8	25.0	24.7	24.9
0.05 mg/L	pH (S.U.)	7.92	7.84	7.91	7.76	8.01	7.81
	Dissolved oxygen (mg/L)	8.1	7.9	8.1	7.2	7.7	7.7
	Salinity (ppt)	25.4	25.6	25.1	25.5	25.2	25.6
	Temperature (°C)	25.0	24.9	24.9	25.0	24.7	24.9
0.1 mg/L	pH (S.U.)	7.92	7.83	7.91	7.71	8.02	7.82
	Dissolved oxygen (mg/L)	8.1	7.9	8.1	7.2	7.7	7.8
	Salinity (ppt)	25.2	25.6	25.1	25.6	25.1	25.6
	Temperature (°C)	25.0	25.1	24.8	25.0	24.7	25.0
0.2 mg/L	pH (S.U.)	7.93	7.84	7.91	7.83	8.03	7.83
	Dissolved oxygen (mg/L)	8.1	8.0	8.1	7.1	7.8	7.9
	Salinity (ppt)	25.3	25.7	25.2	25.6	25.1	25.7
	Temperature (°C)	25.0	24.9	24.9	24.9	24.8	25.0
0.5 mg/L	pH (S.U.)	7.92	7.82	7.92			
	Dissolved oxygen (mg/L)	8.1	8.0	8.2			
	Salinity (ppt)	25.2	25.6	25.1			
	Alkalinity (mg $CaCO_3$/L)	→	04-22-25 M			04-25-25 M	
	Temperature (°C)	25.0	24.9	24.9			
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **142**

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
	Analyst	AP VL	BSL	BSL	BSL	BSL	BL	BL	VL
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.00	7.84	8.03	7.84	8.01	7.89	7.98	7.76
	Dissolved oxygen (mg/L)	7.6	8.0	8.0	7.8	7.9	8.0	7.8	7.3
	Salinity (ppt)	25.0	25.2	24.0	25.1	25.1	25.0	25.0	25.1
	Alkalinity (mg $CaCO_3$/L)	→ 04-12-23 VL		100		→ 04-12-23 VL		100	
	Temperature (°C)	24.9	24.9	24.8	24.9	24.7	24.8	24.8	24.8
0.025 mg/L	pH (S.U.)	7.92	7.83	8.06	7.85	8.03	7.90	8.02	7.78
	Dissolved oxygen (mg/L)	7.9	8.0	7.8	7.9	7.9	8.0	8.1	7.5
	Salinity (ppt)	25.1	25.4	25.0	25.1	25.0	25.3	25.2	25.4
	Temperature (°C)	24.8	24.7	24.8	24.7	24.7	24.7	24.7	25.0
0.05 mg/L	pH (S.U.)	7.91	7.82	8.06	7.84	8.04	7.89	8.01	7.77
	Dissolved oxygen (mg/L)	7.9	8.0	7.9	7.8	7.9	8.2	8.2	7.5
	Salinity (ppt)	25.2	25.4	25.0	25.2	25.0	25.5	25.4	25.5
	Temperature (°C)	24.8	24.8	24.9	24.9	24.7	25.0	24.7	25.0
0.1 mg/L	pH (S.U.)	7.92	7.81	8.06	7.86	8.04	7.89	8.02	7.80
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.8	7.9	8.2	8.2	7.5
	Salinity (ppt)	25.2	25.4	25.0	25.4	25.0	25.5	25.4	25.7
	Temperature (°C)	24.8	24.8	24.9	24.9	24.7	24.9	24.7	25.0
0.2 mg/L	pH (S.U.)	7.93	7.86	8.07	7.89	8.04	7.91	8.02	7.81
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.9	8.0	8.2	8.2	7.5
	Salinity (ppt)	25.2	25.4	25.0	25.3	25.0	25.4	25.4	25.4
	Temperature (°C)	24.8	24.8	24.9	24.8	24.7	24.9	24.7	24.8
0.5 mg/L	pH (S.U.)								
	Dissolved oxygen (mg/L)								
	Salinity (ppt)						04-05-23 VL		
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final