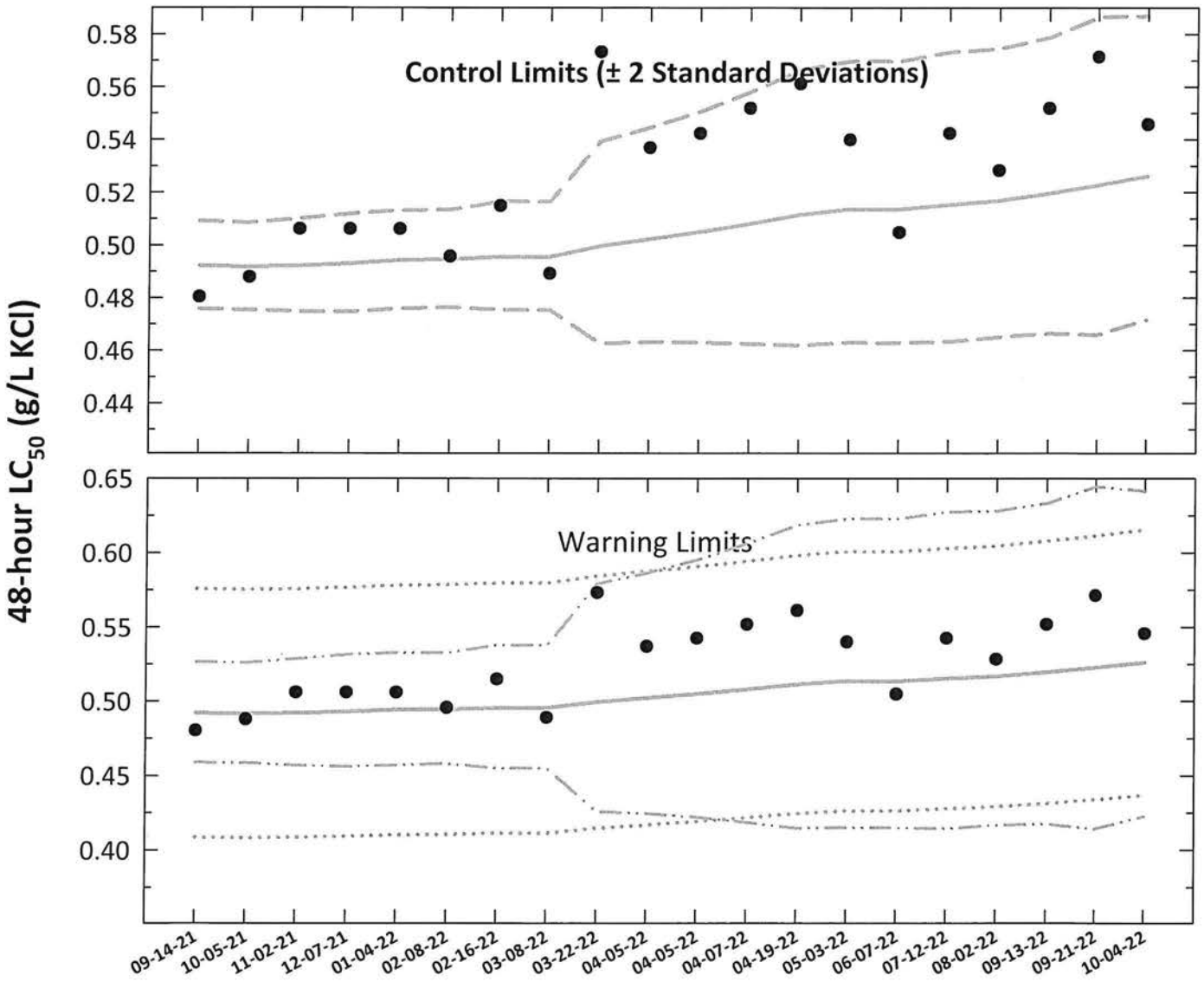
ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **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

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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	09-14-21	0.4804	-0.3184	-0.3079	0.0074	0.4922	0.4758	0.5091	0.4589	0.5266	0.4085	0.5759
2	10-05-21	0.4879	-0.3117	-0.3083	0.0073	0.4917	0.4754	0.5086	0.4586	0.5260	0.4081	0.5753
3	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
4	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
5	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
6	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

AbKCIAC # 259

Dilution Preparation:

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

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

Stock solution INSS #: 2128

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	VL	EC VL	VL
Control, SaltSW	pH (S.U.)	7.51	7.87	7.60
	Dissolved oxygen (mg/L)	7.0	7.1	7.1
	*Salinity (ppt)	25.0	24.9	25.1
	*Alkalinity (mg/L $CaCO_3$)	90		
	*Temperature (°C)	25.0	25.2	25.5
250 mg/L	pH (S.U.)	7.91	7.87	7.59
	Dissolved oxygen (mg/L)	7.8	7.6	7.2
	*Salinity (ppt)	25.3	25.3	25.3
	*Temperature (°C)	25.0	25.1	25.2
375 mg/L	pH (S.U.)	7.59	7.87	7.58
	Dissolved oxygen (mg/L)	7.9	7.5	7.2
	*Salinity (ppt)	25.3	25.3	25.4
	*Temperature (°C)	25.1	25.1	25.0
500 mg/L	pH (S.U.)	7.63	7.88	7.71
	Dissolved oxygen (mg/L)	0.0	7.6	7.1
	*Salinity (ppt)	25.4	25.5	25.5
	*Temperature (°C)	25.1	25.0	25.0
750 mg/L	pH (S.U.)	7.67	7.87	
	Dissolved oxygen (mg/L)	7.9	7.7	
	*Salinity (ppt)	25.5	26.5	
	*Temperature (°C)	25.1	25.0	
1000 mg/L	pH (S.U.)	7.73	7.83	
	Dissolved oxygen (mg/L)	7.9	7.9	
	*Salinity (ppt)	25.0	26.0	
	*Temperature (°C)	25.1	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	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 # 259

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-04-22	* 1014	JL	1220	JL	1D	WHITE	09-28-22A
24	10-05-22			1222	JL			
48 Termination	10-06-22			1225	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	10-03-22
Age (1 to 5 days old):	
Date organisms were born: (time organisms were born between is not provided by supplier)	10-02-22 1200 TO 10-03-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.99
	Temperature (°C) 24.9

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

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	0 100d	0 100d	0 100d	0 100d
48 Termination	10	10	10	10	10	9 1d	8 2d	8 2d	0	0	0	0
Mean Survival	100%		100%		95%		80%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	502.2
Upper 95% confidence limit (mg KCl/L)	602.6
48-hour LC_{50} (mg KCl/L)	545.8

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-24 Hr Survival

Start Date:	10/4/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	10/6/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

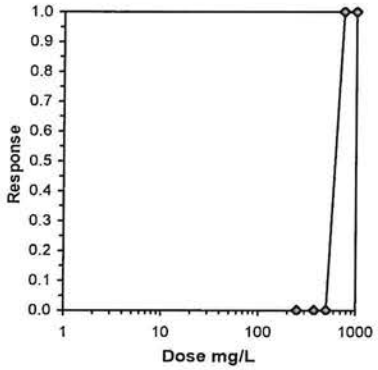
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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										

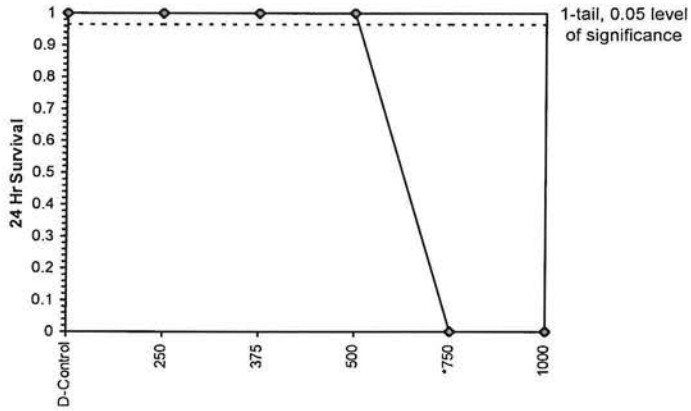
Graphical Method

Trim Level	EC50
0.0%	612.37

612.37



Dose-Response Plot



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Acute Mysid Test-48 Hr Survival

Start Date:	10/4/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/6/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	0.9000
500	0.8000	0.8000
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	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	2	5.916	2.850	0.1469	4	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

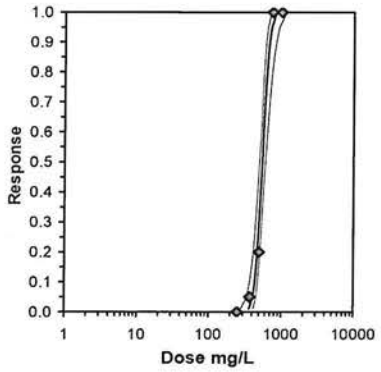
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	500	433.013		0.06555	0.06723	0.56627	0.00266	9.0E-06	4, 5

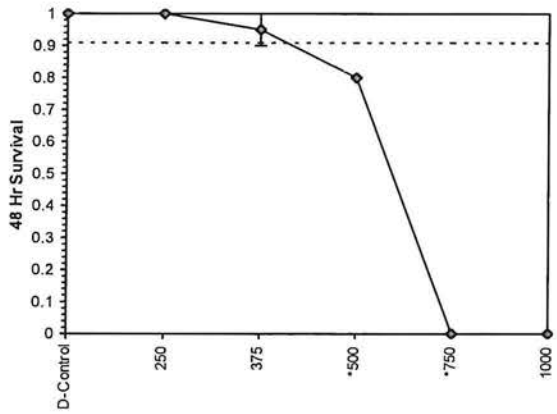
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.7224	2.88377	8.07022	19.3746	0	3.8002	7.81472	0.28386	2.73704	0.07287	7
Intercept	-32.559	7.84845	-47.942	-17.176							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	369.409	282.629	418.198
EC05	3.355	414.162	340.391	457.341
EC10	3.718	440.195	374.759	481.1
EC15	3.964	458.678	399.078	498.832
EC20	4.158	473.92	418.826	514.248
EC25	4.326	487.399	435.88	528.659
EC40	4.747	523.088	478.098	571.409
EC50	5.000	545.804	502.198	602.641
EC60	5.253	569.507	525.084	638.519
EC75	5.674	611.208	561.03	708.497
EC80	5.842	628.592	574.857	739.777
EC85	6.036	649.479	590.837	778.731
EC90	6.282	676.75	610.897	831.595
EC95	6.645	719.288	640.862	918.087
EC99	7.326	806.429	698.931	1108.75



Dose-Response Plot



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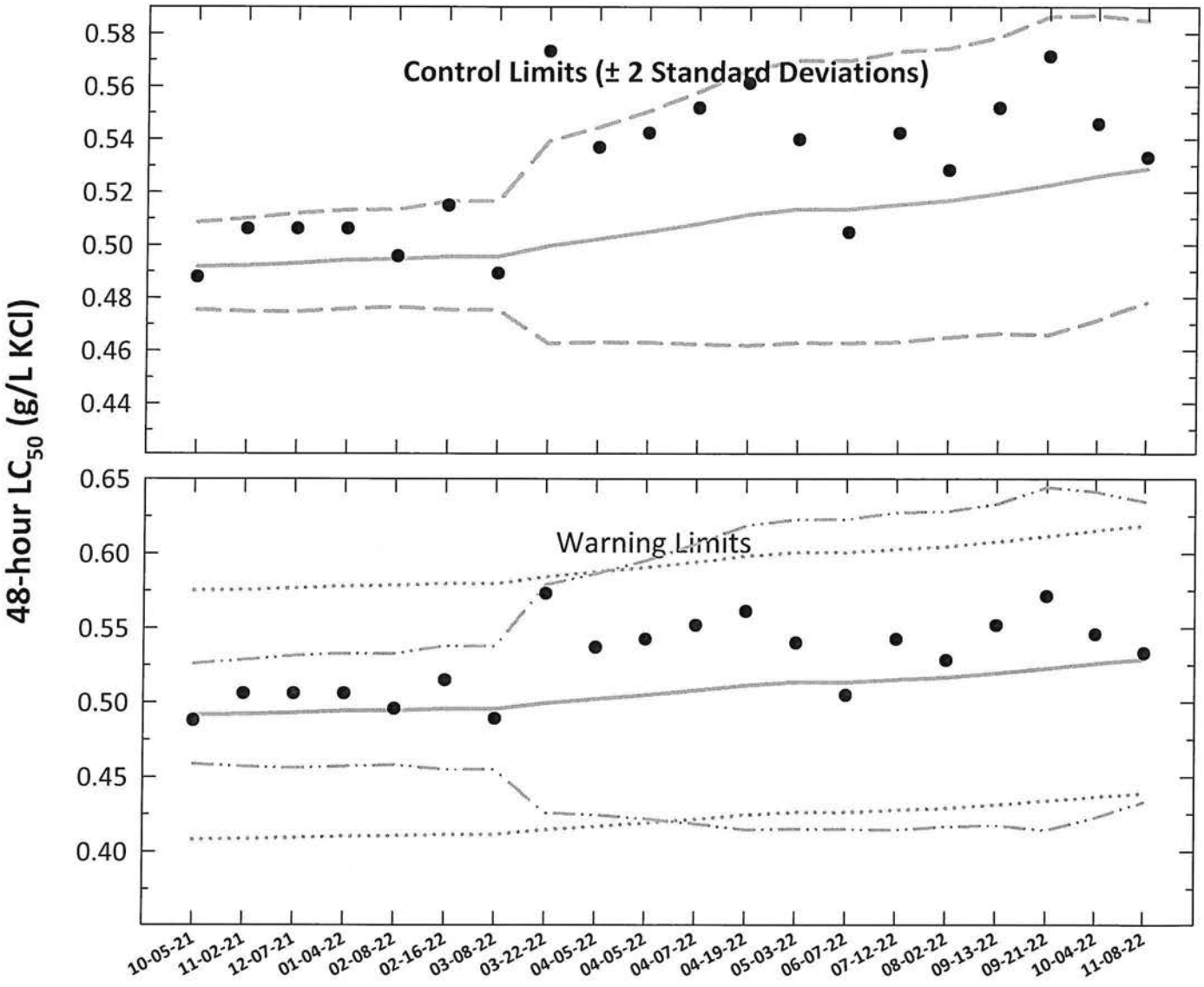

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			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
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2	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
3	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
4	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
5	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
6	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
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9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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 # 260

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 #: 2150

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JH	EC W	EC
Control, SaltSW	pH (S.U.)	7.64	7.99	7.85
	Dissolved oxygen (mg/L)	7.8	7.8	7.5
	*Salinity (ppt)	25.0	25.3	25.5
	*Alkalinity (mg/L $CaCO_3$)	130		
	*Temperature (°C)	25.1	25.3	25.4
250 mg/L	pH (S.U.)	7.67	7.98	7.88
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	*Salinity (ppt)	25.2	25.2	25.4
	*Temperature (°C)	25.0	25.2	25.6
375 mg/L	pH (S.U.)	7.71	7.98	7.92
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	*Salinity (ppt)	25.3	25.4	25.6
	*Temperature (°C)	25.0	25.2	25.6
500 mg/L	pH (S.U.)	7.74	7.99	7.91
	Dissolved oxygen (mg/L)	7.8	7.9	7.4
	*Salinity (ppt)	25.3	25.5	25.7
	*Temperature (°C)	25.0	25.2	25.5
750 mg/L	pH (S.U.)	7.75	7.99	
	Dissolved oxygen (mg/L)	7.8	7.9	
	*Salinity (ppt)	25.4	26.0	
	*Temperature (°C)	25.0	25.3	
1000 mg/L	pH (S.U.)	7.76	7.99	
	Dissolved oxygen (mg/L)	7.8	7.9	
	*Salinity (ppt)	25.5	26.1	
	*Temperature (°C)	25.0	25.3	

11-04-22 JH

*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	130664615

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 # 760

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-08-22	* 1015		1215		6C	LIGHT YELLOW	11-03-22B
24	11-09-22			1214				
48 Termination	11-10-22			1216				

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	11-01-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	11-06-22 1200 TO 11-01-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.94
	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	8 2d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	6 2d	6 3d	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:

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	11/8/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

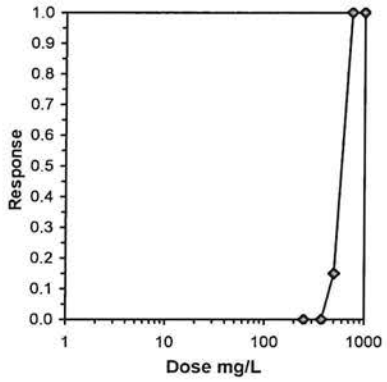
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

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

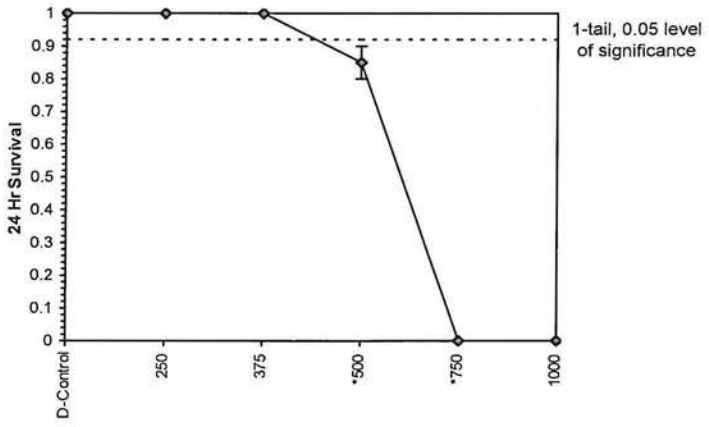
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.05495	0.05636	0.5915	0.00201	4.1E-06	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

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



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Summers

Acute Mysid Test-48 Hr Survival

Start Date:	11/8/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

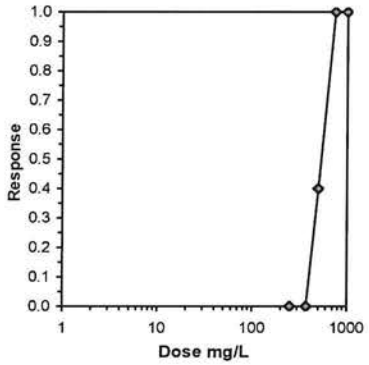
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

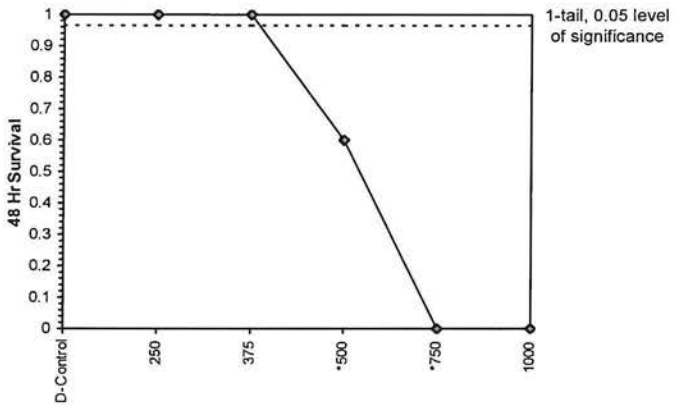
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.60706	0.0001	2.1E-09	4, 5
Treatments vs D-Control										

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

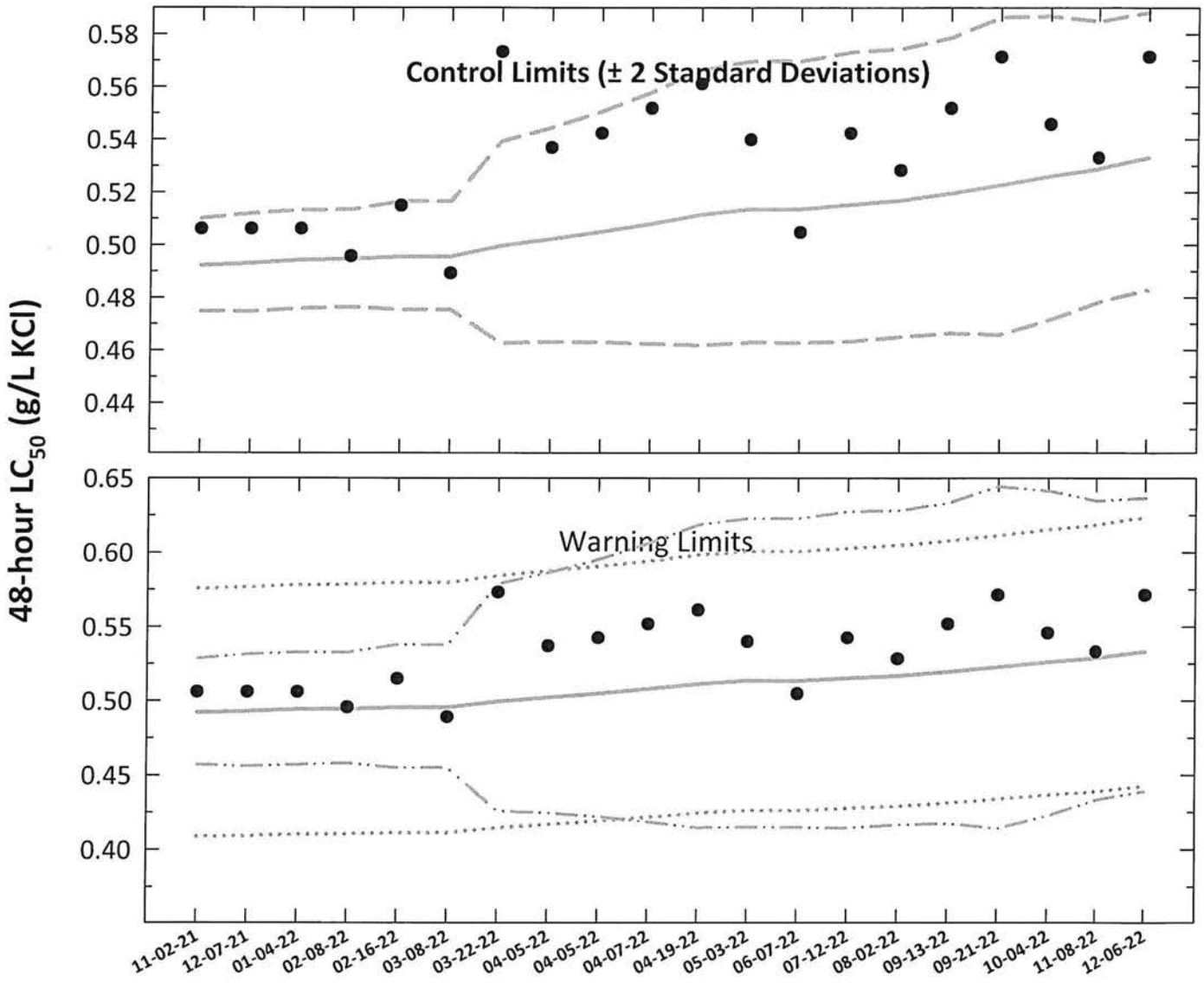


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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	11-02-21	0.5061	-0.2958	-0.3079	0.0078	0.4922	0.4749	0.5101	0.4570	0.5286	0.4085	0.5758
2	12-07-21	0.5061	-0.2958	-0.3072	0.0082	0.4930	0.4747	0.5119	0.4560	0.5314	0.4092	0.5768
3	01-04-22	0.5061	-0.2958	-0.3061	0.0082	0.4942	0.4759	0.5132	0.4572	0.5327	0.4102	0.5782
4	02-08-22	0.4957	-0.3048	-0.3057	0.0081	0.4946	0.4765	0.5134	0.4580	0.5326	0.4105	0.5787
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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 # 761

Dilution Preparation:

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

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

Stock solution INSS #: 2128

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	EC	EC JN	JN
Control, SaltSW	pH (S.U.)	7.91	7.84	7.75
	Dissolved oxygen (mg/L)	7.7	7.7	7.7
	*Salinity (ppt)	24.6	25.0	25.1
	*Alkalinity (mg/L $CaCO_3$)	120		
	*Temperature (°C)	25.2	25.5	25.5
250 mg/L	pH (S.U.)	7.92	7.90	7.74
	Dissolved oxygen (mg/L)	7.7	7.7	7.7
	*Salinity (ppt)	25.0	25.3	25.3
	*Temperature (°C)	25.3	25.4	25.6
375 mg/L	pH (S.U.)	7.95	7.90	7.76
	Dissolved oxygen (mg/L)	7.8	7.7	7.7
	*Salinity (ppt)	24.9	25.4	25.4
	*Temperature (°C)	25.3	25.4	25.6
500 mg/L	pH (S.U.)	7.96	7.90	7.76
	Dissolved oxygen (mg/L)	7.8	7.8	7.0
	*Salinity (ppt)	25.0	25.5	25.6
	*Temperature (°C)	25.2	25.6	25.4
750 mg/L	pH (S.U.)	7.96	7.92	
	Dissolved oxygen (mg/L)	7.8	7.0	
	*Salinity (ppt)	25.0	25.6	
	*Temperature (°C)	25.2	25.6	
1000 mg/L	pH (S.U.)	7.96	7.92	
	Dissolved oxygen (mg/L)	7.8	7.0	
	*Salinity (ppt)	25.3	25.7	
	*Temperature (°C)	25.2	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	130664685

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 # 261

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12.06.22	* 1110	JL	1310	JL	SF	PURPLE	11-30-22A
24	12.07.22			1310	JL			
48 Termination	12.08.22			1308	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	12.05.22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	12.04.22 1200 TO 12.05.22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.84
	Temperature (°C) 24.9

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

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 1d	10	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	8 1d	8 2d	0	0	0	0
Mean Survival	100%		100%		100%		80%		0%		0%	

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

Statistics:

Method	SK 12.12.22
Lower 95% confidence limit (mg KCl/L)	~~537.0~~ 537.0
Upper 95% confidence limit (mg KCl/L)	607.9
48-hour LC_{50} (mg KCl/L)	571.4

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Lemour

Acute Mysid Test-24 Hr Survival

Start Date:	12/6/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/8/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

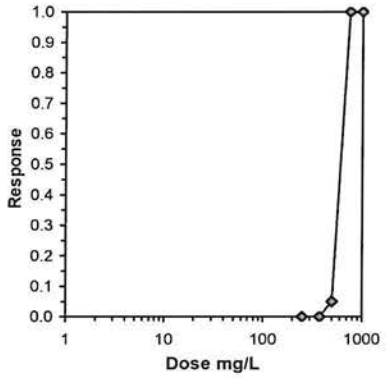
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

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

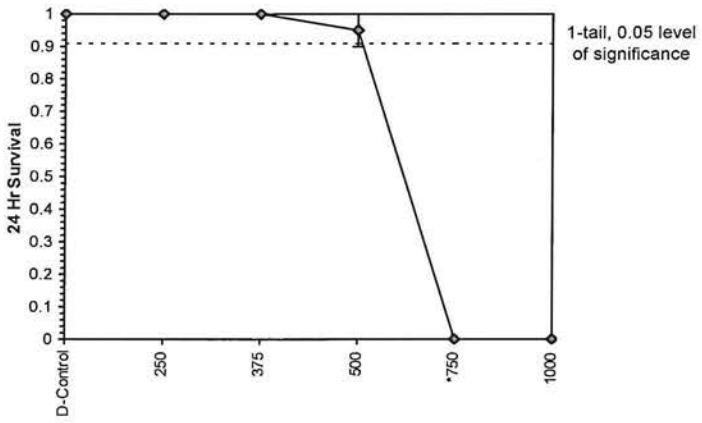
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.06555	0.06723	0.61047	0.00266	7.5E-06	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

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



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-48 Hr Survival

Start Date:	12/6/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	12/8/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

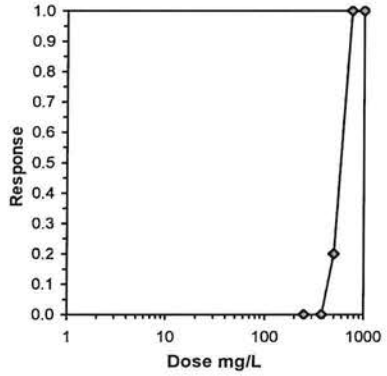
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	2	30.487	2.850	0.0285	4	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

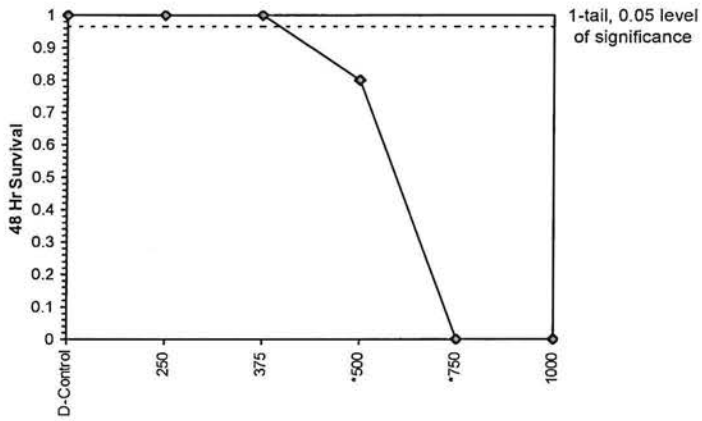
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	500	433.013		0.00967	0.00991	0.589	0.0001	2.3E-09	4, 5

Trimmed Spearman-Karber

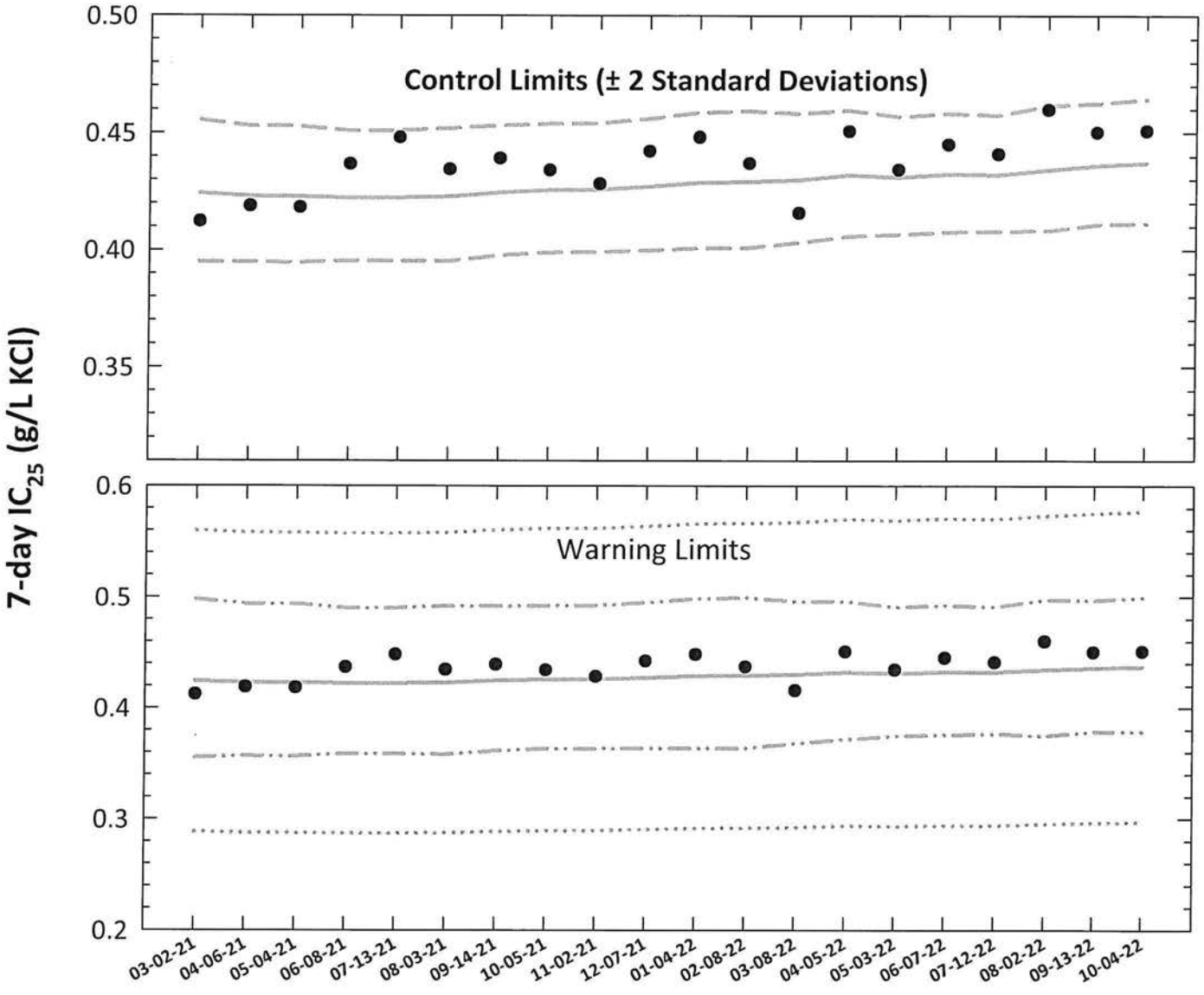
Trim Level	EC50	95% CL	
0.0%	571.36	537.02	607.91
5.0%	575.37	536.19	617.41
10.0%	578.73	531.66	629.96
20.0%	582.11	550.04	616.05
Auto-0.0%	571.36	537.02	607.91



Dose-Response Plot



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



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	03-02-21	0.4122	-0.3849	-0.3725	0.0155	0.4241	0.3949	0.4554	0.3553	0.4980	0.2884	0.5598
2	04-06-21	0.4188	-0.3780	-0.3737	0.0149	0.4229	0.3949	0.4529	0.3567	0.4939	0.2876	0.5582
3	05-04-21	0.4182	-0.3787	-0.3740	0.0149	0.4227	0.3946	0.4527	0.3562	0.4938	0.2874	0.5579
4	06-08-21	0.4366	-0.3599	-0.3745	0.0142	0.4222	0.3954	0.4508	0.3587	0.4900	0.2871	0.5573
5	07-13-21	0.4480	-0.3487	-0.3745	0.0143	0.4222	0.3953	0.4509	0.3585	0.4903	0.2871	0.5573
6	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.4227	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

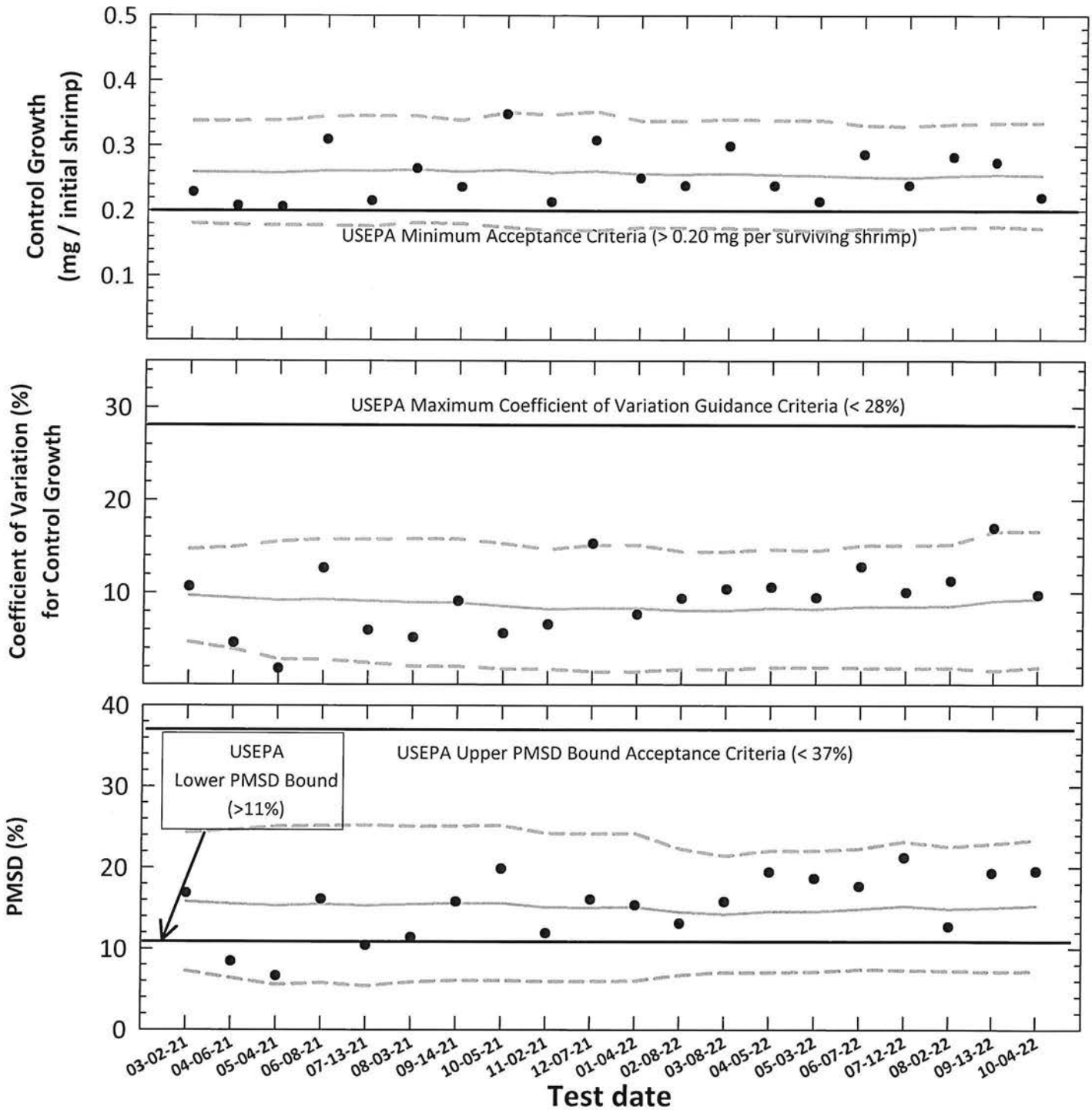
Entered and Reviewed by Jim Sumner

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 (mg/initial shrimp)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	03-02-21	100	0.229	10.6	0.0385	16.8	0.259	0.181	0.338	9.7	4.7	14.7	15.8	7.3	24.3
2	04-06-21	100	0.208	4.6	0.0175	8.4	0.259	0.179	0.339	9.4	3.9	14.9	15.5	6.4	24.6
3	05-04-21	100	0.206	1.8	0.0137	6.7	0.258	0.178	0.339	9.2	2.8	15.6	15.3	5.5	25.0
4	06-08-21	100	0.310	12.7	0.0499	16.1	0.261	0.178	0.345	9.3	2.8	15.8	15.5	5.8	25.1
5	07-13-21	100	0.215	5.9	0.0224	10.4	0.261	0.176	0.346	9.1	2.4	15.8	15.3	5.4	25.2
6	08-03-21	100	0.265	5.2	0.0301	11.4	0.263	0.181	0.345	8.9	2.0	15.8	15.4	5.9	25.0
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 Summer

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: **232**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2128				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	PURPLE
Date and times organisms were born between:	09-27-22 1200 to 09-28-22 1130	Incubator number and shelf location:	5F
Organism source:	AI Batch Ab: 09-28-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 8.03 S.U. Temperature = 25.0 °C	Date / Time in oven:	10-11-22 1055
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	10-12-22 1055
		*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	10-04-22	1014	JL	1200	JL	1125	JL	09-29-22 A
1	10-05-22	0500	JL	1100	JL	0928	JL	↓
2	10-06-22	0500	JL	1130	JL	1100	JL	09-29-22 B
3	10-07-22	0500	JL	1100	JL	0929	JL	↓
4	10-08-22	0600	JL	1200	JL	1125	JL	10-06-22
5	10-09-22	0600	JL	1200	JL	0956	JL	↓
6	10-10-22	0500	JL	1100	JL	0933	JL	↓
7	10-11-22					0948	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	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	561.6
Average weight per initial shrimp:	0.220		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.220	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	450.9

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **232**

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															JA 10-11-22	
*A = Pan weight (mg) Tray color code: Lavender Analyst: SC Date: 10/2822	15.37	14.32	15.67	13.70	15.85	15.54	12.76	14.78	14.02	15.07	13.67	13.26	14.46	14.92	13.46	13.03
*B = Pan + Shrimp weight (mg) Analyst: AJ Date: 101622	16.50	15.47	16.83	14.95	16.85	16.57	13.68	15.94	15.27	16.08	14.69	14.52	15.57	15.83	14.75	13.97
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JA	1.13	1.15	1.16	1.25	1.00	1.03	0.92	1.16	1.25	1.01	1.02	1.26	1.11	0.91	1.29	0.94
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JA	0.226	0.230	0.232	0.250	0.200	0.206	0.184	0.232	0.250	0.202	0.204	0.252	0.222	0.182	0.258	0.188
	Average weight per initial number of shrimp (mg) 0.220								Average weight per initial number of shrimp (mg) 0.220				Percent reduction from control (%) 0.17			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **232**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4 1d	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	5
5	5	5	5	5	5	5	5	5	4 1d	5	4 1d	4 1d	4 1d	5	4	4 1d
6	5	5	5	5	5	5	5	5	4	5	4	4	4	5	3 1d	4
7	5	5	5	5	5	5	5	5	4	3 2d	4	4	4	4 1d	3	4
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																10-21-7
*A = Pan weight (mg) Tray color code: Lavender Analyst: EC Date: 092822	15.88	15.48	14.85	15.46	14.87	14.89	15.69	15.49	13.63	11.89	14.02	13.93	15.86	13.62	15.13	16.36
*B = Pan + Shrimp weight (mg) Analyst: AJ Date: 101622	16.53	16.24	15.86	16.55	15.57	15.65	16.82	16.50	14.42	12.66	14.81	14.91	16.67	14.34	15.79	17.11
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JA	0.65	0.76	1.01	1.09	0.70	0.76	1.13	1.01	0.79	0.77	0.79	0.98	0.81	0.72	0.66	0.75
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JA	0.130	0.152	0.202	0.218	0.140	0.152	0.226	0.202	0.158	0.154	0.158	0.196	0.162	0.144	0.132	0.150
	Average weight per initial number of shrimp (mg) 0.178				Percent reduction from control (%) 19.2%				Average weight per initial number of shrimp (mg) 0.157				Percent reduction from control (%) 28.7%			

*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: **232**

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: lavender * Analyst: EC Date: 09 28 22	15.18	13.03	13.98	15.24	13.94 *	14.00	14.71	15.22	15.94	15.48	13.35	13.73	13.65	15.55	12.92	15.85
*B = Pan + Shrimp weight (mg) Analyst: Date:																
C = Shrimp weight (mg) = B – A Hand calculated Analyst:															10.05.22	
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)	Percent reduction from control (%)	Average weight per initial number of shrimp (mg)	Percent reduction from control (%)
	0	100%	0	100%

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

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: 232

Test dates: October 04-11, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	15.37	16.50	1.13	0.226	100.0	0.220	9.8	Not applicable
	B	5	5	14.32	15.47	1.15	0.230				
	C	5	5	15.67	16.83	1.16	0.232				
	D	5	5	13.70	14.95	1.25	0.250				
	E	5	5	15.85	16.85	1.00	0.200				
	F	5	5	15.54	16.57	1.03	0.206				
	G	5	5	12.76	13.68	0.92	0.184				
	H	5	5	14.78	15.94	1.16	0.232				
250	I	5	5	14.02	15.27	1.25	0.250	100.0	0.220	13.8	0.1
	J	5	5	15.07	16.08	1.01	0.202				
	K	5	5	13.67	14.69	1.02	0.204				
	L	5	5	13.26	14.52	1.26	0.252				
	M	5	5	14.46	15.57	1.11	0.222				
	N	5	5	14.92	15.83	0.91	0.182				
	O	5	5	13.46	14.75	1.29	0.258				
	P	5	5	13.03	13.97	0.94	0.188				
375	Q	5	5	15.88	16.53	0.65	0.130	100.0	0.178	21.4	19.2
	R	5	5	15.48	16.24	0.76	0.152				
	S	5	5	14.85	15.86	1.01	0.202				
	T	5	5	15.46	16.55	1.09	0.218				
	U	5	5	14.87	15.57	0.70	0.140				
	V	5	5	14.89	15.65	0.76	0.152				
	W	5	5	15.69	16.82	1.13	0.226				
	X	5	5	15.49	16.50	1.01	0.202				
500	Y	5	4	13.63	14.42	0.79	0.158	75.0	0.157	11.8	28.7
	Z	5	3	11.89	12.66	0.77	0.154				
	AA	5	4	14.02	14.81	0.79	0.158				
	BB	5	4	13.93	14.91	0.98	0.196				
	CC	5	4	15.86	16.67	0.81	0.162				
	DD	5	4	13.62	14.34	0.72	0.144				
	EE	5	3	15.13	15.79	0.66	0.132				
	FF	5	4	16.36	17.11	0.75	0.150				
750	GG	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	HH	5	0	0.00	0.00	0.00	0.000				
	II	5	0	0.00	0.00	0.00	0.000				
	JJ	5	0	0.00	0.00	0.00	0.000				
	KK	5	0	0.00	0.00	0.00	0.000				
	LL	5	0	0.00	0.00	0.00	0.000				
	MM	5	0	0.00	0.00	0.00	0.000				
	NN	5	0	0.00	0.00	0.00	0.000				
1000	OO	5	0	0.00	0.00	0.00	0.000	0.0	0.000	0.0	100.0
	PP	5	0	0.00	0.00	0.00	0.000				
	QQ	5	0	0.00	0.00	0.00	0.000				
	RR	5	0	0.00	0.00	0.00	0.000				
	SS	5	0	0.00	0.00	0.00	0.000				
	TT	5	0	0.00	0.00	0.00	0.000				
	UU	5	0	0.00	0.00	0.00	0.000				
	VV	5	0	0.00	0.00	0.00	0.000				

Dunnett's MSD value: 0.0430 MSD = Minimum Significant Difference

PMSD: 19.5 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.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	10/4/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/11/2022	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	0.8000	0.6000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

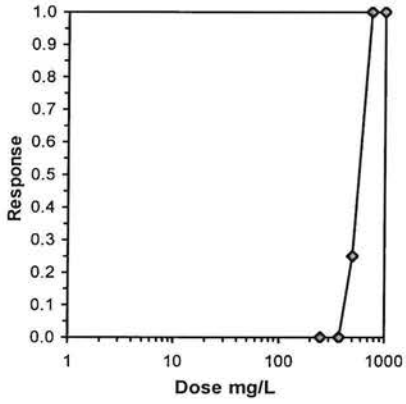
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.7500	0.7500	1.0519	0.8861	1.1071	9.729	8	36.00	48.00	10	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

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

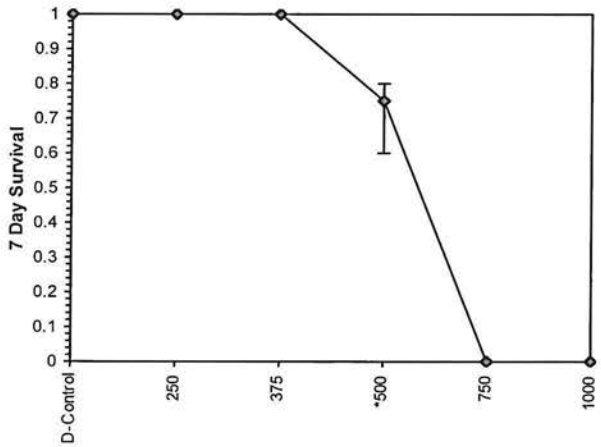
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Trimmed Spearman-Karber

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



Dose-Response Plot



Entered and Reviewed by Jim Summer

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	10/4/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/11/2022	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	0.2260	0.2300	0.2320	0.2500	0.2000	0.2060	0.1840	0.2320
250	0.2500	0.2020	0.2040	0.2520	0.2220	0.1820	0.2580	0.1880
375	0.1300	0.1520	0.2020	0.2180	0.1400	0.1520	0.2260	0.2020
500	0.1580	0.1540	0.1580	0.1960	0.1620	0.1440	0.1320	0.1500
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

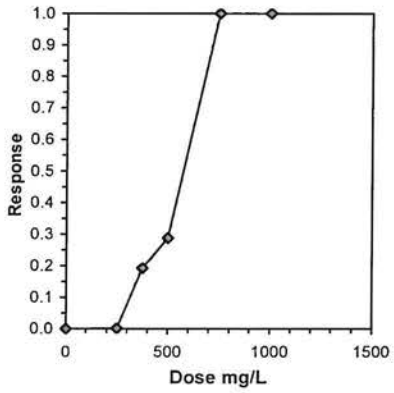
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2200	1.0000	0.2200	0.1840	0.2500	9.743	8				0.2200	1.0000
250	0.2198	0.9989	0.2198	0.1820	0.2580	13.781	8	0.016	2.799	0.0430	0.2198	0.9989
375	0.1778	0.8080	0.1778	0.1300	0.2260	21.429	8	2.753	2.799	0.0430	0.1778	0.8080
500	0.1568	0.7125	0.1568	0.1320	0.1960	11.811	8				0.1568	0.7125
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)	0.93726	0.884	0.01016	-1.3872
Bartlett's Test indicates equal variances (p = 0.36)	2.06909	9.21035		

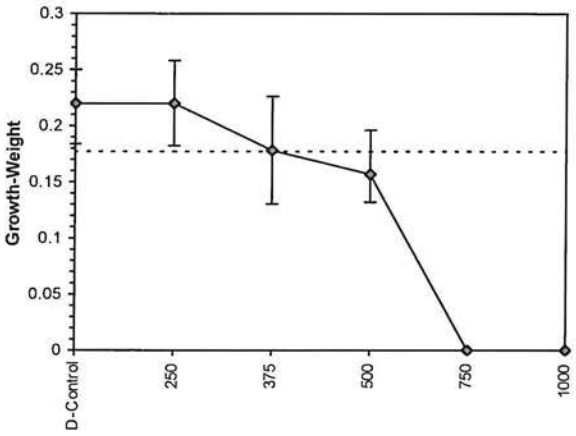
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	>375			0.04296	0.19526	0.00473	0.00094	0.01651	2, 21

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	281.99	44.29	142.28	333.50	-1.4492
IC10	314.73	31.42	262.25	391.05	0.6483
IC15	347.47	32.95	301.29	420.30	0.8951
IC20	385.42	38.07	329.23	454.17	0.6436
IC25	450.89	47.11	349.31	502.93	-0.0847
IC40	539.47	8.93	517.63	552.34	-0.0686
IC50	574.56	7.44	556.35	585.29	-0.0686



Dose-Response Plot



1-tail, 0.01 level of significance

Entered and Reviewed by Jim Summer

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **232**

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.

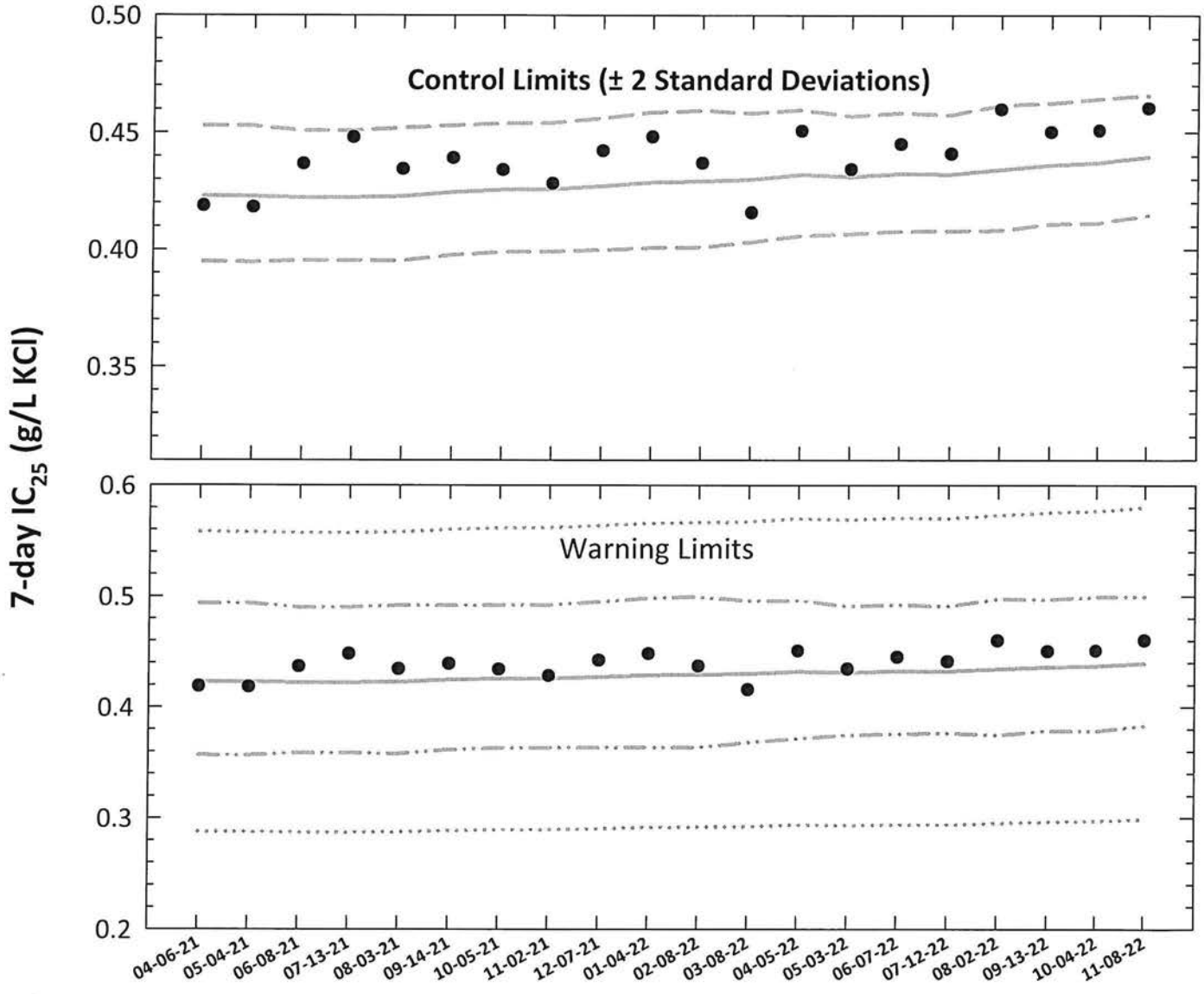
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	VL	EC VL	EC VL	VL	VL	EC VL
CONTROL, Salt SW	pH (S.U.)	7.57	7.77	7.84	7.61	7.77	7.77
	DO (mg/L)	7.6	7.8	8.0	6.9	7.9	7.7
	Salinity (ppt)	25.0	25.0	24.7	24.9	24.8	24.7
	Alkalinity (mg $CaCO_3$/L)	90			VL 10-09-22	100	
	Temperature (°C)	25.5	25.8	25.6	25.9	25.5	25.9
250 mg KCl/L	pH (S.U.)	7.57	7.79	7.80	7.64	7.74	7.76
	DO (mg/L)	7.6	7.7	8.0	6.8	7.9	7.5
	Salinity (ppt)	25.3	25.4	25.2	25.3	25.2	25.3
	Temperature (°C)	25.5	26.0	25.5	26.0	25.5	26.0
375 mg KCl/L	pH (S.U.)	7.59	7.80	7.81	7.61	7.74	7.77
	DO (mg/L)	7.9	7.8	8.0	7.2	7.9	7.6
	Salinity (ppt)	25.3	25.6	25.3	25.3	25.4	25.4
	Temperature (°C)	25.6	25.7	25.5	26.0	25.5	26.0
500 mg KCl/L	pH (S.U.)	7.63	7.80	7.82	7.62	7.75	7.75
	DO (mg/L)	8.0	8.0	8.0	7.5	8.1	7.7
	Salinity (ppt)	25.4	25.5	25.3	25.5	25.5	25.6
	Temperature (°C)	25.6	25.7	25.5	26.0	25.6	26.0
750 mg KCl/L	pH (S.U.)	7.67	7.84				
	DO (mg/L)	7.9	8.0				
	Salinity (ppt)	25.5	25.8				
	Temperature (°C)	25.6	25.7				
1000 mg KCl/L	pH (S.U.)	7.73	7.82			VL 10-05-22	
	DO (mg/L)	7.9	8.1				
	Salinity (ppt)	25.0	26.2				
	Temperature (°C)	25.4	25.8				
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **232**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	EC N	BSL	BSL	N	N	N	N	N
CONTROL, Salt SW	pH (S.U.)	7.90	7.83	7.94	7.65	7.84	7.78	7.87	7.59
	DO (mg/L)	8.0	7.8	7.6	7.3	7.6	7.6	7.8	6.8
	Salinity (ppt)	24.6	24.9	24.8	25.2	25.0	25.0	25.2	24.9
	Alkalinity (mg $CaCO_3$/L)		10-09-22	150				10-09-22	
	Temperature (°C)	25.5	26.2	25.4	26.0	25.5	26.2	25.6	25.9
250 mg KCl/L	pH (S.U.)	7.91	7.89	7.95	7.64	7.78	7.70	7.80	7.49
	DO (mg/L)	8.0	7.4	7.6	7.3	7.6	7.6	7.8	6.8
	Salinity (ppt)	25.2	25.3	25.2	25.7	25.2	25.2	25.2	25.3
	Temperature (°C)	25.6	26.3	25.3	26.1	25.6	26.3	25.6	26.1
375 mg KCl/L	pH (S.U.)	7.93	7.88	7.95	7.65	7.77	7.67	7.80	7.47
	DO (mg/L)	8.1	7.2	7.6	7.4	7.7	7.6	7.8	6.8
	Salinity (ppt)	25.1	25.5	25.3	26.0	25.1	25.3	25.4	25.5
	Temperature (°C)	25.7	26.0	25.3	26.1	25.6	26.1	25.6	26.1
500 mg KCl/L	pH (S.U.)	7.93	7.87	7.97	7.62	7.77	7.68	7.81	7.45
	DO (mg/L)	8.1	7.1	7.6	7.3	7.9	7.8	7.8	6.4
	Salinity (ppt)	25.1	25.5	25.4	26.3	25.3	25.5	25.4	25.6
	Temperature (°C)	25.6	26.0	25.3	26.1	25.6	26.2	25.5	26.1
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)					10-05-22			
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic IC_{25} ± 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	04-06-21	0.4188	-0.3780	-0.3737	0.0149	0.4229	0.3949	0.4529	0.3567	0.4939	0.2876	0.5582
2	05-04-21	0.4182	-0.3787	-0.3740	0.0149	0.4227	0.3946	0.4527	0.3562	0.4938	0.2874	0.5579
3	06-08-21	0.4366	-0.3599	-0.3745	0.0142	0.4222	0.3954	0.4508	0.3587	0.4900	0.2871	0.5573
4	07-13-21	0.4480	-0.3487	-0.3745	0.0143	0.4222	0.3953	0.4509	0.3585	0.4903	0.2871	0.5573
5	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.4227	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

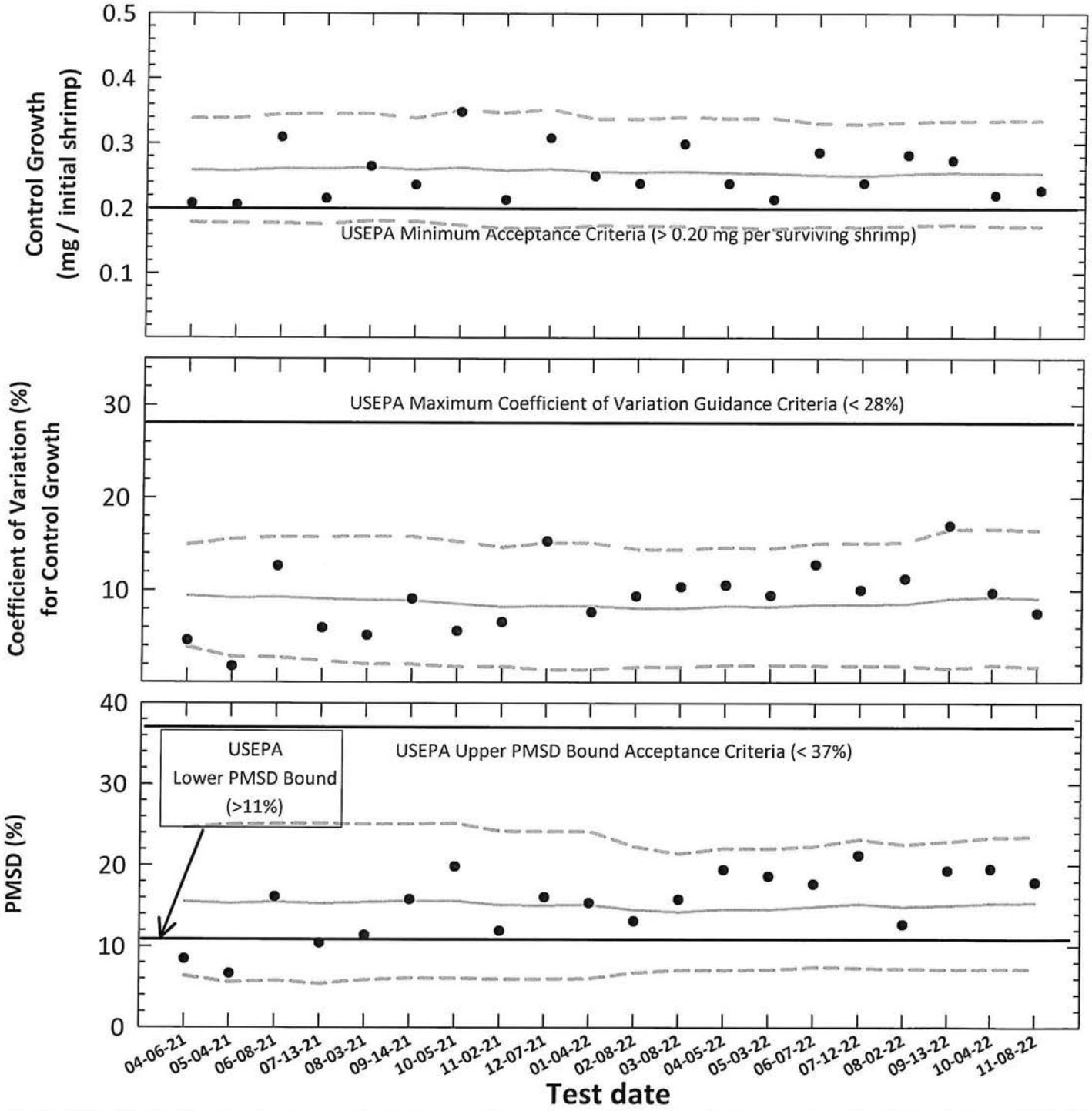
Entered and Reviewed by Jim Sumner

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)

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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	04-06-21	100	0.208	4.6	0.0175	8.4	0.259	0.179	0.339	9.4	3.9	14.9	15.5	6.4	24.6
2	05-04-21	100	0.206	1.8	0.0137	6.7	0.258	0.178	0.339	9.2	2.8	15.6	15.3	5.5	25.0
3	06-08-21	100	0.310	12.7	0.0499	16.1	0.261	0.178	0.345	9.3	2.8	15.8	15.5	5.8	25.1
4	07-13-21	100	0.215	5.9	0.0224	10.4	0.261	0.176	0.346	9.1	2.4	15.8	15.3	5.4	25.2
5	08-03-21	100	0.265	5.2	0.0301	11.4	0.263	0.181	0.345	8.9	2.0	15.8	15.4	5.9	25.0
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 Summer

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: **233**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2150				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	PALE GREEN
Date and times organisms were born between:	11-01-22 1200 to 11-02-22 1130	Incubator number and shelf location:	5D
Organism source:	AI Batch Ab: 11-02-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.73 S.U. Temperature = 25.0 °C	Date / Time in oven:	11-15-22 1115
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	11-16-22 1115
		*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	11-08-22	1015	JL	1245	JL	1130	JL	11-03-22 B
1	11-09-22	0500	JL	1100	JL	0930	JL	↓
2	11-10-22	0500	JL	1115	JL	0930	JL	11-07-22
3	11-11-22	0500	JL	1100	JL	0930	JL	↓
4	11-12-22	0610	JL	1210	JL	0943	JL	11-10-22
5	11-13-22	0600	JL	1200	JL	0933	JL	↓
6	11-14-22	0600	JL	1200	JL	0932	JL	↓
7	11-15-22					1033	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	13066465

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	544.7
Average weight per initial shrimp:	0.228		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.228	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	460.4

ETS
Environmental Testing Solutions, Inc.

AbKClCR Test Number: 233

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	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Lavender Analyst: AJ Date: 10-26-22	14.49	15.36	15.53	15.46	12.90	14.51	14.59	14.66	13.85	14.60	13.63	14.37	14.67	13.50	13.96	15.07
*B = Pan + Shrimp weight (mg) Analyst: JL Date: 11-21-22	15.57	16.59	16.75	16.56	13.96	15.71	15.80	15.67	14.89	15.77	14.74	15.58	15.88	14.52	15.08	16.17
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	1.08	1.23	1.22	1.10	1.06	1.20	1.21	1.01	1.04	1.17	1.11	1.21	1.21	1.02	1.12	1.10
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.216	0.246	0.244	0.220	0.212	0.240	0.242	0.202	0.208	0.234	0.222	0.242	0.242	0.204	0.224	0.220
	Average weight per initial number of shrimp (mg) 0.228								Average weight per initial number of shrimp (mg) 0.225				Percent reduction from control (%) 1.47			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **233**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	5	5	5	5	4[d]	5	5	5	3[fg]	5	4[d]	5	5	4[d]	5	4[d]
4	S	S	S	S	4	S	S	S	3	S	4	S	S	4	S	4
5	S	S	S	S	4	S	S	S	3	S	4	S	S	4	S	4
6	S	S	S	S	4	S	S	S	3	4[sm]	4	S	4[sm]	4	4[sm]	4
7	S	S	S	S	4	S	S	S	3	4	4	4[sm]	4	4	4	4
# females with eggs in brood sac																
# females with developing ova in oviducts																
# Immature females																
# males																K 11-15-22
*A = Pan weight (mg) Tray color code: Lavender Analyst: AJ Date: 10-26-22	13.95	14.76	15.24	14.37	14.41	13.76	15.21	15.31	14.52	13.77	16.43	15.09	15.26	13.58	14.52	15.42
*B = Pan + Shrimp weight (mg) Analyst: JH Date: 11-21-22	15.03	15.29	16.49	15.48	15.33	14.75	16.27	16.33	15.14	14.67	16.96	16.00	16.08	14.29	15.44	16.32
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JH	1.08	0.53	1.25	1.11	0.92	0.99	1.06	1.02	0.62	0.90	0.53	0.91	0.82	0.71	0.92	0.90
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JH	0.216	0.106	0.250	0.222	0.184	0.198	0.212	0.204	0.124	0.180	0.106	0.182	0.164	0.142	0.184	0.180

	Average weight per initial number of shrimp (mg)	Percent reduction from control (%)	Average weight per initial number of shrimp (mg)	Percent reduction from control (%)
	0.199	12.6 %	0.158	30.7 %

*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:

ETS
Environmental Testing Solutions, Inc.

AbKClCR Test Number: **233**

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															JH 11-09-22	
*A = Pan weight (mg) Tray color code: Lavender, *Black Analyst: Date:					*											
*B = Pan + Shrimp weight (mg) Analyst: Date:																
C = Shrimp weight (mg) = B – A Hand calculated Analyst:															KH 11-09-22	
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: 233

Test dates: November 08-15, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.49	15.57	1.08	0.216	100.0	0.228	7.5	Not applicable
	B	5	5	15.36	16.59	1.23	0.246				
	C	5	5	15.53	16.75	1.22	0.244				
	D	5	5	15.46	16.56	1.10	0.220				
	E	5	5	12.90	13.96	1.06	0.212				
	F	5	5	14.51	15.71	1.20	0.240				
	G	5	5	14.59	15.80	1.21	0.242				
	H	5	5	14.66	15.67	1.01	0.202				
250	I	5	5	13.85	14.89	1.04	0.208	100.0	0.225	6.3	1.4
	J	5	5	14.60	15.77	1.17	0.234				
	K	5	5	13.63	14.74	1.11	0.222				
	L	5	5	14.37	15.58	1.21	0.242				
	M	5	5	14.67	15.88	1.21	0.242				
	N	5	5	13.50	14.52	1.02	0.204				
	O	5	5	13.96	15.08	1.12	0.224				
	P	5	5	15.07	16.17	1.10	0.220				
375	Q	5	5	13.95	15.03	1.08	0.216	97.5	0.199	21.2	12.6
	R	5	5	14.76	15.29	0.53	0.106				
	S	5	5	15.24	16.49	1.25	0.250				
	T	5	5	14.37	15.48	1.11	0.222				
	U	5	4	14.41	15.33	0.92	0.184				
	V	5	5	13.76	14.75	0.99	0.198				
	W	5	5	15.21	16.27	1.06	0.212				
	X	5	5	15.31	16.33	1.02	0.204				
500	Y	5	3	14.52	15.14	0.62	0.124	77.5	0.158	19.1	30.7
	Z	5	4	13.77	14.67	0.90	0.180				
	AA	5	4	16.43	16.96	0.53	0.106				
	BB	5	4	15.09	16.00	0.91	0.182				
	CC	5	4	15.26	16.08	0.82	0.164				
	DD	5	4	13.58	14.29	0.71	0.142				
	EE	5	4	14.52	15.44	0.92	0.184				
	FF	5	4	15.42	16.32	0.90	0.180				
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: 0.0407

PMSD: 17.9

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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:	11/8/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

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

			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	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.7750	0.7750	1.0795	0.8861	1.1071	7.240	8	36.00	48.00	9	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

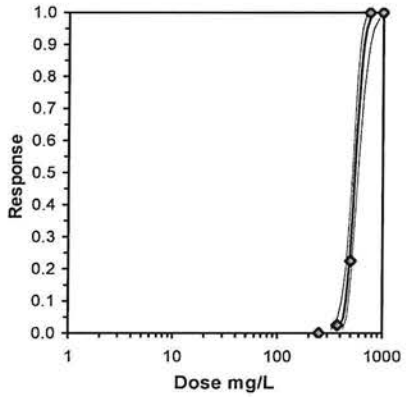
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.46921	0.904	-3.374	11.2121
Equality of variance cannot be confirmed				

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

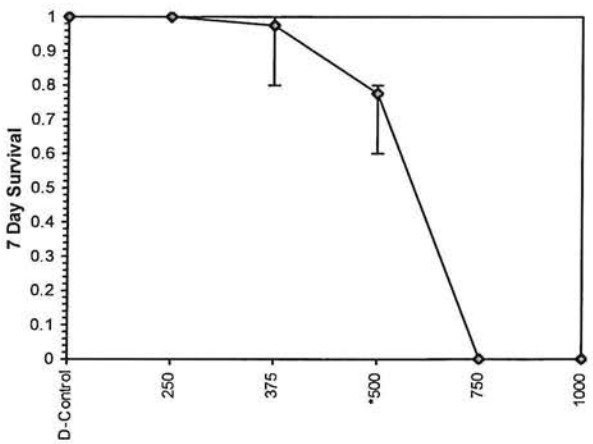
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.5805	2.62498	10.4355	20.7255	0	3.81625	7.81472	0.282	2.73613	0.06418	6
Intercept	-37.63	7.12994	-51.605	-23.656							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	386.202	329.091	421.576
EC05	3.355	427.124	380.226	457.443
EC10	3.718	450.684	409.769	478.832
EC15	3.964	467.309	430.336	494.572
EC20	4.158	480.959	446.859	508.083
EC25	4.326	492.986	461.019	520.555
EC40	4.747	524.645	495.839	556.572
EC50	5.000	544.66	515.799	581.946
EC60	5.253	565.44	535.013	610.239
EC75	5.674	601.75	565.901	663.439
EC80	5.842	616.798	578.003	686.579
EC85	6.036	634.815	592.113	714.964
EC90	6.282	658.232	609.971	752.827
EC95	6.645	694.54	636.853	813.408
EC99	7.326	768.134	689.29	942.178



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:	11/8/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2022	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	0.2160	0.2460	0.2440	0.2200	0.2120	0.2400	0.2420	0.2020
250	0.2080	0.2340	0.2220	0.2420	0.2420	0.2040	0.2240	0.2200
375	0.2160	0.1060	0.2500	0.2220	0.1840	0.1980	0.2120	0.2040
500	0.1240	0.1800	0.1060	0.1820	0.1640	0.1420	0.1840	0.1800
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

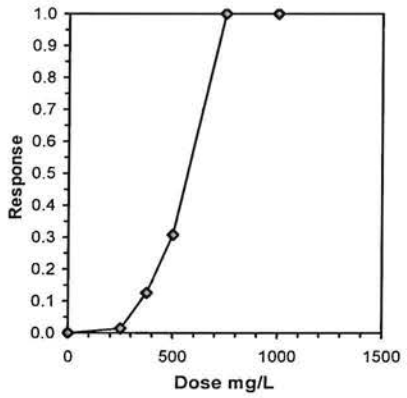
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	0.2278	1.0000	0.2278	0.2020	0.2460	7.531	8			0.2278	1.0000
250	0.2245	0.9857	0.2245	0.2040	0.2420	6.349	8	65.00	43.00	0.2245	0.9857
375	0.1990	0.8738	0.1990	0.1060	0.2500	21.225	8	52.50	43.00	0.1990	0.8738
500	0.1578	0.6926	0.1578	0.1060	0.1840	19.125	8			0.1578	0.6926
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)	0.84327	0.884	-1.7247	6.31321
Bartlett's Test indicates unequal variances (p = 9.44E-03)	9.32622	9.21035		

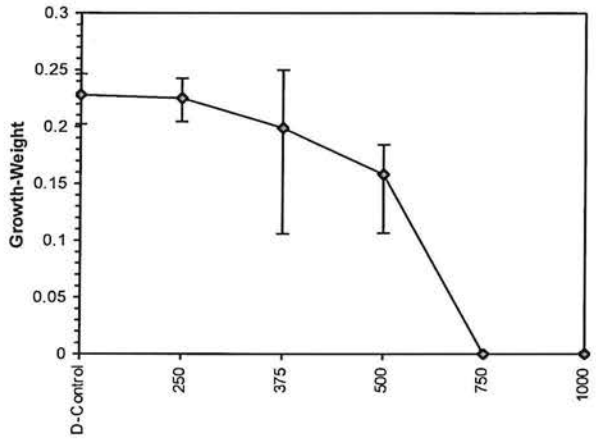
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs D-Control	375	>375		

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	289.89	50.93	168.74	381.04	-0.1380
IC10	345.71	39.25	278.67	406.71	-0.0243
IC15	391.40	34.71	314.92	441.05	-0.4236
IC20	425.91	33.39	341.05	473.63	-0.6550
IC25	460.42	31.51	367.13	505.63	-0.9721
IC40	533.44	14.01	500.76	554.51	-0.5071
IC50	569.53	11.70	542.30	587.09	-0.5217



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKClCR Test Number: **233**

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	JH	EC K	EC K	EC	EC	K
CONTROL, Salt SW	pH (S.U.)	7.64	7.92	7.96	7.89	7.94	7.83
	DO (mg/L)	7.8	7.6	7.7	7.7	7.9	7.1
	Salinity (ppt)	25.0	25.2	24.9	25.1	25.0	25.2
	Alkalinity (mg $CaCO_3$/L)	130			JH 11-13-22	120	
	Temperature (°C)	25.7	25.8	25.4	26.2	25.6	25.9
250 mg KCl/L	pH (S.U.)	7.67	7.95	7.96	7.93	7.97	7.84
	DO (mg/L)	7.8	7.6	7.7	7.6	7.8	7.0
	Salinity (ppt)	25.2	25.3	25.0	25.2	25.1	25.3
	Temperature (°C)	25.7	26.0	25.5	26.3	25.5	26.2
375 mg KCl/L	pH (S.U.)	7.71	7.95	7.98	7.89	8.00	7.80
	DO (mg/L)	7.8	7.7	7.8	7.6	7.8	7.2
	Salinity (ppt)	25.3	25.3	25.2	25.4	25.1	25.6
	Temperature (°C)	25.8	26.0	25.5	26.1	25.5	26.0
500 mg KCl/L	pH (S.U.)	7.74	7.96	7.98	7.94	8.01	7.83
	DO (mg/L)	7.8	7.7	7.9	7.6	7.8	7.1
	Salinity (ppt)	25.3	25.4	25.2	25.5	25.2	25.0
	Temperature (°C)	25.8	26.1	25.5	26.2	25.5	26.0
750 mg KCl/L	pH (S.U.)	7.75	7.95				
	DO (mg/L)	7.8	7.8				
	Salinity (ppt)	25.4	25.6				
	Temperature (°C)	25.7	25.9				
1000 mg KCl/L	pH (S.U.)	7.76	7.95			JH 11-09-22	
	DO (mg/L)	7.8	7.8				
	Salinity (ppt)	25.5	25.7				
	Temperature (°C)	25.7	26.1				
		Initial	Final	Initial	Final	Initial	Final

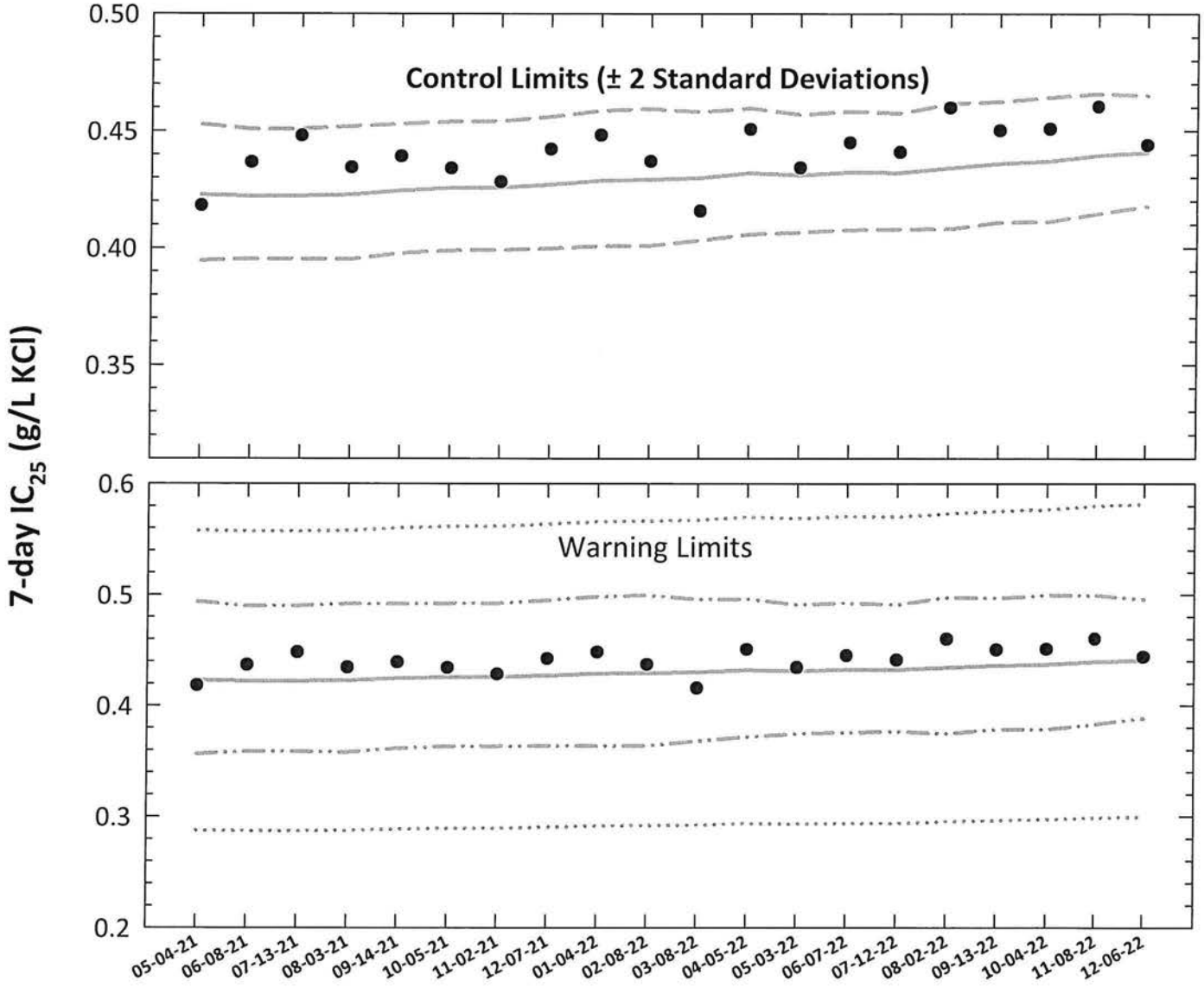
Environmental Testing Solutions, Inc.

AbKClCR Test Number: 233

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 3		4		5		6	
	Analyst	K	BSL	BSL	K	K	EC K	EC K	EC
CONTROL, Salt SW	pH (S.U.)	7.88	8.08	8.20	7.87	7.98	7.91	8.03	7.95
	DO (mg/L)	7.8	7.5	7.7	6.8	7.7	7.6	7.7	7.5
	Salinity (ppt)	25.0	25.6	24.8	25.1	24.4	25.4	24.4	25.8
	Alkalinity (mg $CaCO_3$/L)	K 11-13-22		120				K 11-13-22	
	Temperature (°C)	25.4	26.2	25.3	26.1	25.3	26.0	25.2	26.1
250 mg KCl/L	pH (S.U.)	7.90	8.07	8.19	7.87	7.97	7.92	8.00	7.97
	DO (mg/L)	7.7	7.8	7.7	6.5	7.7	7.5	7.6	7.4
	Salinity (ppt)	25.3	25.7	25.2	25.4	25.1	25.2	25.1	25.3
	Temperature (°C)	25.2	26.1	25.2	26.0	25.5	26.2	25.3	26.0
375 mg KCl/L	pH (S.U.)	7.92	8.08	8.20	7.86	7.98	7.91	8.01	7.97
	DO (mg/L)	7.7	7.7	7.7	7.0	7.9	7.5	7.6	7.4
	Salinity (ppt)	25.3	25.6	25.5	25.7	25.1	25.3	25.2	25.7
	Temperature (°C)	25.2	26.2	25.3	26.0	25.5	26.1	25.3	26.0
500 mg KCl/L	pH (S.U.)	7.93	8.14	8.20	7.86	8.00	7.90	8.01	7.98
	DO (mg/L)	7.8	7.6	7.7	7.4	7.9	7.6	7.7	7.4
	Salinity (ppt)	25.3	25.7	25.7	26.0	25.2	25.5	25.3	25.8
	Temperature (°C)	25.2	26.2	25.3	26.2	25.6	26.1	25.4	26.2
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)						JT 11-09-22		
	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.



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	05-04-21	0.4182	-0.3787	-0.3740	0.0149	0.4227	0.3946	0.4527	0.3562	0.4938	0.2874	0.5579
2	06-08-21	0.4366	-0.3599	-0.3745	0.0142	0.4222	0.3954	0.4508	0.3587	0.4900	0.2871	0.5573
3	07-13-21	0.4480	-0.3487	-0.3745	0.0143	0.4222	0.3953	0.4509	0.3585	0.4903	0.2871	0.5573
4	08-03-21	0.4344	-0.3622	-0.3740	0.0146	0.4227	0.3953	0.4520	0.3578	0.4920	0.2874	0.5579
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

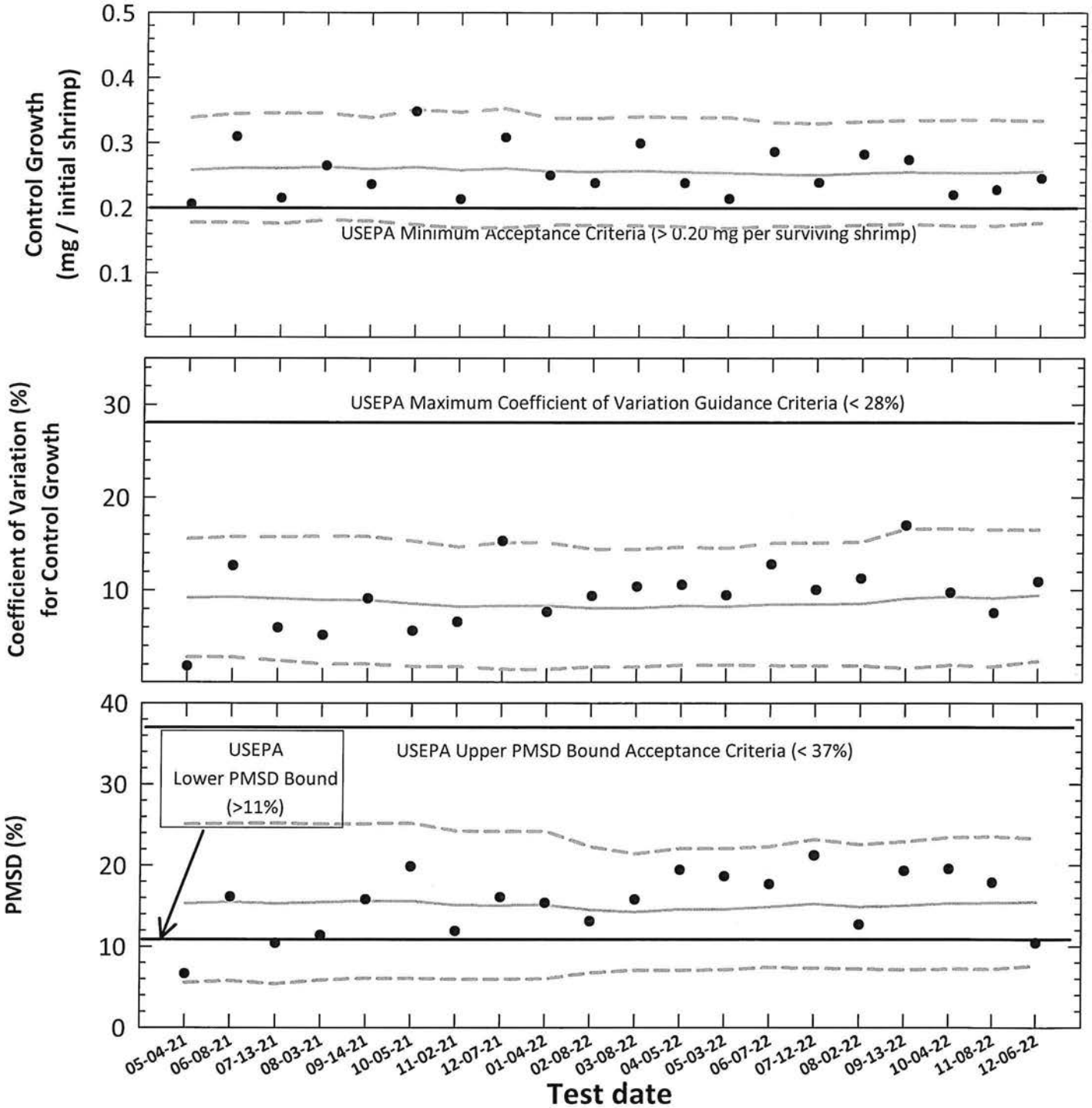
Entered and Reviewed by Jim Sumner

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										
			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	05-04-21	100	0.206	1.8	0.0137	6.7	0.258	0.178	0.339	9.2	2.8	15.6	15.3	5.5	25.0
2	06-08-21	100	0.310	12.7	0.0499	16.1	0.261	0.178	0.345	9.3	2.8	15.8	15.5	5.8	25.1
3	07-13-21	100	0.215	5.9	0.0224	10.4	0.261	0.176	0.346	9.1	2.4	15.8	15.3	5.4	25.2
4	08-03-21	100	0.265	5.2	0.0301	11.4	0.263	0.181	0.345	8.9	2.0	15.8	15.4	5.9	25.0
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: **234**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2128				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	PALE GREEN
Date and times organisms were born between:	11-29-22 1200 to 11-30-22 1130	Incubator number and shelf location:	5D
Organism source:	AI Batch Ab: 11-30-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.80 S.U. Temperature = 25.0 °C	Date / Time in oven:	12-13-22 1112
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	12-14-22 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	12-06-22	1110	JK	1230	JK	1220	JK	11-30-22 A
1	12-07-22	0500	JK	1100	JK	1020	JK	↓
2	12-08-22	0500	JK	1100	JK	1020	JK	11-30-22 B
3	12-09-22	0500	JK	1100	JK	1020	JK	↓
4	12-10-22	0600	JK	1200	JK	1030	JK	12-06-22
5	12-11-22	0600	JK	1200	JK	1040	JK	↓
6	12-12-22	0555	JK	1200	JK	1020	JK	
7	12-13-22					1100	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	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	533.1
Average weight per initial shrimp:	0.246		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.246	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	444.0

ETS
Environmental Testing Solutions, Inc.

AbKClCR Test Number: **234**

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 12.13.22	
*A = Pan weight (mg) Tray color code: Grey Analyst: EC Date: 112922	15.31	14.10	14.49	14.60	13.87	13.73	14.88	15.74	14.87	13.54	15.28	15.82	14.82	13.43	14.88	15.43
*B = Pan + Shrimp weight (mg) Analyst: JH Date: 12.19.22	16.51	15.33	15.68	15.88	15.04	14.75	16.11	17.24	15.89	14.69	16.34	16.89	16.00	14.68	15.53	16.55
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JH	1.20	1.23	1.19	1.28	1.17	1.02	1.23	1.50	1.02	1.15	1.06	1.07	1.18	1.25	1.15	1.12
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JH	0.240	0.246	0.238	0.256	0.234	0.204	0.246	0.300	0.204	0.230	0.212	0.214	0.236	0.250	0.230	0.224
	Average weight per initial number of shrimp (mg) 0.246								Average weight per initial number of shrimp (mg) 0.225				Percent reduction from control (%) 8.47.			

*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: 234

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	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	4 1d	3 2d	4 1d	4 1d	3 2d	3 2d	4 1d	4 1d
6	S	S	S	S	S	S	S	S	3 1d	3	4	3 1d	3	3	3 1d	4
7	S	S	S	S	S	S	S	S	2 1d	3	4	2 1d	3	3	3	4
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Grey Analyst: EC Date: 112922	13.22	13.17	14.72	13.74	12.88	13.72	13.76	13.44	14.12	15.77	14.21	16.18	14.10	15.19	15.05	12.50
*B = Pan + Shrimp weight (mg) Analyst: JH Date: 12-14-22	14.36	14.30	15.88	14.81	13.98	14.77	14.85	14.60	14.57	16.46	15.20	16.84	15.06	15.83	15.91	13.37
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JH	1.14	1.13	1.16	1.07	1.10	1.05	1.09	1.16	0.45	0.69	0.99	0.66	0.96	0.64	0.86	0.87
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JH	0.228	0.226	0.232	0.214	0.220	0.210	0.218	0.232	0.090	0.138	0.198	0.132	0.192	0.128	0.172	0.174
	Average weight per initial number of shrimp (mg) 0.223				Percent reduction from control (%) 9.47.				Average weight per initial number of shrimp (mg) 0.153				Percent reduction from control (%) 37.7 7.			

*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: 234

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 sk	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: Grey *Darkgreen Analyst: EC Date: 11 29 22	15.32	13.20	14.96	13.95	15.23	14.78	15.38	14.28	13.62	14.26	13.83	15.15	14.03	14.66	13.45	14.63
*B = Pan + Shrimp weight (mg) Analyst: Date:																
C = Shrimp weight (mg) = B – A Hand calculated Analyst:																12-07-22
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: 234

Test dates: December 06-13, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	15.31	16.51	1.20	0.240	100.0	0.246	10.9	Not applicable
	B	5	5	14.10	15.33	1.23	0.246				
	C	5	5	14.49	15.68	1.19	0.238				
	D	5	5	14.60	15.88	1.28	0.256				
	E	5	5	13.87	15.04	1.17	0.234				
	F	5	5	13.73	14.75	1.02	0.204				
	G	5	5	14.88	16.11	1.23	0.246				
	H	5	5	15.74	17.24	1.50	0.300				
250	I	5	5	14.87	15.89	1.02	0.204	100.0	0.225	6.6	8.4
	J	5	5	13.54	14.69	1.15	0.230				
	K	5	5	15.28	16.34	1.06	0.212				
	L	5	5	15.82	16.89	1.07	0.214				
	M	5	5	14.82	16.00	1.18	0.236				
	N	5	5	13.43	14.68	1.25	0.250				
	O	5	5	14.38	15.53	1.15	0.230				
	P	5	5	15.43	16.55	1.12	0.224				
375	Q	5	5	13.22	14.36	1.14	0.228	100.0	0.223	3.7	9.4
	R	5	5	13.17	14.30	1.13	0.226				
	S	5	5	14.72	15.88	1.16	0.232				
	T	5	5	13.74	14.81	1.07	0.214				
	U	5	5	12.88	13.98	1.10	0.220				
	V	5	5	13.72	14.77	1.05	0.210				
	W	5	5	13.76	14.85	1.09	0.218				
	X	5	5	13.44	14.60	1.16	0.232				
500	Y	5	2	14.12	14.57	0.45	0.090	60.0	0.153	24.2	37.7
	Z	5	3	15.77	16.46	0.69	0.138				
	AA	5	4	14.21	15.20	0.99	0.198				
	BB	5	2	16.18	16.84	0.66	0.132				
	CC	5	3	14.10	15.06	0.96	0.192				
	DD	5	3	15.19	15.83	0.64	0.128				
	EE	5	3	15.05	15.91	0.86	0.172				
	FF	5	4	12.50	13.37	0.87	0.174				
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: 0.0256

PMSD: 10.4

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:	12/6/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	12/13/2022	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	0.8000	0.4000	0.6000	0.6000	0.6000	0.8000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

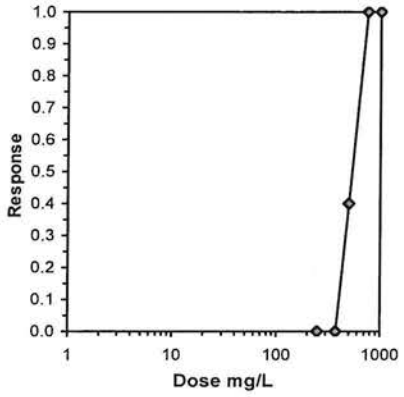
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.6000	0.6000	0.8910	0.6847	1.1071	17.929	8	36.00	48.00	16	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)	0.51509	0.904	0.20753	6.10332
Equality of variance cannot be confirmed				

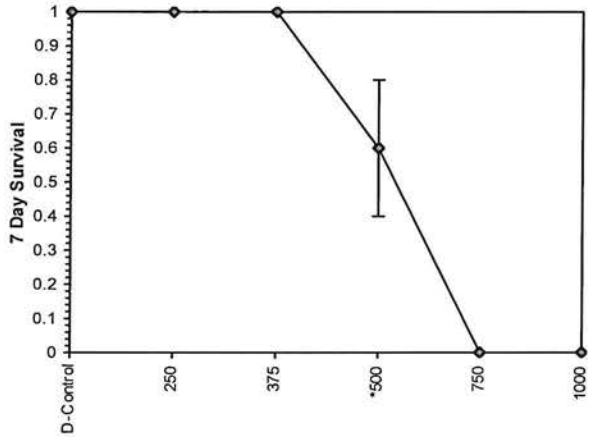
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	533.10	505.23	562.51
5.0%	533.38	502.42	566.25
10.0%	533.65	498.69	571.07
20.0%	534.18	486.77	586.22
Auto-0.0%	533.10	505.23	562.51



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:	12/6/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	12/13/2022	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	0.2400	0.2460	0.2380	0.2560	0.2340	0.2040	0.2460	0.3000
250	0.2040	0.2300	0.2120	0.2140	0.2360	0.2500	0.2300	0.2240
375	0.2280	0.2260	0.2320	0.2140	0.2200	0.2100	0.2180	0.2320
500	0.0900	0.1380	0.1980	0.1320	0.1920	0.1280	0.1720	0.1740
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	0.2455	1.0000	0.2455	0.2040	0.3000	10.910	8				0.2455	1.0000
250	0.2250	0.9165	0.2250	0.2040	0.2500	6.566	8	2.241	2.799	0.0256	0.2250	0.9165
375	0.2225	0.9063	0.2225	0.2100	0.2320	3.714	8	2.514	2.799	0.0256	0.2225	0.9063
500	0.1530	0.6232	0.1530	0.0900	0.1980	24.215	8				0.1530	0.6232
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

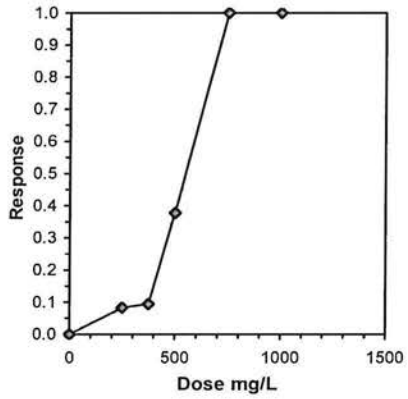
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90328	0.884	0.82689	4.13267
Bartlett's Test indicates equal variances (p = 0.02)	8.25543	9.21035		

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			0.0256	0.10427	0.00127	0.00033	0.03884	2, 21

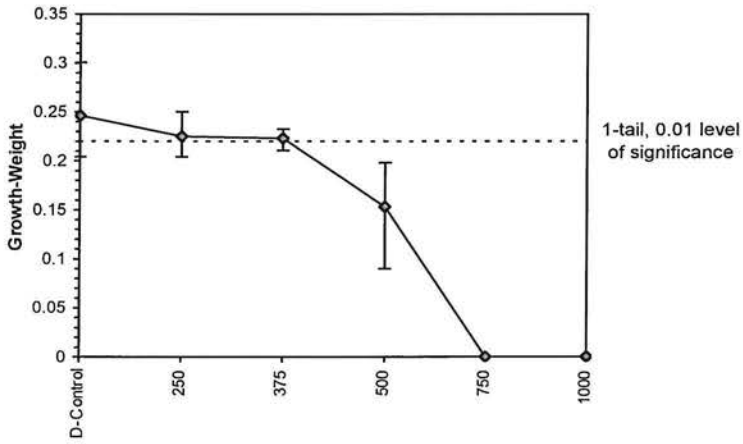
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05*	149.70	79.80	85.12	378.40	1.2174
IC10	377.79	80.64	170.23	406.29	-0.7404
IC15	399.87	31.75	301.12	431.56	-3.5997
IC20	421.94	16.83	394.08	462.88	0.7868
IC25	444.02	18.73	414.98	494.23	0.7424
IC40	509.31	19.47	474.05	547.40	-0.0541
IC50	549.43	17.64	512.30	581.16	-0.3779

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKClCR Test Number: **234**

Daily Chemistry:

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

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	EC	EC M	EC M	M	M	EC
CONTROL, Salt SW	pH (S.U.)	7.91	7.91	8.00	7.60	7.64	7.82
	DO (mg/L)	7.7	7.7	7.8	7.8	7.9	7.1
	Salinity (ppt)	24.6	25.0	24.7	24.9	24.7	24.9
	Alkalinity (mg $CaCO_3$/L)	120		← 12-11-22		130	
	Temperature (°C)	25.6	26.2	25.5	26.3	25.3	26.3
250 mg KCl/L	pH (S.U.)	7.92	7.92	7.97	7.81	7.84	7.83
	DO (mg/L)	7.7	7.7	7.8	7.8	7.8	6.9
	Salinity (ppt)	25.0	25.3	25.2	25.2	25.0	25.1
	Temperature (°C)	25.4	26.0	25.4	26.2	25.4	26.2
375 mg KCl/L	pH (S.U.)	7.95	7.92	7.97	7.81	7.84	7.83
	DO (mg/L)	7.8	7.8	7.8	7.8	7.8	6.9
	Salinity (ppt)	24.9	25.4	25.2	25.2	25.2	25.4
	Temperature (°C)	25.4	26.2	25.4	26.2	25.4	26.2
500 mg KCl/L	pH (S.U.)	7.96	7.95	7.97	7.81	7.84	7.83
	DO (mg/L)	7.8	7.9	7.8	7.8	7.9	7.0
	Salinity (ppt)	25.0	25.5	25.3	25.4	25.3	25.5
	Temperature (°C)	25.5	26.2	25.4	26.2	25.3	26.2
750 mg KCl/L	pH (S.U.)	7.91	7.90				
	DO (mg/L)	7.8	7.9				
	Salinity (ppt)	25.0	25.6				
	Temperature (°C)	25.5	26.2				
1000 mg KCl/L	pH (S.U.)	7.91	7.93				
	DO (mg/L)	7.8	7.9				
	Salinity (ppt)	25.3	25.7			12-01-22	
	Temperature (°C)	25.5	26.2				
		Initial	Final	Initial	Final	Initial	Final

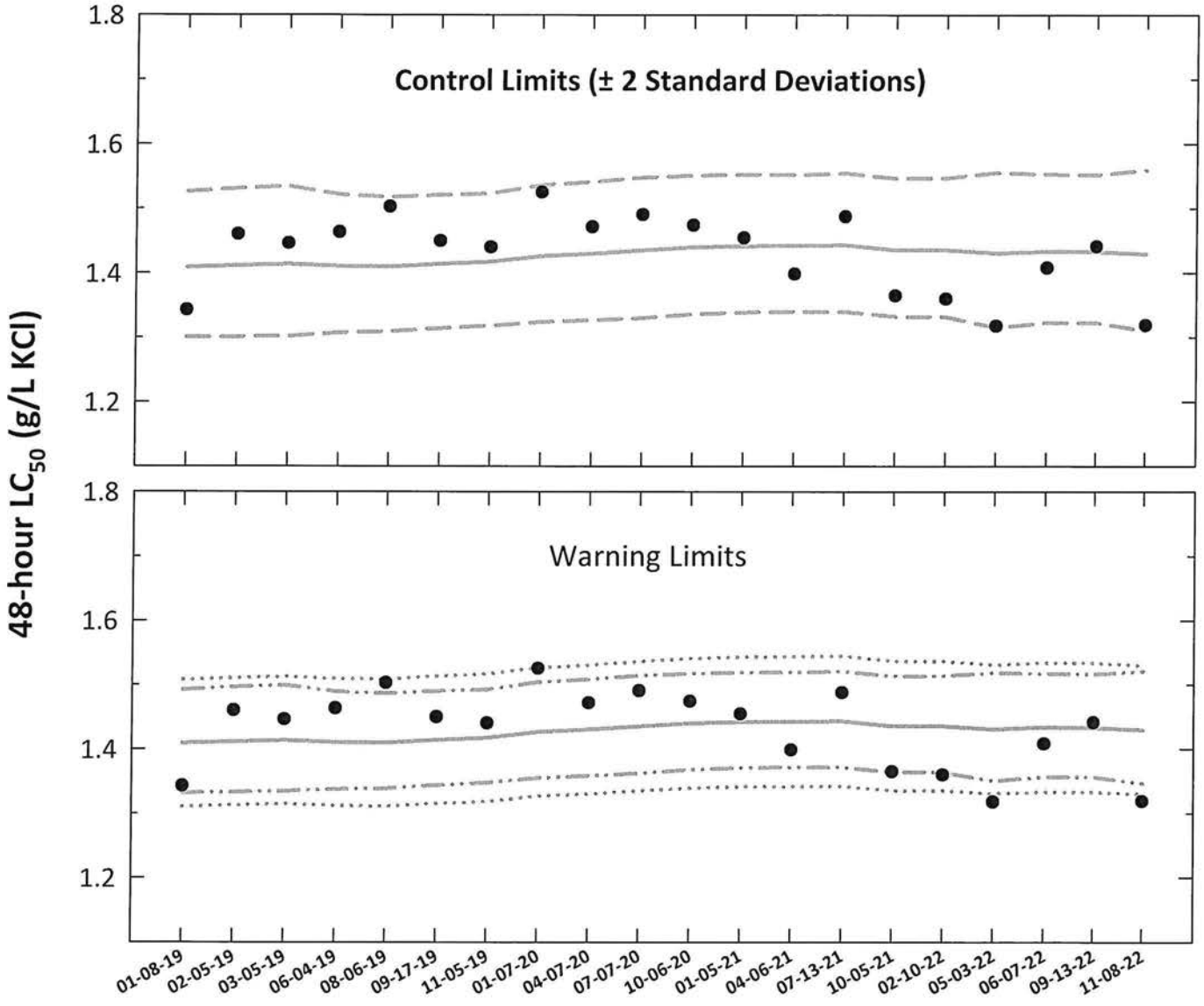
Environmental Testing Solutions, Inc.

AbKClCR Test Number: 234

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	EC	BSL	BSL	BSL	BSL	EC	EC	EC
CONTROL, Salt SW	pH (S.U.)	7.92	7.82	7.94	7.88	7.94	7.71	7.84	7.73
	DO (mg/L)	7.7	7.7	8.0	7.5	7.7	7.5	8.0	7.0
	Salinity (ppt)	24.7	24.8	24.7	25.3	24.9	25.0	25.1	25.1
	Alkalinity (mg $CaCO_3$/L)	N 12-1-22		100				N 12-1-22	
	Temperature (°C)	25.2	26.3	25.3	26.4	25.3	26.4	25.1	26.2
250 mg KCl/L	pH (S.U.)	7.94	7.86	7.94	7.92	7.96	7.81	7.88	7.78
	DO (mg/L)	7.8	7.7	8.0	7.5	7.7	7.5	7.9	7.0
	Salinity (ppt)	25.0	25.1	25.0	25.2	25.2	25.3	25.2	25.4
	Temperature (°C)	25.2	26.2	25.2	26.2	25.2	26.2	25.2	26.4
375 mg KCl/L	pH (S.U.)	7.94	7.83	7.95	7.92	7.96	7.79	7.88	7.77
	DO (mg/L)	7.8	7.8	8.0	7.5	7.8	7.4	7.9	6.9
	Salinity (ppt)	25.2	25.3	25.2	25.5	25.3	25.5	25.4	25.6
	Temperature (°C)	25.2	26.2	25.3	26.2	25.3	26.2	25.2	26.4
500 mg KCl/L	pH (S.U.)	7.95	7.87	7.96	7.92	7.97	7.81	7.89	7.80
	DO (mg/L)	7.8	7.8	8.0	7.5	7.9	7.4	7.9	6.9
	Salinity (ppt)	25.3	25.4	25.3	25.5	25.4	25.7	25.6	25.7
	Temperature (°C)	25.2	26.3	25.3	26.3	25.3	26.2	25.2	26.4
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)						JA 12-07-22		
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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	01-08-19	1.3428	0.1280	0.1488	0.0174	1.4088	1.3005	1.5261	1.3319	1.4920	1.3102	1.5074
2	02-05-19	1.4601	0.1644	0.1496	0.0177	1.4113	1.3007	1.5312	1.3330	1.4962	1.3125	1.5101
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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. ($S_{A.10}$ = 0.07).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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 # 87

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 #: 2150

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
		H	EC N	EC
Control, SaltSW	pH (S.U.)	7.64	7.98	7.91
	Dissolved oxygen (mg/L)	7.8	7.9	7.4
	*Salinity (ppt)	25.0	25.1	25.3
	*Alkalinity (mg/L $CaCO_3$)	130		
	*Temperature (°C)	25.0	25.1	25.0
1000 mg/L	pH (S.U.)	7.82	7.97	7.86
	Dissolved oxygen (mg/L)	7.9	7.9	7.5
	*Salinity (ppt)	25.4	25.8	25.9
	*Temperature (°C)	25.1	24.9	25.0
1250 mg/L	pH (S.U.)	7.85	7.97	7.92
	Dissolved oxygen (mg/L)	7.9	7.9	7.4
	*Salinity (ppt)	25.5	25.9	26.0
	*Temperature (°C)	25.1	25.0	24.8
1500 mg/L	pH (S.U.)	7.96	7.97	7.95
	Dissolved oxygen (mg/L)	7.9	7.9	7.3
	*Salinity (ppt)	25.6	26.0	26.2
	*Temperature (°C)	25.1	25.0	25.0
1750 mg/L	pH (S.U.)	7.97	7.96	
	Dissolved oxygen (mg/L)	7.9	7.9	
	*Salinity (ppt)	25.8	26.1	
	*Temperature (°C)	25.2	25.0	
2000 mg/L	pH (S.U.)	7.97	7.94	
	Dissolved oxygen (mg/L)	7.9	7.9	
	*Salinity (ppt)	26.0	26.4	
	*Temperature (°C)	25.1	25.0	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664655

ETC

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 # 87

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-08-22	* 1015	JH	1222	JH	6C	LIGHT YELLOW	11-03-22B
24	11-09-22			1222	JH			
48 Termination	11-10-22			1219	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):	AI Mb 10-29-22
Age (9 to 14 days old):	10 DAYS OLD
Date organisms were born: (time organisms were born between is not provided by supplier)	10-26-22 1200 TO 10-29-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.41
	Temperature (°C) 24.8

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

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 1d	6 4d	2 8d	1 9d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	8 10d	6	1 1d	1	0	0	0	0
Mean Survival	100%		100%		70%		10%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1253.1
Upper 95% confidence limit (mg KCl/L)	1381.4
48-hour LC_{50} (mg KCl/L)	~~1318~~ 1319.0

Comments:

Test Reviewed by:

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date:	11/8/2022	Test ID:	MbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.9000	0.6000
1500	0.2000	0.1000
1750	0.0000	0.0000
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	0.3512	0	20
1250	0.7500	0.7500	1.0676	0.8861	1.2490	24.041	2	2.795	2.850	0.3512	5	20
*1500	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	8.271	2.850	0.3512	17	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	10.169	2.850	0.3512	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

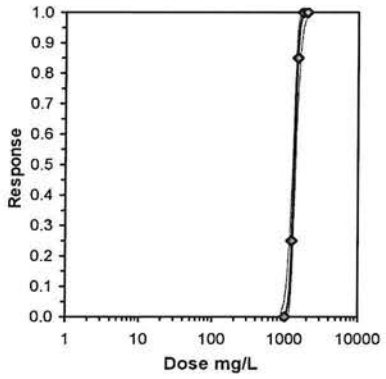
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.21333	0.2188	0.67926	0.01519	4.2E-04	4, 5
Treatments vs D-Control										

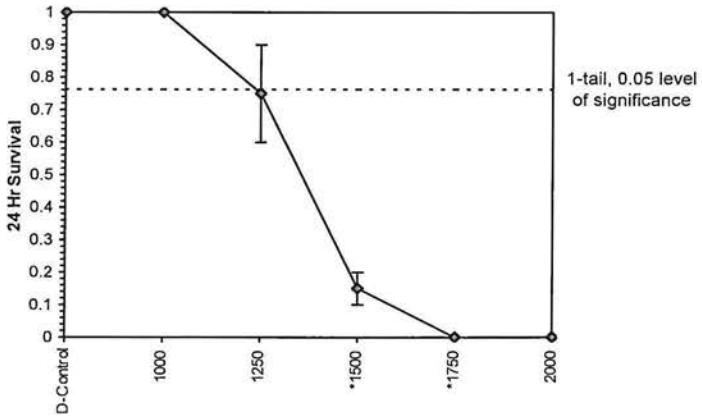
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	23.0061	4.78426	13.629	32.3833	0	0.17492	7.81472	0.98153	3.12802	0.04347	3
Intercept	-66.964	14.9913	-96.347	-37.581							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1063.9	890.676	1152.81
EC05	3.355	1139	996.134	1213.97
EC10	3.718	1181.18	1056.3	1249.15
EC15	3.964	1210.51	1098.18	1274.33
EC20	4.158	1234.35	1132	1295.45
EC25	4.326	1255.17	1161.2	1314.57
EC40	4.747	1309.21	1234.06	1368.55
EC50	5.000	1342.83	1275.91	1406.66
EC60	5.253	1377.31	1315.15	1450.26
EC75	5.674	1436.61	1374.12	1535.69
EC80	5.842	1460.84	1395.76	1573.77
EC85	6.036	1489.6	1420.12	1620.82
EC90	6.282	1526.6	1449.89	1683.75
EC95	6.645	1583.13	1493.03	1784.11
EC99	7.326	1694.88	1573.39	1993.89



Dose-Response Plot



ETS
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Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date:	11/8/2022	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	0.8000	0.6000
1500	0.1000	0.1000
1750	0.0000	0.0000
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	0.1992	0	20
*1250	0.7000	0.7000	0.9966	0.8861	1.1071	15.685	2	5.942	2.850	0.1992	6	20
*1500	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	15.596	2.850	0.1992	18	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	17.927	2.850	0.1992	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

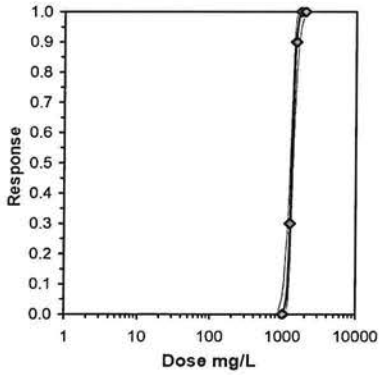
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.0978	0.1003	0.70476	0.00489	2.4E-05	4, 5
Treatments vs D-Control										

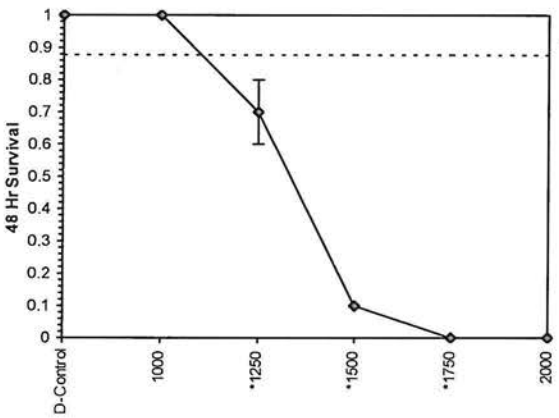
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	23.8988	5.185	13.7362	34.0614	0	0.1066	7.81472	0.99103	3.12023	0.04184	3
Intercept	-69.57	16.2055	-101.33	-37.807							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1054.13	877.495	1141.31
EC05	3.355	1125.66	980.567	1198.93
EC10	3.718	1165.76	1039.34	1232.05
EC15	3.964	1193.62	1080.23	1255.77
EC20	4.158	1216.24	1113.23	1275.69
EC25	4.326	1235.98	1141.7	1293.74
EC40	4.747	1287.16	1212.59	1344.96
EC50	5.000	1318.97	1253.11	1381.39
EC60	5.253	1351.56	1290.85	1423.35
EC75	5.674	1407.53	1346.97	1506.1
EC80	5.842	1430.38	1367.39	1543.1
EC85	6.036	1457.48	1390.3	1588.84
EC90	6.282	1492.31	1418.18	1650.04
EC95	6.645	1545.47	1458.46	1747.62
EC99	7.326	1650.35	1533.19	1951.49



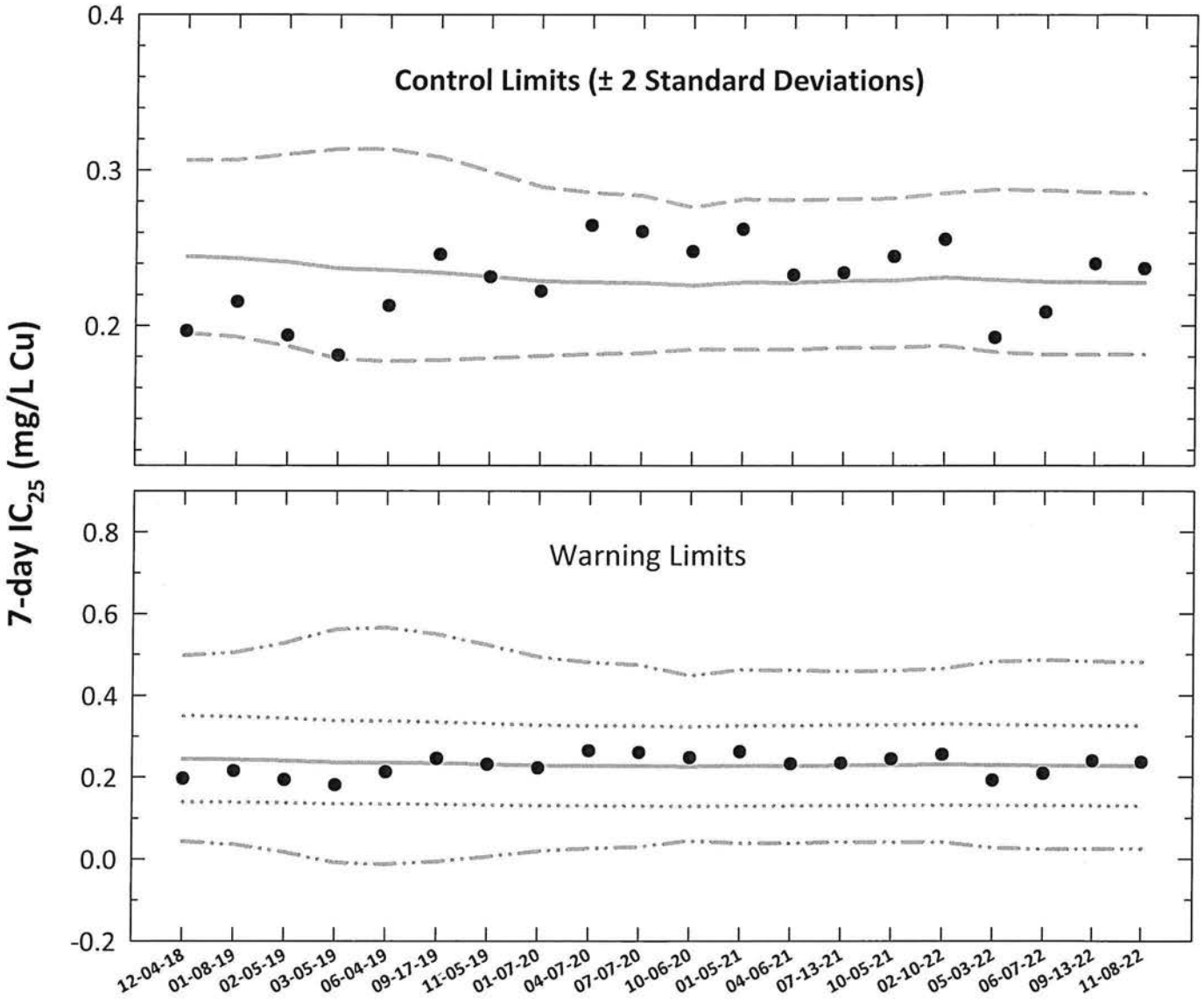
Dose-Response Plot



1-tail, 0.05 level of significance

ETS
Environmental Testing Solutions, Inc.

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	12-04-18	0.1966	-0.7064	-0.6115	0.0489	0.2446	0.1953	0.3064	0.0429	0.4973	0.1394	0.3498
2	01-08-19	0.2154	-0.6668	-0.6140	0.0505	0.2432	0.1928	0.3068	0.0359	0.5048	0.1386	0.3478
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

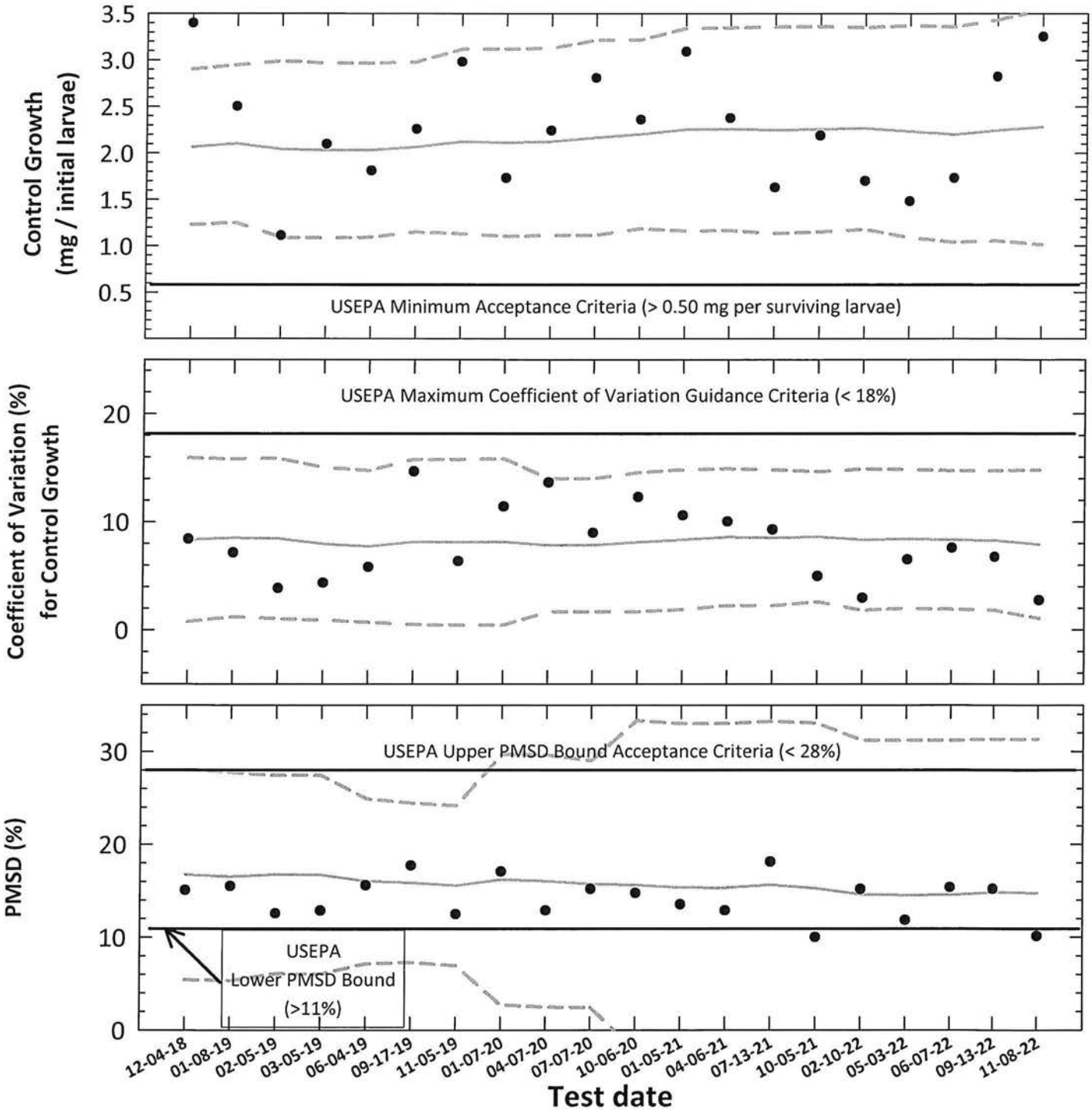
Entered and Reviewed by Jim Summer

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)

Entered and Reviewed by Jim Sumner

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	12-04-18	100	3.399	8.4	0.5125	15.1	2.0668	1.230	2.903	8.3	0.8	15.9	16.7	5.4	28.1
2	01-08-19	100	2.504	7.2	0.3880	15.5	2.102	1.253	2.950	8.5	1.2	15.8	16.5	5.3	27.7
3	02-05-19	100	1.114	3.9	0.1400	12.6	2.042	1.092	2.992	8.5	1.0	15.9	16.7	6.0	27.4
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 Sumner

Environmental Testing Solutions, Inc.

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

Species: *Menidia beryllina*

MbCuCR Test Number: **140**

Dilution preparation information:						***Comments:***
Cu Stock INSS number:		INSS 2151				
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 10-29-22
Hatch dates and times:	10-28-22 1200 to 10-29-22 1130
Transfer vessel information:	pH (S.U.) = 7.41 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:	CHM1149
Drying information for weight determination:	
Date / Time in oven:	11-15-22 1020
*Initial oven temperature:	60 °C
Date / Time out of oven:	11-16-22 1020
*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	11-08-22	1015	JL	1245	JL	1120	JL		11-03-22 B
1	11-09-22	0500	JL	1100	JL	0920	JL		↓
2	11-10-22	0500	JL	1115	JL	0920	JL		11-07-22
3	11-11-22	0500	JL	1100	JL	0920	JL		↓
4	11-12-22	0610	JL	1210	JL	0933	JL		11-10-22
5	11-13-22	0600	JL	1200	JL	0923	JL	11-04-22 JL	↓
6	11-14-22	0600	JL	1200	JL	0922	JL		
7	11-15-22					1020	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	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	7-day LC_{50} (%)	0.29
Average weight per initial larvae:	3.255		NOEC (%)	0.10
Average weight per surviving larvae:	3.255	≥ 0.25mg/larvae	LOEC (%)	0.20
			ChV (%)	0.14
			IC_{25} (%)	0.237

ETS
Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **140**

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: Orange Analyst: EC Date: 102422	15.94	15.21	14.64	14.60	14.84	13.50	14.67	16.47	14.05	15.24	14.70	15.08
*B = Pan + Larvae weight (mg) Analyst: EC Date: 11-18-22	48.03	47.70	46.43	48.41	49.12	45.01	47.96	49.77	49.90	44.03	51.16	47.25
C = Larvae weight (mg) = B – A Analyst: JL	32.09	32.49	31.79	33.81	34.28	31.45	33.29	33.30	35.85	28.79	36.46	32.17
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	3.209	3.249	3.179	3.381	3.428	3.145	3.329	3.330	3.585	3.879	3.646	3.217
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	3.255				3.308	-1.67.			3.332	-2.47.		

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina***

MbCuCR Test Number: **140**

Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	9 id	10	10	0 id	0 id	0 id	0 id
2	10	10	10	10	9 id	9	10	10				
3	10	10	10	10	9	9	10	10				
4	10	10	10	10	9	9	10	10				
5	10	10	10	10	9	9	10	10				
6	10	10	10	10	9	9	9 id	9 id				
7	10	10	10	10	9	9	9	9				
*A = Pan weight (mg) Tray color code: Orange Analyst: EC Date: 102422	15.08	15.15	14.02	13.93	15.21	15.01	13.70	16.10	11.77	12.97	16.28	13.09
*B = Pan + Larvae weight (mg) Analyst: EC Date: 11-18-22	47.88	47.12	48.18	46.24	46.00	42.17	43.49	41.29				
C = Larvae weight (mg) = B – A Analyst: JH	31.80	31.97	33.54	32.31	30.79	27.16	29.79	25.19				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	3.180	3.197	3.354	3.231	3.079	2.716	2.979	2.519	0	0	0	0
Average weight per initial number of larvae (mg)	3.316		-1.9%		2.823		13.3%		0		100%	
Percent reduction from control (%)												

11-18-22 JH

11-20 JH

*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: 140

Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Not for Compliance Assessment, Internal Laboratory QC: Weight / 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	15.94	48.03	32.09	3.209	3.255	2.7	3.209	100.0	3.255	2.7	Not applicable
	B	10	10	15.21	47.70	32.49	3.249			3.249				
	C	10	10	14.64	46.43	31.79	3.179			3.179				
	D	10	10	14.60	48.41	33.81	3.381			3.381				
0.025	E	10	10	14.84	49.12	34.28	3.428	3.308	3.6	3.428	100.0	3.308	3.6	-1.6
	F	10	10	13.56	45.01	31.45	3.145			3.145				
	G	10	10	14.67	47.96	33.29	3.329			3.329				
	H	10	10	16.47	49.77	33.30	3.330			3.330				
0.050	I	10	10	14.05	49.90	35.85	3.585	3.332	10.7	3.585	100.0	3.332	10.7	-2.4
	J	10	10	15.24	44.03	28.79	2.879			2.879				
	K	10	10	14.70	51.16	36.46	3.646			3.646				
	L	10	10	15.08	47.25	32.17	3.217			3.217				
0.100	M	10	10	13.08	47.88	34.80	3.480	3.316	3.9	3.480	100.0	3.316	3.9	-1.9
	N	10	10	15.15	47.12	31.97	3.197			3.197				
	O	10	10	14.64	48.18	33.54	3.354			3.354				
	P	10	10	13.93	46.24	32.31	3.231			3.231				
0.200	Q	10	9	15.21	46.00	30.79	3.421	3.137	9.0	3.079	90.0	2.823	9.0	13.3
	R	10	9	15.01	42.17	27.16	3.018			2.716				
	S	10	9	13.70	43.49	29.79	3.310			2.979				
	T	10	9	16.10	41.29	25.19	2.799			2.519				
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: 0.3294 MSD = Minimum Significant Difference
PMSD: 10.1 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:	11/8/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

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

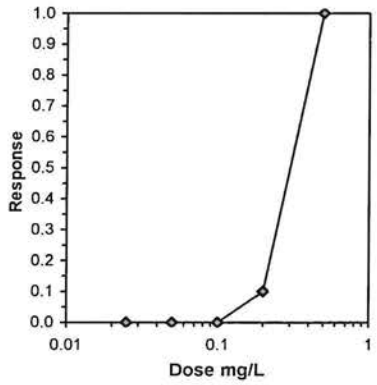
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
0.025	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
0.05	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4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

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.868		
Equality of variance cannot be confirmed				

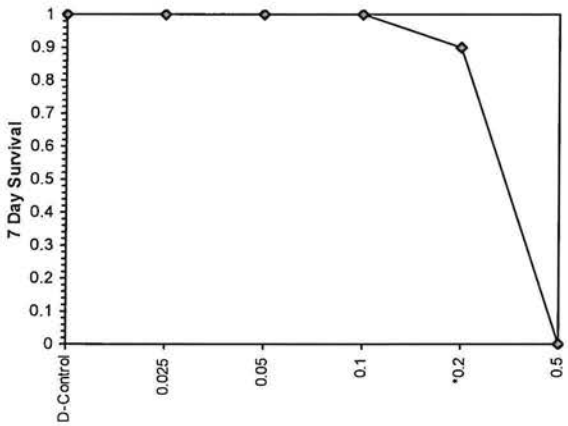
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Trimmed Spearman-Karber

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



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	11/8/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2	3	4
D-Control	3.2090	3.2490	3.1790	3.3810
0.025	3.4280	3.1450	3.3290	3.3300
0.05	3.5850	2.8790	3.6460	3.2170
0.1	3.4800	3.1970	3.3540	3.2310
0.2	3.0790	2.7160	2.9790	2.5190
0.5	0.0000	0.0000	0.0000	0.0000

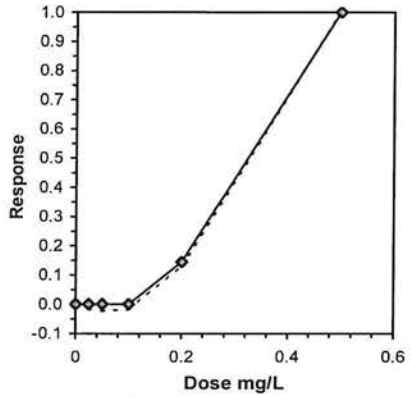
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	3.2545	1.0000	3.2545	3.1790	3.3810	2.737	4				3.3024	1.0000
0.025	3.3080	1.0164	3.3080	3.1450	3.4280	3.572	4	-0.372	2.290	0.3294	3.3024	1.0000
0.05	3.3318	1.0237	3.3318	2.8790	3.6460	10.697	4	-0.537	2.290	0.3294	3.3024	1.0000
0.1	3.3155	1.0187	3.3155	3.1970	3.4800	3.883	4	-0.424	2.290	0.3294	3.3024	1.0000
0.2	2.8233	0.8675	2.8233	2.5190	3.0790	9.001	4				2.8233	0.8549
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)	0.95464	0.844	-0.5806	1.58888
Bartlett's Test indicates equal variances (p = 0.08)	6.66565	11.3449		

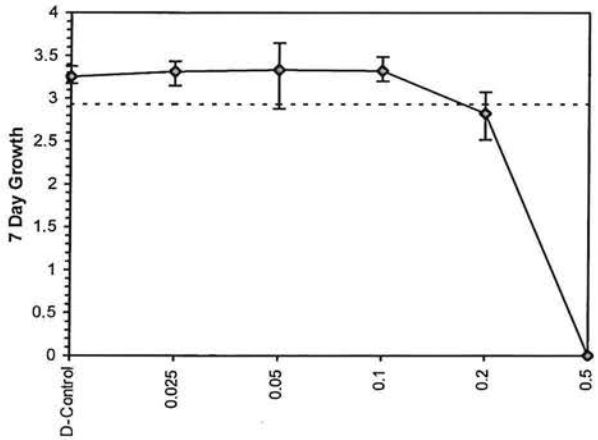
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	0.1	>0.1			0.32936	0.1012	0.00448	0.04137	0.95365	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1345	0.0159	0.0735	0.1640	-1.9037
IC10	0.1689	0.0178	0.1193	0.2257	0.5090
IC15	0.2017	0.0146	0.1454	0.2323	-0.5074
IC20	0.2193	0.0111	0.1775	0.2480	-0.3558
IC25	0.2368	0.0101	0.1996	0.2638	-0.2248
IC40	0.2894	0.0081	0.2597	0.3110	-0.2248
IC50	0.3245	0.0068	0.2997	0.3425	-0.2248



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **140**

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	M	EC IC	EC M	EC	EC	M
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	7.64	7.91	7.96	7.90	7.96	7.85
	Dissolved oxygen (mg/L)	7.8	7.7	7.7	7.5	7.9	7.7
	Salinity (ppt)	25.0	25.2	24.9	25.3	25.0	25.3
	Alkalinity (mg $CaCO_3$/L)	130				120	
	Temperature (°C)	25.0	24.8	24.8	25.0	25.0	24.9
0.025 mg/L	pH (S.U.)	7.63	7.91	8.04	7.88	8.07	7.84
	Dissolved oxygen (mg/L)	7.8	7.6	7.6	7.5	8.0	7.6
	Salinity (ppt)	24.9	25.0	25.0	25.4	25.1	25.4
	Temperature (°C)	25.1	24.9	24.8	25.1	24.9	25.2
0.05 mg/L	pH (S.U.)	7.85	7.91	8.04	7.90	8.07	7.83
	Dissolved oxygen (mg/L)	7.8	7.7	7.6	7.6	8.0	7.6
	Salinity (ppt)	24.9	25.2	25.0	25.4	25.1	25.4
	Temperature (°C)	25.0	24.9	24.9	25.2	24.9	25.2
0.1 mg/L	pH (S.U.)	7.86	7.91	8.04	7.88	8.07	7.84
	Dissolved oxygen (mg/L)	7.8	7.6	7.6	7.6	8.0	7.6
	Salinity (ppt)	24.9	25.2	25.0	25.3	25.1	25.5
	Temperature (°C)	25.0	24.7	24.9	25.0	24.8	25.0
0.2 mg/L	pH (S.U.)	7.88	7.91	8.04	7.87	8.07	7.84
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.6	8.0	7.7
	Salinity (ppt)	24.8	25.0	25.0	25.3	25.1	25.6
	Temperature (°C)	25.0	24.7	24.9	25.0	24.8	25.2
0.5 mg/L	pH (S.U.)	7.89	7.91	8.04			
	Dissolved oxygen (mg/L)	7.9	7.9	7.8			
	Salinity (ppt)	24.8	25.1	25.0			
	Alkalinity (mg $CaCO_3$/L)	~~24.8~~ 11-09-22	22 IC				11-09-22
	Temperature (°C)	25.1	24.7	24.8			
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **140**

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
	Analyst	N	BSL	BSL	N	N	EC N	EC N	EC
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.90 / 7.8	8.09 / 7.9	8.30 / 7.7	7.83 / 7.5	7.90 / 7.7	7.78 / 7.7	8.03 / 7.7	7.85 / 7.6
	Dissolved oxygen (mg/L)								
	Salinity (ppt)	25.0	25.5	24.8	25.1	24.4	25.7	24.4	24.9
	Alkalinity (mg $CaCO_3$/L)	11-03-22		120					11-07-22
	Temperature (°C)	24.8	25.3	24.8	25.3	24.9	25.4	24.9	25.1
0.025 mg/L	pH (S.U.)	8.04	8.08	8.26	7.83	8.07	7.77	8.02	7.87
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.4	7.9	7.7	8.0	7.6
	Salinity (ppt)	25.1	25.5	25.0	25.3	25.1	25.2	24.5	25.0
	Temperature (°C)	24.9	25.2	24.9	25.0	25.0	25.2	24.9	25.0
0.05 mg/L	pH (S.U.)	8.04	8.07	8.27	7.85	8.09	7.79	8.03	7.87
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.4	8.0	7.7	7.9	7.7
	Salinity (ppt)	25.1	25.6	25.0	25.2	25.0	25.5	24.5	25.1
	Temperature (°C)	24.9	25.4	24.9	25.3	25.0	25.4	25.0	25.0
0.1 mg/L	pH (S.U.)	8.05	8.07	8.27	7.84	8.10	2.79	8.03	7.87
	Dissolved oxygen (mg/L)	8.0	7.9	8.1	7.4	8.0	7.7	8.0	7.7
	Salinity (ppt)	25.1	25.5	24.9	25.3	25.0	25.4	24.4	25.1
	Temperature (°C)	24.9	25.4	24.9	25.3	25.0	25.3	25.0	25.2
0.2 mg/L	pH (S.U.)	8.05	8.07	8.27	7.86	8.10	7.82	8.03	7.91
	Dissolved oxygen (mg/L)	8.1	7.8	8.1	7.5	8.0	7.8	8.0	7.7
	Salinity (ppt)	25.0	25.4	24.8	25.3	24.9	25.6	24.4	25.2
	Temperature (°C)	24.8	25.2	25.0	25.2	25.0	25.3	25.0	25.2
0.5 mg/L	pH (S.U.)								
	Dissolved oxygen (mg/L)								
	Salinity (ppt)								
	Temperature (°C)						11-04-22		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final