ETS
Environmental Testing Solutions, Inc.

Surveillance and Corrective Action Report (SCAR)

SURVIELLANCE NUMBER:	SCAR-220324
SURVIELLANCE:	*A. bahia* Acute Reference Toxicant Test #248
DATE OF INSPECTION:	03-24-22
TYPE OF INSPECTION:	Internal
EVALUATORS:	Jim Sumner
LABORATORY PERSONNELL:	Jim Sumner, Alex Salinas

I. DEVIATION / DECREPANCY:

48-hour LC_{50} result above upper control limit.
48-hour LC_{50} = 0.57 mg/L KCl, Upper Control Limit of + 2SD = 0.54 mg/L KCl.
Result was within EPA 10th Percentile CVs.
1st outlier test in the last 20 data points.

This batch of mysids was used for Hubert WTP (NC 0083321) test performed 03-22-22. This compliance test was invalidated. All other testing performed in March 2022 used a batch of mysids where the associated reference test was within control limits (performed the week of March 06).

II. ROOT CAUSE INVESTIGATION:

Reference test met laboratory's acceptance criteria. Test solutions were prepared by J. Sumner and testing was initiated by A. Salinas. Mysids did not appear stressed upon receipt in the laboratory.

The cause of the outlier test was not identified. Outlier test will be maintained in the control chart. 2 follow-up reference tests are scheduled for the week of April 03 to determine if this change in organism sensitivity is maintained.

III. EXECUTION:

03-28-22:
NC and SC certifying authorities were notified of the deviation by e-mail.

04-05-22: 2 reference toxicant tests were initiated on April 05, 2022 using 2 different batches of mysids. A third reference test was initiated on April 07 using the same batch of test organisms as one of the tests initiated on April 05. Each of these tests were within ± SD control limits; however, were above the central tendency typically seen by our laboratory. Chronic mysid testing has not changed in sensitivity.

Placing additional age requirements on the organisms received by our supplier may be providing healthier individuals for our testing program and slightly decreasing their sensitivity.

04-14-22:
NC and SC certifying authorities notified of closing SCAR initiated on 03-24-22 by e-mail.

04-14-22: SCAR has been closed.

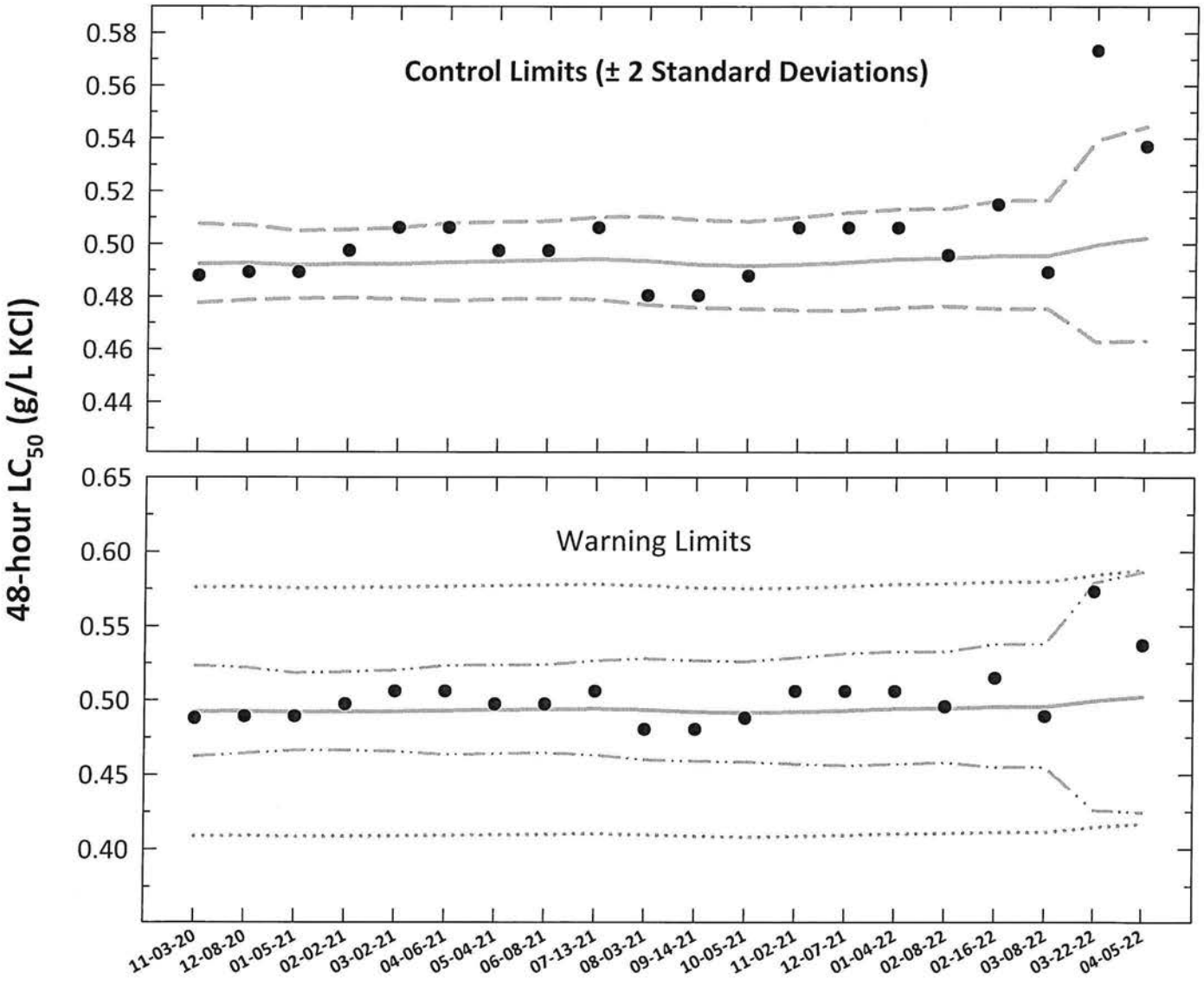
IV. VERIFICATION:

Signature: _____ Date: 04-14-22
Laboratory Supervisor

V. CLOSURE:

Signature: _____ Date: 04-14-22
Owner/Director

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	11-03-20	0.4879	-0.3117	-0.3077	0.0066	0.4924	0.4776	0.5076	0.4624	0.5233	0.4087	0.5761
2	12-08-20	0.4892	-0.3105	-0.3074	0.0063	0.4928	0.4788	0.5072	0.4644	0.5220	0.4090	0.5765
3	01-05-21	0.4892	-0.3105	-0.3081	0.0057	0.4919	0.4793	0.5049	0.4662	0.5183	0.4083	0.5756
4	02-02-21	0.4974	-0.3033	-0.3077	0.0057	0.4923	0.4795	0.5055	0.4663	0.5190	0.4086	0.5760
5	03-02-21	0.5061	-0.2958	-0.3076	0.0059	0.4925	0.4792	0.5061	0.4655	0.5202	0.4087	0.5762
6	04-06-21	0.5061	-0.2958	-0.3072	0.0065	0.4930	0.4785	0.5079	0.4636	0.5233	0.4092	0.5768
7	05-04-21	0.4974	-0.3033	-0.3068	0.0064	0.4934	0.4790	0.5083	0.4642	0.5236	0.4096	0.5773
8	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
9	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
10	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 249

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 #: ~~2079~~ 04.05.21 2078

Chemical Analyses:

Concentration	Analyst	Hours 0	Hours 24	Hours 48
Control, SaltSW	pH (S.U.)	8.02	8.02	7.90
	Dissolved oxygen (mg/L)	7.7	7.7	7.6
	*Salinity (ppt)	25.0	25.0	25.2
	*Alkalinity (mg/L $CaCO_3$)	130		
	*Temperature (°C)	25.2	25.2	25.3
250 mg/L	pH (S.U.)	8.03	8.03	7.89
	Dissolved oxygen (mg/L)	7.7	7.6	7.5
	*Salinity (ppt)	25.2	25.1	25.4
	*Temperature (°C)	25.1	25.4	25.2
375 mg/L	pH (S.U.)	8.04	8.03	7.87
	Dissolved oxygen (mg/L)	7.4	7.6	7.6
	*Salinity (ppt)	25.5	25.4	25.5
	*Temperature (°C)	25.1	25.2	25.2
500 mg/L	pH (S.U.)	8.09	8.04	7.88
	Dissolved oxygen (mg/L)	8.0	7.7	7.6
	*Salinity (ppt)	25.4	25.5	25.7
	*Temperature (°C)	25.0	25.2	25.2
750 mg/L	pH (S.U.)	8.06	8.03	
	Dissolved oxygen (mg/L)	7.6	7.6	
	*Salinity (ppt)	25.4	25.6	
	*Temperature (°C)	25.2	25.2	
1000 mg/L	pH (S.U.)	8.06	8.03	
	Dissolved oxygen (mg/L)	7.9	7.7	
	*Salinity (ppt)	25.6	25.7	
	*Temperature (°C)	25.2	25.3	

04.06.21

*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	13066485

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 # 249

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-05-22	* 1042	JH	1242	JH	6F	LIGHT BLUE	03-30-22A
24	04-06-22			1244	JH			
48 Termination	04-07-22			1243	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):	04-05-22 AK 04-03-22
Age (1 to 5 days old):	2-3 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	04-02-22 1200 TO 04-03-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.99
	Temperature (°C) 24.6

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

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	9 1d	8 2d	7 2d	0	0	0	0
Mean Survival	100%		100%		95%		75%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	494.3
Upper 95% confidence limit (mg KCl/L)	593.3
48-hour LC_{50} (mg KCl/L)	536.9

Comments:

Test Reviewed by: JH

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Acute Mysid Test-24 Hr Survival

Start Date:	4/5/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	4/7/2022	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	0.9000
750	0.0000	0.0000
1000	0.0000	0.0000

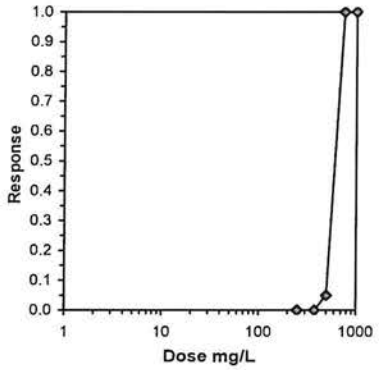
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	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				

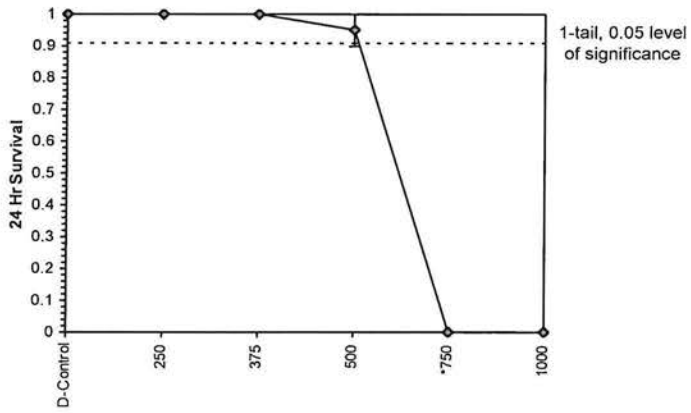
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.06555	0.06723	0.61047	0.00266	7.5E-06	4, 5
Treatments vs D-Control										

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



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Acute Mysid Test-48 Hr Survival

Start Date:	4/5/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	4/7/2022	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	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1803	0	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.288	2.850	0.1803	1	20
*500	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5.736	2.850	0.1803	5	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	19.812	2.850	0.1803	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

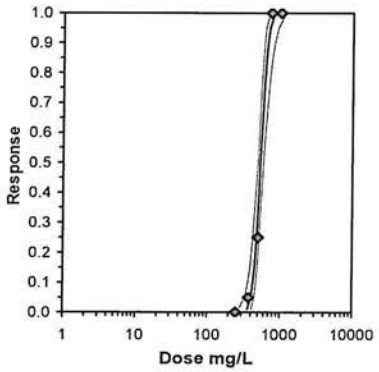
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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		0.08562	0.08782	0.56628	0.004	2.5E-05	4, 5

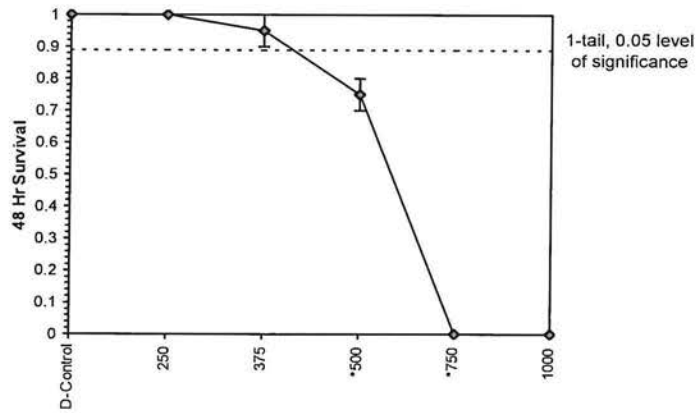
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.6008	2.90462	7.90776	19.2939	0	2.47836	7.81472	0.47921	2.72992	0.07352	7
Intercept	-32.129	7.88247	-47.579	-16.68							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	362.142	274.941	410.453
EC05	3.355	406.43	332.494	448.974
EC10	3.718	432.213	366.856	472.371
EC15	3.964	450.526	391.211	489.856
EC20	4.158	465.633	411.002	505.089
EC25	4.326	478.996	428.092	519.365
EC40	4.747	514.395	470.298	561.98
EC50	5.000	536.938	494.266	593.338
EC60	5.253	560.468	516.928	629.509
EC75	5.674	601.888	552.396	700.271
EC80	5.842	619.161	566.021	731.95
EC85	6.036	639.923	581.767	771.432
EC90	6.282	667.037	601.536	825.062
EC95	6.645	709.352	631.08	912.927
EC99	7.326	796.101	688.38	1107.12



Dose-Response Plot



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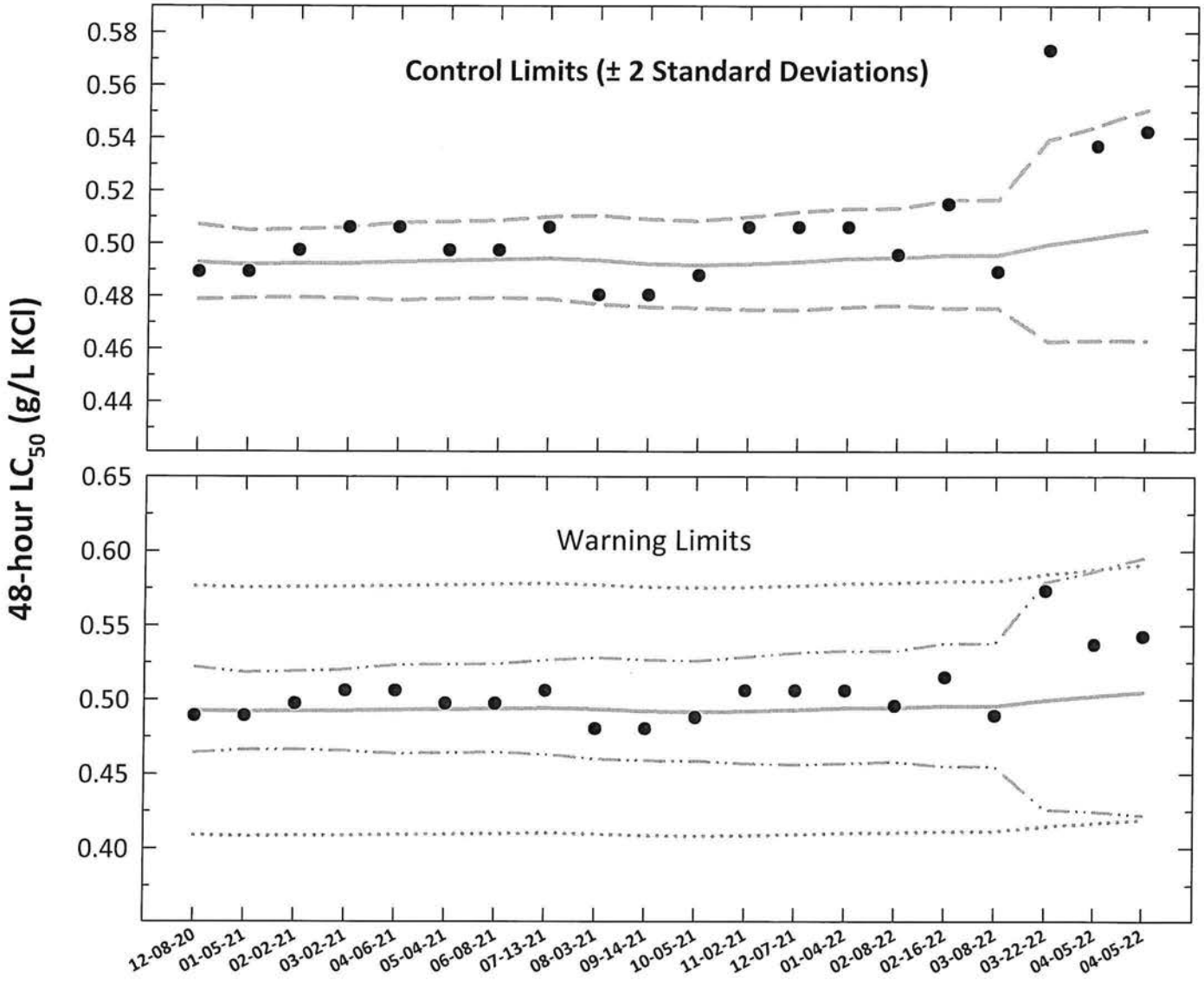

Test date

- ● **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).
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- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
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- ····· **USEPA Warning Limits** (mean logarithmic LC_{50} ± $S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	12-08-20	0.4892	-0.3105	-0.3074	0.0063	0.4928	0.4788	0.5072	0.4644	0.5220	0.4090	0.5765
2	01-05-21	0.4892	-0.3105	-0.3081	0.0057	0.4919	0.4793	0.5049	0.4662	0.5183	0.4083	0.5756
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8	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
9	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 250

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 #: 2079 (04.06.22 N) 2078

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, SaltSW	pH (S.U.)	8.02	8.02	7.99
	Dissolved oxygen (mg/L)	7.7	7.8	7.7
	*Salinity (ppt)	25.0	25.0	25.1
	*Alkalinity (mg/L $CaCO_3$)	130		
	*Temperature (°C)	25.1	25.3	25.2
250 mg/L	pH (S.U.)	8.03	8.03	7.98
	Dissolved oxygen (mg/L)	7.7	7.6	7.7
	*Salinity (ppt)	25.2	25.2	25.3
	*Temperature (°C)	25.2	25.2	25.0
375 mg/L	pH (S.U.)	8.04	8.03	7.97
	Dissolved oxygen (mg/L)	7.4	7.6	7.7
	*Salinity (ppt)	25.5	25.5	25.6
	*Temperature (°C)	25.2	25.2	25.0
500 mg/L	pH (S.U.)	8.09	8.04	7.97
	Dissolved oxygen (mg/L)	8.0	7.6	7.7
	*Salinity (ppt)	25.4	25.6	25.8
	*Temperature (°C)	25.0	25.0	25.3
750 mg/L	pH (S.U.)	8.06	8.03	
	Dissolved oxygen (mg/L)	7.8	7.6	
	*Salinity (ppt)	25.4	25.6	
	*Temperature (°C)	25.2	25.3	
1000 mg/L	pH (S.U.)	8.06	8.03	
	Dissolved oxygen (mg/L)	7.9	7.6	
	*Salinity (ppt)	25.6	25.7	
	*Temperature (°C)	25.2	25.3	

04.06.22 N

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Acute 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 # 250

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-05-22	* 1042	JH	1250	JH	6F	LIGHT BLUE	03-30-22 A
24	04-06-22			1248	JH			
48 Termination	04-07-22			1247	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):	04-04-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	04-03-22 1200 TO 04-04-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.08
	Temperature (°C) 24.5

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	7 3d	6 4d	0	0	0	0
Mean Survival	100%		100%		100%		35%		0%		0%	

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

Statistics:

Method	04-14-22 JH ~~JH~~ SK
Lower 95% confidence limit (mg KCl/L)	503.8
Upper 95% confidence limit (mg KCl/L)	584.0
48-hour LC_{50} (mg KCl/L)	542.4

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-24 Hr Survival

Start Date:	4/5/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	4/7/2022	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

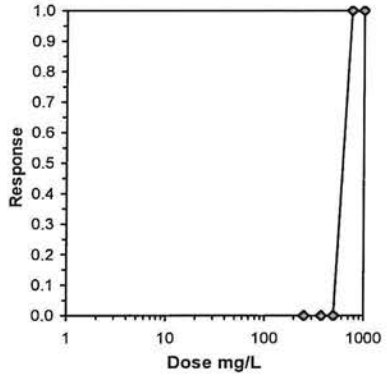
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

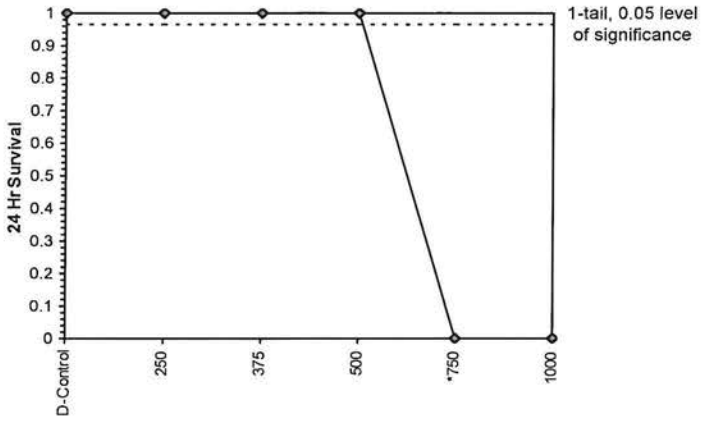
Graphical Method

Trim Level	EC50
0.0%	612.37

612.37



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-48 Hr Survival

Start Date:	4/5/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/7/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

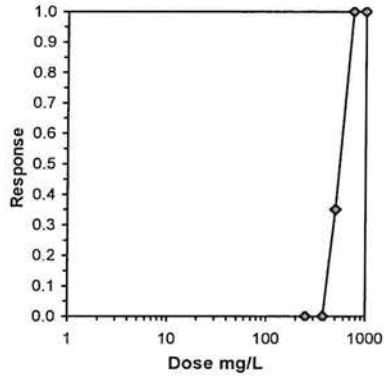
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0947	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0947	0	20
*500	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	14.247	2.850	0.0947	7	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	37.715	2.850	0.0947	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

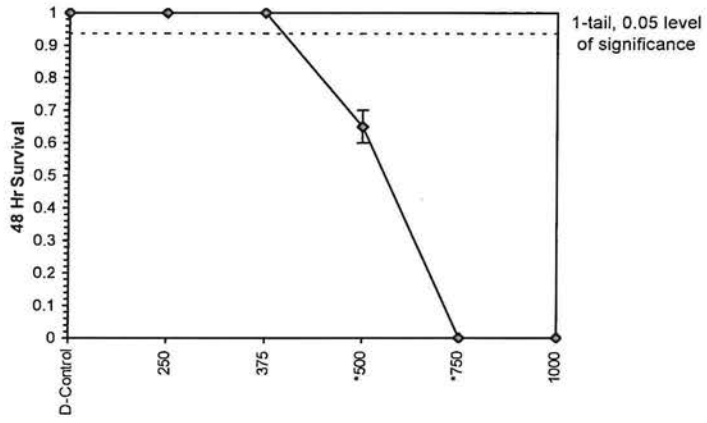
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.03789	0.03886	0.59923	0.0011	8.8E-07	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

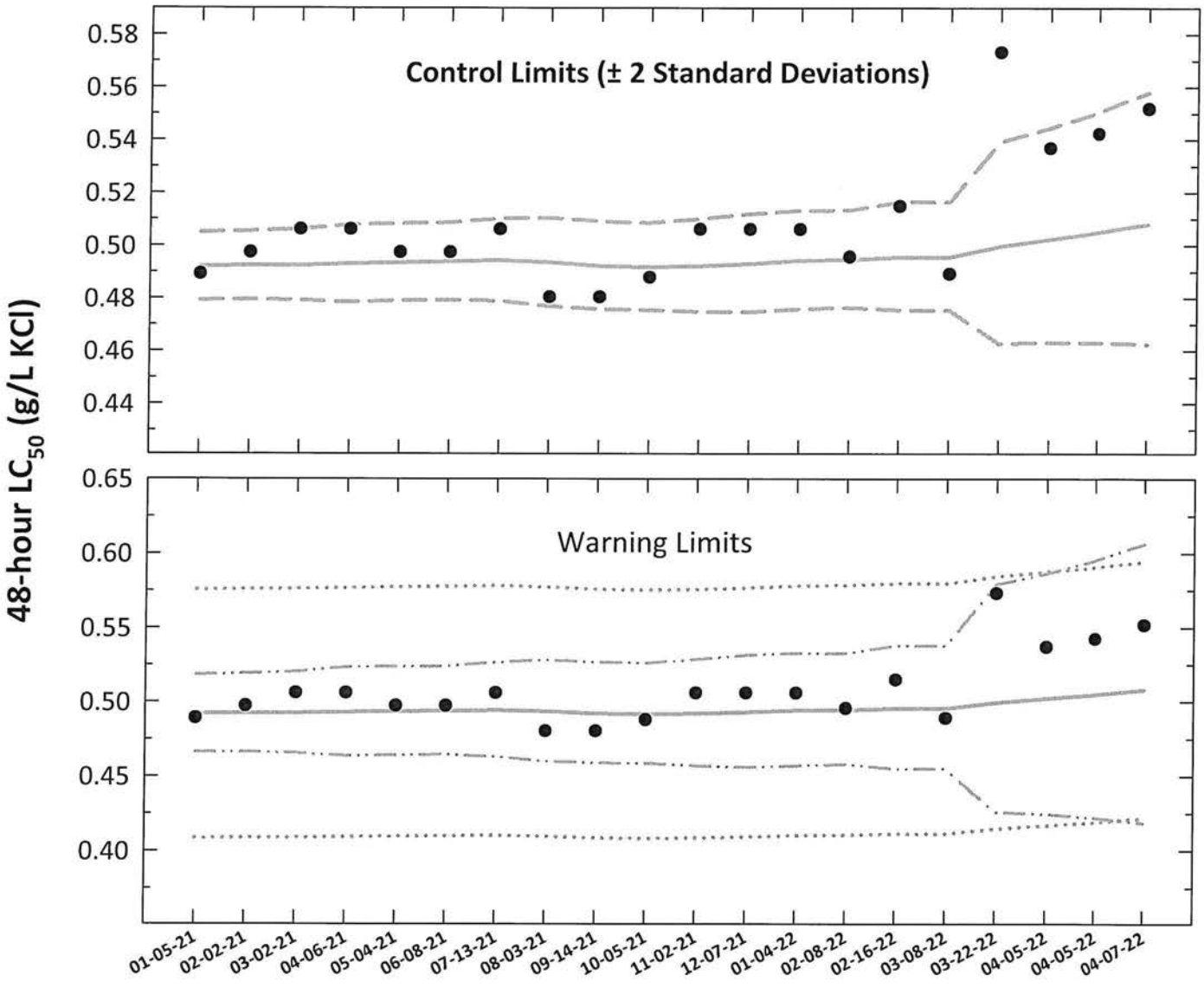
Trim Level	EC50	95% CL	
0.0%	542.42	503.77	584.04
5.0%	543.63	500.55	590.41
10.0%	544.81	495.80	598.66
20.0%	547.01	478.84	624.87
Auto-0.0%	542.42	503.77	584.04



Dose-Response Plot



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



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

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	01-05-21	0.4892	-0.3105	-0.3081	0.0057	0.4919	0.4793	0.5049	0.4662	0.5183	0.4083	0.5756
2	02-02-21	0.4974	-0.3033	-0.3077	0.0057	0.4923	0.4795	0.5055	0.4663	0.5190	0.4086	0.5760
3	03-02-21	0.5061	-0.2958	-0.3076	0.0059	0.4925	0.4792	0.5061	0.4655	0.5202	0.4087	0.5762
4	04-06-21	0.5061	-0.2958	-0.3072	0.0065	0.4930	0.4785	0.5079	0.4636	0.5233	0.4092	0.5768
5	05-04-21	0.4974	-0.3033	-0.3068	0.0064	0.4934	0.4790	0.5083	0.4642	0.5236	0.4096	0.5773
6	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
7	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
8	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 751

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 #: ~~2079~~ 04-05-22 M 2078

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, SaltSW	pH (S.U.)	7.95	7.93	7.92
	Dissolved oxygen (mg/L)	7.8	7.7	7.1
	*Salinity (ppt)	25.0	25.1	25.4
	*Alkalinity (mg/L $CaCO_3$)	130		
	*Temperature (°C)	25.1	25.2	25.5
250 mg/L	pH (S.U.)	7.96	7.89	7.89
	Dissolved oxygen (mg/L)	7.8	7.7	7.2
	*Salinity (ppt)	25.2	25.3	25.8
	*Temperature (°C)	25.0	25.3	25.6
375 mg/L	pH (S.U.)	7.96	7.90	7.89
	Dissolved oxygen (mg/L)	7.8	7.7	7.2
	*Salinity (ppt)	25.3	25.7	26.2
	*Temperature (°C)	25.2	25.3	25.6
500 mg/L	pH (S.U.)	7.98	7.90	7.86
	Dissolved oxygen (mg/L)	7.9	7.7	7.1
	*Salinity (ppt)	25.3	25.7	26.0
	*Temperature (°C)	25.0	25.2	25.4
750 mg/L	pH (S.U.)	7.98	7.92	
	Dissolved oxygen (mg/L)	7.9	7.8	
	*Salinity (ppt)	25.6	25.7	
	*Temperature (°C)	25.0	25.2	04-08-22
1000 mg/L	pH (S.U.)	7.98	7.94	
	Dissolved oxygen (mg/L)	7.9	7.8	
	*Salinity (ppt)	25.8	26.0	
	*Temperature (°C)	25.0	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066485

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 # 251

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-07-22	* 0805	JL	1030	JL	1F	Yellow	04-05-22
24	04-08-22			1030	JL			
48 Termination	04-09-22			1027	JL			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	04-04-22
Age (1 to 5 days old):	3-4 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	04-03-22 1200 TO 04-04-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.07
	Temperature (°C) 24.9

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

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	10	10	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	7 3u	7 3u	0	0	0	0
Mean Survival	100%		100%		100%		70%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	514.1
Upper 95% confidence limit (mg KCl/L)	592.5
48-hour LC_{50} (mg KCl/L)	551.9

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-24 Hr Survival

Start Date:	4/7/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	4/9/2022	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

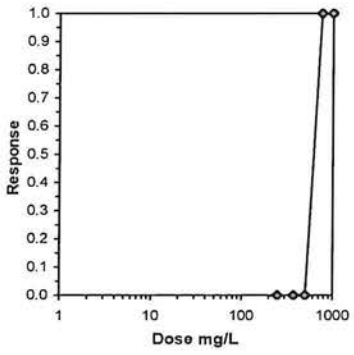
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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										

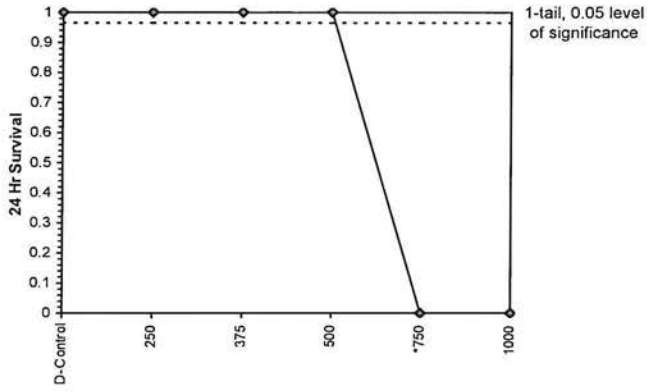
Graphical Method

Trim Level	EC50
0.0%	612.37

612.37



Dose-Response Plot



Environmental Testing Solutions, Inc.

Acute Mysid Test-48 Hr Survival

Start Date:	4/7/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	4/9/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

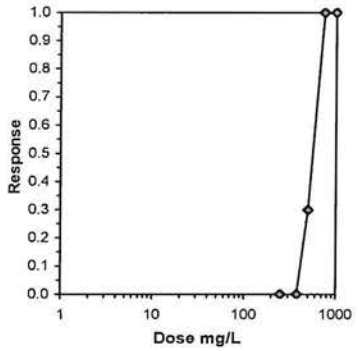
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	42.086	2.850	0.0285	6	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

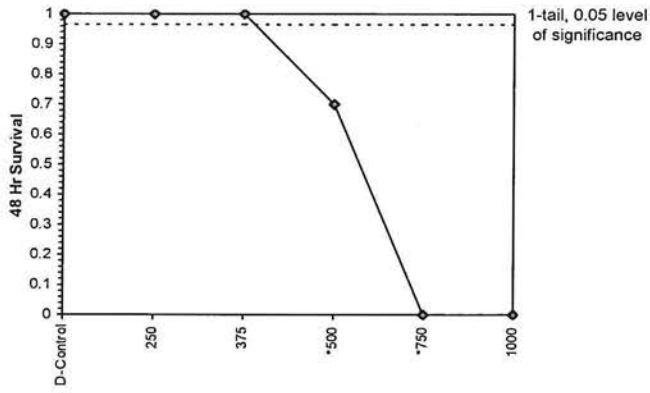
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.5936	0.0001	2.3E-09	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

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

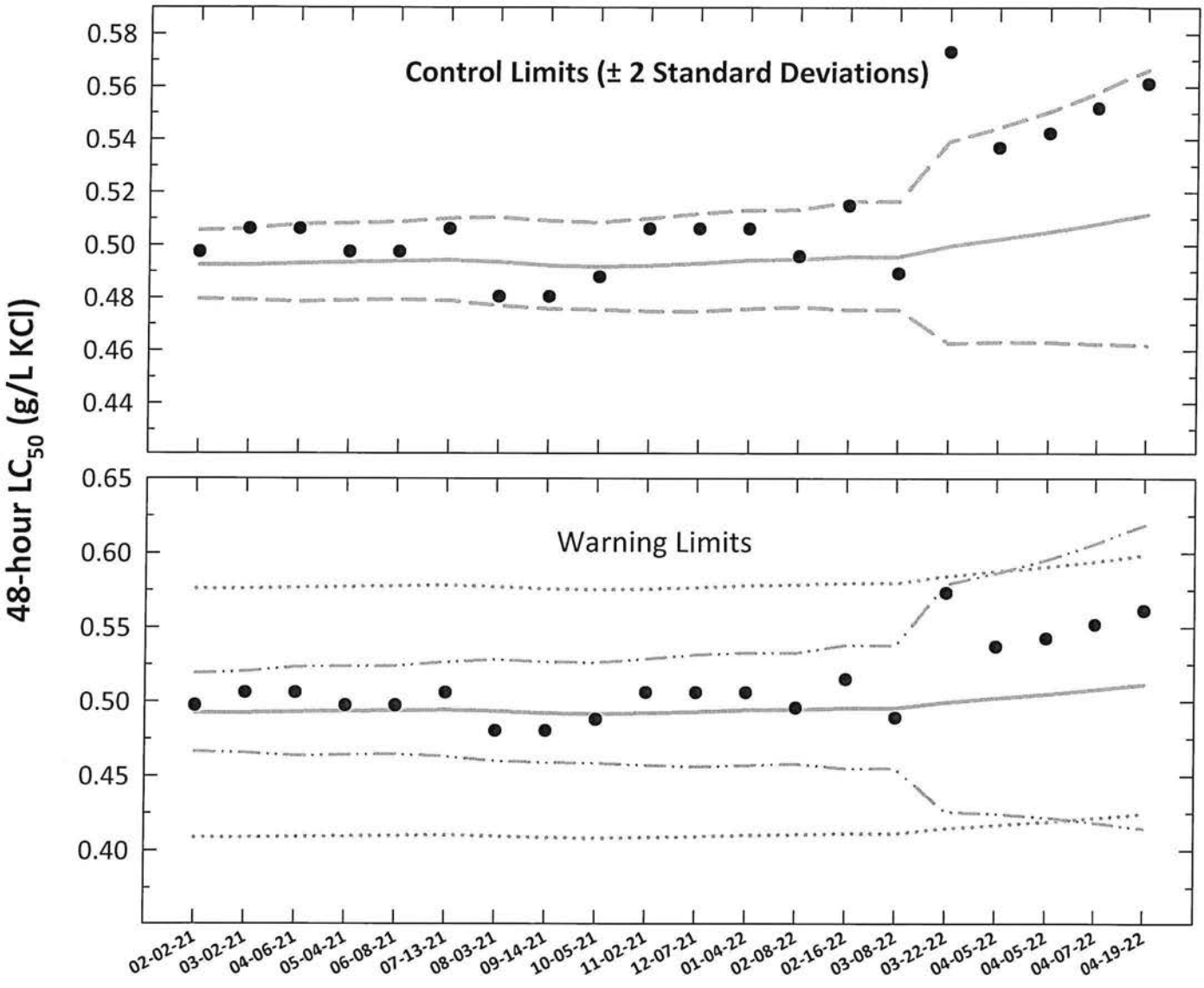


Dose-Response Plot



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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	02-02-21	0.4974	-0.3033	-0.3077	0.0057	0.4923	0.4795	0.5055	0.4663	0.5190	0.4086	0.5760
2	03-02-21	0.5061	-0.2958	-0.3076	0.0059	0.4925	0.4792	0.5061	0.4655	0.5202	0.4087	0.5762
3	04-06-21	0.5061	-0.2958	-0.3072	0.0065	0.4930	0.4785	0.5079	0.4636	0.5233	0.4092	0.5768
4	05-04-21	0.4974	-0.3033	-0.3068	0.0064	0.4934	0.4790	0.5083	0.4642	0.5236	0.4096	0.5773
5	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
6	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
7	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS

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

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

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKClAC # 252

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 #: 2078

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JH	JW	JL
Control, SaltSW	pH (S.U.)	8.26	7.99	7.94
	Dissolved oxygen (mg/L)	7.7	7.6	7.4
	*Salinity (ppt)	24.7	24.8	24.9
	*Alkalinity (mg/L $CaCO_3$)	130		
	*Temperature (°C)	24.4	24.5	24.3
250 mg/L	pH (S.U.)	8.26	8.00	7.98
	Dissolved oxygen (mg/L)	7.7	7.6	7.3
	*Salinity (ppt)	25.0	25.0	25.1
	*Temperature (°C)	24.5	24.6	24.3
375 mg/L	pH (S.U.)	8.25	7.99	7.98
	Dissolved oxygen (mg/L)	7.7	7.7	7.4
	*Salinity (ppt)	25.0	25.1	25.1
	*Temperature (°C)	24.3	24.3	24.4
500 mg/L	pH (S.U.)	8.25	8.00	7.99
	Dissolved oxygen (mg/L)	7.7	7.8	7.6
	*Salinity (ppt)	25.0	25.2	25.2
	*Temperature (°C)	24.3	24.3	24.2
750 mg/L	pH (S.U.)	8.25	7.97	
	Dissolved oxygen (mg/L)	7.8	7.8	
	*Salinity (ppt)	25.1	25.4	
	*Temperature (°C)	24.6	24.5	
1000 mg/L	pH (S.U.)	8.25	7.98	
	Dissolved oxygen (mg/L)	7.9	7.8	
	*Salinity (ppt)	25.3	25.5	
	*Temperature (°C)	24.3	24.5	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066485

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 # 252

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04.19.22	* 1100	A.S	04.19.22 ~~1500~~ 1400	A.S	7B	Yellow	04-09-22
24	04.20.22			1405	A.S			
48 Termination	04.21.22			1400	A.S			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	04.18.22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	04-17-22 1200 TO 04-18-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.23
	Temperature (°C) 24.0°C

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	8 2d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	9 1d	10	10	10	A.S ~~7d~~ ~~6~~ 7 1d 04-21-22	8 1d	0	0	0	0
Mean Survival	100%		95%		100%		04-21-22 ~~80%~~ 75%		0%		0%	

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

Statistics:

Method	TSK
Lower 95% confidence limit (mg KCl/L)	522.6
Upper 95% confidence limit (mg KCl/L)	602.7
48-hour LC_{50} (mg KCl/L)	561.2

Comments:

Test Reviewed by: [signature]

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	4/19/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/21/2022	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:					

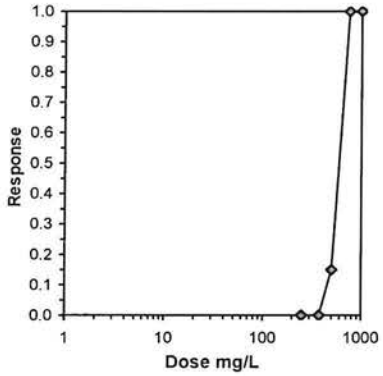
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.8000	0.9000
750	0.0000	0.0000
1000	0.0000	0.0000

			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

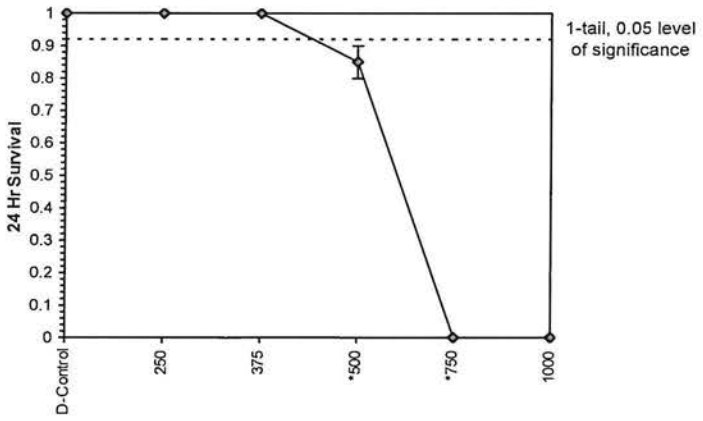
Auxiliary Tests				Statistic		Critical		Skew	Kurt
Normality of the data set cannot be confirmed									
Equality of variance cannot be confirmed									
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	375	500	433.013		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 Sumner

Acute Mysid Test-48 Hr Survival

Start Date:	4/19/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/21/2022	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:					

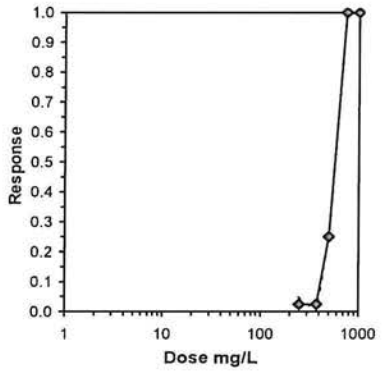
Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	0.9000	1.0000
375	1.0000	1.0000
500	0.7000	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

			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	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.288	2.850	0.1803	1	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1803	0	20
*500	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5.736	2.850	0.1803	5	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	19.812	2.850	0.1803	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

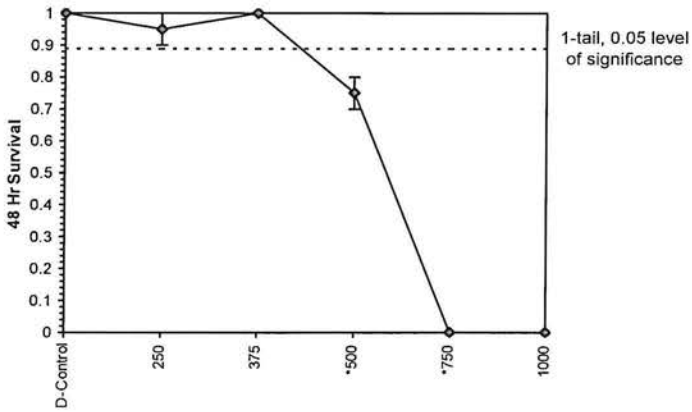
Auxiliary Tests			Statistic		Critical		Skew	Kurt
Normality of the data set cannot be confirmed								
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	375	500	433.013		0.08562	0.08782	0.56628	0.004	2.5E-05	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

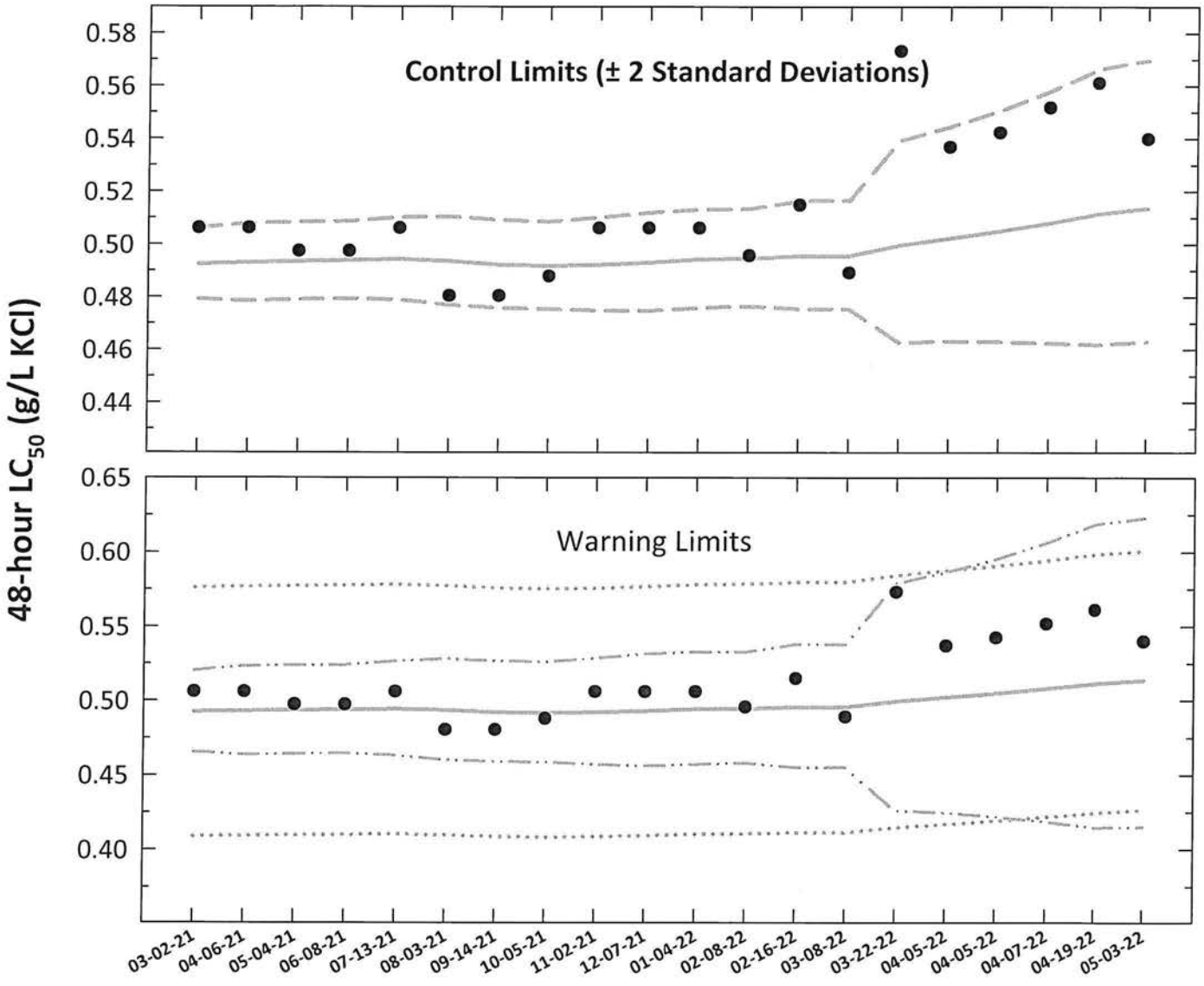
Trim Level	EC50	95% CL	
0.0%			
5.0%	563.05	522.22	607.07
10.0%	566.45	519.22	617.98
20.0%	571.48	499.16	654.27
Auto-2.5%	561.21	522.55	602.74



Dose-Response Plot



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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	03-02-21	0.5061	-0.2958	-0.3076	0.0059	0.4925	0.4792	0.5061	0.4655	0.5202	0.4087	0.5762
2	04-06-21	0.5061	-0.2958	-0.3072	0.0065	0.4930	0.4785	0.5079	0.4636	0.5233	0.4092	0.5768
3	05-04-21	0.4974	-0.3033	-0.3068	0.0064	0.4934	0.4790	0.5083	0.4642	0.5236	0.4096	0.5773
4	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
5	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
6	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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 # 253

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 #: 2078

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, SaltSW	pH (S.U.)	8.23	~~8.12~~ JW 8.00	8.00
	Dissolved oxygen (mg/L)	8.0	8.0	7.4
	*Salinity (ppt)	24.7	25.2	25.3
	*Alkalinity (mg/L $CaCO_3$)	NO		
	*Temperature (°C)	24.9	24.9	25.0
250 mg/L	pH (S.U.)	8.25	8.11	7.98
	Dissolved oxygen (mg/L)	8.0	8.1	7.4
	*Salinity (ppt)	24.9	25.2	25.3
	*Temperature (°C)	25.0	25.1	24.9
375 mg/L	pH (S.U.)	8.25	8.12 JW 8.00	8.00
	Dissolved oxygen (mg/L)	8.0	~~8.12~~ 8.0	7.4
	*Salinity (ppt)	25.0	25.3	25.5
	*Temperature (°C)	24.9	24.8	24.9
500 mg/L	pH (S.U.)	8.26	8.11	8.01
	Dissolved oxygen (mg/L)	8.0	7.8	7.5
	*Salinity (ppt)	25.0	25.3	25.5
	*Temperature (°C)	24.9	24.8	24.9
750 mg/L	pH (S.U.)	8.26	8.11	
	Dissolved oxygen (mg/L)	8.1	7.7	
	*Salinity (ppt)	25.0	25.2	
	*Temperature (°C)	25.0	24.8	
1000 mg/L	pH (S.U.)	8.26	8.12	
	Dissolved oxygen (mg/L)	8.1	7.9	
	*Salinity (ppt)	25.2	25.4	
	*Temperature (°C)	25.0	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	13066485

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 # 253

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-03-22	* 1120	H	1330	H	1E	Yellow	04-27-22B
24	05-04-22			1328	H			
48 Termination	05-05-22			1329	H			

*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 Ab
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	05-01-22 1200 TO 05-02-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.11
	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	8 2d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	9	6 2d	7 2d	0	0	0	0
Mean Survival	100%		100%		95%		65%		0%		0%	

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

Statistics:

Method	TSK 05-12-22 M
Lower 95% confidence limit (mg KCl/L)	~~586.8~~ 496.8 05-12-22 M
Upper 95% confidence limit (mg KCl/L)	586.8
48-hour LC_{50} (mg KCl/L)	539.9

Comments:

Test Reviewed by: H

ETS
Environmental Testing Solutions, Inc.

Acute Mysid Test-24 Hr Survival

Start Date:	5/3/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	5/5/2022	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

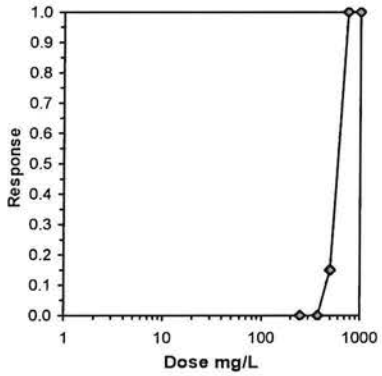
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	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				

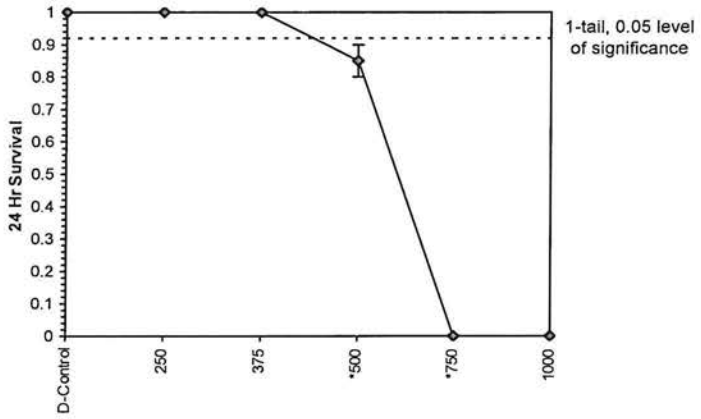
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.05495	0.05636	0.5915	0.00201	4.1E-06	4, 5
Treatments vs D-Control										

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.

Acute Mysid Test-48 Hr Survival

Start Date:	5/3/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/5/2022	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	0.9000	1.0000
375	1.0000	0.9000
500	0.6000	0.7000
750	0.0000	0.0000
1000	0.0000	0.0000

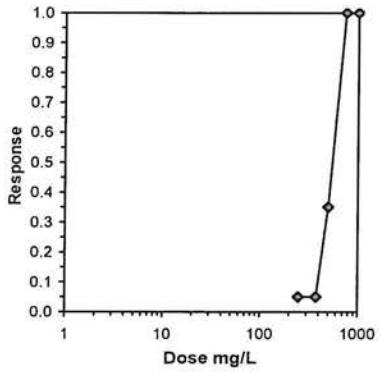
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.017	2.850	0.2283	1	20
375	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.017	2.850	0.2283	1	20
*500	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	5.910	2.850	0.2283	7	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.646	2.850	0.2283	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				

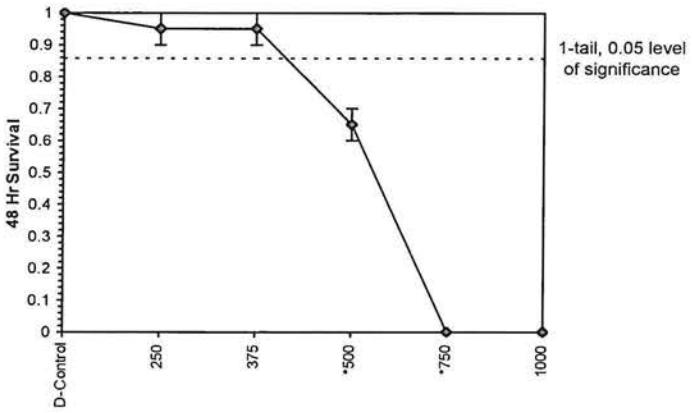
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	500	433.013		0.11749	0.1205	0.54693	0.00642	8.7E-05	4, 5

Trimmed Spearman-Karber

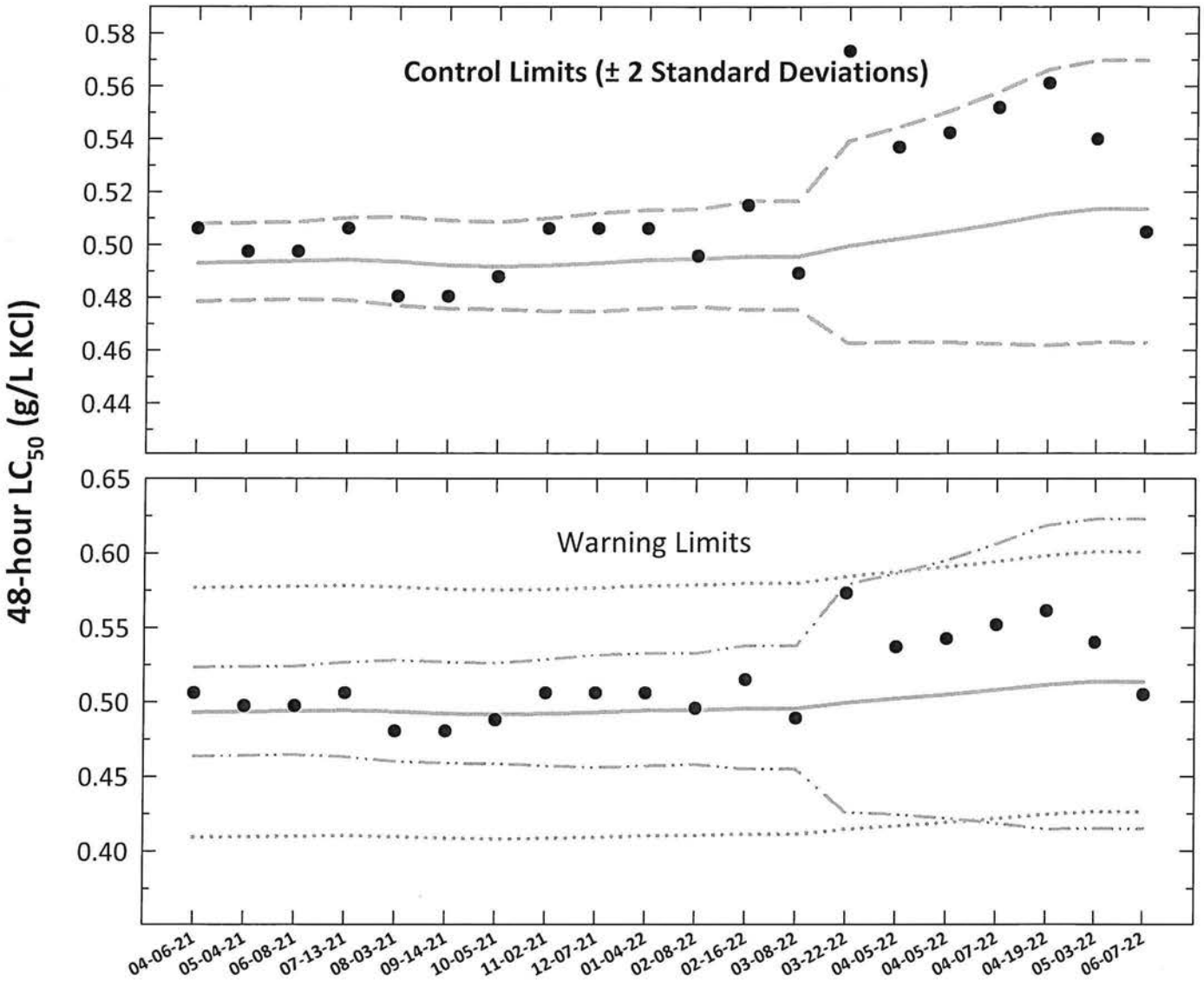
Trim Level	EC50	95% CL	
0.0%			
5.0%	539.92	496.75	586.84
10.0%	541.90	493.78	594.71
20.0%	545.60	479.43	620.92
Auto-5.0%	539.92	496.75	586.84



Dose-Response Plot



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

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	04-06-21	0.5061	-0.2958	-0.3072	0.0065	0.4930	0.4785	0.5079	0.4636	0.5233	0.4092	0.5768
2	05-04-21	0.4974	-0.3033	-0.3068	0.0064	0.4934	0.4790	0.5083	0.4642	0.5236	0.4096	0.5773
3	06-08-21	0.4974	-0.3033	-0.3064	0.0065	0.4938	0.4794	0.5087	0.4645	0.5240	0.4099	0.5778
4	07-13-21	0.5061	-0.2958	-0.3060	0.0069	0.4943	0.4789	0.5102	0.4632	0.5265	0.4103	0.5784
5	08-03-21	0.4804	-0.3184	-0.3067	0.0074	0.4935	0.4770	0.5106	0.4601	0.5281	0.4096	0.5774
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 254

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 #: 2401

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, SaltSW	pH (S.U.)	8.00	7.76	7.72
	Dissolved oxygen (mg/L)	7.6	7.7	7.4
	*Salinity (ppt)	24.9	25.0	25.2
	*Alkalinity (mg/L $CaCO_3$)	96		
	*Temperature (°C)	25.0	25.5	25.1
250 mg/L	pH (S.U.)	8.00	7.78	7.77
	Dissolved oxygen (mg/L)	7.9	7.7	7.2
	*Salinity (ppt)	24.9	25.0	25.6
	*Temperature (°C)	25.2	25.6	25.0
375 mg/L	pH (S.U.)	8.00	7.78	7.78
	Dissolved oxygen (mg/L)	7.8	7.8	7.2
	*Salinity (ppt)	24.9	25.2	25.9
	*Temperature (°C)	25.2	25.6	25.2
500 mg/L	pH (S.U.)	7.99	7.78	7.78
	Dissolved oxygen (mg/L)	7.8	7.7	7.2
	*Salinity (ppt)	24.9	25.2	25.9
	*Temperature (°C)	25.1	25.5	25.2
750 mg/L	pH (S.U.)	7.97	7.80	
	Dissolved oxygen (mg/L)	8.0	7.6	
	*Salinity (ppt)	25.1	25.3	
	*Temperature (°C)	25.1	25.7	
1000 mg/L	pH (S.U.)	7.96	7.80	
	Dissolved oxygen (mg/L)	8.1	7.8	
	*Salinity (ppt)	25.3	25.5	
	*Temperature (°C)	25.3	25.7	

06-08-22

*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	13664665

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 # 254

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-05-22	* 1100	JJ	1300	JJ	SF	PURPLE	06-01-22B
24	06-06-22			1253	JJ			
48 Termination	06-09-22			1300	JJ			

*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):	06-06-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	06-05-22 1200 TO 06-06-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.8
	Temperature (°C) 25.0

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

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	8 2d	8 2d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	6 2d	6 2d	0	0	0	0
Mean Survival	100%		100%		90%		60%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	462.7
Upper 95% confidence limit (mg KCl/L)	558.0
48-hour LC_{50} (mg KCl/L)	504.8

Comments:

Test Reviewed by: JJ

ETS
Environmental Testing Solutions, Inc.

Reviewed and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	6/7/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2022	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

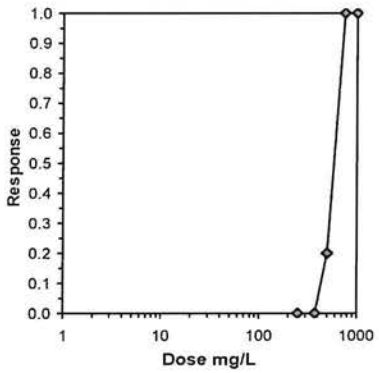
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

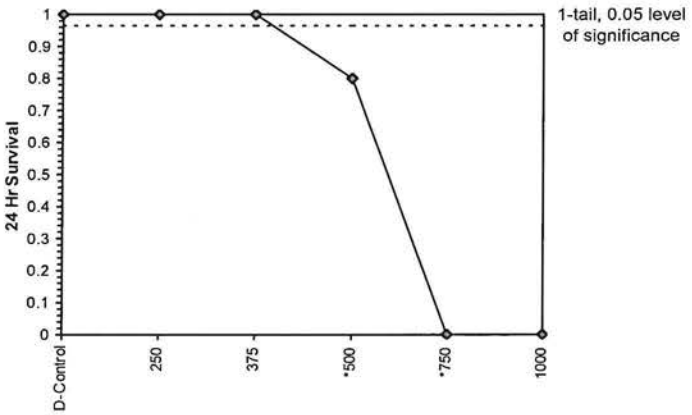
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.589	0.0001	2.3E-09	4, 5
Treatments vs D-Control										

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



Environmental Testing Solutions, Inc.

Acute Mysid Test-48 Hr Survival

Start Date:	6/7/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2022	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	0.9000	0.9000
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	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	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

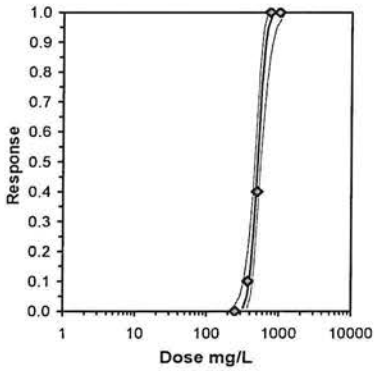
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	250	375	306.186		0.00967	0.00991	0.55969	0.0001	2.6E-09	4, 5
Treatments vs D-Control										

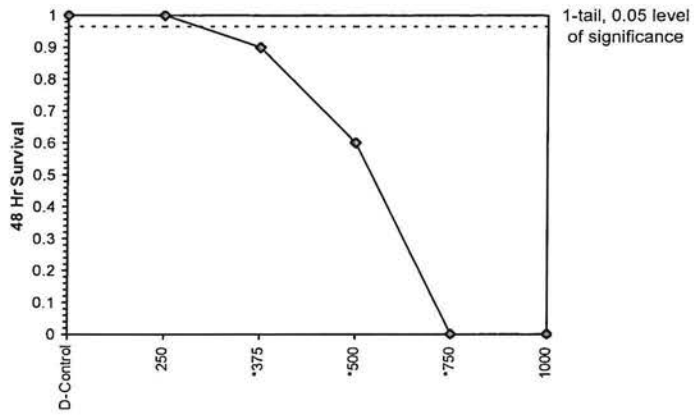
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.8187	2.45238	7.01202	16.6254	0	1.4029	7.81472	0.70485	2.70313	0.08461	6
Intercept	-26.947	6.59765	-39.879	-14.016							
TSCR											

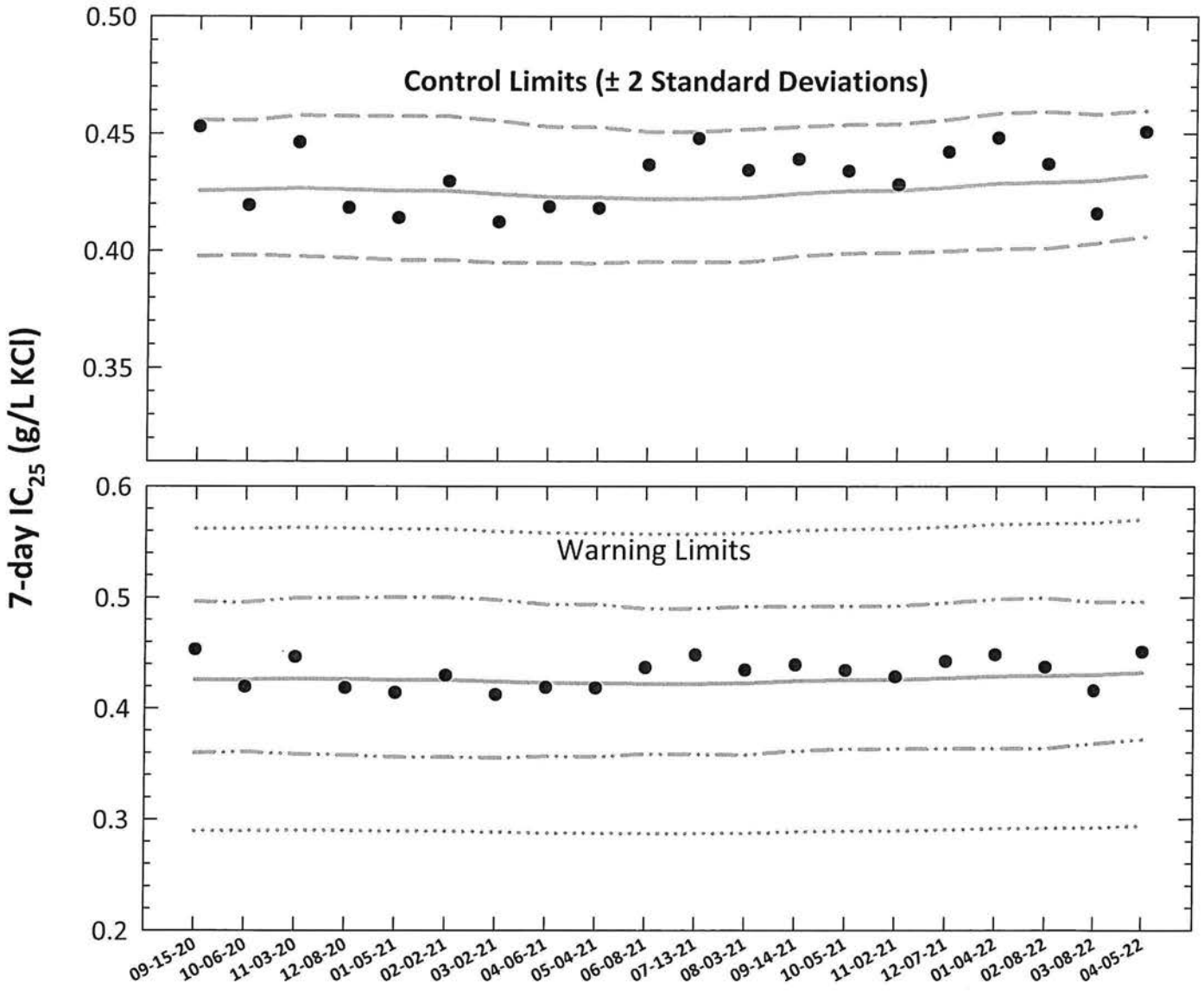
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	320.845	235.823	369.545
EC05	3.355	366.402	292.668	409.316
EC10	3.718	393.276	327.459	433.451
EC15	3.964	412.513	352.538	451.432
EC20	4.158	428.471	373.193	467.052
EC25	4.326	442.652	391.238	481.665
EC40	4.747	480.503	436.593	525.413
EC50	5.000	504.815	462.707	558.008
EC60	5.253	530.357	487.492	596.14
EC75	5.674	575.708	526.368	672.074
EC80	5.842	594.762	541.353	706.503
EC85	6.036	617.77	558.722	749.72
EC90	6.282	647.988	580.627	808.907
EC95	6.645	695.515	613.581	906.958
EC99	7.326	794.271	678.254	1127.84



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	09-15-20	0.4529	-0.3440	-0.3709	0.0148	0.4257	0.3976	0.4557	0.3598	0.4963	0.2895	0.5619
2	10-06-20	0.4194	-0.3774	-0.3706	0.0146	0.4260	0.3982	0.4557	0.3608	0.4957	0.2897	0.5623
3	11-03-20	0.4463	-0.3504	-0.3699	0.0153	0.4267	0.3977	0.4578	0.3587	0.4996	0.2901	0.5632
4	12-08-20	0.4183	-0.3786	-0.3704	0.0154	0.4262	0.3970	0.4575	0.3577	0.4996	0.2898	0.5625
5	01-05-21	0.4140	-0.3830	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
6	02-02-21	0.4296	-0.3669	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

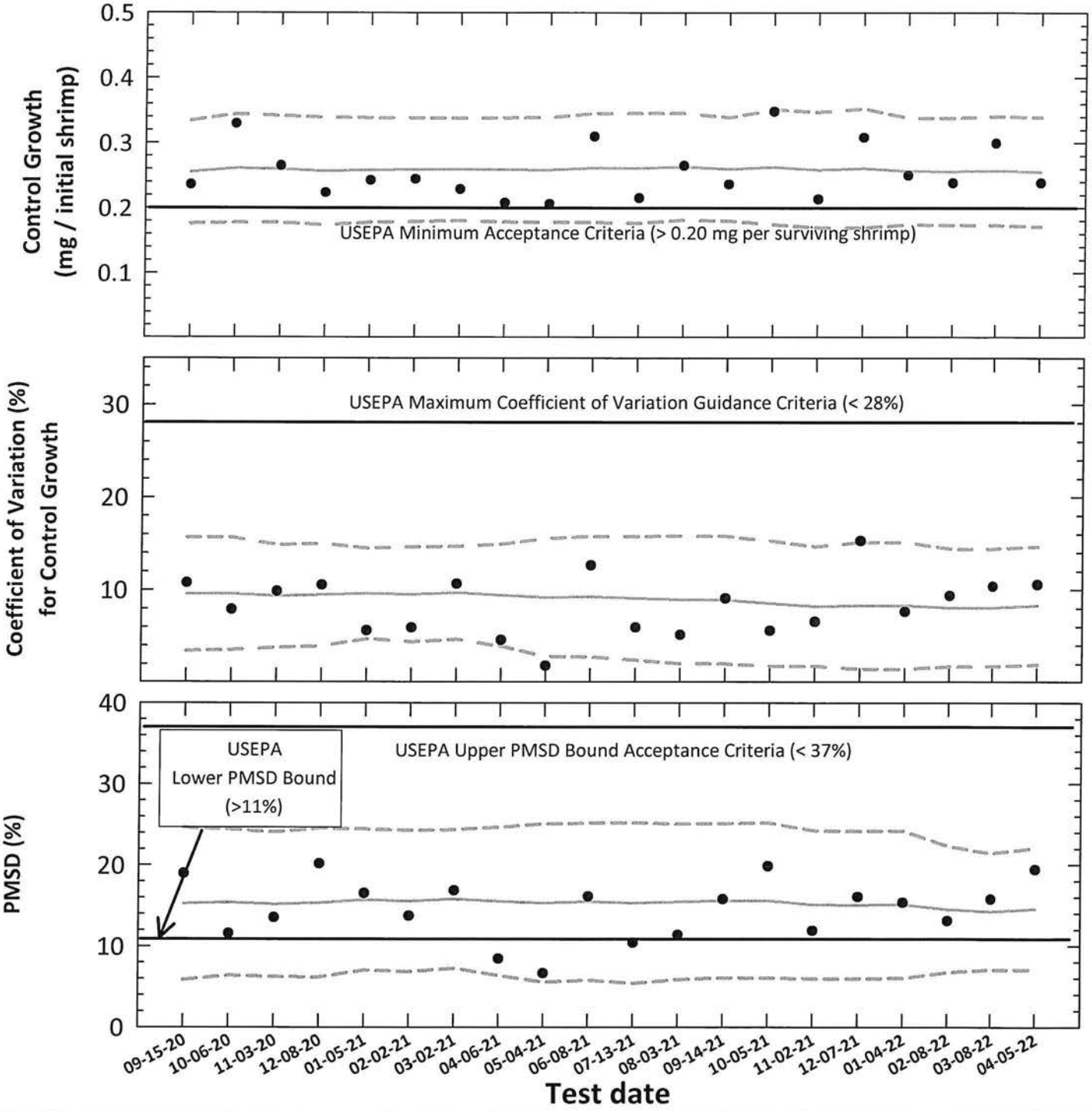
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

——— **Central Tendency** (mean Control Growth, CV or PMSD)

– – – **95% Confidence Interval** (mean Control Growth, CV or PMSD ± 2 Standard Deviations)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
			Mean	CV	MSD	PMSD	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
		(%)	(mg/initial shrimp)	(%)		(%)									
1	09-15-20	100	0.236	10.8	0.0447	19.0	0.255	0.176	0.334	9.5	3.4	15.7	15.2	5.8	24.6
2	10-06-20	100	0.330	7.9	0.0381	11.6	0.261	0.178	0.344	9.6	3.5	15.7	15.4	6.4	24.4
3	11-03-20	100	0.265	9.8	0.0358	13.5	0.260	0.177	0.342	9.3	3.8	14.9	15.1	6.2	24.1
4	12-08-20	100	0.223	10.5	0.0450	20.2	0.256	0.174	0.339	9.5	3.9	15.0	15.3	6.1	24.5
5	01-05-21	100	0.243	5.6	0.0400	16.5	0.258	0.178	0.338	9.6	4.7	14.5	15.7	7.0	24.4
6	02-02-21	100	0.244	5.9	0.0335	13.7	0.259	0.179	0.338	9.5	4.4	14.6	15.5	6.8	24.2
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: **226**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2078				
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 04-05-22 1500	Randomizing template:	Grey
Date and times organisms were born between:	03-28-22 ~~1200~~ to 03-29-22 1130	Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 03-29-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.99 S.U. Temperature = 25.0 °C	Date / Time in oven:	04-12-22 1125
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	04-13-22 1125
		*Final oven temperature:	60 °C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	04-05-22	1042	JK	1400	JK	1200	JK	03-30-22 A
1	04-06-22	0510	JK	1110	JK	1000	JK	↓
2	04-07-22	0530	JK	1230	JK	1140	JK	04-05-22
3	04-08-22	0500	JK	1100	JK	1000	JK	↓
4	04-09-22	0600	JK	1200	JK	1004	JK	04-06-22
5	04-10-22	0600	JK	1200	JK	1000	JK	↓
6	04-11-22	0500	JK	1100	JK	1000	JK	↓
7	04-12-22					1014	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria
% Mortality:	07.	≤ 20%
Average weight per initial shrimp:	0.238	
Average weight per surviving shrimp:	0.238	≥ 0.20 mg/shrimp

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	493.1
NOEC (mg/L KCl)	375
LOEC (mg/L KCl)	500
ChV (mg/L KCl)	433.0
IC_{25} (mg/L KCl)	450.7

ETS Environmental Testing Solutions, Inc.

AbKClCR Test Number: **226**

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Forest green Analyst: JW Date: 03-24-22	14.03	JW 03-24-22 15.* 1560	15.48	14.96	14.46	14.18	15.16	15.32	12.93	15.82	15.63	15.15	15.13	15.02	14.63	13.92
*B = Pan + Shrimp weight (mg) Analyst: AJ Date: 04-13-22	15.34	16.85	16.53	16.10	15.84	15.44	16.25	16.37	14.13	17.02	16.90	16.50	16.30	16.28	15.89	15.21
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	1.31	1.25	1.05	1.14	1.38	1.26	1.09	1.05	1.20	1.20	1.27	1.35	1.17	1.26	1.26	1.29
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.262	0.250	0.210	0.228	0.276	0.252	0.218	0.210	0.240	0.240	0.254	0.270	0.234	0.252	0.252	0.258
	Average weight per initial number of shrimp (mg) 0.238								Average weight per initial number of shrimp (mg) 0.250				Percent reduction from control (%) -4.97			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **226**

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	4 1d	4 1d	S	S
2	S	S	S	S	S	S	S	S	4 1d	4 1d	S	4 1d	4	4	4 1d	4 1d
3	S	S	S	S	S	S	S	S	4	4	4 1d	4	4	4	3 1d	4
4	S	S	S	S	S	S	S	S	3 1d	4	4	3 1d	3 1d	3 1d	1 2d	4
5	S	S	S	S	4 1d	S	4 1d	S	3	4	3 1d	3	2 1d	3	1	4
6	S	S	S	S	4	S	4	S	3	4	3	3	2	3	1	3 1d
7	S	S	S	S	4	4 1d	4	4 1d	3	3 1d	3	3	2	3	1	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: forest green Analyst: JW Date: 03-28-22	13.05	14.71	12.98	13.79	14.99	13.21	15.23	15.02	15.26	15.11	15.84	13.37	14.72	15.15	15.17	15.45
*B = Pan + Shrimp weight (mg) Analyst: A.S Date: 04-15-22	14.45	15.92	14.00	14.87	04-15-22 ~~15~~ 1.9 16.00	14.19	16.33	16.73	16.03	15.94	16.65	14.35	15.21	15.91	15.41	16.47
C = Shrimp weight (mg) = B – A Hand calculated Analyst:	1.40	1.21	1.02	1.08	1.01	0.98	1.10	1.71	0.77	0.83	0.81	0.98	0.49	0.76	0.24	1.02
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst:	0.280	0.242	0.204	0.216	0.202	0.196	0.220	0.342	0.154	0.166	0.162	0.196	0.098	0.152	0.048	0.204
	Average weight per initial number of shrimp (mg) 0.238				Percent reduction from control (%) 0.27.				Average weight per initial number of shrimp (mg) 0.148				Percent reduction from control (%) 38.17			

*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: **226**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd	0 Sd
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males															04-06-22	
*A = Pan weight (mg) Tray color code: _____ Analyst: _____ Date: _____																
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B – A Hand calculated Analyst: _____															04-06-22	
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: 226

Test dates: April 05-12, 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	14.03	15.34	1.31	0.262	100.0	0.238	10.6	Not applicable
	B	5	5	15.60	16.85	1.25	0.250				
	C	5	5	15.48	16.53	1.05	0.210				
	D	5	5	14.96	16.10	1.14	0.228				
	E	5	5	14.46	15.84	1.38	0.276				
	F	5	5	14.18	15.44	1.26	0.252				
	G	5	5	15.16	16.25	1.09	0.218				
	H	5	5	15.32	16.37	1.05	0.210				
250	I	5	5	12.93	14.13	1.20	0.240	100.0	0.250	4.6	-4.9
	J	5	5	15.82	17.02	1.20	0.240				
	K	5	5	15.63	16.90	1.27	0.254				
	L	5	5	15.15	16.50	1.35	0.270				
	M	5	5	15.13	16.30	1.17	0.234				
	N	5	5	15.02	16.28	1.26	0.252				
	O	5	5	14.63	15.89	1.26	0.252				
	P	5	5	13.92	15.21	1.29	0.258				
375	Q	5	5	13.05	14.45	1.40	0.280	90.0	0.238	21.1	0.2
	R	5	5	14.71	15.92	1.21	0.242				
	S	5	5	12.98	14.00	1.02	0.204				
	T	5	5	13.79	14.87	1.08	0.216				
	U	5	4	14.99	16.00	1.01	0.202				
	V	5	4	13.21	14.19	0.98	0.196				
	W	5	4	15.23	16.33	1.10	0.220				
	X	5	4	15.02	16.73	1.71	0.342				
500	Y	5	3	15.26	16.03	0.77	0.154	52.5	0.148	34.9	38.1
	Z	5	3	15.11	15.94	0.83	0.166				
	AA	5	3	15.84	16.65	0.81	0.162				
	BB	5	3	13.37	14.35	0.98	0.196				
	CC	5	2	14.72	15.21	0.49	0.098				
	DD	5	3	15.15	15.91	0.76	0.152				
	EE	5	1	15.17	15.41	0.24	0.048				
	FF	5	3	15.45	16.47	1.02	0.204				
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: 0.0463

PMSD: 19.4

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:	4/5/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/12/2022	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	0.8000	0.8000	0.8000	0.8000
500	0.6000	0.6000	0.6000	0.6000	0.4000	0.6000	0.2000	0.6000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9000	0.9000	1.2262	1.1071	1.3453	10.381	8	52.00	48.00	4	40
*500	0.5250	0.5250	0.8081	0.4636	0.8861	19.305	8	36.00	48.00	19	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

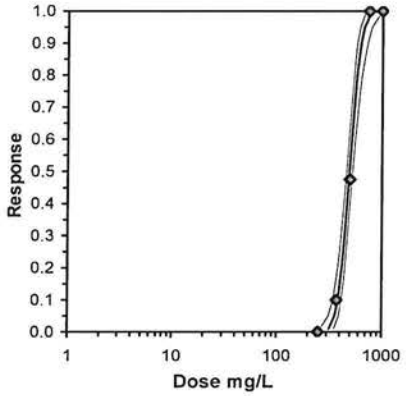
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.81355	0.904	-1.5677	4.22777
Equality of variance cannot be confirmed				

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

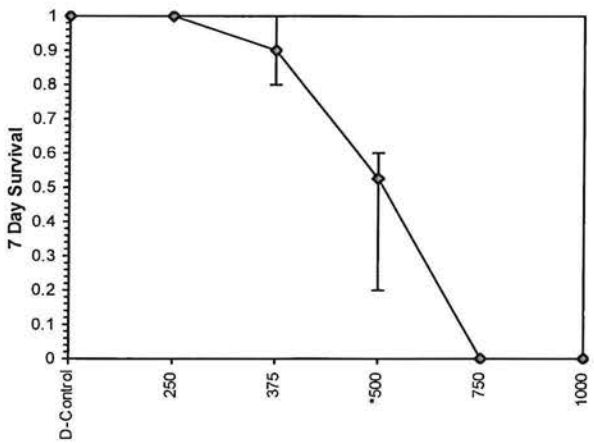
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.1372	1.85846	8.49459	15.7798	0	1.4087	7.81472	0.7035	2.6929	0.08239	5
Intercept	-27.684	4.98111	-37.447	-17.921							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	317.124	262.611	353.161
EC05	3.355	360.893	314.252	392.122
EC10	3.718	386.644	345.171	415.391
EC15	3.964	405.049	367.249	432.435
EC20	4.158	420.299	385.362	446.984
EC25	4.326	433.839	401.181	460.348
EC40	4.747	469.923	441.275	498.866
EC50	5.000	493.061	464.887	526.291
EC60	5.253	517.338	487.866	557.381
EC75	5.674	560.367	525.139	617.227
EC80	5.842	578.42	539.862	643.73
EC85	6.036	600.197	557.125	676.569
EC90	6.282	628.767	579.143	720.89
EC95	6.645	673.632	612.659	792.918
EC99	7.326	766.605	679.34	950.11



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	4/5/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/12/2022	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	0.2620	0.2500	0.2100	0.2280	0.2760	0.2520	0.2180	0.2100
250	0.2400	0.2400	0.2540	0.2700	0.2340	0.2520	0.2520	0.2580
375	0.2800	0.2420	0.2040	0.2160	0.2020	0.1960	0.2200	0.3420
500	0.1540	0.1660	0.1620	0.1960	0.0980	0.1520	0.0480	0.2040
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

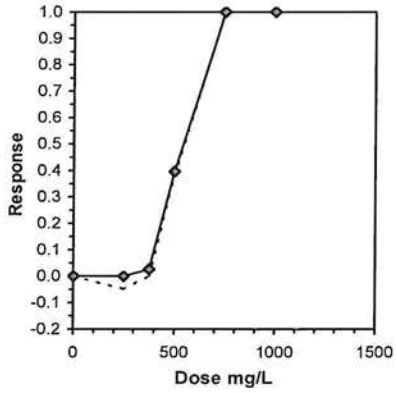
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	0.2383	1.0000	0.2383	0.2100	0.2760	10.556	8			0.2441	1.0000
250	0.2500	1.0493	0.2500	0.2340	0.2700	4.645	8	78.50	43.00	0.2441	1.0000
375	0.2378	0.9979	0.2378	0.1960	0.3420	21.102	8	61.00	43.00	0.2378	0.9739
500	0.1475	0.6191	0.1475	0.0480	0.2040	34.863	8			0.1475	0.6042
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)	0.88501	0.884	1.58353	4.00726
Bartlett's Test indicates unequal variances (p = 2.59E-03)	11.9153	9.21035		

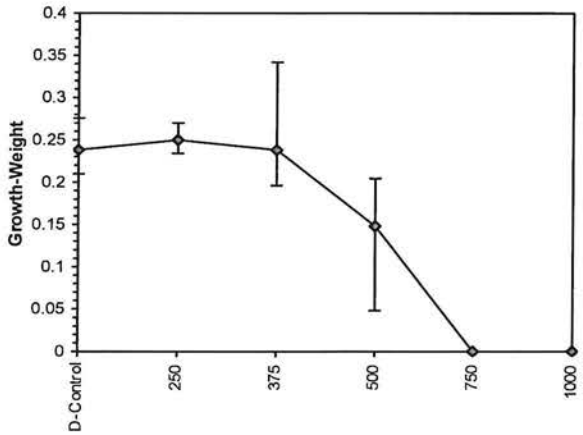
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	>375		
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	383.08	40.03	286.25	395.63	-1.3631
IC10	399.98	25.10	324.61	416.27	-1.7636
IC15	416.89	18.47	362.94	436.90	-1.3007
IC20	433.80	16.37	388.50	457.86	-0.5549
IC25	450.70	17.48	410.40	484.73	0.2346
IC40	501.74	21.48	464.84	543.89	0.3353
IC50	543.11	23.19	492.18	578.24	-0.1703



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKClCR Test Number: **226**

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.

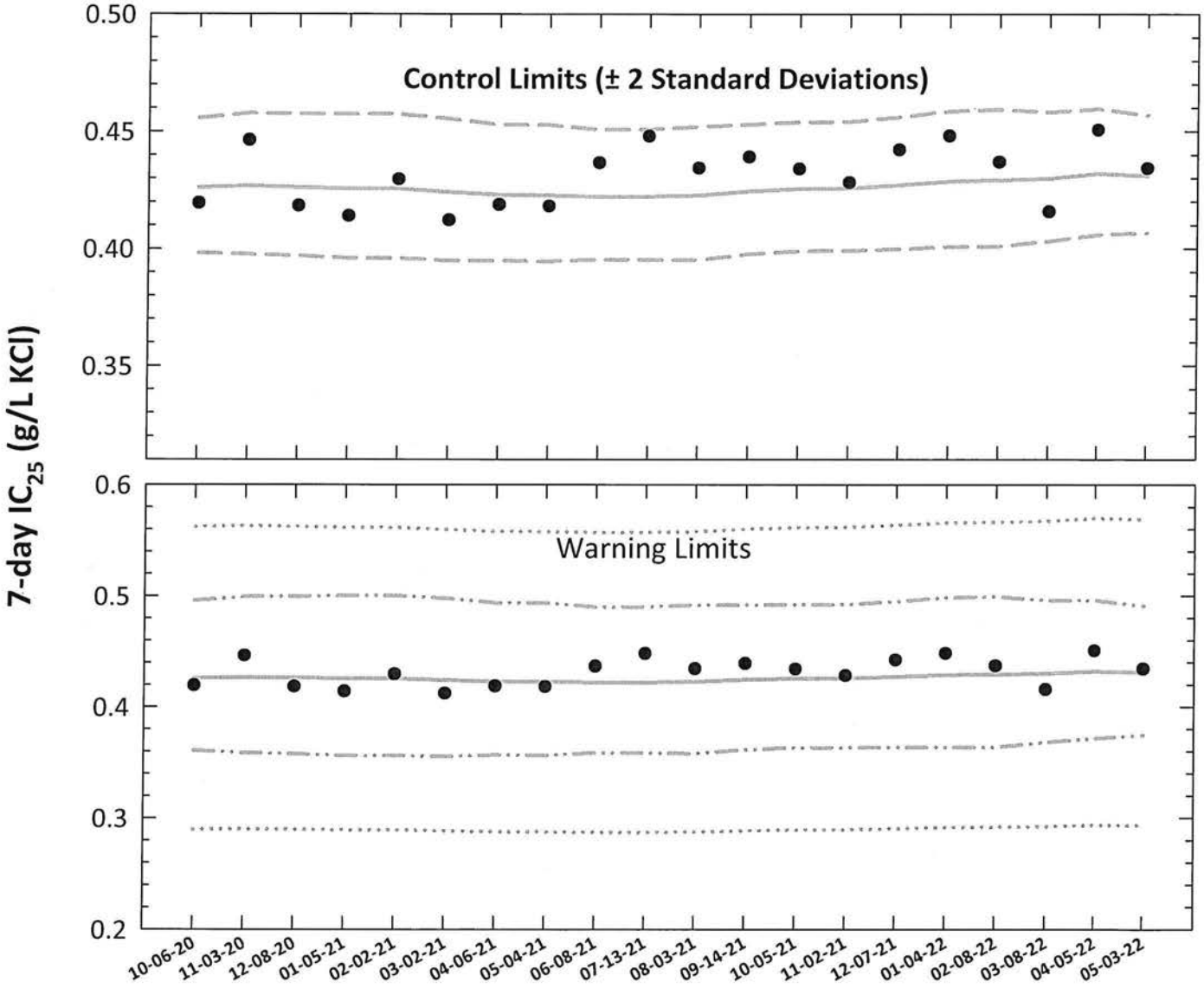
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	JW	JW	JW	K	K	K
CONTROL, Salt SW	pH (S.U.)	8.02	7.94	8.00	7.95	7.95	7.93
	DO (mg/L)	7.7	7.5	7.6	7.7	7.8	7.5
	Salinity (ppt)	25.0	25.1	24.8	25.4	25.0	25.4
	Alkalinity (mg $CaCO_3$/L)	130		→	04-10-22 K	130	
	Temperature (°C)	25.2	25.6	25.3	25.6	25.2	25.7
250 mg KCl/L	pH (S.U.)	8.03	7.95	7.98	7.90	7.96	7.89
	DO (mg/L)	7.7	7.5	7.7	7.7	7.8	7.5
	Salinity (ppt)	25.2	25.3	25.0	25.4	25.2	25.4
	Temperature (°C)	25.3	25.5	25.4	25.6	25.3	25.5
375 mg KCl/L	pH (S.U.)	8.04	7.95	7.98	7.92	7.96	7.91
	DO (mg/L)	7.7	7.4	7.7	7.7	7.8	7.6
	Salinity (ppt)	25.5	25.5	25.2	25.5	25.3	25.3
	Temperature (°C)	25.3	25.5	25.4	25.5	25.3	25.7
500 mg KCl/L	pH (S.U.)	8.09	7.93	7.96	7.92	7.98	7.94
	DO (mg/L)	8.0	7.7	7.7	7.8	7.9	7.7
	Salinity (ppt)	25.4	25.5	25.4	25.7	25.3	25.5
	Temperature (°C)	25.3	25.7	25.4	25.7	25.2	25.6
750 mg KCl/L	pH (S.U.)	8.06	7.96				
	DO (mg/L)	7.6	7.8				
	Salinity (ppt)	25.4	25.4				
	Temperature (°C)	25.2	25.4				
1000 mg KCl/L	pH (S.U.)	8.06	7.99				
	DO (mg/L)	7.9	7.9				
	Salinity (ppt)	25.6	25.6				
	Temperature (°C)	25.4	25.4				
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

AbKClCR Test Number: 226

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	K	K	K
CONTROL, Salt SW	pH (S.U.)	7.93	8.01	8.04	7.89	8.05	7.81	7.96	7.68
	DO (mg/L)	7.8	25.4 (7.4)	7.6	7.2	7.7	7.5	7.8	6.3
	Salinity (ppt)	25.1	~~24.4~~ 04.05.22	25.0	25.4	25.1	25.3	24.7	24.7
	Alkalinity (mg $CaCO_3$/L)	K 04.10.22		130		K		K 04.10.22	
	Temperature (°C)	25.3	25.6	25.2	25.6	25.3	25.7	25.3	25.8
250 mg KCl/L	pH (S.U.)	7.94	7.96	8.10	7.89	8.04	7.83	7.97	7.68
	DO (mg/L)	7.8	25.4 (7.7)	7.7	7.3	7.6	7.3	7.8	6.3
	Salinity (ppt)	25.2	~~24.4~~ 04.04.22	25.3	25.6	25.2	25.4	25.2	25.5
	Temperature (°C)	25.4	25.5	25.3	25.6	25.4	25.5	25.4	25.7
375 mg KCl/L	pH (S.U.)	7.96	7.95	8.10	7.90	8.04	7.84	7.98	7.75
	DO (mg/L)	7.9	7.3	7.6	7.5	7.6	7.4	7.8	6.2
	Salinity (ppt)	25.4	25.7	25.3	25.8	25.4	25.7	25.4	26.1
	Temperature (°C)	25.2	25.5	25.3	25.7	25.4	25.7	25.4	25.7
500 mg KCl/L	pH (S.U.)	7.97	7.99	8.11	7.92	8.04	7.92	8.00	7.80
	DO (mg/L)	8.0	7.2	7.7	7.5	7.6	7.5	7.9	6.5
	Salinity (ppt)	25.5	26.1	25.5	26.0	25.4	25.8	25.6	26.0
	Temperature (°C)	25.4	25.7	25.3	25.5	25.4	25.7	25.4	25.6
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)					K 04.08.22			
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	10-06-20	0.4194	-0.3774	-0.3706	0.0146	0.4260	0.3982	0.4557	0.3608	0.4957	0.2897	0.5623
2	11-03-20	0.4463	-0.3504	-0.3699	0.0153	0.4267	0.3977	0.4578	0.3587	0.4996	0.2901	0.5632
3	12-08-20	0.4183	-0.3786	-0.3704	0.0154	0.4262	0.3970	0.4575	0.3577	0.4996	0.2898	0.5625
4	01-05-21	0.4140	-0.3830	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
5	02-02-21	0.4296	-0.3669	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

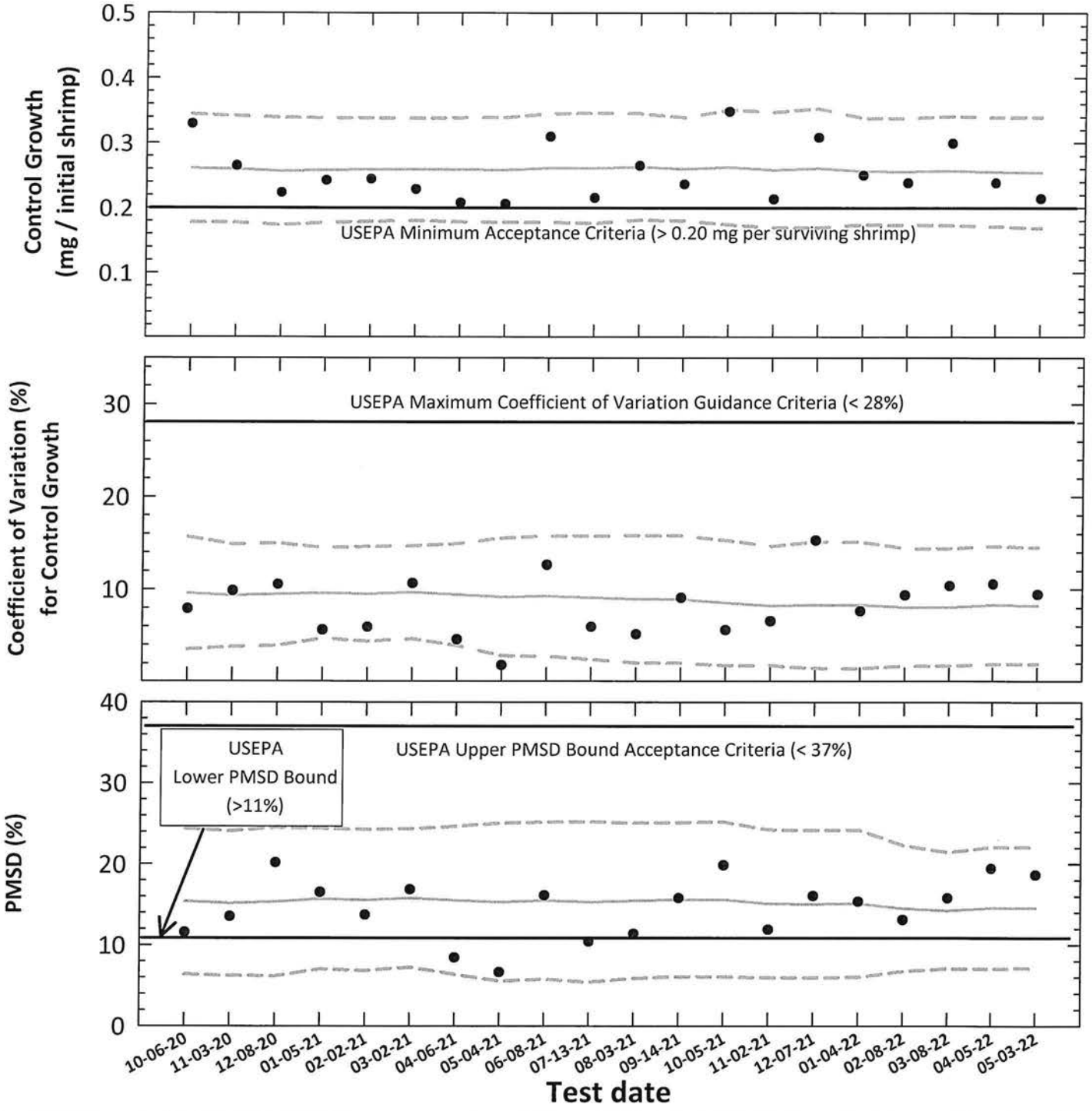
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

——— **Central Tendency** (mean Control Growth, CV or PMSD)

– – – – **95% Confidence Interval** (mean Control Growth, CV or PMSD ± 2 Standard Deviations)

Entered and Reviewed by Jim Sumner

ETS Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
			Mean	CV	MSD	PMSD	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
		(%)	(mg/initial shrimp)	(%)		(%)									
1	10-06-20	100	0.330	7.9	0.0381	11.6	0.261	0.178	0.344	9.6	3.5	15.7	15.4	6.4	24.4
2	11-03-20	100	0.265	9.8	0.0358	13.5	0.260	0.177	0.342	9.3	3.8	14.9	15.1	6.2	24.1
3	12-08-20	100	0.223	10.5	0.0450	20.2	0.256	0.174	0.339	9.5	3.9	15.0	15.3	6.1	24.5
4	01-05-21	100	0.243	5.6	0.0400	16.5	0.258	0.178	0.338	9.6	4.7	14.5	15.7	7.0	24.4
5	02-02-21	100	0.244	5.9	0.0335	13.7	0.259	0.179	0.338	9.5	4.4	14.6	15.5	6.8	24.2
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

Control Survival = USEPA minimum test acceptability criteria ≥ 80% survival.

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

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 28%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 11%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 37%.

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Species: *Americamysis (Mysidopsis) bahia*

AbKClCR Test Number: **227**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:	INSS 2078					
Stock preparation:	50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water					
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	PURPLE
Date and times organisms were born between:	04-26-22 1200 to 04-27-22 1130	Incubator number and shelf location:	SE
Organism source:	AI Batch Ab: 04-27-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 8.10 S.U. Temperature = 25.0 °C	Date / Time in oven:	05-10-22 1200
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	05-11-22 1200
		*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	05-03-22	1120	JK	1310	JK	1227	JK	04-27-22 B
1	05-04-22	0500	JK	1115	JK	1030	JK	↓
2	05-05-22	0500	JK	1245	JK	1030	JK	05-02-22
3	05-06-22	0500	JK	1100	JK	1027	JK	↓
4	05-07-22	0600	JK	1200	JK	1125	JK	05-04-22
5	05-08-22	0500	JK	1100	JK	1027	JK	↓
6	05-09-22	0500	JK	1100	JK	1027	JK	05-05-22
7	05-10-22					1050	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	130664PS

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	494.6
Average weight per initial shrimp:	0.214		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.214	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	434.3

ETS Environmental Testing Solutions, Inc.

AbKClCR Test Number: **227**

Survival and Growth Data

Day	CONTROL								250 mg KCl/L							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: Lavender Analyst: A.I Date: 04-27-22	14.49	14.87	13.37	13.48	14.61	13.93	12.70	13.92	14.06	13.44	15.36	13.87	13.77	14.75	12.87	13.15
*B = Pan + Shrimp weight (mg) Analyst: TG Date: 05-11-22	15.35	15.98	14.46	14.69	15.69	14.94	13.79	15.03	15.15	14.34	16.42	14.83	15.04	16.15	13.98	13.92
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	0.86	1.11	1.09	1.21	1.08	1.01	1.09	1.11	1.09	0.90	1.06	0.96	1.27	1.40	1.11	0.77
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.172	0.222	0.218	0.242	0.216	0.202	0.218	0.222	0.218	0.180	0.212	0.192	0.254	0.280	0.222	0.154
	Average weight per initial number of shrimp (mg) 0.214								Average weight per initial number of shrimp (mg) 0.214				Percent reduction from control (%) 0.0			

*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: **227**

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	4 1d	4 1d	4 1d	3 2d	6	3 2d	2 3d	3 2d
4	S	4 1d	S	S	S	S	S	S	3 1d	4	3 1d	3	4 1d	3	2	3
5	S	4	S	S	S	S	S	S	3	4	3	3	4	3	2	3
6	S	4	S	S	S	S	S	S	3	3 1d 05-04-22	3	2 1d	3 1d	3	2	2 1d
7	S	4	S	S	S	S	S	S	3	2 1d	2 1d	2	3	3	2	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																1 05-10
*A = Pan weight (mg) Tray color code: Lavender Analyst: A.I Date: 04-27-22	15.96	14.63	13.84	13.71	15.59	15.04	16.22	15.20	14.73	14.84	14.79	04-27-22 A.I ~~14.9~~ 15.00	15.01	14.35	15.09	14.09
*B = Pan + Shrimp weight (mg) Analyst: TG Date: 05-17-22	17.04	15.69	14.87	14.88	16.55	15.99	17.34	16.45	15.39	15.19	15.34	15.23	15.74	15.04	15.32	14.71
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JI	1.08	1.06	1.03	1.17	0.96	0.95	1.12	1.25	0.66	0.35	0.55	0.23	0.73	0.69	0.23	0.62
Weight per Initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JI	0.216	0.212	0.206	0.234	0.192	0.190	0.224	0.250	0.132	0.070	0.110	0.046	0.146	0.138	0.046	0.124
	Average weight per initial number of shrimp (mg) 0.216				Percent reduction from control (%) −0.7 %				Average weight per initial number of shrimp (mg) 0.102				Percent reduction from control (%) 52.6 %			

*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: **227**

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															05.04.22	
*A = Pan weight (mg) Tray color code: _____ Analyst: _____ Date: _____																
*B = Pan + Shrimp weight (mg) Analyst: _____ Date: _____																
C = Shrimp weight (mg) = B – A Hand calculated Analyst: _____															05.04.22	
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.

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: 227

Test dates: May 03-10, 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	15.35	0.86	0.172	100.0	0.214	9.4	Not applicable
	B	5	5	14.87	15.98	1.11	0.222				
	C	5	5	13.37	14.46	1.09	0.218				
	D	5	5	13.48	14.69	1.21	0.242				
	E	5	5	14.61	15.69	1.08	0.216				
	F	5	5	13.93	14.94	1.01	0.202				
	G	5	5	12.70	13.79	1.09	0.218				
	H	5	5	13.92	15.03	1.11	0.222				
250	I	5	5	14.06	15.15	1.09	0.218	100.0	0.214	18.8	0.0
	J	5	5	13.44	14.34	0.90	0.180				
	K	5	5	15.36	16.42	1.06	0.212				
	L	5	5	13.87	14.83	0.96	0.192				
	M	5	5	13.77	15.04	1.27	0.254				
	N	5	5	14.75	16.15	1.40	0.280				
	O	5	5	12.87	13.98	1.11	0.222				
	P	5	5	13.15	13.92	0.77	0.154				
375	Q	5	5	15.96	17.04	1.08	0.216	97.5	0.216	9.5	-0.7
	R	5	4	14.63	15.69	1.06	0.212				
	S	5	5	13.84	14.87	1.03	0.206				
	T	5	5	13.71	14.88	1.17	0.234				
	U	5	5	15.59	16.55	0.96	0.192				
	V	5	5	15.04	15.99	0.95	0.190				
	W	5	5	16.22	17.34	1.12	0.224				
	X	5	5	15.20	16.45	1.25	0.250				
500	Y	5	3	14.73	15.39	0.66	0.132	47.5	0.102	40.7	52.6
	Z	5	2	14.84	15.19	0.35	0.070				
	AA	5	2	14.79	15.34	0.55	0.110				
	BB	5	2	15.00	15.23	0.23	0.046				
	CC	5	3	15.01	15.74	0.73	0.146				
	DD	5	3	14.35	15.04	0.69	0.138				
	EE	5	2	15.09	15.32	0.23	0.046				
	FF	5	2	14.09	14.71	0.62	0.124				
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: 0.0399 MSD = Minimum Significant Difference

PMSD: 18.6 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

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	5/3/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	5/10/2022	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	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.4000	0.4000	0.4000	0.6000	0.6000	0.4000	0.4000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.4750	0.4750	0.7602	0.6847	0.8861	13.708	8	36.00	48.00	21	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

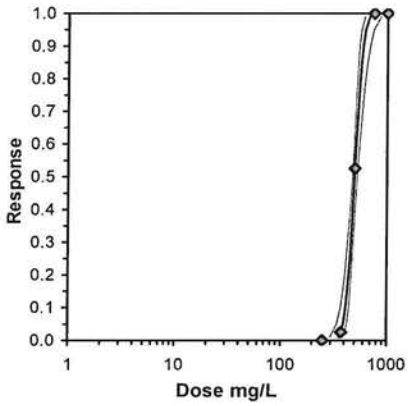
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82366	0.904	-0.6715	3.37374
Equality of variance cannot be confirmed				

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

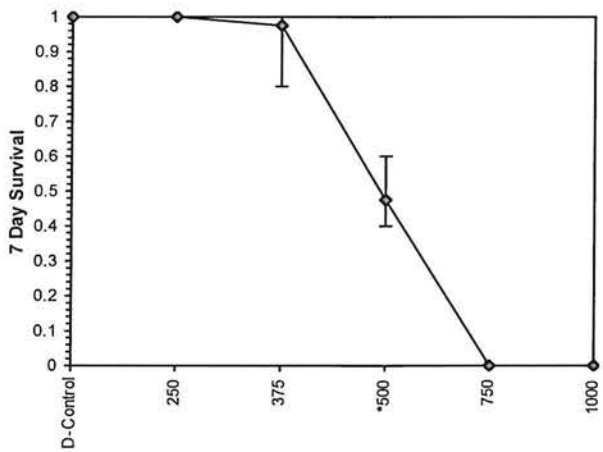
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.6709	3.35252	10.1	23.2418	0	0.06921	7.81472	0.99526	2.69424	0.05998	3
Intercept	-39.915	8.99074	-57.537	-22.294							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	358.671	293.812	393.338
EC05	3.355	394.072	341.769	422.569
EC10	3.718	414.351	369.842	439.761
EC15	3.964	428.619	389.578	452.329
EC20	4.158	440.309	405.537	463.115
EC25	4.326	450.591	419.252	473.133
EC40	4.747	477.578	452.589	503.004
EC50	5.000	494.586	470.903	525.205
EC60	5.253	512.199	487.759	550.857
EC75	5.674	542.876	513.648	600.34
EC80	5.842	555.554	523.547	622.079
EC85	6.036	570.705	534.988	648.825
EC90	6.282	590.358	549.364	684.584
EC95	6.645	620.738	570.857	741.928
EC99	7.326	682.005	612.446	864.208



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	5/3/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	5/10/2022	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:					

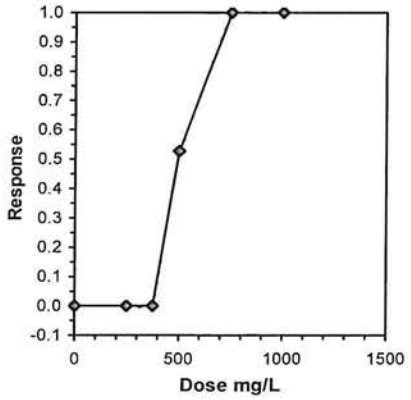
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.1720	0.2220	0.2180	0.2420	0.2160	0.2020	0.2180	0.2220
250	0.2180	0.1800	0.2120	0.1920	0.2540	0.2800	0.2220	0.1540
375	0.2160	0.2120	0.2060	0.2340	0.1920	0.1900	0.2240	0.2500
500	0.1320	0.0700	0.1100	0.0460	0.1460	0.1380	0.0460	0.1240
750	0.0000	0.0000	0.0000	0.0000	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	0.2140	1.0000	0.2140	0.1720	0.2420	9.439	8				0.2145	1.0000
250	0.2140	1.0000	0.2140	0.1540	0.2800	18.758	8	0.000	2.799	0.0399	0.2145	1.0000
375	0.2155	1.0070	0.2155	0.1900	0.2500	9.461	8	-0.105	2.799	0.0399	0.2145	1.0000
500	0.1015	0.4743	0.1015	0.0460	0.1460	40.748	8				0.1015	0.4732
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

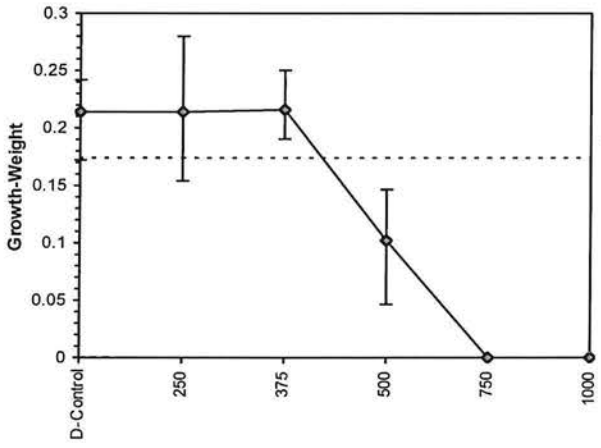
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97257		0.884		0.11979	0.89287
Bartlett's Test indicates equal variances (p = 0.11)					4.41858		9.21035			
Hypothesis Test (1-tail, 0.01)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	375	>375			0.03987	0.18629	6E-06	0.00081	0.99264	2, 21
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	386.86	64.80	160.00	389.34	-2.1409
IC10	398.73	12.70	377.79	403.69	-7.6846
IC15	410.59	7.59	390.18	419.41	-0.2603
IC20	422.46	8.28	402.44	434.22	0.0355
IC25	434.32	9.23	412.90	449.02	0.2405
IC40	469.91	13.06	445.50	493.44	0.5055
IC50	493.64	18.50	465.27	533.67	0.6823



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **227**

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.

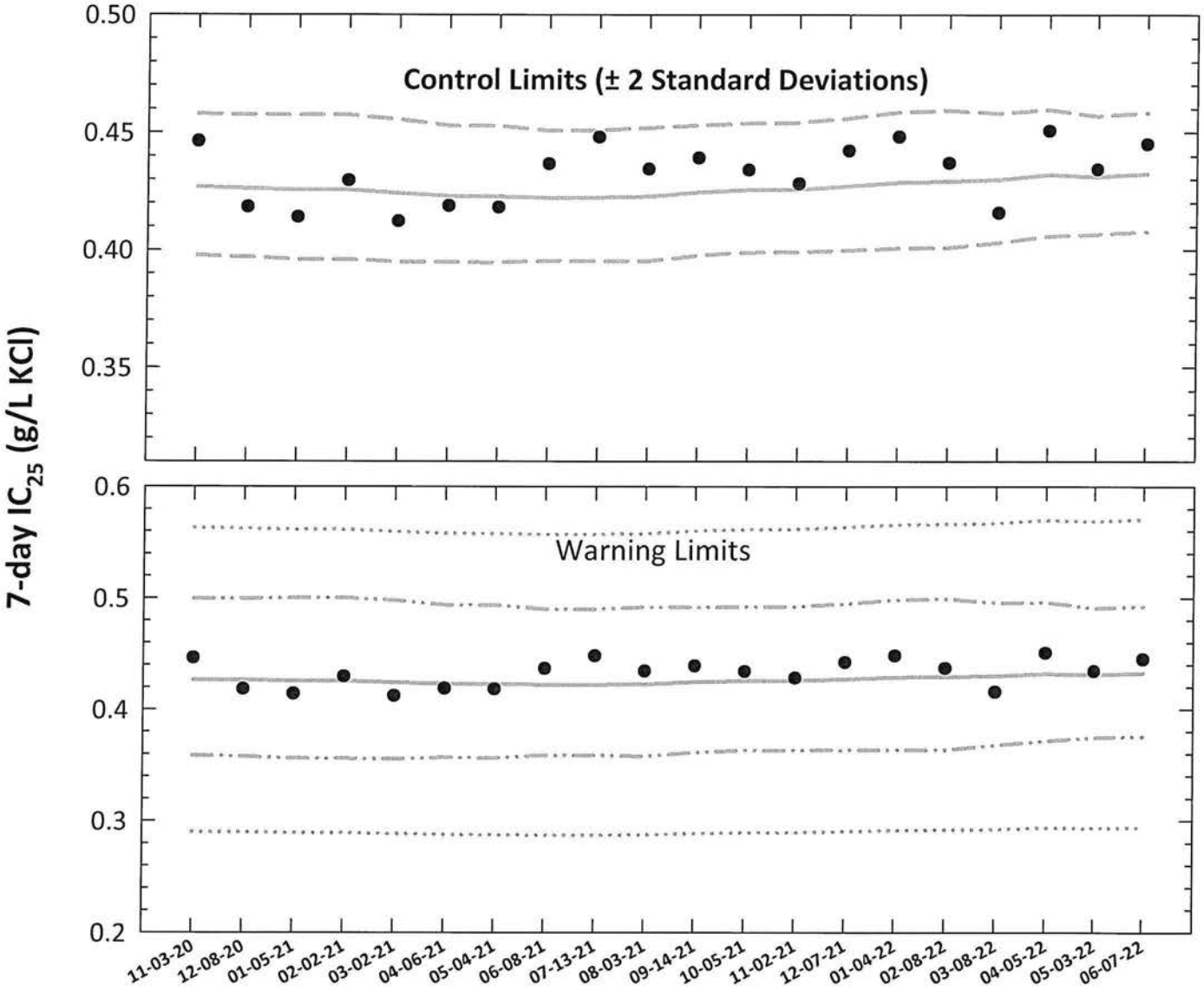
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	JW	JW	JW	JW	JW	JW
CONTROL, Salt SW	pH (S.U.)	8.23	8.05	8.07	7.90	8.01	7.85
	DO (mg/L)	8.0	7.7	7.8	7.1	7.6	6.9
	Salinity (ppt)	24.7	25.0	24.7	25.0	24.7	24.6
	Alkalinity (mg $CaCO_3$/L)	140				140	
	Temperature (°C)	25.0	25.6	25.3	25.6	25.4	25.7
250 mg KCl/L	pH (S.U.)	8.25	8.04	8.07	7.93	8.03	7.86
	DO (mg/L)	8.0	7.5	7.9	7.1	7.7	7.3
	Salinity (ppt)	24.9	25.2	24.9	25.0	24.7	24.9
	Temperature (°C)	25.0	25.5	25.2	25.7	25.4	25.9
375 mg KCl/L	pH (S.U.)	8.25	8.05	8.07	7.94	8.04	7.91
	DO (mg/L)	8.0	7.5	7.9	7.3	7.8	7.3
	Salinity (ppt)	25.0	25.2	25.0	25.1	24.9	25.3
	Temperature (°C)	25.0	25.7	25.3	25.7	25.5	25.9
500 mg KCl/L	pH (S.U.)	8.26	8.06	8.07	7.94	8.05	7.94
	DO (mg/L)	8.0	7.5	7.9	7.5	7.9	7.6
	Salinity (ppt)	25.0	25.3	25.1	25.2	25.0	25.4
	Temperature (°C)	25.1	25.7	25.3	25.6	25.5	25.8
750 mg KCl/L	pH (S.U.)	8.26	8.05				
	DO (mg/L)	8.1	7.6				
	Salinity (ppt)	25.0	25.3				
	Temperature (°C)	25.1	25.6				
1000 mg KCl/L	pH (S.U.)	8.26	8.05				
	DO (mg/L)	8.1	7.8				
	Salinity (ppt)	25.2	25.4				
	Temperature (°C)	25.1	25.6				
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

AbKClCR Test Number: 227

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	N	BSL	BSL	BSL	BSL	N	N	N
CONTROL, Salt SW	pH (S.U.)	8.01	7.93	8.08	7.92	7.98	7.03	7.93	7.69
	DO (mg/L)	8.0	7.4	7.8	7.5	7.7	7.5	7.9	6.8
	Salinity (ppt)	25.0	25.0	24.7	24.8	24.8	25.0	24.7	24.8
	Alkalinity (mg $CaCO_3$/L)	→ 05.14.22 N		140		→ 05.4.22 N		110	
	Temperature (°C)	25.4	25.9	25.3	25.8	25.6	25.8	25.3	25.9
250 mg KCl/L	pH (S.U.)	7.99	7.97	8.07	7.95	8.08	7.00	8.02	7.08
	DO (mg/L)	8.0	7.5	7.9	7.4	7.8	7.3	8.0	6.7
	Salinity (ppt)	24.8	24.9	24.8	24.9	24.8	25.1	24.9	25.0
	Temperature (°C)	25.5	25.7	25.5	25.9	25.6	25.9	25.4	26.0
375 mg KCl/L	pH (S.U.)	8.00	7.98	8.07	7.98	8.08	7.93	8.03	7.08
	DO (mg/L)	8.1	7.6	7.9	7.5	7.9	7.4	8.1	6.6
	Salinity (ppt)	24.8	25.0	25.0	25.2	24.8	25.2	25.1	25.3
	Temperature (°C)	25.5	25.9	25.5	25.7	25.6	25.9	25.4	25.9
500 mg KCl/L	pH (S.U.)	8.00	7.99	8.07	7.99	8.09	7.95	8.04	7.96
	DO (mg/L)	8.2	7.6	7.9	7.6	8.0	7.4	8.2	6.5
	Salinity (ppt)	24.8	25.2	25.1	25.5	25.0	25.3	25.1	25.4
	Temperature (°C)	25.5	25.9	25.5	25.7	25.5	25.7	25.4	25.5
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)						05.05.22		
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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 $IC_{25} \pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	11-03-20	0.4463	-0.3504	-0.3699	0.0153	0.4267	0.3977	0.4578	0.3587	0.4996	0.2901	0.5632
2	12-08-20	0.4183	-0.3786	-0.3704	0.0154	0.4262	0.3970	0.4575	0.3577	0.4996	0.2898	0.5625
3	01-05-21	0.4140	-0.3830	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
4	02-02-21	0.4296	-0.3669	-0.3711	0.0157	0.4255	0.3959	0.4573	0.3560	0.5003	0.2894	0.5617
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

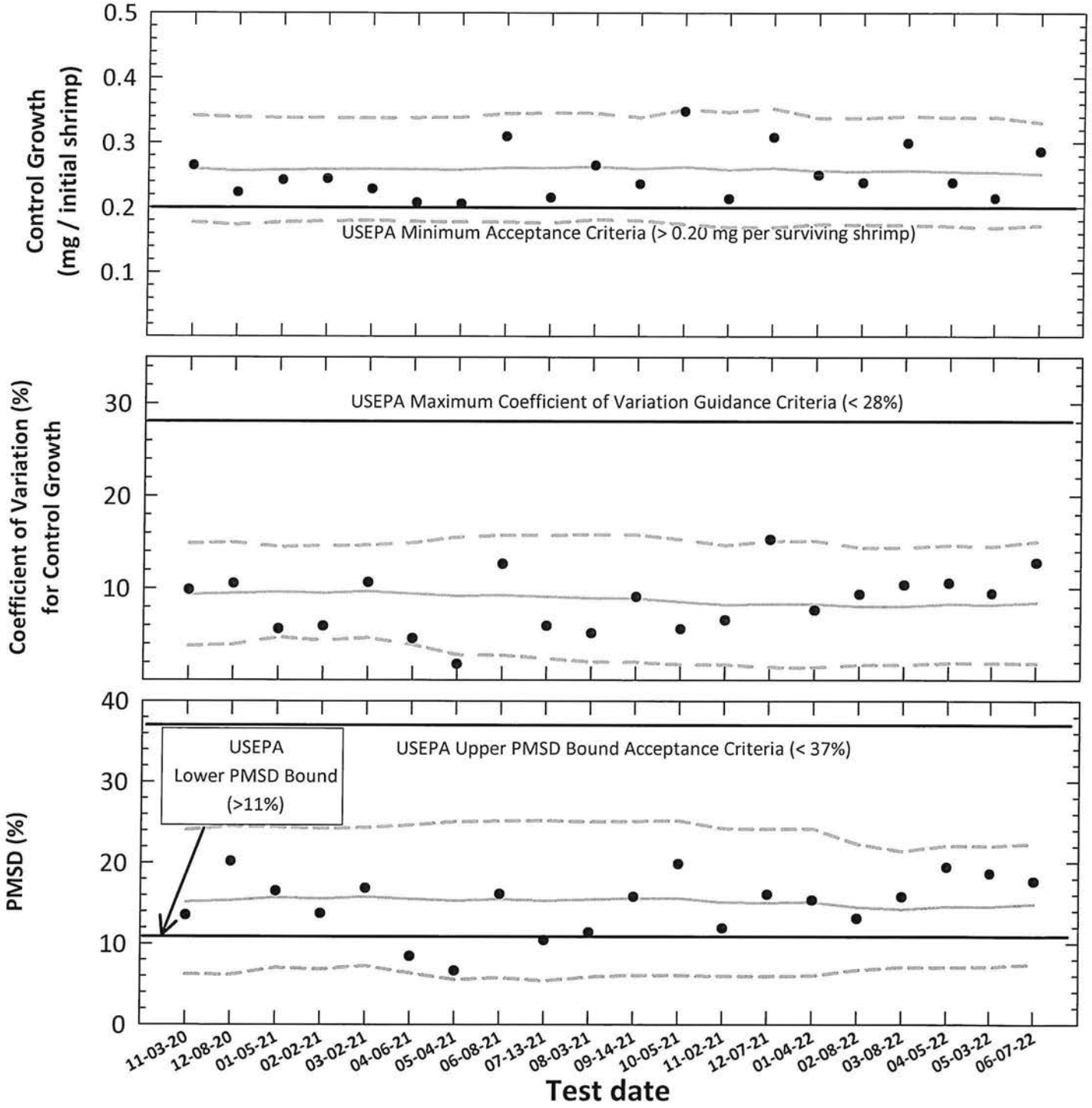
Entered and Reviewed by Jim Sumner

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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ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
			Mean	CV	MSD	PMSD	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
		(%)	(mg/initial shrimp)	(%)		(%)									
1	11-03-20	100	0.265	9.8	0.0358	13.5	0.260	0.177	0.342	9.3	3.8	14.9	15.1	6.2	24.1
2	12-08-20	100	0.223	10.5	0.0450	20.2	0.256	0.174	0.339	9.5	3.9	15.0	15.3	6.1	24.5
3	01-05-21	100	0.243	5.6	0.0400	16.5	0.258	0.178	0.338	9.6	4.7	14.5	15.7	7.0	24.4
4	02-02-21	100	0.244	5.9	0.0335	13.7	0.259	0.179	0.338	9.5	4.4	14.6	15.5	6.8	24.2
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: **228**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2101				
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water				
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	PURPLE
Date and times organisms were born between:	05-31-22 1200 to 06-01-22 1130	Incubator number and shelf location:	5E
Organism source:	AI Batch Ab: 06-01-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.83 S.U. Temperature = 25.1 °C	Date / Time in oven:	06-14-22 1155
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	06-15-22 1155
		*Final oven temperature:	60 °C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		Salt SW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	06-07-22	1100	JH	1230	JH	1207	JH	06-01-22 B
1	06-08-22	0500	JH	1100	JH	1032	JH	↓
2	06-09-22	0500	JH	1100	JH	1010	JH	06-06-22 B
3	06-10-22	0500	JH	1100	JH	1007	JH	↓
4	06-11-22	0530	JH	1215	JH	1025	JH	06-09-22 B
5	06-12-22	0500	JH	1100	JH	1020	JH	↓
6	06-13-22	0500	JH	1100	JH	1010	JH	06-11-22 A
7	06-14-22					1157	JH	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	511.7
Average weight per initial shrimp:	0.287		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.267	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	445.0

ETS Environmental Testing Solutions, Inc.

AbKClCR Test Number: **228**

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	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																0
# females with developing ova in oviducts																
# immature females	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
# males																0
*A = Pan weight (mg) Tray color code: TURQUOISE Analyst: TG Date: 05-24-22	12.78	14.47	14.77	12.87	15.23	14.99	14.99	15.12	13.84	14.49	13.66	15.13	14.64	15.06	12.21	15.77
*B = Pan + Shrimp weight (mg) Analyst: TG Date: 06-21-22	14.33	15.68	16.11	14.01	16.78	16.44	16.57	16.76	15.34	15.86	15.33	16.69	16.15	16.71 ~~16.73~~ TG 06-21-22	13.75	17.38
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JL	1.55	1.21	1.34	1.14	1.55	1.45	1.58	1.64	1.50	1.37	1.67	1.56	1.51	1.65	1.54	1.61
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JL	0.310	0.242	0.268	0.228	0.310	0.290	0.316	0.328	0.300	0.274	0.334	0.312	0.302	0.330	0.308	0.322
	Average weight per initial number of shrimp (mg) 0.287								Average weight per initial number of shrimp (mg) 0.310				Percent reduction from control (%) -8.37.			

*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: **228**

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	3[2d]	4[1d]	5	4[1d]	5	3[2d]	4[1d]	4[1d]
3	5	5	5	5	5	5	4[1d]	5	3	4	4[1d]	4	5	3	4	4
4	5	5	5	5	5	5	4	5	3	4	3[1d]	4	5	3	4	4
5	5	5	4[1d]	5	5	5	4	5	3	4	3	4	4[1d]	3	4	4
6	5	5	4	5	5	5	4	5	3	4	3	4	3[1d]	3	3[1d]	4
7	5	5	4	5	5	5	4	5	3	3[1d]	3	4	3	3	3	2[2d]
# females with eggs in brood sac																
# females with developing ova in oviducts																06
# immature females	5	5	4	5	5	5	4	5	3	3	3	4	3	3	3	2
# males																06
*A = Pan weight (mg) Tray color code: Turquoise Analyst: TG Date: 05-24-22	13.29	14.71	14.17	14.02	14.23	14.59	15.04	14.11 fg TG 05-24-22	14.22	13.88	14.91	13.98	13.79	14.58	15.69	14.80
*B = Pan + Shrimp weight (mg) Analyst: TG Date: 06-21-22	14.49	16.41	15.64	15.71	15.78	16.28	16.24	15.93	15.21	14.69	15.67	14.74	14.23	15.70	16.68	15.61
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JH	1.20	1.70	1.47	1.69	1.55	1.69	1.20	1.82	0.99	0.81	0.76	0.76	0.44	1.12	0.99	0.81
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JH	0.240	0.340	0.294	0.338	0.310	0.338	0.240	0.364	0.198	0.162	0.152	0.152	0.088	0.224	0.198	0.162
	Average weight per initial number of shrimp (mg) 0.308			Percent reduction from control (%) -7.57.					Average weight per initial number of shrimp (mg) 0.167				Percent reduction from control (%) 41.77.			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **228**

Survival and Growth Data

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

06-08-22

06-08-22

*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: 228

Test dates: June 07-14, 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	12.78	14.33	1.55	0.310	100.0	0.287	12.8	Not applicable
	B	5	5	14.47	15.68	1.21	0.242				
	C	5	5	14.77	16.11	1.34	0.268				
	D	5	5	12.87	14.01	1.14	0.228				
	E	5	5	15.23	16.78	1.55	0.310				
	F	5	5	14.99	16.44	1.45	0.290				
	G	5	5	14.99	16.57	1.58	0.316				
	H	5	5	15.12	16.76	1.64	0.328				
250	I	5	5	13.84	15.34	1.50	0.300	100.0	0.310	6.2	-8.3
	J	5	5	14.49	15.86	1.37	0.274				
	K	5	5	13.66	15.33	1.67	0.334				
	L	5	5	15.13	16.69	1.56	0.312				
	M	5	5	14.64	16.15	1.51	0.302				
	N	5	5	15.06	16.71	1.65	0.330				
	O	5	5	12.21	13.75	1.54	0.308				
	P	5	5	15.77	17.38	1.61	0.322				
375	Q	5	5	13.29	14.49	1.20	0.240	95.0	0.308	15.2	-7.5
	R	5	5	14.71	16.41	1.70	0.340				
	S	5	4	14.17	15.64	1.47	0.294				
	T	5	5	14.02	15.71	1.69	0.338				
	U	5	5	14.23	15.78	1.55	0.310				
	V	5	5	14.59	16.28	1.69	0.338				
	W	5	4	15.04	16.24	1.20	0.240				
	X	5	5	14.11	15.93	1.82	0.364				
500	Y	5	3	14.22	15.21	0.99	0.198	60.0	0.167	24.7	41.7
	Z	5	3	13.88	14.69	0.81	0.162				
	AA	5	3	14.91	15.67	0.76	0.152				
	BB	5	4	13.98	14.74	0.76	0.152				
	CC	5	3	13.79	14.23	0.44	0.088				
	DD	5	3	14.58	15.70	1.12	0.224				
	EE	5	3	15.69	16.68	0.99	0.198				
	FF	5	2	14.80	15.61	0.81	0.162				
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: 0.0506

PMSD: 17.7

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	6/7/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	6/14/2022	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	0.8000	1.0000	1.0000	1.0000	0.8000	1.0000
500	0.6000	0.6000	0.6000	0.8000	0.6000	0.6000	0.6000	0.4000
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9500	0.9500	1.2857	1.1071	1.3453	8.574	8	60.00	48.00	2	40
*500	0.6000	0.6000	0.8885	0.6847	1.1071	12.716	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

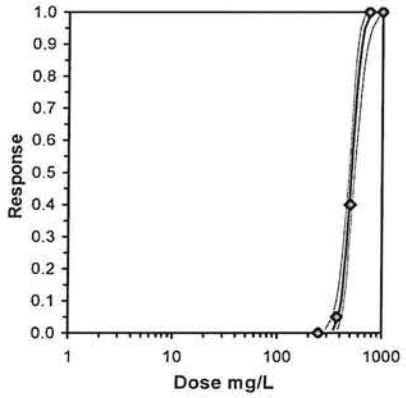
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.69936	0.904	-0.6643	4.25476
Equality of variance cannot be confirmed				

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

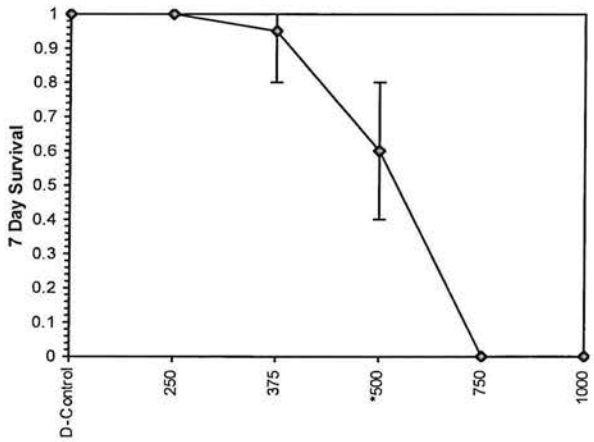
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.7501	2.23101	9.37737	18.1229	0	1.21881	7.81472	0.7485	2.709	0.07273	5
Intercept	-32.249	6.00655	-44.022	-20.476							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	346.589	290.732	382
EC05	3.355	388.488	341.848	418.795
EC10	3.718	412.857	371.941	440.707
EC15	3.964	430.156	393.174	456.777
EC20	4.158	444.421	410.418	470.53
EC25	4.326	457.035	425.334	483.201
EC40	4.747	490.431	462.5	519.871
EC50	5.000	511.685	483.999	545.957
EC60	5.253	533.86	504.734	575.355
EC75	5.674	572.87	538.123	631.344
EC80	5.842	589.129	551.242	655.919
EC85	6.036	608.666	566.575	686.213
EC90	6.282	634.169	586.051	726.852
EC95	6.645	673.949	615.524	792.365
EC99	7.326	755.422	673.558	933.413



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	6/7/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/14/2022	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	0.3100	0.2420	0.2680	0.2280	0.3100	0.2900	0.3160	0.3280
250	0.3000	0.2740	0.3340	0.3120	0.3020	0.3300	0.3080	0.3220
375	0.2400	0.3400	0.2940	0.3380	0.3100	0.3380	0.2400	0.3640
500	0.1980	0.1620	0.1520	0.1520	0.0880	0.2240	0.1980	0.1620
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

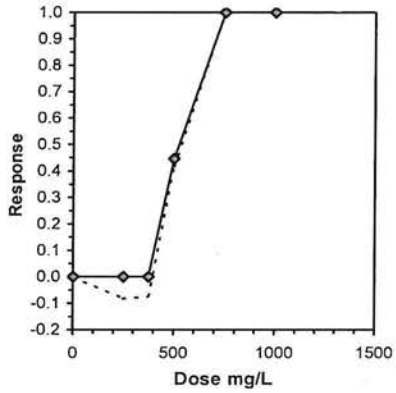
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2865	1.0000	0.2865	0.2280	0.3280	12.827	8				0.3016	1.0000
250	0.3103	1.0829	0.3103	0.2740	0.3340	6.197	8	-1.314	2.799	0.0506	0.3016	1.0000
375	0.3080	1.0750	0.3080	0.2400	0.3640	15.233	8	-1.189	2.799	0.0506	0.3016	1.0000
500	0.1670	0.5829	0.1670	0.0880	0.2240	24.702	8				0.1670	0.5537
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)	0.9323	0.884	-0.6526	-0.358
Bartlett's Test indicates equal variances (p = 0.10)	4.66795	9.21035		

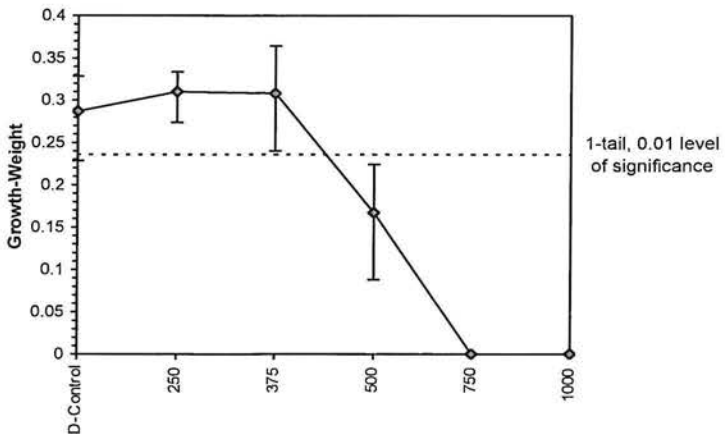
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	>375			0.05059	0.17658	0.00138	0.00131	0.36691	2, 21

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	389.01	19.16	320.59	392.24	-4.5191
IC10	403.01	9.70	379.30	409.48	-4.4644
IC15	417.02	7.71	395.85	426.71	-1.4527
IC20	431.02	8.08	412.76	443.95	-0.1134
IC25	445.03	8.95	429.65	461.53	0.6722
IC40	487.04	12.83	467.50	517.52	0.9607
IC50	524.26	17.41	491.62	556.26	0.1665



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **228**

Daily Chemistry:

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

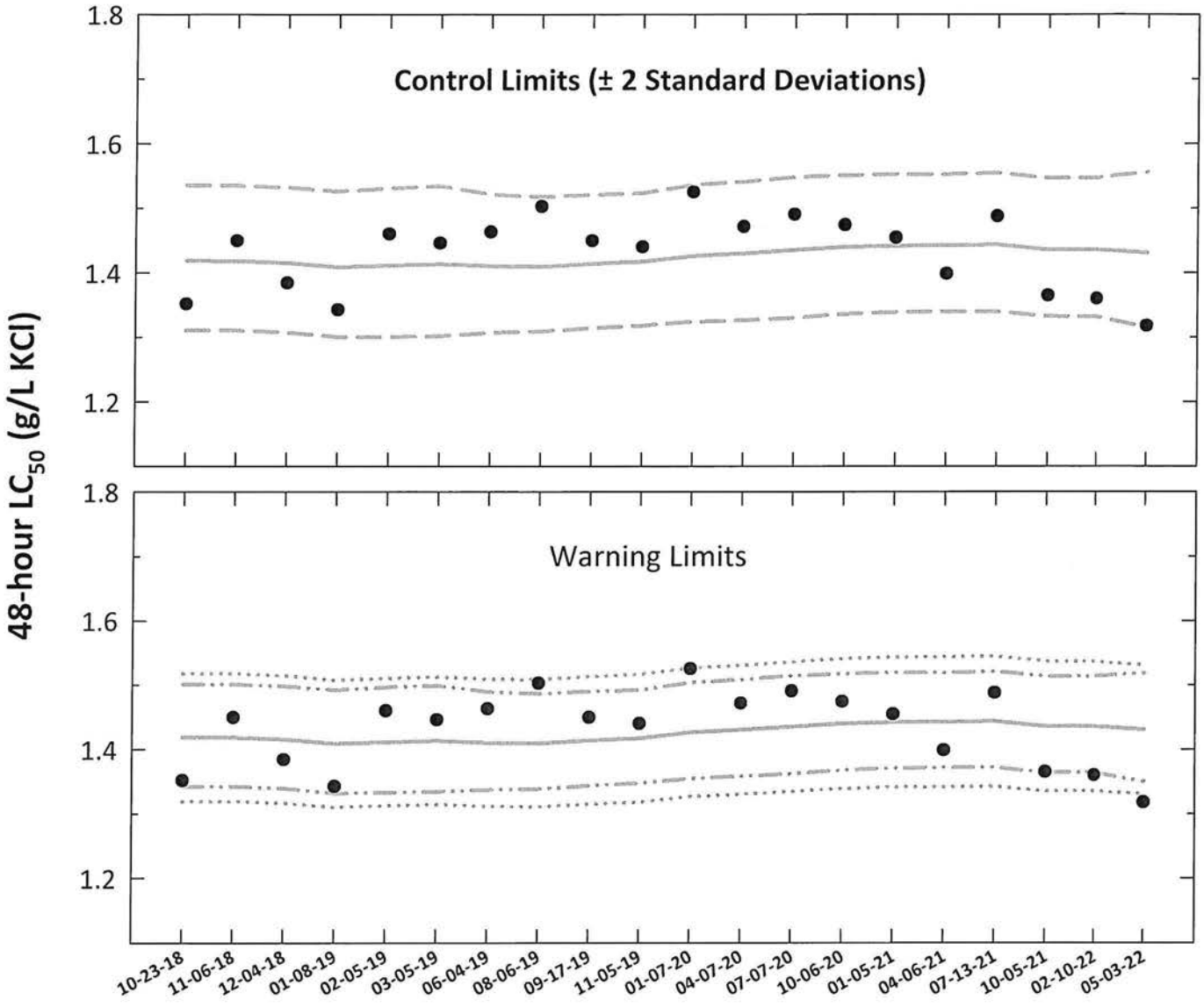
Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	N	JW	JW	A.r N	A.r N	N
CONTROL, Salt SW	pH (S.U.)	8.00	7.75	7.94	~~7.79~~ (7.81)	~~7.84~~ (7.79)	7.72
	DO (mg/L)	7.6	7.7	7.7	7.5	7.7	7.7
	Salinity (ppt)	24.9	25.2	25.0	25.1	24.8	25.1
	Alkalinity (mg $CaCO_3$/L)	96				92	
	Temperature (°C)	25.5	25.9	25.7	26.1	25.7	25.6
250 mg KCl/L	pH (S.U.)	7.95	7.78	7.84	7.81	7.84	7.72
	DO (mg/L)	7.6	7.1	7.8	7.6	7.8	7.6
	Salinity (ppt)	25.0	25.4	24.8	25.1	24.8	25.1
	Temperature (°C)	25.6	25.9	25.7	26.0	25.8	25.5
375 mg KCl/L	pH (S.U.)	7.94	7.76	7.88	7.83	7.87	7.73
	DO (mg/L)	7.6	7.5	7.6	7.5	7.9	7.6
	Salinity (ppt)	24.9	25.4	25.0	25.3	25.1	25.3
	Temperature (°C)	25.7	26.2	25.6	26.0	25.8	25.8
500 mg KCl/L	pH (S.U.)	7.94	7.77	7.88	7.83	7.87	7.74
	DO (mg/L)	7.6	7.4	7.8	7.5	8.0	7.7
	Salinity (ppt)	25.0	25.5	25.0	25.4	25.2	25.4
	Temperature (°C)	25.7	26.0	25.6	26.2	25.9	25.9
750 mg KCl/L	pH (S.U.)	7.94	7.76				
	DO (mg/L)	7.6	7.4				
	Salinity (ppt)	25.3	25.7				
	Temperature (°C)	25.6	26.2				
1000 mg KCl/L	pH (S.U.)	7.93	7.76				
	DO (mg/L)	7.7	7.5			06.08.22	
	Salinity (ppt)	25.4	25.8				
	Temperature (°C)	25.7	26.2				
		Initial	Final	Initial	Final	Initial	Final

AbKClCR Test Number: **228**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	M	BSL	BSL	BSL	BSL	JW	JW	M
CONTROL, Salt SW	pH (S.U.)	7.82	7.79	7.92	7.78	7.97	7.84	7.92	7.52
	DO (mg/L)	7.8	7.5	7.7	7.5	7.8	7.5	7.7	6.4
	Salinity (ppt)	24.9	25.2	24.8	25.1	24.7	24.9	24.8	25.0
	Alkalinity (mg $CaCO_3$/L)	~~06-22 M~~		94		~~06-22 M~~		100	
	Temperature (°C)	25.7	26.1	25.8	25.9	25.7	26.3	25.8	26.2
250 mg KCl/L	pH (S.U.)	7.82	7.80	7.90	7.78	7.90	7.83	7.91	7.59
	DO (mg/L)	7.7	7.6	7.8	7.5	7.7	7.5	7.7	7.0
	Salinity (ppt)	25.0	25.3	25.1	25.2	25.2	25.2	25.1	25.2
	Temperature (°C)	25.8	26.2	25.7	26.2	25.6	26.4	25.8	26.4
375 mg KCl/L	pH (S.U.)	7.83	7.83	7.90	7.77	7.90	7.84	7.92	7.59
	DO (mg/L)	7.7	7.7	7.8	7.5	7.7	7.5	7.8	7.3
	Salinity (ppt)	25.0	25.3	25.3	25.4	25.1	25.3	25.2	25.4
	Temperature (°C)	25.8	26.3	25.7	26.3	25.6	26.4	25.9	26.4
500 mg KCl/L	pH (S.U.)	7.83	7.81	7.90	7.79	7.90	7.84	7.92	7.66
	DO (mg/L)	7.7	7.7	8.0	7.5	7.7	7.5	7.8	7.3
	Salinity (ppt)	25.1	25.6	25.4	25.5	25.2	25.4	25.4	25.6
	Temperature (°C)	25.8	26.3	25.7	26.3	25.6	26.3	25.9	26.5
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)						M 06-09-22		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Menidia beryllina
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

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	10-23-18	1.3518	0.1309	0.1519	0.0172	1.4187	1.3107	1.5357	1.3426	1.5012	1.3194	1.5180
2	11-06-18	1.4497	0.1613	0.1518	0.0172	1.4185	1.3107	1.5352	1.3425	1.5008	1.3192	1.5178
3	12-04-18	1.3844	0.1412	0.1509	0.0172	1.4155	1.3076	1.5322	1.3393	1.4980	1.3164	1.5146
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 # 84

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 #: 2078

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	OW	JW	VL
Control, SaltSW	pH (S.U.)	8.23	8.07	8.04
	Dissolved oxygen (mg/L)	8.0	7.8	7.6
	*Salinity (ppt)	24.7	25.2	25.1
	*Alkalinity (mg/L $CaCO_3$)	140		
	*Temperature (°C)	24.8	24.7	24.9
1000 mg/L	pH (S.U.)	8.26	8.10	8.03
	Dissolved oxygen (mg/L)	8.0	8.0	7.7
	*Salinity (ppt)	25.2	25.3	25.3
	*Temperature (°C)	24.9	24.8	24.9
1250 mg/L	pH (S.U.)	8.26	8.09	8.03
	Dissolved oxygen (mg/L)	8.1	8.0	7.4
	*Salinity (ppt)	25.4	25.6	25.4
	*Temperature (°C)	24.9	24.7	25.0
1500 mg/L	pH (S.U.)	8.26	8.09	8.03
	Dissolved oxygen (mg/L)	8.1	8.0	7.4
	*Salinity (ppt)	25.6	25.8	25.6
	*Temperature (°C)	24.7	24.7	25.0
1750 mg/L	pH (S.U.)	8.27	8.08	
	Dissolved oxygen (mg/L)	8.1	8.0	
	*Salinity (ppt)	25.7	25.8	
	*Temperature (°C)	24.9	24.9	05.04.22
2000 mg/L	pH (S.U.)	8.27	8.07	
	Dissolved oxygen (mg/L)	8.1	8.0	
	*Salinity (ppt)	25.8	26.0	
	*Temperature (°C)	24.9	24.8	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13060165

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 # 84

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	05-03-22	* 1120	JL	1320	JL	1E	Yellow	04-27-22B
24	05-04-22			1324	JL			
48 Termination	05-05-22			1324	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 DAYS OLD
Age (9 to 14 days old):	AI MB 04-14-22
Date organisms were born: (time organisms were born between is not provided by supplier)	04-23-22 1200 TO 04-24-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.07
	Temperature (°C) 24.9

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

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

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1247.8
Upper 95% confidence limit (mg KCl/L)	1384.0
48-hour LC_{50} (mg KCl/L)	1316.0

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date:	5/3/2022	Test ID:	MbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/5/2022	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	0.7000	0.7000
1500	0.3000	0.3000
1750	0.0000	0.0000
2000	0.0000	0.0000

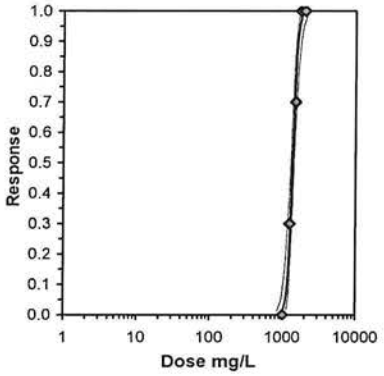
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*1250	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	42.086	2.850	0.0285	6	20
*1500	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	2	83.238	2.850	0.0285	14	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	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		0.00967	0.00991	0.59205	0.0001	2.3E-09	4, 5
Treatments vs D-Control										

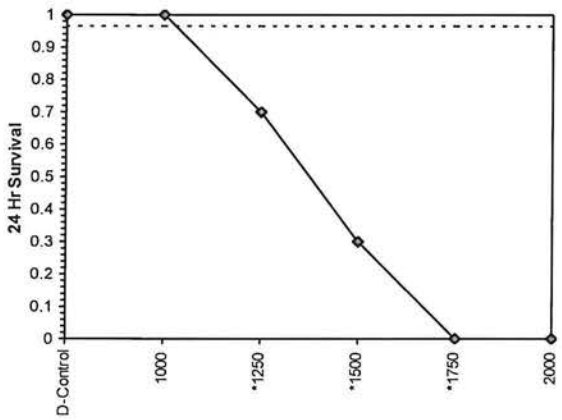
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.4087	3.37457	11.7946	25.0229	0	1.66235	7.81472	0.64534	3.13449	0.05432	5
Intercept	-52.702	10.608	-73.494	-31.91							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1018.87	847.195	1117.58
EC05	3.355	1109.53	964.385	1194.06
EC10	3.718	1161.11	1032.26	1238.26
EC15	3.964	1197.26	1079.97	1269.89
EC20	4.158	1226.79	1118.8	1296.36
EC25	4.326	1252.71	1152.58	1320.25
EC40	4.747	1320.46	1238.18	1387.02
EC50	5.000	1362.98	1288.51	1433.45
EC60	5.253	1406.86	1336.75	1486.02
EC75	5.674	1482.96	1411.37	1588.44
EC80	5.842	1514.29	1439.34	1634.17
EC85	6.036	1551.64	1471.17	1690.83
EC90	6.282	1599.95	1510.46	1766.98
EC95	6.645	1674.33	1568.08	1889.27
EC99	7.326	1823.31	1677.18	2148.31



Dose-Response Plot



1-tail, 0.05 level of significance

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date:	5/3/2022	Test ID:	MbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/5/2022	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	0.7000	0.6000
1500	0.2000	0.1000
1750	0.0000	0.0000
2000	0.0000	0.0000

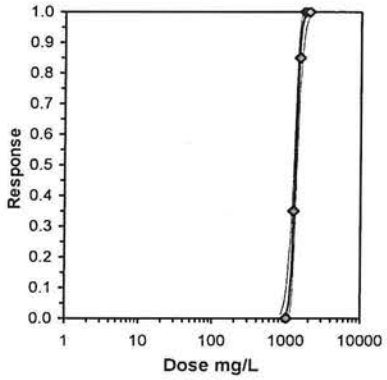
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1591	0	20
*1250	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	8.478	2.850	0.1591	7	20
*1500	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	18.256	2.850	0.1591	17	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	22.445	2.850	0.1591	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	1000	1250	1118.03		0.07271	0.07457	0.66283	0.00312	9.1E-06	4, 5
Treatments vs D-Control										

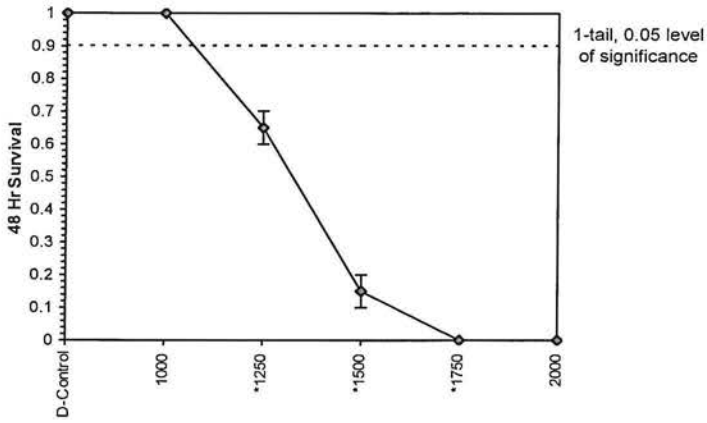
Maximum Likelihood-Probit

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

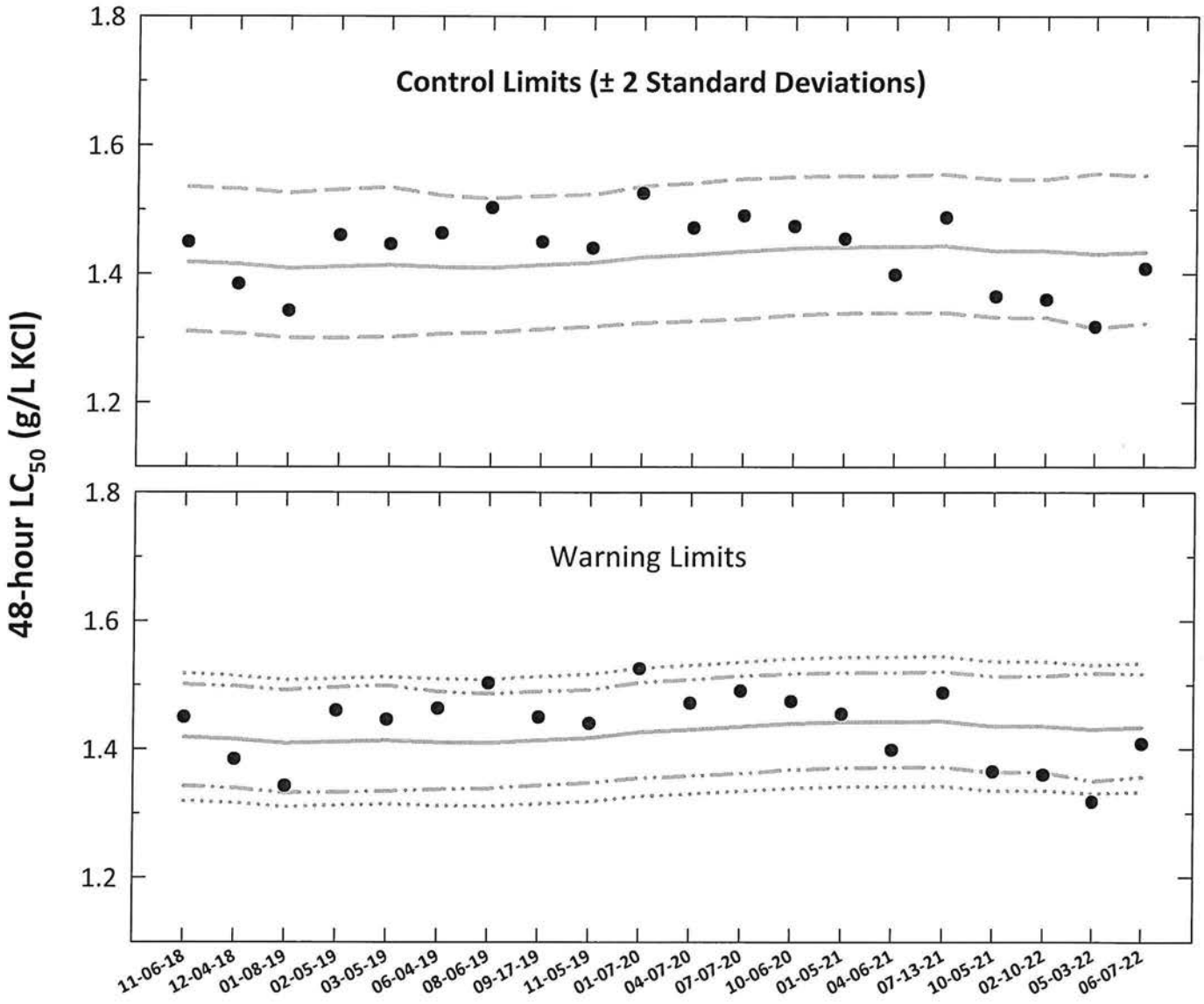
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1017.62	844.477	1110.41
EC05	3.355	1097.73	953.149	1176.54
EC10	3.718	1142.98	1015.64	1214.63
EC15	3.964	1174.56	1059.37	1241.88
EC20	4.158	1200.28	1094.82	1264.72
EC25	4.326	1222.8	1125.56	1285.35
EC40	4.747	1281.42	1202.86	1343.35
EC50	5.000	1318.03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



Dose-Response Plot



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 $LC_{50} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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	11-06-18	1.4497	0.1613	0.1518	0.0172	1.4185	1.3107	1.5352	1.3425	1.5008	1.3192	1.5178
2	12-04-18	1.3844	0.1412	0.1509	0.0172	1.4155	1.3076	1.5322	1.3393	1.4980	1.3164	1.5146
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 Kim 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 # 85

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 #: 2101

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	V	JW	A.S V
Control, SaltSW	pH (S.U.)	8.00	7.81	7.83
	Dissolved oxygen (mg/L)	7.6	7.6	7.3
	*Salinity (ppt)	24.9	25.0	24.9
	*Alkalinity (mg/L $CaCO_3$)	96		
	*Temperature (°C)	24.9	24.7	25.0
1000 mg/L	pH (S.U.)	8.00	7.80	7.83
	Dissolved oxygen (mg/L)	7.9	7.8	7.4
	*Salinity (ppt)	25.1	25.0	25.8
	*Temperature (°C)	25.0	24.9	24.7
1250 mg/L	pH (S.U.)	8.00	7.80	7.83
	Dissolved oxygen (mg/L)	7.9	7.8	7.4
	*Salinity (ppt)	25.1	25.2	26.0
	*Temperature (°C)	25.0	24.9	24.7
1500 mg/L	pH (S.U.)	8.02	7.79	7.83
	Dissolved oxygen (mg/L)	8.0	7.8	7.5
	*Salinity (ppt)	25.5	25.6	26.1
	*Temperature (°C)	25.1	25.0	24.9
1750 mg/L	pH (S.U.)	8.02	7.79	7.84
	Dissolved oxygen (mg/L)	8.0	7.8	7.6
	*Salinity (ppt)	25.5	25.7	26.5
	*Temperature (°C)	25.1	24.8	24.9
2000 mg/L	pH (S.U.)	8.03	7.78	
	Dissolved oxygen (mg/L)	8.0	8.0	
	*Salinity (ppt)	25.6	25.9	
	*Temperature (°C)	25.0	24.9	

06-04-22

*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	13066465

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 # 85

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-07-22	* 1100	JK	1310	JK	7A	PINK	06-01-22B
24	06-08-22			1302	JK			
48 Termination	06-09-22			1310	JK			

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

Test Organism Information:

Organism Source: ~~AI~~ JK	Aquatic Indicators, Inc.
Batch (AI Batch ~~#~~): MB	05-29-22
Age (9 to 14 days old):	10 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	05-28-22 1200 TO 05-29-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.77
	Temperature (°C) 25.0

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

Hours	Control		1000 mg/L		1250 mg/L		1500 mg/L		1750 mg/L		2000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	3d 7	2d 8	9d 1	9d 1	10d 0	10d 0
48 Termination	10	10	10	10	3d 7	2d 8	3d 4	3d 5	1d 0	1d 0	0	0
Mean Survival	100%		100%		75%		45%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1331.7
Upper 95% confidence limit (mg KCl/L)	1480.7
48-hour LC_{50} (mg KCl/L)	1408.1

Comments:

Test Reviewed by: JK

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date:	6/7/2022	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
1000	1.0000	1.0000
1250	1.0000	1.0000
1500	0.7000	0.8000
1750	0.1000	0.1000
2000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
1000	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045	0	20
1250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045	0	20
*1500	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	9.893	2.850	0.1045	5	20
*1750	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	29.724	2.850	0.1045	18	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

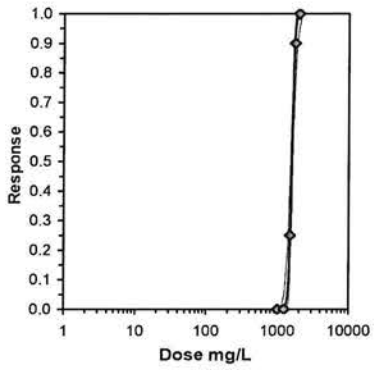
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	1500	1369.31		0.04275	0.04384	0.44902	0.00135	3.0E-06	4, 5
Treatments vs D-Control										

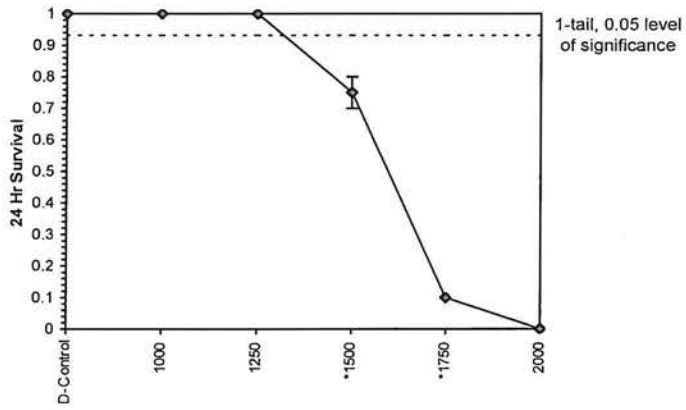
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	30.0201	6.53877	17.2041	42.8361	0	0.05592	7.81472	0.99654	3.19924	0.03331	3
Intercept	-91.042	20.9367	-132.08	-50.006							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1323.58	1144.99	1409.46
EC05	3.355	1394.61	1250.92	1466.04
EC10	3.718	1434.02	1310.21	1498.42
EC15	3.964	1461.23	1350.99	1521.56
EC20	4.158	1483.23	1383.62	1540.99
EC25	4.326	1502.37	1411.55	1558.6
EC40	4.747	1551.69	1480.19	1608.51
EC50	5.000	1582.14	1518.84	1643.88
EC60	5.253	1613.18	1554.54	1684.31
EC75	5.674	1666.14	1607.34	1762.89
EC80	5.842	1687.64	1626.53	1797.58
EC85	6.036	1713.04	1648.04	1840.16
EC90	6.282	1745.55	1674.17	1896.66
EC95	6.645	1794.88	1711.81	1985.79
EC99	7.326	1891.2	1781.19	2168.7



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date:	6/7/2022	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2022	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	0.7000	0.8000
1500	0.4000	0.5000
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	0.1384	0	20
*1250	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	7.471	2.850	0.1384	5	20
*1500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	13.938	2.850	0.1384	11	20
*1750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	25.803	2.850	0.1384	20	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

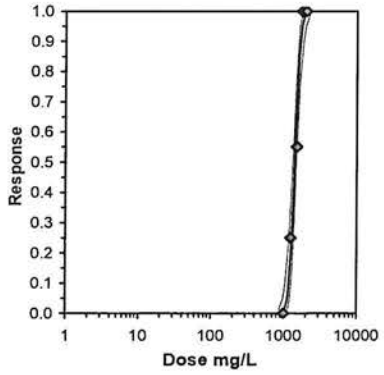
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.06076	0.06232	0.55446	0.00236	7.1E-06	4, 5
Treatments vs D-Control										

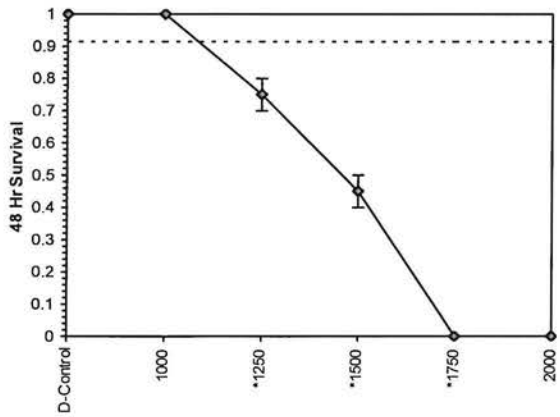
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.8354	3.17587	11.6107	24.0601	0	3.5883	7.81472	0.30949	3.14863	0.05607	6
Intercept	-51.157	10.0288	-70.814	-31.501							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1042.78	869.244	1144.65
EC05	3.355	1138.68	991.576	1226.05
EC10	3.718	1193.36	1062.56	1273.12
EC15	3.964	1231.73	1112.52	1306.82
EC20	4.158	1263.1	1153.22	1335.02
EC25	4.326	1290.65	1188.66	1360.45
EC40	4.747	1362.77	1278.63	1431.44
EC50	5.000	1408.08	1331.68	1480.69
EC60	5.253	1454.89	1382.68	1536.36
EC75	5.674	1536.18	1461.94	1644.6
EC80	5.842	1569.69	1491.79	1692.87
EC85	6.036	1609.67	1525.83	1752.68
EC90	6.282	1661.42	1567.95	1833.05
EC95	6.645	1741.2	1629.89	1962.17
EC99	7.326	1901.34	1747.63	2235.97



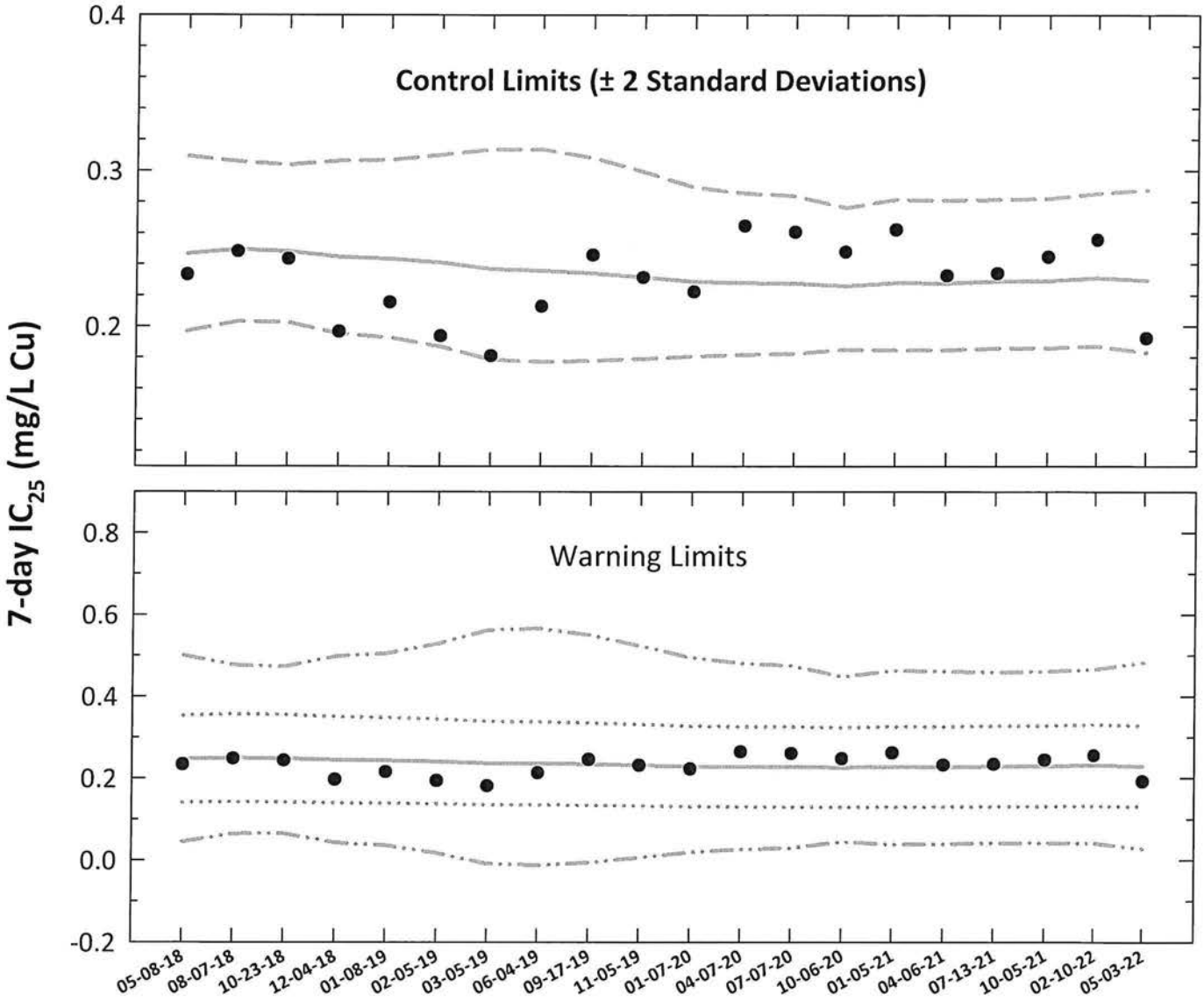
Dose-Response Plot



Reviewed and Reviewed by Jim Sumner

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 $IC_{25} \pm S_{A.75}$ 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	05-08-18	0.2333	-0.6321	-0.6077	0.0491	0.2468	0.1969	0.3093	0.0446	0.5002	0.1407	0.3529
2	08-07-18	0.2482	-0.6052	-0.6032	0.0444	0.2494	0.2032	0.3060	0.0643	0.4765	0.1421	0.3566
3	10-23-18	0.2434	-0.6137	-0.6052	0.0439	0.2482	0.2027	0.3039	0.0650	0.4725	0.1415	0.3549
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

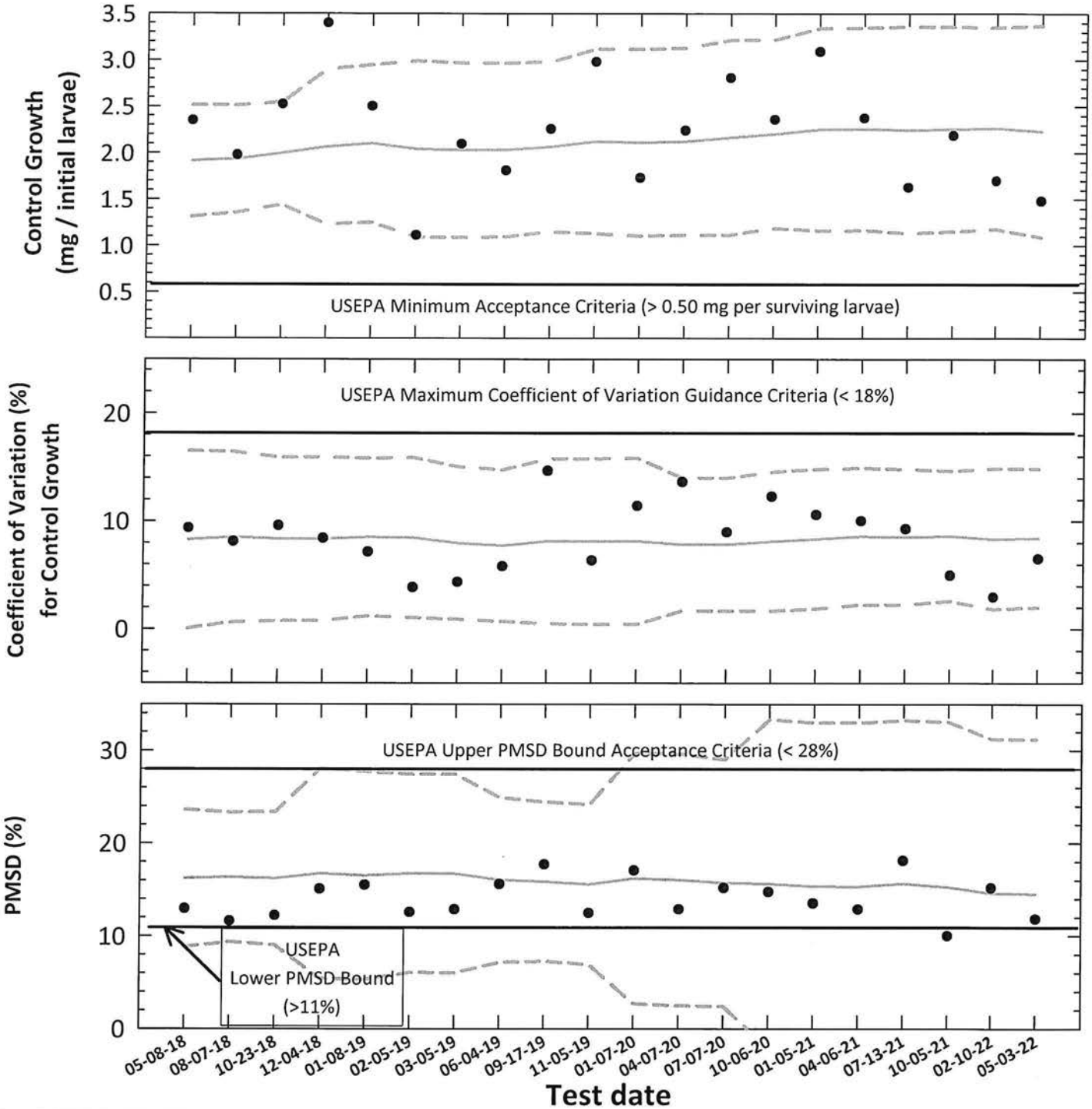
Entered and Reviewed by Jim Sumner

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										
			Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(mg/initial larvae)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	05-08-18	100	2.349	9.4	0.3041	12.9	1.9140	1.313	2.515	8.3	0.1	16.5	16.2	8.8	23.6
2	08-07-18	97.5	1.978	8.1	0.2300	11.6	1.935	1.358	2.512	8.5	0.6	16.4	16.3	9.4	23.3
3	10-23-18	100	2.526	9.6	0.3085	12.2	1.996	1.443	2.550	8.3	0.7	15.9	16.2	9.0	23.4
4	12-04-18	100	3.399	8.4	0.5125	15.1	2.067	1.230	2.903	8.3	0.8	15.9	16.7	5.4	28.1
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

Environmental Testing Solutions, Inc.

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

Species: *Menidia beryllina*

MbCuCR Test Number: **137**

Dilution preparation information:						***Comments:***
Cu Stock INSS number:		INSS 2153				
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 04-24-22
Hatch dates and times:	04-23-22 1200 to 04-24-22 1130
Transfer vessel information:	pH (S.U.) = 8.07 Temperature (°C) = 24.9
Average transfer volume (mL):	< 0.25 mL

Test information:

Randomizing template:	ORANGE
Incubator number and shelf location:	1C
Artemia CHM number:	CHM1149
Drying information for weight determination:	
Date / Time in oven:	05-10-22 1200
*Initial oven temperature:	60 °C
Date / Time out of oven:	05-11-22 1200
*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	05-03-22	1120	JK	1310	JK	1307	JK		04-27-22B
1	05-04-22	0500	JK	1115	JK	1108	JK		↓
2	05-05-22	0500	JK	1245	JK	1110	JK		05-05-22
3	05-06-22	0500	JK	1115	JK	1108	JK	05-05-22 JK	↓
4	05-07-22	0600	JK	1200	JK	1158	JK		05-04-22
5	05-08-22	0500	JK	1110	JK	1107	JK		↓
6	05-09-22	0500	JK	1110	JK	1107	JK		05-05-22
7	05-10-22					1155	JK		

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria
% Mortality:	07.	≤ 20%
Average weight per initial larvae:	1.483	
Average weight per surviving larvae:	1.483	≥ 0.25mg/larvae

Summary of test endpoints:	
7-day LC_{50} (%)	0.282
NOEC (%)	0.1
LOEC (%)	0.2
ChV (%)	0.141
IC_{25} (%)	0.193

ETS
Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina***

MbCuCR Test Number: **137**

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: Peach Analyst: A.S Date: 04-27-22	14.18	14.32	13.93	13.45	13.20	13.18	13.35	04-27-22 A.S ~~15.18~~ 15.19	13.78	14.02	14.49	14.71
*B = Pan + Larvae weight (mg) Analyst: TG Date: 05-17-22	28.11	28.43	29.28	29.38	28.25	29.73	30.29	31.25	32.69	32.55	30.67	33.20
C = Larvae weight (mg) = B – A Analyst:	13.93	14.11	15.35	15.93	15.05	16.55	16.94	16.06	18.91	18.53	16.18	18.49
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst:	1.393	1.411	1.535	1.593	1.505	1.655	1.694	1.606	1.891	1.853	1.618	1.849
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	1.483				1.615		-8.9%		1.803		-21.6%	

*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: **137**

Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	9 id	9 id	3 id	4 id	2 id	4 id
2	10	10	10	10	10	10	9	9	3	3 id	2	3 id
3	10	10	10	10	10	10	9	9	3	3	2	3
4	10	10	10	10	10	10	9	9	2 id	2 id	2	3
5	10	10	10	10	9 id	10	9	9	1 id	2	2	3
6	10	10	10	10	9	8 id	9	8 id	1	2	2	2 id
7	10	10	10	10	8 id	8	8 id	8	1	1 id	1 id	0 2d
*A = Pan weight (mg) Tray color code: Peach Analyst: A.S Date: 04-27-22	14.61	14.46	14.31	13.14	13.52	14.54	15.34	15.19	14.03	14.89	14.36	15.67
*B = Pan + Larvae weight (mg) Analyst: TG Date: 05-17-22	~~32.46~~ 46.11	~~28.43~~	~~29.28~~	~~29.38~~	~~28.25~~	~~29.73~~	46.29 ~~30.29~~	46.99 ~~31.25~~	15.98 ~~32.69~~	17.76 ~~32.55~~	15.78 ~~30.67~~	~~15.67~~ 31.26 ~~33.26~~
	32.18	30.18	31.40	28.00	24.95	26.05						
C = Larvae weight (mg) = B – A Analyst: JH	17.57	15.72	17.09	14.86	11.43	11.51	10.95	13.80	1.95	2.87	1.42	—
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	1.757	1.572	1.709	1.486	1.143	1.151	1.095	1.380	0.195	0.287	0.142	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	1.631		-10.07.		1.192		19.67.		0.156		89.57.	

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

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

Comments:

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: 137

							Not for Compliance Assessment, Internal Laboratory QC							
Concentration (mg/L Cu)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.18	28.11	13.93	1.393	1.483	6.5	1.393	100.0	1.483	6.5	Not applicable
	B	10	10	14.32	28.43	14.11	1.411			1.411				
	C	10	10	13.93	29.28	15.35	1.535			1.535				
	D	10	10	13.45	29.38	15.93	1.593			1.593				
0.025	E	10	10	13.20	28.25	15.05	1.505	1.615	5.1	1.505	100.0	1.615	5.1	-8.9
	F	10	10	13.18	29.73	16.55	1.655			1.655				
	G	10	10	13.35	30.29	16.94	1.694			1.694				
	H	10	10	15.19	31.25	16.06	1.606			1.606				
0.050	I	10	10	13.78	32.69	18.91	1.891	1.803	6.9	1.891	100.0	1.803	6.9	-21.6
	J	10	10	14.02	32.55	18.53	1.853			1.853				
	K	10	10	14.49	30.67	16.18	1.618			1.618				
	L	10	10	14.71	33.20	18.49	1.849			1.849				
0.100	M	10	10	14.61	32.18	17.57	1.757	1.631	7.6	1.757	100.0	1.631	7.6	-10.0
	N	10	10	14.46	30.18	15.72	1.572			1.572				
	O	10	10	14.31	31.40	17.09	1.709			1.709				
	P	10	10	13.14	28.00	14.86	1.486			1.486				
0.200	Q	10	8	13.52	24.95	11.43	1.429	1.490	10.7	1.143	80.0	1.192	10.7	19.6
	R	10	8	14.54	26.05	11.51	1.439			1.151				
	S	10	8	15.34	26.29	10.95	1.369			1.095				
	T	10	8	15.19	28.99	13.80	1.725			1.380				
0.500	U	10	1	14.03	15.98	1.95	1.950	2.080	35.3	0.195	7.5	0.156	76.9	89.5
	V	10	1	14.89	17.76	2.87	2.870			0.287				
	W	10	1	14.36	15.78	1.42	1.420			0.142				
	X	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1756
PMSD: 11.8

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	5/3/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	5/10/2022	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	0.8000	0.8000	0.8000	0.8000
0.5	0.1000	0.1000	0.1000	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	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	4	10.00	10.00	8	40
*0.5	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.46508	0.884	-3.0206	13.9892
Equality of variance cannot be confirmed				

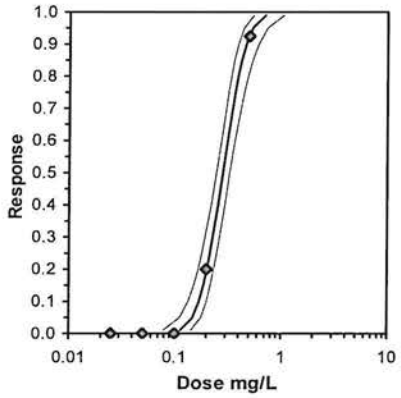
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.1	0.2	0.14142	
Treatments vs D-Control				

Maximum Likelihood-Probit

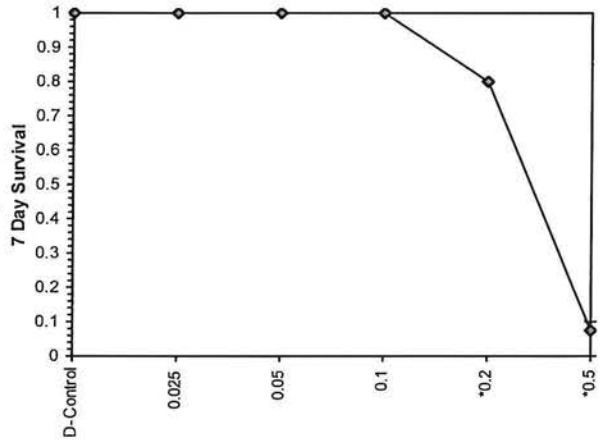
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.91457	0.85149	4.24566	7.58349	0	0.20076	7.81472	0.97747	-0.5496	0.16907	3
Intercept	8.25069	0.51826	7.2349	9.26647							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	0.11404	0.07863	0.14266
EC05	3.355	0.14869	0.11232	0.17776
EC10	3.718	0.17128	0.13528	0.20069
EC15	3.964	0.18843	0.15296	0.2184
EC20	4.158	0.20328	0.16828	0.23408
EC25	4.326	0.21695	0.1823	0.24889
EC40	4.747	0.2556	0.22079	0.29346
EC50	5.000	0.28209	0.24574	0.32668
EC60	5.253	0.31134	0.2718	0.36596
EC75	5.674	0.3668	0.31763	0.44718
EC80	5.842	0.39146	0.33685	0.48571
EC85	6.036	0.42231	0.36014	0.5357
EC90	6.282	0.46459	0.39098	0.60714
EC95	6.645	0.53517	0.44036	0.73302
EC99	7.326	0.69778	0.54733	1.04967



Dose-Response Plot



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ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	5/3/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	5/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

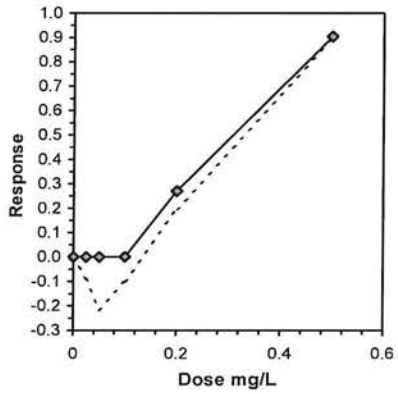
Conc-mg/L	1	2	3	4
D-Control	1.3930	1.4110	1.5350	1.5930
0.025	1.5050	1.6550	1.6940	1.6060
0.05	1.8910	1.8530	1.6180	1.8490
0.1	1.7570	1.5720	1.7090	1.4860
0.2	1.1430	1.1510	1.0950	1.3800
0.5	0.1950	0.2870	0.1420	0.0000

			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	1.4830	1.0000	1.4830	1.3930	1.5930	6.525	4				1.6336	1.0000
0.025	1.6150	1.0890	1.6150	1.5050	1.6940	5.058	4	-1.721	2.290	0.1756	1.6336	1.0000
0.05	1.8028	1.2156	1.8028	1.6180	1.8910	6.912	4	-4.169	2.290	0.1756	1.6336	1.0000
0.1	1.6310	1.0998	1.6310	1.4860	1.7570	7.631	4	-1.930	2.290	0.1756	1.6310	0.9984
0.2	1.1923	0.8039	1.1923	1.0950	1.3800	10.701	4				1.1923	0.7298
0.5	0.1560	0.1052	0.1560	0.0000	0.2870	76.936	4				0.1560	0.0955

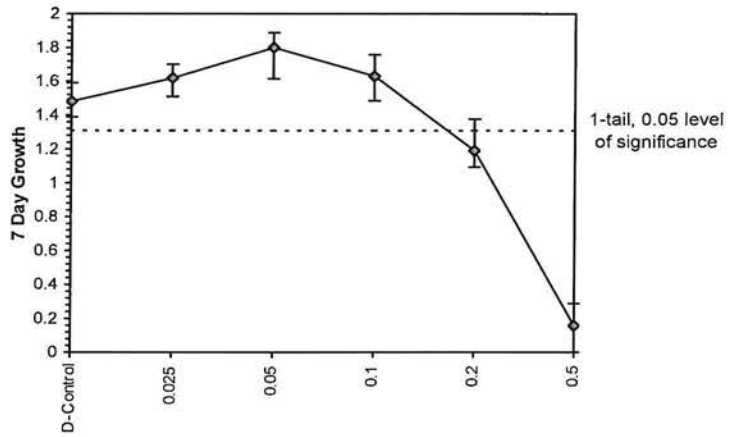
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.9194		0.844		-0.5489	-0.993
Bartlett's Test indicates equal variances (p = 0.89)					0.63813		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs D-Control	0.1	>0.1			0.17563	0.11843	0.06886	0.01176	0.01059	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1180	0.0097	0.0620	0.1267	-1.6489
IC10	0.1366	0.0091	0.0975	0.1532	-0.5330
IC15	0.1553	0.0099	0.1229	0.1810	0.6183
IC20	0.1739	0.0112	0.1436	0.2085	0.8825
IC25	0.1925	0.0127	0.1641	0.2326	0.7636
IC40	0.2614	0.0133	0.2198	0.2964	0.2107
IC50	0.3087	0.0118	0.2738	0.3431	0.1369



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina***

MbCuCR Test Number: **137**

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	JW	JW	JW	K	K	K
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	8.23	8.09	8.07	8.00	8.01	7.93
	Dissolved oxygen (mg/L)	8.0	8.0	7.8	7.3	7.6	7.6
	Salinity (ppt)	24.7	25.3	24.7	25.0	24.7	25.0
	Alkalinity (mg $CaCO_3$/L)	140		→ 05-24-22 K		140	
	Temperature (°C)	24.9	24.9	24.8	24.7	24.7	24.6
0.025 mg/L	pH (S.U.)	8.27	8.09	8.11	8.00	8.12	7.95
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	7.4	8.0	7.9
	Salinity (ppt)	24.8	25.3	24.8	25.1	24.7	25.0
	Temperature (°C)	24.9	24.7	24.9	24.7	24.7	24.7
0.05 mg/L	pH (S.U.)	8.28	8.09	8.12	8.00	8.13	7.94
	Dissolved oxygen (mg/L)	8.0	7.6	7.8	7.4	7.9	7.9
	Salinity (ppt)	24.8	25.1	24.7	25.0	24.7	25.1
	Temperature (°C)	25.0	24.7	25.0	24.8	24.7	24.7
0.1 mg/L	pH (S.U.)	8.28	8.10	8.14	7.99	8.13	7.93
	Dissolved oxygen (mg/L)	8.0	7.7	7.9	7.5	7.8	7.8
	Salinity (ppt)	24.8	24.8	24.6	25.0	24.7	25.1
	Temperature (°C)	25.0	25.0	25.0	24.8	24.7	24.7
0.2 mg/L	pH (S.U.)	8.28	8.09	8.14	7.99	8.14	7.93
	Dissolved oxygen (mg/L)	8.0	7.6	8.0	7.4	7.8	7.8
	Salinity (ppt)	24.8	25.1	24.5	24.9	24.6	25.1
	Temperature (°C)	25.0	24.8	25.0	24.6	24.8	24.9
0.5 mg/L	pH (S.U.)	8.28	8.08	8.14	8.03	8.14	7.95
	Dissolved oxygen (mg/L)	8.0	7.6	8.1	7.4	8.0	7.8
	Salinity (ppt)	24.7	25.1	24.5	24.9	24.6	25.1
	Alkalinity (mg $CaCO_3$/L)	→ 05-23-22 K					
	Temperature (°C)	24.9	24.8	25.0	24.9	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **137**

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
	Analyst	M	BSL	BSL	BSL	BSL	M	M	M
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	8.01	8.00	8.08	8.01	7.98	7.84	7.93	7.76
	Dissolved oxygen (mg/L)	8.0	7.9	7.8	8.0	7.7	7.5	7.9	6.8
	Salinity (ppt)	25.0	25.0	24.7	24.9	24.8	25.3	24.7	25.2
	Alkalinity (mg $CaCO_3$/L)		35.44.22 M	140			35.44.22 M	110	
	Temperature (°C)	24.8	25.0	24.8	24.9	24.7	24.7	24.9	25.0
0.025 mg/L	pH (S.U.)	8.04	7.99	8.11	8.00	8.13	7.87	8.06	7.76
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	8.0	7.9	7.4	8.0	6.9
	Salinity (ppt)	24.8	25.1	24.6	25.1	24.7	25.3	25.2	25.3
	Temperature (°C)	24.9	25.2	24.7	25.0	24.7	24.9	24.9	25.1
0.05 mg/L	pH (S.U.)	8.07	7.97	8.11	8.00	8.14	7.87	8.06	7.79
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	8.0	8.0	7.4	8.0	6.8
	Salinity (ppt)	24.6	25.0	24.5	25.0	24.7	25.2	24.9	25.4
	Temperature (°C)	24.9	25.2	24.8	25.0	24.8	24.9	25.0	25.1
0.1 mg/L	pH (S.U.)	8.08	7.98	8.11	8.00	8.14	7.88	8.07	7.79
	Dissolved oxygen (mg/L)	8.0	8.0	8.1	8.1	8.0	7.5	8.0	6.8
	Salinity (ppt)	24.6	25.0	24.5	25.0	24.6	25.3	25.0	25.5
	Temperature (°C)	25.0	25.0	24.7	24.9	24.8	24.8	25.0	24.9
0.2 mg/L	pH (S.U.)	8.08	7.95	8.12	8.01	8.14	7.91	8.07	7.83
	Dissolved oxygen (mg/L)	8.0	8.1	8.1	8.1	8.0	7.7	8.1	6.7
	Salinity (ppt)	24.5	25.0	24.6	25.0	24.6	25.2	25.0	25.5
	Temperature (°C)	24.9	24.9	24.7	24.9	24.8	24.7	24.9	25.2
0.5 mg/L	pH (S.U.)	8.07	7.98	8.11	8.00	8.14	7.93	8.07	7.80
	Dissolved oxygen (mg/L)	8.1	8.1	8.1	8.1	8.1	7.9	8.2	6.7
	Salinity (ppt)	24.5	25.0	24.5	25.1	24.6	25.2	24.9	25.5
	Temperature (°C)	24.9	24.9	24.7	24.9	24.8	24.9	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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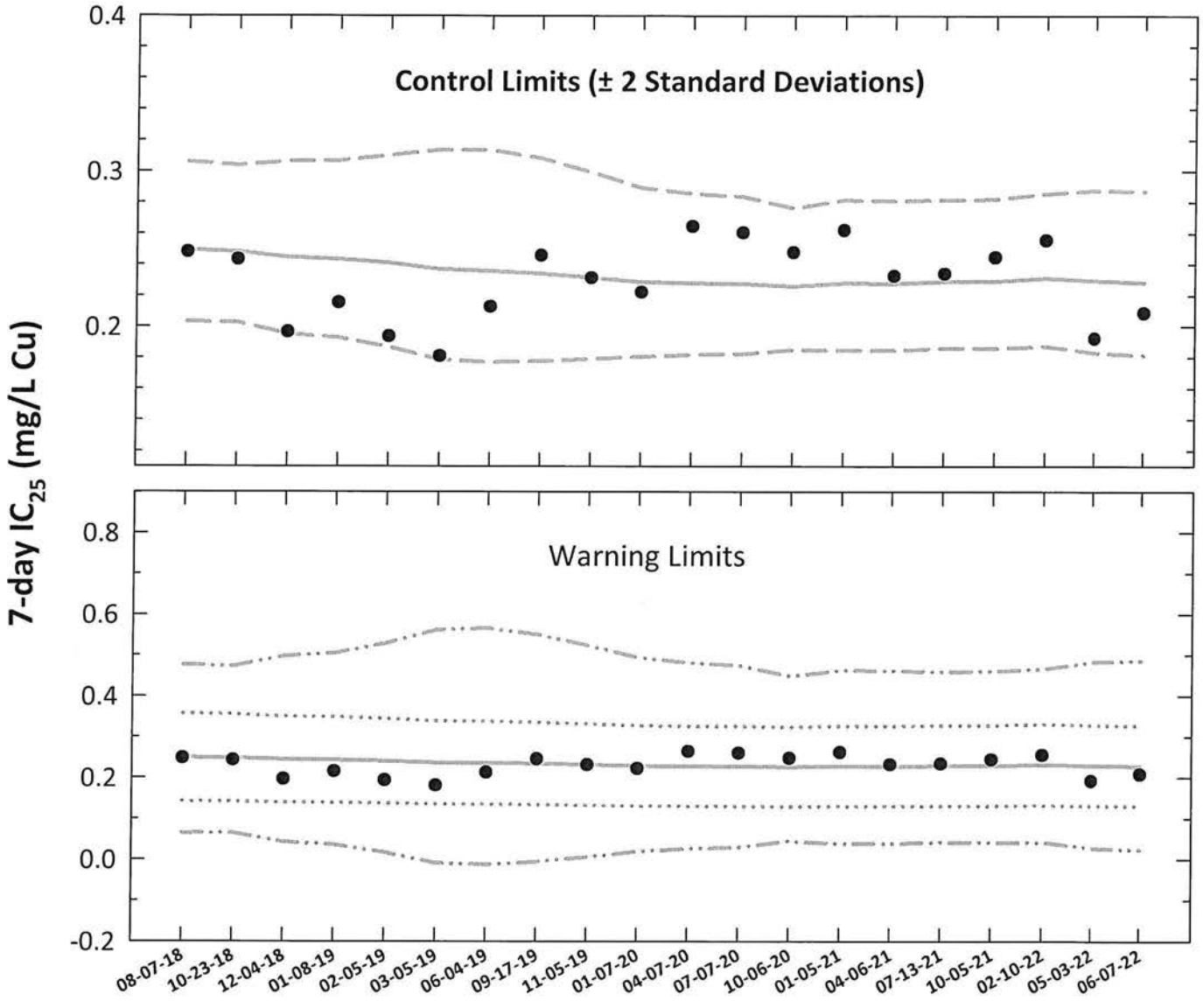
Menidia beryllina
Chronic Reference Toxicant Control Chart
Source: Aquatic Indicators, Inc.



Test date

- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the copper concentration which would cause a 25% reduction in *Menidia* growth (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.75}$ 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	08-07-18	0.2482	-0.6052	-0.6032	0.0444	0.2494	0.2032	0.3060	0.0643	0.4765	0.1421	0.3566
2	10-23-18	0.2434	-0.6137	-0.6052	0.0439	0.2482	0.2027	0.3039	0.0650	0.4725	0.1415	0.3549
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

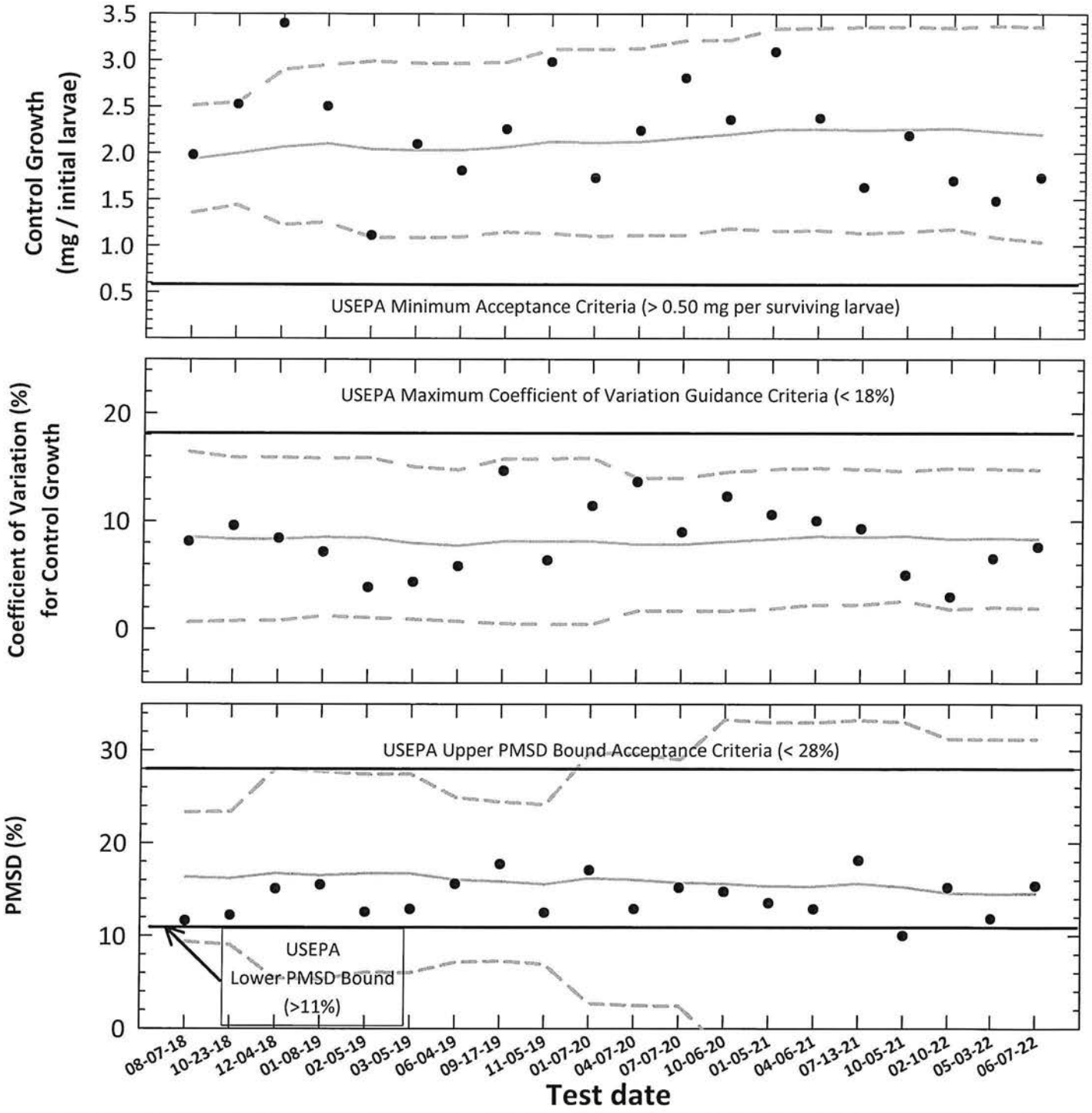
Entered and Reviewed by Jim Summey

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										
			Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(mg/initial larvae)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	08-07-18	97.5	1.978	8.1	0.2300	11.6	1.9352	1.358	2.512	8.5	0.6	16.4	16.3	9.4	23.3
2	10-23-18	100	2.526	9.6	0.3085	12.2	1.996	1.443	2.550	8.3	0.7	15.9	16.2	9.0	23.4
3	12-04-18	100	3.399	8.4	0.5125	15.1	2.067	1.230	2.903	8.3	0.8	15.9	16.7	5.4	28.1
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: **138**

Dilution preparation information:						***Comments:***
Cu Stock INSS number:		INSS 2106				
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 05-29-22
Hatch dates and times:	05-28-22 1200 to 05-29-22 1130
Transfer vessel information:	pH (S.U.) = 7.77 Temperature (°C) = 25.0
Average transfer volume (mL):	< 0.25 mL

Test information:

Randomizing template:	PINK
Incubator number and shelf location:	1B
Artemia CHM number:	CHM1149
Drying information for weight determination:	
Date / Time in oven:	06-14-22 1155
*Initial oven temperature:	60 °C
Date / Time out of oven:	06-15-22 1155
*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	06-07-22	1100	JH	1230	JH	1227	JH		01-01-22B
1	06-08-22	0500	JH	1100	JH	1054	JH		↓
2	06-09-22	0500	JH	1100	JH	1030	JH		06-06-22B
3	06-10-22	0500	JH	1100	JH	1030	JH		↓
4	06-11-22	0530	JH	1215	JH	1035	JH	06-09-22 JH	06-09-22B
5	06-12-22	0500	JH	1100	JH	1030	JH		↓
6	06-13-22	0500	JH	1100	JH	1030	JH		06-01-22A
7	06-14-22					1148	JH		

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	1.733	
Average weight per surviving larvae:	1.733	≥ 0.25mg/larvae

Summary of test endpoints:	
7-day LC_{50} (%)	0.249
NOEC (%)	0.1
LOEC (%)	0.2
ChV (%)	0.141
IC_{25} (%)	0.209

ETS
Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **138**

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: Red Analyst: TG Date: 05-24-22	14.65	15.29	15.38	15.76	13.51	14.53	14.15	13.52	13.50	14.09	15.14	14.44
*B = Pan + Larvae weight (mg) Analyst: TG Date: 06-22-22	32.94	33.08	33.23	31.14	32.15	36.00	31.29	31.14	29.36	32.85	34.06	29.27
C = Larvae weight (mg) = B – A Analyst: JH	18.29	17.79	17.85	15.38	18.64	21.47	17.14	17.62	15.86	18.76	18.92	14.83
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	1.829	1.779	1.785	1.538	1.864	2.147	1.714	1.762	1.586	1.876	1.892	1.483
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	1.733				1.872		-8.07		1.709		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:

ETS
Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina***

MbCuCR Test Number: **138**

Survival and Growth Data

Day	0.1 mg/L				0.2 mg/L				0.5 mg/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	4 6d	5 5d	6 4d	5 5d
2	10	10	10	10	10	9 1d	9 1d	9 1d	3 1d	5	5 1d	4 1d
3	10	10	10	10	10	9	9	9	3	2 3d	3 2d	3 1d
4	10	10	10	10	10	9	9	9	1 2d	1 1d	1 2d	0 3d
5	10	10	10	10	10	9	9	9	1	1	0 1d	0
6	10	10	10	10	9 1d	8	9	9	1	0 1d	0	0
7	10	10	10	10	8 1d	7 1d	7 2d	7 2d	1	0	0	0
*A = Pan weight (mg) Tray color code: Red Analyst: TG Date: 05-24-22	16.44	15.51	14.70	13.07	15.59	14.75	15.84	14.15	15.48	14.49	15.88	14.33
*B = Pan + Larvae weight (mg) Analyst: TG Date: 06-22-22	35.28	35.84	33.84	34.15	34.08	26.49	28.40	27.73	16.42	~~14.51~~	~~15.90~~	~~14.35~~
C = Larvae weight (mg) = B – A Analyst: JH	18.84	20.33	19.14	21.08	18.49	11.74	12.56	13.58	0.94			06-28-22 JH
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	1.884	2.033	1.914	2.108	1.849	1.174	1.256	1.358	0.094	0	0	0

Average weight per initial number of larvae (mg)	Percent reduction from control (%)							
1.985	-14.57.	1.409	18.77.	0.024	98.67.
---	---	---	---	---	---

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 138

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)	Not for Compliance Assessment, Internal Laboratory QC: Mean weight/ Surviving number of larvae (mg)	Not for Compliance Assessment, Internal Laboratory QC: 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	14.65	32.94	18.29	1.829	1.733	7.6	1.829	100.0	1.733	7.6	Not applicable
	B	10	10	15.29	33.08	17.79	1.779			1.779				
	C	10	10	15.38	33.23	17.85	1.785			1.785				
	D	10	10	15.76	31.14	15.38	1.538			1.538				
0.025	E	10	10	13.51	32.15	18.64	1.864	1.872	10.4	1.864	100.0	1.872	10.4	-8.0
	F	10	10	14.53	36.00	21.47	2.147			2.147				
	G	10	10	14.15	31.29	17.14	1.714			1.714				
	H	10	10	13.52	31.14	17.62	1.762			1.762				
0.050	I	10	10	13.50	29.36	15.86	1.586	1.709	12.1	1.586	100.0	1.709	12.1	1.4
	J	10	10	14.09	32.85	18.76	1.876			1.876				
	K	10	10	15.14	34.06	18.92	1.892			1.892				
	L	10	10	14.44	29.27	14.83	1.483			1.483				
0.100	M	10	10	16.44	35.28	18.84	1.884	1.985	5.3	1.884	100.0	1.985	5.3	-14.5
	N	10	10	15.51	35.84	20.33	2.033			2.033				
	O	10	10	14.70	33.84	19.14	1.914			1.914				
	P	10	10	13.07	34.15	21.08	2.108			2.108				
0.200	Q	10	8	15.59	34.08	18.49	2.311	1.931	14.3	1.849	72.5	1.409	21.5	18.7
	R	10	7	14.75	26.49	11.74	1.677			1.174				
	S	10	7	15.84	28.40	12.56	1.794			1.256				
	T	10	7	14.15	27.73	13.58	1.940			1.358				
0.500	U	10	1	15.48	16.42	0.94	0.940	0.940	0.0	0.094	2.5	0.024	200.0	98.6
	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: 0.2665
PMSD: 15.4

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) = 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

Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	6/7/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	6/14/2022	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	0.8000	0.7000	0.7000	0.7000
0.5	0.1000	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	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	40
*0.5	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

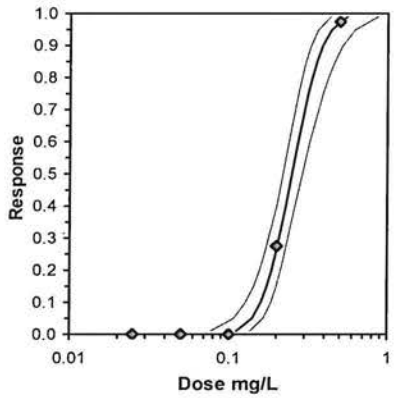
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.64424	0.884	2.22239	6.21159
Equality of variance cannot be confirmed				

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				

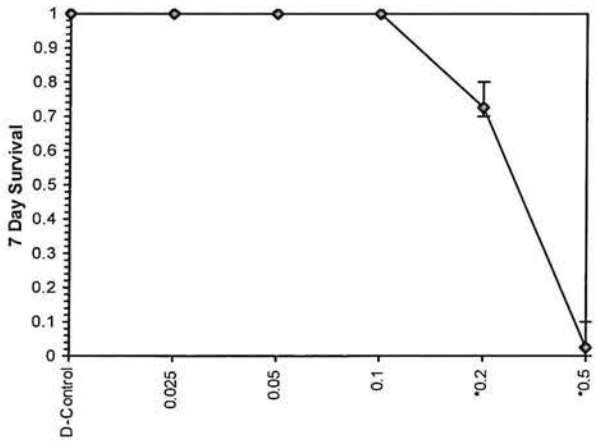
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.69229	1.09416	4.54774	8.83684	0	0.2199	7.81472	0.97431	-0.604	0.14943	3
Intercept	9.04211	0.72903	7.61321	10.471							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	0.11179	0.07742	0.13723
EC05	3.355	0.14133	0.10788	0.16609
EC10	3.718	0.16014	0.12816	0.18473
EC15	3.964	0.17423	0.1435	0.1991
EC20	4.158	0.18631	0.15659	0.21187
EC25	4.326	0.19734	0.16837	0.22399
EC40	4.747	0.22811	0.19968	0.26087
EC50	5.000	0.24889	0.21918	0.28862
EC60	5.253	0.27156	0.23901	0.32143
EC75	5.674	0.3139	0.27298	0.38871
EC80	5.842	0.33248	0.28698	0.42031
EC85	6.036	0.35553	0.3038	0.46103
EC90	6.282	0.38681	0.32585	0.51872
EC95	6.645	0.43832	0.36068	0.6192
EC99	7.326	0.55414	0.43443	0.86697



Dose-Response Plot



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ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	6/7/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	6/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

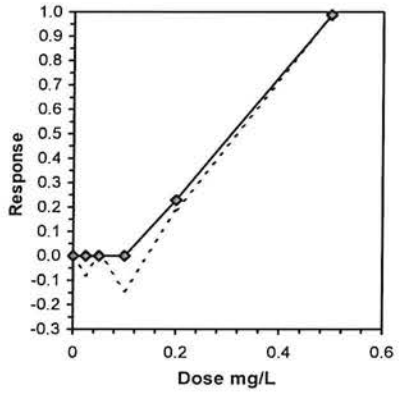
Conc-mg/L	1	2	3	4
D-Control	1.8290	1.7790	1.7850	1.5380
0.025	1.8640	2.1470	1.7140	1.7620
0.05	1.5860	1.8760	1.8920	1.4830
0.1	1.8840	2.0330	1.9140	2.1080
0.2	1.8490	1.1740	1.2560	1.3580
0.5	0.0940	0.0000	0.0000	0.0000

			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	1.7328	1.0000	1.7328	1.5380	1.8290	7.603	4				1.8246	1.0000
0.025	1.8718	1.0802	1.8718	1.7140	2.1470	10.358	4	-1.194	2.290	0.2665	1.8246	1.0000
0.05	1.7093	0.9864	1.7093	1.4830	1.8920	12.065	4	0.202	2.290	0.2665	1.8246	1.0000
0.1	1.9848	1.1454	1.9848	1.8840	2.1080	5.258	4	-2.165	2.290	0.2665	1.8246	1.0000
0.2	1.4093	0.8133	1.4093	1.1740	1.8490	21.478	4				1.4093	0.7724
0.5	0.0235	0.0136	0.0235	0.0000	0.0940	200.000	4				0.0235	0.0129

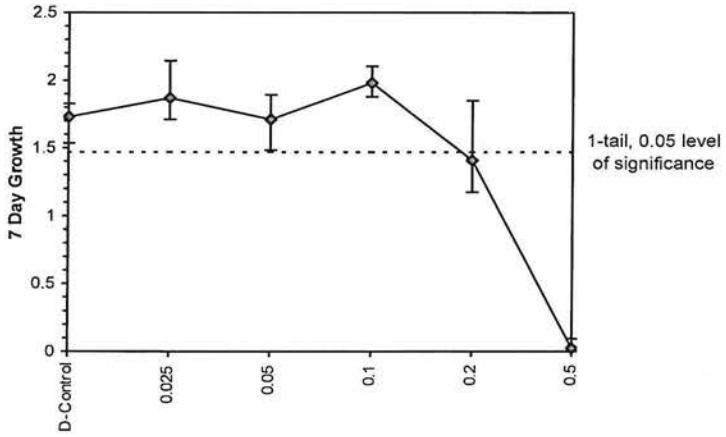
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.96698		0.844		0.17306	-0.9202
Bartlett's Test indicates equal variances (p = 0.68)					1.52259		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	0.1	>0.1			0.26651	0.15381	0.06615	0.02709	0.1146	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1220	0.0189	0.0046	0.1721	-0.8944
IC10	0.1439	0.0217	0.1068	0.2438	1.4777
IC15	0.1659	0.0238	0.1235	0.2572	0.9692
IC20	0.1879	0.0242	0.1374	0.2711	0.6284
IC25	0.2088	0.0244	0.1486	0.2850	0.4071
IC40	0.2681	0.0223	0.2009	0.3288	0.0613
IC50	0.3076	0.0186	0.2514	0.3580	0.0652



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **138**

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	K	JW	JW	A.S K	A.S K	K
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	8.00	7.82	7.94	7.85	7.79	7.81
	Dissolved oxygen (mg/L)	7.6	7.6	7.7	7.8	7.7	7.8
	Salinity (ppt)	24.9	25.1	25.0	25.2	24.8	25.3
	Alkalinity (mg $CaCO_3$/L)	96		←		92	
	Temperature (°C)	24.9	24.7	24.7	24.5	24.8	24.7
0.025 mg/L	pH (S.U.)	7.95	7.81	7.89	7.86	7.88	7.80
	Dissolved oxygen (mg/L)	8.0	7.2	7.6	7.7	8.0	7.9
	Salinity (ppt)	24.9	25.0	25.2	25.2	25.1	25.4
	Temperature (°C)	25.0	25.0	24.7	24.5	24.9	24.7
0.05 mg/L	pH (S.U.)	7.95	7.81	7.91	7.87	7.89	7.80
	Dissolved oxygen (mg/L)	7.9	7.2	7.6	7.7	7.9	7.9
	Salinity (ppt)	24.8	25.0	25.0	25.2	25.1	25.4
	Temperature (°C)	25.0	25.0	24.8	24.7	24.9	24.8
0.1 mg/L	pH (S.U.)	7.95	7.80	7.91	7.87	7.90	7.79
	Dissolved oxygen (mg/L)	7.9	7.4	7.7	7.7	7.9	8.0
	Salinity (ppt)	24.9	25.2	25.0	25.3	25.0	25.4
	Temperature (°C)	24.9	24.8	24.8	24.6	24.9	24.8
0.2 mg/L	pH (S.U.)	7.95	7.74	7.92	7.87	7.90	7.79
	Dissolved oxygen (mg/L)	8.0	6.9	7.7	7.8	8.0	8.1
	Salinity (ppt)	24.8	25.4	25.0	25.2	25.0	25.3
	Temperature (°C)	24.9	24.8	24.9	24.6	24.8	24.6
0.5 mg/L	pH (S.U.)	7.96	7.78	7.92	7.84	7.90	7.78
	Dissolved oxygen (mg/L)	8.1	7.3	7.9	7.8	8.0	8.0
	Salinity (ppt)	24.9	25.5	24.9	25.3	24.9	25.5
	Alkalinity (mg $CaCO_3$/L)						
	Temperature (°C)	24.9	24.8	24.9	24.6	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina***

MbCuCR Test Number: **138**

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
	Analyst	VL	BSL	BSL	BSL	BSL	JW	JW	VL
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.82	7.85	7.92	7.83	7.97	7.94	7.92	7.73
	Dissolved oxygen (mg/L)	7.0	7.9	7.7	7.6	7.8	7.6	7.7	7.5
	Salinity (ppt)	24.9	25.2	24.8	25.2	24.7	25.1	24.8	24.7
	Alkalinity (mg $CaCO_3$/L)			94					
	Temperature (°C)	24.7	24.8	24.8	24.6	24.8	24.7	24.8	24.6
0.025 mg/L	pH (S.U.)	7.91	7.84	7.95	7.82	7.93	7.91	7.99	7.73
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.6	7.8	7.7	7.8	7.7
	Salinity (ppt)	25.1	25.3	24.9	25.2	24.9	25.3	24.8	25.2
	Temperature (°C)	24.7	24.5	24.8	24.7	24.9	24.9	24.8	24.9
0.05 mg/L	pH (S.U.)	7.90	7.82	7.95	7.82	7.94	7.90	7.99	7.74
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.7	7.8	7.6	7.7	7.8
	Salinity (ppt)	25.1	25.4	24.8	25.2	24.9	25.4	24.7	25.1
	Temperature (°C)	24.8	24.7	24.9	24.7	25.0	24.9	24.9	24.9
0.1 mg/L	pH (S.U.)	7.89	7.83	7.95	7.82	7.94	7.90	7.99	7.73
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.7	7.8	7.5	7.7	7.8
	Salinity (ppt)	25.1	25.4	24.8	25.2	24.9	25.4	24.8	25.1
	Temperature (°C)	24.8	24.7	24.9	24.7	25.0	24.6	24.9	24.7
0.2 mg/L	pH (S.U.)	7.89	7.82	7.95	7.81	7.93	7.89	7.99	7.73
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.4	7.8	7.6	7.7	7.8
	Salinity (ppt)	25.1	25.4	24.7	25.3	24.8	25.3	24.7	25.2
	Temperature (°C)	24.7	24.7	24.9	24.7	25.0	24.9	24.9	24.7
0.5 mg/L	pH (S.U.)	7.89	7.82	7.96	7.82	7.93	7.88	7.98	7.77
	Dissolved oxygen (mg/L)	8.1	7.9	8.1	7.3	7.9	7.6	7.8	7.8
	Salinity (ppt)	25.0	25.4	24.7	25.5	24.8	25.3	25.0	25.5
	Temperature (°C)	24.8	24.6	24.8	24.8	25.0	25.0	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final