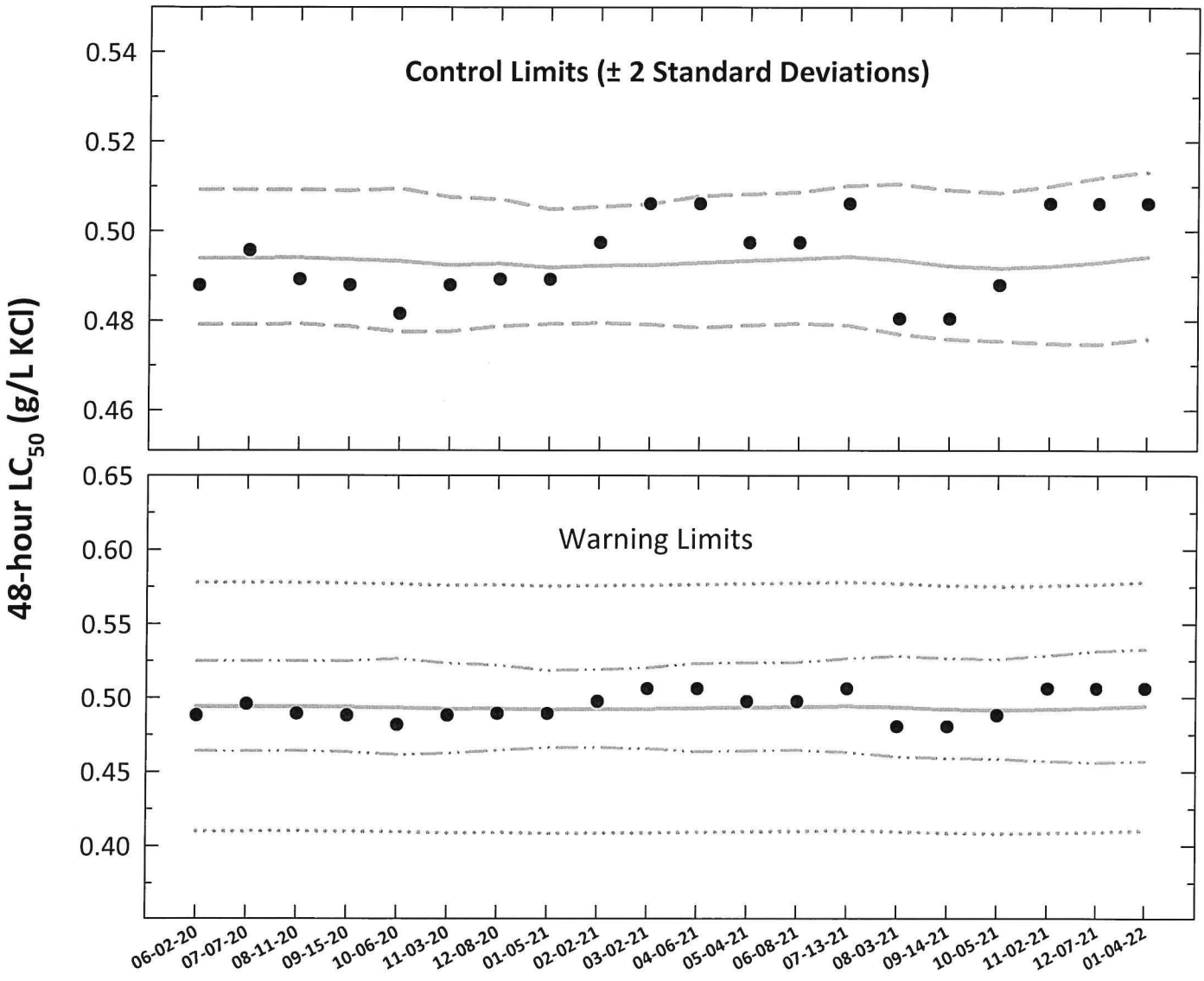
ETS
Environmental Testing Solutions, Inc.

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	06-02-20	0.4879	-0.3117	-0.3063	0.0066	0.4940	0.4792	0.5093	0.4640	0.5249	0.4100	0.5780
2	07-07-20	0.4957	-0.3048	-0.3063	0.0066	0.4940	0.4792	0.5093	0.4640	0.5249	0.4100	0.5780
3	08-11-20	0.4892	-0.3105	-0.3062	0.0066	0.4941	0.4793	0.5092	0.4642	0.5248	0.4101	0.5780
4	09-15-20	0.4879	-0.3117	-0.3066	0.0067	0.4937	0.4787	0.5091	0.4634	0.5249	0.4097	0.5776
5	10-06-20	0.4816	-0.3174	-0.3069	0.0071	0.4933	0.4775	0.5096	0.4613	0.5263	0.4094	0.5771
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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 # 244

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 #: 2063

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	M	M	M
Control, SaltSW	pH (S.U.)	7.72	7.04	7.73
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	*Salinity (ppt)	24.7	25.0	25.2
	*Alkalinity (mg/L $CaCO_3$)	95		
	*Temperature (°C)	25.2	25.3	25.6
250 mg/L	pH (S.U.)	7.73	7.05	7.72
	Dissolved oxygen (mg/L)	8.0	7.9	7.8
	*Salinity (ppt)	24.9	25.2	25.4
	*Temperature (°C)	25.1	25.2	25.5
375 mg/L	pH (S.U.)	7.76	7.05	7.70
	Dissolved oxygen (mg/L)	8.0	8.0	7.8
	*Salinity (ppt)	25.0	25.4	25.6
	*Temperature (°C)	25.1	25.2	25.5
500 mg/L	pH (S.U.) 01.04.22	~~7.74~~	7.05	7.71
	Dissolved oxygen (mg/L)	7.74 7.9	8.0	7.7
	*Salinity (ppt)	25.1	25.4	25.7
	*Temperature (°C)	25.3	25.5	25.6
750 mg/L	pH (S.U.)	7.76	7.05	
	Dissolved oxygen (mg/L)	7.9	8.0	
	*Salinity (ppt)	25.2	25.5	
	*Temperature (°C)	25.1	25.5	
1000 mg/L	pH (S.U.)	7.77	7.04	
	Dissolved oxygen (mg/L)	7.9	8.0	
	*Salinity (ppt)	25.4	25.6	
	*Temperature (°C)	25.1	25.5	

01.04.22

*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	130664679

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 # 244

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	01-04-22	* 1045	JH	1255	JH	7E	Yellow	12-29-21B
24	01-05-22			1300	JH			
48 Termination	01-06-22			1252	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):	01-03-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	01-02-22 1200 TO 01-03-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.64
	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	9 1d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	5 4d	4 5d	0	0	0	0
Mean Survival	100%		100%		100%		45%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	468.5
Upper 95% confidence limit (mg KCl/L)	546.7
48-hour LC_{50} (mg KCl/L)	506.1

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	1/4/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/6/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

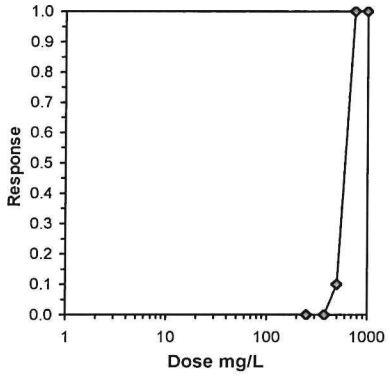
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

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

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

Trimmed Spearman-Karber

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



Dose-Response Plot

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-48 Hr Survival

Start Date:	1/4/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/6/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	1.0000	1.0000
500	0.5000	0.4000
750	0.0000	0.0000
1000	0.0000	0.0000

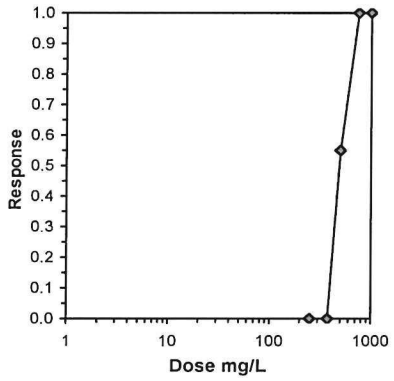
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	21.263	2.850	0.0907	11	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	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				

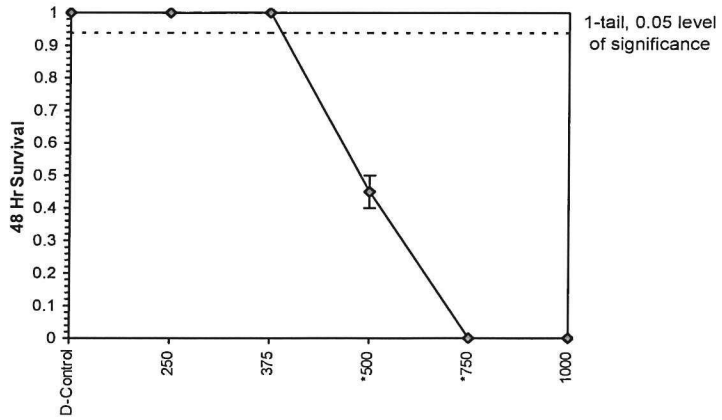
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.03598	0.0369	0.64187	0.00101	6.0E-07	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	506.10	468.54	546.66
5.0%	503.74	462.53	548.62
10.0%	501.39	455.99	551.32
20.0%	496.78	440.29	560.52
Auto-0.0%	506.10	468.54	546.66



Dose-Response Plot



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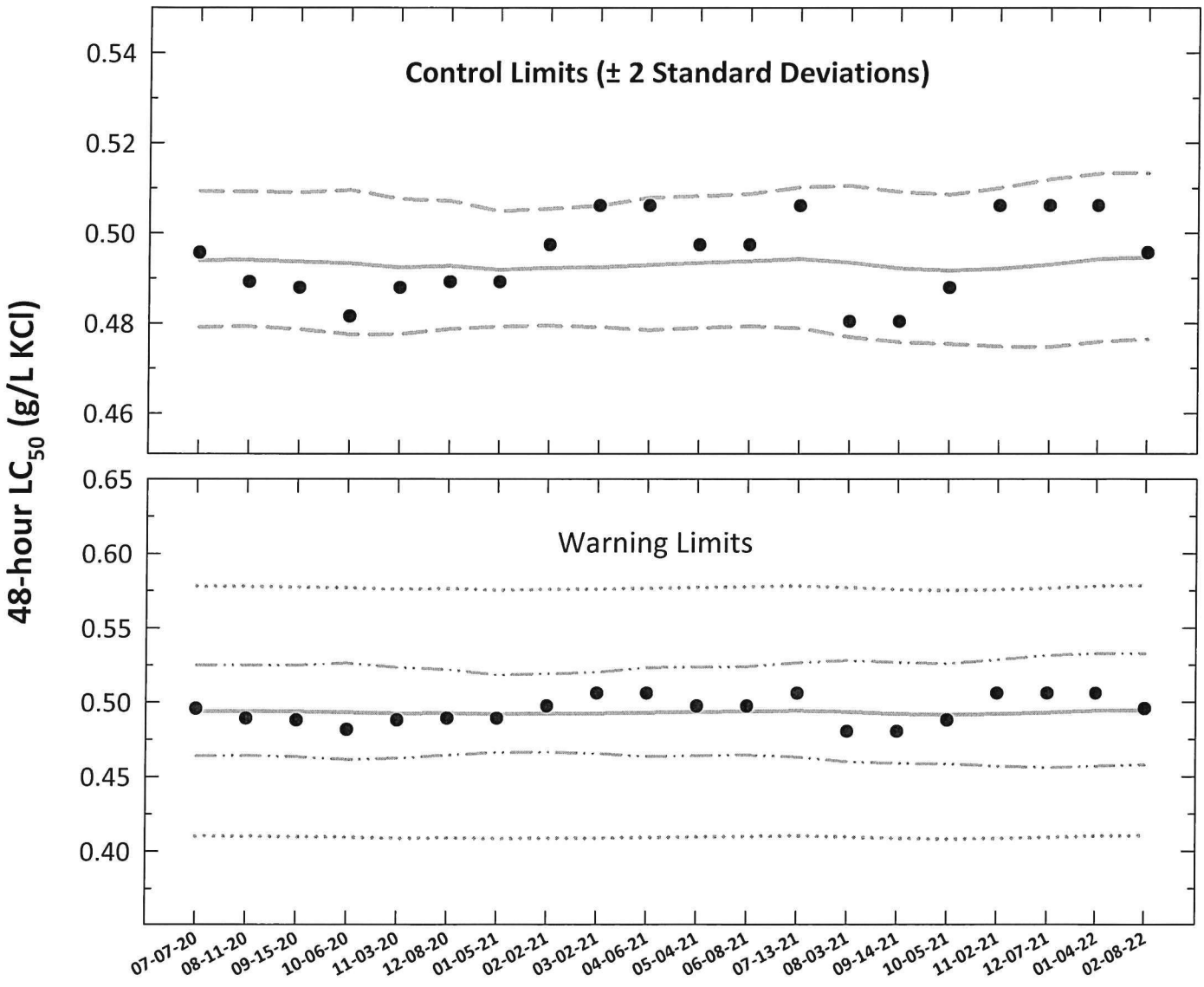

- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control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

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			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	07-07-20	0.4957	-0.3048	-0.3063	0.0066	0.4940	0.4792	0.5093	0.4640	0.5249	0.4100	0.5780
2	08-11-20	0.4892	-0.3105	-0.3062	0.0066	0.4941	0.4793	0.5092	0.4642	0.5248	0.4101	0.5780
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9	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
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17	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
18	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
19	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
20	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

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

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

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

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

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

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

CV = Coefficient of variation.

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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 # 245

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 #: 2063

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JW	JW	K
Control, SaltSW	pH (S.U.)	8.12	7.95	7.89
	Dissolved oxygen (mg/L)	7.9	7.6	7.8
	*Salinity (ppt)	25.0	25.1	25.2
	*Alkalinity (mg/L $CaCO_3$)	99		
	*Temperature (°C)	25.4	25.2	25.6
250 mg/L	pH (S.U.)	8.05	7.95	7.82
	Dissolved oxygen (mg/L)	7.9	7.6	7.8
	*Salinity (ppt)	25.2	25.3	25.4
	*Temperature (°C)	25.5	25.1	25.7
375 mg/L	pH (S.U.)	8.06	7.94	7.78
	Dissolved oxygen (mg/L)	8.0	7.6	7.7
	*Salinity (ppt)	25.2	25.5	25.5
	*Temperature (°C)	25.6	25.2	25.5
500 mg/L	pH (S.U.)	8.05	7.94	7.76
	Dissolved oxygen (mg/L)	8.0	7.6	7.7
	*Salinity (ppt)	25.2	25.6	25.7
	*Temperature (°C)	25.4	25.2	25.5
750 mg/L	pH (S.U.)	8.06	7.92	
	Dissolved oxygen (mg/L)	8.0	7.6	
	*Salinity (ppt)	25.4	25.7	
	*Temperature (°C)	25.4	25.2	
1000 mg/L	pH (S.U.)	8.06	7.95	
	Dissolved oxygen (mg/L)	8.0	7.6	
	*Salinity (ppt)	25.6	25.7	
	*Temperature (°C)	25.3	25.4	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066685

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 # 245

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	02-08-22	* 1200	JH	1400	JH	1C	ORANGE	02-05-22A
24	02-09-22			1355	JH			
48 Termination	02-10-22			1355	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):	02-05-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	02-06-22 1200 TO 02-08-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.06
	Temperature (°C) 25.1

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 1od	0 1od	0 1od	0 1od
48 Termination	10	10	10	10	9 1d	10	5 3d	5 3d	0	0	0	0
Mean Survival	100%		100%		95%		50%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	458.6
Upper 95% confidence limit (mg KCl/L)	547.7
48-hour LC_{50} (mg KCl/L)	~~495.6~~ 495.7

Comments:

Test Reviewed by: _____

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	2/8/2022	Test ID:	AbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

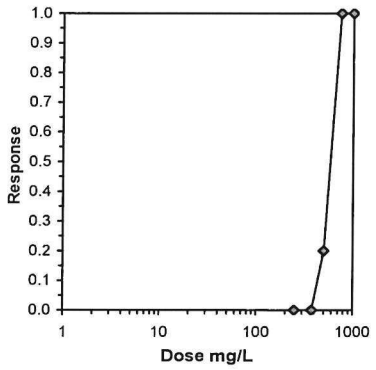
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

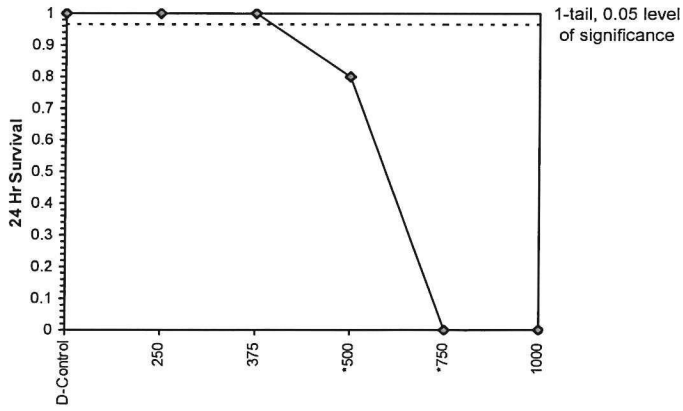
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.589	0.0001	2.3E-09	4, 5
Treatments vs D-Control										

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



ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-48 Hr Survival

Start Date:	2/8/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	1.0000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

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

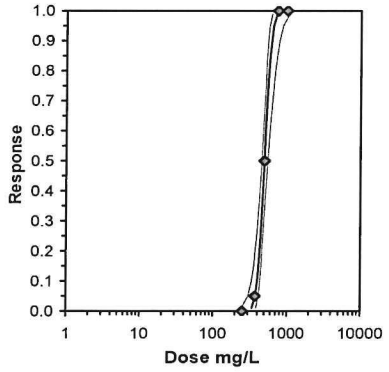
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.06555	0.06723	0.60026	0.00266	7.8E-06	4, 5
Treatments vs D-Control										

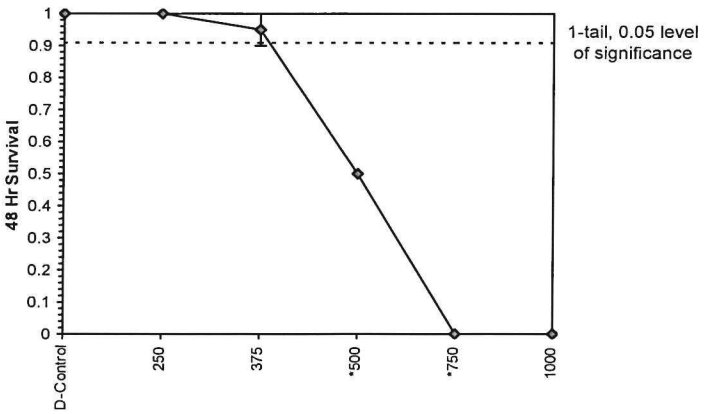
Maximum Likelihood-Probit

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

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

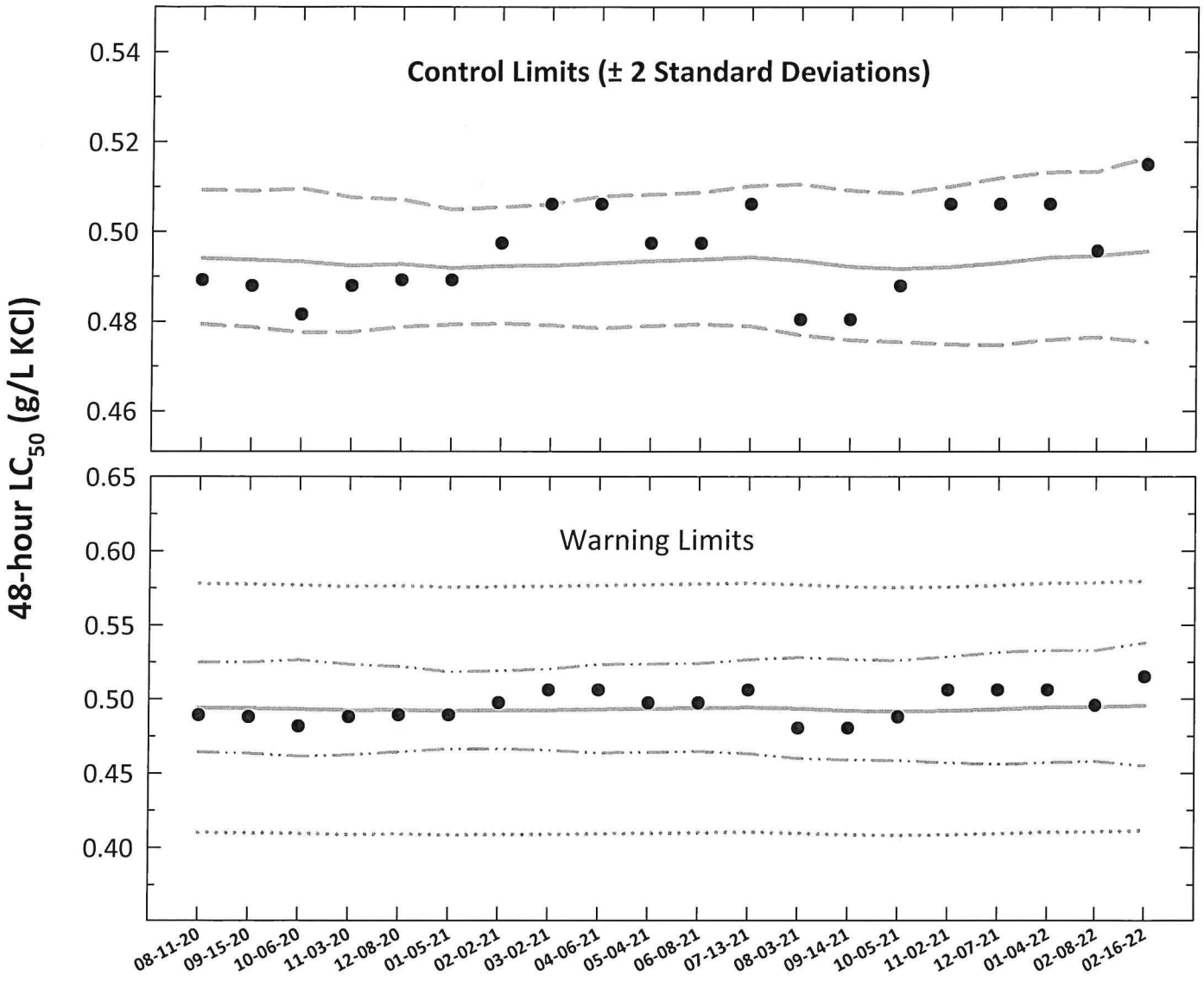


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-11-20	0.4892	-0.3105	-0.3062	0.0066	0.4941	0.4793	0.5092	0.4642	0.5248	0.4101	0.5780
2	09-15-20	0.4879	-0.3117	-0.3066	0.0067	0.4937	0.4787	0.5091	0.4634	0.5249	0.4097	0.5776
3	10-06-20	0.4816	-0.3174	-0.3069	0.0071	0.4933	0.4775	0.5096	0.4613	0.5263	0.4094	0.5771
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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 # 246

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 #: 2063

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, SaltSW	pH (S.U.)	JW	JW	JW
	Dissolved oxygen (mg/L)	7.84	7.83	7.65
	*Salinity (ppt)	7.7	7.5	7.5
	*Alkalinity (mg/L $CaCO_3$)	25.1	25.4	25.4
	*Temperature (°C)			
250 mg/L	pH (S.U.)	25.2	25.2	25.3
	Dissolved oxygen (mg/L)	7.91	7.82	7.67
	*Salinity (ppt)	7.9	7.5	7.4
	*Temperature (°C)	25.1	25.5	25.6
375 mg/L	pH (S.U.)	25.2	25.4	25.1
	Dissolved oxygen (mg/L)	7.92	7.83	7.68
	*Salinity (ppt)	7.9	7.5	7.4
	*Temperature (°C)	25.4	25.6	25.8
500 mg/L	pH (S.U.)	25.4	25.4	25.1
	Dissolved oxygen (mg/L)	7.92	7.85	7.76
	*Salinity (ppt)	7.8	7.6	7.4
	*Temperature (°C)	25.5	25.7	26.1
750 mg/L	pH (S.U.)	25.2	25.3	25.4
	Dissolved oxygen (mg/L)	7.92	7.84	
	*Salinity (ppt)	7.8	7.7	
	*Temperature (°C)	25.6	25.9	
1000 mg/L	pH (S.U.)	25.3	25.3	02-17-22
	Dissolved oxygen (mg/L)	7.92	7.84	
	*Salinity (ppt)	7.8	7.7	
	*Temperature (°C)	25.8	26.2	
		25.3	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

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 # 246

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	02-16-22	* 1100	JH	1300	JH	02-16-22 A SB4C	PALE YELLOW	02-12-22 B
24	02-17-22			1256	JH			
48 Termination	02-18-22			1255	JH			

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

Test Organism Information:

Organism Source:	Aquatic Indicators, Inc.
Batch (AI Batch Ab):	AI Ab 02-15-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	02-14-22 1200 TO 02-15-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01
	Temperature (°C) 24.2

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 1d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	5 4d	5 4d	0	0	0	0
Mean Survival	100%		100%		100%		50%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	476.5
Upper 95% confidence limit (mg KCl/L)	556.4
48-hour LC_{50} (mg KCl/L)	514.9

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	2/16/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/18/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

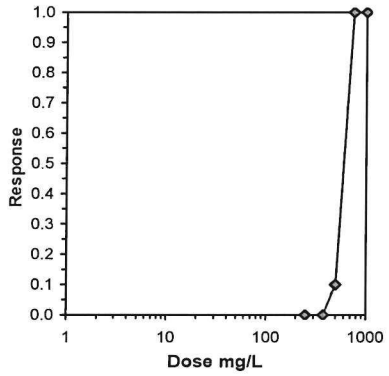
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

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

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

Trimmed Spearman-Karber

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



Dose-Response Plot

Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Summer

Acute Mysid Test-48 Hr Survival

Start Date:	2/16/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/18/2022	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	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

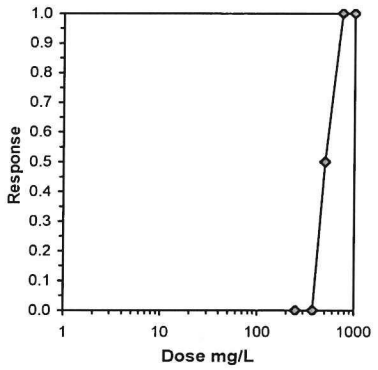
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*500	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	62.662	2.850	0.0285	10	20
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	125.324	2.850	0.0285	20	20
1000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

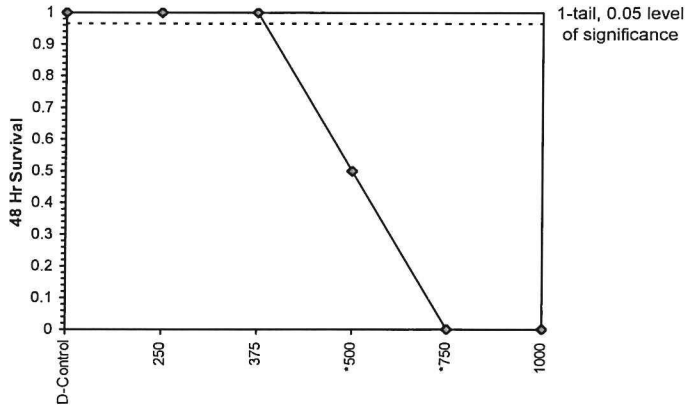
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	500	433.013		0.00967	0.00991	0.62824	0.0001	2.0E-09	4, 5

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	514.94	476.54	556.43
5.0%	513.43	471.14	559.51
10.0%	511.92	464.96	563.62
20.0%	508.91	448.82	577.05
Auto-0.0%	514.94	476.54	556.43

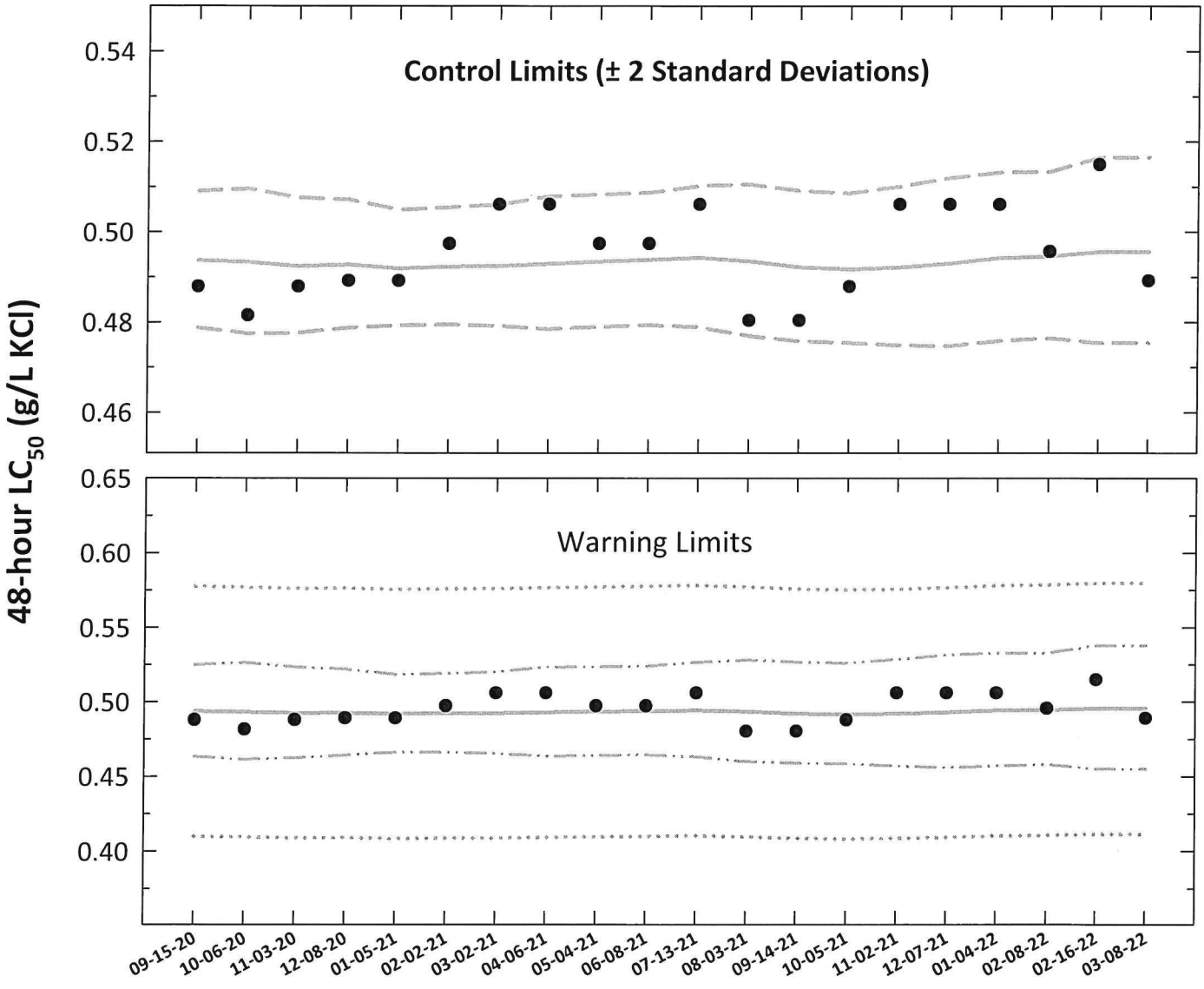


Dose-Response Plot



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	09-15-20	0.4879	-0.3117	-0.3066	0.0067	0.4937	0.4787	0.5091	0.4634	0.5249	0.4097	0.5776
2	10-06-20	0.4816	-0.3174	-0.3069	0.0071	0.4933	0.4775	0.5096	0.4613	0.5263	0.4094	0.5771
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Americamysis bahia*
EPA-821-R-02-012, Method 2007.0

Americamysis bahia Potassium Chloride Acute Reference Toxicant Test

AbKCIAC # 247

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 #: 2063

Chemical Analyses:

Concentration	Analyst	Hours 0	Hours 24	Hours 48
Control, SaltSW	pH (S.U.)	7.93	7.94	7.82
	Dissolved oxygen (mg/L)	8.0	7.9	7.0
	*Salinity (ppt)	24.7	25.4	25.7
	*Alkalinity (mg/L $CaCO_3$)	100		
	*Temperature (°C)	25.4	25.5	25.4
250 mg/L	pH (S.U.)	7.93	7.94	7.82
	Dissolved oxygen (mg/L)	7.9	7.9	7.5
	*Salinity (ppt)	25.2	25.4	25.2
	*Temperature (°C)	25.5	25.2	25.5
375 mg/L	pH (S.U.)	7.96	7.93	7.82
	Dissolved oxygen (mg/L)	7.9	7.9	7.6
	*Salinity (ppt)	25.1	25.4	25.6
	*Temperature (°C)	25.5	25.2	25.5
500 mg/L	pH (S.U.)	7.98	7.93	7.81
	Dissolved oxygen (mg/L)	7.9	8.0	7.6
	*Salinity (ppt)	25.2	25.4	25.6
	*Temperature (°C)	25.3	25.2	25.5
750 mg/L	pH (S.U.)	7.97	7.93	
	Dissolved oxygen (mg/L)	8.0	8.0	
	*Salinity (ppt)	25.4	25.8	
	*Temperature (°C)	25.5	25.2	
1000 mg/L	pH (S.U.)	7.99	7.93	
	Dissolved oxygen (mg/L)	8.0	8.0	
	*Salinity (ppt)	25.6	25.8	
	*Temperature (°C)	25.5	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

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 # 24

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	03-08-22	* 1045	JH	1245	JH	1C	ORANGE	03-03-22A
24	03-09-22			1244	JH			
48 Termination	03-10-22			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):	03-07-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	03-06-22 1200 TO 03-07-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.11
	Temperature (°C) 25.0

Survival Data (number of living organisms):

Hours	Control		250 mg/L		375 mg/L		500 mg/L		750 mg/L		1000 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	9 1d	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	5 4d	5 4d	0	0	0	0
Mean Survival	100%		100%		90%		50%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	449.4
Upper 95% confidence limit (mg KCl/L)	540.3
48-hour LC_{50} (mg KCl/L)	489.2

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	3/8/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

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

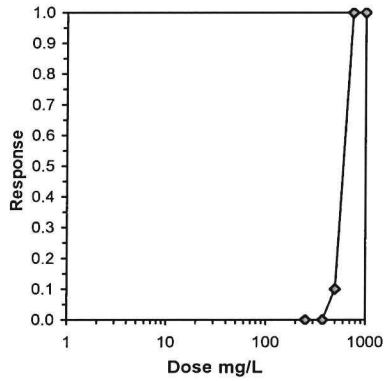
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120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

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

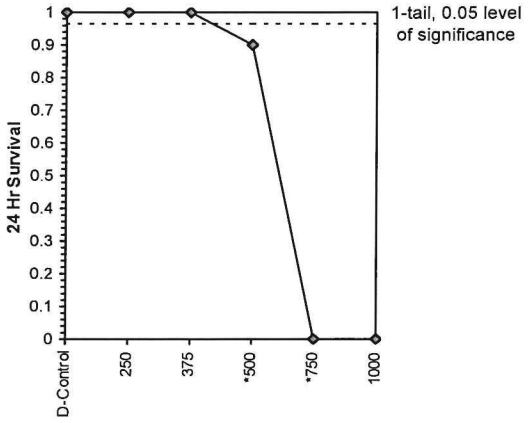
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00967	0.00991	0.59802	0.0001	2.2E-09	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

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



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Jumner

Acute Mysid Test-48 Hr Survival

Start Date:	3/8/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/10/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
250	1.0000	1.0000
375	0.9000	0.9000
500	0.5000	0.5000
750	0.0000	0.0000
1000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	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	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	62.662	2.850	0.0285	10	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

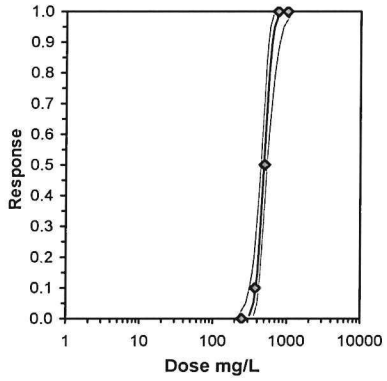
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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	0.57759	0.0001	2.4E-09	4, 5
Treatments vs D-Control										

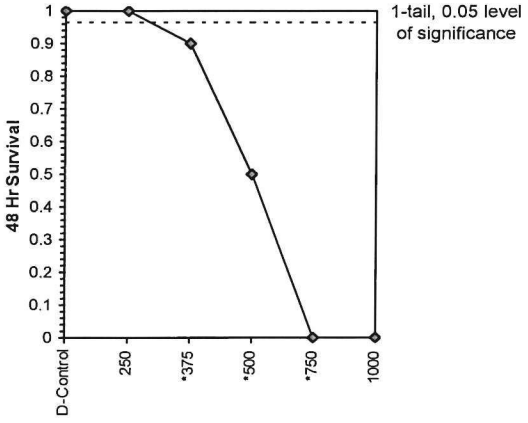
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.2852	2.70352	6.9863	17.5841	0	0.5411	7.81472	0.90977	2.68948	0.0814	5
Intercept	-28.041	7.23729	-42.226	-13.856							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	316.317	228.545	363.918
EC05	3.355	359.415	284.002	400.827
EC10	3.718	384.741	318.008	423.159
EC15	3.964	402.829	342.542	439.798
EC20	4.158	417.809	362.75	454.279
EC25	4.326	431.104	380.387	467.877
EC40	4.747	466.512	424.438	509.067
EC50	5.000	489.198	449.414	540.273
EC60	5.253	512.988	472.755	577.159
EC75	5.674	555.121	508.762	651.101
EC80	5.842	572.785	522.508	684.696
EC85	6.036	594.086	538.382	726.881
EC90	6.282	622.016	558.334	784.667
EC95	6.645	665.846	588.246	880.407
EC99	7.326	756.566	646.669	1096.14



Dose-Response Plot



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.

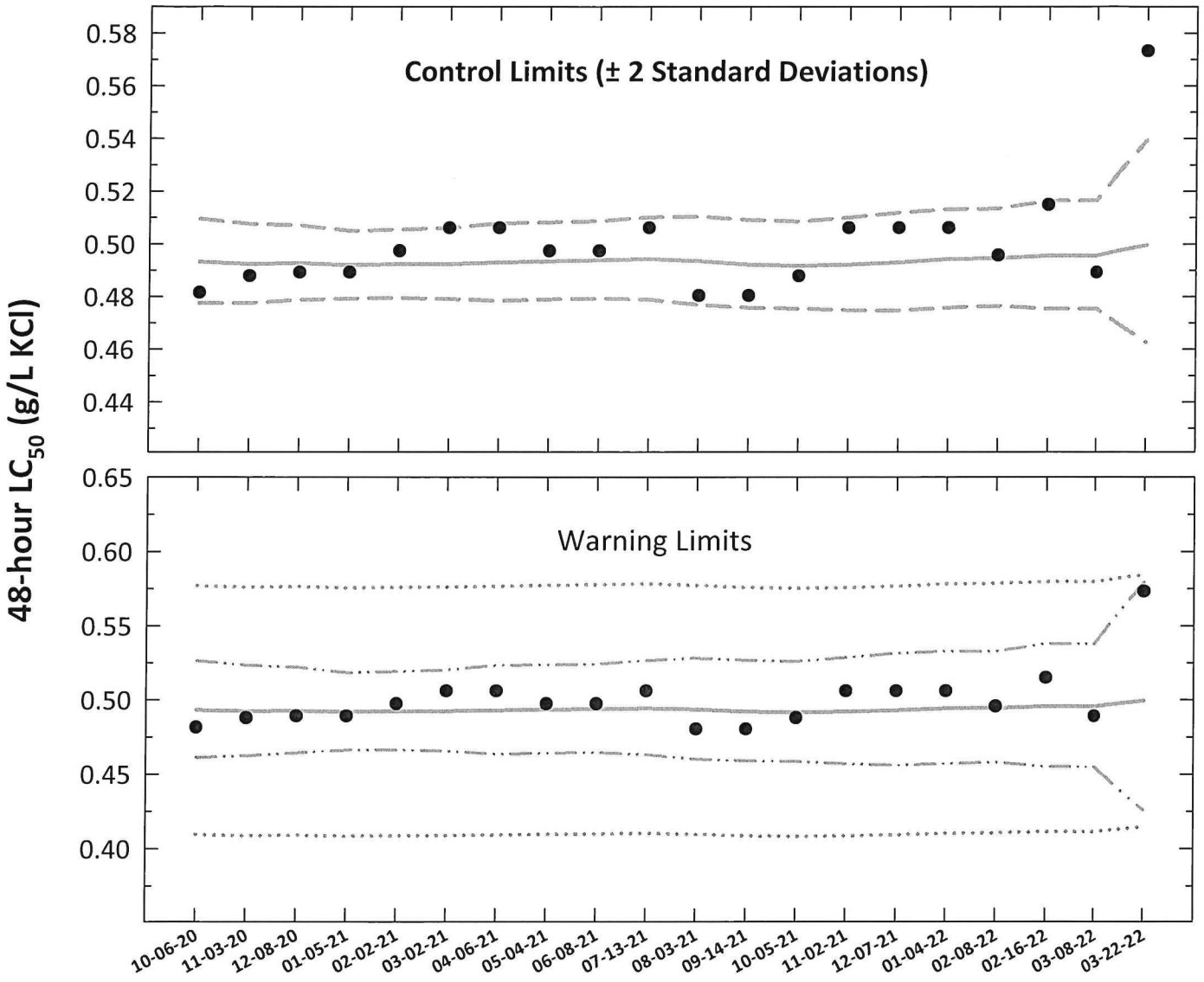
IV. VERIFICATION:

Signature: _____ Date: 03.28.22
Laboratory Supervisor

V. CLOSURE:

Signature: _____ Date: _____
Owner/Director

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	10-06-20	0.4816	-0.3174	-0.3069	0.0071	0.4933	0.4775	0.5096	0.4613	0.5263	0.4094	0.5771
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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 # 248

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	JW	FAS H	H
Control, SaltSW	pH (S.U.)	8.12	~~7.90~~ 7.91	7.94
	Dissolved oxygen (mg/L)	7.7	7.6	7.1
	*Salinity (ppt)	25.3	24.9	25.1
	*Alkalinity (mg/L $CaCO_3$)			
	*Temperature (°C)	24.3	24.4	24.9
250 mg/L	pH (S.U.)	8.14	7.98	7.90
	Dissolved oxygen (mg/L)	7.7	7.5	7.2
	*Salinity (ppt)	24.9	25.2	25.2
	*Temperature (°C)	24.2	24.8	25.1
375 mg/L	pH (S.U.)	8.15	8.00	7.94
	Dissolved oxygen (mg/L)	7.9	7.5	7.1
	*Salinity (ppt)	25.0	~~25.1~~ 25.6	25.2
	*Temperature (°C)	24.2	24.8	25.0
500 mg/L	pH (S.U.)	8.15	8.01	7.92
	Dissolved oxygen (mg/L)	7.9	7.5	7.1
	*Salinity (ppt)	25.1	25.0	25.2
	*Temperature (°C)	24.2	24.5	24.8
750 mg/L	pH (S.U.)	8.15	8.00	
	Dissolved oxygen (mg/L)	7.9	7.5	
	*Salinity (ppt)	25.6	~~25.7~~ 25.7	
	*Temperature (°C)	24.3	24.5	
1000 mg/L	pH (S.U.)	8.14	8.01	
	Dissolved oxygen (mg/L)	8.0	7.5	
	*Salinity (ppt)	25.6	25.7	
	*Temperature (°C)	24.2	24.6	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	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 # 218

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	SaltSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-22-22	* 1205	JL A.S	1430	A.S	7B	Yellow	03-11-22
24	03-23-22			1445	A.S			
48 Termination	03-24-22			1445	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):	03-21-22
Age (1 to 5 days old):	1-2 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	03-20-22 1200 TO 03-21-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.25
	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	A.S 03-24-22 ++10 10	03-24-22 A.S 10 11 *	10	10	10	10	10
24	10	10	10	10	10	03-24-22 A.S 10 ++ 10	03-24-22 A.S 10 11	9 1d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	9 2d	8 1d	0	0	0	0
Mean Survival	100%		100%		100%		80.9%		0%		0%	

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

* 11 ORGANISMS LOADED IN TEST CUP.

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	540.2
Upper 95% confidence limit (mg KCl/L)	608.3
48-hour LC_{50} (mg KCl/L)	573.3

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-24 Hr Survival

Start Date:	3/22/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/24/2022	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

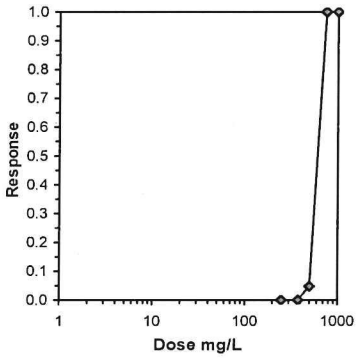
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1536	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1536	0	20
500	0.9500	0.9500	1.3343	1.2490	1.4195	9.032	2	1.443	2.850	0.1536	1	21
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	23.255	2.850	0.1536	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				

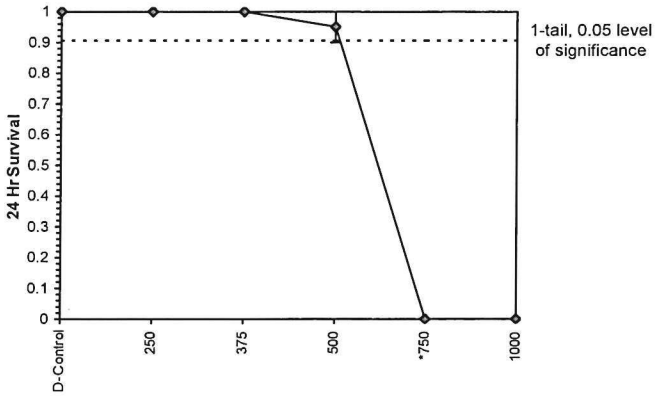
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.06944	0.07122	0.61117	0.0029	9.3E-06	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	602.35	583.26	622.07
5.0%	606.20	593.73	618.92
10.0%	606.20	593.73	618.92
20.0%	606.20	593.73	618.92
Auto-0.0%	602.35	583.26	622.07



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Mysid Test-48 Hr Survival

Start Date:	3/22/2022	Test ID:	AbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/24/2022	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	0.8182	0.8000
750	0.0000	0.0000
1000	0.0000	0.0000

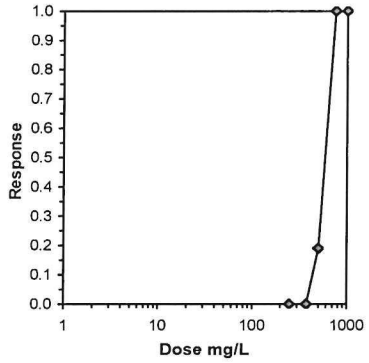
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
250	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0209	0	20
375	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0209	0	20
*500	0.8091	0.8091	1.1187	1.1071	1.1303	1.462	2	40.087	2.850	0.0209	4	21
*750	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	171.288	2.850	0.0209	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				

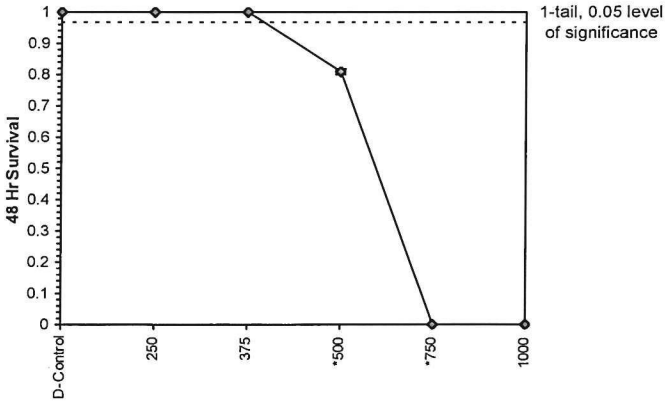
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	375	500	433.013		0.00692	0.0071	0.58914	5.4E-05	4.8E-10	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	573.25	540.20	608.33
5.0%	577.42	539.60	617.89
10.0%	580.84	535.07	630.52
20.0%	583.85	553.70	615.64
Auto-0.0%	573.25	540.20	608.33

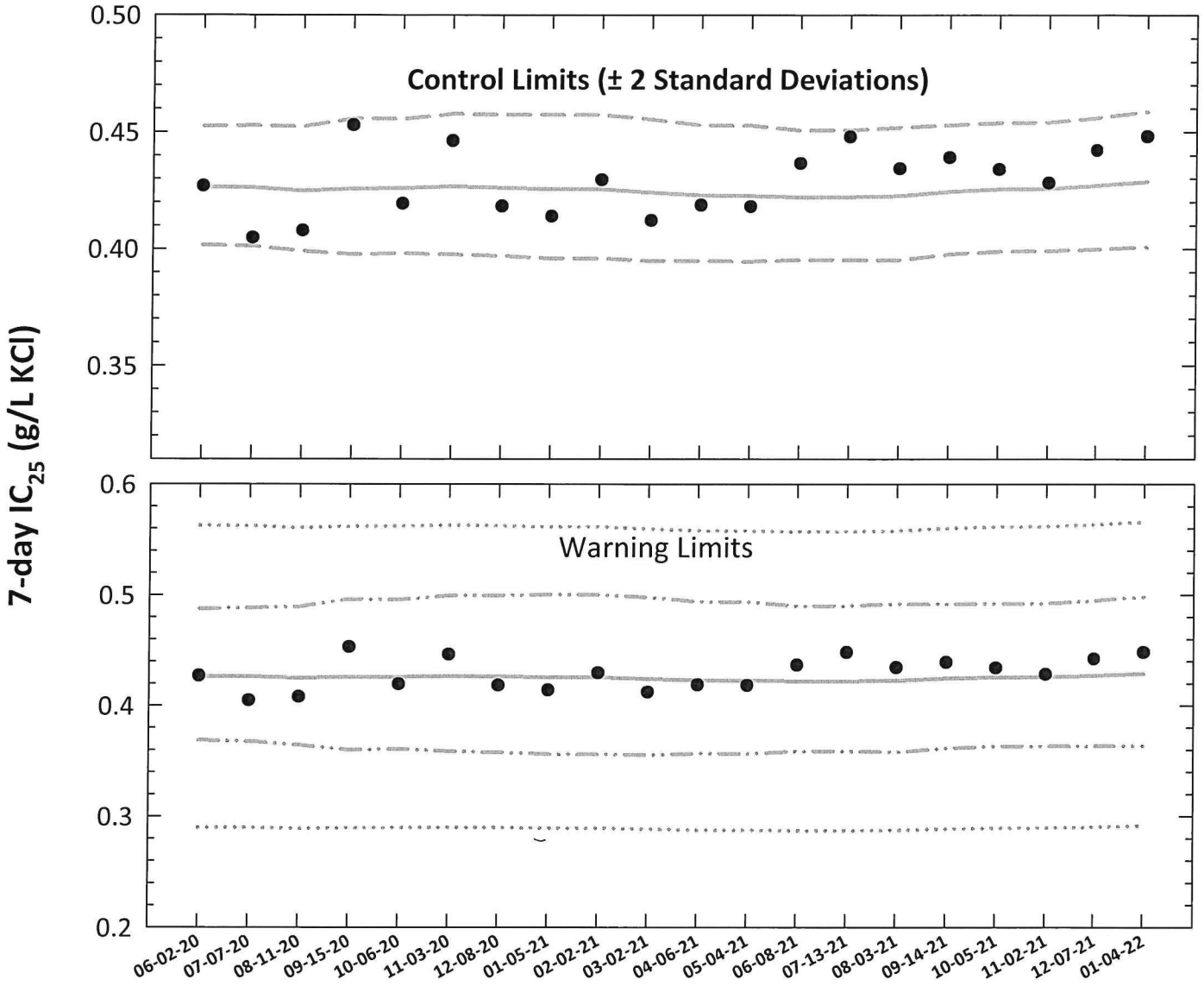


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	06-02-20	0.4270	-0.3696	-0.3702	0.0129	0.4264	0.4018	0.4525	0.3686	0.4876	0.2899	0.5628
2	07-07-20	0.4048	-0.3928	-0.3703	0.0131	0.4262	0.4012	0.4528	0.3676	0.4886	0.2898	0.5626
3	08-11-20	0.4078	-0.3895	-0.3718	0.0136	0.4249	0.3991	0.4523	0.3641	0.4895	0.2889	0.5608
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

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

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

CV = Coefficient of variation.

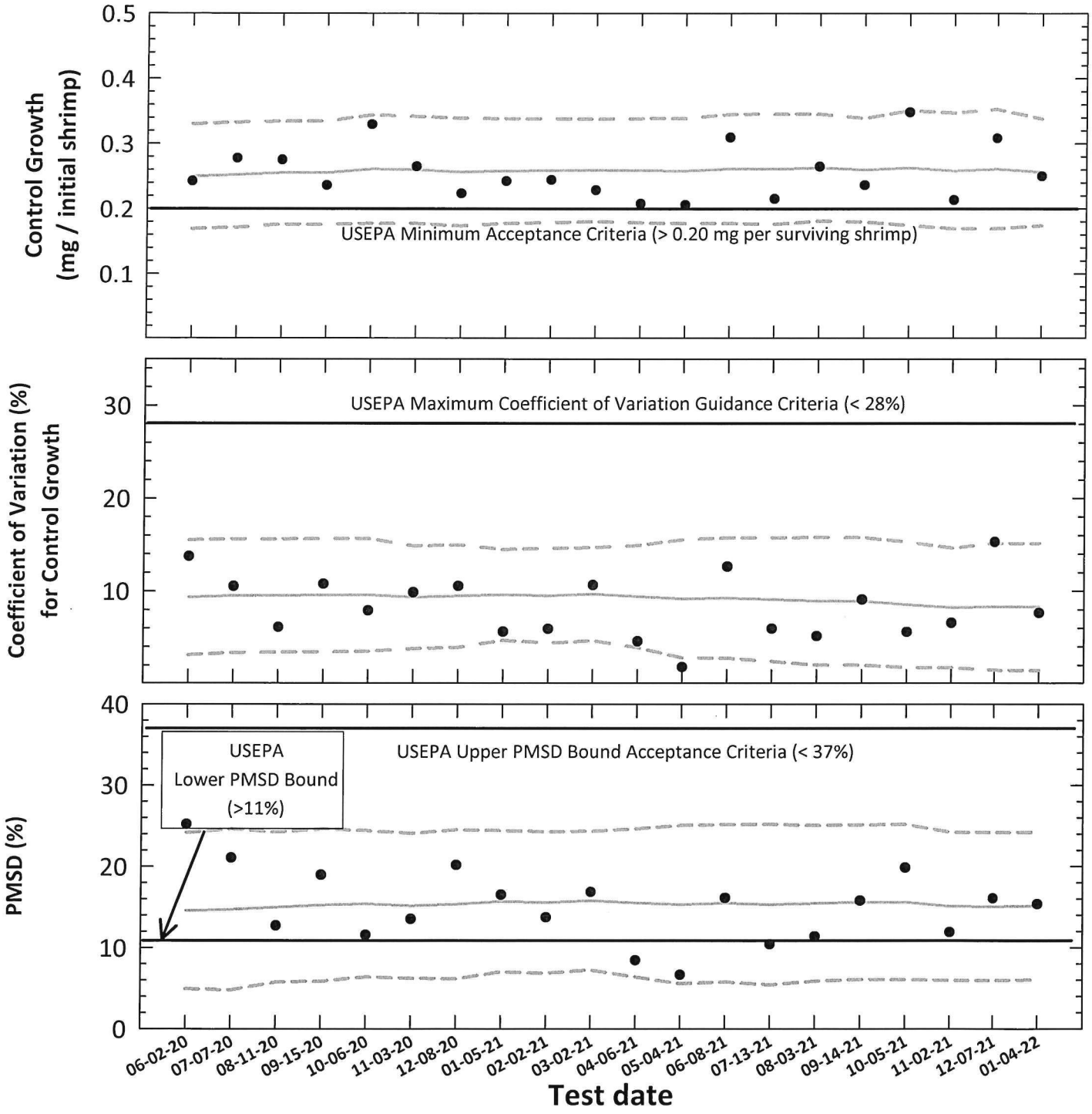
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Americamysis (Mysidopsis) bahia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: Aquatic Indicators, Inc.



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

Central Tendency (mean Control Growth, CV or PMSD)

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

Entered and Reviewed by Jim Sumner

ETS Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial shrimp)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	06-02-20	100	0.243	13.8	0.0611	25.2	0.250	0.169	0.330	9.3	3.1	15.5	14.5	4.9	24.1
2	07-07-20	100	0.278	10.5	0.0584	21.0	0.252	0.172	0.333	9.5	3.3	15.6	14.7	4.8	24.6
3	08-11-20	100	0.275	6.1	0.0349	12.7	0.255	0.176	0.334	9.5	3.4	15.6	15.0	5.7	24.2
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: **223**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2063				
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:	PINK
Date and times organisms were born between:	12-27-21 1200 to 12-28-21 1130	Incubator number and shelf location:	01-04-22 #8 SF
Organism source:	AI Batch Ab: 12-28-21	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 7.57 S.U. Temperature = 25.1 °C	Date / Time in oven:	01-11-22 1150
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	01-12-22 1150
		*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	01-04-22	1045	JL	1320	JL	1220	JL	12-29-21B
1	01-05-22	0505	JL	1105	JL	1020	JL	↓
2	01-06-22	0530	JL	1300	JL	1020	JL	01-03-22
3	01-07-22	0600	JL	1200	JL	1020	JL	↓
4	01-08-22	0700	JL	1300	JL	1025	JL	01-06-22
5	01-09-22	0700	JL	1300	JL	1020	JL	↓
6	01-10-22	0600	JL	1200	JL	1020	JL	
7	01-11-22					1029	JL	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468 S

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	506.3
Average weight per initial shrimp:	0.250		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.250	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	448.2

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **223**

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															JK 01-11-22	
*A = Pan weight (mg) Tray color code: light pink Analyst: SW Date: 12-20-21	13.92	16.37	14.60	13.11	14.99	15.90	13.70	15.87	12.97	14.50	14.34	14.71	14.71	16.07	15.97	15.39
*B = Pan + Shrimp weight (mg) Analyst: A.L Date: 01-14-22	15.30	17.67	15.90	14.43	16.19	17.04	14.96	16.97	14.16	15.86	15.61	16.44	15.86	17.30	17.12	16.52
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JK	1.38	1.30	1.30	1.32	1.20	1.14	1.26	1.10	1.19	1.36	1.27	1.73	1.15	1.23	1.15	1.13
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JK	0.276	0.260	0.260	0.264	0.240	0.228	0.252	0.220	0.238	0.272	0.254	0.346	0.230	0.246	0.230	0.226
	Average weight per initial number of shrimp (mg) 0.250								Average weight per initial number of shrimp (mg) 0.255				Percent reduction from control (%) -2.17.			

*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: **223**

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	4 id	4 id	4 id	S	S	S	4 id
3	S	S	S	S	S	S	S	S	4 id	4	4	4	S	4 id	4 id	4
4	S	S	S	S	S	S	S	S	4	4	4	3 id	4 id	4	4	4
5	S	S	4 id	S	S	S	S	S	4	3 id	4	3	3 id	3 id	4	4
6	S	S	4	S	S	S	S	S	3 id	3	3 id	2 id	3	3	3 id	2 id
7	S	S	4	S	S	S	S	S	3	3	3	2	3	3	3	2
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*A = Pan weight (mg) Tray color code: light pink Analyst: SW Date: 12-20-21	13.20	13.92	14.50	13.91	13.79	14.63	15.62	14.49	12.83	13.27	14.28	14.63	13.60	12.87	14.54	13.26
*B = Pan + Shrimp weight (mg) Analyst: A.I Date: 01-14-22	14.58	15.33	15.67	15.24	15.01	15.92	16.98	15.90	13.61	14.22	14.94	15.13	14.28	13.87	15.31	13.80
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JJ	1.38	1.41	1.17	1.33	1.22	1.29	1.36	1.41	0.78	0.95	0.66	0.50	0.68	1.00	0.77	0.54
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JJ	0.276	0.282	0.234	0.266	0.244	0.258	0.272	0.282	0.156	0.190	0.132	0.100	0.136	0.200	0.154	0.108
	Average weight per initial number of shrimp (mg) 0.264				Percent reduction from control (%) -5.77.				Average weight per initial number of shrimp (mg) 0.147				Percent reduction from control (%) 41.27.			

*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: **223**

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																4
*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:_____																0
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: 223

Test dates: January 04-11, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	13.92	15.30	1.38	0.276	100.0	0.250	7.6	Not applicable
	B	5	5	16.37	17.67	1.30	0.260				
	C	5	5	14.60	15.90	1.30	0.260				
	D	5	5	13.11	14.43	1.32	0.264				
	E	5	5	14.99	16.19	1.20	0.240				
	F	5	5	15.90	17.04	1.14	0.228				
	G	5	5	13.70	14.96	1.26	0.252				
	H	5	5	15.87	16.97	1.10	0.220				
250	I	5	5	12.97	14.16	1.19	0.238	100.0	0.255	15.6	-2.1
	J	5	5	14.50	15.86	1.36	0.272				
	K	5	5	14.34	15.61	1.27	0.254				
	L	5	5	14.71	16.44	1.73	0.346				
	M	5	5	14.71	15.86	1.15	0.230				
	N	5	5	16.07	17.30	1.23	0.246				
	O	5	5	15.97	17.12	1.15	0.230				
	P	5	5	15.39	16.52	1.13	0.226				
375	Q	5	5	13.20	14.58	1.38	0.276	97.5	0.264	6.7	-5.7
	R	5	5	13.92	15.33	1.41	0.282				
	S	5	4	14.50	15.67	1.17	0.234				
	T	5	5	13.91	15.24	1.33	0.266				
	U	5	5	13.79	15.01	1.22	0.244				
	V	5	5	14.63	15.92	1.29	0.258				
	W	5	5	15.62	16.98	1.36	0.272				
	X	5	5	14.49	15.90	1.41	0.282				
500	Y	5	3	12.83	13.61	0.78	0.156	55.0	0.147	24.2	41.2
	Z	5	3	13.27	14.22	0.95	0.190				
	AA	5	3	14.28	14.94	0.66	0.132				
	BB	5	2	14.63	15.13	0.50	0.100				
	CC	5	3	13.60	14.28	0.68	0.136				
	DD	5	3	12.87	13.87	1.00	0.200				
	EE	5	3	14.54	15.31	0.77	0.154				
	FF	5	2	13.26	13.80	0.54	0.108				
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: 0.0384

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) = 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:	1/4/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	1/11/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
250	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
375	1.0000	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000
500	0.6000	0.6000	0.6000	0.4000	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	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.5500	0.5500	0.8357	0.6847	0.8861	11.153	8	36.00	48.00	18	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

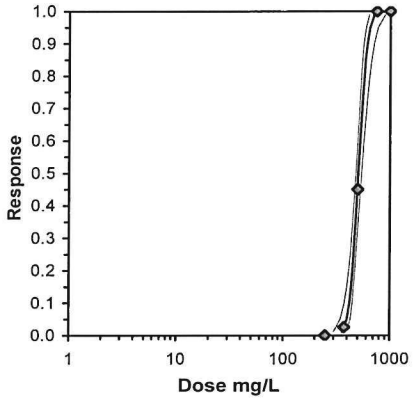
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.63372	0.904	-2.4249	5.84709
Equality of variance cannot be confirmed				

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

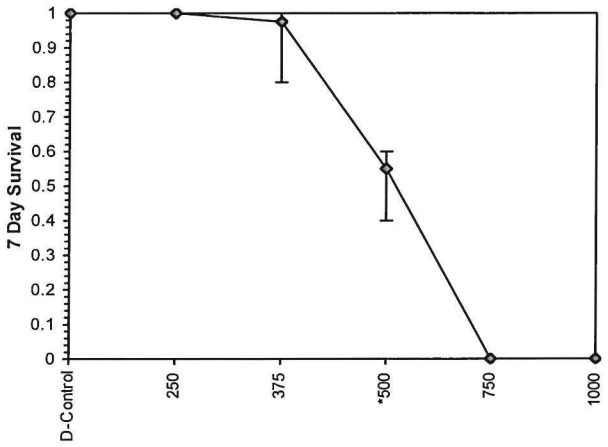
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.8775	3.06249	9.87504	21.88	0	0.24502	7.81472	0.97001	2.70444	0.06298	4
Intercept	-37.94	8.23191	-54.074	-21.805							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	361.343	298.176	396.465
EC05	3.355	398.88	347.877	427.966
EC10	3.718	420.459	376.957	446.62
EC15	3.964	435.674	397.363	460.328
EC20	4.158	448.158	413.826	472.138
EC25	4.326	459.153	427.94	483.133
EC40	4.747	488.07	462.193	515.847
EC50	5.000	506.336	481.151	539.88
EC60	5.253	525.285	498.837	567.354
EC75	5.674	558.366	526.466	619.923
EC80	5.842	572.065	537.141	642.958
EC85	6.036	588.458	549.531	671.29
EC90	6.282	609.752	565.158	709.182
EC95	6.645	642.739	588.611	770.004
EC99	7.326	709.507	634.212	900.007



Dose-Response Plot



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ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-Growth-Weight

Start Date:	1/4/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	1/11/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2760	0.2600	0.2600	0.2640	0.2400	0.2280	0.2520	0.2200
250	0.2380	0.2720	0.2540	0.3460	0.2300	0.2460	0.2300	0.2260
375	0.2760	0.2820	0.2340	0.2660	0.2440	0.2580	0.2720	0.2820
500	0.1560	0.1900	0.1320	0.1000	0.1360	0.2000	0.1540	0.1080
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

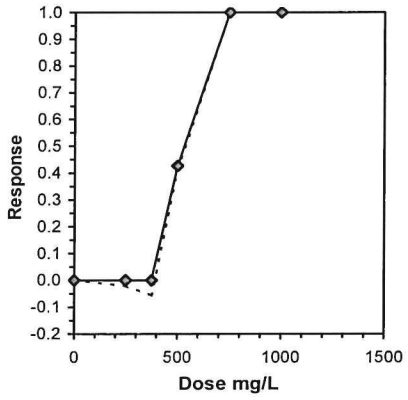
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	0.2500	1.0000	0.2500	0.2200	0.2760	7.649	8			0.2565	1.0000
250	0.2553	1.0210	0.2553	0.2260	0.3460	15.553	8	65.00	43.00	0.2565	1.0000
375	0.2643	1.0570	0.2643	0.2340	0.2820	6.702	8	82.50	43.00	0.2565	1.0000
500	0.1470	0.5880	0.1470	0.1000	0.2000	24.207	8			0.1470	0.5731
750	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8			0.0000	0.0000
1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	8			0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84379	0.884	1.71351	5.23511
Bartlett's Test indicates equal variances (p = 0.06)	5.62474	9.21035		

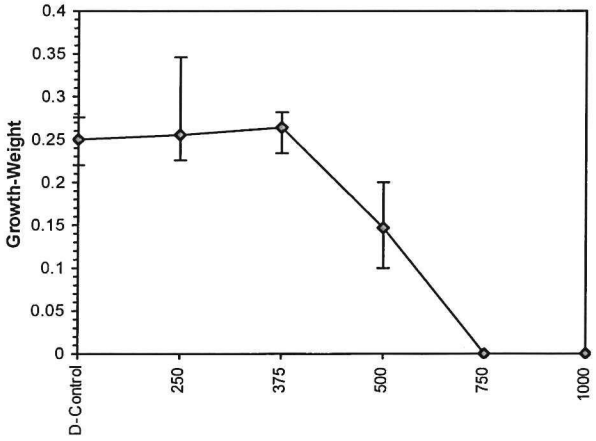
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs D-Control	375	>375		

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	389.64	3.65	379.45	393.27	-3.1088
IC10	404.28	3.98	395.12	411.54	-0.1450
IC15	418.92	5.13	409.08	429.81	0.5176
IC20	433.56	6.47	422.33	448.07	0.7280
IC25	448.20	7.91	435.04	466.34	0.7903
IC40	492.12	12.64	472.38	522.00	0.7244
IC50	531.89	16.53	497.27	560.00	-0.1405



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **223**

Daily Chemistry:

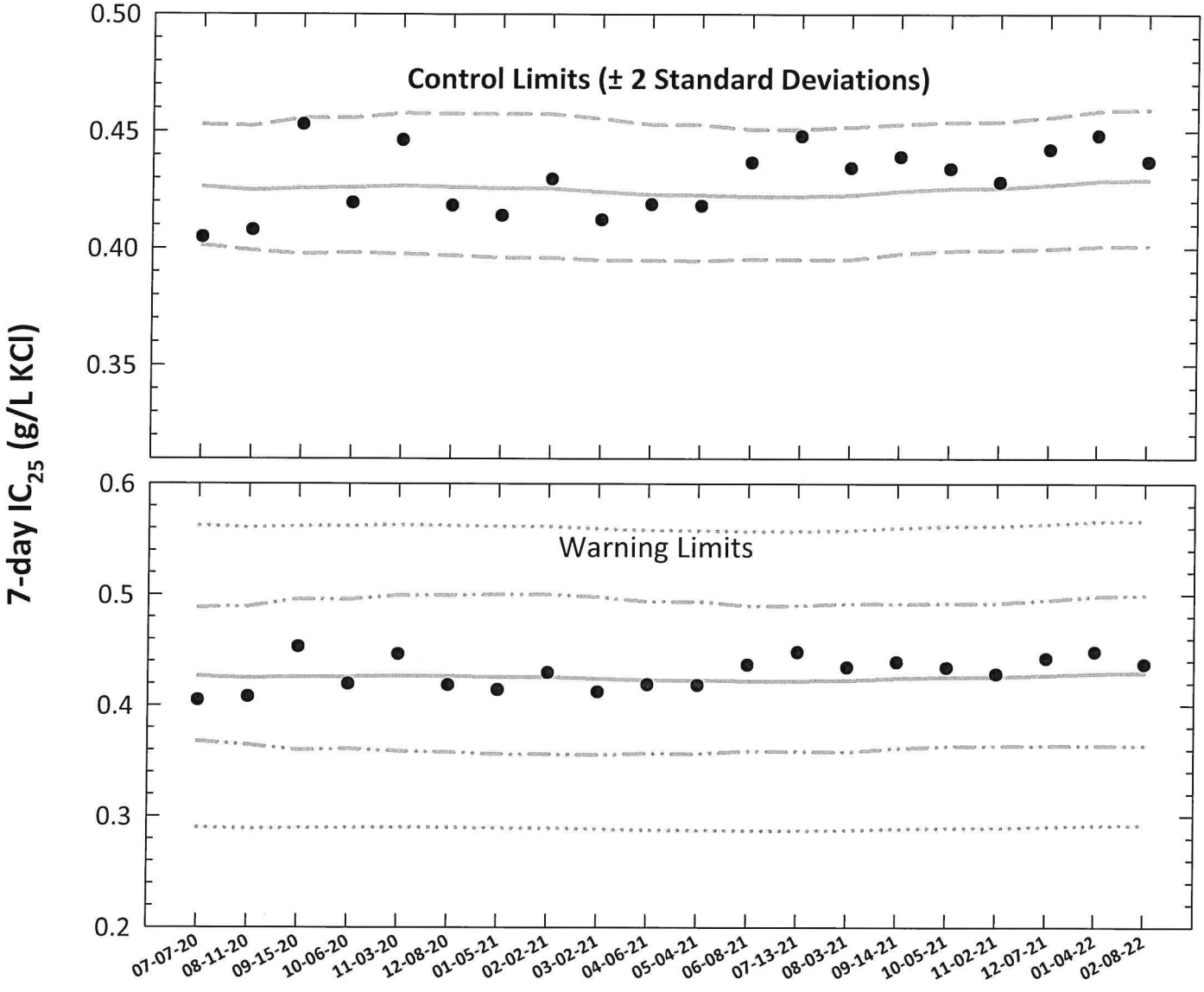
Temperatures performed at the time of test initiation, renewal or termination by the analyst identified in the Daily Renewal Information table located on Page 1. Alkalinity performed by the analyst identified on the bench sheet specific for this analysis and transcribed to this bench sheet.

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 0		1		2	
	Analyst	K	K	K	K	K	K
CONTROL, Salt SW	pH (S.U.)	7.72	7.82	7.79	7.73	7.79	7.68
	DO (mg/L)	8.0	7.8	7.0	7.5	7.9	7.6
	Salinity (ppt)	24.7	25.0	24.8	25.2	24.8	25.5
	Alkalinity (mg $CaCO_3$/L)	95		01-10-22 gl		100	
	Temperature (°C)	25.2	25.6	25.3	25.5	25.2	25.8
250 mg KCl/L	pH (S.U.)	7.73	7.81	7.81	7.70	7.82	7.66
	DO (mg/L)	8.0	7.7	7.8	7.5	7.9	7.6
	Salinity (ppt)	24.9	25.2	25.0	25.2	25.0	25.4
	Temperature (°C)	25.2	25.7	25.4	25.5	25.3	25.5
375 mg KCl/L	pH (S.U.)	7.76	7.81	7.83	7.70	7.82	7.62
	DO (mg/L)	8.0	7.7	7.8	7.5	7.9	7.7
	Salinity (ppt)	25.0	25.4	25.2	25.4	25.3	25.8
	Temperature (°C)	25.1	25.5	25.4	25.6	25.3	25.5
500 mg KCl/L	pH (S.U.)	7.74	7.82	7.85	7.71	7.82	7.63
	DO (mg/L)	7.9	7.7	7.8	7.8	8.0	7.7
	Salinity (ppt)	25.1	25.4	25.2	25.6	25.4	25.3
	Temperature (°C)	25.1	25.5	25.4	25.5	25.3	25.7
750 mg KCl/L	pH (S.U.)	7.76	7.81				
	DO (mg/L)	7.9	7.8				
	Salinity (ppt)	25.2	25.5				
	Temperature (°C)	25.1	25.6				
1000 mg KCl/L	pH (S.U.)	7.77	7.81				
	DO (mg/L)	7.9	7.9			01-05-22 K	
	Salinity (ppt)	25.4	25.6				
	Temperature (°C)	25.1	25.6				
		Initial	Final	Initial	Final	Initial	Final

AbKClCR Test Number: **223**

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.) 3		4		5		6	
	Analyst	N	BSL	BSL	N	N	N	N	JW
CONTROL, Salt SW	pH (S.U.)	7.75	7.65	7.80	7.65	7.83	7.64	7.85	7.66
	DO (mg/L)	7.8	7.0	7.8	7.3	7.7	7.4	7.8	7.5
	Salinity (ppt)	24.8	25.0	24.8	25.4	24.7	25.0	24.7	25.1
	Alkalinity (mg $CaCO_3$/L)			99					
	Temperature (°C)	25.4	25.8	25.3	25.7	25.3	25.6	25.3	25.5
250 mg KCl/L	pH (S.U.)	7.74	7.64	7.87	7.70	7.83	7.63	7.84	7.75
	DO (mg/L)	7.9	7.0	7.8	7.3	7.9	7.4	7.8	7.5
	Salinity (ppt)	25.1	25.3	25.2	25.5	24.8	25.1	24.9	25.1
	Temperature (°C)	25.4	25.7	25.4	25.8	25.2	25.5	25.2	25.7
375 mg KCl/L	pH (S.U.)	7.80	7.64	7.88	7.70	7.83	7.63	7.84	7.76
	DO (mg/L)	8.0	7.0	7.8	7.6	8.0	7.4	7.8	7.6
	Salinity (ppt)	25.2	25.5	25.5	25.8	24.9	25.2	25.3	25.5
	Temperature (°C)	25.5	25.7	25.4	25.6	25.4	25.6	25.2	25.7
500 mg KCl/L	pH (S.U.)	7.81	7.74	7.88	7.78	7.84	7.69	7.85	7.83
	DO (mg/L)	8.0	7.1	7.9	7.6	8.0	7.8	7.9	7.7
	Salinity (ppt)	25.3	26.1	25.5	26.1	25.1	25.4	25.3	25.6
	Temperature (°C)	25.5	25.7	25.4	25.6	25.4	25.6	25.3	25.6
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Americamysis* growth (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic IC_{25} ± 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	07-07-20	0.4048	-0.3928	-0.3703	0.0131	0.4262	0.4012	0.4528	0.3676	0.4886	0.2898	0.5626
2	08-11-20	0.4078	-0.3895	-0.3718	0.0136	0.4249	0.3991	0.4523	0.3641	0.4895	0.2889	0.5608
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

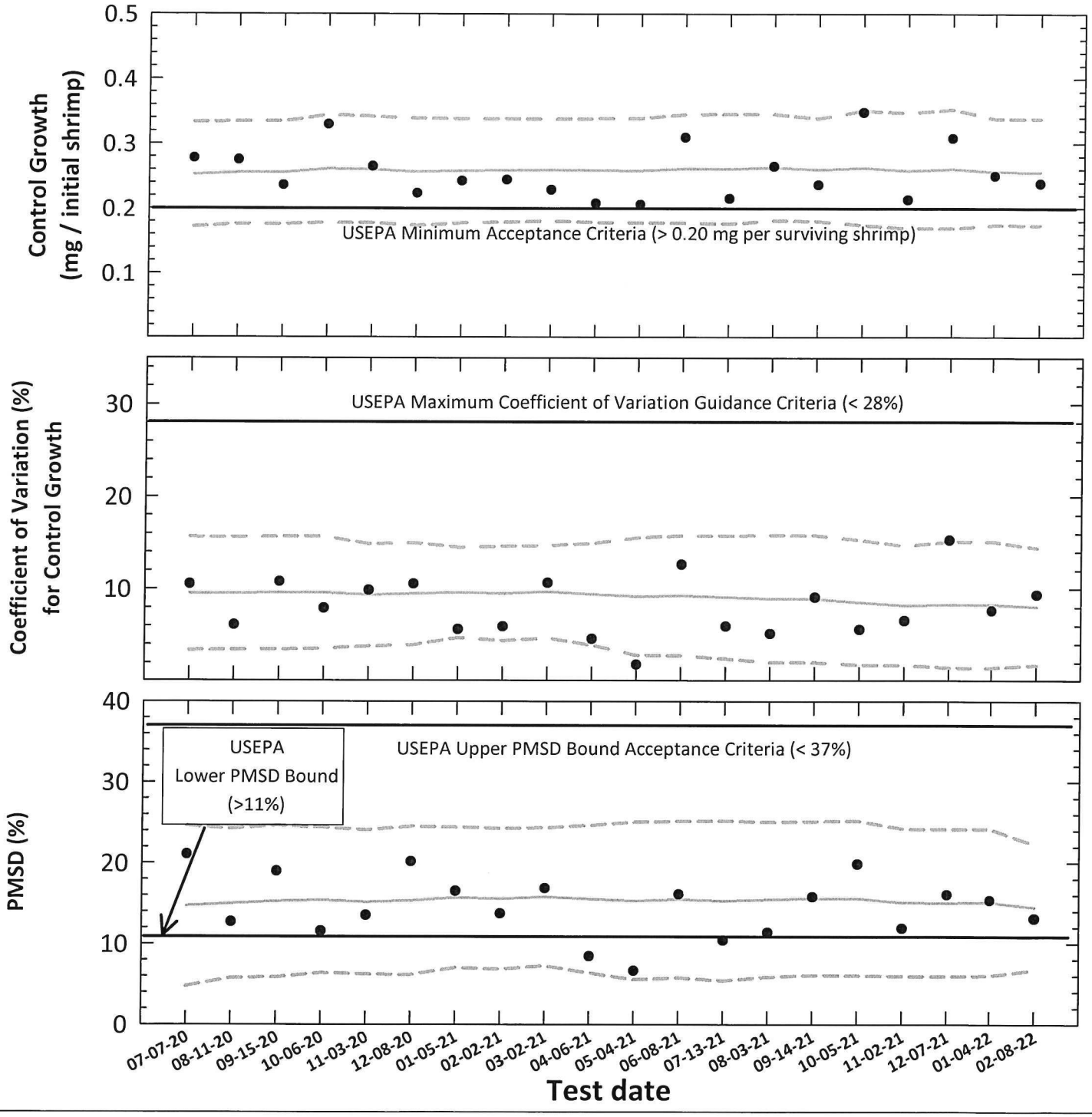
Entered and Reviewed by Jim Sumner

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 (mg/initial shrimp)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	07-07-20	100	0.278	10.5	0.0584	21.0	0.252	0.172	0.333	9.5	3.3	15.6	14.7	4.8	24.6
2	08-11-20	100	0.275	6.1	0.0349	12.7	0.255	0.176	0.334	9.5	3.4	15.6	15.0	5.7	24.2
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: 224

Dilution preparation information:						***Comments:***
KCl Stock INSS number:	INSS 2063					
Stock preparation:	50 g KCl/L: Dissolve 50 g KCl in 1-L Deionized water					
Dilution prep (mg/L)	250	375	500	750	1000	
Stock volume (mL)	7.5	11.25	15	22.5	30	
Diluent volume (mL)	1492.5	1488.75	1485	1477.5	1470	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism information:		***Test information:***	
Organism age:	7-days old	Randomizing template:	GREY
Date and times organisms were born between:	01-31-22 1200 to 02-01-22 1130	Incubator number and shelf location:	SB
Organism source:	AI Batch Ab: 02-01-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 8.06 S.U. Temperature = 25.1 °C	Date / Time in oven:	02-15-22 1125
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	02-16-22 1130
		*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	02-08-22	1200	JH	1300	JH	1230	JH	02-03-22A
1	02-09-22	0530	JH	1130	JH	1030	JH	↓
2	02-10-22	0600	JH	1200	JH	1030	JH	02-08-22
3	02-11-22	0600	JH	1200	JH	1032	JH	↓
4	02-12-22	0600	JH	1200	JH	1030	JH	02-10-22
5	02-13-22	0600	JH	1200	JH	1030	JH	↓
6	02-14-22	0600	JH	1200	JH	1030	JH	02-12-22A
7	02-15-22					1034	JH	

Chemical analyses:

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

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	514.9
Average weight per initial shrimp:	0.238		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.238	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	437.0

Environmental Testing Solutions, Inc.

AbKClCR Test Number: 224

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: Light Pink Analyst: A.S Date: 01-27-22	A.S 01-27-22 ~~15.00~~ 15.01	14.62	14.52	15.98	14.90	15.58	13.19	15.09	A.S 01-27-22 ~~13.43~~ 13.44	15.69	15.39	14.96	15.09	14.82	14.73	13.09
*B = Pan + Shrimp weight (mg) Analyst: JH Date: 02-17-22	16.17	15.87	15.59	17.31	16.23	16.62	14.42	16.21	14.61	16.77	16.77	16.09	16.25	16.00	15.96 ~~15.04~~ 02-17-22 JH	14.32
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JH	1.16	1.25	1.07	1.33	1.33	1.04	1.23	1.12	1.17	1.08	1.38	1.13	1.16	1.18	1.23	1.23
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JH	0.232	0.250	0.214	0.266	0.266	0.208	0.246	0.224	0.234	0.216	0.276	0.226	0.232	0.236	0.246	0.246
	Average weight per initial number of shrimp (mg) 0.238								Average weight per initial number of shrimp (mg) 0.239				Percent reduction from control (%) -0.37			

*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: **224**

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	4 1d	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	3 2d	3 1d	4 1d	3 2d	5	5
3	5	5	5	5	5	5	5	5	5	5	3	3	4	3	4 1d	5
4	5	5	5	5	5	5	5	5	5	4 1d	3	3	3 1d	2 1d	3 1d	4 1d
5	5	5	5	5	5	5	5	5	4 1d	4	3	3	3	2	3	4
6	5	5	5	5	5	5	5	5	2 2d	4	2 1d	2 1d	3	2	2 1d	3 1d
7	5	5	5	5	5	5	5	5	2	4	2	2	3	2	2	3
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																h 02-15-22
*A = Pan weight (mg) Tray color code: light Pink Analyst: A.I Date: 01-27-22	15.07	14.32	15.16	13.89	14.78	14.91	A.I 01-27-22 ~~13.28~~ 13.29	14.82	15.19	15.54	14.49	15.22	13.22	13.87	15.85	13.17
*B = Pan + Shrimp weight (mg) Analyst: JH Date: 02-17-22	16.28	15.65	16.47	15.09	15.72	15.96	14.51	16.01	15.62	16.41	15.07	15.68	13.93	14.40	16.33	13.94
C = Shrimp weight (mg) = B – A Hand calculated Analyst: JH	1.21	1.33	1.31	1.20	0.94	1.05	1.22	1.19	0.43	0.87	0.58	0.46	0.71	0.53	0.47	0.77
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst: JH	0.242	0.266	0.262	0.240	0.188	0.210	0.244	0.238	0.086	0.174	0.116	0.092	0.142	0.106	0.095	0.154
	Average weight per initial number of shrimp (mg) 0.236				Percent reduction from control (%) 0.87.				Average weight per initial number of shrimp (mg) 0.121				Percent reduction from control (%) 49.47.			

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

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

Comments:

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **224**

Survival and Growth Data

Day	750 mg KCl/L								1000 mg KCl/L							
	GG	HH	II	JJ	KK	LL	MM	NN	OO	PP	QQ	RR	SS	TT	UU	VV
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol	0 Sol
2																
3																
4																
5																
6																
7																
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																
*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%
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*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: 224

Test dates: February 08-15, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	15.01	16.17	1.16	0.232	100.0	0.238	9.4	Not applicable
	B	5	5	14.62	15.87	1.25	0.250				
	C	5	5	14.52	15.59	1.07	0.214				
	D	5	5	15.98	17.31	1.33	0.266				
	E	5	5	14.90	16.23	1.33	0.266				
	F	5	5	15.58	16.62	1.04	0.208				
	G	5	5	13.19	14.42	1.23	0.246				
	H	5	5	15.09	16.21	1.12	0.224				
250	I	5	5	13.44	14.61	1.17	0.234	100.0	0.239	7.5	-0.3
	J	5	5	15.69	16.77	1.08	0.216				
	K	5	5	15.39	16.77	1.38	0.276				
	L	5	5	14.96	16.09	1.13	0.226				
	M	5	5	15.09	16.25	1.16	0.232				
	N	5	5	14.82	16.00	1.18	0.236				
	O	5	5	14.73	15.96	1.23	0.246				
	P	5	5	13.09	14.32	1.23	0.246				
375	Q	5	5	15.07	16.28	1.21	0.242	100.0	0.236	10.9	0.8
	R	5	5	14.32	15.65	1.33	0.266				
	S	5	5	15.16	16.47	1.31	0.262				
	T	5	5	13.89	15.09	1.20	0.240				
	U	5	5	14.78	15.72	0.94	0.188				
	V	5	5	14.91	15.96	1.05	0.210				
	W	5	5	13.29	14.51	1.22	0.244				
	X	5	5	14.82	16.01	1.19	0.238				
500	Y	5	2	15.19	15.62	0.43	0.086	50.0	0.121	26.9	49.4
	Z	5	4	15.54	16.41	0.87	0.174				
	AA	5	2	14.49	15.07	0.58	0.116				
	BB	5	2	15.22	15.68	0.46	0.092				
	CC	5	3	13.22	13.93	0.71	0.142				
	DD	5	2	13.87	14.40	0.53	0.106				
	EE	5	2	15.86	16.33	0.47	0.095				
	FF	5	3	13.17	13.94	0.77	0.154				
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: 0.0312

PMSD: 13.1

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	2/8/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	2/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

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

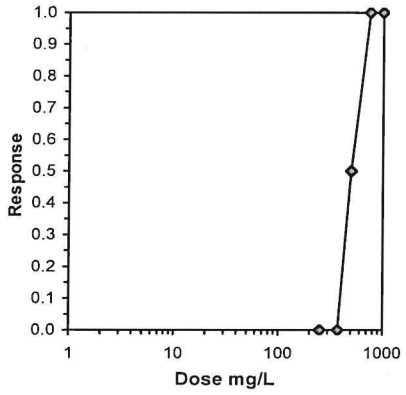
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
*500	0.5000	0.5000	0.7879	0.6847	1.1071	20.036	8	36.00	48.00	20	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.61038	0.904	2.36072	10.4688
Equality of variance cannot be confirmed				

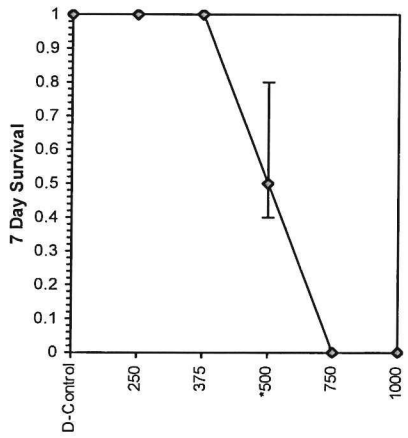
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	375	500	433.013	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	514.94	487.48	543.95
5.0%	513.43	483.15	545.60
10.0%	511.92	478.25	547.96
20.0%	508.91	465.65	556.20
Auto-0.0%	514.94	487.48	543.95



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:	2/8/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2320	0.2500	0.2140	0.2660	0.2660	0.2080	0.2460	0.2240
250	0.2340	0.2160	0.2760	0.2260	0.2320	0.2360	0.2460	0.2460
375	0.2420	0.2660	0.2620	0.2400	0.1880	0.2100	0.2440	0.2380
500	0.0860	0.1740	0.1160	0.0920	0.1420	0.1060	0.0960	0.1540
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

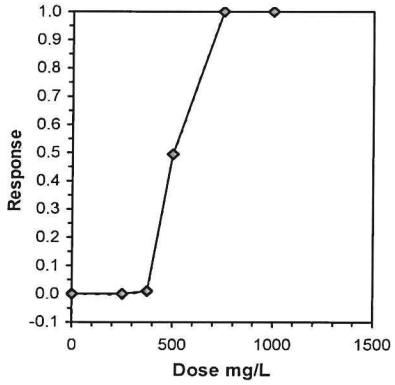
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2383	1.0000	0.2383	0.2080	0.2660	9.363	8				0.2386	1.0000
250	0.2390	1.0031	0.2390	0.2160	0.2760	7.498	8	-0.067	2.799	0.0312	0.2386	1.0000
375	0.2363	0.9916	0.2363	0.1880	0.2660	10.939	8	0.180	2.799	0.0312	0.2363	0.9900
500	0.1208	0.5068	0.1208	0.0860	0.1740	26.706	8				0.1208	0.5060
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)	0.9723	0.884	-0.2467	-0.1951
Bartlett's Test indicates equal variances (p = 0.65)	0.86347	9.21035		

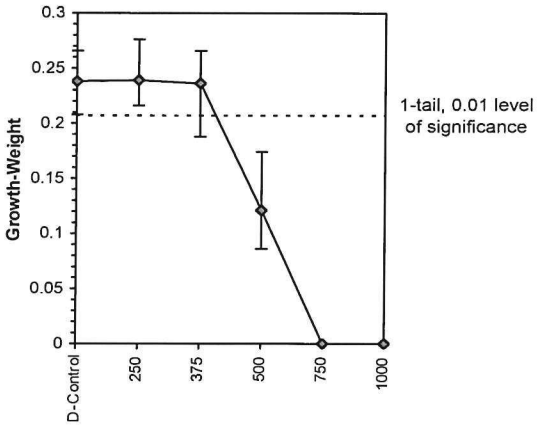
Hypothesis Test (1-tail, 0.01)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	375	>375			0.03115	0.13075	1.6E-05	0.0005	0.96795	2, 21

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	385.34	48.40	214.13	389.56	-2.5542
IC10	398.25	9.68	376.89	404.21	-2.3688
IC15	411.17	7.62	391.12	418.81	-0.6187
IC20	424.08	7.59	404.19	433.88	-0.2304
IC25	436.99	7.86	419.44	448.60	0.1153
IC40	475.73	10.17	458.63	494.16	0.5883
IC50	502.98	15.67	481.84	535.06	0.7022



Dose-Response Plot



Entered and Reviewed by Jim Sumner

AbKClCR Test Number: **224**

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.)	8.12	7.81	~~7.88~~ 8.00	7.72	7.03	7.75
	DO (mg/L)	7.9	7.7	7.7	7.5	7.8	7.8
	Salinity (ppt)	25.0	25.1	24.9	25.2	24.9	25.4
	Alkalinity (mg $CaCO_3$/L)	99				100	
	Temperature (°C)	25.3	25.6	25.2	25.4	25.2	25.6
250 mg KCl/L	pH (S.U.)	8.05	7.83	~~7.85~~ 7.90	7.72	7.80	7.75
	DO (mg/L)	7.9	7.7	7.7	7.3	7.8	7.8
	Salinity (ppt)	25.2	25.3	25.3	25.4	25.2	25.5
	Temperature (°C)	25.6	25.5	25.2	25.5	25.4	25.6
375 mg KCl/L	pH (S.U.)	8.06	7.82	~~7.88~~ 7.96	7.71	7.81	7.73
	DO (mg/L)	8.0	7.7	7.7	7.4	7.9	7.8
	Salinity (ppt)	25.2	25.5	25.3	25.7	25.5	25.2
	Temperature (°C)	25.6	25.5	25.4	25.7	25.4	25.5
500 mg KCl/L	pH (S.U.)	8.05	7.82	~~7.8~~ 7.96	7.70	7.81	7.74
	DO (mg/L)	8.0	7.7	7.8	7.5	8.0	7.8
	Salinity (ppt)	25.2	25.6	25.3	26.0	25.6	26.4
	Temperature (°C)	25.7	25.7	25.4	25.7	25.4	25.6
750 mg KCl/L	pH (S.U.)	8.06	7.81				
	DO (mg/L)	8.0	7.8				
	Salinity (ppt)	25.4	25.7				
	Temperature (°C)	25.5	25.7				
1000 mg KCl/L	pH (S.U.)	8.06	7.84				
	DO (mg/L)	8.0	7.6				
	Salinity (ppt)	25.6	25.7				
	Temperature (°C)	25.7	25.7				
		Initial	Final	Initial	Final	Initial	Final

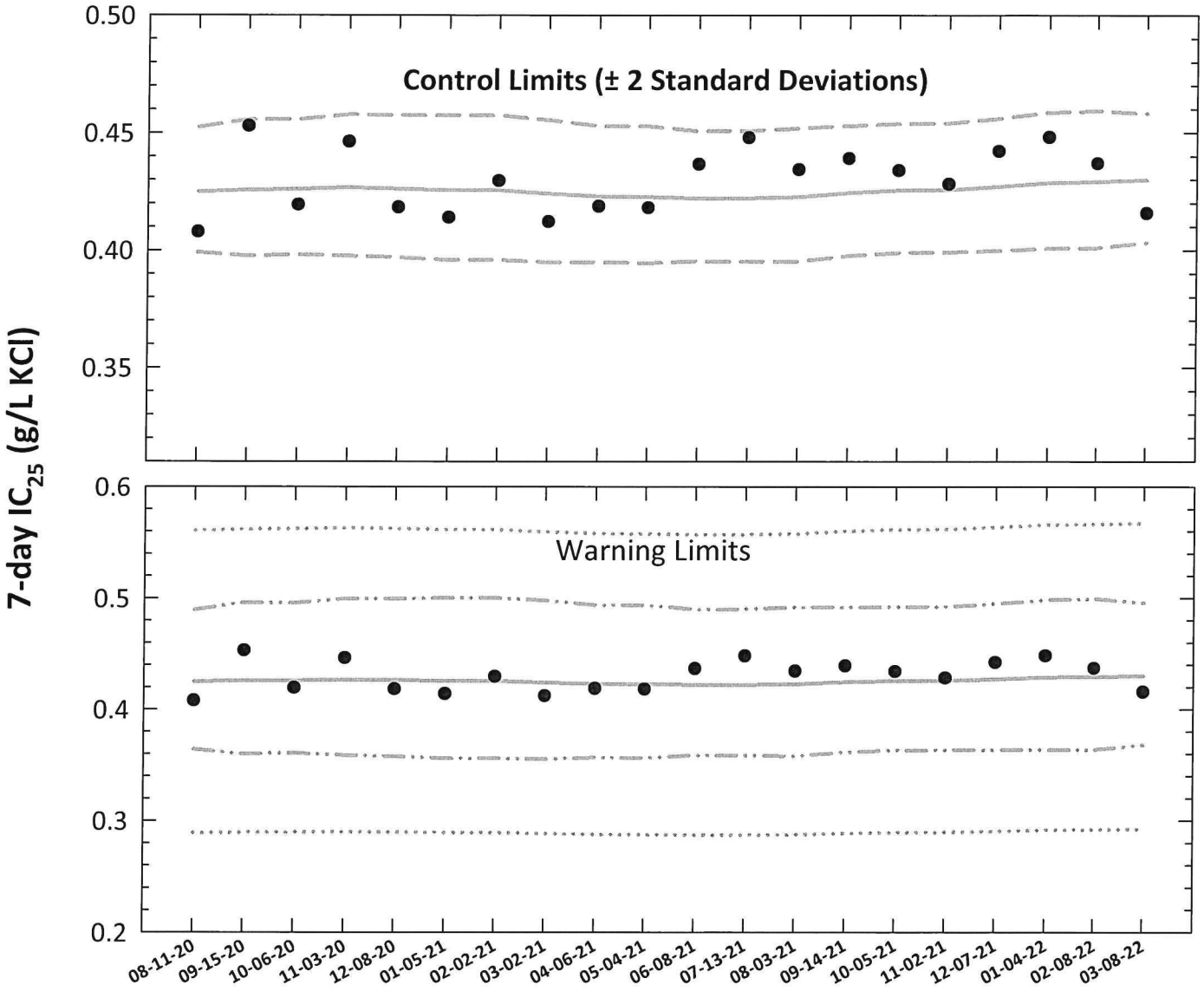
02.09.22

AbKClCR Test Number: 224

Conc.	Parameter	Day (Analyst identified for each day, performed pH, D.O. and salinity measurements only.)							
		3		4		5		6	
	Analyst	N	BSL	BSL	N	N	JW	JW	JW
CONTROL, Salt SW	pH (S.U.)	7.84	7.80	7.94	7.77	7.95	7.86	7.92	7.80
	DO (mg/L)	8.0	7.6	7.8	7.5	7.7	7.5	8.0	7.5
	Salinity (ppt)	25.0	25.3	25.0	25.4	24.7	25.1	24.7	25.9
	Alkalinity (mg $CaCO_3$/L)	———	2-14-22	98		———	2-14-22	100	
	Temperature (°C)	25.2	25.8	25.2	25.3	25.2	25.7	25.2	25.6
250 mg KCl/L	pH (S.U.)	7.85	7.84	7.92	7.79	7.92	7.85	7.90	7.82
	DO (mg/L)	8.1	7.6	8.0	7.4	7.8	7.5	8.0	7.5
	Salinity (ppt)	25.3	25.4	25.3	25.5	25.3	25.5	25.6	25.8
	Temperature (°C)	25.4	25.5	25.2	25.5	25.1	25.5	25.2	25.6
375 mg KCl/L	pH (S.U.)	7.85	7.80	7.93	7.79	7.92	7.86	7.90	7.81
	DO (mg/L)	8.1	7.6	7.9	7.4	7.8	7.5	8.0	7.5
	Salinity (ppt)	25.2	25.6	25.5	25.9	25.4	25.9	25.7	26.1
	Temperature (°C)	25.4	25.8	25.4	25.5	25.2	25.6	25.2	25.6
500 mg KCl/L	pH (S.U.)	7.86	7.77	7.93	7.76	7.92	7.86	7.91	7.83
	DO (mg/L)	8.1	7.6	7.9	7.5	7.9	7.6	8.1	7.4
	Salinity (ppt)	25.3	25.9	25.7	26.0	25.5	25.9	25.8	26.0
	Temperature (°C)	25.4	25.7	25.4	25.4	25.2	25.6	25.2	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)								
	Salinity (ppt)						2-09-22		
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	08-11-20	0.4078	-0.3895	-0.3718	0.0136	0.4249	0.3991	0.4523	0.3641	0.4895	0.2889	0.5608
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

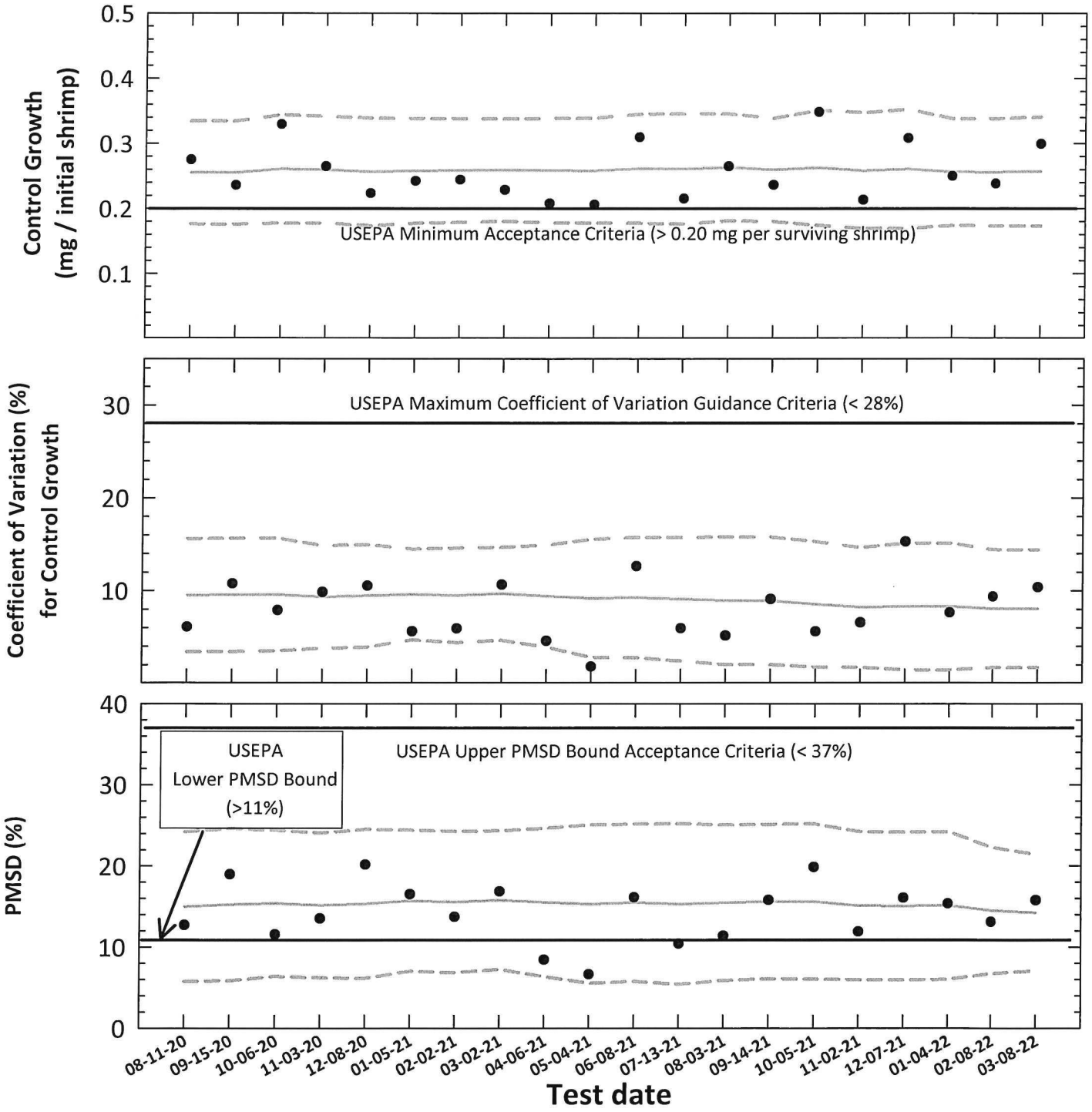
Entered and Reviewed by Jim Sumner

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		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial shrimp)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	08-11-20	100	0.275	6.1	0.0349	12.7	0.255	0.176	0.334	9.5	3.4	15.6	15.0	5.7	24.2
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

MSD = Minimum significant difference.

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

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

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

Entered and Reviewed by Jim Summer

Environmental Testing Solutions, Inc.

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

Species: *Americamysis (Mysidopsis) bahia*

AbKClCR Test Number: **225**

Dilution preparation information:						***Comments:***
KCl Stock INSS number:		INSS 2063				
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:	02-28-22 1200 to 03-01-22 1130	Incubator number and shelf location:	5E
Organism source:	AI Batch Ab: 03-01-22	*Artemia* CHM number:	CHM1149
		Drying information for weight determination:	
Transfer bowl information:	pH = 8.03 S.U. Temperature = 25.0 °C	Date / Time in oven:	03-16-22 1156
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	03-16-22 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	03-08-22	1045	JH	1230	JH	1200	JH	03-03-22 A
1	03-09-22	0530	JH	1130	JH	1006	JH	↓
2	03-10-22	0530	JH	1130	JH	1005	JH	03-09-22
3	03-11-22	0530	JH	1130	JH	1000	JH	↓
4	03-12-22	0630	JH	1230	JH	1011	JH	03-11-22
5	03-13-22	0555	JH	1155	JH	1000	JH	↓
6	03-14-22	0600	JH	1200	JH	1007	JH	↓
7	03-15-22					1112	JH	

Chemical analyses:

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

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	506.3
Average weight per initial shrimp:	0.299		**NOEC** (mg/L KCl)	375
Average weight per surviving shrimp:	0.299	≥ 0.20 mg/shrimp	**LOEC** (mg/L KCl)	500
			ChV (mg/L KCl)	433.0
			IC_{25} (mg/L KCl)	415.8

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **225**

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																h 02-15-22
*A = Pan weight (mg) Tray color code: 1-7&+ blue Analyst: A.S Date: 02-25-22	14.57	13.72	14.04	15.34	15.06	14.65	14.83	13.58	14.81	13.56	12.62	02-25-22 A.S ~~12.67~~ 12.66	15.77	13.99	15.63	14.66
*B = Pan + Shrimp weight (mg) Analyst: A.S Date: 03-18-22	16.02	15.42	~~14.58~~ Continued on 3.14 15.58	16.89	16.66	15.83	16.24	15.12	16.47	15.06	14.01	14.31	17.14	15.78	16.87	15.99
C = Shrimp weight (mg) = B – A Hand calculated Analyst:	1.45	1.70	1.54	1.55	1.60	1.18	1.41	1.54	1.66	1.50	1.39	1.65	1.37	1.79	1.24	1.33
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst:	0.290	0.340	0.308	0.310	0.320	0.236	0.282	0.308	0.332	0.300	0.278	0.330	0.274	0.358	0.248	0.266
	Average weight per initial number of shrimp (mg) 0.299								Average weight per initial number of shrimp (mg) 0.298				Percent reduction from control (%) 0.37			

*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: **225**

Survival and Growth Data

Day	375 mg KCl/L								500 mg KCl/L							
	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	4 1d	4 1d	5	5	4 1d	5
3	5	5	5	5	5	5	5	5	4 1d	4 1d	4	4	4 1d	4 1d	4	4 1d
4	5	5	5	5	5	4 1d	5	5	4	3 1d	4	4	3 1d	2 2d	3 1d	4
5	5	5	5	5	5	4	5	5	4	3	4	4	3	2	3	4
6	5	5	5	5	5	4	5	5	2 2d	2 1d	3 1d	3 1d	3	2	2 1d	4
7	5	5	5	5	5	4	5	5	2	3	3	3	3	2	2	4
# females with eggs in brood sac																
# females with developing ova in oviducts																
# immature females																
# males																1k 3-d
*A = Pan weight (mg) Tray color code: Light blue Analyst: A.S Date: 02-25-22	15.11	14.93	15.43	14.20	16.37	13.12	14.86	16.07	15.98	13.72	15.51	14.60	14.96	13.68	15.96	15.49
*B = Pan + Shrimp weight (mg) Analyst: A.S Date: 02-28-22	16.40	16.36	16.71	15.29	17.98	14.31	16.12	17.36	16.64	14.49	16.40	15.44	15.59	14.17	16.54	16.59
C = Shrimp weight (mg) = B – A Hand calculated Analyst:	1.29	1.43	1.28	1.09	1.61	1.19	1.26	1.29	0.66	0.77	0.89	0.84	0.63	0.49	0.58	1.10
Weight per initial number of shrimp (mg) = C / Initial number of shrimp Hand calculated Analyst:	0.258	0.286	0.256	0.218	0.322	0.238	0.252	0.258	0.132	0.154	0.178	0.168	0.126	0.098	0.116	0.220
	Average weight per initial number of shrimp (mg) 0.261				Percent reduction from control (%) 12.8%				Average weight per initial number of shrimp (mg) 0.149				Percent reduction from control (%) 50.2%			

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

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

Comments:

ETS Environmental Testing Solutions, Inc.

AbKClCR Test Number: **225**

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 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl	0 Scl
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%

*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: 225

Test dates: March 08-15, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	5	5	14.57	16.02	1.45	0.290	100.0	0.299	10.4	Not applicable
	B	5	5	13.72	15.42	1.70	0.340				
	C	5	5	14.04	15.58	1.54	0.308				
	D	5	5	15.34	16.89	1.55	0.310				
	E	5	5	15.06	16.66	1.60	0.320				
	F	5	5	14.65	15.83	1.18	0.236				
	G	5	5	14.83	16.24	1.41	0.282				
	H	5	5	13.58	15.12	1.54	0.308				
250	I	5	5	14.81	16.47	1.66	0.332	100.0	0.298	12.9	0.3
	J	5	5	13.56	15.06	1.50	0.300				
	K	5	5	12.62	14.01	1.39	0.278				
	L	5	5	12.66	14.31	1.65	0.330				
	M	5	5	15.77	17.14	1.37	0.274				
	N	5	5	13.99	15.78	1.79	0.358				
	O	5	5	15.63	16.87	1.24	0.248				
	P	5	5	14.66	15.99	1.33	0.266				
375	Q	5	5	15.11	16.40	1.29	0.258	97.5	0.261	12.0	12.8
	R	5	5	14.93	16.36	1.43	0.286				
	S	5	5	15.43	16.71	1.28	0.256				
	T	5	5	14.20	15.29	1.09	0.218				
	U	5	5	16.37	17.98	1.61	0.322				
	V	5	4	13.12	14.31	1.19	0.238				
	W	5	5	14.86	16.12	1.26	0.252				
	X	5	5	16.07	17.36	1.29	0.258				
500	Y	5	2	15.98	16.64	0.66	0.132	55.0	0.149	26.3	50.2
	Z	5	3	13.72	14.49	0.77	0.154				
	AA	5	3	15.51	16.40	0.89	0.178				
	BB	5	3	14.60	15.44	0.84	0.168				
	CC	5	3	14.96	15.59	0.63	0.126				
	DD	5	2	13.68	14.17	0.49	0.098				
	EE	5	2	15.96	16.54	0.58	0.116				
	FF	5	4	15.49	16.59	1.10	0.220				
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: 0.0472

PMSD: 15.8

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

Entered and Reviewed by Jen Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Mysid Survival and Growth Test-7 Day Survival

Start Date:	3/8/2022	Test ID:	AbKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8			0	40
250	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	8	68.00	48.00	0	40
375	0.9750	0.9750	1.3155	1.1071	1.3453	6.400	8	64.00	48.00	1	40
*500	0.5500	0.5500	0.8382	0.6847	1.1071	17.590	8	36.00	48.00	18	40
750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40
1000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	8			40	40

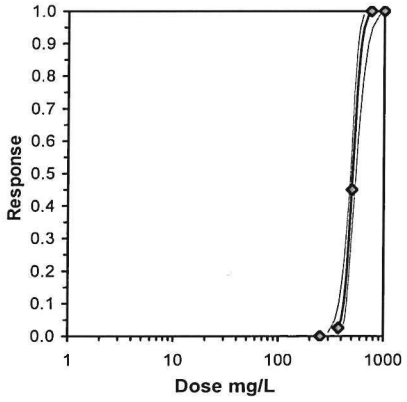
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.72744	0.904	0.01204	4.82568
Equality of variance cannot be confirmed				

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

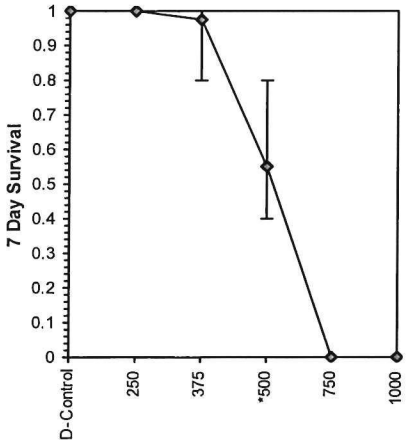
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.8775	3.06249	9.87504	21.88	0	0.24502	7.81472	0.97001	2.70444	0.06298	4
Intercept	-37.94	8.23191	-54.074	-21.805							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	361.343	298.176	396.465
EC05	3.355	398.88	347.877	427.966
EC10	3.718	420.459	376.957	446.62
EC15	3.964	435.674	397.363	460.328
EC20	4.158	448.158	413.826	472.138
EC25	4.326	459.153	427.94	483.133
EC40	4.747	488.07	462.193	515.847
EC50	5.000	506.336	481.151	539.88
EC60	5.253	525.285	498.837	567.354
EC75	5.674	558.366	526.466	619.923
EC80	5.842	572.065	537.141	642.958
EC85	6.036	588.458	549.531	671.29
EC90	6.282	609.752	565.158	709.182
EC95	6.645	642.739	588.611	770.004
EC99	7.326	709.507	634.212	900.007



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:	3/8/2022	Test ID:	AbKClCR	Sample ID:	REF-Ref Toxicant
End Date:	3/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	AB-Americamysis bahia
Comments:					

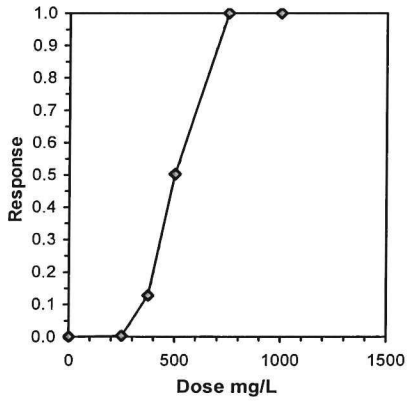
Conc-mg/L	1	2	3	4	5	6	7	8
D-Control	0.2900	0.3400	0.3080	0.3100	0.3200	0.2360	0.2820	0.3080
250	0.3320	0.3000	0.2780	0.3300	0.2740	0.3580	0.2480	0.2660
375	0.2580	0.2860	0.2560	0.2180	0.3220	0.2380	0.2520	0.2580
500	0.1320	0.1540	0.1780	0.1680	0.1260	0.0980	0.1160	0.2200
750	0.0000	0.0000	0.0000	0.0000	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	0.2993	1.0000	0.2993	0.2360	0.3400	10.372	8				0.2993	1.0000
250	0.2983	0.9967	0.2983	0.2480	0.3580	12.851	8	0.059	2.799	0.0472	0.2983	0.9967
375	0.2610	0.8722	0.2610	0.2180	0.3220	11.984	8	2.269	2.799	0.0472	0.2610	0.8722
500	0.1490	0.4979	0.1490	0.0980	0.2200	26.333	8				0.1490	0.4979
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

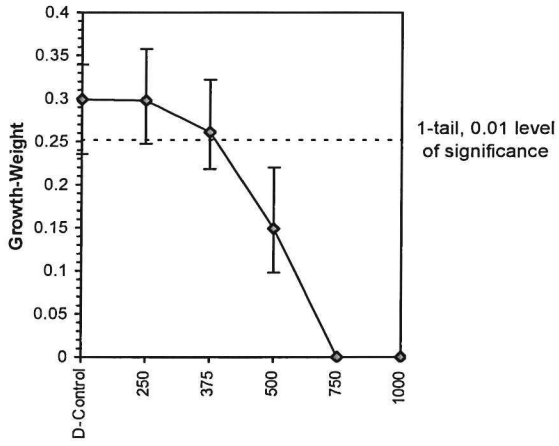
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.98397		0.884		0.10073	-0.2888
Bartlett's Test indicates equal variances (p = 0.82)					0.39218		9.21035			
Hypothesis Test (1-tail, 0.01)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	375	>375			0.04718	0.15766	0.0038	0.00114	0.05485	2, 21
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	296.85	56.05	133.59	378.37	-1.0316
IC10	347.06	34.02	263.04	392.51	-0.3974
IC15	382.41	19.29	331.09	407.67	-0.8063
IC20	399.11	12.48	372.65	423.04	-0.3156
IC25	415.81	11.14	393.04	437.62	0.0353
IC40	465.90	12.91	447.53	498.71	0.7243
IC50	499.30	16.84	478.09	540.48	0.6020



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

AbKClCR Test Number: **225**

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	M.	M	M	M	M	M
CONTROL, Salt SW	pH (S.U.)	7.93	7.83	7.86	7.79	8.01	7.83
	DO (mg/L)	8.0	7.7	7.8	7.3	7.6	7.1
	Salinity (ppt)	24.7	25.1	25.2	25.0	24.9	25.4
	Alkalinity (mg $CaCO_3$/L)	100			03-22 M	140	
	Temperature (°C)	25.4	25.7	25.4	25.6	25.2	25.7
250 mg KCl/L	pH (S.U.)	7.93	7.80	7.87	7.77	7.95	7.77
	DO (mg/L)	7.9	7.8	7.8	7.3	7.7	7.0
	Salinity (ppt)	25.2	25.3	25.2	25.2	25.1	25.3
	Temperature (°C)	25.5	25.6	25.3	25.6	25.3	25.5
375 mg KCl/L	pH (S.U.)	7.96	7.79	7.88	7.81	7.95	7.78
	DO (mg/L)	7.9	7.7	7.8	7.3	7.7	7.4
	Salinity (ppt)	25.1	25.4	25.0	25.5	25.4	25.7
	Temperature (°C)	25.5	25.6	25.3	25.5	25.3	25.7
500 mg KCl/L	pH (S.U.)	7.90	7.84	7.89	7.83	7.95	7.74
	DO (mg/L)	7.9	7.7	7.8	7.5	7.7	7.5
	Salinity (ppt)	25.2	25.4	25.3	25.6	25.5	25.7
	Temperature (°C)	25.3	25.6	25.3	25.7	25.4	25.7
750 mg KCl/L	pH (S.U.)	7.97	7.80				
	DO (mg/L)	8.0	7.8				
	Salinity (ppt)	25.4	25.6				
	Temperature (°C)	25.3	25.7				
1000 mg KCl/L	pH (S.U.)	7.99	7.85				
	DO (mg/L)	8.0	7.9				
	Salinity (ppt)	25.6	25.7				
	Temperature (°C)	25.4	25.5				
		Initial	Final	Initial	Final	Initial	Final

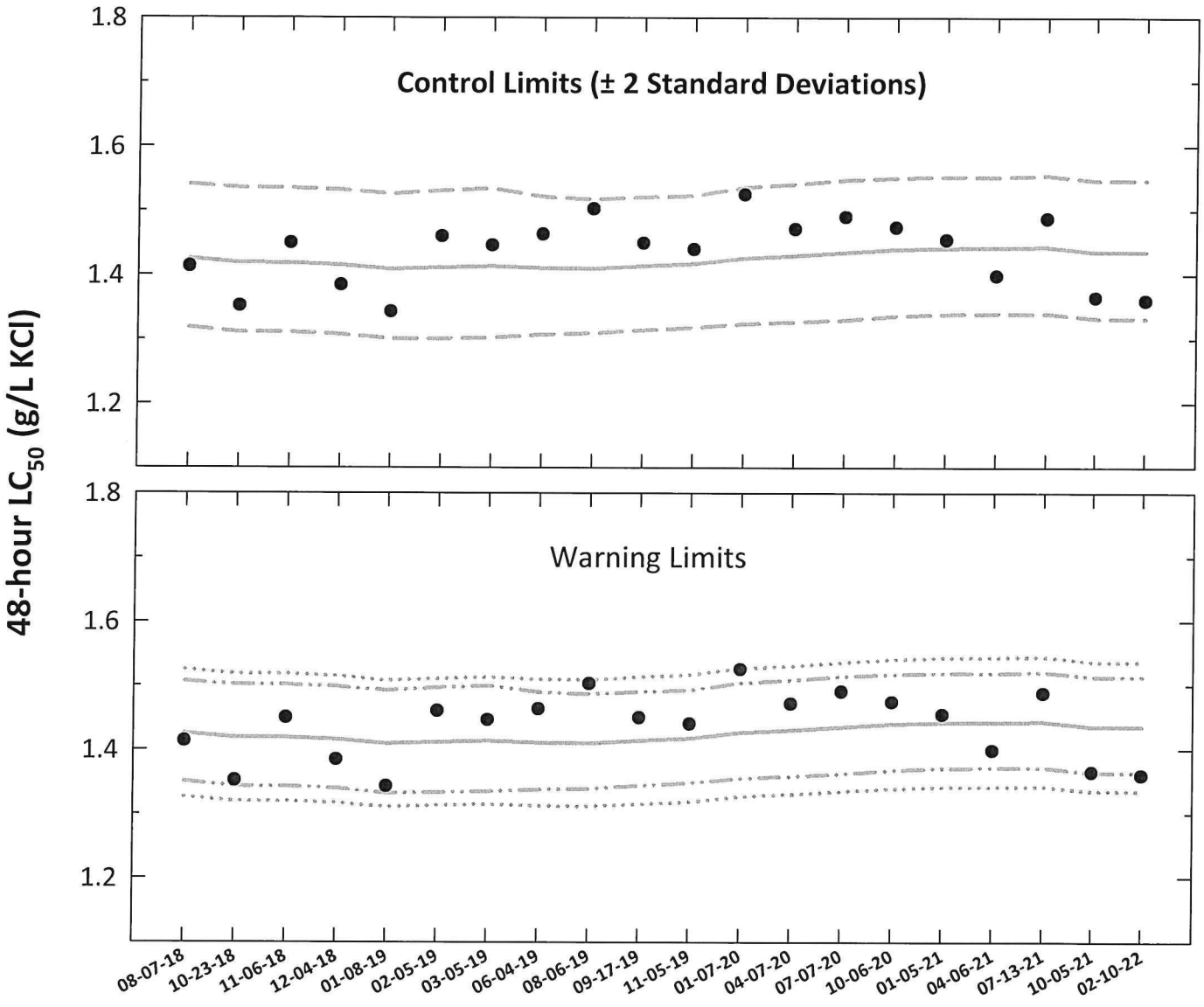
Environmental Testing Solutions, Inc.

AbKClCR Test Number: **225**

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	JW
CONTROL, Salt SW	pH (S.U.)	8.00	7.97	8.06	7.88	8.10	7.96	8.00	7.80
	DO (mg/L)	7.8	7.7	7.7	7.3	7.9	7.1	7.9	7.5
	Salinity (ppt)	25.2	25.2	25.0	24.3	24.7	24.8	24.8	25.0
	Alkalinity (mg $CaCO_3$/L)			120					
	Temperature (°C)	25.3	25.7	25.3	25.6	25.3	25.7	25.4	25.6
250 mg KCl/L	pH (S.U.)	8.00	7.90	8.06	7.87	8.07	7.89	8.03	7.88
	DO (mg/L)	7.7	7.6	7.7	7.3	8.1	7.2	8.0	7.7
	Salinity (ppt)	25.6	25.6	25.7	25.9	25.2	25.3	25.3	25.4
	Temperature (°C)	25.2	25.8	25.4	25.4	25.5	25.7	25.3	25.7
375 mg KCl/L	pH (S.U.)	8.00	7.93	8.06	7.88	8.08	7.91	8.05	7.88
	DO (mg/L)	7.10	7.5	7.7	7.4	8.1	7.5	8.0	7.8
	Salinity (ppt)	25.6	25.8	25.9	26.2	25.3	25.3	25.4	25.7
	Temperature (°C)	25.3	24.6	25.4	25.4	25.5	25.6	25.3	25.7
500 mg KCl/L	pH (S.U.)	8.00	7.93	8.07	7.90	8.10	7.90	8.06	7.91
	DO (mg/L)	7.9	7.4	7.7	7.5	8.2	7.5	8.1	7.8
	Salinity (ppt)	25.9	25.9	25.9	26.3	25.3	25.5	25.5	25.8
	Temperature (°C)	25.3	25.8	25.4	25.6	25.4	25.6	25.5	25.7
750 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
1000 mg KCl/L	pH (S.U.)								
	DO (mg/L)								
	Salinity (ppt)								
	Temperature (°C)								
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-07-18	1.4133	0.1502	0.1539	0.0169	1.4252	1.3183	1.5408	1.3502	1.5063	1.3254	1.5250
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim 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 # 83

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 #: 2063

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	K	K	BSa
Control, SaltSW	pH (S.U.)	7.83	7.87	7.89
	Dissolved oxygen (mg/L)	7.8	7.7	7.8
	*Salinity (ppt)	24.9	25.2	25.3
	*Alkalinity (mg/L $CaCO_3$)	100		
	*Temperature (°C)	25.2	25.2	25.0
1000 mg/L	pH (S.U.)	7.96	7.84	7.85
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	*Salinity (ppt)	25.5	25.7	25.7
	*Temperature (°C)	25.2	25.3	25.2
1250 mg/L	pH (S.U.)	7.96	7.82	7.82
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	*Salinity (ppt)	25.6	25.8	26.0
	*Temperature (°C)	25.3	25.3	25.2
1500 mg/L	pH (S.U.)	7.97	7.81	7.85
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	*Salinity (ppt)	25.8	26.0	26.1
	*Temperature (°C)	25.3	25.4	25.1
1750 mg/L	pH (S.U.)	7.96	7.80	7.86
	Dissolved oxygen (mg/L)	8.1	7.8	7.7
	*Salinity (ppt)	25.9	26.2	26.4
	*Temperature (°C)	25.4	25.1	25.1
2000 mg/L	pH (S.U.)	7.94	7.77	
	Dissolved oxygen (mg/L)	8.1	7.8	
	*Salinity (ppt)	26.1	26.3	
	*Temperature (°C)	25.3	25.4	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Salinity	1.0 ppt	SM 2520 B-2011	YSI PRO30	18D104324
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

ETS

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 # 83

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	SaltSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	02-10-22	* 0550	JH	0750	JH	7C	ORANGE	02-08-22
24	02-11-22			0748	JH			
48 Termination	02-12-22			0751	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. 02-01-22 JH
Batch (AI Batch Ab):	~~AIAb 01-28-22~~ 01-31-22
Age (9 to 14 days old):	10-11 DAYS
Date organisms were born: (time organisms were born between is not provided by supplier)	01-30-22 1200 TO 01-31-22 1130
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.00
	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	6 4d	6 4d	4 6d	5 5d	1 9d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	6	6	4	3 2d	1	0	0	0
Mean Survival	100%		100%		60%		35%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	1277.4
Upper 95% confidence limit (mg KCl/L)	1436.1
48-hour LC_{50} (mg KCl/L)	1359.9

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-24 Hr Survival

Start Date:	2/10/2022	Test ID:	MbKClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/12/2022	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	0.6000	0.6000
1500	0.4000	0.5000
1750	0.1000	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	0.1726	0	20
*1250	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	8.682	2.850	0.1726	8	20
*1500	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	11.175	2.850	0.1726	11	20
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.343	2.850	0.1726	19	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

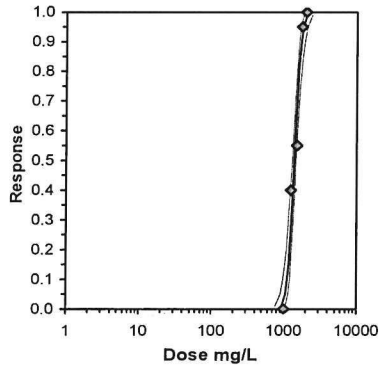
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1250	1118.03		0.08088	0.08295	0.49005	0.00367	2.9E-05	4, 5

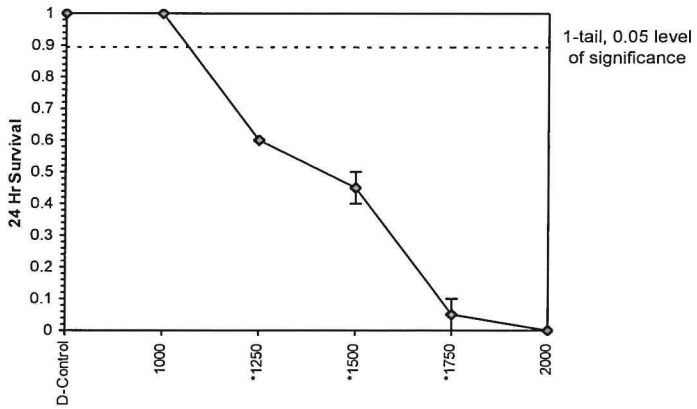
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1159	2.31835	9.57193	18.6598	0	4.52766	7.81472	0.20984	3.14019	0.07084	4
Intercept	-39.327	7.31039	-53.655	-24.998							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	944.898	769.819	1055.51
EC05	3.355	1056	903.498	1152.5
EC10	3.718	1120.47	982.874	1209.19
EC15	3.964	1166.18	1039.53	1249.98
EC20	4.158	1203.83	1086.17	1284.19
EC25	4.326	1237.1	1127.17	1315.1
EC40	4.747	1325.08	1232.9	1401.49
EC50	5.000	1380.98	1296.49	1461.48
EC60	5.253	1439.25	1358.56	1529.42
EC75	5.674	1541.6	1456.92	1662.41
EC80	5.842	1584.2	1494.55	1722.18
EC85	6.036	1635.35	1537.84	1796.68
EC90	6.282	1702.07	1591.94	1897.57
EC95	6.645	1805.98	1672.52	2061.48
EC99	7.326	2018.33	1828.63	2416.26



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Silverside Test-48 Hr Survival

Start Date:	2/10/2022	Test ID:	MbKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/12/2022	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	0.6000	0.6000
1500	0.4000	0.3000
1750	0.1000	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	0.1748	0	20
*1250	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	8.577	2.850	0.1748	8	20
*1500	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	12.718	2.850	0.1748	13	20
*1750	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.109	2.850	0.1748	19	20
2000	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

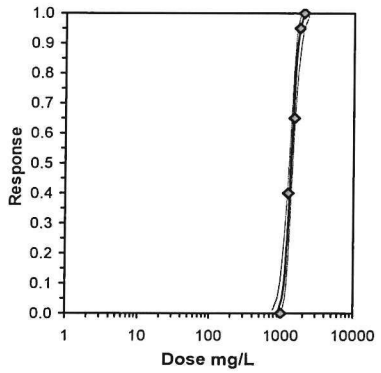
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1250	1118.03		0.08218	0.08429	0.51506	0.00376	2.7E-05	4, 5

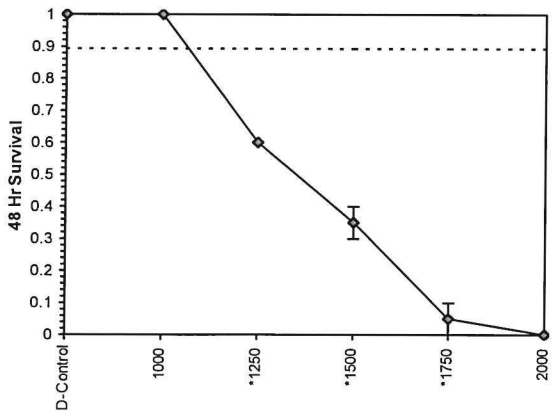
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.6599	2.43666	9.88405	19.4358	0	2.42464	7.81472	0.48906	3.13349	0.06821	4
Intercept	-40.937	7.6667	-55.963	-25.91							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	943.634	771.514	1051.29
EC05	3.355	1050.24	900.836	1144.02
EC10	3.718	1111.92	977.306	1198.11
EC15	3.964	1155.56	1031.75	1236.98
EC20	4.158	1191.47	1076.5	1269.57
EC25	4.326	1223.16	1115.76	1298.99
EC40	4.747	1306.8	1216.8	1381.14
EC50	5.000	1359.85	1277.43	1438.09
EC60	5.253	1415.05	1336.52	1502.5
EC75	5.674	1511.82	1429.96	1628.33
EC80	5.842	1552.03	1465.63	1684.81
EC85	6.036	1600.26	1506.59	1755.14
EC90	6.282	1663.07	1557.69	1850.28
EC95	6.645	1760.73	1633.59	2004.59
EC99	7.326	1959.65	1780.08	2337.45



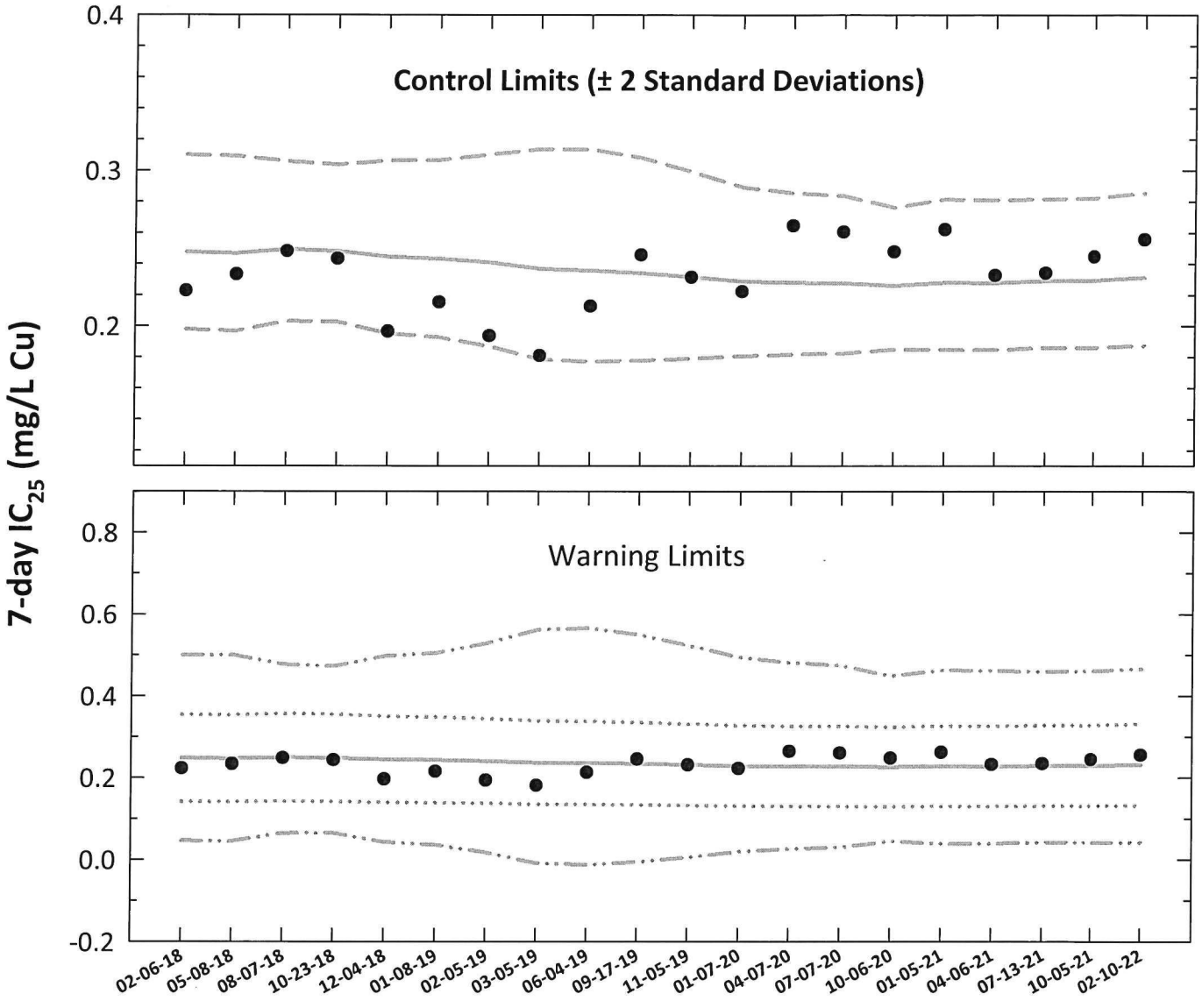
Dose-Response Plot



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (mg/L Cu)	Log_{10} Conversion			Anti-logarithmic Values (mg/L Cu)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	02-06-18	0.2228	-0.6521	-0.6060	0.0487	0.2477	0.1979	0.3101	0.0466	0.4994	0.1412	0.3542
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

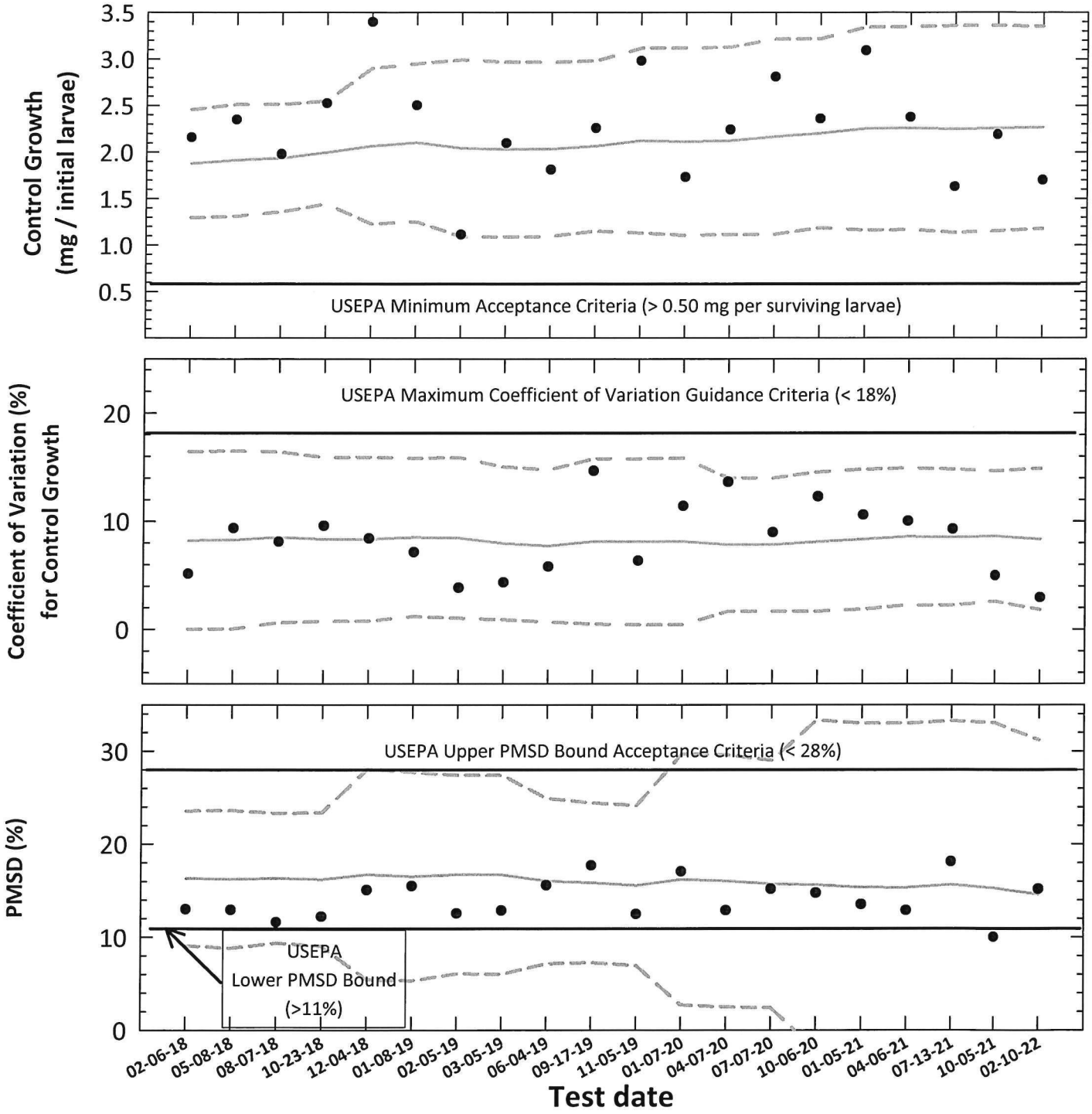
Entered and Reviewed by Jim Sumner

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			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 larvae)	(%)		(%)									
1	02-06-18	100	2.159	5.2	0.2807	13.0	1.8767	1.297	2.456	8.2	0.0	16.4	16.3	9.1	23.6
2	05-08-18	100	2.349	9.4	0.3041	12.9	1.914	1.313	2.515	8.3	0.1	16.5	16.2	8.8	23.6
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 Summer

Environmental Testing Solutions, Inc.

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

Species: *Menidia beryllina*

MbCuCR Test Number: **136**

Dilution preparation information:						***Comments:***
Cu Stock INSS number:		INSS 7072				
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 02-10-22 H
Batch:	Al Mb ~~01-29-22 H~~ 01-31-22
Hatch dates and times:	01-30-22 1200 to 01-31-22 1130
Transfer vessel information:	pH (S.U.) = 8.00 Temperature (°C) = 24.9
Average transfer volume (mL):	< 0.25 mL

Test information:

Randomizing template:	PINK
Incubator number and shelf location:	7B
Artemia CHM number:	CHM1149
Drying information for weight determination:	
Date / Time in oven:	02-17-22 0846
*Initial oven temperature:	60° C
Date / Time out of oven:	02-18-22 0850
*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	02-10-22	0550	H	~~1200~~ 0750 02-10-22	H	0750	H		02-08-22
1	02-11-22	0600	H	1200	H	0850	H		↓
2	02-12-22	0600	H	1200	H	0852	H		02-10-22
3	02-13-22	0600	H	1200	H	0850	H		↓
4	02-14-22	0600	H	1200	H	0850	H		02-12-22A
5	02-15-22	0600	H	1200	H	0800	H		02-12-22B
6	02-16-22	0605	H	1205	H	0811	H		↓
7	02-17-22					0846	H	02-18-22 H	

Chemical analyses:

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

Control information:		Acceptance criteria
% Mortality:	07.	≤ 20%
Average weight per initial larvae:	1.701	
Average weight per surviving larvae:	1.701	≥ 0.25mg/larvae

Summary of test endpoints:	mg/L Cu
7-day LC_{50} (%) 02-11-22 H	0.33
NOEC (%)	0.2
LOEC (%)	0.5
ChV (%)	0.32
IC_{25} (%)	0.256

ETS Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **136**

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: Ruby Analyst: A.S Date: 01-25-22	15.45	14.84	13.52	14.05	15.73	15.49	14.78	13.79	15.83	15.10	16.53	14.90
*B = Pan + Larvae weight (mg) Analyst: A.S Date: 03-01-22	32.95	32.22	30.17	30.56	30.79	32.48	34.47	33.34	33.62	28.67	33.51	32.65
C = Larvae weight (mg) = B – A Analyst: JH	17.50	17.38	16.65	16.51	15.06	16.99	19.69	19.55	17.79	13.57	16.98	17.75
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	1.750	1.738	1.665	1.651	1.506	1.699	1.969	1.955	1.779	1.357	1.698	1.775
Average weight per initial number of larvae (mg)	1.701				1.782		-4.87.		1.652		2.97.	
Percent reduction from control (%)												

*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: **136**

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	9 1d	10	10	10	4 6d	3 7d	4 6d	4 6d
2	10	10	10	10	9	10	10	10	3 1d	2 1d	2 1d	3 1d
3	10	10	10	10	9	10	10	10	2 1d	2	2	3
4	10	10	10	10	9	10	10	10	2	2	2	3
5	10	10	10	10	9	9 1d	10	10	2	1 1d	2	2 1d
6	10	10	10	10	9	9	10	10	2	1	2	2
7	10	10	10	10	9	9	9 1d	10	2	1	1 1d	1 1d
*A = Pan weight (mg) Tray color code: Ruby Analyst: A.S Date: 01-25-22	14.55	16.47	14.40	13.47	13.78	14.68	14.51	13.27	15.47	14.67	14.57	13.47
*B = Pan + Larvae weight (mg) Analyst: A.S Date: 02-01-22	32.18	35.06	31.42	31.10	28.30	30.14	29.57	31.26	17.88	15.43	15.83	14.05
C = Larvae weight (mg) = B – A Analyst:	17.63	18.59	17.02	17.63	14.52	15.46	15.06	17.99	2.41	0.76	1.26	0.58
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst:	1.763	1.859	1.702	1.763	1.452	1.546	1.506	1.799	0.241	0.076	0.126	0.058
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	1.772		-4.27.		1.576		7.47.		0.125		92.67.	

*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: 136

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	15.45	32.95	17.50	1.750	1.701	3.0	1.750	100.0	1.701	3.0	Not applicable
	B	10	10	14.84	32.22	17.38	1.738			1.738				
	C	10	10	13.52	30.17	16.65	1.665			1.665				
	D	10	10	14.05	30.56	16.51	1.651			1.651				
0.025	E	10	10	15.73	30.79	15.06	1.506	1.782	12.5	1.506	100.0	1.782	12.5	-4.8
	F	10	10	15.49	32.48	16.99	1.699			1.699				
	G	10	10	14.78	34.47	19.69	1.969			1.969				
	H	10	10	13.79	33.34	19.55	1.955			1.955				
0.050	I	10	10	15.83	33.62	17.79	1.779	1.652	12.1	1.779	100.0	1.652	12.1	2.9
	J	10	10	15.10	28.67	13.57	1.357			1.357				
	K	10	10	16.53	33.51	16.98	1.698			1.698				
	L	10	10	14.90	32.65	17.75	1.775			1.775				
0.100	M	10	10	14.55	32.18	17.63	1.763	1.772	3.7	1.763	100.0	1.772	3.7	-4.2
	N	10	10	16.47	35.06	18.59	1.859			1.859				
	O	10	10	14.40	31.42	17.02	1.702			1.702				
	P	10	10	13.47	31.10	17.63	1.763			1.763				
0.200	Q	10	9	13.78	28.30	14.52	1.613	1.701	4.6	1.452	92.5	1.576	9.8	7.4
	R	10	9	14.68	30.14	15.46	1.718			1.546				
	S	10	9	14.51	29.57	15.06	1.673			1.506				
	T	10	10	13.27	31.26	17.99	1.799			1.799				
0.500	U	10	2	15.47	17.88	2.41	1.205	0.951	35.1	0.241	12.5	0.125	65.8	92.6
	V	10	1	14.67	15.43	0.76	0.760			0.076				
	W	10	1	14.57	15.83	1.26	1.260			0.126				
	X	10	1	13.47	14.05	0.58	0.580			0.058				

Dunnett's MSD value: 0.2583 MSD = Minimum Significant Difference
PMSD: 15.2 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:	2/10/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	CUSO4
Sample Date:		Protocol:	SWCHR-EPA-821-R-02-014	Test Species:	MB-Menidia beryllina
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
0.025	1.0000	1.0000	1.0000	1.0000
0.05	1.0000	1.0000	1.0000	1.0000
0.1	1.0000	1.0000	1.0000	1.0000
0.2	0.9000	0.9000	0.9000	1.0000
0.5	0.2000	0.1000	0.1000	0.1000

			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	0.9250	0.9250	1.2898	1.2490	1.4120	6.318	4	12.00	10.00	3	40
*0.5	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

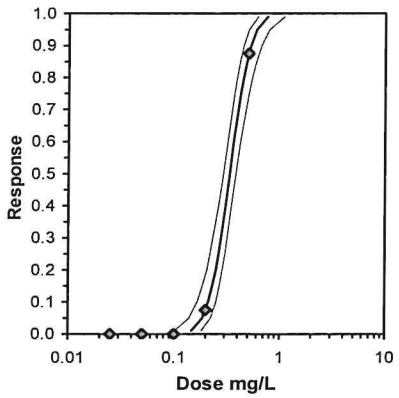
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.63057	0.884	2.15106	5.44198
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.2	0.5	0.31623	
Treatments vs D-Control				

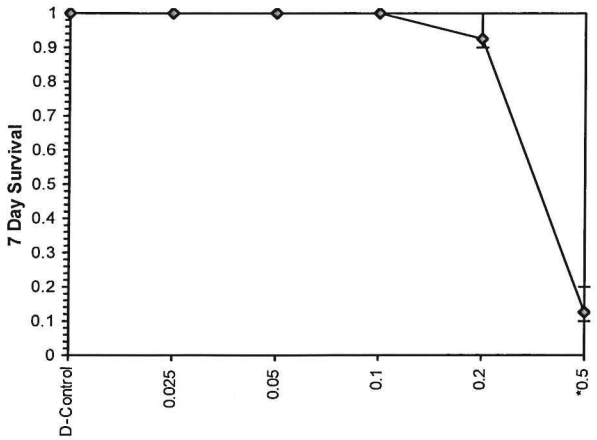
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.53209	0.95759	4.65521	8.40897	0	0.01354	7.81472	0.99958	-0.4775	0.15309	3
Intercept	8.11901	0.49267	7.15337	9.08465							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	0.14668	0.10144	0.1827
EC05	3.355	0.18651	0.14047	0.22276
EC10	3.718	0.21199	0.16653	0.24841
EC15	3.964	0.23112	0.1864	0.26793
EC20	4.158	0.24755	0.20353	0.285
EC25	4.326	0.26257	0.21915	0.30096
EC40	4.747	0.3046	0.26198	0.34796
EC50	5.000	0.33305	0.28972	0.38227
EC60	5.253	0.36416	0.31862	0.4223
EC75	5.674	0.42244	0.36893	0.50408
EC80	5.842	0.44808	0.38976	0.54254
EC85	6.036	0.47993	0.41478	0.59215
EC90	6.282	0.52324	0.44755	0.66252
EC95	6.645	0.59473	0.49931	0.7851
EC99	7.326	0.75623	0.60905	1.08663



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	2/10/2022	Test ID:	MbCuCR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2022	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	1.7500	1.7380	1.6650	1.6510
0.025	1.5060	1.6990	1.9690	1.9550
0.05	1.7790	1.3570	1.6980	1.7750
0.1	1.7630	1.8590	1.7020	1.7630
0.2	1.4520	1.5460	1.5060	1.7990
0.5	0.2410	0.0760	0.1260	0.0580

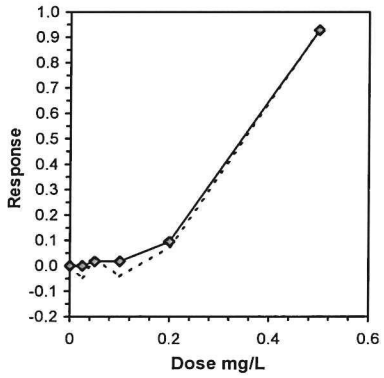
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.7010	1.0000	1.7010	1.6510	1.7500	2.952	4				1.7416	1.0000
0.025	1.7823	1.0478	1.7823	1.5060	1.9690	12.461	4	-0.742	2.360	0.2583	1.7416	1.0000
0.05	1.6523	0.9713	1.6523	1.3570	1.7790	12.125	4	0.445	2.360	0.2583	1.7120	0.9830
0.1	1.7718	1.0416	1.7718	1.7020	1.8590	3.662	4	-0.646	2.360	0.2583	1.7120	0.9830
0.2	1.5758	0.9264	1.5758	1.4520	1.7990	9.756	4	1.144	2.360	0.2583	1.5758	0.9048
0.5	0.1253	0.0736	0.1253	0.0580	0.2410	65.752	4				0.1253	0.0719

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95537	0.868	-0.507	0.27679
Bartlett's Test indicates equal variances (p = 0.12)	7.32431	13.2767		

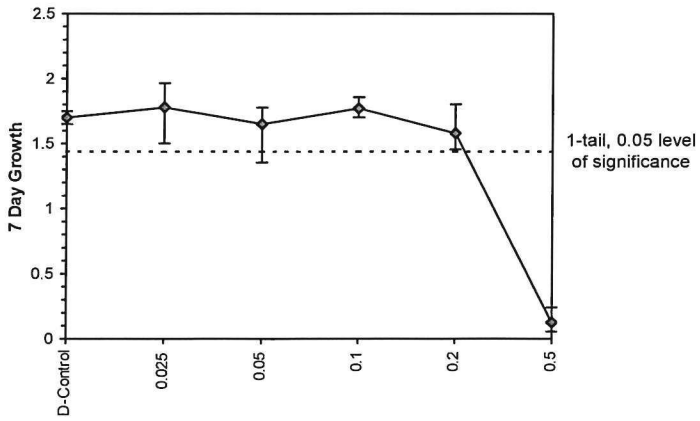
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	0.2	>0.2			0.25833	0.15187	0.02957	0.02396	0.33826	4, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.1422	0.0522	0.0000	0.2471	-0.3375
IC10	0.2017	0.0296	0.0863	0.2389	-1.4601
IC15	0.2197	0.0150	0.1646	0.2556	-0.4423
IC20	0.2377	0.0128	0.1973	0.2723	-0.0244
IC25	0.2557	0.0119	0.2173	0.2887	-0.0292
IC40	0.3098	0.0097	0.2777	0.3384	-0.0499
IC50	0.3458	0.0084	0.3173	0.3706	-0.0580



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **136**

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	K	K	BC	BC	K
Concentration	Parameter						
CONTROL, SaltSW	pH (S.U.)	7.83	7.85	7.86	7.88	7.98 (7.94)	7.77
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.9	7.8	7.9
	Salinity (ppt)	24.9	25.3	25.0	25.4	25.0	25.4
	Alkalinity (mg $CaCO_3$/L)	100		—	02.14.22 K	98	
	Temperature (°C)	25.2	25.2	25.0	25.2	25.0	25.3
0.025 mg/L	pH (S.U.)	7.95	7.83	7.96	7.84	7.99	7.77
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.9	7.9	7.9
	Salinity (ppt)	25.2	25.6	25.2	25.4	25.1	25.4
	Temperature (°C)	25.4	25.4	25.0	25.3	25.1	25.1
0.05 mg/L	pH (S.U.)	7.95	7.82	7.96	7.82	7.99	7.76
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.8	8.0	7.9
	Salinity (ppt)	25.3	25.6	25.1	25.5	24.9	25.3
	Temperature (°C)	25.4	25.3	24.9	25.3	24.9	25.3
0.1 mg/L	pH (S.U.)	7.95	7.83	7.97	7.85	7.99	7.75
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.8	7.9	7.9
	Salinity (ppt)	25.3	25.7	25.1	25.4	25.0	25.4
	Temperature (°C)	25.2	25.3	24.9	25.4	24.9	25.2
0.2 mg/L	pH (S.U.)	7.95	7.82	7.97	7.86	7.99	7.76
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.8	8.0	7.8
	Salinity (ppt)	25.3	25.5	25.1	25.5	25.0	25.3
	Temperature (°C)	25.2	25.6	24.9	25.4	24.9	25.2
0.5 mg/L	pH (S.U.)	7.96	7.81	7.97	7.86	7.99	7.82
	Dissolved oxygen (mg/L)	7.9	7.9	8.1	7.8	8.0	7.8
	Salinity (ppt)	25.3	25.6	25.1	25.3	24.9	25.5
	Alkalinity (mg $CaCO_3$/L)	—	02.16.22 K				
	Temperature (°C)	25.2	25.3	25.0	25.5	25.0	25.1
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Menidia beryllina*** MbCuCR Test Number: **136**

		Day (Analyst identified for each day, performed pH and D.O. measurements only.)							
		3		4		5		6	
	Analyst	K	JW	JW	JW	JW	JW	JW	K
Concentration	Parameter								
CONTROL, SaltSW	pH (S.U.)	7.95	7.94	7.92	7.82	7.86	7.65	7.84	7.72
	Dissolved oxygen (mg/L)	7.7	7.6	8.0	7.5	7.8	7.2	7.7	7.6
	Salinity (ppt)	24.7	25.1	24.7	25.0	24.9	25.6	25.1	25.5
	Alkalinity (mg $CaCO_3$/L)		02-14-22	100		97			02-14-22 H
	Temperature (°C)	24.8	25.3	24.8	25.5	24.9	25.4	24.9	25.1
0.025 mg/L	pH (S.U.)	7.94	7.92	8.02	7.81	7.87	7.65	7.88	7.73
	Dissolved oxygen (mg/L)	8.0	7.7	7.8	7.6	7.7	7.1	7.7	7.6
	Salinity (ppt)	25.3	25.5	25.0	25.3	24.9	25.6	25.1	25.4
	Temperature (°C)	25.0	25.2	24.9	25.3	25.0	25.2	24.9	25.0
0.05 mg/L	pH (S.U.)	7.95	7.91	8.02	7.83	7.88	7.64	7.88	7.74
	Dissolved oxygen (mg/L)	8.0	7.7	7.8	7.6	7.7	7.1	7.8	7.5
	Salinity (ppt)	25.2	25.6	24.9	25.3	24.9	25.7	25.1	25.4
	Temperature (°C)	24.8	25.2	24.9	25.2	25.0	25.2	25.0	25.2
0.1 mg/L	pH (S.U.)	7.95	7.92	8.03	7.84	7.89	7.64	7.89	7.74
	Dissolved oxygen (mg/L)	8.0	7.8	7.8	7.6	7.8	7.1	7.8	7.6
	Salinity (ppt)	25.3	25.5	24.9	25.3	24.9	25.7	25.2	25.6
	Temperature (°C)	24.8	25.2	25.0	25.2	25.0	25.2	25.0	25.2
0.2 mg/L	pH (S.U.)	7.95	7.92	8.03	7.83	7.90	7.63	7.89	7.76
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	7.6	7.8	7.1	7.8	7.6
	Salinity (ppt)	25.2	25.5	24.9	25.3	24.9	25.6	25.1	25.6
	Temperature (°C)	24.8	25.4	24.9	25.3	25.0	25.3	25.2	25.2
0.5 mg/L	pH (S.U.)	7.96	7.91	8.02	7.87	7.91	7.68	7.89	7.78
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	7.7	7.8	7.0	8.0	7.7
	Salinity (ppt)	25.1	25.5	24.7	25.0	24.7	25.6	25.1	25.7
	Temperature (°C)	24.9	25.1	24.9	25.3	25.0	25.1	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final