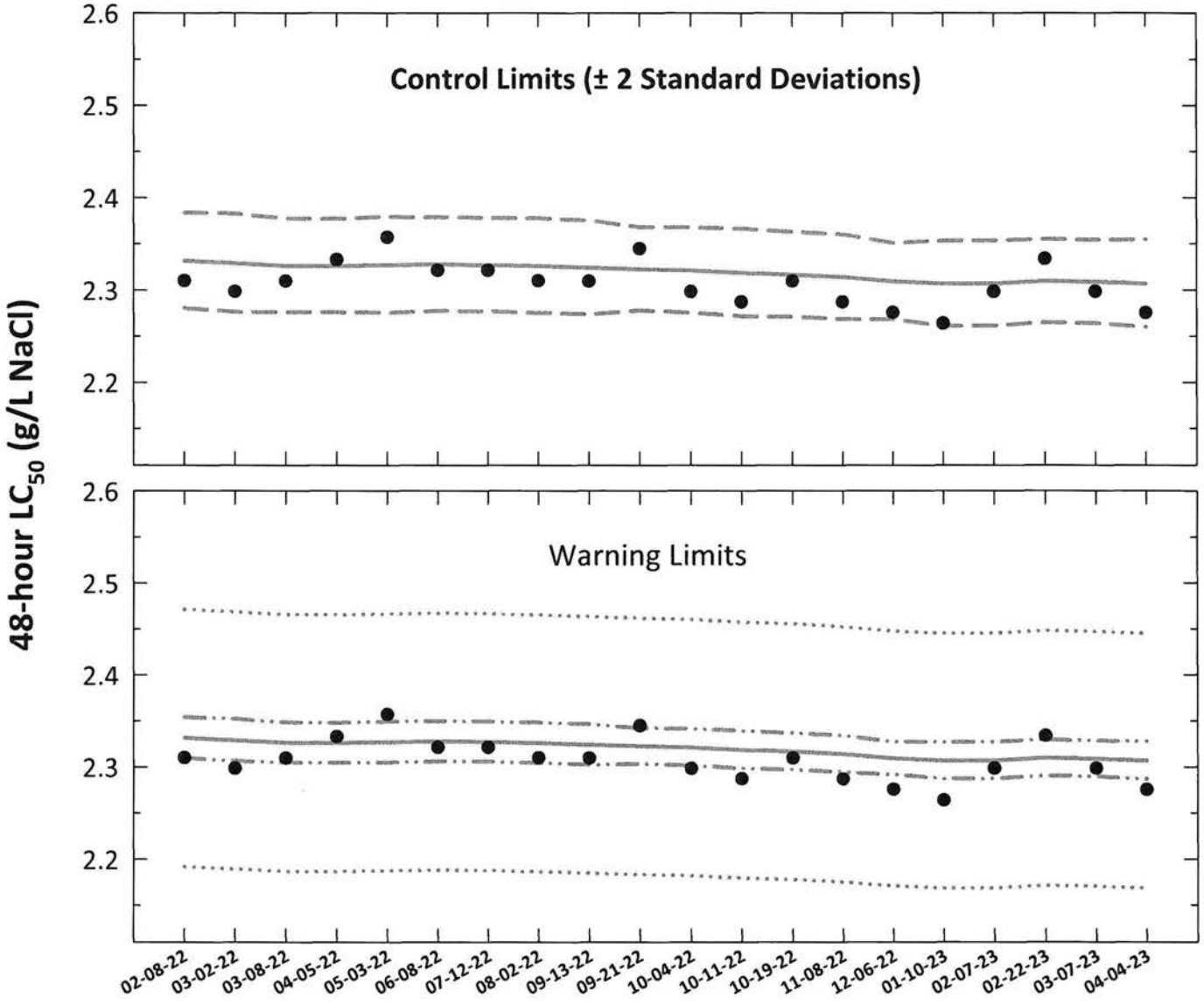
Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium 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, $S_{A.10}$ = 10^{th} percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**02-08-22**	**2.3099**	0.3636	0.3676	0.0048	2.3315	2.2803	2.3837	2.3095	2.3539	2.1916	2.4714
2	**03-02-22**	**2.2985**	0.3614	0.3672	0.0050	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
3	**03-08-22**	**2.3096**	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
4	**04-05-22**	**2.3330**	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
5	**05-03-22**	**2.3569**	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
6	**06-08-22**	**2.3212**	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
7	**07-12-22**	**2.3212**	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
8	**08-02-22**	**2.3099**	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
9	**09-13-22**	**2.3096**	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
10	**09-21-22**	**2.3449**	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
11	**10-04-22**	**2.2984**	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
12	**10-11-22**	**2.2870**	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
13	**10-19-22**	**2.3096**	0.3635	0.3649	0.0043	2.3168	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558
14	**11-08-22**	**2.2868**	0.3592	0.3643	0.0043	2.3139	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527
15	**12-06-22**	**2.2755**	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478
16	**01-10-23**	**2.2639**	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685	2.4454
17	**02-07-23**	**2.2984**	0.3614	0.3630	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271	2.1685	2.4454
18	**02-22-23**	**2.3339**	0.3681	0.3636	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296	2.1713	2.4485
19	**03-07-23**	**2.2984**	0.3614	0.3634	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284	2.1702	2.4472
20	**04-04-23**	**2.2753**	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium 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. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

Entered and Reviewed by Jim Isaacson

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 395

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/l stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2180

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JL	BL JL	JL
Control, MHSW	pH (S.U.)	7.97	7.92	7.95
	Dissolved oxygen (mg/L)	7.6	7.7	7.7
	Conductivity (µmhos/cm)	303		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.9	25.2	25.0
1750 mg/L	pH (S.U.)	7.93	7.88	7.97
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	Conductivity (µmhos/cm)	3410		
	Temperature (°C)	25.1	25.0	25.2
2000 mg/L	pH (S.U.)	7.91	7.90	7.99
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	Conductivity (µmhos/cm)	3790		
	Temperature (°C)	25.0	25.0	24.9
2250 mg/L	pH (S.U.)	7.91	7.89	7.99
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	4090		
	Temperature (°C)	25.0	25.1	24.9
2500 mg/L	pH (S.U.)	7.91	7.89	7.98
	Dissolved oxygen (mg/L)	8.0	7.9	7.8
	Conductivity (µmhos/cm)	4030		
	Temperature (°C)	25.0	25.1	25.1
2750 mg/L	pH (S.U.)	7.92	7.90	7.99
	Dissolved oxygen (mg/L)	8.0	7.9	7.9
	Conductivity (µmhos/cm)	5370		
	Temperature (°C)	25.2	25.2	24.9

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuren only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified or test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numb
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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicat
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130641

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 395

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04.04.23	0545	JL	0745	JL	2B3	BWC	03.28.23B
24	04.05.23			0740	JL			
48 Termination	04.06.23			0742	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	03.28.23 D
Age:	< 24-hours old
Date and time organisms were born between:	04.03.23 1450 TO 04.04.23 0545
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.99
	Temperature (°C): 24.9

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	S	S	S	S	S	S	S
48 Termination	S	S	S	S	S	S	S	S	S	S	S	S
Mean Survival	100%				100%				100%			

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	4 1d	4 1d	S	3 2d	3 2d	3 2d	4 1d
48 Termination	4 1d	2 2d	2 2d	2 2d	0 2d	1 3d	0 4d	1 4d	0 2d	0 2d	0 3d	0 4d
Mean Survival	50%				10%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2206.8
Upper 95% confidence limit (mg NaCl/L)	2341.5
48-hour LC_{50} (mg NaCl/L)	2275.3

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	4/4/2023	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/6/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.8000	0.4000	0.4000	0.4000
2500	0.0000	0.2000	0.0000	0.2000
2750	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	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7903	0.6847	1.1071	26.725	4	10.00	10.00	10	20
*2500	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

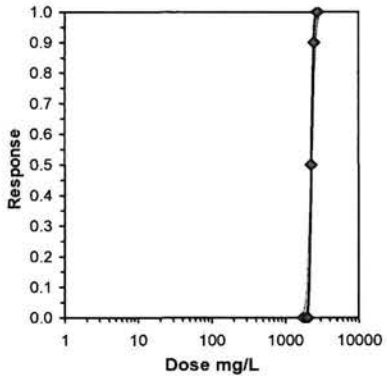
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.75891	0.868	1.64604	4.54327
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

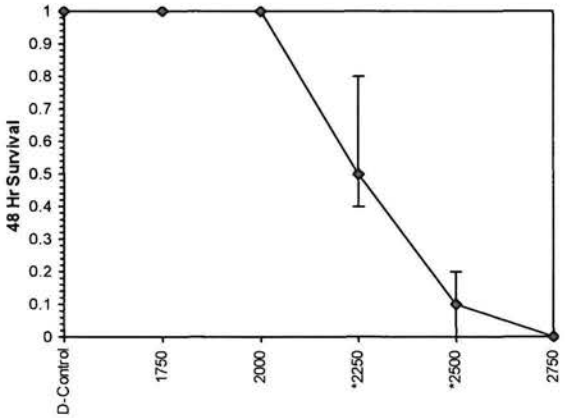
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	36.4002	7.23631	22.2171	50.5834	0	1.16917	7.81472	0.76041	3.35703	0.02747	5
Intercept	-117.2	24.3102	-164.84	-69.549							
TSCR											

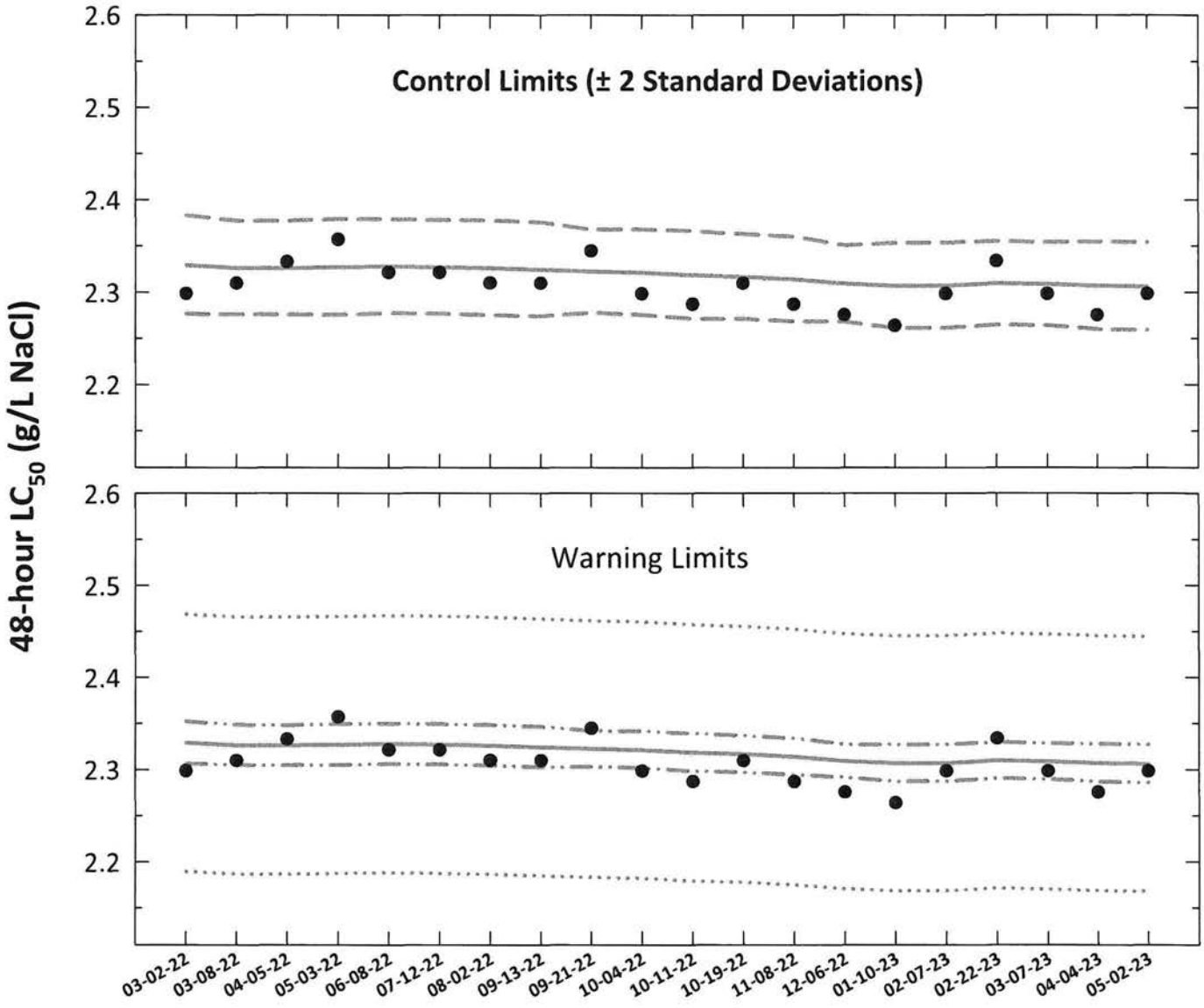
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1963.92	1771.18	2061.96
EC05	3.355	2050.44	1896.88	2131.33
EC10	3.718	2098.1	1966.23	2170.65
EC15	3.964	2130.89	2013.57	2198.55
EC20	4.158	2157.31	2051.25	2221.8
EC25	4.326	2180.24	2083.42	2242.71
EC40	4.747	2239.1	2162.26	2301.04
EC50	5.000	2275.28	2206.78	2341.45
EC60	5.253	2312.03	2248.18	2386.85
EC75	5.674	2374.46	2310.15	2473.42
EC80	5.842	2399.69	2332.86	2511.16
EC85	6.036	2429.45	2358.4	2557.21
EC90	6.282	2467.41	2389.53	2617.88
EC95	6.645	2524.77	2434.41	2712.7
EC99	7.326	2635.99	2517.11	2904.3



Dose-Response Plot



Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**03-02-22**	**2.2985**	0.3614	0.3672	0.0050	2.3291	2.2765	2.3830	2.3065	2.3523	2.1894	2.4689
2	**03-08-22**	**2.3096**	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
3	**04-05-22**	**2.3330**	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
4	**05-03-22**	**2.3569**	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
5	**06-08-22**	**2.3212**	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
6	**07-12-22**	**2.3212**	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
7	**08-02-22**	**2.3099**	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
8	**09-13-22**	**2.3096**	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
9	**09-21-22**	**2.3449**	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
10	**10-04-22**	**2.2984**	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
11	**10-11-22**	**2.2870**	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
12	**10-19-22**	**2.3096**	0.3635	0.3649	0.0043	2.3168	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558
13	**11-08-22**	**2.2868**	0.3592	0.3643	0.0043	2.3139	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527
14	**12-06-22**	**2.2755**	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478
15	**01-10-23**	**2.2639**	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685	2.4454
16	**02-07-23**	**2.2984**	0.3614	0.3630	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271	2.1685	2.4454
17	**02-22-23**	**2.3339**	0.3681	0.3636	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296	2.1713	2.4485
18	**03-07-23**	**2.2984**	0.3614	0.3634	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284	2.1702	2.4472
19	**04-04-23**	**2.2753**	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
20	**05-02-23**	**2.2985**	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272	2.1680	2.4448

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium 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. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sommer

FTS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 346

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2180

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	BL	BL N	N
Control, MHSW	pH (S.U.)	7.92	7.92	7.93
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	82		
	Temperature (°C)	24.9	25.2	25.7
1750 mg/L	pH (S.U.)	7.91	7.89	7.84
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	3280		
	Temperature (°C)	25.0	24.9	25.6
2000 mg/L	pH (S.U.)	7.93	7.85	7.85
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	3790		
	Temperature (°C)	25.0	25.2	25.6
2250 mg/L	pH (S.U.)	7.90	7.85	7.87
	Dissolved oxygen (mg/L)	7.8	7.9	7.8
	Conductivity (µmhos/cm)	3990		
	Temperature (°C)	25.0	25.0	25.6
2500 mg/L	pH (S.U.)	7.90	7.85	7.87
	Dissolved oxygen (mg/L)	7.8	7.9	7.9
	Conductivity (µmhos/cm)	4430		
	Temperature (°C)	25.0	24.9	25.3
2750 mg/L	pH (S.U.)	7.90	7.85	7.89
	Dissolved oxygen (mg/L)	7.9	7.9	8.0
	Conductivity (µmhos/cm)	4940		
	Temperature (°C)	25.0	24.9	25.4

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on the test specific bench sheets 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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066485

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 396

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05-02-23	*0530	JH	0800	JH	2F1	ORANGE	04-26-23 A
24	05-03-23			0756	JH			
48 Termination	05-04-23			0758	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:	In-house Culture
Source (organisms were pooled):	04-25-23 D
Age:	< 24-hours old
Date and time organisms were born between:	05-01-23 1444 TO 05-02-23 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01
	Temperature (°C): 25.0

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 1d	4 1d	5	5	2 3d	3 2d	3 2d	3 2d
48 Termination	3 2d	2 3d	4 1d	2 2d	1 3d	1 3d	0 5d	1 4d	0 2d	0 3d	0 3d	0 3d
Mean Survival	55%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2227.7
Upper 95% confidence limit (mg NaCl/L)	2367.1
48-hour LC_{50} (mg NaCl/L)	2298.5

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	5/2/2023	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/4/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.4000	0.8000	0.4000
2500	0.2000	0.2000	0.0000	0.2000
2750	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	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8407	0.6847	1.1071	23.960	4	10.00	10.00	9	20
*2500	0.1500	0.1500	0.4041	0.2255	0.4636	29.464	4	10.00	10.00	17	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

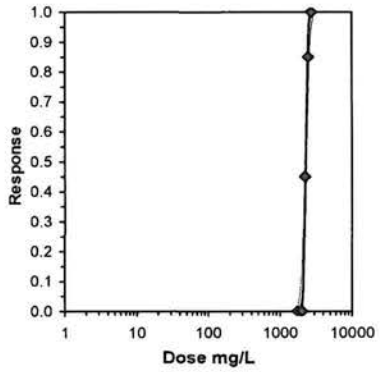
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76831	0.868	0.46336	3.50343
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

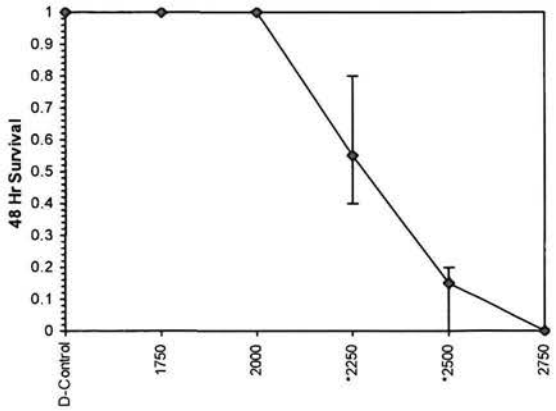
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	33.8498	6.46692	21.1747	46.525	0	1.31508	7.81472	0.72555	3.36145	0.02954	5
Intercept	-108.78	21.7554	-151.42	-66.144							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1962.1	1767.76	2064.38
EC05	3.355	2055.2	1899.71	2139.73
EC10	3.718	2106.62	1972.73	2182.45
EC15	3.964	2142.04	2022.67	2212.75
EC20	4.158	2170.62	2062.49	2237.96
EC25	4.326	2195.44	2096.53	2260.62
EC40	4.747	2259.24	2180.24	2323.62
EC50	5.000	2298.51	2227.74	2367.09
EC60	5.253	2338.47	2272.15	2415.76
EC75	5.674	2406.43	2339.1	2508.31
EC80	5.842	2433.94	2363.79	2548.63
EC85	6.036	2466.41	2391.64	2597.81
EC90	6.282	2507.88	2425.69	2662.64
EC95	6.645	2570.63	2474.96	2764.04
EC99	7.326	2692.6	2566.15	2969.37

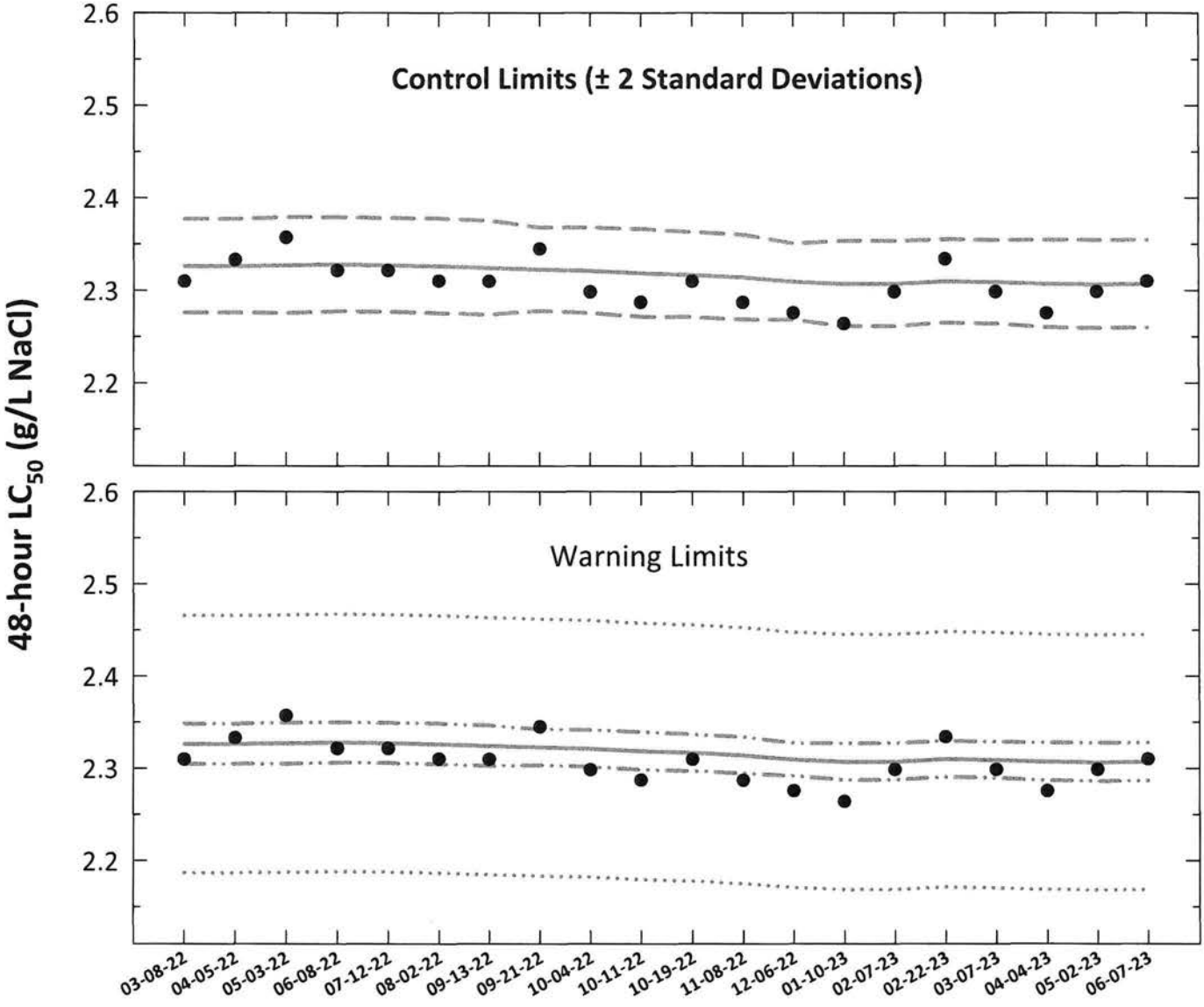


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium 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.

Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**03-08-22**	**2.3096**	0.3635	0.3667	0.0047	2.3262	2.2762	2.3774	2.3047	2.3482	2.1867	2.4658
2	**04-05-22**	**2.3330**	0.3679	0.3667	0.0047	2.3263	2.2762	2.3774	2.3047	2.3483	2.1867	2.4658
3	**05-03-22**	**2.3569**	0.3723	0.3668	0.0048	2.3269	2.2756	2.3793	2.3048	2.3494	2.1873	2.4665
4	**06-08-22**	**2.3212**	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
5	**07-12-22**	**2.3212**	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
6	**08-02-22**	**2.3099**	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
7	**09-13-22**	**2.3096**	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
8	**09-21-22**	**2.3449**	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
9	**10-04-22**	**2.2984**	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
10	**10-11-22**	**2.2870**	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
11	**10-19-22**	**2.3096**	0.3635	0.3649	0.0043	2.3168	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558
12	**11-08-22**	**2.2868**	0.3592	0.3643	0.0043	2.3139	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527
13	**12-06-22**	**2.2755**	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478
14	**01-10-23**	**2.2639**	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685	2.4454
15	**02-07-23**	**2.2984**	0.3614	0.3630	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271	2.1685	2.4454
16	**02-22-23**	**2.3339**	0.3681	0.3636	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296	2.1713	2.4485
17	**03-07-23**	**2.2984**	0.3614	0.3634	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284	2.1702	2.4472
18	**04-04-23**	**2.2753**	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
19	**05-02-23**	**2.2985**	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272	2.1680	2.4448
20	**06-07-23**	**2.3099**	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium 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. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

Entered and Reviewed by

FTS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 397

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2194

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	BL M	M	M
Control, MHSW	pH (S.U.)	7.77	7.75	7.80
	Dissolved oxygen (mg/L)	7.9	7.7	8.0
	Conductivity (µmhos/cm)	314		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	24.9	25.0	25.2
1750 mg/L	pH (S.U.)	7.79	7.75	7.87
	Dissolved oxygen (mg/L)	8.0	7.7	8.0
	Conductivity (µmhos/cm)	3320		
	Temperature (°C)	25.0	24.8	25.0
2000 mg/L	pH (S.U.)	7.80	7.79	7.88
	Dissolved oxygen (mg/L)	8.0	7.7	8.1
	Conductivity (µmhos/cm)	3770		
	Temperature (°C)	24.9	24.9	25.3
2250 mg/L	pH (S.U.)	7.82	7.82	7.88
	Dissolved oxygen (mg/L)	8.0	7.7	8.1
	Conductivity (µmhos/cm)	4240		
	Temperature (°C)	24.9	24.9	25.3
2500 mg/L	pH (S.U.)	7.82	7.82	7.89
	Dissolved oxygen (mg/L)	8.0	7.7	8.1
	Conductivity (µmhos/cm)	4680		
	Temperature (°C)	24.9	25.2	25.0
2750 mg/L	pH (S.U.)	7.83	7.82	7.91
	Dissolved oxygen (mg/L)	8.0	7.8	8.0
	Conductivity (µmhos/cm)	5100		
	Temperature (°C)	25.0	24.9	25.0

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on the test specific bench sheets 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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066615

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 397

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-07-23	0455*		0700		2C3	RED	05-30-23C
24	06-08-23			0700				
48 Termination	06-09-23			0700				

*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:	In-house Culture
Source (organisms were pooled):	05-30-23 B
Age:	< 24-hours old
Date and time organisms were born between:	06-06-23 1602 TO 06-07-23 0455
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.75
	Temperature (°C): 24.9°C

Survival Data (number of living organisms):

Hours	Control				1750 mg/L				2000 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	S	S	S	S	S	S	S
48 Termination	S	S	S	S	S	S	S	S	S	S	S	S
Mean Survival	100%				100%				100%			

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	S	S	S	3 1d	4 1d	4 1d	2 1d
48 Termination	2 1d	2 1d	4 1d	3 1d	2 1d	1 1d	1 1d	0 1d	0 1d	0 1d	0 1d	0 1d
Mean Survival	55%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2236.9
Upper 95% confidence limit (mg NaCl/L)	2381.0
48-hour LC_{50} (mg NaCl/L)	2309.9

Comments:

Test Reviewed by:

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	6/7/2023	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.4000	0.8000	0.6000
2500	0.4000	0.2000	0.2000	0.0000
2750	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	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8407	0.6847	1.1071	23.960	4	10.00	10.00	9	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

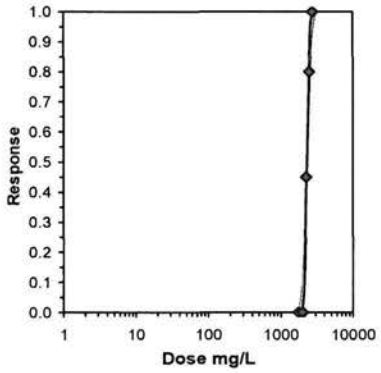
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.74905	0.868	0.45087	2.42089
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

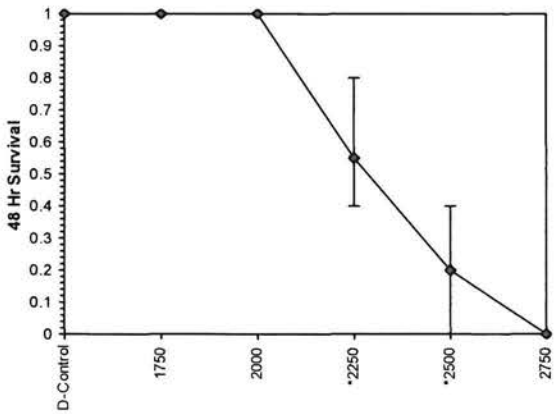
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	31.4761	5.81124	20.0861	42.8661	0	1.98377	7.81472	0.57578	3.3636	0.03177	5
Intercept	-100.87	19.5631	-139.22	-62.529							
TSCR											

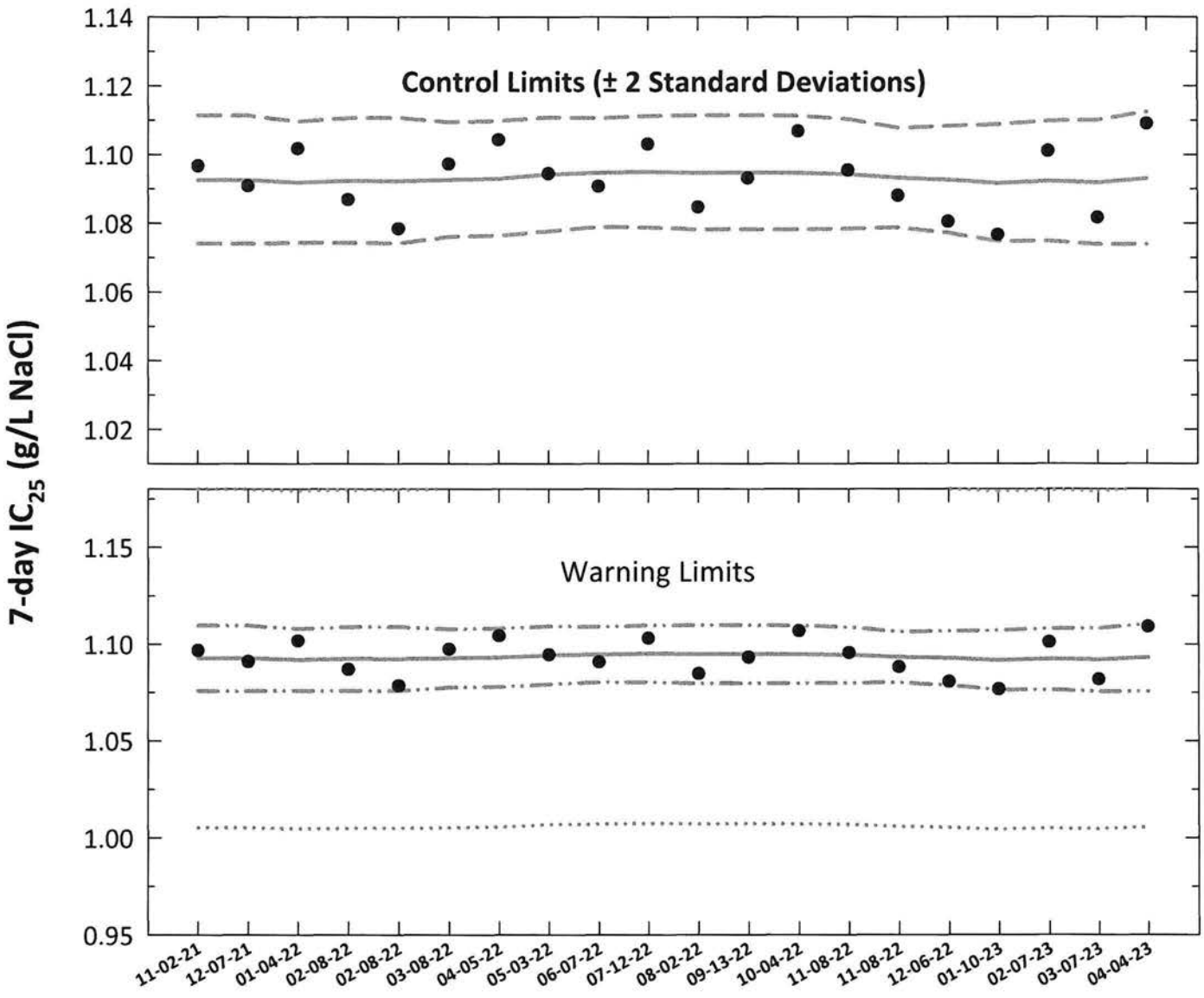
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1948.47	1752	2054.85
EC05	3.355	2048.07	1890.23	2136.13
EC10	3.718	2103.23	1966.98	2182.25
EC15	3.964	2141.28	2019.59	2214.95
EC20	4.158	2172.02	2061.61	2242.15
EC25	4.326	2198.74	2097.59	2266.57
EC40	4.747	2267.53	2186.32	2334.36
EC50	5.000	2309.94	2236.9	2381
EC60	5.253	2353.15	2284.37	2433.11
EC75	5.674	2426.78	2356.4	2532.04
EC80	5.842	2456.63	2383.1	2575.12
EC85	6.036	2491.89	2413.3	2627.67
EC90	6.282	2536.98	2450.33	2696.99
EC95	6.645	2605.31	2504.1	2805.53
EC99	7.326	2738.48	2604.02	3025.86



Dose-Response Plot



Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture



Test date

- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (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} ± $S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Reviewed by Jim Sumner

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	**11-02-21**	**1.0967**	0.0351	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
2	**12-07-21**	**1.0909**	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
3	**01-04-22**	**1.1017**	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
4	**02-08-22**	**1.0869**	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
5	**02-08-22**	**1.0784**	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
6	**03-08-22**	**1.0972**	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
7	**04-05-22**	**1.1043**	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
8	**05-03-22**	**1.0944**	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
9	**06-07-22**	**1.0908**	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
10	**07-12-22**	**1.1030**	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
11	**08-02-22**	**1.0847**	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
12	**09-13-22**	**1.0931**	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
13	**10-04-22**	**1.1068**	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
14	**11-08-22**	**1.0954**	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
15	**11-08-22**	**1.0881**	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
16	**12-06-22**	**1.0805**	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
17	**01-10-23**	**1.0767**	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
18	**02-07-23**	**1.1011**	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
19	**03-07-23**	**1.0816**	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
20	**04-04-23**	**1.1090**	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805

Note: **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (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 $S_{A.10}$ converted to anti-logarithmic values.

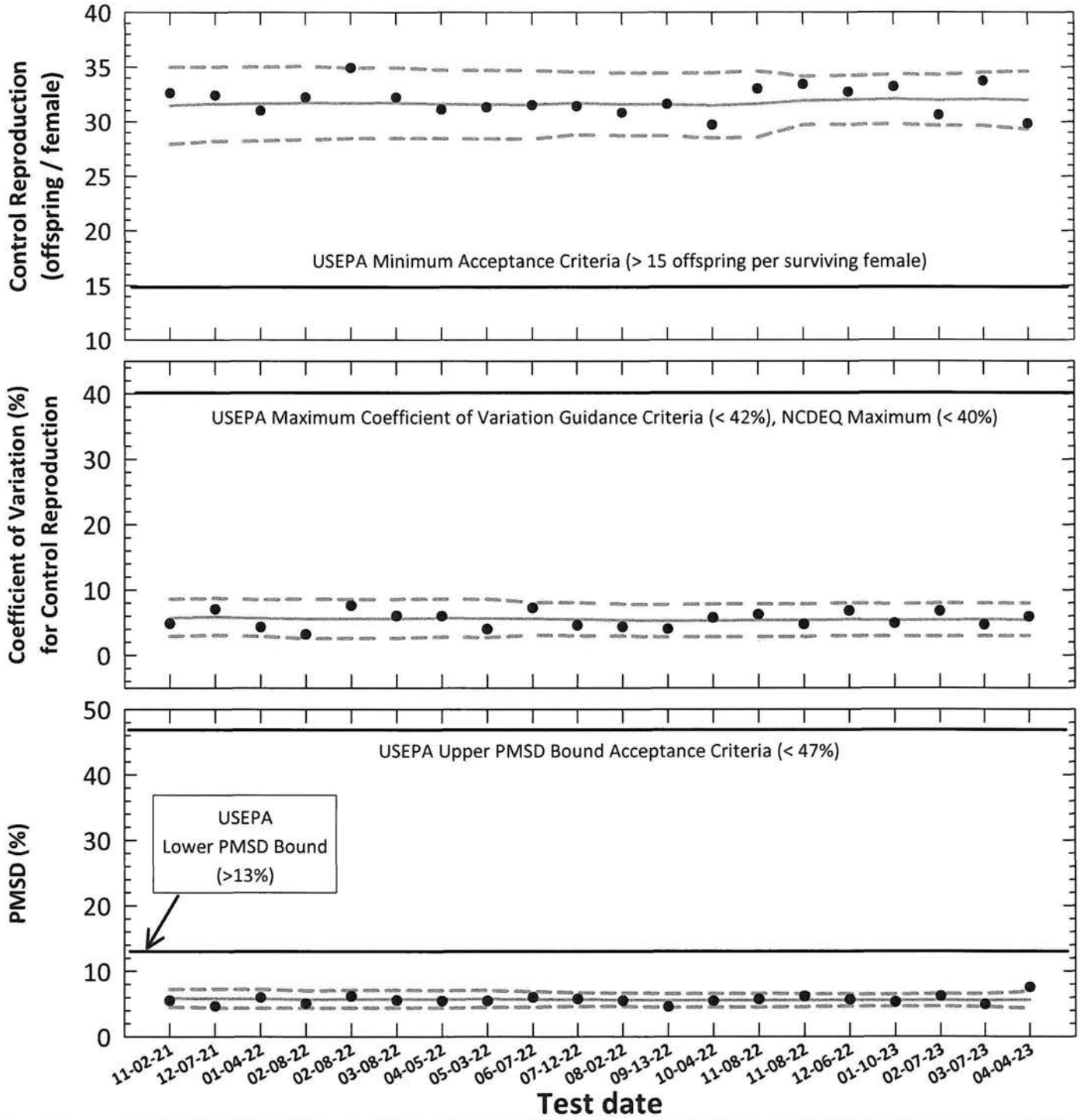
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **Control Reproduction, 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 Reproduction, CV or PMSD)

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

Entered and Reviewed by Jim Sumner

Reviewed by: Jim Sumner

ETS Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival	Control Reproduction		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	2.9	8.6	5.9	4.5	7.2
2	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	3.0	8.7	5.8	4.4	7.3
3	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	8.5	5.8	4.4	7.2
4	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0
5	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1
6	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1
7	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
8	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
9	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
10	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
11	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
12	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
13	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6
14	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6
15	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	4.5	6.5
16	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	4.6	6.5
17	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	4.6	6.4
18	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5
19	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
20	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9

Note:

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

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

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) > 13%.
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) < 47%.

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)
Species: *Ceriodaphnia dubia*

CdNaClCR #: 283

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2180				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	04-04-23 0545 TO 0811									
Culture board:	03-28-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	3	5	6	7	9	10	11	18	19	22
Transfer vessel information:	pH (S.U.): 7.99 Temperature (°C): 24.9									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLACK
Incubator number and shelf location:	2B1

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	04-04-23	0825	03-30-23	03-30-23	03-28-23 B	JH
1	04-05-23	0735	↓	↓	↓	JH
2	04-06-23	0736	↓	↓	03-28-23 D	JH
3	04-07-23	0731	↓	↓	↓	JH
4	04-08-23	0832	↓	↓	03-28-23 E	JH
5	04-09-23	0830	↓	↓	↓	JH
6	04-10-23	0733	↓	↓	↓	JH
7	04-11-23	0800				JH

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	29.8	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	5.97%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1109.0

Reviewed by: Jim Siemers JH

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 283

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	5	4	4	5	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	11	14	12	13	10	10	10	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	11	15	14	12	16	15	16	13	14	13
Total young produced		27	28	30	31	32	32	31	29	28	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	29.8

600 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	5	5	5	3	5	5	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	11	12	12	10	13	11	12	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	12	17	13	17	15	13	13	13	16
Total young produced		32	28	34	30	30	33	29	29	27	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	30.5
% Reduction from Control:	-2.37.

Reviewed by: Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #:** 283

800 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	4	4	6	4	4	4	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	14	11	13	13	10	12	12	11	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	12	17	16	13	13	16	13	15	15
Total young produced		32	31	32	33	32	27	32	29	31	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.0
% Reduction from Control:	-4.0%

1000 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	4	4	4	6	4	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	12	12	10	11	12	13	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	18	14	13	15	13	17	15	14	12
Total young produced		32	33	30	27	30	31	34	30	31	28
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.6
% Reduction from Control:	-2.7%

Reviewed by: Jim Summers

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 283**

1200 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	2	4	2	2	4	2	4	4	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	7	9	6	10	11	10	8	5	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	0	3	10	0	4	5	5	10	11
Total young produced		18	9	16	18	12	19	17	17	19	20
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	16.5
% Reduction from Control:	44.6%

1400 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	1	3	1	2	2	2	1	2	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	2	0	0	0	0	1	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	2	0	0	0	0	0	0	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		3	3	3	2	2	3	1	2	3	4
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.6
% Reduction from Control:	91.37%

Reviewed by: Jon Sumner

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	5	4	4	5	6	4	4	45
5	11	10	11	14	12	13	10	10	10	13	114
6	0	0	0	0	0	0	0	0	0	0	0
7	11	15	14	12	16	15	16	13	14	13	139
Total	**27**	**28**	**30**	**31**	**32**	**32**	**31**	**29**	**28**	**30**	**298**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	4	4	4	6	4	5	5	4	44
5	14	12	12	10	11	12	13	10	12	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	13	18	14	13	15	13	17	15	14	12	144
Total	**32**	**33**	**30**	**27**	**30**	**31**	**34**	**30**	**31**	**28**	**306**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0.	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	6	5	5	5	3	5	5	4	4	5	47
5	11	11	12	12	10	13	11	12	10	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	15	12	17	13	17	15	13	13	13	16	144
Total	**32**	**28**	**34**	**30**	**30**	**33**	**29**	**29**	**27**	**33**	**305**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	2	4	2	2	4	2	4	4	2	29
5	10	7	9	6	10	11	10	8	5	7	83
6	0	0	0	0	0	0	0	0	0	0	0
7	5	0	3	10	0	4	5	5	10	11	53
Total	**18**	**9**	**16**	**18**	**12**	**19**	**17**	**17**	**19**	**20**	**165**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	4	6	4	4	4	5	4	45
5	10	14	11	13	13	10	12	12	11	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	17	12	17	16	13	13	16	13	15	15	147
Total	**32**	**31**	**32**	**33**	**32**	**27**	**32**	**29**	**31**	**31**	**310**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	1	3	1	2	2	2	1	2	3	3	20
5	2	0	0	0	0	1	0	0	0	0	3
6	0	0	2	0	0	0	0	0	0	1	3
7	0	0	0	0	0	0	0	0	0	0	0
Total	**3**	**3**	**3**	**2**	**2**	**3**	**1**	**2**	**3**	**4**	**26**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Ceriodaphnia dubia Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1002.0

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

Test number: CdNaClCR #283

Test dates: April 04-11, 2023

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	27	28	30	31	32	32	31	29	28	30	**100**	**29.8**	**5.9**	**Not applicable**
600	32	28	34	30	30	33	29	29	27	33	**100**	**30.5**	**7.8**	**-2.3**
800	32	31	32	33	32	27	32	29	31	31	**100**	**31.0**	**5.7**	**-4.0**
1000	32	33	30	27	30	31	34	30	31	28	**100**	**30.6**	**6.9**	**-2.7**
1200	18	9	16	18	12	19	17	17	19	20	**100**	**16.5**	**20.8**	**44.6**
1400	3	3	3	2	2	3	1	2	3	4	**100**	**2.6**	**32.4**	**91.3**

Dunnett's MSD value: 2.241

PMSD: 7.5

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 (10^{th} percentile) = 13%.

Upper PMSD bound determined by USEPA (90^{th} percentile) = 47%.

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 Summer

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	4/4/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	4/11/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	27.000	28.000	30.000	31.000	32.000	32.000	31.000	29.000	28.000	30.000
600	32.000	28.000	34.000	30.000	30.000	33.000	29.000	29.000	27.000	33.000
800	32.000	31.000	32.000	33.000	32.000	27.000	32.000	29.000	31.000	31.000
1000	32.000	33.000	30.000	27.000	30.000	31.000	34.000	30.000	31.000	28.000
1200	18.000	9.000	16.000	18.000	12.000	19.000	17.000	17.000	19.000	20.000
1400	3.000	3.000	3.000	2.000	2.000	3.000	1.000	2.000	3.000	4.000

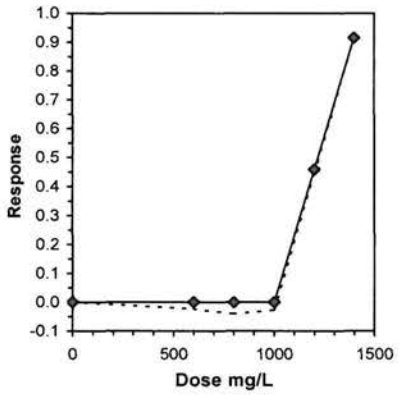
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	29.800	1.0000	29.800	27.000	32.000	5.876	10			30.475	1.0000
600	30.500	1.0235	30.500	27.000	34.000	7.766	10	113.50	75.00	30.475	1.0000
800	31.000	1.0403	31.000	27.000	33.000	5.690	10	126.00	75.00	30.475	1.0000
1000	30.600	1.0268	30.600	27.000	34.000	6.924	10	115.50	75.00	30.475	1.0000
*1200	16.500	0.5537	16.500	9.000	20.000	20.848	10	55.00	75.00	16.500	0.5414
*1400	2.600	0.0872	2.600	1.000	4.000	32.434	10	55.00	75.00	2.600	0.0853

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.07786	1.035	-0.9508	1.75959
Bartlett's Test indicates unequal variances (p = 8.20E-03)	15.567	15.0863		

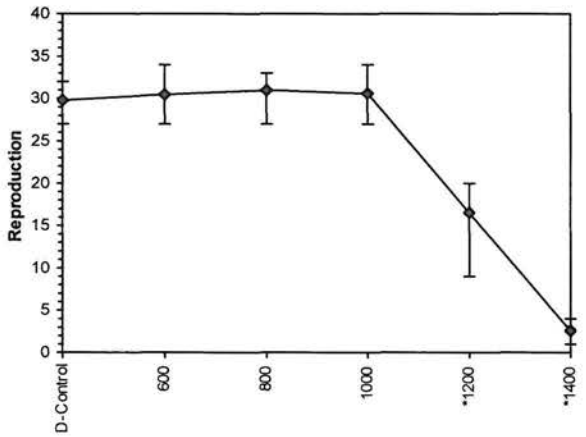
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1021.81	5.69741	1005.61	1024.16	-2.8529
IC10	1043.61	4.86507	1029.52	1048.31	-0.9503
IC15	1065.42	5.27108	1053.23	1072.47	-0.2598
IC20	1087.23	6.16929	1075.11	1096.62	0.0008
IC25	1109.03	7.38216	1095.05	1121.62	0.0443
IC40	1174.45	11.8641	1153.82	1196.14	0.0433
IC50	1218.17	12.3362	1192.37	1236.36	-0.3622



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

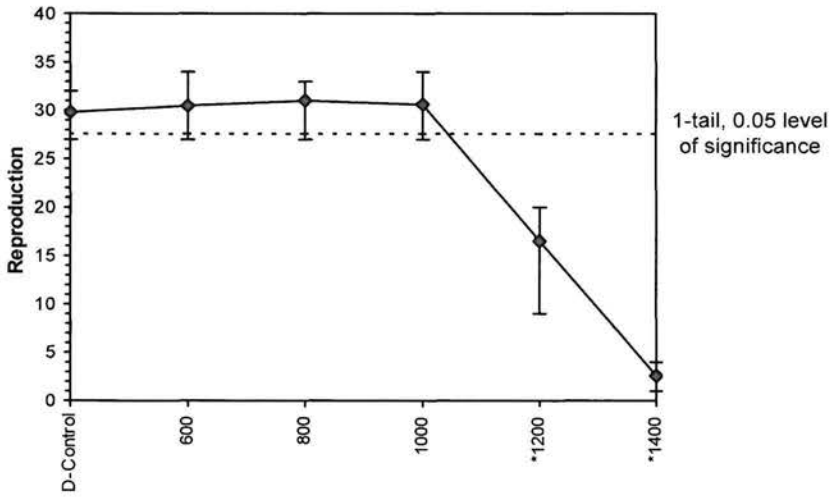
Start Date:	4/4/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	4/11/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	27.000	28.000	30.000	31.000	32.000	32.000	31.000	29.000	28.000	30.000
600	32.000	28.000	34.000	30.000	30.000	33.000	29.000	29.000	27.000	33.000
800	32.000	31.000	32.000	33.000	32.000	27.000	32.000	29.000	31.000	31.000
1000	32.000	33.000	30.000	27.000	30.000	31.000	34.000	30.000	31.000	28.000
1200	18.000	9.000	16.000	18.000	12.000	19.000	17.000	17.000	19.000	20.000
1400	3.000	3.000	3.000	2.000	2.000	3.000	1.000	2.000	3.000	4.000

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	29.800	1.0000	29.800	27.000	32.000	5.876	10			
600	30.500	1.0235	30.500	27.000	34.000	7.766	10	-0.714	2.287	2.241
800	31.000	1.0403	31.000	27.000	33.000	5.690	10	-1.224	2.287	2.241
1000	30.600	1.0268	30.600	27.000	34.000	6.924	10	-0.816	2.287	2.241
*1200	16.500	0.5537	16.500	9.000	20.000	20.848	10	13.569	2.287	2.241
*1400	2.600	0.0872	2.600	1.000	4.000	32.434	10	27.750	2.287	2.241

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)					1.07786		1.035		-0.9508	1.75959
Bartlett's Test indicates unequal variances (p = 8.20E-03)					15.567		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	1000	1200	1095.45		2.24133	0.07521	1362.32	4.8037	1.9E-37	5, 54
Treatments vs D-Control										

Dose-Response Plot



Entered and Reviewed by Jim Sumner

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* CdNaClCR #: 283

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	[illegible]	BL [illegible]	BL [illegible]	[illegible]	[illegible]	BL [illegible]
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.97	7.97	7.94	7.97	7.98	8.01
	Dissolved oxygen (mg/L)	7.6	8.0	8.0	7.7	7.4	7.9
	Conductivity (µmhos/cm)	303		306		297	
	Alkalinity (mg $CaCO_3$/L)	59				59	
	Hardness (mg $CaCO_3$/L)	86		X 04-12-23		91	
	Temperature (°C)	24.8	25.2	24.7	25.0	24.7	24.7
600 mg NaCl/L	pH (S.U.)	7.94	7.91	7.94	7.94	7.95	7.95
	Dissolved oxygen (mg/L)	7.6	8.0	8.0	7.7	7.4	7.9
	Conductivity (µmhos/cm)	1430		1430		1370	
	Temperature (°C)	24.9	25.0	24.8	24.7	24.8	24.9
800 mg NaCl/L	pH (S.U.)	7.94	7.91	7.94	7.94	7.95	8.32
	Dissolved oxygen (mg/L)	7.6	8.0	8.0	7.7	7.6	7.9
	Conductivity (µmhos/cm)	1750		1770		1730	
	Temperature (°C)	24.9	25.0	24.8	24.7	24.8	25.1
1000 mg NaCl/L	pH (S.U.)	7.95	7.90	7.93	7.94	7.94	8.22
	Dissolved oxygen (mg/L)	7.6	8.0	8.0	7.7	7.7	7.9
	Conductivity (µmhos/cm)	2100		2130		2090	
	Temperature (°C)	25.0	25.1	24.8	24.9	24.7	25.1
1200 mg NaCl/L	pH (S.U.)	7.95	7.90	7.93	7.93	7.95	7.93
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	7.7	7.7	8.0
	Conductivity (µmhos/cm)	2480		2490		2430	
	Temperature (°C)	25.0	25.3	24.9	24.9	24.7	25.1
1400 mg NaCl/L	pH (S.U.)	7.96	7.89	7.94	7.93	7.97	7.91
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.7	7.8	8.0
	Conductivity (µmhos/cm)	2900		2850		2790	
	Temperature (°C)	25.0	25.0	24.9	24.8	24.7	24.8
		Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

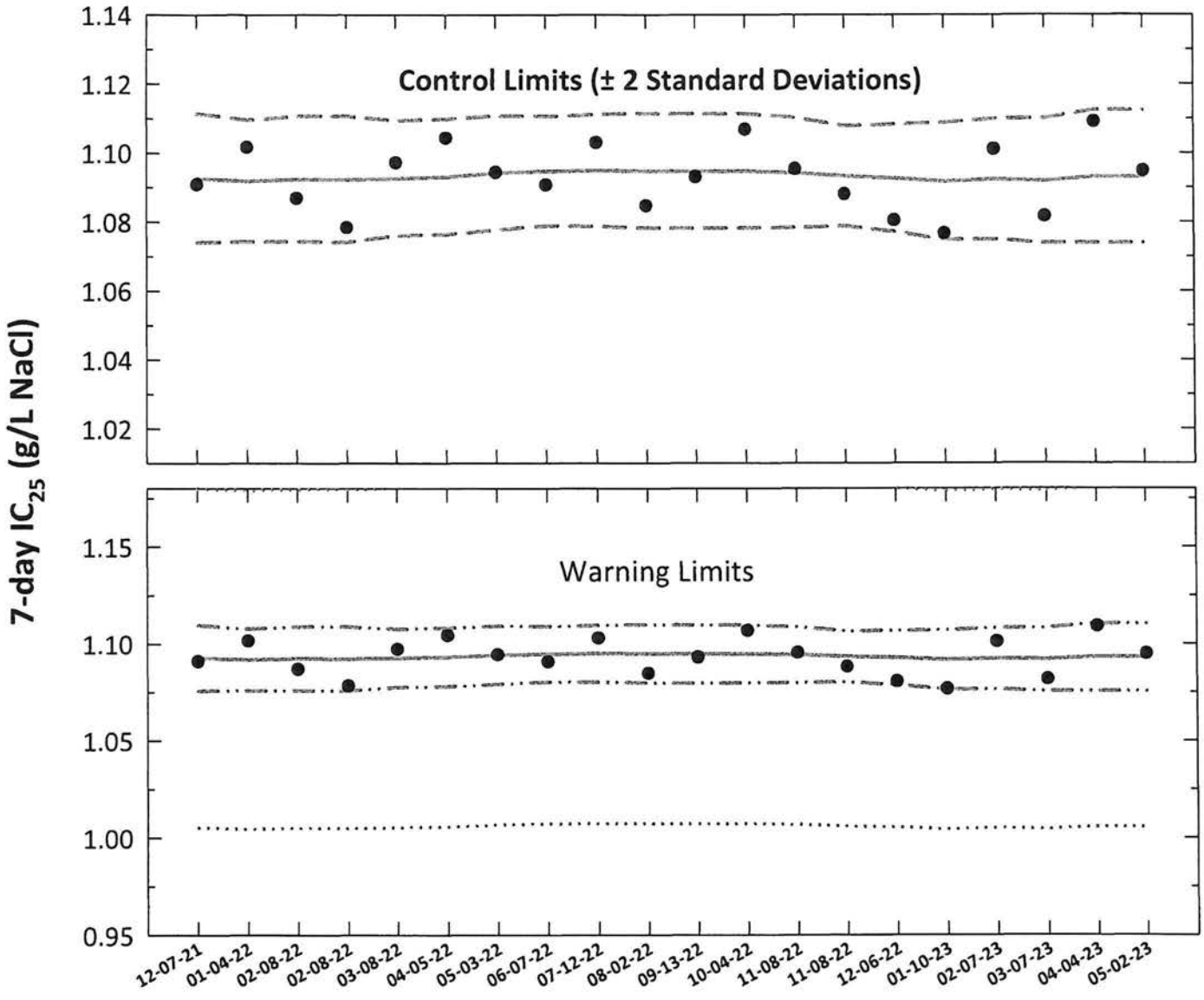
CdNaClCR #: 283

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL M	BSL	BSL	BSL	BSL	BL	BL	M
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.87	8.15	8.04	8.08	7.98	8.06	8.04	8.05
	Dissolved oxygen (mg/L)	8.0	8.0	7.8	8.0	7.9	7.7	7.8	8.0
	Conductivity (µmhos/cm)	313		318		308		308	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	25.1	24.8	25.1	24.8	25.1	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.94	8.10	8.07	8.03	8.04	8.01	8.03	7.96
	Dissolved oxygen (mg/L)	8.0	8.0	7.7	8.0	8.0	7.7	7.7	8.0
	Conductivity (µmhos/cm)	1440		1390		1410		1460	
	Temperature (°C)	24.7	25.0	24.9	25.0	24.9	25.1	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.93	8.11	8.09	8.02	8.05	8.00	8.04	7.96
	Dissolved oxygen (mg/L)	8.0	8.0	7.8	8.0	8.0	7.8	7.7	8.0
	Conductivity (µmhos/cm)	1760		1720		1770		1840	
	Temperature (°C)	24.7	25.2	24.9	25.2	24.9	25.0	24.8	25.2
1000 mg NaCl/L	pH (S.U.)	7.93	8.10	8.08	8.01	8.04	8.00	8.03	7.95
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	8.0	8.0	7.7	7.7	8.0
	Conductivity (µmhos/cm)	2130		2070		2150		2210	
	Temperature (°C)	24.7	25.2	24.9	25.2	25.0	25.0	24.9	25.2
1200 mg NaCl/L	pH (S.U.)	7.93	8.10	8.08	8.01	8.04	7.99	8.03	7.94
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	8.0	8.0	7.7	7.7	8.1
	Conductivity (µmhos/cm)	2490		2450		2480		2590	
	Temperature (°C)	24.9	25.0	24.9	24.8	25.0	25.2	24.9	25.0
1400 mg NaCl/L	pH (S.U.)	7.93	8.08	8.06	8.00	8.04	8.00	8.03	7.95
	Dissolved oxygen (mg/L)	8.1	8.0	8.0	8.0	8.0	7.7	7.7	8.1
	Conductivity (µmhos/cm)	2790		2790		2820		2960	
	Temperature (°C)	24.9	25.0	25.0	25.2	25.0	24.9	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture



Test date

- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (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} ± $S_{A.10}$ 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.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	12-07-21	1.0909	0.0401	0.0385	0.0037	1.0926	1.0741	1.1114	1.0757	1.1095	1.0052	1.1800
2	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
3	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
4	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
5	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
6	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
7	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
8	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
9	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
10	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
11	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
12	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
13	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
14	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
15	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
16	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
17	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
18	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
19	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
20	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804

Note:
7-day IC_{25} = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (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 $S_{A.10}$ converted to anti-logarithmic values.
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).
CV = Coefficient of variation.

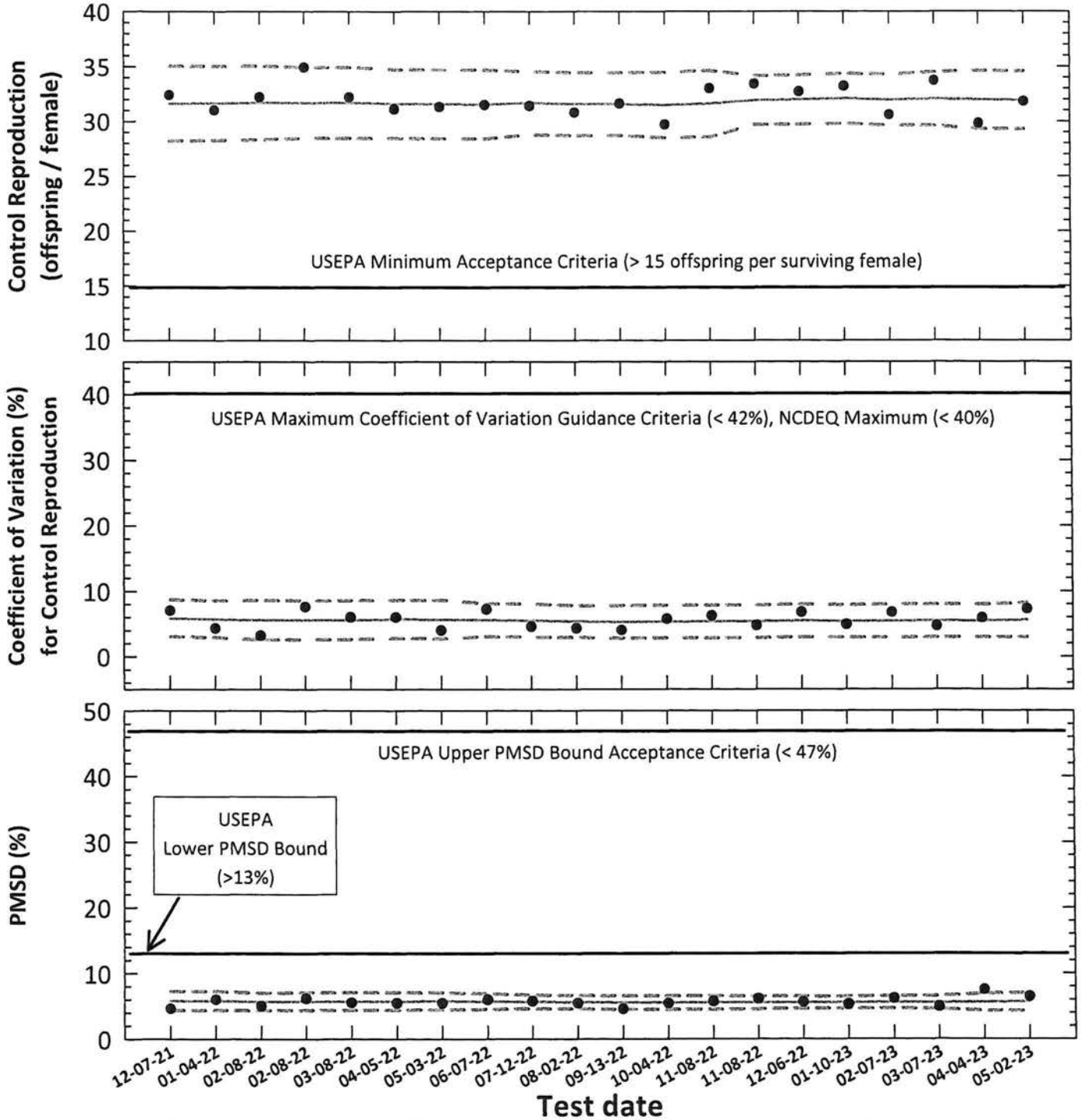
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **Control Reproduction, 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 Reproduction, CV or PMSD)

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Reproduction		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
			Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	3.0	8.7	5.8	4.4	7.3
2	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	8.5	5.8	4.4	7.2
3	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0
4	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1
5	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1
6	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
7	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
8	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
9	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
10	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
11	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
12	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6
13	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6
14	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	4.5	6.5
15	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	4.6	6.5
16	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	4.6	6.4
17	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5
18	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
19	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9
20	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0

Note:

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

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

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) > 13%.
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) < 47%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)
Species: *Ceriodaphnia dubia*

CdNaClCR #: 284

Dilution preparation information:						*Comments:*
NaCl Stock INSS number:		INSS 2180				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	05-02-23 0530 TO 0810									
Culture board:	04-25-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	7	8	10	11	12	17	18	22	25	29
Transfer vessel information:	pH (S.U.): 8.01 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	RED
Incubator number and shelf location:	2B1

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	05-02-23	0821	04-27-23	04-27-23	04-26-23A	
1	05-03-23	0745	↓	↓	↓	
2	05-04-23	0750	↓	↓	04-26-23E	
3	05-05-23	0742	↓	↓	↓	
4	05-06-23	0852	↓	↓	05-04-23A	
5	05-07-23	0842	↓	↓	↓	
6	05-08-23	0841	↓	↓	↓	
7	05-09-23	0725				

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130646ES

Control information:		Acceptance criteria	*Summary of test endpoints:*	
% of Male Adults:	0%	≤ 20%	7-day LC_{50} (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	31.8	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.5
% CV:	7.27%	< 40.0 %	IC_{25} (mg/L NaCl)	1094.8

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 284

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	3	4	4	5	4	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	12	14	10	14	11	11	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	17	15	16	16	17	19	13	15	14
Total young produced		33	31	30	34	30	36	34	30	31	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.8

600 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	6	5	4	4	4	6	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	14	10	12	12	13	10	12	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	16	18	16	13	18	15	15	15	19
Total young produced		33	36	33	32	29	35	31	31	32	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.7
% Reduction from Control:	-2.8%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 284

800 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	6	3	6	5	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	11	13	11	14	12	12	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	17	13	17	17	13	14	16	14
Total young produced		29	30	34	29	34	36	30	31	32	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.4
% Reduction from Control:	1.3%

1000 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	5	4	3	3	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	11	13	12	13	12	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	15	14	15	18	15	13	17	16	17
Total young produced		32	32	30	33	34	31	28	30	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.3
% Reduction from Control:	1.6%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 284

1200 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	3	5	3	4	2	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	7	5	8	6	7	4	10	7	6	9
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	9	3	6	5	9	5	3	8	3
Total young produced		16	18	16	15	17	16	19	12	18	16
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	16.3
% Reduction from Control:	48.7%

1400 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	3	2	3	4	2	2	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	1	1	1	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		4	5	4	4	2	3	4	2	2	2
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	3.2
% Reduction from Control:	89.9 %

ETS
Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	3	4	4	5	4	6	4	4	42
5	13	11	12	14	10	14	11	11	12	11	119
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	15	16	16	17	19	13	15	14	157
Total	**33**	**31**	**30**	**34**	**30**	**36**	**34**	**30**	**31**	**29**	**318**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	5	4	3	3	3	5	5	41
5	10	13	11	13	12	13	12	10	10	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	14	15	18	15	13	17	16	17	158
Total	**32**	**32**	**30**	**33**	**34**	**31**	**28**	**30**	**31**	**32**	**313**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	6	5	4	4	4	6	4	5	5	48
5	13	14	10	12	12	13	10	12	12	11	119
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	18	16	13	18	15	15	15	19	160
Total	**33**	**36**	**33**	**32**	**29**	**35**	**31**	**31**	**32**	**35**	**327**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	3	5	3	4	2	4	4	38
5	7	5	8	6	7	4	10	7	6	9	69
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	3	6	5	9	5	3	8	3	56
Total	**16**	**18**	**16**	**15**	**17**	**16**	**19**	**12**	**18**	**16**	**163**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	6	3	6	5	5	5	4	5	47
5	10	11	11	13	11	14	12	12	12	10	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	17	13	17	17	13	14	16	14	151
Total	**29**	**30**	**34**	**29**	**34**	**36**	**30**	**31**	**32**	**29**	**314**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	3	3	2	3	4	2	2	2	29
5	0	1	1	1	0	0	0	0	0	0	3
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**4**	**5**	**4**	**4**	**2**	**3**	**4**	**2**	**2**	**2**	**32**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Ceriodaphnia dubia Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1002.0

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

Test number: CdNaClCR #284

Test dates: May 02-09, 2023

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	33	31	30	34	30	36	34	30	31	29	100	31.8	7.2	Not applicable
600	33	36	33	32	29	35	31	31	32	35	100	32.7	6.6	-2.8
800	29	30	34	29	34	36	30	31	32	29	100	31.4	8.0	1.3
1000	32	32	30	33	34	31	28	30	31	32	100	31.3	5.4	1.6
1200	16	18	16	15	17	16	19	12	18	16	100	16.3	11.9	48.7
1400	4	5	4	4	2	3	4	2	2	2	100	3.2	35.5	89.9

Dunnett's MSD value: 2.054

PMSD: 6.5

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 (10^{th} percentile) = 13%.

Upper PMSD bound determined by USEPA (90^{th} percentile) = 47%.

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 Summer

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	5/2/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	31.000	30.000	34.000	30.000	36.000	34.000	30.000	31.000	29.000
600	33.000	36.000	33.000	32.000	29.000	35.000	31.000	31.000	32.000	35.000
800	29.000	30.000	34.000	29.000	34.000	36.000	30.000	31.000	32.000	29.000
1000	32.000	32.000	30.000	33.000	34.000	31.000	28.000	30.000	31.000	32.000
1200	16.000	18.000	16.000	15.000	17.000	16.000	19.000	12.000	18.000	16.000
1400	4.000	5.000	4.000	4.000	2.000	3.000	4.000	2.000	2.000	2.000

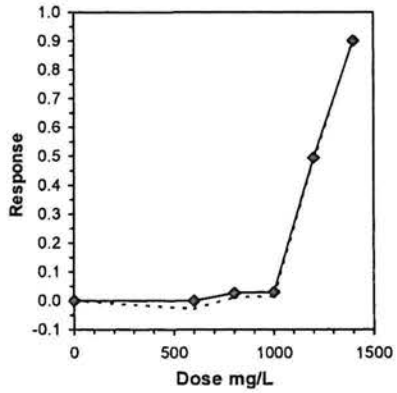
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.800	1.0000	31.800	29.000	36.000	7.232	10				32.250	1.0000
600	32.700	1.0283	32.700	29.000	36.000	6.614	10	-1.002	2.287	2.054	32.250	1.0000
800	31.400	0.9874	31.400	29.000	36.000	7.972	10	0.445	2.287	2.054	31.400	0.9736
1000	31.300	0.9843	31.300	28.000	34.000	5.441	10	0.557	2.287	2.054	31.300	0.9705
*1200	16.300	0.5126	16.300	12.000	19.000	11.942	10	17.254	2.287	2.054	16.300	0.5054
*1400	3.200	0.1006	3.200	2.000	5.000	35.478	10	31.836	2.287	2.054	3.200	0.0992

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.74815	1.035	0.18706	-0.268
Bartlett's Test indicates equal variances (p = 0.32)	5.83263	15.0863		

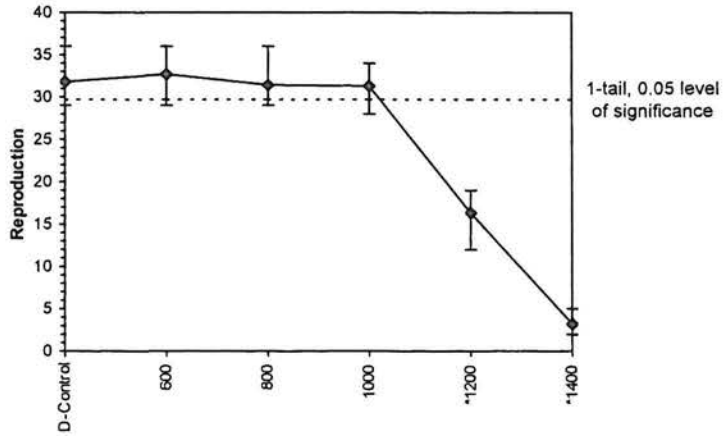
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		2.05423	0.0646	1470.59	4.03519	2.7E-40	5, 54

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1008.83	71.9184	766.917	1020.07	-2.3428
IC10	1030.33	7.02039	1013.9	1041.41	-0.2782
IC15	1051.83	6.48317	1036.41	1062.3	-0.0996
IC20	1073.33	6.14688	1059.11	1083.7	0.0801
IC25	1094.83	6.04515	1082.87	1104.79	0.2073
IC40	1159.33	7.13198	1145.12	1172.68	0.1756
IC50	1202.67	8.64762	1185.48	1218.41	-0.0253



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 284

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	BL	BL M	BL M	M	M	M
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.92	7.97	7.92	7.96	7.96	7.94
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.7	7.6	7.7
	Conductivity (µmhos/cm)	293		313		294	
	Alkalinity (mg $CaCO_3$/L)	60				63	
	Hardness (mg $CaCO_3$/L)	82			* 05-11-23 M	86	
	Temperature (°C)	24.9	25.1	24.8	24.9	24.8	24.9
600 mg NaCl/L	pH (S.U.)	7.88	7.90	7.92	7.87	7.96	7.87
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.6	7.6	7.7
	Conductivity (µmhos/cm)	1410		1450		1390	
	Temperature (°C)	24.9	24.9	24.9	25.1	24.9	25.1
800 mg NaCl/L	pH (S.U.)	7.90	7.87	7.92	7.86	7.95	7.87
	Dissolved oxygen (mg/L)	7.9	8.1	8.0	7.6	7.7	7.7
	Conductivity (µmhos/cm)	1770		1820		1750	
	Temperature (°C)	25.0	25.2	24.9	24.8	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.91	7.87	7.89	7.86	7.94	7.85
	Dissolved oxygen (mg/L)	(7.9) BL 05.02.23 1770 (2100)	8.1	8.0	7.7	7.7	7.7
	Conductivity (µmhos/cm)	↓		2170		2100	
	Temperature (°C)	24.9	25.2	24.9	24.8	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.91	7.86	7.90	7.86	7.93	7.85
	Dissolved oxygen (mg/L)	7.9	8.1	8.1	7.6	7.7	7.6
	Conductivity (µmhos/cm)	2470		2560		2480	
	Temperature (°C)	24.9	25.2	25.0	25.1	25.0	24.9
1400 mg NaCl/L	pH (S.U.)	7.91	7.85	7.89	7.86	7.93	7.85
	Dissolved oxygen (mg/L)	05-02-23 M 2850 (7.9)	8.0	8.1	7.6	7.7	7.6
	Conductivity (µmhos/cm)	2850		2920		2830	
	Temperature (°C)	24.9	25.0	25.0	25.0	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final

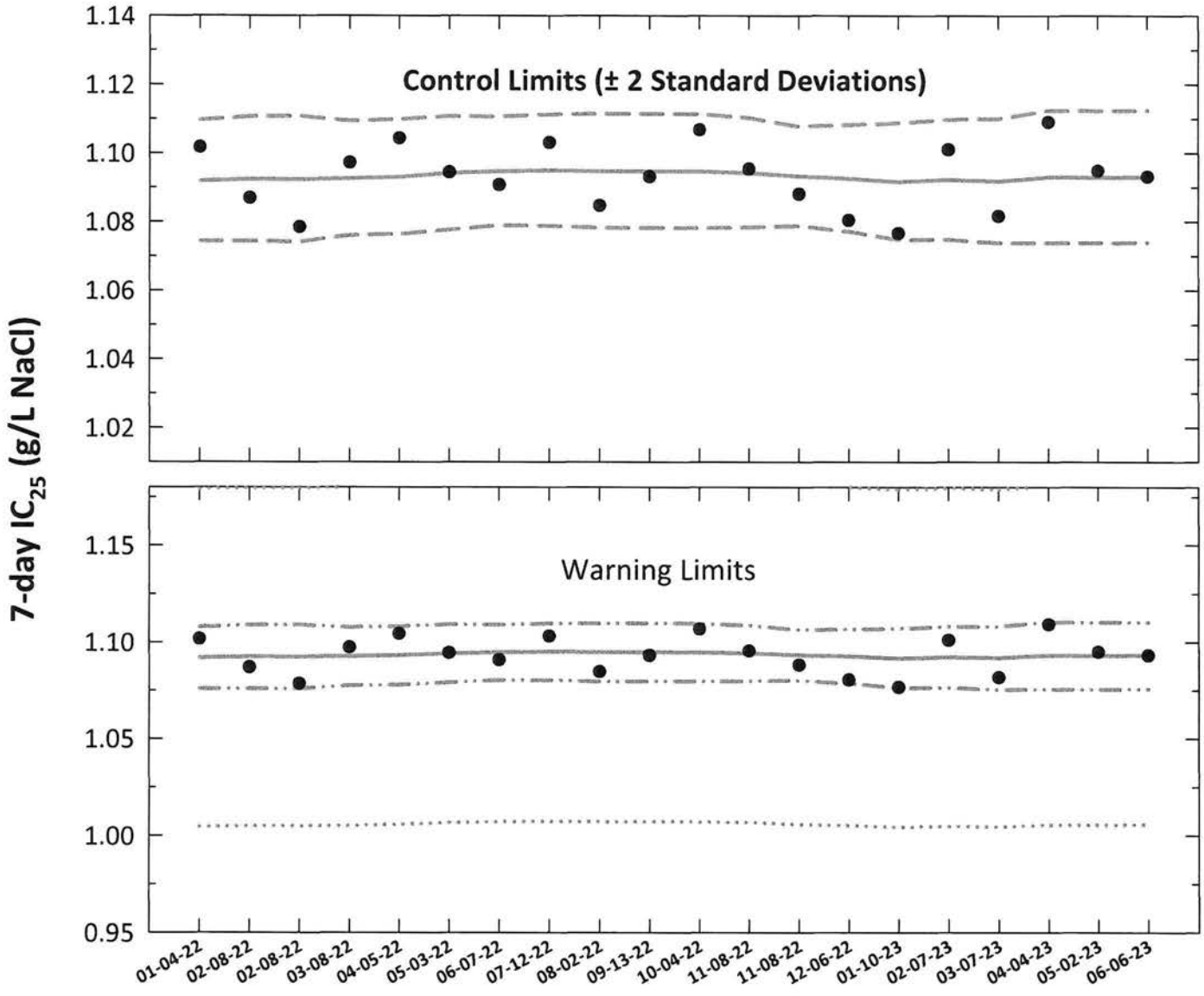
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 284

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	VL	BSL	BSL	BSL	BSL	BL VL	BLVL	VL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.01	8.00	7.89	7.99	7.92	7.97	7.90	8.13
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	8.0	7.9	7.8	8.0	7.9
	Conductivity (µmhos/cm)	308		306		300		306	
	Alkalinity (mg $CaCO_3$/L)			60					
	Hardness (mg $CaCO_3$/L)		05-11-23 H	64					05-11-23 H
	Temperature (°C)	24.8	25.2	24.8	25.2	24.8	25.2	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.98	7.93	7.90	7.92	7.90	7.92	7.91	7.97
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	8.0	8.0	7.8	8.0	7.9
	Conductivity (µmhos/cm)	1420		1420		1450		1460	
	Temperature (°C)	24.9	25.0	24.8	25.0	24.7	25.0	24.9	25.1
800 mg NaCl/L	pH (S.U.)	7.97	7.92	7.92	7.90	7.90	7.91	7.94	7.95
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	7.9	8.0	7.7	8.0	7.9
	Conductivity (µmhos/cm)	1760		1740		1790		1840	
	Temperature (°C)	25.0	24.9	24.9	25.2	24.7	25.0	24.8	25.1
1000 mg NaCl/L	pH (S.U.)	7.97	7.91	7.92	7.88	7.90	7.91	7.94	7.93
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	8.0	8.0	7.7	8.0	7.9
	Conductivity (µmhos/cm)	2120		2130		2150		2210	
	Temperature (°C)	25.0	24.9	25.0	24.9	24.7	24.9	24.8	25.2
1200 mg NaCl/L	pH (S.U.)	7.96	7.90	7.91	7.87	7.90	7.91	7.94	7.92
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.9	8.0	7.8	8.1	7.9
	Conductivity (µmhos/cm)	2470		2480		2520		2590	
	Temperature (°C)	25.0	25.1	25.0	24.9	24.7	25.2	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.97	7.89	7.91	7.86	7.89	7.92	7.94	7.90
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.8	8.0	7.9	8.1	7.9
	Conductivity (µmhos/cm)	2070		2840		2910		2950	
	Temperature (°C)	25.0	24.8	24.9	25.1	24.7	24.9	24.8	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (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} ± $S_{A.10}$ 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.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	01-04-22	1.1017	0.0378	0.0382	0.0035	1.0919	1.0744	1.1096	1.0758	1.1079	1.0045	1.1792
2	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
3	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
4	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
5	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
6	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
7	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
8	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
9	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
10	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
11	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
12	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
13	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
14	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
15	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
16	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
17	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
18	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
19	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
20	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805

Note:

7-day IC_{25} = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (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 $S_{A.10}$ converted to anti-logarithmic values.

$S_{A.10}$ = Standard deviation corresponding to the 10^{th} percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

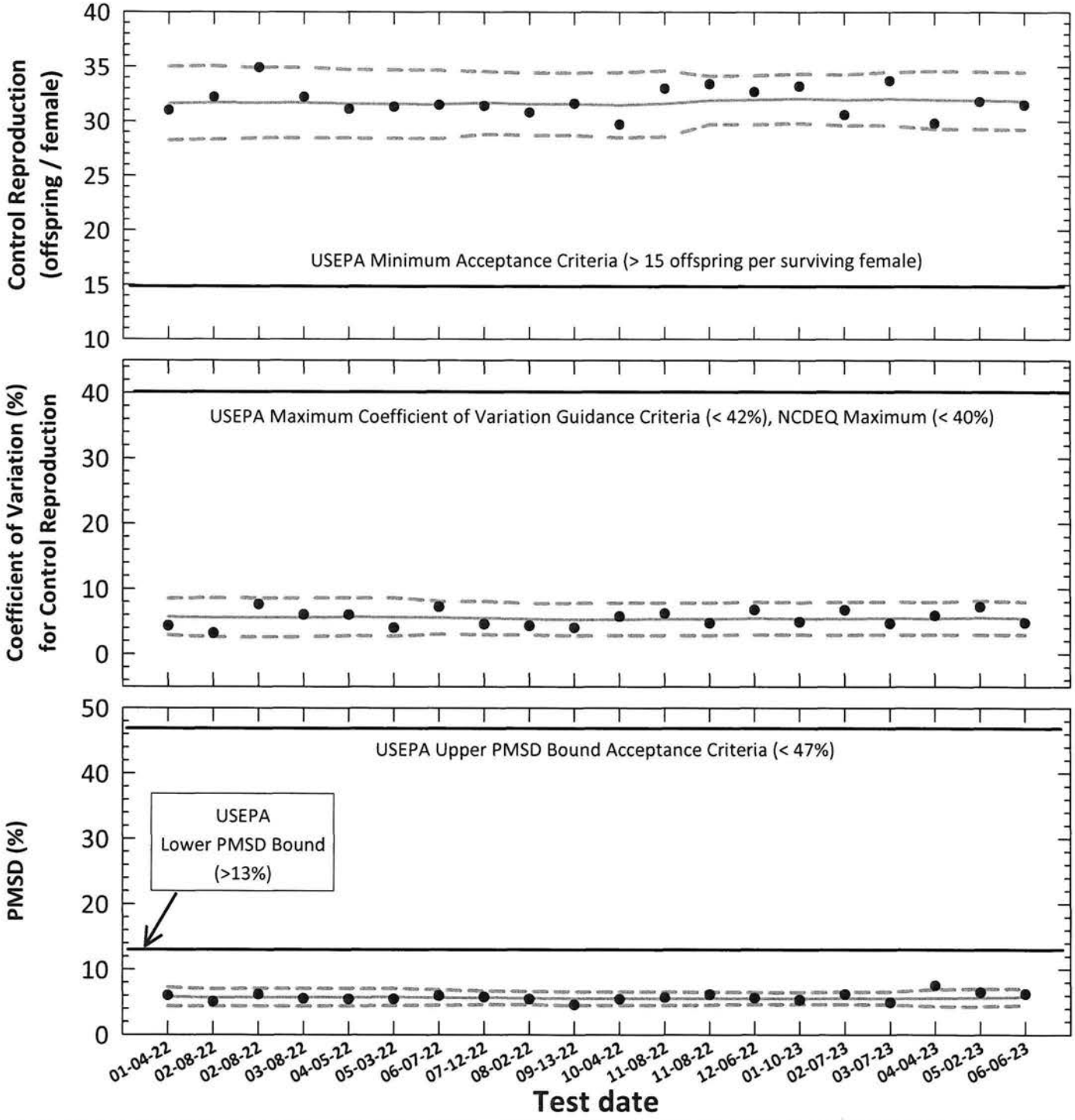
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **Control Reproduction, 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 Reproduction, CV or PMSD)

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control	Control Reproduction		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		Survival (%)	Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	2.9	8.5	5.8	4.4	7.2
2	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0
3	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1
4	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1
5	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
6	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
7	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
8	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
9	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
10	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
11	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6
12	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6
13	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	4.5	6.5
14	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	4.6	6.5
15	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	4.6	6.4
16	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5
17	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
18	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9
19	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0
20	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0

Note:

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

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

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) > 13%.
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) < 47%.

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)
Species: *Ceriodaphnia dubia*

CdNaClCR #: 285 - New Algae

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2194				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	06-06-23 0530 TO 0800									
Culture board:	05-30-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	8	9	10	18	23	24	29	31
Transfer vessel information:	pH (S.U.): 7.75 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	GREEN
Incubator number and shelf location:	ZB1

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	06-06-23	0810	05-24-23	05-31-23	05-30-23 C	H
1	06-07-23	0750	↓	↓	↓	H
2	06-08-23	0811	↓	↓	06-05-23 A	H
3	06-09-23	0810	↓	↓	↓	H
4	06-10-23	0910	↓	↓	06-05-23B	H
5	06-11-23	0910	↓	↓	↓	H
6	06-12-23	0850	↓	↓	↓	H
7	06-13-23	0724				H

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	31.5	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.8%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1093.1

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Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 285 - New Algae

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	4	6	6	3	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	13	10	10	13	9	13	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	16	13	17	18	14	18	15	16	15
Total young produced		29	32	30	33	34	30	32	32	32	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.5

600 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	5	6	5	4	4	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	13	12	10	10	10	13	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	17	19	17	18	15	19	14	16	16
Total young produced		30	35	36	36	35	29	33	30	33	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	33.0
% Reduction from Control:	-4.8%

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Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 285 - New Algae

800 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	4	5	4	5	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	11	12	10	13	13	11	13	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	17	15	19	14	14	18	16	13	16
Total young produced		33	32	31	34	31	32	34	34	30	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.5
% Reduction from Control:	3.27.

1000 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	6	4	4	5	4	3	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	10	12	13	13	13	11	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	17	14	14	13	18	15	14	14	14
Total young produced		29	30	28	32	30	35	33	29	27	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.3
% Reduction from Control:	3.87.

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 285 - New Algae

1200 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	5	4	4	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	8	7	10	9	9	7	11	8	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	3	3	6	2	6	5	5	7	4	5
Total young produced		17	14	17	17	19	18	16	21	15	19
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.3
% Reduction from Control:	45.1%

1400 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	2	3	2	4	2	3	3	3	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	1	0	0	0	0	0	0	1	0
Total young produced		2	3	3	2	4	2	3	3	4	1
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.7
% Reduction from Control:	91.4%

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Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	6	6	3	5	4	4	4	46
5	12	10	13	10	10	13	9	13	12	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	13	16	13	17	18	14	18	15	16	15	155
Total	**29**	**32**	**30**	**33**	**34**	**30**	**32**	**32**	**32**	**31**	**315**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	6	4	4	5	4	3	6	43
5	10	10	10	12	13	13	13	11	10	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	14	14	13	18	15	14	14	14	148
Total	**29**	**30**	**28**	**32**	**30**	**35**	**33**	**29**	**27**	**30**	**303**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	6	5	4	4	6	4	4	48
5	11	12	12	13	12	10	10	10	13	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	19	17	18	15	19	14	16	16	166
Total	**30**	**35**	**36**	**36**	**35**	**29**	**33**	**30**	**33**	**33**	**330**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	5	4	4	4	3	3	4	38
5	10	8	7	10	9	9	7	11	8	10	89
6	0	0	0	0	0	0	0	0	0	0	0
7	3	3	6	2	6	5	5	7	4	5	46
Total	**17**	**14**	**17**	**17**	**19**	**18**	**16**	**21**	**15**	**19**	**173**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	5	4	5	5	5	5	5	46
5	11	11	12	10	13	13	11	13	12	13	119
6	0	0	0	0	0	0	0	0	0	0	0
7	18	17	15	19	14	14	18	16	13	16	160
Total	**33**	**32**	**31**	**34**	**31**	**32**	**34**	**34**	**30**	**34**	**325**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	2	3	2	4	2	3	3	3	1	25
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	1	0	0	0	0	0	0	1	0	2
Total	**2**	**3**	**3**	**2**	**4**	**2**	**3**	**3**	**4**	**1**	**27**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Ceriodaphnia dubia Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1002.0

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

Test number: CdNaClCR #285 - New Algae Slant

Test dates: June 06-13, 2023

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	29	32	30	33	34	30	32	32	32	31	**100**	**31.5**	**4.8**	**Not applicable**
600	30	35	36	36	35	29	33	30	33	33	**100**	**33.0**	**7.8**	**-4.8**
800	33	32	31	34	31	32	34	34	30	34	**100**	**32.5**	**4.6**	**-3.2**
1000	29	30	28	32	30	35	33	29	27	30	**100**	**30.3**	**7.9**	**3.8**
1200	17	14	17	17	19	18	16	21	15	19	**100**	**17.3**	**11.9**	**45.1**
1400	2	3	3	2	4	2	3	3	4	1	**100**	**2.7**	**35.1**	**91.4**

Dunnett's MSD value: 1.965

PMSD: 6.2

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (10^{th} percentile) = 13%.

Upper PMSD bound determined by USEPA (90^{th} percentile) = 47%.

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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	6/6/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/13/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:	New Algae Slant				

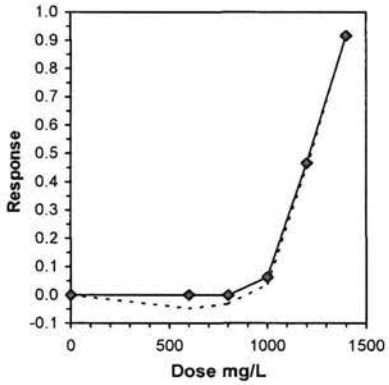
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	29.000	32.000	30.000	33.000	34.000	30.000	32.000	32.000	32.000	31.000
600	30.000	35.000	36.000	36.000	35.000	29.000	33.000	30.000	33.000	33.000
800	33.000	32.000	31.000	34.000	31.000	32.000	34.000	34.000	30.000	34.000
1000	29.000	30.000	28.000	32.000	30.000	35.000	33.000	29.000	27.000	30.000
1200	17.000	14.000	17.000	17.000	19.000	18.000	16.000	21.000	15.000	19.000
1400	2.000	3.000	3.000	2.000	4.000	2.000	3.000	3.000	4.000	1.000

			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	31.500	1.0000	31.500	29.000	34.000	4.791	10				32.333	1.0000
600	33.000	1.0476	33.000	29.000	36.000	7.824	10	-1.746	2.287	1.965	32.333	1.0000
800	32.500	1.0317	32.500	30.000	34.000	4.644	10	-1.164	2.287	1.965	32.333	1.0000
1000	30.300	0.9619	30.300	27.000	35.000	7.941	10	1.397	2.287	1.965	30.300	0.9371
*1200	17.300	0.5492	17.300	14.000	21.000	11.893	10	16.528	2.287	1.965	17.300	0.5351
*1400	2.700	0.0857	2.700	1.000	4.000	35.136	10	33.521	2.287	1.965	2.700	0.0835

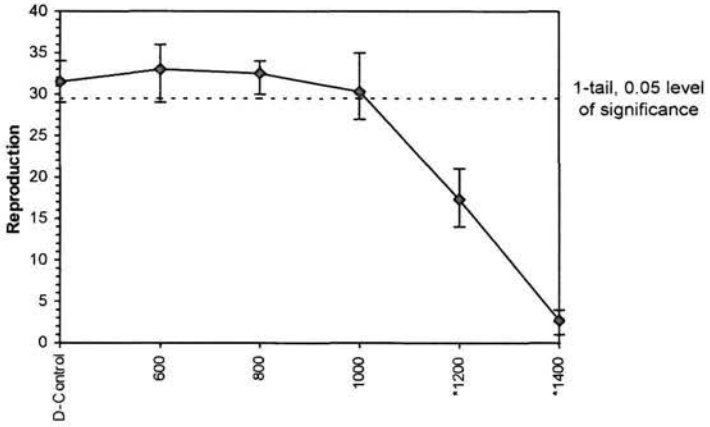
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)					0.59727		1.035		0.07015	-0.1237
Bartlett's Test indicates equal variances (p = 0.07)					10.3343		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	1000	1200	1095.45		1.9646	0.06237	1491.91	3.69074	1.8E-41	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	959.016	45.7796	868.87	1014.41	-0.1868
IC10	1018.46	14.3624	977.595	1036.57	-0.9276
IC15	1043.33	10.5267	1020.95	1059.4	-0.0307
IC20	1068.21	9.53976	1049.08	1083.89	0.0079
IC25	1093.08	8.92026	1075.2	1108.5	0.0378
IC40	1167.69	9.77108	1150.96	1188.49	0.2605
IC50	1215.53	8.80783	1199.02	1232.32	-0.0828



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **285 - New Algae**

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	BL	BL N	BL N	N	N	BL N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.74	7.81	7.77	7.09	7.87	7.88
	Dissolved oxygen (mg/L)	7.5	8.0	7.9	7.6	7.7	7.8
	Conductivity (µmhos/cm)	314		314		297	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	84			06-13-23	84	
	Temperature (°C)	24.9	25.1	24.8	25.2	24.7	25.2
600 mg NaCl/L	pH (S.U.)	7.82	7.85	7.86	7.06	7.90	7.87
	Dissolved oxygen (mg/L)	7.5	7.9	7.9	7.6	7.6	7.8
	Conductivity (µmhos/cm)	1450		1470		1400	
	Temperature (°C)	25.0	24.9	24.9	24.9	24.7	25.2
800 mg NaCl/L	pH (S.U.)	7.84	7.85	7.86	7.06	7.89	7.87
	Dissolved oxygen (mg/L)	7.5	7.8	8.0	7.6	7.6	7.8
	Conductivity (µmhos/cm)	1820		1880		1090	
	Temperature (°C)	25.0	25.0	24.8	24.9	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.84	7.84	7.86	7.07	7.89	7.86
	Dissolved oxygen (mg/L)	7.6	7.8	8.0	7.6	7.6	7.7
	Conductivity (µmhos/cm)	2180		2200		2050	
	Temperature (°C)	25.0	25.2	24.8	24.9	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.84	283	7.87	7.07	7.00	7.86
	Dissolved oxygen (mg/L)	7.6	7.8	7.8	7.6	7.4	7.8
	Conductivity (µmhos/cm)	2530		2570		2440	
	Temperature (°C)	24.9	24.8	24.9	25.1	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.84	7.83	7.87	7.00	7.90	7.85
	Dissolved oxygen (mg/L)	7.6	7.9	7.8	7.5	7.6	7.8
	Conductivity (µmhos/cm)	2900 ~~2910~~ 06-06-23		2910		2760	
	Temperature (°C)	24.9	24.8	24.9	24.9	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

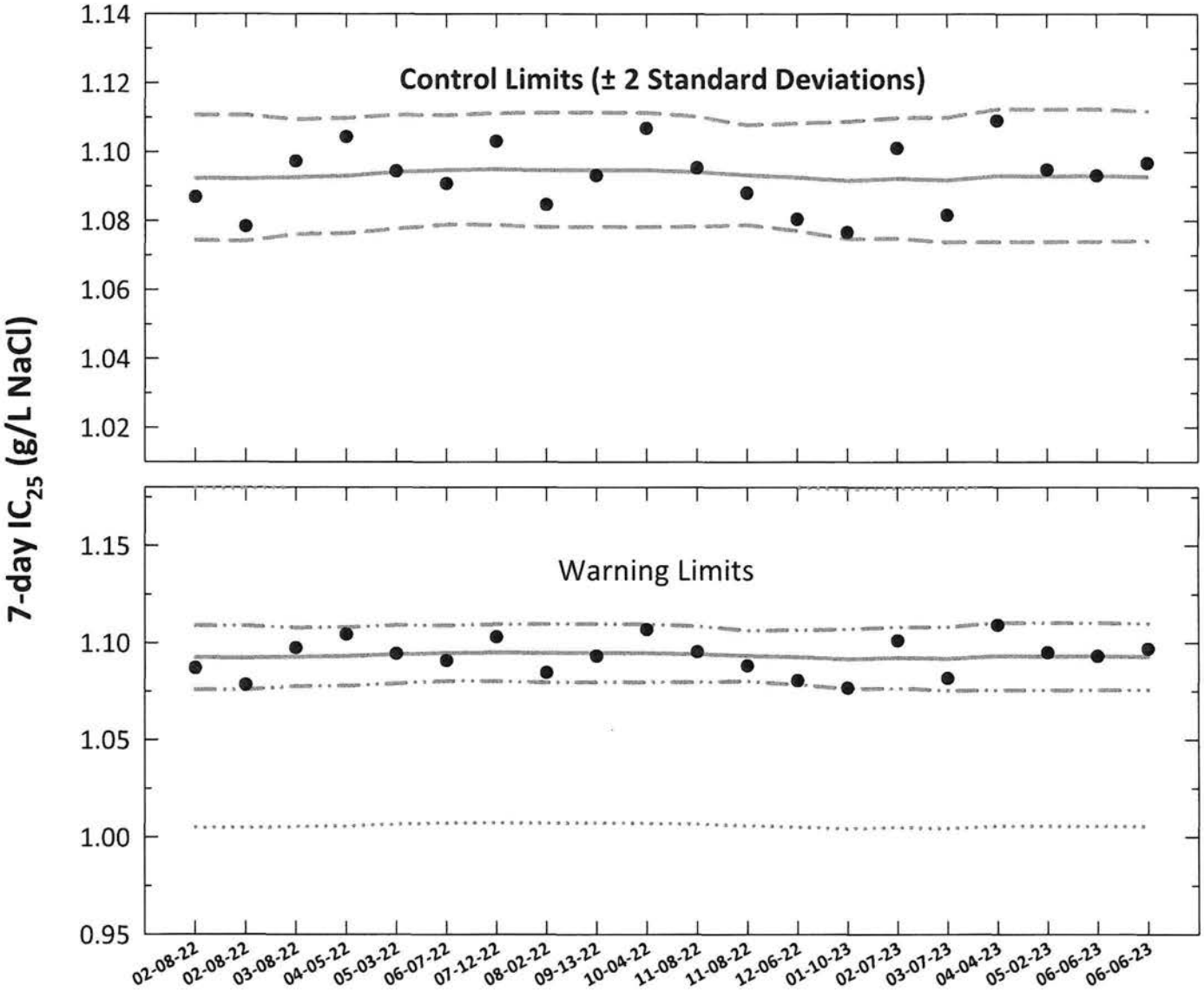
Species: ***Ceriodaphnia dubia***

CdNaClCR #: 285 - New Algae

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL N	BSL	BSL	BSL	BSL	BL N	BL N	N
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.90	7.96	7.77	7.90	7.78	7.90	7.81	7.99
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.9	7.7	7.7	7.7	7.8
	Conductivity (µmhos/cm)	311		300		303		310	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	24.4	24.8	25.0	24.8	25.2	24.7	25.1
600 mg NaCl/L	pH (S.U.)	7.93	7.93	7.91	7.89	7.92	7.92	7.92	7.91
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.9	8.0	7.7	7.7	7.8
	Conductivity (µmhos/cm)	1470		1420		1450		1430	
	Temperature (°C)	24.8	24.7	24.7	24.8	24.9	24.9	24.7	24.8
800 mg NaCl/L	pH (S.U.)	7.93	7.93	7.93	7.89	7.92	7.92	7.94	7.90
	Dissolved oxygen (mg/L)	7.8	7.9	8.0	7.8	8.0	7.7	7.7	7.9
	Conductivity (µmhos/cm)	1800		1720		1750		1790	
	Temperature (°C)	24.9	24.7	24.7	24.8	24.9	24.9	24.8	24.8
1000 mg NaCl/L	pH (S.U.)	7.93	7.93	7.94	7.89	7.92	7.94	7.94	7.88
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.8	8.0	7.7	7.8	8.0
	Conductivity (µmhos/cm)	2180		2080		2150		2130	
	Temperature (°C)	24.9	24.7	24.7	25.1	24.8	25.1	24.7	24.8
1200 mg NaCl/L	pH (S.U.)	7.92	7.92	7.94	7.89	7.92	7.93	7.94	7.87
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.8	8.0	7.7	7.9	8.0
	Conductivity (µmhos/cm)	2570		2440		2520		2500	
	Temperature (°C)	24.9	24.8	24.8	24.9	24.8	24.8	24.7	25.0
1400 mg NaCl/L	pH (S.U.)	7.92	7.92	7.95	7.88	7.91	7.92	7.94	7.88
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.8	8.0	7.8	7.9	8.0
	Conductivity (µmhos/cm)	2880		2800		2880		2880	
	Temperature (°C)	24.9	24.7	24.8	25.1	24.8	24.8	24.7	25.0
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (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} ± $S_{A.10}$ 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.

Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	02-08-22	1.0869	0.0421	0.0384	0.0036	1.0924	1.0744	1.1107	1.0759	1.1089	1.0050	1.1798
2	02-08-22	1.0784	0.0362	0.0383	0.0036	1.0923	1.0742	1.1107	1.0757	1.1089	1.0049	1.1797
3	03-08-22	1.0972	0.0328	0.0385	0.0033	1.0927	1.0761	1.1094	1.0775	1.1078	1.0052	1.1801
4	04-05-22	1.1043	0.0403	0.0386	0.0033	1.0930	1.0765	1.1099	1.0779	1.1082	1.0056	1.1805
5	05-03-22	1.0944	0.0431	0.0391	0.0033	1.0942	1.0777	1.1108	1.0792	1.1092	1.0066	1.1817
6	06-07-22	1.0908	0.0377	0.0393	0.0031	1.0947	1.0789	1.1106	1.0803	1.1090	1.0071	1.1822
7	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
8	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
9	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
10	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
11	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
12	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
13	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
14	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
15	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
16	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
17	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
18	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
19	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
20	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802

Note:

7-day IC_{25} = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (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 $S_{A.10}$ converted to anti-logarithmic values.

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

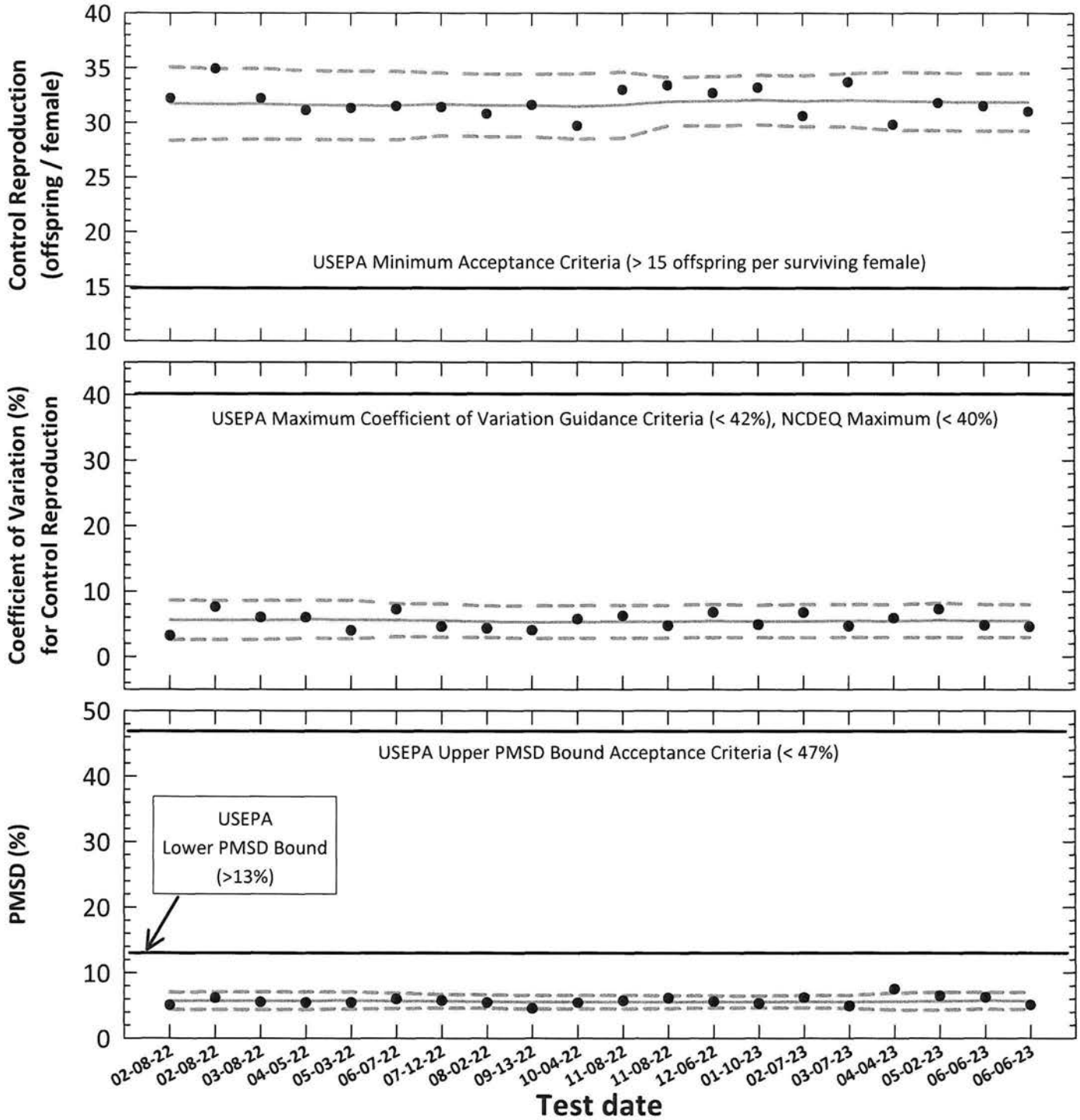
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **Control Reproduction, 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 Reproduction, CV or PMSD)

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

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ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control	Control Reproduction		Test										
		Survival	Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(offspring/female)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	2.6	8.6	5.7	4.4	7.0
2	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	2.6	8.5	5.7	4.4	7.1
3	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	2.6	8.6	5.7	4.4	7.1
4	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
5	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
6	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
7	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
8	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
9	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
10	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6
11	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6
12	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	4.5	6.5
13	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	4.6	6.5
14	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	4.6	6.4
15	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5
16	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
17	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9
18	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0
19	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0
20	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0

Note:

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

Control Mean Reproduction = USEPA minimum test acceptability criteria ≥ 15 offspring/surviving female.

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

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) > 13%.
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) < 47%.

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)
Species: *Ceriodaphnia dubia*

CdNaClCR #: 286

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2194				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	06-06-23 0530 TO 0800									
Culture board:	05-30-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	8	9	10	18	23	24	29	31
Transfer vessel information:	pH (S.U.): 7.75 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	GOLD
Incubator number and shelf location:	2B2

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	06-06-23	0820	05-24-23 NEW	05-31-23	05-30-23 C	JH
1	06-07-23	0800	↓	↓	↓	JH
2	06-08-23	0823			06-05-23 A	JH
3	06-09-23	0820			↓	JH
4	06-10-23	0920			06-05-23B	JH
5	06-11-23	0920			↓	JH
6	06-12-23	0900	↓	↓		JH
7	06-13-23	0739				JH

*Organisms fed daily 100 µL *Selenastrum* and 100 µL YWT per replicate using HandyStep repeat pipettor SN 17E59354.

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664PS

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	31.0	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.6%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1096.7

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 286

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	3	4	3	3	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	9	10	10	12	12	12	11	12	9
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	17	19	18	15	18	16	16	14	16
Total young produced		29	30	33	31	31	33	31	32	31	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.0

600 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	4	3	5	4	5	5	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	9	10	10	10	13	11	11	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	15	19	17	18	19	14	18	17	17
Total young produced		33	32	32	30	33	33	32	34	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.2
% Reduction from Control:	-3.9%

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 286

800 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	3	4	4	4	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	9	10	10	14	12	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	19	16	15	15	17	13	16	16	17
Total young produced		34	35	31	28	29	31	32	33	30	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.5
% Reduction from Control:	-1.6%

1000 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	3	5	4	5	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	10	9	11	12	10	12	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	15	13	16	16	14	16	12	15	17
Total young produced		30	30	26	30	31	31	30	28	29	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	29.6
% Reduction from Control:	4.5%

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 286**

1200 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	2	4	4	4	3	4	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	10	8	11	9	7	10	8	8	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	4	4	9	3	7	6	6	4	4	7
Total young produced		15	18	19	18	20	17	19	16	15	17
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.4
% Reduction from Control:	43.9%

1400 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	1	3	2	2	1	1	3	1	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		2	1	3	2	2	1	1	3	1	3
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

Note: Adult mortality (L = live, D = dead), SB = split brood (single brood split between two days), CO = carry over (offspring carried over with adult during transfer).

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.9
% Reduction from Control:	93.9%

ETS
Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	3	4	3	3	5	5	4	40
5	10	9	10	10	12	12	12	11	12	9	107
6	0	0	0	0	0	0	0	0	0	0	0
7	14	17	19	18	15	18	16	16	14	16	163
Total	**29**	**30**	**33**	**31**	**31**	**33**	**31**	**32**	**31**	**29**	**310**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	3	5	4	5	4	4	4	4	40
5	12	12	10	9	11	12	10	12	10	10	108
6	0	0	0	0	0	0	0	0	0	0	0
7	14	15	13	16	16	14	16	12	15	17	148
Total	**30**	**30**	**26**	**30**	**31**	**31**	**30**	**28**	**29**	**31**	**296**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	3	5	4	5	5	3	5	42
5	10	13	9	10	10	10	13	11	11	10	107
6	0	0	0	0	0	0	0	0	0	0	0
7	19	15	19	17	18	19	14	18	17	17	173
Total	**33**	**32**	**32**	**30**	**33**	**33**	**32**	**34**	**31**	**32**	**322**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	2	4	4	4	3	4	3	3	34
5	8	10	8	11	9	7	10	8	8	7	86
6	0	0	0	0	0	0	0	0	0	0	0
7	4	4	9	3	7	6	6	4	4	7	54
Total	**15**	**18**	**19**	**18**	**20**	**17**	**19**	**16**	**15**	**17**	**174**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	3	4	4	4	5	5	4	4	42
5	11	12	12	9	10	10	14	12	10	11	111
6	0	0	0	0	0	0	0	0	0	0	0
7	18	19	16	15	15	17	13	16	16	17	162
Total	**34**	**35**	**31**	**28**	**29**	**31**	**32**	**33**	**30**	**32**	**315**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	2	2	1	1	3	1	3	19
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**1**	**3**	**2**	**2**	**1**	**1**	**3**	**1**	**3**	**19**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Ceriodaphnia dubia Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1002.0

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

Test number: CdNaClCR #286

Test dates: June 06-13, 2023

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	29	30	33	31	31	33	31	32	31	29	100	31.0	4.6	Not applicable
600	33	32	32	30	33	33	32	34	31	32	100	32.2	3.5	-3.9
800	34	35	31	28	29	31	32	33	30	32	100	31.5	6.9	-1.6
1000	30	30	26	30	31	31	30	28	29	31	100	29.6	5.3	4.5
1200	15	18	19	18	20	17	19	16	15	17	100	17.4	9.8	43.9
1400	2	1	3	2	2	1	1	3	1	3	100	1.9	46.1	93.9

Dunnett's MSD value: 1.573

PMSD: 5.1

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 (10^{th} percentile) = 13%.

Upper PMSD bound determined by USEPA (90^{th} percentile) = 47%.

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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	6/6/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/13/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

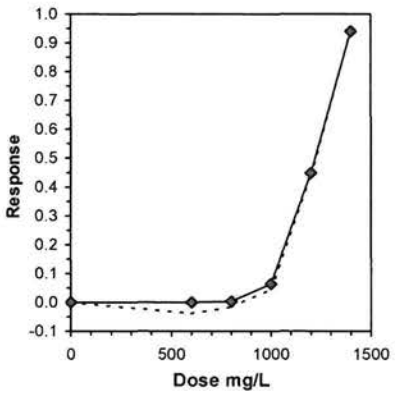
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	29.000	30.000	33.000	31.000	31.000	33.000	31.000	32.000	31.000	29.000
600	33.000	32.000	32.000	30.000	33.000	33.000	32.000	34.000	31.000	32.000
800	34.000	35.000	31.000	28.000	29.000	31.000	32.000	33.000	30.000	32.000
1000	30.000	30.000	26.000	30.000	31.000	31.000	30.000	28.000	29.000	31.000
1200	15.000	18.000	19.000	18.000	20.000	17.000	19.000	16.000	15.000	17.000
1400	2.000	1.000	3.000	2.000	2.000	1.000	1.000	3.000	1.000	3.000

			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	31.000	1.0000	31.000	29.000	33.000	4.562	10				31.600	1.0000
600	32.200	1.0387	32.200	30.000	34.000	3.526	10	-1.744	2.287	1.573	31.600	1.0000
800	31.500	1.0161	31.500	28.000	35.000	6.899	10	-0.727	2.287	1.573	31.500	0.9968
1000	29.600	0.9548	29.600	26.000	31.000	5.330	10	2.035	2.287	1.573	29.600	0.9367
*1200	17.400	0.5613	17.400	15.000	20.000	9.843	10	19.768	2.287	1.573	17.400	0.5506
*1400	1.900	0.0613	1.900	1.000	3.000	46.084	10	42.297	2.287	1.573	1.900	0.0601

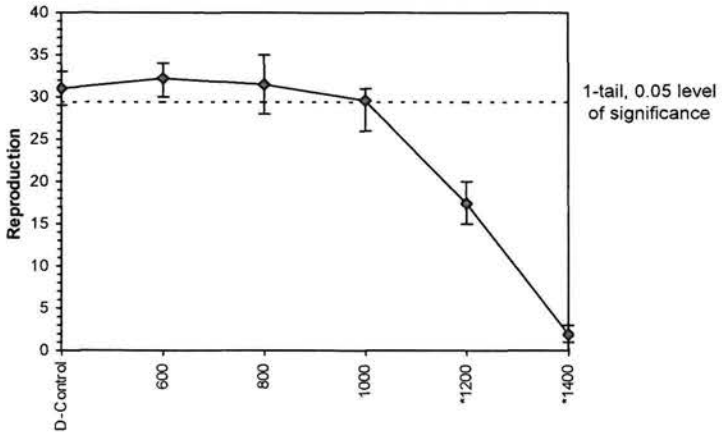
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)					0.62206		1.035		-0.2792	0.09834
Bartlett's Test indicates equal variances (p = 0.15)					8.17356		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	1000	1200	1095.45		1.57321	0.05075	1471.59	2.36667	0.0E+00	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	955.789	47.0477	840.217	1006.61	-0.9329
IC10	1019.02	9.00451	1000.6	1030.87	-1.4963
IC15	1044.92	7.14986	1028.76	1056.08	-0.3753
IC20	1070.82	6.59086	1056.49	1081.67	-0.2135
IC25	1096.72	6.3896	1083.19	1107.35	-0.0285
IC40	1174.43	7.96253	1159.39	1189.36	0.1984
IC50	1220.65	6.51793	1206.55	1231.08	-0.3685



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 286**

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	BL	BL N	BL N	N	N	BL N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.74	7.84	7.77	7.87	7.87	7.87
	Dissolved oxygen (mg/L)	7.5	7.0	7.9	7.5	7.7	7.9
	Conductivity (µmhos/cm)	314		314		197	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	84			N 06-13-23	84	
	Temperature (°C)	24.8	25.1	24.8	25.0	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.82	7.86	7.86	7.85	7.90	7.87
	Dissolved oxygen (mg/L)	7.5	7.0	7.9	7.5	7.6	7.0
	Conductivity (µmhos/cm)	1450		1450		1400	
	Temperature (°C)	24.9	24.9	24.9	24.8	24.8	24.8
800 mg NaCl/L	pH (S.U.)	7.84	7.85	7.86	7.87	7.89	7.87
	Dissolved oxygen (mg/L)	7.5	7.9	8.0	7.5	7.6	7.0
	Conductivity (µmhos/cm)	1820		1820		1690	
	Temperature (°C)	24.9	24.9	24.9	25.0	24.9	24.8
1000 mg NaCl/L	pH (S.U.)	7.84	7.85	7.86	7.86	7.89	7.86
	Dissolved oxygen (mg/L)	7.6	7.9	8.0	7.6	7.6	7.0
	Conductivity (µmhos/cm)	2180		2200		2050	
	Temperature (°C)	25.0	25.0	25.0	25.1	24.9	24.8
1200 mg NaCl/L	pH (S.U.)	7.84	7.84	7.87	7.88	7.88	7.87
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.6	7.6	7.9
	Conductivity (µmhos/cm)	2530		2570		2440	
	Temperature (°C)	25.0	25.0	25.0	24.9	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.84	7.84	7.87	7.87	7.90	7.86
	Dissolved oxygen (mg/L)	7.6	8.0	7.9	7.6	7.4	7.9
	Conductivity (µmhos/cm)	2900		2910		2700	
	Temperature (°C)	24.9	25.2	25.0	24.8	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

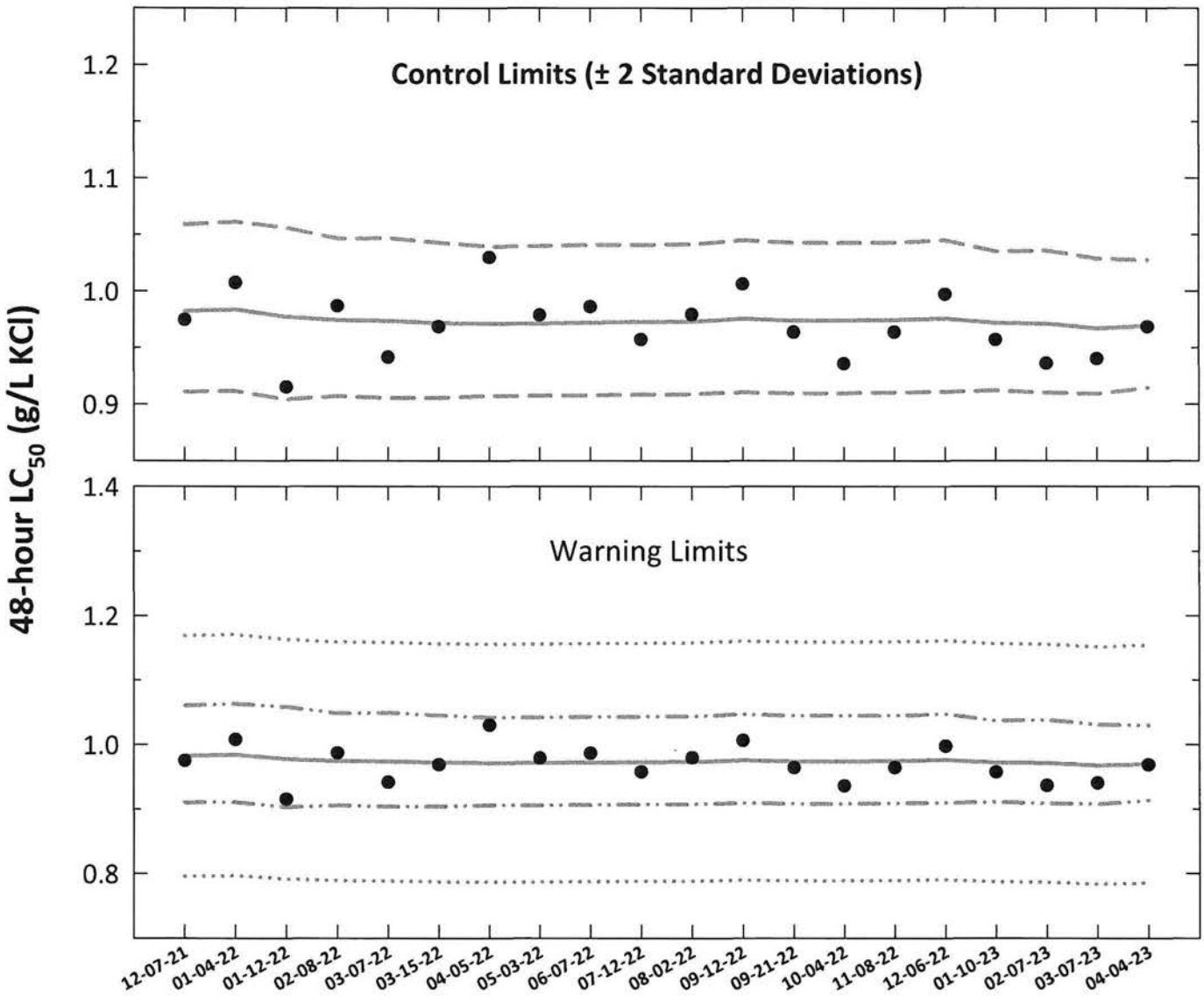
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 286**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL	BSL	BSL	BSL	BSL	BL	BL	
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.90	7.96	7.77	7.90	7.78	7.86	7.81	7.97
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.9	7.7	7.8	7.7	7.8
	Conductivity (µmhos/cm)	311		300		303		310	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	25.1	24.8	25.0	24.7	24.6	24.6	25.1
600 mg NaCl/L	pH (S.U.)	7.93	7.94	7.91	7.89	7.92	7.93	7.92	7.91
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.9	8.0	7.8	7.7	7.8
	Conductivity (µmhos/cm)	1420		1420		1450		1430	
	Temperature (°C)	24.8	25.1	24.7	24.8	24.9	24.3	24.6	24.8
800 mg NaCl/L	pH (S.U.)	7.93	7.93	7.93	7.88	7.92	7.92	7.94	7.90
	Dissolved oxygen (mg/L)	7.8	7.9	8.0	7.8	8.0	7.8	7.7	7.9
	Conductivity (µmhos/cm)	1800		1720		1750		1790	
	Temperature (°C)	24.8	24.8	24.7	24.8	24.9	24.3	24.8	24.9
1000 mg NaCl/L	pH (S.U.)	7.93	7.92	7.94	7.88	7.92	7.91	7.94	7.88
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.8	8.0	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2180		2080		2150		2130	
	Temperature (°C)	24.8	24.7	24.7	24.8	24.7	24.4	24.7	24.9
1200 mg NaCl/L	pH (S.U.)	7.92	7.93	7.94	7.87	7.92	7.93	7.94	7.88
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.7	8.0	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2570		2440		2520		2500	
	Temperature (°C)	24.8	24.7	24.8	24.9	24.7	24.4	24.8	25.0
1400 mg NaCl/L	pH (S.U.)	7.92	7.93	7.95	7.87	7.91	7.95	7.94	7.88
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	7.7	8.0	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2880		2800		2880		2880	
	Temperature (°C)	24.9	24.9	24.8	24.8	24.7	24.4	24.8	25.0
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



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} ± $S_{A.75}$ 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.

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**12-07-21**	**0.9747**	-0.0111	-0.0078	0.0163	0.9823	0.9111	1.0590	0.9099	1.0604	0.7957	1.1689
2	**01-04-22**	**1.0075**	0.0033	-0.0072	0.0165	0.9836	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705
3	**01-12-22**	**0.9151**	-0.0385	-0.0100	0.0168	0.9773	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630
4	**02-08-22**	**0.9869**	-0.0057	-0.0112	0.0155	0.9745	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597
5	**03-07-22**	**0.9416**	-0.0261	-0.0116	0.0157	0.9737	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587
6	**03-15-22**	**0.9685**	-0.0139	-0.0124	0.0153	0.9718	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564
7	**04-05-22**	**1.0297**	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
8	**05-03-22**	**0.9788**	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562
9	**06-07-22**	**0.9862**	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
10	**07-12-22**	**0.9572**	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
11	**08-02-22**	**0.9794**	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
12	**09-12-22**	**1.0063**	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
13	**09-21-22**	**0.9638**	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
14	**10-04-22**	**0.9358**	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
15	**11-08-22**	**0.9637**	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595
16	**12-06-22**	**0.9971**	-0.0013	-0.0106	0.0149	0.9758	0.9111	1.0450	0.9095	1.0468	0.7904	1.1612
17	**01-10-23**	**0.9572**	-0.0190	-0.0123	0.0137	0.9720	0.9126	1.0352	0.9109	1.0370	0.7873	1.1566
18	**02-07-23**	**0.9362**	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379	0.7866	1.1557
19	**03-07-23**	**0.9402**	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308	0.7834	1.1509
20	**04-04-23**	**0.9683**	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293	0.7851	1.1535

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 $S_{A.75}$ converted to anti-logarithmic values.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.19).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 148

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
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 K stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2176

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	K	BL K	K
Control, MHSW	pH (S.U.)	7.97	7.93	8.04
	Dissolved oxygen (mg/L)	7.6	7.5	7.6
	Conductivity (µmhos/cm)	303		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.8	24.6	24.9
500 mg/L	pH (S.U.)	8.03	7.89	7.94
	Dissolved oxygen (mg/L)	8.0	7.7	7.7
	Conductivity (µmhos/cm)	1100		
	Temperature (°C)	24.8	24.7	24.6
750 mg/L	pH (S.U.)	8.00	7.88	7.91
	Dissolved oxygen (mg/L)	8.0	7.7	7.7
	Conductivity (µmhos/cm)	1580		
	Temperature (°C)	24.9	24.7	24.6
1000 mg/L	pH (S.U.)	7.98	7.85	7.84
	Dissolved oxygen (mg/L)	8.0	7.7	7.7
	Conductivity (µmhos/cm)	1990		
	Temperature (°C)	24.9	24.5	24.8
1250 mg/L	pH (S.U.)	7.98	283	7.90
	Dissolved oxygen (mg/L)	8.0	7.7	7.7
	Conductivity (µmhos/cm)	2430		
	Temperature (°C)	25.0	24.7	24.6
1500 mg/L	pH (S.U.)	7.98	7.88	
	Dissolved oxygen (mg/L)	8.0	7.8	
	Conductivity (µmhos/cm)	2890		
	Temperature (°C)	25.0	24.8	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identi on the test specific bench sheets 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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 148

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	04-04-23	* 0505	JJ	0705	JJ	7F	GREEN	03-24-23B
24	04-05-23			0700	JJ			
48 Termination	04-06-23			0703	JJ			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	03-23-23
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	03-28-23 1203 TO 03-29-23 0950
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.04
	Temperature (°C): 24.5

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

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

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 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	6 4d	6 4d	1 4d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	3 3d	5 1d	1	2	0	0
Mean Survival	100%		100%		90%		40%		15%		0%	

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	888.2
Upper 95% confidence limit (mg KCl/L)	1043.8
48-hour LC_{50} (mg KCl/L)	968.3

Comments:

Test Reviewed by: JA

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.6000
1250	0.1000	0.2000
1500	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
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	11.721	2.850	0.1279	8	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	22.716	2.850	0.1279	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

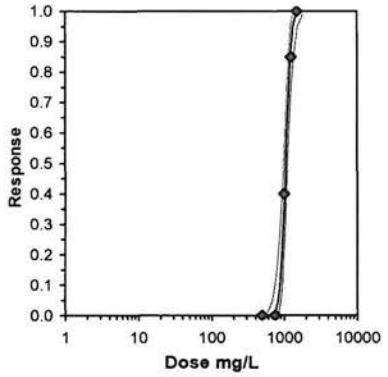
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.05495	0.05636	0.41903	0.00201	9.6E-06	4, 5
Treatments vs D-Control										

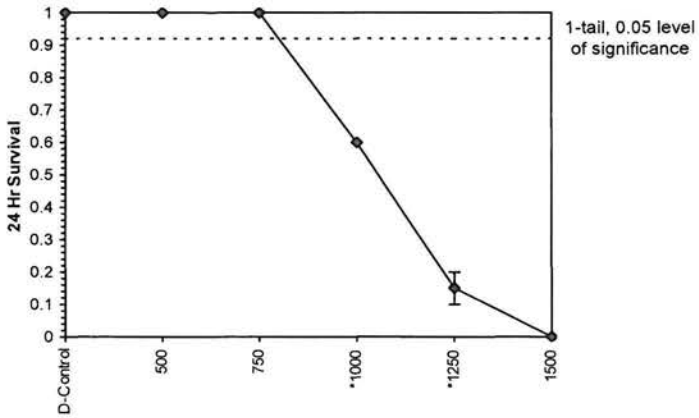
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.1171	3.20353	9.83815	22.396	0	0.69627	7.81472	0.87408	3.02272	0.06205	4
Intercept	-43.717	9.72207	-62.773	-24.662							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	755.756	593.827	846.04
EC05	3.355	833.04	693.657	911.183
EC10	3.718	877.419	752.63	949.114
EC15	3.964	908.69	794.561	976.411
EC20	4.158	934.336	828.965	999.364
EC25	4.326	956.914	859.092	1020.17
EC40	4.747	1016.26	936.132	1078.91
EC50	5.000	1053.71	981.768	1120.41
EC60	5.253	1092.55	1025.52	1168.16
EC75	5.674	1160.3	1092.79	1263.36
EC80	5.842	1188.34	1117.82	1306.59
EC85	6.036	1221.88	1146.19	1360.67
EC90	6.282	1265.43	1181.12	1434.1
EC95	6.645	1332.84	1232.24	1553.55
EC99	7.326	1469.14	1329.16	1811.94



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.3000	0.5000
1250	0.1000	0.2000
1500	0.0000	0.0000

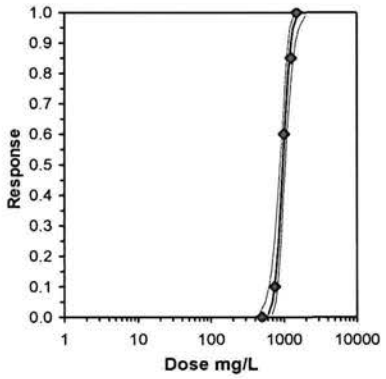
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2253	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.062	2.850	0.2253	2	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	9.230	2.850	0.2253	12	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	12.896	2.850	0.2253	17	20
1500	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	750	1000	866.025		0.11538	0.11834	0.43337	0.00625	1.4E-04	4, 5
Treatments vs D-Control										

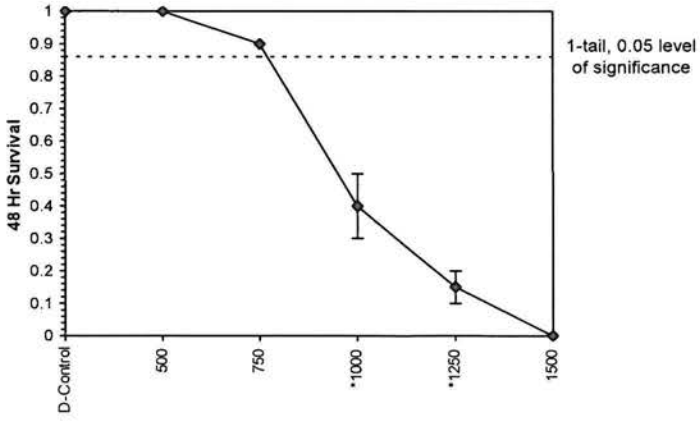
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.3411	2.00231	7.41661	15.2657	0	0.89	7.81472	0.82784	2.986	0.08817	3
Intercept	-28.865	6.01764	-40.659	-17.07							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	603.77	453.278	699.353
EC05	3.355	693.366	557.331	778.894
EC10	3.718	746.443	621.322	826.149
EC15	3.964	784.53	667.936	860.492
EC20	4.158	816.182	706.88	889.538
EC25	4.326	844.353	741.511	915.958
EC40	4.747	919.725	832.472	990.82
EC50	5.000	968.271	888.184	1043.8
EC60	5.253	1019.38	943.086	1104.9
EC75	5.674	1110.37	1030.61	1227.87
EC80	5.842	1148.7	1064.17	1284.46
EC85	6.036	1195.04	1102.82	1355.98
EC90	6.282	1256.02	1151.25	1454.43
EC95	6.645	1352.17	1223.74	1617.93
EC99	7.326	1552.82	1365.76	1985.2



Dose-Response Plot



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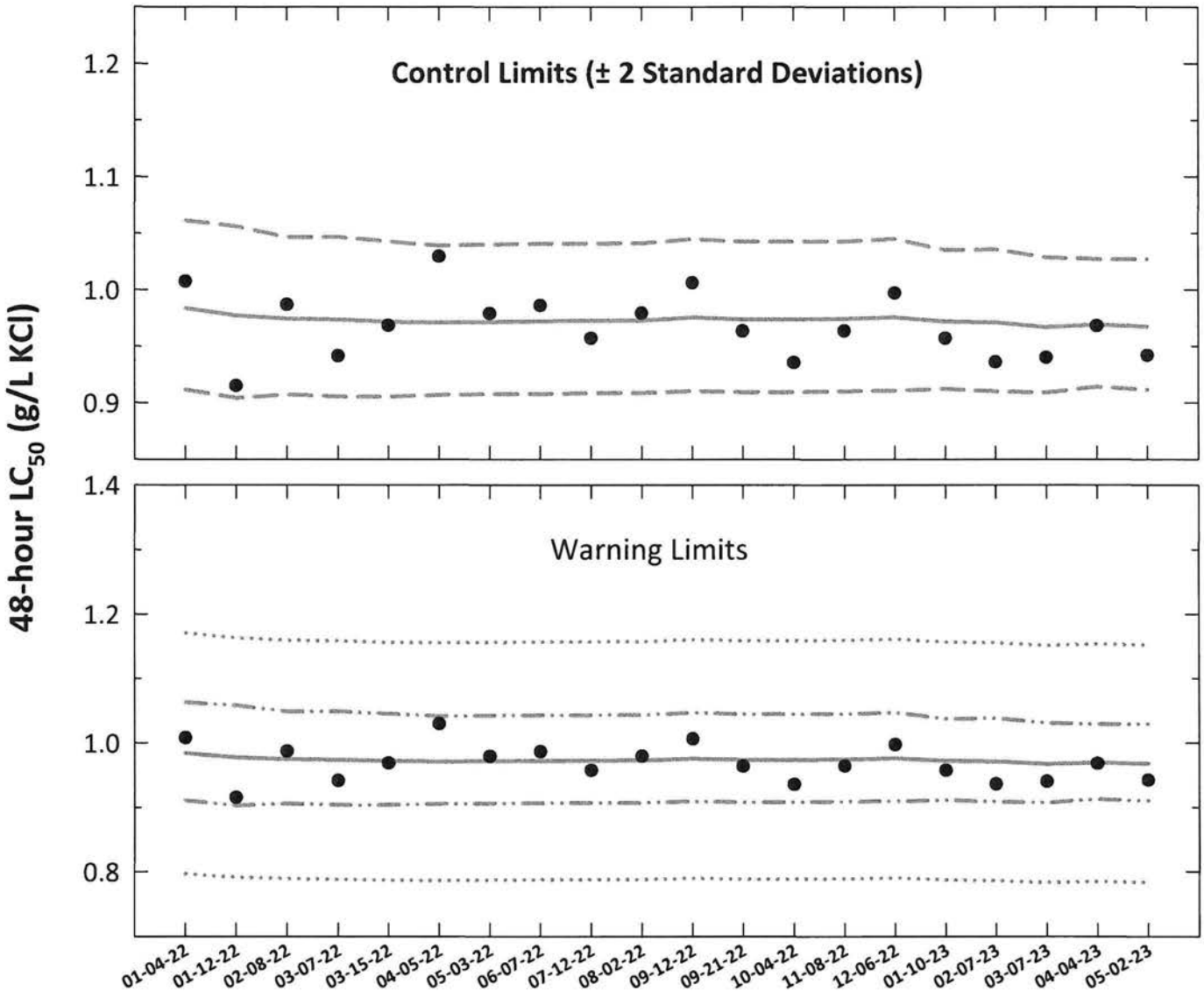
Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 149

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
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 K stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2176

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	BL	BL N	N
Control, MHSW	pH (S.U.)	7.92	7.92	8.02
	Dissolved oxygen (mg/L)	7.8	7.7	7.9
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	82		
	Temperature (°C)	24.7	24.6	24.5
500 mg/L	pH (S.U.)	8.05	7.88	7.92
	Dissolved oxygen (mg/L)	7.8	7.7	7.9
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	24.7	24.4	24.6
750 mg/L	pH (S.U.)	8.04	7.86	7.89
	Dissolved oxygen (mg/L)	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1540		
	Temperature (°C)	24.8	24.4	24.6
1000 mg/L	pH (S.U.)	8.03	7.83	7.88
	Dissolved oxygen (mg/L)	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1970		
	Temperature (°C)	24.8	24.7	24.6
1250 mg/L	pH (S.U.)	8.02	7.81	7.89
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	2400		
	Temperature (°C)	24.9	24.6	24.5
1500 mg/L	pH (S.U.)	8.02	7.82	
	Dissolved oxygen (mg/L)	8.0	7.9	
	Conductivity (µmhos/cm)	2810		
	Temperature (°C)	24.9	24.6	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numbe
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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066446

ETC

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 149

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05-02-23	* 0505	JH	0705	JH	1F	PINK	04-26-23A
24	05-03-23			0655	JH			
48 Termination	05-04-23			0703	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:	In-house culture
Spawning date:	04-21-23
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	04-26-23 1545 TO 04-27-23 0837
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.11
	Temperature (°C): 24.0° C

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

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

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 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	4 6d	5 5d	1 9d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	10	9 1d	3 1d	3 2d	1	0 1d	0	0
Mean Survival	100%		100%		95%		30%		5%		0%	

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	874.0
Upper 95% confidence limit (mg KCl/L)	1007.0
48-hour LC_{50} (mg KCl/L)	942.0

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**01-04-22**	**1.0075**	0.0033	-0.0072	0.0165	0.9836	0.9116	1.0614	0.9104	1.0626	0.7967	1.1705
2	**01-12-22**	**0.9151**	-0.0385	-0.0100	0.0168	0.9773	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630
3	**02-08-22**	**0.9869**	-0.0057	-0.0112	0.0155	0.9745	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597
4	**03-07-22**	**0.9416**	-0.0261	-0.0116	0.0157	0.9737	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587
5	**03-15-22**	**0.9685**	-0.0139	-0.0124	0.0153	0.9718	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564
6	**04-05-22**	**1.0297**	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
7	**05-03-22**	**0.9788**	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562
8	**06-07-22**	**0.9862**	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
9	**07-12-22**	**0.9572**	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
10	**08-02-22**	**0.9794**	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
11	**09-12-22**	**1.0063**	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
12	**09-21-22**	**0.9638**	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
13	**10-04-22**	**0.9358**	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
14	**11-08-22**	**0.9637**	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595
15	**12-06-22**	**0.9971**	-0.0013	-0.0106	0.0149	0.9758	0.9111	1.0450	0.9095	1.0468	0.7904	1.1612
16	**01-10-23**	**0.9572**	-0.0190	-0.0123	0.0137	0.9720	0.9126	1.0352	0.9109	1.0370	0.7873	1.1566
17	**02-07-23**	**0.9362**	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379	0.7866	1.1557
18	**03-07-23**	**0.9402**	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308	0.7834	1.1509
19	**04-04-23**	**0.9683**	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293	0.7851	1.1535
20	**05-02-23**	**0.9420**	-0.0260	-0.0143	0.0129	0.9677	0.9117	1.0270	0.9098	1.0290	0.7838	1.1515

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 $S_{A.75}$ converted to anti-logarithmic values.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.19).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.4000	0.5000
1250	0.1000	0.1000
1500	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
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	21.263	2.850	0.0907	11	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	34.245	2.850	0.0907	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

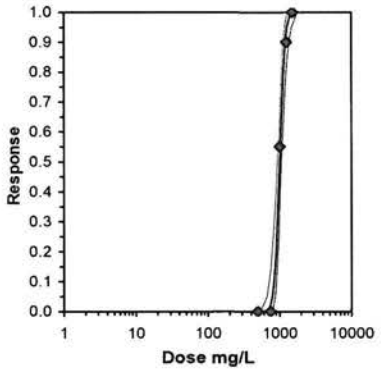
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	750	1000	866.025		0.03598	0.0369	0.51117	0.00101	1.1E-06	4, 5

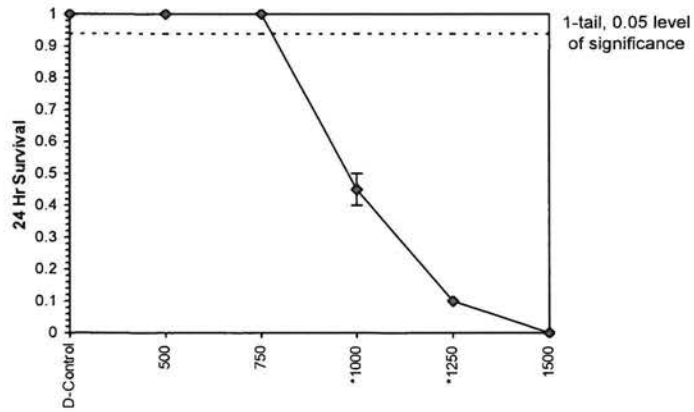
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.2237	3.23436	9.88433	22.563	0	1.26017	7.81472	0.73861	3.00326	0.06164	5
Intercept	-43.724	9.75376	-62.841	-24.606							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	724.209	568.642	810.983
EC05	3.355	797.756	663.652	873.095
EC10	3.718	839.969	719.716	909.281
EC15	3.964	869.705	759.549	935.332
EC20	4.158	894.087	792.214	957.242
EC25	4.326	915.549	820.807	977.101
EC40	4.747	971.941	893.904	1033.15
EC50	5.000	1007.53	937.235	1072.66
EC60	5.253	1044.41	978.852	1118.03
EC75	5.674	1108.74	1042.98	1208.28
EC80	5.842	1135.36	1066.88	1249.24
EC85	6.036	1167.19	1093.95	1300.48
EC90	6.282	1208.51	1127.25	1370.07
EC95	6.645	1272.45	1175.95	1483.32
EC99	7.326	1401.68	1268.09	1728.32



Dose-Response Plot



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Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.3000	0.3000
1250	0.1000	0.0000
1500	0.0000	0.0000

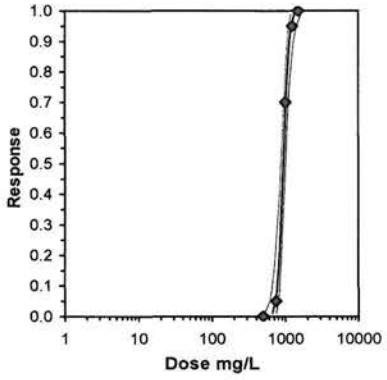
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2077	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.118	2.850	0.2077	1	20
*1000	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	2	11.421	2.850	0.2077	14	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	16.077	2.850	0.2077	19	20
1500	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	750	1000	866.025		0.10341	0.10606	0.60127	0.00531	4.3E-05	4, 5
Treatments vs D-Control										

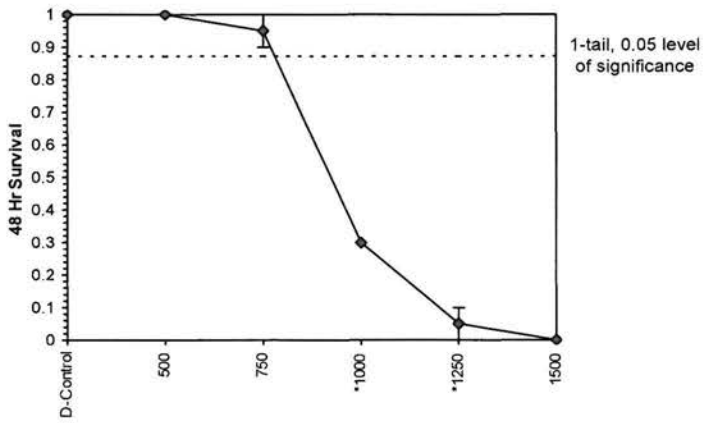
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.2983	2.94601	9.52413	21.0725	0	0.55053	7.81472	0.90766	2.97405	0.06537	3
Intercept	-40.498	8.79622	-57.739	-23.257							
TSCR											

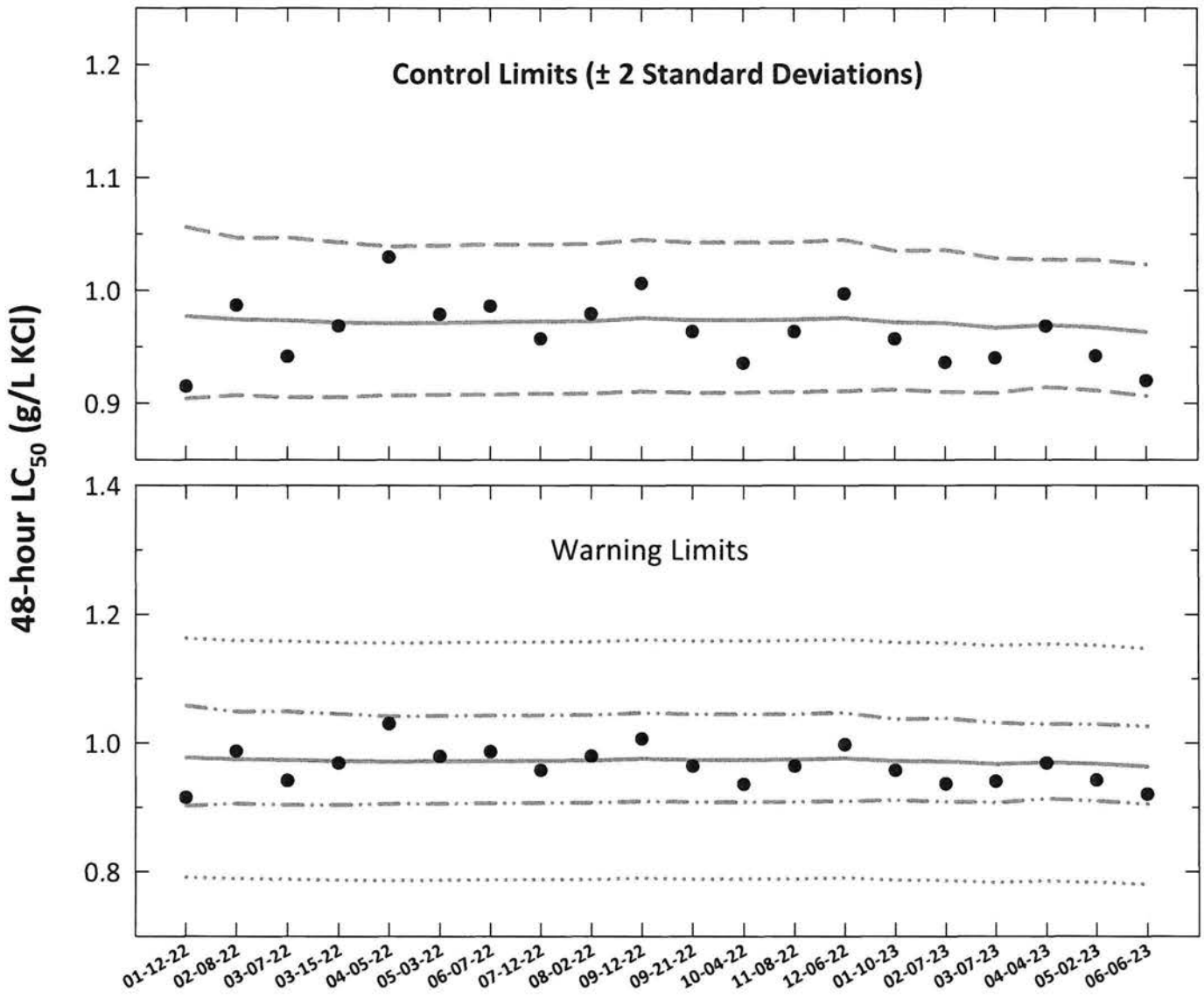
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	663.719	521.26	746.093
EC05	3.355	735.413	611.74	807.561
EC10	3.718	776.746	665.301	843.548
EC15	3.964	805.938	703.414	869.533
EC20	4.158	829.919	734.696	891.433
EC25	4.326	851.061	762.096	911.31
EC40	4.747	906.754	832.24	967.457
EC50	5.000	941.998	873.961	1006.96
EC60	5.253	978.611	914.261	1052.1
EC75	5.674	1042.65	977.088	1141.29
EC80	5.842	1069.21	1000.75	1181.64
EC85	6.036	1101.03	1027.71	1232.08
EC90	6.282	1142.41	1061.04	1300.61
EC95	6.645	1206.61	1110.04	1412.3
EC99	7.326	1336.95	1203.33	1654.91



Dose-Response Plot



Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ 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.

Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**01-12-22**	**0.9151**	-0.0385	-0.0100	0.0168	0.9773	0.9044	1.0560	0.9027	1.0579	0.7916	1.1630
2	**02-08-22**	**0.9869**	-0.0057	-0.0112	0.0155	0.9745	0.9075	1.0465	0.9058	1.0484	0.7894	1.1597
3	**03-07-22**	**0.9416**	-0.0261	-0.0116	0.0157	0.9737	0.9056	1.0469	0.9038	1.0489	0.7887	1.1587
4	**03-15-22**	**0.9685**	-0.0139	-0.0124	0.0153	0.9718	0.9055	1.0429	0.9036	1.0450	0.7871	1.1564
5	**04-05-22**	**1.0297**	0.0127	-0.0127	0.0147	0.9711	0.9074	1.0393	0.9055	1.0414	0.7866	1.1556
6	**05-03-22**	**0.9788**	-0.0093	-0.0125	0.0148	0.9716	0.9078	1.0400	0.9059	1.0420	0.7870	1.1562
7	**06-07-22**	**0.9862**	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
8	**07-12-22**	**0.9572**	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
9	**08-02-22**	**0.9794**	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
10	**09-12-22**	**1.0063**	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
11	**09-21-22**	**0.9638**	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
12	**10-04-22**	**0.9358**	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
13	**11-08-22**	**0.9637**	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595
14	**12-06-22**	**0.9971**	-0.0013	-0.0106	0.0149	0.9758	0.9111	1.0450	0.9095	1.0468	0.7904	1.1612
15	**01-10-23**	**0.9572**	-0.0190	-0.0123	0.0137	0.9720	0.9126	1.0352	0.9109	1.0370	0.7873	1.1566
16	**02-07-23**	**0.9362**	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379	0.7866	1.1557
17	**03-07-23**	**0.9402**	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308	0.7834	1.1509
18	**04-04-23**	**0.9683**	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293	0.7851	1.1535
19	**05-02-23**	**0.9420**	-0.0260	-0.0143	0.0129	0.9677	0.9117	1.0270	0.9098	1.0290	0.7838	1.1515
20	**06-06-23**	**0.9201**	-0.0362	-0.0162	0.0131	0.9633	0.9068	1.0233	0.9046	1.0256	0.7803	1.1463

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 $S_{A.75}$ converted to anti-logarithmic values.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.19).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 150

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
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 K stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2176

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, MHSW	pH (S.U.)	7.74	7.82	7.77
	Dissolved oxygen (mg/L)	7.5	7.6	7.6
	Conductivity (µmhos/cm)	314		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	64		
	Temperature (°C)	24.8	25.0	24.9
500 mg/L	pH (S.U.)	7.81	7.82	7.71
	Dissolved oxygen (mg/L)	7.7	7.5	7.6
	Conductivity (µmhos/cm)	1150		
	Temperature (°C)	24.7	24.8	24.7
750 mg/L	pH (S.U.)	7.85	7.84	7.76
	Dissolved oxygen (mg/L)	7.8	7.5	7.6
	Conductivity (µmhos/cm)	1640		
	Temperature (°C)	24.7	24.9	24.7
1000 mg/L	pH (S.U.)	7.86	7.83	7.81
	Dissolved oxygen (mg/L)	7.8	7.5	7.7
	Conductivity (µmhos/cm)	1990		
	Temperature (°C)	24.7	24.9	25.0
1250 mg/L	pH (S.U.)	7.86	7.83	7.85
	Dissolved oxygen (mg/L)	7.9	7.5	7.7
	Conductivity (µmhos/cm)	2390		
	Temperature (°C)	24.8	25.1	24.7
1500 mg/L	pH (S.U.)	7.87	7.82	
	Dissolved oxygen (mg/L)	7.9	7.6	
	Conductivity (µmhos/cm)	3040		
	Temperature (°C)	24.8	24.9	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identif on the test specific bench sheets 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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664

ETS

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 150

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-06-23	* 0505	JH	0705	JH	1D	WHITE	05-30-23C
24	06-07-23			0652	JH			
48 Termination	06-08-23			0700	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:	In-house culture
Spawning date:	05-24-23
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	05-30-23 1353 ~~06-06-23 M~~ TO 05-31-23 0807
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.14
	Temperature (°C): 24.0°C

EPA loading requirement for freshwater species of < 0.40 g/L at 25.0°C has been documented by ETS to never be exceeded using 1 to 14 day old *P. promelas*.

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

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 1d	8 2d	4 6d	4 6d	2 8d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	9	8	4	3 1d	1 1d	0 1d	0	0
Mean Survival	100%		100%		85%		35%		5%		0%	

Comment codes: d = dead, u = unhealthy, bs = bent spines, s = stressed

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	844.8
Upper 95% confidence limit (mg KCl/L)	990.5
48-hour LC_{50} (mg KCl/L)	920.1

Comments:

Test Reviewed by: JH

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.8000
1000	0.4000	0.4000
1250	0.2000	0.1000
1500	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
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1809	0	20
*750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.686	2.850	0.1809	3	20
*1000	0.4000	0.4000	0.6847	0.6847	0.6847	0.000	2	11.461	2.850	0.1809	12	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	16.063	2.850	0.1809	17	20
1500	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				

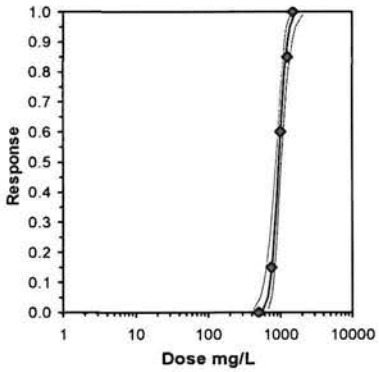
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.08599	0.08819	0.41909	0.00403	5.3E-05	4, 5
Treatments vs D-Control										

Maximum Likelihood-Probit

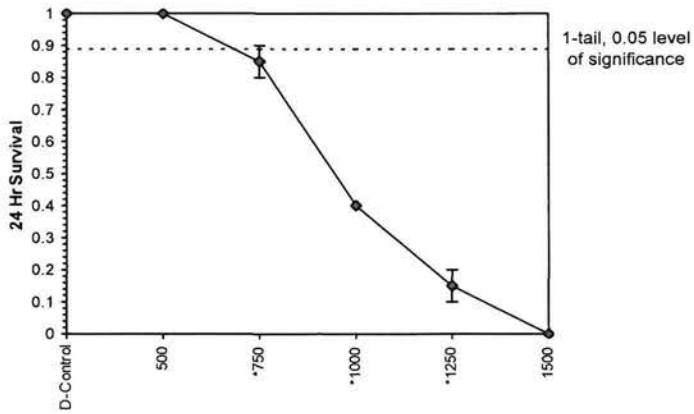
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	10.4498	1.82299	6.87676	14.0229	0	0.8245	7.81472	0.8436	2.97861	0.0957	4
Intercept	-26.126	5.47033	-36.848	-15.404							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	570.144	419.74	667.75
EC05	3.355	662.522	524.664	750.607
EC10	3.718	717.739	590.036	800.117
EC15	3.964	757.571	638.032	836.208
EC20	4.158	790.799	678.364	866.792
EC25	4.326	820.465	714.406	894.654
EC40	4.747	900.246	809.85	973.8
EC50	5.000	951.931	868.839	1030.01
EC60	5.253	1006.58	927.314	1095.12
EC75	5.674	1104.46	1021.1	1227.15
EC80	5.842	1145.89	1057.22	1288.31
EC85	6.036	1196.15	1098.95	1365.94
EC90	6.282	1262.54	1151.4	1473.34
EC95	6.645	1367.76	1230.29	1652.96
EC99	7.326	1589.38	1386.14	2061.41



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

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

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.8000
1000	0.4000	0.3000
1250	0.1000	0.0000
1500	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
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2166	0	20
*750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.079	2.850	0.2166	3	20
*1000	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	10.263	2.850	0.2166	13	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	15.421	2.850	0.2166	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

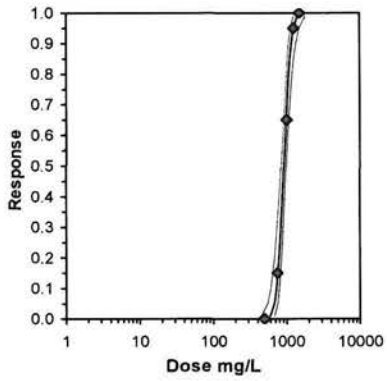
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.10938	0.11219	0.54029	0.00577	6.9E-05	4, 5
Treatments vs D-Control										

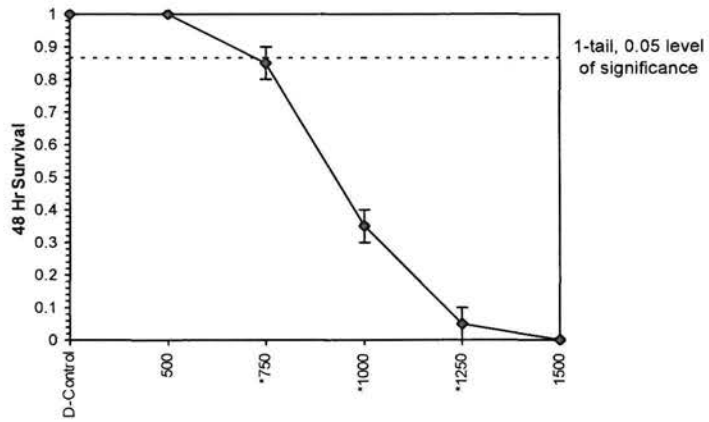
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.3217	2.25704	7.89789	16.7455	0	0.17339	7.81472	0.98177	2.96382	0.08116	3
Intercept	-31.519	6.73083	-44.712	-18.327							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	595.686	450.016	685.315
EC05	3.355	676.591	546.247	756.335
EC10	3.718	724.121	604.819	798.313
EC15	3.964	758.061	647.219	828.746
EC20	4.158	786.167	682.478	854.444
EC25	4.326	811.108	713.711	877.794
EC40	4.747	877.522	795.226	943.838
EC50	5.000	920.066	844.8	990.469
EC60	5.253	964.673	893.404	1044.13
EC75	5.674	1043.66	970.325	1151.75
EC80	5.842	1076.77	999.607	1201.12
EC85	6.036	1116.69	1033.18	1263.4
EC90	6.282	1169.03	1075.01	1348.89
EC95	6.645	1251.16	1137.18	1490.25
EC99	7.326	1421.09	1257.71	1805.06



Dose-Response Plot



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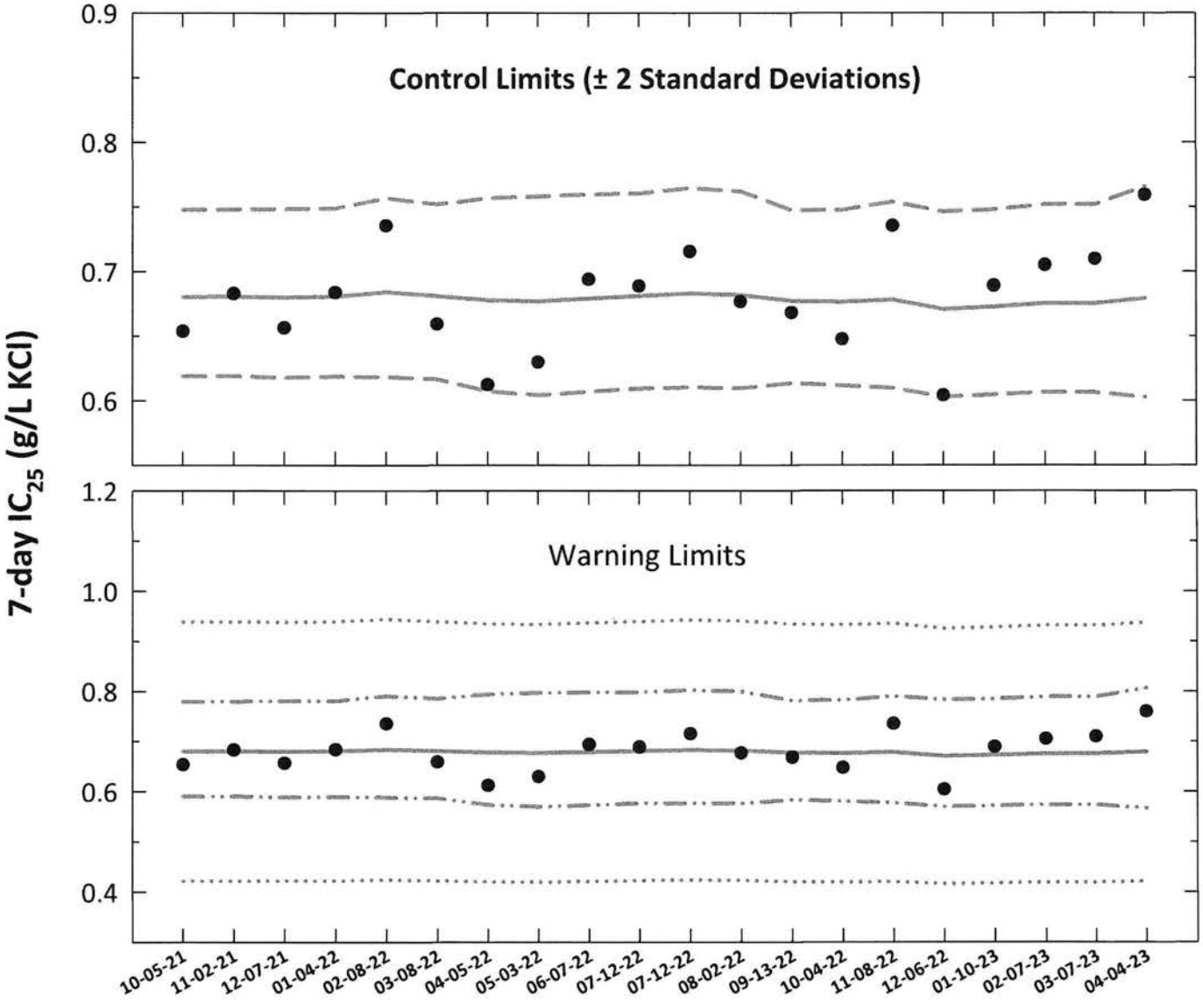
Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture



Test date

- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* 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)

Reviewed by: Jim Sumner

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**10-05-21**	**0.6538**	-0.1846	-0.1672	0.0205	0.6805	0.6190	0.7480	0.5902	0.7797	0.4219	0.9390
2	**11-02-21**	**0.6829**	-0.1656	-0.1670	0.0205	0.6808	0.6193	0.7483	0.5905	0.7799	0.4221	0.9395
3	**12-07-21**	**0.6565**	-0.1828	-0.1675	0.0208	0.6800	0.6180	0.7484	0.5887	0.7805	0.4216	0.9385
4	**01-04-22**	**0.6838**	-0.1651	-0.1670	0.0207	0.6808	0.6188	0.7490	0.5897	0.7810	0.4221	0.9395
5	**02-08-22**	**0.7354**	-0.1335	-0.1649	0.0219	0.6841	0.6184	0.7567	0.5881	0.7903	0.4241	0.9440
6	**03-08-22**	**0.6594**	-0.1808	-0.1668	0.0216	0.6811	0.6167	0.7522	0.5866	0.7855	0.4223	0.9399
7	**04-05-22**	**0.6124**	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
8	**05-03-22**	**0.6299**	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
9	**06-07-22**	**0.6939**	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372
10	**07-12-22**	**0.6887**	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
11	**07-12-22**	**0.7153**	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
12	**08-02-22**	**0.6766**	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
13	**09-13-22**	**0.6682**	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
14	**10-04-22**	**0.6477**	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
15	**11-08-22**	**0.7354**	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
16	**12-06-22**	**0.6041**	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
17	**01-10-23**	**0.6890**	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
18	**02-07-23**	**0.7050**	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
19	**03-07-23**	**0.7096**	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318
20	**04-04-23**	**0.7592**	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373

Note:

7-day IC_{25} = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* 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 ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

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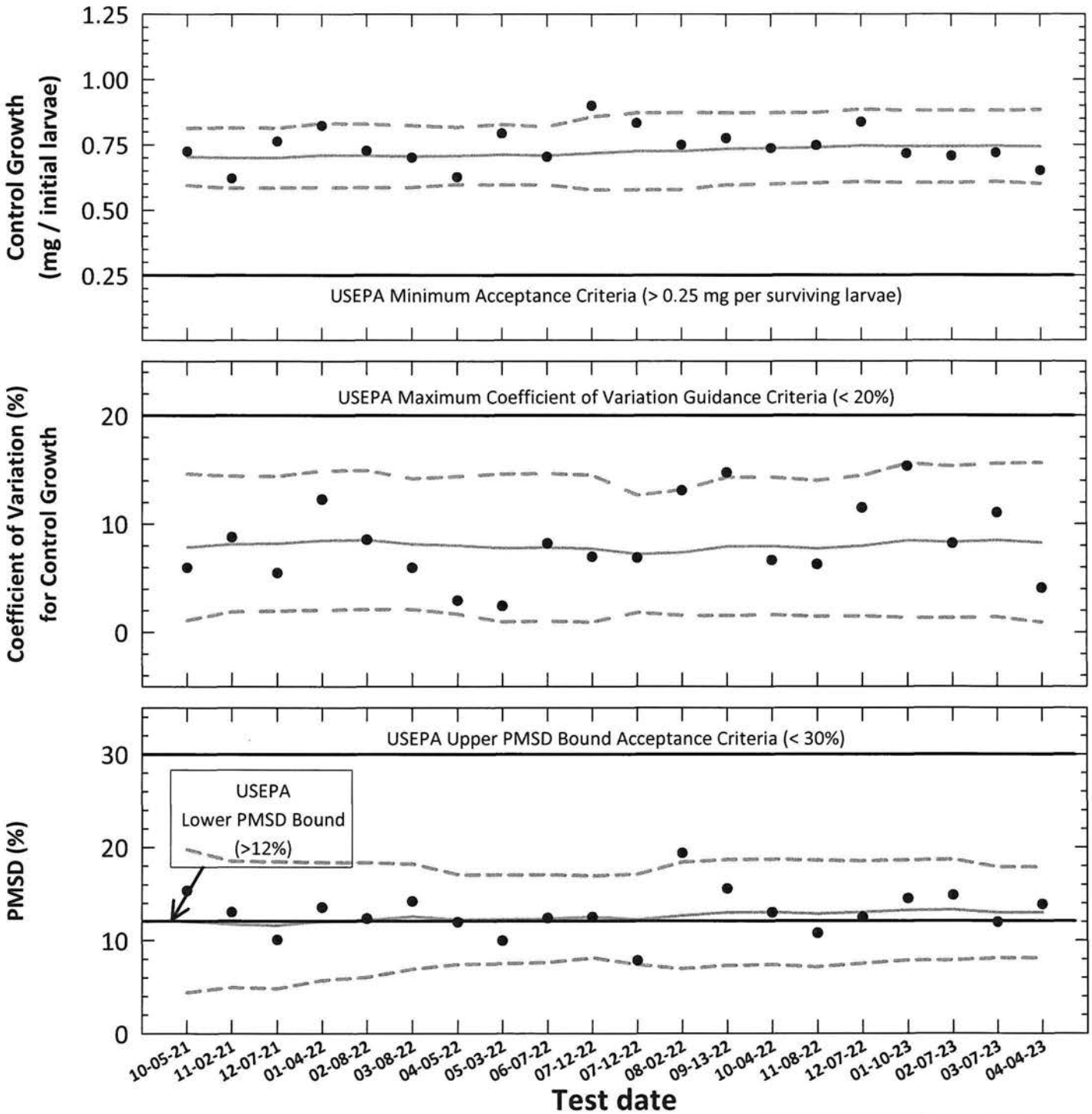
ETS
Environmental Testing Solutions, Inc.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **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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Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
2	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
3	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
4	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
5	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
6	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
7	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
8	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
9	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
10	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
11	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
12	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4
13	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	14.3	13.0	7.3	18.7
14	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	14.3	13.0	7.4	18.7
15	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	14.0	12.9	7.1	18.6
16	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	14.4	13.0	7.5	18.5
17	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	15.6	13.2	7.9	18.6
18	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3	7.9	18.7
19	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8
20	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

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) > 12%.
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) < 30%.

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-02-013, Method 1000.0)

Species: *Pimephales promelas*

PpKClCR Test Number: **101**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2176				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	03-29-23	*Artemia* CHM number:	CHM1222
Hatch dates and times:	04-03-23 1446 TO 04-04-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.04 S.U. Temperature = 24.5 °C	Date / Time in oven:	04-11-23 0750
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	04-12-23 0750
		*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		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	04-04-23	0505	JH	1105	JH	0715	JH	03-28-23 B
1	04-05-23	0500	JH	1100	JH	0700	JH	↓
2	04-06-23	0500	JH	1100	JH	0700	JH	03-28-23 D
3	04-07-23	0500	JH	1100	JH	0700	JH	↓
4	04-08-23	0600	JH	1200	JH	0800	JH	03-28-23 E
5	04-09-23	0600	JH	1200	JH	0800	JH	↓
6	04-10-23	0500	JH	1100	JH	0700	JH	
7	04-11-23					0615	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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
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)	794.1
Average weight per initial larvae:	0.651		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.651	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	759.2

Reviewed by: Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **101**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/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: light pink Analyst: BL Date: 03-22-23	14.19	14.09	15.75	14.47	15.27	13.36	12.56	15.61	16.37	13.49	15.23	14.96
*B = Pan + Larvae weight (mg) Analyst: JL Date: 04-17-23	20.64	20.52	22.63	20.73	22.37	21.12	19.56	23.54	21.08	20.75	22.68	22.87
C = Larvae weight (mg) = B – A Analyst: JL	6.45	6.43	6.88	6.26	7.10	7.76	7.00	7.93	7.71	7.26	7.45	7.91
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.645	0.643	0.688	0.626	0.710	0.776	0.700	0.793	0.771	0.726	0.745	0.791
Average weight per initial number of larvae (mg)	0.651				0.745		-14.5%		0.758		-16.6%	
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:

Reviewed by: Jim Summer JL

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **101**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/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	8 2d	8 2d	8 2d	8 2d	5 5d	5 5d	4 6d	4 6d
2	10	10	10	10	7 1d	7 1d	7 1d	7 1d	4 1d	4 1d	4	4
3	10	10	10	10	7	7	7	7	4	3 1d	3 1d	4
4	10	10	10	10	7	7	7	7	3 1d	3	3	3 1d
5	10	10	10	10	7	7	7	6 1d	2 1d	3	2 1d	3
6	9 1d	10	10	10	7	7	7	6	2	2 1d	2	3
7	9	10	10	10	7	7	7	6 1fg	2 2fg	2 2fg	2 2fg	2 2fg d
*A = Pan weight (mg) Tray color code: light pink Analyst: BL Date: 03-22-23	12.24	16.03	14.33	14.32	14.02	15.77	14.75	13.64	15.32	14.13	14.27	13.63
*B = Pan + Larvae weight (mg) Analyst: JJ Date: 04-17-23	18.86	22.47	22.60	22.30	19.25	21.57	20.04	19.83	17.71	16.14	16.66	15.32
C = Larvae weight (mg) = B – A Analyst: JJ	6.62	6.44	8.27	7.98	5.23	5.80	5.29	6.19	2.39	2.01	2.39	1.69
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JJ	0.662	0.644	0.827	0.798	0.523	0.580	0.529	0.619	0.239	0.201	0.239	0.169
Average weight per initial number of larvae (mg)	0.733		-12.6%		0.563		13.5%		0.212		67.4%	
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:

Reviewed by: Jim Sumner JS

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **101**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 d	2 d	2 d	2 d
2	1 d	1 d	1 d	1 d
3	1	0 d	1	0 d
4	1	0	0 d	0
5	0 d	0	0	0
6				
7				04-10-23
*A = Pan weight (mg) Tray color code: light pink Analyst: BL Date: 03-22-23	12.99	15.42	15.07	14.64
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B – A Analyst: _____				04-10-23
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: _____	0	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	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:

Reviewed by: Jon Sumner

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1000.0

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

Test number: 101

Test dates: April 04-11, 2023

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 / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.19	20.64	6.45	0.645	0.651	4.1	0.645	100.0	0.651	4.1	Not applicable
	B	10	10	14.09	20.52	6.43	0.643			0.643				
	C	10	10	15.75	22.63	6.88	0.688			0.688				
	D	10	10	14.47	20.73	6.26	0.626			0.626				
300	E	10	10	15.27	22.37	7.10	0.710	0.745	6.3	0.710	100.0	0.745	6.3	-14.5
	F	10	10	13.36	21.12	7.76	0.776			0.776				
	G	10	10	12.56	19.56	7.00	0.700			0.700				
	H	10	10	15.61	23.54	7.93	0.793			0.793				
450	I	10	10	16.37	24.08	7.71	0.771	0.758	3.8	0.771	100.0	0.758	3.8	-16.6
	J	10	10	13.49	20.75	7.26	0.726			0.726				
	K	10	10	15.23	22.68	7.45	0.745			0.745				
	L	10	10	14.96	22.87	7.91	0.791			0.791				
600	M	10	9	12.24	18.86	6.62	0.736	0.751	10.8	0.662	97.5	0.733	12.7	-12.6
	N	10	10	16.03	22.47	6.44	0.644			0.644				
	O	10	10	14.33	22.60	8.27	0.827			0.827				
	P	10	10	14.32	22.30	7.98	0.798			0.798				
750	Q	10	7	14.02	19.25	5.23	0.747	0.841	15.7	0.523	67.5	0.563	8.1	13.5
	R	10	7	15.77	21.57	5.80	0.829			0.580				
	S	10	7	14.75	20.04	5.29	0.756			0.529				
	T	10	6	13.64	19.83	6.19	1.032			0.619				
900	U	10	2	15.32	17.71	2.39	1.195	1.060	15.9	0.239	20.0	0.212	15.9	67.4
	V	10	2	14.13	16.14	2.01	1.005			0.201				
	W	10	2	14.27	16.66	2.39	1.195			0.239				
	X	10	2	13.63	15.32	1.69	0.845			0.169				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.0900 MSD = Minimum Significant Difference

PMSD: 13.8 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) = 12%.
Upper PMSD bound determined by USEPA (90th percentile) = 30%.
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.

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Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	4/4/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	4/11/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	1.0000	1.0000	1.0000
750	0.7000	0.7000	0.7000	0.6000
900	0.2000	0.2000	0.2000	0.2000
1050	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40
*900	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	32	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

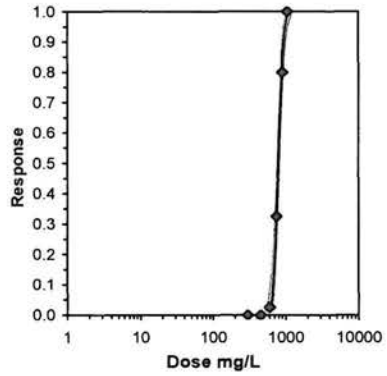
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.64644	0.884	-2.2738	6.76086
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

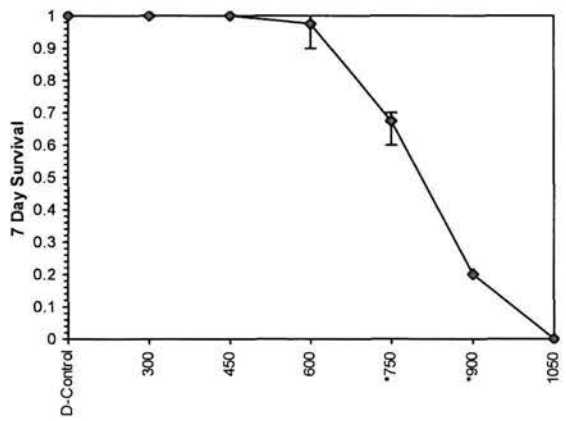
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.5035	2.29618	13.003	22.0041	0	1.11326	9.48773	0.89216	2.89988	0.05713	4
Intercept	-45.758	6.67849	-58.848	-32.668							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	584.76	518.7	629.637
EC05	3.355	639.605	583.74	677.905
EC10	3.718	670.916	621.206	705.673
EC15	3.964	692.902	647.499	725.421
EC20	4.158	710.889	668.903	741.826
EC25	4.326	726.692	687.552	756.499
EC40	4.747	768.087	735.126	796.672
EC50	5.000	794.117	763.563	823.747
EC60	5.253	821.029	791.395	853.576
EC75	5.674	867.799	836.018	909.803
EC80	5.842	887.09	853.291	934.361
EC85	6.036	910.117	873.27	964.492
EC90	6.282	939.943	898.354	1004.59
EC95	6.645	985.955	935.807	1068.32
EC99	7.326	1078.43	1008.24	1201.45



Dose-Response Plot



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ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/4/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	4/11/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	0.6450	0.6430	0.6880	0.6260
300	0.7100	0.7760	0.7000	0.7930
450	0.7710	0.7260	0.7450	0.7910
600	0.6620	0.6440	0.8270	0.7980
750	0.5230	0.5800	0.5290	0.6190
900	0.2390	0.2010	0.2390	0.1690
1050	0.0000	0.0000	0.0000	0.0000

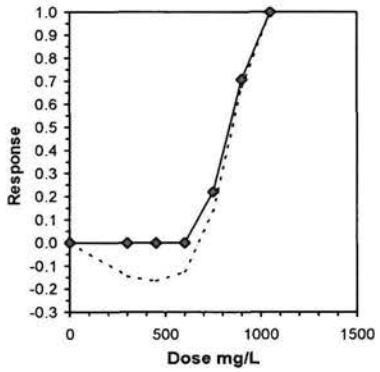
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.6505	1.0000	0.6505	0.6260	0.6880	4.060	4				0.7216	1.0000
300	0.7448	1.1449	0.7448	0.7000	0.7930	6.257	4	-2.398	2.290	0.0900	0.7216	1.0000
450	0.7583	1.1656	0.7583	0.7260	0.7910	3.769	4	-2.741	2.290	0.0900	0.7216	1.0000
600	0.7328	1.1264	0.7328	0.6440	0.8270	12.710	4	-2.092	2.290	0.0900	0.7216	1.0000
750	0.5628	0.8651	0.5628	0.5230	0.6190	8.066	4				0.5628	0.7799
900	0.2120	0.3259	0.2120	0.1690	0.2390	15.945	4				0.2120	0.2938
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98704	0.844	0.06936	-0.4382
Bartlett's Test indicates equal variances (p = 0.13)	5.63506	11.3449		

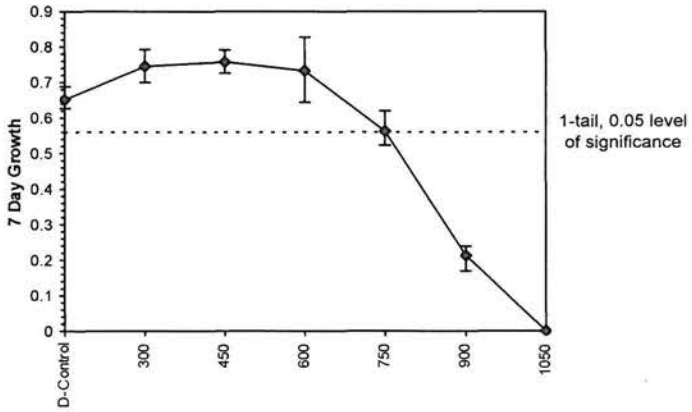
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.09001	0.13838	0.00941	0.00309	0.0702	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	634.08	28.44	457.54	646.73	-2.4275
IC10	668.15	18.76	555.07	693.84	-1.9158
IC15	702.23	16.46	618.57	740.75	-0.4982
IC20	736.30	15.54	679.93	768.48	-0.1299
IC25	759.23	9.41	720.53	778.73	-0.6632
IC40	805.51	5.93	785.36	821.16	-0.1435
IC50	836.37	5.29	818.91	851.44	-0.0893



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **101**

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	K	BL K	BL K	K	K	BL K
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.97	7.91	7.94	7.69	7.98	7.81
	Dissolved oxygen (mg/L)	7.6	7.6	8.0	7.0	7.6	7.3
	Conductivity (µmhos/cm)	303		306		297	
	Alkalinity (mg $CaCO_3$/L)	59				59	
	Hardness (mg $CaCO_3$/L)	86				91	
	Temperature (°C)	24.7	24.7	24.8	24.6	24.7	24.5
300 mg KCl/L	pH (S.U.)	8.03	7.86	7.97	7.62	8.01	7.77
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.0	7.9	7.2
	Conductivity (µmhos/cm)	839		825		800	
	Temperature (°C)	24.8	24.6	24.7	24.8	24.9	24.6
450 mg KCl/L	pH (S.U.)	8.00	7.86	7.97	7.61	8.01	7.77
	Dissolved oxygen (mg/L)	8.0	7.7	7.9	7.0	7.9	7.2
	Conductivity (µmhos/cm)	1070		1090		1060	
	Temperature (°C)	24.8	24.8	24.8	24.6	24.9	24.4
600 mg KCl/L	pH (S.U.)	8.00	7.84	7.97	7.61	8.02	7.76
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.0	7.9	7.2
	Conductivity (µmhos/cm)	1310		1320		1320	
	Temperature (°C)	24.7	24.8	24.8	24.9	24.9	24.4
750 mg KCl/L	pH (S.U.)	8.00	7.84	7.96	7.63	8.02	7.74
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.0	7.9	7.2
	Conductivity (µmhos/cm)	1560		1580		1570	
	Temperature (°C)	24.7	24.7	24.8	24.9	24.8	24.3
900 mg KCl/L	pH (S.U.)	7.99	7.84	7.96	7.70	8.02	7.74
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.1	8.0	7.2
	Conductivity (µmhos/cm)	1800		1830		1820	
	Temperature (°C)	24.7	24.7	24.6	24.8	24.9	24.6
1050 mg KCl/L	pH (S.U.)	7.99	7.85	7.95	7.71	8.03	7.82
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.2	8.0	7.2
	Conductivity (µmhos/cm)	2070		2090		2090	
	Temperature (°C)	24.8	24.7	24.6	24.9	24.8	24.4
		Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Summers

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

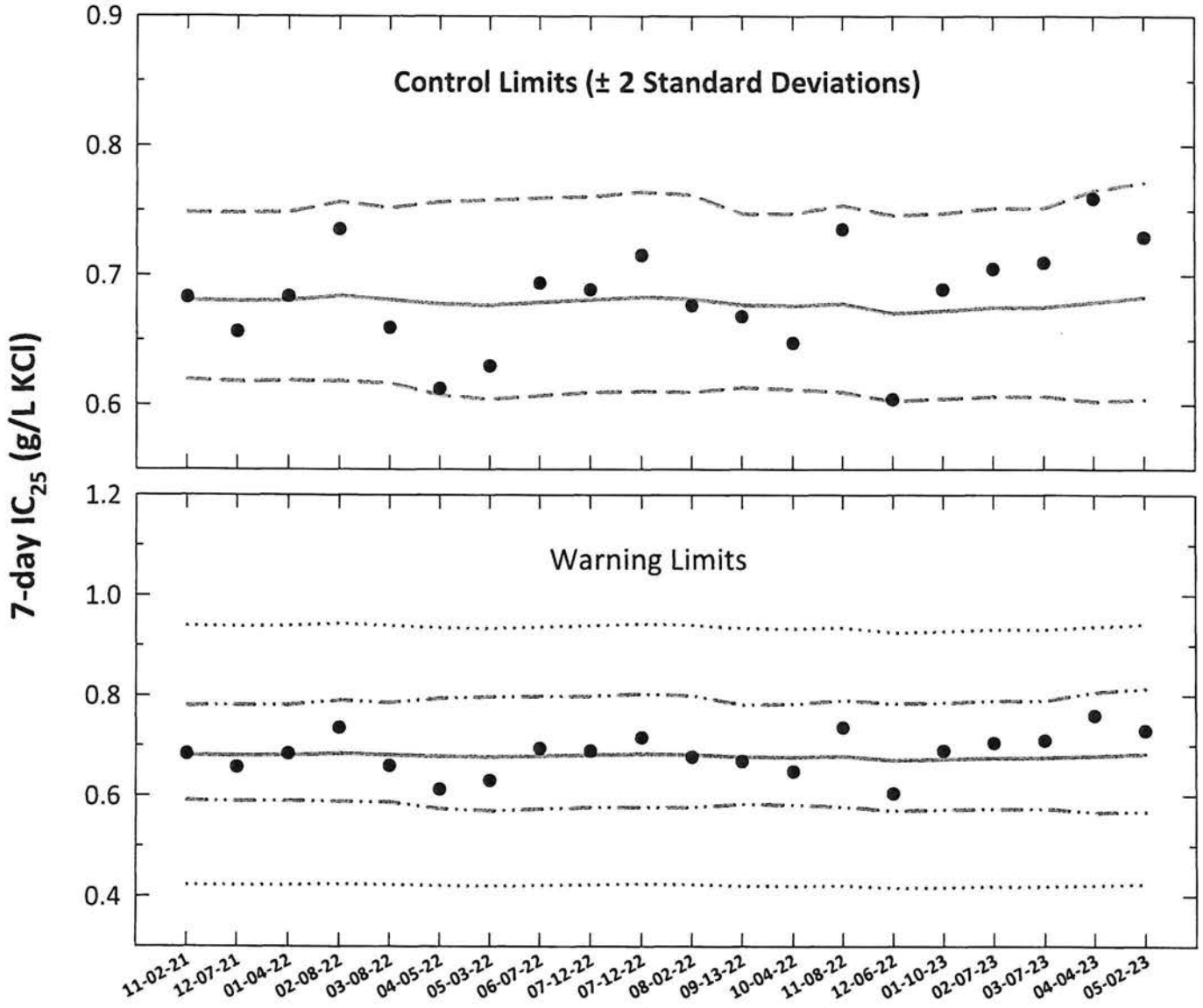
PpKClCR Test Number: **101**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL JL	BSL	BSL	BSL	BSL	BL	BL	JL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.87	7.76	8.04	7.78	7.98	7.94	8.04	7.58
	Dissolved oxygen (mg/L)	8.0	7.3	7.8	7.6	7.9	7.5	7.7	6.8
	Conductivity (µmhos/cm)	313		318		308		308	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.7	24.6	24.7	25.1	24.8	24.9	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.97	7.86	8.12	7.79	8.07	7.91	8.10	7.46
	Dissolved oxygen (mg/L)	7.9	7.3	8.0	7.5	8.0	7.6	7.6	6.7
	Conductivity (µmhos/cm)	855		811		850		864	
	Temperature (°C)	24.6	24.5	24.7	25.0	24.8	25.1	24.9	24.6
450 mg KCl/L	pH (S.U.)	7.97	7.86	8.13	7.80	8.07	7.92	8.08	7.57
	Dissolved oxygen (mg/L)	7.9	7.4	8.0	7.5	8.0	7.6	7.6	6.6
	Conductivity (µmhos/cm)	1090		1040		1120		1120	
	Temperature (°C)	24.7	24.5	24.8	25.2	24.7	24.9	24.8	24.6
600 mg KCl/L	pH (S.U.)	8.41	7.80	8.12	7.70	8.07	7.90	8.07	7.53
	Dissolved oxygen (mg/L)	7.9	7.5	8.0	7.6	8.0	7.7	7.6	6.6
	Conductivity (µmhos/cm)	1350		1310		1380		1380	
	Temperature (°C)	24.7	24.7	24.7	25.2	24.8	25.0	24.8	24.5
750 mg KCl/L	pH (S.U.)	8.63	7.75	8.12	7.68	8.07	7.88	8.06	7.53
	Dissolved oxygen (mg/L)	7.9	7.5	8.0	7.2	8.0	7.7	7.7	6.6
	Conductivity (µmhos/cm)	1600		1550		1620		1610	
	Temperature (°C)	24.7	24.4	24.8	25.2	24.7	25.0	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.99	7.73	8.11	7.72	8.08	7.87	8.06	7.61
	Dissolved oxygen (mg/L)	7.9	7.4	8.0	7.3	8.0	7.7	7.7	6.6
	Conductivity (µmhos/cm)	1830		1810		1950		1900	
	Temperature (°C)	24.7	24.6	24.8	25.0	24.7	25.0	24.8	24.5
1050 mg KCl/L	pH (S.U.)	7.97	7.80	8.11	7.79	8.07			
	Dissolved oxygen (mg/L)	8.0	7.4	8.0	7.6	8.0			
	Conductivity (µmhos/cm)	2120		2080		2110			
	Temperature (°C)	24.8	24.6	24.8	25.0	24.7	25.1	24.8	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**11-02-21**	**0.6829**	-0.1656	-0.1670	0.0205	0.6808	0.6193	0.7483	0.5905	0.7799	0.4221	0.9395
2	**12-07-21**	**0.6565**	-0.1828	-0.1675	0.0208	0.6800	0.6180	0.7484	0.5887	0.7805	0.4216	0.9385
3	**01-04-22**	**0.6838**	-0.1651	-0.1670	0.0207	0.6808	0.6188	0.7490	0.5897	0.7810	0.4221	0.9395
4	**02-08-22**	**0.7354**	-0.1335	-0.1649	0.0219	0.6841	0.6184	0.7567	0.5881	0.7903	0.4241	0.9440
5	**03-08-22**	**0.6594**	-0.1808	-0.1668	0.0216	0.6811	0.6167	0.7522	0.5866	0.7855	0.4223	0.9399
6	**04-05-22**	**0.6124**	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
7	**05-03-22**	**0.6299**	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
8	**06-07-22**	**0.6939**	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372
9	**07-12-22**	**0.6887**	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
10	**07-12-22**	**0.7153**	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
11	**08-02-22**	**0.6766**	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
12	**09-13-22**	**0.6682**	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
13	**10-04-22**	**0.6477**	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
14	**11-08-22**	**0.7354**	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
15	**12-06-22**	**0.6041**	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
16	**01-10-23**	**0.6890**	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
17	**02-07-23**	**0.7050**	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
18	**03-07-23**	**0.7096**	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318
19	**04-04-23**	**0.7592**	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373
20	**05-02-23**	**0.7295**	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424

Note:

7-day IC_{25} = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* 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 ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

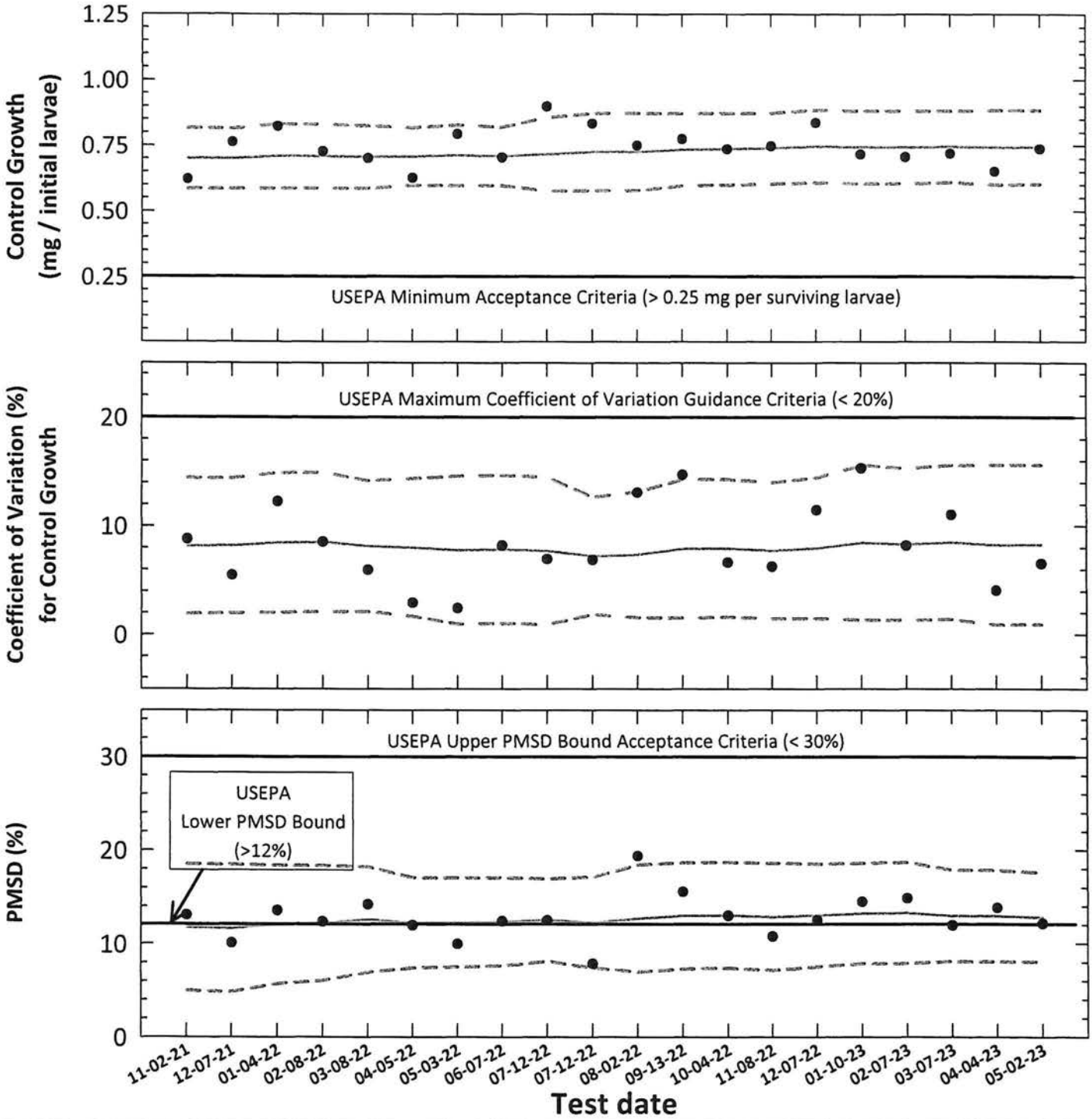
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **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.

Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
2	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
3	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
4	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
5	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
6	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
7	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
8	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
9	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
10	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
11	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4
12	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	14.3	13.0	7.3	18.7
13	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	14.3	13.0	7.4	18.7
14	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	14.0	12.9	7.1	18.6
15	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	14.4	13.0	7.5	18.5
16	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	15.6	13.2	7.9	18.6
17	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3	7.9	18.7
18	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8
19	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9
20	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

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) > 12%.
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) < 30%.

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-02-013, Method 1000.0)

Species: *Pimephales promelas*

PpKClCR Test Number: **102**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2176				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	04-26-23	*Artemia* CHM number:	CHM1222
Hatch dates and times:	05-01-23 1430 TO 05-02-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.11 S.U. Temperature = 24.0 °C	Date / Time in oven:	05-09-23 0705
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	05-10-23 0705
		*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		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	05-02-23	0505	JH	1230	JH	0715	JH	04-26-23 A
1	05-03-23	0500	JH	1100	JH	0700	JH	↓
2	05-04-23	0500	JH	1200	JH	0700	JH	04-26-23 E
3	05-05-23	0500	JH	1100	JH	0700	JH	↓
4	05-06-23	0610	JH	1210	JH	0810	JH	05-04-23 A
5	05-07-23	0600	JH	1200	JH	0800	JH	↓
6	05-08-23	0600	JH	1200	JH	0800	JH	↓
7	05-09-23					0616	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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066485

Control information:		Acceptance criteria
% Mortality:	07.	≤ 20%
Average weight per initial larvae:	0.757	
Average weight per surviving larvae:	0.737	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	832.9
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	729.5

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **102**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/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: Forest Green Analyst: BL Date: 04.20.23	14.16	15.91	14.77	13.68	13.83	15.53	13.30	12.48	14.82	15.74	15.29	14.67
*B = Pan + Larvae weight (mg) Analyst: BL Date: 05.11.23	21.88	22.57	22.38	21.17	22.04	23.19	21.42	20.35	21.69	22.49	22.45	21.78
C = Larvae weight (mg) = B – A Analyst: JL	7.72	6.66	7.61	7.49	8.21	7.66	8.12	7.87	6.87	6.75	7.16	7.11
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.772	0.666	0.761	0.749	0.821	0.766	0.812	0.787	0.687	0.675	0.716	0.711
Average weight per initial number of larvae (mg)	0.737				0.797		-8.17.		0.697		5.47.	
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: ***Pimephales promelas***

PpKClCR Test Number: **102**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	9 1d	9 1d	8 1d	8 1d
2	10	10	10	10	10	10	10	10	7 1d	8 1d	8	8
3	10	10	10	10	10	10	9 1d	10	6 1d	8	6 1d	8
4	10	10	10	10	10	10	8 1d	9 1d	5 1d	7 1d	5 1d	8
5	10	10	10	10	10	9 1d	7 1d	8 1d	4 1d	4 1d	4 1d	4 1d
6	10	10	10	10	10	9	7	8	3 1d	3 1d	4	4
7	10	10	10	10	8 1d	7 1d	7	8	3	3	3 1d	4
*A = Pan weight (mg) Tray color code: Forest Green Analyst: BL Date: 04·20·23	13.07	14.76	14.30	13.26	14.47	13.69	15.72	15.40	14.81	14.84	13.85	13.64
*B = Pan + Larvae weight (mg) Analyst: BL Date: 05·11·23	18.97	22.40	21.84	21.29	20.74	18.74	20.86	21.07	17.28	17.43	16.62	16.13
C = Larvae weight (mg) = B – A Analyst:	5.90	7.64	7.54	8.03	6.27	5.05	5.14	5.67	2.47	2.59	2.77	2.49
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst:	0.590	0.764	0.754	0.803	0.627	0.505	0.514	0.567	0.247	0.259	0.277	0.249
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.728		1.37.		0.553		24.9 7.		0.258		65.07.	

*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: *Pimephales promelas* PpKClCR Test Number: **102**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 7d	3 7d	3 7d	3 7d
2	2 1d	2 1d	3	2 1d
3	2	2	2 1d	1 1d
4	1 1d	1 1d	2	1
5	1	0 1d	2	0 1d
6	0 1d	0	1 1d	0
7	0	0	1	0
*A = Pan weight (mg) Tray color code: Forest Green Analyst: BL Date: 04·20·23	14.76	14.67	14.50	14.81
*B = Pan + Larvae weight (mg) Analyst: BL Date: 05·11·23		05·04·23	15.60	04·04·23
C = Larvae weight (mg) = B – A Analyst: JL		05·04·23	1.10	04·04·23
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0	0	0.110	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.028		96.37	

*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:

ETS
Environmental Testing Solutions, Inc.

Pimephales promelas Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1000.0

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

Test number: 102

Test dates: May 02-09, 2023

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 / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.16	21.88	7.72	0.772	0.737	6.5	0.772	100.0	0.737	6.5	Not applicable
	B	10	10	15.91	22.57	6.66	0.666			0.666				
	C	10	10	14.77	22.38	7.61	0.761			0.761				
	D	10	10	13.68	21.17	7.49	0.749			0.749				
300	E	10	10	13.83	22.04	8.21	0.821	0.797	3.1	0.821	100.0	0.797	3.1	-8.1
	F	10	10	15.53	23.19	7.66	0.766			0.766				
	G	10	10	13.30	21.42	8.12	0.812			0.812				
	H	10	10	12.48	20.35	7.87	0.787			0.787				
450	I	10	10	14.82	21.69	6.87	0.687	0.697	2.8	0.687	100.0	0.697	2.8	5.4
	J	10	10	15.74	22.49	6.75	0.675			0.675				
	K	10	10	15.29	22.45	7.16	0.716			0.716				
	L	10	10	14.67	21.78	7.11	0.711			0.711				
600	M	10	10	13.07	18.97	5.90	0.590	0.728	12.9	0.590	100.0	0.728	12.9	1.3
	N	10	10	14.76	22.40	7.64	0.764			0.764				
	O	10	10	14.30	21.84	7.54	0.754			0.754				
	P	10	10	13.26	21.29	8.03	0.803			0.803				
750	Q	10	8	14.47	20.74	6.27	0.784	0.737	4.5	0.627	75.0	0.553	10.2	24.9
	R	10	7	13.69	18.74	5.05	0.721			0.505				
	S	10	7	15.72	20.86	5.14	0.734			0.514				
	T	10	8	15.40	21.07	5.67	0.709			0.567				
900	U	10	3	14.81	17.28	2.47	0.823	0.808	16.1	0.247	32.5	0.258	5.3	65.0
	V	10	3	14.84	17.43	2.59	0.863			0.259				
	W	10	3	13.85	16.62	2.77	0.923			0.277				
	X	10	4	13.64	16.13	2.49	0.623			0.249				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.275	200.0	0.000	2.5	0.028	0.0	96.3
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	1	14.50	15.60	1.10	1.100			0.110				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.0895 MSD = Minimum Significant Difference

PMSD: 12.1 PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) = 12%.
Upper PMSD bound determined by USEPA (90th percentile) = 30%.
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.

Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	5/2/2023	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.8000	0.7000	0.7000	0.8000
900	0.3000	0.3000	0.3000	0.4000
1050	0.0000	0.0000	0.1000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	40
*900	0.3250	0.3250	0.6059	0.5796	0.6847	8.671	4	10.00	10.00	27	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

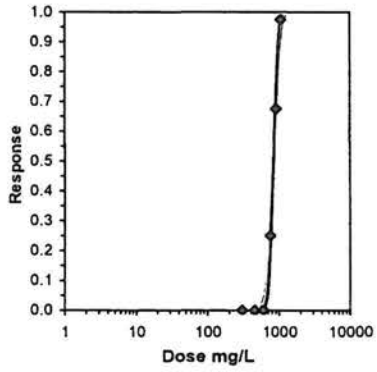
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.80734	0.896	1.35476	2.79204
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

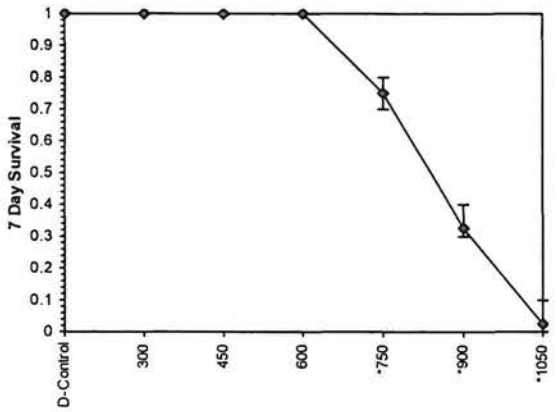
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.5423	2.34079	12.9543	22.1302	0	1.24691	9.48773	0.87032	2.92061	0.05701	3
Intercept	-46.234	6.85212	-59.664	-32.804							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	613.761	544.144	660.573
EC05	3.355	671.194	612.676	710.896
EC10	3.718	703.976	652.162	739.849
EC15	3.964	726.994	679.87	760.449
EC20	4.158	745.824	702.417	777.576
EC25	4.326	762.366	722.049	792.911
EC40	4.747	805.695	772.02	835.031
EC50	5.000	832.938	801.757	863.562
EC60	5.253	861.103	830.736	895.113
EC75	5.674	910.044	877.013	954.718
EC80	5.842	930.229	894.905	980.75
EC85	6.036	954.322	915.605	1012.67
EC90	6.282	985.526	941.609	1055.12
EC95	6.645	1033.66	980.468	1122.54
EC99	7.326	1130.39	1055.7	1263.28



Dose-Response Plot



Entered and Reviewed by Jim Summer

FTS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	5/2/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	5/9/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	0.7720	0.6660	0.7610	0.7490
300	0.8210	0.7660	0.8120	0.7870
450	0.6870	0.6750	0.7160	0.7110
600	0.5900	0.7640	0.7540	0.8030
750	0.6270	0.5050	0.5140	0.5670
900	0.2470	0.2590	0.2770	0.2490
1050	0.0000	0.0000	0.1100	0.0000

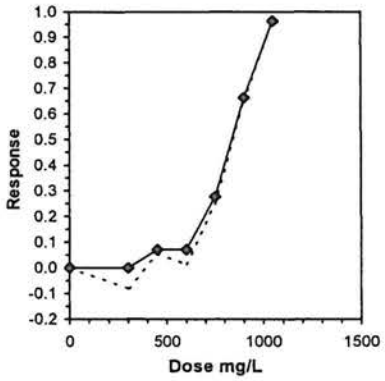
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7370	1.0000	0.7370	0.6660	0.7720	6.548	4				0.7668	1.0000
300	0.7965	1.0807	0.7965	0.7660	0.8210	3.127	4	-1.523	2.290	0.0895	0.7668	1.0000
450	0.6973	0.9461	0.6973	0.6750	0.7160	2.797	4	1.017	2.290	0.0895	0.7125	0.9292
600	0.7278	0.9874	0.7278	0.5900	0.8030	12.949	4	0.237	2.290	0.0895	0.7125	0.9292
750	0.5533	0.7507	0.5533	0.5050	0.6270	10.170	4				0.5533	0.7216
900	0.2580	0.3501	0.2580	0.2470	0.2770	5.314	4				0.2580	0.3365
1050	0.0275	0.0373	0.0275	0.0000	0.1100	200.000	4				0.0275	0.0359

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.86233	0.844	-1.5376	3.36398
Bartlett's Test indicates equal variances (p = 0.06)	7.58365	11.3449		

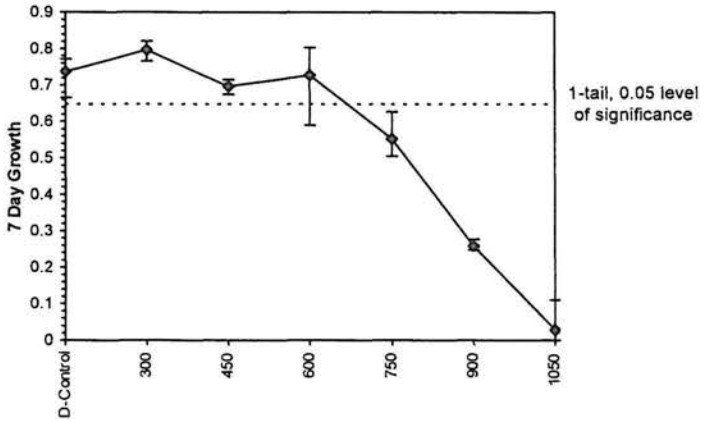
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.08946	0.12139	0.0069	0.00305	0.13354	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	406.00	96.99	342.11	749.32	0.8440
IC10	621.12	68.30	318.76	680.79	-1.4533
IC15	657.23	28.23	505.96	723.10	-1.6803
IC20	693.34	24.28	611.34	779.28	-0.2060
IC25	729.45	21.16	665.21	786.32	-0.2097
IC40	797.35	9.43	768.10	824.67	-0.0141
IC50	836.30	6.35	817.42	854.12	-0.0009



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **102**

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	BL	BL	BL			
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.92	~~8.0~~ (7.85) DL 05.03.23	7.92	7.68	7.96	7.74
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.6	7.6	7.5
	Conductivity (µmhos/cm)	293		313		194	
	Alkalinity (mg $CaCO_3$/L)	60				63	
	Hardness (mg $CaCO_3$/L)	82				86	
	Temperature (°C)	24.7	24.6	24.7	24.5	24.7	24.5
300 mg KCl/L	pH (S.U.)	8.00	7.79	8.01	7.62	7.99	7.66
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.5	7.7	7.5
	Conductivity (µmhos/cm)	833		859		834	
	Temperature (°C)	24.9	24.4	24.8	24.7	24.8	24.7
450 mg KCl/L	pH (S.U.)	7.99	7.78	8.00	7.62	7.97	7.61
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.5	7.7	7.6
	Conductivity (µmhos/cm)	1100		1110		1080	
	Temperature (°C)	24.9	24.7	24.8	24.3	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.99	7.78	7.99	7.61	7.97	7.60
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.5	7.8	7.4
	Conductivity (µmhos/cm)	1240		1310		1270	
	Temperature (°C)	24.9	24.8	24.8	24.6	24.7	24.3
750 mg KCl/L	pH (S.U.)	7.99	7.79	7.99	7.60	7.96	7.60
	Dissolved oxygen (mg/L)	8.0	7.8	7.8	7.5	7.9	7.7
	Conductivity (µmhos/cm)	1580		1590		1560	
	Temperature (°C)	24.8	24.8	24.8	24.6	24.7	24.6
900 mg KCl/L	pH (S.U.)	7.98	7.79	7.99	7.59	7.97	7.59
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	7.5	7.9	7.7
	Conductivity (µmhos/cm)	1770		1840		1800	
	Temperature (°C)	24.8	24.6	24.8	24.4	24.7	24.5
1050 mg KCl/L	pH (S.U.)	7.98	7.84	7.98	7.71	7.98	7.69
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.5	7.9	7.6
	Conductivity (µmhos/cm)	2070		2110		2070	
	Temperature (°C)	24.8	24.6	24.8	24.5	24.7	24.5
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **102**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	BSL	BSL	BSL	BSL	BL K	BL K	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.01	7.77	7.89	7.70	7.92	~~8.04~~ 7.96*	7.90	7.44
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.8	7.9	7.3	8.0	6.6
	Conductivity (µmhos/cm)	308		306		300		306	
	Alkalinity (mg $CaCO_3$/L)			60					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.7	24.5	24.7	24.4	24.7	24.8	24.7	24.3
300 mg KCl/L	pH (S.U.)	8.02	7.77	8.00	7.68	8.00	~~8.03~~ 7.98*	7.94	7.28
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.6	8.0	7.3	7.9	6.7
	Conductivity (µmhos/cm)	840		832		830		840	
	Temperature (°C)	24.7	24.4	24.8	24.6	24.8	24.5	24.8	24.4
450 mg KCl/L	pH (S.U.)	7.99	7.76	7.99	7.59	7.99	~~8.02~~ 7.98*	7.98	7.27
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.2	8.0	7.3	7.9	6.3
	Conductivity (µmhos/cm)	1110		1090		1080		1100	
	Temperature (°C)	24.8	24.4	24.8	24.3	24.8	24.5	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.98	7.75	7.99	7.59	7.98	~~8.01~~ 7.99*	8.00	7.33
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.4	8.0	7.2	8.0	6.1
	Conductivity (µmhos/cm)	1300		1330		1340		1350	
	Temperature (°C)	24.7	24.4	24.8	24.3	24.9	24.6	24.8	24.2
750 mg KCl/L	pH (S.U.)	7.99	7.75	7.98	7.68	7.97	~~8.01~~ 7.99*	8.00	7.33
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	7.5	8.0	7.2*	8.0	6.5
	Conductivity (µmhos/cm)	1590		1590		1580		1600	
	Temperature (°C)	24.8	24.6	24.7	24.7	24.9	24.6	24.7	24.2
900 mg KCl/L	pH (S.U.)	8.00	7.78	7.98	7.66	7.98	~~8.01~~ 7.99*	8.01	7.35
	Dissolved oxygen (mg/L)	8.1	7.7	8.0	7.4	8.0	7.2*	8.0	6.1
	Conductivity (µmhos/cm)	1830		1840		1850		1850	
	Temperature (°C)	24.8	24.6	24.7	24.7	25.0	24.7	24.7	24.2
1050 mg KCl/L	pH (S.U.)	8.00	7.75	7.98	7.65	7.98	7.79	7.99	7.32
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	7.5	8.0	7.1	8.1	5.7
	Conductivity (µmhos/cm)	2080		2120		2100		2120	
	Temperature (°C)	24.9	24.6	24.7	24.6	24.9	24.7	24.7	24.4
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

*BL 05.08.23 CONFIRMED VALUES K

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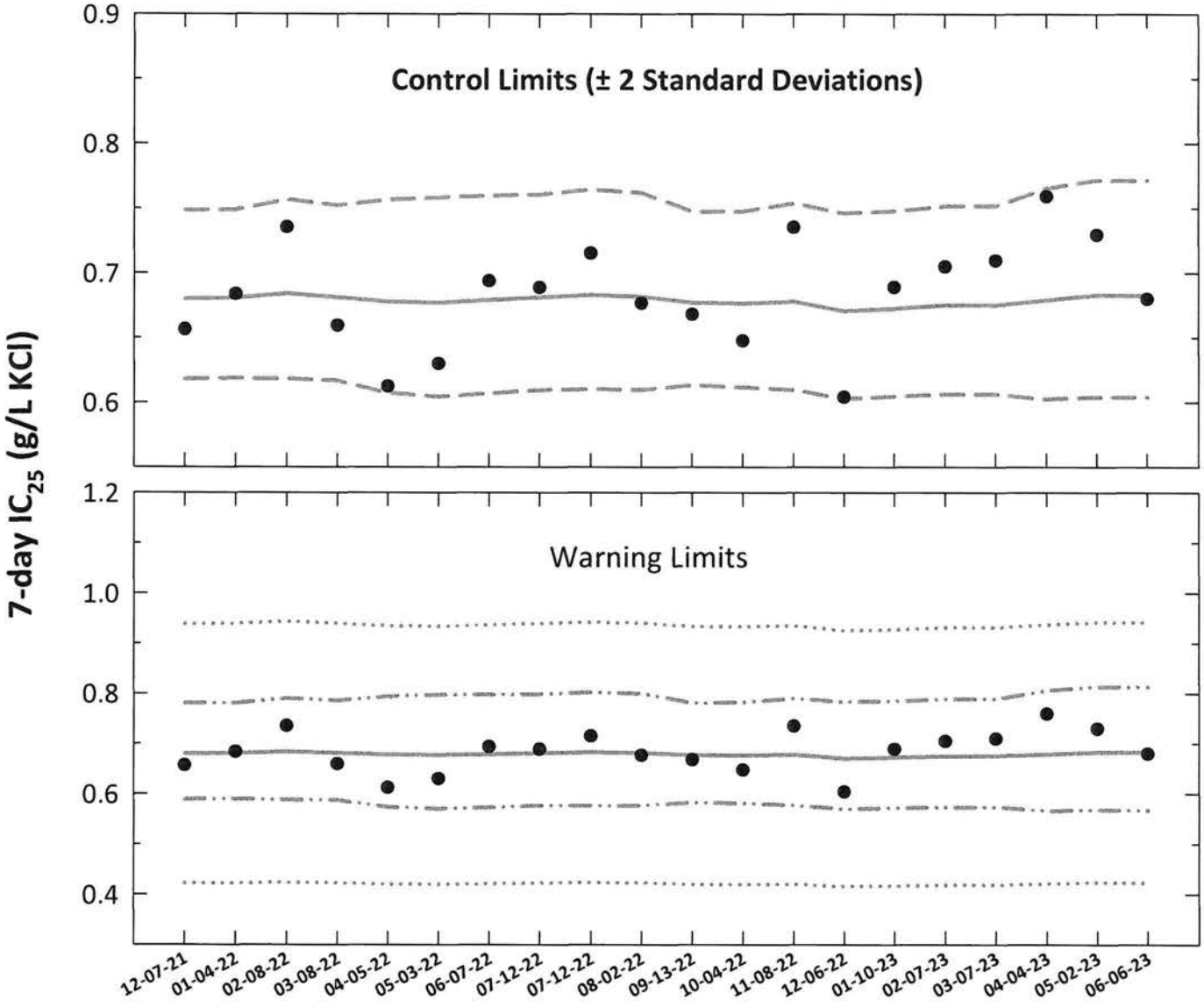
Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture



Test date

- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* 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.

Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture

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	**12-07-21**	**0.6565**	-0.1828	-0.1675	0.0208	0.6800	0.6180	0.7484	0.5887	0.7805	0.4216	0.9385
2	**01-04-22**	**0.6838**	-0.1651	-0.1670	0.0207	0.6808	0.6188	0.7490	0.5897	0.7810	0.4221	0.9395
3	**02-08-22**	**0.7354**	-0.1335	-0.1649	0.0219	0.6841	0.6184	0.7567	0.5881	0.7903	0.4241	0.9440
4	**03-08-22**	**0.6594**	-0.1808	-0.1668	0.0216	0.6811	0.6167	0.7522	0.5866	0.7855	0.4223	0.9399
5	**04-05-22**	**0.6124**	-0.2130	-0.1688	0.0239	0.6779	0.6073	0.7568	0.5737	0.7943	0.4203	0.9356
6	**05-03-22**	**0.6299**	-0.2007	-0.1695	0.0246	0.6769	0.6044	0.7581	0.5698	0.7969	0.4197	0.9341
7	**06-07-22**	**0.6939**	-0.1587	-0.1681	0.0244	0.6791	0.6070	0.7598	0.5729	0.7979	0.4210	0.9372
8	**07-12-22**	**0.6887**	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
9	**07-12-22**	**0.7153**	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
10	**08-02-22**	**0.6766**	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
11	**09-13-22**	**0.6682**	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
12	**10-04-22**	**0.6477**	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
13	**11-08-22**	**0.7354**	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
14	**12-06-22**	**0.6041**	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
15	**01-10-23**	**0.6890**	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
16	**02-07-23**	**0.7050**	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
17	**03-07-23**	**0.7096**	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318
18	**04-04-23**	**0.7592**	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373
19	**05-02-23**	**0.7295**	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424
20	**06-06-23**	**0.6800**	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422

Note:

7-day IC_{25} = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* 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 ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
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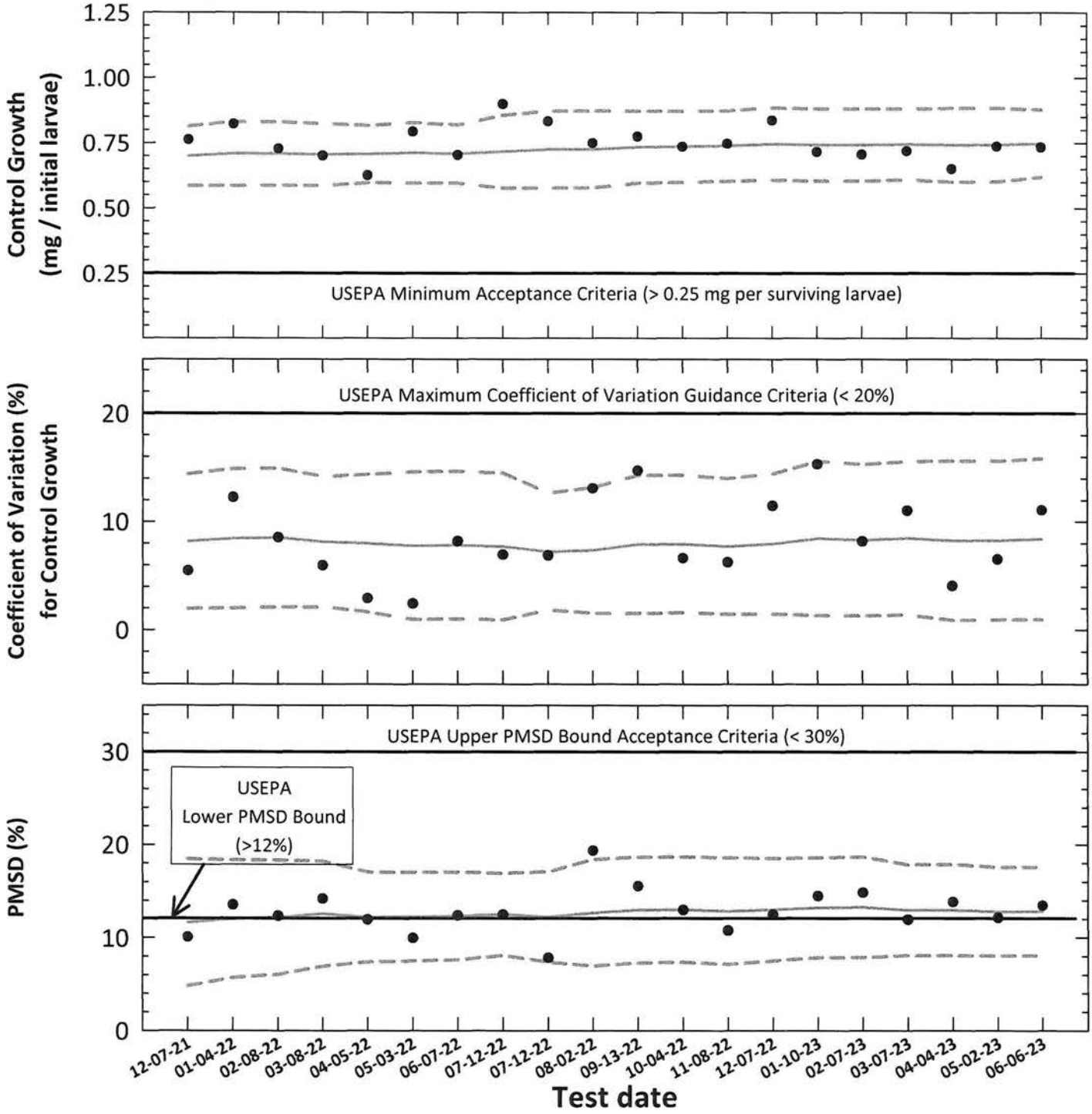
Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **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
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Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
2	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
3	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
4	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
5	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
6	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
7	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
8	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
9	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
10	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4
11	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	14.3	13.0	7.3	18.7
12	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	14.3	13.0	7.4	18.7
13	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	14.0	12.9	7.1	18.6
14	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	14.4	13.0	7.5	18.5
15	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	15.6	13.2	7.9	18.6
16	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3	7.9	18.7
17	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8
18	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9
19	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6
20	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

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) > 12%.
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) < 30%.

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Potassium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1000.0)

Species: *Pimephales promelas*

PpKClCR Test Number: **103**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2176				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	05-30-23	*Artemia* CHM number:	CHM1222
Hatch dates and times:	06-05-23 1201 TO 06-06-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.14 S.U. Temperature = 24.0 °C	Date / Time in oven:	06-13-23 0715
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	06-14-23 0715
		*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		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	06-06-23	0505	JH	1230	JH	0715	JH	05-30-23 C
1	06-07-23	0500	JH	1100	JH	0710	JH	↓
2	06-08-23	0500	JH	1230	JH	0710	JH	06-05-23 A
3	06-09-23	0500	JH	1100	JH	0710	JH	↓
4	06-10-23	0610	JH	1210	JH	0810	JH	06-05-23 B
5	06-11-23	0600	JH	1200	JH	0800	JH	↓
6	06-12-23	0600	JH	1200	JH	0800	JH	↓
7	06-13-23				JH	0615	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
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria
% Mortality:	01.	≤ 20%
Average weight per initial larvae:	0.734	
Average weight per surviving larvae:	0.734	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	797.8
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	680.0

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **103**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/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: Light pink Analyst: BL Date: 05·19·23	15.47	13.42	15.14	13.25	13.99	15.39	13.86	13.84	14.71	14.97	14.40	14.80
*B = Pan + Larvae weight (mg) Analyst: BL Date: 06·15·23	23.41	20.01	23.27	19.93	20.80	23.18	20.89	22.04	21.70	22.67	22.43	21.94
C = Larvae weight (mg) = B – A Analyst:	7.94	6.59	8.13	6.68	6.81	7.79	7.03	8.20	6.99	7.70	8.03	7.14
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst:	0.794	0.659	0.813	0.668	0.681	0.779	0.703	0.820	0.699	0.770	0.803	0.714
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.734				0.746		−1.7%		0.747		−1.8%	

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

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

Comments:

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **103**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	7 2d	7 3d	6 1d	6 1d
2	10	10	10	10	10	10	10	10	7	7	6	6
3	10	10	10	10	10	10	10	10	7	7	5 1d	6
4	10	10	10	10	10	10	10	10	7	5 2d	5	6
5	10	10	10	10	8 2d	8 2d	8 2d	8 2d	4 3d	4 1d	4 1d	5 1d
6	10	10	10	10	7 1d	7 1d	7 1d	7 1d	3 1d	3 1d	2 2d	3 2d
7	10	10	10	10	6 1d	6 1d	7	6 1d	2 1d	1 2d	2	2 1d
*A = Pan weight (mg) Tray color code: Light pink Analyst: BL Date: 05·19·23	13.81	15.25	14.16	13.90	14.02	13.95	14.83	13.60	12.57	14.91	15.07	15.56
*B = Pan + Larvae weight (mg) Analyst: BL Date: 06·15·23	20.64	21.36	21.20	20.32	18.69	18.76	19.74	17.85	14.30	15.81	16.58	16.77
C = Larvae weight (mg) = B – A Analyst:	6.83	6.11	7.04	6.42	4.67	4.81	4.91	4.25	1.73	0.90	1.51	1.21
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst:	0.683	0.611	0.704	0.642	0.467	0.481	0.491	0.425	0.173	0.090	0.151	0.121
Average weight per initial number of larvae (mg)	0.660 ~~0.466~~ 06.20.23				0.466				0.134			
Percent reduction from control (%)	10.0%				36.5%				81.8%			

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

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

Comments:

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **103**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	5 5d	3 7d	3 7d	5 5d
2	3 7d	3	3	3 7d
3	3	2 10d	3	3
4	1 12d	2	3	3
5	1	1 10d	1 12d	1 10d
6	1	1	0	1
7	1	1	0	0 14d
*A = Pan weight (mg) Tray color code: Light pink Analyst: BL Date: 05.19.23	15.59	13.64	15.23	12.72
*B = Pan + Larvae weight (mg) Analyst: BL Date: 06.15.23	16.46	14.16	→ 14 06.10 23 M	
C = Larvae weight (mg) = B – A Analyst: JL	0.87	0.52	→ 14 06.20 23 M	
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.087	0.052	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.035		95.37	

*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.

Pimephales promelas Chronic Reference Toxicant Test
EPA-821-R-02-013, Method 1000.0

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

Test number: 103

Test dates: June 06-13, 2023

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 / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	15.47	23.41	7.94	0.794	0.734	11.1	0.794	100.0	0.734	11.1	Not applicable
	B	10	10	13.42	20.01	6.59	0.659			0.659				
	C	10	10	15.14	23.27	8.13	0.813			0.813				
	D	10	10	13.25	19.93	6.68	0.668			0.668				
300	E	10	10	13.99	20.80	6.81	0.681	0.746	8.7	0.681	100.0	0.746	8.7	-1.7
	F	10	10	15.39	23.18	7.79	0.779			0.779				
	G	10	10	13.86	20.89	7.03	0.703			0.703				
	H	10	10	13.84	22.04	8.20	0.820			0.820				
450	I	10	10	14.71	21.70	6.99	0.699	0.747	6.5	0.699	100.0	0.747	6.5	-1.8
	J	10	10	14.97	22.67	7.70	0.770			0.770				
	K	10	10	14.40	22.43	8.03	0.803			0.803				
	L	10	10	14.80	21.94	7.14	0.714			0.714				
600	M	10	10	13.81	20.64	6.83	0.683	0.660	6.3	0.683	100.0	0.660	6.3	10.0
	N	10	10	15.25	21.36	6.11	0.611			0.611				
	O	10	10	14.16	21.20	7.04	0.704			0.704				
	P	10	10	13.90	20.32	6.42	0.642			0.642				
750	Q	10	6	14.02	18.69	4.67	0.778	0.747	6.7	0.467	62.5	0.466	6.2	36.5
	R	10	6	13.95	18.76	4.81	0.802			0.481				
	S	10	7	14.83	19.74	4.91	0.701			0.491				
	T	10	6	13.60	17.85	4.25	0.708			0.425				
900	U	10	2	12.57	14.30	1.73	0.865	0.781	17.0	0.173	17.5	0.134	27.0	81.8
	V	10	1	14.91	15.81	0.90	0.900			0.090				
	W	10	2	15.07	16.58	1.51	0.755			0.151				
	X	10	2	15.56	16.77	1.21	0.605			0.121				
1050	Y	10	1	15.59	16.46	0.87	0.870	0.695	35.6	0.087	5.0	0.035	0.0	95.3
	Z	10	1	13.64	14.16	0.52	0.520			0.052				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.0988 MSD = Minimum Significant Difference

PMSD: 13.5 PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) = 12%.
Upper PMSD bound determined by USEPA (90th percentile) = 30%.
Lower and upper PMSD bounds were determined from the 10th and 90th percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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

Entered and Reviewed by

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	6/6/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/13/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.6000	0.6000	0.7000	0.6000
900	0.2000	0.1000	0.2000	0.2000
1050	0.1000	0.1000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.6250	0.6250	0.9123	0.8861	0.9912	5.759	4	10.00	10.00	15	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	40
*1050	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

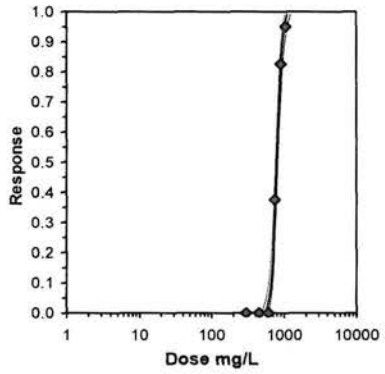
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8411	0.896	-0.319	1.36011
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

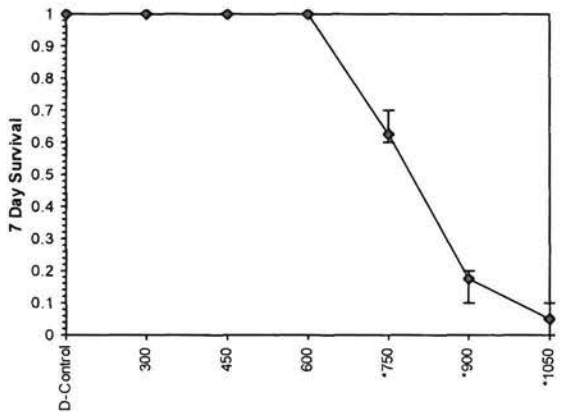
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.2713	2.08942	12.176	20.3665	0	2.26753	9.48773	0.68669	2.9019	0.06146	4
Intercept	-42.218	6.08147	-54.137	-30.298							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	574.022	506.728	620.39
EC05	3.355	632.138	574.91	671.849
EC10	3.718	665.487	614.437	701.568
EC15	3.964	688.976	642.283	722.749
EC20	4.158	708.234	665.012	740.371
EC25	4.326	725.184	684.86	756.153
EC40	4.747	769.717	735.667	799.459
EC50	5.000	797.813	766.15	828.724
EC60	5.253	826.935	796.067	861.035
EC75	5.674	877.716	844.231	922.094
EC80	5.842	898.722	862.952	948.817
EC85	6.036	923.843	884.663	981.649
EC90	6.282	956.451	912.004	1025.43
EC95	6.645	1006.91	952.986	1095.19
EC99	7.326	1108.85	1032.71	1241.74



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:	6/6/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/13/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

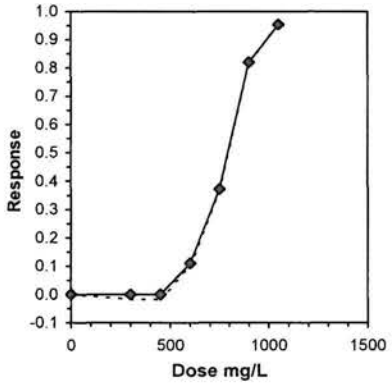
Conc-mg/L	1	2	3	4
D-Control	0.7940	0.6590	0.8130	0.6680
300	0.6810	0.7790	0.7030	0.8200
450	0.6990	0.7700	0.8030	0.7140
600	0.6830	0.6110	0.7040	0.6420
750	0.4670	0.4805	0.4910	0.4250
900	0.1730	0.0900	0.1510	0.1210
1050	0.0870	0.0520	0.0000	0.0000

			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	0.7335	1.0000	0.7335	0.6590	0.8130	11.082	4				0.7419	1.0000
300	0.7458	1.0167	0.7458	0.6810	0.8200	8.704	4	-0.284	2.290	0.0988	0.7419	1.0000
450	0.7465	1.0177	0.7465	0.6990	0.8030	6.497	4	-0.301	2.290	0.0988	0.7419	1.0000
600	0.6600	0.8998	0.6600	0.6110	0.7040	6.302	4	1.703	2.290	0.0988	0.6600	0.8896
750	0.4659	0.6351	0.4659	0.4250	0.4910	6.218	4				0.4659	0.6279
900	0.1338	0.1823	0.1338	0.0900	0.1730	27.008	4				0.1338	0.1803
1050	0.0348	0.0474	0.0348	0.0000	0.0870	122.573	4				0.0348	0.0468

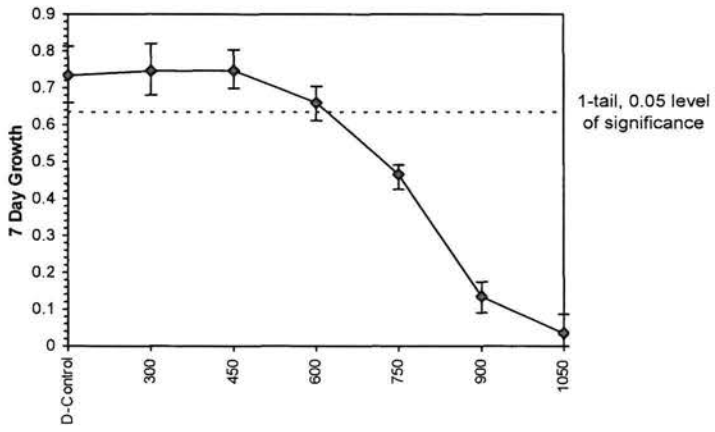
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.90256		0.844		0.071	-1.671
Bartlett's Test indicates equal variances (p = 0.71)					1.39888		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	600	>600			0.09884	0.13475	0.00685	0.00373	0.19376	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	517.93	87.06	0.00	650.88	-2.2873
IC10	585.85	43.56	423.09	649.13	-1.9890
IC15	622.69	23.86	514.99	666.53	-1.2300
IC20	651.36	17.77	577.36	689.44	-0.5403
IC25	680.02	15.89	620.38	717.11	-0.3503
IC40	759.36	9.58	718.54	776.76	-0.7160
IC50	792.87	7.24	766.14	809.50	-0.3520



Dose-Response Plot



Entered and Reviewed by Jim Surmeier

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **103**

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 and hardness 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, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	BL	BL N	BL N	N	N	BL N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.74	7.74	7.77	7.72	7.87	7.70
	Dissolved oxygen (mg/L)	7.5	7.8	7.9	7.1	7.7	7.5
	Conductivity (µmhos/cm)	314		314		297	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	84				84	
	Temperature (°C)	24.9	24.6	24.8	24.2	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.88	7.77	7.85	7.69	7.94	7.65
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.0	7.6	7.5
	Conductivity (µmhos/cm)	872		862		903	
	Temperature (°C)	24.7	24.7	24.9	24.5	24.8	24.3
450 mg KCl/L	pH (S.U.)	7.88	7.76	7.88	7.69	7.94	7.70
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.0	7.6	7.5
	Conductivity (µmhos/cm)	1120		1110		1050	
	Temperature (°C)	24.7	24.5	24.8	24.7	24.8	24.5
600 mg KCl/L	pH (S.U.)	7.88	7.77	7.88	7.68	7.93	7.68
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.1	7.6	7.4
	Conductivity (µmhos/cm)	1380		1370		1300	
	Temperature (°C)	24.7	24.5	24.8	24.4	24.9	24.5
750 mg KCl/L	pH (S.U.)	7.87	7.76	7.89	7.68	7.93	7.74
	Dissolved oxygen (mg/L)	7.6	7.7	8.0	7.1	7.7	7.3
	Conductivity (µmhos/cm)	1600		1630		1500	
	Temperature (°C)	24.8	24.5	24.8	24.4	24.7	24.7
900 mg KCl/L	pH (S.U.)	7.88	7.76	7.89	7.68	7.94	7.70
	Dissolved oxygen (mg/L)	7.6	7.7	8.0	7.1	7.8	7.3
	Conductivity (µmhos/cm)	1870		1880		1790	
	Temperature (°C)	24.6	24.8	24.7	24.6	24.7	24.7
1050 mg KCl/L	pH (S.U.)	7.88	7.77	7.89	7.69	7.94	7.69
	Dissolved oxygen (mg/L)	7.6	7.8	8.0	7.1	7.8	7.2
	Conductivity (µmhos/cm)	2160		2150		2050	
	Temperature (°C)	24.7	24.7	24.7	24.6	24.9	24.6
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **103**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL M	BSC	BSC	BSC	BSC	BL M	BL M	M
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.90	7.74	7.77	7.64	7.78	7.67	7.81	7.42
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.3	7.7	7.4	7.7	6.2
	Conductivity (µmhos/cm)	311		300		303		310	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)	M 04-18-23		84				M 04-18-23	
	Temperature (°C)	24.8	24.5	24.7	24.4	24.7	24.7	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.92	7.75	7.87	7.67	7.89	7.64	8.01	7.35
	Dissolved oxygen (mg/L)	7.8	7.7	8.0	7.3	7.7	7.4	7.9	6.2
	Conductivity (µmhos/cm)	849		802		832		850	
	Temperature (°C)	24.7	24.7	24.8	24.6	24.6	24.3	24.7	24.6
450 mg KCl/L	pH (S.U.)	7.93	7.77	7.93	7.67	7.94	7.64	8.01	7.35
	Dissolved oxygen (mg/L)	7.7	7.7	8.1	7.3	7.8	7.5	7.9	5.7
	Conductivity (µmhos/cm)	1110		1060		1080		1100	
	Temperature (°C)	24.7	24.7	24.9	24.6	24.6	24.3	24.7	24.4
600 mg KCl/L	pH (S.U.)	7.92	7.76	7.96	7.60	7.96	7.62	8.00	7.30
	Dissolved oxygen (mg/L)	7.7	7.8	8.1	7.2	7.8	7.5	7.9	5.6
	Conductivity (µmhos/cm)	1370		1340		1340		1360	
	Temperature (°C)	24.8	24.6	24.9	24.5	24.6	24.6	24.8	24.5
750 mg KCl/L	pH (S.U.)	7.91	7.77	7.97	7.57	7.96	7.61	8.00	7.30
	Dissolved oxygen (mg/L)	7.7	7.8	8.1	7.1	7.9	7.4	8.0	5.8
	Conductivity (µmhos/cm)	1610		1570		1560		1590	
	Temperature (°C)	24.9	24.3	24.8	24.5	24.6	24.2	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.94	7.77	7.97	7.68	7.95	7.62	8.00	7.42
	Dissolved oxygen (mg/L)	7.8	7.8	8.1	6.9	7.9	7.5	8.0	5.9
	Conductivity (µmhos/cm)	1890		1850		1860		1870	
	Temperature (°C)	24.9	24.6	24.8	24.5	24.7	24.5	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.94	7.75	7.98	7.65	7.96	7.63	8.00	7.46
	Dissolved oxygen (mg/L)	7.8	7.7	8.1	7.3	7.9	7.5	8.0	4.4
	Conductivity (µmhos/cm)	2160		2100		2080		2120	
	Temperature (°C)	24.8	24.6	24.8	24.4	24.7	24.5	24.8	24.4
		Initial	Final	Initial	Final	Initial	Final	Initial	Final