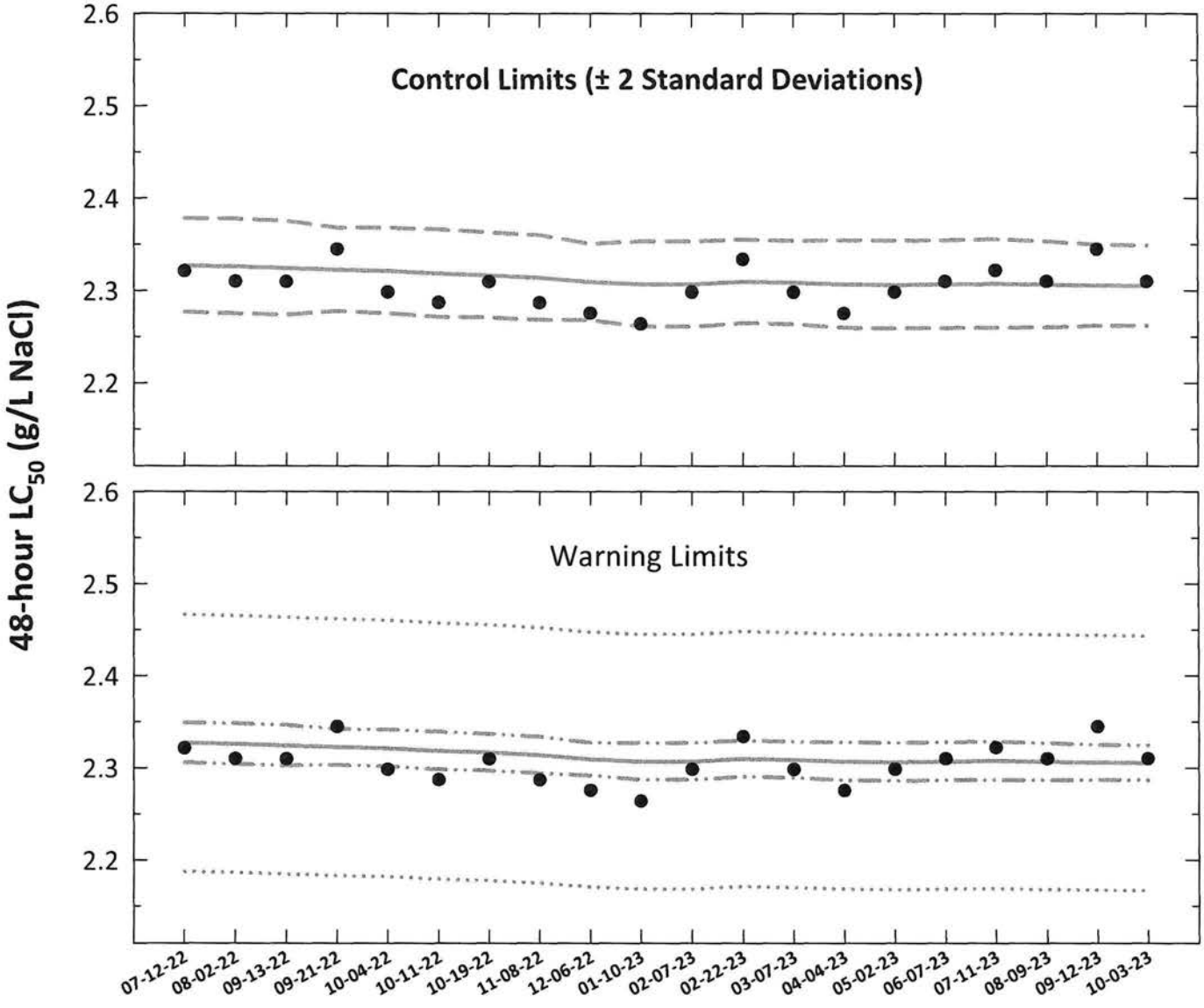
ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**07-11-23**	**2.3220**	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
18	**08-09-23**	**2.3099**	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
19	**09-12-23**	**2.3449**	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
20	**10-03-23**	**2.3099**	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436

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 Erin Sumner

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 # 401

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 Na stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2216

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	M	M	M
Control, MHSW	pH (S.U.)	7.83	7.92	7.91
	Dissolved oxygen (mg/L)	7.6	7.8	7.8
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.8	25.1	25.0
1750 mg/L	pH (S.U.)	7.94	7.85	7.90
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	3330		
	Temperature (°C)	24.8	24.9	24.8
2000 mg/L	pH (S.U.)	7.93	7.85	7.91
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	3720		
	Temperature (°C)	24.9	24.9	24.9
2250 mg/L	pH (S.U.)	7.93	7.85	7.90
	Dissolved oxygen (mg/L)	7.8	7.9	7.8
	Conductivity (µmhos/cm)	4150		
	Temperature (°C)	24.9	25.2	24.9
2500 mg/L	pH (S.U.)	7.94	7.85	7.90
	Dissolved oxygen (mg/L)	7.8	7.9	7.9
	Conductivity (µmhos/cm)	4600		
	Temperature (°C)	24.8	25.0	25.0
2750 mg/L	pH (S.U.)	7.94	7.84	7.95
	Dissolved oxygen (mg/L)	7.9	8.0	7.9
	Conductivity (µmhos/cm)	5530		
	Temperature (°C)	24.8	25.2	24.9

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuremen 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 th 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: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 401

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-03-23	0520	JL	0825	JL	283	Blue	09-27-23B
24	10-04-23			0821	JL			
48 Termination	10-05-23			0820	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):	09-26-23 D
Age:	< 24-hours old
Date and time organisms were born between:	10-02-23 1427 TO 10-03-23 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.95
	Temperature (°C): 25.1

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	5	4 2d	4 1d	5	3 2d	3 2d	4 1d	4 1d
48 Termination	3 2d	2 3d	4 1d	2 3d	1 4d	1 3d	1 3d	1 4d	0 3d	0 3d	0 4d	0 4d
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)	2361.0
48-hour LC_{50} (mg NaCl/L)	2309.9

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	10/3/2023	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/5/2023	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	0.2000	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	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

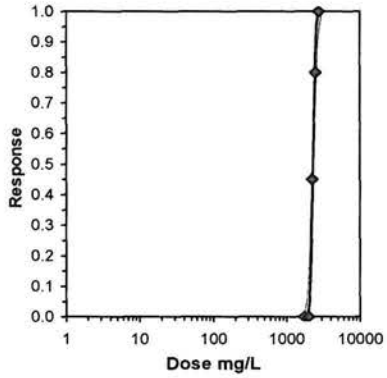
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.57538	0.868	1.30394	7.42774
Equality of variance cannot be confirmed				

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

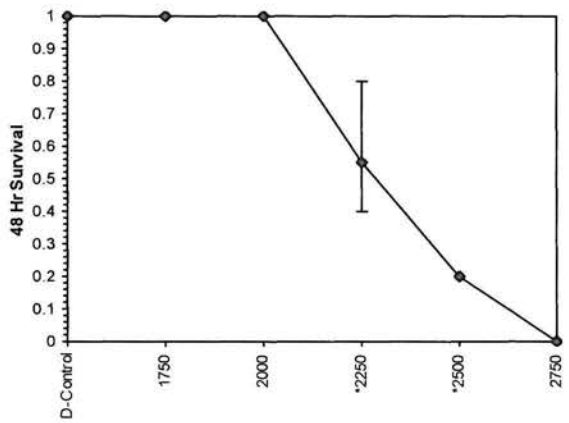
Maximum Likelihood-Probit

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



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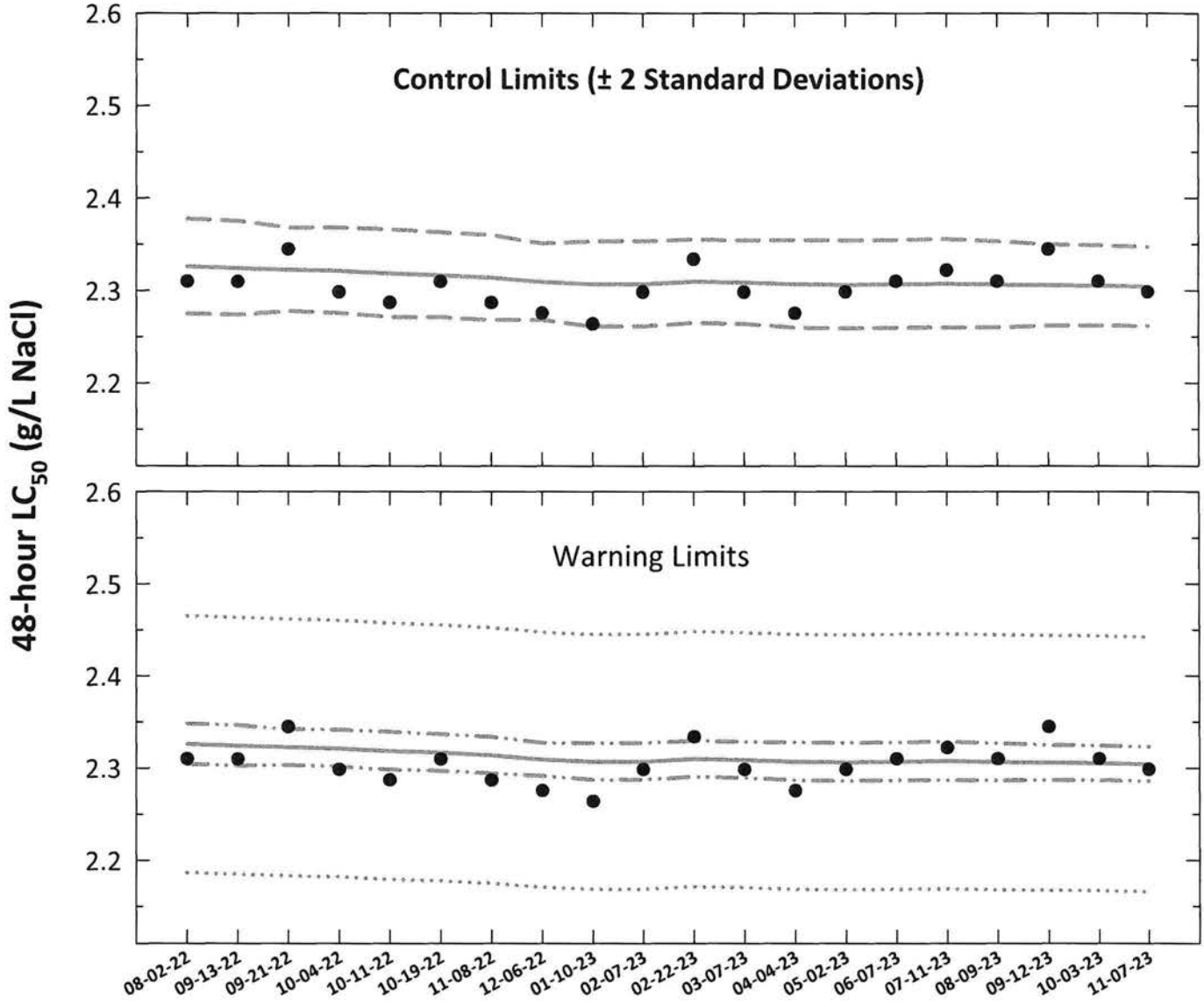


- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
17	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
18	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
19	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
20	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424

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.

Reviewed and Reviewed by Jim Sweeten

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 # 402

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 I stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2234

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	VL	BZ	BZ
Control, MHSW	pH (S.U.)	7.90	7.93	7.94
	Dissolved oxygen (mg/L)	7.7	7.9	7.7
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	25.2	25.3	24.9
1750 mg/L	pH (S.U.)	7.94	7.93	7.93
	Dissolved oxygen (mg/L)	7.7	7.9	7.7
	Conductivity (µmhos/cm)	3330		
	Temperature (°C)	25.0	25.2	25.0
2000 mg/L	pH (S.U.)	7.93	7.93	7.93
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	3050		
	Temperature (°C)	25.0	25.2	25.0
2250 mg/L	pH (S.U.)	7.92	7.92	7.94
	Dissolved oxygen (mg/L)	7.7	7.9	7.9
	Conductivity (µmhos/cm)	4110		
	Temperature (°C)	24.9	25.0	24.9
2500 mg/L	pH (S.U.)	7.91	7.92	7.94
	Dissolved oxygen (mg/L)	7.7	7.9	7.9
	Conductivity (µmhos/cm)	4570		
	Temperature (°C)	25.1	25.0	24.9
2750 mg/L	pH (S.U.)	7.91	7.93	7.94
	Dissolved oxygen (mg/L)	7.8	0.0	7.9
	Conductivity (µmhos/cm)	5090		
	Temperature (°C)	24.9	25.0	25.1

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measureme 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 t 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	13066461

ETC

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 # 402

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-07-23	0515	JH	0755	JH	2	RED	10-31-23B
24	11-08-23			0750	JH			
48 Termination	11-09-23			0755	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):	10-31-23 D
Age:	< 24-hours old
Date and time organisms were born between:	11-06-23 1350 TO 11-07-23 0515
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.98
	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	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	4 4d	S	4 4d	4 4d	4 4d	4 4d
48 Termination	3 3d	3 3d	3 3d	2 3d	1 4d	0 5d	1 3d	1 4d	0 4d	0 4d	0 4d	0 4d
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:	11/7/2023	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/9/2023	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	0.6000	0.6000	0.6000	0.4000
2500	0.2000	0.0000	0.2000	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	0.8357	0.6847	0.8861	12.047	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

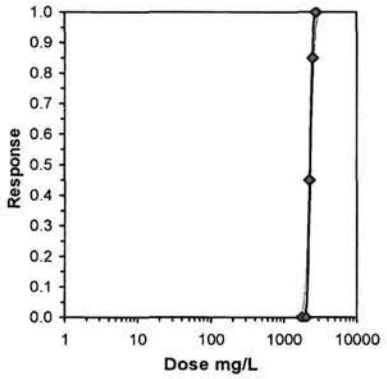
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.67149	0.868	-1.9978	4.27708
Equality of variance cannot be confirmed				

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

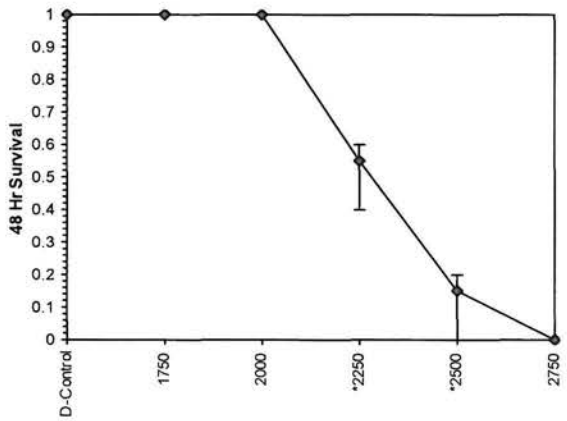
Maximum Likelihood-Probit

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

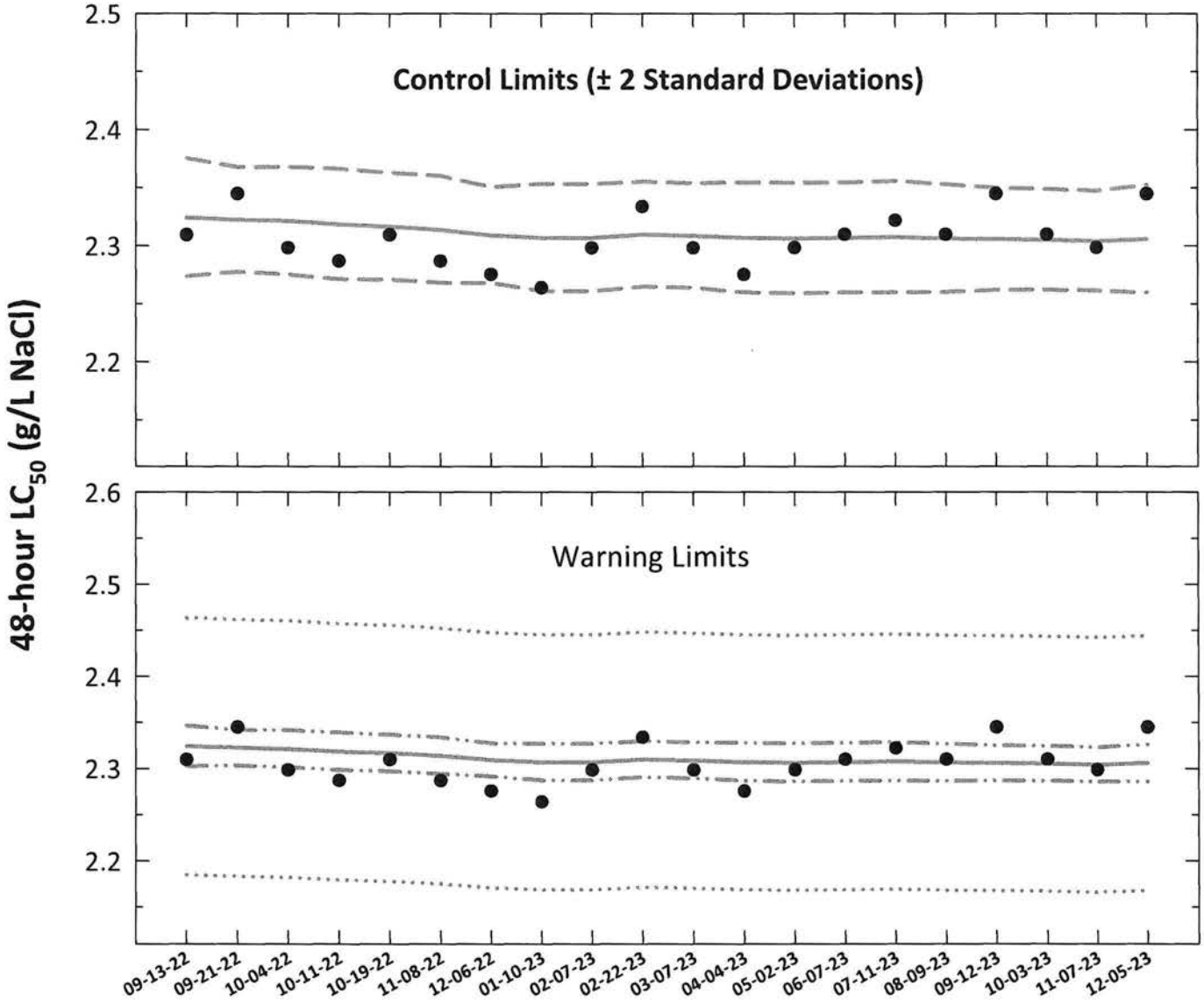


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**07-11-23**	**2.3220**	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
16	**08-09-23**	**2.3099**	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
17	**09-12-23**	**2.3449**	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
18	**10-03-23**	**2.3099**	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
19	**11-07-23**	**2.2985**	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
20	**12-05-23**	**2.3449**	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442

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 Swanson

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 # 403

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 #: 2234

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	BL PM	BL	N
Control, MHSW	pH (S.U.)	7.75	8.00	7.87
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	312		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	91		
	Temperature (°C)	24.9	25.2	25.0
1750 mg/L	pH (S.U.)	7.84	7.97	7.86
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	3530		
	Temperature (°C)	25.0	25.3	25.2
2000 mg/L	pH (S.U.)	7.83	7.97	7.89
	Dissolved oxygen (mg/L)	7.7	8.0	7.6
	Conductivity (µmhos/cm)	3860		
	Temperature (°C)	24.8	25.2	25.2
2250 mg/L	pH (S.U.)	7.83	7.96	7.90
	Dissolved oxygen (mg/L)	7.8	8.0	7.7
	Conductivity (µmhos/cm)	4300		
	Temperature (°C)	24.8	25.2	25.1
2500 mg/L	pH (S.U.)	7.82	7.96	7.92
	Dissolved oxygen (mg/L)	7.8	8.1	7.7
	Conductivity (µmhos/cm)	4880		
	Temperature (°C)	24.8	25.4	24.9
2750 mg/L	pH (S.U.)	7.82	7.96	7.93
	Dissolved oxygen (mg/L)	7.9	8.0	7.6
	Conductivity (µmhos/cm) ≤300	~~6920~~ 5.13 h		
	Temperature (°C)	25.0	25.4	25.1

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurem 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 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 applicabl
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468

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 # 403

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12-05-23	* 0520	JH	0730	JH	2B3	BULK	11-29-23B
24	12-06-23			0730	JH			
48 Termination	12-07-23			0728	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):	11-28-23 D
Age:	< 24-hours old
Date and time organisms were born between:	12-04-23 1427 TO 12-05-23 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86
	Temperature (°C): 25.1°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	4 1d	S	S	4 1d	4 1d	3 2d	3 2d	3 2d
48 Termination	3 2d	4 1d	4 1d	2 3d	2 2d	1 1d	1 1d	1 3d	0 1d	0 3d	0 3d	0 3d
Mean Survival	65%				23%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2271.6
Upper 95% confidence limit (mg NaCl/L)	2416.3
48-hour LC_{50} (mg NaCl/L)	2344.9

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	12/5/2023	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/7/2023	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	0.8000	0.8000	0.4000
2500	0.4000	0.2000	0.2000	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	0.6500	0.6500	0.9463	0.6847	1.1071	21.467	4	10.00	10.00	7	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

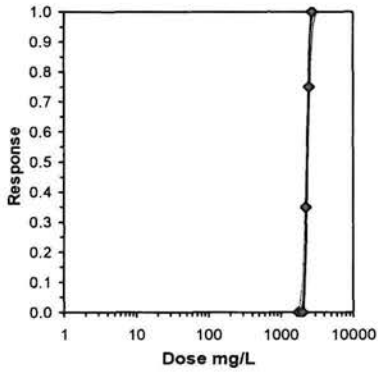
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76164	0.868	-0.432	3.36576
Equality of variance cannot be confirmed				

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

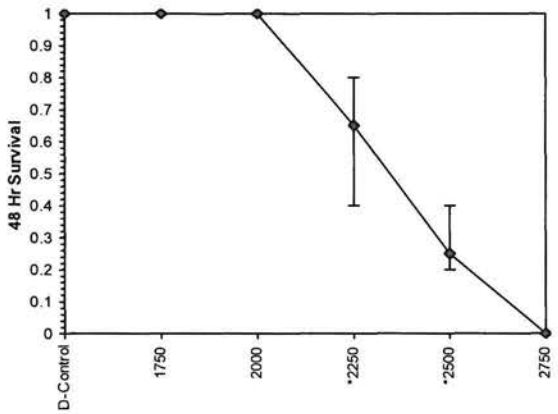
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	31.7967	5.91059	20.2119	43.3815	0	1.4914	7.81472	0.68426	3.37012	0.03145	5
Intercept	-102.16	19.935	-141.23	-63.086							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1981.32	1782.02	2088.57
EC05	3.355	2081.56	1921.73	2170.17
EC10	3.718	2137.05	1999.28	2216.47
EC15	3.964	2175.32	2052.43	2249.29
EC20	4.158	2206.22	2094.86	2276.61
EC25	4.326	2233.09	2131.18	2301.14
EC40	4.747	2302.24	2220.69	2369.31
EC50	5.000	2344.87	2271.62	2416.29
EC60	5.253	2388.29	2319.33	2468.85
EC75	5.674	2462.24	2391.58	2568.72
EC80	5.842	2492.23	2418.34	2612.2
EC85	6.036	2527.63	2448.59	2665.24
EC90	6.282	2572.9	2485.68	2735.18
EC95	6.645	2641.49	2539.52	2844.65
EC99	7.326	2775.12	2639.56	3066.72

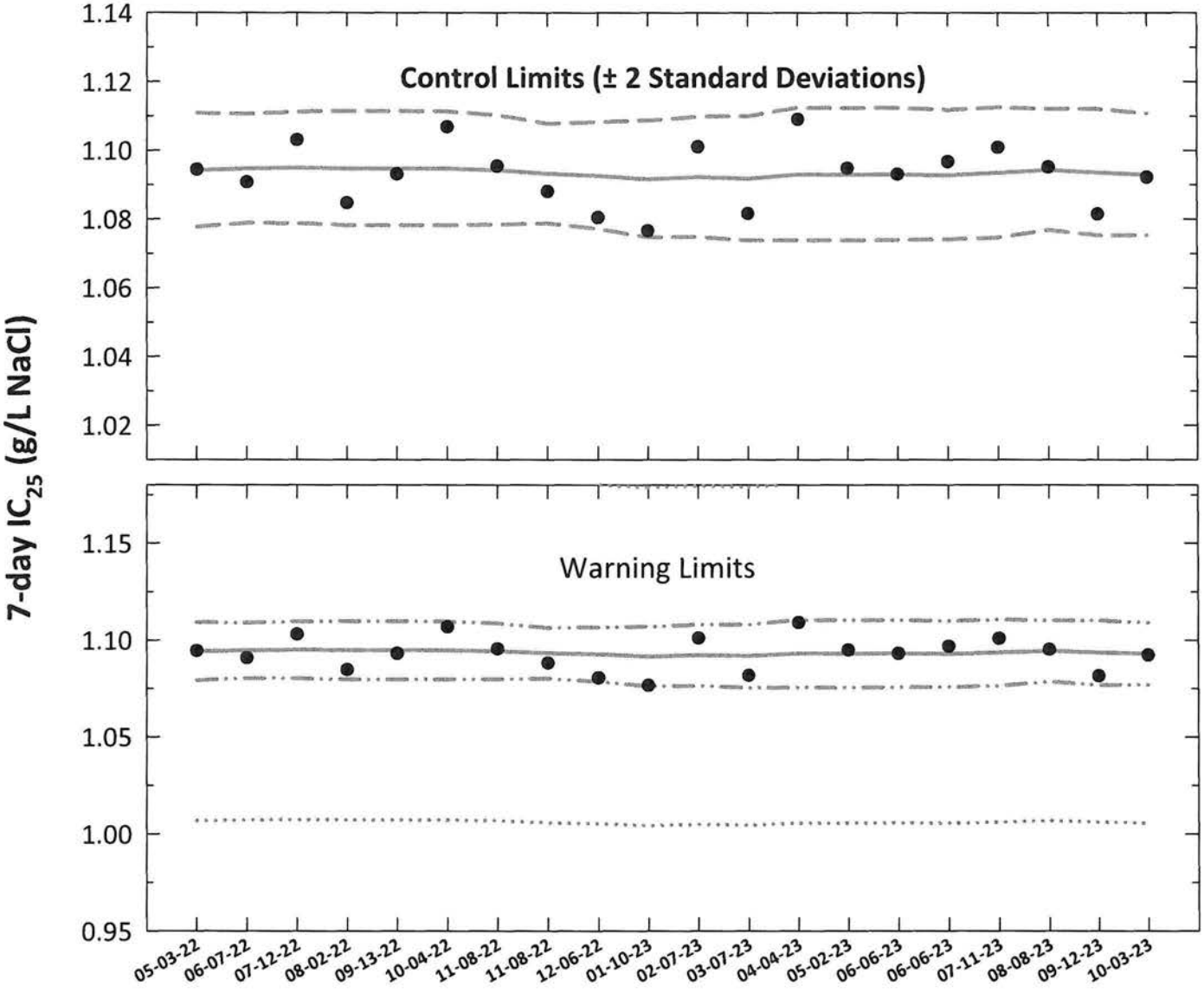


Dose-Response Plot



ETS
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 $IC_{25} \pm S_{A.10}$ 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.

Reviewed by Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
18	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
19	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
20	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	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.

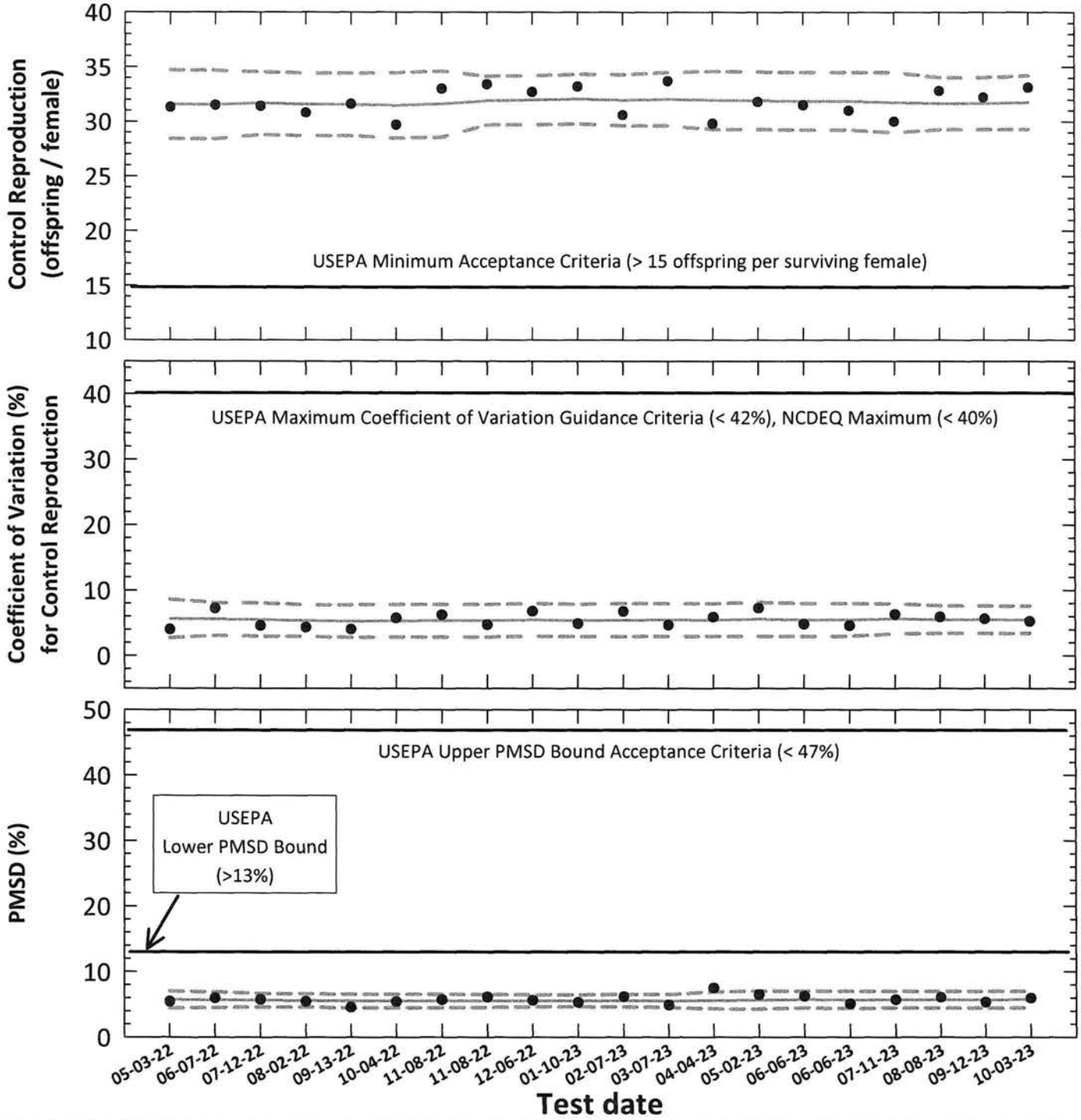
$S_{A.10}$ = Standard deviation corresponding to the 10^{th} percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

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.

Reviewed by: Jim Sumner

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 Mean (offspring/female)	Control Reproduction CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
18	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
19	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
20	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	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

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 290

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 7216				
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:	10-03-23 0820 TO 0830									
Culture board:	09-26-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	3	8	9	10	17	18	21	24
Transfer vessel information:	pH (S.U.): 7.95 Temperature (°C): 25.1									
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	10-03-23	0841	09-28-23	09-28-23	09-27-23 B	JH
1	10-04-23	0645	↓	↓	↓	JH
2	10-05-23	0645	↓	↓	10-02-23 A	JH
3	10-06-23	0642	↓	↓	↓	JH
4	10-07-23	0908	↓	↓	10-05-23 B	JH
5	10-08-23	0853	↓	↓	↓	JH
6	10-09-23	10-09-23 JH ~~0851~~ 0851	↓	↓	↓	JH
7	10-10-23	0734				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:	33.1	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	5.27%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1092.2

Reviewed by: Jim Sumner JH

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 290

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	6	5	5	6	4	4	4	6	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	10	12	11	13	10	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	13	17	18	17	15	17	19	16	19	18
Total young produced		32	32	33	35	30	34	33	34	36	32
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:	33.1

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	4	6	5	5	6	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	11	12	13	13	12	10	11	11	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	18	19	18	19	15	18	15	16	15
Total young produced		35	33	37	36	37	33	33	31	32	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:	34.0
% Reduction from Control:	-2.7 %

Reviewed by: Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 290

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	6	4	6	6	5	6	4	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	13	11	13	10	10	11	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	16	15	18	16	19	19	18	16	15
Total young produced		33	30	34	35	34	35	33	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:	32.9
% Reduction from Control:	0.6%

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	5	3	5	5	4	4	3	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	10	10	13	11	11	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	16	14	14	18	15	19	15	14	18	18
Total young produced		31	29	29	33	32	34	29	28	35	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:	31.2
% Reduction from Control:	5.7%

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 290**

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	6	3	3	4	5	3	3	4	2	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	7	10	7	7	11	6	8	9	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	5	9	3	3	10	3	10	5	8	4
Total young produced		19	19	16	14	22	17	19	17	19	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:	18.1
% Reduction from Control:	45.3%

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	3	2	3	1	2	2	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	1	0	0	1	1	2
	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	1	0
Total young produced		2	1	3	3	3	3	1	3	4	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:	2.7
% Reduction from Control:	91.8%

Reviewed by: Jim Summer

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	6	5	5	6	4	4	4	6	5	4	49
5	13	10	10	12	11	13	10	12	12	10	113
6	0	0	0	0	0	0	0	0	0	0	0
7	13	17	18	17	15	17	19	16	19	18	169
Total	**32**	**32**	**33**	**35**	**30**	**34**	**33**	**34**	**36**	**32**	**331**

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	5	5	4	4	3	3	5	5	42
5	10	12	10	10	13	11	11	11	12	9	109
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	14	18	15	19	15	14	18	18	161
Total	**31**	**29**	**29**	**33**	**32**	**34**	**29**	**28**	**35**	**32**	**312**

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	4	6	5	5	6	5	5	5	5	52
5	11	11	12	13	13	12	10	11	11	13	117
6	0	0	0	0	0	0	0	0	0	0	0
7	18	18	19	18	19	15	18	15	16	15	171
Total	**35**	**33**	**37**	**36**	**37**	**33**	**33**	**31**	**32**	**33**	**340**

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	6	3	3	4	5	3	3	4	2	4	37
5	8	7	10	7	7	11	6	8	9	11	84
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	3	3	10	3	10	5	8	4	60
Total	**19**	**19**	**16**	**14**	**22**	**17**	**19**	**17**	**19**	**19**	**181**

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	6	4	6	6	5	6	4	4	4	5	50
5	12	10	13	11	13	10	10	11	10	12	112
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	15	18	16	19	19	18	16	15	167
Total	**33**	**30**	**34**	**35**	**34**	**35**	**33**	**33**	**30**	**32**	**329**

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	3	2	3	1	2	2	2	21
5	0	0	0	0	1	0	0	1	1	2	5
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	1	0	1
Total	**2**	**1**	**3**	**3**	**3**	**3**	**1**	**3**	**4**	**4**	**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 #290

Test dates: October 03-10, 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	32	32	33	35	30	34	33	34	36	32	100	33.1	5.2	Not applicable
600	35	33	37	36	37	33	33	31	32	33	100	34.0	6.2	-2.7
800	33	30	34	35	34	35	33	33	30	32	100	32.9	5.4	0.6
1000	31	29	29	33	32	34	29	28	35	32	100	31.2	7.7	5.7
1200	19	19	16	14	22	17	19	17	19	19	100	18.1	12.1	45.3
1400	2	1	3	3	3	3	1	3	4	4	100	2.7	39.2	91.8

Dunnett's MSD value: 1.970

PMSD: 6.0

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:	10/3/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	10/10/2023	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	32.000	32.000	33.000	35.000	30.000	34.000	33.000	34.000	36.000	32.000
600	35.000	33.000	37.000	36.000	37.000	33.000	33.000	31.000	32.000	33.000
800	33.000	30.000	34.000	35.000	34.000	35.000	33.000	33.000	30.000	32.000
1000	31.000	29.000	29.000	33.000	32.000	34.000	29.000	28.000	35.000	32.000
1200	19.000	19.000	16.000	14.000	22.000	17.000	19.000	17.000	19.000	19.000
1400	2.000	1.000	3.000	3.000	3.000	3.000	1.000	3.000	4.000	4.000

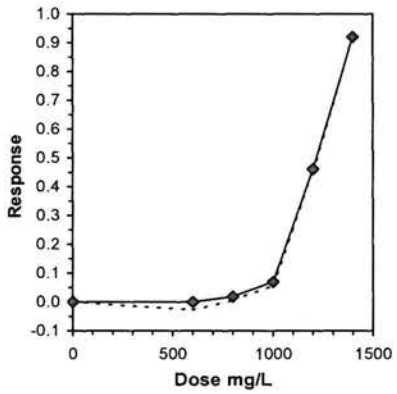
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	33.100	1.0000	33.100	30.000	36.000	5.223	10				33.550	1.0000
600	34.000	1.0272	34.000	31.000	37.000	6.201	10	-1.045	2.287	1.970	33.550	1.0000
800	32.900	0.9940	32.900	30.000	35.000	5.447	10	0.232	2.287	1.970	32.900	0.9806
1000	31.200	0.9426	31.200	28.000	35.000	7.674	10	2.205	2.287	1.970	31.200	0.9300
*1200	18.100	0.5468	18.100	14.000	22.000	12.062	10	17.411	2.287	1.970	18.100	0.5395
*1400	2.700	0.0816	2.700	1.000	4.000	39.235	10	35.286	2.287	1.970	2.700	0.0805

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.66336	1.035	-0.0279	-0.4517
Bartlett's Test indicates equal variances (p = 0.31)	5.96623	15.0863		

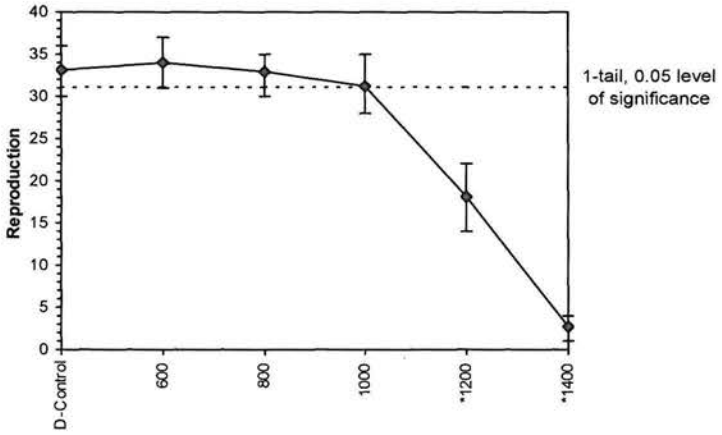
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.97002	0.05952	1583.39	3.71111	4.3E-42	5, 54

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	920.882	67.8966	768.301	1012.36	-0.3119
IC10	1015.34	16.5488	966.783	1036.05	-1.7681
IC15	1040.95	10.1265	1021.22	1059.99	0.0071
IC20	1066.56	9.12713	1049.06	1083.42	0.0414
IC25	1092.18	8.48615	1075.55	1107.39	0.0539
IC40	1169.01	9.26584	1151.38	1187.39	0.0881
IC50	1217.21	8.18476	1197.55	1231.27	-0.3841



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 290

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	N	N	N	N	N	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.83	7.88	7.76	7.80	7.83	7.84
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.7	7.8	7.8
	Conductivity (µmhos/cm)	295		310		298	
	Alkalinity (mg $CaCO_3$/L)	60				61	
	Hardness (mg $CaCO_3$/L)	86		X 10-11-23 H		86	
	Temperature (°C)	24.9	25.2	24.8	25.1	24.8	24.8
600 mg NaCl/L	pH (S.U.)	7.91	7.84	7.79	7.87	7.86	7.84
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.6	7.8	7.7
	Conductivity (µmhos/cm)	1400		1460		1410	
	Temperature (°C)	25.0	25.0	24.9	24.9	24.9	24.8
800 mg NaCl/L	pH (S.U.)	7.91	7.83	7.79	7.86	7.86	7.85
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.6	7.7	7.6
	Conductivity (µmhos/cm)	1730		1820		1750	
	Temperature (°C)	25.0	24.9	25.0	24.9	24.8	25.0
1000 mg NaCl/L	pH (S.U.)	7.92	7.82	7.79	7.86	7.87	7.85
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.6	7.7	7.7
	Conductivity (µmhos/cm)	2110		2130		2130	
	Temperature (°C)	25.0	24.9	25.0	24.9	24.8	24.8
1200 mg NaCl/L	pH (S.U.)	7.91	7.82	7.80	7.87	7.86	7.85
	Dissolved oxygen (mg/L)	7.9	7.6	7.9	7.6	7.7	7.7
	Conductivity (µmhos/cm)	2470		2540		2480	
	Temperature (°C)	25.0	24.9	24.9	25.2	24.9	24.8
1400 mg NaCl/L	pH (S.U.)	7.92	7.82	7.80	7.87	7.86	7.85
	Dissolved oxygen (mg/L)	7.9	7.6	7.9	7.6	7.7	7.7
	Conductivity (µmhos/cm)	2860		2950		2850	
	Temperature (°C)	25.0	25.2	25.0	24.8	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

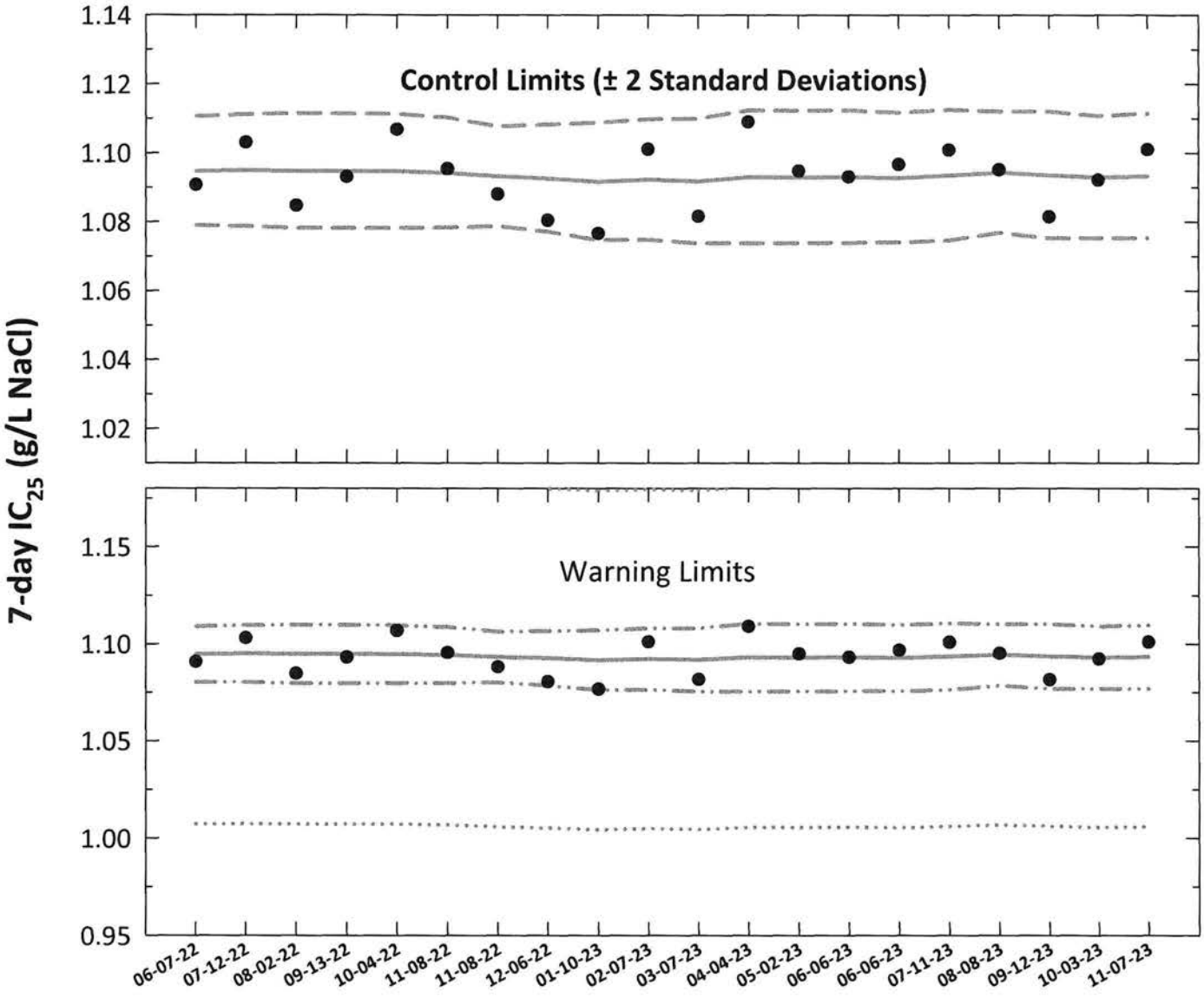
CdNaClCR #: 290

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	M	BSL	BSL	BSL	BSL	BL	BL	M
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.89	7.99	7.79	8.05	7.91	7.99	7.91	8.11
	Dissolved oxygen (mg/L)	7.9	8.0	7.9	7.8	7.8	8.0	8.0	8.0
	Conductivity (µmhos/cm)	301		310		316		301	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.8	25.1	24.8	24.9	24.8	24.8	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.91	8.00	7.97	8.05	8.02	8.01	7.99	8.06
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.8	7.8	8.0	8.0	8.0
	Conductivity (µmhos/cm)	1390		1430		1420		1450	
	Temperature (°C)	25.0	24.8	24.9	25.2	24.7	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.91	8.00	7.99	8.05	8.06	8.02	8.01	8.05
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	7.8	7.9	8.0	8.0	8.0
	Conductivity (µmhos/cm)	1790		1780		1810		1840	
	Temperature (°C)	25.0	24.8	24.8	25.0	24.7	25.0	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.92	8.00	8.00	8.04	8.06	8.02	8.03	8.04
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.8	7.9	8.0	8.0	8.0
	Conductivity (µmhos/cm)	2140		2120		2140		2180	
	Temperature (°C)	25.0	24.9	24.8	25.2	24.7	24.9	25.0	24.8
1200 mg NaCl/L	pH (S.U.)	7.91	7.99	8.00	8.05	8.06	8.02	8.03	8.03
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.8	8.0	8.1	8.1	8.0
	Conductivity (µmhos/cm)	2510		2510		2560		2630	
	Temperature (°C)	24.8	24.9	24.8	25.2	24.9	24.9	25.0	24.8
1400 mg NaCl/L	pH (S.U.)	7.91	7.98	8.01	8.04	8.06	8.02	8.03	8.03
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.8	8.0	8.1	8.1	8.0
	Conductivity (µmhos/cm)	2900		2800		2860		2930	
	Temperature (°C)	24.9	25.1	24.9	25.3	24.9	25.1	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Reviewed by: Kim Summer

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
17	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
18	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
19	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
20	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807

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.

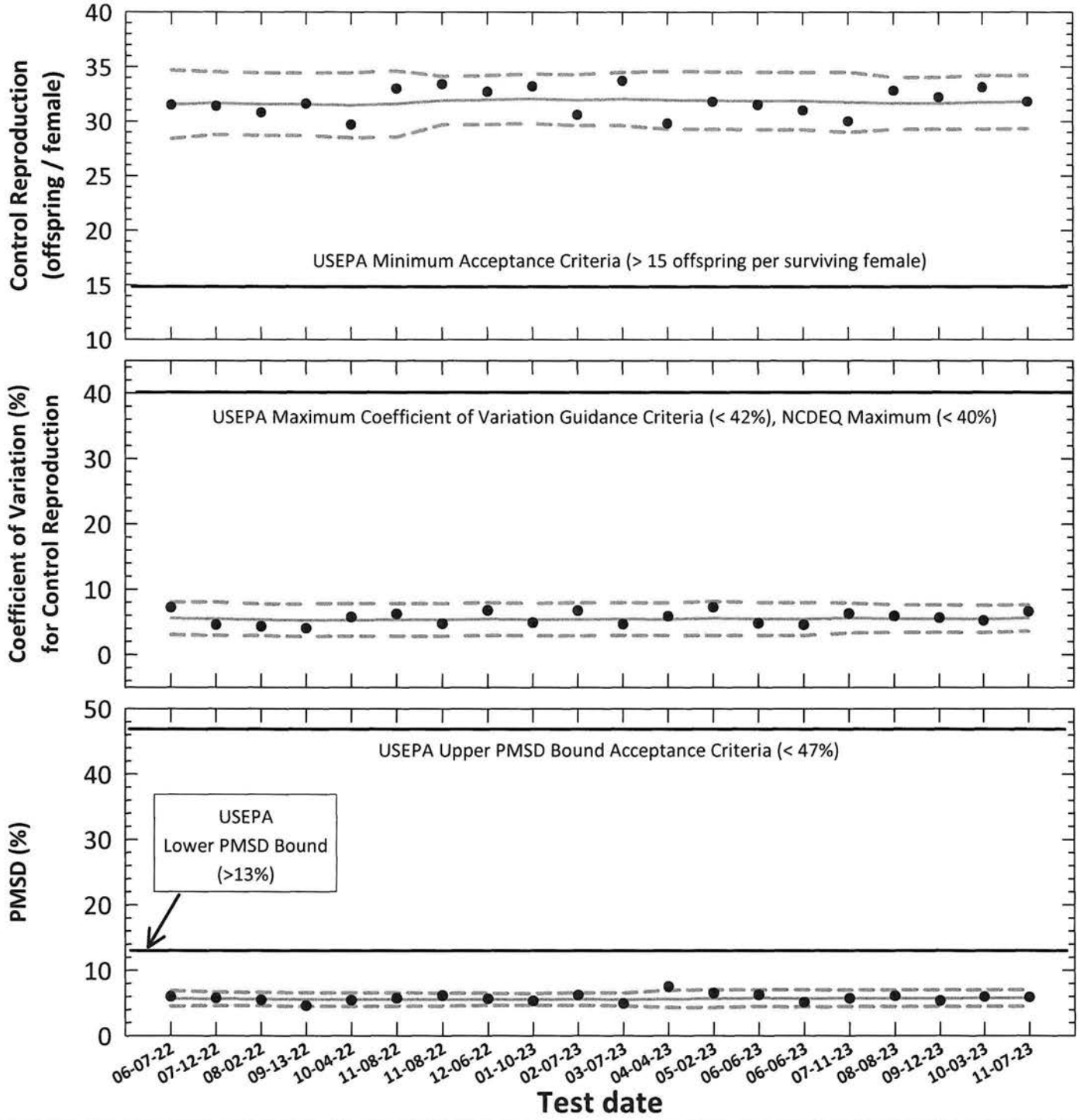
Entered and Reviewed by Jim Sumner

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)

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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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
17	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
18	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
19	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
20	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	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 #: 291

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2234				
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:	11-07-23 0515 TO 0800									
Culture board:	10-31-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	3	5	8	11	12	19	22	23	28	29
Transfer vessel information:	pH (S.U.): 7.98 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLUE
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	11-07-23	0811	11-02-23	10-26-23	10-31-23 B	JJ
1	11-08-23	0712	↓	↓	↓	JK
2	11-09-23	0712			10-31-23 D	JK
3	11-10-23	0711			↓	JK
4	11-11-23	0910			11-08-23 A	JK
5	11-12-23	0711			↓	JK
6	11-13-23	0724	↓	↓		JK
7	11-14-23	0719				JK

*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	13066685

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:	6.67%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1101.0

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 291**

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	5	5	5	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	11	11	12	12	10	10	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	19	17	17	18	14	15	15	19	14
Total young produced		31	34	31	33	35	31	29	29	34	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:	31.8

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	4	5	4	6	3	6	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	12	12	14	11	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	18	17	17	19	15	17	13	16	18	15
Total young produced		36	32	34	35	35	31	29	30	32	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:	~~31.7~~ 32.7
% Reduction from Control:	~~0.3%~~ -2.8%

11-24-15

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 291

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	4	5	4	3	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	12	12	10	12	13	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	15	17	18	16	18	19	14	19	15
Total young produced		30	31	33	34	33	32	34	31	35	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:	32.2
% 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	6	3	3	5	5	5	4	5	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	10	13	9	12	12	12	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	13	14	17	14	18	15	14	13	13	16
Total young produced		28	33	30	30	32	32	31	29	29	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:	30.3
% Reduction from Control:	4.7%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 291

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	3	4	3	5	3	3	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	7	10	9	5	11	9	6	9	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	4	6	6	10	3	7	8	8	4
Total young produced		19	14	19	19	18	19	19	17	20	18
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:	18.2
% Reduction from Control:	42.8%

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	1	3	2	2	2	1	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	1	0	1	1	0	0	0	0	0	0
Total young produced		3	1	4	2	3	2	2	2	1	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:	2.1
% Reduction from Control:	93.4%

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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	5	3	3	5	5	5	4	4	5	5	44
5	11	12	11	11	12	12	10	10	10	12	111
6	0	0	0	0	0	0	0	0	0	0	0
7	15	19	17	17	18	14	15	15	19	14	163
Total	**31**	**34**	**31**	**33**	**35**	**31**	**29**	**29**	**34**	**31**	**318**

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	4	5	4	6	3	6	4	4	5	46
5	13	11	12	12	14	11	10	10	10	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	18	17	17	19	15	17	13	16	18	15	165
Total	**36**	**32**	**34**	**35**	**35**	**31**	**29**	**30**	**32**	**33**	**327**

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	4	5	4	3	4	6	4	42
5	11	12	12	12	12	10	12	13	10	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	17	18	16	18	19	14	19	15	166
Total	**30**	**31**	**33**	**34**	**33**	**32**	**34**	**31**	**35**	**29**	**322**

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	6	3	3	5	5	5	4	5	3	43
5	11	13	10	13	9	12	12	12	11	10	113
6	0	0	0	0	0	0	0	0	0	0	0
7	13	14	17	14	18	15	14	13	13	16	147
Total	**28**	**33**	**30**	**30**	**32**	**32**	**31**	**29**	**29**	**29**	**303**

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	3	4	3	5	3	3	3	5	36
5	10	7	10	9	5	11	9	6	9	9	85
6	0	0	0	0	0	0	0	0	0	0	0
7	5	4	6	6	10	3	7	8	8	4	61
Total	**19**	**14**	**19**	**19**	**18**	**19**	**19**	**17**	**20**	**18**	**182**

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	1	3	2	2	2	1	1	18
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	1	0	1	1	0	0	0	0	0	0	3
Total	**3**	**1**	**4**	**2**	**3**	**2**	**2**	**2**	**1**	**1**	**21**

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 #291

Test dates: November 07-14, 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	31	34	31	33	35	31	29	29	34	31	100	31.8	6.6	Not applicable
600	36	32	34	35	35	31	29	30	32	33	100	32.7	7.1	-2.8
800	30	31	33	34	33	32	34	31	35	29	100	32.2	6.0	-1.3
1000	28	33	30	30	32	32	31	29	29	29	100	30.3	5.4	4.7
1200	19	14	19	19	18	19	19	17	20	18	100	18.2	9.3	42.8
1400	3	1	4	2	3	2	2	2	1	1	100	2.1	47.4	93.4

Dunnett's MSD value: 1.867

PMSD: 5.9

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (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.

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ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	11/7/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/14/2023	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	31.000	34.000	31.000	33.000	35.000	31.000	29.000	29.000	34.000	31.000
600	36.000	32.000	34.000	35.000	35.000	31.000	29.000	30.000	32.000	33.000
800	30.000	31.000	33.000	34.000	33.000	32.000	34.000	31.000	35.000	29.000
1000	28.000	33.000	30.000	30.000	32.000	32.000	31.000	29.000	29.000	29.000
1200	19.000	14.000	19.000	19.000	18.000	19.000	19.000	17.000	20.000	18.000
1400	3.000	1.000	4.000	2.000	3.000	2.000	2.000	2.000	1.000	1.000

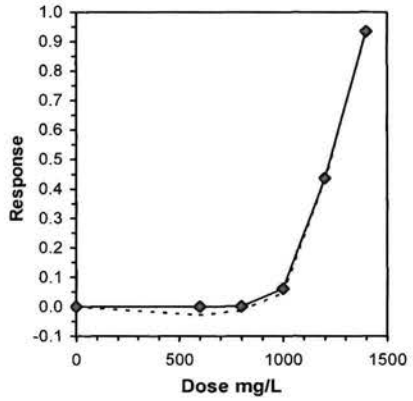
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.800	1.0000	31.800	29.000	35.000	6.596	10				32.250	1.0000
600	32.700	1.0283	32.700	29.000	36.000	7.070	10	-1.103	2.287	1.867	32.250	1.0000
800	32.200	1.0126	32.200	29.000	35.000	6.001	10	-0.490	2.287	1.867	32.200	0.9984
1000	30.300	0.9528	30.300	28.000	33.000	5.401	10	1.838	2.287	1.867	30.300	0.9395
*1200	18.200	0.5723	18.200	14.000	20.000	9.267	10	16.661	2.287	1.867	18.200	0.5643
*1400	2.100	0.0660	2.100	1.000	4.000	47.354	10	36.385	2.287	1.867	2.100	0.0651

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.73155	1.035	-0.2237	-0.3897
Bartlett's Test indicates equal variances (p = 0.28)	6.26644	15.0863		

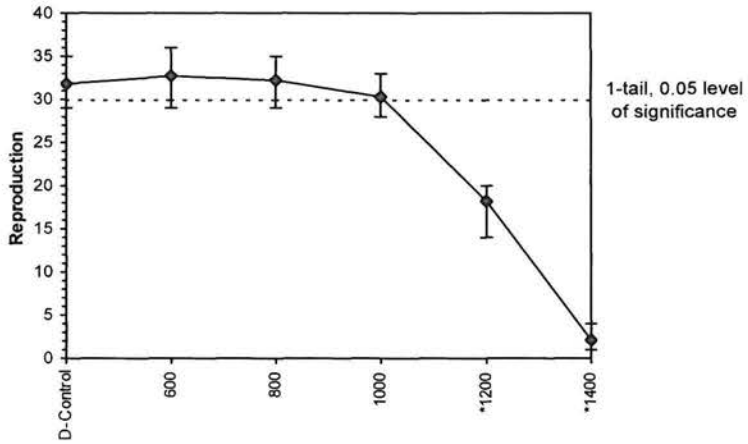
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.86654	0.0587	1509.79	3.33148	8.8E-43	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	964.474	46.9677	845.429	1008.67	-0.6681
IC10	1021.07	9.81845	992.409	1033.54	-0.9068
IC15	1047.73	8.02666	1027.85	1058.74	-0.2441
IC20	1074.38	7.38425	1057.48	1085.97	-0.2080
IC25	1101.03	7.02486	1084.65	1114.04	-0.1411
IC40	1180.99	7.88299	1164.96	1194.87	0.0936
IC50	1225.78	5.73121	1212.72	1234.75	-0.2126



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 291**

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	M	BL	BL	BL	BL	BL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.90	7.88	7.76	7.87	7.81	7.92
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.8	7.9	7.9
	Conductivity (µmhos/cm)	293		315		303	
	Alkalinity (mg $CaCO_3$/L)	62				61	
	Hardness (mg $CaCO_3$/L)	88				84	
	Temperature (°C)	24.8	25.2	24.7	25.0	24.9	25.8
600 mg NaCl/L	pH (S.U.)	7.95	7.89	7.89	7.89	7.91	7.97
	Dissolved oxygen (mg/L)	7.6	7.8	7.9	7.7	7.9	7.9
	Conductivity (µmhos/cm)	1380		1470		1430	
	Temperature (°C)	24.9	25.0	24.8	25.3	25.0	25.0
800 mg NaCl/L	pH (S.U.)	7.94	7.89	7.90	7.89	7.91	7.98
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.7	7.9	7.9
	Conductivity (µmhos/cm)	1720		1850		1790	
	Temperature (°C)	24.9	25.0	24.7	25.0	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.94	7.88	7.90	7.89	7.92	7.98
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.7	7.9	7.9
	Conductivity (µmhos/cm)	2040		2180		2120	
	Temperature (°C)	24.8	25.2	24.7	25.2	25.0	25.0
1200 mg NaCl/L	pH (S.U.)	7.94	7.89	7.90	7.88	7.92	7.98
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.7	7.9	7.9
	Conductivity (µmhos/cm)	2420		2570		2490	
	Temperature (°C)	24.8	25.0	24.7	25.4	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.94	7.87	7.90	7.87	7.92	7.98
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.7	7.9	7.9
	Conductivity (µmhos/cm)	1950		2950		2880	
	Temperature (°C)	24.8	24.9	24.8	25.2	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 291

		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	BL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.80	7.97	7.97	7.97	7.61	7.98	7.95	8.02
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.8	7.8	7.9	7.7	7.7
	Conductivity (µmhos/cm)	316		290		293		311	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	25.2	24.8	24.9	24.8	25.1	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.96	7.99	7.86	7.97	7.89	8.04	8.02	7.96
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.9	7.9	8.0	7.7	7.7
	Conductivity (µmhos/cm)	1490		1340		1380		1430	
	Temperature (°C)	24.9	25.1	24.9	25.1	24.9	24.8	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.98	7.98	7.95	7.98	7.99	8.04	8.03	7.95
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.9	7.9	8.0	7.8	7.9
	Conductivity (µmhos/cm)	1910		1760		1780		1860	
	Temperature (°C)	24.9	25.1	24.9	24.8	24.9	24.8	25.0	25.0
1000 mg NaCl/L	pH (S.U.)	7.97	7.97	7.96	7.97	7.90	8.05	8.04	7.95
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.9	8.0	7.9	7.8	7.9
	Conductivity (µmhos/cm)	2260		2060		2100		11/23 1801 (2180)	
	Temperature (°C)	25.0	24.9	24.9	24.8	24.9	25.0	25.0	25.0
1200 mg NaCl/L	pH (S.U.)	7.99	7.97	7.97	7.96	7.95	8.04	8.05	7.95
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.9	8.0	8.1	7.8	7.9
	Conductivity (µmhos/cm)	2650		2430		2490		2560	
	Temperature (°C)	25.0	24.9	25.0	25.0	24.8	25.0	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	7.98	7.96	7.97	7.96	7.94	8.04	8.05	7.94
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	8.0	8.0	8.1	7.8	7.9
	Conductivity (µmhos/cm)	3050		2840		2890		3040	
	Temperature (°C)	25.0	24.9	25.0	24.8	24.9	25.1	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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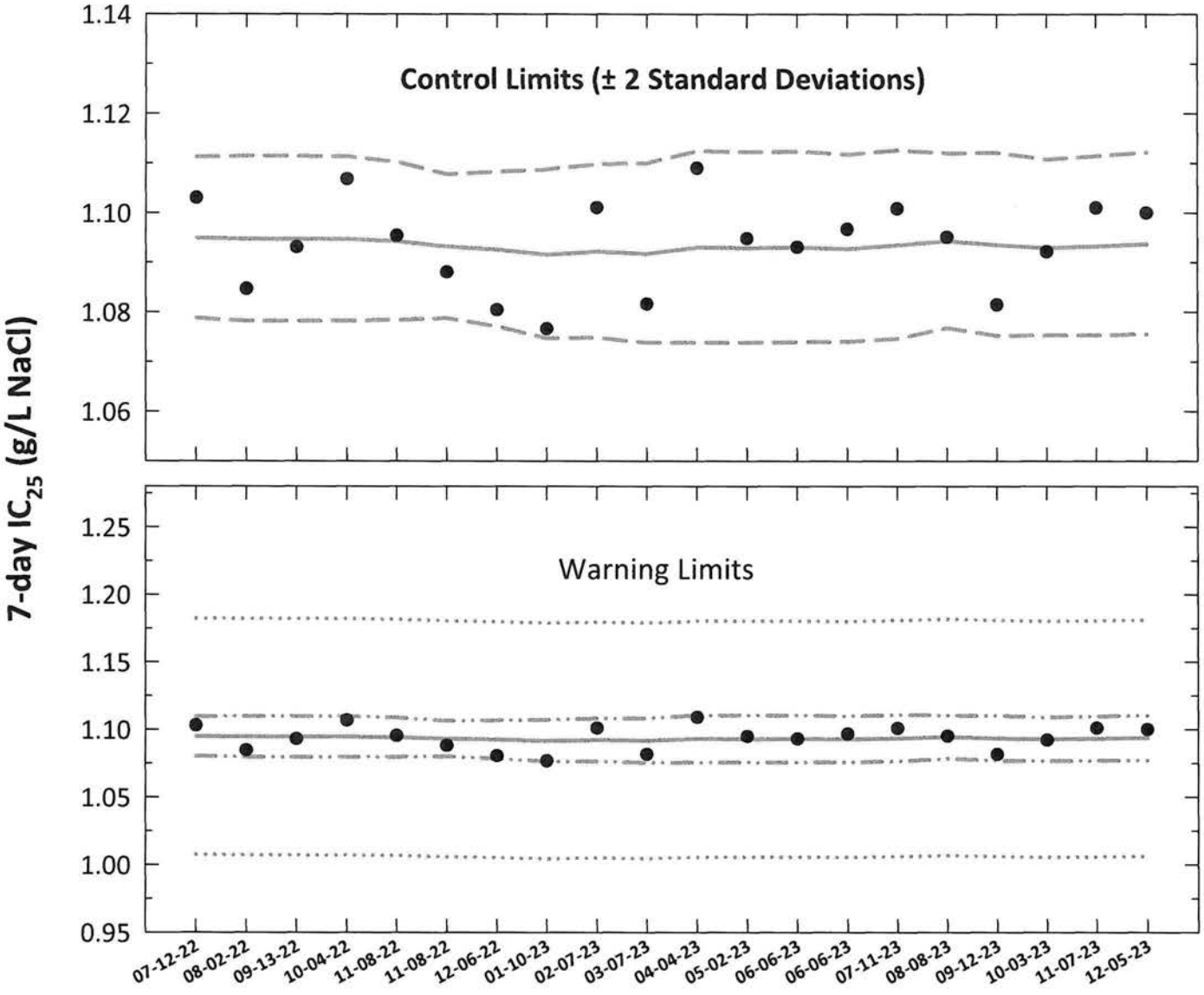
Environmental Testing Solutions, Inc.

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)

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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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
16	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
17	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
18	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
19	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
20	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812

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.

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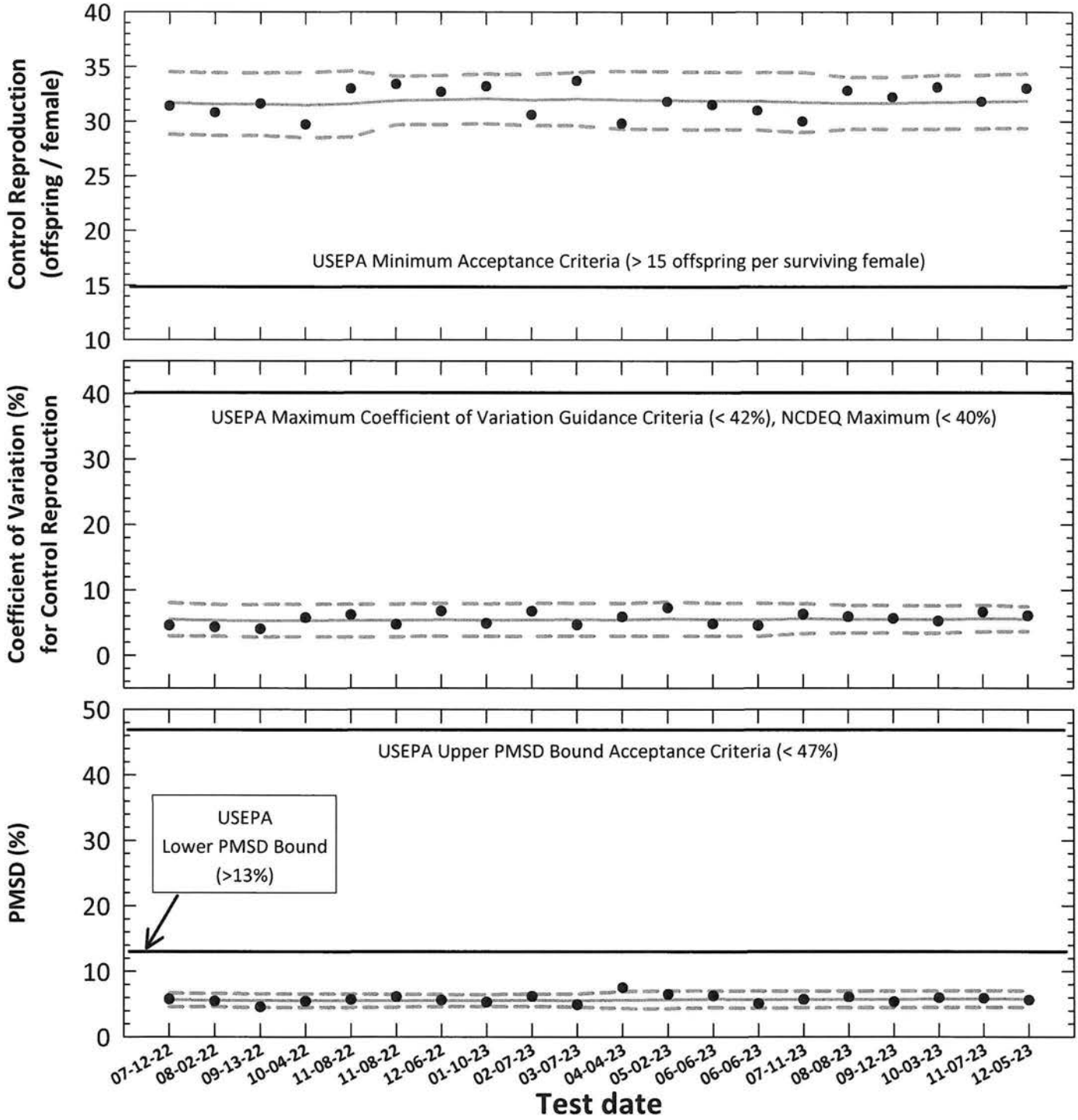
ETS
Environmental Testing Solutions, Inc.

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										
		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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
16	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
17	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
18	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
19	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
20	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	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 #: 292

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 7234				
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:	12-05-23 0520 TO 0750									
Culture board:	11-28-23 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	8	11	12	19	20	21	29	30
Transfer vessel information:	pH (S.U.): 7.86 Temperature (°C): 25.1									
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	12-05-23	0800	11-30-23	11-27-23	11-29-23 B	JH
1	12-06-23	0720	↓	↓	↓	JH
2	12-07-23	0720	↓	↓	12-02-23 B	JH
3	12-08-23	0700	↓	↓	↓	JH
4	12-09-23	0810	↓	↓	12-02-23 C	JH
5	12-10-23	0800	↓	↓	↓	JH
6	12-11-23	0711	↓	↓	↓	JH
7	12-12-23	0700				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	13066468S

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:	33.0	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	6.17%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1100.0

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 292

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	6	4	6	6	5	3	6	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	13	13	12	10	10	12	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	14	17	17	16	14	18	16	16	16	19
Total young produced		33	32	36	35	31	31	32	33	31	36
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:	33.0

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	6	5	5	5	6	3	4	5	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	12	11	11	12	13	13	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	18	18	16	16	19	15	16	18	19
Total young produced		33	36	33	33	33	33	31	34	37	37
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:	34.0
% Reduction from Control:	-3.0%

Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 292**

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	5	6	6	5	6	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	12	11	12	13	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	16	15	16	15	19	18	16	15	19	19
Total young produced		32	31	33	33	36	35	35	30	34	37
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:	33.6
% Reduction from Control:	-1.8%

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	5	5	5	5	4	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	13	14	12	12	11	11	11	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	14	15	15	17	16	14	18	17	18	15
Total young produced		30	31	33	36	33	30	33	32	35	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:	32.5
% Reduction from Control:	1.5%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 292

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	4	3	5	3	3	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	5	8	7	9	4	9	10	7	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	5	9	7	7	5	8	5	5	5	4
Total young produced		17	18	19	17	19	15	17	20	17	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:	17.8
% Reduction from Control:	46.1%

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	1	3	1	3	2	2	2	4	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	1	1	0	1	0	0	0	2	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		3	2	3	2	3	2	2	4	4	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:	2.7
% Reduction from Control:	91.8%

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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	6	4	6	6	5	3	6	5	5	4	50
5	13	11	13	13	12	10	10	12	10	13	117
6	0	0	0	0	0	0	0	0	0	0	0
7	14	17	17	16	14	18	16	16	16	19	163
Total	**33**	**32**	**36**	**35**	**31**	**31**	**32**	**33**	**31**	**36**	**330**

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	5	5	5	5	4	4	4	6	4	46
5	12	11	13	14	12	12	11	11	11	13	120
6	0	0	0	0	0	0	0	0	0	0	0
7	14	15	15	17	16	14	18	17	18	15	159
Total	**30**	**31**	**33**	**36**	**33**	**30**	**33**	**32**	**35**	**32**	**325**

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	6	3	4	5	6	6	51
5	12	13	10	12	11	11	12	13	13	12	119
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	18	16	16	19	15	16	18	19	170
Total	**33**	**36**	**33**	**33**	**33**	**33**	**31**	**34**	**37**	**37**	**340**

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	4	3	5	3	3	5	5	5	41
5	8	5	8	7	9	4	9	10	7	10	77
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	7	7	5	8	5	5	5	4	60
Total	**17**	**18**	**19**	**17**	**19**	**15**	**17**	**20**	**17**	**19**	**178**

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	5	6	6	5	6	5	5	5	52
5	11	12	12	12	11	12	13	10	10	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	16	15	16	15	19	18	16	15	19	19	168
Total	**32**	**31**	**33**	**33**	**36**	**35**	**35**	**30**	**34**	**37**	**336**

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	1	3	2	2	2	4	2	22
5	1	1	0	1	0	0	0	2	0	0	5
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	**3**	**2**	**3**	**2**	**3**	**2**	**2**	**4**	**4**	**2**	**27**

Entered and Reviewed by Jim Suntuner

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 #292

Test dates: December 05-12, 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	32	36	35	31	31	32	33	31	36	**100**	**33.0**	**6.1**	**Not applicable**
600	33	36	33	33	33	33	31	34	37	37	**100**	**34.0**	**5.9**	**-3.0**
800	32	31	33	33	36	35	35	30	34	37	**100**	**33.6**	**6.6**	**-1.8**
1000	30	31	33	36	33	30	33	32	35	32	**100**	**32.5**	**6.0**	**1.5**
1200	17	18	19	17	19	15	17	20	17	19	**100**	**17.8**	**8.3**	**46.1**
1400	3	2	3	2	3	2	2	4	4	2	**100**	**2.7**	**30.5**	**91.8**

Dunnett's MSD value: 1.849

PMSD: 5.6

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (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:	12/5/2023	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	12/12/2023	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	32.000	36.000	35.000	31.000	31.000	32.000	33.000	31.000	36.000
600	33.000	36.000	33.000	33.000	33.000	33.000	31.000	34.000	37.000	37.000
800	32.000	31.000	33.000	33.000	36.000	35.000	35.000	30.000	34.000	37.000
1000	30.000	31.000	33.000	36.000	33.000	30.000	33.000	32.000	35.000	32.000
1200	17.000	18.000	19.000	17.000	19.000	15.000	17.000	20.000	17.000	19.000
1400	3.000	2.000	3.000	2.000	3.000	2.000	2.000	4.000	4.000	2.000

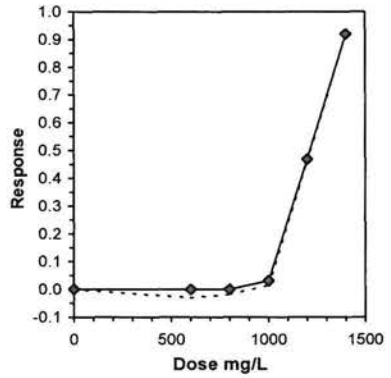
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	33.000	1.0000	33.000	31.000	36.000	6.061	10				33.533	1.0000
600	34.000	1.0303	34.000	31.000	37.000	5.882	10	-1.236	2.287	1.849	33.533	1.0000
800	33.600	1.0182	33.600	30.000	37.000	6.610	10	-0.742	2.287	1.849	33.533	1.0000
1000	32.500	0.9848	32.500	30.000	36.000	6.024	10	0.618	2.287	1.849	32.500	0.9692
*1200	17.800	0.5394	17.800	15.000	20.000	8.291	10	18.794	2.287	1.849	17.800	0.5308
*1400	2.700	0.0818	2.700	2.000	4.000	30.492	10	37.465	2.287	1.849	2.700	0.0805

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	1.0229	1.035	0.25231	-0.5214
Bartlett's Test indicates equal variances (p = 0.12)	8.69375	15.0863		

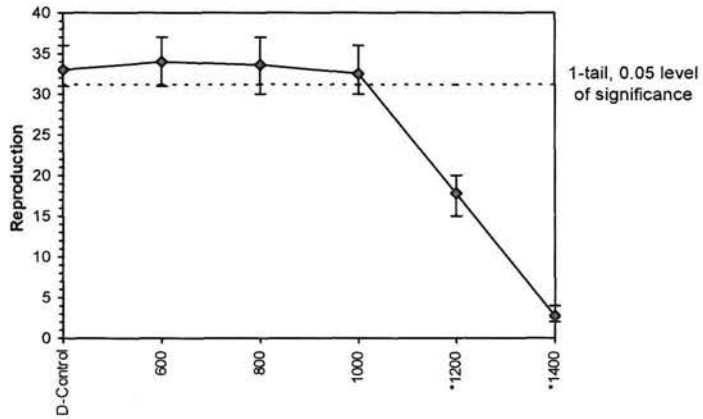
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.84934	0.05604	1644.36	3.27037	5.7E-44	5, 54

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1008.75	36.8529	898.145	1021.91	-3.8819
IC10	1031.56	7.77062	1014.64	1043.99	-0.2231
IC15	1054.38	7.16749	1038.82	1066.19	-0.1860
IC20	1077.19	6.6831	1062.08	1088.34	-0.1376
IC25	1100	6.34472	1085.75	1110.84	-0.0857
IC40	1168.44	6.39015	1156.63	1180.84	-0.0330
IC50	1213.69	6.34892	1198.75	1225.31	-0.4358



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETC
Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 292**

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 PM	BL	BL	N	N	BL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.75	8.01	7.85	7.96	8.01	7.86
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.6	7.6	7.7
	Conductivity (µmhos/cm)	312		307		295	
	Alkalinity (mg $CaCO_3$/L)	60				60	
	Hardness (mg $CaCO_3$/L)	91				86	
	Temperature (°C)	24.8	25.1	24.9	25.2	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.78	7.97	7.95	7.93	8.00	7.87
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.6	7.10	7.8
	Conductivity (µmhos/cm)	1510		1400		1360	
	Temperature (°C)	24.9	25.0	24.9	25.0	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.78	7.97	7.96	7.92	7.99	7.87
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.6	7.6	7.8
	Conductivity (µmhos/cm)	1880		1720		1700	
	Temperature (°C)	25.0	25.0	25.0	25.0	24.8	25.2
1000 mg NaCl/L	pH (S.U.)	7.79	7.95	7.97	7.93	7.98	7.89
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.6	7.6	7.8
	Conductivity (µmhos/cm)	~~2270~~ 2100		2080		2040	
	Temperature (°C)	25.0	25.2	25.0	25.0	24.8	25.1
1200 mg NaCl/L	pH (S.U.)	7.79	7.95	7.97	7.93	7.99	7.88
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.6	7.6	7.8
	Conductivity (µmhos/cm)	~~2700~~ 2400		2420		2370	
	Temperature (°C)	24.9	25.2	25.0	25.1	24.8	25.1
1400 mg NaCl/L	pH (S.U.)	7.84	7.94	7.97	7.93	7.98	7.88
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.6	7.6	7.9
	Conductivity (µmhos/cm)	~~3290~~ 2800		2750		2720	
	Temperature (°C)	24.9	25.2	25.0	25.1	25.0	25.1
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

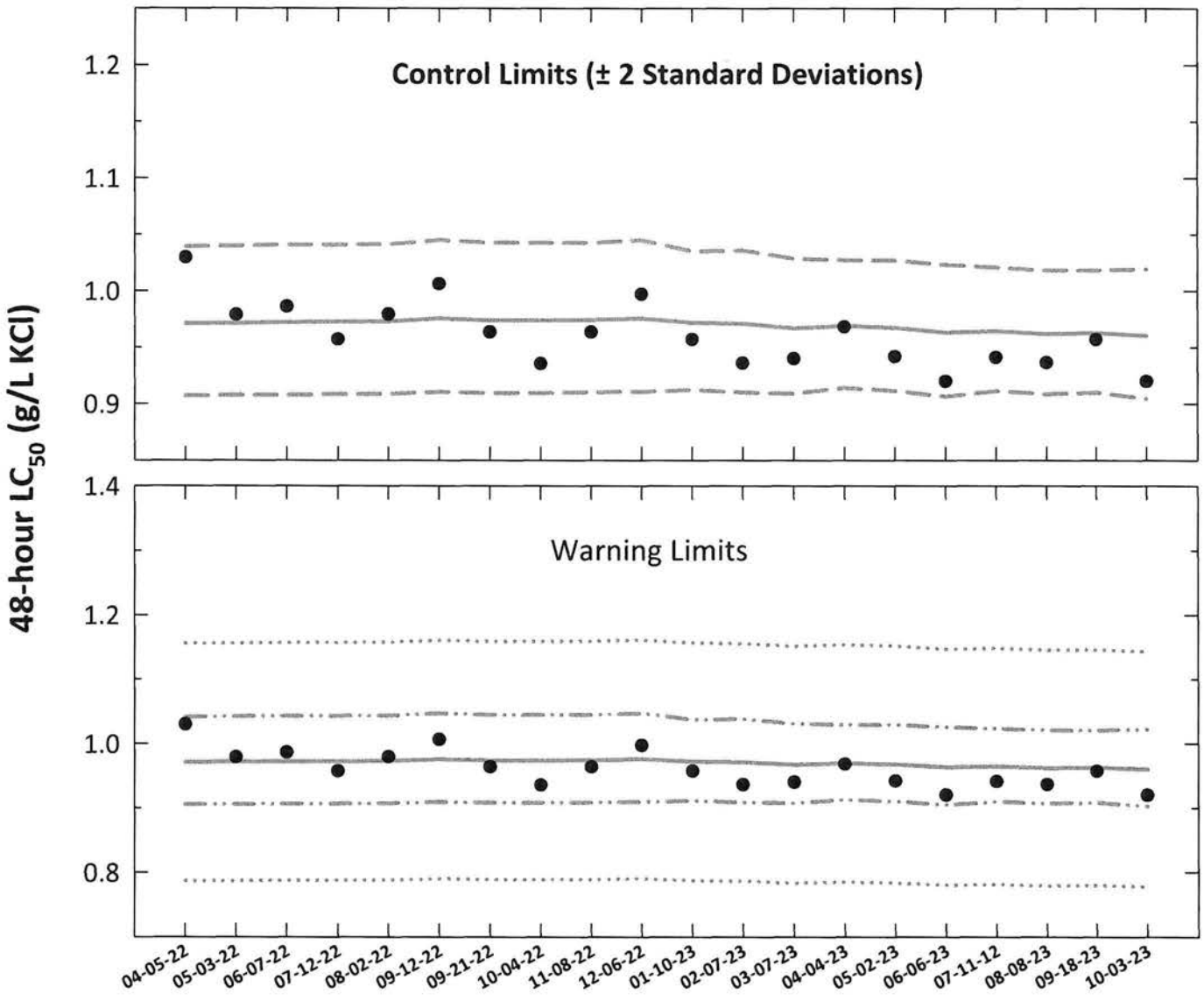
Species: ***Ceriodaphnia dubia***

CdNaClCR #: 292

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL	PM/BSV	PM/BSV	PM/BSV	PM/BSV	PM	PM	K
Concentration	Parameter		PM 12-7-23						
CONTROL, MHSW	pH (S.U.)	7.88	~~7.94~~ (7.94)	7.64	7.90	7.60	7.96	7.68	8.18
	Dissolved oxygen (mg/L)	7.7	8.1	7.9	8.0	8.0	7.7	7.6	7.6
	Conductivity (µmhos/cm)	307		292		300		297	
	Alkalinity (mg $CaCO_3$/L)			60					
	Hardness (mg $CaCO_3$/L)			91					
	Temperature (°C)	24.8	25.0	24.7	25.1	24.7	24.9	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.91	7.87	~~7.80~~ (7.80)	7.89	7.84	7.92	7.85	8.13
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	8.0	8.0	7.7	7.6	7.6
	Conductivity (µmhos/cm)	1470		1380		1440		1410	
	Temperature (°C)	24.9	25.2	24.8	24.9	24.8	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.91	7.87	7.84	7.89	7.90	7.91	7.90	8.09
	Dissolved oxygen (mg/L)	7.8	8.1	8.1	8.0	8.0	7.8	7.6	7.7
	Conductivity (µmhos/cm)	1780		1710		1800		1790	
	Temperature (°C)	24.9	25.0	24.8	24.9	24.9	25.0	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.91	7.86	7.84	7.89	7.91	7.92	7.91	8.07
	Dissolved oxygen (mg/L)	7.9	8.1	8.1	8.0	8.0	7.8	7.6	7.6
	Conductivity (µmhos/cm)	2140		2070		2210		2140	
	Temperature (°C)	24.9	24.9	24.9	24.9	24.8	25.1	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.91	7.84	7.88	7.88	7.93	7.91	7.94	8.01
	Dissolved oxygen (mg/L)	7.9	8.1	8.1	8.0	8.0	7.9	7.6	7.10
	Conductivity (µmhos/cm)	2510		2360		2580		2490	
	Temperature (°C)	24.8	24.9	24.7	25.1	24.9	24.8	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.90	7.83	7.86	7.90	7.93	7.92	7.94	8.04
	Dissolved oxygen (mg/L)	7.9	8.1	8.1	8.0	8.0	7.8	7.6	7.6
	Conductivity (µmhos/cm)	2870		2730		2960		2860	
	Temperature (°C)	24.8	25.1	24.9	25.0	24.9	24.8	25.0	25.2
		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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
18	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
19	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
20	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429

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 Summer

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 # 154

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 #: 2227

Chemical Analyses:

Concentration		Hours 0	Hours 24	Hours 48
	Analyst	N	N	N
Control, MHSW	pH (S.U.)	7.88	7.93	7.82
	Dissolved oxygen (mg/L)	7.6	7.8	7.9
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.8	24.7	24.5
500 mg/L	pH (S.U.)	8.02	7.88	7.85
	Dissolved oxygen (mg/L)	7.7	7.8	7.9
	Conductivity (µmhos/cm)	1180		
	Temperature (°C)	24.7	24.6	24.3
750 mg/L	pH (S.U.)	8.01	7.90	7.86
	Dissolved oxygen (mg/L)	7.7	7.8	7.8
	Conductivity (µmhos/cm)	1590		
	Temperature (°C)	24.7	24.6	24.3
1000 mg/L	pH (S.U.)	8.00	7.89	7.85
	Dissolved oxygen (mg/L)	7.7	7.8	7.7
	Conductivity (µmhos/cm)	2010		
	Temperature (°C)	24.7	24.4	24.6
1250 mg/L	pH (S.U.)	7.99	7.88	7.84
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	2380		
	Temperature (°C)	24.7	24.7	24.5
1500 mg/L	pH (S.U.)	7.98	7.88	
	Dissolved oxygen (mg/L)	7.8	7.9	6.04.18
	Conductivity (µmhos/cm)	2950		
	Temperature (°C)	24.7	24.5	

*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 applicabl
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066461

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 # 154

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-03-23	* 0510	JH	0710	JH	1D	WHITE	09-22-23 B
24	10-04-23			0700	JH			
48 Termination	10-05-23			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:	09-20-23
Age (1 to 14 days old):	6-7 DAYS OLD
Hatch date and times:	09-26-23 1203 TO 09-27-23 0824
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.06
	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	9 1d	5 5d	6 4d	1 2d	1 2d	0 10d	0 10d
48 Termination	10	10	10	10	8 1d	9	3 3d	4 2d	1	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:	10/3/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/5/2023	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	0.5000	0.6000
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	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	5.119	2.850	0.0907	2	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.101	2.850	0.0907	9	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

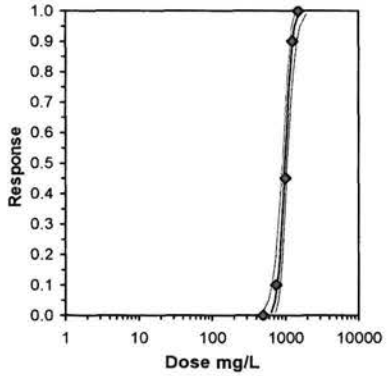
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.03598	0.0369	0.43896	0.00101	1.6E-06	4, 5
Treatments vs D-Control										

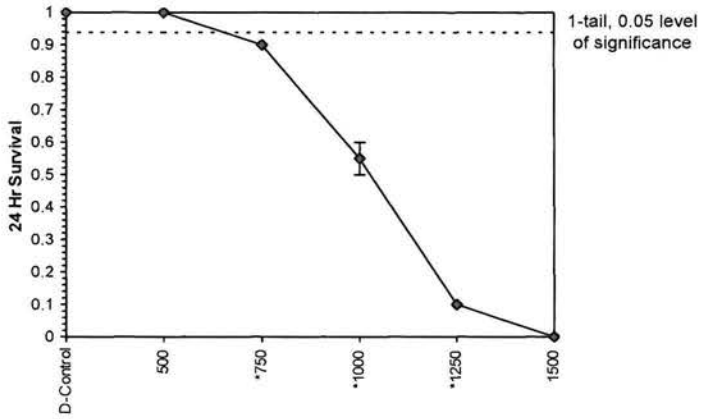
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.2473	2.18401	7.96662	16.5279	0	0.97492	7.81472	0.80732	2.9958	0.08165	3
Intercept	-31.69	6.57958	-44.586	-18.794							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	639.517	488.803	733.461
EC05	3.355	726.937	592.371	810.39
EC10	3.718	778.325	655.333	855.874
EC15	3.964	815.033	700.877	888.85
EC20	4.158	845.438	738.73	916.696
EC25	4.326	872.425	772.242	941.996
EC40	4.747	944.31	859.618	1013.54
EC50	5.000	990.377	912.7	1064.01
EC60	5.253	1038.69	964.737	1122.01
EC75	5.674	1124.28	1047.27	1237.92
EC80	5.842	1160.16	1078.79	1290.93
EC85	6.036	1203.45	1115.03	1357.67
EC90	6.282	1260.2	1160.31	1449.13
EC95	6.645	1349.29	1227.79	1600.07
EC99	7.326	1533.73	1359.1	1935.48



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:	10/3/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/5/2023	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	0.8000	0.9000
1000	0.3000	0.4000
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				

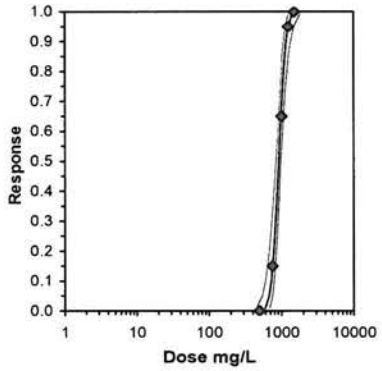
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.10938	0.11219	0.54029	0.00577	6.9E-05	4, 5
Treatments vs D-Control										

Maximum Likelihood-Probit

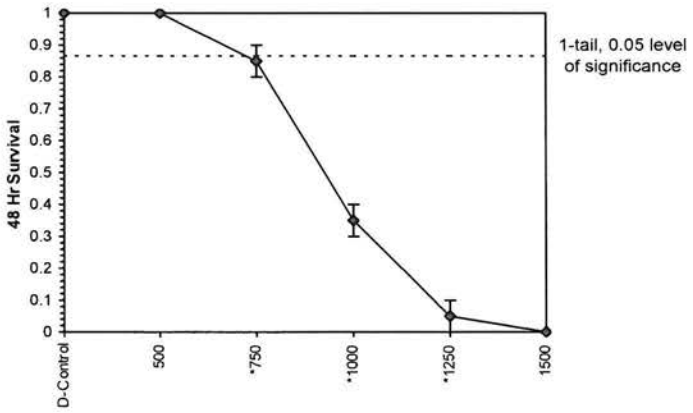
Parameter	Value	SE	95% Fiducial Limits	
Slope	12.3217	2.25704	7.89789	16.7455
Intercept	-31.519	6.73083	-44.712	-18.327
TSCR				

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0	0.17339	7.81472	0.98177	2.96382	0.08116	3

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



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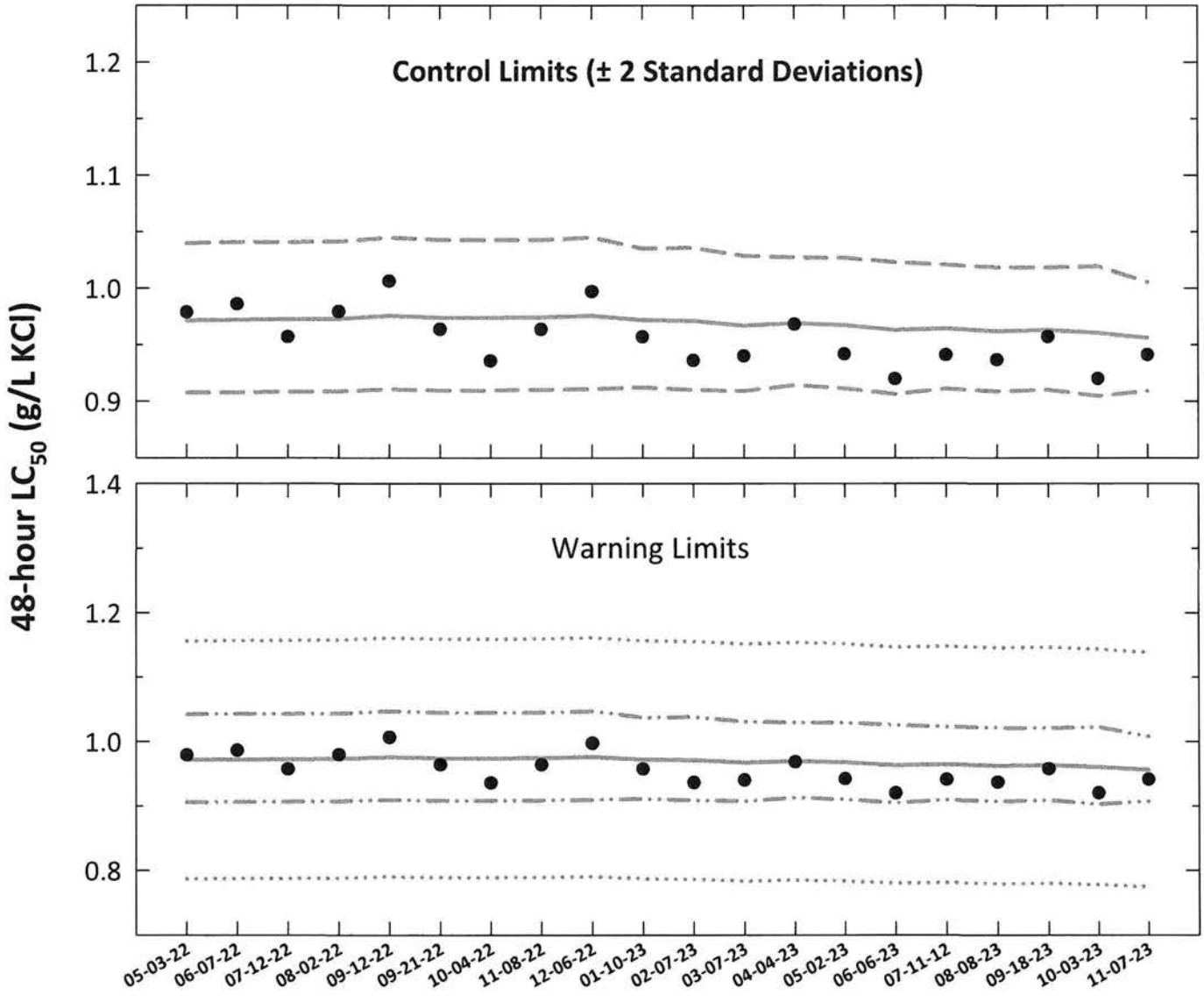
Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



- ● **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} ± $S_{A.75}$ 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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**07-11-12**	**0.9413**	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
17	**08-08-23**	**0.9366**	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
18	**09-18-23**	**0.9572**	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
19	**10-03-23**	**0.9201**	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
20	**11-07-23**	**0.9413**	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378

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 # 155

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 KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2227

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	K	BL	BL
Control, MHSW	pH (S.U.)	7.90	7.91	7.89
	Dissolved oxygen (mg/L)	7.7	7.8	7.6
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.7	24.6	24.3
500 mg/L	pH (S.U.)	7.90	7.89	7.90
	Dissolved oxygen (mg/L)	7.9	7.8	7.5
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	24.6	24.6	24.4
750 mg/L	pH (S.U.)	7.96	7.89	7.90
	Dissolved oxygen (mg/L)	7.8	7.8	7.6
	Conductivity (µmhos/cm)	1570		
	Temperature (°C)	24.6	24.6	24.4
1000 mg/L	pH (S.U.)	7.95	7.88	7.89
	Dissolved oxygen (mg/L)	7.9	7.9	7.6
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	24.6	24.7	24.4
1250 mg/L	pH (S.U.)	7.95	7.87	7.89
	Dissolved oxygen (mg/L)	7.9	7.9	7.6
	Conductivity (µmhos/cm)	2360		
	Temperature (°C)	24.6	24.9	24.2
1500 mg/L	pH (S.U.)	7.95	7.86	
	Dissolved oxygen (mg/L)	8.0	8.0	
	Conductivity (µmhos/cm)	2800		
	Temperature (°C)	24.6	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 th analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifie 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	130d646

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 # 155

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-07-23	* 0505	JL	0705	JL	1C	Yellow	10-31-23B
24	11-08-23			0655	JL			
48 Termination	11-09-23			0705	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
Spawning date:	10-25-23
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	10-31-23 1147 TO 11-01-23 0801
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.98
	Temperature (°C): 24.0

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	9 1d	5 5d	5 5d	1 9d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	8 1d	9	5	4 1d	0 1d	1	0	0
Mean Survival	100%		100%		85%		45%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	864.6
Upper 95% confidence limit (mg KCl/L)	1013.3
48-hour LC_{50} (mg KCl/L)	941.3

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	11/7/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/9/2023	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	0.5000	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	0.0285	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	62.662	2.850	0.0285	10	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	109.027	2.850	0.0285	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

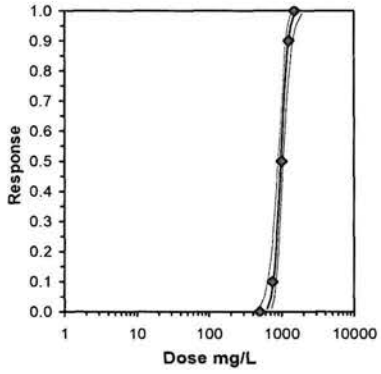
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.00967	0.00991	0.45056	0.0001	4.5E-09	4, 5
Treatments vs D-Control										

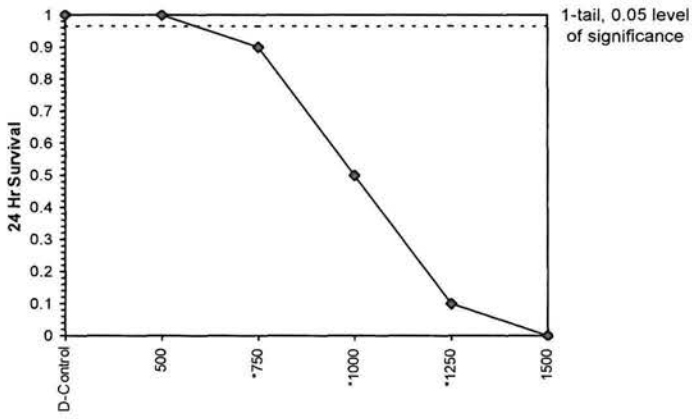
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.2001	2.17755	7.93208	16.4681	0	0.52701	7.81472	0.91292	2.99096	0.08197	3
Intercept	-31.49	6.55042	-44.329	-18.651							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	631.358	481.687	724.713
EC05	3.355	718.019	584.235	801.02
EC10	3.718	768.979	646.617	846.147
EC15	3.964	805.389	691.761	878.868
EC20	4.158	835.553	729.292	906.5
EC25	4.326	862.33	762.529	931.605
EC40	4.747	933.669	849.239	1002.59
EC50	5.000	979.398	901.956	1052.67
EC60	5.253	1027.37	953.665	1110.22
EC75	5.674	1112.36	1035.71	1225.27
EC80	5.842	1148.01	1067.06	1277.91
EC85	6.036	1191	1103.09	1344.22
EC90	6.282	1247.39	1148.09	1435.12
EC95	6.645	1335.93	1215.17	1585.22
EC99	7.326	1519.3	1345.7	1919.01



Dose-Response Plot



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Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	11/7/2023	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/9/2023	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	0.8000	0.9000
1000	0.5000	0.4000
1250	0.0000	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	0.2148	0	20
*750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.103	2.850	0.2148	3	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	8.980	2.850	0.2148	11	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	15.543	2.850	0.2148	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

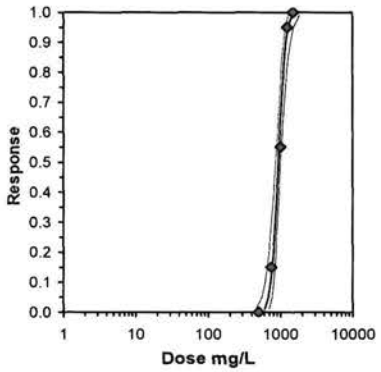
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	500	750	612.372		0.10822	0.111	0.50926	0.00568	7.7E-05	4, 5

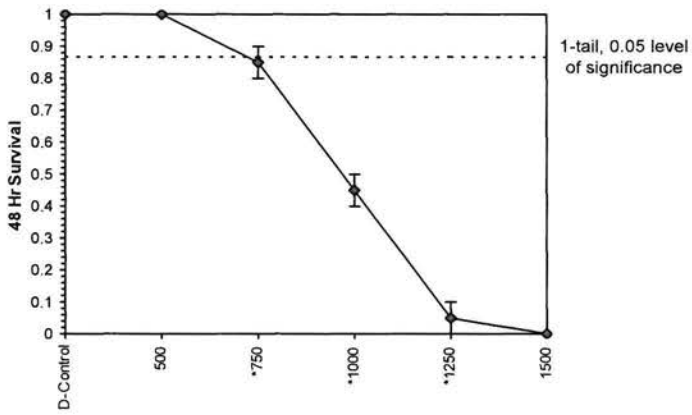
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.0612	2.17343	7.8013	16.3211	0	0.94761	7.81472	0.81393	2.97373	0.08291	3
Intercept	-30.867	6.50354	-43.614	-18.12							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	603.746	456.725	695.29
EC05	3.355	687.634	555.76	769.21
EC10	3.718	737.019	616.162	812.955
EC15	3.964	772.328	659.942	844.684
EC20	4.158	801.593	696.384	871.484
EC25	4.326	827.581	728.691	895.836
EC40	4.747	896.866	813.136	964.708
EC50	5.000	941.31	864.592	1013.32
EC60	5.253	987.957	915.132	1069.23
EC75	5.674	1070.67	995.347	1181.32
EC80	5.842	1105.38	1025.97	1232.74
EC85	6.036	1147.27	1061.14	1297.6
EC90	6.282	1202.23	1105.04	1386.68
EC95	6.645	1288.57	1170.42	1534.05
EC99	7.326	1467.61	1297.58	1862.76

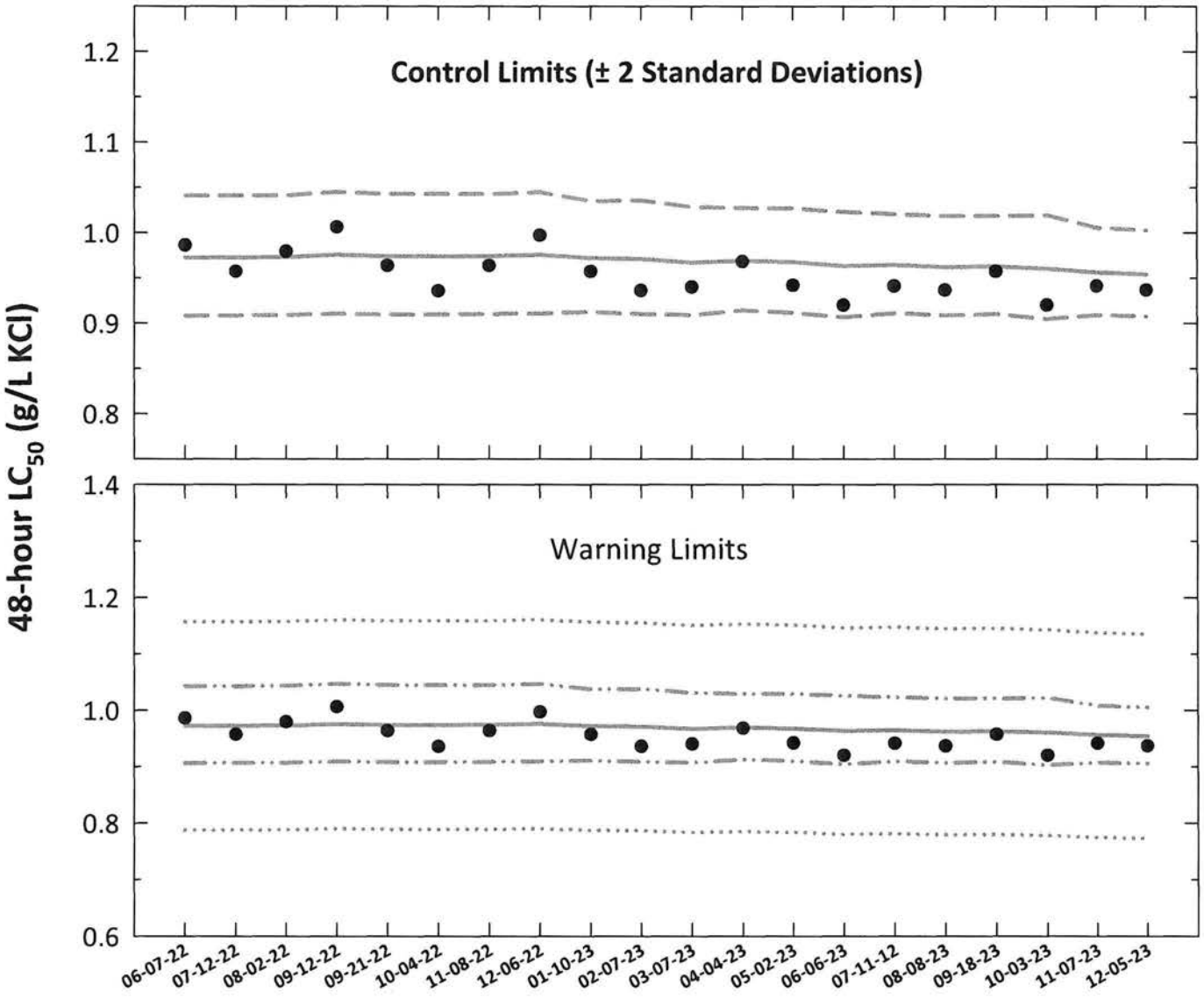


Dose-Response Plot



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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
16	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
17	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
18	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
19	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
20	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353

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 #: 2249

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	BL	BL	VL
Control, MHSW	pH (S.U.)	7.75	8.01	8.01
	Dissolved oxygen (mg/L)	7.7	7.7	7.5
	Conductivity (µmhos/cm)	312		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	91		
	Temperature (°C)	24.7	24.9	24.3
500 mg/L	pH (S.U.)	7.96	7.96	7.94
	Dissolved oxygen (mg/L)	7.8	7.7	7.4
	Conductivity (µmhos/cm)	1220		
	Temperature (°C)	24.6	25.0	24.3
750 mg/L	pH (S.U.)	7.95	7.95	7.90
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	Conductivity (µmhos/cm)	1640		
	Temperature (°C)	24.6	25.0	24.2
1000 mg/L	pH (S.U.)	7.93	7.92	7.90
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	Conductivity (µmhos/cm)	2080		
	Temperature (°C)	24.8	25.0	24.6
1250 mg/L	pH (S.U.)	7.92	7.91	7.90
	Dissolved oxygen (mg/L)	7.8	7.9	7.4
	Conductivity (µmhos/cm)	2530		
	Temperature (°C)	24.5	24.7	24.5
1500 mg/L	pH (S.U.)	7.92	7.90	
	Dissolved oxygen (mg/L)	7.8	7.8	
	Conductivity (µmhos/cm)	3080		
	Temperature (°C)	24.8	24.7	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by t analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifi 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	130646P(

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 # 156

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12-05-23	* 0805	JH	0705	JH	1B	ORANGE	11-29-23 B
24	12-06-23			0705	JH			
48 Termination	12-07-23			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:	11-22-23
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	11-28-23 1124 TO 11-29-23 0821
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.98
	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	5 sd	4 bd	2 sd	0 10d	0 10d	0 10d
48 Termination	10	10	10	10	9 sd	7 sd	5	4	2	0	0	0
Mean Survival	100%		100%		80%		45%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	853.8
Upper 95% confidence limit (mg KCl/L)	1014.5
48-hour LC_{50} (mg KCl/L)	936.8

Comments:

Test Reviewed by: JH

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	12/5/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/7/2023	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	0.7000
1000	0.5000	0.4000
1250	0.2000	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	0.3711	0	20
750	0.8000	0.8000	1.1201	0.9912	1.2490	16.280	2	2.242	2.850	0.3711	4	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	5.198	2.850	0.3711	11	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	8.453	2.850	0.3711	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

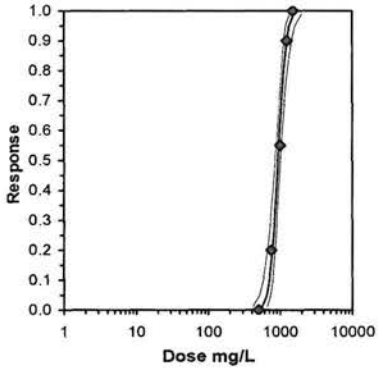
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.2305	0.23641	0.44927	0.01696	0.00146	4, 5
Treatments vs D-Control										

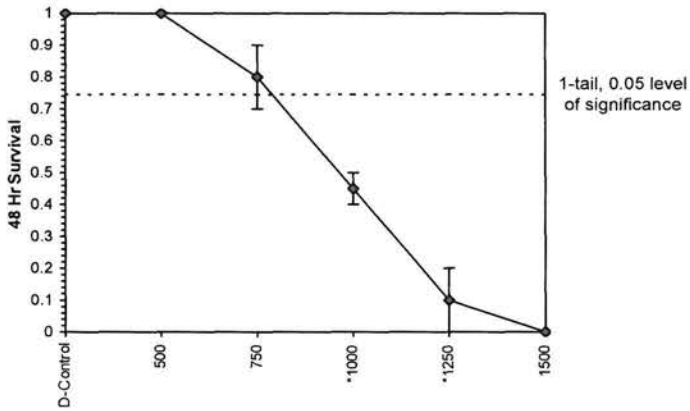
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	10.3517	1.80371	6.81642	13.887	0	1.01568	7.81472	0.79746	2.97166	0.0966	4
Intercept	-25.762	5.40202	-36.35	-15.174							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	558.373	409.648	655.225
EC05	3.355	649.768	513.067	737.359
EC10	3.718	704.457	577.605	786.469
EC15	3.964	743.932	625.037	822.277
EC20	4.158	776.878	664.927	852.624
EC25	4.326	806.302	700.601	880.268
EC40	4.747	885.485	795.21	958.768
EC50	5.000	936.818	853.805	1014.49
EC60	5.253	991.127	911.991	1079.01
EC75	5.674	1088.46	1005.46	1209.93
EC80	5.842	1129.69	1041.49	1270.65
EC85	6.036	1179.72	1083.1	1347.78
EC90	6.282	1245.82	1135.43	1454.59
EC95	6.645	1350.68	1214.12	1633.42
EC99	7.326	1571.76	1369.64	2040.82



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	12/5/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/7/2023	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	0.5000	0.4000
1250	0.2000	0.0000
1500	0.0000	0.0000

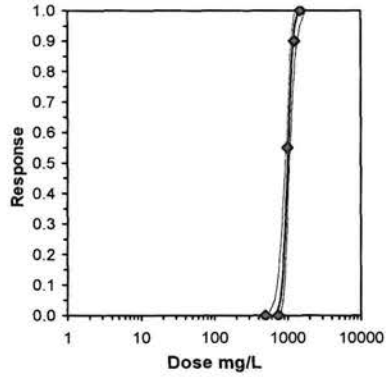
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2894	0	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	6.668	2.850	0.2894	11	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	10.842	2.850	0.2894	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	750	1000	866.025		0.16274	0.16691	0.51898	0.01031	3.1E-04	4, 5
Treatments vs D-Control										

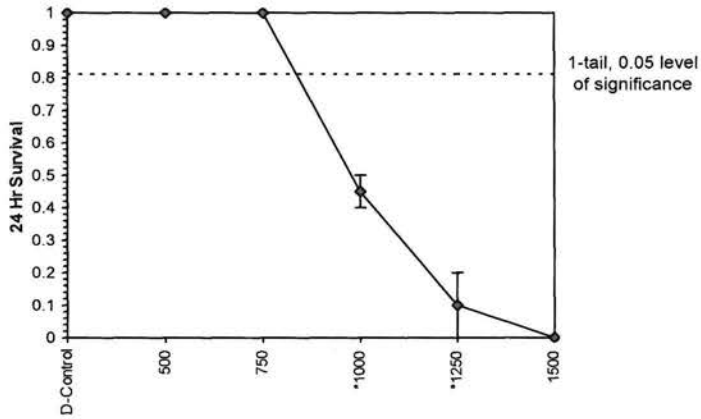
Maximum Likelihood-Probit

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



ETS
Environmental Testing Solutions, Inc.

Surveillance and Corrective Action Report (SCAR)

SURVIELLANCE NUMBER: SCAR-231218

SURVIELLANCE: *P. promelas* Acute Reference Toxicant Test #157

DATE OF INSPECTION: 12-18-23

TYPE OF INSPECTION: Internal

EVALUATORS: Jim Sumner

LABORATORY PERSONNELL: Jim Sumner, Jaydon Perez

I. DEVIATION / DECREPANCY:

LC50 result for *P. promelas* reference toxicant test performed 12/11-13/23 was above upper control limit.

- LC50 = 1018.6 mg/L KCl, 95% confidence interval = 918.8 – 1098.2 mg/L KCl
- Upper Control Limit of ± 2SD = 1010.7 mg/L KCl.
- Result was within EPA 75th Percentile CVs.
- 1st outlier test in the last 20 data points.

Previous *P. promelas* reference toxicant test performed 12/05-07/23 was within control limits.

II. ROOT CAUSE INVESTIGATION:

Due to organism availability, additional reference toxicant tests could not be performed.

Guidance from EPA-821-R-02-012, Section 4.15.4 was followed. Only 1 of 20 tests fell outside control limits and the result was not "well" outside the control limits (95% confidence interval overlapped control limits and result was with EPA 75th Percentile CVs). Therefore, the result was maintained in the control chart and this outlier was not believed to have jeopardized the validity of compliance tests.

III. EXECUTION:

12-18-23: NC and SC certifying authorities were notified of the deviation by e-mail.

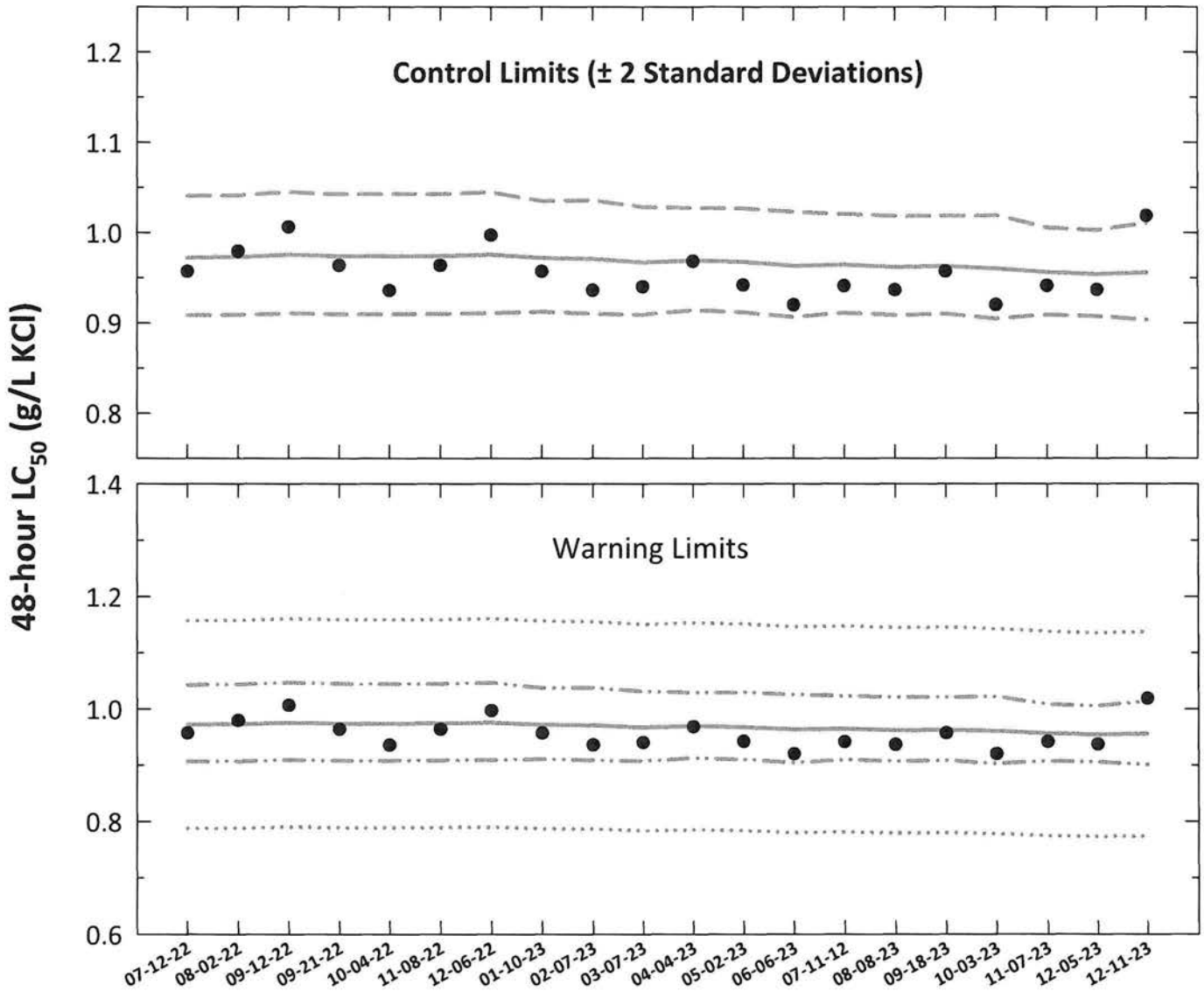
IV. VERIFICATION:

Signature: _____ Laboratory Analyst Date: 12-18-23

V. CLOSURE:

Signature: _____ QA Officer Date: 12-18-23

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} ± $S_{A.75}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
15	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
16	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
17	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
18	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
19	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
20	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372

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 # 157

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 KC stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2249

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JP JR	JL	BL
Control, MHSW	pH (S.U.)	7.68	7.80	7.82
	Dissolved oxygen (mg/L)	7.6	7.7	7.5
	Conductivity (µmhos/cm)	297		
	Alkalinity (mg/L $CaCO_3$)	64		
	Hardness (mg/L $CaCO_3$)	82		
	Temperature (°C)	24.6	24.8	24.2
500 mg/L	pH (S.U.)	7.68	7.80	7.86
	Dissolved oxygen (mg/L)	7.8	7.6	7.5
	Conductivity (µmhos/cm)	1220		
	Temperature (°C)	24.4	24.6	24.4
750 mg/L	pH (S.U.)	7.70	7.79	7.86
	Dissolved oxygen (mg/L)	7.9	7.6	7.6
	Conductivity (µmhos/cm)	1640		
	Temperature (°C)	24.8	24.5	24.1
1000 mg/L	pH (S.U.)	7.74	7.77	7.85
	Dissolved oxygen (mg/L)	7.9	7.7	7.6
	Conductivity (µmhos/cm)	2060		
	Temperature (°C)	25.0	24.8	24.3
1250 mg/L	pH (S.U.)	7.75	7.73	7.87
	Dissolved oxygen (mg/L)	7.9	7.7	7.6
	Conductivity (µmhos/cm)	2490		
	Temperature (°C)	25.4	24.7	24.3
1500 mg/L	pH (S.U.)	7.77	7.74	7.85
	Dissolved oxygen (mg/L)	8.0	7.6	7.6
	Conductivity (µmhos/cm)	2900		
	Temperature (°C)	25.2	24.4	24.2

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by th analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifie 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 # 157

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12-11-23	*0820	JP	1140	JP	2F	Yellow	12-08-23A
24	12-12-23			1141	JP			
48 Termination	12-13-23			1142	JP			

*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:	11-28-23
Age (1 to 14 days old):	6 to 7 days old
Hatch date and times:	12-04-23 1355 to 12-05-23 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.66
	Temperature (°C): 24.6 °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	12-12-23 ~~10~~ 9 1d	10	10	10	10	9 1d	7 3d	7 3d	3 7d	2 8	2 8d	2 8d
48 Termination	9	10	10	10	10	8 1d	4 3d	7	2 1d	1 1d	0 2d	0 2d
Mean Survival	95%		100%		90%		55%		15%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	918.8
Upper 95% confidence limit (mg KCl/L)	1098.2
48-hour LC_{50} (mg KCl/L)	1018.6

Comments:

Test Reviewed by:

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	12/11/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/13/2023	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	0.9000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.7000	0.7000
1250	0.3000	0.2000
1500	0.2000	0.2000

			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	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2				1	20
500	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-1.094	2.830	0.2108	0	20
750	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2	0.000	2.830	0.2108	1	20
*1000	0.7000	0.7368	0.9912	0.9912	0.9912	0.000	2	4.556	2.830	0.2108	6	20
*1250	0.2500	0.2632	0.5216	0.4636	0.5796	15.723	2	10.860	2.830	0.2108	15	20
*1500	0.2000	0.2105	0.4636	0.4636	0.4636	0.000	2	11.639	2.830	0.2108	16	20

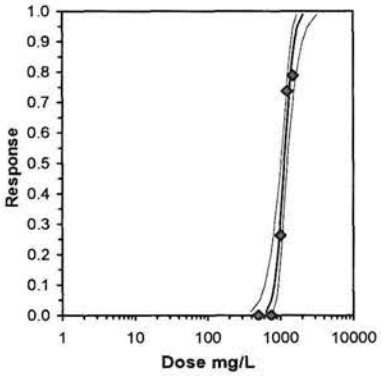
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.1334	0.1414	0.36177	0.00555	3.8E-05	5, 6
Treatments vs D-Control										

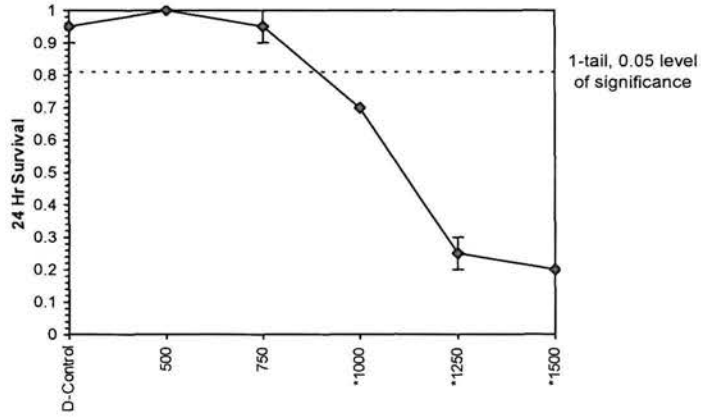
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.12605	2.04191	5.12391	13.1282	0.05	2.18907	7.81472	0.53411	3.05815	0.10958	5
Intercept	-22.909	6.26236	-35.183	-10.635							
TSCR	0.02244	0.02334	-0.0233	0.06819							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	635.671	386.181	778.856
EC05	3.355	754.932	521.898	882.218
EC10	3.718	827.404	611.723	944.461
EC15	3.964	880.19	680.036	990.184
EC20	4.158	924.535	738.871	1029.29
EC25	4.326	964.355	792.43	1065.36
EC40	4.747	1072.47	937.154	1171.97
EC50	5.000	1143.26	1026.11	1253.93
EC60	5.253	1218.73	1110.83	1356.94
EC75	5.674	1355.36	1237.54	1584.62
EC80	5.842	1413.73	1284.44	1694.79
EC85	6.036	1484.96	1338.15	1837.32
EC90	6.282	1579.69	1405.51	2038.76
EC95	6.645	1731.34	1507.08	2385.83
EC99	7.326	2056.17	1709.5	3219.74



Dose-Response Plot



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Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	12/11/2023	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/13/2023	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	0.9000	1.0000
500	1.0000	1.0000
750	1.0000	0.8000
1000	0.4000	0.7000
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	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2				1	20
500	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-0.533	2.850	0.4355	0	20
750	0.9000	0.9474	1.2596	1.1071	1.4120	17.115	2	0.464	2.850	0.4355	2	20
*1000	0.5500	0.5789	0.8379	0.6847	0.9912	25.859	2	3.223	2.850	0.4355	9	20
*1250	0.1500	0.1579	0.3927	0.3218	0.4636	25.550	2	6.137	2.850	0.4355	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

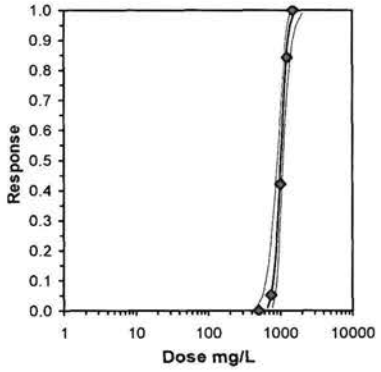
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.33466	0.35474	0.36532	0.02335	0.00494	4, 5
Treatments vs D-Control										

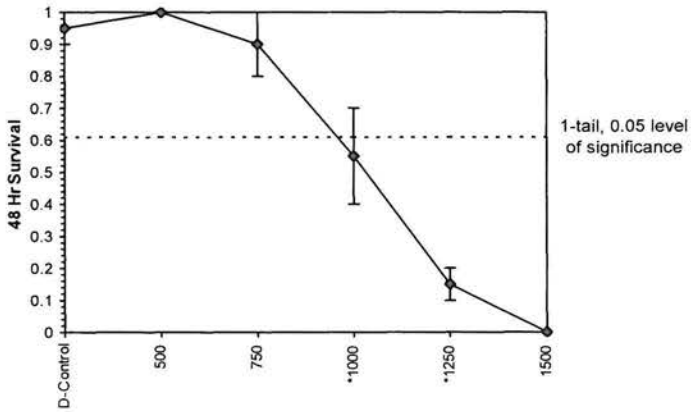
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.4691	2.62137	7.33126	17.607	0.05	1.23578	7.81472	0.74444	3.00799	0.0802	4
Intercept	-32.507	7.95114	-48.091	-16.923							
TSCR	0.02868	0.02582	-0.0219	0.0793							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	662.857	461.478	776.796
EC05	3.355	751.751	569.23	852.773
EC10	3.718	803.915	635.841	897.35
EC15	3.964	841.14	684.582	929.482
EC20	4.158	871.95	725.48	956.485
EC25	4.326	899.281	762.013	980.914
EC40	4.747	972.009	858.873	1049.61
EC50	5.000	1018.56	918.817	1098.18
EC60	5.253	1067.35	977.892	1154.94
EC75	5.674	1153.67	1069.66	1273.41
EC80	5.842	1189.83	1103.45	1329.69
EC85	6.036	1233.41	1141.38	1401.87
EC90	6.282	1290.52	1187.66	1502.47
EC95	6.645	1380.07	1255.03	1671.22
EC99	7.326	1565.15	1383.19	2053.37



Dose-Response Plot



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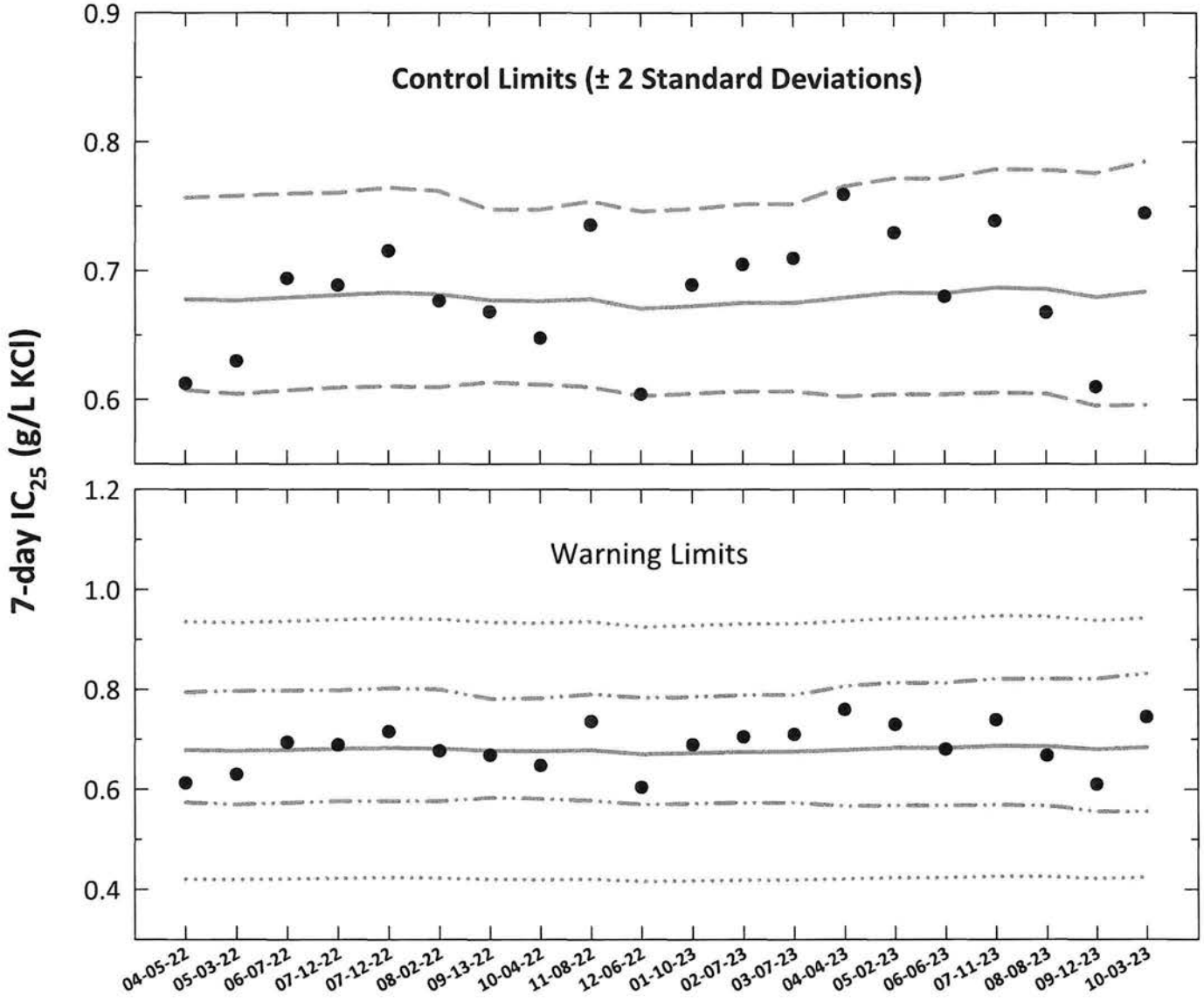
Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture



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 $IC_{25} \pm S_{A.75}$ 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

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
18	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
19	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
20	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436

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.

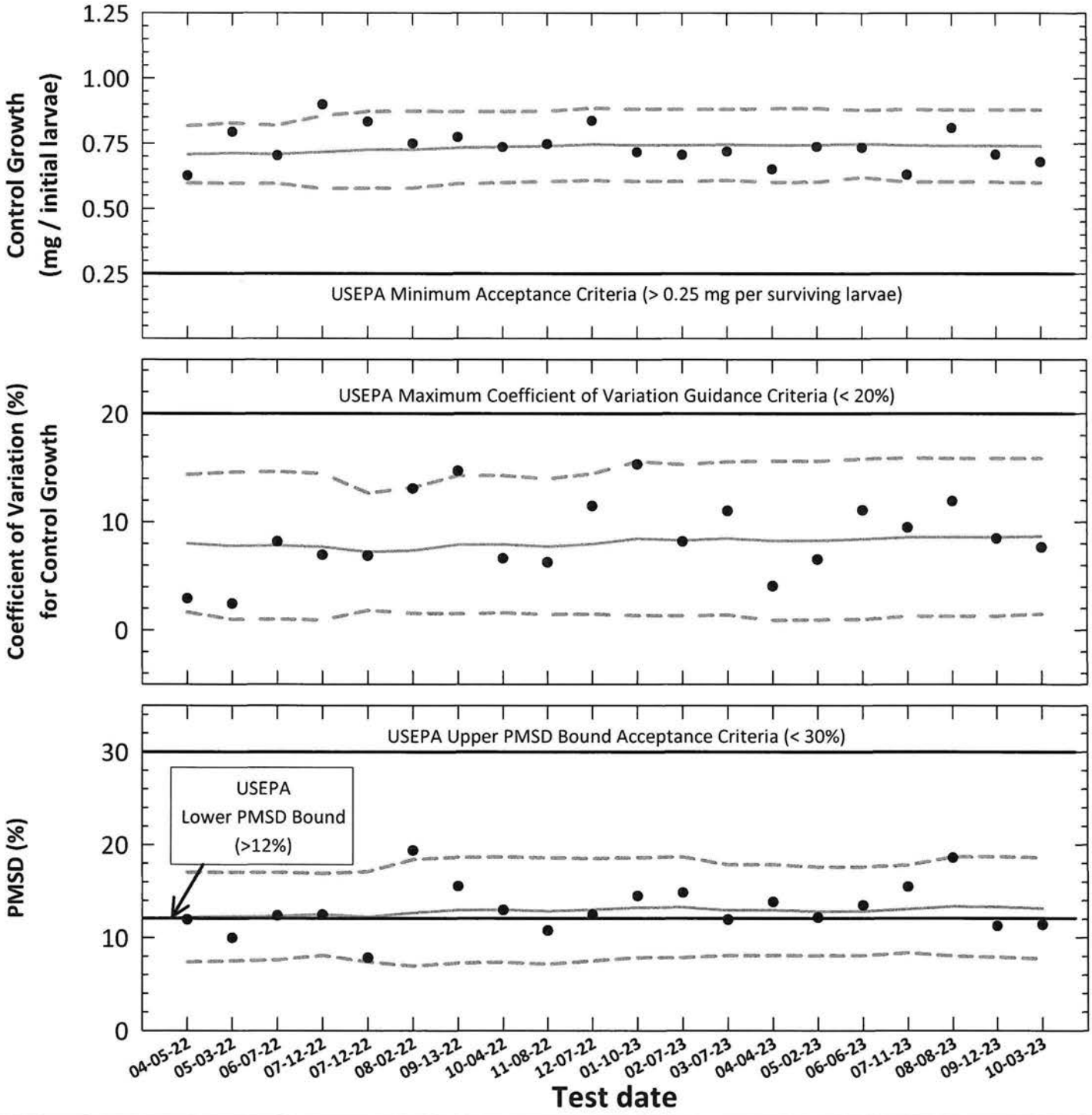
Entered and Reviewed by Jim Sumner

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.

Reviewed by: Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
18	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
19	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
20	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6

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 Summer

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **107**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 7227				
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:	09-26-23	*Artemia* CHM number:	CHM1222
Hatch dates and times:	10-02-23 1400 TO 10-03-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.06 S.U. Temperature = 24.0 °C	Date / Time in oven:	10-10-23 0720
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	10-11-23 0720
		*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	10-03-23	0510	JK	1115	JK	0723	JK	09-27-23 B
1	10-04-23	0500	JK	1100	JK	0700	JK	↓
2	10-05-23	0500	JK	1100	JK	0700	JK	10-02-23 A
3	10-06-23	0500	JK	1100	JK	0700	JK	↓
4	10-07-23	0615	JK	1215	JK	0815	JK	10-05-23 B
5	10-08-23	0600	JK	1200	JK	0800	JK	↓
6	10-09-23	0500	JK	1100	JK	0717	JK	↓
7	10-10-23					0625	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
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:	07.	≤ 20%	7-day LC_{50} (mg/L KCl)	837.8
Average weight per initial larvae:	0.678		NOEC (mg/L KCl)	600
Average weight per surviving larvae:	0.678	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	744.7

Reviewed by: Jim Sumner JK

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **107**

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 (16)	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: Lavender Analyst: BL Date: 09.25.23	13.13	12.41	13.65	12.89	14.54	15.14	13.24	14.12	11.41	14.16	14.86	13.55
*B = Pan + Larvae weight (mg) Analyst: BL Date: 10.13.23	19.34	18.93	21.03	19.91	23.22	24.15	21.40	21.97	18.65	20.93	22.30	20.94
C = Larvae weight (mg) = B – A Analyst: JH	6.21	6.52	7.38	7.02	8.68	9.01	8.16	7.85	7.24	6.77	7.44	6.99
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.621	0.652	0.738	0.702	0.868	0.901	0.816	0.785	0.724	0.677	0.744	0.699
Average weight per initial number of larvae (mg)	0.678				0.843		-24.27.		0.711		-4.87.	
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 JH

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **107**

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	9 1d	9 1d	9 1d	9 1d	6 4d	7 3d	6 4d	6 4d
2	10	10	10	10	9	9	9	9	5 1d	6 1d	6	5 1d
3	10	10	10	10	8 1d	9	9	9	5	6	6	5
4	10	10	10	10	7 1d	9	9	9	5	5 1d	5 1d	5
5	10	10	10	10	7	9	9	8 1d	5	4 1d	5	5
6	10	10	10	10	7	9	9	8	4 1d	3 1d	3	4 1d
7	10	10	10	10	7	8 1d	7 2d	8	Lg 3 1d	Lg 3	3	2 2d
*A = Pan weight (mg) Tray color code: Lavender Analyst: BL Date: 09·25·23	13.00	12.98	14.52	15.31	13.46	12.86	13.82	15.33	13.90	13.94	11.54	15.72
*B = Pan + Larvae weight (mg) Analyst: BL Date: 10·13·23	20.13	20.43	22.68	22.26	18.85	18.66	19.13	21.41	16.74	16.76	13.77	17.40
C = Larvae weight (mg) = B – A Analyst: JH	7.13	7.45	8.16	6.95	5.39	5.80	5.31	6.08	2.84	2.82	2.23	1.68
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.713	0.745	0.816	0.695	0.539	0.580	0.531	0.608	0.284	0.282	0.223	0.168
Average weight per initial number of larvae (mg)	0.742				0.565				0.239			
Percent reduction from control (%)			-9.47.				16.87.				64.77.	

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

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

Comments:

Reviewed by: Jim Sumner JH

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **107**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	4 6d	3 7d	3 7d	4 6d
2	4	2 1d	3	3 1d
3	3 1d	2	2 1d	3
4	3	1 1d	2	3
5	1 2d	1	1 1d	2 1d
6	1	1	1	2
7	~~6~~ 1	~~6~~ 1	~~6~~ 1	~~6~~ 1 1d
*A = Pan weight (mg) Tray color code: Lavender Analyst: BL Date: 09.25.23	12.89	14.69	14.21	15.23
*B = Pan + Larvae weight (mg) Analyst: BL Date: 10.13.23	14.25	15.78	15.28	16.23
C = Larvae weight (mg) = B – A Analyst: JL	1.36	1.09	1.07	1.00
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.136 Removed	0.109	0.107	0.100
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.113		83.37	

*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:

Reviewed by: Jim Sumner JL

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: 107

Test dates: October 03-10, 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	13.13	19.34	6.21	0.621	0.678	7.7	0.621	100.0	0.678	7.7	Not applicable
	B	10	10	12.41	18.93	6.52	0.652			0.652				
	C	10	10	13.65	21.03	7.38	0.738			0.738				
	D	10	10	12.89	19.91	7.02	0.702			0.702				
300	E	10	10	14.54	23.22	8.68	0.868	0.843	6.2	0.868	100.0	0.843	6.2	-24.2
	F	10	10	15.14	24.15	9.01	0.901			0.901				
	G	10	10	13.24	21.40	8.16	0.816			0.816				
	H	10	10	14.12	21.97	7.85	0.785			0.785				
450	I	10	10	11.41	18.65	7.24	0.724	0.711	4.1	0.724	100.0	0.711	4.1	-4.8
	J	10	10	14.16	20.93	6.77	0.677			0.677				
	K	10	10	14.86	22.30	7.44	0.744			0.744				
	L	10	10	13.55	20.54	6.99	0.699			0.699				
600	M	10	10	13.00	20.13	7.13	0.713	0.742	7.2	0.713	100.0	0.742	7.2	-9.4
	N	10	10	12.98	20.43	7.45	0.745			0.745				
	O	10	10	14.52	22.68	8.16	0.816			0.816				
	P	10	10	15.31	22.26	6.95	0.695			0.695				
750	Q	10	7	13.46	18.85	5.39	0.770	0.753	2.6	0.539	75.0	0.565	6.4	16.8
	R	10	8	12.86	18.66	5.80	0.725			0.580				
	S	10	7	13.82	19.13	5.31	0.759			0.531				
	T	10	8	15.33	21.41	6.08	0.760			0.608				
900	U	10	3	13.90	16.74	2.84	0.947	0.868	11.1	0.284	27.5	0.239	23.1	64.7
	V	10	3	13.94	16.76	2.82	0.940			0.282				
	W	10	3	11.54	13.77	2.23	0.743			0.223				
	X	10	2	15.72	17.40	1.68	0.840			0.168				
1050	Y	10	1	12.89	14.25	1.36	1.360	1.130	14.0	0.136	10.0	0.113	14.0	83.3
	Z	10	1	14.69	15.78	1.09	1.090			0.109				
	AA	10	1	14.21	15.28	1.07	1.070			0.107				
	BB	10	1	15.23	16.23	1.00	1.000			0.100				

Dunnett's MSD value: 0.0772

PMSD: 11.4

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	10/3/2023	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/10/2023	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	0.7000	0.8000	0.7000	0.8000
900	0.3000	0.3000	0.3000	0.2000
1050	0.1000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
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	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40
*1050	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	36	40

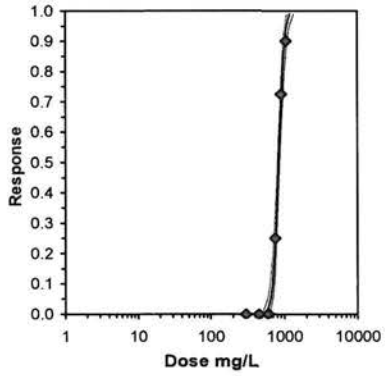
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.72791	0.896	-0.9064	3.00264
Equality of variance cannot be confirmed				

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

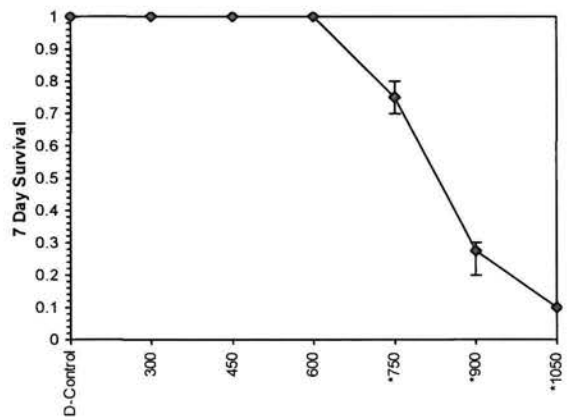
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.1169	1.97882	11.2384	18.9953	0	1.58635	9.48773	0.81124	2.92314	0.06615	4
Intercept	-39.189	5.79436	-50.546	-27.832							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	587.829	514.381	638.148
EC05	3.355	652.128	589.84	695.006
EC10	3.718	689.232	633.932	728.003
EC15	3.964	715.452	665.121	751.606
EC20	4.158	737	690.64	771.311
EC25	4.326	756.003	712.954	789.023
EC40	4.747	806.089	770.063	838.049
EC50	5.000	837.803	804.196	871.594
EC60	5.253	870.766	837.549	908.963
EC75	5.674	928.454	891.143	980.062
EC80	5.842	952.393	912.027	1011.26
EC85	6.036	981.078	936.319	1049.63
EC90	6.282	1018.4	967.028	1100.87
EC95	6.645	1076.34	1013.29	1182.76
EC99	7.326	1194.08	1103.94	1355.82



Dose-Response Plot



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ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	10/3/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	10/10/2023	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:					

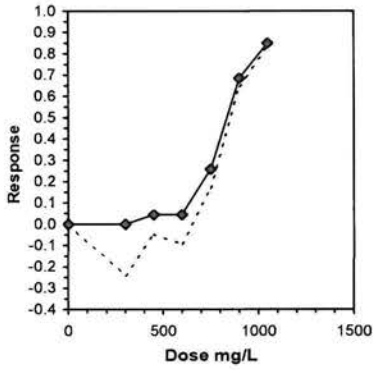
Conc-mg/L	1	2	3	4
D-Control	0.6210	0.6520	0.7380	0.7020
300	0.8680	0.9010	0.8160	0.7850
450	0.7240	0.6770	0.7440	0.6990
600	0.7130	0.7450	0.8160	0.6950
750	0.5390	0.5800	0.5310	0.6080
900	0.2840	0.2820	0.2230	0.1680
1050	0.1360	0.1090	0.1070	0.1000

			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	0.6783	1.0000	0.6783	0.6210	0.7380	7.661	4				0.7604	1.0000
300	0.8425	1.2422	0.8425	0.7850	0.9010	6.160	4	-4.872	2.290	0.0772	0.7604	1.0000
450	0.7110	1.0483	0.7110	0.6770	0.7440	4.107	4	-0.971	2.290	0.0772	0.7266	0.9556
600	0.7423	1.0944	0.7423	0.6950	0.8160	7.186	4	-1.898	2.290	0.0772	0.7266	0.9556
750	0.5645	0.8323	0.5645	0.5310	0.6080	6.391	4				0.5645	0.7424
900	0.2393	0.3527	0.2393	0.1680	0.2840	23.109	4				0.2393	0.3146
1050	0.1130	0.1666	0.1130	0.1000	0.1360	13.992	4				0.1130	0.1486

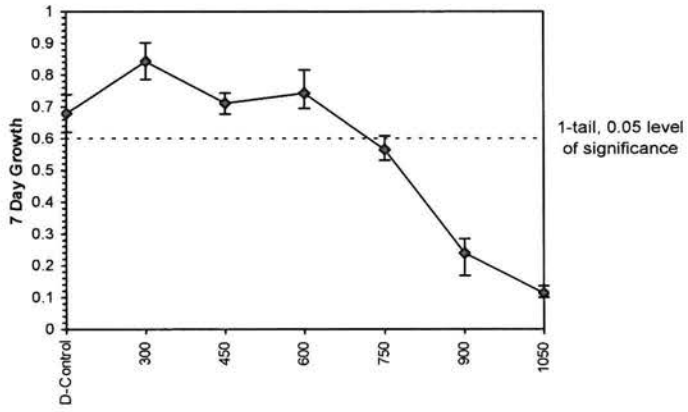
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.9397		0.844		0.29164	-1.1259
Bartlett's Test indicates equal variances (p = 0.78)					1.08998		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	600	>600			0.0772	0.11382	0.02016	0.00227	0.00227	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	603.95	99.75	244.97	649.00	-0.3333
IC10	639.12	25.87	578.88	681.08	-4.5536
IC15	674.30	15.43	623.46	713.36	-0.0758
IC20	709.48	15.37	665.66	754.40	0.0481
IC25	744.65	12.91	701.22	771.09	-0.4516
IC40	799.93	7.24	777.87	820.04	-0.0595
IC50	835.00	7.88	810.59	858.24	-0.1297



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **107**

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]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.83	7.89	7.76	7.80	7.83	7.61
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.5	7.8	6.6
	Conductivity (µmhos/cm)	295		310		298	
	Alkalinity (mg $CaCO_3$/L)	60				61	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.8	24.5	24.7	24.5	24.7	24.5
300 mg KCl/L	pH (S.U.)	7.93	7.86	7.97	7.75	7.99	7.60
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.5	7.9	7.0
	Conductivity (µmhos/cm)	932		958		931	
	Temperature (°C)	24.8	24.4	24.7	24.3	24.8	24.5
450 mg KCl/L	pH (S.U.)	7.92	7.86	7.94	7.74	7.98	7.59
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.4	7.9	7.1
	Conductivity (µmhos/cm)	1080		1110		1070	
	Temperature (°C)	24.9	24.7	24.7	24.7	24.7	24.6
600 mg KCl/L	pH (S.U.)	7.93	7.85	7.96	7.74	7.97	7.58
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.4	7.9	6.9
	Conductivity (µmhos/cm)	1310		1360		1310	
	Temperature (°C)	24.9	24.4	24.8	24.3	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.94	7.85	7.97	7.73	7.96	7.58
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.4	7.9	6.8
	Conductivity (µmhos/cm)	1580		1620		1610	
	Temperature (°C)	24.8	24.4	24.8	24.6	24.7	24.5
900 mg KCl/L	pH (S.U.)	7.94	7.86	7.97	7.74	7.96	7.60
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.4	8.0	7.3
	Conductivity (µmhos/cm)	1940		1890		1920	
	Temperature (°C)	24.8	24.6	24.8	24.6	24.7	24.5
1050 mg KCl/L	pH (S.U.)	4.95	7.86	7.97	7.75	7.90	7.61
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.5	8.0	6.7
	Conductivity (µmhos/cm)	2110		2150		2110	
	Temperature (°C)	24.8	24.6	24.8	24.6	24.7	24.6
		Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Sumner

Environmental Testing Solutions, Inc.

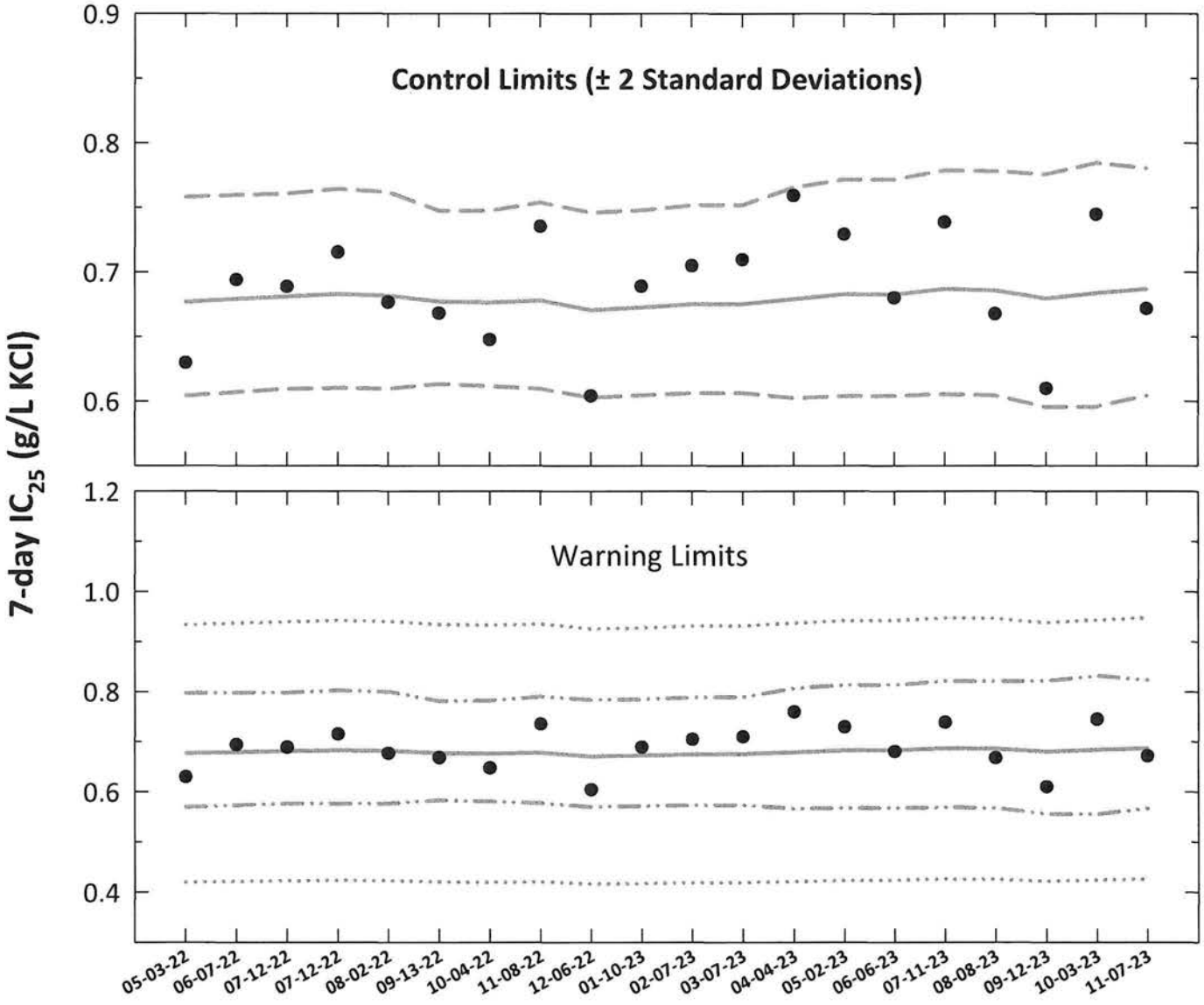
Species: *Pimephales promelas* PpKClCR Test Number: **107**

		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	BL	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.89	7.70	7.79	7.73	7.91	7.86	7.91	7.43
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.6	7.8	7.0	8.0	6.6
	Conductivity (µmhos/cm)	301		310		316		301	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.6	24.5	24.8	24.6	24.7	24.5	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.90	7.71	7.93	7.71	8.02	7.84	8.05	7.44
	Dissolved oxygen (mg/L)	7.7	7.6	7.9	7.6	7.6	6.9	7.9	6.6
	Conductivity (µmhos/cm)	832		828		839		856	
	Temperature (°C)	24.6	24.4	24.8	24.3	24.7	24.4	24.8	24.8
450 mg KCl/L	pH (S.U.)	7.91	7.74	7.97	7.71	8.06	7.87	8.05	7.45
	Dissolved oxygen (mg/L)	7.7	7.6	7.9	7.5	7.6	7.0	7.9	6.6
	Conductivity (µmhos/cm)	1060		1060		1120		1110	
	Temperature (°C)	24.6	24.4	24.7	24.3	24.8	24.4	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.92	7.74	7.99	7.74	8.07	7.86	8.06	7.45
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.3	7.7	7.2	7.8	6.4
	Conductivity (µmhos/cm)	1330		1320		1370		1370	
	Temperature (°C)	24.7	24.7	24.7	24.2	24.8	24.2	24.7	24.8
750 mg KCl/L	pH (S.U.)	7.95	7.76	7.99	7.74	8.08	7.86	8.04	7.43
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.1	7.7	7.2	7.9	6.3
	Conductivity (µmhos/cm)	1570		1570		1560		1650	
	Temperature (°C)	24.7	24.7	24.7	24.5	24.7	24.3	24.8	24.4
900 mg KCl/L	pH (S.U.)	7.96	7.76	7.99	7.74	8.10	7.85	8.06	7.47
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.1	7.8	7.3	7.9	6.6
	Conductivity (µmhos/cm)	1820		1840		1910		1890	
	Temperature (°C)	24.7	24.3	24.7	24.6	24.8	24.6	24.9	24.8
1050 mg KCl/L	pH (S.U.)	7.97	7.77	8.00	7.74	8.10	7.85	8.06	7.48
	Dissolved oxygen (mg/L)	7.9	7.6	7.9	7.1	7.9	7.3	8.0	6.1
	Conductivity (µmhos/cm)	2100		2110		2110		2160	
	Temperature (°C)	24.6	24.7	24.8	24.6	24.8	24.3	24.9	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Reviewed by: Jim Sommer

ETS
Environmental Testing Solutions, Inc.

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



- ● **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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
17	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
18	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
19	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
20	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479

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.

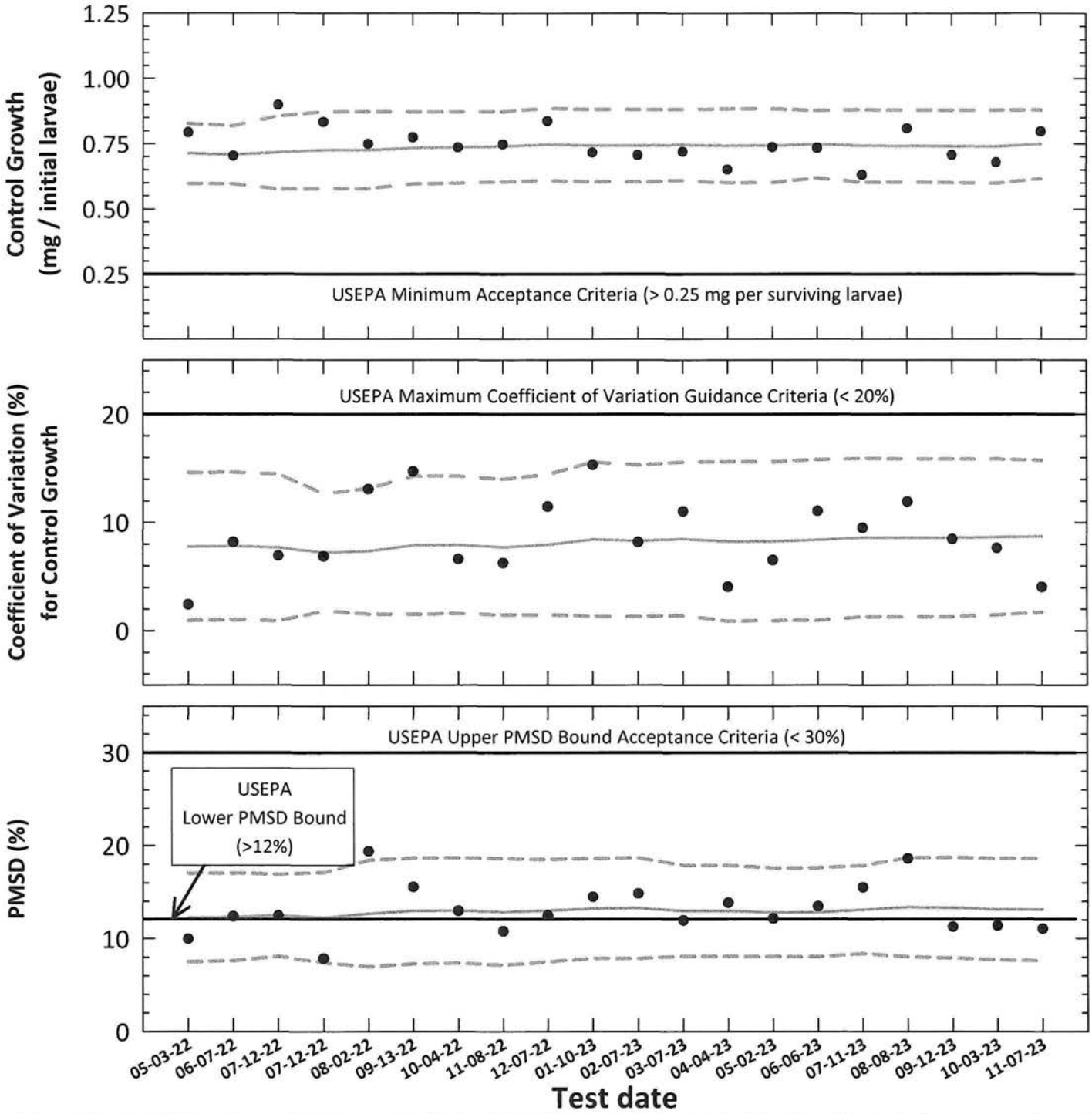
Entered and Reviewed by Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
17	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
18	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
19	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
20	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6

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: **108**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2227				
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:	10-31-23	*Artemia* CHM number:	CHM1222
Hatch dates and times:	11-06-23 1152 TO 11-07-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 7.98 S.U. Temperature = 24.0 °C	Date / Time in oven:	11-14-23 0705
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	11-15-23 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	11-07-23	0505	JK	1105	JK	0720	JK	10-31-23 B
1	11-08-23	0500	JK	1100	JK	0735	JK	↓
2	11-09-23	0500	JK	1230	JK	0736	JK	10-31-23 D
3	11-10-23	0500	JK	1200	JK	0735	JK	↓
4	11-11-23	0600	JK	1200	JK	0830 0730	JK	11-06-23 A
5	11-12-23	0600	JK	1200	JK	0800	JK	↓
6	11-13-23	0600	JK	1200	JK	0800	JK	
7	11-14-23					0620	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
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	130667685

Control information:		Acceptance criteria	***Summary of test endpoints:***		
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	>100%	751.2
Average weight per initial larvae: 0.797	0.789		**NOEC** (mg/L KCl)	100%	600
Average weight per surviving larvae: 0.797	0.789	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	>100%	750
			ChV (mg/L KCl)	>100%	670.8
			IC_{25} (mg/L KCl)	>100%	671.1

11-21-23 JK 11-22-23

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **108**

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 lg	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: Forest Green Analyst: AP Date: 10-20-23	12.92	12.21	15.06	13.53	13.39	15.49	14.11	14.67	15.73	15.49	14.61	15.65
*B = Pan + Larvae weight (mg) Analyst: AP pm Date: 11-16-23	20.60	20.02	23.02	AP 11-16-23 ~~22.00~~ 21.95	23.11	23.11	22.34	23.13	24.65	23.90	23.22	24.53
C = Larvae weight (mg) = B – A Analyst: JH	7.68	7.81	7.96	8.42	9.72	7.62	8.23	8.46	8.92	8.41	8.61	8.88
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.768	0.781	0.796	0.842	0.972	0.762	0.823	0.846	0.892	0.841	0.861	0.888
Average weight per initial number of larvae (mg)	0.797				0.851		-6.87.		0.871		-9.37.	
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:

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **108**

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	9 1d	9 1d	8 2d	9 1d	3 7d	3 7d	3 7d	3 7d
2	10	10	10	10	9	7 2d	7 1d	8 1d	2 1d	2 1d	2 1d	1 2d
3	10	10	10	10	9	7	7	7 1d	2	2	2	1
4	10	10	10	10	7 2d	7	6 1d	7	1 1d	2	1 1d	1
5	10	10	10	10	7	6 1d	6	7	1	2	1	1
6	10	10	10	10	7	5 1d	6	5 2d	1	2	1	1
7	10	10	9 1d	9 1d	6 1d	5	5 1d	5 2Lg	1 Lg	2	1	1
*A = Pan weight (mg) Tray color code: Forest Green Analyst: JP Date: 10-20-23	12.66	14.50	15.05	14.63	12.29	15.14	15.43	14.51	14.74	14.51	14.08	13.88
*B = Pan + Larvae weight (mg) Analyst: PM Date: 11-16-23	20.19	22.81	22.18	22.30	17.52	19.57	19.86	19.64	15.96	16.26	14.73	14.54
C = Larvae weight (mg) = B – A Analyst: JL	7.53	8.31	7.13	7.67	5.23	4.43	4.44	5.13	1.22	1.75	0.65	0.72
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.753	0.831	0.713	0.767	0.523	0.443	0.444	0.513	0.122	0.175	0.065	0.072
Average weight per initial number of larvae (mg)	0.766		3.97.		0.481		39.77.		0.109		86.47.	
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: **108**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 sd	1 sd	2 sd	2 sd
2	0 2d	1	1 2d	1 2d
3	0	0 3d	1	1
4	0	0	1	1
5	0	0	1	1
6	0	0	1	0 6d
7	0	0	0 7d	0
*A = Pan weight (mg) Tray color code: Forest Green Analyst: JP Date: 10-20-23	13.27	14.09	13.67	12.95
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B – A Analyst: _____				11-14-23 JP
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:

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: 108

Test dates: November 07-14, 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	12.92	20.60	7.68	0.768	0.797	4.0	0.768	100.0	0.797	4.0	Not applicable
	B	10	10	12.21	20.02	7.81	0.781			0.781				
	C	10	10	15.06	23.02	7.96	0.796			0.796				
	D	10	10	13.53	21.95	8.42	0.842			0.842				
300	E	10	10	13.39	23.11	9.72	0.972	0.851	10.4	0.972	100.0	0.851	10.4	-6.8
	F	10	10	15.49	23.11	7.62	0.762			0.762				
	G	10	10	14.11	22.34	8.23	0.823			0.823				
	H	10	10	14.67	23.13	8.46	0.846			0.846				
450	I	10	10	15.73	24.65	8.92	0.892	0.871	2.8	0.892	100.0	0.871	2.8	-9.3
	J	10	10	15.49	23.90	8.41	0.841			0.841				
	K	10	10	14.61	23.22	8.61	0.861			0.861				
	L	10	10	15.65	24.53	8.88	0.888			0.888				
600	M	10	10	12.66	20.19	7.53	0.753	0.807	5.4	0.753	95.0	0.766	6.4	3.9
	N	10	10	14.50	22.81	8.31	0.831			0.831				
	O	10	9	15.05	22.18	7.13	0.792			0.713				
	P	10	9	14.63	22.30	7.67	0.852			0.767				
750	Q	10	6	12.29	17.52	5.23	0.872	0.918	7.9	0.523	52.5	0.481	9.0	39.7
	R	10	5	15.14	19.57	4.43	0.886			0.443				
	S	10	5	15.42	19.86	4.44	0.888			0.444				
	T	10	5	14.51	19.64	5.13	1.026			0.513				
900	U	10	1	14.74	15.96	1.22	1.220	0.866	29.3	0.122	12.5	0.109	47.1	86.4
	V	10	2	14.51	16.26	1.75	0.875			0.175				
	W	10	1	14.08	14.73	0.65	0.650			0.065				
	X	10	1	13.82	14.54	0.72	0.720			0.072				
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: 0.0880 MSD = Minimum Significant Difference

PMSD: 11.0 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.

Reviewed and Approved by: [illegible]

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	11/7/2023	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	11/14/2023	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	0.9000	0.9000
750	0.6000	0.5000	0.5000	0.5000
900	0.1000	0.2000	0.1000	0.1000
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	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.5250	0.5250	0.8106	0.7854	0.8861	6.210	4	10.00	10.00	19	40
*900	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

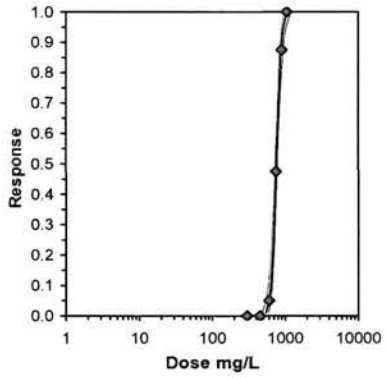
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84852	0.884	0.69577	0.7878
Equality of variance cannot be confirmed				

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

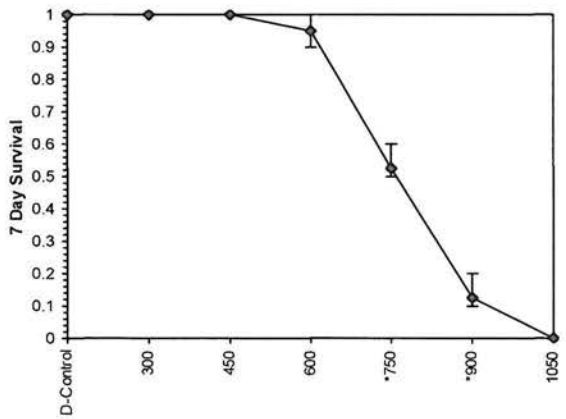
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.6378	2.13408	12.455	20.8206	0	0.53584	9.48773	0.96992	2.87923	0.0601	3
Intercept	-42.904	6.16546	-54.988	-30.82							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	548.788	485.184	592.669
EC05	3.355	603.067	548.858	640.776
EC10	3.718	634.164	585.684	668.532
EC15	3.964	656.046	611.592	688.298
EC20	4.158	673.974	632.722	704.731
EC25	4.326	689.745	651.164	719.436
EC40	4.747	731.14	698.369	759.692
EC50	5.000	757.23	726.725	786.796
EC60	5.253	784.251	754.601	816.627
EC75	5.674	831.319	799.545	872.847
EC80	5.842	850.771	817.009	897.429
EC85	6.036	874.021	837.245	927.625
EC90	6.282	904.179	862.696	967.878
EC95	6.645	950.802	900.775	1032
EC99	7.326	1044.84	974.653	1166.53



Dose-Response Plot



Entered and Reviewed by Jim Surmeier

ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	11/7/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/14/2023	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:					

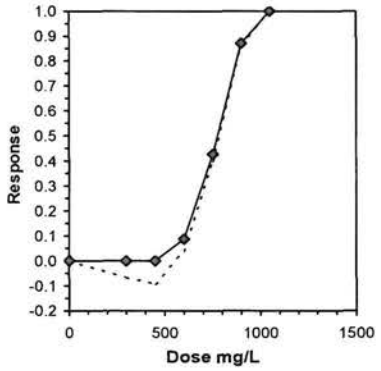
Conc-mg/L	1	2	3	4
D-Control	0.7680	0.7810	0.7960	0.8420
300	0.9720	0.7620	0.8230	0.8460
450	0.8920	0.8410	0.8610	0.8880
600	0.7530	0.8310	0.7130	0.7670
750	0.5230	0.4430	0.4440	0.5130
900	0.1220	0.1750	0.0650	0.0720
1050	0.0000	0.0000	0.0000	0.0000

			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	0.7968	1.0000	0.7968	0.7680	0.8420	4.049	4				0.8393	1.0000
300	0.8508	1.0678	0.8508	0.7620	0.9720	10.375	4	-1.406	2.290	0.0880	0.8393	1.0000
450	0.8705	1.0926	0.8705	0.8410	0.8920	2.758	4	-1.920	2.290	0.0880	0.8393	1.0000
600	0.7660	0.9614	0.7660	0.7130	0.8310	6.397	4	0.800	2.290	0.0880	0.7660	0.9126
750	0.4808	0.6034	0.4808	0.4430	0.5230	8.988	4				0.4808	0.5728
900	0.1085	0.1362	0.1085	0.0650	0.1750	47.083	4				0.1085	0.1293
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

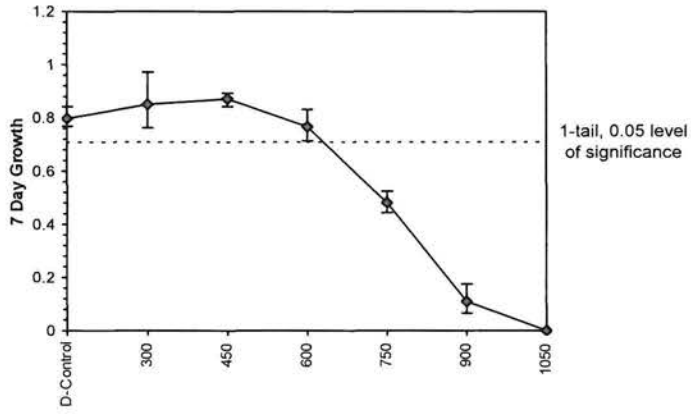
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.94156		0.844		0.82269	1.83827
Bartlett's Test indicates equal variances (p = 0.17)					5.03724		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	600	>600			0.08798	0.11043	0.00926	0.00295	0.06532	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	535.84	31.79	482.19	660.33	0.7260
IC10	605.57	19.72	529.03	647.07	-0.8764
IC15	627.64	11.97	591.36	666.34	-0.3396
IC20	649.71	10.74	616.28	683.05	-0.2356
IC25	671.78	10.11	639.70	699.63	-0.2360
IC40	737.98	10.33	709.91	769.28	-0.1045
IC50	774.61	7.32	753.18	795.66	-0.2225



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **108**

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	BL	BL	BL	BL
Concentration	Parameter			110823			
CONTROL, MHSW	pH (S.U.)	7.90	7.84	~~7.91~~ BL 7.76	7.75	7.81	7.81
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.6	7.9	7.5
	Conductivity (µmhos/cm)	293		315		303	
	Alkalinity (mg $CaCO_3$/L)	62				61	
	Hardness (mg $CaCO_3$/L)	88				84	
	Temperature (°C)	24.8	24.6	24.7	24.5	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.94	7.87	7.93	7.77	7.94	7.80
	Dissolved oxygen (mg/L)	7.9	7.7	7.8	7.5	7.8	7.5
	Conductivity (µmhos/cm)	810		859		841	
	Temperature (°C)	24.9	24.2	24.9	24.5	24.7	24.3
450 mg KCl/L	pH (S.U.)	7.94	7.87	7.93	7.77	7.94	7.81
	Dissolved oxygen (mg/L)	7.9	7.7	7.8	7.5	7.8	7.5
	Conductivity (µmhos/cm)	1030		1120		1090	
	Temperature (°C)	24.8	24.5	24.7	24.2	24.7	24.6
600 mg KCl/L	pH (S.U.)	7.94	7.86	7.93	7.77	7.95	7.80
	Dissolved oxygen (mg/L)	7.9	7.7	7.8	7.5	7.8	7.4
	Conductivity (µmhos/cm)	1290		1380		1350	
	Temperature (°C)	24.8	24.5	24.7	24.3	24.8	24.5
750 mg KCl/L	pH (S.U.)	7.94	7.86	7.93	7.77	7.95	7.81
	Dissolved oxygen (mg/L)	7.9	7.7	7.8	7.5	7.8	7.4
	Conductivity (µmhos/cm)	1540		1640		1580	
	Temperature (°C)	24.8	24.4	24.8	24.5	24.8	24.5
900 mg KCl/L	pH (S.U.)	7.94	7.86	7.93	7.77	7.96	7.84
	Dissolved oxygen (mg/L)	8.0	7.8	7.8	7.6	7.8	7.6
	Conductivity (µmhos/cm)	1810		1900		1850	
	Temperature (°C)	25.0	24.4	24.7	24.2	24.8	24.7
1050 mg KCl/L	pH (S.U.)	7.94	7.86	7.94	7.79	7.96	7.82
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	7.5	7.8	7.6
	Conductivity (µmhos/cm)	2170		2150		2120	
	Temperature (°C)	25.0	24.3	24.7	24.3	24.7	24.4
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **108**

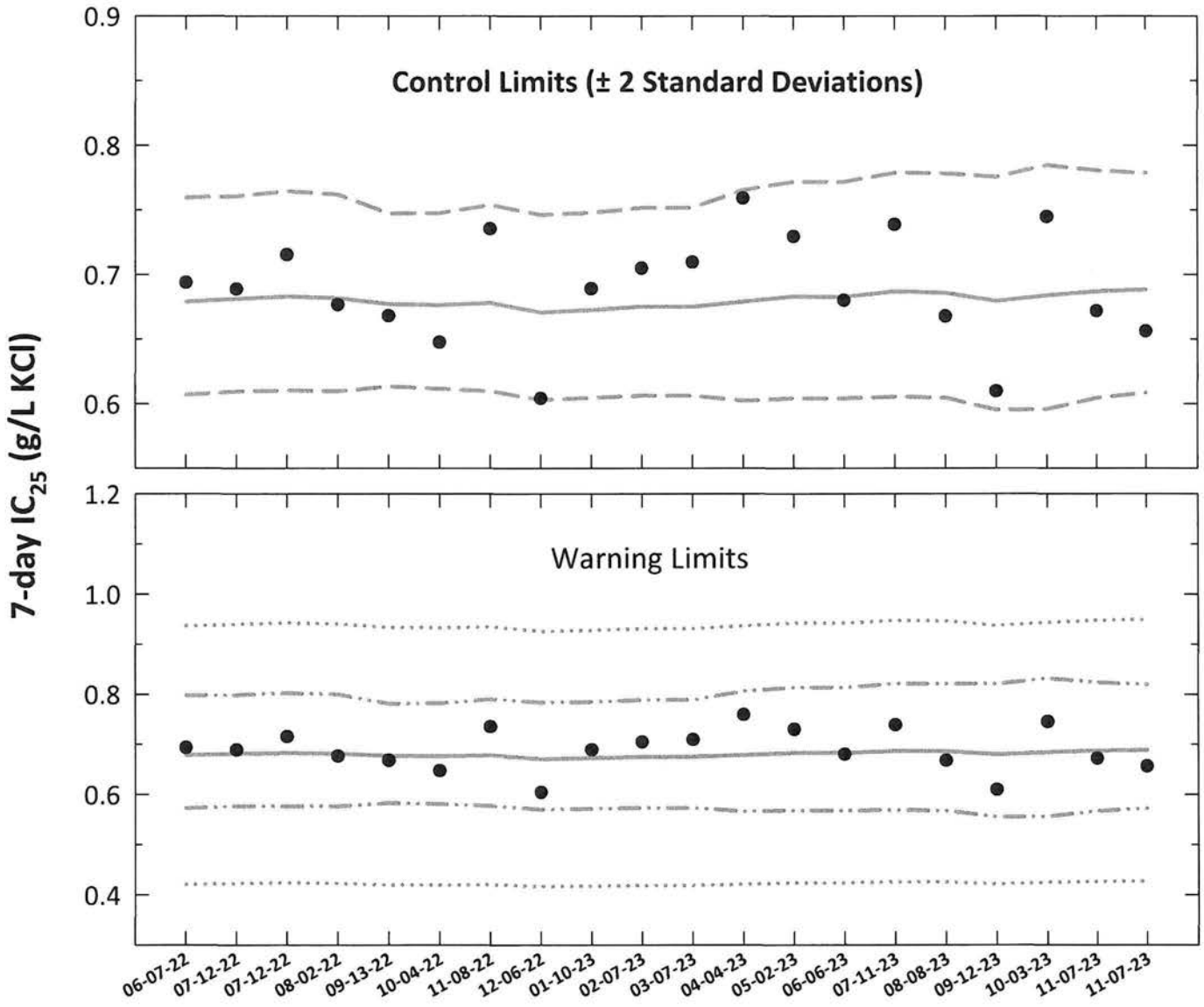
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL	BSL	BSL	BSL	BSL	BL	BL	
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.80	7.74	7.67	7.61	7.61	7.74	7.95	7.32
	Dissolved oxygen (mg/L)	7.9	8.0	7.9	7.2	7.8	7.4	7.7	6.1
	Conductivity (µmhos/cm)	316		290		293		311	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.9	24.7	24.7	24.4	24.6	24.5	24.7	24.5
300 mg KCl/L	pH (S.U.)	~~7.94~~ 8.02*	7.81	7.98	7.63	7.98	7.71	~~8.02~~ 8.04*	7.38
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.0	7.9	7.2	7.7	6.0
	Conductivity (µmhos/cm)	882		834		823		850	
	Temperature (°C)	24.8	24.3	24.7	24.5	24.8	24.6	24.7	24.7
450 mg KCl/L	pH (S.U.)	~~7.98~~ 8.03*	7.81	8.00	7.63	8.00	7.72	~~8.03~~ 8.05*	7.37
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	6.9	7.9	7.2	7.8	5.9
	Conductivity (µmhos/cm)	1140		1080		1060		1100	
	Temperature (°C)	24.8	24.3	24.7	24.5	24.8	24.5	24.7	24.7
600 mg KCl/L	pH (S.U.)	~~7.99~~ 8.03*	7.81	8.00	7.63	8.00	7.72	~~8.04~~ 8.05*	7.38
	Dissolved oxygen (mg/L)	7.0	7.9	8.0	7.1	8.0	7.3	7.8	5.4
	Conductivity (µmhos/cm)	1410		1320		1320		1370	
	Temperature (°C)	24.9	24.6	24.8	24.3	24.8	24.8	24.8	24.7
750 mg KCl/L	pH (S.U.)	~~7.99~~ 8.02*	7.80	8.00	7.62	7.98	7.80	~~8.05~~ 8.07*	7.38
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.2	8.0	7.3	7.8	5.3
	Conductivity (µmhos/cm)	1680		1560		1560		1620	
	Temperature (°C)	24.9	24.6	24.7	24.6	24.7	24.6	24.8	24.6
900 mg KCl/L	pH (S.U.)	8.02	7.80	8.00	7.67	7.97	7.74	8.07	7.38
	Dissolved oxygen (mg/L)	7.9	7.9	~~8.0~~ 8.0	7.2	8.0	7.4	7.8	5.3
	Conductivity (µmhos/cm)	1950		1820		1800		1900	
	Temperature (°C)	24.9	24.4	24.8	24.6	24.7	24.6	24.8	24.6
1050 mg KCl/L	pH (S.U.)	8.03	7.83	8.00	7.67	7.97	7.76	8.06	7.40
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.2	8.0	7.4	7.8	5.5
	Conductivity (µmhos/cm)	2240		2050		2030		2140	
	Temperature (°C)	24.9	24.4	24.8	24.6	24.7	24.6	24.8	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BL 11.10.23

* DL 11-13-23

ETS
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} ± $S_{A.75}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
16	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
17	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
18	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
19	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
20	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499

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.

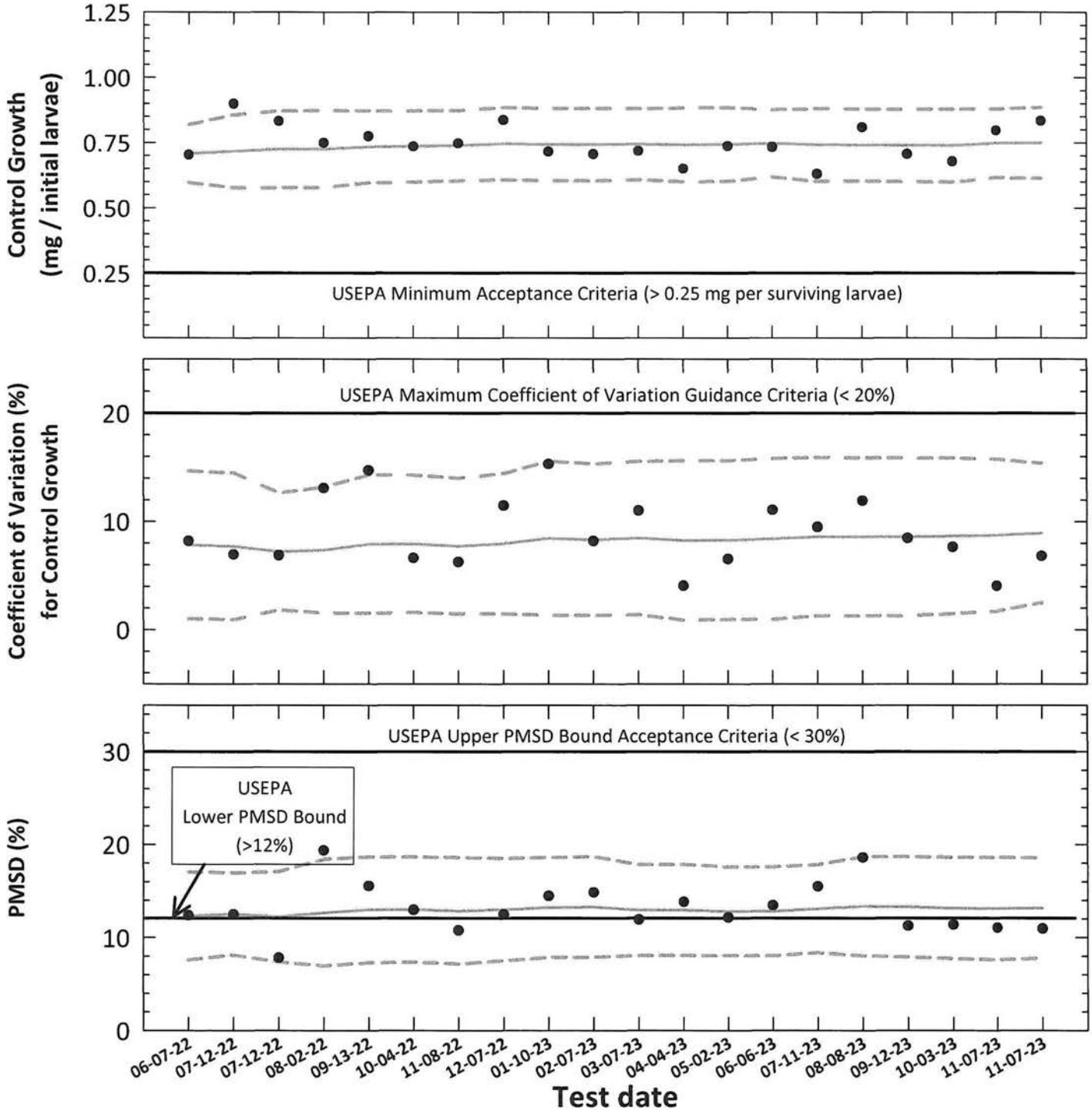
Entered and Reviewed by Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
16	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
17	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
18	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
19	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
20	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6

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: **109 - New Artemia**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2227				
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:	BLUE
Age:	< 24-hours old	Incubator number and shelf location:	7C
Spawn date:	10-31-23	*Artemia* CHM number:	CHM1294
Hatch dates and times:	11-06-23 1152 TO 11-07-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 7.98 S.U. Temperature = 24.0 °C	Date / Time in oven:	11-14-23 0705
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	11-15-23 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	11-07-23	0505	JH	1105	JH	0720	JH	10-31-23 B
1	11-08-23	0500	JH	1100	JH	0745	JH	↓
2	11-09-23	0500	JH	1230	JH	0746	JH	10-31-23 D
3	11-10-23	0500	JH	1200	JH	0745	JH	↓
4	11-11-23	0600	JH	1200	JH	0840	JH	11-01-23 A
5	11-12-23	0600	JH	1200	JH	0810	JH	↓
6	11-13-23	0600	JH	1200	JH	0810	JH	↓
7	11-14-23					0634	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:	0.833	
Average weight per surviving larvae:	0.833	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	766.1
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	656.2

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **109 - New Artemia**

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 16G	10	10	10	10
*A = Pan weight (mg) Tray color code: Light Pink Analyst: JO Date: 10-23-23	14.32	14.60	14.40	14.04	15.18	14.82	15.67	13.67	11.96	13.26	14.46	15.08
*B = Pan + Larvae weight (mg) Analyst: PM Date: 11-15-23	21.80	23.20	23.07	22.62	23.70	23.79	24.05	23.46	20.13	21.87	22.09	22.39
C = Larvae weight (mg) = B – A Analyst: JH	7.48	8.60	8.67	8.58	8.52	8.97	8.38	9.79	8.17	8.61	7.57	7.31
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.748	0.860	0.867	0.858	0.852	0.897	0.838	0.979	0.817	0.861	0.757	0.731
Average weight per initial number of larvae (mg)	0.833				0.892				0.792			
Percent reduction from control (%)							−7.07.				5.07.	

*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:

FTS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **109 - New Artemia**

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	9 1d	5 5d	5 5d	5 5d	7 3d
2	10	10	10	10	8	7 1d	7 1d	9	3 2d	4 1d	4 1d	4 3d
3	10	10	10	10	7 1d	7	6 1d	8 1d	3	3 1d	2 2d	3 1d
4	10	10	10	10	7	7	6	8	3	3	2	3
5	10	10	10	10	6 1d	7	6	7 1d	3	2 2d	2	1 2d
6	10	10	10	10	6	6 1d	5 1d	6 1d	2 2d 2 3rd	2	2	1
7	10	10	10	10	4 2d 1 Lg	5 1d 1 Lg	5	6	2	1 1d Lg	1 1d Lg	1 Lg
*A = Pan weight (mg) Tray color code: light pink Analyst: JD Date: 10-23-23	13.89	14.19	14.09	14.64	14.44	13.86	13.60	14.63	13.01	12.57	14.78	15.48
*B = Pan + Larvae weight (mg) Analyst: PM Date: 11-18-23	21.28	21.77	21.51	22.98	18.07	18.54	17.87	19.79	14.40	13.52	15.75	16.41
C = Larvae weight (mg) = B – A Analyst:	7.39	7.59	7.42	8.34	3.63	4.68	4.27	5.16	1.39	0.95	0.97	0.93
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst:	0.739	0.759	0.742	0.834	0.363	0.468	0.427	0.516	0.139	0.095	0.097	0.093
Average weight per initial number of larvae (mg)	0.769		7.8%		0.444		46.8%		0.106		87.3%	
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: **109 - New Artemia**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 sk	2 sol	2 sol	2 sol
2	0 2d	0 2d	2	1 d
3	0	0	1 d	1
4	0	0	1	1
5	0	0	1	1
6	0	0	0 d	0 d
7	0	0	0	0
*A = Pan weight (mg) Tray color code: Light pink Analyst: JP Date: 10-23-23	14.66	12.21	JP 10-23-23 ~~14.~~ 15.05	14.65
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B – A Analyst: _____				
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:

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: 109 - New Artemia

Test dates: November 07-14, 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	14.32	21.80	7.48	0.748	0.833	6.8	0.748	100.0	0.833	6.8	Not applicable
	B	10	10	14.60	23.20	8.60	0.860			0.860				
	C	10	10	14.40	23.07	8.67	0.867			0.867				
	D	10	10	14.04	22.62	8.58	0.858			0.858				
300	E	10	10	15.18	23.70	8.52	0.852	0.892	7.1	0.852	100.0	0.892	7.1	-7.0
	F	10	10	14.82	23.79	8.97	0.897			0.897				
	G	10	10	15.67	24.05	8.38	0.838			0.838				
	H	10	10	13.67	23.46	9.79	0.979			0.979				
450	I	10	10	11.96	20.13	8.17	0.817	0.792	7.4	0.817	100.0	0.792	7.4	5.0
	J	10	10	13.26	21.87	8.61	0.861			0.861				
	K	10	10	14.46	22.03	7.57	0.757			0.757				
	L	10	10	15.08	22.39	7.31	0.731			0.731				
600	M	10	10	13.89	21.28	7.39	0.739	0.769	5.8	0.739	100.0	0.769	5.8	7.8
	N	10	10	14.18	21.77	7.59	0.759			0.759				
	O	10	10	14.09	21.51	7.42	0.742			0.742				
	P	10	10	14.64	22.98	8.34	0.834			0.834				
750	Q	10	4	14.44	18.07	3.63	0.908	0.889	4.4	0.363	50.0	0.444	14.6	46.8
	R	10	5	13.86	18.54	4.68	0.936			0.468				
	S	10	5	13.60	17.87	4.27	0.854			0.427				
	T	10	6	14.63	19.79	5.16	0.860			0.516				
900	U	10	2	13.01	14.40	1.39	0.695	0.886	14.5	0.139	12.5	0.106	20.8	87.3
	V	10	1	12.57	13.52	0.95	0.950			0.095				
	W	10	1	14.78	15.75	0.97	0.970			0.097				
	X	10	1	15.48	16.41	0.93	0.930			0.093				
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: 0.0913 MSD = Minimum Significant Difference

PMSD: 11.0 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.

Approved and Reviewed by Jim Summers

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	11/7/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/14/2023	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	0.4000	0.5000	0.5000	0.6000
900	0.2000	0.1000	0.1000	0.1000
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	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.5000	0.5000	0.7854	0.6847	0.8861	10.467	4	10.00	10.00	20	40
*900	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

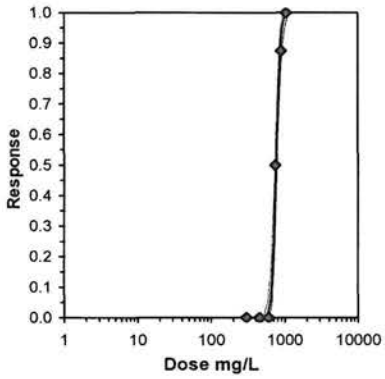
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.65229	0.884	0.84253	4.64571
Equality of variance cannot be confirmed				

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

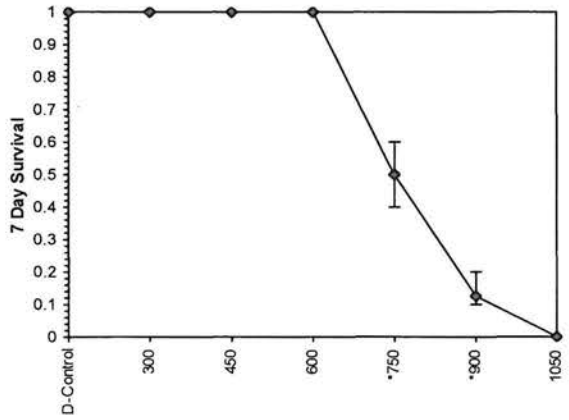
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	19.5475	2.69255	14.2701	24.8249	0	2.54659	9.48773	0.63631	2.88428	0.05116	5
Intercept	-51.38	7.78823	-66.645	-36.116							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	582.466	519.025	624.355
EC05	3.355	631.152	577.925	666.742
EC10	3.718	658.749	611.558	691.011
EC15	3.964	678.047	635.039	708.228
EC20	4.158	693.786	654.078	722.509
EC25	4.326	707.58	670.612	735.268
EC40	4.747	743.567	712.565	770.139
EC50	5.000	766.092	737.486	793.596
EC60	5.253	789.299	761.757	819.399
EC75	5.674	829.442	800.389	867.936
EC80	5.842	845.933	815.24	889.094
EC85	6.036	865.57	832.346	915.016
EC90	6.282	890.926	853.718	949.443
EC95	6.645	929.881	885.436	1003.97
EC99	7.326	1007.61	946.227	1117.1



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	11/7/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/14/2023	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	0.7480	0.8600	0.8670	0.8580
300	0.8520	0.8970	0.8380	0.9790
450	0.8170	0.8610	0.7570	0.7310
600	0.7390	0.7590	0.7420	0.8340
750	0.3630	0.4680	0.4270	0.5160
900	0.1390	0.0950	0.0970	0.0930
1050	0.0000	0.0000	0.0000	0.0000

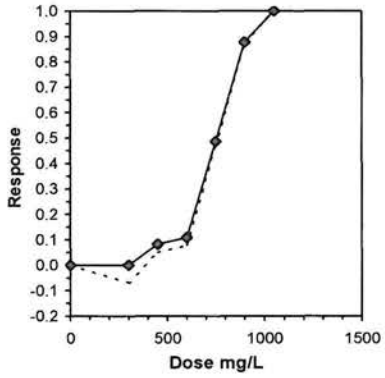
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8333	1.0000	0.8333	0.7480	0.8670	6.836	4				0.8624	1.0000
300	0.8915	1.0699	0.8915	0.8380	0.9790	7.126	4	-1.461	2.290	0.0913	0.8624	1.0000
450	0.7915	0.9499	0.7915	0.7310	0.8610	7.414	4	1.047	2.290	0.0913	0.7915	0.9178
600	0.7685	0.9223	0.7685	0.7390	0.8340	5.796	4	1.625	2.290	0.0913	0.7685	0.8911
750	0.4435	0.5323	0.4435	0.3630	0.5160	14.618	4				0.4435	0.5143
900	0.1060	0.1272	0.1060	0.0930	0.1390	20.812	4				0.1060	0.1229
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)	0.96725	0.844	0.13922	-0.8948
Bartlett's Test indicates equal variances (p = 0.95)	0.34217	11.3449		

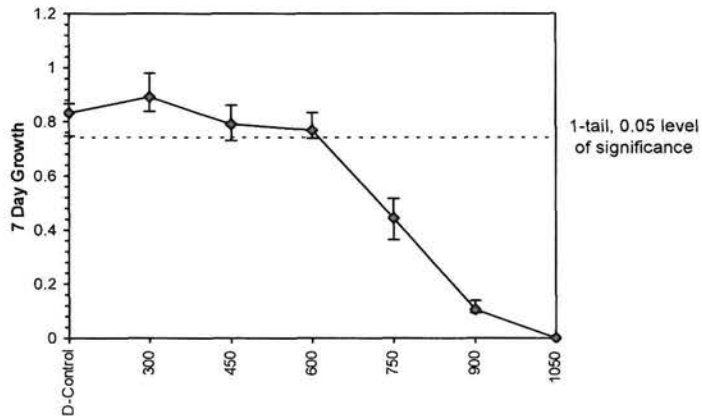
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.09127	0.10954	0.01166	0.00318	0.04379	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	391.26	54.58	329.28	633.38	1.5939
IC10	550.19	74.81	317.96	656.53	-0.2593
IC15	616.38	27.35	512.73	646.46	-4.6835
IC20	636.28	9.58	603.24	666.51	-0.0722
IC25	656.18	9.50	622.77	686.52	-0.0330
IC40	715.88	11.79	683.13	752.80	0.1960
IC50	755.47	12.23	720.08	788.33	-0.1288



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **109 – New Artemia**

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	VL	BL	BL	BL	BL	BL
Concentration	Parameter			110823			
CONTROL, MHSW	pH (S.U.)	7.90	7.81	~~7.92~~ 7.76	7.71	7.81	7.77
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.5	7.9	7.5
	Conductivity (µmhos/cm)	293		315		303	
	Alkalinity (mg $CaCO_3$/L)	62				61	
	Hardness (mg $CaCO_3$/L)	88				84	
	Temperature (°C)	24.8	24.3	24.7	24.4	24.7	24.5
300 mg KCl/L	pH (S.U.)	7.94	7.83	7.93	7.73	7.94	7.78
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.4	7.8	7.5
	Conductivity (µmhos/cm)	810		859		841	
	Temperature (°C)	24.8	24.4	24.7	24.2	24.8	24.5
450 mg KCl/L	pH (S.U.)	7.94	7.85	7.93	7.72	7.94	7.76
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.5	7.8	7.6
	Conductivity (µmhos/cm)	1030		1120		1090	
	Temperature (°C)	24.9	24.4	24.6	24.4	24.9	24.6
600 mg KCl/L	pH (S.U.)	7.94	7.84	7.93	7.72	7.95	7.79
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.5	7.8	7.6
	Conductivity (µmhos/cm)	1290		1380		1350	
	Temperature (°C)	24.9	24.6	24.6	24.5	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.94	7.84	7.93	7.72	7.95	7.78
	Dissolved oxygen (mg/L)	7.9	7.9	7.8	7.5	7.8	7.6
	Conductivity (µmhos/cm)	1540		1640		1550	
	Temperature (°C)	24.8	24.3	24.7	24.5	24.8	24.3
900 mg KCl/L	pH (S.U.)	7.94	7.84	7.93	7.72	7.96	7.78
	Dissolved oxygen (mg/L)	8.0	7.8	7.8	7.5	7.8	7.6
	Conductivity (µmhos/cm)	1810		1900		1850	
	Temperature (°C)	25.0	24.3	24.7	24.6	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.94	7.84	7.94	7.73	7.96	7.82
	Dissolved oxygen (mg/L)	8.0	AB	7.9	7.5	7.8	7.6
	Conductivity (µmhos/cm)	2170		2150		2120	
	Temperature (°C)	25.0	24.3	24.8	24.6	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final

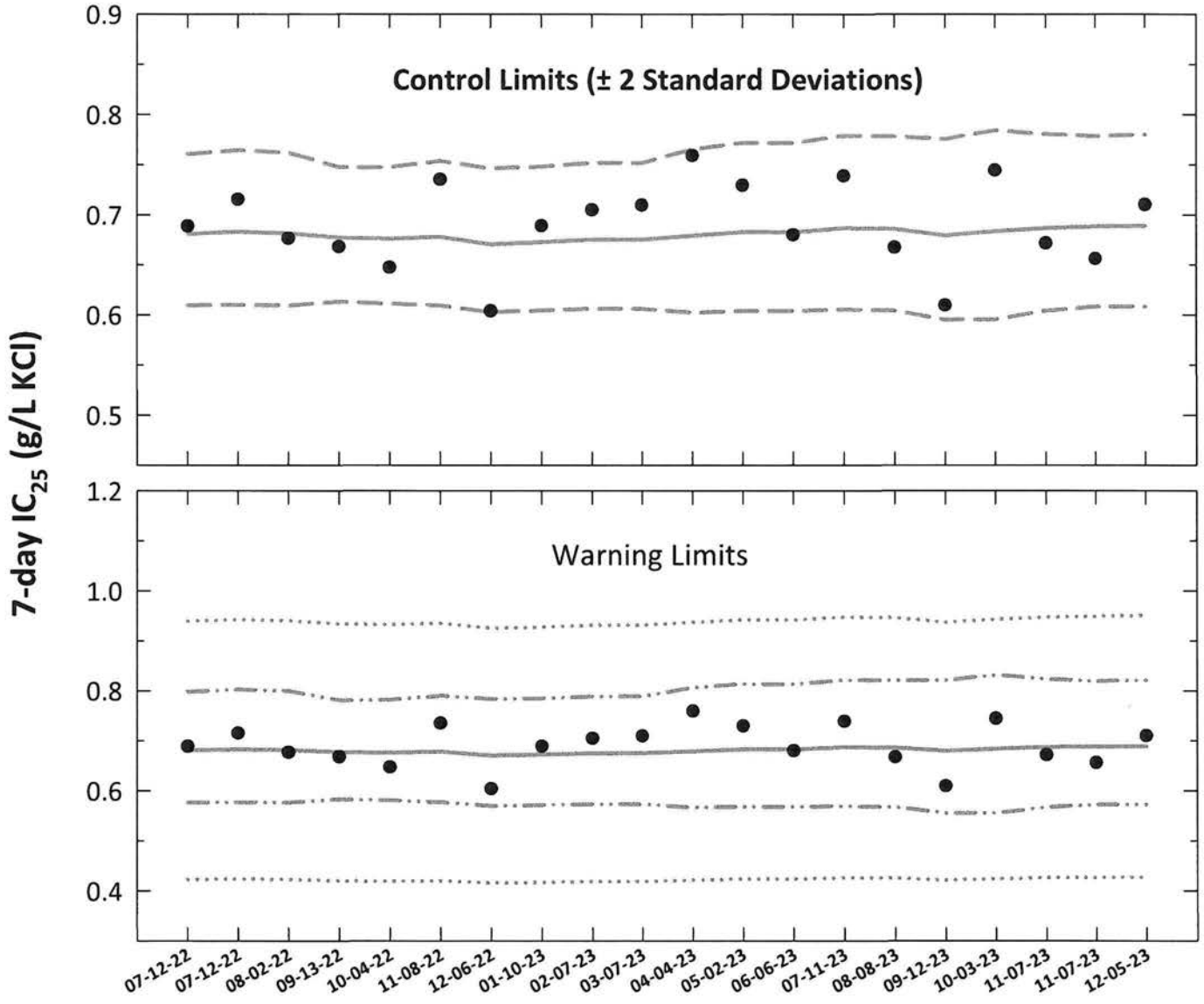
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*

PpKClCR Test Number: **109 - New Artemia**

		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	JL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.80	7.77	7.67	7.62	7.61	7.68	7.95	7.33
	Dissolved oxygen (mg/L)	7.9	8.0	7.7	7.3	7.8	7.3	7.7	5.5
	Conductivity (µmhos/cm)	316		290		293		311	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.7	24.5	24.8	24.6	24.6	24.5	24.7	24.5
300 mg KCl/L	pH (S.U.)	8.02	7.78	7.98	7.62	7.98	7.67	8.04	7.30
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.4	7.9	7.2	7.7	5.4
	Conductivity (µmhos/cm)	882		834		823		850	
	Temperature (°C)	24.8	24.5	24.8	24.7	24.7	24.5	24.8	24.7
450 mg KCl/L	pH (S.U.)	8.03	7.78	8.00	7.61	8.00	7.67	8.05	7.31
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.4	7.9	7.3	7.8	5.3
	Conductivity (µmhos/cm)	1140		1080		1060		1100	
	Temperature (°C)	24.9	24.5	24.7	24.7	24.7	24.7	24.8	24.3
600 mg KCl/L	pH (S.U.)	8.03	7.74	8.00	7.58	8.00	7.70	8.05	7.33
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.3	8.0	7.3	7.8	5.2
	Conductivity (µmhos/cm)	1410		1320		1320		1370	
	Temperature (°C)	24.9	24.4	24.7	24.3	24.7	24.4	24.8	24.3
750 mg KCl/L	pH (S.U.)	8.02	7.75	8.00	7.59	7.98	7.69	8.07	7.34
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.1	8.0	7.3	7.8	5.2
	Conductivity (µmhos/cm)	1680		1560		1560		1620	
	Temperature (°C)	24.9	24.4	24.7	24.7	24.7	24.4	24.9	24.5
900 mg KCl/L	pH (S.U.)	8.02	7.79	8.00	7.56	7.97	7.67	8.07	7.37
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.0	8.0	7.3	7.8	5.3
	Conductivity (µmhos/cm)	1950		1820		1800		1900	
	Temperature (°C)	25.0	24.6	24.7	24.8	24.8	24.4	24.7	24.5
1050 mg KCl/L	pH (S.U.)	8.03	7.80	8.00	7.69	7.97	7.73	8.06	
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	6.8	8.0	7.4	7.8	
	Conductivity (µmhos/cm)	2240		2050		2030		2140	
	Temperature (°C)	25.0	24.6	24.8	24.5	24.8	24.5	24.7	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**07-11-23**	**0.7387**	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
15	**08-08-23**	**0.6677**	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
16	**09-12-23**	**0.6098**	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
17	**10-03-23**	**0.7447**	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
18	**11-07-23**	**0.6718**	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
19	**11-07-23**	**0.6562**	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
20	**12-05-23**	**0.7102**	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510

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.

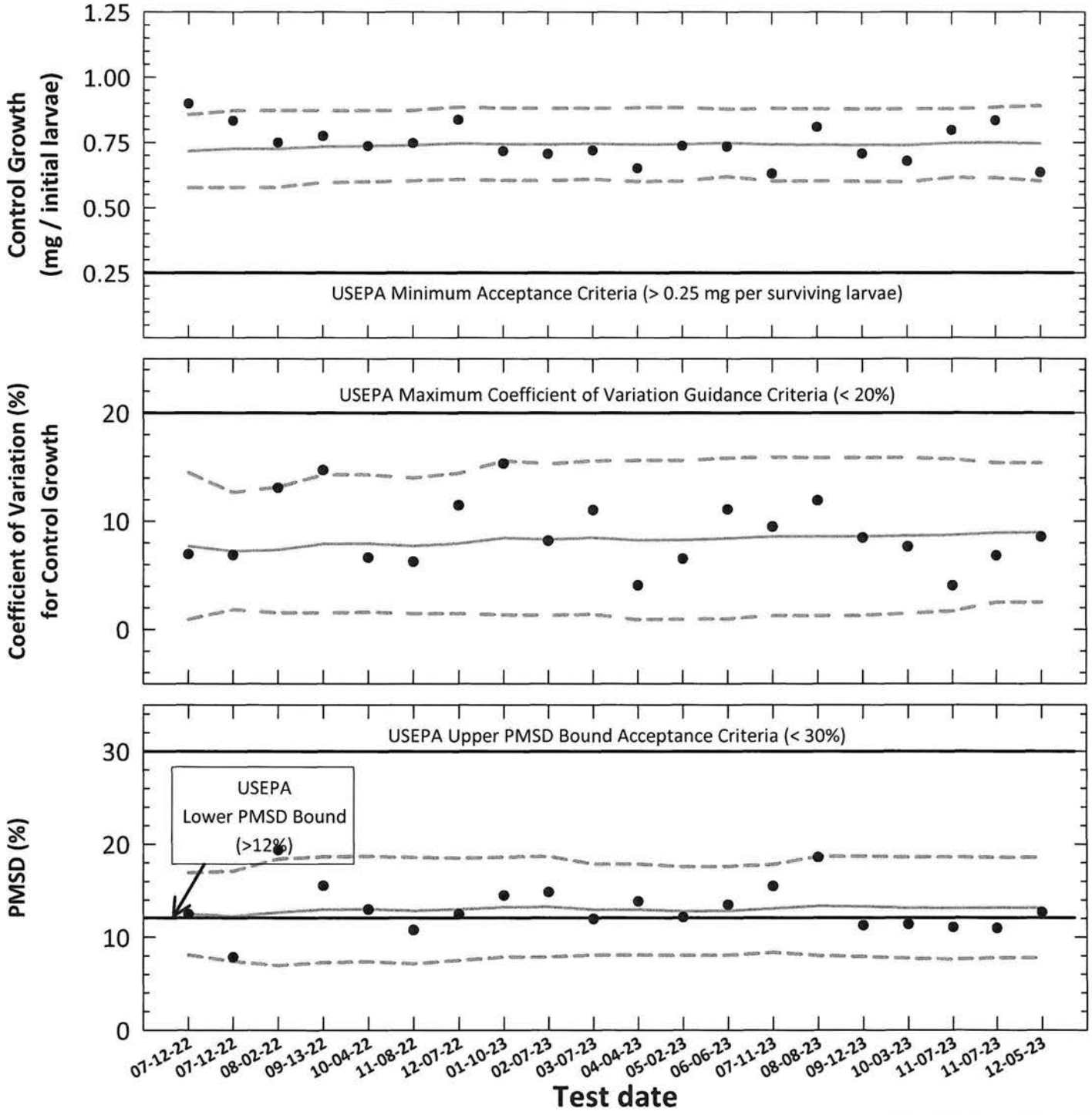
Entered and Reviewed by Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
15	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
16	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
17	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
18	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
19	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
20	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6

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: **110**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2249				
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:	11-28-23	*Artemia* CHM number:	CHM1294
Hatch dates and times:	12-04-23 1355 TO 12-05-23 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 7.98 S.U. Temperature = 24.0 °C	Date / Time in oven:	12-12-23 0635
		*Initial oven temperature:	60° C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	12-13-23 0635
		*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	12-05-23	0505	JH	1415	JH	0717	JH	11-29-23 B
1	12-06-23	0500	JH	1100	JH	0700	JH	↓
2	12-07-23	0500	JH	1315	JH	0710	JH	12-07-23 B
3	12-08-23	0600	JH	1205	JH	0800	JH	↓
4	12-09-23	0600	JH	1235	JH	0800	JH	12-07-23 C
5	12-10-23	0550	JH	1150	JH	0750	JH	↓
6	12-11-23	0500	JH	1115	JH	0700	JH	↓
7	12-12-23					0632	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:	07	≤ 20%
Average weight per initial larvae:	0.635	
Average weight per surviving larvae:	0.635	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	815.1
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	710.2

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **110**

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: Turquoise Analyst: JHC Date: 11-21-23	12.15	12.87	14.37	13.00	13.68	15.03	13.05	14.36	15.16	14.60	14.83	15.05
*B = Pan + Larvae weight (mg) Analyst: BL Date: 12.14.23	19.00	19.41	19.95	19.43	21.14	22.97	20.77	22.48	21.97	21.32	21.67	22.57
C = Larvae weight (mg) = B – A Analyst: JH	6.85	6.54	5.58	6.43	7.46	7.94	7.72	8.12	6.82	6.72	6.64	7.52
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.685	0.654	0.558	0.643	0.746	0.794	0.772	0.812	0.682	0.672	0.664	0.752
Average weight per initial number of larvae (mg)	0.635				0.781		-23.07.		0.697		-9.87.	
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:

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **110**

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	9 fg	10	10	10	6 sm	5 sm	5 sm	5 sm
2	10	10	10	10	8 fg	10	10	10	6	5	5	5
3	10	10	10	10	7 fg	10	10	10	6	5	5	5
4	10	10	10	10	7	10	10	10	5 fg	4 fg	4 fg	5
5	10	9 fg	10	10	7	9 fg	8 fg	8 fg	5	3 fg	3 fg	4 fg
6	10	9	10	10	7	9	8	8	5	3	3	4
7	10	9	9 fg	9 fg	7	8 fg	8	8	lg 3 fg	3	2 fg	lg 4
*A = Pan weight (mg) Tray color code: Turquoise Analyst: JHC Date: 11-21-23	14.57	~~14.08~~ 14.09 (11-21-23 JHC)	12.93	15.00	14.37	14.08	15.59	16.05	15.04	13.05	13.98	12.66
*B = Pan + Larvae weight (mg) Analyst: BL Date: 12-14-23	21.00	19.58	19.76	20.45	18.51	19.67	20.52	21.56	17.48	15.38	15.40	16.07
C = Larvae weight (mg) = B – A Analyst: JL	6.43	5.49	6.83	5.45	4.14	5.59	4.93	5.51	2.44	2.33	1.42	3.41
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.643	0.549	0.683	0.545	0.414	0.559	0.493	0.551	0.244	0.233	0.142	0.341
Average weight per initial number of larvae (mg)	0.605				0.504				0.240			
Percent reduction from control (%)		4.77.				20.67.				62.27.		

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

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

Comments:

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **110**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 sd	2 sd	2 sd	2 sd
2	2	2	2	2
3	2	2	2	1 id
4	2	1 id	1 id	0 id
5	2	1	0 id	0
6	2	1	0	0
7	1 id	0 id	0	0
*A = Pan weight (mg) Tray color code: Turquoise Analyst: JHC Date: 11-21-23	12.95	14.91	14.00	14.34
*B = Pan + Larvae weight (mg) Analyst: BL Date: 12-14-23	13.68			11-12-24
C = Larvae weight (mg) = B – A Analyst: JJ	0.73			12-2-24
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JJ	0.073	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.018		97.1 %	

*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: 110

Test dates: December 05-12, 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	12.15	19.00	6.85	0.685	0.635	8.6	0.685	100.0	0.635	8.6	Not applicable
	B	10	10	12.87	19.41	6.54	0.654			0.654				
	C	10	10	14.37	19.95	5.58	0.558			0.558				
	D	10	10	13.00	19.43	6.43	0.643			0.643				
300	E	10	10	13.68	21.14	7.46	0.746	0.781	3.6	0.746	100.0	0.781	3.6	-23.0
	F	10	10	15.03	22.97	7.94	0.794			0.794				
	G	10	10	13.05	20.77	7.72	0.772			0.772				
	H	10	10	14.36	22.48	8.12	0.812			0.812				
450	I	10	10	15.16	21.97	6.81	0.681	0.697	5.3	0.681	100.0	0.697	5.3	-9.8
	J	10	10	14.60	21.32	6.72	0.672			0.672				
	K	10	10	14.83	21.67	6.84	0.684			0.684				
	L	10	10	15.05	22.57	7.52	0.752			0.752				
600	M	10	10	14.57	21.00	6.43	0.643	0.654	11.0	0.643	92.5	0.605	11.4	4.7
	N	10	9	14.09	19.58	5.49	0.610			0.549				
	O	10	9	12.93	19.76	6.83	0.759			0.683				
	P	10	9	15.00	20.45	5.45	0.606			0.545				
750	Q	10	7	14.37	18.51	4.14	0.591	0.649	8.2	0.414	77.5	0.504	13.3	20.6
	R	10	8	14.08	19.67	5.59	0.699			0.559				
	S	10	8	15.59	20.52	4.93	0.616			0.493				
	T	10	8	16.05	21.56	5.51	0.689			0.551				
900	U	10	3	15.04	17.48	2.44	0.813	0.788	7.7	0.244	30.0	0.240	33.9	62.2
	V	10	3	13.05	15.38	2.33	0.777			0.233				
	W	10	2	13.98	15.40	1.42	0.710			0.142				
	X	10	4	12.66	16.07	3.41	0.853			0.341				
1050	Y	10	1	12.95	13.68	0.73	0.730	0.730	0.0	0.073	2.5	0.018	200.0	97.1
	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: 0.0805 MSD = Minimum Significant Difference

PMSD: 12.7 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 Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	12/5/2023	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	12/12/2023	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	0.9000	0.9000	0.9000
750	0.7000	0.8000	0.8000	0.8000
900	0.3000	0.3000	0.2000	0.4000
1050	0.1000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
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	0.9250	0.9250	1.2898	1.2490	1.4120	6.318	4	12.00	10.00	3	40
*750	0.7750	0.7750	1.0782	0.9912	1.1071	5.379	4	10.00	10.00	9	40
*900	0.3000	0.3000	0.5769	0.4636	0.6847	15.654	4	10.00	10.00	28	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

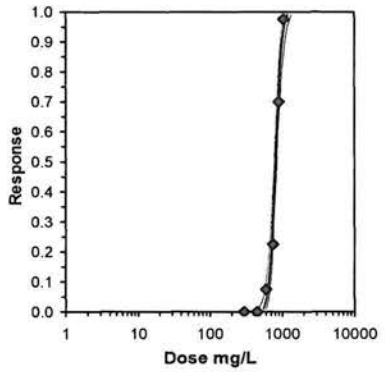
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.85318	0.896	0.67869	1.64457
Equality of variance cannot be confirmed				

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

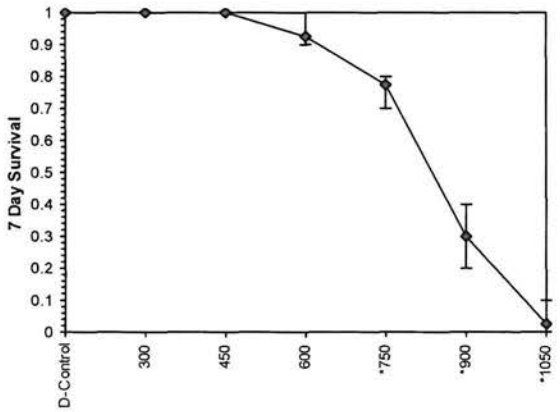
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.7681	1.74935	10.3393	17.1968	0	4.63087	9.48773	0.32731	2.9112	0.07263	3
Intercept	-35.082	5.10382	-45.085	-25.078							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	552.369	479.546	603.341
EC05	3.355	619.053	556.535	662.892
EC10	3.718	657.832	601.951	697.649
EC15	3.964	685.36	634.264	722.586
EC20	4.158	708.057	660.819	743.443
EC25	4.326	728.127	684.125	762.215
EC40	4.747	781.26	744.17	814.259
EC50	5.000	815.073	780.349	849.927
EC60	5.253	850.35	815.919	889.731
EC75	5.674	912.401	873.494	965.756
EC80	5.842	938.264	896.056	999.253
EC85	6.036	969.337	922.38	1040.57
EC90	6.282	1009.9	955.775	1095.94
EC95	6.645	1073.16	1006.32	1184.87
EC99	7.326	1202.72	1106.09	1374.54



Dose-Response Plot



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ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	12/5/2023	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	12/12/2023	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	0.6850	0.6540	0.5580	0.6430
300	0.7460	0.7940	0.7720	0.8120
450	0.6810	0.6720	0.6840	0.7520
600	0.6430	0.5490	0.6830	0.5450
750	0.4140	0.5590	0.4930	0.5510
900	0.2440	0.2330	0.1420	0.3410
1050	0.0730	0.0000	0.0000	0.0000

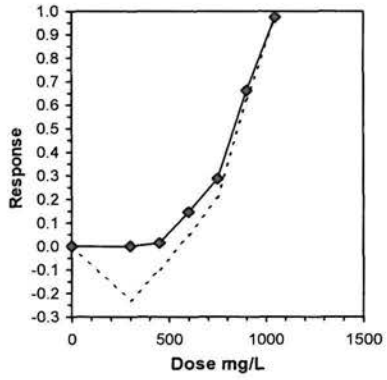
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.6350	1.0000	0.6350	0.5580	0.6850	8.555	4				0.7080	1.0000
300	0.7810	1.2299	0.7810	0.7460	0.8120	3.649	4	-4.155	2.290	0.0805	0.7080	1.0000
450	0.6973	1.0980	0.6973	0.6720	0.7520	5.286	4	-1.772	2.290	0.0805	0.6973	0.9848
600	0.6050	0.9528	0.6050	0.5450	0.6830	11.397	4	0.854	2.290	0.0805	0.6050	0.8545
750	0.5043	0.7941	0.5043	0.4140	0.5590	13.281	4				0.5043	0.7122
900	0.2400	0.3780	0.2400	0.1420	0.3410	33.910	4				0.2400	0.3390
1050	0.0183	0.0287	0.0183	0.0000	0.0730	200.000	4				0.0183	0.0258

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98195	0.844	-0.0491	-0.7623
Bartlett's Test indicates equal variances (p = 0.51)	2.30759	11.3449		

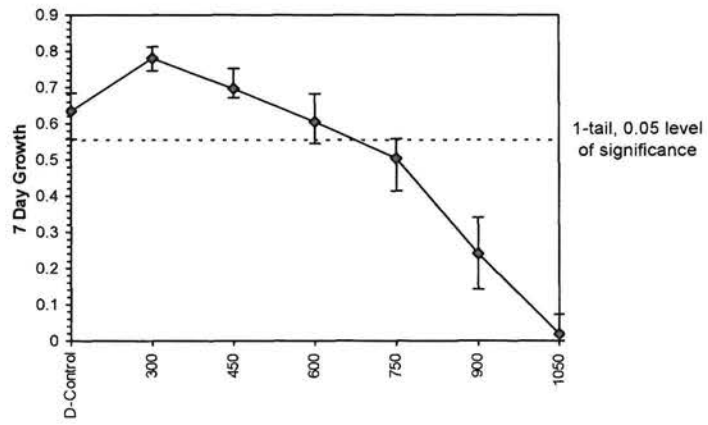
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.08046	0.12671	0.0242	0.00247	0.00151	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	490.08	32.83	367.02	615.28	0.4181
IC10	547.64	37.30	463.80	679.52	0.4893
IC15	604.76	37.93	500.26	719.18	-0.0089
IC20	657.47	41.59	525.76	786.18	-0.1434
IC25	710.17	35.98	592.41	795.51	-0.4593
IC40	795.10	13.22	746.37	833.73	-0.3761
IC50	835.29	13.99	793.41	883.34	0.2743



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **110**

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 PM	BL	BL	N	N	BL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.75	7.94	7.85	7.79	8.01	7.78
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.3	7.6	7.2
	Conductivity (µmhos/cm)	312		307		295	
	Alkalinity (mg $CaCO_3$/L)	60				60	
	Hardness (mg $CaCO_3$/L)	91				86	
	Temperature (°C)	24.7	24.5	24.7	24.4	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.93	7.93	8.07	7.74	8.06	7.76
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.3	7.6	7.3
	Conductivity (µmhos/cm)	866		843		804	
	Temperature (°C)	24.8	24.7	24.7	24.3	24.8	24.3
450 mg KCl/L	pH (S.U.)	7.91	7.91	8.06	7.74	8.04	7.75
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.4	7.6	7.2
	Conductivity (µmhos/cm)	1120		1010		1040	
	Temperature (°C)	24.7	24.7	24.7	24.6	24.9	24.3
600 mg KCl/L	pH (S.U.)	7.90	7.90	8.05	7.74	8.03	7.75
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.4	7.6	7.2
	Conductivity (µmhos/cm)	1380		1350		1290	
	Temperature (°C)	24.7	24.6	24.7	24.6	24.9	24.5
750 mg KCl/L	pH (S.U.)	7.90	7.88	8.04	7.81	8.03	7.74
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.4	7.7	7.4
	Conductivity (µmhos/cm)	1640		1620		1580	
	Temperature (°C)	24.7	24.6	24.8	24.5	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.90	7.88	8.03	7.78	8.02	7.74
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.5	7.9	7.3
	Conductivity (µmhos/cm)	1910		1870		1820	
	Temperature (°C)	24.6	24.6	24.8	24.5	24.8	24.7
1050 mg KCl/L	pH (S.U.)	7.91	7.89	8.04	7.79	8.05	7.73
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.4	7.9	7.3
	Conductivity (µmhos/cm)	2340		2150		2080	
	Temperature (°C)	24.8	24.6	24.9	24.5	24.8	24.7
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: 110

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	BL	B2/ PM	PM/BSL	PM/BSL	PM/BSL	PM	PM	M
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.88	7.80	7.64	7.65	7.60	7.71	7.68	7.54
	Dissolved oxygen (mg/L)	7.7		7.9	8.0	8.0	7.5	7.6	6.3
	Conductivity (µmhos/cm)	307		292		300		297	
	Alkalinity (mg $CaCO_3$/L)			60					
	Hardness (mg $CaCO_3$/L)			91					
	Temperature (°C)	24.7	24.5	24.6	24.7	24.8	24.4	24.7	24.5
300 mg KCl/L	pH (S.U.)	8.01	7.80	8.02	7.68	7.78	7.67	7.94	7.57
	Dissolved oxygen (mg/L)	7.8		7.9	8.0	7.7 (8.0)	7.4	7.9 (7.7)	6.2
	Conductivity (µmhos/cm)	843		793		855		835	
	Temperature (°C)	24.8	24.6	24.7	24.5	24.9	24.4	24.8	24.5
450 mg KCl/L	pH (S.U.)	8.00	7.79	8.02	7.66	7.86	7.68	7.93	7.50
	Dissolved oxygen (mg/L)	7.8		7.9	7.9	7.8 (8.0)	7.4	7.7	6.2
	Conductivity (µmhos/cm)	1100		1050		1100		1070	
	Temperature (°C)	24.8	24.6	24.7	24.5	24.9	24.7	24.7	24.6
600 mg KCl/L	pH (S.U.)	8.00	7.79	8.03	7.66	7.88	7.70	7.95	7.48
	Dissolved oxygen (mg/L)	7.8		8.1	8.0	7.9	7.4	7.7	6.3
	Conductivity (µmhos/cm)	1360		1300		1360		1330	
	Temperature (°C)	24.7	24.6	24.6	24.6	25.0	24.3	24.7	24.3
750 mg KCl/L	pH (S.U.)	7.99	7.78	7.98	7.68	7.91	7.69	7.95	7.48
	Dissolved oxygen (mg/L)	7.8		8.1	7.9	8.0	7.4	7.8	6.3
	Conductivity (µmhos/cm)	1600		1510		1650		1570	
	Temperature (°C)	24.7	24.6	24.6	24.6	24.9	24.3	24.8	24.3
900 mg KCl/L	pH (S.U.)	7.99	7.78	7.99	7.66	7.92	7.72	7.96	7.48
	Dissolved oxygen (mg/L)	7.8		8.1	7.8	8.0	7.5	7.8	6.7
	Conductivity (µmhos/cm)	1860		1780		1880		1820	
	Temperature (°C)	24.7	24.7	24.8	24.6	24.9	24.6	24.8	24.3
1050 mg KCl/L	pH (S.U.)	7.99	7.77	7.99	7.68	7.91	7.70	7.97	7.49
	Dissolved oxygen (mg/L)	7.8		8.1	7.9	8.0	7.5	7.8	6.7
	Conductivity (µmhos/cm)	2150		2040		2150		2100	
	Temperature (°C)	24.7	24.6	24.6	24.5	24.8	24.6	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final