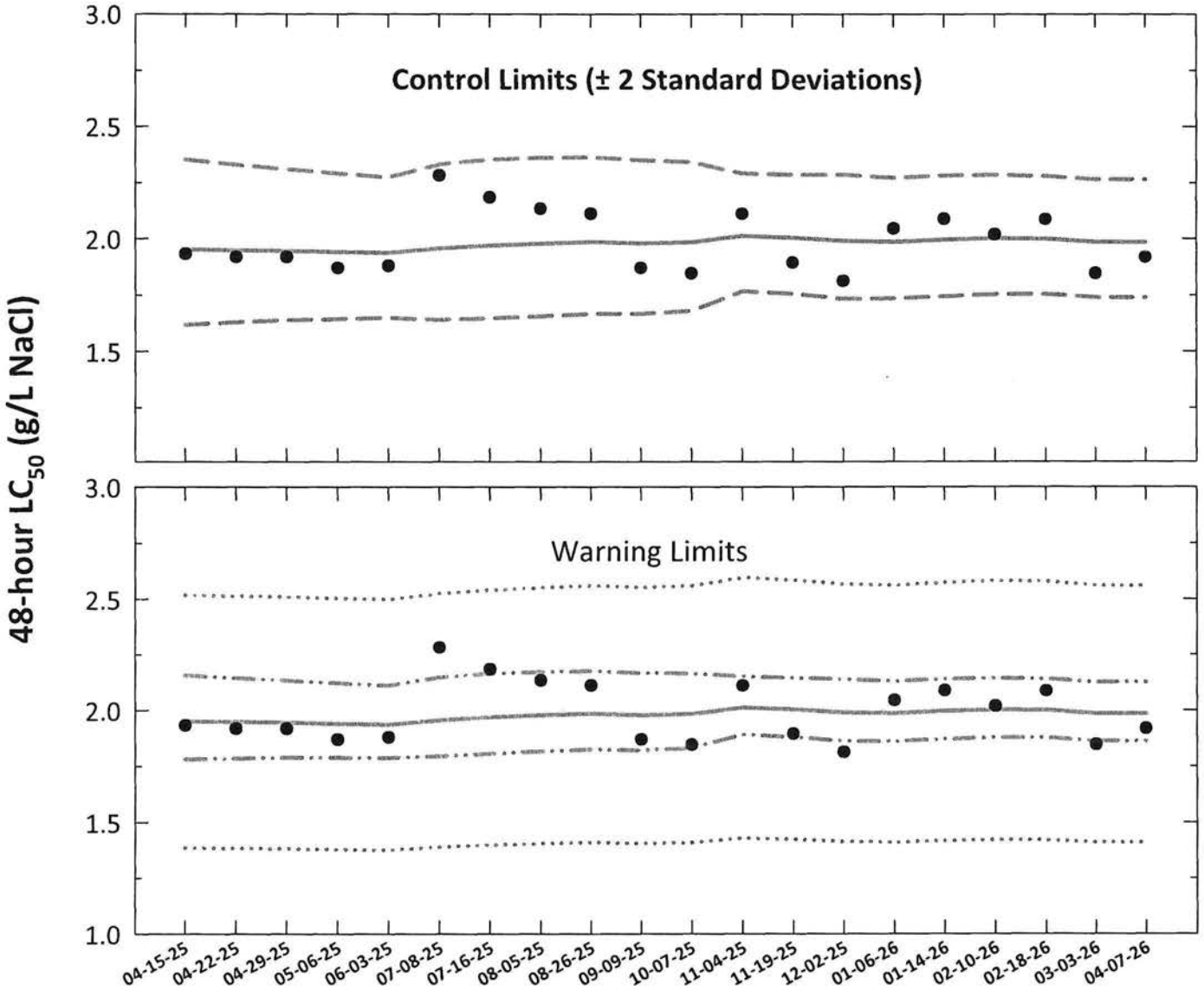
Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.3855	2.5173
2	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
3	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
4	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
5	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
6	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
7	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
8	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
9	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
10	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
11	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
12	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
13	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
14	12-02-25	1.8126	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
15	01-06-26	2.0449	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
16	01-14-26	2.0879	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739
17	02-10-26	2.0189	0.3051	0.3013	0.0287	2.0012	1.7531	2.2845	1.8772	2.1428	1.4209	2.5816
18	02-18-26	2.0862	0.3193	0.3009	0.0284	1.9994	1.7541	2.2790	1.8767	2.1392	1.4196	2.5792
19	03-03-26	1.8463	0.2663	0.2978	0.0286	1.9850	1.7400	2.2644	1.8616	2.1258	1.4093	2.5606
20	04-07-26	1.9184	0.2829	0.2976	0.0287	1.9842	1.7387	2.2644	1.8605	2.1254	1.4088	2.5597

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

CV = Coefficient of variation.

Entered and Reviewed by Jim 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 # 32

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
Total volume (mL)	200	200	200	200	200

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

Stock solution INSS #: 2466

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.79	7.90	7.84
	Dissolved oxygen (mg/L)	8.6	8.2	8.3
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	85		
	Temperature (°C)	24.9	25.3	25.2
1000 mg/L	pH (S.U.)	7.96	7.96	7.89
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	2130		
	Temperature (°C)	25.0	25.0	25.3
1500 mg/L	pH (S.U.)	7.96	7.96	7.91
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	2980		
	Temperature (°C)	25.0	25.0	25.3
2000 mg/L	pH (S.U.)	7.95	7.96	7.91
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	3850		
	Temperature (°C)	24.9	25.2	25.1
2500 mg/L	pH (S.U.)	7.96	7.97	7.92
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	4710		
	Temperature (°C)	25.0	25.2	25.3
3000 mg/L	pH (S.U.)	7.95	7.97	
	Dissolved oxygen (mg/L)	8.5	8.2	
	Conductivity (µmhos/cm)	5530		
	Temperature (°C)	25.1	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13666470

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 # 32

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-07-26	*1515	JP	0928	JP	284	RED	03-31-26A
24	04-08-26			0937	JP			
48 Termination	04-09-26			0931	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
Source (organisms were pooled):	03-31-26D
Age:	< 24-hours old
Date and time organisms were born between:	04-06-26 1230 to 04-07-26 1515
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86
	Temperature (°C): 25.2°C

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

Hours	Control				1000 mg/L				1500 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	2000 mg/L				2500 mg/L				3000 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	1^{4d}	1^{4d}	2^{3d}	1^{4d}	0^{5d}	0^{5d}	0^{5d}	0^{5d}
48 Termination	1^{4d}	0^{5d}	3^{2d}	4^{1d}	0^{1d}	0^{1d}	0^{2d}	0^{1d}	0	0	0	0
Mean Survival	40%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	2026.8
Upper 95% confidence limit (mg NaCl/L)	1814.0
48-hour LC_{50} (mg NaCl/L)	1918.4

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	3/3/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/5/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	0.8000	1.0000	1.0000
2500	0.6000	0.2000	0.8000	0.4000
3000	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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2500	0.5000	0.5000	0.7854	0.4636	1.1071	35.048	4	10.00	10.00	10	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

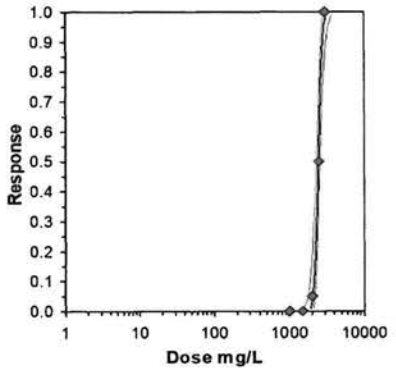
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.77684	0.905	-0.175	4.5884
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

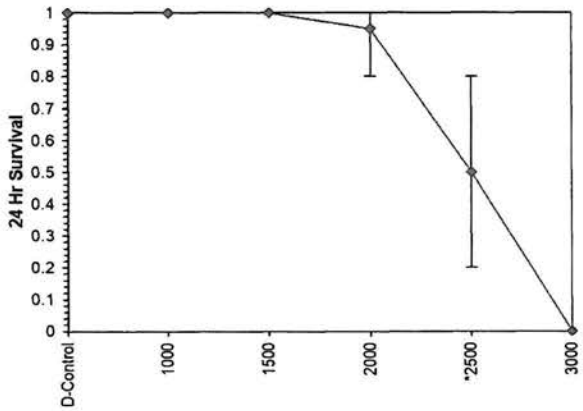
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	21.7796	4.61963	12.7251	30.8341	0	1.41886	7.81472	0.70112	3.3893	0.04591	5
Intercept	-68.818	15.6867	-99.564	-38.072							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1916.39	1576.7	2089.69
EC05	3.355	2059.56	1777.38	2206.51
EC10	3.718	2140.21	1892.56	2273.89
EC15	3.964	2196.39	1973.01	2322.21
EC20	4.158	2242.1	2038.13	2362.8
EC25	4.326	2282.07	2094.47	2399.6
EC40	4.747	2385.97	2235.51	2503.75
EC50	5.000	2450.74	2316.81	2577.51
EC60	5.253	2517.27	2393.18	2662.21
EC75	5.674	2631.88	2507.95	2829.13
EC80	5.842	2678.8	2550.01	2903.92
EC85	6.036	2734.54	2597.32	2996.6
EC90	6.282	2806.33	2655.05	3120.99
EC95	6.645	2916.22	2738.64	3320.19
EC99	7.326	3134.08	2894.31	3739.46



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	4/7/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/9/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.2000	0.0000	0.6000	0.8000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

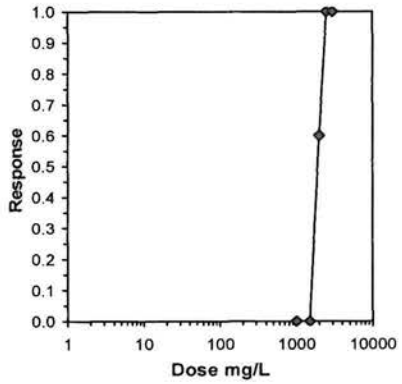
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.4000	0.4000	0.6706	0.2255	1.1071	59.520	4	10.00	10.00	12	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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)	0.7154	0.844	-0.0513	4.09861
Equality of variance cannot be confirmed				

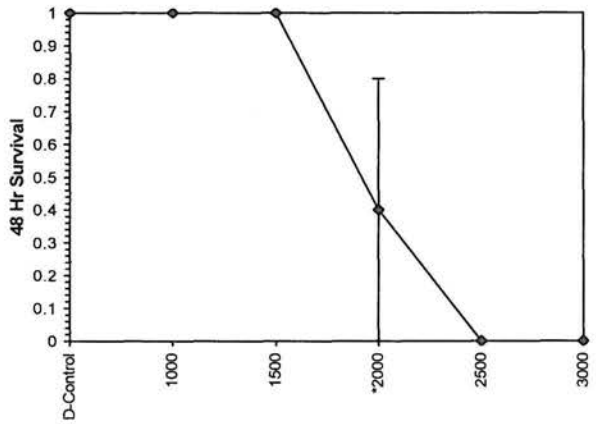
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	1918.36	1813.96	2028.77
5.0%	1916.57	1801.36	2039.14
10.0%	1914.79	1786.89	2051.85
20.0%	1911.36	1748.75	2089.09
Auto-0.0%	1918.36	1813.96	2028.77



Dose-Response Plot



Ceriodaphnia dubia
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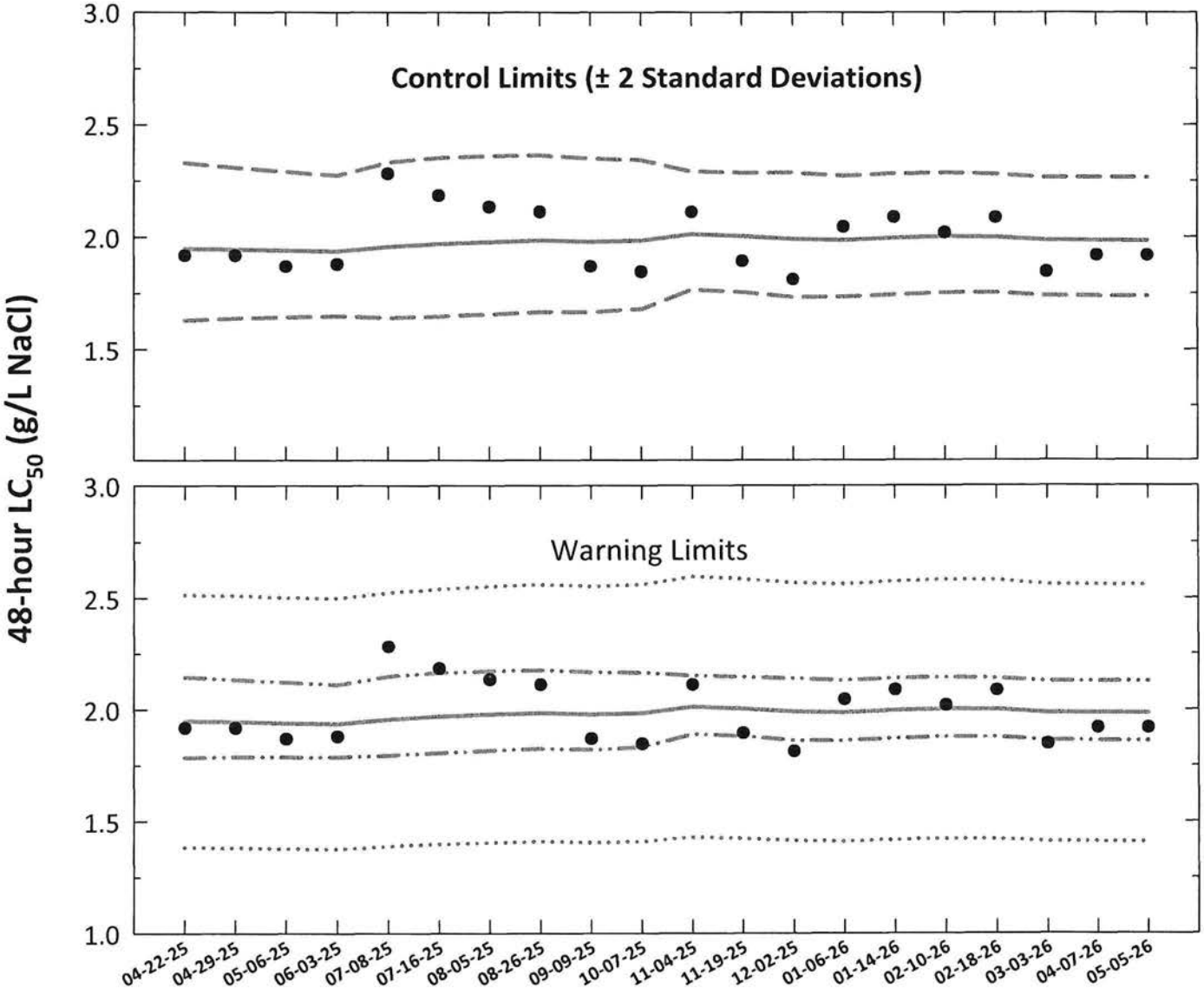


- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits**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

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**04-22-25**	**1.9184**	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
2	**04-29-25**	**1.9184**	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
3	**05-06-25**	**1.8700**	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
4	**06-03-25**	**1.8799**	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
5	**07-08-25**	**2.2819**	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
6	**07-16-25**	**2.1842**	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
7	**08-05-25**	**2.1340**	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
8	**08-26-25**	**2.1117**	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
9	**09-09-25**	**1.8700**	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
10	**10-07-25**	**1.8463**	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
11	**11-04-25**	**2.1111**	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
12	**11-19-25**	**1.8940**	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
13	**12-02-25**	**1.8126**	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
14	**01-06-26**	**2.0449**	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
15	**01-14-26**	**2.0879**	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739
16	**02-10-26**	**2.0189**	0.3051	0.3013	0.0287	2.0012	1.7531	2.2845	1.8772	2.1428	1.4209	2.5816
17	**02-18-26**	**2.0862**	0.3193	0.3009	0.0284	1.9994	1.7541	2.2790	1.8767	2.1392	1.4196	2.5792
18	**03-03-26**	**1.8463**	0.2663	0.2978	0.0286	1.9850	1.7400	2.2644	1.8616	2.1258	1.4093	2.5606
19	**04-07-26**	**1.9184**	0.2829	0.2976	0.0287	1.9842	1.7387	2.2644	1.8605	2.1254	1.4088	2.5597
20	**05-05-26**	**1.9184**	0.2829	0.2974	0.0288	1.9835	1.7374	2.2644	1.8594	2.1251	1.4083	2.5587

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

CV = Coefficient of variation.

Entered and Reviewed by Jim 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 # 33

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 N stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2479

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	VL	XL	XL
Control, MHSW	pH (S.U.)	7.96	7.84	7.79
	Dissolved oxygen (mg/L)	8.2	8.1	8.0
	Conductivity (µmhos/cm)	302		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.9	25.2	25.2
1000 mg/L	pH (S.U.)	8.05	7.88	7.81
	Dissolved oxygen (mg/L)	8.2	8.1	8.1
	Conductivity (µmhos/cm)	2010		
	Temperature (°C)	25.0	25.0	25.3
1500 mg/L	pH (S.U.)	8.04	7.88	7.81
	Dissolved oxygen (mg/L)	8.2	8.2	8.1
	Conductivity (µmhos/cm)	2790		
	Temperature (°C)	25.1	25.3	25.3
2000 mg/L	pH (S.U.)	8.04	7.88	7.81
	Dissolved oxygen (mg/L)	8.2	8.2	8.1
	Conductivity (µmhos/cm)	3680		
	Temperature (°C)	24.9	24.9	25.0
2500 mg/L	pH (S.U.)	8.04	7.89	7.81
	Dissolved oxygen (mg/L)	8.3	8.2	8.1
	Conductivity (µmhos/cm)	4530		
	Temperature (°C)	24.9	24.9	25.1
3000 mg/L	pH (S.U.)	8.03	7.89	7.82
	Dissolved oxygen (mg/L)	8.3	8.2	8.1
	Conductivity (µmhos/cm)	5310		
	Temperature (°C)	25.2	25.3	24.9

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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN25010005030
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664

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 # 33

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05.05.26	*0430	JP	0802	JP	2	GREEN	04.28.26A
24	05.06.26			0816	JP			
48 Termination	05.07.26			0821	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
Source (organisms were pooled):	04-28-26 D
Age:	< 24-hours old
Date and time organisms were born between:	05.04.26 1240 TO 05.05.26 0430
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.70
	Temperature (°C): 24.2°C

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

Hours	Control				1000 mg/L				1500 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	2000 mg/L				2500 mg/L				3000 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	4 1d	5	5	5	1 2d	1 2d	1 2d	2 2d	0 2d	0 2d	1 2d	0 2d
48 Termination	1 3d	3 2d	2 2d	2 3d	0 1d	0 1d	0 1d	0 2d	0	0	0 1d	0
Mean Survival	40%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1814.0
Upper 95% confidence limit (mg NaCl/L)	2029.8
48-hour LC_{50} (mg NaCl/L)	1918.4

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	5/5/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/7/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.8000	1.0000	1.0000	1.0000
2500	0.2000	0.2000	0.2000	0.4000
3000	0.0000	0.0000	0.2000	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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
*3000	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20

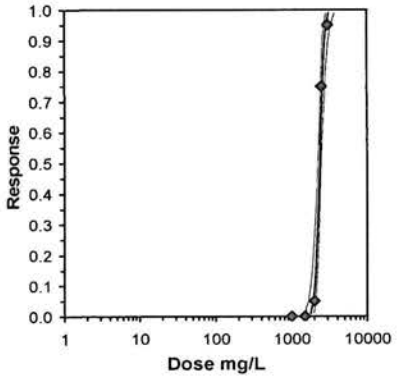
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.85308	0.884	0.49918	2.40009
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

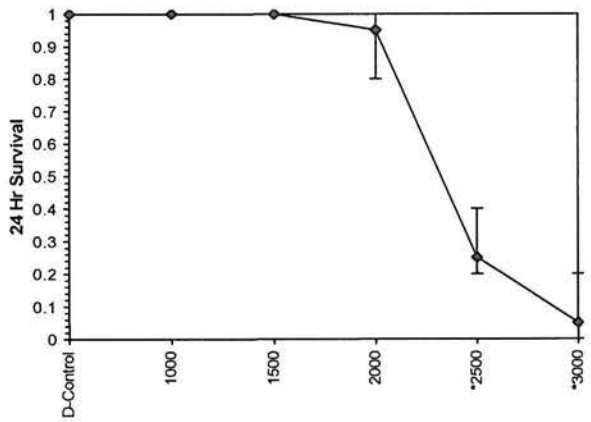
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	19.4843	3.90282	11.8347	27.1338	0	1.36404	7.81472	0.71399	3.37441	0.05132	3
Intercept	-60.748	13.1935	-86.607	-34.889							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1798.92	1476.82	1973.05
EC05	3.355	1949.79	1679.74	2098.56
EC10	3.718	2035.33	1796.95	2171.27
EC15	3.964	2095.15	1879.12	2223.53
EC20	4.158	2143.94	1945.8	2267.46
EC25	4.326	2186.71	2003.62	2307.3
EC40	4.747	2298.29	2148.94	2419.92
EC50	5.000	2368.14	2233.3	2499.32
EC60	5.253	2440.12	2313.23	2589.97
EC75	5.674	2564.63	2435.19	2767.5
EC80	5.842	2615.79	2480.51	2846.82
EC85	6.036	2676.71	2531.85	2945.13
EC90	6.282	2755.38	2594.96	3077.21
EC95	6.645	2876.26	2687.05	3289.26
EC99	7.326	3117.49	2860.26	3738.26



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	5/5/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/7/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.2000	0.6000	0.4000	0.4000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

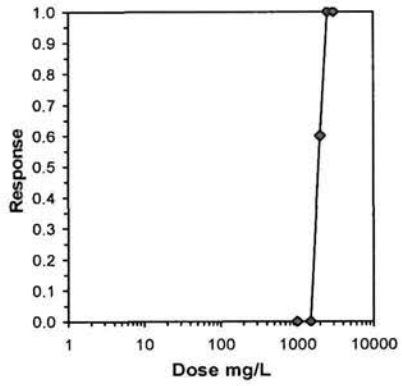
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.4000	0.4000	0.6798	0.4636	0.8861	25.383	4	10.00	10.00	12	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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)	0.52449	0.844	-0.2187	7.51217
Equality of variance cannot be confirmed				

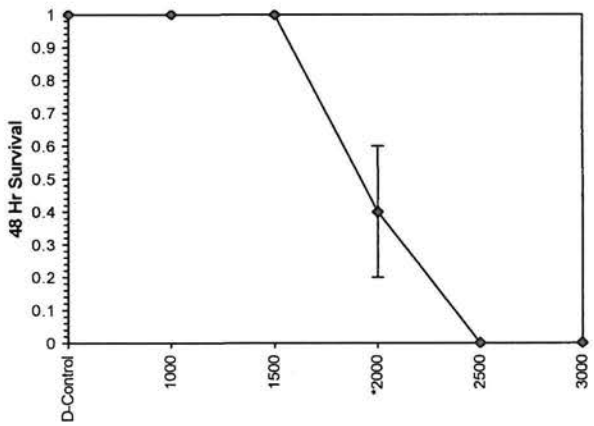
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	1918.36	1813.96	2028.77
5.0%	1916.57	1801.36	2039.14
10.0%	1914.79	1786.89	2051.85
20.0%	1911.36	1748.75	2089.09
Auto-0.0%	1918.36	1813.96	2028.77

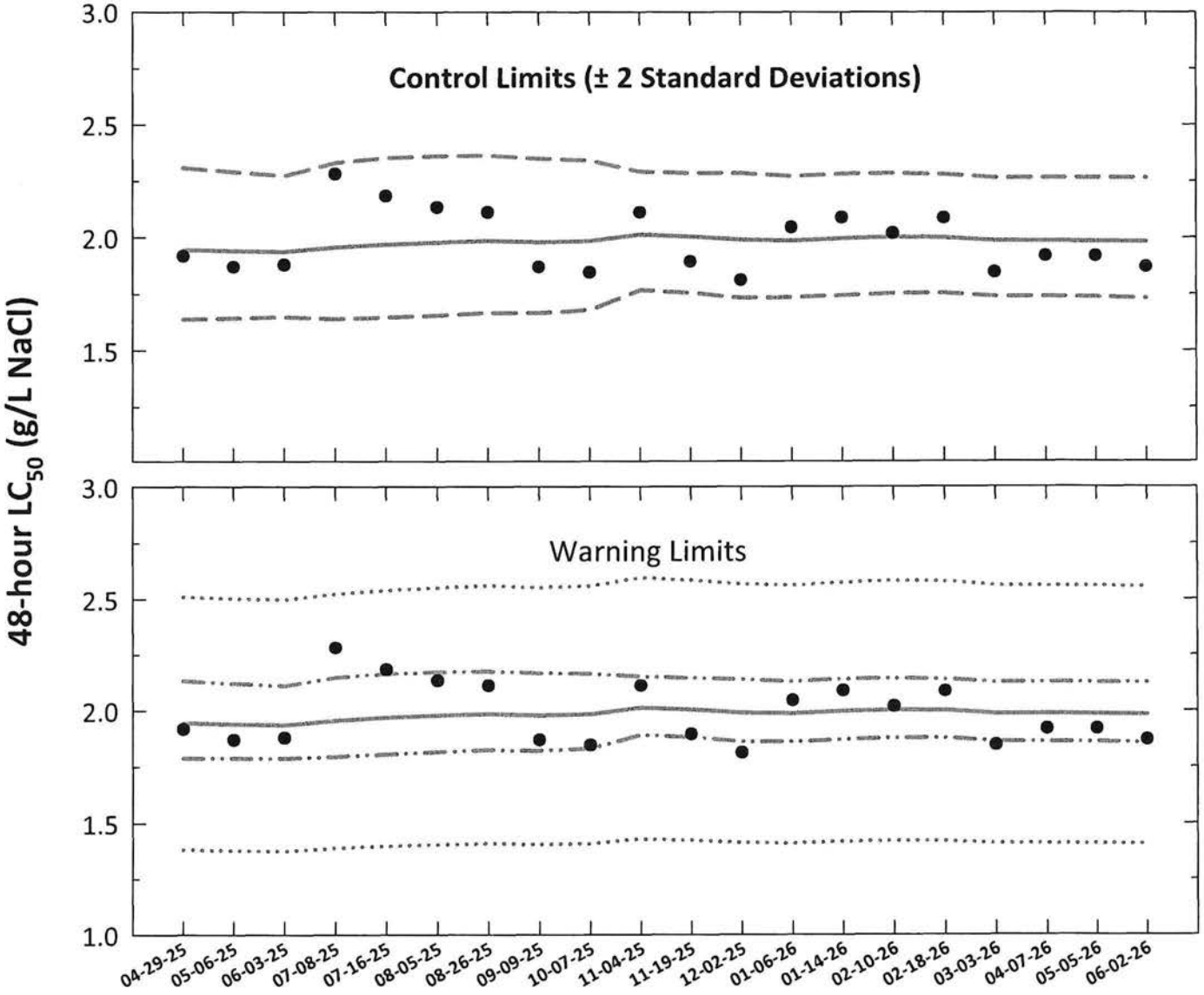


Dose-Response Plot



Environmental Testing Solutions, Inc.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
2	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
3	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
4	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
5	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
6	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
7	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
8	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
9	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
10	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
11	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
12	12-02-25	1.8126	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
13	01-06-26	2.0449	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
14	01-14-26	2.0879	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739
15	02-10-26	2.0189	0.3051	0.3013	0.0287	2.0012	1.7531	2.2845	1.8772	2.1428	1.4209	2.5816
16	02-18-26	2.0862	0.3193	0.3009	0.0284	1.9994	1.7541	2.2790	1.8767	2.1392	1.4196	2.5792
17	03-03-26	1.8463	0.2663	0.2978	0.0286	1.9850	1.7400	2.2644	1.8616	2.1258	1.4093	2.5606
18	04-07-26	1.9184	0.2829	0.2976	0.0287	1.9842	1.7387	2.2644	1.8605	2.1254	1.4088	2.5597
19	05-05-26	1.9184	0.2829	0.2974	0.0288	1.9835	1.7374	2.2644	1.8594	2.1251	1.4083	2.5587
20	06-02-26	1.8700	0.2718	0.2969	0.0292	1.9810	1.7320	2.2656	1.8553	2.1247	1.4065	2.5554

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

CV = Coefficient of variation.

Entered and Reviewed by Jim 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 # 34

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 #: 2479

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.72	7.85	7.82
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.6	25.2	24.8
1000 mg/L	pH (S.U.)	7.80	7.86	7.84
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	2090		
	Temperature (°C)	24.6	25.0	25.2
1500 mg/L	pH (S.U.)	7.82	7.87	7.86
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	2880		
	Temperature (°C)	24.7	25.0	24.7
2000 mg/L	pH (S.U.)	7.82	7.87	7.87
	Dissolved oxygen (mg/L)	8.7	8.2	8.2
	Conductivity (µmhos/cm)	3680		
	Temperature (°C)	24.6	25.0	24.6
2500 mg/L	pH (S.U.)	7.84	7.88	7.88
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	4570		
	Temperature (°C)	24.7	25.1	24.9
3000 mg/L	pH (S.U.)	7.85	7.89	7.89
	Dissolved oxygen (mg/L)	8.6	8.3	8.3
	Conductivity (µmhos/cm)	5450		
	Temperature (°C)	24.5	25.1	24.6

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

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 # 34

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-02-26	* 0530	ZP	0830	ZP	2B2	RD	05-27-26A
24	06-03-26			0838	ZP			
48 Termination	06-04-26			0823	ZP			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	05-26-26 D
Age:	< 24-hours old
Date and time organisms were born between:	06-01-26 1245 TO 06-02-26 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.85
	Temperature (°C): 25.3

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

Hours	Control				1000 mg/L				1500 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	2000 mg/L				2500 mg/L				3000 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	1^{4d}	S	S	S	1^{4d}	3^{2d}	2^{3d}	1^{4d}	0^{5d}	0^{5d}	0^{5d}	1^{4d}
48 Termination	0^{1d}	2^{3d}	3^{2d}	1^{4d}	0^{1d}	0^{3d}	6^{2d}	0^{1d}	0	0	0	0^{1d}
Mean Survival	~~15%~~ 30%				0%				0%			

Comment codes: d = dead, u = unhealthy @06-04-26

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1774.6
Upper 95% confidence limit (mg NaCl/L)	1970.5
48-hour LC_{50} (mg NaCl/L)	1870.0

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	6/2/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	6/4/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.2000	1.0000	1.0000	1.0000
2500	0.2000	0.6000	0.4000	0.2000
3000	0.0000	0.0000	0.0000	0.2000

			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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.8000	0.8000	1.1249	0.4636	1.3453	39.188	4	16.00	10.00	4	20
*2500	0.3500	0.3500	0.6245	0.4636	0.8861	32.527	4	10.00	10.00	13	20
*3000	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20

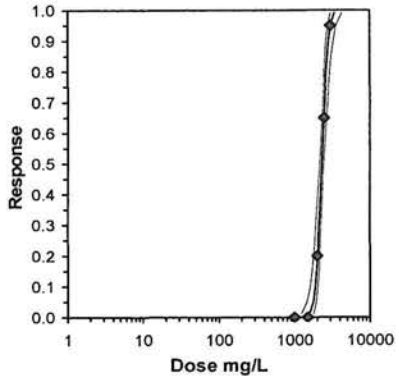
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7533	0.884	-1.9531	7.4892
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

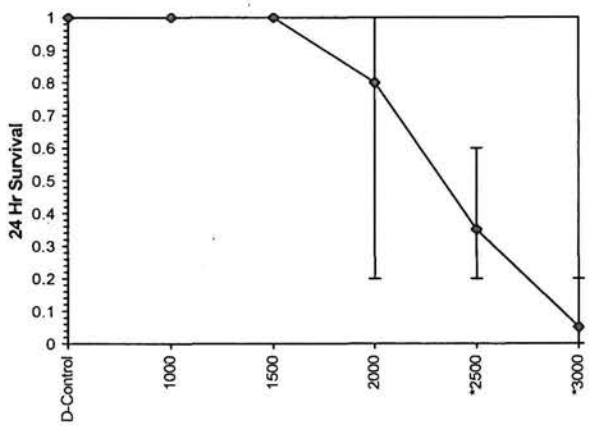
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1798	2.73721	8.81489	19.5447	0	0.2103	7.81472	0.97591	3.36583	0.07052	3
Intercept	-42.727	9.23531	-60.828	-24.626							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1591.36	1236.13	1795.47
EC05	3.355	1777.58	1470.66	1953.94
EC10	3.718	1885.61	1611.14	2046.88
EC15	3.964	1962.17	1711.8	2114.08
EC20	4.158	2025.24	1794.79	2170.84
EC25	4.326	2080.96	1867.7	2222.52
EC40	4.747	2228.24	2054.6	2370
EC50	5.000	2321.83	2165.08	2475.75
EC60	5.253	2419.34	2270.63	2598.59
EC75	5.674	2590.58	2433.18	2844.68
EC80	5.842	2661.85	2494.26	2956.49
EC85	6.036	2747.4	2564.08	3096.39
EC90	6.282	2858.96	2650.88	3286.59
EC95	6.645	3032.7	2779.53	3597.21
EC99	7.326	3387.58	3027.51	4275.95



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	6/2/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	6/4/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.0000	0.4000	0.6000	0.2000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

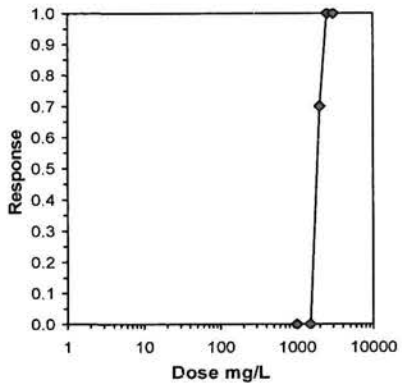
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.3000	0.3000	0.5650	0.2255	0.8861	50.368	4	10.00	10.00	14	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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)	0.68087	0.844	-0.1975	5.49067
Equality of variance cannot be confirmed				

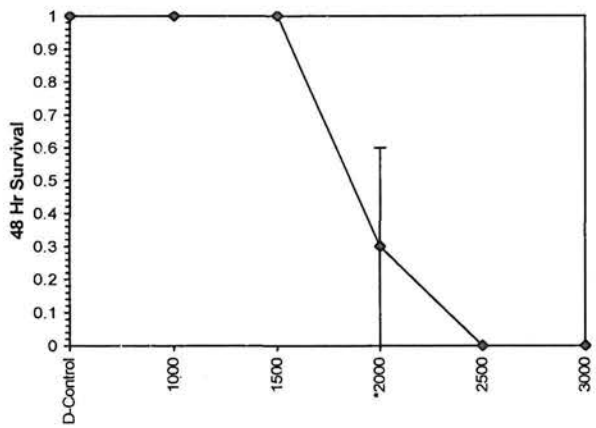
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	1869.98	1774.62	1970.47
5.0%	1863.6	1759.25	1974.14
10.0%	1857.58	1744.15	1978.38
20.0%	1847.3	1715	1989.81
Auto-0.0%	1869.98	1774.62	1970.47

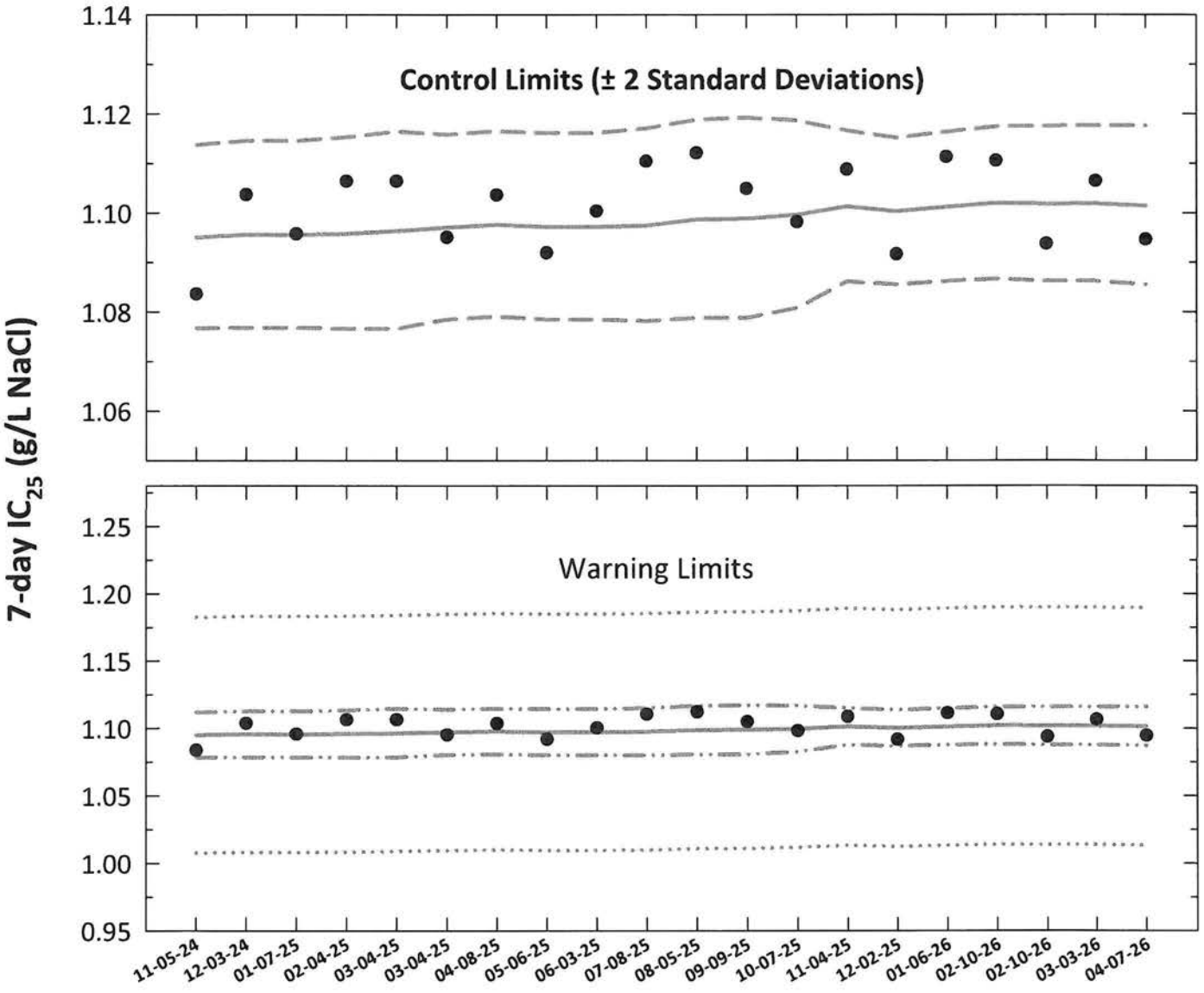


Dose-Response Plot



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} ± $S_{A.10}$ 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	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
2	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
3	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
4	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
5	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
6	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
7	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
8	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
9	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
10	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
11	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
12	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
13	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
14	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
15	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
16	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893
17	02-10-26	1.1106	0.0455	0.0422	0.0030	1.1020	1.0867	1.1175	1.0881	1.1158	1.0138	1.1901
18	02-10-26	1.0938	0.0389	0.0421	0.0031	1.1018	1.0863	1.1176	1.0877	1.1159	1.0137	1.1900
19	03-03-26	1.1065	0.0439	0.0421	0.0031	1.1019	1.0863	1.1177	1.0877	1.1160	1.0137	1.1900
20	04-07-26	1.0947	0.0393	0.0420	0.0032	1.1015	1.0856	1.1176	1.0870	1.1159	1.0134	1.1896

Note:
7-day IC_{25} = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
CT = Central tendency of the IC_{25} values.
S = Standard deviation of the IC_{25} values.
Control Limits = Mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values.
Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).
CV = Coefficient of variation.

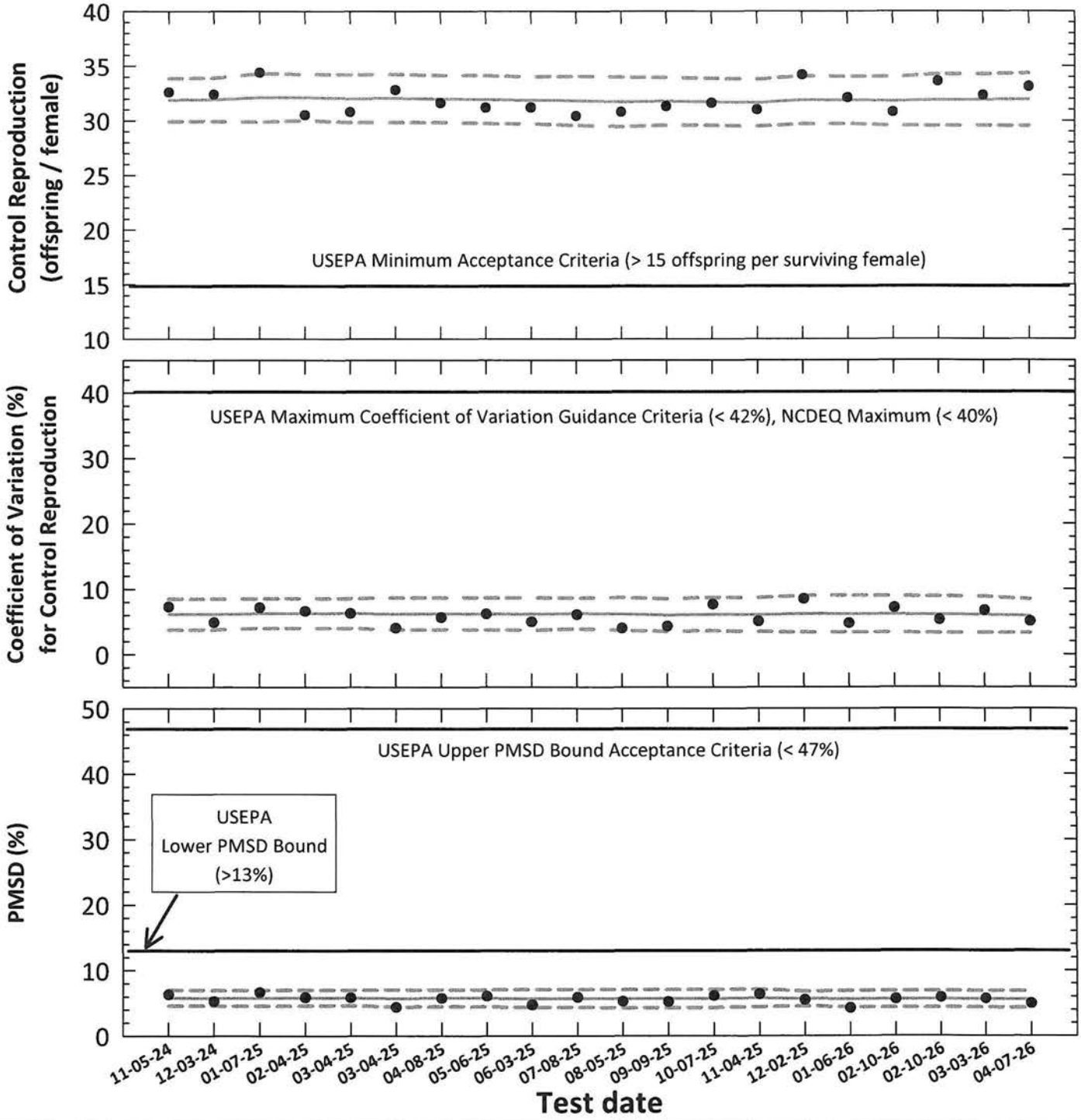
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control	Control Reproduction		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		Survival (%)	Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
2	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
3	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
4	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
5	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
6	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
7	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
8	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
9	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
10	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
11	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
12	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
13	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
14	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
15	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
16	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9
17	02-10-26	100	30.8	7.1	1.734	5.6	31.8	29.6	34.0	6.1	3.3	8.9	5.6	4.3	6.9
18	02-10-26	100	33.6	5.3	1.962	5.8	31.9	29.5	34.3	6.1	3.3	8.9	5.6	4.3	6.9
19	03-03-26	100	32.3	6.7	1.823	5.6	31.9	29.5	34.2	6.0	3.3	8.8	5.6	4.3	6.8
20	04-07-26	100	33.1	5.0	1.630	4.9	31.9	29.5	34.4	5.9	3.3	8.4	5.6	4.3	6.8

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 #: 323

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2466				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	04-07-26 0515 TO 0800									
Culture board:	03-31-26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	3	4	7	8	12	13	14	15	23
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:	BLUE
Incubator number and shelf location:	281

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	04-07-26	0815	03-26-26	03-26-26	03-30-26A	
1	04-08-26	0615	↓	↓	↓	
2	04-09-26	0715	↓	↓	03-30-26C	
3	04-10-26	0616	↓	↓	↓	
4	04-11-26	0700	↓	↓	04-01-26	
5	04-12-26	0640	↓	↓	↓	
6	04-13-26	0745	↓	↓	↓	
7	04-14-26	0616				

*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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	13066465

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)	1095.4
% CV:	5.0%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1094.7

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 323**

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	5	4	5	6	4	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	12	10	10	12	12	12	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	16	18	15	KP	16	19	17	19
Total young produced		33	31	32	33	31	34	32	36	34	35
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	5	5	5	4	6	5	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	12	11	12	12	13	13	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	15	15	17	18	16	16	16	15	16	19
Total young produced		33	30	34	33	34	33	34	32	32	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:	33.2
% Reduction from Control:	-0.37%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 323**

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	5	5	4	5	5	6	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	13	10	10	12	10	12	11	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	13	15	15	17	18	16	19	15	19	18
Total young produced		29	33	33	31	33	33	35	31	34	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.7
% Reduction from Control:	1.2%

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	4	4	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	10	10	9	12	9	11	11	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	12	15	17	16	13	18	14	16	17
Total young produced		28	30	30	32	29	29	31	29	33	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.4
% Reduction from Control:	8.2%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 323

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	5	4	4	5	5	5	3	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	10	5	10	8	7	11	6	5	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	5	10	6	6	5	5	9	11	5
Total young produced		17	20	19	20	19	17	21	18	19	17
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.7
% Reduction from Control:	43.5%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	1	3	2	2	2	1	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	0	0	0	0	0	0	0	0	0	0
Total young produced		2	1	3	2	2	2	1	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:	1.7
% Reduction from Control:	94.9%

ETS
Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	5	6	4	4	5	5	5	48
5	13	11	12	10	10	12	12	12	12	11	115
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	16	18	15	18	16	19	17	19	168
Total	**33**	**31**	**32**	**33**	**31**	**34**	**32**	**36**	**34**	**35**	**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	4	5	5	5	4	4	4	4	6	4	45
5	11	13	10	10	9	12	9	11	11	12	108
6	0	0	0	0	0	0	0	0	0	0	0
7	13	12	15	17	16	13	18	14	16	17	151
Total	**28**	**30**	**30**	**32**	**29**	**29**	**31**	**29**	**33**	**33**	**304**

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	5	5	4	6	5	5	4	5	5	49
5	13	10	12	11	12	12	13	13	11	13	120
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	17	18	16	16	16	15	16	19	163
Total	**33**	**30**	**34**	**33**	**34**	**33**	**34**	**32**	**32**	**37**	**332**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	5	4	4	5	5	5	3	3	3	40
5	9	10	5	10	8	7	11	6	5	9	80
6	0	0	0	0	0	0	0	0	0	0	0
7	5	5	10	6	6	5	5	9	11	5	67
Total	**17**	**20**	**19**	**20**	**19**	**17**	**21**	**18**	**19**	**17**	**187**

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	5	5	4	5	5	6	4	4	6	50
5	10	13	13	10	10	12	10	12	11	11	112
6	0	0	0	0	0	0	0	0	0	0	0
7	13	15	15	17	18	16	19	15	19	18	165
Total	**29**	**33**	**33**	**31**	**33**	**33**	**35**	**31**	**34**	**35**	**327**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	2	2	2	1	2	1	1	17
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**1**	**3**	**2**	**2**	**2**	**1**	**2**	**1**	**1**	**17**

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: 323

Test dates: April 07-14, 2026

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	33	31	32	33	31	34	32	36	34	35	**100**	**33.1**	**5.0**	**Not applicable**
600	33	30	34	33	34	33	34	32	32	37	**100**	**33.2**	**5.5**	**-0.3**
800	29	33	33	31	33	33	35	31	34	35	**100**	**32.7**	**5.8**	**1.2**
1000	28	30	30	32	29	29	31	29	33	33	**100**	**30.4**	**5.8**	**8.2**
1200	17	20	19	20	19	17	21	18	19	17	**100**	**18.7**	**7.6**	**43.5**
1400	2	1	3	2	2	2	1	2	1	1	**100**	**1.7**	**39.7**	**94.9**

Dunnett's MSD value: 1.630

PMSD: 4.9

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.

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	4/7/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	4/14/2026	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	31.000	32.000	33.000	31.000	34.000	32.000	36.000	34.000	35.000
600	33.000	30.000	34.000	33.000	34.000	33.000	34.000	32.000	32.000	37.000
800	29.000	33.000	33.000	31.000	33.000	33.000	35.000	31.000	34.000	35.000
1000	28.000	30.000	30.000	32.000	29.000	29.000	31.000	29.000	33.000	33.000
1200	17.000	20.000	19.000	20.000	19.000	17.000	21.000	18.000	19.000	17.000
1400	2.000	1.000	3.000	2.000	2.000	2.000	1.000	2.000	1.000	1.000

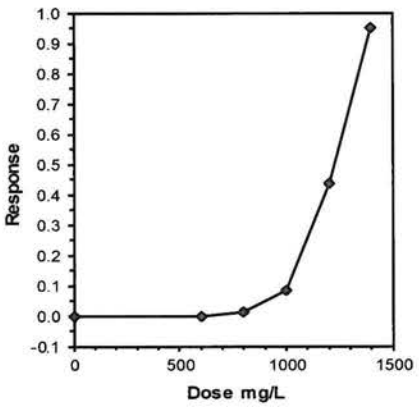
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	33.100	1.0000	33.100	31.000	36.000	5.025	10				33.150	1.0000
600	33.200	1.0030	33.200	30.000	37.000	5.462	10	-0.140	2.287	1.630	33.150	1.0000
800	32.700	0.9879	32.700	29.000	35.000	5.775	10	0.561	2.287	1.630	32.700	0.9864
*1000	30.400	0.9184	30.400	28.000	33.000	5.843	10	3.788	2.287	1.630	30.400	0.9170
*1200	18.700	0.5650	18.700	17.000	21.000	7.584	10	20.201	2.287	1.630	18.700	0.5641
*1400	1.700	0.0514	1.700	1.000	3.000	39.703	10	44.049	2.287	1.630	1.700	0.0513

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.82657	1.035	0.08722	0.01388
Bartlett's Test indicates equal variances (p = 0.11)	9.08701	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		1.63004	0.04925	1607.75	2.54074	0.0E+00	5, 54
Treatments vs D-Control										

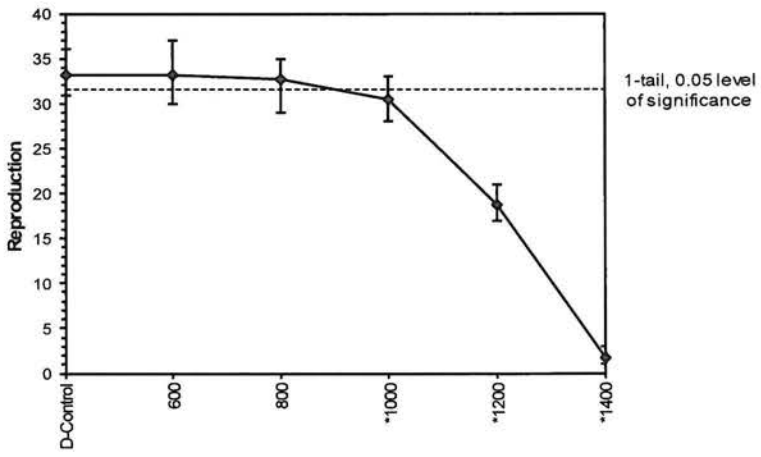
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	905	52.4757	773.383	1001.91	-0.3568
IC10	1009.66	18.3783	958.785	1029	-2.1658
IC15	1037.99	9.06402	1018.44	1054.36	-0.0868
IC20	1066.32	8.1493	1048.35	1080.34	-0.0818
IC25	1094.66	7.48041	1078.63	1106.92	-0.1002
IC40	1179.66	7.50595	1165.2	1192.92	-0.1039
IC50	1225	4.80611	1214.81	1232.95	-0.3191



While hypothesis test results indicate a significant difference in reproduction for the 1000 mg/L concentration, the PMSD was below the lower bound (13%) established by EPA. Guidance in EPA 833-R-00-003 (June 2000) Section 6.4.2 for determining the NOEC was followed, and it was concluded that the hypothesis test yielded a Type I Error. The ChV was recalculated to be 1095.4 mg/L.

Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 323**

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.79	7.91	7.75	7.89	7.76	7.90
	Dissolved oxygen (mg/L)	8.6	8.1	8.4	8.2	8.5	8.1
	Conductivity (µmhos/cm)	306		312		310	
	Alkalinity (mg $CaCO_3$/L)	60				62	
	Hardness (mg $CaCO_3$/L)	85				85	
	Temperature (°C)	24.9	25.3	24.9	25.1	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.88	7.92	7.87	7.89	7.86	7.91
	Dissolved oxygen (mg/L)	8.6	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1390		1400		1400	
	Temperature (°C)	25.0	25.0	25.0	25.3	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.90	7.92	7.89	7.89	7.87	7.93
	Dissolved oxygen (mg/L)	8.6	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1740		1790		1770	
	Temperature (°C)	25.0	25.2	24.9	25.1	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.91	7.92	7.90	7.89	7.89	7.93
	Dissolved oxygen (mg/L)	8.5	8.1	8.3	8.2	8.4	8.1
	Conductivity (µmhos/cm)	2090		2140		2150	
	Temperature (°C)	25.0	25.2	24.9	24.9	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.92	7.93	7.91	7.90	7.90	7.93
	Dissolved oxygen (mg/L)	8.5	8.1	8.3	8.2	8.4	8.1
	Conductivity (µmhos/cm)	2450		2510		2520	
	Temperature (°C)	25.0	25.1	24.9	24.9	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.94	7.95	7.92	7.91	7.91	7.93
	Dissolved oxygen (mg/L)	8.5	8.1	8.3	8.2	8.3	8.1
	Conductivity (µmhos/cm)	2810		2870		2850	
	Temperature (°C)	25.0	25.1	24.9	25.2	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 323

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.83	7.91	7.72	8.05	7.77	7.90	7.75	8.06
	Dissolved oxygen (mg/L)	8.4	8.3	8.6	8.0	8.4	8.1	8.5	7.6
	Conductivity (µmhos/cm)	314		299		313		308	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)			87					
	Temperature (°C)	24.9	25.0	24.9	25.1	24.8	24.9	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.89	7.94	7.74	8.05	7.89	7.93	7.85	7.99
	Dissolved oxygen (mg/L)	8.4	8.3	8.5	8.1	8.3	8.2	8.4	7.6
	Conductivity (µmhos/cm)	1390		1350		1380		1380	
	Temperature (°C)	25.0	25.2	25.0	24.9	24.9	24.9	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.90	7.95	7.85	8.04	7.98	7.94	7.88	7.99
	Dissolved oxygen (mg/L)	8.4	8.3	8.5	8.1	8.3	8.3	8.4	7.6
	Conductivity (µmhos/cm)	1780		1720		1790		1820	
	Temperature (°C)	25.0	25.2	25.0	24.9	24.9	24.9	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.91	7.95	7.88	8.05	8.00	7.95	7.90	7.99
	Dissolved oxygen (mg/L)	8.3	8.2	8.4	8.1	8.3	8.3	8.4	7.6
	Conductivity (µmhos/cm)	2140		2070		2140		2150	
	Temperature (°C)	25.0	25.2	25.0	25.2	24.8	25.2	24.8	25.2
1200 mg NaCl/L	pH (S.U.)	7.92	7.95	7.89	8.06	8.01	7.95	7.92	7.98
	Dissolved oxygen (mg/L)	8.3	8.2	8.3	8.1	8.3	8.2	8.4	7.6
	Conductivity (µmhos/cm)	2520		2480		2520		2610	
	Temperature (°C)	24.9	25.2	24.9	24.9	24.8	25.2	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.94	7.95	7.90	8.05	8.00	7.94	7.94	7.98
	Dissolved oxygen (mg/L)	8.3	8.2	8.4	8.0	8.3	8.2	8.4	7.6
	Conductivity (µmhos/cm)	2880		2830		2840		2980	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.8	25.0	24.8	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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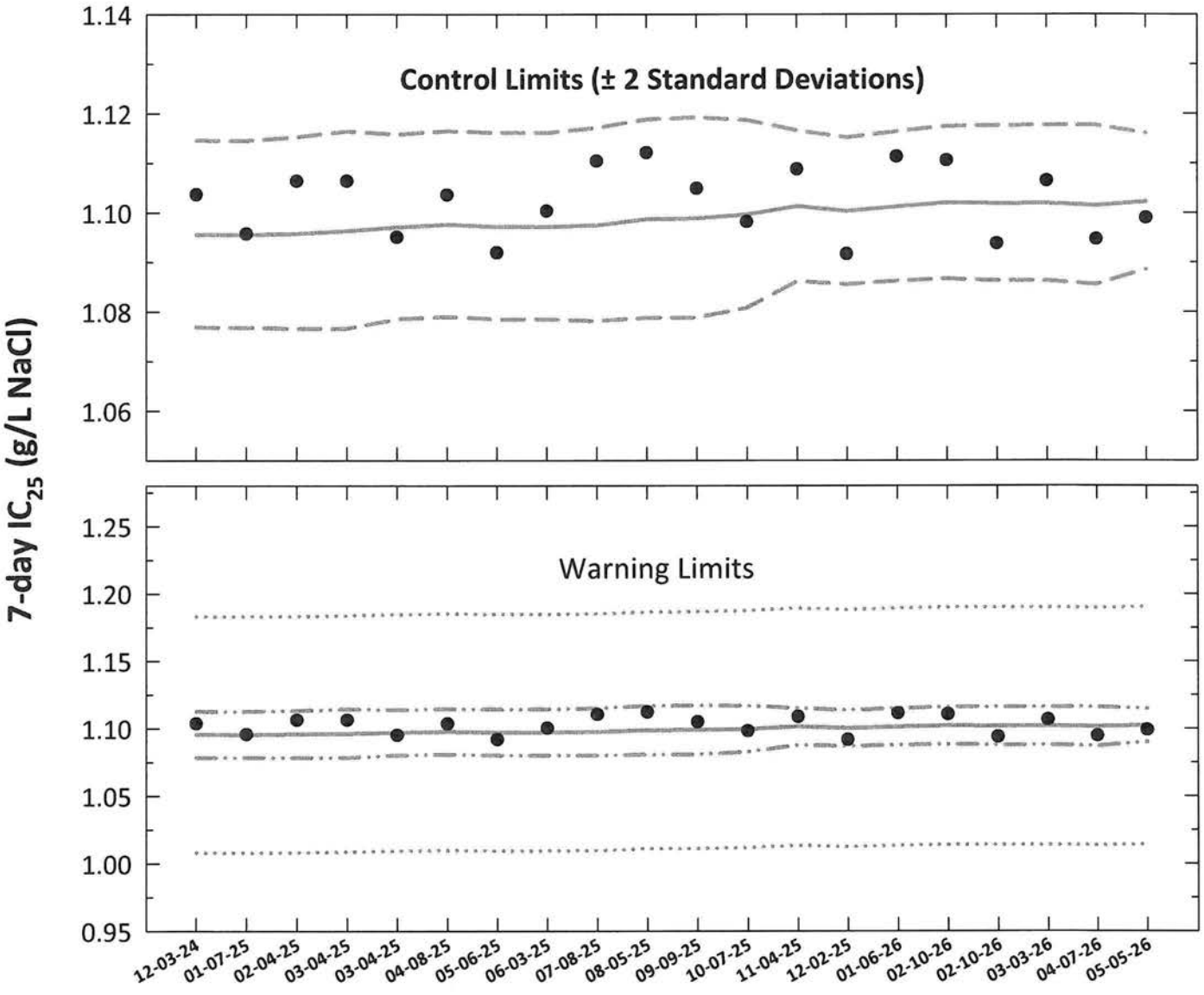
Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture



- ● **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	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
2	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
3	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
4	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
5	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
6	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
7	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
8	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
9	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
10	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
11	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
12	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
13	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
14	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
15	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893
16	02-10-26	1.1106	0.0455	0.0422	0.0030	1.1020	1.0867	1.1175	1.0881	1.1158	1.0138	1.1901
17	02-10-26	1.0938	0.0389	0.0421	0.0031	1.1018	1.0863	1.1176	1.0877	1.1159	1.0137	1.1900
18	03-03-26	1.1065	0.0439	0.0421	0.0031	1.1019	1.0863	1.1177	1.0877	1.1160	1.0137	1.1900
19	04-07-26	1.0947	0.0393	0.0420	0.0032	1.1015	1.0856	1.1176	1.0870	1.1159	1.0134	1.1896
20	05-05-26	1.0990	0.0410	0.0423	0.0027	1.1022	1.0886	1.1160	1.0899	1.1146	1.0141	1.1904

Note:

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

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

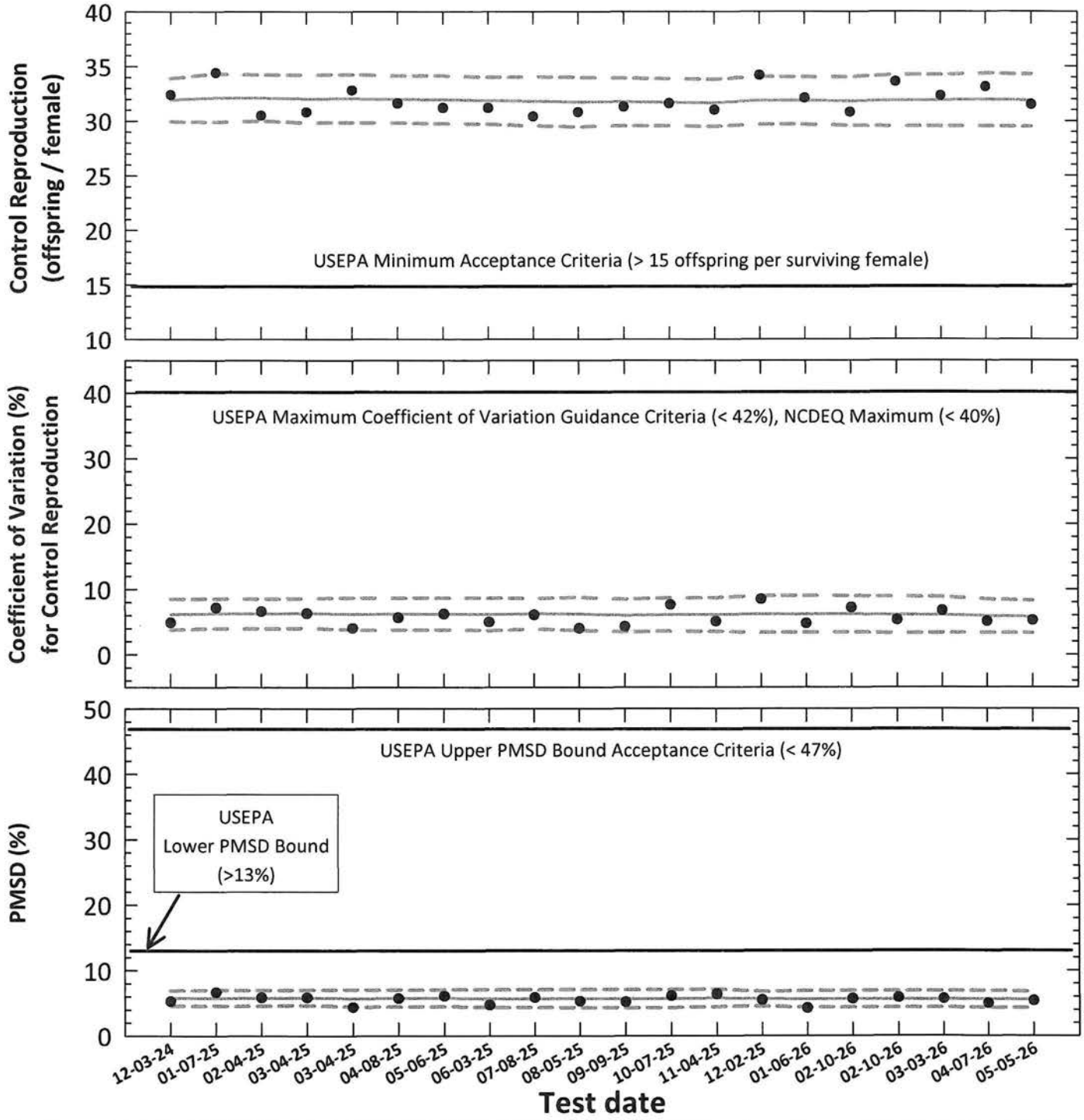
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

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ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control 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	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
2	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
3	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
4	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
5	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
6	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
7	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
8	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
9	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
10	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
11	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
12	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
13	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
14	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
15	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9
16	02-10-26	100	30.8	7.1	1.734	5.6	31.8	29.6	34.0	6.1	3.3	8.9	5.6	4.3	6.9
17	02-10-26	100	33.6	5.3	1.962	5.8	31.9	29.5	34.3	6.1	3.3	8.9	5.6	4.3	6.9
18	03-03-26	100	32.3	6.7	1.823	5.6	31.9	29.5	34.2	6.0	3.3	8.8	5.6	4.3	6.8
19	04-07-26	100	33.1	5.0	1.630	4.9	31.9	29.5	34.4	5.9	3.3	8.4	5.6	4.3	6.8
20	05-05-26	100	31.5	5.2	1.689	5.4	31.9	29.5	34.3	5.8	3.3	8.2	5.5	4.3	6.8

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 Summer

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 324

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2479				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	05-05-26 0430 TO 0740									
Culture board:	04-28-26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	5	8	11	12	13	17	18	23
Transfer vessel information:	pH (S.U.): 7.70 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	05-05-26	0752	04-29-26	04-29-26	04-29-26A	JK
1	05-06-26	0630	↓	↓	↓	JK
2	05-07-26	0632	↓	↓	04-29-26B	JK
3	05-08-26	0630	↓	↓	↓	JK
4	05-09-26	0700	↓	↓	05-07-26A	JK
5	05-10-26	0630	↓	↓	↓	JK
6	05-11-26	0711	↓	↓	↓	JK
7	05-12-26	0633				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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	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)	71400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	31.5	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	5.2%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1099.0

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 324**

CONTROL ***Survival and Reproduction Data***

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

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

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

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.4
% Reduction from Control:	-2.9%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 324

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	5	4	6	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	12	11	11	13	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	12	15	19	14	14	16	13	17	15	19
Total young produced		29	32	34	31	29	33	30	34	30	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	~~32.4~~ 31.6
% Reduction from Control:	-0.3%

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	5	4	3	5	5	5	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	9	12	12	12	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	14	14	16	12	15	16	16	13	18
Total young produced		31	28	27	30	29	32	32	30	28	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:	30.0
% Reduction from Control:	4.8%

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Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 324

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.8
% Reduction from Control:	43.5%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	1	3	1	1	2	2	2	2	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	2	0	1	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	1	0	0	0	0
Total young produced		3	3	2	1	2	3	2	2	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:	2.6
% Reduction from Control:	91.7%

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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	4	4	6	4	6	6	4	4	5	48
5	13	10	13	10	12	12	11	10	10	10	111
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	16	14	17	13	17	17	16	16	156
Total	**33**	**29**	**33**	**30**	**33**	**31**	**34**	**31**	**30**	**31**	**315**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	3	5	5	5	4	4	5	5	45
5	12	10	10	9	12	12	12	10	10	10	107
6	0	0	0	0	0	0	0	0	0	0	0
7	14	14	14	16	12	15	16	16	13	18	148
Total	**31**	**28**	**27**	**30**	**29**	**32**	**32**	**30**	**28**	**33**	**300**

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	4	4	4	6	6	4	4	5	48
5	13	11	13	10	12	10	10	13	13	12	117
6	0	0	0	0	0	0	0	0	0	0	0
7	14	17	16	18	16	13	16	16	16	17	159
Total	**33**	**33**	**33**	**32**	**32**	**29**	**32**	**33**	**33**	**34**	**324**

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	5	3	3	4	4	4	2	4	4	4	37
5	10	5	5	9	7	3	8	4	10	10	71
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	10	4	4	11	9	10	6	2	70
Total	**20**	**17**	**18**	**17**	**15**	**18**	**19**	**18**	**20**	**16**	**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	5	4	6	4	4	5	5	47
5	12	13	10	12	11	11	13	13	10	10	115
6	0	0	0	0	0	0	0	0	0	0	0
7	12	15	19	14	14	16	13	17	15	19	154
Total	**29**	**32**	**34**	**31**	**29**	**33**	**30**	**34**	**30**	**34**	**316**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	1	3	1	1	2	2	2	2	4	4	22
5	2	0	1	0	0	0	0	0	0	0	3
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	1	0	0	0	0	1
Total	**3**	**3**	**2**	**1**	**2**	**3**	**2**	**2**	**4**	**4**	**26**

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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: 324

Test dates: May 05-12, 2026

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	29	33	30	33	31	34	31	30	31	100	31.5	5.2	Not applicable
600	33	33	33	32	32	29	32	33	33	34	100	32.4	4.2	-2.9
800	29	32	34	31	29	33	30	34	30	34	100	31.6	6.5	-0.3
1000	31	28	27	30	29	32	32	30	28	33	100	30.0	6.7	4.8
1200	20	17	18	17	15	18	19	18	20	16	100	17.8	9.1	43.5
1400	3	3	2	1	2	3	2	2	4	4	100	2.6	37.2	91.7

Dunnett's MSD value: 1.689

PMSD: 5.4

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 (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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Statistical Analyses

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Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	5/5/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	5/12/2026	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	29.000	33.000	30.000	33.000	31.000	34.000	31.000	30.000	31.000
600	33.000	33.000	33.000	32.000	32.000	29.000	32.000	33.000	33.000	34.000
800	29.000	32.000	34.000	31.000	29.000	33.000	30.000	34.000	30.000	34.000
1000	31.000	28.000	27.000	30.000	29.000	32.000	32.000	30.000	28.000	33.000
1200	20.000	17.000	18.000	17.000	15.000	18.000	19.000	18.000	20.000	16.000
1400	3.000	3.000	2.000	1.000	2.000	3.000	2.000	2.000	4.000	4.000

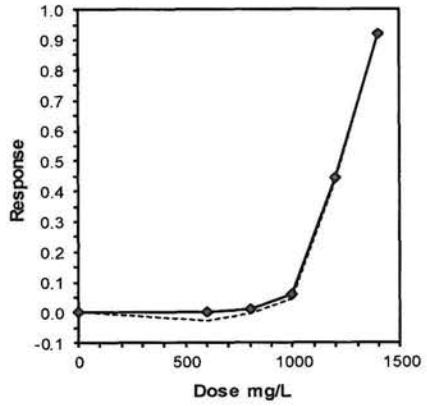
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.500	1.0000	31.500	29.000	34.000	5.238	10				31.950	1.0000
600	32.400	1.0286	32.400	29.000	34.000	4.166	10	-1.218	2.287	1.689	31.950	1.0000
800	31.600	1.0032	31.600	29.000	34.000	6.537	10	-0.135	2.287	1.689	31.600	0.9890
1000	30.000	0.9524	30.000	27.000	33.000	6.667	10	2.031	2.287	1.689	30.000	0.9390
*1200	17.800	0.5651	17.800	15.000	20.000	9.097	10	18.548	2.287	1.689	17.800	0.5571
*1400	2.600	0.0825	2.600	1.000	4.000	37.157	10	39.127	2.287	1.689	2.600	0.0814

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.61878	1.035	-0.1584	-0.699
Bartlett's Test indicates equal variances (p = 0.31)	5.92916	15.0863		

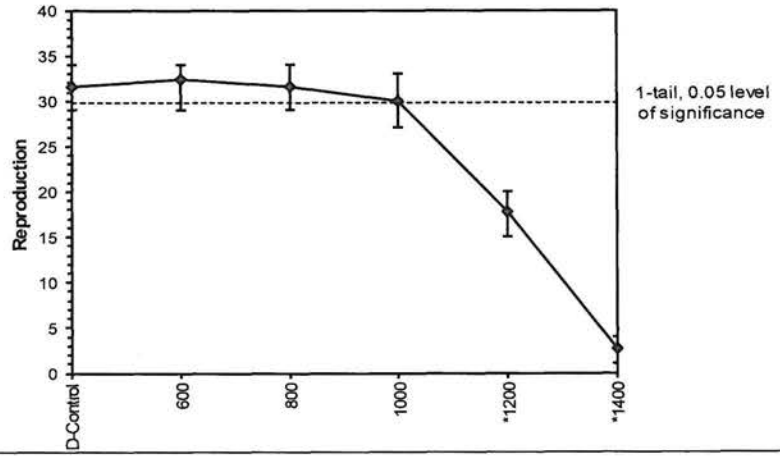
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.68897	0.05362	1432.74	2.72778	1.8E-44	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	955.938	59.0039	794.107	1013.76	-0.7496
IC10	1020.41	10.4	1002.12	1037.84	-0.5805
IC15	1046.6	8.60086	1030.45	1062.81	0.0683
IC20	1072.79	7.74719	1057.18	1087.14	0.1275
IC25	1098.98	7.14257	1085.63	1112.42	0.1728
IC40	1177.54	7.3276	1164.67	1190.93	0.1261
IC50	1224.01	5.69912	1212.58	1233.44	-0.1305



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 324**

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	✓	XL	XL	XL	XL	✓
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.96	7.87	7.74	7.81	7.71	7.93
	Dissolved oxygen (mg/L)	8.2	8.1	8.3	8.0	8.3	7.7
	Conductivity (µmhos/cm)	302		309		307	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	88				88	
	Temperature (°C)	25.1	25.3	24.8	25.3	24.9	25.0
600 mg NaCl/L	pH (S.U.)	7.96	7.86	7.79	7.81	7.78	7.92
	Dissolved oxygen (mg/L)	8.2	8.1	8.3	8.1	8.3	7.7
	Conductivity (µmhos/cm)	1360		1380		1390	
	Temperature (°C)	25.1	25.1	24.9	25.3	25.0	25.2
800 mg NaCl/L	pH (S.U.)	7.98	7.86	7.81	7.82	7.79	7.92
	Dissolved oxygen (mg/L)	8.2	8.1	8.2	8.1	8.3	7.7
	Conductivity (µmhos/cm)	1710		1760		1770	
	Temperature (°C)	25.1	25.1	25.0	25.0	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.99	7.87	7.82	7.82	7.79	7.93
	Dissolved oxygen (mg/L)	8.2	8.1	8.2	8.1	8.2	7.8
	Conductivity (µmhos/cm)	2060		2130		2130	
	Temperature (°C)	25.0	25.2	24.9	24.9	25.0	25.3
1200 mg NaCl/L	pH (S.U.)	8.00	7.88	7.83	7.83	7.80	7.93
	Dissolved oxygen (mg/L)	8.2	8.1	8.2	8.1	8.3	7.7
	Conductivity (µmhos/cm)	2390		2450		2460	
	Temperature (°C)	25.0	25.2	24.9	25.0	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	8.00	7.89	7.85	7.83	7.81	7.93
	Dissolved oxygen (mg/L)	8.2	8.2	8.3	8.1	8.3	7.7
	Conductivity (µmhos/cm)	2730		2770		2820	
	Temperature (°C)	25.0	25.2	25.1	25.1	25.0	25.3
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 324

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	VL	BSL	BSL	BSL	BSL	XL	XL	VL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.95	7.78	7.74	8.03	7.85	7.91	7.82	7.78
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.4	7.8
	Conductivity (µmhos/cm)	305		309		306		312	
	Alkalinity (mg $CaCO_3$/L)			60					
	Hardness (mg $CaCO_3$/L)			64					
	Temperature (°C)	24.9	25.1	24.9	25.3	24.9	25.3	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.99	7.87	7.75	8.03	7.83	7.91	7.84	7.81
	Dissolved oxygen (mg/L)	8.3	8.1	8.2	8.0	8.2	8.0	8.2	7.8
	Conductivity (µmhos/cm)	1330		1410		1390		1440	
	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.1	24.8	25.0
800 mg NaCl/L	pH (S.U.)	7.99	7.90	7.87	8.04	7.93	7.92	7.86	7.81
	Dissolved oxygen (mg/L)	8.3	8.1	8.2	8.0	8.2	8.0	8.2	7.8
	Conductivity (µmhos/cm)	1710		1800		1780		1820	
	Temperature (°C)	25.0	25.2	25.0	25.2	25.0	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.99	7.90	7.89	8.03	7.95	7.93	7.88	7.83
	Dissolved oxygen (mg/L)	8.3	8.0	8.2	8.0	8.2	8.0	8.3	7.8
	Conductivity (µmhos/cm)	2080		2170		2140		2170	
	Temperature (°C)	24.9	25.2	24.9	25.2	25.0	24.9	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.99	7.93	7.91	8.03	7.96	7.93	7.89	7.85
	Dissolved oxygen (mg/L)	8.3	8.0	8.2	8.0	8.2	8.0	8.3	7.8
	Conductivity (µmhos/cm)	2400		2510		2460		2550	
	Temperature (°C)	24.9	25.0	24.9	25.1	25.0	24.9	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	8.01	7.93	7.93	8.03	7.97	7.94	7.91	7.87
	Dissolved oxygen (mg/L)	8.3	8.1	8.2	8.0	8.2	8.0	8.3	7.8
	Conductivity (µmhos/cm)	2760		2890		2880		2960	
	Temperature (°C)	25.1	25.3	24.9	25.0	25.0	25.2	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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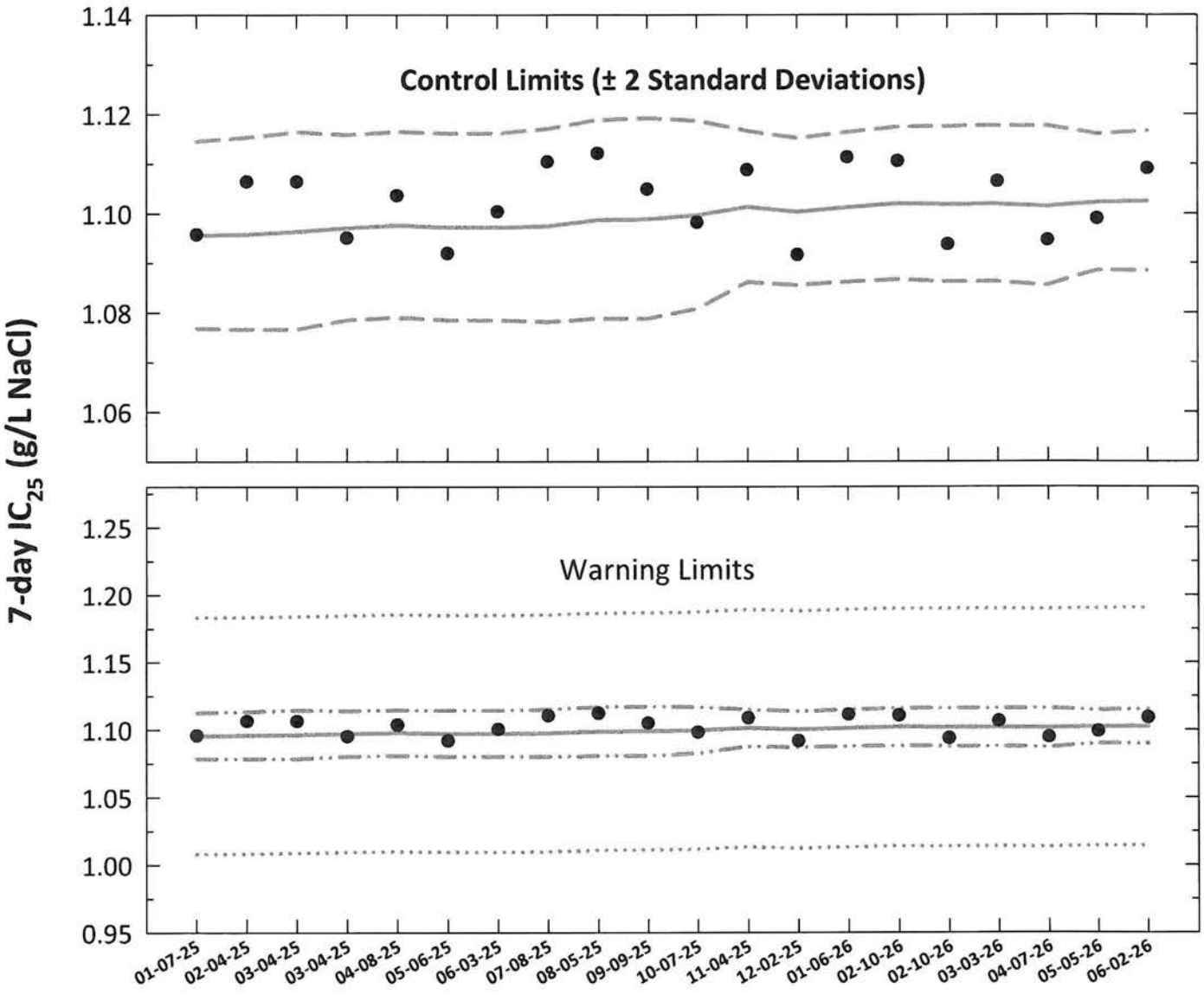
Ceriodaphnia dubia
Chronic Reference Toxicant Control Chart
Source: In-house Culture



- ● **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 $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

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

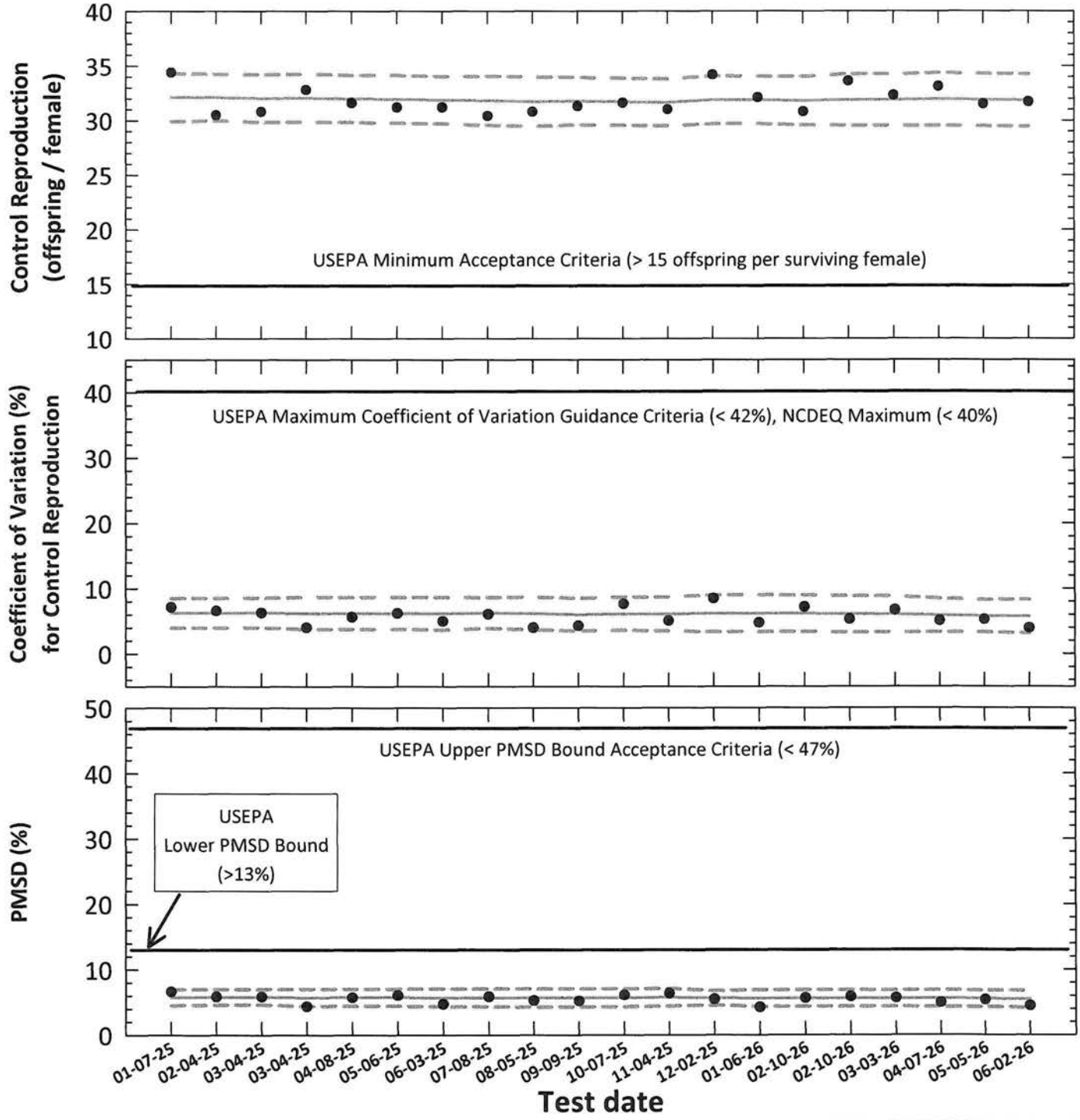
Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
2	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
3	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
4	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
5	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
6	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
7	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
8	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
9	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
10	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
11	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
12	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
13	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
14	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893
15	02-10-26	1.1106	0.0455	0.0422	0.0030	1.1020	1.0867	1.1175	1.0881	1.1158	1.0138	1.1901
16	02-10-26	1.0938	0.0389	0.0421	0.0031	1.1018	1.0863	1.1176	1.0877	1.1159	1.0137	1.1900
17	03-03-26	1.1065	0.0439	0.0421	0.0031	1.1019	1.0863	1.1177	1.0877	1.1160	1.0137	1.1900
18	04-07-26	1.0947	0.0393	0.0420	0.0032	1.1015	1.0856	1.1176	1.0870	1.1159	1.0134	1.1896
19	05-05-26	1.0990	0.0410	0.0423	0.0027	1.1022	1.0886	1.1160	1.0899	1.1146	1.0141	1.1904
20	06-02-26	1.1091	0.0450	0.0424	0.0028	1.1025	1.0886	1.1166	1.0899	1.1152	1.0143	1.1907

Note:
7-day IC_{25} = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
CT = Central tendency of the IC_{25} values.
S = Standard deviation of the IC_{25} values.
Control Limits = Mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values.
Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).
CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control	Control Reproduction		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		Survival (%)	Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
2	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
3	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
4	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
5	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
6	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
7	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
8	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
9	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
10	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
11	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
12	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
13	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
14	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9
15	02-10-26	100	30.8	7.1	1.734	5.6	31.8	29.6	34.0	6.1	3.3	8.9	5.6	4.3	6.9
16	02-10-26	100	33.6	5.3	1.962	5.8	31.9	29.5	34.3	6.1	3.3	8.9	5.6	4.3	6.9
17	03-03-26	100	32.3	6.7	1.823	5.6	31.9	29.5	34.2	6.0	3.3	8.8	5.6	4.3	6.8
18	04-07-26	100	33.1	5.0	1.630	4.9	31.9	29.5	34.4	5.9	3.3	8.4	5.6	4.3	6.8
19	05-05-26	100	31.5	5.2	1.689	5.4	31.9	29.5	34.3	5.8	3.3	8.2	5.5	4.3	6.8
20	06-02-26	100	31.7	3.9	1.418	4.5	31.8	29.5	34.2	5.7	3.1	8.3	5.5	4.1	6.8

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 #: 325

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2479				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	06·02·26 0530 TO 0751									
Culture board:	05·26·26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	4	5	7	12	15	16	19	22	26	27
Transfer vessel information:	pH (S.U.): 7.85 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	GOLD
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	06-02-26	0800	05·26·26	05·26·26	05·27·26 A	
1	06-03-26	0600	↓	↓	↓	
2	06-04-26	0600			06·01·26 A	
3	06-05-26	0605			↓	
4	06-06-26	0730			06·04·26 A	
5	06-07-26	0630			↓	
6	06-08-26	0735	↓	↓		
7	06-09-26	0604				

*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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664645

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:	31.7	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	3.97%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1109.1

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 325

CONTROL ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.7

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	5	4	6	5	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	14	12	12	12	12	13	13	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	16	16	14	12	15	17	17	19	13
Total young produced		33	34	33	30	30	32	35	35	34	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.7
% Reduction from Control:	-3.2%

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Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 325**

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	3	5	5	5	4	4	4	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	12	12	11	13	11	12	12	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	18	14	19	13	19	16	15	15	15
Total young produced		33	33	31	35	31	34	32	31	31	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:	32.4
% Reduction from Control:	-2.2%

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	3	5	4	4	6	4	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	11	12	13	12	12	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	14	14	16	12	15	15	16	14	14
Total young produced		29	31	29	32	31	31	31	33	29	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:	30.8
% Reduction from Control:	2.8%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 325

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.7
% Reduction from Control:	41.0%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	1	1	2	1	3	1	1	4	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	1	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		2	1	2	1	3	1	1	4	1	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:	1.8
% Reduction from Control:	94.3%

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Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	4	5	5	3	5	5	4	46
5	13	13	12	13	9	10	10	10	12	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	16	13	16	15	19	17	17	17	13	14	157
Total	**33**	**32**	**33**	**32**	**33**	**32**	**30**	**32**	**30**	**30**	**317**

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	5	4	6	5	5	5	4	5	49
5	13	14	12	12	12	12	13	13	11	13	125
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	16	14	12	15	17	17	19	13	153
Total	**33**	**34**	**33**	**30**	**30**	**32**	**35**	**35**	**34**	**31**	**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	5	3	5	5	5	4	4	4	6	6	47
5	14	12	12	11	13	11	12	12	10	12	119
6	0	0	0	0	0	0	0	0	0	0	0
7	14	18	14	19	13	19	16	15	15	15	158
Total	**33**	**33**	**31**	**35**	**31**	**34**	**32**	**31**	**31**	**33**	**324**

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	3	5	4	4	6	4	4	5	5	5	45
5	10	12	11	12	13	12	12	10	10	13	115
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	14	16	12	15	15	18	14	14	148
Total	**29**	**31**	**29**	**32**	**31**	**31**	**31**	**33**	**29**	**32**	**308**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	4	3	3	2	4	4	4	2	33
5	9	10	6	10	8	7	5	9	6	9	79
6	0	0	0	0	0	0	0	0	0	0	0
7	5	6	8	6	6	10	11	5	9	9	75
Total	**17**	**20**	**18**	**19**	**17**	**19**	**20**	**18**	**19**	**20**	**187**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	1	1	2	1	3	1	1	4	1	2	17
5	1	0	0	0	0	0	0	0	0	0	1
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**1**	**2**	**1**	**3**	**1**	**1**	**4**	**1**	**2**	**18**

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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: 325

Test dates: June 02-09, 2026

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	33	32	33	32	30	32	30	30	100	31.7	3.9	Not applicable
600	33	34	33	30	30	32	35	35	34	31	100	32.7	5.8	-3.2
800	33	33	31	35	31	34	32	31	31	33	100	32.4	4.4	-2.2
1000	29	31	29	32	31	31	31	33	29	32	100	30.8	4.5	2.8
1200	17	20	18	19	17	19	20	18	19	20	100	18.7	6.2	41.0
1400	2	1	2	1	3	1	1	4	1	2	100	1.8	57.4	94.3

Dunnett's MSD value: 1.418

PMSD: 4.5

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

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Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	6/2/2026	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2026	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	33.000	32.000	33.000	32.000	30.000	32.000	30.000	30.000
600	33.000	34.000	33.000	30.000	30.000	32.000	35.000	35.000	34.000	31.000
800	33.000	33.000	31.000	35.000	31.000	34.000	32.000	31.000	31.000	33.000
1000	29.000	31.000	29.000	32.000	31.000	31.000	31.000	33.000	29.000	32.000
1200	17.000	20.000	18.000	19.000	17.000	19.000	20.000	18.000	19.000	20.000
1400	2.000	1.000	2.000	1.000	3.000	1.000	1.000	4.000	1.000	2.000

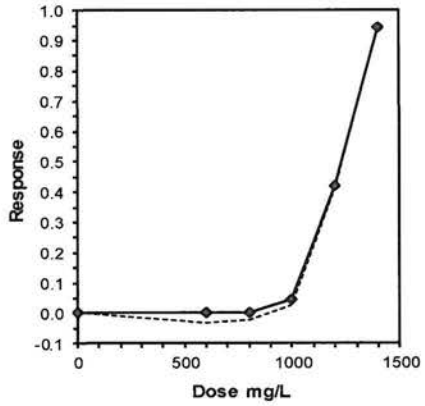
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	31.700	1.0000	31.700	30.000	33.000	3.948	10			32.267	1.0000
600	32.700	1.0315	32.700	30.000	35.000	5.775	10	123.00	75.00	32.267	1.0000
800	32.400	1.0221	32.400	31.000	35.000	4.413	10	117.50	75.00	32.267	1.0000
1000	30.800	0.9716	30.800	29.000	33.000	4.540	10	85.50	75.00	30.800	0.9545
*1200	18.700	0.5899	18.700	17.000	20.000	6.201	10	55.00	75.00	18.700	0.5795
*1400	1.800	0.0568	1.800	1.000	4.000	57.378	10	55.00	75.00	1.800	0.0558

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.25452	1.035	-0.0747	-0.795
Bartlett's Test indicates equal variances (p = 0.55)	3.96652	15.0863		

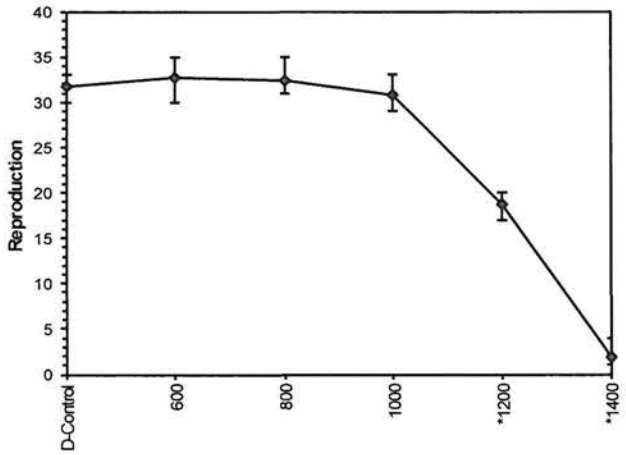
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1002.42	26.1897	928.216	1013.47	-1.0240
IC10	1029.09	6.90613	1013.55	1039.22	-0.2035
IC15	1055.76	6.2269	1040.88	1066.19	-0.1659
IC20	1082.42	5.71897	1069.48	1092.47	-0.1252
IC25	1109.09	5.43063	1097.6	1118.7	-0.0822
IC40	1189.09	6.01298	1176.7	1200.25	0.0194
IC50	1230.37	3.93603	1221.92	1236.8	-0.1825



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	6/2/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2026	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:					

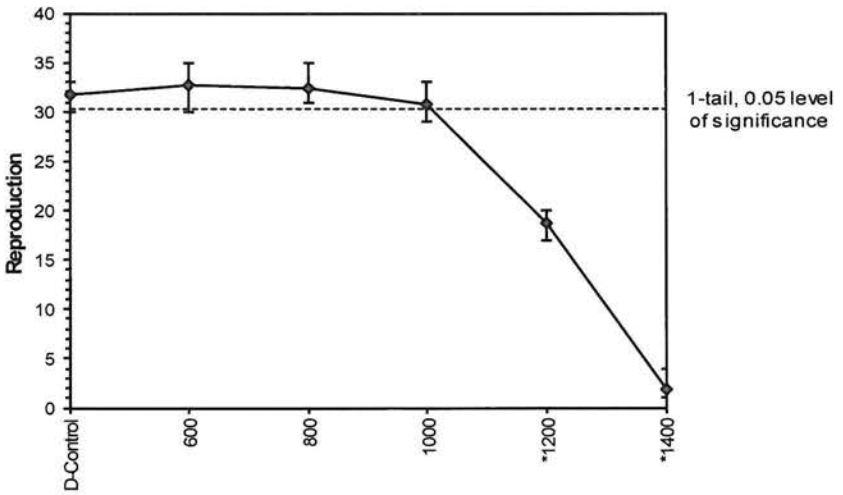
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	32.000	33.000	32.000	33.000	32.000	30.000	32.000	30.000	30.000
600	33.000	34.000	33.000	30.000	30.000	32.000	35.000	35.000	34.000	31.000
800	33.000	33.000	31.000	35.000	31.000	34.000	32.000	31.000	31.000	33.000
1000	29.000	31.000	29.000	32.000	31.000	31.000	31.000	33.000	29.000	32.000
1200	17.000	20.000	18.000	19.000	17.000	19.000	20.000	18.000	19.000	20.000
1400	2.000	1.000	2.000	1.000	3.000	1.000	1.000	4.000	1.000	2.000

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	31.700	1.0000	31.700	30.000	33.000	3.948	10			
600	32.700	1.0315	32.700	30.000	35.000	5.775	10	-1.612	2.287	1.418
800	32.400	1.0221	32.400	31.000	35.000	4.413	10	-1.128	2.287	1.418
1000	30.800	0.9716	30.800	29.000	33.000	4.540	10	1.451	2.287	1.418
*1200	18.700	0.5899	18.700	17.000	20.000	6.201	10	20.956	2.287	1.418
*1400	1.800	0.0568	1.800	1.000	4.000	57.378	10	48.200	2.287	1.418

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.25452	1.035	-0.0747	-0.795
Bartlett's Test indicates equal variances (p = 0.55)	3.96652	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		1.4185	0.04475	1539.82	1.92407	0.0E+00	5, 54

Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 325

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.

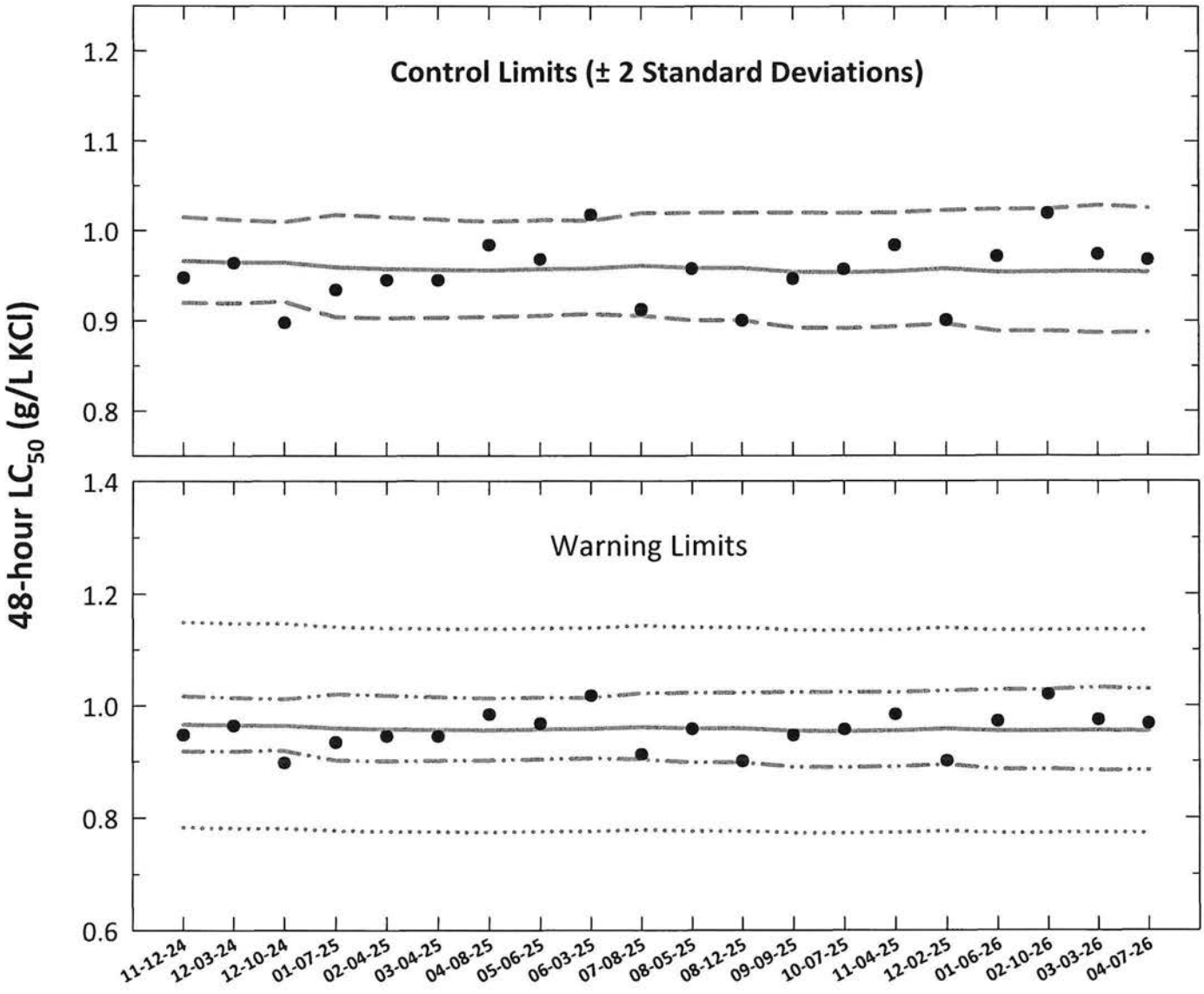
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.72	7.87	7.74	7.85	7.76	7.88
	Dissolved oxygen (mg/L)	8.5	8.2	8.4	8.2	8.5	8.0
	Conductivity (µmhos/cm)	306		310		303	
	Alkalinity (mg $CaCO_3$/L)	60				62	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.9	25.0	24.9	25.2	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.81	7.86	7.82	7.85	7.81	7.88
	Dissolved oxygen (mg/L)	8.6	8.2	8.4	8.2	8.5	8.1
	Conductivity (µmhos/cm)	1370		1390		1360	
	Temperature (°C)	24.9	25.2	25.0	25.0	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.82	7.87	7.83	7.86	7.82	7.87
	Dissolved oxygen (mg/L)	8.6	8.2	8.4	8.2	8.5	8.0
	Conductivity (µmhos/cm)	1750		1790		1770	
	Temperature (°C)	25.0	25.2	25.0	25.0	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.84	7.88	7.84	7.87	7.83	7.88
	Dissolved oxygen (mg/L)	8.6	8.2	8.4	8.2	8.5	8.0
	Conductivity (µmhos/cm)	2120		2140		2120	
	Temperature (°C)	25.0	25.1	25.0	24.9	25.0	25.3
1200 mg NaCl/L	pH (S.U.)	7.85	7.88	7.84	7.87	7.84	7.89
	Dissolved oxygen (mg/L)	8.6	8.2	8.4	8.2	8.5	8.1
	Conductivity (µmhos/cm)	2440		2510		2460	
	Temperature (°C)	25.0	25.3	25.0	24.9	25.0	25.3
1400 mg NaCl/L	pH (S.U.)	7.86	7.89	7.87	7.88	7.86	7.89
	Dissolved oxygen (mg/L)	8.5	8.2	8.4	8.2	8.5	8.1
	Conductivity (µmhos/cm)	2830		2870		2820	
	Temperature (°C)	25.0	25.1	25.0	25.1	24.9	25.4
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 325**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.78	7.83	7.77	7.96	7.82	7.86	7.77	8.20
	Dissolved oxygen (mg/L)	8.4	8.3	8.5	8.0	8.4	8.0	8.4	7.7
	Conductivity (µmhos/cm)	307		292		287		309	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.9	25.2	24.9	25.2	24.9	25.2	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.83	7.96	7.81	8.00	7.90	7.87	7.80	8.18
	Dissolved oxygen (mg/L)	8.3	8.3	8.6	8.1	8.6	8.0	8.3	7.7
	Conductivity (µmhos/cm)	1450		1370		1380		1460	
	Temperature (°C)	25.0	25.0	24.8	25.0	24.9	25.2	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.84	7.96	7.89	8.00	7.98	7.88	7.81	8.14
	Dissolved oxygen (mg/L)	8.3	8.3	8.5	8.1	8.6	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1780		1680		1710		1820	
	Temperature (°C)	25.0	25.0	24.8	25.0	25.0	25.3	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.86	7.96	7.92	8.00	7.99	7.88	7.83	8.12
	Dissolved oxygen (mg/L)	8.3	8.3	8.5	8.1	8.4	8.0	8.3	7.6
	Conductivity (µmhos/cm)	2120		2030		2050		2160	
	Temperature (°C)	25.0	25.3	24.8	25.2	25.0	25.1	25.0	25.0
1200 mg NaCl/L	pH (S.U.)	7.87	7.97	7.93	8.01	8.00	7.89	7.86	8.08
	Dissolved oxygen (mg/L)	8.3	8.2	8.5	8.2	8.6	8.0	8.3	7.6
	Conductivity (µmhos/cm)	2490		2380		2400		2530	
	Temperature (°C)	24.8	25.1	24.9	25.2	25.0	25.1	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.89	7.97	7.93	8.01	8.00	7.89	7.88	8.08
	Dissolved oxygen (mg/L)	8.3	8.2	8.5	8.2	8.6	8.1	8.3	7.6
	Conductivity (µmhos/cm)	2860		2740		2750		2870	
	Temperature (°C)	24.8	25.1	24.9	25.3	25.0	25.3	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	11-12-24	0.9473	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
2	12-03-24	0.9637	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
3	12-10-24	0.8977	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
4	01-07-25	0.9340	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
5	02-04-25	0.9448	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
6	03-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
7	04-08-25	0.9839	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
8	05-06-25	0.9680	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
9	06-03-25	1.0177	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
10	07-08-25	0.9124	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
11	08-05-25	0.9576	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
12	08-12-25	0.9003	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
13	09-09-25	0.9465	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
14	10-07-25	0.9572	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
15	11-04-25	0.9840	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
16	12-02-25	0.9007	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
17	01-06-26	0.9716	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
18	02-10-26	1.0197	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
19	03-03-26	0.9741	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367
20	04-07-26	0.9680	-0.0141	-0.0204	0.0157	0.9541	0.8874	1.0258	0.8842	1.0293	0.7728	1.1354

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 # 190

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 #: 2465

Chemical Analyses:

Concentration	Analyst	Hours 0	Hours 24	Hours 48
Control, MHSW	pH (S.U.)	7.79	7.95	7.92
	Dissolved oxygen (mg/L)	8.6	8.2	8.2
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	85		
	Temperature (°C)	24.4	25.0	24.6
500 mg/L	pH (S.U.)	7.97	7.95	7.91
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	24.3	25.1	24.9
750 mg/L	pH (S.U.)	7.98	7.96	7.91
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	1580		
	Temperature (°C)	24.6	25.1	24.5
1000 mg/L	pH (S.U.)	7.98	7.97	7.92
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	1970		
	Temperature (°C)	24.5	25.1	24.8
1250 mg/L	pH (S.U.)	7.99	7.97	7.92
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	2350		
	Temperature (°C)	24.7	24.8	24.9
1500 mg/L	pH (S.U.)	8.00	7.98	
	Dissolved oxygen (mg/L)	8.5	8.2	
	Conductivity (µmhos/cm)	2790		
	Temperature (°C)	24.4	24.8	

04-08-26

*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 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066470

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 # 190

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-07-26	* 0605 / 0910	JP	1130	JP	1E	Green	03-30-26A
24	04-08-26			1130	JP			
48 Termination	04-09-26			1122	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:	03-26-26
Age (1 to 14 days old):	5 to 6 days
Hatch date and times:	04-01-26 1121 to 04-02-26 0750
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.05
	Temperature (°C): 25.8 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[12]	10	5[12]	6[12]	1[9c]	1[4c]	0[12c]	0[12c]
48 Termination	10	10	10	10	9	9[12]	5	5	0[12]	1	0	0
Mean Survival	100%		100%		90%		50%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	894.0
Upper 95% confidence limit (mg KCl/L)	1038.1
48-hour LC_{50} (mg KCl/L)	968.0

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:	4/7/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/9/2026	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	1.0000
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	0.1726	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.345	2.850	0.1726	1	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	17.998	2.850	0.1726	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

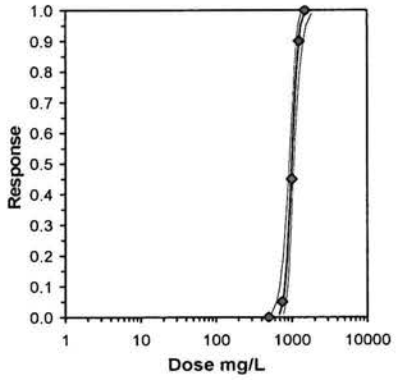
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08088	0.08295	0.45815	0.00367	3.4E-05	4, 5
Treatments vs D-Control										

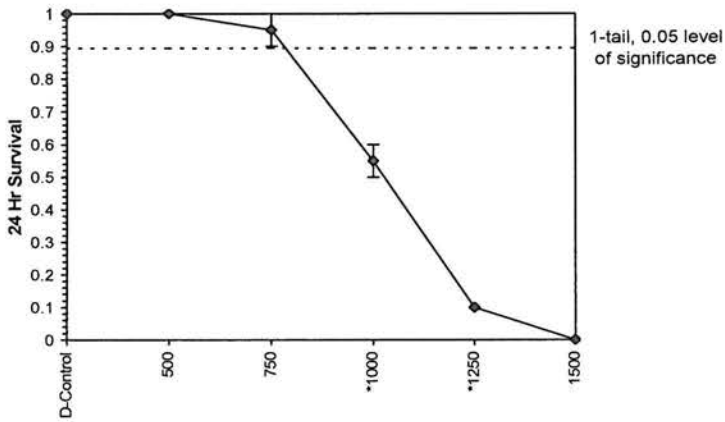
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.878	2.55514	8.86995	18.8861	0	0.33293	7.81472	0.95372	3.00364	0.07206	3
Intercept	-36.685	7.71138	-51.799	-21.57							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	685.512	534.371	776.263
EC05	3.355	767.576	634.876	847.382
EC10	3.718	815.266	695.02	889.131
EC15	3.964	849.106	738.116	919.289
EC20	4.158	876.999	773.682	944.697
EC25	4.326	901.659	804.984	967.743
EC40	4.747	966.915	885.801	1032.74
EC50	5.000	1008.42	934.355	1078.43
EC60	5.253	1051.72	981.578	1130.72
EC75	5.674	1127.83	1055.76	1234.51
EC80	5.842	1159.55	1083.87	1281.67
EC85	6.036	1197.64	1116.01	1340.78
EC90	6.282	1247.35	1155.95	1421.36
EC95	6.645	1324.85	1215.02	1553.3
EC99	7.326	1483.45	1328.6	1842.3



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	4/7/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/9/2026	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	0.0000	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	0.1469	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	3.162	2.850	0.1469	2	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	12.159	2.850	0.1469	10	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	22.737	2.850	0.1469	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

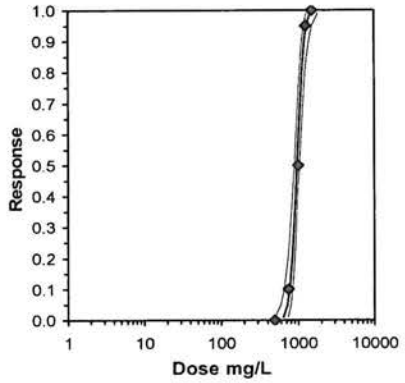
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.06555	0.06723	0.51142	0.00266	1.2E-05	4, 5
Treatments vs D-Control										

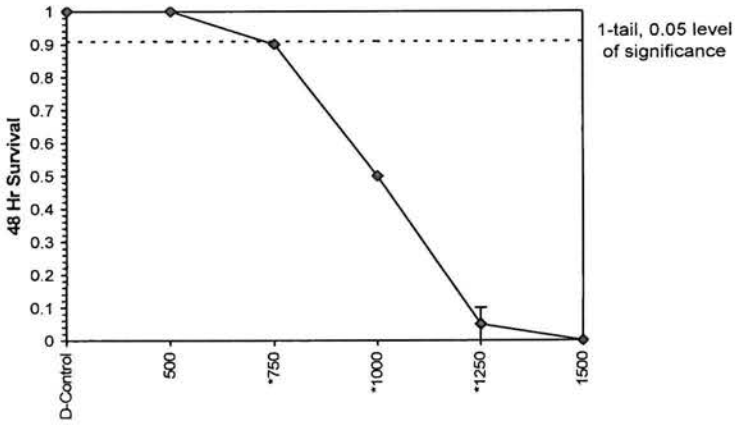
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2148	2.40897	8.4932	17.9364	0	0.94914	7.81472	0.81355	2.98587	0.07567	3
Intercept	-34.458	7.23006	-48.629	-20.287							
TSCR											

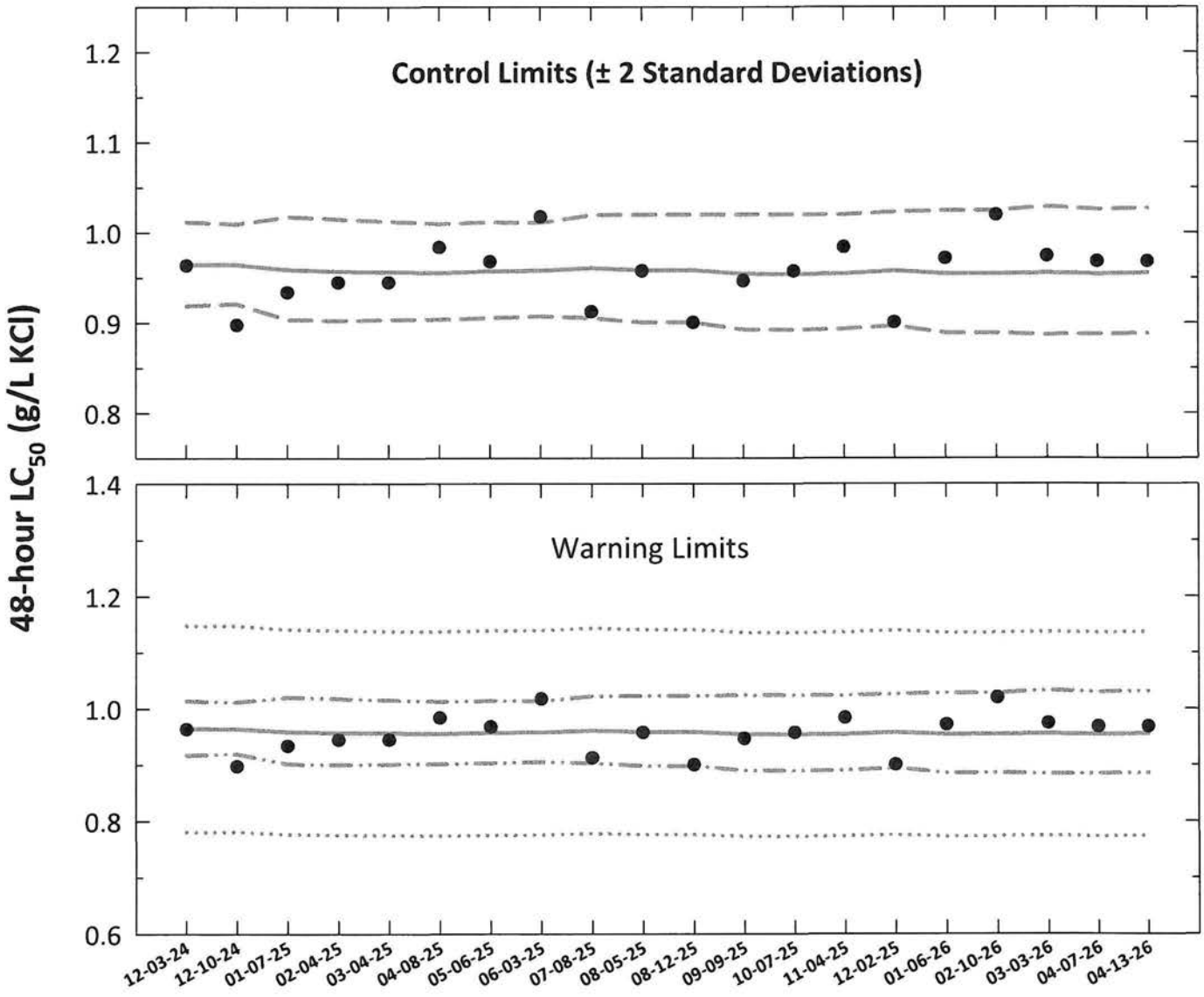
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	645.405	498.603	734.866
EC05	3.355	726.78	596.936	805.88
EC10	3.718	774.274	656.126	847.698
EC15	3.964	808.06	698.686	877.957
EC20	4.158	835.96	733.899	903.478
EC25	4.326	860.663	764.958	926.643
EC40	4.747	926.194	845.444	992.045
EC50	5.000	967.996	894.019	1038.06
EC60	5.253	1011.68	941.438	1090.77
EC75	5.674	1088.71	1016.28	1195.58
EC80	5.842	1120.89	1044.74	1243.31
EC85	6.036	1159.59	1077.35	1303.25
EC90	6.282	1210.19	1117.94	1385.13
EC95	6.645	1289.27	1178.14	1519.66
EC99	7.326	1451.83	1294.34	1816.06



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	12-03-24	0.9637	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
2	12-10-24	0.8977	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
3	01-07-25	0.9340	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
4	02-04-25	0.9448	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
5	03-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
6	04-08-25	0.9839	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
7	05-06-25	0.9680	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
8	06-03-25	1.0177	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
9	07-08-25	0.9124	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
10	08-05-25	0.9576	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
11	08-12-25	0.9003	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
12	09-09-25	0.9465	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
13	10-07-25	0.9572	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
14	11-04-25	0.9840	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
15	12-02-25	0.9007	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
16	01-06-26	0.9716	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
17	02-10-26	1.0197	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
18	03-03-26	0.9741	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367
19	04-07-26	0.9680	-0.0141	-0.0204	0.0157	0.9541	0.8874	1.0258	0.8842	1.0293	0.7728	1.1354
20	04-13-26	0.9680	-0.0141	-0.0199	0.0158	0.9551	0.8882	1.0271	0.8851	1.0305	0.7737	1.1366

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

ETC

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

Pimephales promelas **Potassium Chloride Acute Reference Toxicant Test**

PpKClAC # (19) ~~2478~~ 04-13.26

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 #: 2478

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	✓	XL
Control, MHSW	pH (S.U.)	7.75	7.52	7.95
	Dissolved oxygen (mg/L)	8.6	7.2	8.2
	Conductivity (µmhos/cm)	308		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	85		
	Temperature (°C)	24.6	24.8	24.9
500 mg/L	pH (S.U.)	7.90	7.49	7.94
	Dissolved oxygen (mg/L)	8.3	7.3	8.1
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	24.5	24.5	24.6
750 mg/L	pH (S.U.)	7.92	7.62	7.94
	Dissolved oxygen (mg/L)	8.3	7.3	8.1
	Conductivity (µmhos/cm)	1570		
	Temperature (°C)	25.1	24.5	24.6
1000 mg/L	pH (S.U.)	7.93	7.63	7.93
	Dissolved oxygen (mg/L)	8.2	6.7	8.1
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	25.0	24.7	24.8
1250 mg/L	pH (S.U.)	7.94	7.62	7.92
	Dissolved oxygen (mg/L)	8.2	6.7	8.1
	Conductivity (µmhos/cm)	2420		
	Temperature (°C)	24.9	24.7	24.8
1500 mg/L	pH (S.U.)	7.95	7.62	
	Dissolved oxygen (mg/L)	8.2	6.4	
	Conductivity (µmhos/cm)	2830		
	Temperature (°C)	24.9	24.6	

04-14-26

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066470

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 # 191

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	04-13-26	* 0600	JP	0840	JP	1C	Yellow	04-0926A
24	04-14-26			0840	JP			
48 Termination	04-15-26			0840	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:	03-31-26
Age (1 to 14 days old):	6 to 7 days old
Hatch date and times:	04-06-26 1330 to 04-07-26 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.05
	Temperature (°C): 25.2°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	10	7 3d	6 4d	0 10d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	9	9 1d	6 1d	4 2d	0	1 1d	0	0
Mean Survival	100%		100%		90%		50%		5%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	894.0
Upper 95% confidence limit (mg KCl/L)	1038.1
48-hour LC_{50} (mg KCl/L)	968.0

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:	4/13/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	4/15/2026	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	1.0000
1000	0.7000	0.6000
1250	0.0000	0.2000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.2952	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.781	2.830	0.2952	1	20
*1000	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	4.539	2.830	0.2952	7	20
*1250	0.1000	0.1000	0.3112	0.1588	0.4636	69.269	2	10.554	2.830	0.2952	18	20
*1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	12.016	2.830	0.2952	20	20

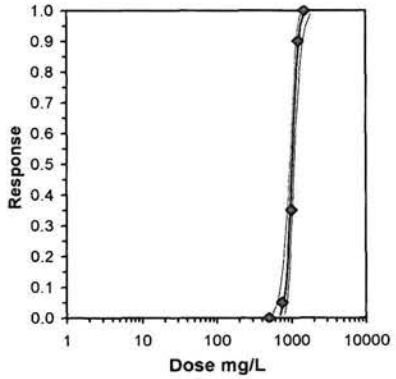
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.1673	0.17159	0.64112	0.01088	5.1E-05	5, 6
Treatments vs D-Control										

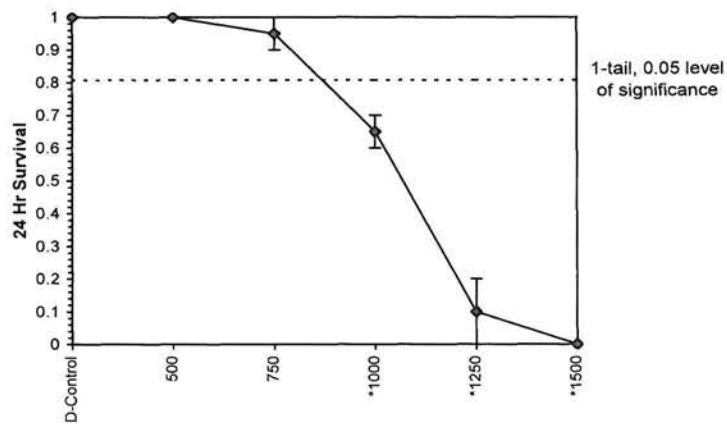
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.2779	2.66356	9.05734	19.4985	0	1.27554	7.81472	0.73495	3.01328	0.07004	3
Intercept	-38.023	8.06293	-53.827	-22.22							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	708.508	553.572	800.291
EC05	3.355	790.816	655.37	871.222
EC10	3.718	838.533	716.131	912.782
EC15	3.964	872.345	759.602	942.773
EC20	4.158	900.186	795.437	968.027
EC25	4.326	924.779	826.945	990.925
EC40	4.747	989.77	908.143	1055.5
EC50	5.000	1031.05	956.789	1100.94
EC60	5.253	1074.04	1003.96	1152.99
EC75	5.674	1149.52	1077.74	1256.39
EC80	5.842	1180.93	1105.6	1303.36
EC85	6.036	1218.62	1137.41	1362.22
EC90	6.282	1267.76	1176.85	1442.37
EC95	6.645	1344.25	1235.09	1573.41
EC99	7.326	1500.42	1346.79	1859.67



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	4/13/2026	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	4/15/2026	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	0.6000	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	0.2335	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	1.989	2.850	0.2335	2	20
*1000	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	7.649	2.850	0.2335	10	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	14.304	2.850	0.2335	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

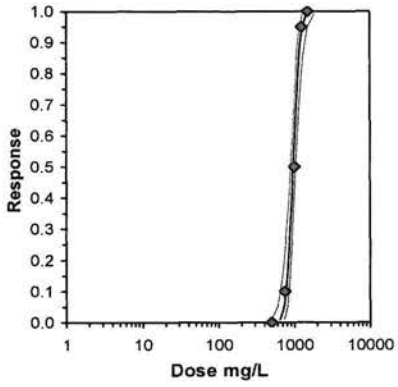
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12113	0.12423	0.51142	0.00671	1.1E-04	4, 5
Treatments vs D-Control										

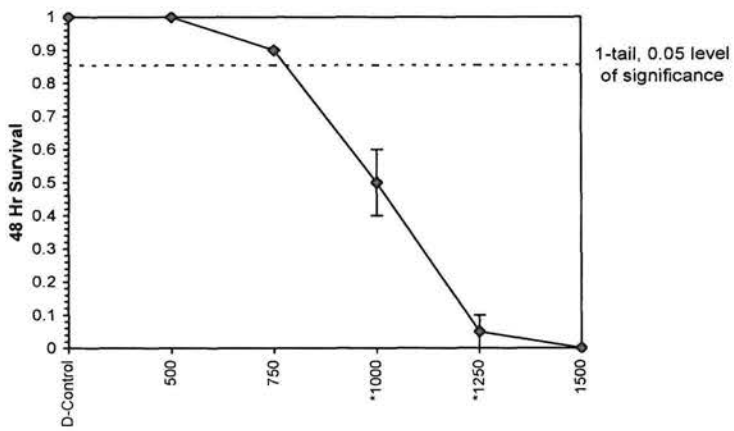
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2148	2.40897	8.4932	17.9364	0	0.94914	7.81472	0.81355	2.98587	0.07567	3
Intercept	-34.458	7.23006	-48.629	-20.287							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	645.405	498.603	734.866
EC05	3.355	726.78	596.936	805.88
EC10	3.718	774.274	656.126	847.698
EC15	3.964	808.06	698.686	877.957
EC20	4.158	835.96	733.899	903.478
EC25	4.326	860.663	764.958	926.643
EC40	4.747	926.194	845.444	992.045
EC50	5.000	967.996	894.019	1038.06
EC60	5.253	1011.68	941.438	1090.77
EC75	5.674	1088.71	1016.28	1195.58
EC80	5.842	1120.89	1044.74	1243.31
EC85	6.036	1159.59	1077.35	1303.25
EC90	6.282	1210.19	1117.94	1385.13
EC95	6.645	1289.27	1178.14	1519.66
EC99	7.326	1451.83	1294.34	1816.06

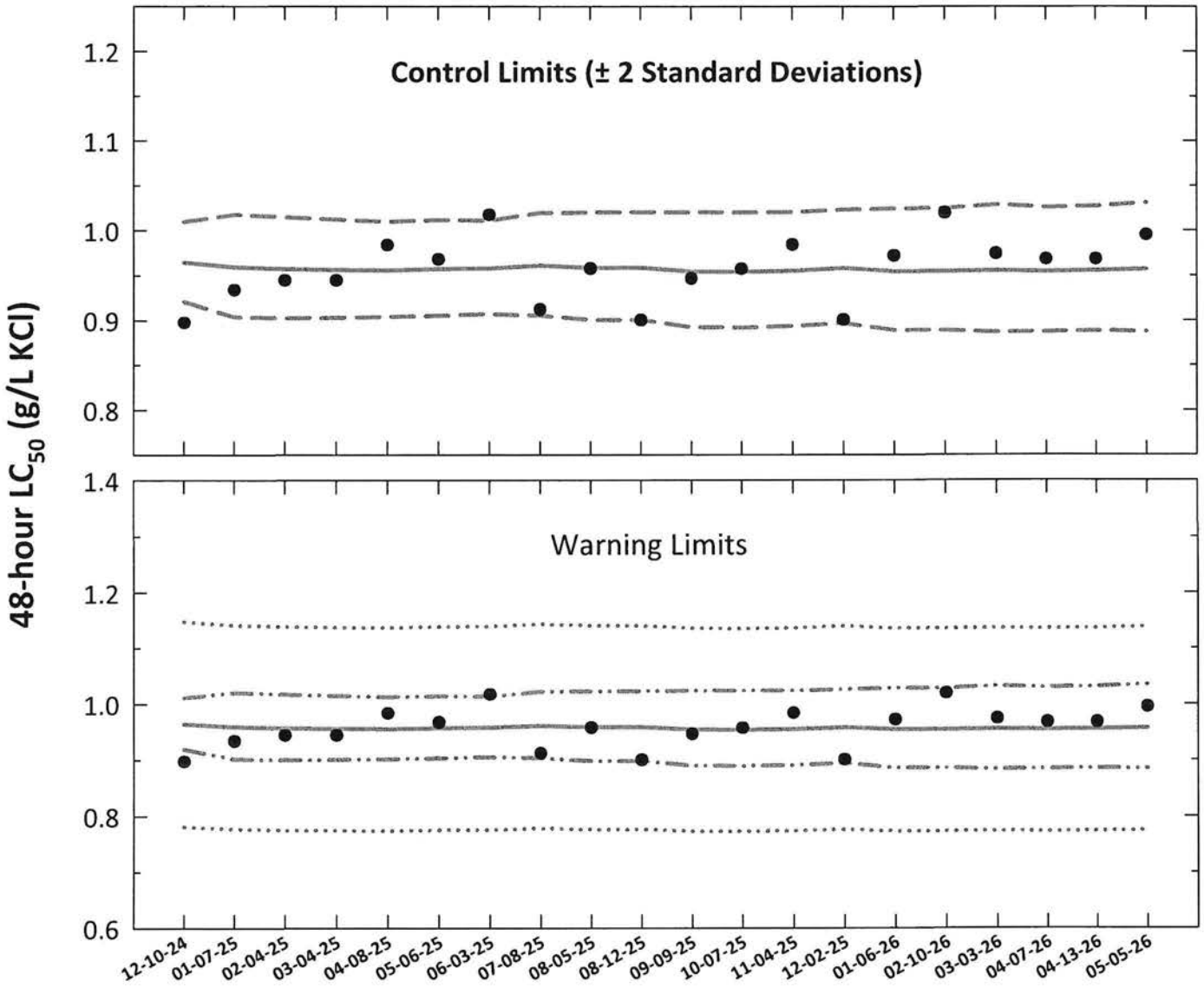


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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	**12-10-24**	**0.8977**	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
2	**01-07-25**	**0.9340**	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
3	**02-04-25**	**0.9448**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
4	**03-04-25**	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
5	**04-08-25**	**0.9839**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
6	**05-06-25**	**0.9680**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
7	**06-03-25**	**1.0177**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
8	**07-08-25**	**0.9124**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
9	**08-05-25**	**0.9576**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
10	**08-12-25**	**0.9003**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
11	**09-09-25**	**0.9465**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
12	**10-07-25**	**0.9572**	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
13	**11-04-25**	**0.9840**	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
14	**12-02-25**	**0.9007**	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
15	**01-06-26**	**0.9716**	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
16	**02-10-26**	**1.0197**	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
17	**03-03-26**	**0.9741**	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367
18	**04-07-26**	**0.9680**	-0.0141	-0.0204	0.0157	0.9541	0.8874	1.0258	0.8842	1.0293	0.7728	1.1354
19	**04-13-26**	**0.9680**	-0.0141	-0.0199	0.0158	0.9551	0.8882	1.0271	0.8851	1.0305	0.7737	1.1366
20	**05-05-26**	**0.9950**	-0.0022	-0.0192	0.0163	0.9567	0.8877	1.0310	0.8845	1.0344	0.7749	1.1384

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 # 192

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 #: 2478

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	KL	XL	XL
Control, MHSW	pH (S.U.)	7.96	7.89	7.82
	Dissolved oxygen (mg/L)	8.2	8.0	8.0
	Conductivity (µmhos/cm)	302		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	25.6	25.3	25.0
500 mg/L	pH (S.U.)	8.06	7.86	7.79
	Dissolved oxygen (mg/L)	8.2	8.0	8.0
	Conductivity (µmhos/cm)	1110		
	Temperature (°C)	25.8	25.2	25.2
750 mg/L	pH (S.U.)	8.05	7.87	7.79
	Dissolved oxygen (mg/L)	8.2	8.0	8.0
	Conductivity (µmhos/cm)	1550		
	Temperature (°C)	25.9	25.4	25.5
1000 mg/L	pH (S.U.)	8.05	7.85	7.80
	Dissolved oxygen (mg/L)	8.2	7.8	7.9
	Conductivity (µmhos/cm)	1440		
	Temperature (°C)	25.8	25.4	25.1
1250 mg/L	pH (S.U.)	8.06	7.83	7.80
	Dissolved oxygen (mg/L)	8.2	7.7	7.9
	Conductivity (µmhos/cm)	2350		
	Temperature (°C)	25.9	25.2	25.6
1500 mg/L	pH (S.U.)	8.07	7.82	
	Dissolved oxygen (mg/L)	8.3	7.6	
	Conductivity (µmhos/cm)	2770		
	Temperature (°C)	25.9	25.2	

15-08-25 M

*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 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066470

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 # 192

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05-05-26	* 0500	JP	0828	JP	E 11	White	04-29-26A
24	05-06-26			0835	JP			
48 Termination	05-07-26			0830	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:	04-23-26
Age (1 to 14 days old):	5 to 6 days
Hatch date and times:	04-29-26 1139 to 04-30-26 0600
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.90
	Temperature (°C): 25.0°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	6 4d	7 3d	2 8d	1 9d	0 12d	0 12d
48 Termination	10	10	10	10	9 12d	10	5 12d	7	0 12d	0 12d	0	0
Mean Survival	100%		100%		95%		60%		0%		0%	

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

Statistics:

Method	probit
Lower 95% confidence limit (mg KCl/L)	926.1
Upper 95% confidence limit (mg KCl/L)	1058.2
48-hour LC_{50} (mg KCl/L)	994.9

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:	5/5/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/7/2026	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.7000
1250	0.2000	0.1000
1500	0.0000	0.0000

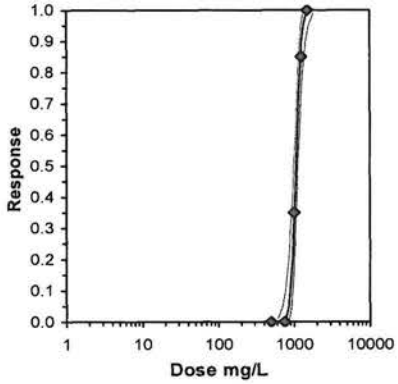
			Transform: Arcsin Square Root					Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0	20
1000	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	7	20
1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	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				

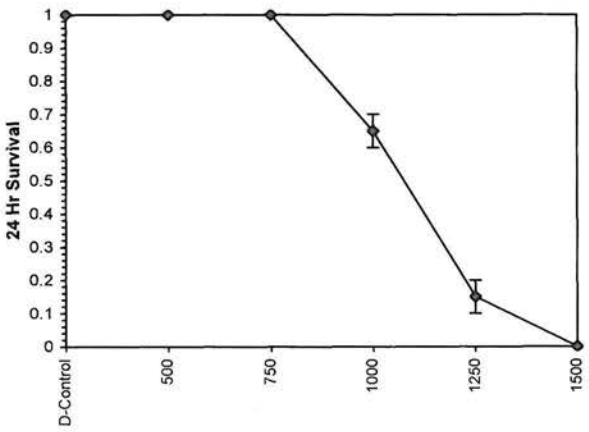
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.8087	3.41655	10.1123	23.5052	0	0.45103	7.81472	0.92951	3.02777	0.05949	4
Intercept	-45.893	10.3825	-66.243	-25.543							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	775.112	610.381	864.895
EC05	3.355	850.959	709.996	928.314
EC10	3.718	894.381	768.646	965.178
EC15	3.964	924.923	810.262	991.691
EC20	4.158	949.938	844.353	1013.98
EC25	4.326	971.938	874.161	1034.19
EC40	4.747	1029.66	950.156	1091.34
EC50	5.000	1066.02	994.957	1131.84
EC60	5.253	1103.67	1037.71	1178.54
EC75	5.674	1169.21	1102.98	1271.8
EC80	5.842	1196.29	1127.15	1314.14
EC85	6.036	1228.64	1154.48	1367.07
EC90	6.282	1270.6	1188.04	1438.83
EC95	6.645	1335.44	1237.06	1555.37
EC99	7.326	1466.11	1329.68	1806.62



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:	5/5/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/7/2026	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	1.0000
1000	0.5000	0.7000
1250	0.0000	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	0.2366	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.982	2.850	0.2366	1	20
*1000	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	6.310	2.850	0.2366	8	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.099	2.850	0.2366	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

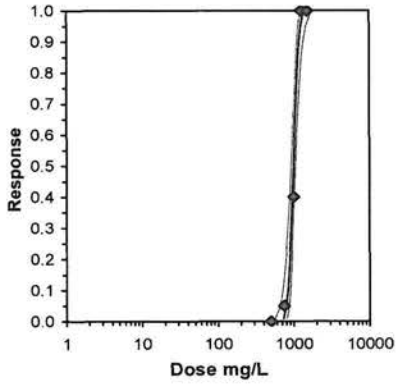
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12332	0.12648	0.58038	0.00689	9.0E-05	4, 5
Treatments vs D-Control										

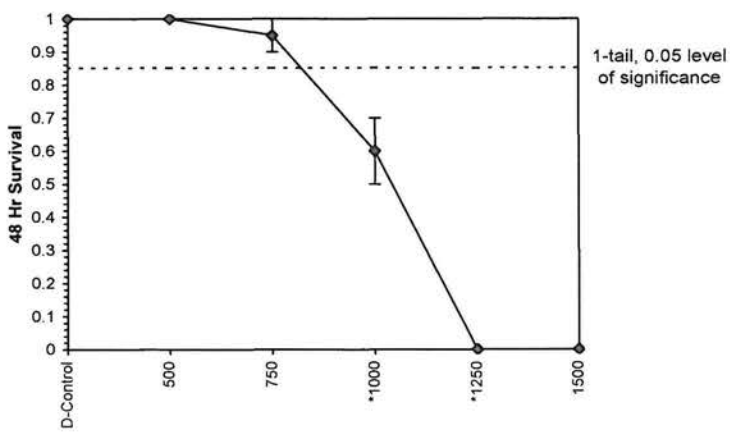
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.1625	3.55509	10.1945	24.1304	0	3.22985	7.81472	0.35753	2.99782	0.05827	6
Intercept	-46.45	10.6997	-67.421	-25.479							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	728.226	570.842	813.013
EC05	3.355	797.948	663.071	871.255
EC10	3.718	837.806	717.288	905.116
EC15	3.964	865.816	755.722	929.476
EC20	4.158	888.744	787.183	949.964
EC25	4.326	908.897	814.678	968.539
EC40	4.747	961.73	884.751	1021.05
EC50	5.000	994.982	926.087	1058.19
EC60	5.253	1029.38	965.58	1100.98
EC75	5.674	1089.22	1025.91	1186.38
EC80	5.842	1113.92	1048.21	1225.19
EC85	6.036	1143.42	1073.38	1273.75
EC90	6.282	1181.64	1104.2	1339.65
EC95	6.645	1240.67	1149.04	1446.75
EC99	7.326	1359.45	1233.37	1677.75

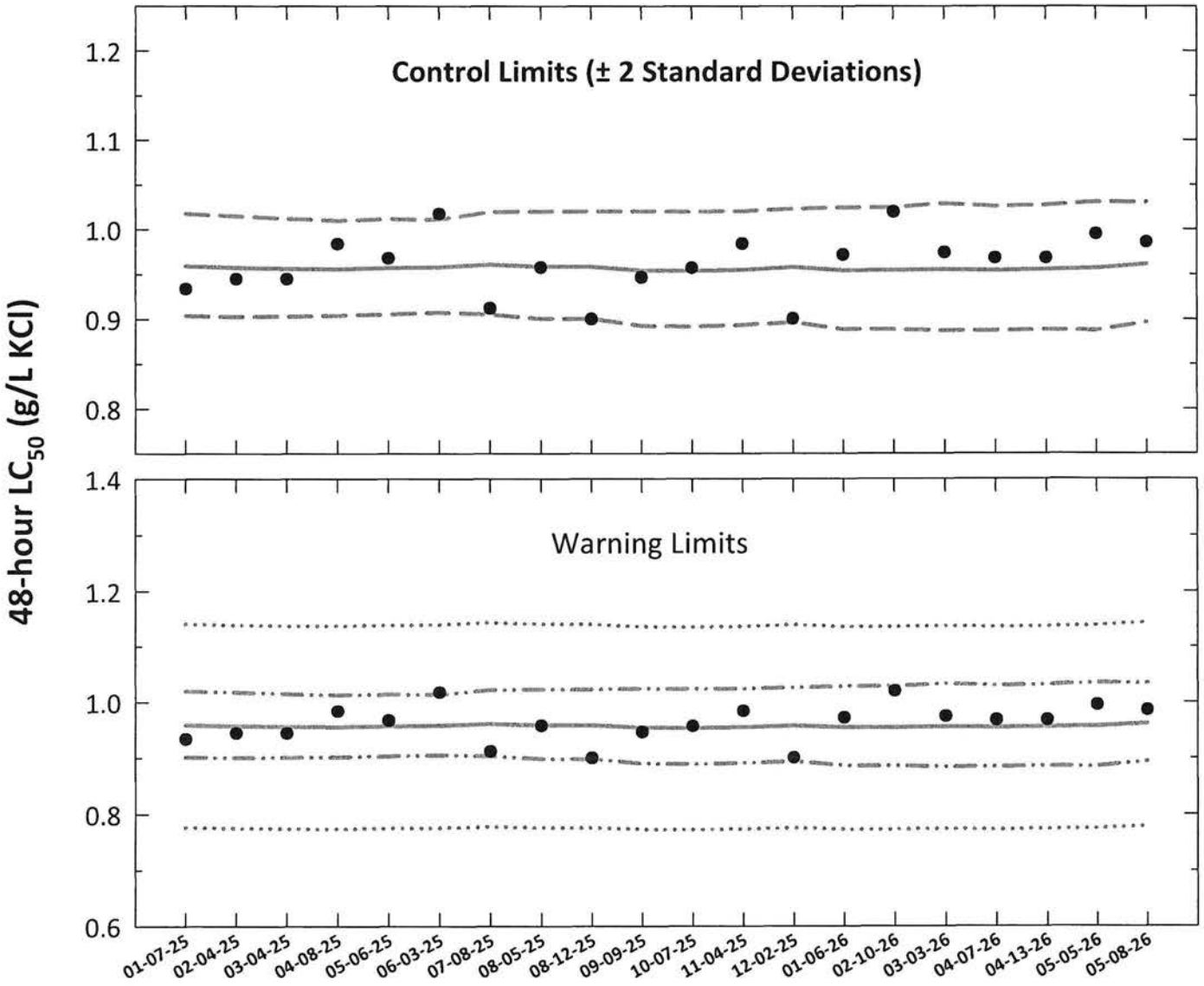


Dose-Response Plot



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 Summer

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**01-07-25**	**0.9340**	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
2	**02-04-25**	**0.9448**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
3	**03-04-25**	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
4	**04-08-25**	**0.9839**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
5	**05-06-25**	**0.9680**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
6	**06-03-25**	**1.0177**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
7	**07-08-25**	**0.9124**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
8	**08-05-25**	**0.9576**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
9	**08-12-25**	**0.9003**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
10	**09-09-25**	**0.9465**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
11	**10-07-25**	**0.9572**	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
12	**11-04-25**	**0.9840**	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
13	**12-02-25**	**0.9007**	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
14	**01-06-26**	**0.9716**	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
15	**02-10-26**	**1.0197**	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
16	**03-03-26**	**0.9741**	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367
17	**04-07-26**	**0.9680**	-0.0141	-0.0204	0.0157	0.9541	0.8874	1.0258	0.8842	1.0293	0.7728	1.1354
18	**04-13-26**	**0.9680**	-0.0141	-0.0199	0.0158	0.9551	0.8882	1.0271	0.8851	1.0305	0.7737	1.1366
19	**05-05-26**	**0.9950**	-0.0022	-0.0192	0.0163	0.9567	0.8877	1.0310	0.8845	1.0344	0.7749	1.1384
20	**05-08-26**	**0.9857**	-0.0063	-0.0172	0.0151	0.9611	0.8965	1.0304	0.8939	1.0332	0.7785	1.1438

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 # 193

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 #: 2478

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	BSL	BSL
Control, MHSW	pH (S.U.)	8.05	7.86	7.87
	Dissolved oxygen (mg/L)	8.3	7.9	7.8
	Conductivity (µmhos/cm)	305		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	24.1	25.2	24.9
500 mg/L	pH (S.U.)	7.80	7.86	7.92
	Dissolved oxygen (mg/L)	8.3	8.0	7.8
	Conductivity (µmhos/cm)	1130		
	Temperature (°C)	24.2	25.0	25.3
750 mg/L	pH (S.U.)	7.85	7.88	7.92
	Dissolved oxygen (mg/L)	8.3	8.0	7.9
	Conductivity (µmhos/cm)	1550		
	Temperature (°C)	24.7	25.0	25.0
1000 mg/L	pH (S.U.)	7.87	7.87	7.93
	Dissolved oxygen (mg/L)	8.3	7.8	7.9
	Conductivity (µmhos/cm)	1980		
	Temperature (°C)	24.1	24.9	25.0
1250 mg/L	pH (S.U.)	7.88	7.86	7.93
	Dissolved oxygen (mg/L)	8.3	7.7	7.8
	Conductivity (µmhos/cm)	2380		
	Temperature (°C)	24.7	24.9	25.1
1500 mg/L	pH (S.U.)	7.90	7.84	
	Dissolved oxygen (mg/L)	8.3	7.5	
	Conductivity (µmhos/cm)	2770		
	Temperature (°C)	24.2	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 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066474

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 # 193

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	05-02-26	* 0500	JP	0837	JP	6D	Yellow	05-04-26A
24	05-03-26			0835	"			
48 Termination	05-04-26			0835	"			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	04-25-26
Age (1 to 14 days old):	6 to 7 days
Hatch date and times:	05-01-26 1121 to 05-02-26 0839
Average transfer volume:	< 0.25 mL
Transfer bowl information:	**pH** (S.U.): 7.90
	Temperature (°C): 24.2 °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	7 3d	6 4d	0 10d	1	0 10d	0 10d
48 Termination	10	10	10	10	9 4d	10	6 4d	4 4d	0	1	0	0
Mean Survival	100%		100%		95%		50%		5%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	914.9
Upper 95% confidence limit (mg KCl/L)	1052.4
48-hour LC_{50} (mg KCl/L)	985.7

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:	5/8/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/10/2026	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	0.7000	0.6000
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	0.1748	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1748	0	20
*1000	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	7.720	2.850	0.1748	7	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.109	2.850	0.1748	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

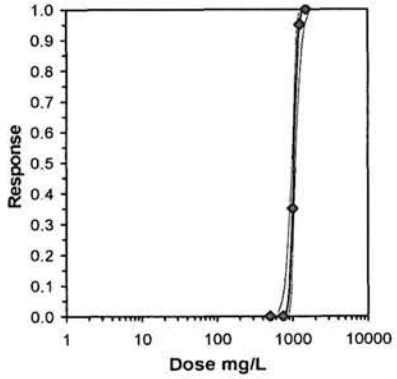
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08218	0.08429	0.5279	0.00376	2.5E-05	4, 5
Treatments vs D-Control										

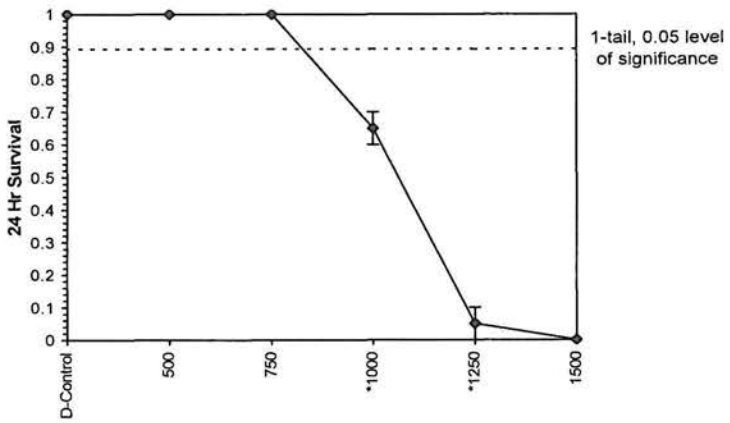
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	21.4313	5.18429	11.2701	31.5925	0	0.03557	7.81472	0.99823	3.01869	0.04666	3
Intercept	-59.694	15.6806	-90.428	-28.96							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	813.086	634.929	894.023
EC05	3.355	874.854	727.146	942.96
EC10	3.718	909.677	780.747	971.27
EC15	3.964	933.952	818.455	991.661
EC20	4.158	953.707	849.118	1008.89
EC25	4.326	970.987	875.727	1024.62
EC40	4.747	1015.93	942.342	1070.09
EC50	5.000	1043.96	980.316	1103.47
EC60	5.253	1072.77	1015.22	1143.05
EC75	5.674	1122.43	1065.8	1223.63
EC80	5.842	1142.76	1083.84	1260.31
EC85	6.036	1166.93	1103.92	1306.04
EC90	6.282	1198.07	1128.22	1367.76
EC95	6.645	1245.76	1163.17	1467.22
EC99	7.326	1340.4	1227.9	1678.87



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:	5/8/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	5/10/2026	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	1.0000
1000	0.6000	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	0.2758	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.842	2.850	0.2758	1	20
*1000	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	6.475	2.850	0.2758	10	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	12.107	2.850	0.2758	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

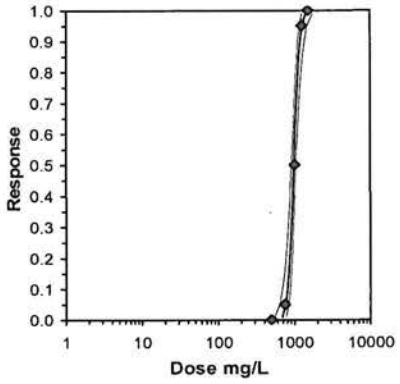
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.15228	0.15619	0.53276	0.00937	2.3E-04	4, 5
Treatments vs D-Control										

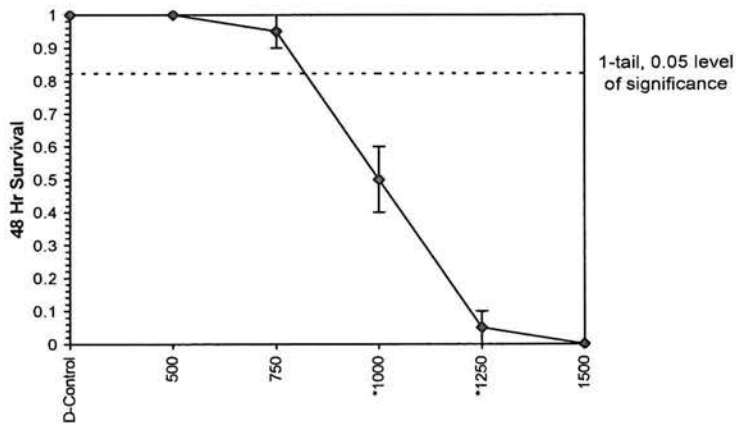
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.0595	2.86984	9.43462	20.6844	0	0.3061	7.81472	0.95888	2.99373	0.0664	3
Intercept	-40.084	8.62891	-56.997	-23.171							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	690.637	541.973	777.15
EC05	3.355	766.485	637.16	842.198
EC10	3.718	810.267	693.631	880.25
EC15	3.964	841.21	733.88	907.697
EC20	4.158	866.644	766.962	930.805
EC25	4.326	889.076	795.979	951.757
EC40	4.747	948.209	870.454	1010.84
EC50	5.000	985.66	914.883	1052.35
EC60	5.253	1024.59	957.866	1099.83
EC75	5.674	1092.74	1024.91	1193.83
EC80	5.842	1121.02	1050.15	1236.43
EC85	6.036	1154.91	1078.91	1289.75
EC90	6.282	1199.02	1114.47	1362.23
EC95	6.645	1267.51	1166.79	1480.47
EC99	7.326	1406.71	1266.54	1737.61



Dose-Response Plot



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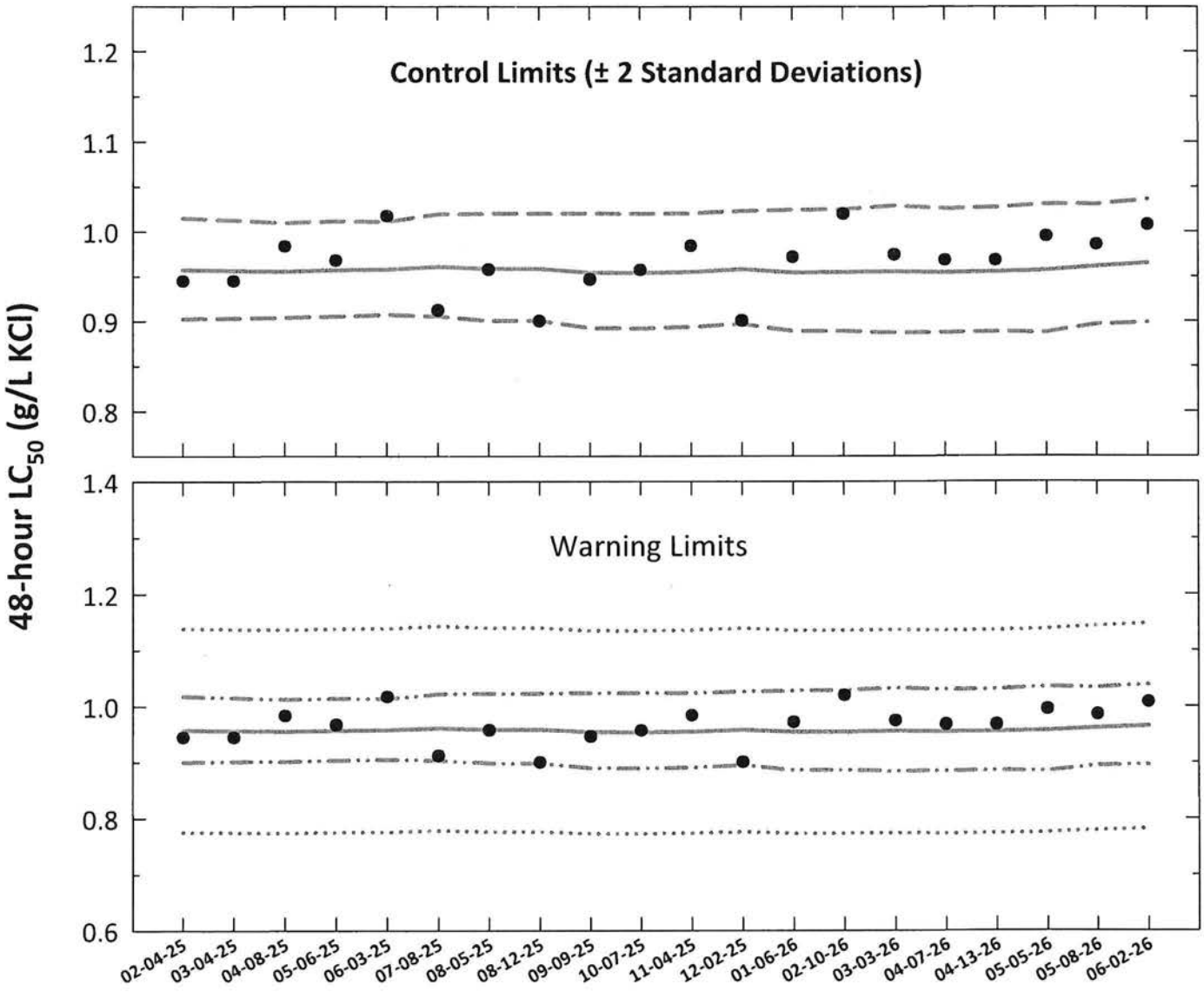
Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**02-04-25**	**0.9448**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
2	**03-04-25**	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
3	**04-08-25**	**0.9839**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
4	**05-06-25**	**0.9680**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
5	**06-03-25**	**1.0177**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
6	**07-08-25**	**0.9124**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
7	**08-05-25**	**0.9576**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
8	**08-12-25**	**0.9003**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
9	**09-09-25**	**0.9465**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
10	**10-07-25**	**0.9572**	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
11	**11-04-25**	**0.9840**	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
12	**12-02-25**	**0.9007**	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
13	**01-06-26**	**0.9716**	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
14	**02-10-26**	**1.0197**	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
15	**03-03-26**	**0.9741**	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367
16	**04-07-26**	**0.9680**	-0.0141	-0.0204	0.0157	0.9541	0.8874	1.0258	0.8842	1.0293	0.7728	1.1354
17	**04-13-26**	**0.9680**	-0.0141	-0.0199	0.0158	0.9551	0.8882	1.0271	0.8851	1.0305	0.7737	1.1366
18	**05-05-26**	**0.9950**	-0.0022	-0.0192	0.0163	0.9567	0.8877	1.0310	0.8845	1.0344	0.7749	1.1384
19	**05-08-26**	**0.9857**	-0.0063	-0.0172	0.0151	0.9611	0.8965	1.0304	0.8939	1.0332	0.7785	1.1438
20	**06-02-26**	**1.0079**	0.0034	-0.0156	0.0155	0.9648	0.8984	1.0361	0.8960	1.0387	0.7815	1.1481

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 # 194

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 #: 2478

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.72	7.90	7.87
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	25.3	25.3	25.0
500 mg/L	pH (S.U.)	7.88	7.89	7.85
	Dissolved oxygen (mg/L)	8.6	8.2	8.1
	Conductivity (µmhos/cm)	1150		
	Temperature (°C)	25.7	25.5	24.9
750 mg/L	pH (S.U.)	7.89	7.88	7.85
	Dissolved oxygen (mg/L)	8.6	8.1	8.1
	Conductivity (µmhos/cm)	1560		
	Temperature (°C)	25.7	25.5	25.3
1000 mg/L	pH (S.U.)	7.89	7.86	7.84
	Dissolved oxygen (mg/L)	8.6	8.1	8.1
	Conductivity (µmhos/cm)	1980		
	Temperature (°C)	25.7	25.4	25.0
1250 mg/L	pH (S.U.)	7.90	7.86	7.84
	Dissolved oxygen (mg/L)	8.6	8.0	8.0
	Conductivity (µmhos/cm)	2410		
	Temperature (°C)	25.7	25.4	24.9
1500 mg/L	pH (S.U.)	7.90	7.84	
	Dissolved oxygen (mg/L)	8.5	7.8	
	Conductivity (µmhos/cm)	2810		
	Temperature (°C)	25.7	25.6	

9-26-24

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13766479

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 # 194

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-02-26	*0905	JP	0907	JP	1B	orange	05-27-26A
24	06-03-26			0901	JP			
48 Termination	06-04-26			0910	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:	05-20-26
Age (1 to 14 days old):	5 to 6 days
Hatch date and times:	05-27-26 1201 to 05-28-26 0946
Average transfer volume:	< 0.25 mL
Transfer bowl information:	**pH** (S.U.): 7.96
	Temperature (°C): 24.7°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 s2	7 s2	1 s2	1 s2	0 s2	0 s2
48 Termination	10	10	10	10	9 s2	10	5	7	1	0 s2	0	0
Mean Survival	100%		100%		95%		60%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	936.3
Upper 95% confidence limit (mg KCl/L)	1074.8
48-hour LC_{50} (mg KCl/L)	1007.9

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:	6/2/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref
End Date:	6/4/2026	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	0.7000
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	0.1854	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1854	0	20
*1000	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	8.049	2.850	0.1854	8	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	16.756	2.850	0.1854	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

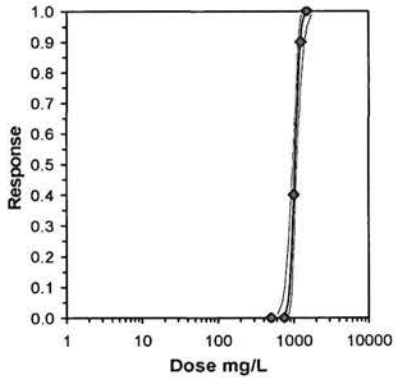
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08888	0.09116	0.47099	0.00423	4.5E-05	4, 5
Treatments vs D-Control										

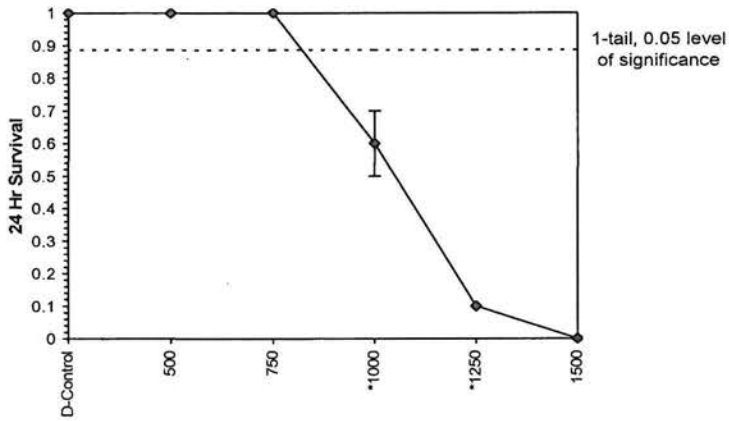
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.6867	3.74573	10.345	25.0283	0	0.312	7.81472	0.95776	3.01832	0.05654	4
Intercept	-48.384	11.3463	-70.623	-26.145							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	770.523	604.072	857.946
EC05	3.355	842.009	700.262	917.05
EC10	3.718	882.791	756.746	951.345
EC15	3.964	911.416	796.759	975.997
EC20	4.158	934.828	829.496	996.727
EC25	4.326	955.391	858.088	1015.53
EC40	4.747	1009.24	930.801	1068.82
EC50	5.000	1043.08	973.483	1106.74
EC60	5.253	1078.05	1013.99	1150.67
EC75	5.674	1138.81	1075.25	1238.83
EC80	5.842	1163.86	1097.75	1278.95
EC85	6.036	1193.76	1123.07	1329.12
EC90	6.282	1232.47	1154.02	1397.16
EC95	6.645	1292.16	1199	1507.55
EC99	7.326	1412.04	1283.48	1745.05



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:	6/2/2026	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	6/4/2026	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	1.0000
1000	0.5000	0.7000
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	0.2784	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.834	2.850	0.2784	1	20
*1000	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	5.361	2.850	0.2784	8	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.993	2.850	0.2784	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

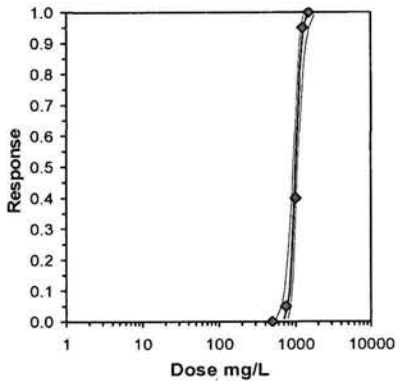
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.15429	0.15825	0.51121	0.00955	2.7E-04	4, 5
Treatments vs D-Control										

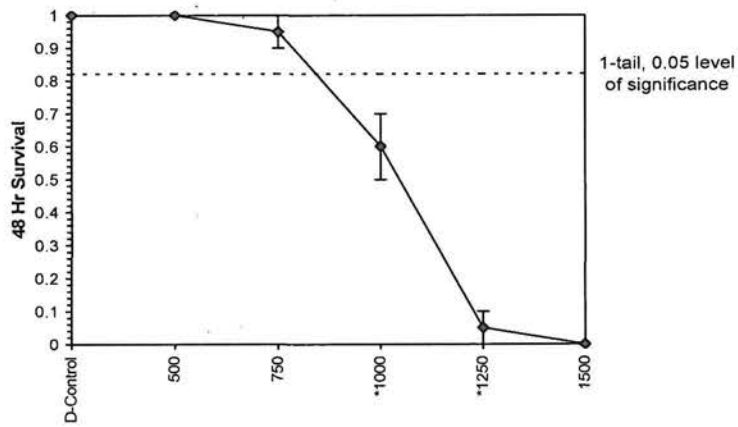
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.2619	2.93363	9.51201	21.0118	0	1.29951	7.81472	0.72925	3.0034	0.06552	3
Intercept	-40.838	8.84931	-58.182	-23.493							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	709.533	556.747	797.648
EC05	3.355	786.368	653.728	863.28
EC10	3.718	830.673	711.225	901.625
EC15	3.964	861.968	752.193	929.262
EC20	4.158	887.678	785.862	952.519
EC25	4.326	910.346	815.389	973.599
EC40	4.747	970.065	891.146	1033.03
EC50	5.000	1007.86	936.295	1074.83
EC60	5.253	1047.13	979.9	1122.7
EC75	5.674	1115.82	1047.69	1217.67
EC80	5.842	1144.32	1073.14	1260.76
EC85	6.036	1178.45	1102.11	1314.67
EC90	6.282	1222.84	1137.88	1387.97
EC95	6.645	1291.74	1190.44	1507.48
EC99	7.326	1431.62	1290.53	1767.14

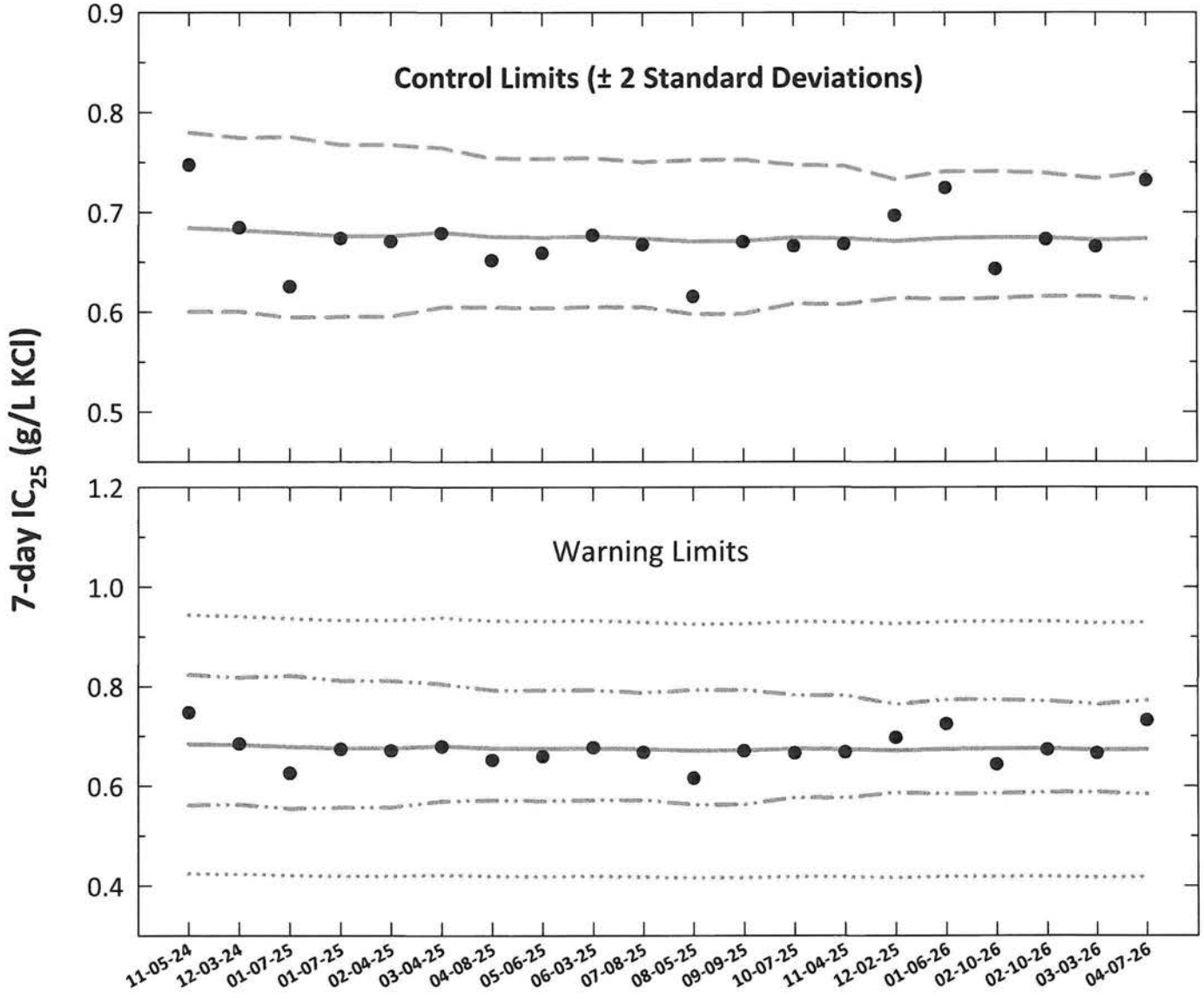


Dose-Response Plot



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
2	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
3	01-07-25	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
4	01-07-25	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
5	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
6	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
7	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
8	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
9	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
10	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
11	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
12	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
13	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
14	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
15	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
16	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302
17	02-10-26	0.6431	-0.1917	-0.1709	0.0204	0.6746	0.6141	0.7411	0.5849	0.7732	0.4183	0.9310
18	02-10-26	0.6727	-0.1722	-0.1710	0.0199	0.6745	0.6156	0.7391	0.5871	0.7703	0.4182	0.9308
19	03-03-26	0.6656	-0.1768	-0.1725	0.0191	0.6722	0.6157	0.7339	0.5881	0.7640	0.4168	0.9277
20	04-07-26	0.7319	-0.1355	-0.1715	0.0205	0.6737	0.6131	0.7403	0.5838	0.7726	0.4177	0.9298

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.

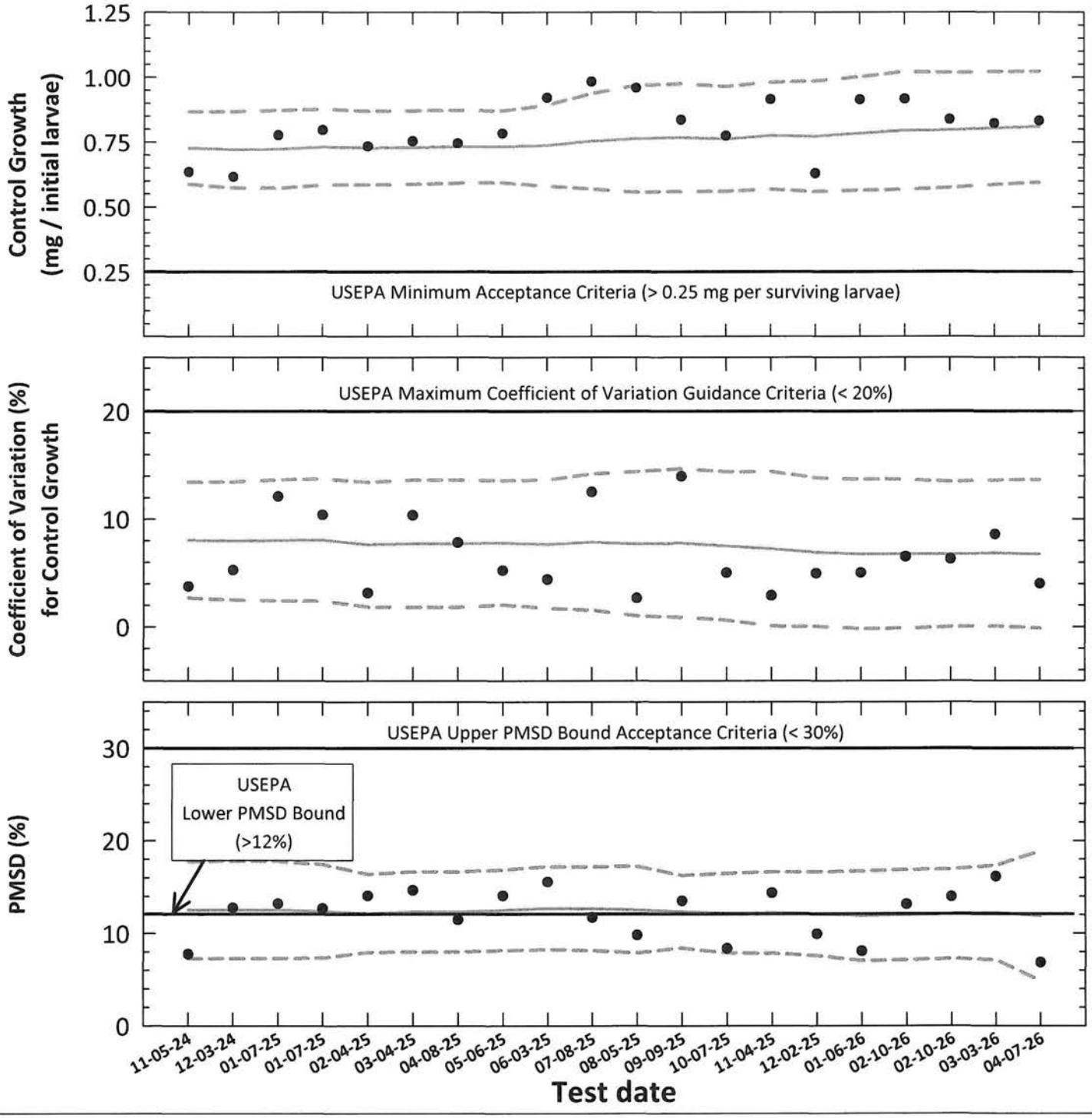
Entered and Reviewed by Jim Sumner

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)

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ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
2	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
3	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
4	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
5	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
6	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
7	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
8	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
9	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
10	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
11	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
12	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
13	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
14	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
15	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
16	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7
17	02-10-26	100	0.916	6.5	0.1204	13.1	0.795	0.568	1.022	6.8	-0.1	13.7	12.0	7.1	16.9
18	02-10-26	100	0.838	6.3	0.1170	14.0	0.797	0.575	1.019	6.8	0.0	13.5	12.1	7.3	16.9
19	03-03-26	100	0.822	8.6	0.1323	16.1	0.803	0.586	1.020	6.8	0.0	13.6	12.2	7.1	17.3
20	04-07-26	100	0.832	4.0	0.0569	6.8	0.809	0.595	1.022	6.7	-0.1	13.6	11.9	4.8	18.9

Note:

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

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

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

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

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

Entered and Reviewed by Jim Sumner

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: **140**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2465				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	03-31-26	*Artemia* CHM number:	CHM1432
Hatch dates and times:	04-06-26 1330 TO 04-07-26 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.05 S.U. Temperature = 24.2 °C	Date / Time in oven:	04-14-26 0515
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	04-15-26 0515
		*Final oven temperature:	60 °C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	04-07-26	0505	JK	1420	JK	0710	JK	03-30-26 A
1	04-08-26	0500	JK	1400	JK	0700	JK	↓
2	04-09-26	0500	JK	1230	JK	0736	JK	03-30-26 C
3	04-10-26	0500	JK	1100	JK	0700	JK	↓
4	04-11-26	0600	JK	1235	JK	0812	JK	04-01-26
5	04-12-26	0600	JK	1100	JK	0600	JK	↓
6	04-13-26	0600	JK	1100	JK	0615	JK	↓
7	04-14-26					0512	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130666685

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	0.832	
Average weight per surviving larvae:	0.832	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	827.7
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	731.9

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **140**

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: Grey Analyst: XL Date: 031726	14.05	13.70	12.52	13.98	11.19	13.02	11.87	10.88	10.95	13.13	9.65	12.47
*B = Pan + Larvae weight (mg) Analyst: XL Date: 041726	22.46	21.57	21.19	22.31	20.58	22.30	22.05	20.20	19.91	22.20	18.65	21.91
C = Larvae weight (mg) = B – A Analyst: H	8.41	7.87	8.67	8.33	9.39	9.28	10.18	9.32	8.96	9.07	9.00	9.44
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: H	0.841	0.787	0.867	0.833	0.939	0.928	1.018	0.932	0.896	0.907	0.900	0.944
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.832				0.954		-14.7%		0.912		-9.6%	

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

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

Comments:

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **140**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	9 1d	9 1d	9 1d	9 1d
2	10	10	10	10	10	10	10	9 1d	9	9	9	8 1d
3	10	10	10	10	10	10	10	9	8 1d	7 2d	9	8
4	10	10	10	10	10	9 1d	9 1d	9	8	6 1d	8 1d	8
5	10	10	10	10	8 2d	9	8 1d	8 1d	6 2d	6	7 1d	7 1d
6	10	10	10	10	7 1d	8 1d	8	8	6	6	7	7
7	10	10	10	9 1d	7	7 1d	8	7 1d	3 3d	2 4d	3 4d	3 4d
*A = Pan weight (mg) Tray color code: Grey Analyst: XL Date: 03-17-26	12.11	13.86	13.43	11.88	11.95	14.86	14.23	12.97	12.56	14.26	12.14	11.40
*B = Pan + Larvae weight (mg) Analyst: XL Date: 04-17-26	21.38	22.25	22.01	20.43	17.82	21.26	22.19	18.65	15.35	16.01	14.73	14.17
C = Larvae weight (mg) = B – A Analyst: JL	9.27	8.38	8.58	8.55	5.87	6.40	7.96	5.68	2.79	1.75	2.59	2.77
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.927	0.838	0.858	0.855	0.587	0.640	0.796	0.568	0.279	0.175	0.259	0.277
Average weight per initial number of larvae (mg)	0.870				0.648				0.248			
Percent reduction from control (%)			-4.5%				22.1%				70.3%	

*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: **140**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	5 5d	6 4d	6 4d	5 5d
2	5	5 1d	5 1d	4 1d
3	4 1d	4 1d	4 1d	3 1d
4	2 2d	3 1d	3 1d	3
5	1 1d	2 1d	2 1d	2 1d
6	1	2	2	2
7	1	2	1 1d	0 2d
*A = Pan weight (mg) Tray color code: Grey Analyst: XL Date: 031726	10.13	10.10	10.13	8.66
*B = Pan + Larvae weight (mg) Analyst: XL Date: 04-1726	11.02	12.34	11.13	8.66 JH 04-20-26
C = Larvae weight (mg) = B – A Analyst: JH	0.89	2.24	1.00	0
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.089	0.224	0.100	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.103		87.6%	

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 140

Test dates: April 07-14, 2026

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	14.05	22.46	8.41	0.841	0.832	4.0	0.841	100.0	0.832	4.0	Not applicable
	B	10	10	13.70	21.57	7.87	0.787			0.787				
	C	10	10	12.52	21.19	8.67	0.867			0.867				
	D	10	10	13.98	22.31	8.33	0.833			0.833				
300	E	10	10	11.19	20.58	9.39	0.939	0.954	4.5	0.939	100.0	0.954	4.5	-14.7
	F	10	10	13.02	22.30	9.28	0.928			0.928				
	G	10	10	11.87	22.05	10.18	1.018			1.018				
	H	10	10	10.88	20.20	9.32	0.932			0.932				
450	I	10	10	10.95	19.91	8.96	0.896	0.912	2.4	0.896	100.0	0.912	2.4	-9.6
	J	10	10	13.13	22.20	9.07	0.907			0.907				
	K	10	10	9.65	18.65	9.00	0.900			0.900				
	L	10	10	12.47	21.91	9.44	0.944			0.944				
600	M	10	10	12.11	21.38	9.27	0.927	0.894	6.0	0.927	97.5	0.870	4.5	-4.5
	N	10	10	13.86	22.25	8.39	0.839			0.839				
	O	10	10	13.43	22.01	8.58	0.858			0.858				
	P	10	9	11.88	20.43	8.55	0.950			0.855				
750	Q	10	7	11.95	17.82	5.87	0.839	0.890	9.3	0.587	72.5	0.648	16.0	22.1
	R	10	7	14.86	21.26	6.40	0.914			0.640				
	S	10	8	14.23	22.19	7.96	0.995			0.796				
	T	10	7	12.97	18.65	5.68	0.811			0.568				
900	U	10	3	12.56	15.35	2.79	0.930	0.898	3.7	0.279	27.5	0.248	19.9	70.3
	V	10	2	14.26	16.01	1.75	0.875			0.175				
	W	10	3	12.14	14.73	2.59	0.863			0.259				
	X	10	3	11.40	14.17	2.77	0.923			0.277				
1050	Y	10	1	10.13	11.02	0.89	0.890	1.003	11.5	0.089	10.0	0.103	89.2	87.6
	Z	10	2	10.10	12.34	2.24	1.120			0.224				
	AA	10	1	10.13	11.13	1.00	1.000			0.100				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.0569 MSD = Minimum Significant Difference

PMSD: 6.8 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Tashner

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	4/7/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/14/2026	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	0.9000
750	0.7000	0.7000	0.8000	0.7000
900	0.3000	0.2000	0.3000	0.3000
1050	0.1000	0.2000	0.1000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	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	0.3165	0.1588	0.4636	39.374	4	10.00	10.00	36	40

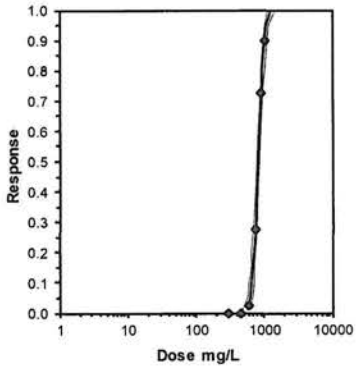
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.85419	0.896	-0.5169	3.08946
Equality of variance cannot be confirmed				

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

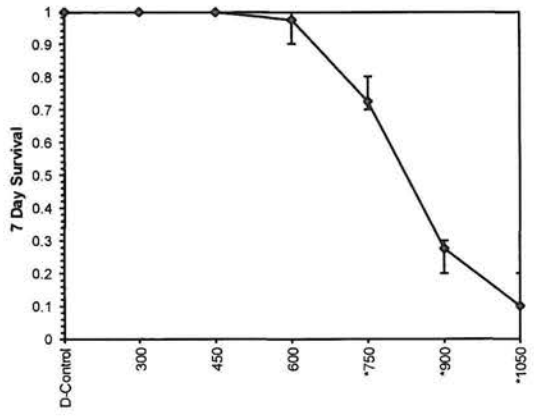
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.5637	1.74538	10.1427	16.9846	0	0.48112	9.48773	0.97531	2.91786	0.07373	3
Intercept	-34.577	5.1007	-44.574	-24.58							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	557.631	482.742	609.787
EC05	3.355	626.024	561.87	670.767
EC10	3.718	665.849	608.642	706.407
EC15	3.964	694.141	641.95	732.006
EC20	4.158	717.481	669.333	753.444
EC25	4.326	738.129	693.367	772.766
EC40	4.747	792.833	755.226	826.516
EC50	5.000	827.676	792.401	863.53
EC60	5.253	864.049	828.867	904.966
EC75	5.674	928.085	887.821	984.258
EC80	5.842	954.794	910.937	1019.21
EC85	6.036	986.899	937.929	1062.33
EC90	6.282	1028.83	972.208	1120.13
EC95	6.645	1094.28	1024.15	1213.03
EC99	7.326	1228.49	1126.87	1411.49



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Summer

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	4/7/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	4/14/2026	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	0.8410	0.7870	0.8670	0.8330
300	0.9390	0.9280	1.0180	0.9320
450	0.8960	0.9070	0.9000	0.9440
600	0.9270	0.8390	0.8580	0.8550
750	0.5870	0.6400	0.7960	0.5680
900	0.2790	0.1750	0.2590	0.2770
1050	0.0890	0.2240	0.1000	0.0000

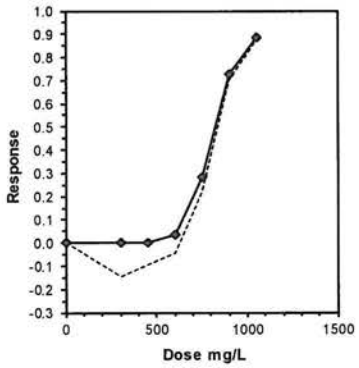
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8320	1.0000	0.8320	0.7870	0.8670	4.006	4				0.8993	1.0000
300	0.9543	1.1469	0.9543	0.9280	1.0180	4.479	4	-4.916	2.290	0.0569	0.8993	1.0000
450	0.9118	1.0959	0.9118	0.8960	0.9440	2.410	4	-3.207	2.290	0.0569	0.8993	1.0000
600	0.8698	1.0454	0.8698	0.8390	0.9270	4.492	4	-1.518	2.290	0.0569	0.8698	0.9671
750	0.6478	0.7785	0.6478	0.5680	0.7960	15.966	4				0.6478	0.7203
900	0.2475	0.2975	0.2475	0.1750	0.2790	19.864	4				0.2475	0.2752
1050	0.1033	0.1241	0.1033	0.0000	0.2240	89.214	4				0.1033	0.1148

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90285	0.844	0.88276	-0.0684
Bartlett's Test indicates equal variances (p = 0.76)	1.17813	11.3449		

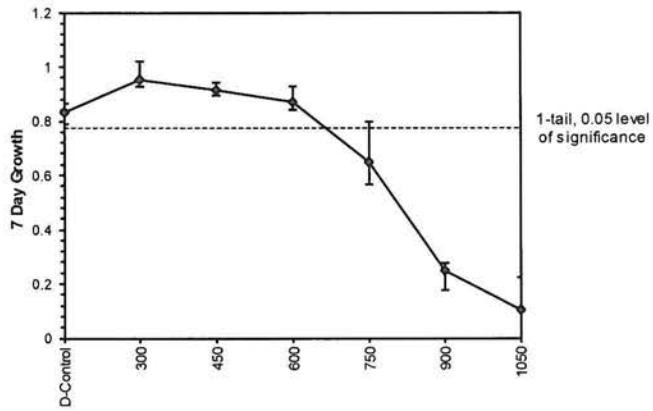
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.05694	0.06844	0.01115	0.00124	0.00212	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	610.39	19.78	531.12	650.20	-0.8672
IC10	640.78	17.07	604.71	710.57	2.2468
IC15	671.16	21.83	633.14	779.86	1.6518
IC20	701.54	23.72	656.58	797.84	0.8972
IC25	731.93	21.97	678.18	801.19	0.3309
IC40	790.53	12.90	753.80	832.12	0.1665
IC50	824.23	9.90	794.15	854.13	0.0484



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **140**

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.79	7.85	7.75	7.76	7.76	7.78
	Dissolved oxygen (mg/L)	8.6	8.0	8.4	8.0	8.5	7.8
	Conductivity (µmhos/cm)	306		312		310	
	Alkalinity (mg $CaCO_3$/L)	60				62	
	Hardness (mg $CaCO_3$/L)	85				85	
	Temperature (°C)	24.7	25.0	24.8	25.1	24.8	25.0
300 mg KCl/L	pH (S.U.)	7.93	7.86	7.96	7.74	7.92	7.78
	Dissolved oxygen (mg/L)	8.6	8.1	8.4	7.8	8.4	7.7
	Conductivity (µmhos/cm)	841		855		853	
	Temperature (°C)	24.7	24.7	24.9	24.7	24.9	24.7
450 mg KCl/L	pH (S.U.)	7.95	7.86	7.97	7.75	7.93	7.78
	Dissolved oxygen (mg/L)	8.6	8.1	8.4	7.9	8.4	7.6
	Conductivity (µmhos/cm)	1090		1120		1100	
	Temperature (°C)	24.8	24.6	24.9	24.7	25.0	24.7
600 mg KCl/L	pH (S.U.)	7.96	7.87	7.97	7.75	7.94	7.77
	Dissolved oxygen (mg/L)	8.6	8.1	8.4	7.9	8.4	7.6
	Conductivity (µmhos/cm)	1330		1360		1350	
	Temperature (°C)	24.8	24.9	25.0	24.8	25.0	24.6
750 mg KCl/L	pH (S.U.)	7.96	7.87	7.98	7.76	7.96	7.77
	Dissolved oxygen (mg/L)	8.5	8.1	8.4	7.9	8.4	7.6
	Conductivity (µmhos/cm)	1580		1600		1600	
	Temperature (°C)	24.9	24.6	25.0	25.0	25.0	24.6
900 mg KCl/L	pH (S.U.)	7.97	7.87	7.98	7.77	7.96	7.77
	Dissolved oxygen (mg/L)	8.5	8.1	8.4	7.9	8.4	7.6
	Conductivity (µmhos/cm)	1830		1870		1840	
	Temperature (°C)	24.8	24.6	25.0	24.6	25.0	24.7
1050 mg KCl/L	pH (S.U.)	7.98	7.87	7.99	7.77	~~7.96~~ 7.97	7.78
	Dissolved oxygen (mg/L)	8.5	8.1	8.4	8.0	8.4	7.7
	Conductivity (µmhos/cm)	2100		2140		2110	
	Temperature (°C)	24.8	24.7	25.0	24.6	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **140**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	R
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.83	7.79	7.72	7.75	7.77	7.78	7.75	7.56
	Dissolved oxygen (mg/L)	8.4	7.8	8.6	7.5	8.4	7.8	8.5	6.3
	Conductivity (µmhos/cm)	314		299		313		308	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)			87					
	Temperature (°C)	24.8	24.5	24.7	25.0	24.8	25.0	24.8	24.5
300 mg KCl/L	pH (S.U.)	7.94	7.76	7.75	7.75	8.03	7.74	7.94	7.55
	Dissolved oxygen (mg/L)	8.3	7.7	8.6	7.3	8.3	7.5	8.4	6.1
	Conductivity (µmhos/cm)	842		815		857		848	
	Temperature (°C)	24.7	24.7	24.7	24.9	24.8	24.8	24.7	24.8
450 mg KCl/L	pH (S.U.)	7.95	7.75	7.88	7.75	8.05	7.73	7.95	7.52
	Dissolved oxygen (mg/L)	8.3	7.8	8.5	7.4	8.3	7.6	8.4	6.2
	Conductivity (µmhos/cm)	1110		1070		1110		1100	
	Temperature (°C)	24.7	24.7	24.8	24.9	24.8	24.8	24.7	24.9
600 mg KCl/L	pH (S.U.)	7.96	7.77	7.92	7.75	8.06	7.73	7.96	7.50
	Dissolved oxygen (mg/L)	8.3	7.8	8.5	7.4	8.4	7.6	8.5	6.1
	Conductivity (µmhos/cm)	1350		1310		1350		1340	
	Temperature (°C)	24.7	24.6	24.8	24.9	24.9	24.8	24.9	24.7
750 mg KCl/L	pH (S.U.)	7.97	7.78	7.93	7.76	8.06	7.74	7.97	7.49
	Dissolved oxygen (mg/L)	8.3	7.9	8.5	7.4	8.4	7.7	8.5	6.1
	Conductivity (µmhos/cm)	1600		1560		1590		1610	
	Temperature (°C)	24.7	24.6	24.6	25.2	24.9	24.9	24.9	24.7
900 mg KCl/L	pH (S.U.)	7.98	7.77	7.94	7.75	8.06	7.73	7.97	7.49
	Dissolved oxygen (mg/L)	8.3	7.7	8.4	7.5	8.4	7.6	8.5	5.8
	Conductivity (µmhos/cm)	1860		1810		1820		1860	
	Temperature (°C)	24.6	24.6	24.6	25.0	24.7	24.7	24.9	24.6
1050 mg KCl/L	pH (S.U.)	7.99	7.77	7.96	7.75	8.06	7.75	7.97	7.50
	Dissolved oxygen (mg/L)	8.3	7.6	8.4	7.4	8.4	7.7	8.5	6.2
	Conductivity (µmhos/cm)	2100		2040		2090		2130	
	Temperature (°C)	24.6	24.8	24.7	25.0	24.7	24.9	24.7	24.1
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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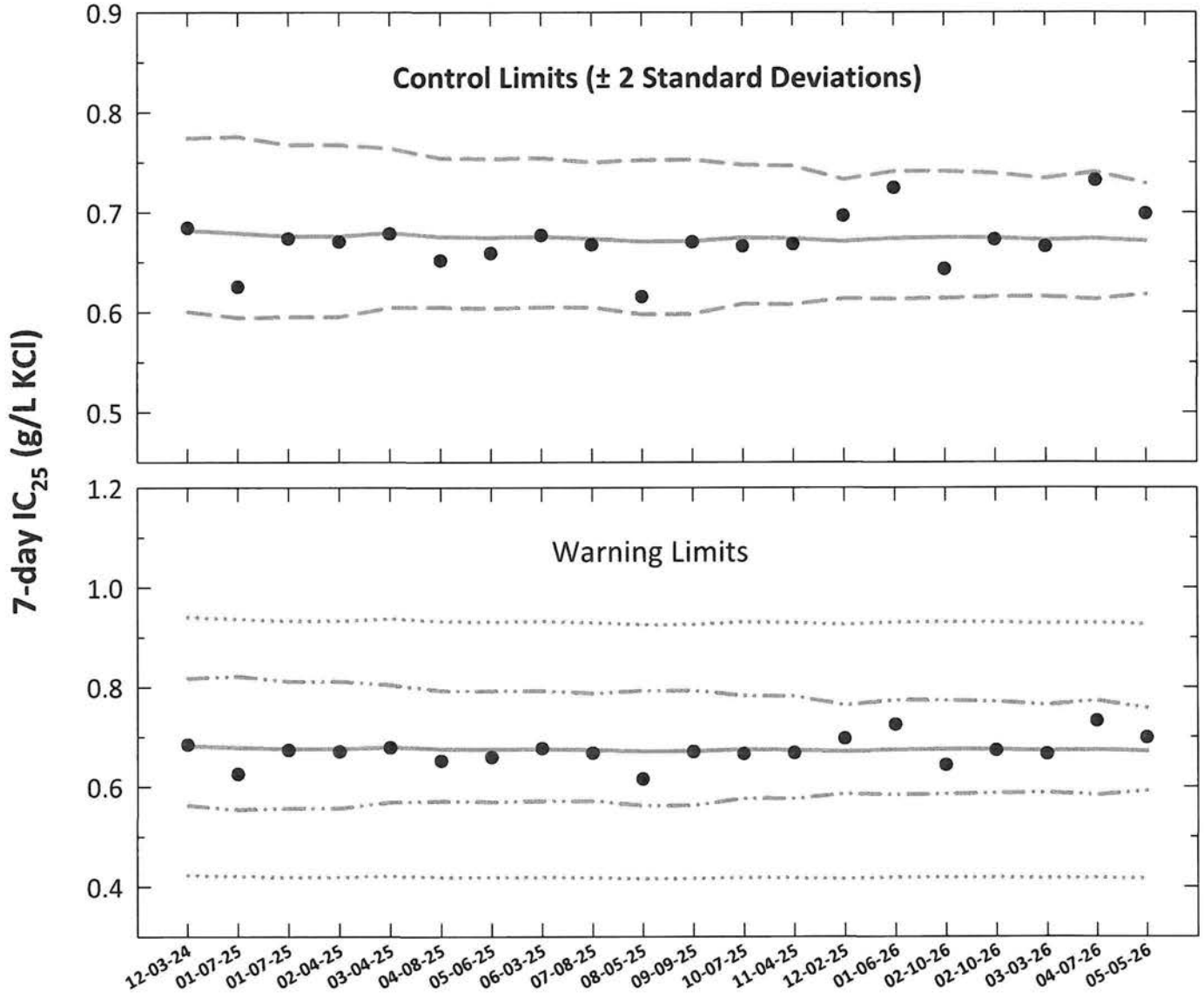
Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture



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)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
2	01-07-25	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
3	01-07-25	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
4	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
5	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
6	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
7	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
8	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
9	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
10	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
11	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
12	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
13	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
14	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
15	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302
16	02-10-26	0.6431	-0.1917	-0.1709	0.0204	0.6746	0.6141	0.7411	0.5849	0.7732	0.4183	0.9310
17	02-10-26	0.6727	-0.1722	-0.1710	0.0199	0.6745	0.6156	0.7391	0.5871	0.7703	0.4182	0.9308
18	03-03-26	0.6656	-0.1768	-0.1725	0.0191	0.6722	0.6157	0.7339	0.5881	0.7640	0.4168	0.9277
19	04-07-26	0.7319	-0.1355	-0.1715	0.0205	0.6737	0.6131	0.7403	0.5838	0.7726	0.4177	0.9298
20	05-05-26	0.6986	-0.1558	-0.1730	0.0180	0.6715	0.6181	0.7294	0.5920	0.7578	0.4163	0.9266

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.

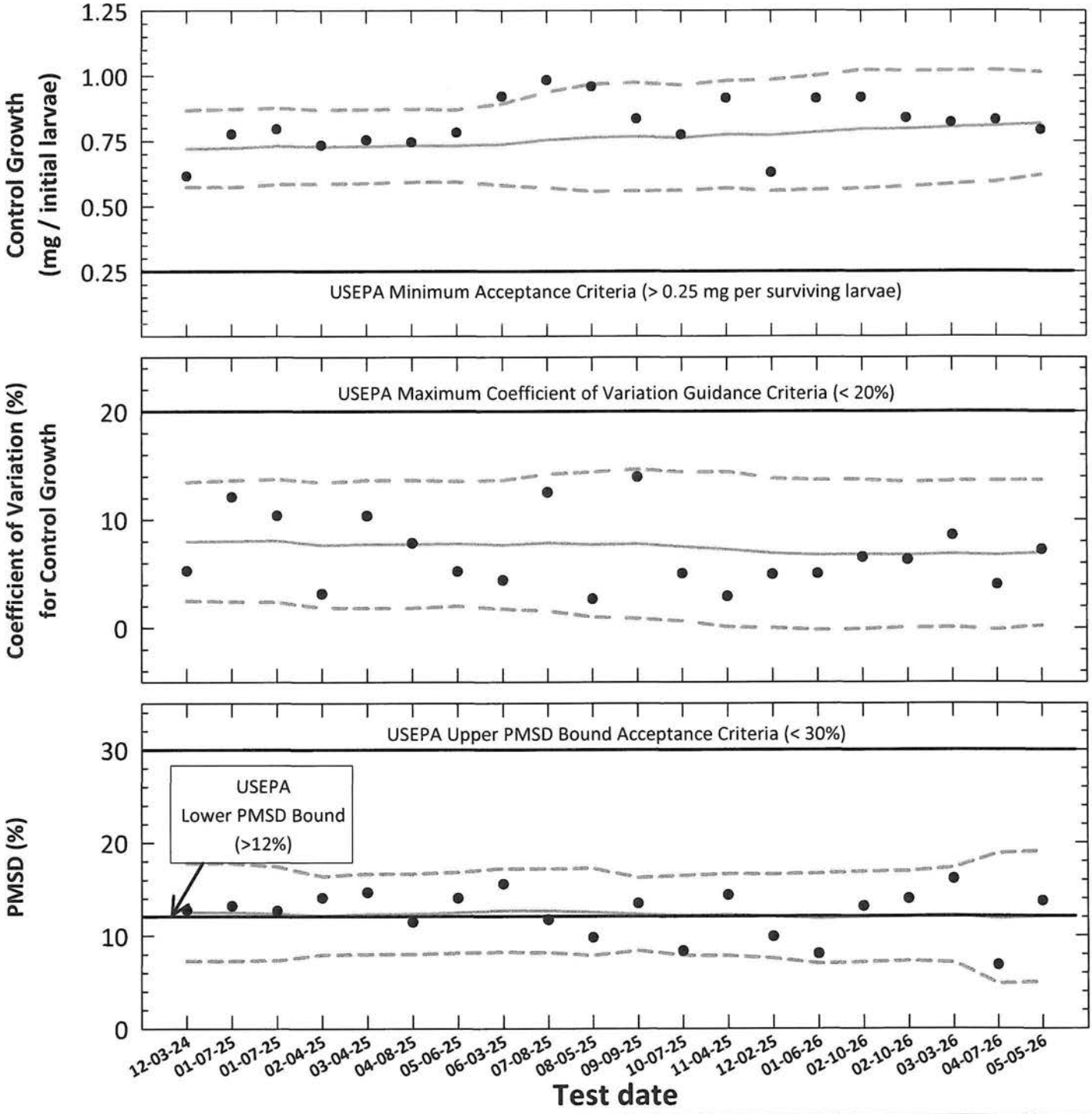
Entered and Reviewed by Jim Sumner

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	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
2	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
3	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
4	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
5	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
6	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
7	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
8	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
9	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
10	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
11	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
12	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
13	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
14	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
15	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7
16	02-10-26	100	0.916	6.5	0.1204	13.1	0.795	0.568	1.022	6.8	-0.1	13.7	12.0	7.1	16.9
17	02-10-26	100	0.838	6.3	0.1170	14.0	0.797	0.575	1.019	6.8	0.0	13.5	12.1	7.3	16.9
18	03-03-26	100	0.822	8.6	0.1323	16.1	0.803	0.586	1.020	6.8	0.0	13.6	12.2	7.1	17.3
19	04-07-26	100	0.832	4.0	0.0569	6.8	0.809	0.595	1.022	6.7	-0.1	13.6	11.9	4.8	18.9
20	05-05-26	100	0.792	7.2	0.1086	13.7	0.817	0.619	1.014	6.9	0.2	13.7	12.0	5.0	19.0

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

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: **141**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2478				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	04-28-26	*Artemia* CHM number:	CHM1432
Hatch dates and times:	05-04-26 1455 TO 05-05-26 0450	***Drying information for weight determination:***	
Transfer vessel information:	pH = 7.90 S.U. Temperature = 24.5 °C	Date / Time in oven:	05-12-26 0617
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	05-13-26 0620
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	05-05-26	0500	JH	1425	JH	0700	JH	04-29-26 A
1	05-06-26	0500	JH	1300	JH	0700	JH	↓
2	05-07-26	0500	JH	1300	JH	0730	JH	04-29-26 B
3	05-08-26	0500	JH	1110	JH	0700	JH	↓
4	05-09-26	0600	JH	1200	JH	0800	JH	05-07-26 A
5	05-10-26	0600	JH	1100	JH	0800	JH	↓
6	05-11-26	0500	JH	1200	JH	0745	JH	
7	05-12-26					0531	JH	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	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)	835.3
Average weight per initial larvae:	0.792		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.792	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	698.6

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*

PpKClCR Test Number: **141**

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 lg	10 lg	10 2lg	10	10	10	10
*A = Pan weight (mg) Tray color code: forest Green Analyst: XL Date: 04-27-26	13.40	12.81	13.92	14.98	12.60	13.04	14.57	14.88	13.55	14.01	13.23	13.38
*B = Pan + Larvae weight (mg) Analyst: EM Date: 05-18-26	20.63	20.89	21.70	23.58	22.44	22.45	24.22	26.57	21.52	22.79	21.02	22.20
C = Larvae weight (mg) = B – A Analyst: JH	7.23	8.08	7.78	8.60	9.84	9.41	9.65	11.69	7.97	8.78	7.79	8.82
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.723	0.808	0.778	0.860	0.984	0.941	0.965	1.169	0.797	0.878	0.779	0.882
Average weight per initial number of larvae (mg)	0.792				1.015		- 28.1%		0.834		- 5.3%	
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: **141**

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 1d	9 1d	5 1d	6 1d	6 1d	6 1d
2	10	10	10	10	9	8 1d	8	9	5	6	6	6
3	10	10	10	10	9	8	8	9	5	6	6	6
4	10	10	10	10	8 1d	8	8	9	5	5 1d	5 1d	4
5	10	10	10	10	8	8	8	8 1d	3 2d	5	4 1d	4
6	9 1d	10	10	10	8	8	8	8	3	5	4	4
7	9	10	10	10	7 1d	7 1d	8	7 1d	3	2 3d	4	3 1d
*A = Pan weight (mg) Tray color code: Forest Green Analyst: XL Date: 04-27-26	13.44	13.32	11.69	11.15	13.43	13.19	12.01	13.56	12.51	12.87	13.11	13.60
*B = Pan + Larvae weight (mg) Analyst: EM Date: 05-18-26	21.41	21.92	19.92	19.75	19.40	19.24	18.02	19.36	15.37	15.07	17.06	16.35
C = Larvae weight (mg) = B – A Analyst: JL	7.97	8.60	8.23	8.60	5.97	6.05	6.01	5.80	2.86	2.20	3.95	2.75
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.797	0.860	0.823	0.860	0.597	0.605	0.601	0.580	0.286	0.220	0.395	0.275
Average weight per initial number of larvae (mg)	0.835		-5.47.		0.596		24.8 7.		0.294		62.9 7.	
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: **141**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	4 cd	4 cd	4 cd	3 cd
2	3 cd	3 id	3 id	3
3	3	2 id	3	3
4	3	2	2 id	2 id
5	2 id	2	2	2
6	1 id	2	2	2
7	1	2	1 id	1 id
*A = Pan weight (mg) Tray color code: Forest Green Analyst: XL Date: 04·27·26	13.50	12.78	12.58	13.08
*B = Pan + Larvae weight (mg) Analyst: EM Date: 05-18-26	14.84	14.58	13.29	14.24
C = Larvae weight (mg) = B – A Analyst: JL	1.34	1.80	0.71	1.16
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.134	0.180	0.071	0.116
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.125		84.27	

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 141

Test dates: May 05-12, 2026

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	13.40	20.63	7.23	0.723	0.792	7.2	0.723	100.0	0.792	7.2	Not applicable
	B	10	10	12.81	20.89	8.08	0.808			0.808				
	C	10	10	13.92	21.70	7.78	0.778			0.778				
	D	10	10	14.98	23.58	8.60	0.860			0.860				
300	E	10	10	12.60	22.44	9.84	0.984	1.015	10.3	0.984	100.0	1.015	10.3	-28.1
	F	10	10	13.04	22.45	9.41	0.941			0.941				
	G	10	10	14.57	24.22	9.65	0.965			0.965				
	H	10	10	14.88	26.57	11.69	1.169			1.169				
450	I	10	10	13.55	21.52	7.97	0.797	0.834	6.4	0.797	100.0	0.834	6.4	-5.3
	J	10	10	14.01	22.79	8.78	0.878			0.878				
	K	10	10	13.23	21.02	7.79	0.779			0.779				
	L	10	10	13.38	22.20	8.82	0.882			0.882				
600	M	10	9	13.44	21.41	7.97	0.886	0.857	3.0	0.797	97.5	0.835	3.7	-5.4
	N	10	10	13.32	21.92	8.60	0.860			0.860				
	O	10	10	11.69	19.92	8.23	0.823			0.823				
	P	10	10	11.15	19.75	8.60	0.860			0.860				
750	Q	10	7	13.43	19.40	5.97	0.853	0.824	6.2	0.597	72.5	0.596	1.8	24.8
	R	10	7	13.19	19.24	6.05	0.864			0.605				
	S	10	8	12.01	18.02	6.01	0.751			0.601				
	T	10	7	13.56	19.36	5.80	0.829			0.580				
900	U	10	3	12.51	15.37	2.86	0.953	0.989	8.0	0.286	30.0	0.294	24.9	62.9
	V	10	2	12.87	15.07	2.20	1.100			0.220				
	W	10	4	13.11	17.06	3.95	0.988			0.395				
	X	10	3	13.60	16.35	2.75	0.917			0.275				
1050	Y	10	1	13.50	14.84	1.34	1.340	1.028	27.1	0.134	12.5	0.125	36.0	84.2
	Z	10	2	12.78	14.58	1.80	0.900			0.180				
	AA	10	1	12.58	13.29	0.71	0.710			0.071				
	BB	10	1	13.08	14.24	1.16	1.160			0.116				

Dunnett's MSD value: 0.1086 MSD = Minimum Significant Difference

PMSD: 13.7 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.

Reviewed and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	5/5/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	5/12/2026	Lab ID:	ETS-Envir. Testing Sol	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	1.0000	1.0000	1.0000
750	0.7000	0.7000	0.8000	0.7000
900	0.3000	0.2000	0.4000	0.3000
1050	0.1000	0.2000	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	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	40
*900	0.3000	0.3000	0.5769	0.4636	0.6847	15.654	4	10.00	10.00	28	40
*1050	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

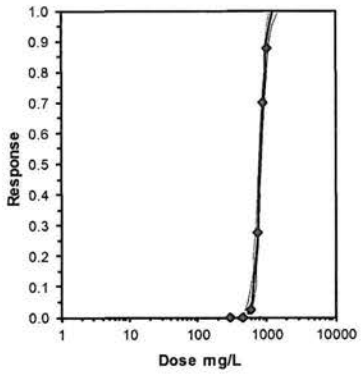
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.86822	0.896	-0.0508	1.67657
Equality of variance cannot be confirmed				

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

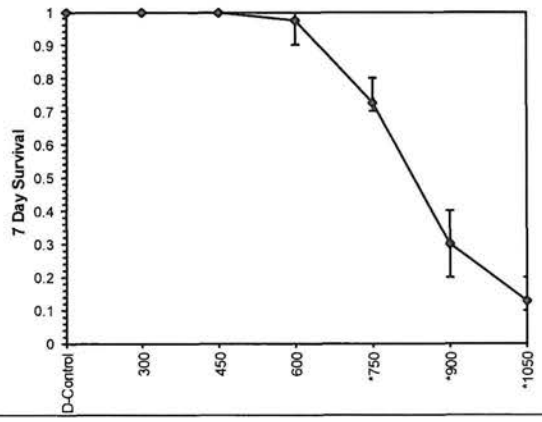
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.8531	1.66906	9.58174	16.1245	0	0.60433	9.48773	0.96258	2.92186	0.0778	3
Intercept	-32.555	4.8806	-42.121	-22.989							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	550.63	473.125	604.614
EC05	3.355	622.131	555.621	668.444
EC10	3.718	663.969	604.706	705.905
EC15	3.964	693.774	639.786	732.891
EC20	4.158	718.415	668.69	755.549
EC25	4.326	740.25	694.096	776.025
EC40	4.747	798.26	759.55	833.316
EC50	5.000	835.324	798.849	873.079
EC60	5.253	874.11	837.357	917.827
EC75	5.674	942.61	899.687	1003.81
EC80	5.842	971.259	924.202	1041.8
EC85	6.036	1005.75	952.895	1088.73
EC90	6.282	1050.9	989.432	1151.76
EC95	6.645	1121.58	1044.99	1253.38
EC99	7.326	1267.22	1155.42	1471.77



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	5/5/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	5/12/2026	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	0.7230	0.8080	0.7780	0.8600
300	0.9840	0.9410	0.9650	1.1690
450	0.7970	0.8780	0.7790	0.8820
600	0.7970	0.8600	0.8230	0.8600
750	0.5970	0.6050	0.6010	0.5800
900	0.2860	0.2200	0.3950	0.2750
1050	0.1340	0.1800	0.0710	0.1160

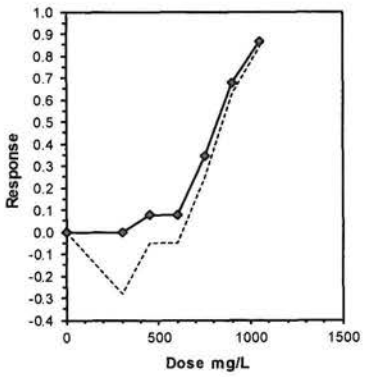
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7923	1.0000	0.7923	0.7230	0.8600	7.228	4				0.9035	1.0000
300	1.0148	1.2808	1.0148	0.9410	1.1690	10.281	4	-4.692	2.290	0.1086	0.9035	1.0000
450	0.8340	1.0527	0.8340	0.7790	0.8820	6.432	4	-0.880	2.290	0.1086	0.8345	0.9236
600	0.8350	1.0540	0.8350	0.7970	0.8600	3.683	4	-0.902	2.290	0.1086	0.8345	0.9236
750	0.5958	0.7520	0.5958	0.5800	0.6050	1.846	4				0.5958	0.6594
900	0.2940	0.3711	0.2940	0.2200	0.3950	24.919	4				0.2940	0.3254
1050	0.1253	0.1581	0.1253	0.0710	0.1800	36.010	4				0.1253	0.1386

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91862	0.844	1.077	1.44823
Bartlett's Test indicates equal variances (p = 0.29)	3.78538	11.3449		

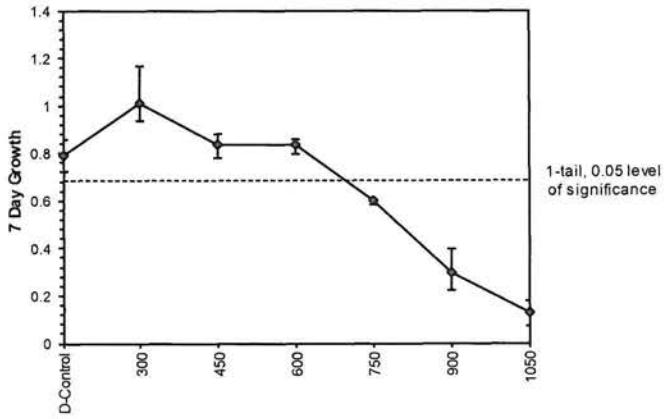
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.10859	0.13706	0.03935	0.0045	0.00239	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	398.21	83.53	334.57	742.02	1.1060
IC10	613.41	73.33	298.94	654.29	-1.4441
IC15	641.80	15.12	581.59	678.64	-0.5477
IC20	670.18	13.97	615.59	705.37	-0.5061
IC25	698.56	13.00	648.40	732.03	-0.4597
IC40	776.67	9.16	750.06	806.24	0.1096
IC50	821.58	11.46	795.00	868.29	0.7949



Dose-Response Plot



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Species: *Pimephales promelas* PpKClCR Test Number: **141**

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	XL	XL	XL	XL	VL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.96	7.78	7.74	7.66	7.71	7.60
	Dissolved oxygen (mg/L)	8.2	7.8	8.3	7.5	8.3	6.1
	Conductivity (µmhos/cm)	302		309		307	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	88				88	
	Temperature (°C)	24.9	25.2	24.8	25.0	24.8	24.9
300 mg KCl/L	pH (S.U.)	8.01	7.77	7.90	7.64	7.84	7.50
	Dissolved oxygen (mg/L)	8.2	7.8	8.3	7.3	8.3	6.1
	Conductivity (µmhos/cm)	879 → 050526 8.77µL		836		836	
	Temperature (°C)	24.9	25.0	24.8	24.8	24.9	25.1
450 mg KCl/L	pH (S.U.)	8.00	7.78	7.90	7.63	7.85	7.50
	Dissolved oxygen (mg/L)	8.2	7.8	8.3	7.3	8.3	5.8
	Conductivity (µmhos/cm)	1070		1090		1100	
	Temperature (°C)	25.0	25.0	24.9	24.9	24.9	25.1
600 mg KCl/L	pH (S.U.)	8.01	7.78	7.91	7.64	7.86	7.50
	Dissolved oxygen (mg/L)	8.2	7.7	8.3	7.5	8.3	5.8
	Conductivity (µmhos/cm)	1310		1350		1350	
	Temperature (°C)	25.0	24.9	24.8	24.9	24.7	24.8
750 mg KCl/L	pH (S.U.)	8.01	7.79	7.91	7.65	7.86	7.50
	Dissolved oxygen (mg/L)	8.2	7.8	8.3	7.5	8.3	5.8
	Conductivity (µmhos/cm)	1560		1590		1600	
	Temperature (°C)	25.0	24.9	24.8	25.1	24.9	24.8
900 mg KCl/L	pH (S.U.)	8.01	7.79	7.92	7.65	7.88	7.50
	Dissolved oxygen (mg/L)	8.2	7.8	8.4	7.5	8.4	5.9
	Conductivity (µmhos/cm)	1800		1820		1840	
	Temperature (°C)	25.0	24.9	24.8	25.1	24.9	24.8
1050 mg KCl/L	pH (S.U.)	8.02	7.79	7.93	7.66	7.89	7.57
	Dissolved oxygen (mg/L)	8.2	7.9	8.3	7.6	8.4	6.3
	Conductivity (µmhos/cm)	2060		2080		2100	
	Temperature (°C)	25.0	25.1	24.8	24.8	24.8	25.0
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **141**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	BSL	BSL	BSL	BSL	XL	XL	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.95	7.74	7.74	7.74	7.85	7.82	7.82	7.82
	Dissolved oxygen (mg/L)	8.3	7.7	8.3	7.3	8.3	7.9	8.4	5.7
	Conductivity (µmhos/cm)	305		309		306		312	
	Alkalinity (mg $CaCO_3$/L)			60					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.8	25.2	24.9	25.2	24.9	25.1	24.9	24.7
300 mg KCl/L	pH (S.U.)	7.96	7.76	7.74	7.76	7.88	7.79	7.93	7.49
	Dissolved oxygen (mg/L)	8.2	7.7	8.4	7.4	8.4	7.9	8.3	5.3
	Conductivity (µmhos/cm)	824		810		828		862	
	Temperature (°C)	24.9	24.8	25.0	25.2	25.1	24.9	24.8	24.9
450 mg KCl/L	pH (S.U.)	7.98	7.77	7.84	7.76	7.99	7.80	7.93	7.49
	Dissolved oxygen (mg/L)	8.2	7.7	8.4	7.4	8.3	7.8	8.3	5.3
	Conductivity (µmhos/cm)	1080		1070		1080		1110	
	Temperature (°C)	24.9	24.8	25.0	25.0	24.9	25.2	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.98	7.78	7.87	7.74	8.01	7.78	7.94	7.47
	Dissolved oxygen (mg/L)	8.3	7.7	8.4	7.4	8.3	7.6	8.3	5.2
	Conductivity (µmhos/cm)	1330		1320		1330		1370	
	Temperature (°C)	25.0	25.1	24.9	25.0	24.9	24.8	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.99	7.76	7.90	7.75	8.01	7.77	7.94	7.49
	Dissolved oxygen (mg/L)	8.3	7.6	8.3	7.4	8.3	7.7	8.3	5.4
	Conductivity (µmhos/cm)	1580		1560		1590		1620	
	Temperature (°C)	24.9	25.1	24.9	25.1	25.0	24.8	25.0	24.8
900 mg KCl/L	pH (S.U.)	8.00	7.77	7.91	7.74	8.03	7.76	7.95	7.57
	Dissolved oxygen (mg/L)	8.3	7.7	8.4	7.4	8.4	7.6	8.3	6.1
	Conductivity (µmhos/cm)	1800		1800		1850		1890	
	Temperature (°C)	24.9	24.9	25.1	24.9	25.0	24.9	25.0	24.8
1050 mg KCl/L	pH (S.U.)	8.01	7.81	7.91	7.73	8.05	7.76	7.96	7.49
	Dissolved oxygen (mg/L)	8.3	7.9	8.4	7.4	8.4	7.6	8.3	5.0
	Conductivity (µmhos/cm)	2080		2060		2090		2140	
	Temperature (°C)	24.9	24.9	24.9	24.9	25.0	24.9	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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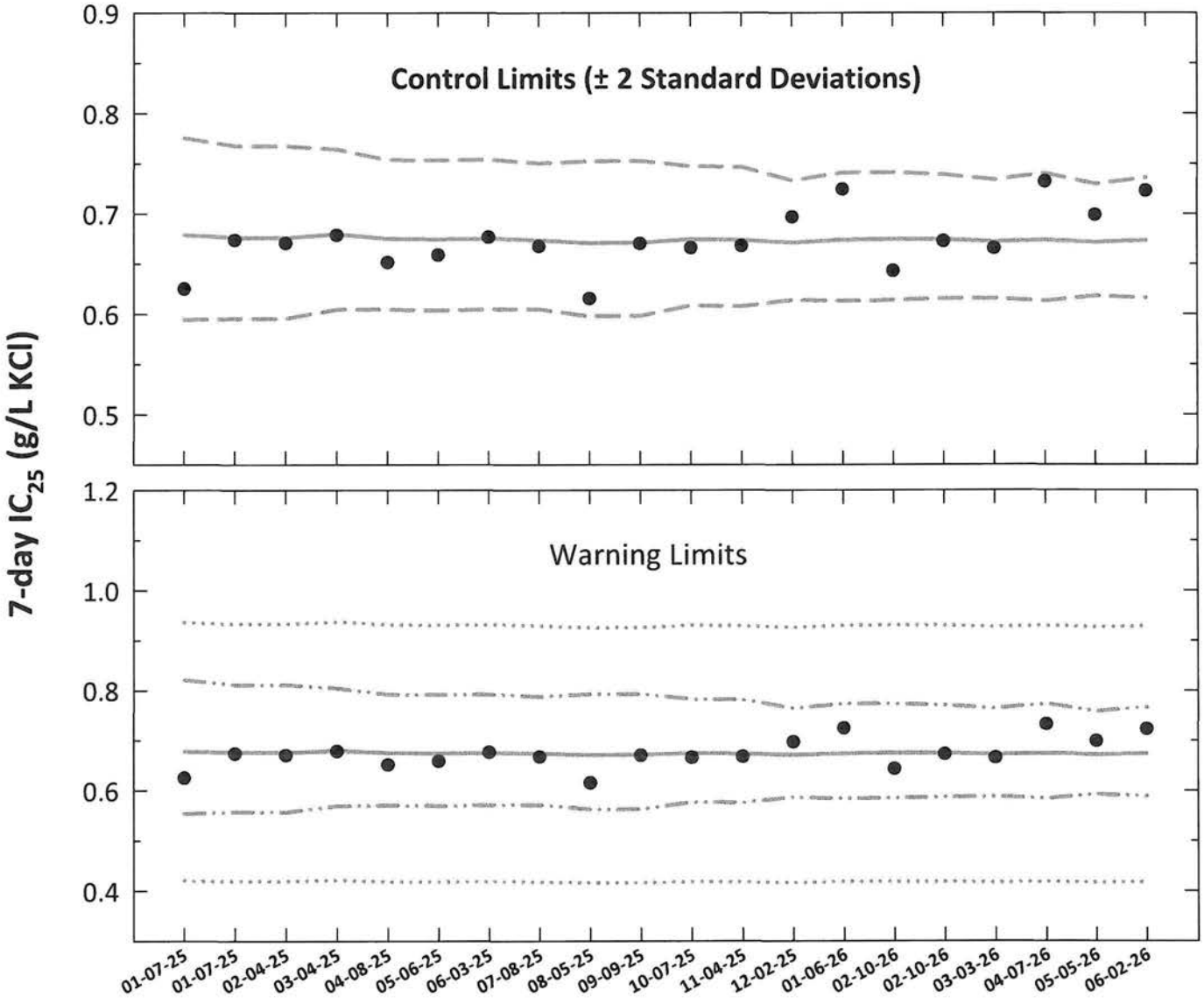
Pimephales promelas
Chronic Reference Toxicant Control Chart
Source: In-house Culture



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)

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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	01-07-25	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
2	01-07-25	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
3	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
4	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
5	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
6	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
7	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
8	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
9	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
10	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
11	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
12	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
13	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
14	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302
15	02-10-26	0.6431	-0.1917	-0.1709	0.0204	0.6746	0.6141	0.7411	0.5849	0.7732	0.4183	0.9310
16	02-10-26	0.6727	-0.1722	-0.1710	0.0199	0.6745	0.6156	0.7391	0.5871	0.7703	0.4182	0.9308
17	03-03-26	0.6656	-0.1768	-0.1725	0.0191	0.6722	0.6157	0.7339	0.5881	0.7640	0.4168	0.9277
18	04-07-26	0.7319	-0.1355	-0.1715	0.0205	0.6737	0.6131	0.7403	0.5838	0.7726	0.4177	0.9298
19	05-05-26	0.6986	-0.1558	-0.1730	0.0180	0.6715	0.6181	0.7294	0.5920	0.7578	0.4163	0.9266
20	06-02-26	0.7227	-0.1410	-0.1718	0.0193	0.6733	0.6161	0.7358	0.5884	0.7661	0.4174	0.9292

Note:

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

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

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

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

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

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

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ETS
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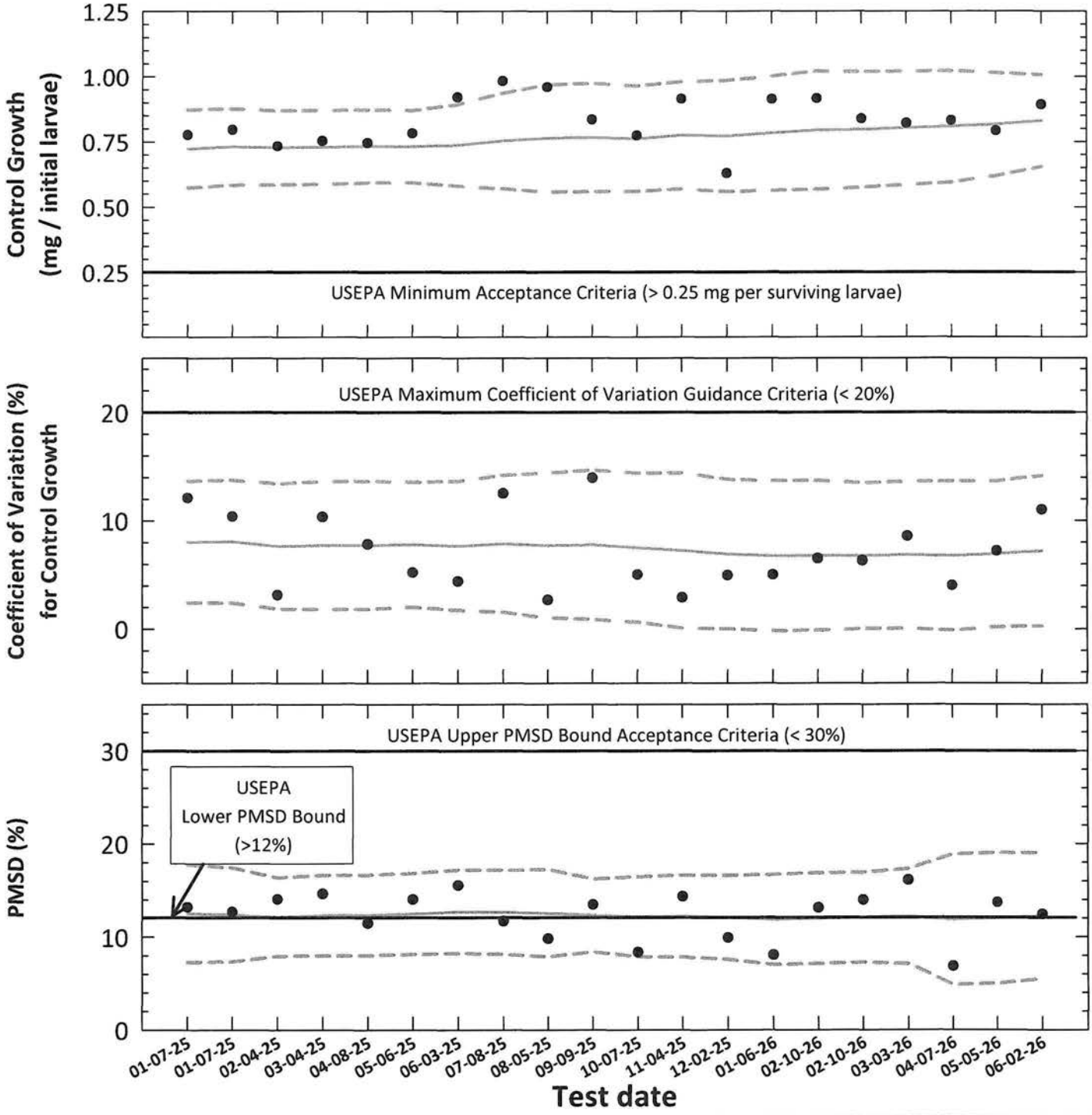
Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

Central Tendency (mean Control Growth, CV or PMSD)

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

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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	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
2	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
3	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
4	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
5	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
6	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
7	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
8	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
9	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
10	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
11	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
12	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
13	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
14	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7
15	02-10-26	100	0.916	6.5	0.1204	13.1	0.795	0.568	1.022	6.8	-0.1	13.7	12.0	7.1	16.9
16	02-10-26	100	0.838	6.3	0.1170	14.0	0.797	0.575	1.019	6.8	0.0	13.5	12.1	7.3	16.9
17	03-03-26	100	0.822	8.6	0.1323	16.1	0.803	0.586	1.020	6.8	0.0	13.6	12.2	7.1	17.3
18	04-07-26	100	0.832	4.0	0.0569	6.8	0.809	0.595	1.022	6.7	-0.1	13.6	11.9	4.8	18.9
19	05-05-26	100	0.792	7.2	0.1086	13.7	0.817	0.619	1.014	6.9	0.2	13.7	12.0	5.0	19.0
20	06-02-26	100	0.892	11.0	0.1110	12.4	0.830	0.654	1.006	7.2	0.3	14.1	12.2	5.5	19.0

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 (90^{th} 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 (10^{th} 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 (90^{th} 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

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: 142

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS 2478				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	05.25.26	*Artemia* CHM number:	CHM1432
Hatch dates and times:	06-01-26 1400 TO 06-02-26 0500	Drying information for weight determination:	
Transfer vessel information:	pH = 7.96 S.U. Temperature = 24.3 °C	Date / Time in oven:	06-09-26 0545
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	06-10-26 0545
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	06-02-26	0505	JH	1500	JH	0705	JH	05.27.26 A
1	06-03-26	0500	JH	1345	JH	0700	JH	↓
2	06-04-26	0500	JH	1230	JH	0700	JH	06.01.26 A
3	06-05-26	0500	JH	1100	JH	0700	JH	↓
4	06-06-26	0700	JH	1215	JH	0900	JH	06.04.26A
5	06-07-26	0600	JH	1100	JH	0800	JH	↓
6	06-08-26	0500	JH	1100	JH	0745	JH	↓
7	06-09-26					0530	JH	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066685

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	0.892	
Average weight per surviving larvae:	0.892	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	842.2
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	722.7

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **142**

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: Ruby Analyst: JP Date: 05-26-26	9.63	14.85	14.20	14.58	13.17	10.33	12.36	12.28	10.24	9.84	(9.91) 9.93	9.93
*B = Pan + Larvae weight (mg) Analyst: JJ Date: 06-11-26	17.34	23.39	23.98	24.24	22.03	20.20	22.47	21.02	18.91	18.17	17.22	18.41
C = Larvae weight (mg) = B – A Analyst: JK	7.71	8.54	9.78	9.66	8.86	9.87	10.11	8.74	8.67	8.33	7.31	8.48
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JK	0.771	0.854	0.978	0.966	0.886	0.987	1.011	0.874	0.867	0.833	0.731	0.848
Average weight per initial number of larvae (mg)	0.892				0.940		-5.37.		0.820		8.17.	
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: **142**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	9 1d	9 1d	9 1d	7 3d	7 3d	7 3d	7 3d
2	10	10	10	10	10	9	9	9	6 1d	6 1d	7	7
3	10	10	10	10	10	9	9	9	5 1d	6	5 Bd	7
4	10	10	10	10	9 1d	9	8 1d	9	5	6	5	5 2d
5	10	10	10	10	8 1d	9	8	9	3 2d	4 2d	4 1d	3 2d
6	10	10	10	10	8	8 1d	8	8 1d	3	4	4	3
7	10	10	10	9 1d	7 1d	7 1d	8	7 1d	3	2 2d	2 2d	3
*A = Pan weight (mg) Tray color code: Ruby Analyst: JP Date: 05-26-26	9.83	11.87	9.23	15.25	14.03	10.95	10.01	9.64	9.72	9.95	13.97	13.11
*B = Pan + Larvae weight (mg) Analyst: JP Date: 06.11.26	18.00	20.23	17.49	23.04	20.54	17.07	17.33	16.08	12.24	12.38	16.38	16.42
C = Larvae weight (mg) = B – A Analyst: JP	8.17	8.36	8.26	7.79	6.46	6.12	7.32	6.44	2.52	2.49	2.41	3.31
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0.817	0.836	0.826	0.779	0.646	0.612	0.732	0.644	0.252	0.249	0.241	0.331
Average weight per initial number of larvae (mg)	0.815				0.659				0.267			
Percent reduction from control (%)		8.77.				26.27.				70.17.		

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **142**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 8d	3 7d	3 7d	3 7d
2	2	2 1d	3	3
3	1 1d	1 1d	3	3
4	1	1	3	3
5	0 1d	1	2 1d	0 8d
6	0	1	1 1d	0
7	0	1	0 1d	0
*A = Pan weight (mg) Tray color code: Ruby Analyst: JB Date: 06-26-26	12.22	13.04	13.53	9.92
*B = Pan + Larvae weight (mg) Analyst: JL Date: 06-11-26		14.20		
C = Larvae weight (mg) = B – A Analyst: JL	06.09.26 JL	1.16		06.09.26 JL
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0	0.116	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.029		96.7%	

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 142

Test dates: June 02-09, 2026

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	9.63	17.34	7.71	0.771	0.892	11.0	0.771	100.0	0.892	11.0	Not applicable
	B	10	10	14.85	23.39	8.54	0.854			0.854				
	C	10	10	14.20	23.98	9.78	0.978			0.978				
	D	10	10	14.58	24.24	9.66	0.966			0.966				
300	E	10	10	13.17	22.03	8.86	0.886	0.940	7.4	0.886	100.0	0.940	7.4	-5.3
	F	10	10	10.33	20.20	9.87	0.987			0.987				
	G	10	10	12.36	22.47	10.11	1.011			1.011				
	H	10	10	12.28	21.02	8.74	0.874			0.874				
450	I	10	10	10.24	18.91	8.67	0.867	0.820	7.4	0.867	100.0	0.820	7.4	8.1
	J	10	10	9.84	18.17	8.33	0.833			0.833				
	K	10	10	9.91	17.22	7.31	0.731			0.731				
	L	10	10	9.93	18.41	8.48	0.848			0.848				
600	M	10	10	9.83	18.00	8.17	0.817	0.836	2.5	0.817	97.5	0.815	3.1	8.7
	N	10	10	11.87	20.23	8.36	0.836			0.836				
	O	10	10	9.23	17.49	8.26	0.826			0.826				
	P	10	9	15.25	23.04	7.79	0.866			0.779				
750	Q	10	7	14.08	20.54	6.46	0.923	0.908	2.5	0.646	72.5	0.659	7.8	26.2
	R	10	7	10.95	17.07	6.12	0.874			0.612				
	S	10	8	10.01	17.33	7.32	0.915			0.732				
	T	10	7	9.64	16.08	6.44	0.920			0.644				
900	U	10	3	9.72	12.24	2.52	0.840	1.091	16.0	0.252	25.0	0.267	16.2	70.1
	V	10	2	9.95	12.38	2.43	1.215			0.243				
	W	10	2	13.97	16.38	2.41	1.205			0.241				
	X	10	3	13.11	16.42	3.31	1.103			0.331				
1050	Y	10	0	0.00	0.00	0.00	0.000	1.160	0.0	0.000	2.5	0.029	200.0	96.7
	Z	10	1	13.04	14.20	1.16	1.160			0.116				
	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: 0.1110 MSD = Minimum Significant Difference

PMSD: 12.4 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 Jim Sumner

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	6/2/2026	Test ID:	PpKCICR	Sample ID	REF-Ref Toxicant
End Date:	6/9/2026	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	0.9000
750	0.7000	0.7000	0.8000	0.7000
900	0.3000	0.2000	0.2000	0.3000
1050	0.0000	0.1000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	40
*900	0.2500	0.2500	0.5216	0.4636	0.5796	12.838	4	10.00	10.00	30	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

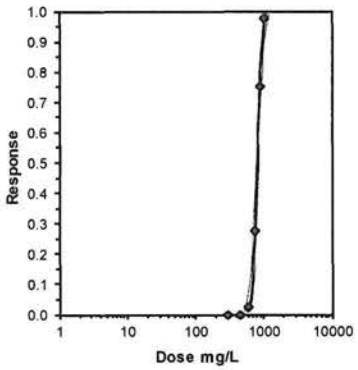
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93505	0.896	0.20522	1.40043
Equality of variance cannot be confirmed				

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

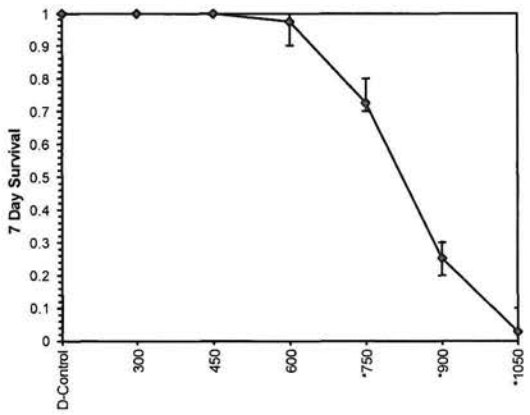
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.1057	2.08056	12.0278	20.1836	0	0.37197	9.48773	0.98471	2.90969	0.06209	3
Intercept	-41.862	6.07012	-53.76	-29.965							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	582.431	513.454	629.826
EC05	3.355	642.035	583.467	682.545
EC10	3.718	676.263	624.103	713.011
EC15	3.964	700.382	652.747	734.736
EC20	4.158	720.164	676.135	752.822
EC25	4.326	737.579	696.559	769.03
EC40	4.747	783.352	748.826	813.581
EC50	5.000	812.246	780.144	843.767
EC60	5.253	842.205	810.836	877.162
EC75	5.674	894.471	860.193	940.366
EC80	5.842	916.102	879.377	968.04
EC85	6.036	941.975	901.635	1002.04
EC90	6.282	975.571	929.681	1047.39
EC95	6.645	1027.58	971.756	1119.67
EC99	7.326	1132.74	1053.71	1271.61



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	6/2/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	6/9/2026	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	0.7710	0.8540	0.9780	0.9660
300	0.8860	0.9870	1.0110	0.8740
450	0.8670	0.8330	0.7310	0.8480
600	0.8170	0.8360	0.8260	0.7790
750	0.6460	0.6120	0.7320	0.6440
900	0.2520	0.2430	0.2410	0.3310
1050	0.0000	0.1160	0.0000	0.0000

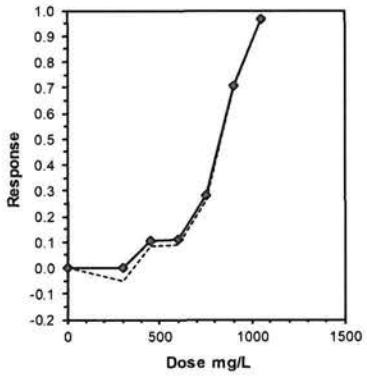
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8923	1.0000	0.8923	0.7710	0.9780	11.011	4				0.9159	1.0000
300	0.9395	1.0530	0.9395	0.8740	1.0110	7.405	4	-0.975	2.290	0.1110	0.9159	1.0000
450	0.8198	0.9187	0.8198	0.7310	0.8670	7.414	4	1.495	2.290	0.1110	0.8198	0.8950
600	0.8145	0.9129	0.8145	0.7790	0.8360	3.058	4	1.604	2.290	0.1110	0.8145	0.8893
750	0.6585	0.7380	0.6585	0.6120	0.7320	7.808	4				0.6585	0.7190
900	0.2668	0.2990	0.2668	0.2410	0.3310	16.157	4				0.2668	0.2913
1050	0.0290	0.0325	0.0290	0.0000	0.1160	200.000	4				0.0290	0.0317

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95547	0.844	-0.4526	-0.6859
Bartlett's Test indicates equal variances (p = 0.26)	3.99948	11.3449		

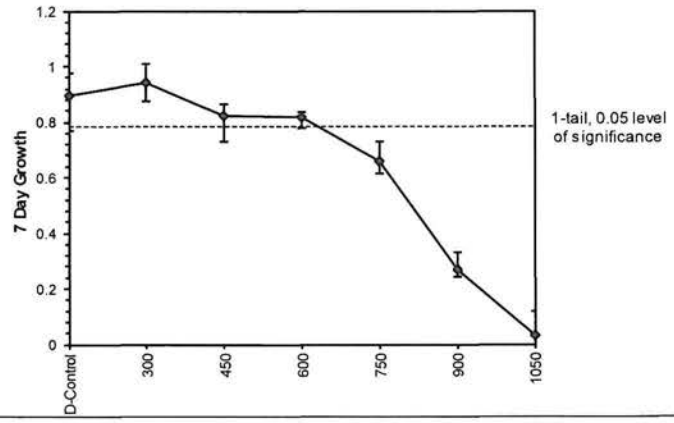
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.11103	0.12444	0.01451	0.0047	0.06803	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	371.46	49.90	276.27	622.46	1.0579
IC10	442.92	90.51	341.60	754.08	0.3973
IC15	634.62	53.02	312.12	709.33	-2.4270
IC20	678.65	30.73	599.89	774.40	-1.7207
IC25	722.69	23.39	649.82	785.60	0.0036
IC40	791.73	8.70	766.96	819.60	0.2829
IC50	826.79	7.37	803.32	850.61	0.3006



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **142**

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.72	7.83	7.74	7.72	7.76	7.75
	Dissolved oxygen (mg/L)	8.5	8.0	8.4	7.8	8.5	7.8
	Conductivity (µmhos/cm)	306		310		303	
	Alkalinity (mg $CaCO_3$/L)	60				62	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.8	25.2	24.7	24.5	24.7	25.3
300 mg KCl/L	pH (S.U.)	7.86	7.79	7.89	7.73	7.88	7.76
	Dissolved oxygen (mg/L)	8.6	7.9	8.4	7.8	8.4	7.7
	Conductivity (µmhos/cm)	821		832		810	
	Temperature (°C)	24.8	25.0	24.9	24.9	24.6	25.5
450 mg KCl/L	pH (S.U.)	7.87	7.79	7.90	7.72	7.84	7.75
	Dissolved oxygen (mg/L)	8.6	7.8	8.4	7.6	8.5	7.7
	Conductivity (µmhos/cm)	1090		1070		1060	
	Temperature (°C)	24.9	25.0	24.7	24.7	24.6	25.5
600 mg KCl/L	pH (S.U.)	7.88	7.78	7.90	7.73	7.90	7.75
	Dissolved oxygen (mg/L)	8.6	7.8	8.4	7.7	8.5	7.7
	Conductivity (µmhos/cm)	1330		1350		1320	
	Temperature (°C)	24.9	24.7	24.7	24.7	24.7	25.2
750 mg KCl/L	pH (S.U.)	7.89	7.78	7.91	7.73	7.90	7.74
	Dissolved oxygen (mg/L)	8.6	7.9	8.4	7.7	8.5	7.5
	Conductivity (µmhos/cm)	1560		1600		1570	
	Temperature (°C)	24.9	25.1	24.8	24.8	24.7	25.2
900 mg KCl/L	pH (S.U.)	7.89	7.78	7.92	7.74	7.91	7.74
	Dissolved oxygen (mg/L)	8.6	7.9	8.4	7.8	8.5	7.5
	Conductivity (µmhos/cm)	1810		1840		1810	
	Temperature (°C)	24.9	24.8	24.8	24.4	24.7	25.2
1050 mg KCl/L	pH (S.U.)	7.90	7.79	7.92	7.74	7.92	7.74
	Dissolved oxygen (mg/L)	8.6	7.9	8.5	7.8	8.5	7.6
	Conductivity (µmhos/cm)	2040		2090		2050	
	Temperature (°C)	24.9	24.8	24.8	24.4	24.8	25.3
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **142**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.78	7.67	7.77	7.62	7.82	7.78	7.77	7.56
	Dissolved oxygen (mg/L)	8.4	~~8.4~~ 7.6	8.5	7.2	8.6	7.8	8.4	6.4
	Conductivity (µmhos/cm)	307		292		287		309	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.9	24.7	24.7	25.1	24.7	25.2	24.8	25.3
300 mg KCl/L	pH (S.U.)	7.90	7.63	7.96	7.63	8.02	7.76	7.90	7.91
	Dissolved oxygen (mg/L)	8.3	7.0	8.4	7.2	8.6	7.8	8.3	5.8
	Conductivity (µmhos/cm)	845		796		800		841	
	Temperature (°C)	25.0	25.1	24.7	24.7	24.8	24.9	24.8	25.0
450 mg KCl/L	pH (S.U.)	7.91	7.68	7.96	7.64	8.02	7.76	7.90	7.84
	Dissolved oxygen (mg/L)	8.3	7.0	8.5	7.3	8.6	7.7	8.3	5.4
	Conductivity (µmhos/cm)	1090		1030		1040		1070	
	Temperature (°C)	25.0	24.8	24.6	24.7	24.8	24.9	24.9	25.0
600 mg KCl/L	pH (S.U.)	7.92	7.67	7.97	7.64	8.03	7.75	7.91	7.53
	Dissolved oxygen (mg/L)	8.3	7.5	8.5	7.3	8.6	7.7	8.3	5.6
	Conductivity (µmhos/cm)	1340		1270		1290		1340	
	Temperature (°C)	25.2	24.9	24.8	24.8	24.7	25.1	24.9	24.7
750 mg KCl/L	pH (S.U.)	7.93	7.66	7.96	7.65	8.03	7.75	7.92	7.53
	Dissolved oxygen (mg/L)	8.3	7.5	8.5	7.4	8.6	7.7	8.3	6.2
	Conductivity (µmhos/cm)	1580		1500		1520		1590	
	Temperature (°C)	25.0	24.9	24.6	24.6	24.7	24.9	24.9	24.7
900 mg KCl/L	pH (S.U.)	7.93	7.68	7.96	7.65	8.04	7.75	7.92	7.53
	Dissolved oxygen (mg/L)	8.4	7.0	8.5	7.3	8.6	7.7	8.3	5.9
	Conductivity (µmhos/cm)	1850		~~1700~~ 1750		1780		1860	
	Temperature (°C)	25.0	24.9	24.6	24.6	24.7	25.0	25.0	24.9
1050 mg KCl/L	pH (S.U.)	7.94	7.68	7.95	7.66	8.04	7.75	7.93	7.81
	Dissolved oxygen (mg/L)	8.4	7.4	8.5	7.2	8.6	7.7	8.3	6.2
	Conductivity (µmhos/cm)	2110		2020		2030		2130	
	Temperature (°C)	25.0	25.0	24.7	24.6	24.6	25.0	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final