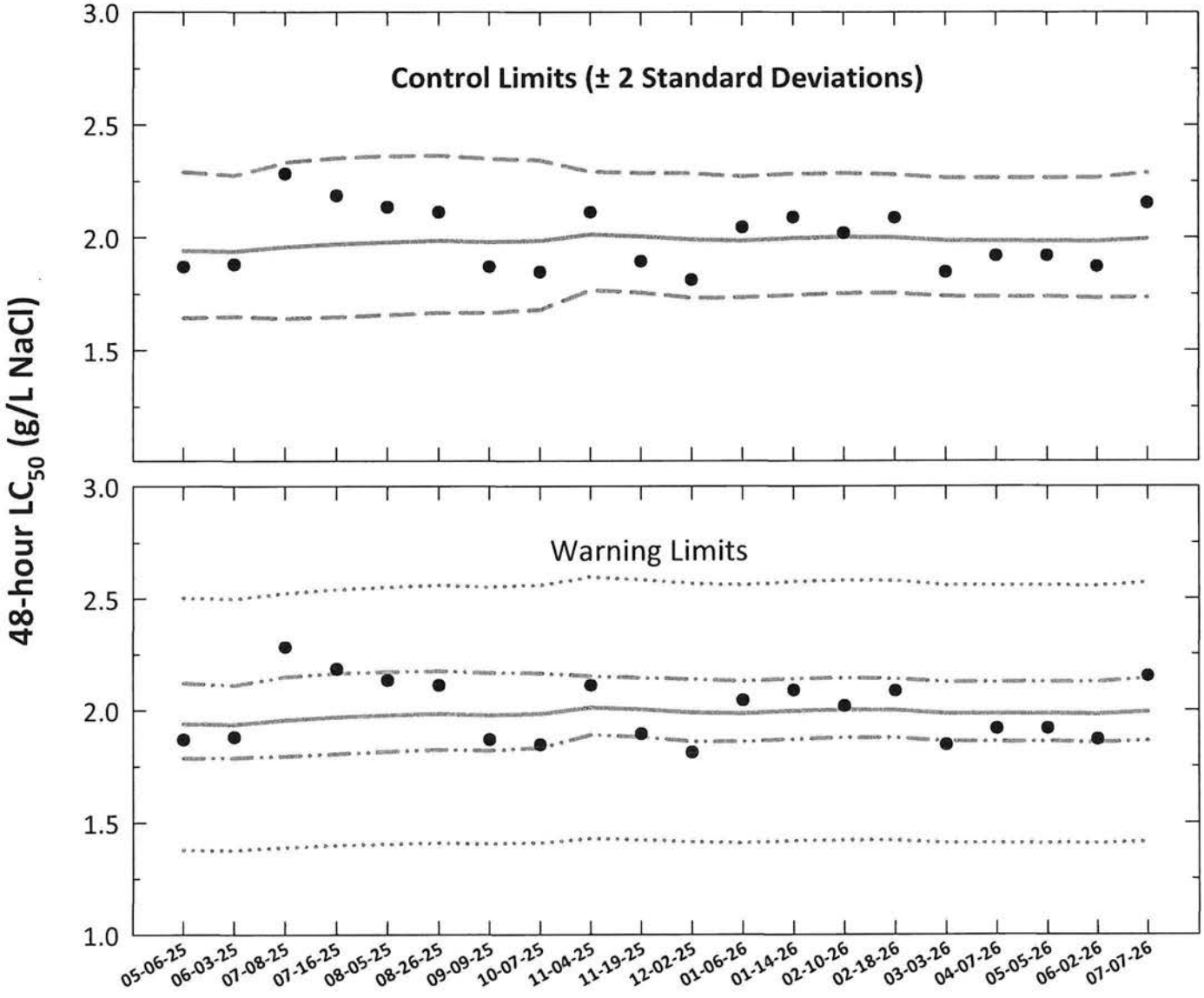
ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic LC_{50} ± $S_{A.75}$ 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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**07-07-26**	**2.1520**	0.3328	0.2994	0.0300	1.9924	1.7351	2.2878	1.8632	2.1407	1.4146	2.5702

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 # 35

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

Stock solution INSS #: ~~2486~~ 07.09.26 2479

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
Control, MHSW	pH (S.U.)	7.76	7.88	7.85
	Dissolved oxygen (mg/L)	8.6	8.2	8.4
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.8	25.2	25.1
1000 mg/L	pH (S.U.)	7.90	7.92	7.88
	Dissolved oxygen (mg/L)	8.7	8.2	8.4
	Conductivity (µmhos/cm)	2080		
	Temperature (°C)	24.5	25.3	25.2
1500 mg/L	pH (S.U.)	7.91	7.93	7.89
	Dissolved oxygen (mg/L)	8.6	8.2	8.3
	Conductivity (µmhos/cm)	3020		
	Temperature (°C)	24.4	25.3	25.2
2000 mg/L	pH (S.U.)	7.91	7.93	7.90
	Dissolved oxygen (mg/L)	8.6	8.2	8.4
	Conductivity (µmhos/cm)	3840		
	Temperature (°C)	24.5	25.0	24.8
2500 mg/L	pH (S.U.)	7.92	7.93	7.90
	Dissolved oxygen (mg/L)	8.6	8.2	8.3
	Conductivity (µmhos/cm)	4670		
	Temperature (°C)	24.9	25.0	24.7
3000 mg/L	pH (S.U.)	7.92	7.93	
	Dissolved oxygen (mg/L)	8.6	8.2	
	Conductivity (µmhos/cm)	5630		X 07.09.26
	Temperature (°C)	24.9	24.9	

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by 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

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 # 35

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-07-26	* 0650	JP	0905	JP	2B2	Red	06-30-26A
24	07-08-26			0850	JP			
48 Termination	07-09-26			0901	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):	06-30-26 D
Age:	< 24-hours old
Date and time organisms were born between:	07-06-26 1226 TO 07-07-26 0650
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01
	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	5	5	2 [3d]	2 [3d]	0 [5d]	0 [5d]	0 [5d]	0 [5d]
48 Termination	4 [1d]	3 [2d]	5	5	0 [5d]	0 [5d]	0 [2d]	0 [2d]	0	0	0	0
Mean Survival	85%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	2066.0
Upper 95% confidence limit (mg NaCl/L)	2241.6
48-hour LC_{50} (mg NaCl/L)	2152.0

Comments:

Test Reviewed by: [signature]

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	7/7/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/9/2026	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	1.0000	1.0000	1.0000	1.0000
2500	1.0000	1.0000	0.4000	0.4000
3000	0.0000	0.0000	0.0000	0.0000

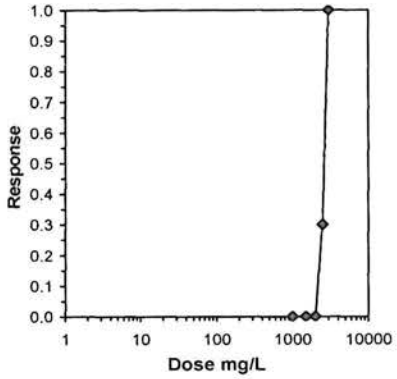
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2500	0.7000	0.7000	1.0150	0.6847	1.3453	37.574	4	14.00	10.00	6	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)	0.63123	0.868	-1E-15	2.98039
Equality of variance cannot be confirmed				

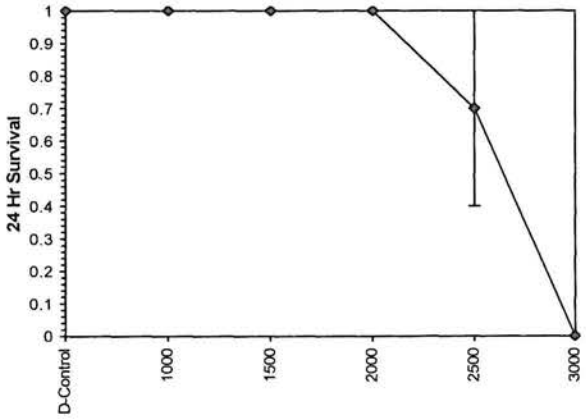
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2500	3000	2738.61	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	2577.01	2472.14	2686.34
5.0%	2589.85	2471.53	2713.84
10.0%	2602.05	2463.7	2748.16
20.0%	2623.09	2412.62	2851.93
Auto-0.0%	2577.01	2472.14	2686.34



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

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

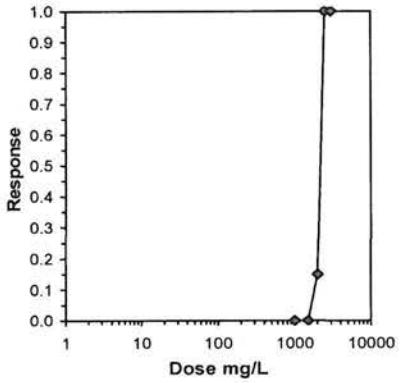
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.8500	0.8500	1.1709	0.8861	1.3453	18.840	4	14.00	10.00	3	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)	0.6604	0.844	-1.014	5.17655
Equality of variance cannot be confirmed				

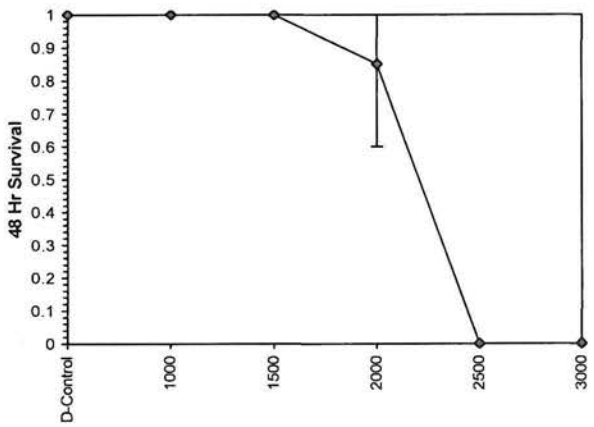
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	2152.02	2066.01	2241.61
5.0%	2172.4	2070.41	2279.41
10.0%	2186.81	2052.4	2330.02
20.0%	2192.47	2139.07	2247.21
Auto-0.0%	2152.02	2066.01	2241.61

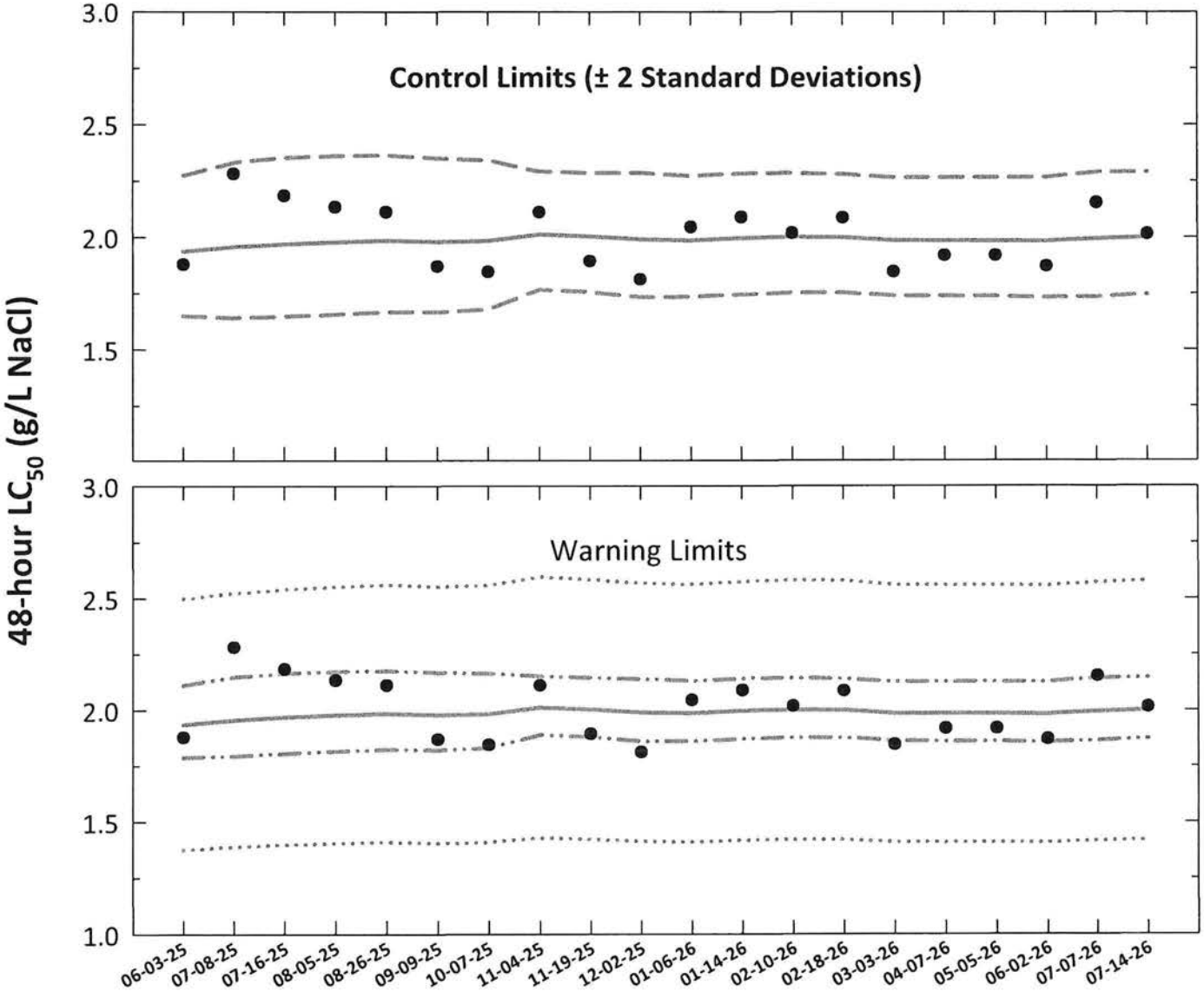


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	07-07-26	2.1520	0.3328	0.2994	0.0300	1.9924	1.7351	2.2878	1.8632	2.1407	1.4146	2.5702
20	07-14-26	2.0151	0.3043	0.3010	0.0293	1.9998	1.7472	2.2890	1.8735	2.1444	1.4199	2.5798

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 # 36

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 #: 2497

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.74	7.94	7.87
	Dissolved oxygen (mg/L)	8.5	8.4	8.3
	Conductivity (µmhos/cm)	314		
	Alkalinity (mg/L $CaCO_3$)	88 (86)		
	Hardness (mg/L $CaCO_3$)	80 (86)		
	Temperature (°C)	25.0	25.2	24.9
1000 mg/L	pH (S.U.)	7.81	7.94	7.92
	Dissolved oxygen (mg/L)	8.5	8.4	8.3
	Conductivity (µmhos/cm)	2140		
	Temperature (°C)	25.0	24.9	24.7
1500 mg/L	pH (S.U.)	7.83	7.93	7.92
	Dissolved oxygen (mg/L)	8.5	8.3	8.3
	Conductivity (µmhos/cm)	3020		
	Temperature (°C)	24.9	24.9	24.7
2000 mg/L	pH (S.U.)	7.84	7.93	7.92
	Dissolved oxygen (mg/L)	8.6	8.3	8.3
	Conductivity (µmhos/cm)	3930		
	Temperature (°C)	24.9	25.1	24.7
2500 mg/L	pH (S.U.)	7.85	7.92	7.91
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	4760		
	Temperature (°C)	25.1	24.8	25.0
3000 mg/L	pH (S.U.)	7.86	7.91	7.93
	Dissolved oxygen (mg/L)	8.6	8.3	8.2
	Conductivity (µmhos/cm)	5690		
	Temperature (°C)	25.1	24.8	24.7

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by 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

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 # 36

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-12-26	* 0800	JP	1010	JP	2B	Rec	07-09-26A
24	07-13-26			1007	JP			
48 Termination	07-14-26			1010	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):	07-07-26 D
Age:	< 24-hours old
Date and time organisms were born between:	07-13-26 1231 to 07-14-26 0800
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01
	Temperature (°C): 24.6°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 12	4 12	5	4 12	4 12	4 12	0 25	5	0 25	0 25	0 25	1 24
48 Termination	1 36	2 24	3 24	3 12	0 24	1 24	0	1 24	0	0	0	0 48
Mean Survival	45%				10%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	1874.5
Upper 95% confidence limit (mg NaCl/L)	2145.3
48-hour LC_{50} (mg NaCl/L)	2015.1

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	7/14/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/16/2026	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	0.8000	1.0000	0.8000
2500	0.8000	0.8000	0.0000	1.0000
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	0.8500	0.8500	1.1667	1.1071	1.3453	10.206	4	12.00	10.00	3	20
2500	0.6500	0.6500	0.9463	0.2255	1.3453	52.146	4	12.00	10.00	7	20
*3000	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20

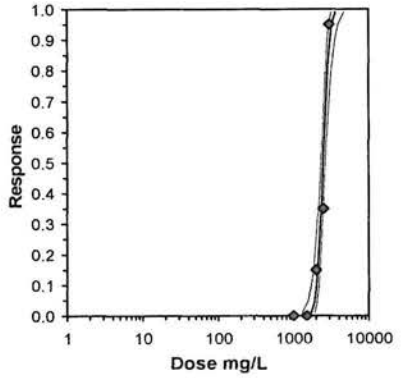
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.67369	0.884	-2.0775	9.97885
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2500	3000	2738.61	
Treatments vs D-Control				

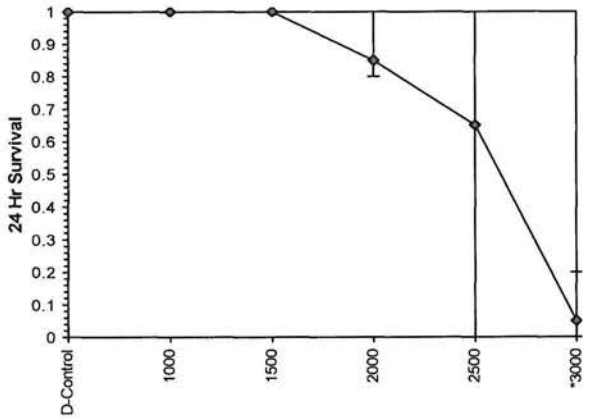
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.2045	2.90329	8.51407	19.895	0	4.04289	7.81472	0.25687	3.39588	0.0704	3
Intercept	-43.237	9.86125	-62.565	-23.909							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1706.48	1310.44	1923.74
EC05	3.355	1905.8	1568.66	2090.91
EC10	3.718	2021.41	1723.84	2189.27
EC15	3.964	2103.35	1835.07	2260.75
EC20	4.158	2170.83	1926.66	2321.52
EC25	4.326	2230.45	2006.9	2377.3
EC40	4.747	2388.03	2210.23	2539.83
EC50	5.000	2488.15	2327.64	2659.61
EC60	5.253	2592.46	2437.49	2800.79
EC75	5.674	2775.62	2604.22	3084.45
EC80	5.842	2851.85	2666.82	3212.87
EC85	6.036	2943.34	2738.52	3373.21
EC90	6.282	3062.65	2827.96	3590.85
EC95	6.645	3248.43	2961.01	3946.05
EC99	7.326	3627.86	3218.32	4723.56



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	7/14/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/16/2026	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	0.4000	0.6000	0.6000
2500	0.0000	0.2000	0.0000	0.2000
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	0.4500	0.4500	0.7301	0.4636	0.8861	27.587	4	10.00	10.00	11	20
*2500	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

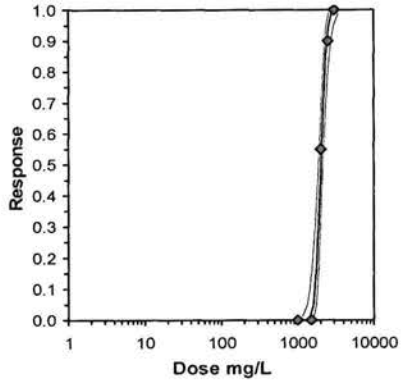
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82498	0.868	-0.7347	2.2229
Equality of variance cannot be confirmed				

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

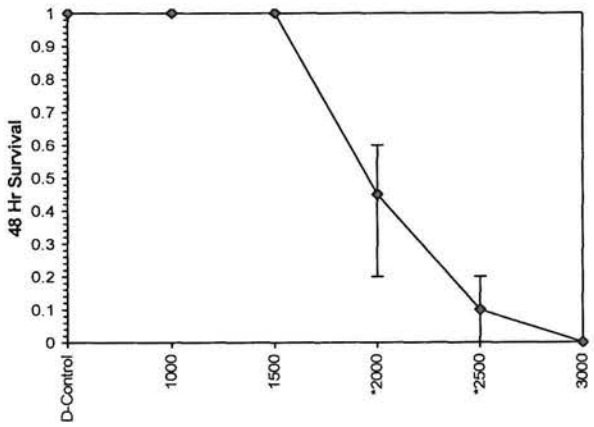
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.2237	3.23436	9.88433	22.563	0	1.26017	7.81472	0.73861	3.30429	0.06164	5
Intercept	-48.608	10.7272	-69.633	-27.582							
TSCR											

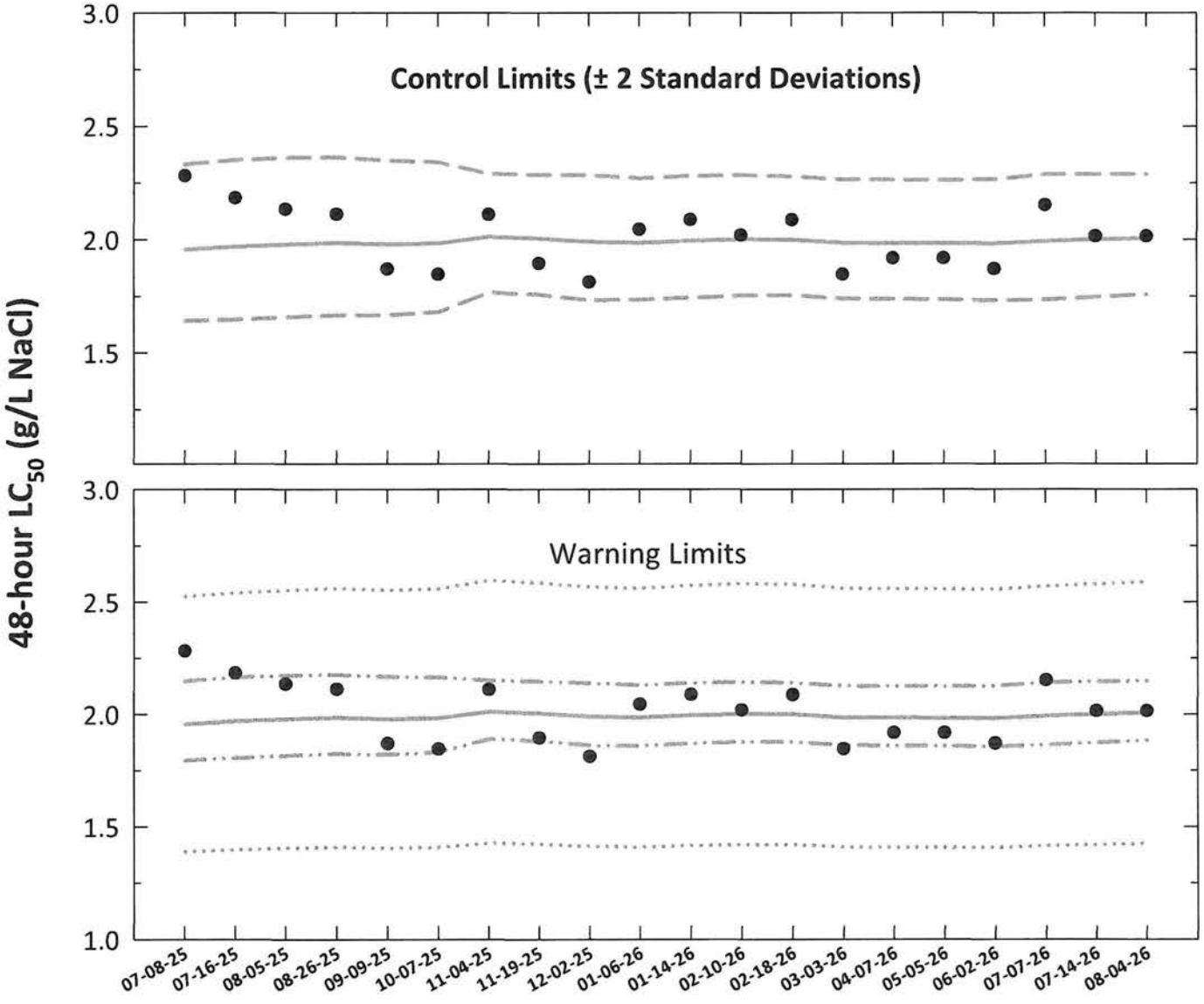
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1448.42	1137.28	1621.97
EC05	3.355	1595.51	1327.3	1746.19
EC10	3.718	1679.94	1439.43	1818.56
EC15	3.964	1739.41	1519.1	1870.66
EC20	4.158	1788.17	1584.43	1914.48
EC25	4.326	1831.1	1641.61	1954.2
EC40	4.747	1943.88	1787.81	2066.29
EC50	5.000	2015.05	1874.47	2145.33
EC60	5.253	2088.82	1957.7	2236.07
EC75	5.674	2217.48	2085.97	2416.56
EC80	5.842	2270.71	2133.75	2498.48
EC85	6.036	2334.37	2187.91	2600.97
EC90	6.282	2417.01	2254.51	2740.15
EC95	6.645	2544.91	2351.9	2966.64
EC99	7.326	2803.36	2536.17	3456.65



Dose-Response Plot



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



- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	07-07-26	2.1520	0.3328	0.2994	0.0300	1.9924	1.7351	2.2878	1.8632	2.1407	1.4146	2.5702
19	07-14-26	2.0151	0.3043	0.3010	0.0293	1.9998	1.7472	2.2890	1.8735	2.1444	1.4199	2.5798
20	08-04-26	2.0151	0.3043	0.3025	0.0286	2.0068	1.7588	2.2897	1.8832	2.1478	1.4248	2.5887

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

ETC

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 37

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 #: 2497

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.76	7.81	7.84
	Dissolved oxygen (mg/L)	8.5	8.0	8.1
	Conductivity (µmhos/cm)	304		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	25.1	25.2	25.3
1000 mg/L	pH (S.U.)	7.92	7.86	7.87
	Dissolved oxygen (mg/L)	8.6	8.0	8.1
	Conductivity (µmhos/cm)	2030		
	Temperature (°C)	25.2	25.0	25.0
1500 mg/L	pH (S.U.)	7.92	7.88	7.88
	Dissolved oxygen (mg/L)	8.6	8.0	8.1
	Conductivity (µmhos/cm)	2870		
	Temperature (°C)	25.2	25.3	25.2
2000 mg/L	pH (S.U.)	7.93	7.89	7.90
	Dissolved oxygen (mg/L)	8.7	8.0	8.1
	Conductivity (µmhos/cm)	3750		
	Temperature (°C)	25.0	25.3	25.2
2500 mg/L	pH (S.U.)	7.92	7.88	7.91
	Dissolved oxygen (mg/L)	8.7	8.0	8.1
	Conductivity (µmhos/cm)	4640		
	Temperature (°C)	25.2	25.1	25.2
3000 mg/L	pH (S.U.)	7.92	7.89	7.91
	Dissolved oxygen (mg/L)	8.6	8.0	8.1
	Conductivity (µmhos/cm)	5520		
	Temperature (°C)	25.2	25.2	25.1

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	130604685

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 # 37

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	06-04-26	* 0520	JL	0756	JL	2F1	RCD	01-30-26A
24	06-05-26			0755	JH			
48 Termination	06-06-26			0755	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	05-25-26 D
Age:	< 24-hours old
Date and time organisms were born between:	06-03-26 1425 TO 06-04-26 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.91
	Temperature (°C): 25.1°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	4 1d	4 1d	4 1d	1 1d	2 2d	4 1d	2 2d	1 1d	0 5d	0 5d	0 5d
48 Termination	3 2d	3 1d	2 2d	1 2d	1	0 2d	1 3d	0 2d	0 1d	0	0	0
Mean Survival	45%				10%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	1874.5
Upper 95% confidence limit (mg NaCl/L)	2145.3
48-hour LC_{50} (mg NaCl/L)	2015.1

Comments:

Test Reviewed by: JL

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	8/4/2026	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/6/2026	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	0.8000	0.8000
2500	0.2000	0.4000	0.8000	0.4000
3000	0.2000	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	0.8500	0.8500	1.1667	1.1071	1.3453	10.206	4	12.00	10.00	3	20
*2500	0.4500	0.4500	0.7351	0.4636	1.1071	36.604	4	10.00	10.00	11	20
*3000	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20

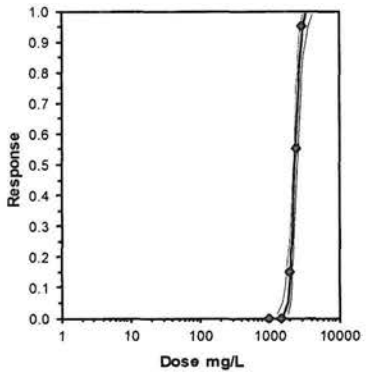
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.73874	0.884	1.30338	5.30123
Equality of variance cannot be confirmed				

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

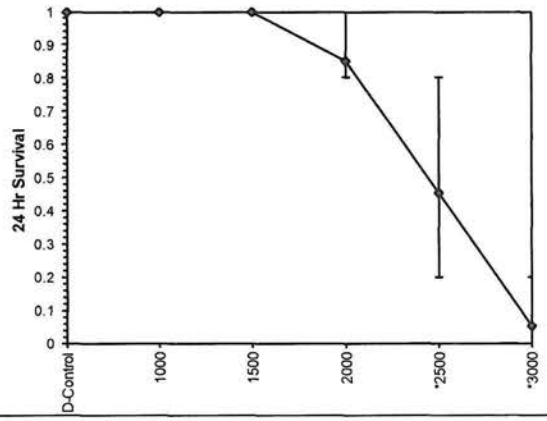
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.7921	2.91775	9.07335	20.5109	0	0.61256	7.81472	0.89355	3.37929	0.0676	3
Intercept	-44.987	9.87645	-64.345	-25.629							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1667.34	1301.88	1872.61
EC05	3.355	1853.94	1541.13	2030.09
EC10	3.718	1961.81	1683.82	2122.32
EC15	3.964	2038.11	1785.74	2189.04
EC20	4.158	2100.86	1869.56	2245.44
EC25	4.326	2156.24	1943.01	2296.86
EC40	4.747	2302.33	2130.15	2444.26
EC50	5.000	2394.94	2239.75	2550.54
EC60	5.253	2491.27	2343.65	2674.32
EC75	5.674	2660.06	2502.58	2921.9
EC80	5.842	2730.18	2562.14	3034.03
EC85	6.036	2814.24	2630.15	3174.02
EC90	6.282	2923.69	2714.66	3363.89
EC95	6.645	3093.8	2839.79	3673.03
EC99	7.326	3440.04	3080.43	4345.44



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	8/4/2026	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/6/2026	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	0.6000	0.6000	0.4000	0.2000
2500	0.2000	0.0000	0.2000	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	0.4500	0.4500	0.7301	0.4636	0.8861	27.587	4	10.00	10.00	11	20
*2500	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

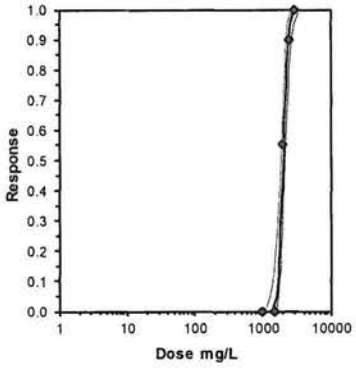
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82498	0.868	-0.7347	2.2229
Equality of variance cannot be confirmed				

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

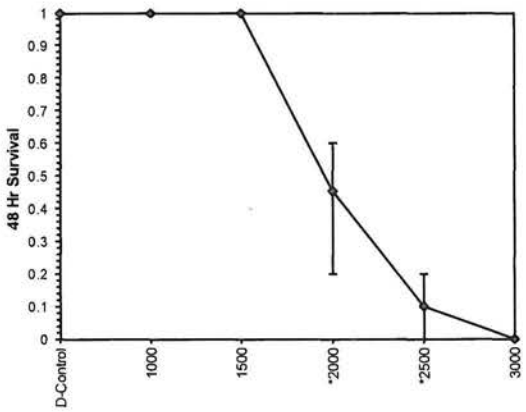
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.2237	3.23436	9.88433	22.563	0	1.26017	7.81472	0.73861	3.30429	0.06164	5
Intercept	-48.608	10.7272	-69.633	-27.582							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1448.42	1137.28	1621.97
EC05	3.355	1595.51	1327.3	1746.19
EC10	3.718	1679.94	1439.43	1818.56
EC15	3.964	1739.41	1519.1	1870.66
EC20	4.158	1788.17	1584.43	1914.48
EC25	4.326	1831.1	1641.61	1954.2
EC40	4.747	1943.88	1787.81	2066.29
EC50	5.000	2015.05	1874.47	2145.33
EC60	5.253	2088.82	1957.7	2236.07
EC75	5.674	2217.48	2085.97	2416.56
EC80	5.842	2270.71	2133.75	2498.48
EC85	6.036	2334.37	2187.91	2600.97
EC90	6.282	2417.01	2254.51	2740.15
EC95	6.645	2544.91	2351.9	2966.64
EC99	7.326	2803.36	2536.17	3456.65

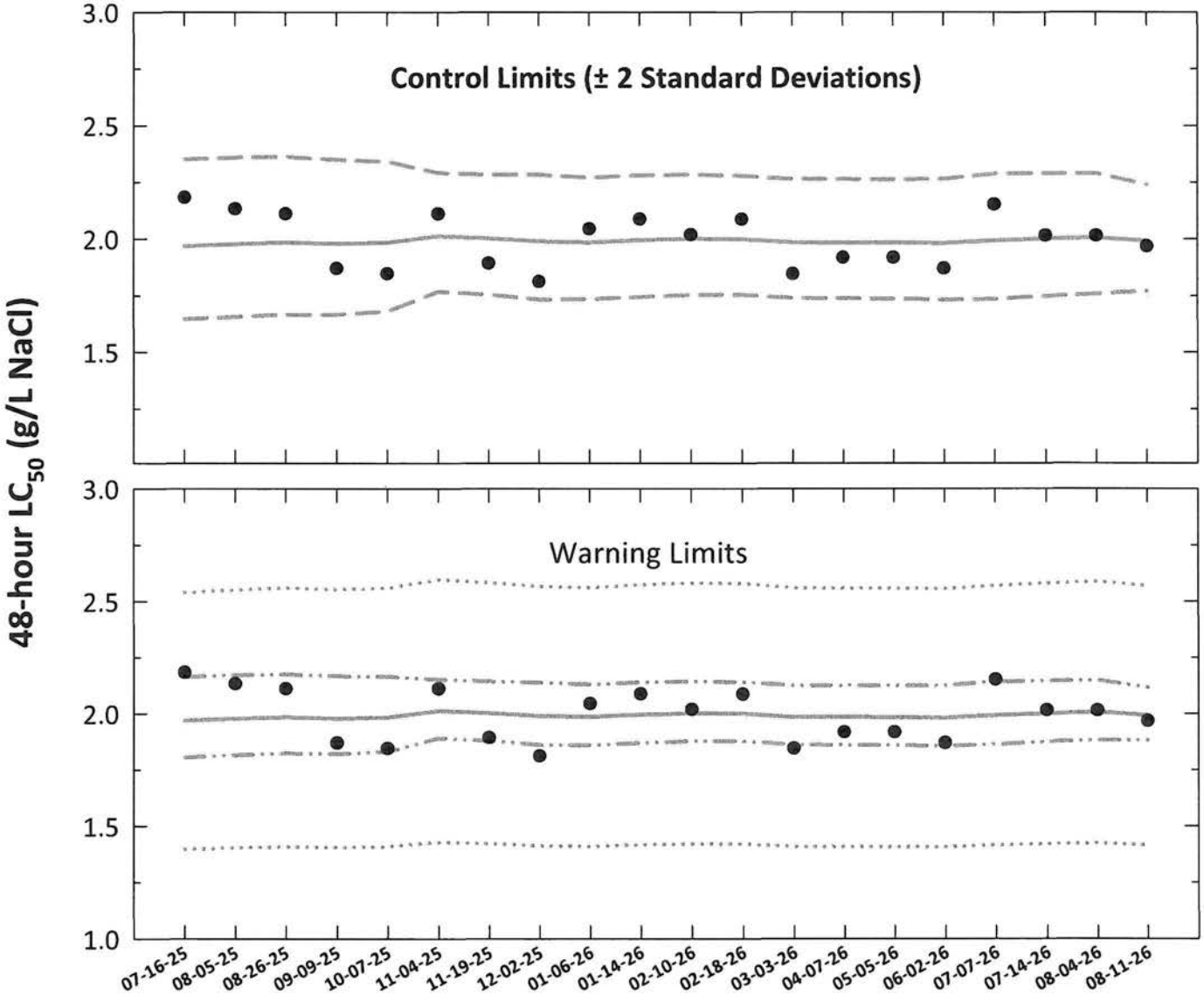


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	07-07-26	2.1520	0.3328	0.2994	0.0300	1.9924	1.7351	2.2878	1.8632	2.1407	1.4146	2.5702
18	07-14-26	2.0151	0.3043	0.3010	0.0293	1.9998	1.7472	2.2890	1.8735	2.1444	1.4199	2.5798
19	08-04-26	2.0151	0.3043	0.3025	0.0286	2.0068	1.7588	2.2897	1.8832	2.1478	1.4248	2.5887
20	08-11-26	1.9680	0.2940	0.2993	0.0255	1.9920	1.7714	2.2400	1.8813	2.1165	1.4143	2.5697

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 # 38

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 #: 2497

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	VL	XL	XL
Control, MHSW	pH (S.U.)	7.93	7.96	7.81
	Dissolved oxygen (mg/L)	8.3	8.2	8.4
	Conductivity (µmhos/cm)	222		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.9	25.3	25.3
1000 mg/L	pH (S.U.)	7.97	7.97	7.90
	Dissolved oxygen (mg/L)	8.3	8.2	8.4
	Conductivity (µmhos/cm)	1990		
	Temperature (°C)	25.1	25.0	25.0
1500 mg/L	pH (S.U.)	7.97	7.96	7.90
	Dissolved oxygen (mg/L)	8.3	8.1	8.4
	Conductivity (µmhos/cm)	2910		
	Temperature (°C)	24.9	25.0	25.0
2000 mg/L	pH (S.U.)	7.97	7.96	7.90
	Dissolved oxygen (mg/L)	8.3	8.1	8.4
	Conductivity (µmhos/cm)	3740		
	Temperature (°C)	24.9	25.2	25.2
2500 mg/L	pH (S.U.)	7.98	7.96	7.89
	Dissolved oxygen (mg/L)	8.3	8.2	8.4
	Conductivity (µmhos/cm)	4580		
	Temperature (°C)	25.2	25.2	25.2
3000 mg/L	pH (S.U.)	7.98	7.95	7.89
	Dissolved oxygen (mg/L)	8.3	8.1	8.3
	Conductivity (µmhos/cm)	5430		
	Temperature (°C)	25.0	25.2	25.1

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 th test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13066466

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 # 38

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-11-26	* 0510	JK	0712	JK	281	RED	08-06-26B
24	08-12-26			0710	JK			
48 Termination	08-13-26			0710	JK			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	08-04-26 D
Age:	< 24-hours old
Date and time organisms were born between:	08-10-26 1413 TO 08-11-26 0510
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.00
	Temperature (°C): 24.9

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

Hours	Control				1000 mg/L				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	S	S	S	S	4 1d	3 2d	4 1d	4 1d	1 4d	0 5d	1 4d	0 5d
48 Termination	3 2d	2 2d	2 2d	3 2d	0 4d	0 3d	0 4d	0 4d	0 1d	0	0 1d	0
Mean Survival	50%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1858.7
Upper 95% confidence limit (mg NaCl/L)	2083.7
48-hour LC_{50} (mg NaCl/L)	1968.0

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	8/11/2026	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/13/2026	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	1.0000	1.0000	1.0000
2500	0.8000	0.6000	0.8000	0.8000
3000	0.2000	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	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2500	0.7500	0.7500	1.0519	0.8861	1.1071	10.508	4	10.00	10.00	5	20
*3000	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20

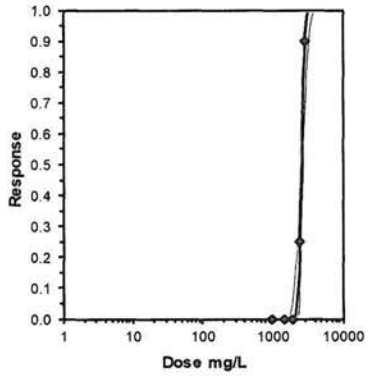
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77007	0.884	-0.7431	2.00567
Equality of variance cannot be confirmed				

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

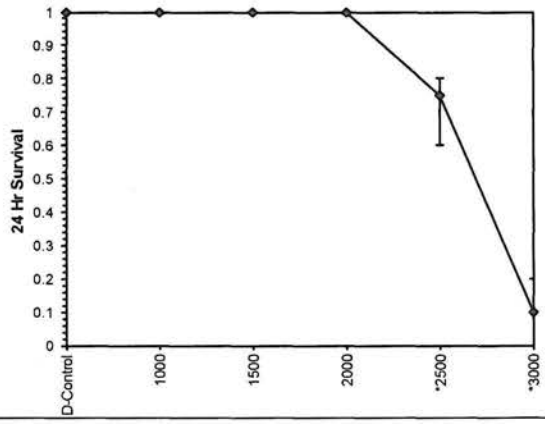
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	25.0114	5.82943	13.5857	36.4371	0	0.02136	7.81472	0.99917	3.42543	0.03998	3
Intercept	-80.675	19.9793	-119.83	-41.515							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2149.9	1773.92	2322.62
EC05	3.355	2289.1	1984.7	2432.67
EC10	3.718	2366.96	2104.84	2496.15
EC15	3.964	2420.98	2188.32	2541.84
EC20	4.158	2464.79	2255.54	2580.45
EC25	4.326	2503.01	2313.34	2615.74
EC40	4.747	2601.96	2455.67	2717.82
EC50	5.000	2663.36	2535.27	2792.34
EC60	5.253	2726.21	2607.84	2879.47
EC75	5.674	2833.98	2713.49	3052.27
EC80	5.842	2877.92	2751.6	3129.35
EC85	6.036	2930	2794.28	3224.44
EC90	6.282	2996.87	2846.23	3351.39
EC95	6.645	3098.8	2921.25	3553.35
EC99	7.326	3299.44	3060.42	3974.58



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	8/11/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/13/2026	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	0.6000	0.4000	0.4000	0.6000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

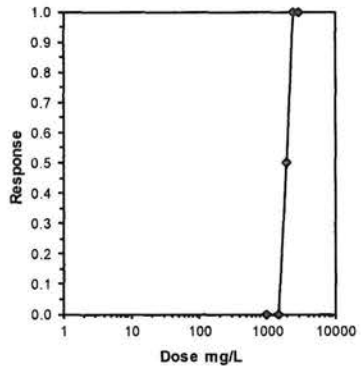
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.5000	0.5000	0.7854	0.6847	0.8861	14.802	4	10.00	10.00	10	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)	0.69656	0.844	2.6E-15	1.8956
Equality of variance cannot be confirmed				

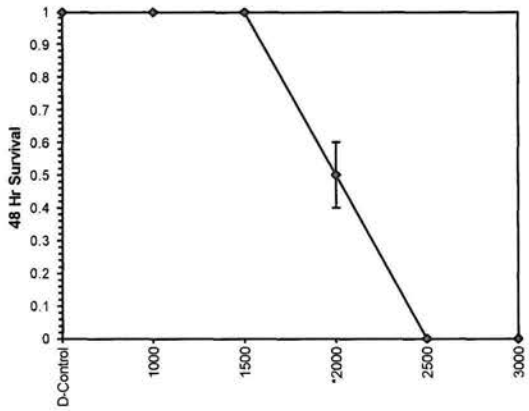
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

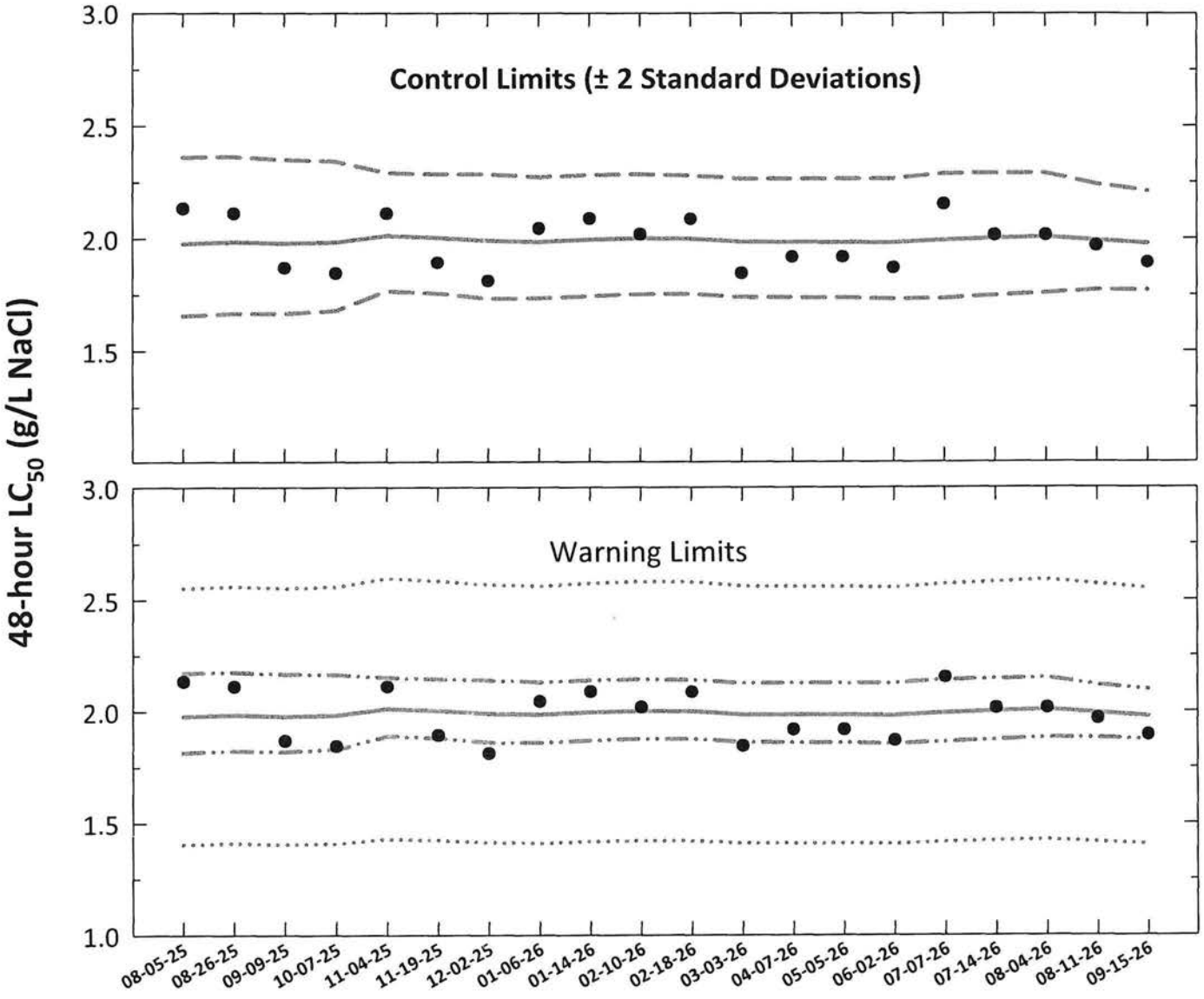
Trim Level	EC50	95% CL	
0.0%	1967.99	1858.74	2083.66
5.0%	1971.17	1849.82	2100.48
10.0%	1974.35	1837.65	2121.22
20.0%	1980.73	1797.43	2182.73
Auto-0.0%	1967.99	1858.74	2083.66



Dose-Response Plot



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



- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- – · – · **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic LC_{50} ± $S_{A.75}$ 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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**07-07-26**	**2.1520**	0.3328	0.2994	0.0300	1.9924	1.7351	2.2878	1.8632	2.1407	1.4146	2.5702
17	**07-14-26**	**2.0151**	0.3043	0.3010	0.0293	1.9998	1.7472	2.2890	1.8735	2.1444	1.4199	2.5798
18	**08-04-26**	**2.0151**	0.3043	0.3025	0.0286	2.0068	1.7588	2.2897	1.8832	2.1478	1.4248	2.5887
19	**08-11-26**	**1.9680**	0.2940	0.2993	0.0255	1.9920	1.7714	2.2400	1.8813	2.1165	1.4143	2.5697
20	**09-15-26**	**1.8940**	0.2774	0.2962	0.0241	1.9778	1.7702	2.2099	1.8728	2.0952	1.4043	2.5514

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

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 # 39

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 #: 2497

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	✓	XL	XL
Control, MHSW	pH (S.U.)	8.01	7.85	7.86
	Dissolved oxygen (mg/L)	8.3	8.2	8.2
	Conductivity (µmhos/cm)	292		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	80		
	Temperature (°C)	24.9	25.2	25.2
1000 mg/L	pH (S.U.)	8.01	7.86	7.87
	Dissolved oxygen (mg/L)	8.2	8.3	8.3
	Conductivity (µmhos/cm)	2020		
	Temperature (°C)	24.9	25.0	25.0
1500 mg/L	pH (S.U.)	7.97	7.87	7.88
	Dissolved oxygen (mg/L)	8.2	8.3	8.3
	Conductivity (µmhos/cm)	2790		
	Temperature (°C)	25.0	25.3	25.3
2000 mg/L	pH (S.U.)	7.99	7.87	7.89
	Dissolved oxygen (mg/L)	8.2	8.3	8.3
	Conductivity (µmhos/cm)	3660		
	Temperature (°C)	~~24.8~~ 25.0 MA-X36	25.1	25.3
2500 mg/L	pH (S.U.)	7.99	7.88	7.90
	Dissolved oxygen (mg/L)	8.3	8.3	8.3
	Conductivity (µmhos/cm)	4470		
	Temperature (°C)	25.2	25.1	25.3
3000 mg/L	pH (S.U.)	7.98	7.88	7.90
	Dissolved oxygen (mg/L)	8.3	8.3	8.2
	Conductivity (µmhos/cm)	5300		
	Temperature (°C)	24.9	25.1	25.0

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13366475

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 # 39

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09.15.26	* 0530	JP	0947	JP	2	Green	09-09-26A
24	09.16.26			~~0947~~ 0946 09.16.26	JP			
48 Termination	09.17.26			0942	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):	09.08.26 1)
Age:	< 24-hours old
Date and time organisms were born between:	09.14.26 1243 TO 09.15.26 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.95
	Temperature (°C): 24.3°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	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	S	4 1d	S	S	0 5d	3 2d	2 3d	3 2d	0 5d	2 3d	0 5d	1 4d
48 Termination	4 1d	1 3d	2 3d	0 5d	0	0 3d	0 2d	0 3d	0	0 2d	0	0 1d
Mean Survival	35%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1793.6
Upper 95% confidence limit (mg NaCl/L)	2000.1
48-hour LC_{50} (mg NaCl/L)	1894.0

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	9/15/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/17/2026	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	0.0000	0.6000	0.4000	0.6000
3000	0.0000	0.4000	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	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2500	0.4000	0.4000	0.6706	0.2255	0.8861	46.456	4	10.00	10.00	12	20
*3000	0.1500	0.1500	0.3998	0.2255	0.6847	55.174	4	10.00	10.00	17	20

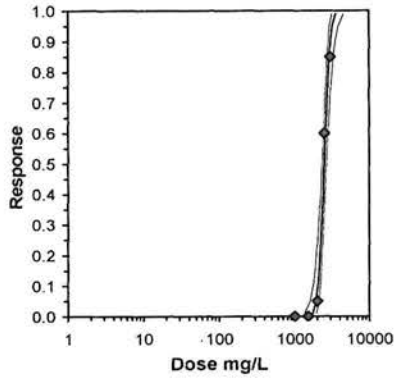
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82548	0.884	-0.9518	3.45246
Equality of variance cannot be confirmed				

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

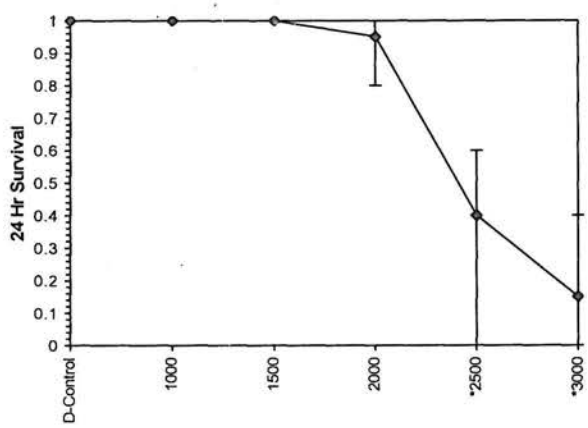
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.9852	3.05057	9.00611	20.9644	0	1.13287	7.81472	0.76915	3.39543	0.06673	3
Intercept	-45.881	10.3639	-66.194	-25.568							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1738.54	1352.57	1949.5
EC05	3.355	1930.47	1603.1	2110.08
EC10	3.718	2041.3	1752.54	2204.25
EC15	3.964	2119.65	1859.21	2272.54
EC20	4.158	2184.06	1946.81	2330.45
EC25	4.326	2240.88	2023.4	2383.48
EC40	4.747	2390.68	2217.22	2537.08
EC50	5.000	2485.58	2329.25	2649.36
EC60	5.253	2584.25	2434.27	2781.02
EC75	5.674	2757.01	2593.62	3044.5
EC80	5.842	2828.73	2653.27	3163.53
EC85	6.036	2914.69	2721.45	3311.9
EC90	6.282	3026.56	2806.25	3512.84
EC95	6.645	3200.32	2931.99	3839.66
EC99	7.326	3553.62	3173.99	4550.17



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	9/15/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/17/2026	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	0.2000	0.4000	0.0000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

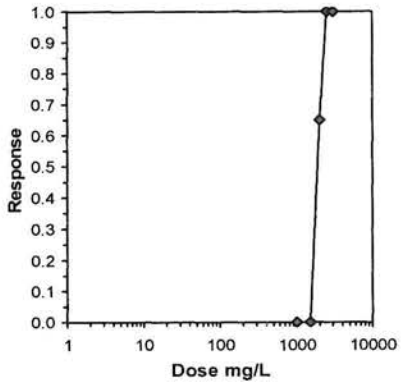
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.3500	0.3500	0.6203	0.2255	1.1071	60.437	4	10.00	10.00	13	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)	0.63769	0.844	0.81406	6.521
Equality of variance cannot be confirmed				

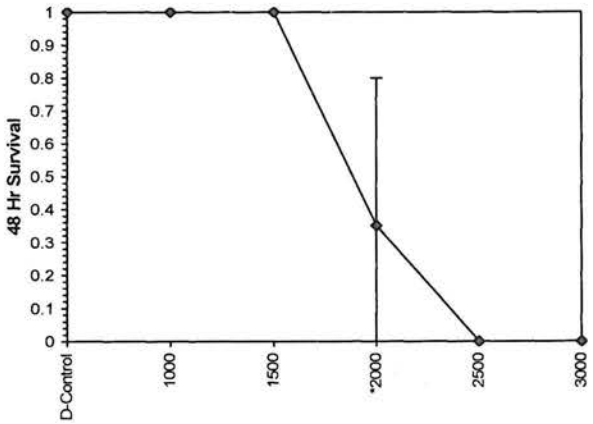
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	1894.02	1793.59	2000.07
5.0%	1889.87	1779.46	2007.13
10.0%	1885.84	1764.37	2015.68
20.0%	1878.39	1729.31	2040.32
Auto-0.0%	1894.02	1793.59	2000.07

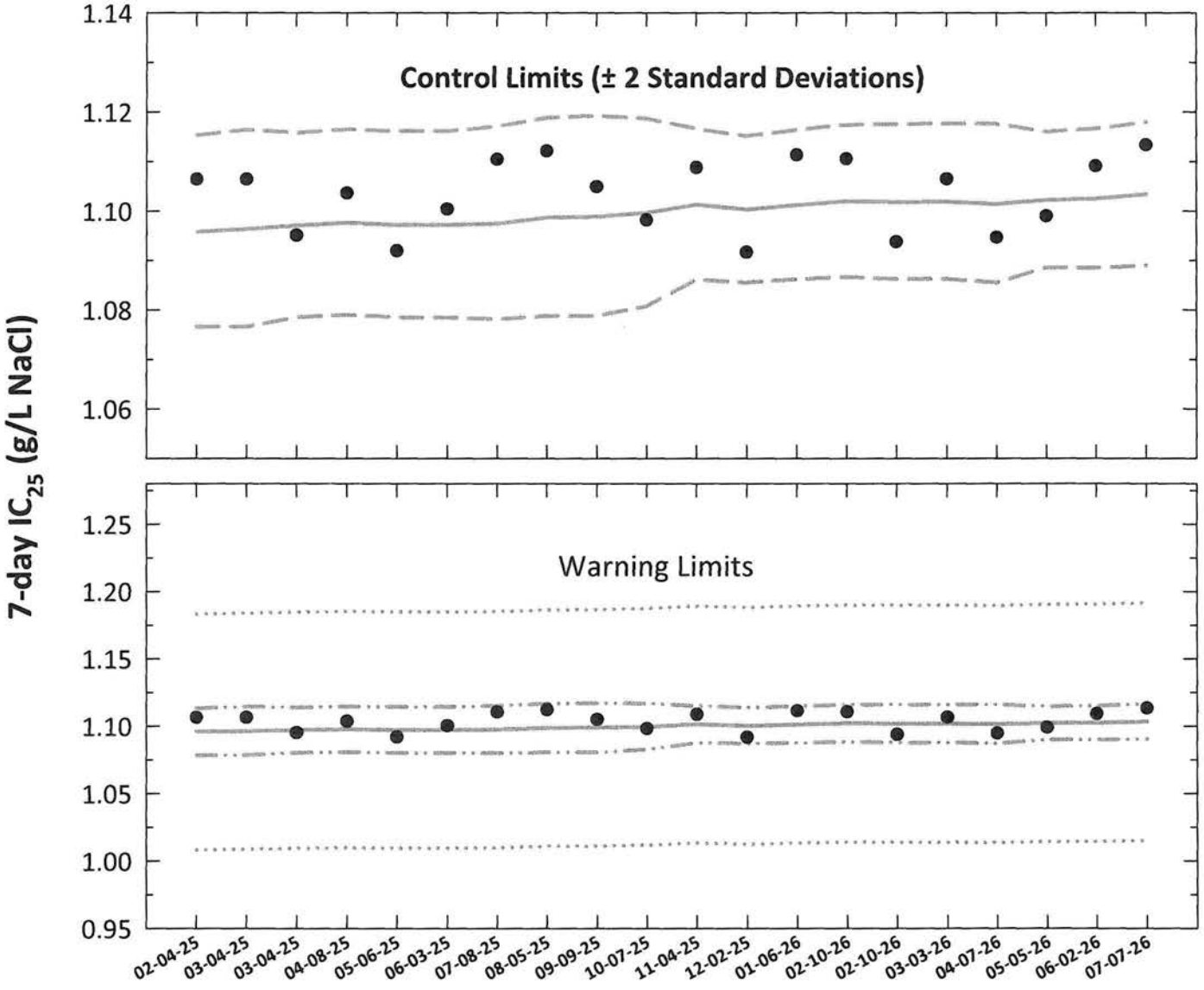


Dose-Response Plot



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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
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	07-07-26	1.1133	0.0466	0.0427	0.0028	1.1034	1.0890	1.1179	1.0904	1.1164	1.0151	1.1917

Note:

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

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

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

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

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

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

CV = Coefficient of variation.

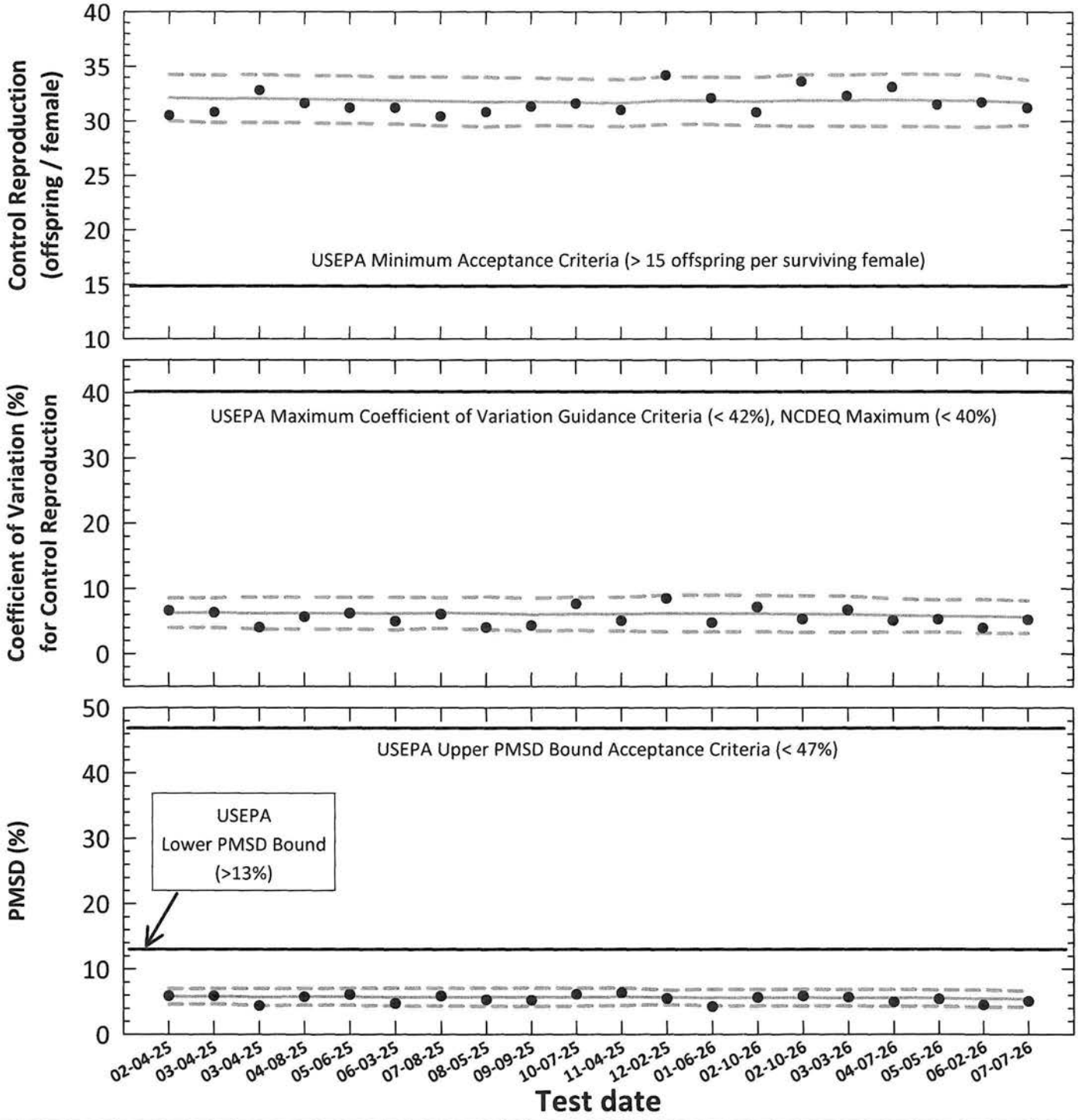
Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



- **Control Reproduction, Coefficient of Variation (CV) or Percent Minimum Significant Difference (PMSD)**

PMSD is the percent minimum significant difference between the control and treatment that can be declared statistically significant. The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival	Control Reproduction		Test			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	07-07-26	100	31.2	5.2	1.568	5.0	31.7	29.6	33.8	5.6	3.1	8.1	5.4	4.2	6.6

Note:

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

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

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 13%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 47%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 326

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:	07.07.26 0650 TO 0856									
Culture board:	06.30.26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	10	11	12	13	18	19	23	27	28	30
Transfer vessel information:	pH (S.U.): 8.01 Temperature (°C): ~~8.0~~ 25.0 ← 25.0°C									
Average transfer volume (mL):	< 0.25 mL 07.02.26 JL									

Test randomization and location:

Randomizing template color:	ORANGE
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	07-07-26	0907	06.25.26	06.25.26	06.30.26 A	JL
1	07-08-26	0715	↓	↓	↓	JL
2	07-09-26	0715			06.30.26 B	JL
3	07-10-26	0710			↓	JL
4	07-11-26	0730			06.30.26 C	JL
5	07-12-26	0710			↓	JL
6	07-13-26	0756	↓	↓		JL
7	07-14-26	0710				JL

*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)	7140 0
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	31.2	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	5.27%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1113.3

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 326

CONTROL ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.2

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	4	5	6	5	4	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	10	12	12	11	12	12	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	15	17	19	16	16	17	13	17	14	13
Total young produced		31	35	33	33	34	33	29	33	30	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:	32.2
% Reduction from Control:	-3.27%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 326

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	4	4	6	6	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	12	13	12	10	10	10	13	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	16	15	17	15	15	18	17	18	16
Total young produced		30	32	31	34	33	31	34	31	35	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:	32.2
% Reduction from Control:	-3.2%

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	4	4	4	5	4	5	5	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	12	11	11	13	10	10	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	16	14	14	15	13	14	18	14	16	16
Total young produced		32	28	30	30	29	31	33	29	32	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.7
% Reduction from Control:	1.6%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 326**

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.7
% Reduction from Control:	-0.17%

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	3	1	2	2	1	1	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	2	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	2	0	0	0	1	0	0	0	0	0
Total young produced		3	3	3	1	3	2	1	1	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:	2.0
% Reduction from Control:	93.67%

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Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	4	5	5	6	4	6	6	50
5	12	10	13	12	12	10	10	10	13	13	115
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	15	15	12	18	16	16	15	12	147
Total	**31**	**29**	**32**	**31**	**29**	**33**	**32**	**30**	**34**	**31**	**312**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	4	5	4	5	5	3	5	43
5	12	10	12	11	11	13	10	10	13	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	14	15	13	14	18	14	16	16	150
Total	**32**	**28**	**30**	**30**	**29**	**31**	**33**	**29**	**32**	**33**	**307**

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	4	5	6	5	4	4	4	6	48
5	11	13	10	12	12	11	12	12	12	12	117
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	19	16	16	17	13	17	14	13	157
Total	**31**	**35**	**33**	**33**	**34**	**33**	**29**	**33**	**30**	**31**	**322**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	2	2	4	4	4	3	4	2	34
5	9	11	7	10	10	8	6	9	12	10	92
6	0	0	0	0	0	0	0	0	0	0	0
7	5	5	11	8	4	6	7	6	2	7	61
Total	**19**	**20**	**20**	**20**	**18**	**18**	**17**	**18**	**18**	**19**	**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	5	4	4	4	6	6	6	4	4	4	47
5	12	12	12	13	12	10	10	10	13	11	115
6	0	0	0	0	0	0	0	0	0	0	0
7	13	16	15	17	15	15	18	17	18	16	160
Total	**30**	**32**	**31**	**34**	**33**	**31**	**34**	**31**	**35**	**31**	**322**

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	3	1	2	2	1	1	1	2	15
5	0	2	0	0	0	0	0	0	0	0	2
6	0	0	0	0	0	0	0	0	0	0	0
7	2	0	0	0	1	0	0	0	0	0	3
Total	**3**	**3**	**3**	**1**	**3**	**2**	**1**	**1**	**1**	**2**	**20**

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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: 326

Test dates: July 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	31	29	32	31	29	33	32	30	34	31	100	31.2	5.2	Not applicable
600	31	35	33	33	34	33	29	33	30	31	100	32.2	5.8	-3.2
800	30	32	31	34	33	31	34	31	35	31	100	32.2	5.2	-3.2
1000	32	28	30	30	29	31	33	29	32	33	100	30.7	5.8	1.6
1200	19	20	20	20	18	18	17	18	18	19	100	18.7	5.7	40.1
1400	3	3	3	1	3	2	1	1	1	2	100	2.0	47.1	93.6

Dunnett's MSD value: 1.568

PMSD: 5.0

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

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Statistical Analyses

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Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	7/7/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	7/14/2026	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	31.000	29.000	32.000	31.000	29.000	33.000	32.000	30.000	34.000	31.000
600	31.000	35.000	33.000	33.000	34.000	33.000	29.000	33.000	30.000	31.000
800	30.000	32.000	31.000	34.000	33.000	31.000	34.000	31.000	35.000	31.000
1000	32.000	28.000	30.000	30.000	29.000	31.000	33.000	29.000	32.000	33.000
1200	19.000	20.000	20.000	20.000	18.000	18.000	17.000	18.000	18.000	19.000
1400	3.000	3.000	3.000	1.000	3.000	2.000	1.000	1.000	1.000	2.000

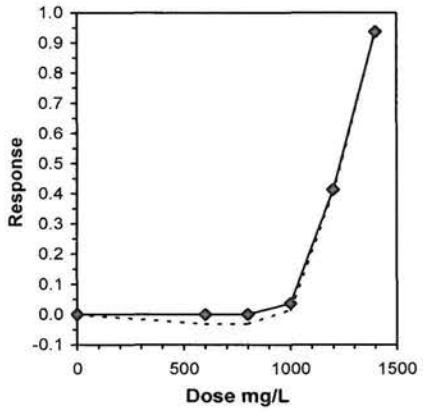
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.200	1.0000	31.200	29.000	34.000	5.190	10				31.867	1.0000
600	32.200	1.0321	32.200	29.000	35.000	5.819	10	-1.458	2.287	1.568	31.867	1.0000
800	32.200	1.0321	32.200	30.000	35.000	5.238	10	-1.458	2.287	1.568	31.867	1.0000
1000	30.700	0.9840	30.700	28.000	33.000	5.756	10	0.729	2.287	1.568	30.700	0.9634
*1200	18.700	0.5994	18.700	17.000	20.000	5.665	10	18.226	2.287	1.568	18.700	0.5868
*1400	2.000	0.0641	2.000	1.000	3.000	47.140	10	42.576	2.287	1.568	2.000	0.0628

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.97196	1.035	0.02332	-0.728
Bartlett's Test indicates equal variances (p = 0.28)	6.29634	15.0863		

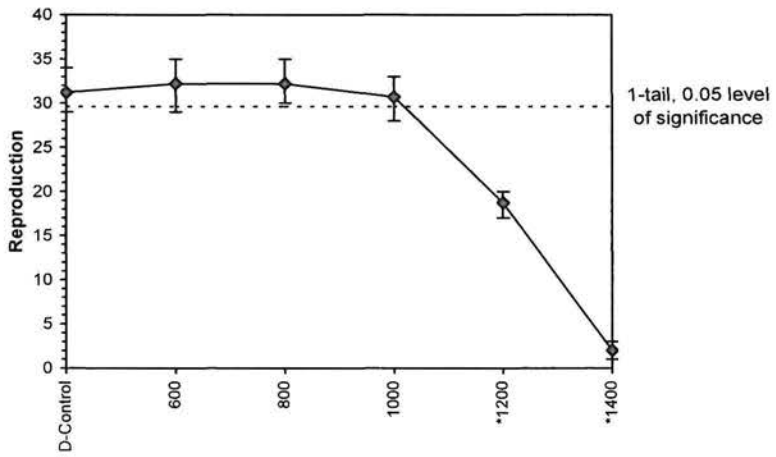
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.56828	0.05027	1483.6	2.35185	0.0E+00	5, 54

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1007.11	21.1433	943.62	1024.62	-2.1570
IC10	1033.67	8.44539	1017.61	1049.36	-0.1772
IC15	1060.22	7.53803	1046.38	1074.86	-0.1617
IC20	1086.78	6.76317	1073.64	1099.83	-0.1462
IC25	1113.33	6.17094	1101.02	1125.11	-0.1331
IC40	1193	5.76507	1181.35	1202.79	-0.2283
IC50	1233.13	3.7303	1225.77	1239.66	-0.1696



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc

Species: *Ceriodaphnia dubia* **CdNaClCR #: 326**

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.)	7.76	7.88	7.72	7.87	7.77	7.88
	Dissolved oxygen (mg/L)	8.6	8.1	8.3	8.2	8.5	8.0
	Conductivity (µmhos/cm)	306		314		303	
	Alkalinity (mg $CaCO_3$/L)	60				61	
	Hardness (mg $CaCO_3$/L)	88				88	
	Temperature (°C)	24.8	25.2	24.9	25.0	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.87	7.88	7.83	7.88	7.82	7.90
	Dissolved oxygen (mg/L)	8.7	8.0	8.3	8.2	8.5	8.0
	Conductivity (µmhos/cm)	1420		1480		1420	
	Temperature (°C)	24.9	25.0	24.9	25.0	25.1	25.0
800 mg NaCl/L	pH (S.U.)	7.89	7.89	7.84	7.88	7.84	7.90
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.2	8.5	8.0
	Conductivity (µmhos/cm)	1810		1870		1790	
	Temperature (°C)	24.9	25.3	25.0	24.8	25.1	24.9
1000 mg NaCl/L	pH (S.U.)	7.90	7.89	7.86	7.88	7.85	7.90
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.2	8.4	8.0
	Conductivity (µmhos/cm)	2150		2230		2160	
	Temperature (°C)	24.9	25.3	24.9	24.8	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.92	7.89	7.88	7.89	7.87	7.91
	Dissolved oxygen (mg/L)	8.6	8.0	8.2	8.2	8.4	8.0
	Conductivity (µmhos/cm)	2520		2570		2510	
	Temperature (°C)	25.0	25.1	24.9	25.1	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.92	7.89	7.89	7.90	7.88	7.92
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.2	8.5	8.0
	Conductivity (µmhos/cm)	2570		2600		2530	
	Temperature (°C)	25.0	25.3	24.9	24.9	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final

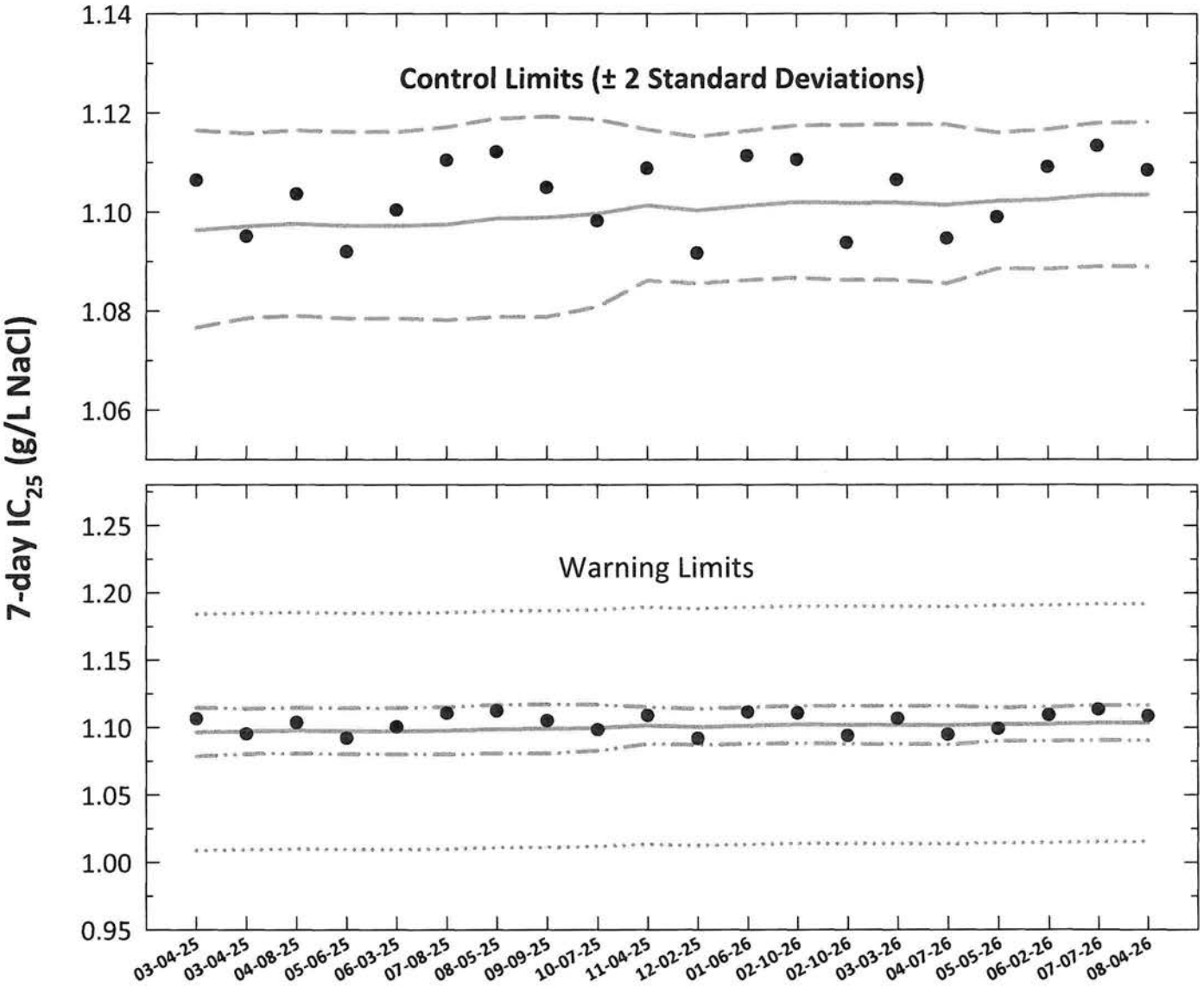
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Species: *Ceriodaphnia dubia* **CdNaClCR #: 326**

		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 JM	XL JM	JM
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.80	7.87	7.70	7.97	7.78	7.81	7.72	8.11
	Dissolved oxygen (mg/L)	8.3	8.4	8.6	8.1	8.5	8.1	8.3	7.7
	Conductivity (µmhos/cm)	309		298		298		310	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	25.1	25.3	24.9	25.2	24.7	25.3	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.85	7.92	7.76	8.00	7.83	7.83	7.73	8.12
	Dissolved oxygen (mg/L)	8.3	8.4	8.6	8.2	8.5	8.1	8.3	7.6
	Conductivity (µmhos/cm)	1440		1380		1390		1470	
	Temperature (°C)	25.0	25.0	24.9	25.1	24.8	25.0	25.0	25.0
800 mg NaCl/L	pH (S.U.)	7.86	7.94	7.86	8.01	7.94	7.84	7.75	8.09
	Dissolved oxygen (mg/L)	8.3	8.4	8.6	8.2	8.5	8.1	8.3	7.6
	Conductivity (µmhos/cm)	1840		1750		1770		1870	
	Temperature (°C)	25.0	25.0	25.0	25.1	24.8	25.0	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.88	7.94	7.88	8.01	7.95	7.84	7.78	8.07
	Dissolved oxygen (mg/L)	8.3	8.4	8.6	8.2	8.5	8.1	8.3	7.6
	Conductivity (µmhos/cm)	2190		2090		2090		2210	
	Temperature (°C)	25.0	25.0	25.0	25.3	24.9	25.2	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.90	7.95	7.89	8.02	7.96	7.85	7.79	8.06
	Dissolved oxygen (mg/L)	8.2	8.4	8.6	8.2	8.5	8.1	8.3	7.6
	Conductivity (µmhos/cm)	2550		2460		2460		2610	
	Temperature (°C)	24.9	25.2	25.0	25.0	24.9	25.2	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	7.91	7.95	7.89	8.02	7.97	7.86	7.81	8.05
	Dissolved oxygen (mg/L)	8.3	8.4	8.6	8.2	8.5	8.1	8.3	7.4
	Conductivity (µmhos/cm)	2720		2430		2620		2760	
	Temperature (°C)	24.9	25.0	25.0	25.0	24.9	25.2	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



Test date

- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	07-07-26	1.1133	0.0466	0.0427	0.0028	1.1034	1.0890	1.1179	1.0904	1.1164	1.0151	1.1917
20	08-04-26	1.1085	0.0447	0.0428	0.0029	1.1035	1.0890	1.1182	1.0904	1.1166	1.0152	1.1918

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.

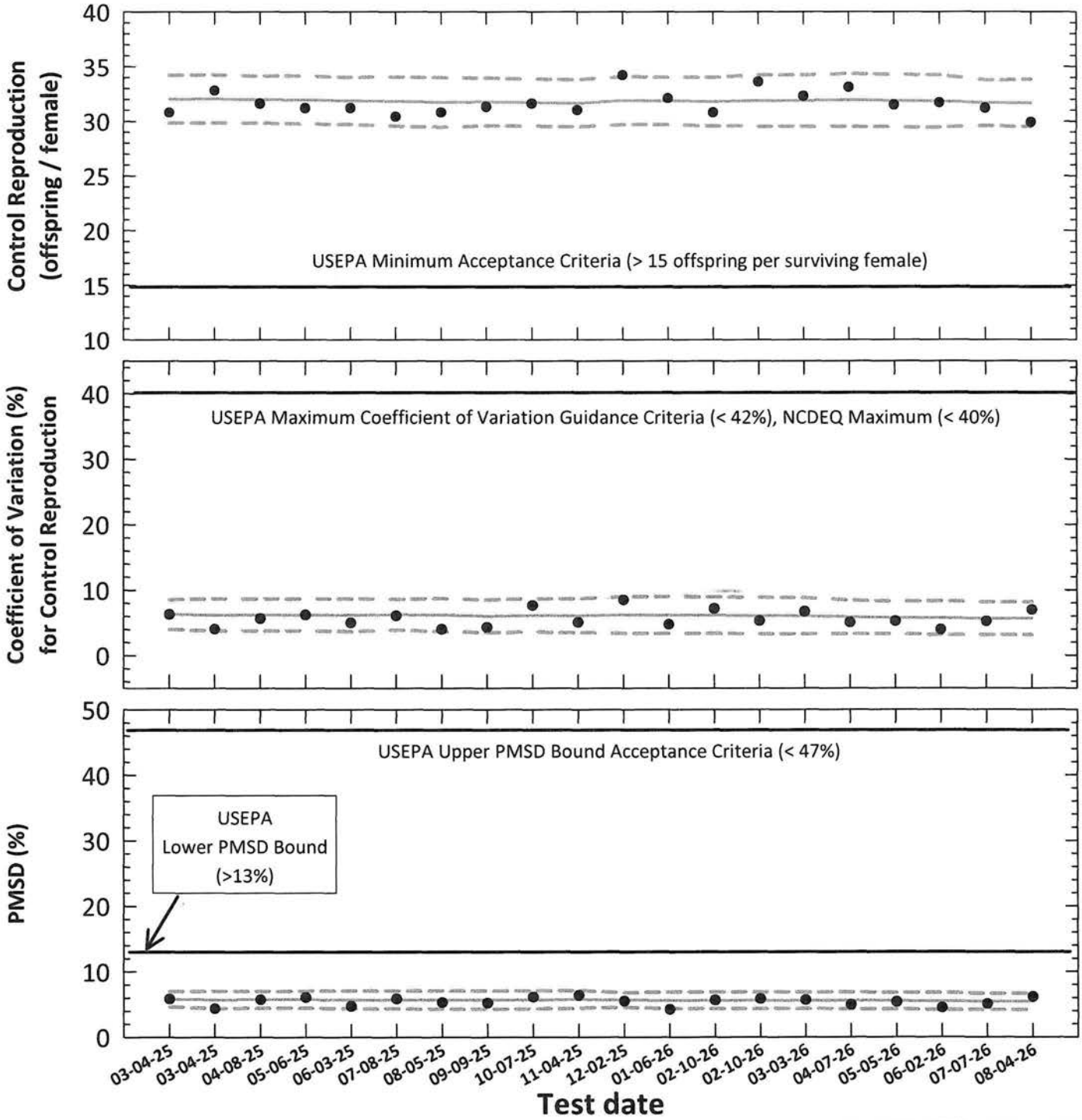
Entered and Reviewed by Jim Sumner

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										
		Survival	Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(offspring/female)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	07-07-26	100	31.2	5.2	1.568	5.0	31.7	29.6	33.8	5.6	3.1	8.1	5.4	4.2	6.6
20	08-04-26	100	29.9	7.0	1.829	6.1	31.7	29.5	33.8	5.6	3.1	8.2	5.4	4.2	6.6

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 #: 327

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2497				
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:	08.04.26 0620 TO 0800									
Culture board:	07.28.26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	3	4	8	9	13	17	18	22
Transfer vessel information:	pH (S.U.): 7.91 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLACK
Incubator number and shelf location:	2B3

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	08-04-26	0813	07.29.26	07.29.26	07.30.26A	
1	08-05-26	0620	↓	↓	↓	
2	08-06-26	0615			07.31.26D	
3	08-07-26	0610			↓	
4	08-08-26	0800			08.03.26A	
5	08-09-26	0630			↓	
6	08-10-26	0710	↓	↓		
7	08-11-26	0628				

*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	190664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	29.9	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	7.0%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1108.5

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 327**

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	5	5	3	5	4	4	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	11	10	10	10	12	10	11	11	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	17	12	15	16	16	13	12	15	17
Total young produced		31	33	27	28	31	32	27	29	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:	29.9

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	4	5	5	5	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	12	12	10	10	11	10	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	16	17	14	18	18	13	16	18	15
Total young produced		37	32	33	31	33	33	29	31	32	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.3
% Reduction from Control:	-8.0%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 327**

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	6	6	5	4	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	12	11	12	10	12	10	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	17	18	16	13	18	19	15	15	15
Total young produced		36	34	36	33	30	32	35	29	30	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:	32.6
% Reduction from Control:	-9.0%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.1
% Reduction from Control:	-0.77%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 327

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.3
% Reduction from Control:	38.8%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.4
% Reduction from Control:	92.0%

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	4	5	5	3	5	4	4	6	4	4	44
5	11	11	10	10	10	12	10	11	11	10	106
6	0	0	0	0	0	0	0	0	0	0	0
7	16	17	12	15	16	16	13	12	15	17	149
Total	**31**	**33**	**27**	**28**	**31**	**32**	**27**	**29**	**30**	**31**	**299**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	5	5	3	4	4	4	4	42
5	12	11	13	10	10	13	10	11	11	11	112
6	0	0	0	0	0	0	0	0	0	0	0
7	13	15	13	16	17	12	15	14	15	17	147
Total	**30**	**29**	**31**	**31**	**32**	**28**	**29**	**29**	**30**	**32**	**301**

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	4	5	5	5	5	5	4	5	48
5	13	12	12	12	10	10	11	10	10	12	112
6	0	0	0	0	0	0	0	0	0	0	0
7	18	16	17	14	18	18	13	16	18	15	163
Total	**37**	**32**	**33**	**31**	**33**	**33**	**29**	**31**	**32**	**32**	**323**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	3	4	4	4	4	2	4	5	37
5	9	7	6	8	5	10	7	5	5	8	70
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	9	6	11	4	5	12	10	5	76
Total	**18**	**19**	**18**	**18**	**20**	**18**	**16**	**19**	**19**	**18**	**183**

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	6	6	5	4	4	4	5	5	48
5	12	13	12	11	12	10	12	10	10	11	113
6	0	0	0	0	0	0	0	0	0	0	0
7	19	17	18	16	13	18	19	15	15	15	165
Total	**36**	**34**	**36**	**33**	**30**	**32**	**35**	**29**	**30**	**31**	**326**

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	3	1	1	2	1	0	0	2	2	1	13
5	0	0	0	0	0	2	1	0	0	0	3
6	0	0	0	0	0	0	0	0	0	0	0
7	1	3	1	0	2	0	0	0	0	1	8
Total	**4**	**4**	**2**	**2**	**3**	**2**	**1**	**2**	**2**	**2**	**24**

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: 327

Test dates: August 04-11, 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	31	33	27	28	31	32	27	29	30	31	100	29.9	7.0	Not applicable
600	37	32	33	31	33	33	29	31	32	32	100	32.3	6.4	-8.0
800	36	34	36	33	30	32	35	29	30	31	100	32.6	7.9	-9.0
1000	30	29	31	31	32	28	29	29	30	32	100	30.1	4.6	-0.7
1200	18	19	18	18	20	18	16	19	19	18	100	18.3	5.8	38.8
1400	4	4	2	2	3	2	1	2	2	2	100	2.4	40.3	92.0

Dunnett's MSD value: 1.829

PMSD: 6.1

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	8/4/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	8/11/2026	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	31.000	33.000	27.000	28.000	31.000	32.000	27.000	29.000	30.000	31.000
600	37.000	32.000	33.000	31.000	33.000	33.000	29.000	31.000	32.000	32.000
800	36.000	34.000	36.000	33.000	30.000	32.000	35.000	29.000	30.000	31.000
1000	30.000	29.000	31.000	31.000	32.000	28.000	29.000	29.000	30.000	32.000
1200	18.000	19.000	18.000	18.000	20.000	18.000	16.000	19.000	19.000	18.000
1400	4.000	4.000	2.000	2.000	3.000	2.000	1.000	2.000	2.000	2.000

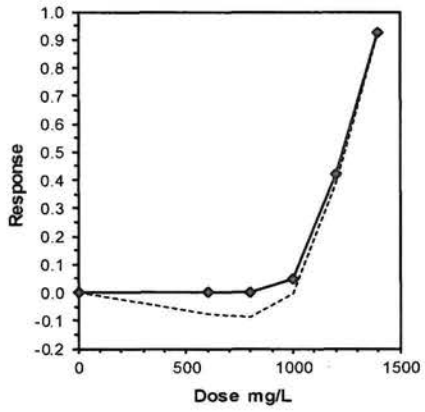
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	29.900	1.0000	29.900	27.000	33.000	6.953	10				31.600	1.0000
600	32.300	1.0803	32.300	29.000	37.000	6.370	10	-3.000	2.287	1.829	31.600	1.0000
800	32.600	1.0903	32.600	29.000	36.000	7.947	10	-3.375	2.287	1.829	31.600	1.0000
1000	30.100	1.0067	30.100	28.000	32.000	4.553	10	-0.250	2.287	1.829	30.100	0.9525
*1200	18.300	0.6120	18.300	16.000	20.000	5.789	10	14.500	2.287	1.829	18.300	0.5791
*1400	2.400	0.0803	2.400	1.000	4.000	40.254	10	34.375	2.287	1.829	2.400	0.0759

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.84418	1.035	0.21366	0.25421
Bartlett's Test indicates equal variances (p = 0.02)	12.8716	15.0863		

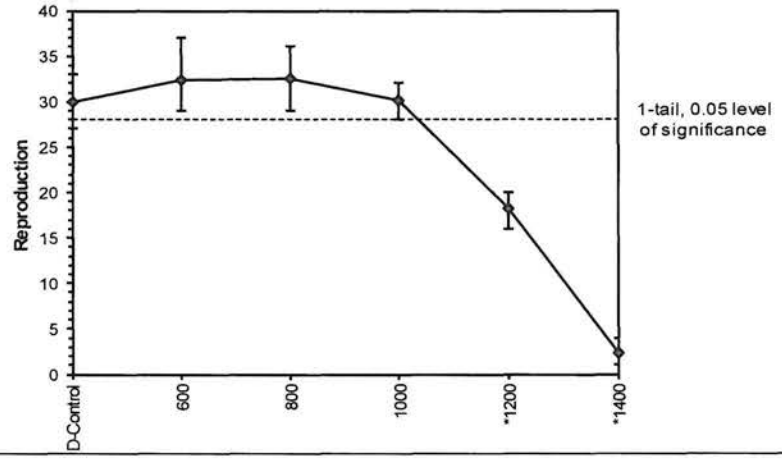
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.82933	0.06118	1426.99	3.2	1.3E-42	5, 54

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1001.36	28.8503	919.574	1018.51	-1.1328
IC10	1028.14	8.83236	1009.53	1044.41	-0.2464
IC15	1054.92	7.92317	1038.64	1068.58	-0.1836
IC20	1081.69	7.14439	1067.73	1094.05	-0.1291
IC25	1108.47	6.54274	1095	1120.01	-0.1026
IC40	1188.81	6.23092	1176.64	1200.24	-0.2127
IC50	1231.45	4.15849	1222.41	1238.82	-0.3986



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 327**

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.

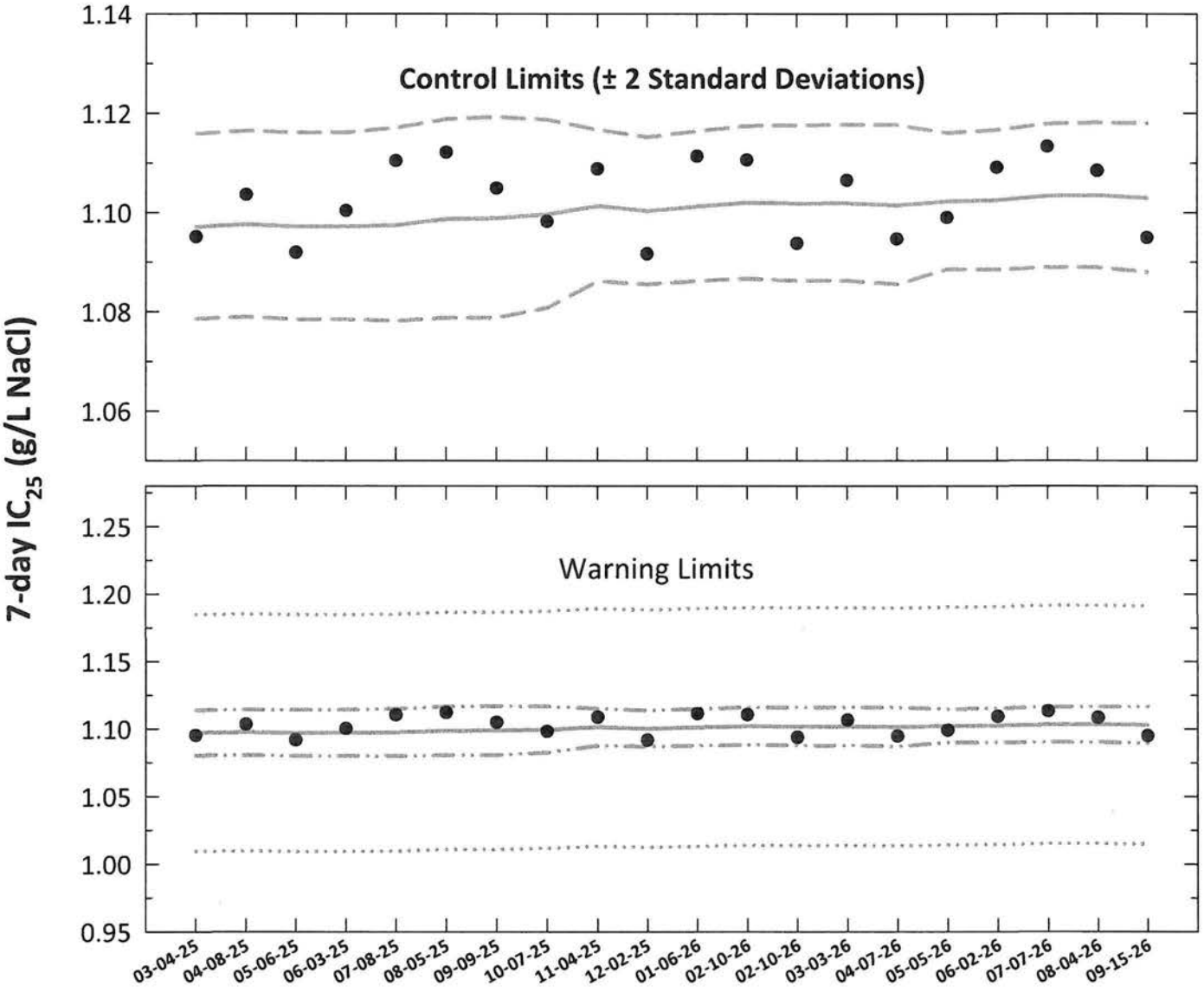
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.76	7.85	7.71	7.83	7.74	7.90
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	8.1	8.4	8.1
	Conductivity (µmhos/cm)	304		307		308	
	Alkalinity (mg $CaCO_3$/L)	63				63	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.9	25.2	24.9	25.0	24.9	25.1
600 mg NaCl/L	pH (S.U.)	7.83	7.85	7.80	7.85	7.81	7.92
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	8.1	8.4	8.1
	Conductivity (µmhos/cm)	1430		1440		1420	
	Temperature (°C)	25.1	24.9	24.8	25.3	25.0	24.8
800 mg NaCl/L	pH (S.U.)	7.85	7.85	7.83	7.86	7.82	7.93
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	8.1	8.4	8.0
	Conductivity (µmhos/cm)	1820		1830		1830	
	Temperature (°C)	25.2	24.9	24.8	25.1	25.0	24.8
1000 mg NaCl/L	pH (S.U.)	7.87	7.86	7.84	7.87	7.84	7.94
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	8.1	8.4	8.0
	Conductivity (µmhos/cm)	2120		2170		2160	
	Temperature (°C)	25.0	25.0	24.9	25.1	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.89	7.86	7.86	7.87	7.85	7.94
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	8.1	8.3	8.0
	Conductivity (µmhos/cm)	2510		2530		2520	
	Temperature (°C)	25.0	25.0	24.9	25.1	24.9	25.2
1400 mg NaCl/L	pH (S.U.)	7.90	7.85	7.86	7.88	7.87	7.95
	Dissolved oxygen (mg/L)	8.5	7.9	8.1	8.1	8.3	8.0
	Conductivity (µmhos/cm)	2960		2960		2910	
	Temperature (°C)	25.1	24.9	24.9	25.0	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 327**

		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.)	7.77	7.99	8.01	8.00	7.77	7.89	7.81	7.78
	Dissolved oxygen (mg/L)	8.3	8.3	8.2	8.1	8.5	8.1	8.3	7.6
	Conductivity (µmhos/cm)	307		307		296		308	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			86					
	Temperature (°C)	24.9	25.1	24.8	25.0	24.9	25.2	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.83	7.99	7.76	8.01	7.77	7.90	7.87	7.80
	Dissolved oxygen (mg/L)	8.3	8.3	8.5	8.1	8.4	8.1	8.3	7.6
	Conductivity (µmhos/cm)	1480		1390		1390		1420	
	Temperature (°C)	25.0	24.9	24.8	25.0	25.0	25.1	24.9	24.9
800 mg NaCl/L	pH (S.U.)	7.86	7.99	7.83	8.00	7.86	7.91	7.89	7.84
	Dissolved oxygen (mg/L)	8.2	8.3	8.5	8.1	8.4	8.1	8.3	7.6
	Conductivity (µmhos/cm)	1890		1780		1770		1840	
	Temperature (°C)	25.0	24.9	24.9	25.2	25.0	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.87	7.98	7.84	7.99	7.89	7.91	7.90	7.85
	Dissolved oxygen (mg/L)	8.2	8.3	8.5	8.1	8.4	8.1	8.3	7.6
	Conductivity (µmhos/cm)	2260		2100		2090		2170	
	Temperature (°C)	24.9	24.9	24.9	25.2	25.0	24.9	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.89	7.98	7.85	7.99	7.89	7.92	7.91	7.81
	Dissolved oxygen (mg/L)	8.2	8.3	8.5	8.1	8.4	8.1	8.3	7.6
	Conductivity (µmhos/cm)	2610		2440		2440		2530	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.9	24.9	25.0	24.9
1400 mg NaCl/L	pH (S.U.)	7.90	7.98	7.87	7.99	7.91	7.92	7.93	7.88
	Dissolved oxygen (mg/L)	8.2	8.3	8.5	8.1	8.4	8.1	8.3	7.6
	Conductivity (µmhos/cm)	3030		2800		2810		2930	
	Temperature (°C)	24.9	25.2	24.9	24.9	24.9	25.2	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic IC_{25} ± $S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

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ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	07-07-26	1.1133	0.0466	0.0427	0.0028	1.1034	1.0890	1.1179	1.0904	1.1164	1.0151	1.1917
19	08-04-26	1.1085	0.0447	0.0428	0.0029	1.1035	1.0890	1.1182	1.0904	1.1166	1.0152	1.1918
20	09-15-26	1.0950	0.0394	0.0425	0.0030	1.1029	1.0880	1.1180	1.0894	1.1164	1.0147	1.1912

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.

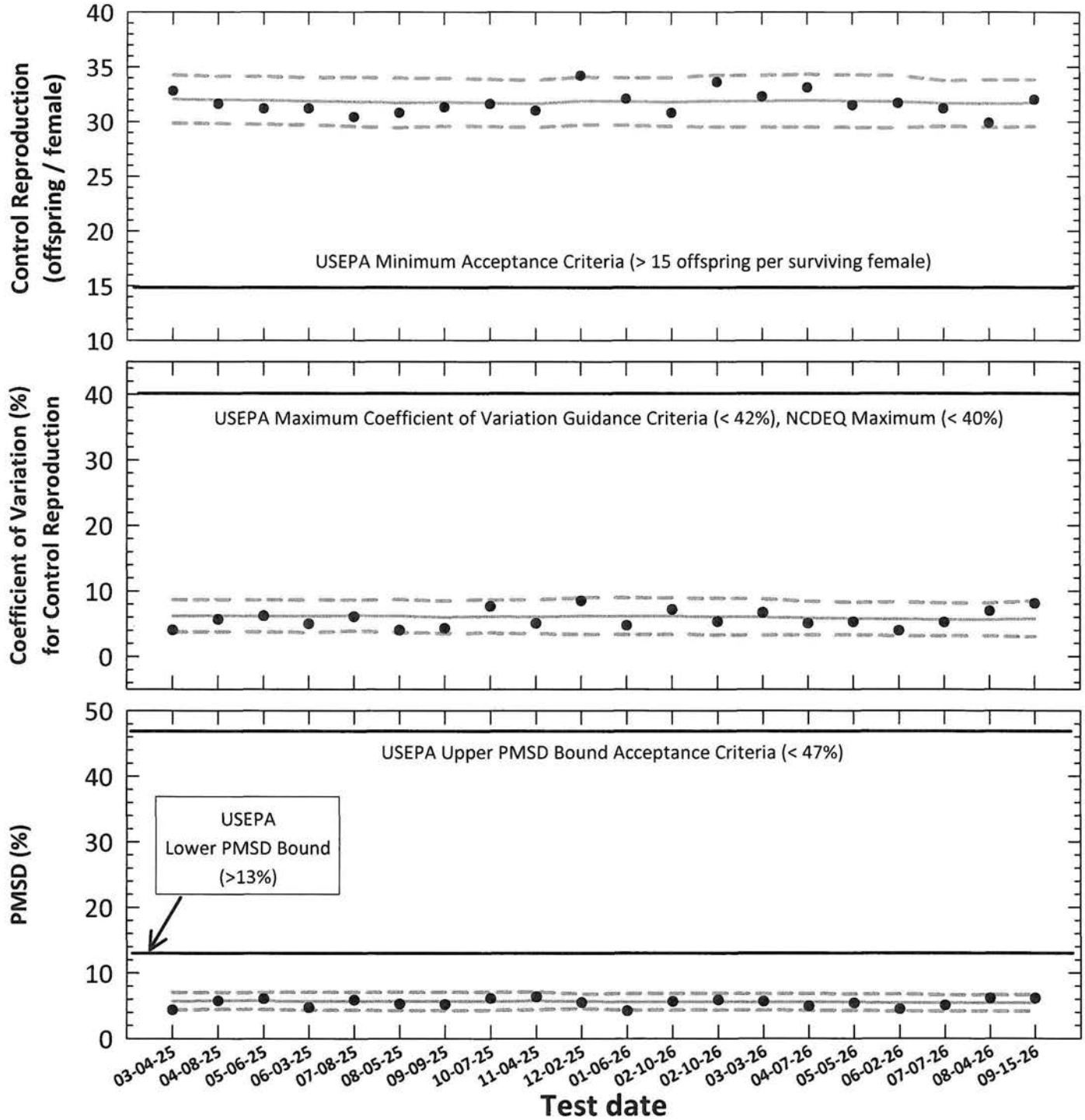
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ETS
Environmental Testing Solutions, Inc.

Ceriodaphnia dubia

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control	Control Reproduction		Test										
		Survival	Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(offspring/female)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	07-07-26	100	31.2	5.2	1.568	5.0	31.7	29.6	33.8	5.6	3.1	8.1	5.4	4.2	6.6
19	08-04-26	100	29.9	7.0	1.829	6.1	31.7	29.5	33.8	5.6	3.1	8.2	5.4	4.2	6.6
20	09-15-26	100	32.0	8.1	1.959	6.1	31.7	29.6	33.8	5.7	3.0	8.5	5.4	4.1	6.7

Note:

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

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

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 13%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 47%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 328

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2497				
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:	09.15.26 0530 - 0830									
Culture board:	09.08.26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	4	5	8	11	12	13	17	22	24	27
Transfer vessel information:	pH (S.U.): 7.95 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	09-15-26	0843	09.27.26	09.27.26	09.09.26A	JK
1	09-16-26	0643	↓	↓	↓	JK
2	09-17-26	0645	↓	↓	09.09.26B	JK
3	09-18-26	0645	↓	↓	↓	JK
4	09-19-26	0700	↓	↓	09.14.26A	JK
5	09-20-26	0700	↓	↓	↓	JK
6	09-21-26	0714	↓	↓	↓	JK
7	~~09-21-26~~ 09.22.26	0644				K

*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)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	32.0	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	8.17%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1095.0

Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 328**

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	4	5	3	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	11	12	12	10	13	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	18	14	13	19	15	15	15	18	17
Total young produced		34	35	29	31	35	29	33	28	33	33
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:	32.0

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	6	5	5	4	6	4	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	12	12	11	10	12	11	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	14	19	16	15	19	15	17	17	16
Total young produced		34	33	36	33	30	35	31	32	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:	32.8
% Reduction from Control:	-2.5%.

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 328

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	6	5	5	5	5	6	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	12	10	10	10	10	12	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	19	18	16	18	14	14	17	19
Total young produced		33	32	37	33	31	33	29	32	35	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:	32.8
% Reduction from Control:	-2.5%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	4	3	4	4	5	5	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	9	12	12	10	9	11	11	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	15	17	16	17	17	14	17	18	16
Total young produced		29	29	30	31	33	31	28	33	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:	31.0
% Reduction from Control:	3.1%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 328

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	1	4	3	2	2	4	2	2	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	6	5	9	4	6	4	7	8	5	8
	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	10	5	11	9	12	5	10	10	7
Total young produced		14	16	18	18	17	18	16	20	17	17
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.1
% Reduction from Control:	46.6%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	1	1	1	3	2	1	2	2	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	2	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	1	1	0	0	0	0	0	0
Total young produced		1	3	2	4	2	1	2	2	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:	2.0
% Reduction from Control:	93.8%

ETS
Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	6	4	4	5	3	5	6	46
5	11	13	11	12	12	10	13	10	10	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	18	18	14	13	19	15	15	15	18	17	162
Total	**34**	**35**	**29**	**31**	**35**	**29**	**33**	**28**	**33**	**33**	**320**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	3	4	4	5	5	4	5	42
5	12	10	9	12	12	10	9	11	11	10	106
6	0	0	0	0	0	0	0	0	0	0	0
7	13	15	17	16	17	17	14	17	18	18	162
Total	**29**	**29**	**30**	**31**	**33**	**31**	**28**	**33**	**33**	**33**	**310**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	6	5	5	4	6	4	4	4	6	49
5	10	13	12	12	11	10	12	11	10	11	112
6	0	0	0	0	0	0	0	0	0	0	0
7	19	14	19	16	15	19	15	17	17	16	167
Total	**34**	**33**	**36**	**33**	**30**	**35**	**31**	**32**	**31**	**33**	**328**

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	1	4	3	2	2	4	2	2	2	25
5	6	5	9	4	6	4	7	8	5	8	62
6	0	0	0	0	0	0	0	0	0	0	0
7	5	10	5	11	9	12	5	10	10	7	84
Total	**14**	**16**	**18**	**18**	**17**	**18**	**16**	**20**	**17**	**17**	**171**

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	6	5	5	5	5	6	6	4	51
5	13	10	12	10	10	10	10	12	12	10	109
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	19	18	16	18	14	14	17	19	168
Total	**33**	**32**	**37**	**33**	**31**	**33**	**29**	**32**	**35**	**33**	**328**

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	1	3	2	1	2	2	1	2	16
5	0	2	0	0	0	0	0	0	0	0	2
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	1	1	0	0	0	0	0	0	2
Total	**1**	**3**	**2**	**4**	**2**	**1**	**2**	**2**	**1**	**2**	**20**

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: 328

Test dates: September 15-22, 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	34	35	29	31	35	29	33	28	33	33	**100**	**32.0**	**8.1**	**Not applicable**
600	34	33	36	33	30	35	31	32	31	33	**100**	**32.8**	**5.7**	**-2.5**
800	33	32	37	33	31	33	29	32	35	33	**100**	**32.8**	**6.6**	**-2.5**
1000	29	29	30	31	33	31	28	33	33	33	**100**	**31.0**	**6.3**	**3.1**
1200	14	16	18	18	17	18	16	20	17	17	**100**	**17.1**	**9.3**	**46.6**
1400	1	3	2	4	2	1	2	2	1	2	**100**	**2.0**	**47.1**	**93.8**

Dunnett's MSD value: 1.959

PMSD: 6.1

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/15/2026	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	9/22/2026	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	34.000	35.000	29.000	31.000	35.000	29.000	33.000	28.000	33.000	33.000
600	34.000	33.000	36.000	33.000	30.000	35.000	31.000	32.000	31.000	33.000
800	33.000	32.000	37.000	33.000	31.000	33.000	29.000	32.000	35.000	33.000
1000	29.000	29.000	30.000	31.000	33.000	31.000	28.000	33.000	33.000	33.000
1200	14.000	16.000	18.000	18.000	17.000	18.000	16.000	20.000	17.000	17.000
1400	1.000	3.000	2.000	4.000	2.000	1.000	2.000	2.000	1.000	2.000

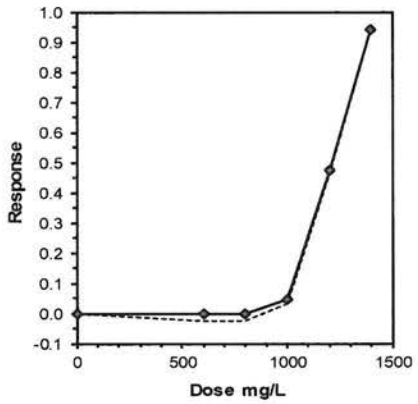
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	32.000	1.0000	32.000	28.000	35.000	8.069	10				32.533	1.0000
600	32.800	1.0250	32.800	30.000	36.000	5.713	10	-0.934	2.287	1.959	32.533	1.0000
800	32.800	1.0250	32.800	29.000	37.000	6.555	10	-0.934	2.287	1.959	32.533	1.0000
1000	31.000	0.9688	31.000	28.000	33.000	6.270	10	1.167	2.287	1.959	31.000	0.9529
*1200	17.100	0.5344	17.100	14.000	20.000	9.328	10	17.395	2.287	1.959	17.100	0.5256
*1400	2.000	0.0625	2.000	1.000	4.000	47.140	10	35.024	2.287	1.959	2.000	0.0615

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.87536	1.035	-0.0906	-0.2733
Bartlett's Test indicates equal variances (p = 0.14)	8.36438	15.0863		

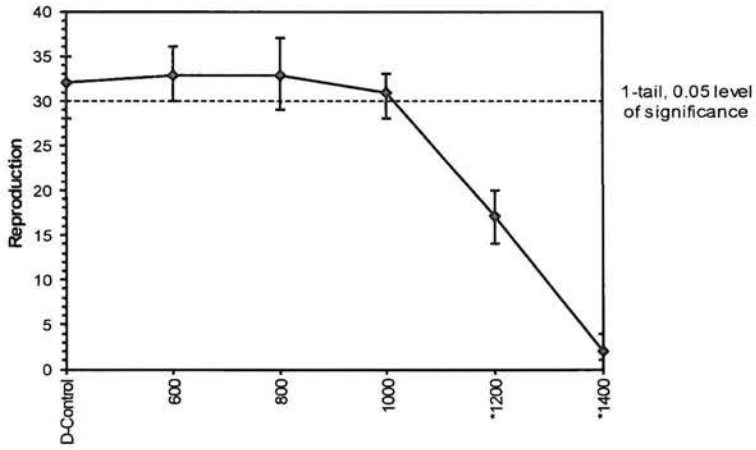
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.95868	0.06121	1594.42	3.66852	2.6E-42	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1001.34	39.8591	887.3	1017.31	-1.2066
IC10	1024.75	9.23038	1004.95	1039.72	-0.4844
IC15	1048.15	8.11987	1030.92	1062.52	-0.2249
IC20	1071.56	7.30513	1056.71	1084.68	-0.0800
IC25	1094.96	6.69991	1082.12	1107.14	0.0487
IC40	1165.18	6.63383	1153.56	1178.59	0.1126
IC50	1211.04	6.54035	1197.87	1222.36	-0.2667



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 328

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
Analyst		[illegible]	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.01	7.84	7.71	7.85	7.74	7.85
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	8.0	8.4	8.0
	Conductivity (µmhos/cm)	292		311		312	
	Alkalinity (mg $CaCO_3$/L)	61				63	
	Hardness (mg $CaCO_3$/L)	80			[illegible]	84	
	Temperature (°C)	24.9	25.1	24.9	25.0	24.6	25.2
600 mg NaCl/L	pH (S.U.)	7.99	7.83	7.77	7.83	7.78	7.84
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.4	8.0
	Conductivity (µmhos/cm)	1360		1420		1440	
	Temperature (°C)	25.0	25.0	25.0	24.9	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.99	7.82	7.78	7.83	7.79	7.84
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	1700		1840		1820	
	Temperature (°C)	25.0	25.3	25.0	24.9	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.98	7.82	7.80	7.84	7.81	7.85
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	2050		2200		2200	
	Temperature (°C)	25.0	25.3	25.0	25.2	24.9	25.1
1200 mg NaCl/L	pH (S.U.)	7.98	7.82	7.81	7.84	7.82	7.86
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	2400		2580		2550	
	Temperature (°C)	25.0	25.0	25.0	25.2	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	7.99	7.83	7.82	7.84	7.83	7.86
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.4	8.0
	Conductivity (µmhos/cm)	2770		2940		2950	
	Temperature (°C)	25.0	25.0	25.0	24.8	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

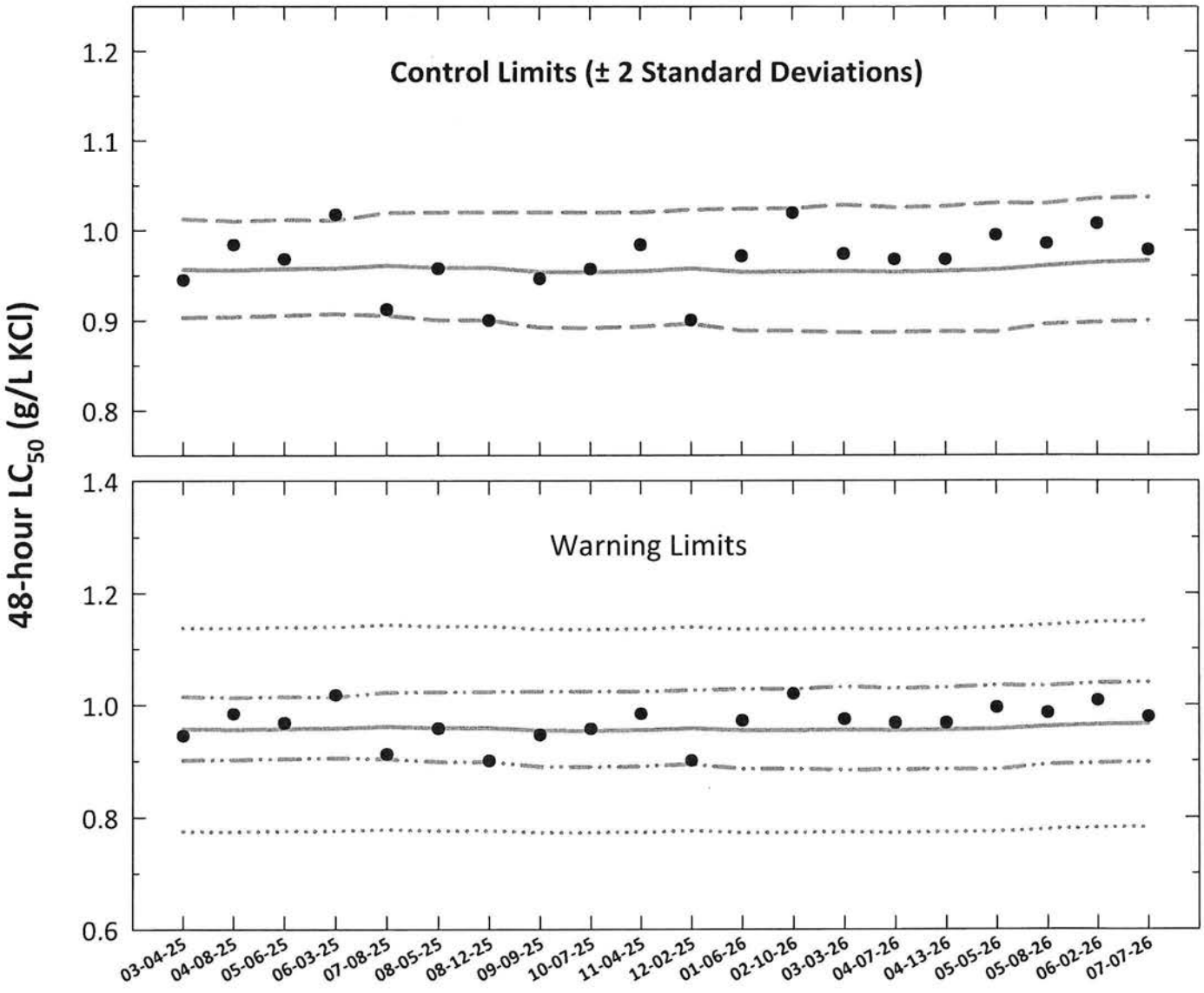
CdNaClCR #: 328

		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.)	7.76	8.01	7.73	~~7.92~~ (8.02)*	7.78	7.92	7.82	7.93
	Dissolved oxygen (mg/L)	8.3	8.0	8.4	~~8.3~~ (7.9)*	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	309		306		302		306	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	25.2	24.8	25.1	24.9	25.2	24.8	25.3
600 mg NaCl/L	pH (S.U.)	7.79	8.00	7.75	~~7.94~~ (8.03)*	7.82	7.91	7.84	7.92
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	~~8.3~~ (7.9)*	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	1440		1450		1420		1420	
	Temperature (°C)	24.9	25.3	24.9	25.2	25.0	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.81	8.01	7.82	7.95	7.90	7.92	7.86	7.94
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	~~8.3~~ (7.9)*	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	1790		1830		1770		1830	
	Temperature (°C)	24.9	25.0	24.9	25.2	25.0	24.9	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.82	8.02	7.83	7.96	7.92	7.93	7.89	7.93
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	~~8.3~~ (7.9)*	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2180		2180		2140		2170	
	Temperature (°C)	24.9	25.3	24.9	25.1	25.0	24.9	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.83	8.01	7.85	7.94	7.94	7.93	7.91	7.93
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	7.9	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2550		2560		2510		2550	
	Temperature (°C)	25.0	25.3	25.0	24.9	25.0	25.3	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.84	8.00	7.86	~~8.0~~ 7.92*	7.94	7.94	7.92	7.93
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	7.9	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2890		2880		2920		2910	
	Temperature (°C)	25.0	25.1	25.0	24.9	25.0	24.9	25.0	25.3
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 09.20.26 verified correct

ETS
Environmental Testing Solutions, Inc.

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



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	07-07-26	0.9788	-0.0093	-0.0148	0.0154	0.9665	0.9004	1.0375	0.8981	1.0400	0.7829	1.1502

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 # 195

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 #: 2486

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.76	7.90	7.89
	Dissolved oxygen (mg/L)	8.6	8.1	8.3
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.1	25.2	24.7
500 mg/L	pH (S.U.)	7.93	7.90	7.87
	Dissolved oxygen (mg/L)	8.6	8.1	8.2
	Conductivity (µmhos/cm)	1200		
	Temperature (°C)	24.1	25.0	25.1
750 mg/L	pH (S.U.)	7.94	7.91	7.87
	Dissolved oxygen (mg/L)	8.6	8.1	8.2
	Conductivity (µmhos/cm)	1620		
	Temperature (°C)	25.0	24.9	24.8
1000 mg/L	pH (S.U.)	7.94	7.91	7.86
	Dissolved oxygen (mg/L)	8.6	8.0	8.1
	Conductivity (µmhos/cm)	2020		
	Temperature (°C)	24.2	24.7	24.6
1250 mg/L	pH (S.U.)	7.95	7.85	7.86
	Dissolved oxygen (mg/L)	8.6	7.8	8.0
	Conductivity (µmhos/cm)	2470		
	Temperature (°C)	24.7	24.7	24.4
1500 mg/L	pH (S.U.)	7.96	7.84	7.85
	Dissolved oxygen (mg/L)	8.6	7.7	8.0
	Conductivity (µmhos/cm)	2970		
	Temperature (°C)	24.1	24.6	24.9

*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	170664705

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 # 195

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-07-26	* 0535	JP	0802	JP	7E	Green	06-30-26A
24	07-08-26			0758	JP			
48 Termination	07-09-26			0810	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:	06-24-26
Age (1 to 14 days old):	5-6 DAYS
Hatch date and times:	07-01-26 1150 TO 07-02-26 0815
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.06
	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	9 d	9 d	6 d	~~4~~ 5 s	1 d	0 d	1 d	0 d
48 Termination	10	10	10	10	9	9	6	5	1	0	0 d	0
Mean Survival	100%		100%		90%		55%		5%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	1049.4
Upper 95% confidence limit (mg KCl/L)	904.41
48-hour LC_{50} (mg KCl/L)	978.8

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:	7/7/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/9/2026	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	0.6000	0.5000
1250	0.1000	0.0000
1500	0.1000	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	0.2055	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.245	2.830	0.2055	2	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	7.937	2.830	0.2055	9	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	16.139	2.830	0.2055	19	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	16.139	2.830	0.2055	19	20

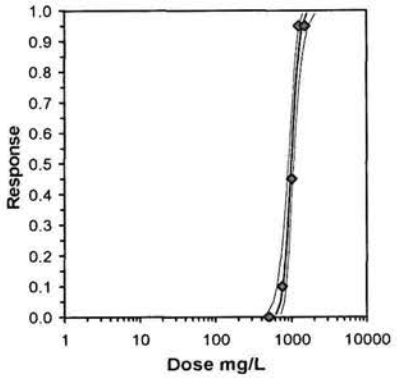
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.10191	0.10452	0.60831	0.00527	7.1E-06	5, 6
Treatments vs D-Control										

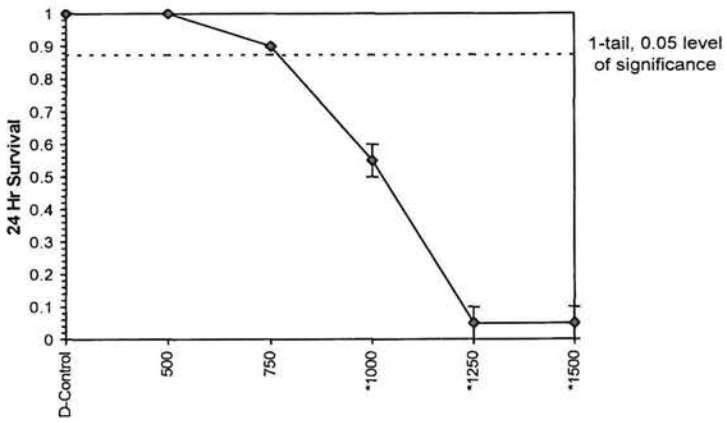
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.1834	1.96802	7.3261	15.0407	0	2.25139	7.81472	0.5219	2.99634	0.08942	3
Intercept	-28.509	5.93319	-40.138	-16.88							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	614.221	460.689	711.983
EC05	3.355	706.745	567.926	794.18
EC10	3.718	761.638	634.007	843.065
EC15	3.964	801.063	682.197	878.618
EC20	4.158	833.847	722.487	908.708
EC25	4.326	863.04	758.331	936.096
EC40	4.747	941.215	852.514	1013.83
EC50	5.000	991.614	910.18	1068.96
EC60	5.253	1044.71	966.963	1132.68
EC75	5.674	1139.34	1057.45	1261.07
EC80	5.842	1179.23	1092.18	1320.17
EC85	6.036	1227.49	1132.23	1394.88
EC90	6.282	1291.03	1182.46	1497.74
EC95	6.645	1391.3	1257.78	1668.65
EC99	7.326	1600.89	1405.7	2053.09



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:	7/7/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/9/2026	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	0.6000	0.5000
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	0.1726	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.690	2.850	0.1726	2	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.343	2.850	0.1726	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

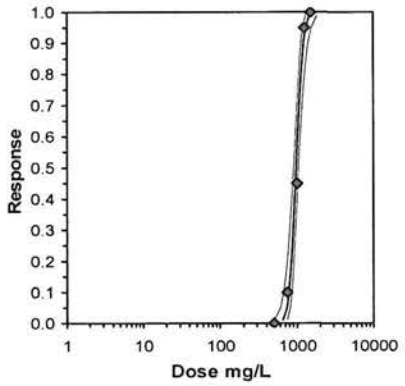
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08088	0.08295	0.50064	0.00367	2.7E-05	4, 5
Treatments vs D-Control										

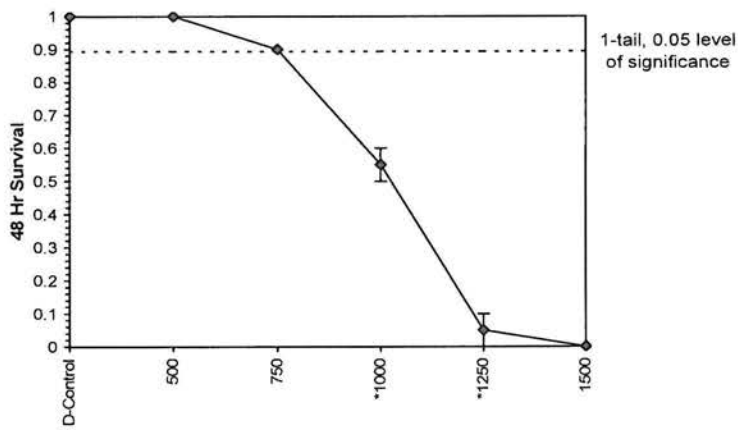
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2013	2.39931	8.49862	17.9039	0	1.63607	7.81472	0.65124	2.99071	0.07575	3
Intercept	-34.481	7.21256	-48.618	-20.345							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	652.364	504.505	742.597
EC05	3.355	734.706	603.946	814.467
EC10	3.718	782.769	663.804	856.785
EC15	3.964	816.962	706.844	887.404
EC20	4.158	845.199	742.456	913.226
EC25	4.326	870.2	773.866	936.663
EC40	4.747	936.528	855.269	1002.82
EC50	5.000	978.84	904.4	1049.36
EC60	5.253	1023.06	952.365	1102.67
EC75	5.674	1101.04	1028.07	1208.65
EC80	5.842	1133.61	1056.88	1256.9
EC85	6.036	1172.79	1089.88	1317.5
EC90	6.282	1224.02	1130.97	1400.26
EC95	6.645	1304.1	1191.93	1536.21
EC99	7.326	1468.7	1309.64	1835.7

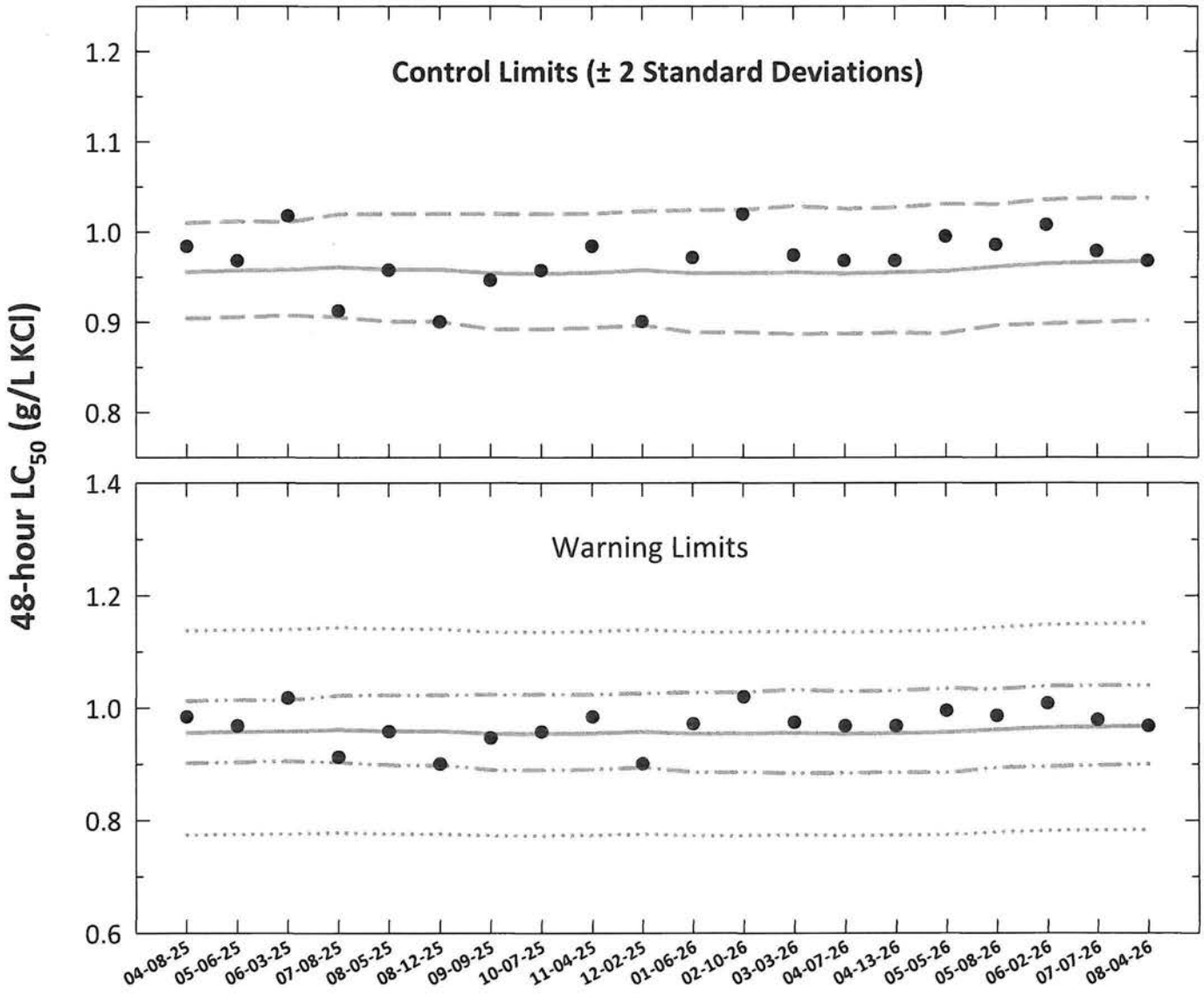


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	07-07-26	0.9788	-0.0093	-0.0148	0.0154	0.9665	0.9004	1.0375	0.8981	1.0400	0.7829	1.1502
20	08-04-26	0.9680	-0.0141	-0.0143	0.0152	0.9677	0.9022	1.0379	0.9000	1.0403	0.7838	1.1516

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 # 196

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 #: 2486

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.76	7.88	7.87
	Dissolved oxygen (mg/L)	8.5	8.0	8.0
	Conductivity (µmhos/cm)	304		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	24.9	24.9	24.7
500 mg/L	pH (S.U.)	7.94	7.87	7.87
	Dissolved oxygen (mg/L)	8.6	8.0	8.0
	Conductivity (µmhos/cm)	1180		
	Temperature (°C)	24.7	24.8	24.9
750 mg/L	pH (S.U.)	7.95	7.87	7.86
	Dissolved oxygen (mg/L)	8.6	7.9	7.9
	Conductivity (µmhos/cm)	1580		
	Temperature (°C)	24.7	25.0	25.0
1000 mg/L	pH (S.U.)	7.96	7.87	7.86
	Dissolved oxygen (mg/L)	8.6	7.8	7.9
	Conductivity (µmhos/cm)	2040		
	Temperature (°C)	24.7	25.0	24.8
1250 mg/L	pH (S.U.)	7.96	7.85	7.87
	Dissolved oxygen (mg/L)	8.6	7.6	7.9
	Conductivity (µmhos/cm)	2470		
	Temperature (°C)	24.8	25.0	24.8
1500 mg/L	pH (S.U.)	7.97	7.85	
	Dissolved oxygen (mg/L)	8.6	7.80	
	Conductivity (µmhos/cm)	2890		
	Temperature (°C)	24.6	24.7	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13066468

ETS

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 196

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08.04.26	* 0505	JK	0705	JK	7F	GREEN	07.30.26A
24	08.05.26			0700	JK			
48 Termination	08.06.26			0700	JK			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	07.22.26
Age (1 to 14 days old):	6-7 DAYS
Hatch date and times:	07.28.26 1154 TO 07.29.26 0607
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.02
	Temperature (°C): 24.0°C

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

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

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 1d	10	8 2d	7 2d	1 9d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	9	9 1d	5 2d	5 2d	1	0 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: JK

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	8/4/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/6/2026	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	0.8000	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	0.1803	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.288	2.850	0.1803	1	20
*1000	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	5.736	2.850	0.1803	5	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	17.236	2.850	0.1803	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

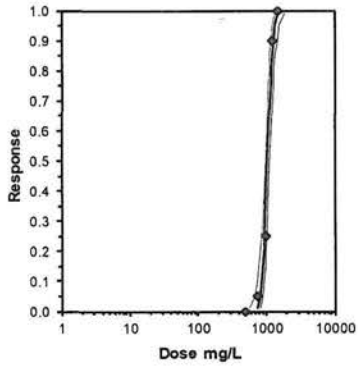
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08562	0.08782	0.42799	0.004	5.0E-05	4, 5
Treatments vs D-Control										

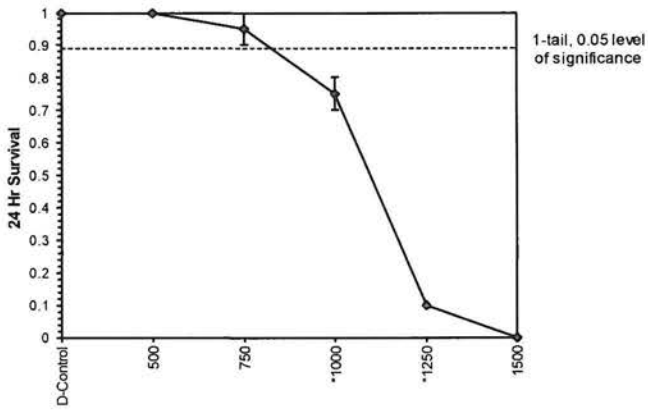
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.8792	2.83523	9.3222	20.4363	0	3.51076	7.81472	0.31937	3.0229	0.06721	4
Intercept	-39.978	8.60757	-56.849	-23.108							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	735.44	576.087	827.89
EC05	3.355	817.239	678.783	897.804
EC10	3.718	864.501	739.86	938.668
EC15	3.964	897.924	783.463	968.124
EC20	4.158	925.406	819.35	992.912
EC25	4.326	949.653	850.858	1015.38
EC40	4.747	1013.61	931.828	1078.78
EC50	5.000	1054.13	980.128	1123.47
EC60	5.253	1096.28	1026.76	1174.76
EC75	5.674	1170.11	1099.21	1276.8
EC80	5.842	1200.77	1126.42	1323.14
EC85	6.036	1237.52	1157.42	1381.17
EC90	6.282	1285.36	1195.76	1460.1
EC95	6.645	1359.7	1252.23	1588.89
EC99	7.326	1510.93	1360.12	1869.17



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:	8/4/2026	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/6/2026	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	0.1000	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	0.1469	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

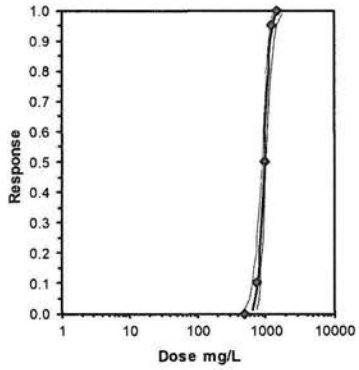
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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										

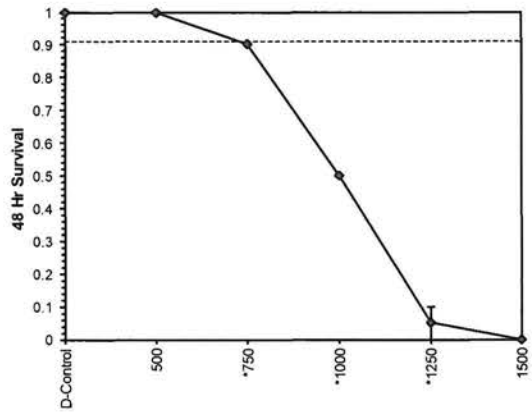
Maximum Likelihood-Probit

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

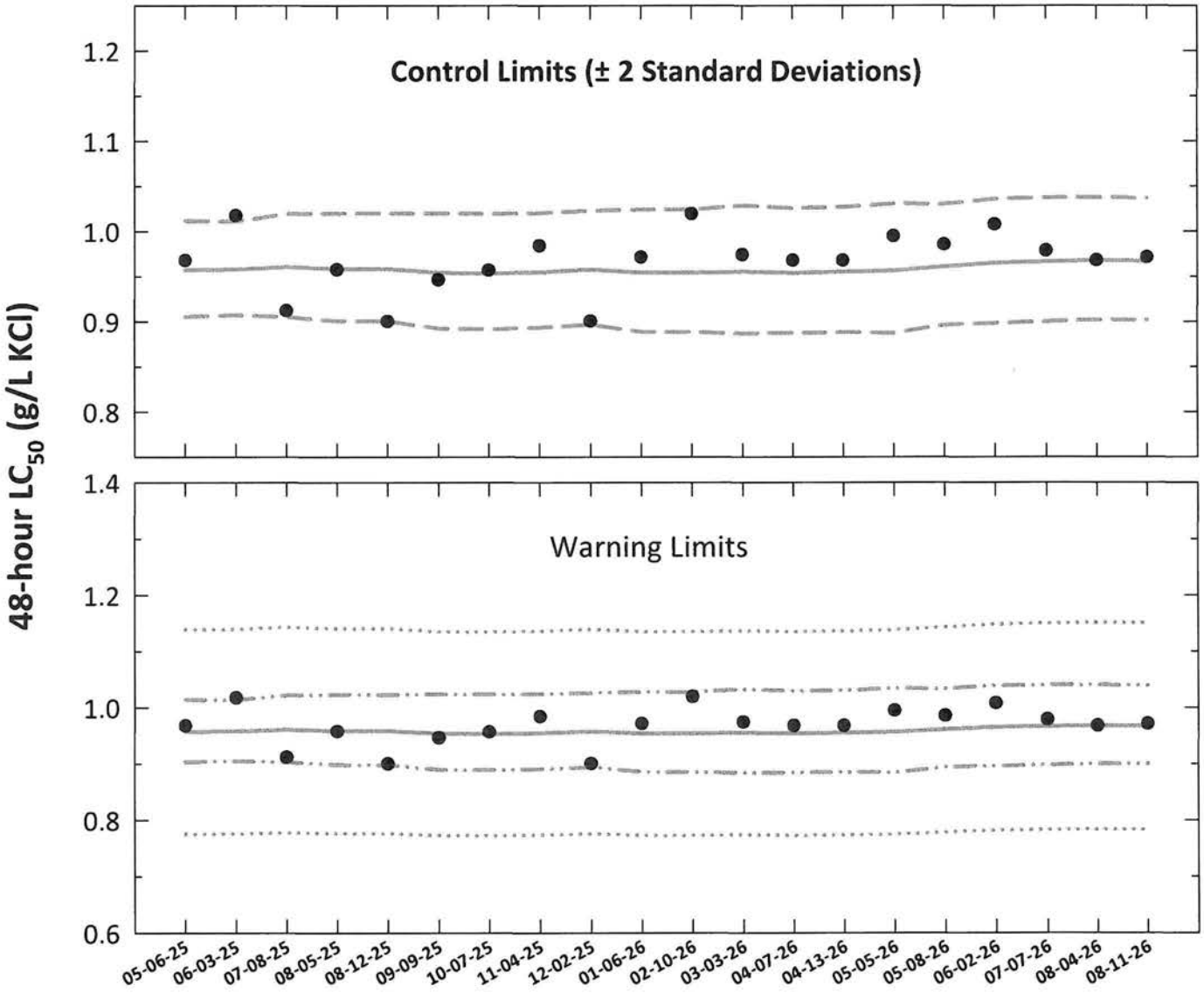


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- – – – **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	07-07-26	0.9788	-0.0093	-0.0148	0.0154	0.9665	0.9004	1.0375	0.8981	1.0400	0.7829	1.1502
19	08-04-26	0.9680	-0.0141	-0.0143	0.0152	0.9677	0.9022	1.0379	0.9000	1.0403	0.7838	1.1516
20	08-11-26	0.9715	-0.0126	-0.0145	0.0151	0.9671	0.9020	1.0369	0.8998	1.0392	0.7833	1.1508

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

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

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 197

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KC stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2466

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JL	XL	XL
Control, MHSW	pH (S.U.)	7.93	7.95	7.83
	Dissolved oxygen (mg/L)	8.3	8.1	8.4
	Conductivity (µmhos/cm)	299		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.9	24.5	24.9
500 mg/L	pH (S.U.)	7.99	7.92	7.86
	Dissolved oxygen (mg/L)	8.2	8.1	8.4
	Conductivity (µmhos/cm)	1140		
	Temperature (°C)	25.0	24.6	24.7
750 mg/L	pH (S.U.)	7.99	7.92	7.85
	Dissolved oxygen (mg/L)	8.2	8.1	8.3
	Conductivity (µmhos/cm)	1580		
	Temperature (°C)	25.0	24.5	24.7
1000 mg/L	pH (S.U.)	8.00	7.92	7.87
	Dissolved oxygen (mg/L)	8.2	8.1	8.2
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	24.9	24.5	25.0
1250 mg/L	pH (S.U.)	8.01	7.91	7.87
	Dissolved oxygen (mg/L)	8.3	8.0	8.3
	Conductivity (µmhos/cm)	2520		
	Temperature (°C)	24.9	24.7	24.8
1500 mg/L	pH (S.U.)	8.02	7.92	
	Dissolved oxygen (mg/L)	8.3	8.0	
	Conductivity (µmhos/cm)	2820		
	Temperature (°C)	25.1	24.7	

*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 th test specific bench sheets and transcribed to this bench sheet.

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13066468(

ETS

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 197

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-11-26	* 0811	JH	1013	JH	7B	Yellow	08-06-26B
24	08-12-26			1015	JH			
48 Termination	08-13-26			1015	JH			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	07-30-26
Age (1 to 14 days old):	5-6 DAYS
Hatch date and times:	08-05-26 1340 TO 08-06-26 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.10
	Temperature (°C): 24.2

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 5d	7 3d	0 10d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	10	10	5	4 4d	0	0 1d	0	0
Mean Survival	100%		100%		100%		45%		0%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	917.8
Upper 95% confidence limit (mg KCl/L)	1028.3
48-hour LC_{50} (mg KCl/L)	971.5

Comments:

Test Reviewed by: JH

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	8/11/2026	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/13/2026	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	0.0000	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	0.2366	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2366	0	20
*1000	0.6000	0.6000	0.8883	0.7854	0.9912	16.379	2	6.310	2.850	0.2366	8	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	14.117	2.850	0.2366	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

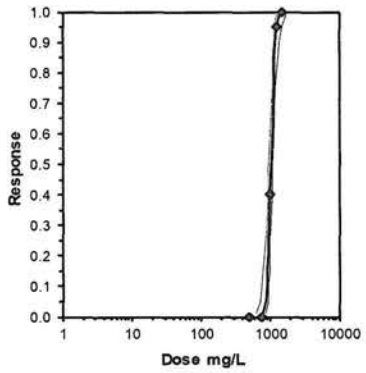
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12332	0.12648	0.53618	0.00689	1.1E-04	4, 5
Treatments vs D-Control										

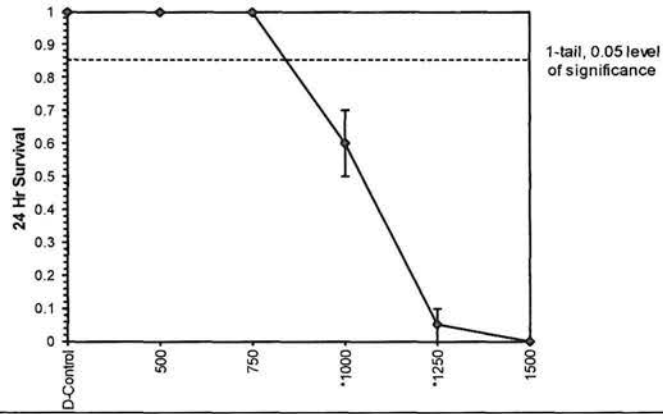
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	20.4558	4.89777	10.8562	30.0555	0	0.07876	7.81472	0.99426	3.01378	0.04889	3
Intercept	-56.649	14.8004	-85.658	-27.64							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	794.419	613.92	877.815
EC05	3.355	857.759	706.832	928.213
EC10	3.718	893.563	761.106	957.352
EC15	3.964	918.561	799.422	978.306
EC20	4.158	938.927	830.674	995.971
EC25	4.326	956.758	857.879	1012.06
EC40	4.747	1003.21	926.446	1058.32
EC50	5.000	1032.23	965.93	1092.09
EC60	5.253	1062.09	1002.5	1132.11
EC75	5.674	1113.65	1055.71	1214.03
EC80	5.842	1134.8	1074.69	1251.55
EC85	6.036	1159.96	1095.79	1298.46
EC90	6.282	1192.41	1121.3	1361.98
EC95	6.645	1242.18	1157.95	1464.72
EC99	7.326	1341.22	1225.87	1684.41



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	8/11/2026	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/13/2026	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	0.4000
1250	0.0000	0.0000
1500	0.0000	0.0000

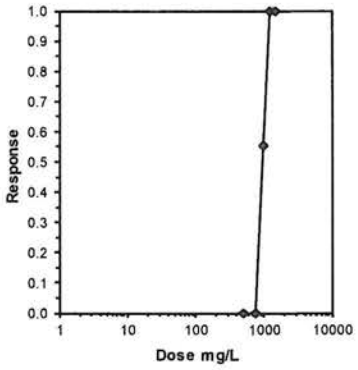
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	21.263	2.850	0.0907	11	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	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				

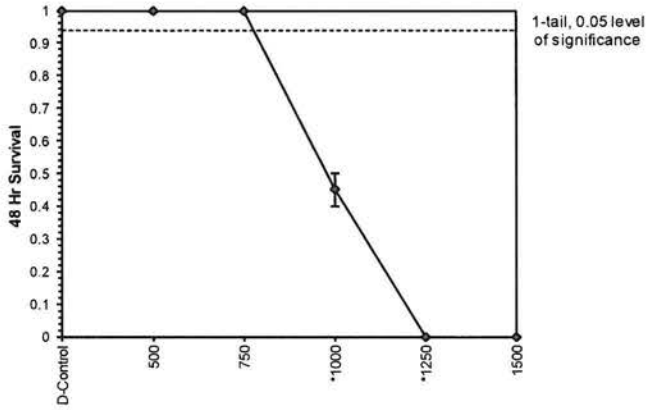
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.03598	0.0369	0.64187	0.00101	6.0E-07	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

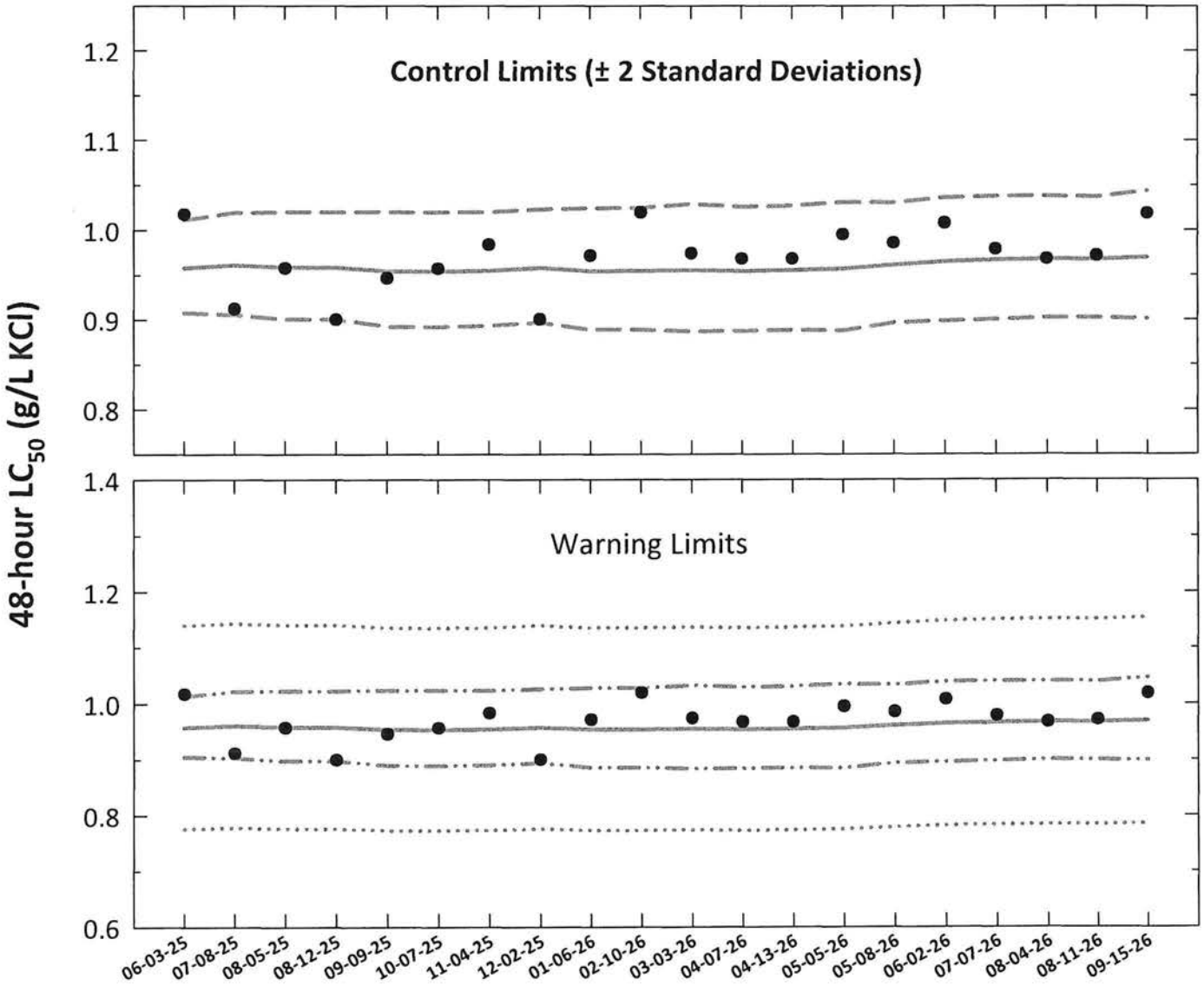
Trim Level	EC50	95% CL	
0.0%	971.51	917.84	1028.31
5.0%	971.83	912.41	1035.13
10.0%	972.16	905.69	1043.51
20.0%	972.81	885.89	1068.25
Auto-0.0%	971.51	917.84	1028.31



Dose-Response Plot



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



Test date

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**06-03-25**	**1.0177**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**07-07-26**	**0.9788**	-0.0093	-0.0148	0.0154	0.9665	0.9004	1.0375	0.8981	1.0400	0.7829	1.1502
18	**08-04-26**	**0.9680**	-0.0141	-0.0143	0.0152	0.9677	0.9022	1.0379	0.9000	1.0403	0.7838	1.1516
19	**08-11-26**	**0.9715**	-0.0126	-0.0145	0.0151	0.9671	0.9020	1.0369	0.8998	1.0392	0.7833	1.1508
20	**09-15-26**	**1.0186**	0.0080	-0.0134	0.0159	0.9695	0.9009	1.0434	0.8987	1.0457	0.7853	1.1538

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 # 198

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 #: 2501

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	VL	XL	XL
Control, MHSW	pH (S.U.)	8.01	7.87	7.81
	Dissolved oxygen (mg/L)	8.3	8.1	8.0
	Conductivity (µmhos/cm)	292		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	80		
	Temperature (°C)	25.7	24.7	24.7
500 mg/L	pH (S.U.)	8.04	7.87	7.83
	Dissolved oxygen (mg/L)	8.3	8.2	8.0
	Conductivity (µmhos/cm)	1120		
	Temperature (°C)	25.7	24.9	24.9
750 mg/L	pH (S.U.)	8.03	7.88	7.84
	Dissolved oxygen (mg/L)	8.3	8.1	8.1
	Conductivity (µmhos/cm)	1550		
	Temperature (°C)	25.8	24.5	24.9
1000 mg/L	pH (S.U.)	8.02	7.88	7.84
	Dissolved oxygen (mg/L)	8.3	8.1	8.0
	Conductivity (µmhos/cm)	1940		
	Temperature (°C)	25.8	24.9	25.1
1250 mg/L	pH (S.U.)	8.03	7.89	7.86
	Dissolved oxygen (mg/L)	8.4	8.1	8.0
	Conductivity (µmhos/cm)	2330		
	Temperature (°C)	25.9	24.9	24.9
1500 mg/L	pH (S.U.)	8.04	7.88	
	Dissolved oxygen (mg/L)	3.4	8.0	
	Conductivity (µmhos/cm)	2770		
	Temperature (°C)	25.8	25.1	

*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	130664705

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	9/15/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/17/2026	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	0.9000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.7000
1250	0.2000	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	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2				1	20
500	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-1.329	2.850	0.1748	0	20
750	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-1.329	2.850	0.1748	0	20
*1000	0.6500	0.6842	0.9386	0.8861	0.9912	7.916	2	6.391	2.850	0.1748	7	20
*1250	0.2000	0.2105	0.4636	0.4636	0.4636	0.000	2	14.137	2.850	0.1748	16	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

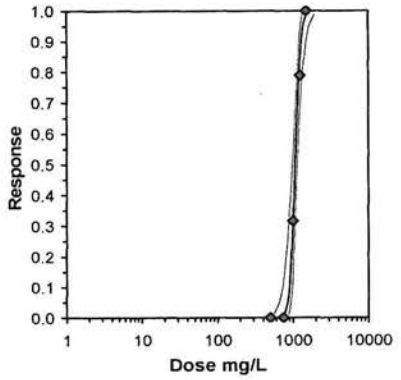
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.10596	0.11232	0.3391	0.00376	7.6E-05	4, 5
Treatments vs D-Control										

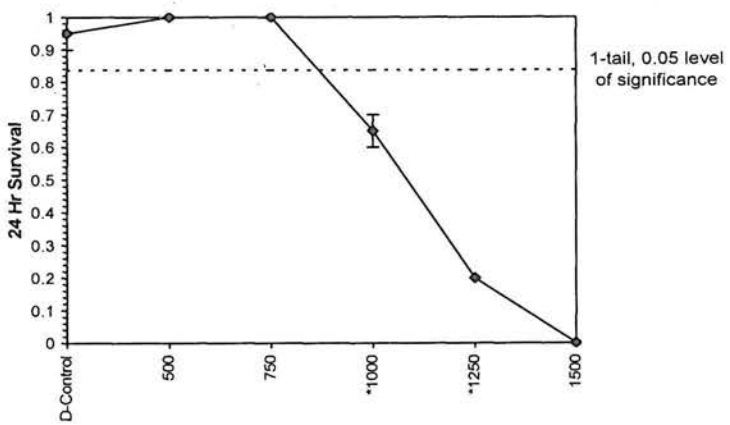
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.8125	3.62798	8.70167	22.9234	0.05	1.51611	7.81472	0.67856	3.0337	0.06324	7
Intercept	-42.971	11.0716	-64.671	-21.27							
TSCR	0.01719	0.01809	-0.0183	0.05266							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	770.166	556.594	877.231
EC05	3.355	850.516	664.203	942.754
EC10	3.718	896.723	729.049	980.713
EC15	3.964	929.308	775.773	1007.92
EC20	4.158	956.048	814.526	1030.72
EC25	4.326	979.602	848.792	1051.34
EC40	4.747	1041.56	937.923	1109.55
EC50	5.000	1080.7	991.581	1151.21
EC60	5.253	1121.31	1043	1200.51
EC75	5.674	1192.23	1119.63	1304.19
EC80	5.842	1221.6	1147	1353.16
EC85	6.036	1256.75	1177.34	1415.47
EC90	6.282	1302.42	1213.92	1501.32
EC95	6.645	1373.18	1266.53	1643.04
EC99	7.326	1516.44	1364.81	1955.41



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	9/15/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/17/2026	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	0.9000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.5000	0.6000
1250	0.2000	0.1000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	0.9500	1.0000	1.3305	1.2490	1.4120	8.661	2				1	20
500	1.0000	1.0526	1.4120	1.4120	1.4120	0.000	2	-1.081	2.850	0.2148	0	20
750	0.9000	0.9474	1.2490	1.2490	1.2490	0.000	2	1.081	2.850	0.2148	2	20
*1000	0.5500	0.5789	0.8357	0.7854	0.8861	8.518	2	6.563	2.850	0.2148	9	20
*1250	0.1500	0.1579	0.3927	0.3218	0.4636	25.550	2	12.440	2.850	0.2148	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

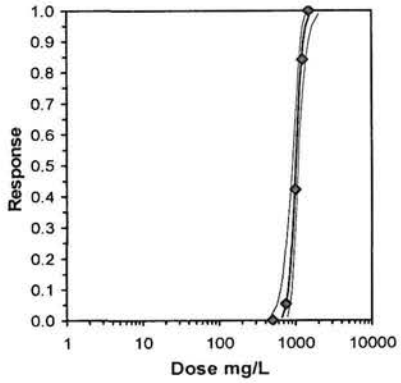
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.13659	0.14479	0.36357	0.00568	1.8E-04	4, 5
Treatments vs D-Control										

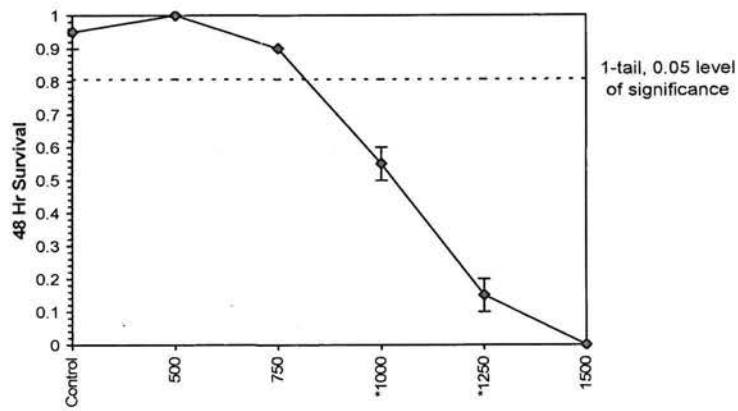
Maximum Likelihood-Probit

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

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

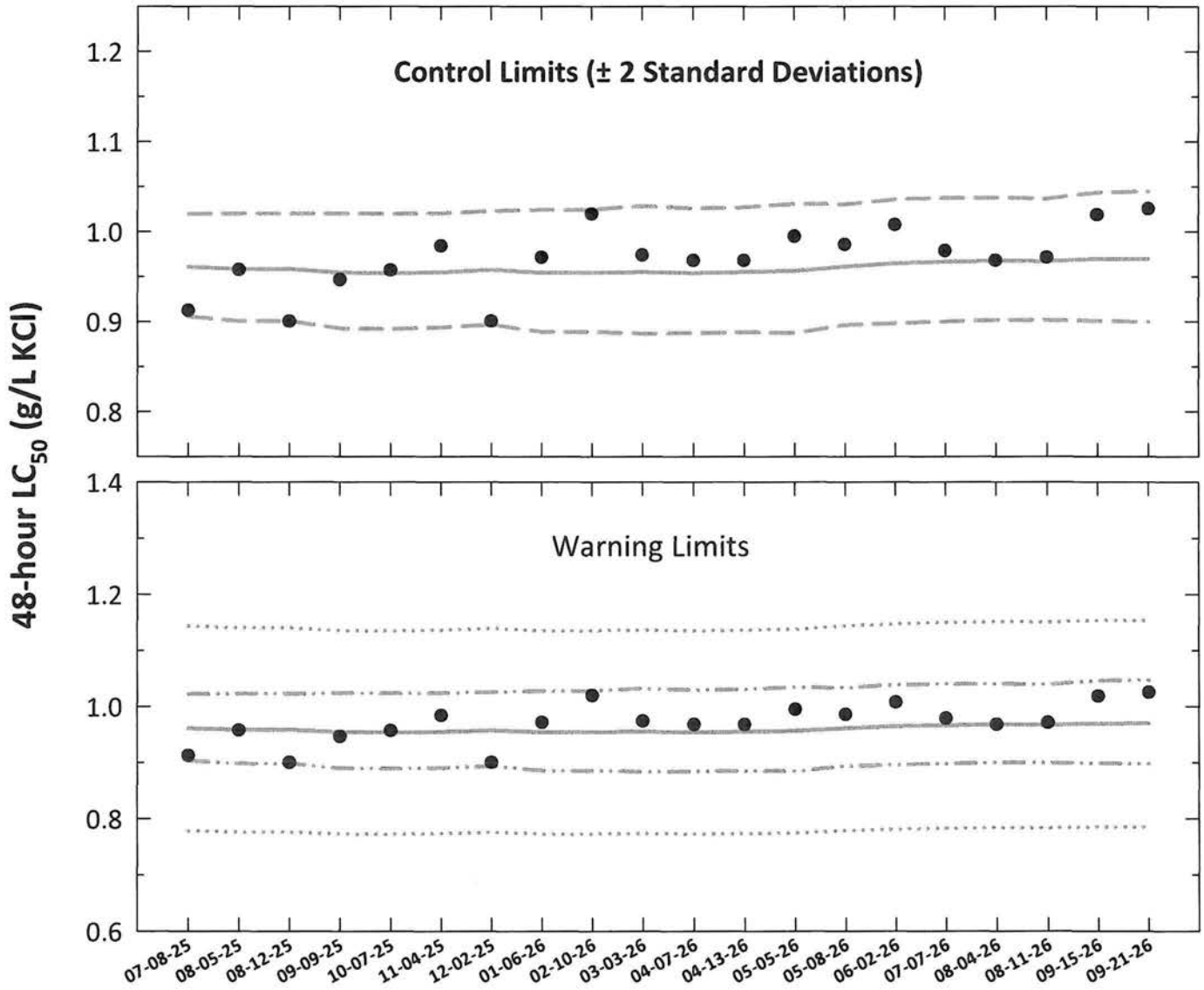


Dose-Response Plot



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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	07-07-26	0.9788	-0.0093	-0.0148	0.0154	0.9665	0.9004	1.0375	0.8981	1.0400	0.7829	1.1502
17	08-04-26	0.9680	-0.0141	-0.0143	0.0152	0.9677	0.9022	1.0379	0.9000	1.0403	0.7838	1.1516
18	08-11-26	0.9715	-0.0126	-0.0145	0.0151	0.9671	0.9020	1.0369	0.8998	1.0392	0.7833	1.1508
19	09-15-26	1.0186	0.0080	-0.0134	0.0159	0.9695	0.9009	1.0434	0.8987	1.0457	0.7853	1.1538
20	09-21-26	1.0256	0.0110	-0.0133	0.0162	0.9699	0.9002	1.0450	0.8980	1.0474	0.7856	1.1542

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

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

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 199

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 #: 2501

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	N	XL
Control, MHSW	pH (S.U.)	7.82	7.85	7.72
	Dissolved oxygen (mg/L)	8.2	7.4	8.3
	Conductivity (µmhos/cm)	306		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	24.9	24.7	25.0
500 mg/L	pH (S.U.)	7.79	7.89	7.74
	Dissolved oxygen (mg/L)	8.3	7.3	8.3
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	24.9	24.4	24.6
750 mg/L	pH (S.U.)	7.81	7.89	7.76
	Dissolved oxygen (mg/L)	8.3	7.3	8.2
	Conductivity (µmhos/cm)	1540		
	Temperature (°C)	24.7	24.9	25.1
1000 mg/L	pH (S.U.)	7.82	7.90	7.79
	Dissolved oxygen (mg/L)	8.3	7.3	8.2
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	24.8	24.6	24.8
1250 mg/L	pH (S.U.)	7.84	7.89	7.81
	Dissolved oxygen (mg/L)	8.3	7.3	8.1
	Conductivity (µmhos/cm)	2380		
	Temperature (°C)	24.6	24.6	24.7
1500 mg/L	pH (S.U.)	7.85	7.88	
	Dissolved oxygen (mg/L)	8.3	7.1	
	Conductivity (µmhos/cm)	2780		
	Temperature (°C)	24.9	24.8	

2109-22-26

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuremer 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 # 199

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09-21-26	* 0500	JP	0932	JP	1B	orange	0917-25A
24	09-22-26			0945	JP			
48 Termination	09-23-26			0938	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:	09-09-26
Age (1 to 14 days old):	09-21-26 5 to 6 days
Hatch date and times:	09-15-26 1150 to 09-16-26 0425
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.06
	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	9 d	9 d	10	10	10	10	5 d	8 d	1 d	0 d	0 d	0 d
48 Termination	9	9	10	10	10	10	5	6 s	1	0	0	0
Mean Survival	90%		100%		100%		55%		5%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	935.3
Upper 95% confidence limit (mg KCl/L)	1088.5
48-hour LC_{50} (mg KCl/L)	1025.6

Comments:

Test Reviewed by:

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	9/21/2026	Test ID:	PpKClAC	Sample ID:	PPKCLAC
End Date:	9/23/2026	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	0.9000	0.9000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.5000	0.8000
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	0.9000	1.0000	1.2490	1.2490	1.2490	0.000	2				2	20
500	1.0000	1.1111	1.4120	1.4120	1.4120	0.000	2	-1.429	2.850	0.3251	0	20
750	1.0000	1.1111	1.4120	1.4120	1.4120	0.000	2	-1.429	2.850	0.3251	0	20
1000	0.6500	0.7222	0.9463	0.7854	1.1071	24.043	2	2.655	2.850	0.3251	7	20
*1250	0.0500	0.0556	0.2403	0.1588	0.3218	47.963	2	8.845	2.850	0.3251	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

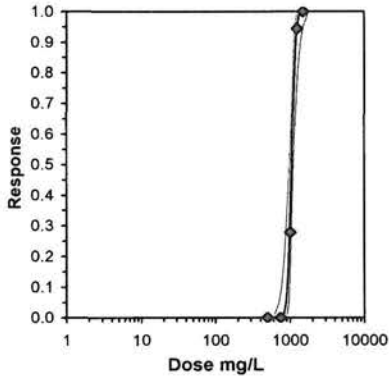
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.26317	0.29242	0.48407	0.01301	6.5E-04	4, 5
Treatments vs D-Control										

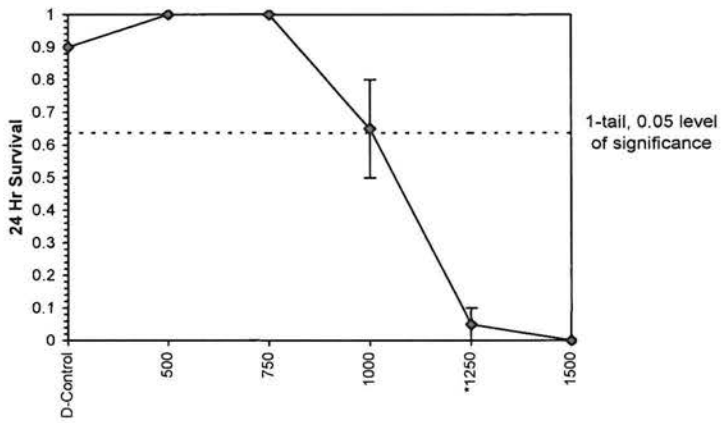
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	21.8077	5.72525	10.5862	33.0292	0.1	1.41062	7.81472	0.70305	3.02107	0.04586	4
Intercept	-60.883	17.3416	-94.872	-26.893							
TSCR	0.03345	0.02337	-0.0124	0.07926							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	821.105	613.989	907.679
EC05	3.355	882.366	709.554	955.248
EC10	3.718	916.87	765.564	982.737
EC15	3.964	940.91	805.189	1002.53
EC20	4.158	960.464	837.558	1019.25
EC25	4.326	977.563	865.767	1034.53
EC40	4.747	1022.01	936.912	1078.89
EC50	5.000	1049.72	977.715	1111.9
EC60	5.253	1078.18	1015.07	1151.82
EC75	5.674	1127.2	1067.97	1235.53
EC80	5.842	1147.27	1086.35	1274.35
EC85	6.036	1171.12	1106.53	1323.12
EC90	6.282	1201.82	1130.63	1389.36
EC95	6.645	1248.82	1164.88	1496.83
EC99	7.326	1341.99	1227.59	1727.46



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:	9/21/2026	Test ID:	PpKClAC	Sample ID:	PPKCLAC
End Date:	9/23/2026	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	0.9000	0.9000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.5000	0.6000
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	0.9000	1.0000	1.2490	1.2490	1.2490	0.000	2				2	20
500	1.0000	1.1111	1.4120	1.4120	1.4120	0.000	2	-2.690	2.850	0.1726	0	20
750	1.0000	1.1111	1.4120	1.4120	1.4120	0.000	2	-2.690	2.850	0.1726	0	20
*1000	0.5500	0.6111	0.8357	0.7854	0.8861	8.518	2	6.823	2.850	0.1726	9	20
*1250	0.0500	0.0556	0.2403	0.1588	0.3218	47.963	2	16.653	2.850	0.1726	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

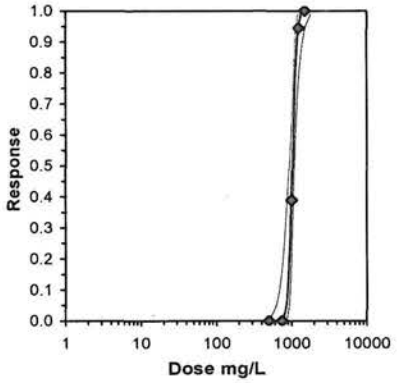
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12515	0.13905	0.50064	0.00367	2.7E-05	4, 5
Treatments vs D-Control										

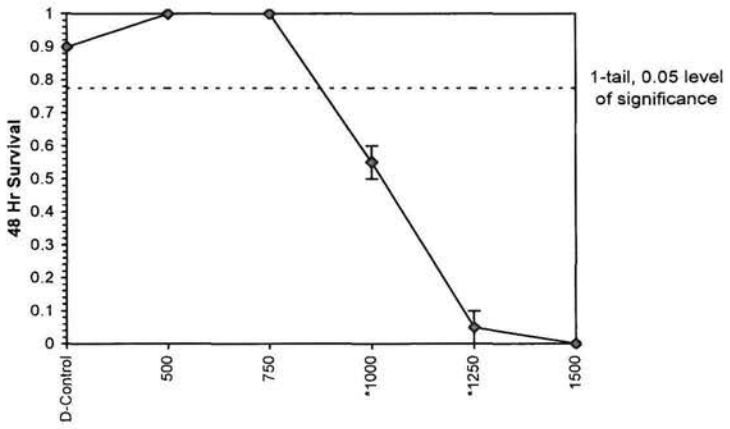
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	19.862	5.6685	8.75176	30.9723	0.1	1.52698	7.81472	0.67606	3.01098	0.05035	9
Intercept	-54.804	17.1518	-88.422	-21.187							
TSCR	0.03368	0.02404	-0.0134	0.08079							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	783.159	526.271	882.355
EC05	3.355	847.542	627.624	931.165
EC10	3.718	884	688.727	959.225
EC15	3.964	909.481	732.775	979.308
EC20	4.158	930.255	769.317	996.171
EC25	4.326	948.454	801.628	1011.49
EC40	4.747	995.909	885.464	1055.55
EC50	5.000	1025.59	935.303	1088.5
EC60	5.253	1056.16	981.666	1129.65
EC75	5.674	1109.01	1045.73	1222.38
EC80	5.842	1130.7	1066.92	1267.62
EC85	6.036	1156.53	1089.56	1325.61
EC90	6.282	1189.87	1116	1405.81
EC95	6.645	1241.05	1152.89	1538.31
EC99	7.326	1343.08	1219.7	1829.99

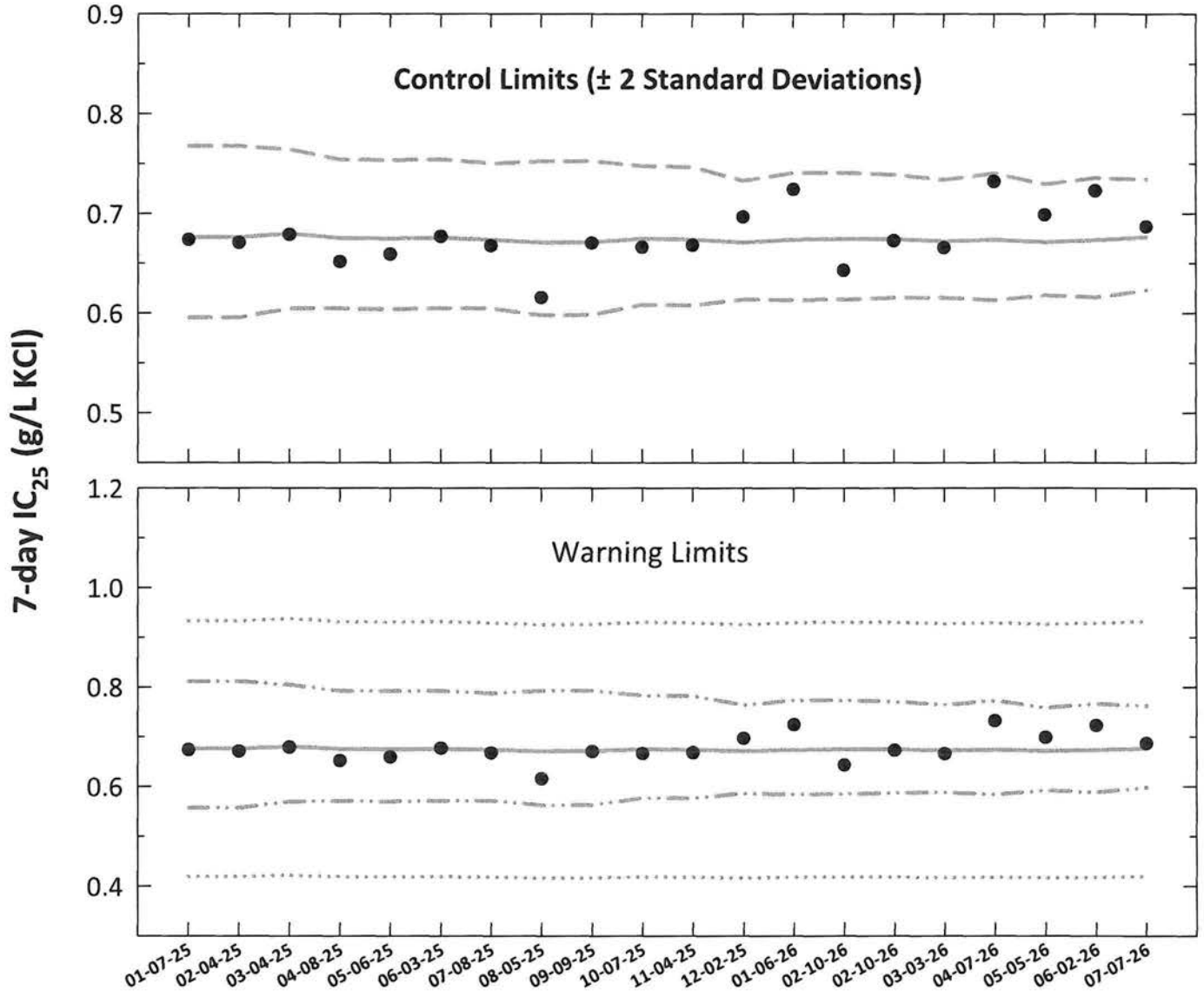


Dose-Response Plot



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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	07-07-26	0.6868	-0.1632	-0.1698	0.0178	0.6765	0.6232	0.7343	0.5977	0.7620	0.4194	0.9335

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.

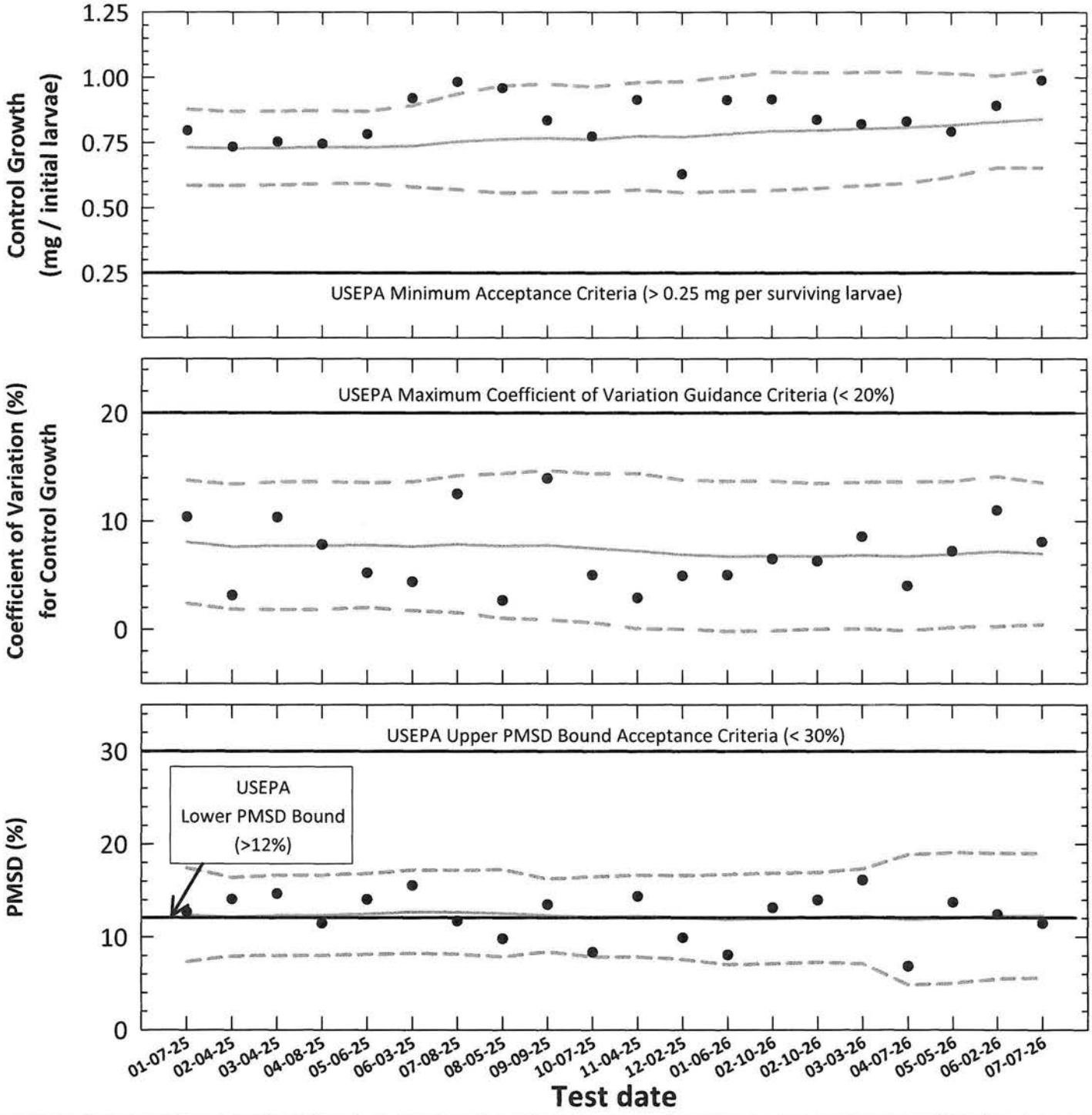
Entered and Reviewed by Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	07-07-26	100	0.989	8.1	0.1133	11.5	0.841	0.653	1.029	7.0	0.4	13.6	12.3	5.6	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 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: **143**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2486				
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:	06·29·26	*Artemia* CHM number:	CHM1432
Hatch dates and times:	07·06·26 1400 TO 07·07·26 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.06 S.U. Temperature = 25.4 °C	Date / Time in oven:	07·14·26 0545
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	07·15·26 0545
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-98585

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	07-07-26	0505	JK	1420	JK	0705	JK	06·30·26 A
1	07-08-26	0500	JK	1325	JK	0700	JK	↓
2	07-09-26	0500	JK	1330	JK	0730	JK	06·30·26 B
3	07-10-26	0500	JK	1100	JK	0720	JK	↓
4	07-11-26	0700	JK	1200	JK	0900	JK	06·30·26 C
5	07-12-26	0600	JK	1100	JK	0810	JK	↓
6	07-13-26	0600	JK	1200	JK	0810	JK	
7	07-14-26					0545	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	13066468 S

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	781.8
Average weight per initial larvae:	0.989		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.989	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	686.8

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **143**

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 (2Lg)	10 (1Lg)	10 (1Lg)	10	10	10	10
*A = Pan weight (mg) Tray color code: Ruby Analyst: XL Date: 06/22/26	13.54	12.46	13.34	13.64	12.95	13.31	11.85	13.85	13.66	14.12	13.84	13.49
*B = Pan + Larvae weight (mg) Analyst: JF Date: 07-16-26	22.37	22.59	23.19	24.39	23.58	25.77	23.21	25.45	23.11	24.04	24.25	23.54
C = Larvae weight (mg) = B – A Analyst: JF	8.83	10.13	9.85	10.75	10.63	12.46	11.36	11.60	9.45	9.92	10.41	10.05
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JF	0.883	1.013	0.985	1.075	1.063	1.246	1.136	1.160	0.945	0.992	1.041	1.005
Average weight per initial number of larvae (mg)	0.989				1.151		-16.47.		0.996		-0.77.	
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: **143**

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	8 2d	8 2d	8 2d	8 2d
2	10	10	10	10	9 1d	10	10	9 1d	8	7 1d	7 1d	7 1d
3	10	10	10	10	9	9 1d	9 1d	9	8	7	7	7
4	10	10	10	10	9	7 2d	9	8 1d	7 1d	6 1d	7	6 1d
5	10	10	10	10	7 2d	7	8 1d	7 1d	5 2d	4 1d	4 3d	5 1d
6	10	9 1d	10	9 1d	7	7	8	7	2 3d	3 1d	4	3 2d
7	10	9	10	9	6 1d	6 1d	6 2d	7	2	1 2d	2 2d	2 1d
*A = Pan weight (mg) Tray color code: Ruby Analyst: XL Date: 06-22-26	13.81	13.39	13.23	14.62	14.05	13.92	14.88	13.84	12.57	12.92	11.85	12.58
*B = Pan + Larvae weight (mg) Analyst: JL Date: 07-16-26	23.73	22.27	23.71	23.60	20.44	19.72	22.19	21.96	14.91	14.27	14.18	14.36
C = Larvae weight (mg) = B – A Analyst: JL	9.92	8.88	10.48	8.98	6.39	5.80	7.31	6.12	2.34	1.35	2.33	1.78
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.992	0.888	1.048	0.898	0.639	0.580	0.731	0.612	0.234	0.135	0.233	0.178
Average weight per initial number of larvae (mg)	0.957				0.691				0.195			
Percent reduction from control (%)			3.32				30.27.				80.37.	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **143**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 7d	3 2d	3 7d	3 7d
2	1 2d	1 2d	1 2d	2 1d
3	1	0 1d	1	2
4	0 1d	0	0 1d	1 1d
5	0	0	0	1
6	0	0	0	1
7	0	0	0	1
*A = Pan weight (mg) Tray color code: Ruby Analyst: XL Date: 06/22/26	12.85	15.11	13.53	14.77
*B = Pan + Larvae weight (mg) Analyst: 07-16-26 Date: M				15.97
C = Larvae weight (mg) = B – A Analyst: M			07/16/26 *	1.20
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0	0	0	0.120
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.030		97.0%	

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 143

Test dates: July 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	13.54	22.37	8.83	0.883	0.989	8.1	0.883	100.0	0.989	8.1	Not applicable
	B	10	10	12.46	22.59	10.13	1.013			1.013				
	C	10	10	13.34	23.19	9.85	0.985			0.985				
	D	10	10	13.64	24.39	10.75	1.075			1.075				
300	E	10	10	12.95	23.58	10.63	1.063	1.151	6.6	1.063	100.0	1.151	6.6	-16.4
	F	10	10	13.31	25.77	12.46	1.246			1.246				
	G	10	10	11.85	23.21	11.36	1.136			1.136				
	H	10	10	13.85	25.45	11.60	1.160			1.160				
450	I	10	10	13.66	23.11	9.45	0.945	0.996	4.0	0.945	100.0	0.996	4.0	-0.7
	J	10	10	14.12	24.04	9.92	0.992			0.992				
	K	10	10	13.84	24.25	10.41	1.041			1.041				
	L	10	10	13.49	23.54	10.05	1.005			1.005				
600	M	10	10	13.81	23.73	9.92	0.992	1.006	2.8	0.992	95.0	0.957	8.0	3.3
	N	10	9	13.39	22.27	8.88	0.987			0.888				
	O	10	10	13.23	23.71	10.48	1.048			1.048				
	P	10	9	14.62	23.60	8.98	0.998			0.898				
750	Q	10	6	14.05	20.44	6.39	1.065	1.103	10.0	0.639	62.5	0.691	14.8	30.2
	R	10	6	13.92	19.72	5.80	0.967			0.580				
	S	10	6	14.88	22.19	7.31	1.218			0.731				
	T	10	7	13.84	21.96	8.12	1.160			0.812				
900	U	10	2	12.57	14.91	2.34	1.170	1.144	16.6	0.234	17.5	0.195	24.5	80.3
	V	10	1	12.92	14.27	1.35	1.350			0.135				
	W	10	2	11.85	14.18	2.33	1.165			0.233				
	X	10	2	12.58	14.36	1.78	0.890			0.178				
1050	Y	10	0	0.00	0.00	0.00	0.000	1.200	0.0	0.000	2.5	0.030	200.0	97.0
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	1	14.77	15.97	1.20	1.200			0.120				

Dunnett's MSD value: 0.1133 MSD = Minimum Significant Difference

PMSD: 11.5 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Santrock

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	7/7/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	7/14/2026	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	0.9000	1.0000	0.9000
750	0.6000	0.6000	0.6000	0.7000
900	0.2000	0.1000	0.2000	0.2000
1050	0.0000	0.0000	0.0000	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	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.6250	0.6250	0.9123	0.8861	0.9912	5.759	4	10.00	10.00	15	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

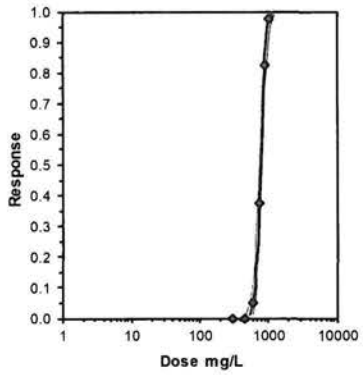
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.929	0.896	0.29906	0.6495
Equality of variance cannot be confirmed				

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

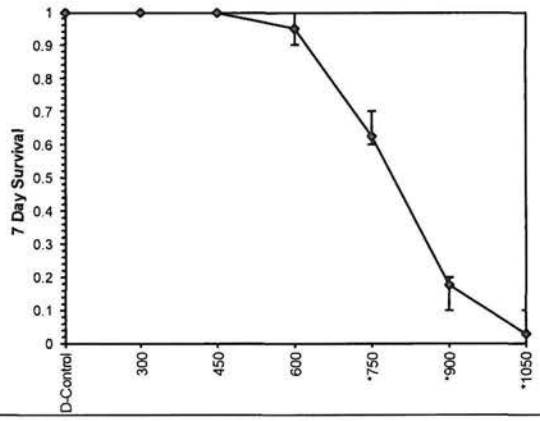
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.9888	1.87908	11.3058	18.6718	0	0.13439	9.48773	0.99784	2.89307	0.06672	3
Intercept	-38.364	5.45459	-49.055	-27.673							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	546.847	479.595	593.977
EC05	3.355	607.201	549.486	647.836
EC10	3.718	642.053	590.32	679.087
EC15	3.964	666.691	619.221	701.415
EC20	4.158	686.944	642.894	720.023
EC25	4.326	704.81	663.626	736.709
EC40	4.747	751.916	716.963	782.579
EC50	5.000	781.757	749.151	813.64
EC60	5.253	812.782	780.883	847.995
EC75	5.674	867.104	832.259	913.115
EC80	5.842	889.655	852.322	941.703
EC85	6.036	916.682	875.652	976.901
EC90	6.282	951.858	905.122	1023.96
EC95	6.645	1006.49	949.473	1099.26
EC99	7.326	1117.58	1036.3	1258.56



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:	7/7/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	7/14/2026	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	0.8830	1.0130	0.9850	1.0750
300	1.0630	1.2460	1.1360	1.1600
450	0.9450	0.9920	1.0410	1.0050
600	0.9920	0.8880	1.0480	0.8980
750	0.6390	0.5800	0.7310	0.8120
900	0.2340	0.1350	0.2330	0.1780
1050	0.0000	0.0000	0.0000	0.1200

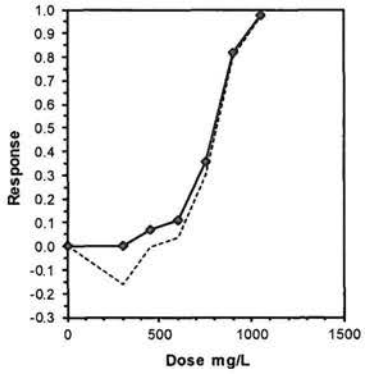
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.9890	1.0000	0.9890	0.8830	1.0750	8.094	4				1.0701	1.0000
300	1.1513	1.1641	1.1513	1.0630	1.2460	6.553	4	-3.279	2.290	0.1133	1.0701	1.0000
450	0.9958	1.0068	0.9958	0.9450	1.0410	3.985	4	-0.136	2.290	0.1133	0.9958	0.9305
600	0.9565	0.9671	0.9565	0.8880	1.0480	8.041	4	0.657	2.290	0.1133	0.9565	0.8938
750	0.6905	0.6982	0.6905	0.5800	0.8120	14.784	4				0.6905	0.6453
900	0.1950	0.1972	0.1950	0.1350	0.2340	24.512	4				0.1950	0.1822
1050	0.0300	0.0303	0.0300	0.0000	0.1200	200.000	4				0.0300	0.0280

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95705	0.844	-0.04	-0.8604
Bartlett's Test indicates equal variances (p = 0.71)	1.38788	11.3449		

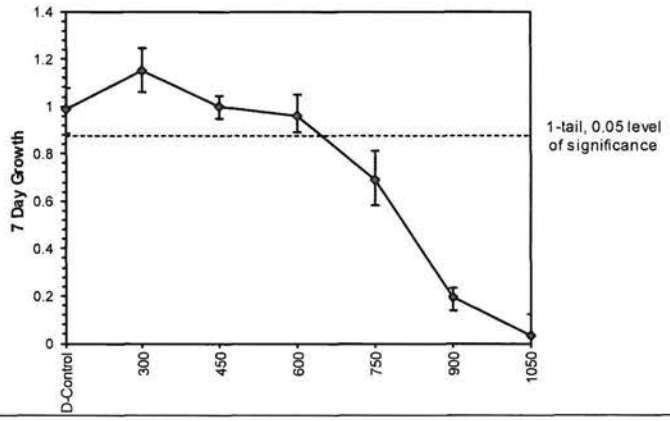
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.11332	0.11458	0.03036	0.0049	0.00869	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	407.91	59.09	340.94	716.20	1.5004
IC10	574.73	62.59	346.54	668.18	-0.4888
IC15	626.44	24.21	516.43	685.91	-1.0495
IC20	656.62	19.66	597.65	717.84	-0.0105
IC25	686.79	21.69	632.18	763.17	0.2065
IC40	764.66	15.08	709.74	798.83	-0.6600
IC50	797.05	11.19	759.67	825.13	-0.4288



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **143**

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.)	7.76	7.83	7.72	7.73	7.77	7.79
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.7	8.5	7.7
	Conductivity (µmhos/cm)	306		314		303	
	Alkalinity (mg $CaCO_3$/L)	60				61	
	Hardness (mg $CaCO_3$/L)	88				88	
	Temperature (°C)	24.8	25.1	24.9	24.8	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.91	7.82	7.90	7.74	7.90	7.78
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.7	8.5	7.7
	Conductivity (µmhos/cm)	837		847		830	
	Temperature (°C)	24.9	24.9	25.0	25.2	24.7	24.6
450 mg KCl/L	pH (S.U.)	7.92	7.81	7.92	7.74	7.91	7.79
	Dissolved oxygen (mg/L)	8.7	7.8	8.3	7.8	8.5	7.6
	Conductivity (µmhos/cm)	1100		1120		1080	
	Temperature (°C)	24.9	24.9	24.9	24.8	24.8	24.5
600 mg KCl/L	pH (S.U.)	7.92	7.82	7.92	7.74	7.91	7.79
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.8	8.5	7.6
	Conductivity (µmhos/cm)	1340		1400		1330	
	Temperature (°C)	25.0	25.3	24.9	24.8	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.93	7.83	7.93	7.73	7.92	7.78
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.8	8.5	7.4
	Conductivity (µmhos/cm)	1580		1620		1570	
	Temperature (°C)	25.0	25.0	24.9	24.8	24.9	24.7
900 mg KCl/L	pH (S.U.)	7.94	7.84	7.94	7.74	7.93	7.78
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.8	8.5	7.6
	Conductivity (µmhos/cm)	1840		1880		1830	
	Temperature (°C)	25.0	25.0	25.1	25.1	24.9	24.9
1050 mg KCl/L	pH (S.U.)	7.94	7.85	7.94	7.76	7.93	7.79
	Dissolved oxygen (mg/L)	8.6	7.9	8.3	7.9	8.5	7.6
	Conductivity (µmhos/cm)	2130		2160		2100	
	Temperature (°C)	25.0	25.0	25.1	24.8	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final

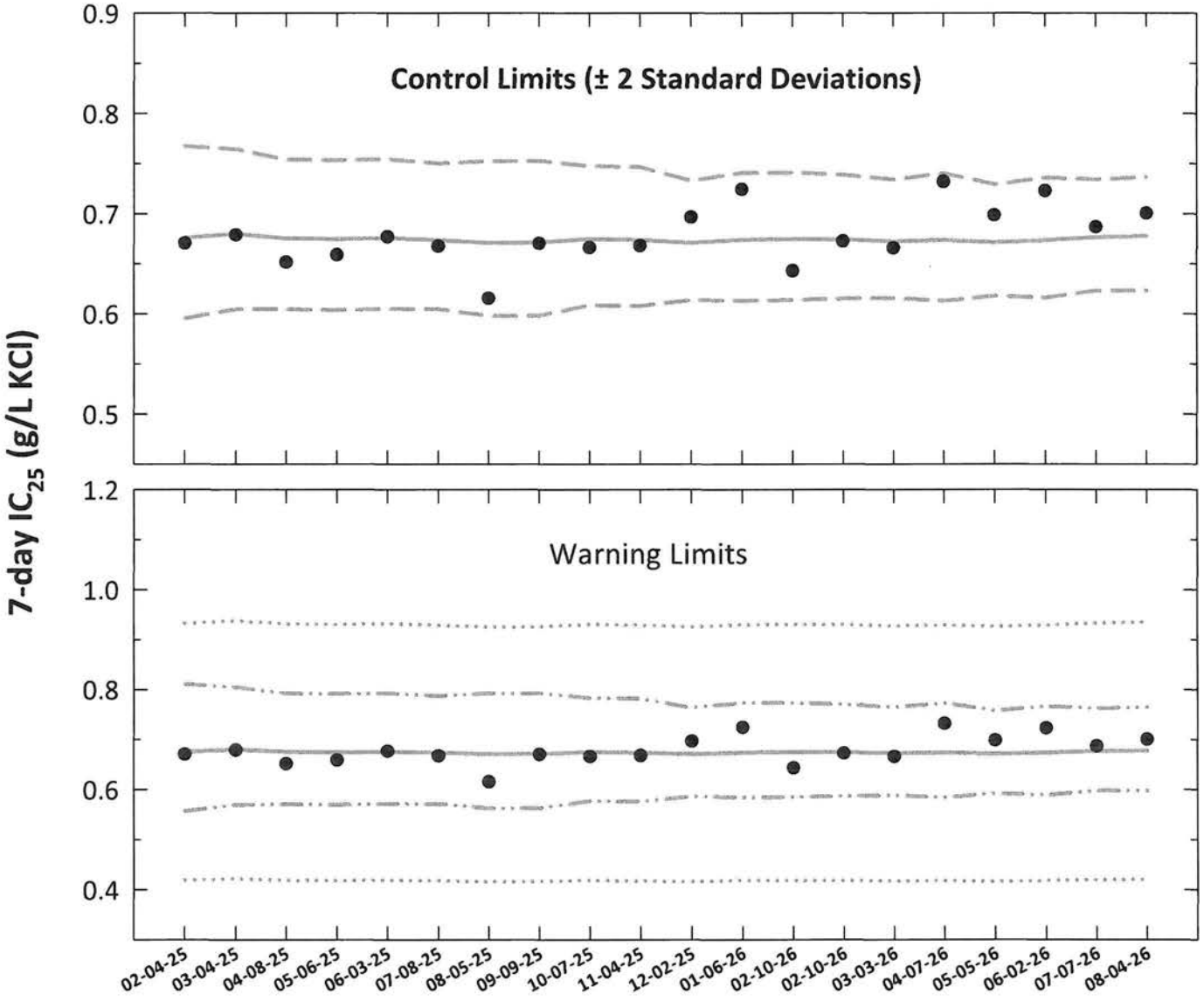
ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **143**

		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 JM	XL JM	JM
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.80	7.78	7.70	7.84	7.78	7.73	7.72	7.56
	Dissolved oxygen (mg/L)	8.3	8.2	8.6	7.7	8.5	7.6	8.3	5.9
	Conductivity (µmhos/cm)	309		298		298		310	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.7	25.2	24.6	25.1	24.7	24.7	24.6	24.9
300 mg KCl/L	pH (S.U.)	7.92	7.81	7.90	7.81	7.98	7.69	7.81	7.56
	Dissolved oxygen (mg/L)	8.2	8.2	8.6	7.7	8.5	7.6	8.3	6.3
	Conductivity (µmhos/cm)	846		816		811		850	
	Temperature (°C)	24.6	24.9	24.9	24.7	24.7	24.7	24.9	24.6
450 mg KCl/L	pH (S.U.)	7.93	7.83	7.92	7.81	7.99	7.69	7.82	7.56
	Dissolved oxygen (mg/L)	8.3	8.2	8.6	7.7	8.5	7.6	8.3	6.1
	Conductivity (µmhos/cm)	1110		1060		1060		1110	
	Temperature (°C)	24.6	24.9	24.9	24.7	24.6	24.9	24.9	24.6
600 mg KCl/L	pH (S.U.)	7.94	7.84	7.93	7.81	8.00	7.69	7.82	7.57
	Dissolved oxygen (mg/L)	8.3	8.1	8.6	7.7	8.5	7.7	8.3	6.2
	Conductivity (µmhos/cm)	1370		1310		1300		1360	
	Temperature (°C)	24.7	24.7	25.0	24.7	24.6	24.9	24.9	24.7
750 mg KCl/L	pH (S.U.)	7.94	7.84	7.94	7.80	8.01	7.71	7.83	7.57
	Dissolved oxygen (mg/L)	8.3	8.1	8.6	7.7	8.5	7.8	8.3	6.2
	Conductivity (µmhos/cm)	1630		1560		1540		1630	
	Temperature (°C)	24.7	24.7	25.0	24.9	24.7	25.0	24.6	24.7
900 mg KCl/L	pH (S.U.)	7.95	7.84	7.95	7.80	8.01	7.71	7.84	7.58
	Dissolved oxygen (mg/L)	8.3	8.1	8.6	7.7	8.5	7.6	8.3	6.2
	Conductivity (µmhos/cm)	1870		1800		1800		1900	
	Temperature (°C)	24.7	25.0	25.0	24.9	24.7	24.7	24.6	24.9
1050 mg KCl/L	pH (S.U.)	7.96	7.86	7.95	7.81	8.01	7.69	7.84	7.58
	Dissolved oxygen (mg/L)	8.3	8.0	8.6	7.5	8.5	7.5	8.3	6.1
	Conductivity (µmhos/cm)	2130		2070		2030		2170	
	Temperature (°C)	24.7	24.8	25.0	25.0	24.7	24.7	24.8	24.7
		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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	07-07-26	0.6868	-0.1632	-0.1698	0.0178	0.6765	0.6232	0.7343	0.5977	0.7620	0.4194	0.9335
20	08-04-26	0.7004	-0.1546	-0.1689	0.0181	0.6778	0.6235	0.7368	0.5977	0.7648	0.4202	0.9353

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.

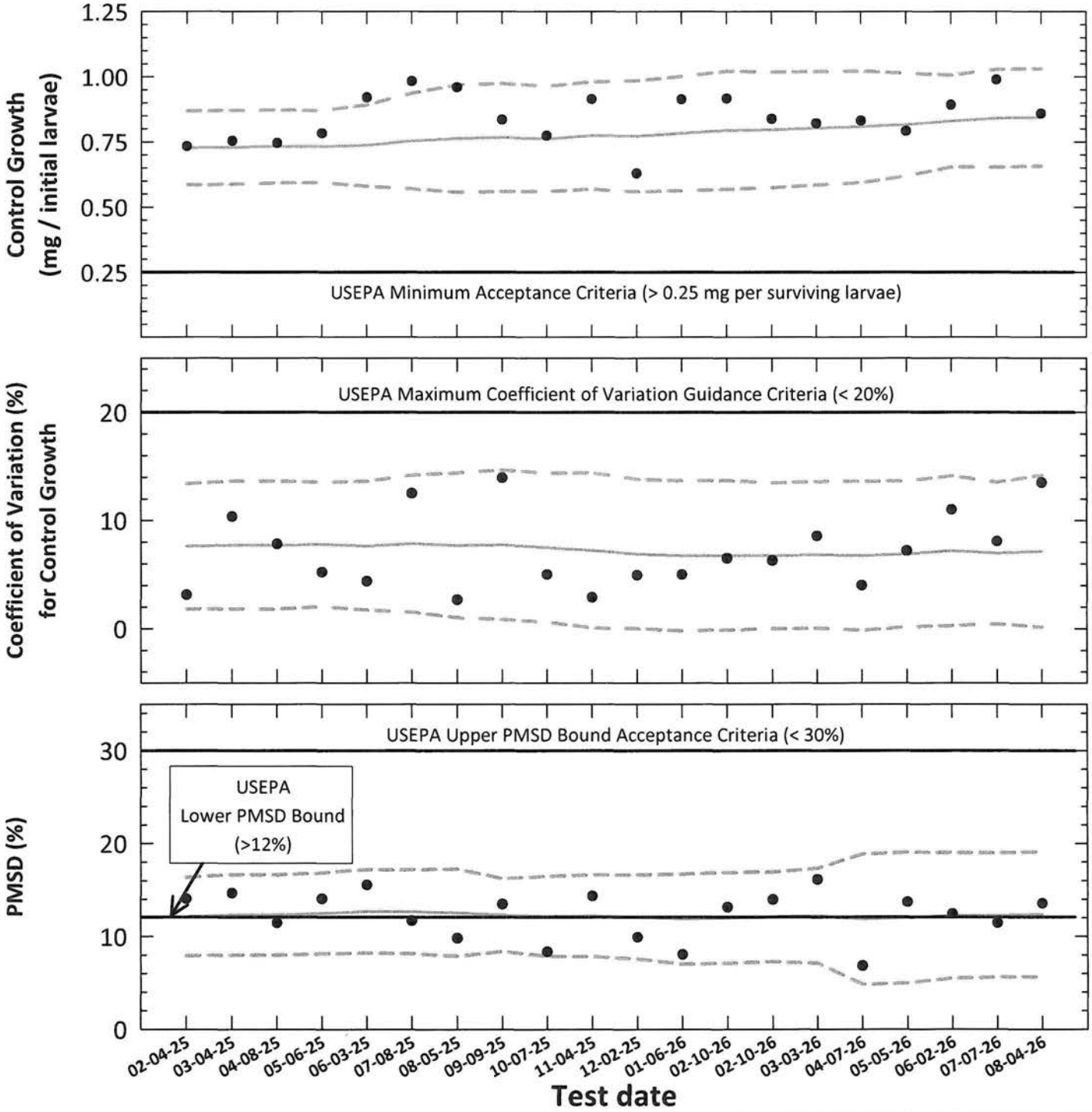
Entered and Reviewed by Jim Sumner

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Pimephales promelas

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Organism Source: In-house Culture



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

Central Tendency (mean Control Growth, CV or PMSD)

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

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ETS
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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	07-07-26	100	0.989	8.1	0.1133	11.5	0.841	0.653	1.029	7.0	0.4	13.6	12.3	5.6	19.0
20	08-04-26	100	0.858	13.5	0.1163	13.6	0.844	0.658	1.031	7.2	0.1	14.2	12.3	5.6	19.1

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: **144**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2486				
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:	
Organism source:	In-house culture
Age:	< 24-hours old
Spawn date:	08-03-26 1400 TO 08-04-26 0500
Hatch dates and times:	07-28-26
Transfer vessel information:	pH = 8.02 S.U. Temperature = 24.0 °C
Average transfer volume:	< 0.25 mL

Test information:	
Randomizing template:	YELLOW
Incubator number and shelf location:	7B
Artemia CHM number:	CHM1432
Drying information for weight determination:	
Date / Time in oven:	08-11-26 0612
*Initial oven temperature:	60°C
Date / Time out of oven:	08-12-26 0615
*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	08-04-26	0505	JK	1330	JK	0715	JK	07-30-26 A
1	08-05-26	0500	JK	1100	JK	0712	JK	↓
2	08-06-26	0500	JK	1130	JK	0710	JK	07-31-26 D
3	08-07-26	0500	JK	1100	JK	0700	JK	↓
4	08-08-26	0600	JK	1200	JK	0835	JK	08-03-26 A
5	08-09-26	0600	JK	1200	JK	0800	JK	↓
6	08-10-26	0500	JK	1100	JK	0745	JK	↓
7	08-11-26					0527	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	130664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	820.6
Average weight per initial larvae:	0.858		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.858	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	700.4

ETS
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: 144

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	ISM 10	10	10	10	10	10	10 IG	10	10	10	10	10
*A = Pan weight (mg) Tray color code: Ruby Analyst: JG 20 Date: 09-22-26	12.42	13.74	8.42	14.14	13.80	14.47	13.14	13.41	10.38	9.16	9.94	11.95
*B = Pan + Larvae weight (mg) Analyst: JL Date: 09.13.26	19.37	21.96	17.58	23.74	23.37	24.13	23.72	22.78	19.11	17.58	18.29	19.67
C = Larvae weight (mg) = B – A Analyst: JL	6.95	8.62	9.16	9.60	9.57	9.66	10.58	9.37	8.73	8.42	8.35	7.72
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.695	0.862	0.916	0.960	0.957	0.966	1.058	0.937	0.873	0.842	0.835	0.772
Average weight per initial number of larvae (mg)	0.858				0.980		-14.17		0.831		3.27	
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: **144**

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	6 4d	7 3d	6 4d	6 4d
2	10	10	10	10	10	9 1d	10	10	5 1d	6 1d	6	6
3	10	10	10	10	10	9	10	10	5	6	6	6
4	9 1d	10	10	10	10	9	10	10	5	5 1d	6	5 1d
5	9	10	10	10	10	9	10	10	4 1d	5	6	5
6	9	10	10	10	7 3d	9	8 2d	8 2d	4	4 1d	5 1d	5
7	9	10	10	10	7	6 3d	7 1d	7 1d	3 3d	2 3d	2 3d	3 3d
*A = Pan weight (mg) Tray color code: Ruby Analyst: JG Date: 07-22-26	10.36	8.60	13.13	10.42	13.36	9.54	7.85	8.64	8.74	14.43	9.52	10.13
*B = Pan + Larvae weight (mg) Analyst: JJ Date: 07.13.26	18.53	17.71	22.33	18.89	19.71	15.58	13.43	15.02	11.42	16.65	11.85	13.50
C = Larvae weight (mg) = B – A Analyst: JJ	8.17	9.11	9.20	8.47	6.35	6.04	5.58	6.38	2.68	2.22	2.33	3.37
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JJ	0.817	0.911	0.920	0.847	0.635	0.604	0.558	0.638	0.268	0.222	0.233	0.337
Average weight per initial number of larvae (mg)	0.874				0.609				0.265			
Percent reduction from control (%)			-1.8%				29.1%				69.1%	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **144**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	5 sd	5 sd	4 sd	5 sd
2	4 d	4 d	4	4 d
3	2 d	3 d	4	4
4	2	3	3 d	3 d
5	2	2 d	2 d	2 d
6	2	2	2	2
7	2	1 d	1 d	1 d
*A = Pan weight (mg) Tray color code: Ruby Analyst: JM Date: 07-22-26	13.66	10.83	13.40	13.32
*B = Pan + Larvae weight (mg) Analyst: JL Date: 08-13-26	16.07	12.01	14.27	14.39
C = Larvae weight (mg) = B – A Analyst: JL	2.41	1.18	0.87	1.07
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.241	0.118	0.087	0.107
Average weight per initial number of larvae (mg)	0.138		63.97	
Percent reduction from control (%)				

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

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

Comments:

ETS
Environmental Testing Solutions, Inc.

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

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

Test number: 144

Test dates: August 04-11, 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	12.42	19.37	6.95	0.695	0.858	13.5	0.695	100.0	0.858	13.5	Not applicable
	B	10	10	13.34	21.96	8.62	0.862			0.862				
	C	10	10	8.42	17.58	9.16	0.916			0.916				
	D	10	10	14.14	23.74	9.60	0.960			0.960				
300	E	10	10	13.80	23.37	9.57	0.957	0.980	5.5	0.957	100.0	0.980	5.5	-14.1
	F	10	10	14.47	24.13	9.66	0.966			0.966				
	G	10	10	13.14	23.72	10.58	1.058			1.058				
	H	10	10	13.41	22.78	9.37	0.937			0.937				
450	I	10	10	10.38	19.11	8.73	0.873	0.831	5.1	0.873	100.0	0.831	5.1	3.2
	J	10	10	9.16	17.58	8.42	0.842			0.842				
	K	10	10	9.94	18.29	8.35	0.835			0.835				
	L	10	10	11.95	19.67	7.72	0.772			0.772				
600	M	10	9	10.36	18.53	8.17	0.908	0.896	3.7	0.817	97.5	0.874	5.7	-1.8
	N	10	10	8.60	17.71	9.11	0.911			0.911				
	O	10	10	13.13	22.33	9.20	0.920			0.920				
	P	10	10	10.42	18.89	8.47	0.847			0.847				
750	Q	10	7	13.36	19.71	6.35	0.907	0.906	9.5	0.635	67.5	0.609	6.1	29.1
	R	10	6	9.54	15.58	6.04	1.007			0.604				
	S	10	7	7.85	13.43	5.58	0.797			0.558				
	T	10	7	8.64	15.02	6.38	0.911			0.638				
900	U	10	3	8.74	11.42	2.68	0.893	1.073	11.4	0.268	25.0	0.265	19.6	69.1
	V	10	2	14.43	16.65	2.22	1.110			0.222				
	W	10	2	9.52	11.85	2.33	1.165			0.233				
	X	10	3	10.13	13.50	3.37	1.123			0.337				
1050	Y	10	2	13.66	16.07	2.41	1.205	1.081	14.1	0.241	12.5	0.138	50.4	83.9
	Z	10	1	10.83	12.01	1.18	1.180			0.118				
	AA	10	1	13.40	14.27	0.87	0.870			0.087				
	BB	10	1	13.32	14.39	1.07	1.070			0.107				

Dunnett's MSD value: 0.1163 MSD = Minimum Significant Difference

PMSD: 13.6 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	8/4/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	8/11/2026	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	0.6000	0.7000	0.7000
900	0.3000	0.2000	0.2000	0.3000
1050	0.2000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40
*900	0.2500	0.2500	0.5216	0.4636	0.5796	12.838	4	10.00	10.00	30	40
*1050	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

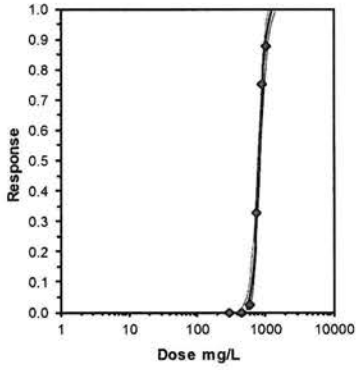
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93741	0.896	-0.4084	1.34457
Equality of variance cannot be confirmed				

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

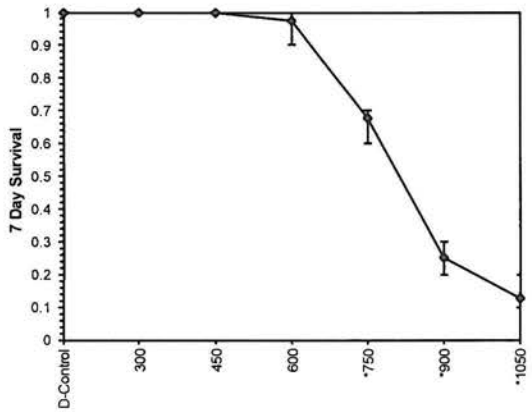
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.7182	1.62283	9.53747	15.8989	0	1.65923	9.48773	0.79811	2.91411	0.07863	3
Intercept	-32.062	4.73606	-41.345	-22.78							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	538.508	462.831	591.711
EC05	3.355	609.223	543.952	655.075
EC10	3.718	650.642	592.263	692.277
EC15	3.964	680.166	626.818	719.069
EC20	4.158	704.584	655.315	741.551
EC25	4.326	726.229	680.391	761.848
EC40	4.747	783.767	745.171	818.495
EC50	5.000	820.554	784.245	857.667
EC60	5.253	859.067	822.664	901.667
EC75	5.674	927.13	885.008	986.205
EC80	5.842	955.612	909.544	1023.59
EC85	6.036	989.918	938.261	1069.81
EC90	6.282	1034.84	974.832	1131.92
EC95	6.645	1105.19	1030.45	1232.15
EC99	7.326	1250.32	1141.06	1447.77



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:	8/4/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	8/11/2026	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	0.6950	0.8620	0.9160	0.9600
300	0.9570	0.9660	1.0580	0.9370
450	0.8730	0.8420	0.8350	0.7720
600	0.8170	0.9110	0.9200	0.8470
750	0.6350	0.6040	0.5580	0.6380
900	0.2680	0.2220	0.2330	0.3370
1050	0.2410	0.1180	0.0870	0.1070

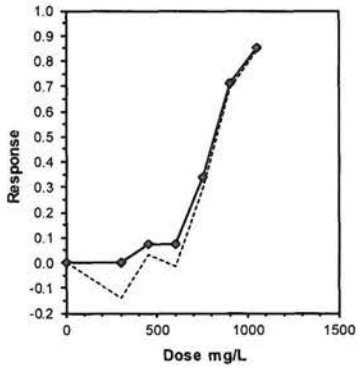
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8583	1.0000	0.8583	0.6950	0.9600	13.513	4				0.9189	1.0000
300	0.9795	1.1413	0.9795	0.9370	1.0580	5.484	4	-2.388	2.290	0.1163	0.9189	1.0000
450	0.8305	0.9677	0.8305	0.7720	0.8730	5.100	4	0.547	2.290	0.1163	0.8521	0.9274
600	0.8738	1.0181	0.8738	0.8170	0.9200	5.708	4	-0.305	2.290	0.1163	0.8521	0.9274
750	0.6088	0.7093	0.6088	0.5580	0.6380	6.104	4				0.6088	0.6625
900	0.2650	0.3088	0.2650	0.2220	0.3370	19.567	4				0.2650	0.2884
1050	0.1383	0.1611	0.1383	0.0870	0.2410	50.410	4				0.1383	0.1505

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95194	0.844	-0.83	1.57919
Bartlett's Test indicates equal variances (p = 0.30)	3.70116	11.3449		

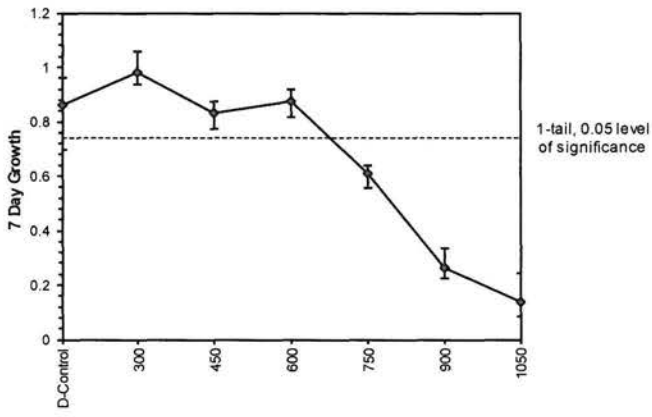
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.11626	0.13546	0.01699	0.00515	0.05787	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	403.24	93.66	327.60	754.35	1.1881
IC10	615.49	82.36	289.80	663.66	-1.1799
IC15	643.81	16.20	589.39	692.83	0.0597
IC20	672.13	15.15	624.39	722.11	0.1258
IC25	700.44	14.50	658.29	748.28	0.1910
IC40	775.06	9.00	744.25	802.31	-0.0116
IC50	815.15	8.14	786.94	841.82	0.1708



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **144**

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.)	7.76	7.81	7.71	7.76	7.74	7.84
	Dissolved oxygen (mg/L)	8.5	7.6	8.2	7.7	8.4	7.8
	Conductivity (µmhos/cm)	304		307		308	
	Alkalinity (mg $CaCO_3$/L)	63				63	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.8	25.1	24.7	24.9	24.8	25.1
300 mg KCl/L	pH (S.U.)	7.89	7.77	7.86	7.73	7.87	7.84
	Dissolved oxygen (mg/L)	8.5	7.6	8.2	7.7	8.3	7.8
	Conductivity (µmhos/cm)	846		860		844	
	Temperature (°C)	24.7	24.7	24.9	24.8	24.9	24.8
450 mg KCl/L	pH (S.U.)	7.90	7.77	7.88	7.72	7.88	7.84
	Dissolved oxygen (mg/L)	8.5	7.6	8.2	7.6	8.3	7.7
	Conductivity (µmhos/cm)	1100		1120		1100	
	Temperature (°C)	24.7	24.7	24.9	24.8	24.9	24.8
600 mg KCl/L	pH (S.U.)	7.91	7.76	7.90	7.72	7.89	7.83
	Dissolved oxygen (mg/L)	8.5	7.5	8.2	7.6	8.4	7.7
	Conductivity (µmhos/cm)	1350		1370		1370	
	Temperature (°C)	24.7	25.0	24.8	25.0	25.0	25.0
750 mg KCl/L	pH (S.U.)	7.92	7.75	7.91	7.71	7.90	7.87
	Dissolved oxygen (mg/L)	8.5	7.5	8.2	7.5	8.4	7.8
	Conductivity (µmhos/cm)	1580		1630		1620	
	Temperature (°C)	24.9	24.8	24.8	25.0	25.0	25.0
900 mg KCl/L	pH (S.U.)	7.93	7.76	7.91	7.72	7.90	7.88
	Dissolved oxygen (mg/L)	8.5	7.7	8.2	7.8	8.4	7.9
	Conductivity (µmhos/cm)	1860		1900		1870	
	Temperature (°C)	24.9	24.8	24.7	24.8	25.0	25.0
1050 mg KCl/L	pH (S.U.)	7.93	7.78	7.92	7.73	7.91	7.90
	Dissolved oxygen (mg/L)	8.5	7.8	8.2	7.8	8.4	7.8
	Conductivity (µmhos/cm)	2170		2200		2160	
	Temperature (°C)	24.9	24.8	24.8	24.9	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

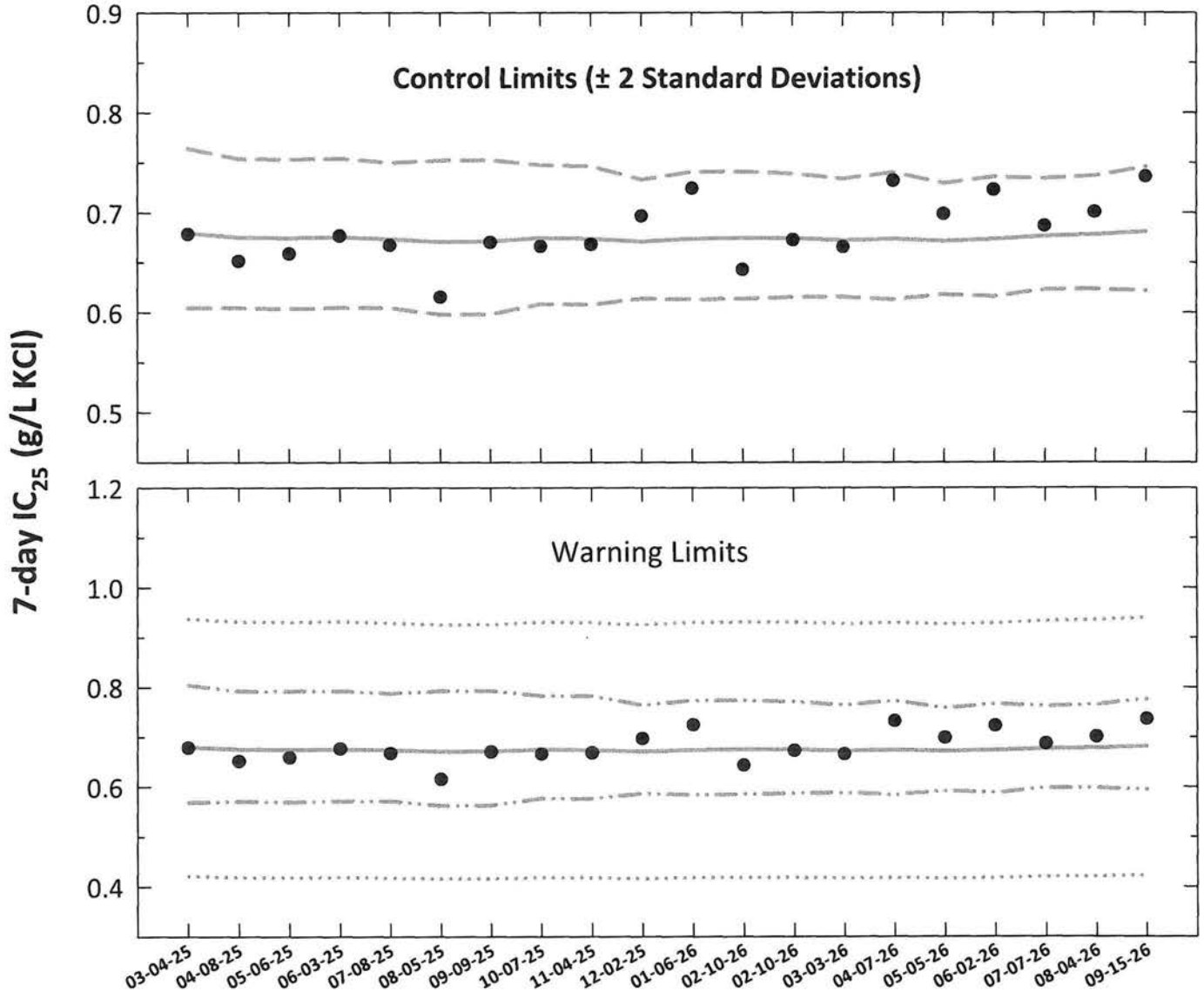
Species: *Pimephales promelas*

PpKClCR Test Number: **144**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	[illegible]	[illegible]	BSL	BSL	XL	XL	[illegible]
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.77	7.73	8.01	7.66	7.77	7.74	7.81	7.82
	Dissolved oxygen (mg/L)	8.3	7.2	8.2	7.3	8.5	7.6	8.3	6.2
	Conductivity (µmhos/cm)	307		307		296		308	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			86					
	Temperature (°C)	24.7	24.7	24.8	24.9	24.8	24.7	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.95	7.71	8.01	7.72	7.79	7.74	7.93	7.76
	Dissolved oxygen (mg/L)	8.2	7.4	8.3	7.3	8.5	7.7	8.3	5.6
	Conductivity (µmhos/cm)	884		917		815		860	
	Temperature (°C)	24.7	25.0	24.9	24.6	24.9	24.8	24.9	24.9
450 mg KCl/L	pH (S.U.)	7.97	7.71	8.01	7.75	7.90	7.74	7.94	7.77
	Dissolved oxygen (mg/L)	8.2	7.4	8.3	7.4	8.5	7.6	8.3	6.3
	Conductivity (µmhos/cm)	1140		1070		1070		1100	
	Temperature (°C)	24.9	24.7	24.8	24.6	24.9	25.0	24.9	24.7
600 mg KCl/L	pH (S.U.)	7.98	7.72	8.01	7.76	7.94	7.75	7.95	7.75
	Dissolved oxygen (mg/L)	8.2	7.5	8.4	7.4	8.5	7.6	8.3	6.1
	Conductivity (µmhos/cm)	1410		1330		1310		1340	
	Temperature (°C)	24.8	24.7	24.8	24.7	24.8	24.8	25.0	24.7
750 mg KCl/L	pH (S.U.)	7.99	7.73	8.01	7.77	7.95	7.74	7.96	7.74
	Dissolved oxygen (mg/L)	8.2	7.5	8.4	7.5	8.5	7.5	8.3	5.7
	Conductivity (µmhos/cm)	1690		1580		1540		1580	
	Temperature (°C)	24.9	24.7	24.8	24.7	24.8	24.8	25.0	24.6
900 mg KCl/L	pH (S.U.)	7.99	7.72	8.01	7.77	7.95	7.74	7.96	7.73
	Dissolved oxygen (mg/L)	8.2	7.5	8.4	7.5	1800 8.5	7.6	8.3	6.5
	Conductivity (µmhos/cm)	1990		1930		8.5 1800		1890	
	Temperature (°C)	24.9	24.9	24.9	24.7	24.8	25.1	24.9	24.8
1050 mg KCl/L	pH (S.U.)	8.00	7.72	8.01	7.89	7.97	7.76	7.97	7.73
	Dissolved oxygen (mg/L)	8.2	7.4	8.4	7.3	8.5	7.7	8.3	5.9
	Conductivity (µmhos/cm)	2250		2160		2030		2150	
	Temperature (°C)	24.9	24.8	24.9	24.9	24.9	24.9	24.9	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	07-07-26	0.6868	-0.1632	-0.1698	0.0178	0.6765	0.6232	0.7343	0.5977	0.7620	0.4194	0.9335
19	08-04-26	0.7004	-0.1546	-0.1689	0.0181	0.6778	0.6235	0.7368	0.5977	0.7648	0.4202	0.9353
20	09-15-26	0.7359	-0.1332	-0.1669	0.0198	0.6809	0.6217	0.7458	0.5940	0.7762	0.4222	0.9397

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.

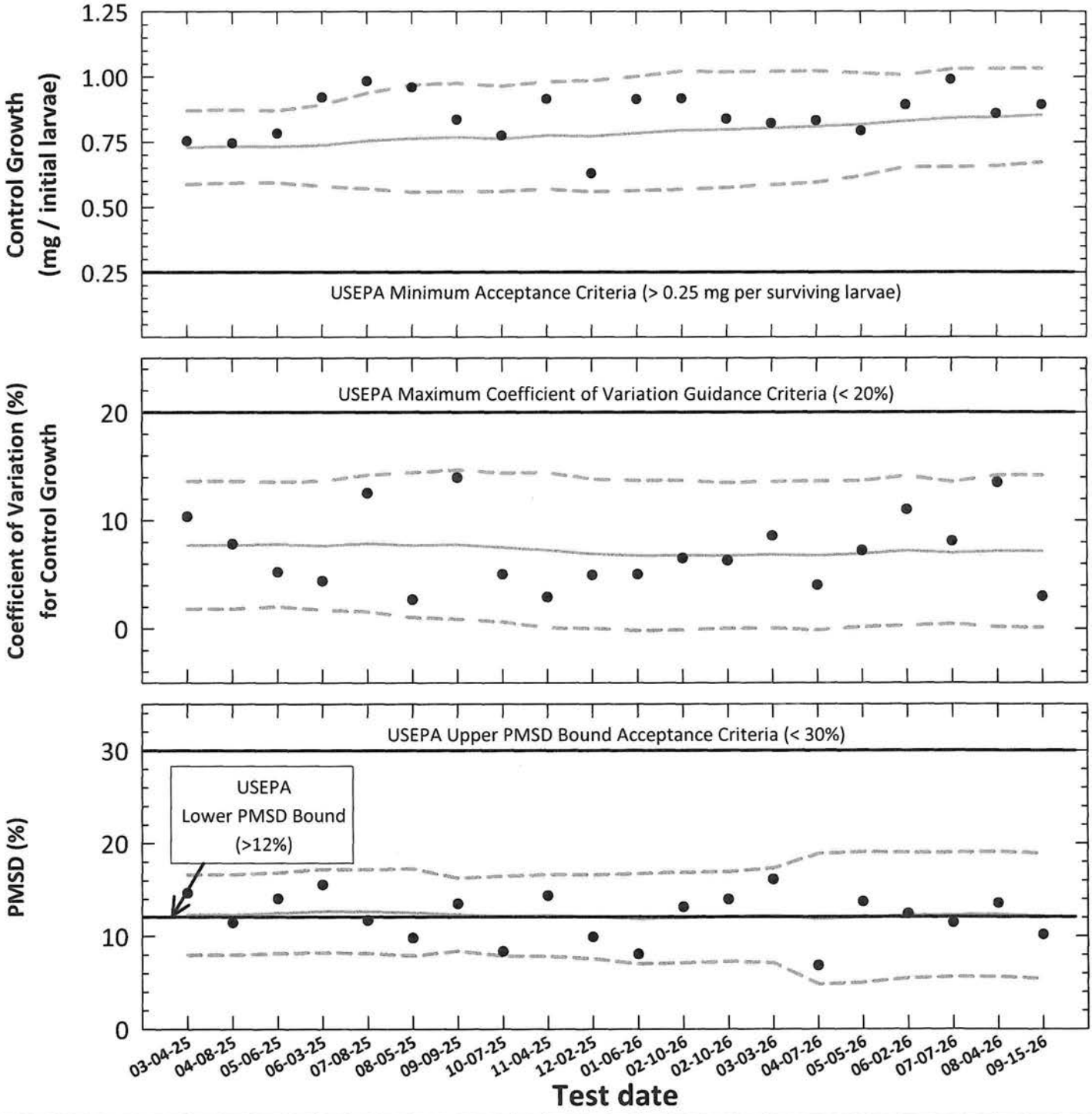
Entered and Reviewed by Jim Sumner

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	07-07-26	100	0.989	8.1	0.1133	11.5	0.841	0.653	1.029	7.0	0.4	13.6	12.3	5.6	19.0
19	08-04-26	100	0.858	13.5	0.1163	13.6	0.844	0.658	1.031	7.2	0.1	14.2	12.3	5.6	19.1
20	09-15-26	100	0.893	3.0	0.0908	10.2	0.852	0.672	1.032	7.2	0.1	14.2	12.2	5.4	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 Summer

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **145**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 7501				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	YELLOW
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	09-08-26	*Artemia* CHM number:	CHM1432
Hatch dates and times:	09-14-26 1435 TO 09-15-26 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.06 S.U. Temperature = 24.1 °C	Date / Time in oven:	09-23-26 0615
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	09-23-26 0615
		*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	09-15-26	0505	JH	1300	JH	0705	JH	09-09-26 A
1	09-16-26	0500	JH	1330	JH	0715	JH	↓
2	09-17-26	0500	JH	1200	JH	0730	JH	09-09-26 B
3	09-18-26	0500	JH	1100	JH	0715	JH	↓
4	09-19-26	0600	JH	1100	JH	0800	JH	09-14-26 A
5	09-20-26	0600	JH	1100	JH	0800	JH	↓
6	09-21-26	0500	JH	1100	JH	0745	JH	↓
7	~~09-21-26~~ 09-22-26					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	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	**7-day LC_{50}** (mg/L KCl)	836.9
Average weight per initial larvae:	0.893		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.893	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	735.9

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **145**

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: JP Date: 08-25-26	13.31	14.06	8.90	13.82	13.92	13.10	11.87	14.50	17.10 ~~14.05~~	14.47	10.52	13.94
*B = Pan + Larvae weight (mg) Analyst: JH Date: 09-24-26	22.31	22.66	17.77	23.06	24.26	23.48	22.50	24.93	21.52	22.76	19.80	21.15
C = Larvae weight (mg) = B – A Analyst: JH	9.00	8.60	8.87	9.24	10.34	10.38	10.63	10.43	8.42	8.29	9.28	8.21
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.900	0.860	0.887	0.924	1.034	1.038	1.063	1.043	0.842	0.829	0.928	0.821
Average weight per initial number of larvae (mg)	0.893				1.045		-17.07.		0.855		4.27.	
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: **145**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	7 2d	8 2d	8 2d	8 2d
2	10	10	10	10	10	10	10	10	6 1d	7 1d	7 1d	7 1d
3	10	10	10	10	10	9H	10	10	6	6 1d	7	7
4	10	9 1d	10	9 1d	10	9	9 1d	10	5 1d	5 1d	7	5 2d
5	10	9	10	9	9 1d	9	8 1d	10	5	5	5 2d	4 1d
6	10	9	10	9	8 1d	8 1d	8	9 1d	4 1d	4 1d	4 1d	4
7	10	9	10	9	7 1d	7 1d	8	8 1d	3 1d	4	4	3 1d
*A = Pan weight (mg) Tray color code: Grey Analyst: JP Date: 08-25-26	12.29	11.97	13.05	13.43	13.11	11.09	14.03	11.85	15.41	12.93	14.68	13 (12.99)
*B = Pan + Larvae weight (mg) Analyst: JH Date: 09.14.26	21.18	16.56	21.51	21.72	19.18	17.71	21.63	20.28	18.41	16.23	18.76	15.39
C = Larvae weight (mg) = B – A Analyst: JH	8.89	6.69	8.46	8.29	6.07	6.62	7.60	8.43	3.00	3.40	4.08	2.40
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.889	0.669	0.846	0.829	0.607	0.662	0.760	0.843	0.300	0.340	0.408	0.240
Average weight per initial number of larvae (mg)	0.808		9.5%		0.718		19.6%		0.322		63.9%	
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: **145**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	7 3d	7 3d	7 3d	7 3d
2	6 1d	6 1d	6 1d	6 1d
3	6	4 2d	4 2d	4 2d
4	3 3d	3 1d	2 2d	3 1d
5	2 1d	2 1d	2	2 1d
6	1 1d	2	1 1d	1 1d
7	1	1 1d	1	1
*A = Pan weight (mg) Tray color code: Grey Analyst: AP Date: 08-25-26	12.85	11.46	13.11	9.73
*B = Pan + Larvae weight (mg) Analyst: N Date: 09.04.26	13.69	12.18	14.16	9.67
C = Larvae weight (mg) = B – A Analyst: N	0.84	0.72	1.05	0.94
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: N	0.084	0.072	0.105	0.094
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.089		90.1%	

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

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

Comments:

ETS
Environmental Testing Solutions, Inc.

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

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

Test number: 145

Test dates: September 15-22, 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	13.31	22.31	9.00	0.900	0.893	3.0	0.900	100.0	0.893	3.0	Not applicable
	B	10	10	14.06	22.66	8.60	0.860			0.860				
	C	10	10	8.90	17.77	8.87	0.887			0.887				
	D	10	10	13.82	23.06	9.24	0.924			0.924				
300	E	10	10	13.92	24.26	10.34	1.034	1.045	1.2	1.034	100.0	1.045	1.2	-17.0
	F	10	10	13.10	23.48	10.38	1.038			1.038				
	G	10	10	11.87	22.50	10.63	1.063			1.063				
	H	10	10	14.50	24.93	10.43	1.043			1.043				
450	I	10	10	13.10	21.52	8.42	0.842	0.855	5.8	0.842	100.0	0.855	5.8	4.2
	J	10	10	14.47	22.76	8.29	0.829			0.829				
	K	10	10	10.52	19.80	9.28	0.928			0.928				
	L	10	10	12.94	21.15	8.21	0.821			0.821				
600	M	10	10	12.29	21.18	8.89	0.889	0.850	9.1	0.889	95.0	0.808	11.9	9.5
	N	10	9	11.87	18.56	6.69	0.743			0.669				
	O	10	10	13.05	21.51	8.46	0.846			0.846				
	P	10	9	13.43	21.72	8.29	0.921			0.829				
750	Q	10	7	13.11	19.18	6.07	0.867	0.954	8.0	0.607	75.0	0.718	14.6	19.6
	R	10	7	11.09	17.71	6.62	0.946			0.662				
	S	10	8	14.03	21.63	7.60	0.950			0.760				
	T	10	8	11.85	20.28	8.43	1.054			0.843				
900	U	10	3	15.41	18.41	3.00	1.000	0.918	11.9	0.300	35.0	0.322	21.9	63.9
	V	10	4	12.83	16.23	3.40	0.850			0.340				
	W	10	4	14.68	18.76	4.08	1.020			0.408				
	X	10	3	12.99	15.39	2.40	0.800			0.240				
1050	Y	10	1	12.85	13.69	0.84	0.840	0.888	15.9	0.084	10.0	0.089	15.9	90.1
	Z	10	1	11.46	12.18	0.72	0.720			0.072				
	AA	10	1	13.11	14.16	1.05	1.050			0.105				
	BB	10	1	8.73	9.67	0.94	0.940			0.094				

Dunnett's MSD value: 0.0908 MSD = Minimum Significant Difference

PMSD: 10.2 PMSD = Percent Minimum Significant Difference

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

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Summer

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	9/15/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	9/22/2026	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	0.9000	1.0000	0.9000
750	0.7000	0.7000	0.8000	0.8000
900	0.3000	0.4000	0.4000	0.3000
1050	0.1000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	40
*900	0.3500	0.3500	0.6322	0.5796	0.6847	9.597	4	10.00	10.00	26	40
*1050	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	36	40

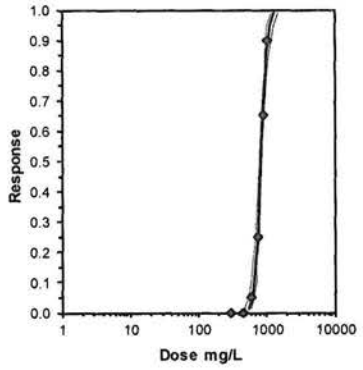
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.86126	0.896	2.1E-16	-0.1027
Equality of variance cannot be confirmed				

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

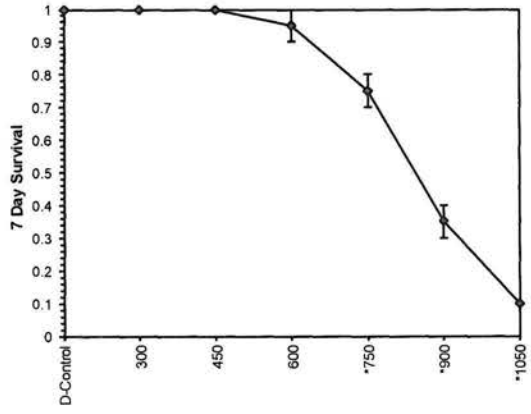
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.4128	1.61636	9.24472	15.5809	0	0.42265	9.48773	0.98058	2.92265	0.08056	3
Intercept	-31.278	4.72596	-40.541	-22.015							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	543.548	464.802	598.495
EC05	3.355	616.795	549.072	663.981
EC10	3.718	659.795	599.434	702.52
EC15	3.964	690.488	635.514	730.334
EC20	4.158	715.897	665.29	753.723
EC25	4.326	738.44	691.492	774.892
EC40	4.747	798.442	759.067	834.319
EC50	5.000	836.861	799.65	875.744
EC60	5.253	877.129	839.415	922.497
EC75	5.674	948.4	903.871	1012.56
EC80	5.842	978.264	929.278	1052.41
EC85	6.036	1014.26	959.057	1101.7
EC90	6.282	1061.45	997.044	1167.99
EC95	6.645	1135.45	1054.93	1275.1
EC99	7.326	1288.45	1170.38	1506.26



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:	9/15/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	9/22/2026	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	0.9000	0.8600	0.8870	0.9240
300	1.0340	1.0380	1.0630	1.0430
450	0.8420	0.8290	0.9280	0.8210
600	0.8890	0.6690	0.8460	0.8290
750	0.6070	0.6620	0.7600	0.8430
900	0.3000	0.3400	0.4080	0.2400
1050	0.0840	0.0720	0.1050	0.0940

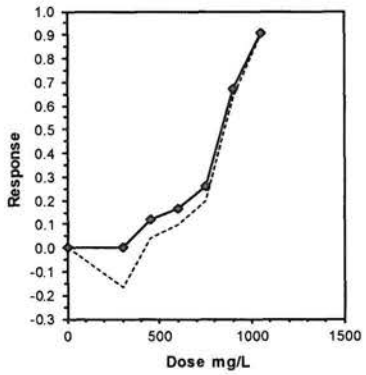
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8928	1.0000	0.8928	0.8600	0.9240	2.988	4				0.9686	1.0000
300	1.0445	1.1700	1.0445	1.0340	1.0630	1.232	4	-3.827	2.290	0.0908	0.9686	1.0000
450	0.8550	0.9577	0.8550	0.8210	0.9280	5.781	4	0.952	2.290	0.0908	0.8550	0.8827
600	0.8083	0.9053	0.8083	0.6690	0.8890	11.903	4	2.131	2.290	0.0908	0.8083	0.8344
750	0.7180	0.8043	0.7180	0.6070	0.8430	14.573	4				0.7180	0.7413
900	0.3220	0.3607	0.3220	0.2400	0.4080	21.907	4				0.3220	0.3324
1050	0.0888	0.0994	0.0888	0.0720	0.1050	15.865	4				0.0888	0.0916

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89741	0.844	-1.0754	3.4284
Bartlett's Test indicates equal variances (p = 0.02)	9.48684	11.3449		

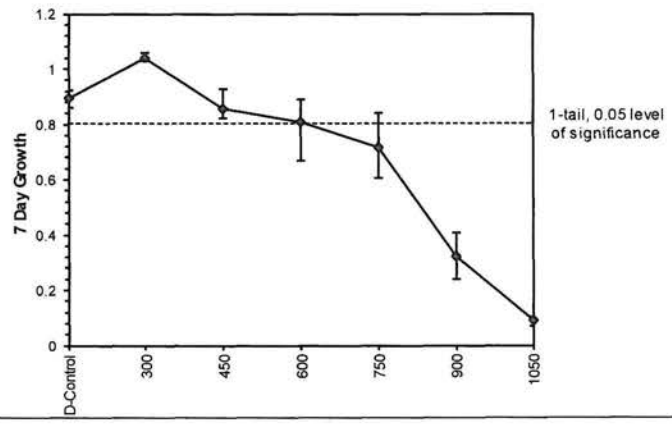
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.0908	0.1017	0.04183	0.00314	4.0E-04	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	363.94	16.56	339.88	441.15	1.8283
IC10	427.87	39.88	379.77	620.48	2.1830
IC15	551.61	68.59	383.77	748.56	0.0921
IC20	655.43	62.01	463.61	821.04	-0.1267
IC25	735.92	45.75	513.02	796.73	-1.2735
IC40	801.83	11.95	762.07	837.78	-0.1983
IC50	838.52	9.80	808.29	869.57	0.1797



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **145**

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	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.01	7.83	7.71	7.79	7.74	7.77
	Dissolved oxygen (mg/L)	8.3	8.0	8.4	7.9	8.4	7.8
	Conductivity (µmhos/cm)	292		311		312	
	Alkalinity (mg $CaCO_3$/L)	61				63	
	Hardness (mg $CaCO_3$/L)	80				84	
	Temperature (°C)	24.9	25.1	24.6	25.0	24.7	25.1
300 mg KCl/L	pH (S.U.)	8.02	7.82	7.86	7.74	7.87	7.74
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	7.7	8.4	7.7
	Conductivity (µmhos/cm)	797		847		844	
	Temperature (°C)	25.0	24.9	24.6	24.7	24.7	25.2
450 mg KCl/L	pH (S.U.)	8.02	7.80	7.87	7.73	7.88	7.73
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	7.6	8.4	7.6
	Conductivity (µmhos/cm)	1030		1090		1080	
	Temperature (°C)	25.0	24.9	24.5	24.7	24.7	25.0
600 mg KCl/L	pH (S.U.)	8.02	7.80	7.87	7.73	7.88	7.72
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	7.7	8.4	7.5
	Conductivity (µmhos/cm)	1270		1350		1350	
	Temperature (°C)	25.0	25.0	24.5	24.6	24.8	25.0
750 mg KCl/L	pH (S.U.)	8.02	7.81	7.88	7.72	7.89	7.71
	Dissolved oxygen (mg/L)	8.3	7.9	8.3	7.6	8.4	7.5
	Conductivity (µmhos/cm)	1520		1620		1610	
	Temperature (°C)	25.0	25.2	24.5	24.9	24.8	25.2
900 mg KCl/L	pH (S.U.)	8.02	7.80	7.89	7.72	7.90	7.71
	Dissolved oxygen (mg/L)	8.3	7.9	8.3	7.8	8.4	7.4
	Conductivity (µmhos/cm)	1770		1890		1860	
	Temperature (°C)	24.9	25.2	24.7	24.9	24.8	25.2
1050 mg KCl/L	pH (S.U.)	8.03	7.80	7.89	7.73	7.90	7.71
	Dissolved oxygen (mg/L)	8.3	7.9	8.3	7.8	8.4	7.6
	Conductivity (µmhos/cm)	2020		2150		2110	
	Temperature (°C)	24.9	25.0	24.7	24.7	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **145**

		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	AL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.76	7.69	7.73	7.69	7.78	7.79	7.82	7.41
	Dissolved oxygen (mg/L)	8.3	7.4	8.4	7.5	8.3	7.6	8.2	5.4
	Conductivity (µmhos/cm)	309		306		302		306	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.7	24.6	24.7	25.0	24.8	25.2	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.88	7.70	7.71	7.70	7.81	7.75	7.94	7.40
	Dissolved oxygen (mg/L)	8.3	7.5	8.4	7.4	8.5	7.6	8.2	5.7
	Conductivity (µmhos/cm)	839		839		865		835	
	Temperature (°C)	24.6	24.9	24.8	25.1	24.9	24.9	24.9	24.9
450 mg KCl/L	pH (S.U.)	7.88	7.70	7.81	7.70	7.87	7.74	7.95	7.41
	Dissolved oxygen (mg/L)	8.3	7.6	8.4	7.3	8.5	7.5	8.2	5.7
	Conductivity (µmhos/cm)	1090		1090		1070		1090	
	Temperature (°C)	24.6	24.9	24.8	24.7	24.9	25.1	24.8	24.9
600 mg KCl/L	pH (S.U.)	7.89	7.71	7.86	7.71	7.88	7.73	7.95	7.41
	Dissolved oxygen (mg/L)	8.4	7.6	8.4	7.3	8.4	7.3	8.2	5.5
	Conductivity (µmhos/cm)	1340		1360		1310		1340	
	Temperature (°C)	24.8	25.0	24.7	24.9	25.0	24.9	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.90	7.73	7.87	7.72	7.92	7.72	7.96	7.44
	Dissolved oxygen (mg/L)	8.4	7.4	8.4	7.2	8.4	7.3	8.2	6.9
	Conductivity (µmhos/cm)	1580		1610		1560		1600	
	Temperature (°C)	24.8	24.8	24.7	24.9	25.0	24.9	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.90	7.73	7.88	7.74	7.94	7.75	7.96	7.44
	Dissolved oxygen (mg/L)	8.4	7.4	8.5	7.7	8.4	7.7	8.3	5.8
	Conductivity (µmhos/cm)	1850		1920		1810		1850	
	Temperature (°C)	24.8	24.8	24.7	25.0	25.0	25.0	24.9	24.6
1050 mg KCl/L	pH (S.U.)	7.91	7.78	7.90	7.74	7.96	7.76	7.97	7.46
	Dissolved oxygen (mg/L)	8.4	7.6	8.4	7.4	8.4	7.6	8.3	6.4
	Conductivity (µmhos/cm)	2130		2120		2070		2130	
	Temperature (°C)	24.7	24.6	24.9	25.0	25.0	24.9	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final