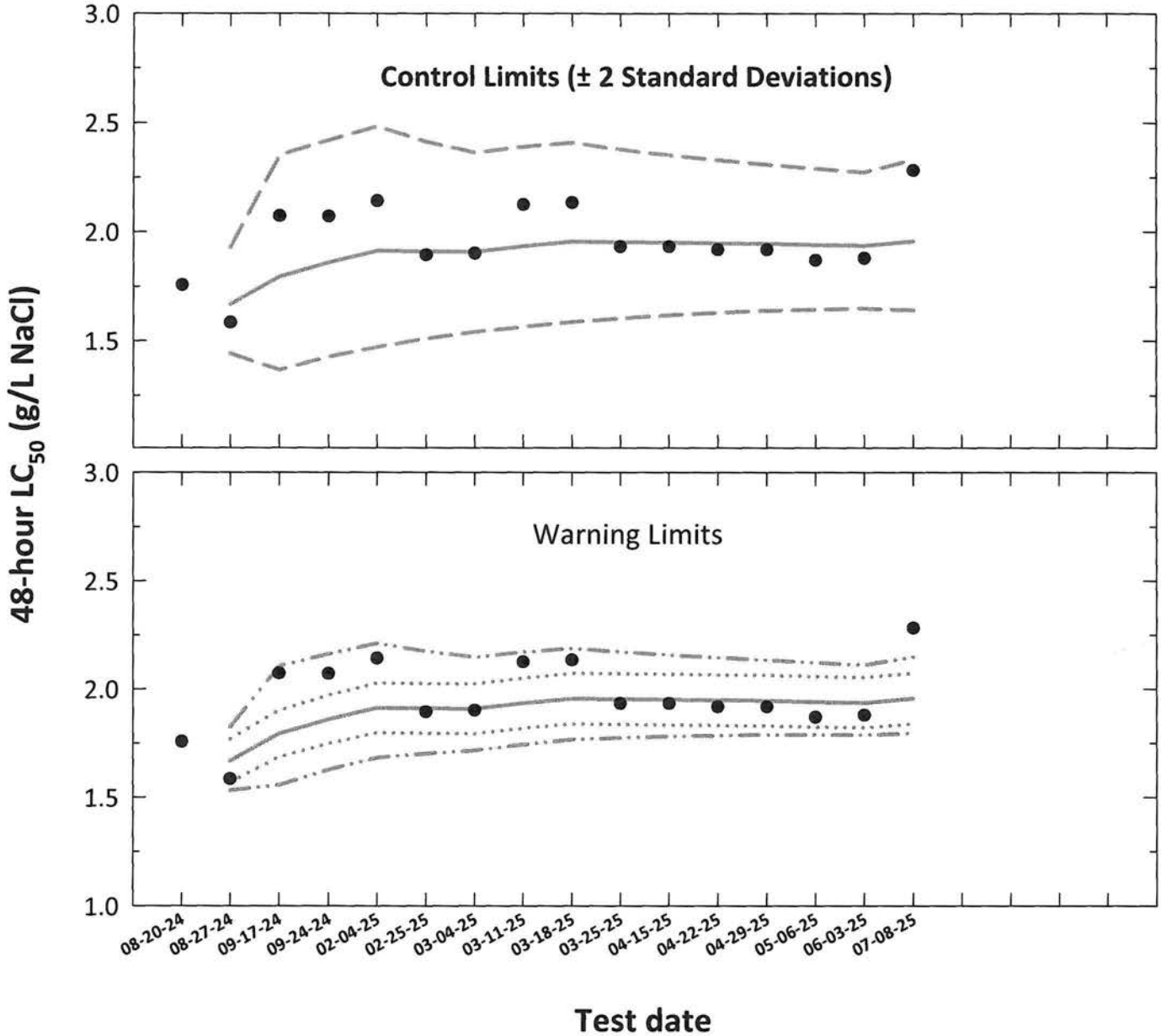
Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-20-24	1.7569	0.2447									
2	08-27-24	1.5847	0.2000	0.2224	0.0317	1.6686	1.4422	1.9306	1.5329	1.8256	1.5685	1.7687
3	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
4	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
5	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
6	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
7	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
8	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
9	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
10	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
11	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
12	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
13	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
14	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
15	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
16	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
17												
18												
19												
20												

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

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

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

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

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

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

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 # 18

Dilution Preparation:

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

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

Stock solution INSS #: 2393

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.76	7.79	7.91
	Dissolved oxygen (mg/L)	8.5	8.3	8.1
	Conductivity (µmhos/cm)	~~2507~~ 298		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.5	24.9	25.2
1000 mg/L	pH (S.U.)	7.86	7.87	7.90
	Dissolved oxygen (mg/L)	8.6	8.3	8.1
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	24.9	25.1	24.7
1500 mg/L	pH (S.U.)	7.87	7.87	7.91
	Dissolved oxygen (mg/L)	8.7	8.2	8.1
	Conductivity (µmhos/cm)	2750		
	Temperature (°C)	24.4	24.8	24.7
2000 mg/L	pH (S.U.)	7.87	7.88	7.90
	Dissolved oxygen (mg/L)	8.7	8.3	8.1
	Conductivity (µmhos/cm)	3560		
	Temperature (°C)	24.6	24.7	24.9
2500 mg/L	pH (S.U.)	7.87	7.87	7.90
	Dissolved oxygen (mg/L)	8.7	8.3	8.1
	Conductivity (µmhos/cm)	4310		
	Temperature (°C)	24.6	24.9	25.0
3000 mg/L	pH (S.U.)	7.86	7.86	
	Dissolved oxygen (mg/L)	8.7	8.3	
	Conductivity (µmhos/cm)	5140		
	Temperature (°C)	24.6	24.7	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	1306642

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 # 18

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-08-25	*0600	JL	0930	JP	2B3	BIACK	07-21-25B
24	07-09-25			0921	JP			
48 Termination	07-10-25			0927	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):	07-01-25 D
Age:	< 24-hours old
Date and time organisms were born between:	07-07-25 1350 TO 07-08-25 0600
Average transfer volume:	< 0.25 mL
Transfer bowl Information:	pH (S.U.): 7.82
	Temperature (°C): 24.9°C

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

Hours	Control				1000 mg/L				1500 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	1 d	3 d	3 d	0 d	0 d	0 d	0 d	0 d
48 Termination	5	5	5	5	0 d	1 u	1 u	0	0	0	0	0
Mean Survival	100%				10%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	2220.6
Upper 95% confidence limit (mg NaCl/L)	2344.8
48-hour LC_{50} (mg NaCl/L)	2281.9

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	7/8/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/10/2025	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	0.2000	0.6000	0.6000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

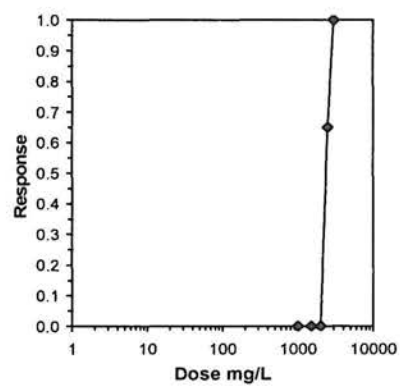
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2500	0.3500	0.3500	0.6153	0.2255	0.8861	53.207	4	10.00	10.00	13	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)	0.62512	0.868	-0.6117	5.12774
Equality of variance cannot be confirmed				

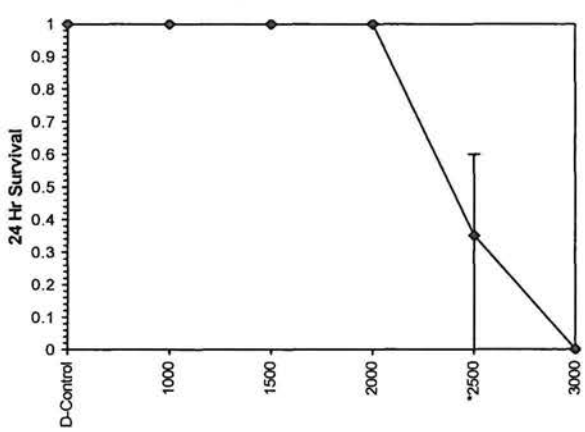
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	2400.5	2298.9	2506.58
5.0%	2395.7	2283.96	2512.91
10.0%	2391.05	2268.14	2520.63
20.0%	2382.44	2231.96	2543.07
Auto-0.0%	2400.5	2298.9	2506.58



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	7/8/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/10/2025	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	0.0000	0.2000	0.2000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

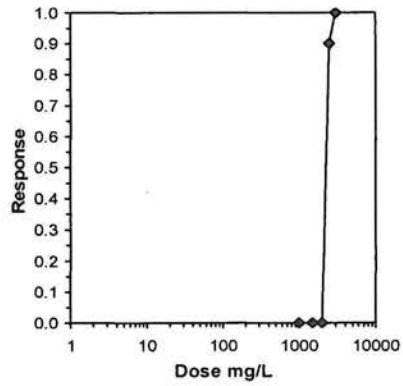
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	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)	0.63123	0.868	-8E-16	2.98039
Equality of variance cannot be confirmed				

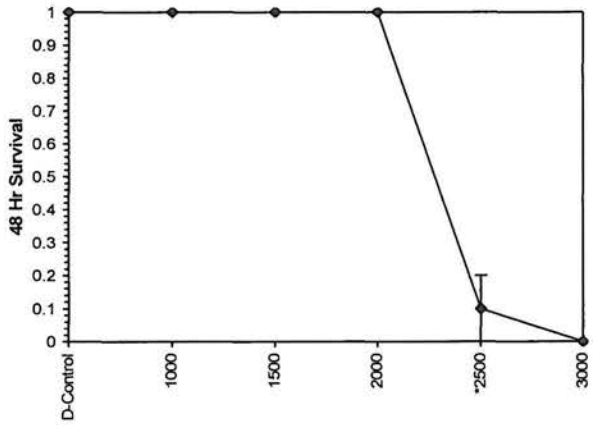
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Karber

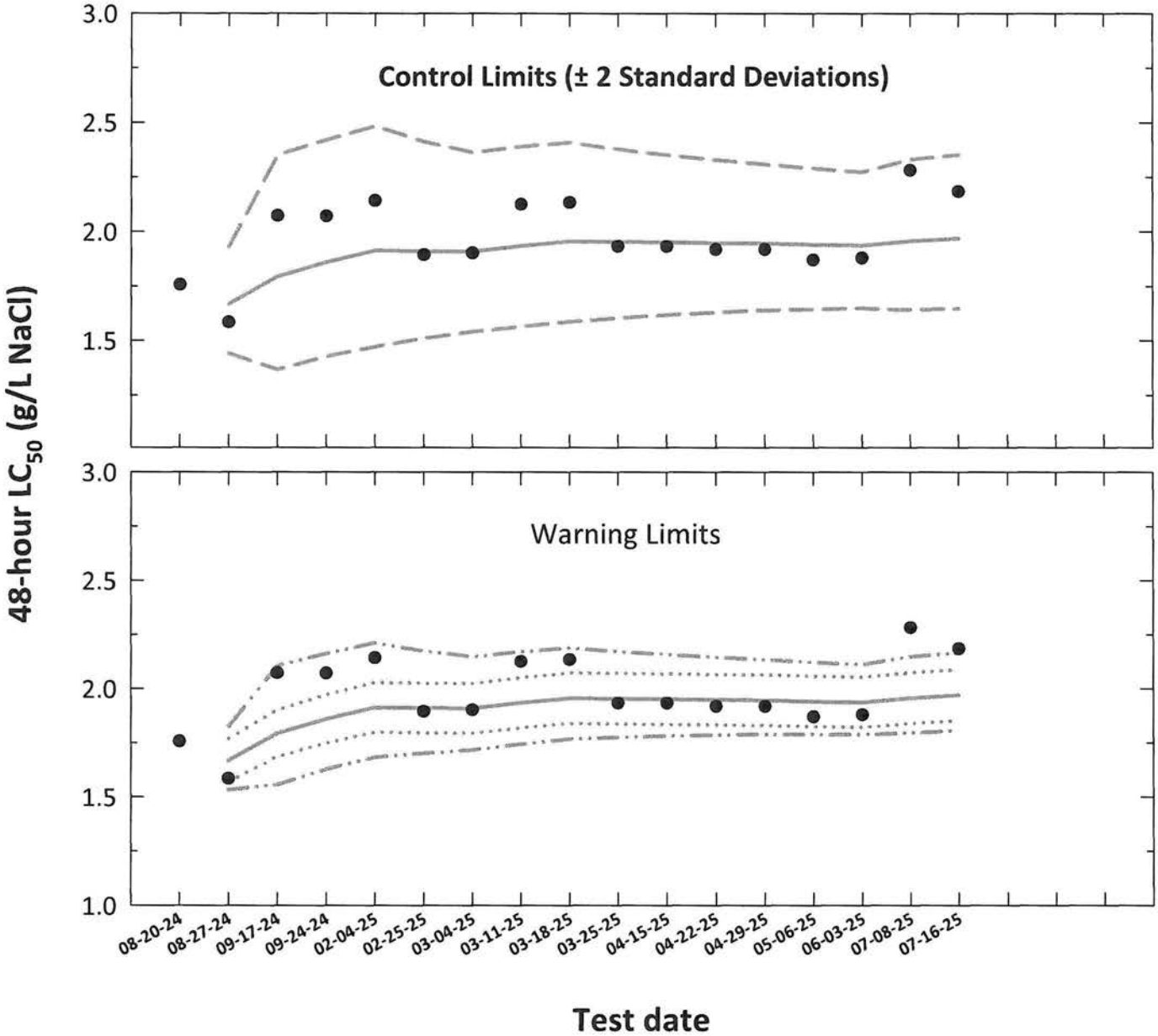
Trim Level	EC50	95% CL	
0.0%	2281.86	2220.63	2344.78
5.0%	2268.92	2208.75	2330.72
10.0%	2263.96	2222.51	2306.19
20.0%	2263.96	2222.51	2306.19
Auto-0.0%	2281.86	2220.63	2344.78



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-20-24	1.7569	0.2447									
2	08-27-24	1.5847	0.2000	0.2224	0.0317	1.6686	1.4422	1.9306	1.5329	1.8256	1.5685	1.7687
3	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
4	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
5	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
6	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
7	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
8	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
9	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
10	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
11	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
12	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
13	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
14	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
15	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
16	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
17	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
18												
19												
20												

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

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

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

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

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

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

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 # 19

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

Stock solution INSS #: 2393

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.80	7.79	7.93
	Dissolved oxygen (mg/L)	8.2	8.5	8.2
	Conductivity (µmhos/cm)	298		
	Alkalinity (mg/L $CaCO_3$)	64		
	Hardness (mg/L $CaCO_3$)	82		
	Temperature (°C)	24.7	25.2	25.2
1000 mg/L	pH (S.U.)	7.86	7.88	7.92
	Dissolved oxygen (mg/L)	8.2	8.6	8.2
	Conductivity (µmhos/cm)	2070		
	Temperature (°C)	24.9	25.2	25.0
1500 mg/L	pH (S.U.)	7.87	7.89	7.93
	Dissolved oxygen (mg/L)	8.2	8.6	8.2
	Conductivity (µmhos/cm)	2820		
	Temperature (°C)	24.6	25.0	25.0
2000 mg/L	pH (S.U.)	7.87	7.89	7.94
	Dissolved oxygen (mg/L)	8.2	8.6	8.2
	Conductivity (µmhos/cm)	3670		
	Temperature (°C)	24.6	25.0	25.0
2500 mg/L	pH (S.U.)	7.87	7.88	7.93
	Dissolved oxygen (mg/L)	8.2	8.6	8.3
	Conductivity (µmhos/cm)	4530		
	Temperature (°C)	24.5	24.8	24.9
3000 mg/L	pH (S.U.)	7.87	7.87	7.92
	Dissolved oxygen (mg/L)	8.2	8.5	8.3
	Conductivity (µmhos/cm)	5450		
	Temperature (°C)	24.8	25.0	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numbe
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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 applicabl
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306646

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 # 19

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-16-25	* 0400	H	0645	H	2B1	RED	07.02.25A
24	07-17-25			0650	H			
48 Termination	07-18-25			0645	H			

*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):	07-08-25 D
Age:	< 24-hours old
Date and time organisms were born between:	07-15-25 1415 TO 07-16-25 0400
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.90
	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	5	5	5	5	5	5	5	5	5	5	5	5
48 Termination	5	5	5	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	1 4d	3 3d	2 3d	1 4d	0 5d	0 5d	0 5d	0 5d
48 Termination	4 1d	4 1d	4 1d	4 1d	1	0 3d	0 3d	1	0	0	0	0
Mean Survival	80%				10%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2064.5
Upper 95% confidence limit (mg NaCl/L)	2304.4
48-hour LC_{50} (mg NaCl/L)	2184.2

Comments:

Test Reviewed by: H

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	7/16/2025	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	7/18/2025	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	0.2000	0.6000	0.4000	0.2000
3000	0.0000	0.0000	0.0000	0.0000

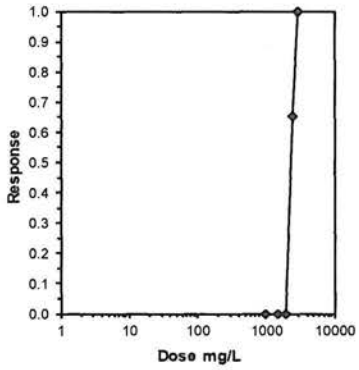
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2500	0.3500	0.3500	0.6245	0.4636	0.8861	32.527	4	10.00	10.00	13	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)	0.59309	0.868	1.08791	6.72643
Equality of variance cannot be confirmed				

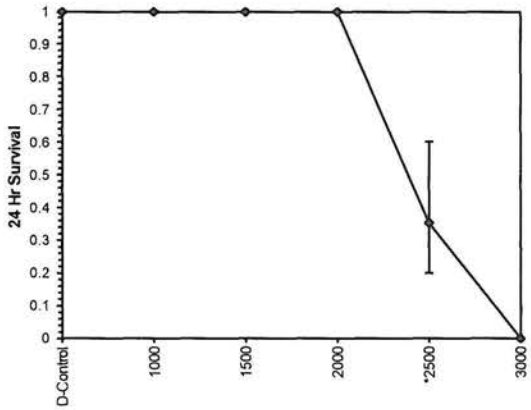
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	2400.5	2298.9	2506.58
5.0%	2395.7	2283.96	2512.91
10.0%	2391.05	2268.14	2520.63
20.0%	2382.44	2231.96	2543.07
Auto-0.0%	2400.5	2298.9	2506.58



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	7/16/2025	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	7/18/2025	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	0.8000	0.8000
2500	0.2000	0.0000	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	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	4	10.00	10.00	4	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

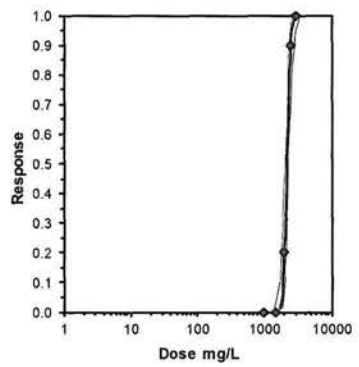
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.63123	0.868	-8E-16	2.98039
Equality of variance cannot be confirmed				

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

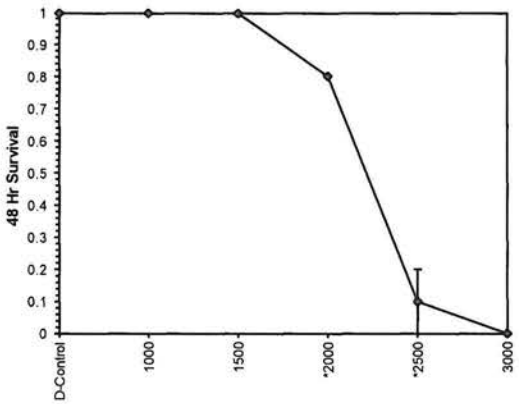
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	22.223	4.82452	12.7669	31.679	0	0.02881	7.81472	0.99871	3.33929	0.045	3
Intercept	-69.209	16.1258	-100.82	-37.602							
TSCR											

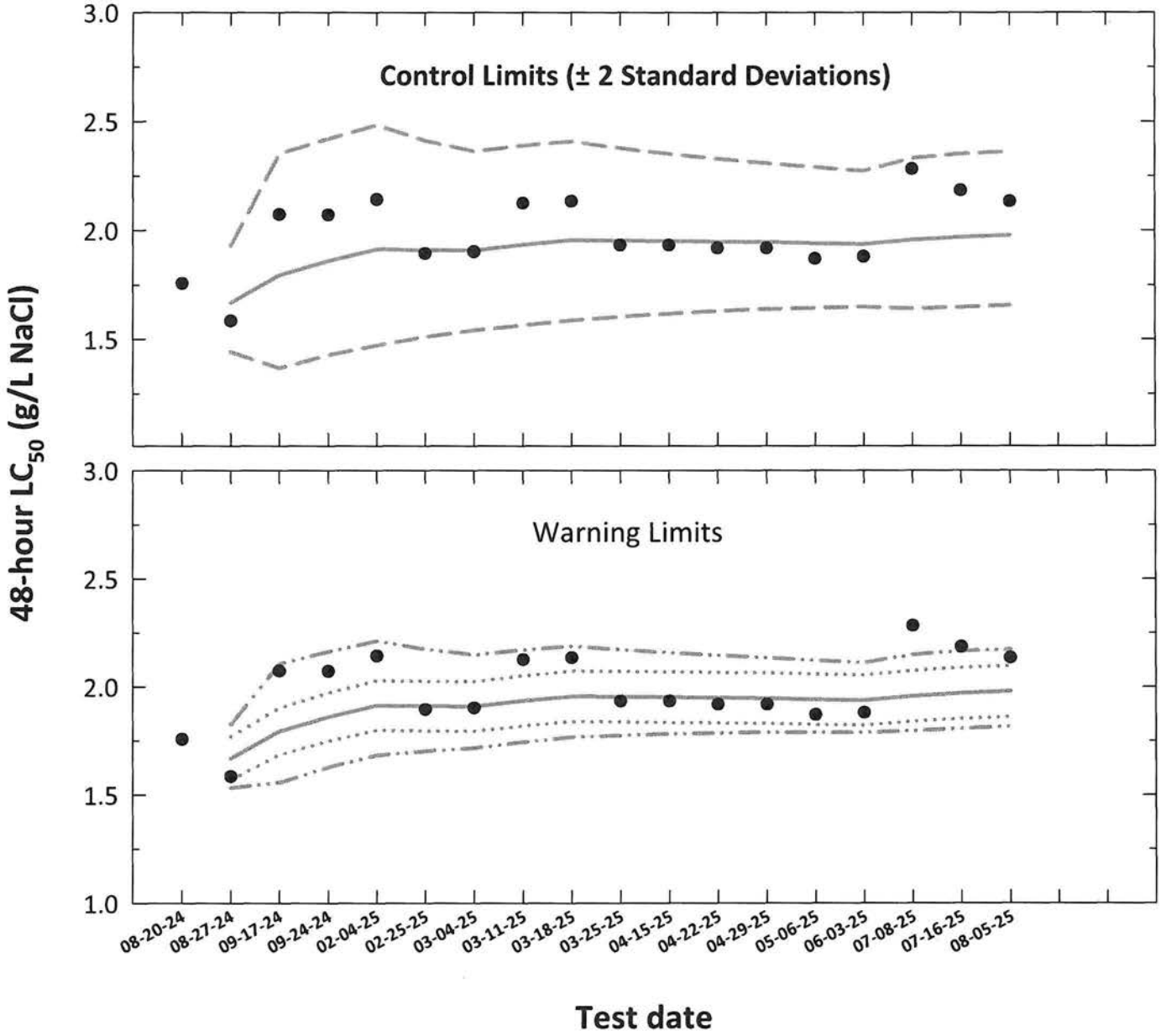
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1716.38	1413	1868.8
EC05	3.355	1841.95	1591.55	1971.41
EC10	3.718	1912.61	1693.69	2030.9
EC15	3.964	1961.81	1764.8	2073.78
EC20	4.158	2001.81	1822.17	2109.98
EC25	4.326	2036.78	1871.62	2142.97
EC40	4.747	2127.62	1994.41	2237.18
EC50	5.000	2184.21	2064.45	2304.37
EC60	5.253	2242.3	2129.87	2381.47
EC75	5.674	2342.32	2228.11	2532.46
EC80	5.842	2383.23	2264.23	2599.72
EC85	6.036	2431.82	2304.93	2682.86
EC90	6.282	2494.38	2354.69	2794.2
EC95	6.645	2590.06	2426.83	2972.19
EC99	7.326	2779.56	2561.21	3346.29



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-20-24	1.7569	0.2447									
2	08-27-24	1.5847	0.2000	0.2224	0.0317	1.6686	1.4422	1.9306	1.5329	1.8256	1.5685	1.7687
3	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
4	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
5	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
6	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
7	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
8	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
9	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
10	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
11	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
12	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
13	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
14	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
15	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
16	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
17	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
18	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
19												
20												

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

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

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

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

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

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

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 # 20

Dilution Preparation:

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

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

Stock solution INSS #: 2393

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	[illegible]	XL	XL
Control, MHSW	pH (S.U.)	7.77	7.81	7.82
	Dissolved oxygen (mg/L)	8.4	8.3	8.1
	Conductivity (µmhos/cm)	297		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	91		
	Temperature (°C)	25.2	24.9	25.2
1000 mg/L	pH (S.U.)	7.92	7.83	7.82
	Dissolved oxygen (mg/L)	8.4	8.3	8.1
	Conductivity (µmhos/cm)	1980		
	Temperature (°C)	25.2	24.9	25.0
1500 mg/L	pH (S.U.)	7.92	7.83	7.83
	Dissolved oxygen (mg/L)	8.4	8.3	8.1
	Conductivity (µmhos/cm)	2780		
	Temperature (°C)	25.2	24.8	25.0
2000 mg/L	pH (S.U.)	7.94	7.84	7.83
	Dissolved oxygen (mg/L)	8.4	8.3	8.1
	Conductivity (µmhos/cm)	3580		
	Temperature (°C)	25.2	24.8	25.0
2500 mg/L	pH (S.U.)	7.94	7.83	7.83
	Dissolved oxygen (mg/L)	8.4	8.4	8.1
	Conductivity (µmhos/cm)	4360		
	Temperature (°C)	25.2	25.0	25.2
3000 mg/L	pH (S.U.)	7.94	7.83	
	Dissolved oxygen (mg/L)	8.4	8.4	
	Conductivity (µmhos/cm)	5300		
	Temperature (°C)	25.1	24.9	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	1306647

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 # 20

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-05-25	* 0650 ✓ 0850	JL	0912	JP	2B2	BIACK	07-31-25B
24	08-06-25			0910	JP			
48 Termination	08-07-25			0906	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):	07-29-25 D
Age:	< 24-hours old
Date and time organisms were born between:	08-04-25 1433 TO 08-05-25 0650
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.27
	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	4 d	5	5	4 d	2 d	5	0 d	5	0 d	0 d	0 d	0 d
48 Termination	4	5	3 d	3 d	0 d	1 d	0	0 d	0	0	0	0
Mean Survival	75 %				5 %				0 %			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	2021.4
Upper 95% confidence limit (mg NaCl/L)	2252.2
48-hour LC_{50} (mg NaCl/L)	2134.0

Comments:

Test Reviewed by: JP

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	8/5/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/7/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.8000	1.0000	1.0000	0.8000
2500	0.4000	1.0000	0.0000	1.0000
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	0.9000	0.9000	1.2262	1.1071	1.3453	11.212	4	14.00	10.00	2	20
2500	0.6000	0.6000	0.9002	0.2255	1.3453	60.771	4	14.00	10.00	8	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

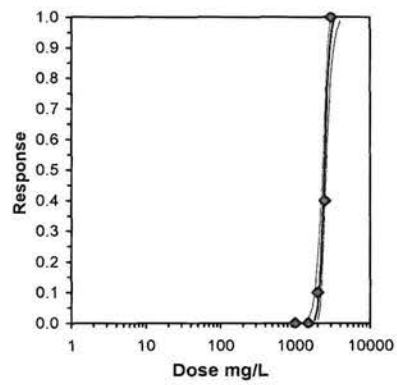
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76215	0.868	-0.7311	4.72254
Equality of variance cannot be confirmed				

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

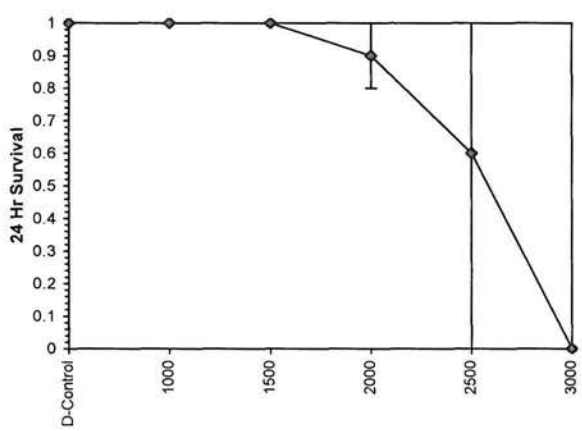
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.0539	3.64112	10.9173	25.1905	0	4.00689	7.81472	0.26072	3.39149	0.05539	5
Intercept	-56.23	12.3673	-80.47	-31.99							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1830.79	1481.12	2019.58
EC05	3.355	1997.03	1703.44	2157.75
EC10	3.718	2091.75	1833.01	2238.01
EC15	3.964	2158.17	1924.31	2295.8
EC20	4.158	2212.46	1998.65	2344.5
EC25	4.326	2260.13	2063.28	2388.77
EC40	4.747	2384.85	2225.99	2514.79
EC50	5.000	2463.16	2320.28	2604.64
EC60	5.253	2544.05	2409.22	2708.17
EC75	5.674	2684.44	2544.34	2912.53
EC80	5.842	2742.27	2594.55	3004.19
EC85	6.036	2811.26	2651.54	3117.94
EC90	6.282	2900.54	2721.82	3271.07
EC95	6.645	3038.09	2824.84	3517.67
EC99	7.326	3313.97	3019.94	4043.23



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	8/5/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/7/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.8000	1.0000	0.6000	0.6000
2500	0.0000	0.2000	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	0.7500	0.7500	1.0561	0.8861	1.3453	20.748	4	12.00	10.00	5	20
*2500	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

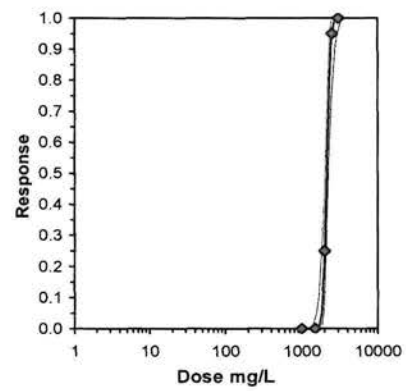
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77454	0.868	1.17369	3.74531
Equality of variance cannot be confirmed				

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

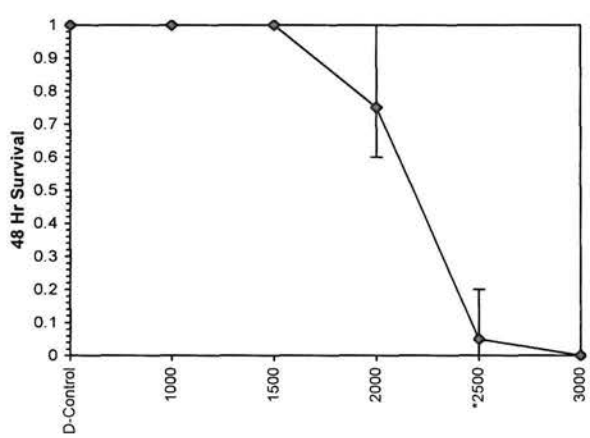
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	24.0509	5.63628	13.0038	35.098	0	0.00647	7.81472	0.99986	3.3292	0.04158	3
Intercept	-75.07	18.7675	-111.85	-38.286							
TSCR											

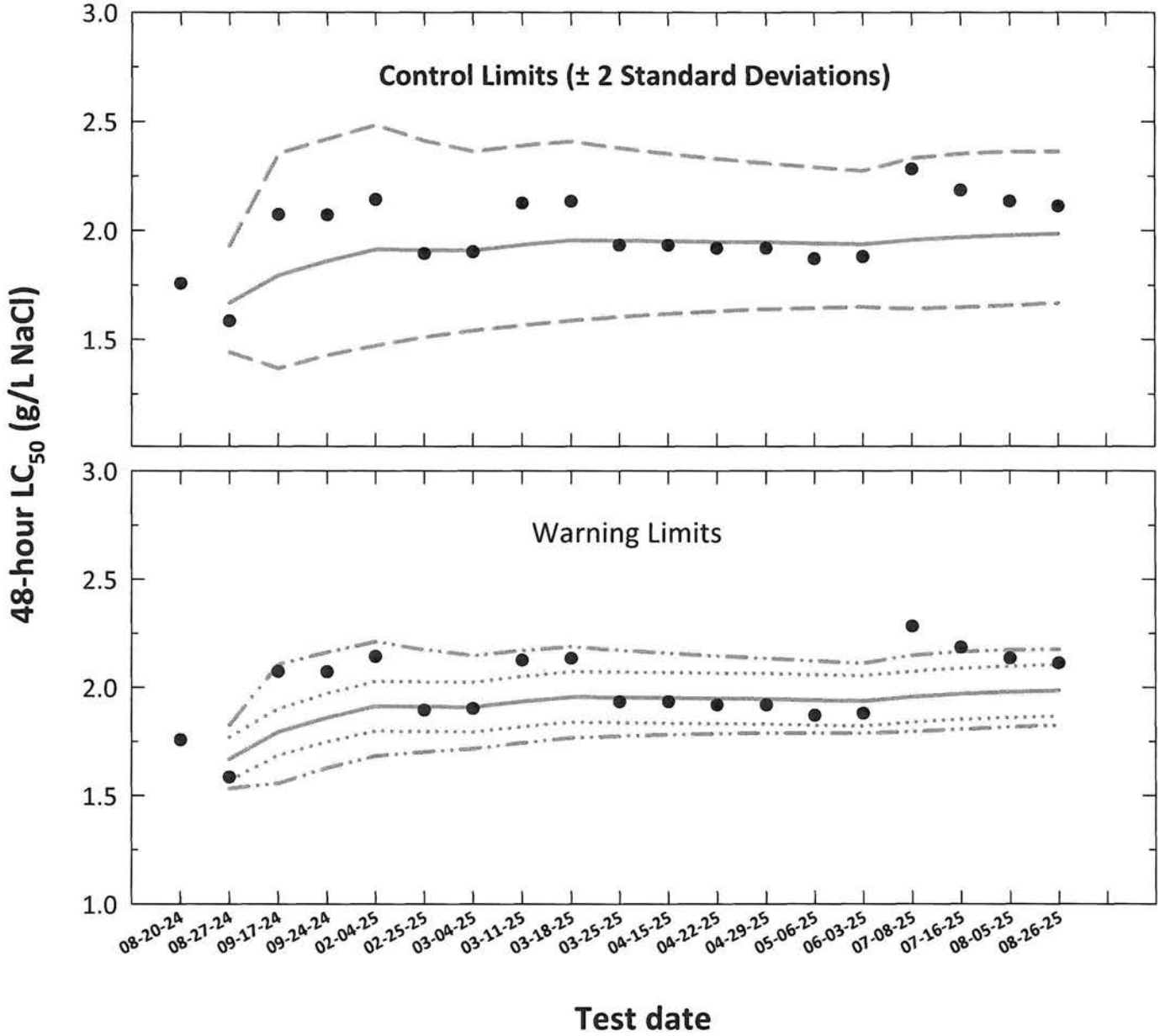
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1707.94	1397.63	1852.19
EC05	3.355	1823.09	1570.73	1944.47
EC10	3.718	1887.62	1669.45	1998.11
EC15	3.964	1932.44	1737.98	2036.96
EC20	4.158	1968.82	1793.07	2069.96
EC25	4.326	2000.58	1840.37	2100.23
EC40	4.747	2082.89	1956.48	2188.17
EC50	5.000	2134.02	2021.4	2252.19
EC60	5.253	2186.42	2080.92	2326.51
EC75	5.674	2276.37	2168.53	2472.74
EC80	5.842	2313.09	2200.38	2537.82
EC85	6.036	2356.64	2236.14	2618.12
EC90	6.282	2412.59	2279.73	2725.46
EC95	6.645	2497.98	2342.72	2896.63
EC99	7.326	2666.4	2459.54	3255.23



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-20-24	1.7569	0.2447									
2	08-27-24	1.5847	0.2000	0.2224	0.0317	1.6686	1.4422	1.9306	1.5329	1.8256	1.5685	1.7687
3	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
4	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
5	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
6	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
7	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
8	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
9	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
10	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
11	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
12	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
13	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
14	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
15	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
16	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
17	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
18	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
19	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.8658	2.1040
20												

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

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

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

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

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

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

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 # 21

Dilution Preparation:

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

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

Stock solution INSS #: 2411

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.85	7.84	7.99
	Dissolved oxygen (mg/L)	8.3	8.4	8.3
	Conductivity (µmhos/cm)	304		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	94		
	Temperature (°C)	24.1	24.8	25.1
1000 mg/L	pH (S.U.)	7.93	7.92	8.01
	Dissolved oxygen (mg/L)	8.3	8.4	8.3
	Conductivity (µmhos/cm)	2070		
	Temperature (°C)	24.2	25.0	24.9
1500 mg/L	pH (S.U.)	7.92	7.94	8.02
	Dissolved oxygen (mg/L)	8.3	8.4	8.3
	Conductivity (µmhos/cm)	2900		
	Temperature (°C)	24.4	24.7	24.9
2000 mg/L	pH (S.U.)	7.93	7.94	8.01
	Dissolved oxygen (mg/L)	8.3	8.4	8.3
	Conductivity (µmhos/cm)	3780		
	Temperature (°C)	24.5	24.7	25.2
2500 mg/L	pH (S.U.)	7.93	7.95	8.01
	Dissolved oxygen (mg/L)	8.3	8.4	8.3
	Conductivity (µmhos/cm)	4430		
	Temperature (°C)	24.3	24.9	25.2
3000 mg/L	pH (S.U.)	7.94	7.93	8.00
	Dissolved oxygen (mg/L)	8.3	8.4	8.3
	Conductivity (µmhos/cm)	5570		
	Temperature (°C)	24.1	24.7	25.0

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	136647

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 # 21

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-26-25	*0630	ZP	0925	ZP	1B	Block	08-11-25 A
24	08-27-25			0920	ZP			
48 Termination	08-28-25			0911	ZP			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	08-19-25 A + B
Age:	< 24-hours old
Date and time organisms were born between:	~~08-26-25 1230 to 08-27-25 0600~~ K 08-02-25 08-25-25 1230 to 08-26-25 0600
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.36
	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	5	5	3 d	5	5	5	5	5	5	5	5	5
48 Termination	5	5	3	5	5	5	5	5	5	5	5	5
Mean Survival	90%				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	2 3d	2 3d	3 2d	3 2d	0 5d	0 5d	4 1d	0 5d
48 Termination	3 2d	5	3 2d	4 1d	0 2d	0 2d	0 3d	0 3d	0	0	0 4d	0
Mean Survival	75%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	2013.4
Upper 95% confidence limit (mg NaCl/L)	2214.7
48-hour LC_{50} (mg NaCl/L)	2111.7

Comments:

Test Reviewed by: K

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	8/26/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/28/2025	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	0.6000	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	0.4000	0.4000	0.6000	0.6000
3000	0.0000	0.0000	0.8000	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	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4			2	20
1000	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0	20
1500	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0	20
2000	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0	20
2500	0.5000	0.5556	0.7854	0.6847	0.8861	14.802	4	11.00	10.00	10	20
3000	0.2000	0.2222	0.4459	0.2255	1.1071	98.855	4	11.00	10.00	16	20

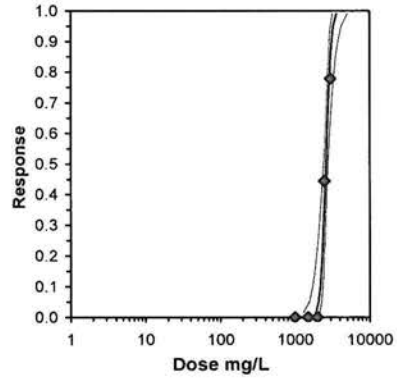
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77839	0.884	1.67057	6.99
Equality of variance cannot be confirmed				

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

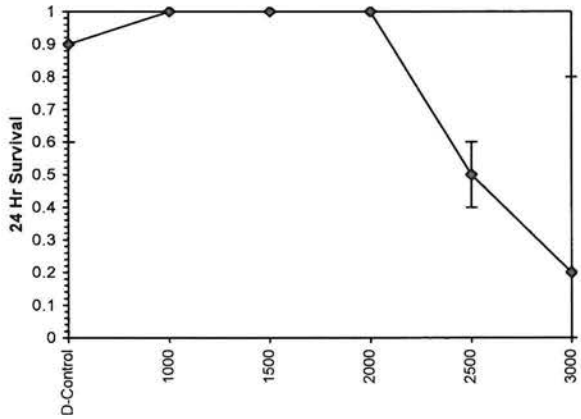
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.4413	4.35203	7.91134	24.9713	0.1	3.41573	7.81472	0.33186	3.41587	0.06082	6
Intercept	-51.161	14.8922	-80.35	-21.973							
TSCR	0.02628	0.02016	-0.0132	0.0658							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1880.97	1283.86	2137.37
EC05	3.355	2069.34	1559.95	2284.13
EC10	3.718	2177.35	1728.52	2369.33
EC15	3.964	2253.39	1850.76	2430.8
EC20	4.158	2315.72	1952.46	2482.81
EC25	4.326	2370.56	2042.4	2530.48
EC40	4.747	2514.58	2273.9	2670.98
EC50	5.000	2605.4	2407.81	2779.64
EC60	5.253	2699.5	2528.51	2916.86
EC75	5.674	2863.51	2694.77	3216.3
EC80	5.842	2931.33	2752.24	3357.45
EC85	6.036	3012.4	2815.85	3536
EC90	6.282	3117.61	2892.86	3780.89
EC95	6.645	3280.34	3004.36	4184.46
EC99	7.326	3608.85	3214.02	5078.97



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	8/26/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/28/2025	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	0.6000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.6000	1.0000	0.6000	0.8000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

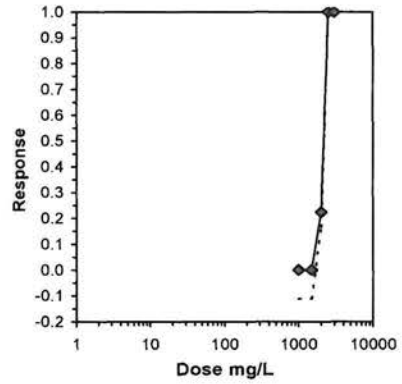
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4			2	20
1000	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0	20
1500	1.0000	1.1111	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0	20
2000	0.7500	0.8333	1.0561	0.8861	1.3453	20.748	4	14.50	10.00	5	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 normal distribution (p > 0.01)	0.87934	0.844	-0.5821	2.00066
Equality of variance cannot be confirmed				

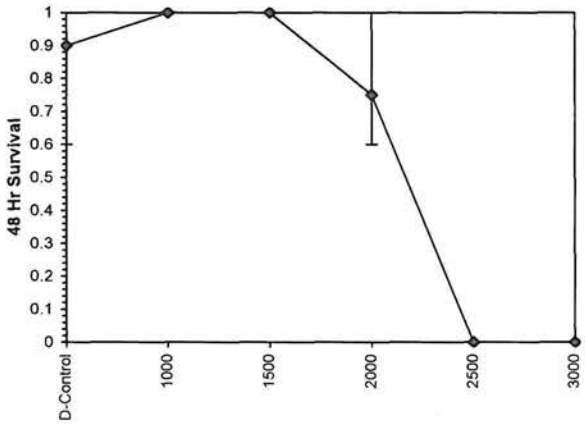
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Karber

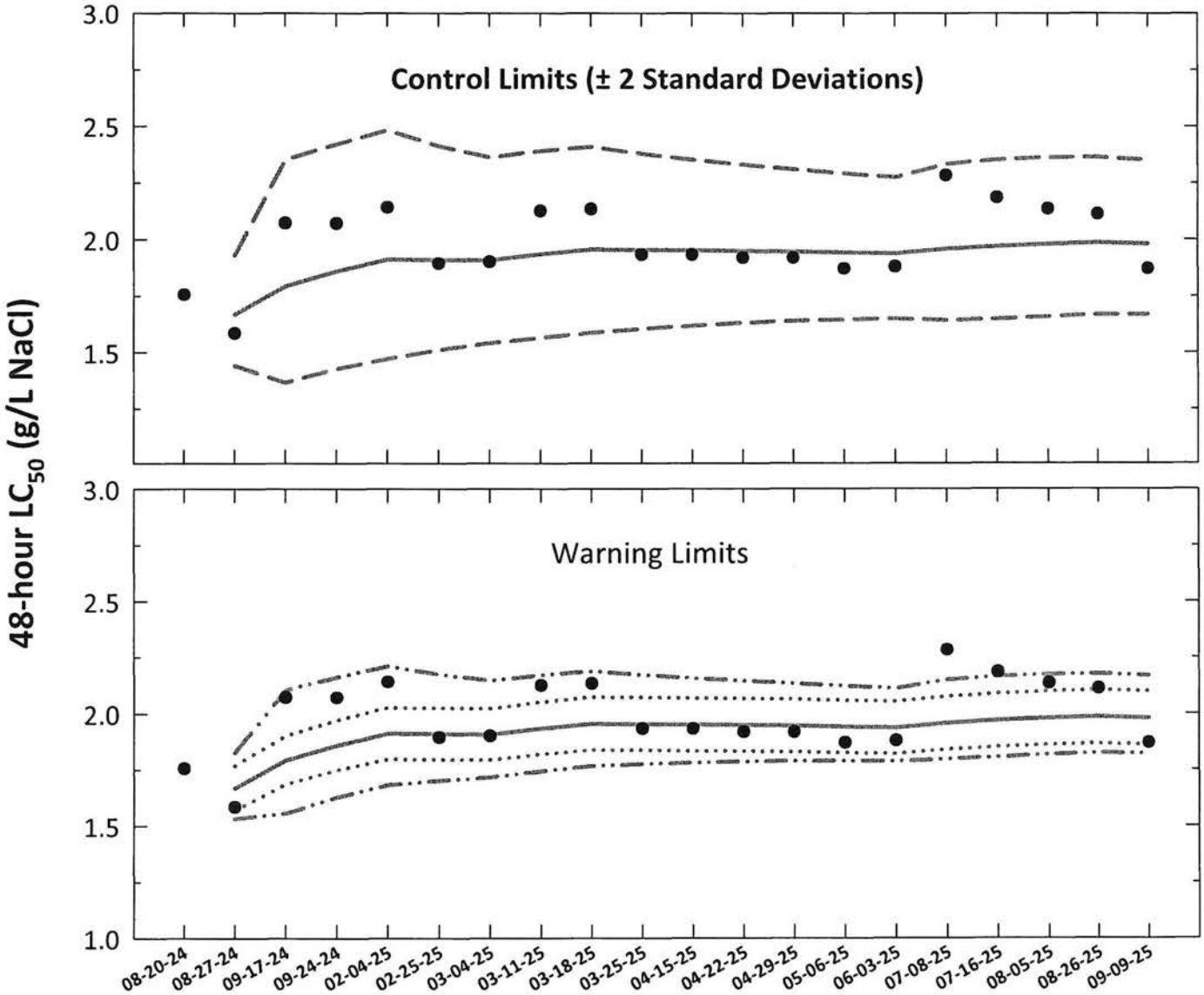
Trim Level	EC50	95% CL	
0.0%	2111.65	2013.43	2214.67
5.0%	2129.12	2016.57	2247.96
10.0%	2144.48	2007.94	2290.29
20.0%	2164.1	1933.48	2422.22
Auto-0.0%	2111.65	2013.43	2214.67



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-20-24	1.7569	0.2447									
2	08-27-24	1.5847	0.2000	0.2224	0.0317	1.6686	1.4422	1.9306	1.5329	1.8256	1.5685	1.7687
3	09-17-24	2.0733	0.3167	0.2538	0.0589	1.7938	1.3679	2.3525	1.5564	2.1053	1.6862	1.9015
4	09-24-24	2.0711	0.3162	0.2694	0.0573	1.8595	1.4281	2.4211	1.6275	2.1615	1.7479	1.9710
5	02-04-25	2.1422	0.3309	0.2817	0.0567	1.9129	1.4730	2.4841	1.6829	2.2115	1.7981	2.0276
6	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.7951	2.0243
7	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.7941	2.0231
8	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.8183	2.0504
9	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.8382	2.0729
10	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.8361	2.0705
11	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.8343	2.0685
12	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.8317	2.0656
13	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.8295	2.0631
14	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.8243	2.0572
15	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.8204	2.0528
16	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.8392	2.0740
17	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.8511	2.0874
18	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.8594	2.0968
19	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.8658	2.1040
20	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.8603	2.0977

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

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

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

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

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

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

CV = Coefficient of variation.

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 # 22

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 #: 2411

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.97	7.84	7.80
	Dissolved oxygen (mg/L)	8.3	8.2	8.2
	Conductivity (µmhos/cm)	307		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	25.3	24.8	25.2
1000 mg/L	pH (S.U.)	7.94	7.89	7.87
	Dissolved oxygen (mg/L)	8.3	8.2	8.2
	Conductivity (µmhos/cm)	2150		
	Temperature (°C)	24.9	25.0	25.2
1500 mg/L	pH (S.U.)	7.93	7.89	7.87
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	3050		
	Temperature (°C)	24.9	24.7	25.3
2000 mg/L	pH (S.U.)	7.93	7.89	7.87
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	3860		
	Temperature (°C)	25.0	24.7	25.1
2500 mg/L	pH (S.U.)	7.92	7.89	7.88
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	4680		
	Temperature (°C)	25.0	25.0	24.9
3000 mg/L	pH (S.U.)	7.92	7.88	
	Dissolved oxygen (mg/L)	8.4	8.2	
	Conductivity (µmhos/cm)	5580		
	Temperature (°C)	25.1	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 G-2021	YSI Model 52CE	08A100271
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

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 # 22

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09-09-25	* 0600	JH	0813	JP	284	GREEN	09-01-25A
24	09-10-25			0824	JP			
48 Termination	09-11-25			0821	JP			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	09-02-25A JAY
Age:	< 24-hours old
Date and time organisms were born between:	09-08-25 to 09-09-25 1208 ~~0851 to 09-09-25 H~~ 09-09-25 H ← 09-08-25 0851 TO 120
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.91
	Temperature (°C): 24.1°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	2 3d	4 1d	5	2 3d	3 2d	2 3d	0 5d	1 4d	0 5d	0 5d	0 5d	0 5d
48 Termination	0 2d	3 1d	2 3d	1 1d	0 3d	0 2d	0	0 1d	0	0	0	0
Mean Survival	30%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1774.6
Upper 95% confidence limit (mg NaCl/L)	~~1989.9~~ 1970.5 09-23-25
48-hour LC_{50} (mg NaCl/L)	1870.0

Comments:

Test Reviewed by: JP

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	9/9/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/11/2025	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	0.4000	0.8000	1.0000	0.4000
2500	0.6000	0.4000	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	0.6500	0.6500	0.9555	0.6847	1.3453	34.266	4	12.00	10.00	7	20
*2500	0.3000	0.3000	0.5650	0.2255	0.8861	50.368	4	10.00	10.00	14	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

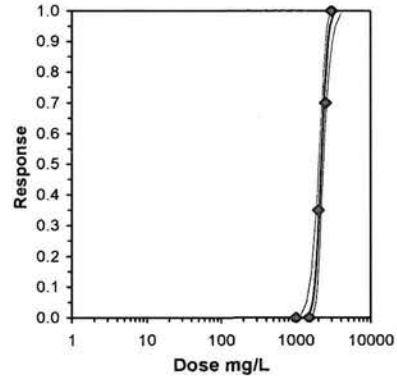
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84272	0.868	0.20196	1.25519
Equality of variance cannot be confirmed				

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

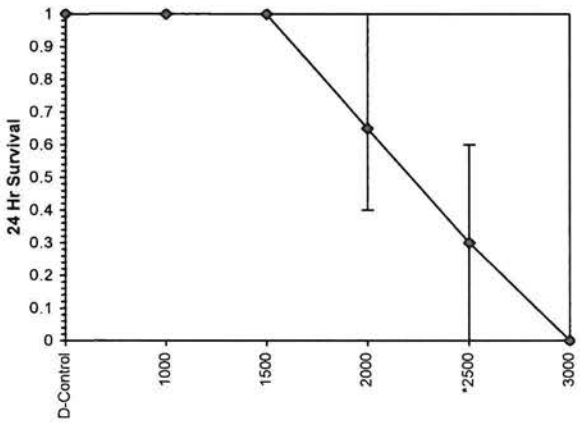
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1611	2.66876	8.9303	19.3918	0	2.11704	7.81472	0.54847	3.34163	0.07062	5
Intercept	-42.321	8.94981	-59.863	-24.779							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1504.35	1172.41	1699.02
EC05	3.355	1680.64	1391.62	1850.18
EC10	3.718	1782.91	1522.75	1938.76
EC15	3.964	1855.41	1616.67	2002.71
EC20	4.158	1915.12	1694.15	2056.6
EC25	4.326	1967.88	1762.28	2105.52
EC40	4.747	2107.35	1937.69	2244.03
EC50	5.000	2195.98	2042.35	2342.15
EC60	5.253	2288.33	2143.32	2455.24
EC75	5.674	2450.52	2300.22	2680.94
EC80	5.842	2518.02	2359.33	2783.57
EC85	6.036	2599.06	2426.82	2912.15
EC90	6.282	2704.74	2510.6	3087.17
EC95	6.645	2869.33	2634.53	3373.28
EC99	7.326	3205.57	2872.84	3998.54



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	9/9/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/11/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.0000	0.6000	0.4000	0.2000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

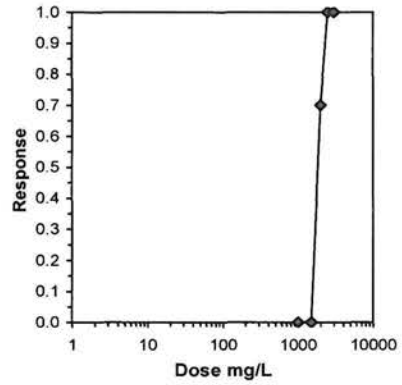
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.3000	0.3000	0.5650	0.2255	0.8861	50.368	4	10.00	10.00	14	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.68087	0.844	-0.1975	5.49067
Equality of variance cannot be confirmed				

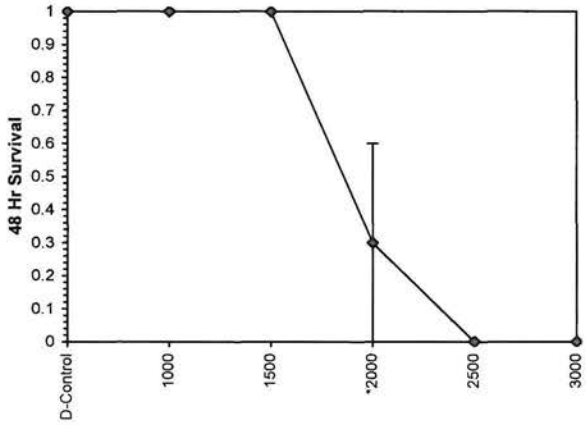
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

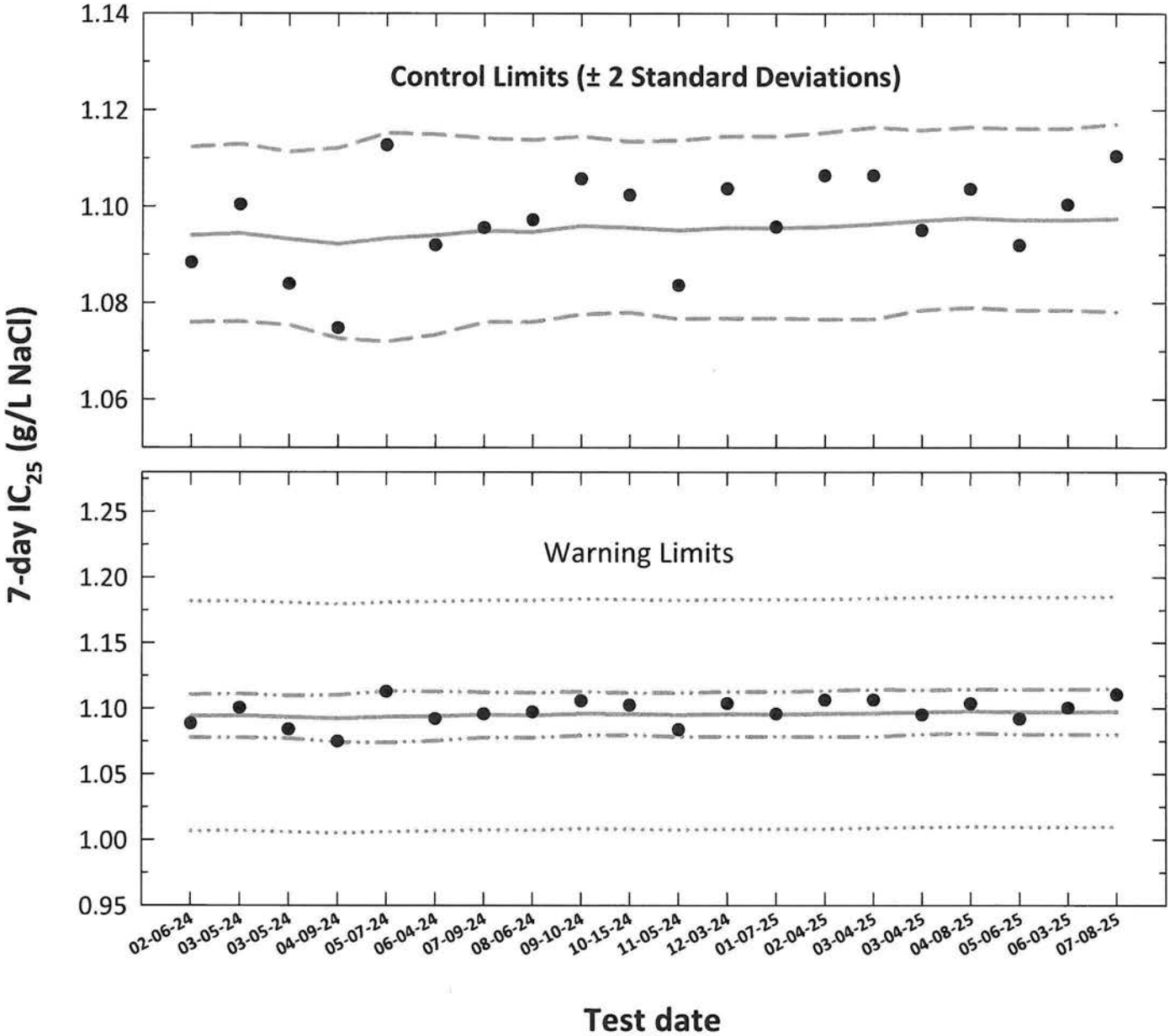
Trim Level	EC50	95% CL	
0.0%	1869.98	1774.62	1970.47
5.0%	1863.6	1759.25	1974.14
10.0%	1857.58	1744.15	1978.38
20.0%	1847.3	1715	1989.81
Auto-0.0%	1869.98	1774.62	1970.47



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760	1.1123	1.0775	1.1105	1.0065	1.1815
2	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
3	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
4	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
5	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
6	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
7	07-09-24	1.0956	0.0396	0.0394	0.0038	1.0949	1.0760	1.1142	1.0777	1.1122	1.0073	1.1825
8	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
9	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
10	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
11	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
12	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
13	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

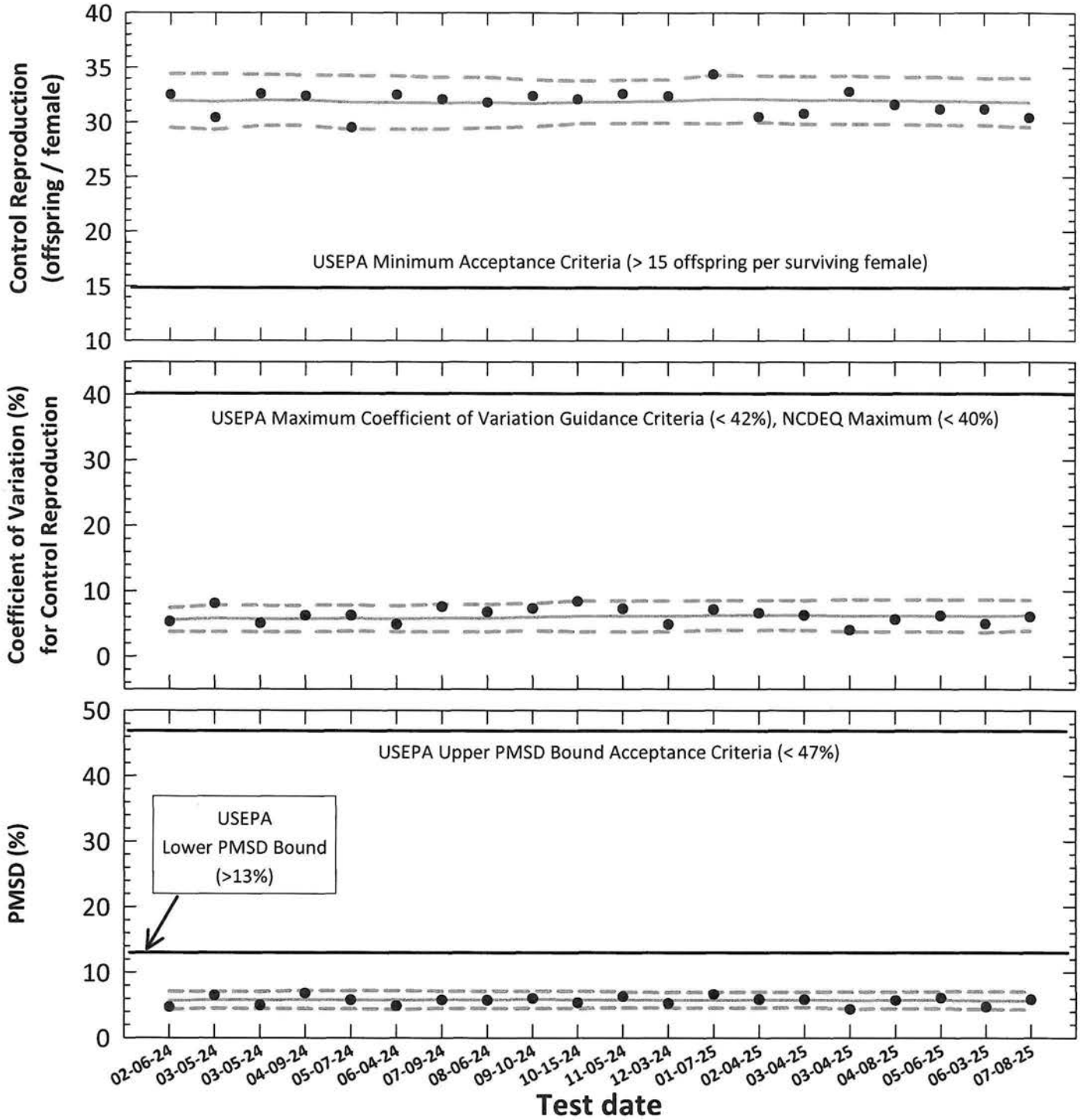
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

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 Mean (offspring/female)	Control Reproduction CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
1	02-06-24	100	32.5	5.3	1.526	4.7	31.9	29.5	34.4	5.6	3.7	7.4	5.7	4.4	7.0
2	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	5.8	4.5	7.1
3	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
4	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
5	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
6	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
7	07-09-24	100	32.1	7.6	1.846	5.8	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5	7.1
8	08-06-24	100	31.8	6.8	1.827	5.7	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
9	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
10	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
11	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
12	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
13	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 #: 313

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2393				
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:	07-08-25 0600 TO 0800									
Culture board:	07-01-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	4	5	7	10	12	13	17	22	23	24
Transfer vessel information:	pH (S.U.): 7.82 Temperature (°C): 24.8									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLACK
Incubator number and shelf location:	281

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	07-08-25	0810	06-26-25	06-26-25	07-01-25B	JH
1	07-09-25	0620	↓	↓	↓	JH
2	07-10-25	0620	↓	↓	07-01-25 A	JH
3	07-11-25	0610	↓	↓	↓	JH
4	07-12-25	0700	↓	↓	07-01-25 C	JH
5	07-13-25	0630	↓	↓	↓	JH
6	07-14-25	0659	↓	↓	↓	JH
7	07-15-25	0710				JH

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	130604685

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:	30.4	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	6.0%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1110.4

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 313**

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	4	5	4	6	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	12	11	11	10	10	10	10	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	15	15	17	12	15	14	14	14	16
Total young produced		32	31	31	32	27	30	28	30	30	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:	30.4

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.9
% Reduction from Control:	-1.6%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 313**

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.3
% Reduction from Control:	-3.0%

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	4	6	4	3	4	5	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	11	10	10	12	12	12	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	12	13	15	14	14	17	14	12	16	15
Total young produced		26	30	30	27	28	34	31	28	31	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	29.5
% Reduction from Control:	3.0%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 313**

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.0
% Reduction from Control:	40.8%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.9
% Reduction from Control:	93.8%

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	6	4	4	4	5	4	6	6	4	47
5	12	10	12	11	11	10	10	10	10	13	109
6	0	0	0	0	0	0	0	0	0	0	0
7	16	15	15	17	12	15	14	14	14	16	148
Total	**32**	**31**	**31**	**32**	**27**	**30**	**28**	**30**	**30**	**33**	**304**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	3	4	5	5	4	5	5	45
5	10	11	11	10	10	12	12	12	10	10	108
6	0	0	0	0	0	0	0	0	0	0	0
7	12	13	15	14	14	17	14	12	16	15	142
Total	**26**	**30**	**30**	**27**	**28**	**34**	**31**	**28**	**31**	**30**	**295**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	5	4	4	6	4	6	6	50
5	12	11	10	10	12	12	12	10	12	11	112
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	13	17	13	14	12	16	17	15	147
Total	**31**	**32**	**28**	**32**	**29**	**30**	**30**	**30**	**35**	**32**	**309**

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	5	4	4	3	3	4	4	6	40
5	9	7	7	10	4	8	9	5	11	6	76
6	0	0	0	0	0	0	0	0	0	0	0
7	4	9	6	6	10	7	3	11	4	4	64
Total	**17**	**19**	**18**	**20**	**18**	**18**	**15**	**20**	**19**	**16**	**180**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	5	5	5	6	4	6	5	5	50
5	10	12	12	10	10	10	10	10	13	11	108
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	14	16	14	17	18	13	15	15	155
Total	**32**	**32**	**31**	**31**	**29**	**33**	**32**	**29**	**33**	**31**	**313**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	1	1	1	3	1	2	1	1	14
5	0	1	0	0	0	0	0	0	2	0	3
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	2	0	0	0	0	0	2
Total	**2**	**2**	**1**	**1**	**3**	**3**	**1**	**2**	**3**	**1**	**19**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

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

Test number: 313

Test dates: July 08-15, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	32	31	31	32	27	30	28	30	30	33	100	30.4	6.0	Not applicable
600	31	32	28	32	29	30	30	30	35	32	100	30.9	6.4	-1.6
800	32	32	31	31	29	33	32	29	33	31	100	31.3	4.5	-3.0
1000	26	30	30	27	28	34	31	28	31	30	100	29.5	7.9	3.0
1200	17	19	18	20	18	18	15	20	19	16	100	18.0	9.1	40.8
1400	2	2	1	1	3	3	1	2	3	1	100	1.9	46.1	93.8

Dunnett's MSD value: 1.776

PMSD: 5.8

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Upper PMSD bound determined by USEPA (90th 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:	7/8/2025	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	7/15/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	32.000	31.000	31.000	32.000	27.000	30.000	28.000	30.000	30.000	33.000
600	31.000	32.000	28.000	32.000	29.000	30.000	30.000	30.000	35.000	32.000
800	32.000	32.000	31.000	31.000	29.000	33.000	32.000	29.000	33.000	31.000
1000	26.000	30.000	30.000	27.000	28.000	34.000	31.000	28.000	31.000	30.000
1200	17.000	19.000	18.000	20.000	18.000	18.000	15.000	20.000	19.000	16.000
1400	2.000	2.000	1.000	1.000	3.000	3.000	1.000	2.000	3.000	1.000

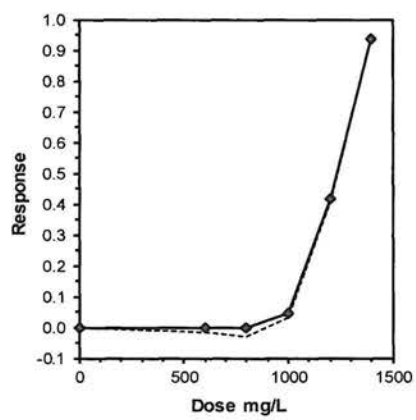
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	30.400	1.0000	30.400	27.000	33.000	6.046	10				30.867	1.0000
600	30.900	1.0164	30.900	28.000	35.000	6.373	10	-0.644	2.287	1.776	30.867	1.0000
800	31.300	1.0296	31.300	29.000	33.000	4.531	10	-1.159	2.287	1.776	30.867	1.0000
1000	29.500	0.9704	29.500	26.000	34.000	7.869	10	1.159	2.287	1.776	29.500	0.9557
*1200	18.000	0.5921	18.000	15.000	20.000	9.072	10	15.969	2.287	1.776	18.000	0.5832
*1400	1.900	0.0625	1.900	1.000	3.000	46.084	10	36.703	2.287	1.776	1.900	0.0616

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.60655	1.035	0.05692	0.36712
Bartlett's Test indicates equal variances (p = 0.15)	8.20101	15.0863		

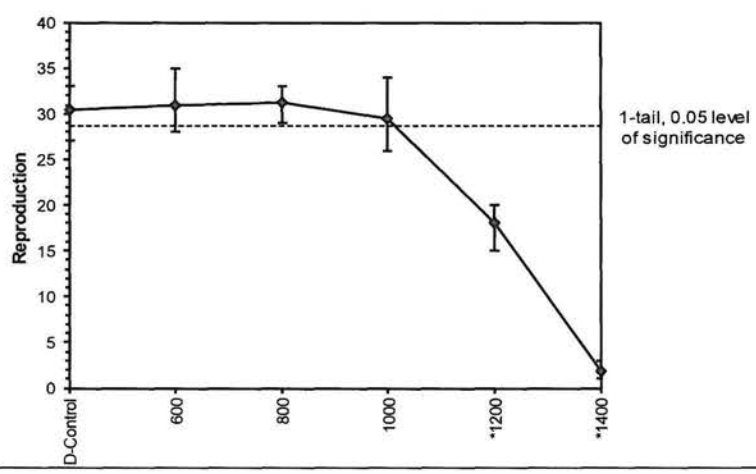
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.77561	0.05841	1391.71	3.01481	5.4E-43	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1003.07	36.2337	898.011	1024.16	-1.4976
IC10	1029.91	11.9032	1004.58	1048.99	-0.9498
IC15	1056.75	10.1902	1034.22	1073.77	-0.5462
IC20	1083.59	9.2659	1061.94	1099.08	-0.5671
IC25	1110.43	8.65762	1091.36	1124.42	-0.5850
IC40	1190.96	8.78389	1171.65	1204.86	-0.5336
IC50	1231.88	5.78721	1219.19	1241.62	-0.5856



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 313**

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.

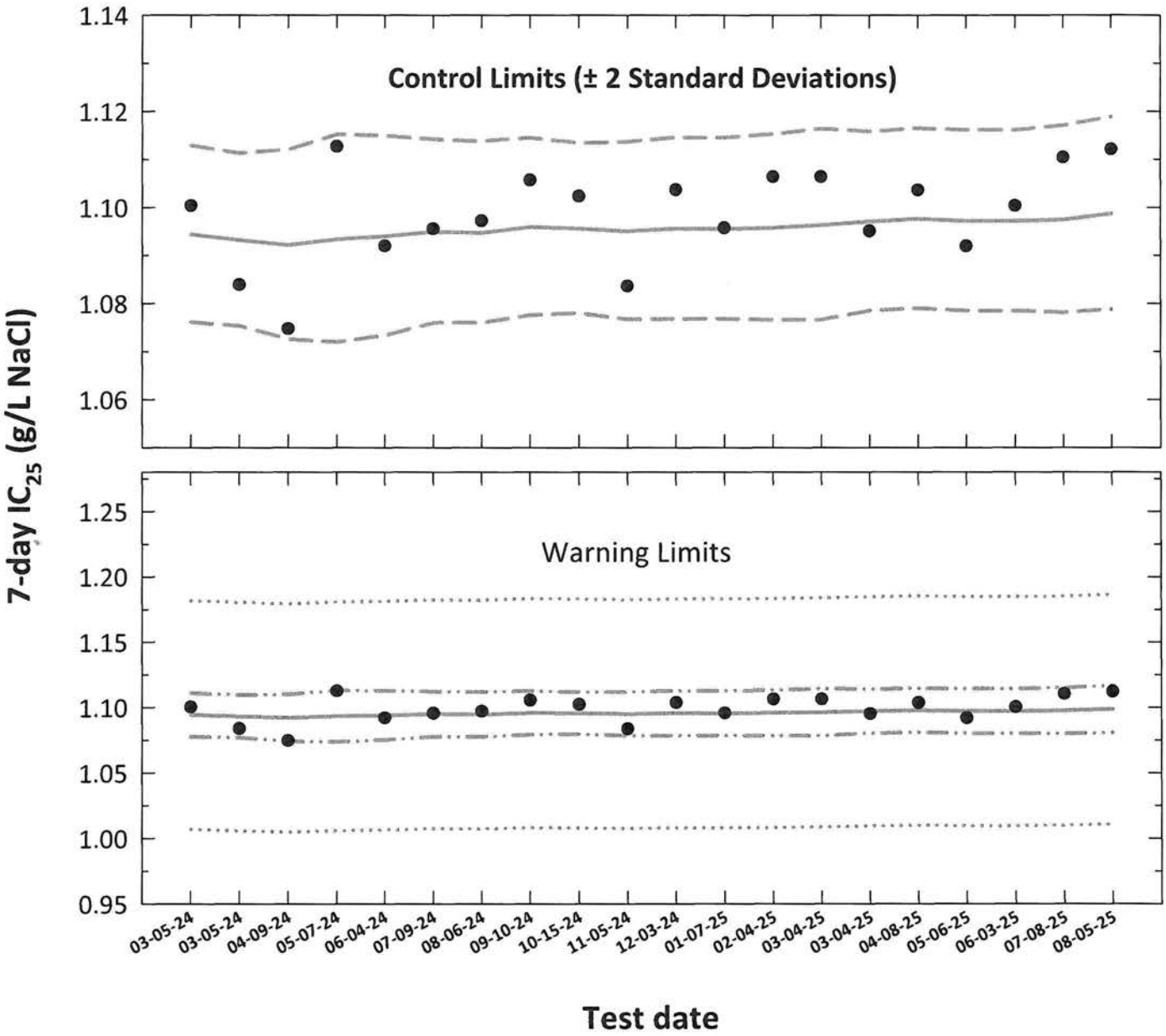
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	XL	XL	XL	XL	XL	K
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.76	7.84	7.79	7.91	7.81	7.92
	Dissolved oxygen (mg/L)	8.5	8.0	8.4	8.0	8.3	8.1
	Conductivity (µmhos/cm)	XL-070525 ~~289~~ 298		299		306	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	86			7-16-25 K	86	
	Temperature (°C)	24.8	25.1	24.8	25.2	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.84	7.83	7.84	7.91	7.85	7.88
	Dissolved oxygen (mg/L)	8.5	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	1360		1390		1400	
	Temperature (°C)	24.9	24.8	24.9	25.0	24.8	25.0
800 mg NaCl/L	pH (S.U.)	7.86	7.84	7.85	7.90	7.86	7.88
	Dissolved oxygen (mg/L)	8.6	8.0	8.4	8.0	8.2	8.1
	Conductivity (µmhos/cm)	1730		1750		1770	
	Temperature (°C)	24.9	25.0	24.9	25.0	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.88	7.84	7.86	7.90	7.88	7.88
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.2	8.0
	Conductivity (µmhos/cm)	2100		2110		2130	
	Temperature (°C)	24.8	24.9	25.0	24.9	24.9	25.1
1200 mg NaCl/L	pH (S.U.)	7.89	7.84	7.87	7.90	7.89	7.87
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.2	8.0
	Conductivity (µmhos/cm)	2430		2450		2480	
	Temperature (°C)	24.8	24.9	25.0	24.9	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.89	7.84	7.88	7.90	7.90	7.88
	Dissolved oxygen (mg/L)	8.6	8.0	8.3	8.0	8.2	8.0
	Conductivity (µmhos/cm)	2750		2770		2790	
	Temperature (°C)	24.8	24.9	25.0	24.9	24.9	25.1
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 313**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	BSL	BSL	BSL	BSL	XL	XL	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.96	7.93	7.86	7.96	7.81	7.90	7.84	8.23
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	8.0	8.5	8.0	8.3	7.6
	Conductivity (µmhos/cm)	296		292		289		303	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)	N 07-16-25	N	88				N 07-16-25	N
	Temperature (°C)	24.8	25.0	24.8	25.2	24.8	25.0	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.95	7.96	7.88	7.98	7.91	7.93	7.93	8.10
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	8.0	8.5	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1300		1380		1350		1430	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.9	25.3	24.9	24.8
800 mg NaCl/L	pH (S.U.)	7.93	7.96	7.96	7.98	8.01	7.93	7.94	8.08
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	8.0	8.5	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1650		1730		1710		1830	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.9	25.1	24.9	24.8
1000 mg NaCl/L	pH (S.U.)	7.94	7.95	7.97	7.98	8.01	7.94	7.95	8.08
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	8.0	8.5	8.0	8.3	7.5
	Conductivity (µmhos/cm)	1990		2030		2080		2200	
	Temperature (°C)	25.0	24.9	24.9	24.9	25.0	25.2	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.93	7.95	7.99	8.01	8.02	7.93	7.96	8.06
	Dissolved oxygen (mg/L)	8.4	8.1	8.4	8.0	8.5	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2320		2430		2410		2560	
	Temperature (°C)	24.9	24.9	25.0	24.9	24.9	25.2	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.93	7.94	8.00	8.00	8.02	7.93	7.97	8.06
	Dissolved oxygen (mg/L)	8.4	8.1	8.5	8.0	8.5	8.0	8.4	7.5
	Conductivity (µmhos/cm)	2640		2820		2720		2910	
	Temperature (°C)	25.0	25.1	25.0	24.9	24.9	25.0	24.8	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 $IC_{25} \pm S_{A.10}$ 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	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
2	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
3	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
4	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
5	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
6	07-09-24	1.0956	0.0396	0.0394	0.0038	1.0949	1.0760	1.1142	1.0777	1.1122	1.0073	1.1825
7	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
8	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
9	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
10	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
11	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
12	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

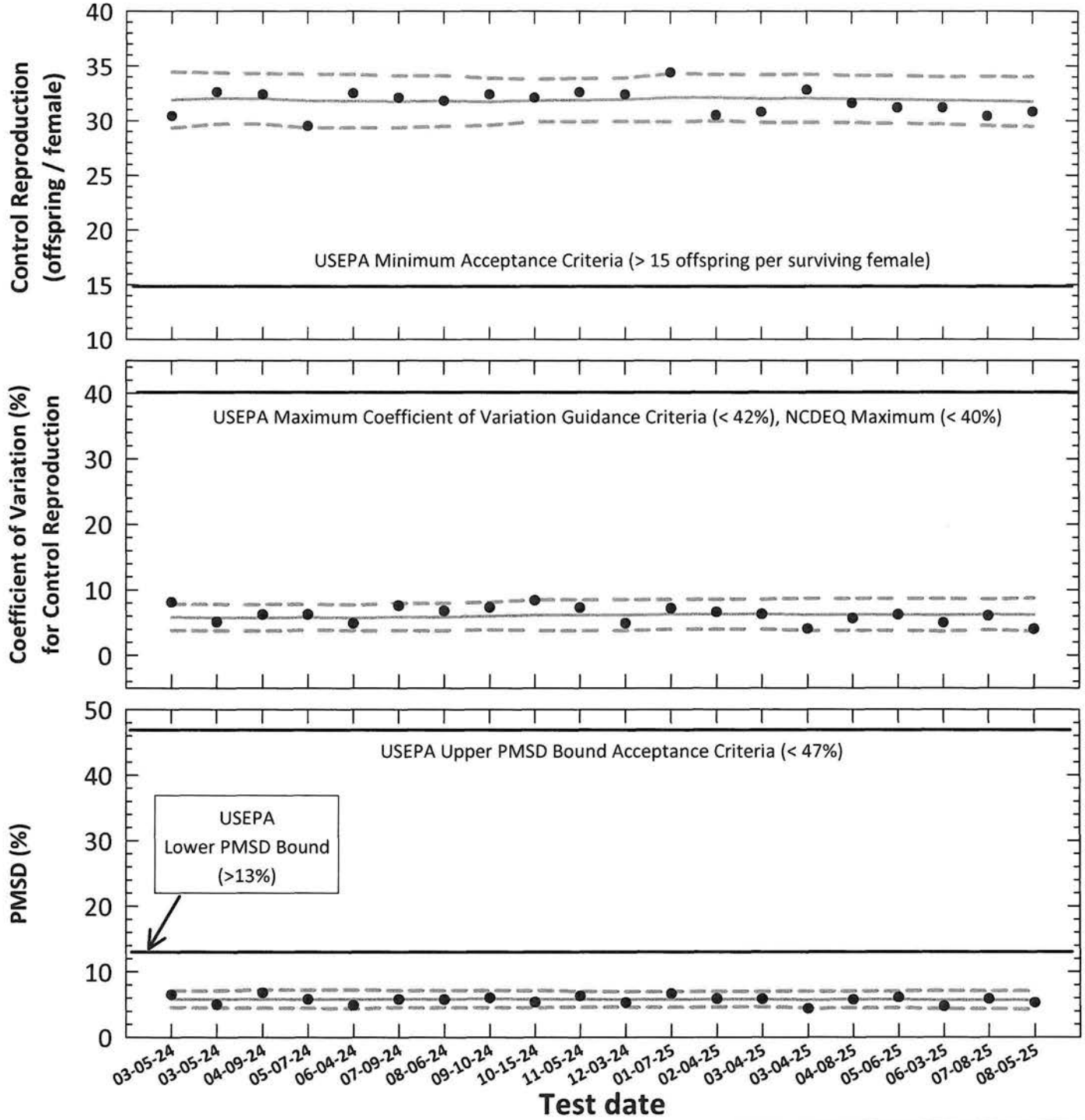
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

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 Mean (offspring/female)	Control Reproduction CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	5.8	4.5	7.1
2	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
3	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
4	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
5	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
6	07-09-24	100	32.1	7.6	1.846	5.8	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5	7.1
7	08-06-24	100	31.8	6.8	1.827	5.7	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
8	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
9	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
10	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
11	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
12	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 #: 314

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2393				
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:	08-05-25 0530 TO 0740									
Culture board:	07-29-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	3	7	11	12	13	20	23	24
Transfer vessel information:	pH (S.U.): 8.27 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLACK
Incubator number and shelf location:	2 B 1

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	08-05-25	0800	08-01-25	08-01-25	07-31-25 B	JH
1	08-06-25	0605	↓	↓	↓	JH
2	08-07-25	0610	↓	↓	07-31-25 C	JH
3	08-08-25	0620	↓	↓	↓	JH
4	08-09-25	0720	↓	↓	08-04-25A	JH
5	08-10-25	0710	↓	↓	↓	JH
6	08-11-25	0634	↓	↓	↓	JH
7	08-12-25	0600				JH

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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:	30.8	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	4.0 %	< 40.0 %	**IC_{25}** (mg/L NaCl)	1112.1

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 314**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	5	3	5	4	3	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	13	10	11	11	12	10	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	14	18	14	15	18	17	12	17
Total young produced		31	30	32	31	30	30	33	30	29	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.8

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.5
% Reduction from Control:	-2.3%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 314**

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.7
% Reduction from Control:	-2.9%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.9
% Reduction from Control:	-0.3%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 314**

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	4	4	3	4	4	4	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	8	7	8	5	9	5	6	6	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	6	3	5	11	4	10	7	9	7
Total young produced		20	18	14	17	19	17	19	17	18	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:	17.7
% Reduction from Control:	-0.3%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.1
% Reduction from Control:	93.2%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	5	3	5	4	3	3	5	5	43
5	11	10	13	10	11	11	12	10	12	10	110
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	14	18	14	15	18	17	12	17	155
Total	**31**	**30**	**32**	**31**	**30**	**30**	**33**	**30**	**29**	**32**	**308**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	5	5	6	4	4	4	5	46
5	12	11	13	10	10	10	10	10	12	12	110
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	14	14	15	13	17	14	16	17	153
Total	**32**	**33**	**31**	**29**	**30**	**29**	**31**	**28**	**32**	**34**	**309**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	5	5	3	5	5	5	4	46
5	10	12	12	11	11	10	13	10	11	11	111
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	14	16	19	17	13	18	14	15	158
Total	**30**	**33**	**31**	**32**	**35**	**30**	**31**	**33**	**30**	**30**	**315**

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	4	4	3	4	4	4	3	4	39
5	10	8	7	8	5	9	5	6	6	7	71
6	0	0	0	0	0	0	0	0	0	0	0
7	5	6	3	5	11	4	10	7	9	7	67
Total	**20**	**18**	**14**	**17**	**19**	**17**	**19**	**17**	**18**	**18**	**177**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	5	5	4	4	5	5	5	48
5	10	12	12	12	13	11	11	10	12	11	114
6	0	0	0	0	0	0	0	0	0	0	0
7	19	15	15	15	16	13	19	15	14	14	155
Total	**33**	**33**	**32**	**32**	**34**	**28**	**34**	**30**	**31**	**30**	**317**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	2	2	1	1	1	3	3	19
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	1	0	0	0	1	0	0	0	0	2
Total	**2**	**2**	**3**	**2**	**2**	**2**	**1**	**1**	**3**	**3**	**21**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

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

Test number: 314

Test dates: August 05-12, 2025

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	30	32	31	30	30	33	30	29	32	**100**	**30.8**	**4.0**	**Not applicable**
600	30	33	31	32	35	30	31	33	30	30	**100**	**31.5**	**5.4**	**-2.3**
800	33	33	32	32	34	28	34	30	31	30	**100**	**31.7**	**6.1**	**-2.9**
1000	32	33	31	29	30	29	31	28	32	34	**100**	**30.9**	**6.2**	**-0.3**
1200	20	18	14	17	19	17	19	17	18	18	**100**	**17.7**	**9.2**	**42.5**
1400	2	2	3	2	2	2	1	1	3	3	**100**	**2.1**	**35.1**	**93.2**

Dunnett's MSD value: 1.623

PMSD: 5.3

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%.

Upper PMSD bound determined by USEPA (90th 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 Summer

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	8/5/2025	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	8/12/2025	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	30.000	32.000	31.000	30.000	30.000	33.000	30.000	29.000	32.000
600	30.000	33.000	31.000	32.000	35.000	30.000	31.000	33.000	30.000	30.000
800	33.000	33.000	32.000	32.000	34.000	28.000	34.000	30.000	31.000	30.000
1000	32.000	33.000	31.000	29.000	30.000	29.000	31.000	28.000	32.000	34.000
1200	20.000	18.000	14.000	17.000	19.000	17.000	19.000	17.000	18.000	18.000
1400	2.000	2.000	3.000	2.000	2.000	2.000	1.000	1.000	3.000	3.000

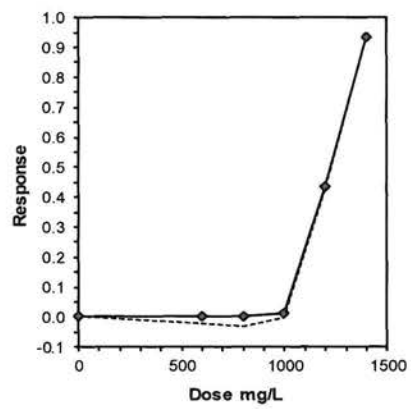
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	30.800	1.0000	30.800	29.000	33.000	3.991	10				31.333	1.0000
600	31.500	1.0227	31.500	30.000	35.000	5.447	10	-0.986	2.287	1.623	31.333	1.0000
800	31.700	1.0292	31.700	28.000	34.000	6.140	10	-1.268	2.287	1.623	31.333	1.0000
1000	30.900	1.0032	30.900	28.000	34.000	6.188	10	-0.141	2.287	1.623	30.900	0.9862
*1200	17.700	0.5747	17.700	14.000	20.000	9.245	10	18.451	2.287	1.623	17.700	0.5649
*1400	2.100	0.0682	2.100	1.000	3.000	35.136	10	40.424	2.287	1.623	2.100	0.0670

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.56261	1.035	-0.1123	0.10413
Bartlett's Test indicates equal variances (p = 0.11)	9.04435	15.0863		

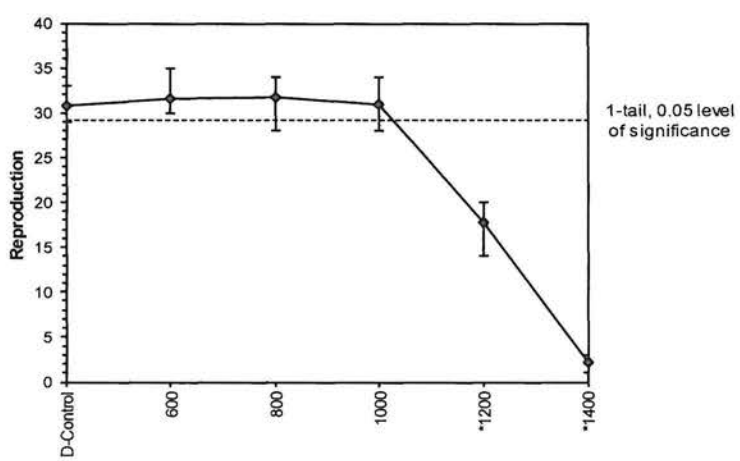
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.62349	0.05271	1457.22	2.52037	1.4E-45	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1017.17	12.6802	993.449	1024.06	-5.9826
IC10	1040.91	6.36064	1025.46	1048.35	-0.9472
IC15	1064.65	6.01704	1050.79	1072.79	-0.7349
IC20	1088.38	5.90182	1074.37	1097.22	-0.5088
IC25	1112.12	6.02808	1098.12	1121.66	-0.3307
IC40	1183.33	7.61971	1167.87	1197.47	-0.1585
IC50	1226.07	6.23606	1212.13	1236.39	-0.4513



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 314**

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.)	7.99	7.81	7.74	7.76	7.76	7.86
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.4	8.0
	Conductivity (µmhos/cm)	297		299		298	
	Alkalinity (mg $CaCO_3$/L)	63				63	
	Hardness (mg $CaCO_3$/L)	91			08-11-24	91	
	Temperature (°C)	24.9	25.3	24.9	25.3	24.8	25.0
600 mg NaCl/L	pH (S.U.)	8.00	7.80	7.84	7.77	7.83	7.88
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.4	8.0
	Conductivity (µmhos/cm)	1310		1370		1380	
	Temperature (°C)	24.9	25.0	25.0	25.1	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.99	7.81	7.83	7.77	7.83	7.88
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.4	7.9
	Conductivity (µmhos/cm)	1650		1750		1760	
	Temperature (°C)	25.0	25.0	24.9	24.9	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	8.00	7.81	7.83	7.77	7.83	7.89
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.4	7.9
	Conductivity (µmhos/cm)	1990		2090		2110	
	Temperature (°C)	25.0	24.9	24.9	24.9	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	8.00	7.80	7.84	7.77	7.83	7.89
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.1	8.4	7.9
	Conductivity (µmhos/cm)	2330		2450		2450	
	Temperature (°C)	25.0	24.9	24.9	25.0	25.0	24.8
1400 mg NaCl/L	pH (S.U.)	8.00	7.81	7.84	7.76	7.84	7.90
	Dissolved oxygen (mg/L)	8.4	8.1	8.3	8.0	8.4	7.9
	Conductivity (µmhos/cm)	2650		2750		2760	
	Temperature (°C)	25.0	25.1	25.0	25.0	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final

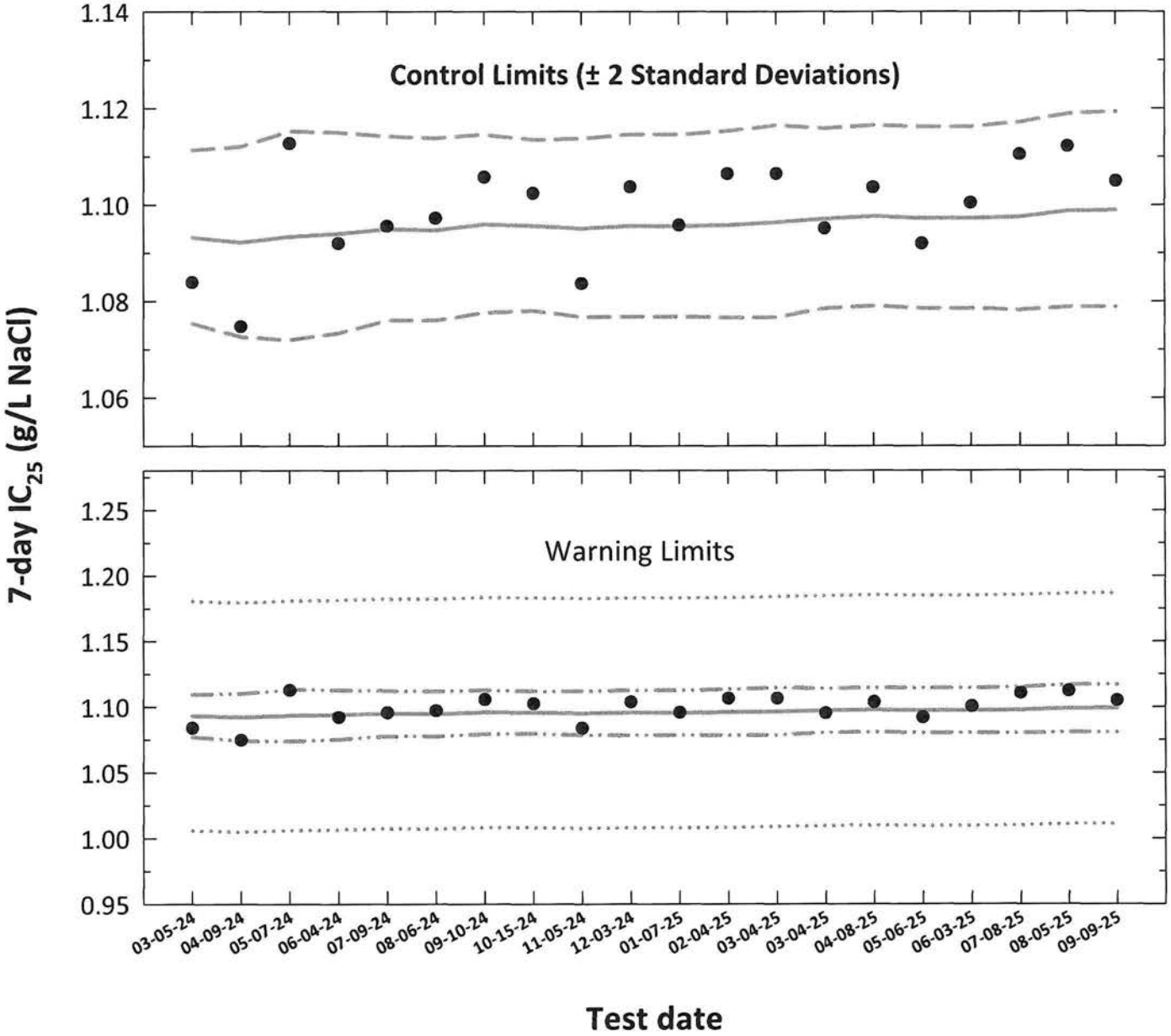
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 314

		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	[illegible]
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.87	7.98	7.88	7.99	7.87	7.89	7.84	7.91
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	8.0	8.4	8.1	8.3	7.6
	Conductivity (µmhos/cm)	301		290		292		299	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)		02-11-25 [illegible]	91					05-11-25 [illegible]
	Temperature (°C)	24.8	25.1	24.9	25.3	24.9	25.1	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.94	7.97	8.02	8.00	7.89	7.90	7.93	7.92
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1370		1350		1360		1370	
	Temperature (°C)	24.9	24.9	25.0	24.9	25.0	24.8	25.0	25.2
800 mg NaCl/L	pH (S.U.)	7.94	7.97	8.02	8.00	7.98	7.91	7.92	7.94
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.3	7.6
	Conductivity (µmhos/cm)	1730		1710		1710		1760	
	Temperature (°C)	24.9	24.9	24.9	24.9	25.0	24.8	25.0	25.1
1000 mg NaCl/L	pH (S.U.)	7.95	7.97	8.02	8.00	7.99	7.91	7.95	7.94
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2070		2060		2070		2120	
	Temperature (°C)	24.8	25.1	24.9	25.1	25.0	24.8	24.9	25.1
1200 mg NaCl/L	pH (S.U.)	7.96	7.97	8.00	8.00	7.99	7.92	7.96	7.94
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2440		2420		2410		2490	
	Temperature (°C)	24.9	24.8	25.0	25.2	25.2	25.1	25.0	25.3
1400 mg NaCl/L	pH (S.U.)	7.97	7.97	8.00	8.00	8.00	7.93	7.98	7.95
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.2	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2800		2710		2480		2800	
	Temperature (°C)	24.9	25.0	25.0	25.0	24.9	24.9	25.0	25.2
		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 $IC_{25} \pm S_{A.10}$ 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	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
2	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
3	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
4	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
5	07-09-24	1.0956	0.0396	0.0394	0.0038	1.0949	1.0760	1.1142	1.0777	1.1122	1.0073	1.1825
6	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
7	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
8	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
9	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
10	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
11	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

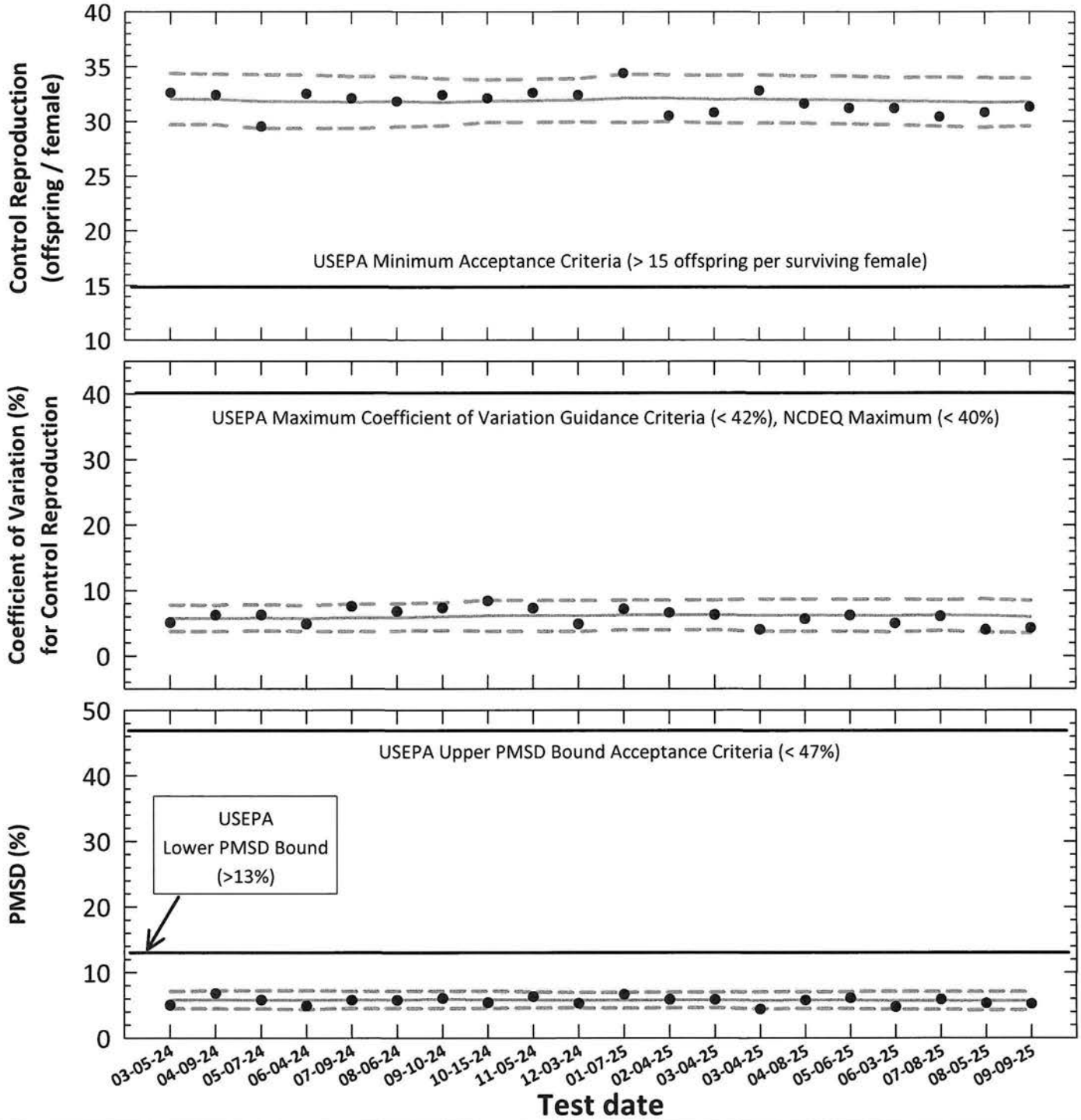
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

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 Mean (offspring/female)	Control Reproduction CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
2	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
3	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
4	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
5	07-09-24	100	32.1	7.6	1.846	5.8	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5	7.1
6	08-06-24	100	31.8	6.8	1.827	5.7	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
7	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
8	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
9	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
10	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
11	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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 #: 315

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2411				
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:	09-09-25 0530 TO 0743									
Culture board:	09-02-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	3	4	8	11	12	14	18	20	21	22
Transfer vessel information:	pH (S.U.): 7.91 Temperature (°C): 24.9									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	09-09-25	0755	08-28-25	08-29-25	09-04-25 A	JH
1	09-10-25	0640	↓	↓	↓	JH
2	09-11-25	0630	↓	↓	09-04-25 B	JH
3	09-12-25	0630	↓	↓	↓	JH
4	09-13-25	0740	↓	↓	09-04-25 E	JH
5	09-14-25	0738	↓	↓	↓	JH
6	09-15-25	0652	↓	↓	↓	JH
7	09-16-25	0600				JH

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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:	31.3	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.37%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1104.9

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 315**

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	4	6	4	4	4	6	6	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	12	11	14	12	10	10	10	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	16	14	14	15	18	16	15	15	16
Total young produced		30	31	36	31	33	34	30	31	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.3

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	6	5	5	5	6	6	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	13	12	11	12	10	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	13	15	14	14	17	13	17	15	15
Total young produced		32	27	34	31	30	34	29	33	31	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.3
% Reduction from Control:	0.0

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 315**

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	4	5	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	10	13	12	12	11	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	18	15	16	14	16	17	15	16	16
Total young produced		31	33	31	30	31	33	34	30	33	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.8
% Reduction from Control:	-1.6%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.0
% Reduction from Control:	4.7%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 315**

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	2	5	4	3	3	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	5	10	8	4	11	6	9	9	7
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	9	4	4	10	5	11	6	4	7
Total young produced		17	18	16	17	18	19	20	18	17	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:	17.8
% Reduction from Control:	43.1%

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	1	0	2	2	3	1	3	1	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	2	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	2	1	1	1	0	0	1
Total young produced		2	1	2	4	3	4	2	3	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:	2.2
% Reduction from Control:	93.0%

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	4	6	4	4	4	6	6	3	46
5	12	10	12	11	14	12	10	10	10	13	114
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	14	14	15	18	16	15	15	16	153
Total	**30**	**31**	**30**	**31**	**33**	**34**	**30**	**31**	**31**	**32**	**313**

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	6	4	4	4	3	5	5	5	3	3	42
5	10	10	10	12	10	11	13	10	10	10	106
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	14	14	16	13	15	15	17	15	152
Total	**34**	**29**	**28**	**30**	**29**	**29**	**33**	**30**	**30**	**28**	**300**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	6	5	5	5	6	6	4	5	51
5	12	10	13	12	11	12	10	10	12	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	15	14	14	17	13	17	15	15	148
Total	**32**	**27**	**34**	**31**	**30**	**34**	**29**	**33**	**31**	**32**	**313**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	2	5	4	3	3	3	4	4	35
5	9	5	10	8	4	11	6	9	9	7	78
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	4	4	10	5	11	6	4	7	65
Total	**17**	**18**	**16**	**17**	**18**	**19**	**20**	**18**	**17**	**18**	**178**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	4	4	5	5	4	5	5	45
5	12	11	12	10	13	12	12	11	12	11	116
6	0	0	0	0	0	0	0	0	0	0	0
7	14	18	15	16	14	16	17	15	16	16	157
Total	**31**	**33**	**31**	**30**	**31**	**33**	**34**	**30**	**33**	**32**	**318**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	0	2	2	3	1	3	1	1	16
5	0	0	2	0	0	0	0	0	0	0	2
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	2	1	1	1	0	0	1	6
Total	**2**	**1**	**2**	**4**	**3**	**4**	**2**	**3**	**1**	**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: 315

Test dates: September 09-16, 2025

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	30	31	30	31	33	34	30	31	31	32	**100**	**31.3**	**4.3**	**Not applicable**
600	32	27	34	31	30	34	29	33	31	32	**100**	**31.3**	**7.1**	**0.0**
800	31	33	31	30	31	33	34	30	33	32	**100**	**31.8**	**4.4**	**-1.6**
1000	34	29	28	30	29	29	33	30	30	28	**100**	**30.0**	**6.7**	**4.2**
1200	17	18	16	17	18	19	20	18	17	18	**100**	**17.8**	**6.4**	**43.1**
1400	2	1	2	4	3	4	2	3	1	2	**100**	**2.4**	**44.8**	**92.3**

Dunnett's MSD value: 1.622

PMSD: 5.2

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Upper PMSD bound determined by USEPA (90th 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:	9/9/2025	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	9/16/2025	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	30.000	31.000	30.000	31.000	33.000	34.000	30.000	31.000	31.000	32.000
600	32.000	27.000	34.000	31.000	30.000	34.000	29.000	33.000	31.000	32.000
800	31.000	33.000	31.000	30.000	31.000	33.000	34.000	30.000	33.000	32.000
1000	34.000	29.000	28.000	30.000	29.000	29.000	33.000	30.000	30.000	28.000
1200	17.000	18.000	16.000	17.000	18.000	19.000	20.000	18.000	17.000	18.000
1400	2.000	1.000	2.000	4.000	3.000	4.000	2.000	3.000	1.000	2.000

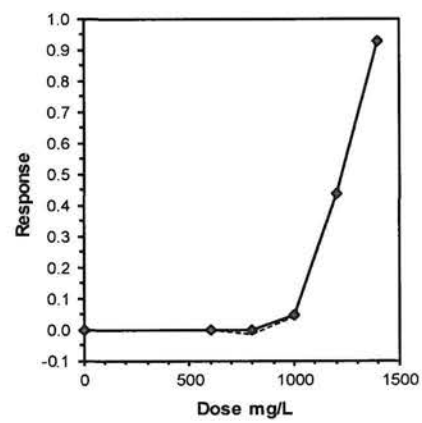
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.300	1.0000	31.300	30.000	34.000	4.273	10				31.467	1.0000
600	31.300	1.0000	31.300	27.000	34.000	7.072	10	0.000	2.287	1.622	31.467	1.0000
800	31.800	1.0160	31.800	30.000	34.000	4.398	10	-0.705	2.287	1.622	31.467	1.0000
1000	30.000	0.9585	30.000	28.000	34.000	6.667	10	1.833	2.287	1.622	30.000	0.9534
*1200	17.800	0.5687	17.800	16.000	20.000	6.378	10	19.036	2.287	1.622	17.800	0.5657
*1400	2.400	0.0767	2.400	1.000	4.000	44.790	10	40.750	2.287	1.622	2.400	0.0763

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.89619	1.035	0.26029	0.52632
Bartlett's Test indicates equal variances (p = 0.17)	7.82519	15.0863		

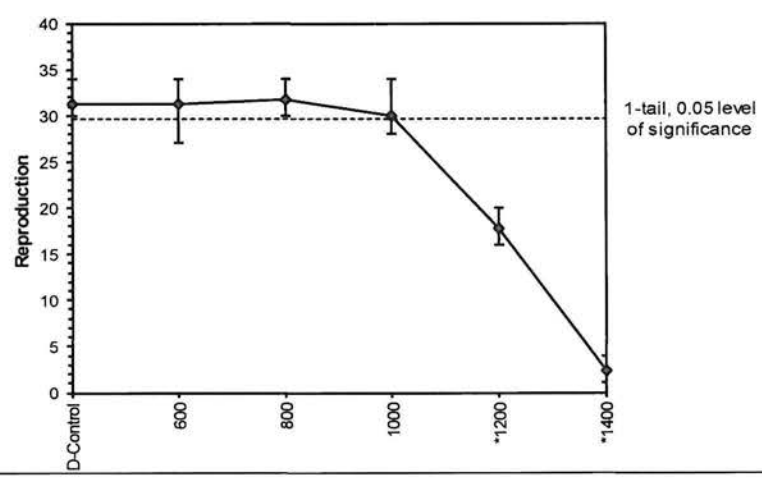
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.6217	0.05181	1416.72	2.51481	2.8E-45	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1001.75	32.6954	904.595	1018.8	-0.9365
IC10	1027.54	8.96355	1009.28	1043.02	-0.0724
IC15	1053.33	7.85637	1036.52	1067.91	-0.0122
IC20	1079.13	6.89146	1064.32	1091.38	0.0292
IC25	1104.92	6.1363	1092.2	1115.41	0.0332
IC40	1182.3	5.87316	1170.84	1192.48	0.1558
IC50	1226.84	4.26774	1217.57	1233.98	0.0145



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 315**

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.)	7.97	7.87	7.81	7.79	7.78	7.85
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.3	8.0
	Conductivity (µmhos/cm)	307		306		305	
	Alkalinity (mg $CaCO_3$/L)	60				63	
	Hardness (mg $CaCO_3$/L)	84			09-15-25	86	
	Temperature (°C)	24.9	25.1	24.8	25.3	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.97	7.87	7.90	7.83	7.87	7.84
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.0	8.3	8.0
	Conductivity (µmhos/cm)	1420		1400		1390	
	Temperature (°C)	25.0	25.0	24.9	25.0	25.0	25.1
800 mg NaCl/L	pH (S.U.)	7.96	7.86	7.89	7.83	7.87	7.85
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.0	8.3	7.9
	Conductivity (µmhos/cm)	1790		1790		1780	
	Temperature (°C)	24.9	25.0	24.9	25.2	25.0	24.9
1000 mg NaCl/L	pH (S.U.)	7.96	7.86	7.90	7.83	7.88	7.86
	Dissolved oxygen (mg/L)	8.4	7.9	8.2	8.0	8.3	7.9
	Conductivity (µmhos/cm)	2130		2140		2140	
	Temperature (°C)	24.9	25.2	24.9	25.2	24.8	24.9
1200 mg NaCl/L	pH (S.U.)	7.96	7.85	7.90	7.83	7.89	7.86
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.0	8.3	7.9
	Conductivity (µmhos/cm)	2500		2490		2480	
	Temperature (°C)	25.0	25.2	25.0	25.2	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.97	7.85	7.90	7.84	7.90	7.85
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.0	8.3	7.9
	Conductivity (µmhos/cm)	2840		2850		2820	
	Temperature (°C)	25.0	25.1	24.8	25.3	24.8	25.0
		Initial	Final	Initial	Final	Initial	Final

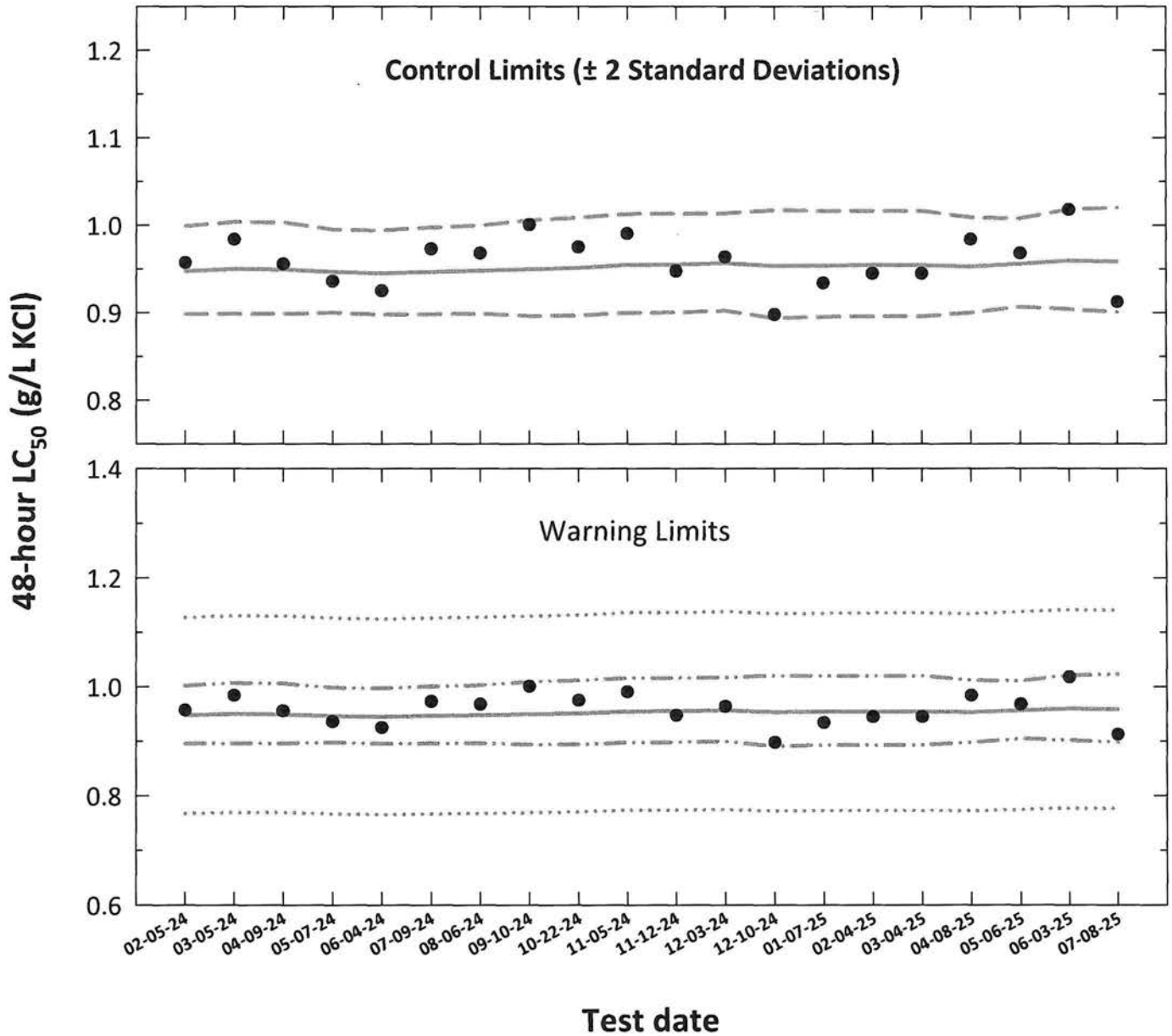
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 315

		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	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.78	7.94	7.83	7.98	7.84	7.91	7.83	8.05
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.5	8.0	8.3	7.5
	Conductivity (µmhos/cm)	303		298		289		304	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)	K	09-15-25 K	88				K	09-15-25 K
	Temperature (°C)	24.8	25.3	24.7	25.2	24.8	25.3	24.9	25.3
600 mg NaCl/L	pH (S.U.)	7.86	7.94	7.86	7.97	7.96	7.95	7.94	8.01
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.3	8.0	8.3	7.5
	Conductivity (µmhos/cm)	1380		1390		1380		1420	
	Temperature (°C)	24.9	25.1	24.7	25.0	24.9	25.2	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.86	7.95	7.93	7.98	8.00	7.94	7.97	7.99
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.3	7.5
	Conductivity (µmhos/cm)	1780		1730		1720		1810	
	Temperature (°C)	25.0	24.9	24.8	24.9	24.9	25.2	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.86	7.95	7.95	7.98	8.00	7.94	7.99	7.97
	Dissolved oxygen (mg/L)	8.3	8.1	8.3	8.0	8.3	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2130		2080		2060		2170	
	Temperature (°C)	24.9	25.2	24.8	24.9	25.0	25.1	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.87	7.94	7.95	7.98	8.00	7.94	7.99	7.97
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.4	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2470		2440		2440		2550	
	Temperature (°C)	24.9	24.9	24.8	25.1	25.0	25.3	25.0	25.4
1400 mg NaCl/L	pH (S.U.)	7.87	7.94	7.96	7.97	8.01	7.94	8.00	7.96
	Dissolved oxygen (mg/L)	8.3	8.0	8.3	8.0	8.3	8.0	8.3	7.5
	Conductivity (µmhos/cm)	2840		2820		2800		2940	
	Temperature (°C)	24.9	24.9	24.7	24.8	25.0	25.2	24.8	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	02-05-24	0.9572	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
2	03-05-24	0.9839	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
3	04-09-24	0.9556	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
4	05-07-24	0.9358	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263
5	06-04-24	0.9251	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970	0.7653	1.1244
6	07-09-24	0.9729	-0.0119	-0.0238	0.0114	0.9467	0.8985	0.9975	0.8958	1.0004	0.7668	1.1266
7	08-06-24	0.9680	-0.0141	-0.0232	0.0115	0.9481	0.8991	0.9998	0.8964	1.0026	0.7679	1.1282
8	09-10-24	1.0007	0.0003	-0.0224	0.0125	0.9496	0.8964	1.0060	0.8936	1.0090	0.7692	1.1301
9	10-22-24	0.9751	-0.0109	-0.0217	0.0128	0.9513	0.8970	1.0088	0.8942	1.0118	0.7705	1.1320
10	11-05-24	0.9904	-0.0042	-0.0201	0.0128	0.9548	0.9000	1.0130	0.8974	1.0157	0.7734	1.1362
11	11-12-24	0.9473	-0.0235	-0.0200	0.0128	0.9551	0.9005	1.0130	0.8979	1.0157	0.7736	1.1366
12	12-03-24	0.9637	-0.0160	-0.0193	0.0127	0.9565	0.9023	1.0138	0.8998	1.0165	0.7747	1.1382
13	12-10-24	0.8977	-0.0469	-0.0207	0.0141	0.9534	0.8936	1.0172	0.8906	1.0203	0.7722	1.1345
14	01-07-25	0.9340	-0.0296	-0.0204	0.0138	0.9541	0.8955	1.0165	0.8927	1.0196	0.7728	1.1354
15	02-04-25	0.9448	-0.0247	-0.0203	0.0137	0.9543	0.8958	1.0166	0.8930	1.0196	0.7730	1.1356
16	03-04-25	0.9448	-0.0247	-0.0203	0.0137	0.9543	0.8958	1.0166	0.8930	1.0196	0.7730	1.1356
17	04-08-25	0.9839	-0.0070	-0.0209	0.0124	0.9530	0.9003	1.0089	0.8977	1.0117	0.7720	1.1341
18	05-06-25	0.9680	-0.0141	-0.0195	0.0115	0.9560	0.9068	1.0079	0.9045	1.0103	0.7744	1.1377
19	06-03-25	1.0177	0.0076	-0.0180	0.0129	0.9595	0.9040	1.0183	0.9017	1.0208	0.7772	1.1418
20	07-08-25	0.9124	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406

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 # 180

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 #: 2394

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XPXL	XL	XL
Control, MHSW	pH (S.U.)	7.76	7.79	7.88
	Dissolved oxygen (mg/L)	8.5	8.2	8.0
	Conductivity (µmhos/cm)	KL 07 06 22 ~~269~~ 298		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.8	24.6	24.6
500 mg/L	pH (S.U.)	7.85	7.85	7.86
	Dissolved oxygen (mg/L)	8.6	8.3	8.0
	Conductivity (µmhos/cm)	1120		
	Temperature (°C)	24.5	24.4	24.8
750 mg/L	pH (S.U.)	7.86	7.85	7.86
	Dissolved oxygen (mg/L)	8.6	8.2	8.0
	Conductivity (µmhos/cm)	1500		
	Temperature (°C)	24.3	24.4	24.7
1000 mg/L	pH (S.U.)	7.88	7.84	7.86
	Dissolved oxygen (mg/L)	8.6	8.2	8.0
	Conductivity (µmhos/cm)	1890		
	Temperature (°C)	24.4	24.6	24.7
1250 mg/L	pH (S.U.)	7.90	7.84	
	Dissolved oxygen (mg/L)	8.6	8.2	
	Conductivity (µmhos/cm)	2270		
	Temperature (°C)	24.6	24.3	
1500 mg/L	pH (S.U.)	7.91	7.85	KL 07-10-25
	Dissolved oxygen (mg/L)	8.6	8.2	
	Conductivity (µmhos/cm)	2460		
	Temperature (°C)	24.7	24.5	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	170604 6

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 # 180

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-08-25	*0505	ZP	0846	ZP	1B	orange	07-01-25B
24	07-09-25			0839	ZP			
48 Termination	07-10-25			0841	ZP			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	06-25-25
Age (1 to 14 days old):	5 to 6 days old
Hatch date and times:	07-02-25 1150 to 07-03-25 0850
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.01
	Temperature (°C): 25.2 C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 d	9 d	2 bd	4 bd	0 bd	0 bd	0 bd	0 bd
48 Termination	10	10	10	10	9	9	2	4	0	0	0	0
Mean Survival	100%		100%		90%		30%		0%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	847.5
Upper 95% confidence limit (mg KCl/L)	975.2
48-hour LC_{50} (mg KCl/L)	912.4

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:	7/8/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/10/2025	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	0.2000	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	0.1992	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.331	2.850	0.1992	2	20
*1000	0.3000	0.3000	0.5742	0.4636	0.6847	27.225	2	11.985	2.850	0.1992	14	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	17.927	2.850	0.1992	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

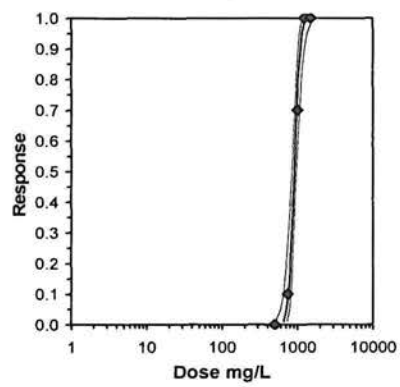
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.0978	0.1003	0.64149	0.00489	3.0E-05	4, 5
Treatments vs D-Control										

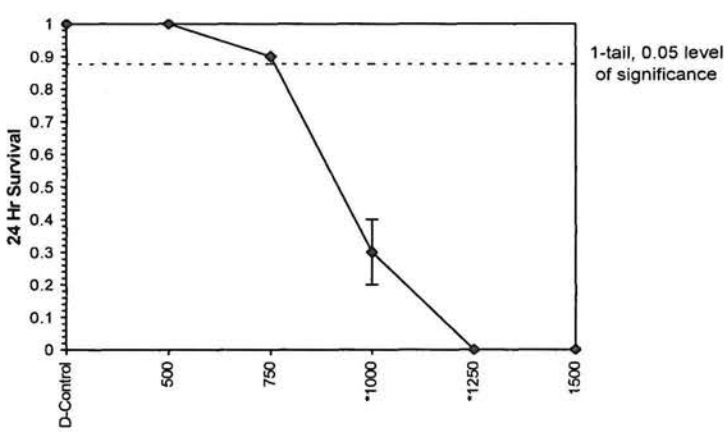
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.0964	3.17807	9.86736	22.3254	0	0.50143	7.81472	0.91858	2.96018	0.06213	4
Intercept	-42.648	9.43861	-61.148	-24.148							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	654.111	515.891	732.334
EC05	3.355	721.091	601.92	789.44
EC10	3.718	759.557	652.568	822.857
EC15	3.964	786.663	688.474	846.999
EC20	4.158	808.894	717.855	867.366
EC25	4.326	828.466	743.518	885.874
EC40	4.747	879.91	808.87	938.273
EC50	5.000	912.384	847.483	975.214
EC60	5.253	946.057	884.606	1017.43
EC75	5.674	1004.8	942.214	1100.65
EC80	5.842	1029.12	963.845	1138.19
EC85	6.036	1058.2	988.454	1185.05
EC90	6.282	1095.96	1018.82	1248.57
EC95	6.645	1154.42	1063.35	1351.84
EC99	7.326	1272.64	1147.78	1575.21



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	7/8/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/10/2025	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	0.2000	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	0.1992	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.331	2.850	0.1992	2	20
*1000	0.3000	0.3000	0.5742	0.4636	0.6847	27.225	2	11.985	2.850	0.1992	14	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	17.927	2.850	0.1992	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

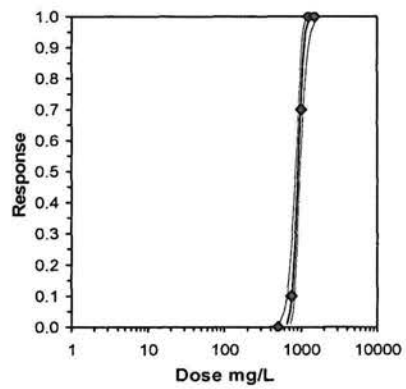
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.0978	0.1003	0.64149	0.00489	3.0E-05	4, 5
Treatments vs D-Control										

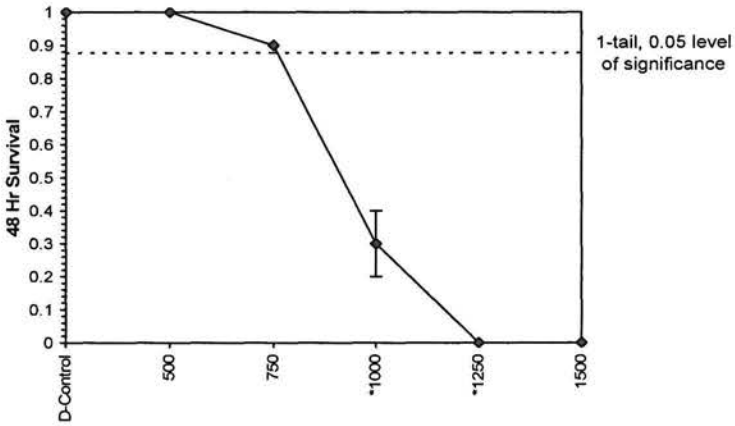
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.0964	3.17807	9.86736	22.3254	0	0.50143	7.81472	0.91858	2.96018	0.06213	4
Intercept	-42.648	9.43861	-61.148	-24.148							
TSCR											

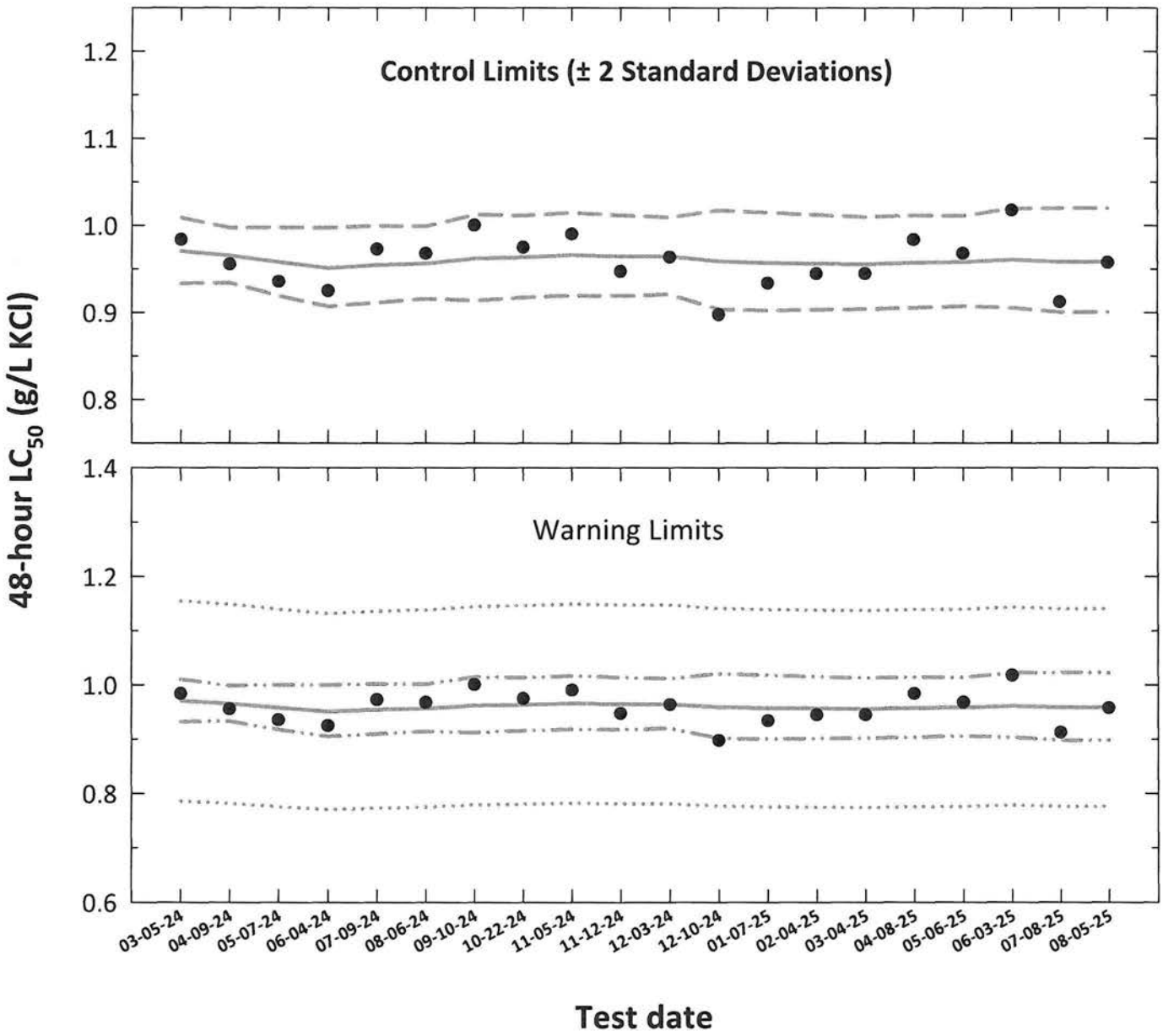
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	654.111	515.891	732.334
EC05	3.355	721.091	601.92	789.44
EC10	3.718	759.557	652.568	822.857
EC15	3.964	786.663	688.474	846.999
EC20	4.158	808.894	717.855	867.366
EC25	4.326	828.466	743.518	885.874
EC40	4.747	879.91	808.87	938.273
EC50	5.000	912.384	847.483	975.214
EC60	5.253	946.057	884.606	1017.43
EC75	5.674	1004.8	942.214	1100.65
EC80	5.842	1029.12	963.845	1138.19
EC85	6.036	1058.2	988.454	1185.05
EC90	6.282	1095.96	1018.82	1248.57
EC95	6.645	1154.42	1063.35	1351.84
EC99	7.326	1272.64	1147.78	1575.21



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	03-05-24	0.9839	-0.0070	-0.0130	0.0084	0.9705	0.9334	1.0090	0.9323	1.0101	0.7861	1.1549
2	04-09-24	0.9556	-0.0197	-0.0153	0.0071	0.9655	0.9343	0.9977	0.9332	0.9988	0.7820	1.1489
3	05-07-24	0.9358	-0.0288	-0.0187	0.0089	0.9580	0.9193	0.9982	0.9176	1.0000	0.7759	1.1400
4	06-04-24	0.9251	-0.0338	-0.0217	0.0103	0.9513	0.9073	0.9975	0.9050	0.9998	0.7705	1.1320
5	07-09-24	0.9729	-0.0119	-0.0201	0.0100	0.9549	0.9118	1.0000	0.9097	1.0021	0.7734	1.1363
6	08-06-24	0.9680	-0.0141	-0.0192	0.0094	0.9567	0.9161	0.9992	0.9142	1.0011	0.7749	1.1385
7	09-10-24	1.0007	0.0003	-0.0168	0.0111	0.9621	0.9141	1.0127	0.9122	1.0147	0.7793	1.1449
8	10-22-24	0.9751	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
9	11-05-24	0.9904	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
10	11-12-24	0.9473	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
11	12-03-24	0.9637	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
12	12-10-24	0.8977	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
13	01-07-25	0.9340	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
14	02-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
15	03-04-25	0.9448	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
16	04-08-25	0.9839	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
17	05-06-25	0.9680	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
18	06-03-25	1.0177	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
19	07-08-25	0.9124	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
20	08-05-25	0.9576	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406

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 # 181

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 #: 2394

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	VL	XL	XL
Control, MHSW	pH (S.U.)	7.99	7.86	7.82
	Dissolved oxygen (mg/L)	8.4	8.3	8.0
	Conductivity (µmhos/cm)	297		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	87		
	Temperature (°C)	25.4	24.8	25.3
500 mg/L	pH (S.U.)	8.00	7.83	7.79
	Dissolved oxygen (mg/L)	8.4	8.3	8.0
	Conductivity (µmhos/cm)	1130		
	Temperature (°C)	25.4	24.9	25.2
750 mg/L	pH (S.U.)	8.00	7.82	7.80
	Dissolved oxygen (mg/L)	8.4	8.3	8.0
	Conductivity (µmhos/cm)	1520		
	Temperature (°C)	25.5	24.6	25.2
1000 mg/L	pH (S.U.)	8.01	7.82	7.79
	Dissolved oxygen (mg/L)	8.4	8.2	8.0
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	25.5	25.0	25.4
1250 mg/L	pH (S.U.)	8.01	7.80	7.80
	Dissolved oxygen (mg/L)	8.4	8.1	8.0
	Conductivity (µmhos/cm)	2320		
	Temperature (°C)	25.6	25.0	25.2
1500 mg/L	pH (S.U.)	8.01	7.80	
	Dissolved oxygen (mg/L)	8.4	8.1	08.03.24
	Conductivity (µmhos/cm)	2630		
	Temperature (°C)	25.6	25.1	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664

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 # 181

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-06-25	* 0505	JP	0830	JP	15	Yellow	07-31-25A
24	08-06-25			0830	JP			
48 Termination	08-07-25			0815	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:	07-24-25
Age (1 to 14 days old):	5 to 6 days old
Hatch date and times:	07-30-25 1143 to 07-31-25 0833
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.21
	Temperature (°C): 25.2 °C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 d	9 d	6 d	4 6d	1 9d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	9	9	~~4 2d~~ 08-07-25 4	4	1	1 1d	0	0
Mean Survival	100%		100%		90%		40%		10%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	880.8
Upper 95% confidence limit (mg KCl/L)	1030.0
48-hour LC_{50} (mg KCl/L)	957.6

Comments:

Test Reviewed by: JP

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	8/5/2025	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	8/7/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.6000	0.4000
1250	0.1000	0.2000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2220	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.092	2.850	0.2220	2	20
*1000	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	8.044	2.850	0.2220	10	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	13.085	2.850	0.2220	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

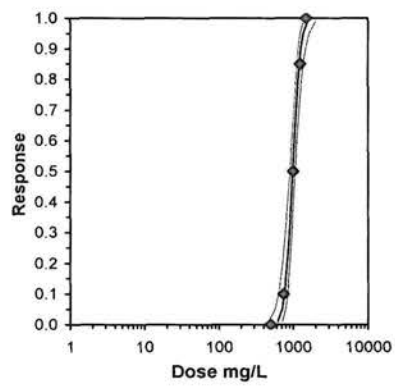
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.11312	0.11603	0.40189	0.00607	1.6E-04	4, 5
Treatments vs D-Control										

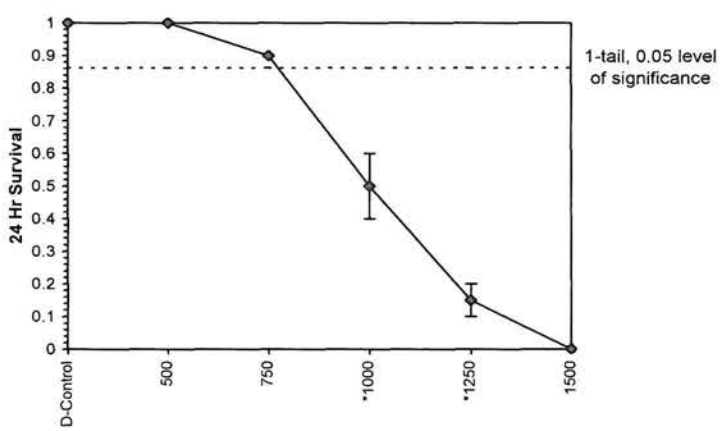
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.4402	2.01873	7.48343	15.3969	0	0.63348	7.81472	0.88873	2.9958	0.08741	4
Intercept	-29.272	6.08432	-41.198	-17.347							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	620.087	467.401	716.82
EC05	3.355	711.251	573.63	797.621
EC10	3.718	765.208	638.861	845.602
EC15	3.964	803.907	686.331	880.468
EC20	4.158	836.054	725.958	909.957
EC25	4.326	864.657	761.169	936.784
EC40	4.747	941.145	853.511	1012.84
EC50	5.000	990.38	909.943	1066.71
EC60	5.253	1042.19	965.457	1128.85
EC75	5.674	1134.38	1053.83	1253.79
EC80	5.842	1173.19	1087.71	1311.21
EC85	6.036	1220.11	1126.74	1383.72
EC90	6.282	1281.81	1175.65	1483.43
EC95	6.645	1379.05	1248.87	1648.82
EC99	7.326	1581.8	1392.33	2019.65



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	8/5/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/7/2025	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	0.4000	0.4000
1250	0.1000	0.1000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0285	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	16.297	2.850	0.0285	2	20
*1000	0.4000	0.4000	0.6847	0.6847	0.6847	0.000	2	72.730	2.850	0.0285	12	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	109.027	2.850	0.0285	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

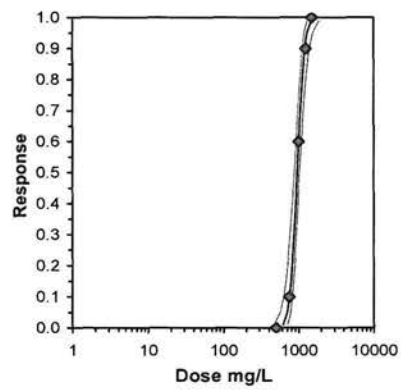
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.00967	0.00991	0.47985	0.0001	3.8E-09	4, 5
Treatments vs D-Control										

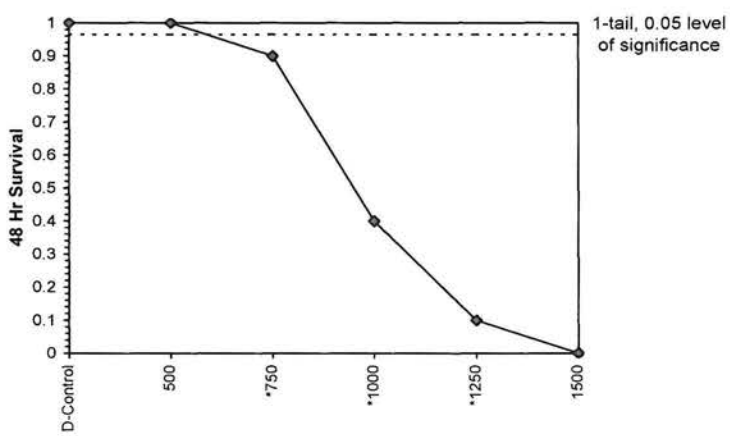
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.1839	2.18654	7.89829	16.4695	0	0.3087	7.81472	0.95838	2.98119	0.08208	3
Intercept	-31.323	6.55745	-44.175	-18.47							
TSCR											

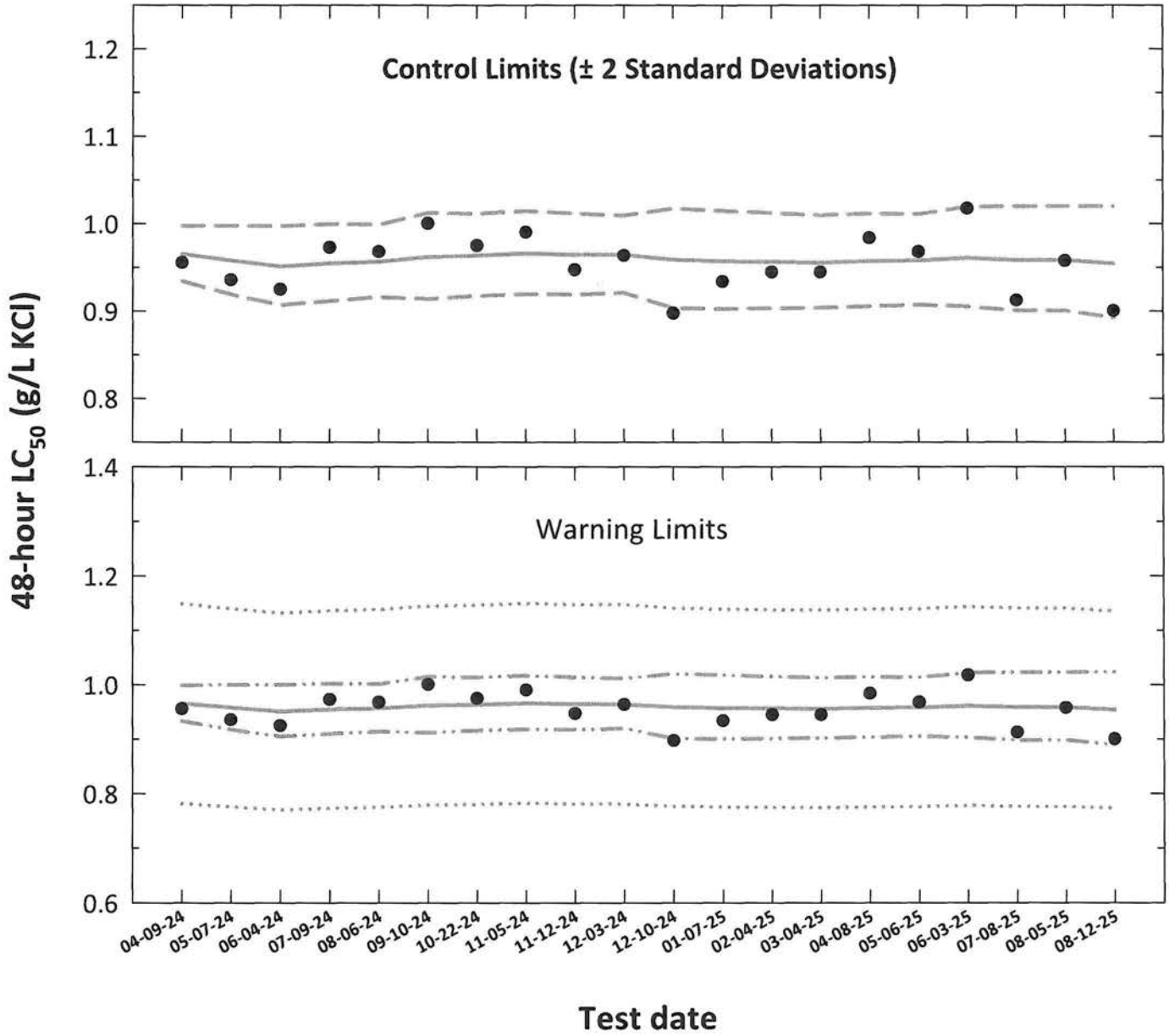
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	616.957	469.117	709.006
EC05	3.355	701.76	569.45	783.68
EC10	3.718	751.635	630.523	827.847
EC15	3.964	787.273	674.737	859.872
EC20	4.158	816.798	711.507	886.917
EC25	4.326	843.008	744.079	911.489
EC40	4.747	912.845	829.104	980.964
EC50	5.000	957.615	880.838	1029.97
EC60	5.253	1004.58	931.608	1086.29
EC75	5.674	1087.8	1012.17	1199
EC80	5.842	1122.71	1042.93	1250.62
EC85	6.036	1164.81	1078.27	1315.67
EC90	6.282	1220.04	1122.39	1404.91
EC95	6.645	1306.75	1188.1	1552.37
EC99	7.326	1486.37	1315.88	1880.59



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	04-09-24	**0.9556**	-0.0197	-0.0153	0.0071	0.9655	0.9343	0.9977	0.9332	0.9988	0.7820	1.1489
2	05-07-24	**0.9358**	-0.0288	-0.0187	0.0089	0.9580	0.9193	0.9982	0.9176	1.0000	0.7759	1.1400
3	06-04-24	**0.9251**	-0.0338	-0.0217	0.0103	0.9513	0.9073	0.9975	0.9050	0.9998	0.7705	1.1320
4	07-09-24	**0.9729**	-0.0119	-0.0201	0.0100	0.9549	0.9118	1.0000	0.9097	1.0021	0.7734	1.1363
5	08-06-24	**0.9680**	-0.0141	-0.0192	0.0094	0.9567	0.9161	0.9992	0.9142	1.0011	0.7749	1.1385
6	09-10-24	**1.0007**	0.0003	-0.0168	0.0111	0.9621	0.9141	1.0127	0.9122	1.0147	0.7793	1.1449
7	10-22-24	**0.9751**	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
8	11-05-24	**0.9904**	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
9	11-12-24	**0.9473**	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
10	12-03-24	**0.9637**	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
11	12-10-24	**0.8977**	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
12	01-07-25	**0.9340**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
13	02-04-25	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
14	03-04-25	**0.9448**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
15	04-08-25	**0.9839**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
16	05-06-25	**0.9680**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
17	06-03-25	**1.0177**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
18	07-08-25	**0.9124**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
19	08-05-25	**0.9576**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
20	08-12-25	**0.9003**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356

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 # 182

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 #: 2412

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	K	K	XL
Control, MHSW	pH (S.U.)	8.11	7.83	7.72
	Dissolved oxygen (mg/L)	8.3	8.2	8.3
	Conductivity (µmhos/cm)	301		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	85		
	Temperature (°C)	24.3	25.3	25.4
500 mg/L	pH (S.U.)	8.03	7.83	7.79
	Dissolved oxygen (mg/L)	8.3	8.2	8.3
	Conductivity (µmhos/cm)	1090		
	Temperature (°C)	24.1	25.1	25.1
750 mg/L	pH (S.U.)	8.03	7.84	7.80
	Dissolved oxygen (mg/L)	8.3	8.2	8.3
	Conductivity (µmhos/cm)	1500		
	Temperature (°C)	24.9	25.4	25.2
1000 mg/L	pH (S.U.)	8.04	7.83	7.80
	Dissolved oxygen (mg/L)	8.3	8.2	8.3
	Conductivity (µmhos/cm)	1880		
	Temperature (°C)	24.9	25.0	25.0
1250 mg/L	pH (S.U.)	8.06	7.83	7.81
	Dissolved oxygen (mg/L)	8.4	8.1	8.2
	Conductivity (µmhos/cm)	2300		
	Temperature (°C)	25.0	25.1	25.3
1500 mg/L	pH (S.U.)	8.07	7.83	
	Dissolved oxygen (mg/L)	8.4	8.0	08-15-25
	Conductivity (µmhos/cm)	2600		
	Temperature (°C)	24.9	25.3	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664

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 # 182

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-12-25	* 0812	2P	1037	2P	1B	orange	~~08-09-25~~ 08-09-25c 2P 08-14-25
24	08-13-25			1028	2P			
48 Termination	08-14-25			1032	2P			

*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:	07-30-25
Age (1 to 14 days old):	6 to 7 days old
Hatch date and times:	08-05-25 1200 to 08-06-25 0905
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.84
	Temperature (°C): 24.4 °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 d	1 d	1 d	0 d	0 d
48 Termination	10	10	10	9 d	9	8 d	4 d	3 d	1	1	0	0
Mean Survival	100%		95%		85%		35%		10%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	812.5
Upper 95% confidence limit (mg KCl/L)	982.9
48-hour LC_{50} (mg KCl/L)	900.3

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:	8/12/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/14/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.6000	0.4000
1250	0.1000	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	0.1815	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.559	2.850	0.1815	2	20
*1000	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	9.841	2.850	0.1815	10	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	17.122	2.850	0.1815	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

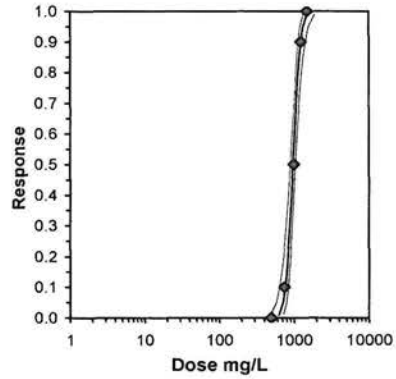
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08637	0.08859	0.45056	0.00405	4.5E-05	4, 5
Treatments vs D-Control										

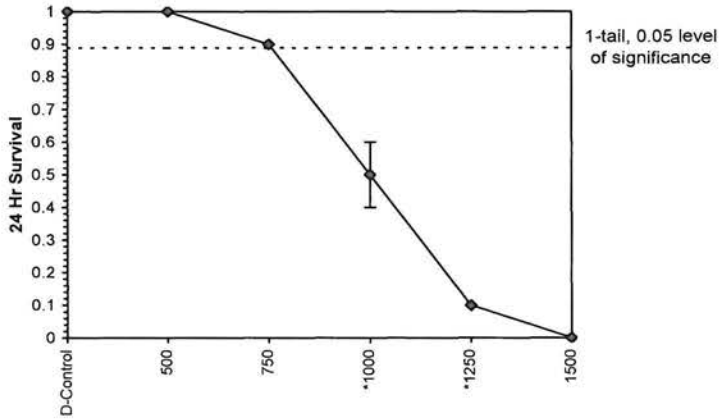
Maximum Likelihood-Probit

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

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



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	8/12/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/14/2025	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	0.9000
750	0.9000	0.8000
1000	0.4000	0.3000
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	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.072	2.850	0.2166	1	20
*750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	3.079	2.850	0.2166	3	20
*1000	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	10.263	2.850	0.2166	13	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	14.349	2.850	0.2166	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

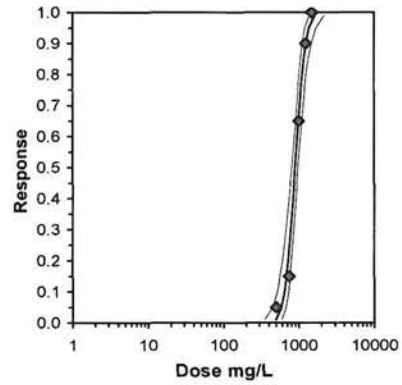
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.10938	0.11219	0.45145	0.00577	1.1E-04	4, 5
Treatments vs D-Control										

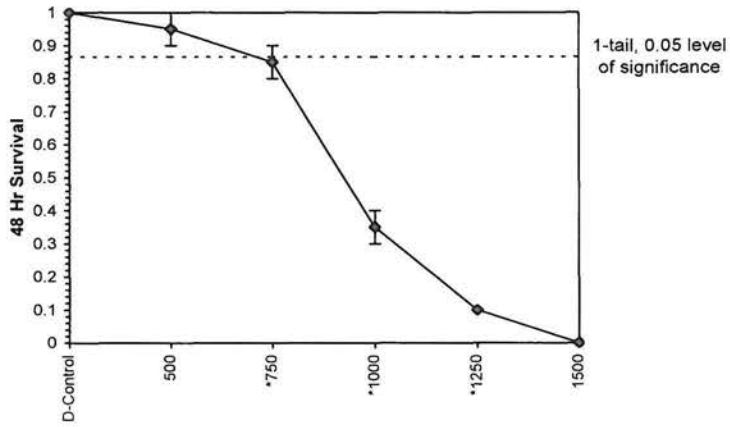
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	8.86602	1.48763	5.95027	11.7818	0	3.92776	7.81472	0.26937	2.95439	0.11279	4
Intercept	-21.194	4.43773	-29.892	-12.496							
TSCR											

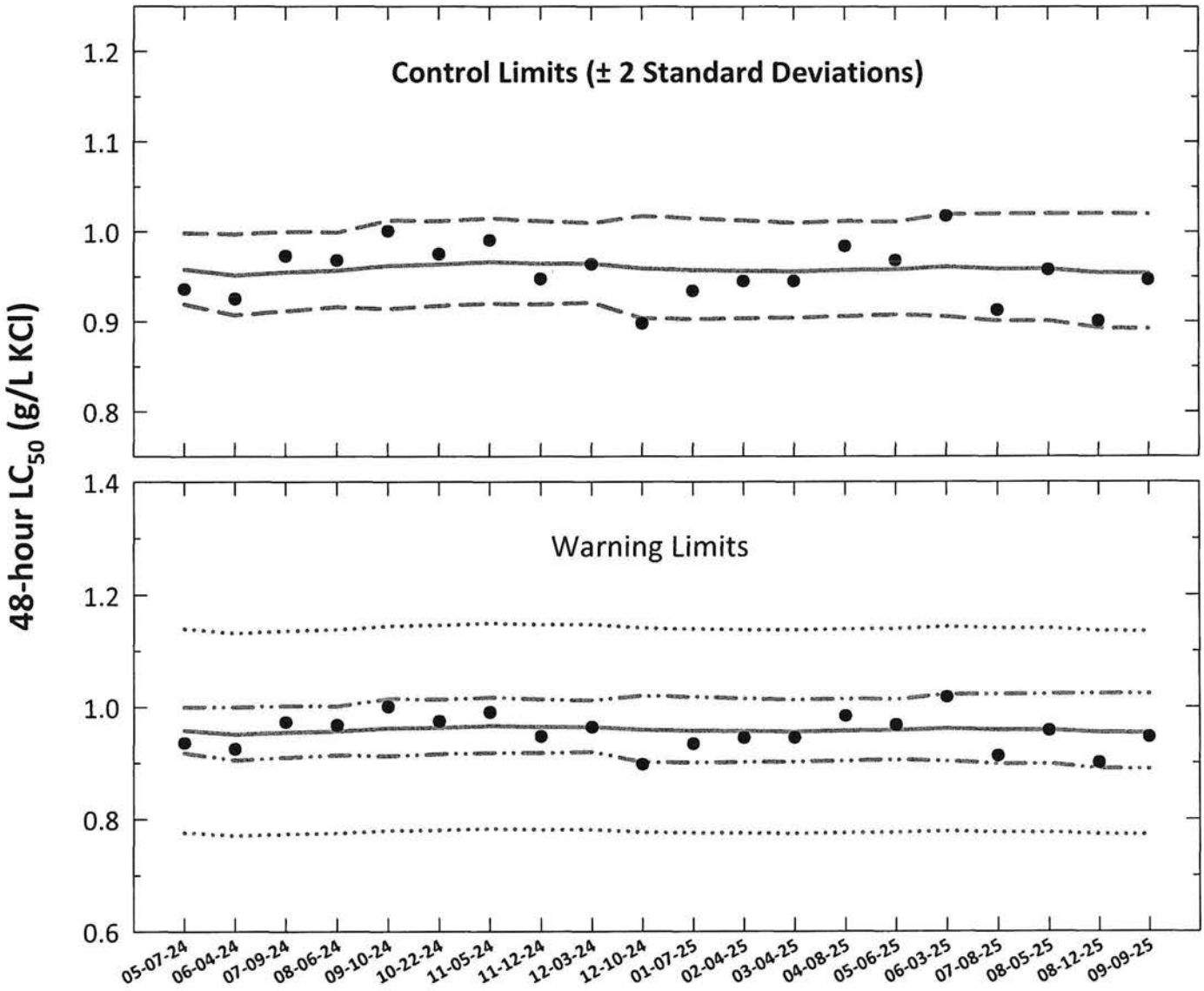
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	492.038	349.245	589.937
EC05	3.355	587.306	452.136	677.706
EC10	3.718	645.418	518.008	730.915
EC15	3.964	687.841	567.164	770.005
EC20	4.158	723.537	608.974	803.302
EC25	4.326	755.635	646.717	833.752
EC40	4.747	842.971	748.408	920.736
EC50	5.000	900.301	812.546	982.893
EC60	5.253	961.53	877.103	1055.32
EC75	5.674	1072.66	982.54	1203.95
EC80	5.842	1120.25	1023.72	1273.65
EC85	6.036	1178.38	1071.66	1362.86
EC90	6.282	1255.84	1132.45	1487.59
EC95	6.645	1380.1	1224.95	1699.34
EC99	7.326	1647.32	1411.14	2193.83



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**05-07-24**	**0.9358**	-0.0288	-0.0187	0.0089	0.9580	0.9193	0.9982	0.9176	1.0000	0.7759	1.1400
2	**06-04-24**	**0.9251**	-0.0338	-0.0217	0.0103	0.9513	0.9073	0.9975	0.9050	0.9998	0.7705	1.1320
3	**07-09-24**	**0.9729**	-0.0119	-0.0201	0.0100	0.9549	0.9118	1.0000	0.9097	1.0021	0.7734	1.1363
4	**08-06-24**	**0.9680**	-0.0141	-0.0192	0.0094	0.9567	0.9161	0.9992	0.9142	1.0011	0.7749	1.1385
5	**09-10-24**	**1.0007**	0.0003	-0.0168	0.0111	0.9621	0.9141	1.0127	0.9122	1.0147	0.7793	1.1449
6	**10-22-24**	**0.9751**	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
7	**11-05-24**	**0.9904**	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
8	**11-12-24**	**0.9473**	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
9	**12-03-24**	**0.9637**	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
10	**12-10-24**	**0.8977**	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
11	**01-07-25**	**0.9340**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
12	**02-04-25**	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
13	**03-04-25**	**0.9448**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
14	**04-08-25**	**0.9839**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
15	**05-06-25**	**0.9680**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
16	**06-03-25**	**1.0177**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
17	**07-08-25**	**0.9124**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
18	**08-05-25**	**0.9576**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
19	**08-12-25**	**0.9003**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
20	**09-09-25**	**0.9465**	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351

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

PpKCIAC # 183

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 #: 2412

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.97	7.83	7.78
	Dissolved oxygen (mg/L)	8.3	8.2	8.0
	Conductivity (µmhos/cm)	307		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	24.3	24.7	24.6
500 mg/L	pH (S.U.)	7.96	7.84	7.79
	Dissolved oxygen (mg/L)	8.4	8.2	8.0
	Conductivity (µmhos/cm)	1170		
	Temperature (°C)	24.6	24.9	24.9
750 mg/L	pH (S.U.)	7.96	7.86	7.82
	Dissolved oxygen (mg/L)	8.4	8.2	8.0
	Conductivity (µmhos/cm)	1590		
	Temperature (°C)	24.2	24.6	24.9
1000 mg/L	pH (S.U.)	7.96	7.86	7.83
	Dissolved oxygen (mg/L)	8.4	8.2	7.9
	Conductivity (µmhos/cm)	2020		
	Temperature (°C)	24.2	24.6	25.0
1250 mg/L	pH (S.U.)	7.97	7.86	7.85
	Dissolved oxygen (mg/L)	8.4	8.2	7.9
	Conductivity (µmhos/cm)	2430		
	Temperature (°C)	24.5	24.6	24.6
1500 mg/L	pH (S.U.)	7.97	7.85	
	Dissolved oxygen (mg/L)	8.4	8.2	11-09-21
	Conductivity (µmhos/cm)	2840		
	Temperature (°C)	24.5	24.8	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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

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 # 183

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09.04.25	* 0500	H	0834	JP	7F	GREEN	09.04.25A
24	09.10.25			0840	JP			
48 Termination	09.11.25			0839	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:	08-27-25
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	09.03.25 1215 TO 09.04.25 0850
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.12
	Temperature (°C): 24.1°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 12	6 4d	5 5d	1 9d	1 9d	10 10d	0 10d
48 Termination	10	10	10	10	9 12	9	5 12	3 22	0 1d	1	0	0
Mean Survival	100%		100%		90%		40%		5%		0%	

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

Statistics:

Method	Probit
Lower 95% confidence limit (mg KCl/L)	873.7
Upper 95% confidence limit (mg KCl/L)	1015.4
48-hour LC_{50} (mg KCl/L)	946.5

Comments:

Test Reviewed by: [signature]

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	9/9/2025	Test ID:	PpKClAC	Sample ID:	PpKClAC
End Date:	9/11/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.6000	0.5000
1250	0.1000	0.1000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.345	2.850	0.1726	1	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	9.513	2.850	0.1726	9	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	17.998	2.850	0.1726	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

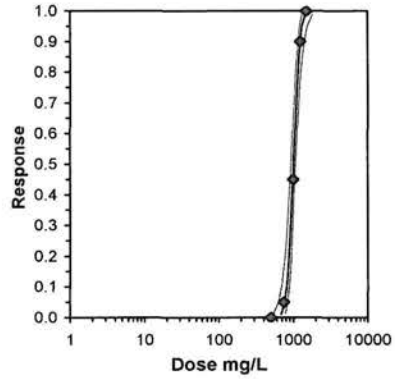
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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

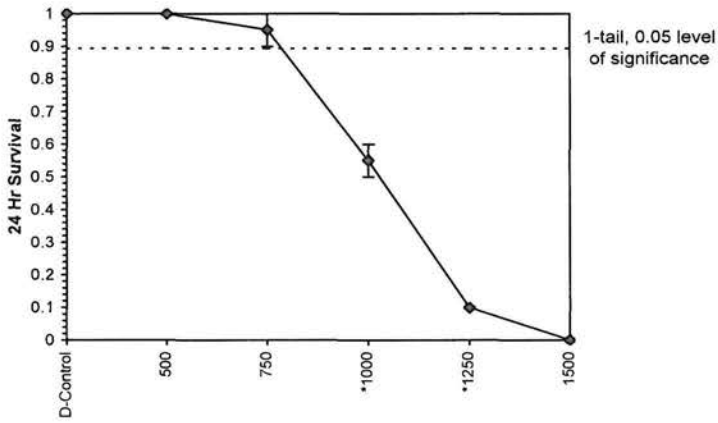
Maximum Likelihood-Probit

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

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



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	9/9/2025	Test ID:	PpKClAC	Sample ID:	PpKClAC
End Date:	9/11/2025	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	0.3000
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	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	1.963	2.850	0.2366	2	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	8.789	2.850	0.2366	12	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

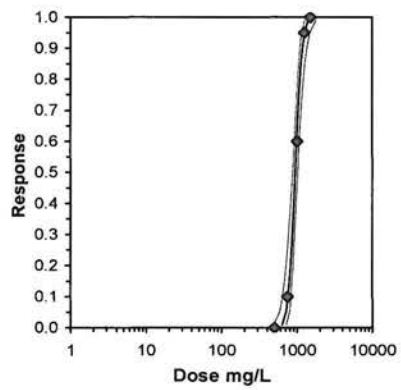
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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	0.53976	0.00689	1.1E-04	4, 5
Treatments vs D-Control										

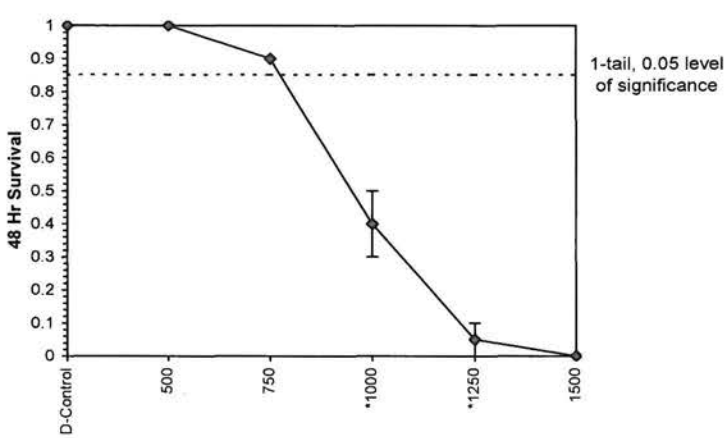
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.3529	2.46093	8.52944	18.1763	0	0.16871	7.81472	0.98248	2.9761	0.07489	3
Intercept	-34.74	7.36144	-49.168	-20.311							
TSCR											

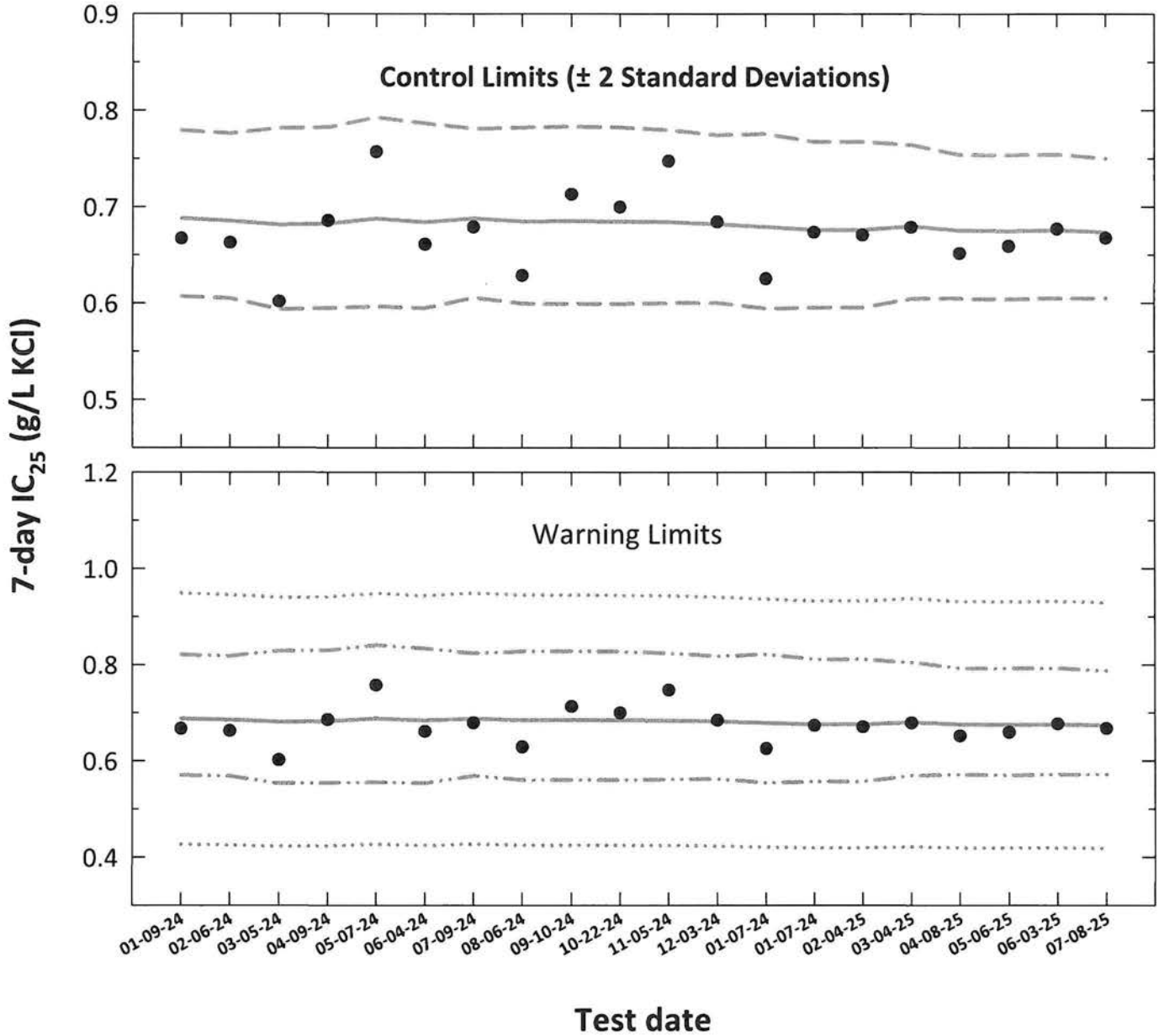
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	633.699	488.728	721.416
EC05	3.355	712.723	584.63	790.25
EC10	3.718	758.802	642.309	830.774
EC15	3.964	791.563	683.76	860.096
EC20	4.158	818.606	718.039	884.832
EC25	4.326	842.542	748.26	907.289
EC40	4.747	906.006	826.509	970.723
EC50	5.000	946.464	873.679	1015.39
EC60	5.253	988.729	919.679	1066.57
EC75	5.674	1063.2	992.165	1168.39
EC80	5.842	1094.29	1019.69	1214.76
EC85	6.036	1131.68	1051.21	1273
EC90	6.282	1180.54	1090.4	1352.56
EC95	6.645	1256.86	1148.45	1483.24
EC99	7.326	1413.6	1260.33	1771.06



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

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	01-09-24	0.6671	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
2	02-06-24	0.6628	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
3	03-05-24	0.6019	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
4	04-09-24	0.6855	-0.1640	-0.1660	0.0298	0.6823	0.5949	0.7825	0.5542	0.8292	0.4230	0.9416
5	05-07-24	0.7569	-0.1210	-0.1627	0.0309	0.6876	0.5965	0.7927	0.5551	0.8404	0.4263	0.9489
6	06-04-24	0.6610	-0.1798	-0.1650	0.0303	0.6840	0.5949	0.7864	0.5537	0.8338	0.4241	0.9439
7	07-09-24	0.6789	-0.1682	-0.1624	0.0276	0.6880	0.6060	0.7811	0.5688	0.8233	0.4265	0.9494
8	08-06-24	0.6287	-0.2015	-0.1644	0.0289	0.6848	0.5994	0.7823	0.5602	0.8272	0.4246	0.9450
9	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994	0.7832	0.5600	0.8283	0.4248	0.9456
10	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
11	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
12	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
13	01-07-24	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
14	01-07-24	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
15	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
16	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
17	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
18	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
19	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
20	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

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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

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

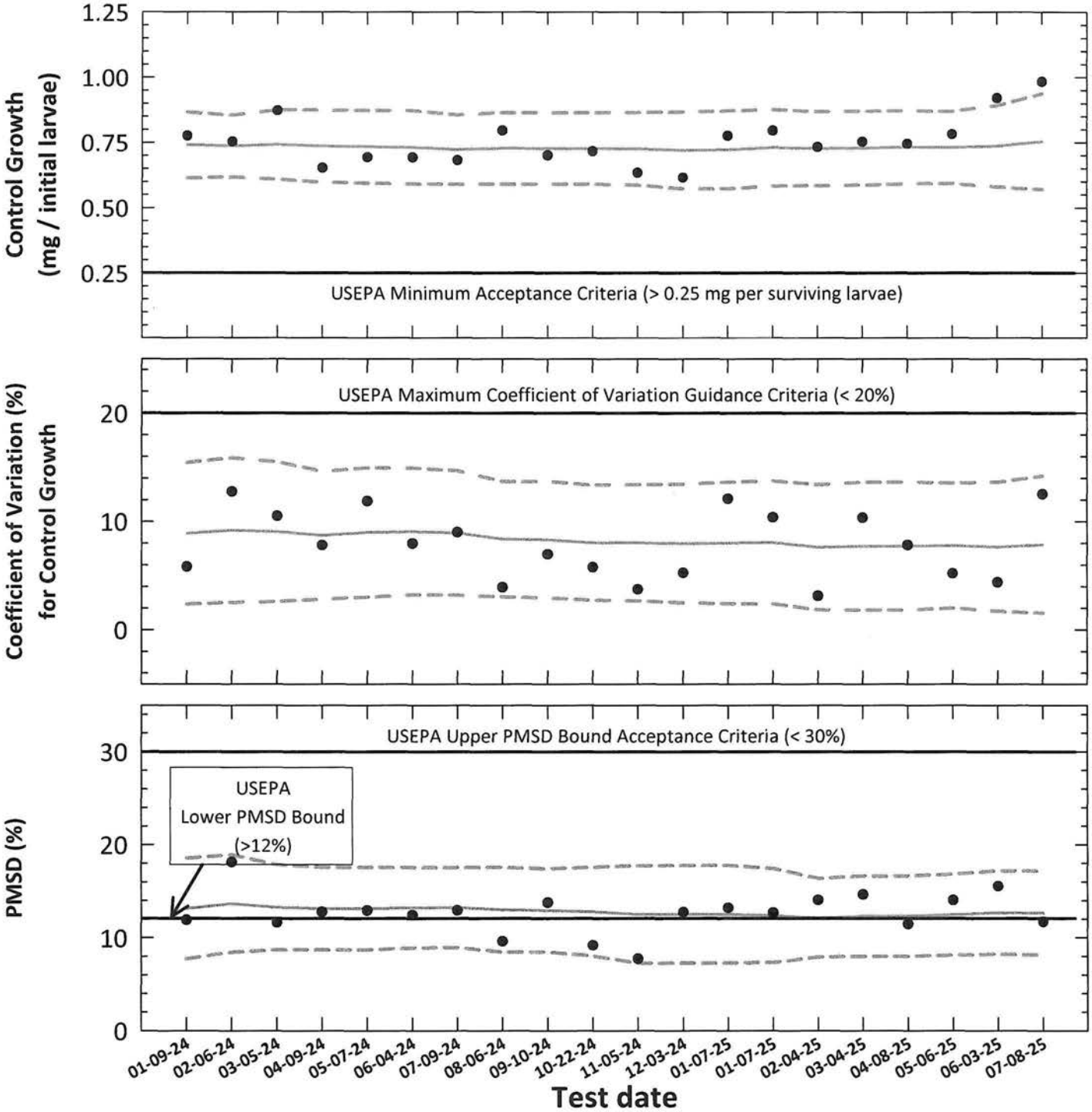
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75} = 0.38$).

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 Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	01-09-24	100	0.776	5.8	0.0923	11.9	0.740	0.614	0.866	8.9	2.4	15.4	13.1	7.7	18.6
2	02-06-24	100	0.753	12.8	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
3	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
4	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
5	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
6	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.2	8.9	17.5
7	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.2	8.9	17.5
8	08-06-24	100	0.796	3.9	0.0766	9.6	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
9	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
10	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
11	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
12	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
13	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

MSD = Minimum significant difference.

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **130**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2394				
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:	06·30·25	*Artemia* CHM number:	CHM1385
Hatch dates and times:	07·07·25 1425 TO 07·08·25 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.01 S.U. Temperature = 24.0 °C	Date / Time in oven:	07·15·25 0640
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	07·16·25 0640
		*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	07-08-25	0505	JL	1440	JL	0705	JL	07.01.25 B
1	07-09-25	0500	JL	1215	JL	0700	JL	↓
2	07-10-25	0500	JL	1215	JL	0712	JL	07-07-25 A
3	07-11-25	0500	JL	1100	JL	0710	JL	↓
4	07-12-25	0600	JL	1200	JL	0800	JL	07-07-25 B 07-07-25 JL C
5	07-13-25	0600	JL	1200	JL	0800	JL	↓
6	07-14-25	0500	JL	1100	JL	0717	JL	↓
7	07-15-25					0605	JL	

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 G-2021	YSI Model 52CE	08A100271
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:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	784.5
Average weight per initial larvae:	0.983		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.983	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	667.4

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **130**

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: Lavender Analyst: XL Date: 07-17-25	10.12	10.45	10.30	10.72	15.38	14.23	10.20	12.68	10.39	13.18	13.27	10.26
*B = Pan + Larvae weight (mg) Analyst: XL Date: 07-18-25	20.15	21.08	20.92	18.75	26.46	24.49	20.93	23.03	19.43	22.51	22.93	20.28
C = Larvae weight (mg) = B – A Analyst: JA	10.03	10.63	10.62	8.03	11.08	10.26	10.73	10.35	9.04	9.33	9.66	10.02
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JA	1.003	1.063	1.062	0.803	1.108	1.026	1.073	1.035	0.904	0.933	0.966	1.002
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.983				1.061		-7.9%		0.951		3.2%	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **130**

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	5 5d	6 4d	6 4d
2	10	10	10	10	10	9 1d	9 1d	9 1d	5 1d	4 1d	4 2d	4 2d
3	10	10	10	10	9 1d	8 1d	9	9	4 1d	4	3 1d	3 1d
4	10	10	10	10	9	8	8 1d	9	2 2d	4	3	3
5	9 1d	10	8 2d	10	8 1d	7 1d	8	8 1d	2	4	3	3
6	9	10	8	10	8	7	8	8	2	4	3	3
7	9	10	8	9 1d	7 1d	7	7 1d	6 2d	2	2 2d	3	3
*A = Pan weight (mg) Tray color code: Lavender Analyst: XL Date: 06-17-25	14.14	14.24	14.29	13.26	10.26	12.27	15.58	12.49	12.47	10.12	13.56	10.76
*B = Pan + Larvae weight (mg) Analyst: XL Date: 07-18-25	22.99	23.84	23.35	21.85	15.57	19.04	21.85	18.11	14.56	12.21	16.37	13.95
C = Larvae weight (mg) = B – A Analyst: XL	8.85	9.60	9.06	8.59	5.31	6.77	6.27	5.62	2.09	2.09	2.81	3.19
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: XL	0.885	0.960	0.906	0.859	0.531	0.677	0.627	0.562	0.209	0.209	0.281	0.319
Average weight per initial number of larvae (mg)	0.903				0.599				0.255			
Percent reduction from control (%)			8.2%				39.0%				74.1%	

*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: **130**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 sk	3 2d	2 2d	3 2d
2	2	3	2	3
3	1 1d	1 2d	1 1d	2 1d
4	1	1	1	2
5	1	0 1d	1	2
6	1	0	0 1d	0 2d
7	0 1d	0	0	0
*A = Pan weight (mg) Tray color code: Lavender Analyst: XL Date: 06-17-25	16.11	14.21	13.35	11.05
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B – A Analyst: _____				* 07-15-25
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0		100%	

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

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

Comments:

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: 130

Test dates: July 08-15, 2025

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	10.12	20.15	10.03	1.003	0.983	12.5	1.003	100.0	0.983	12.5	Not applicable
	B	10	10	10.45	21.08	10.63	1.063			1.063				
	C	10	10	10.30	20.92	10.62	1.062			1.062				
	D	10	10	10.72	18.75	8.03	0.803			0.803				
300	E	10	10	15.38	26.46	11.08	1.108	1.061	3.6	1.108	100.0	1.061	3.6	-7.9
	F	10	10	14.23	24.49	10.26	1.026			1.026				
	G	10	10	10.20	20.93	10.73	1.073			1.073				
	H	10	10	12.68	23.03	10.35	1.035			1.035				
450	I	10	10	10.39	19.43	9.04	0.904	0.951	4.4	0.904	100.0	0.951	4.4	3.2
	J	10	10	13.18	22.51	9.33	0.933			0.933				
	K	10	10	13.27	22.93	9.66	0.966			0.966				
	L	10	10	10.26	20.28	10.02	1.002			1.002				
600	M	10	9	14.14	22.99	8.85	0.983	1.008	8.4	0.885	90.0	0.903	4.8	8.2
	N	10	10	14.24	23.84	9.60	0.960			0.960				
	O	10	8	14.29	23.35	9.06	1.133			0.906				
	P	10	9	13.26	21.85	8.59	0.954			0.859				
750	Q	10	7	10.26	15.57	5.31	0.759	0.890	10.4	0.531	67.5	0.599	10.9	39.0
	R	10	7	12.27	19.04	6.77	0.967			0.677				
	S	10	7	15.58	21.85	6.27	0.896			0.627				
	T	10	6	12.49	18.11	5.62	0.937			0.562				
900	U	10	2	12.47	14.56	2.09	1.045	1.023	5.7	0.209	25.0	0.255	21.5	74.1
	V	10	2	10.12	12.21	2.09	1.045			0.209				
	W	10	3	13.56	16.37	2.81	0.937			0.281				
	X	10	3	10.76	13.95	3.19	1.063			0.319				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1150 — MSD = Minimum Significant Difference

PMSD: 11.7 — PMSD = Percent Minimum Significant Difference

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

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

Prepared and Reviewed by Jim Sommer

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	7/8/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	7/15/2025	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	0.8000	0.9000
750	0.7000	0.7000	0.7000	0.6000
900	0.2000	0.2000	0.3000	0.3000
1050	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9000	0.9000	1.2543	1.1071	1.4120	9.935	4	12.00	10.00	4	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	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

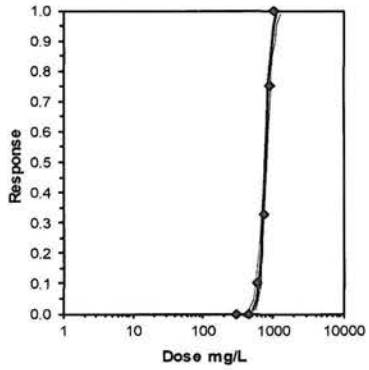
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82582	0.884	0.08772	4.07068
Equality of variance cannot be confirmed				

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

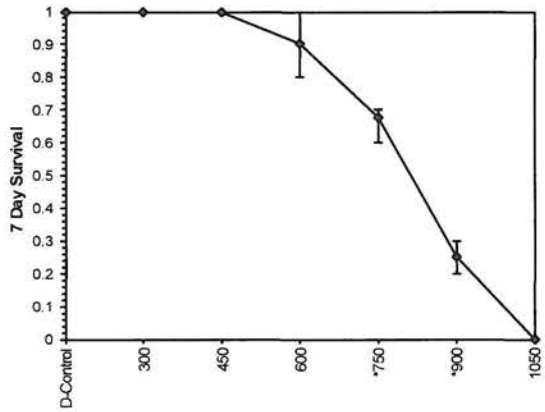
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.5527	1.67933	10.2612	16.8442	0	4.40662	9.48773	0.35376	2.89457	0.07379	4
Intercept	-34.229	4.87685	-43.788	-24.671							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	528.351	458.653	577.799
EC05	3.355	593.208	532.899	636.046
EC10	3.718	630.978	576.755	670.064
EC15	3.964	657.81	607.993	694.463
EC20	4.158	679.947	633.695	714.856
EC25	4.326	699.531	656.286	733.189
EC40	4.747	751.417	714.703	783.841
EC50	5.000	784.467	750.121	818.375
EC60	5.253	818.971	785.129	856.788
EC75	5.674	879.717	842.049	930.083
EC80	5.842	905.055	864.385	962.413
EC85	6.036	935.512	890.448	1002.33
EC90	6.282	975.294	923.51	1055.89
EC95	6.645	1037.39	973.547	1142.03
EC99	7.326	1164.73	1072.38	1326.05



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	7/8/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	7/15/2025	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:					

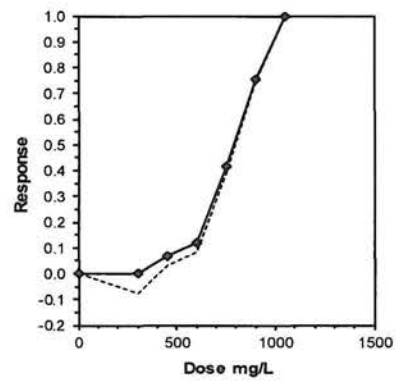
Conc-mg/L	1	2	3	4
D-Control	1.0030	1.0630	1.0620	0.8030
300	1.1080	1.0260	1.0730	1.0350
450	0.9040	0.9330	0.9660	1.0020
600	0.8850	0.9600	0.9060	0.8590
750	0.5310	0.6770	0.6270	0.5620
900	0.2090	0.2090	0.2810	0.3190
1050	0.0000	0.0000	0.0000	0.0000

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.9828	1.0000	0.9828	0.8030	1.0630	12.523	4				1.0216	1.0000
300	1.0605	1.0791	1.0605	1.0260	1.1080	3.550	4	-1.548	2.290	0.1150	1.0216	1.0000
450	0.9513	0.9679	0.9513	0.9040	1.0020	4.443	4	0.627	2.290	0.1150	0.9513	0.9311
600	0.9025	0.9183	0.9025	0.8590	0.9600	4.752	4	1.597	2.290	0.1150	0.9025	0.8834
750	0.5993	0.6098	0.5993	0.5310	0.6770	10.926	4				0.5993	0.5866
900	0.2545	0.2590	0.2545	0.2090	0.3190	21.525	4				0.2545	0.2491
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

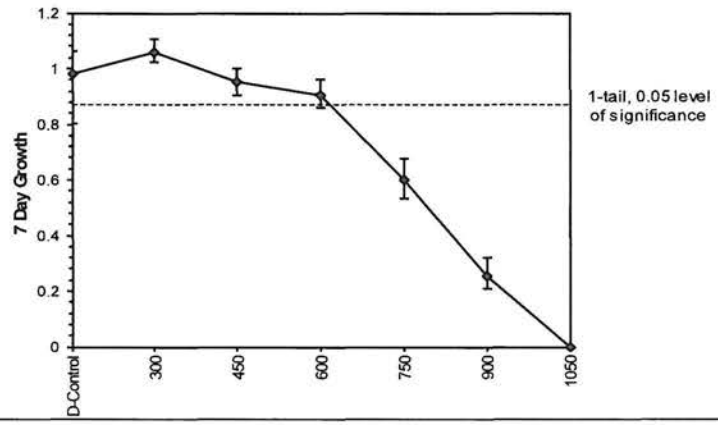
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88548	0.844	-1.3684	3.36946
Bartlett's Test indicates equal variances (p = 0.12)	5.8462	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.11504	0.11706	0.01758	0.00505	0.05024	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	408.88	61.77	321.78	723.04	1.4157
IC10	547.81	67.69	346.68	676.09	-0.3885
IC15	616.88	26.54	506.16	669.93	-1.8092
IC20	642.14	15.01	598.55	692.15	0.2362
IC25	667.41	14.31	629.69	717.63	0.3592
IC40	743.21	14.16	700.54	786.41	0.0319
IC50	788.48	11.34	751.43	821.43	-0.1717



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **130**

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.

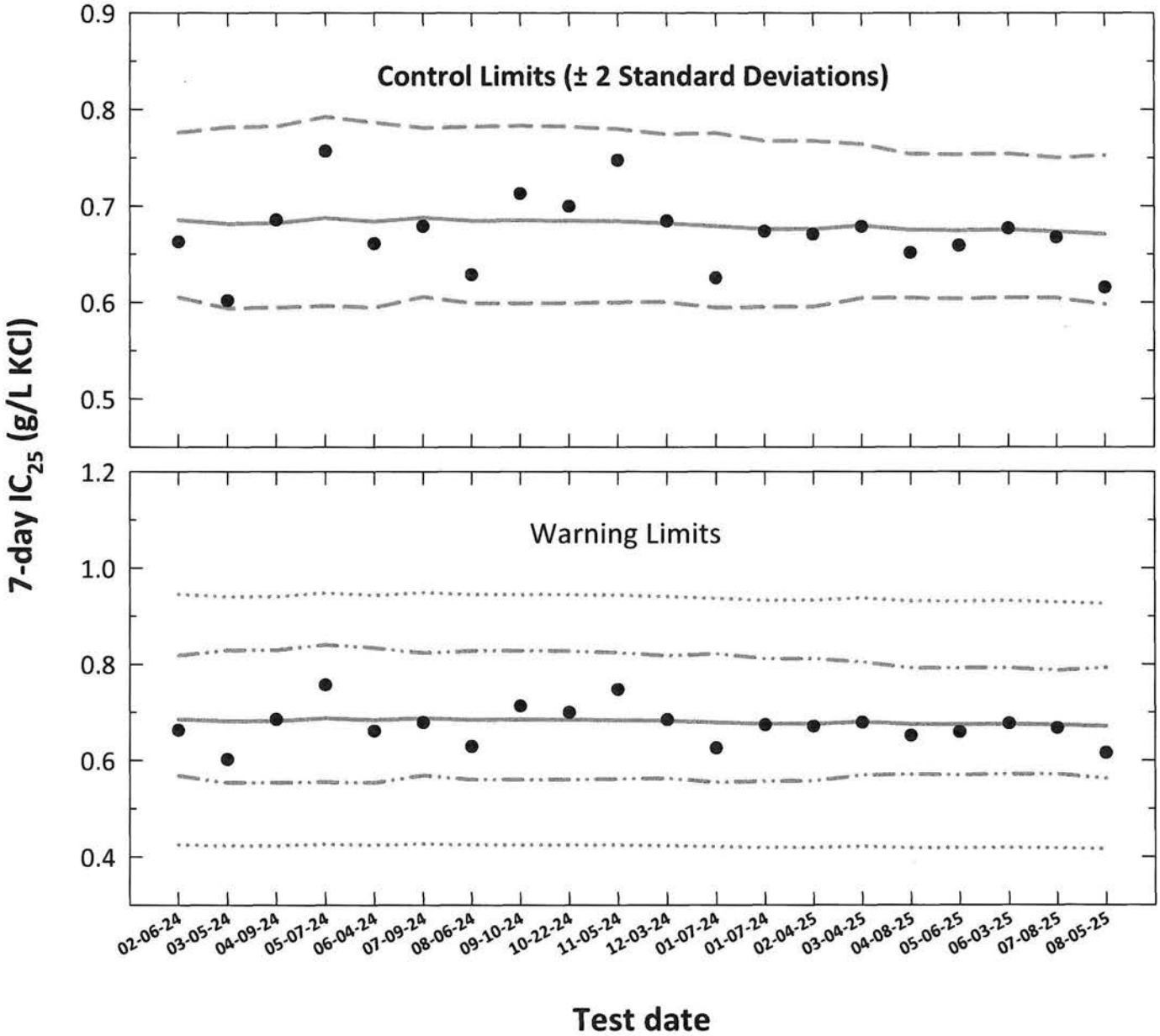
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.76	7.74	7.79	7.74	7.81	7.63
	Dissolved oxygen (mg/L)	8.5	7.9	8.4	7.8	8.3	6.4
	Conductivity (µmhos/cm)	AL 070823 ~~287~~ 298		299		306	
	Alkalinity (mg $CaCO_3$/L)	62				62	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.9	24.5	24.6	24.5	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.85	7.73	7.86	7.70	7.90	7.59
	Dissolved oxygen (mg/L)	8.6	7.8	8.4	7.6	8.3	6.6
	Conductivity (µmhos/cm)	816		810		827	
	Temperature (°C)	25.0	24.6	24.6	24.5	24.7	24.9
450 mg KCl/L	pH (S.U.)	7.87	7.76	7.87	7.71	7.91	7.57
	Dissolved oxygen (mg/L)	8.6	7.9	8.4	7.7	8.3	6.6
	Conductivity (µmhos/cm)	1040		1060		1070	
	Temperature (°C)	24.9	24.6	24.6	24.6	24.6	25.0
600 mg KCl/L	pH (S.U.)	7.87	7.77	7.88	7.72	7.90	7.57
	Dissolved oxygen (mg/L)	8.6	7.8	8.4	7.7	8.3	6.7
	Conductivity (µmhos/cm)	1290		1290		1300	
	Temperature (°C)	24.9	24.7	24.7	24.6	24.6	24.8
750 mg KCl/L	pH (S.U.)	7.89	7.77	7.89	7.71	7.91	7.58
	Dissolved oxygen (mg/L)	8.6	7.8	8.4	7.6	8.3	6.7
	Conductivity (µmhos/cm)	1510		1530		1550	
	Temperature (°C)	24.8	24.7	24.7	24.3	24.6	24.8
900 mg KCl/L	pH (S.U.)	7.90	7.76	7.89	7.74	7.91	7.57
	Dissolved oxygen (mg/L)	8.6	7.7	8.4	7.8	8.3	6.7
	Conductivity (µmhos/cm)	1760		1780		1790	
	Temperature (°C)	25.0	24.3	24.7	24.7	24.6	24.9
1050 mg KCl/L	pH (S.U.)	7.90	7.78	7.90	7.75	7.92	7.56
	Dissolved oxygen (mg/L)	8.6	7.9	8.4	7.8	8.3	6.5
	Conductivity (µmhos/cm)	2010		2050		2050	
	Temperature (°C)	25.0	24.8	24.8	24.7	24.6	24.9
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **130**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	BSL	BSL	BSL	BSL	XL	XL	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.96	7.67	7.80	7.67	7.81	7.67	7.84	7.43
	Dissolved oxygen (mg/L)	8.3	7.5	8.4	7.5	8.5	7.7	8.3	5.7
	Conductivity (µmhos/cm)	290		292		289		303	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)	n	7-15-25 N	88				n	7-15-25 N
	Temperature (°C)	24.8	24.8	24.9	24.6	24.8	24.8	24.7	24.3
300 mg KCl/L	pH (S.U.)	7.89	7.74	7.99	7.66	8.03	7.67	7.93	7.41
	Dissolved oxygen (mg/L)	8.3	7.6	8.3	7.4	8.5	7.6	8.3	5.6
	Conductivity (µmhos/cm)	830		793		781		826	
	Temperature (°C)	24.9	24.9	24.8	24.7	24.9	24.6	24.8	24.6
450 mg KCl/L	pH (S.U.)	7.89	7.79	7.99	7.65	8.03	7.66	7.94	7.41
	Dissolved oxygen (mg/L)	8.3	7.7	8.4	7.4	8.6	7.4	8.3	5.5
	Conductivity (µmhos/cm)	1020		1030		1020		1060	
	Temperature (°C)	24.9	24.9	24.8	24.7	24.8	24.5	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.90	7.80	7.99	7.62	8.04	7.65	7.95	7.40
	Dissolved oxygen (mg/L)	8.3	7.8	8.4	6.9	8.6	7.4	8.4	5.1
	Conductivity (µmhos/cm)	1250		1290		1260		1330	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.8	24.8	24.7	24.6
750 mg KCl/L	pH (S.U.)	7.92	7.71	7.99	7.62	8.04	7.64	7.96	7.40
	Dissolved oxygen (mg/L)	8.3	7.5	8.4	7.0	8.6	7.3	8.4	5.0
	Conductivity (µmhos/cm)	1480		1510		1510		1560	
	Temperature (°C)	25.0	24.7	24.9	24.6	24.8	24.6	24.7	24.7
900 mg KCl/L	pH (S.U.)	7.92	7.73	8.00	7.71	8.04	7.64	7.96	7.39
	Dissolved oxygen (mg/L)	8.4	7.5	8.4	7.3	8.6	7.3	8.4	5.0
	Conductivity (µmhos/cm)	1720		1750		1740		1810	
	Temperature (°C)	24.9	24.9	24.9	24.6	24.9	24.8	24.7	24.5
1050 mg KCl/L	pH (S.U.)	7.93	7.74	8.01	7.74	8.04	7.62	7.96	7.43
	Dissolved oxygen (mg/L)	8.4	7.7	8.4	7.6	8.6	7.1	8.4	6.0
	Conductivity (µmhos/cm)	1970		2030		2000		2090	
	Temperature (°C)	24.9	24.9	24.9	24.9	24.9	24.8	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	02-06-24	**0.6628**	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
2	03-05-24	**0.6019**	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
3	04-09-24	**0.6855**	-0.1640	-0.1660	0.0298	0.6823	0.5949	0.7825	0.5542	0.8292	0.4230	0.9416
4	05-07-24	**0.7569**	-0.1210	-0.1627	0.0309	0.6876	0.5965	0.7927	0.5551	0.8404	0.4263	0.9489
5	06-04-24	**0.6610**	-0.1798	-0.1650	0.0303	0.6840	0.5949	0.7864	0.5537	0.8338	0.4241	0.9439
6	07-09-24	**0.6789**	-0.1682	-0.1624	0.0276	0.6880	0.6060	0.7811	0.5688	0.8233	0.4265	0.9494
7	08-06-24	**0.6287**	-0.2015	-0.1644	0.0289	0.6848	0.5994	0.7823	0.5602	0.8272	0.4246	0.9450
8	09-10-24	**0.7128**	-0.1470	-0.1642	0.0290	0.6852	0.5994	0.7832	0.5600	0.8283	0.4248	0.9456
9	10-22-24	**0.6997**	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
10	11-05-24	**0.7473**	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
11	12-03-24	**0.6844**	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
12	01-07-24	**0.6255**	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
13	01-07-24	**0.6737**	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

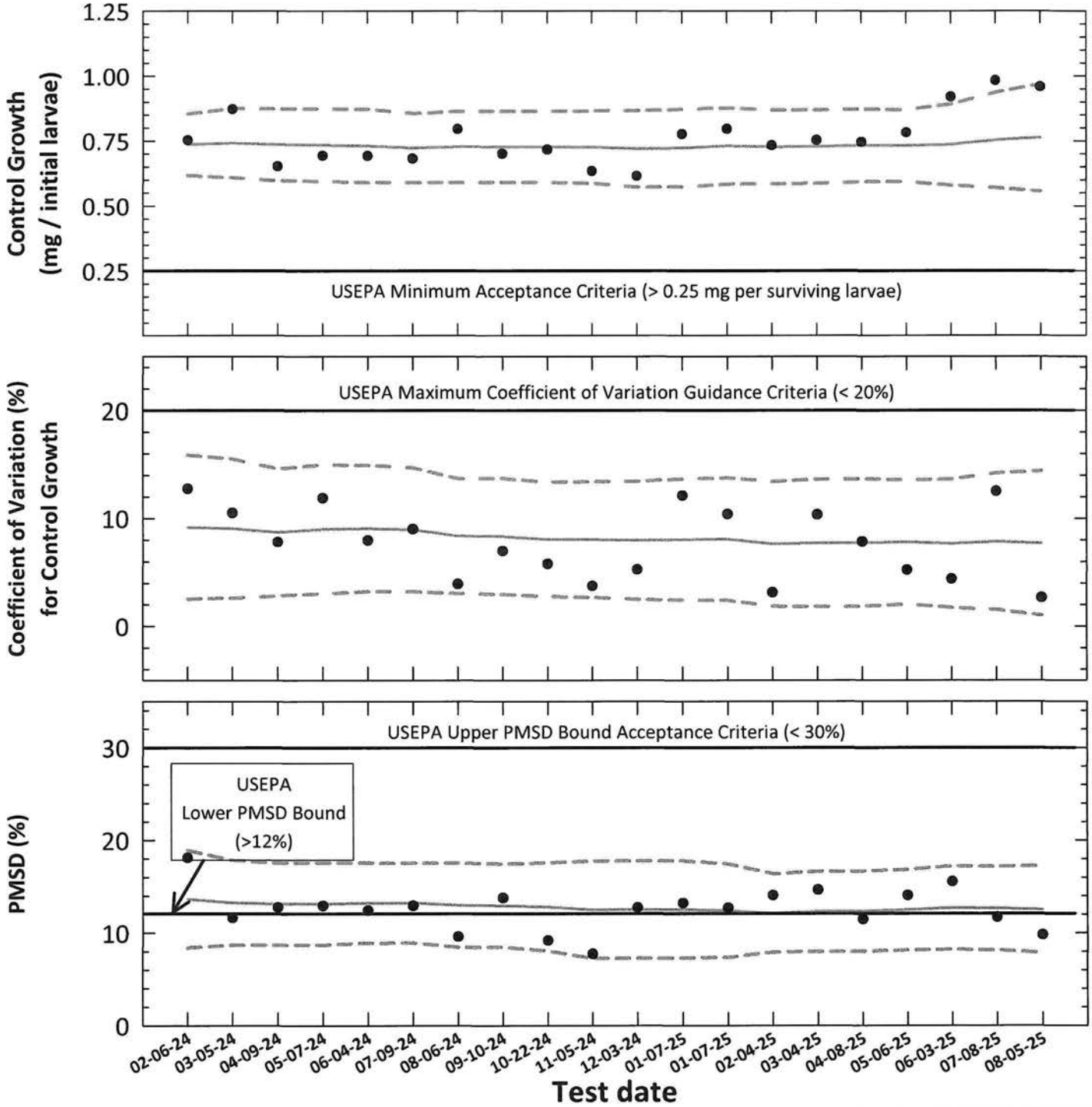
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

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 Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	02-06-24	100	0.753	12.8	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
2	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
3	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
4	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
5	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.2	8.9	17.5
6	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.2	8.9	17.5
7	08-06-24	100	0.796	3.9	0.0766	9.6	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
8	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
9	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
10	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
11	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
12	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

MSD = Minimum significant difference.

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **131**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2394				
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:	07-29-25	*Artemia* CHM number:	CHM1385
Hatch dates and times:	08-04-25 1442 TO 08-05-25 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.24 S.U. Temperature = 24.0 °C	Date / Time in oven:	08-12-25 0730
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	08-13-25 0730
		*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	08-05-25	0505	K	1430	K	0705	K	07-31-25 B
1	08-06-25	0500	K	1100	K	0700	K	↓
2	08-07-25	0500	K	1130	K	0710	K	07-31-25 C
3	08-08-25	0600	K	1100	K	0500	K	↓
4	08-09-25	0600	K	1100	K	0500	K	08-04-25 A
5	08-10-25	0600	K	1100	K	0500	K	↓
6	08-11-25	0500	K	1100	K	0710	K	
7	08-12-25					0644	K	

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 G-2021	YSI Model 52CE	08A100271
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:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	764.3
Average weight per initial larvae:	0.959		**NOEC** (mg/L KCl)	450
Average weight per surviving larvae:	0.959	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	600
			ChV (mg/L KCl)	519.6
			IC_{25} (mg/L KCl)	615.6

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **131**

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: Magenta Analyst: EM Date: 07-22-25	12.21	14.36	11.62	14.91	13.67	14.46	12.74	14.69	14.39	13.73	10.06	13.36
*B = Pan + Larvae weight (mg) Analyst: JH Date: 08-14-25	21.65	24.13	21.46	24.22	23.48	25.58	23.14	25.64	24.00	22.58	18.43	22.25
C = Larvae weight (mg) = B – A Analyst: ZP	9.44	9.77	9.84	9.31	9.81 ~~8.81~~ ZP 08-18-25	11.12	10.40	10.95	9.61	8.85	8.37	8.89
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: ZP	0.944	0.977	0.984	0.931	0.981	1.112	1.040	1.095	0.961	0.885	0.837	0.889
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.959				1.057		−10.2%		0.893		6.9%	

*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: **131**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	9 1d	9 1d	9 1d	9 1d	6 4d	6 4d	6 4d	6 4d
2	10	10	10	10	8 1d	8 1d	8 1d	7 2d	5 1d	6	4 2d	5 1d
3	10	10	10	8 2d	8	8	8	6 1d	4 1d	6	3 1d	5
4	10	10	10	8	6 2d	7 1d	6 2d	6	4	4 2d	3	4 1d
5	10	9 1d	10	8	5 1d	6 1d	5 1d	5 1d	2 2d	4	2 1d	2 2d
6	10	9	10	8	4 1d	6	5	5	2	4	2	2
7	10	9	9 1d	8	4	5 1d	5	5	2	4	2	2
*A = Pan weight (mg) Tray color code: Magenta Analyst: Em Date: 07-22-25	11.92	13.46	13.60	12.99	14.22	13.25	10.55	11.78	12.84	9.79	14.26	14.06
*B = Pan + Larvae weight (mg) Analyst: JV Date: 08.14.25	20.21	21.08	22.40	19.90	18.24	18.06	14.86	16.97	15.01	14.55	16.27	16.29
C = Larvae weight (mg) = B – A Analyst: JP	8.29	7.62	8.80	6.91	4.02	4.81	4.31	5.19	2.17	4.76	2.01	2.23
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0.829	0.762	0.880	0.691	0.402	0.481	0.431	0.519	0.217	0.476	0.201	0.223
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.791	17.6%			0.458	52.2%			0.279		~~70.2%~~ JP 08-18-2 70.9%	

*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: **131**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	7d 3	7d 3	8d 2	9d 1
2	1d 2	2d 1	1d 1	1
3	2	1	1	1
4	1d	1	1	1
5	1	1	1	1
6	1	0 1d	1	1
7	0 1d	0	1	1
*A = Pan weight (mg) Tray color code: Magenta Analyst: EM Date: 07-22-25	13.35	10.28	10.32	10.68
*B = Pan + Larvae weight (mg) Analyst: JL Date: 08-14-25	08-14-25 JL		11.66	12.11
C = Larvae weight (mg) = B – A Analyst: JP	08-14-25 JL		1.34	1.43
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0	0	0.134	0.143
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.069		92.8%	

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

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

Comments:

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: 131

Test dates: August 05-12, 2025

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	12.21	21.65	9.44	0.944	0.959	2.7	0.944	100.0	0.959	2.7	Not applicable
	B	10	10	14.36	24.13	9.77	0.977			0.977				
	C	10	10	11.62	21.46	9.84	0.984			0.984				
	D	10	10	14.91	24.22	9.31	0.931			0.931				
300	E	10	10	13.67	23.48	9.81	0.981	1.057	5.6	0.981	100.0	1.057	5.6	-10.2
	F	10	10	14.46	25.58	11.12	1.112			1.112				
	G	10	10	12.74	23.14	10.40	1.040			1.040				
	H	10	10	14.69	25.64	10.95	1.095			1.095				
450	I	10	10	14.39	24.00	9.61	0.961	0.893	5.7	0.961	100.0	0.893	5.7	6.9
	J	10	10	13.73	22.58	8.85	0.885			0.885				
	K	10	10	10.06	18.43	8.37	0.837			0.837				
	L	10	10	13.36	22.25	8.89	0.889			0.889				
600	M	10	10	11.92	20.21	8.29	0.829	0.879	7.6	0.829	90.0	0.791	10.4	17.6
	N	10	9	13.46	21.08	7.62	0.847			0.762				
	O	10	9	13.60	22.40	8.80	0.978			0.880				
	P	10	8	12.99	19.90	6.91	0.864			0.691				
750	Q	10	4	14.22	18.24	4.02	1.005	0.967	7.9	0.402	47.5	0.458	11.3	52.2
	R	10	5	13.25	18.06	4.81	0.962			0.481				
	S	10	5	10.55	14.86	4.31	0.862			0.431				
	T	10	5	11.78	16.97	5.19	1.038			0.519				
900	U	10	2	12.84	15.01	2.17	1.085	1.099	7.0	0.217	25.0	0.279	47.1	70.9
	V	10	4	9.79	14.55	4.76	1.190			0.476				
	W	10	2	14.26	16.27	2.01	1.005			0.201				
	X	10	2	14.06	16.29	2.23	1.115			0.223				
1050	Y	10	0	0.00	0.00	0.00	0.000	1.385	4.6	0.000	5.0	0.069	115.6	92.8
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	1	10.32	11.66	1.34	1.340			0.134				
	BB	10	1	10.68	12.11	1.43	1.430			0.143				

Dunnett's MSD value: 0.0941 MSD = Minimum Significant Difference

PMSD: 9.8 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jon Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	8/5/2025	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	8/12/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	0.9000	0.9000	0.8000
750	0.4000	0.5000	0.5000	0.5000
900	0.2000	0.4000	0.2000	0.2000
1050	0.0000	0.0000	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	0.9000	0.9000	1.2543	1.1071	1.4120	9.935	4	12.00	10.00	4	40
*750	0.4750	0.4750	0.7602	0.6847	0.7854	6.622	4	10.00	10.00	21	40
*900	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	30	40
*1050	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

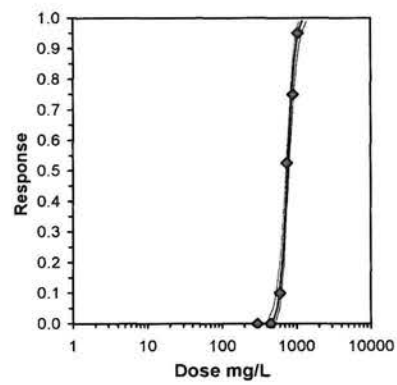
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.88134	0.896	0.61258	1.729
Equality of variance cannot be confirmed				

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

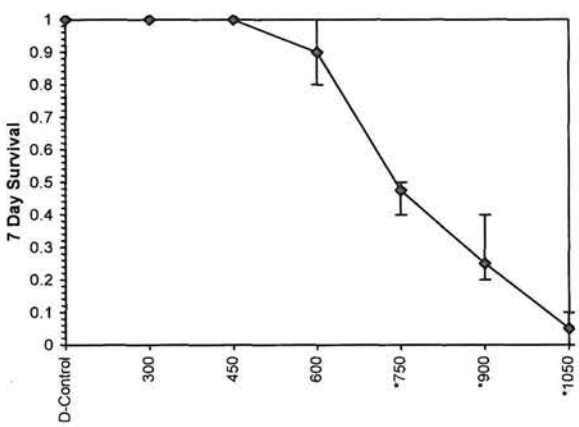
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.597	1.40182	8.84944	14.3446	0	1.35263	9.48773	0.85238	2.88329	0.08623	3
Intercept	-28.438	4.05686	-36.389	-20.486							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	481.603	410.728	533.139
EC05	3.355	551.383	488.885	596.633
EC10	3.718	592.626	535.928	634.143
EC15	3.964	622.182	569.812	661.224
EC20	4.158	646.719	597.918	683.965
EC25	4.326	668.54	622.785	704.49
EC40	4.747	726.845	687.764	761.592
EC50	5.000	764.342	727.595	800.87
EC60	5.253	803.774	767.251	844.898
EC75	5.674	873.874	832.345	929.794
EC80	5.842	903.359	858.116	967.569
EC85	6.036	938.985	888.353	1014.47
EC90	6.282	985.814	926.967	1077.82
EC95	6.645	1059.55	985.928	1180.71
EC99	7.326	1213.07	1104.08	1404.46



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	8/5/2025	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	8/12/2025	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	0.9440	0.9770	0.9840	0.9310
300	0.9810	1.1120	1.0400	1.0950
450	0.9610	0.8850	0.8370	0.8890
600	0.8290	0.7620	0.8800	0.6910
750	0.4020	0.4810	0.4310	0.5190
900	0.2170	0.4760	0.2010	0.2230
1050	0.0000	0.0000	0.1340	0.1430

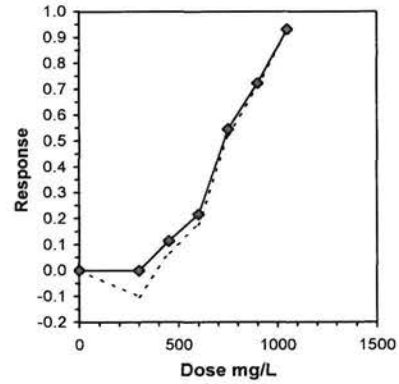
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.9590	1.0000	0.9590	0.9310	0.9840	2.664	4				1.0080	1.0000
300	1.0570	1.1022	1.0570	0.9810	1.1120	5.606	4	-2.385	2.290	0.0941	1.0080	1.0000
450	0.8930	0.9312	0.8930	0.8370	0.9610	5.725	4	1.606	2.290	0.0941	0.8930	0.8859
*600	0.7905	0.8243	0.7905	0.6910	0.8800	10.382	4	4.100	2.290	0.0941	0.7905	0.7842
750	0.4583	0.4778	0.4583	0.4020	0.5190	11.349	4				0.4583	0.4546
900	0.2793	0.2912	0.2793	0.2010	0.4760	47.089	4				0.2793	0.2770
1050	0.0693	0.0722	0.0693	0.0000	0.1430	115.592	4				0.0693	0.0687

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98395	0.844	-0.1749	-0.406
Bartlett's Test indicates equal variances (p = 0.38)	3.08717	11.3449		

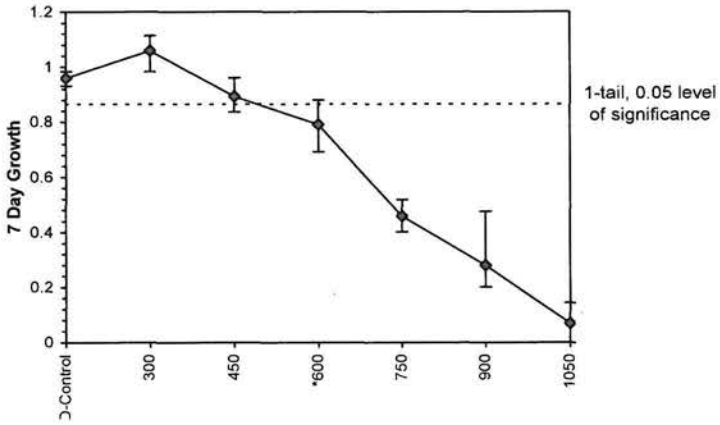
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.09411	0.09814	0.05026	0.00338	2.4E-04	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	365.74	17.71	336.04	449.63	2.0034
IC10	431.48	24.86	372.07	519.28	0.4374
IC15	502.98	39.14	404.66	665.88	0.6105
IC20	576.73	32.68	469.95	651.97	-0.3161
IC25	615.58	18.65	540.91	658.84	-0.8219
IC40	683.84	10.93	646.85	713.67	-0.1556
IC50	729.35	10.37	698.96	764.86	0.2795



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **131**

Daily Chemistry:

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

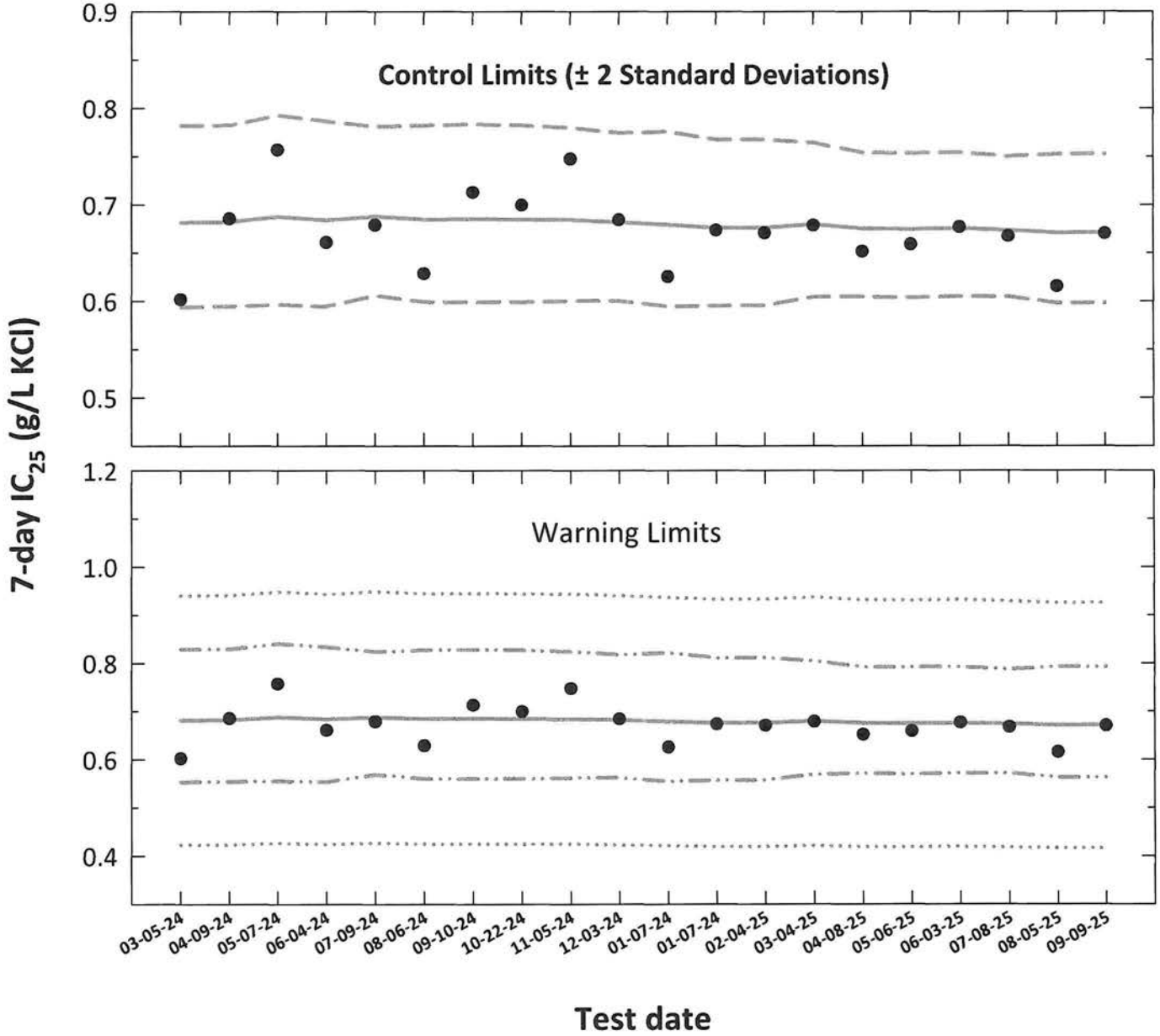
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	N	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.97	7.72	7.74	7.57	7.76	7.58
	Dissolved oxygen (mg/L)	8.4	7.8	8.3	7.8	8.4	N 09/05/25 ~~8.~~ 7.4
	Conductivity (µmhos/cm)	297		299		298	
	Alkalinity (mg $CaCO_3$/L)	63				63	
	Hardness (mg $CaCO_3$/L)	91				91	
	Temperature (°C)	24.9	24.6	24.8	24.6	24.8	24.8
300 mg KCl/L	pH (S.U.)	8.00	7.64	7.84	7.57	7.81	7.56
	Dissolved oxygen (mg/L)	8.4	7.8	8.4	7.8	8.4	7.1
	Conductivity (µmhos/cm)	802		817		818	
	Temperature (°C)	24.8	24.9	24.7	24.6	24.9	25.0
450 mg KCl/L	pH (S.U.)	8.00	7.69	7.84	7.58	7.82	7.59
	Dissolved oxygen (mg/L)	8.4	7.9	8.4	7.8	8.4	7.2
	Conductivity (µmhos/cm)	1050		1070		1070	
	Temperature (°C)	24.9	24.8	24.7	24.8	24.9	24.7
600 mg KCl/L	pH (S.U.)	8.00	7.70	7.83	7.62	7.83	7.61
	Dissolved oxygen (mg/L)	8.4	7.9	8.4	7.8	8.4	7.2
	Conductivity (µmhos/cm)	1310		1310		1310	
	Temperature (°C)	24.9	24.8	24.7	24.5	25.0	24.7
750 mg KCl/L	pH (S.U.)	8.00	7.72	7.85	7.62	7.83	7.61
	Dissolved oxygen (mg/L)	8.4	7.9	8.4	7.7	8.4	7.2
	Conductivity (µmhos/cm)	1530		1560		1560	
	Temperature (°C)	24.9	25.0	24.8	24.9	25.0	24.9
900 mg KCl/L	pH (S.U.)	8.02	7.72	7.86	7.62	7.83	7.61
	Dissolved oxygen (mg/L)	8.4	7.9	8.4	7.7	8.4	7.4
	Conductivity (µmhos/cm)	1770		1800		1810	
	Temperature (°C)	25.0	24.8	24.8	24.9	25.0	24.6
1050 mg KCl/L	pH (S.U.)	8.02	7.75	7.86	7.69	7.84	7.72
	Dissolved oxygen (mg/L)	8.4	8.1	8.4	7.8	8.4	7.6
	Conductivity (µmhos/cm)	2040		2060		2070	
	Temperature (°C)	24.8	24.8	24.8	25.0	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **131**

		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	[illegible]
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.87	7.44	7.88	7.61	7.87	7.62	7.84	7.57
	Dissolved oxygen (mg/L)	8.3	7.4	8.4	7.3	8.4	7.5	8.3	6.4
	Conductivity (µmhos/cm)	301		290		292		299	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)		08-11-25	91					08-11-25
	Temperature (°C)	24.8	25.0	24.8	24.9	24.9	25.1	24.9	25.1
300 mg KCl/L	pH (S.U.)	7.90	7.45	7.98	7.61	8.03	7.61	7.92	7.46
	Dissolved oxygen (mg/L)	8.2	7.4	8.3	7.3	~~5.00~~ 8.4 08-10-25	7.4	8.3	5.7
	Conductivity (µmhos/cm)	801		796		800		807	
	Temperature (°C)	24.9	24.9	24.7	25.1	24.9	24.9	24.8	25.0
450 mg KCl/L	pH (S.U.)	7.90	7.45	8.01	7.59	8.04	7.61	7.92	7.43
	Dissolved oxygen (mg/L)	8.2	7.1	8.3	6.8	8.4	7.1	8.3	5.8
	Conductivity (µmhos/cm)	1050		1050		1050		1060	
	Temperature (°C)	24.9	24.9	24.7	25.1	25.0	24.9	24.8	25.0
600 mg KCl/L	pH (S.U.)	7.91	7.47	8.01	7.59	8.03	7.60	7.93	7.44
	Dissolved oxygen (mg/L)	8.3	7.2	8.4	6.9	8.4	7.1	8.3	5.8
	Conductivity (µmhos/cm)	1300		1260		1290		1310	
	Temperature (°C)	24.8	25.1	24.7	24.8	25.0	25.2	24.9	25.2
750 mg KCl/L	pH (S.U.)	7.92	7.49	8.01	7.57	8.04	7.60	7.94	7.44
	Dissolved oxygen (mg/L)	8.3	7.2	8.4	6.6	8.4	7.2	8.3	5.8
	Conductivity (µmhos/cm)	1550		1550		1560		1570	
	Temperature (°C)	24.8	25.1	24.9	24.8	25.0	25.2	24.9	25.2
900 mg KCl/L	pH (S.U.)	7.94	7.70	8.02	7.49	8.03	7.63	7.95	7.46
	Dissolved oxygen (mg/L)	8.3	7.2	8.3	6.9	8.4	7.2	8.3	6.5
	Conductivity (µmhos/cm)	1790		1800		1780		1790	
	Temperature (°C)	25.0	24.8	24.9	24.8	24.9	24.8	25.0	25.2
1050 mg KCl/L	pH (S.U.)	7.95	7.73	8.02	7.77	8.03	7.66	7.96	7.50
	Dissolved oxygen (mg/L)	8.3	7.3	8.3	7.2	8.4	7.4	8.3	5.6
	Conductivity (µmhos/cm)	2030		2010		2020		2080	
	Temperature (°C)	24.8	24.8	24.8	25.0	24.9	25.0	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- — — — **Control Limits** (mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.75}$ 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	03-05-24	**0.6019**	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
2	04-09-24	**0.6855**	-0.1640	-0.1660	0.0298	0.6823	0.5949	0.7825	0.5542	0.8292	0.4230	0.9416
3	05-07-24	**0.7569**	-0.1210	-0.1627	0.0309	0.6876	0.5965	0.7927	0.5551	0.8404	0.4263	0.9489
4	06-04-24	**0.6610**	-0.1798	-0.1650	0.0303	0.6840	0.5949	0.7864	0.5537	0.8338	0.4241	0.9439
5	07-09-24	**0.6789**	-0.1682	-0.1624	0.0276	0.6880	0.6060	0.7811	0.5688	0.8233	0.4265	0.9494
6	08-06-24	**0.6287**	-0.2015	-0.1644	0.0289	0.6848	0.5994	0.7823	0.5602	0.8272	0.4246	0.9450
7	09-10-24	**0.7128**	-0.1470	-0.1642	0.0290	0.6852	0.5994	0.7832	0.5600	0.8283	0.4248	0.9456
8	10-22-24	**0.6997**	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
9	11-05-24	**0.7473**	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
10	12-03-24	**0.6844**	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
11	01-07-24	**0.6255**	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
12	01-07-24	**0.6737**	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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.

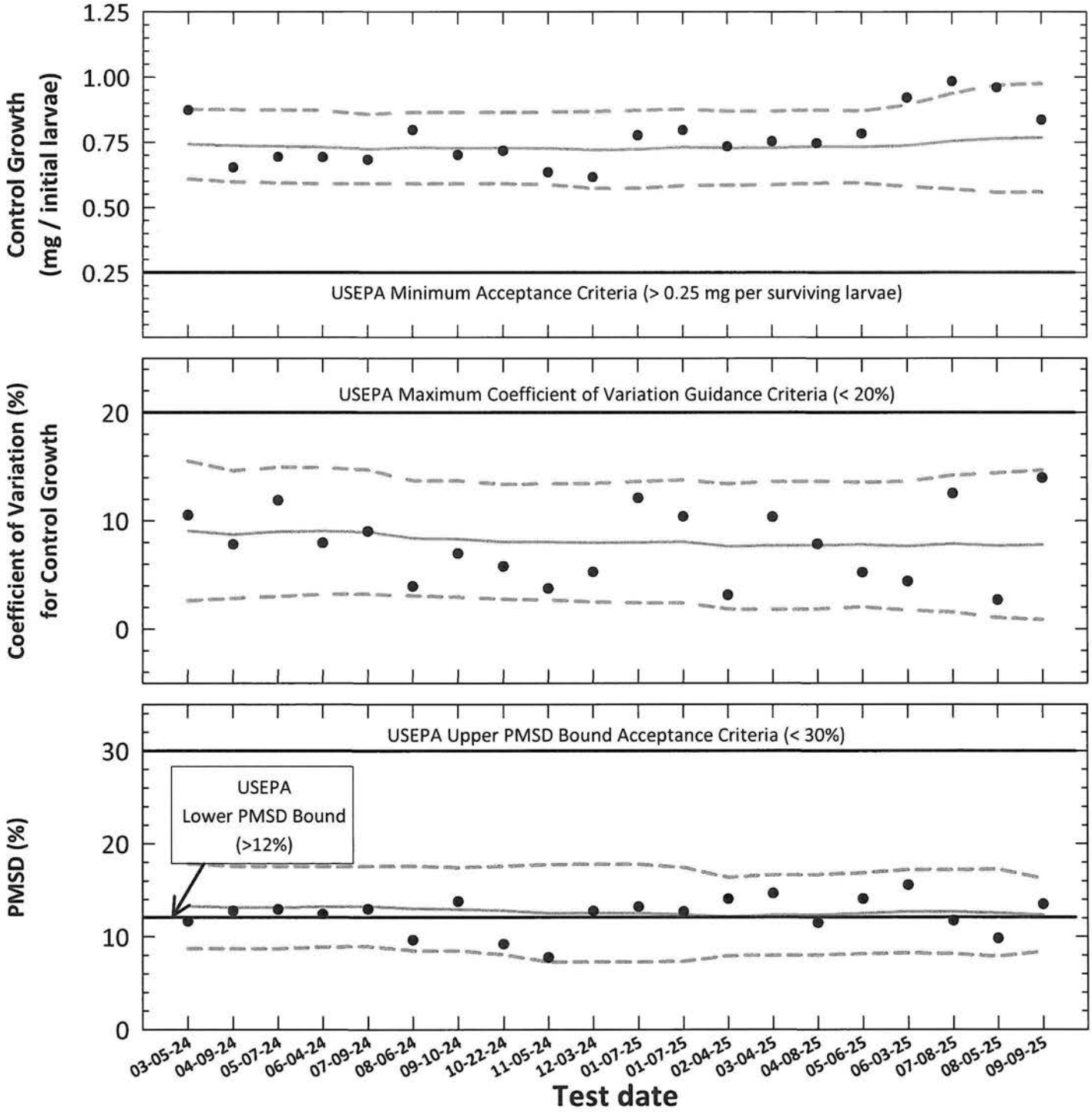
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

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 Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
2	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
3	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
4	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.2	8.9	17.5
5	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.2	8.9	17.5
6	08-06-24	100	0.796	3.9	0.0766	9.6	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
7	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
8	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
9	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
10	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
11	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

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: **132**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2412				
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:	09-01-25	*Artemia* CHM number:	CHM1385
Hatch dates and times:	09-02-25 1400 TO 09-03-25 0455	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.12 S.U. Temperature = 24.1 °C	Date / Time in oven:	09-16-25 0545
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	09-17-25 0545
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	09-09-25	0500	K	1445	K	0700	K	09-04-25A
1	09-10-25	0500	K	1315	K	0710	K	↓
2	09-11-25	0500	K	1330	K	0700	K	09-04-25 B
3	09-12-25	0500	K	1100	K	0700	K	↓
4	09-13-25	0600	K	1130	K	0800	K	09-04-25 E
5	09-14-25	0600	K	1130	K	0800	K	↓
6	09-15-25	0500	K	1100	K	0713	K	↓
7	09-16-25					0530	K	

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 G-2021	YSI Model 52CE	08A100271
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:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	742.1
Average weight per initial larvae:	0.835		**NOEC** (mg/L KCl)	450
Average weight per surviving larvae:	0.835	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	600
			ChV (mg/L KCl)	519.6
			IC_{25} (mg/L KCl)	670.3

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **132**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: Forest Green Analyst: EM Date: 08-19-25	11.98	10.66	14.99	13.76	15.98	10.42	10.46	14.14	14.39	14.49	10.26	14.44
*B = Pan + Larvae weight (mg) Analyst: JK Date: 09-18-25	20.12	19.76	21.78	23.13	25.47	19.47	20.11	24.14	22.52	23.21	18.73	23.21
C = Larvae weight (mg) = B – A Analyst: J	8.14	9.10	6.79	9.37	9.49	9.05	9.65	10.00	8.13	8.72	8.47	8.77
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: M	0.814	0.910	0.679	0.937	0.949	0.905	0.965	1.000	0.813	0.872	0.847	0.877
Average weight per initial number of larvae (mg)	0.835				0.955				0.852			
Percent reduction from control (%)							-14.37.				-2.17.	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **132**

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 3d	6 4d	7 3d	7 3d
2	10	10	10	10	10	10	9 1d	9 1d	6 1d	6	5 2d	6 1d
3	10	10	10	10	9 1d	9 1d	8 1d	8 1d	4 2d	5 1d	4 1d	4 2d
4	10	10	10	10	7 2d	7 1d	7 1d	7 1d	3 1d	5	4	3 1d
5	10	9 1d	9 1d	10	7	6 1d	6 1d	7	2 1d	2 3d	2 2d	2 1d
6	10	9	8 1d	10	6 1d	6	6	7	1 1d	2	2	2
7	9 1d	9	8	9 1d	5 1d	5 1d	6	5 2d	1	2	1 1d	1 1d
*A = Pan weight (mg) Tray color code: Forest Green Analyst: EM Date: 08-19-25	9.93	13.96	14.92	12.31	12.67	12.38	12.25	14.01	11.53	13.46	13.59	13.00
*B = Pan + Larvae weight (mg) Analyst: JH Date: 09-18-25	18.38	22.73	22.18	19.50	17.75	17.03	18.12	19.78	12.40	15.70	14.67	13.71
C = Larvae weight (mg) = B – A Analyst: JH	8.45	8.77	7.26	7.19	5.08	4.65	5.87	5.77	0.87	2.24	1.08	0.71
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.845	0.877	0.726	0.719	0.508	0.465	0.587	0.577	0.087	0.224	0.108	0.071
Average weight per initial number of larvae (mg)	0.792				0.534				0.123			
Percent reduction from control (%)		5.2%				36.0%				85.3%		

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **132**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 7d	3 7d	3 7d	3 7d
2	2 1d	3	2 1d	2 1d
3	2	2 1d	1 1d	2
4	2	1 1d	1	1 1d
5	0 2d	1	1	1
6	0	1	1	0 1d
7	0	0 1d	0 1d	0
*A = Pan weight (mg) Tray color code: Forest Green Analyst: EM Date: 08-19-25	13.73	14.56	13.25	13.33
*B = Pan + Larvae weight (mg) Analyst: ____ Date: ____				
C = Larvae weight (mg) = B – A Analyst: ____				08-26-25
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: ____	0	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0		100%	

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

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

Comments:

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: 132

Test dates: September 09-16, 2025

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	11.98	20.12	8.14	0.814	0.835	14.0	0.814	100.0	0.835	14.0	Not applicable
	B	10	10	10.66	19.76	9.10	0.910			0.910				
	C	10	10	14.99	21.78	6.79	0.679			0.679				
	D	10	10	13.76	23.13	9.37	0.937			0.937				
300	E	10	10	15.98	25.47	9.49	0.949	0.955	4.1	0.949	100.0	0.955	4.1	-14.3
	F	10	10	10.42	19.47	9.05	0.905			0.905				
	G	10	10	10.46	20.11	9.65	0.965			0.965				
	H	10	10	14.14	24.14	10.00	1.000			1.000				
450	I	10	10	14.39	22.52	8.13	0.813	0.852	3.4	0.813	100.0	0.852	3.4	-2.1
	J	10	10	14.49	23.21	8.72	0.872			0.872				
	K	10	10	10.26	18.73	8.47	0.847			0.847				
	L	10	10	14.44	23.21	8.77	0.877			0.877				
600	M	10	9	9.93	18.38	8.45	0.939	0.905	8.4	0.845	87.5	0.792	10.2	5.2
	N	10	9	13.96	22.73	8.77	0.974			0.877				
	O	10	8	14.92	22.18	7.26	0.908			0.726				
	P	10	9	12.31	19.50	7.19	0.799			0.719				
750	Q	10	5	12.67	17.75	5.08	1.016	1.020	9.4	0.508	52.5	0.534	10.9	36.0
	R	10	5	12.38	17.03	4.65	0.930			0.465				
	S	10	6	12.25	18.12	5.87	0.978			0.587				
	T	10	5	14.01	19.78	5.77	1.154			0.577				
900	U	10	1	11.53	12.40	0.87	0.870	0.945	20.2	0.087	12.5	0.123	56.6	85.3
	V	10	2	13.46	15.70	2.24	1.120			0.224				
	W	10	1	13.59	14.67	1.08	1.080			0.108				
	X	10	1	13.00	13.71	0.71	0.710			0.071				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1126 MSD = Minimum Significant Difference

PMSD: 13.5 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Swisher

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	9/9/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	9/16/2025	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	0.9000	0.8000	0.9000
750	0.5000	0.5000	0.6000	0.5000
900	0.1000	0.2000	0.1000	0.1000
1050	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*600	0.8750	0.8750	1.2136	1.1071	1.2490	5.846	4	10.00	10.00	5	40
*750	0.5250	0.5250	0.8106	0.7854	0.8861	6.210	4	10.00	10.00	19	40
*900	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

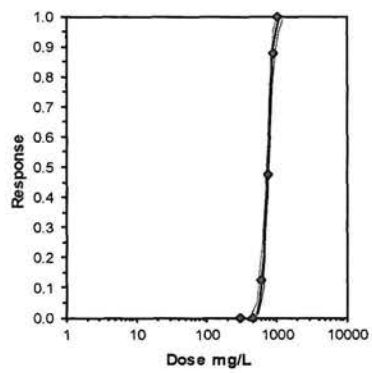
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.86899	0.884	0.27239	2.83008
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	450	600	519.615	
Treatments vs D-Control				

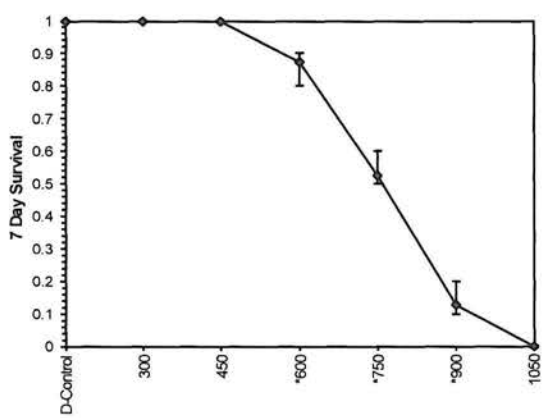
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.1802	1.7472	10.7557	17.6047	0	1.55134	9.48773	0.81751	2.87044	0.07052	3
Intercept	-35.703	5.03685	-45.576	-25.831							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	508.612	443.417	554.917
EC05	3.355	568.128	511.608	608.381
EC10	3.718	602.652	551.677	639.503
EC15	3.964	627.123	580.139	661.773
EC20	4.158	647.279	603.517	680.345
EC25	4.326	665.086	624.045	697.005
EC40	4.747	712.159	677.113	742.794
EC50	5.000	742.067	709.348	773.776
EC60	5.253	773.232	741.287	808.043
EC75	5.674	827.959	793.267	873.13
EC80	5.842	850.737	813.626	901.791
EC85	6.036	878.079	837.331	937.157
EC90	6.282	913.734	867.316	984.566
EC95	6.645	969.26	912.529	1060.69
EC99	7.326	1082.68	1001.36	1222.7



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:	9/9/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	9/16/2025	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	0.8140	0.9100	0.6790	0.9370
300	0.9490	0.9050	0.9650	1.0000
450	0.8130	0.8720	0.8470	0.8770
600	0.8450	0.8770	0.7260	0.7190
750	0.5080	0.4650	0.5870	0.5770
900	0.0870	0.2240	0.1080	0.0710
1050	0.0000	0.0000	0.0000	0.0000

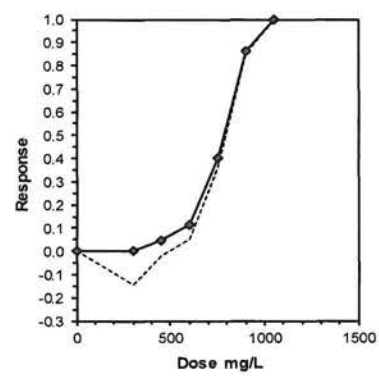
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8350	1.0000	0.8350	0.6790	0.9370	13.967	4				0.8949	1.0000
300	0.9548	1.1434	0.9548	0.9050	1.0000	4.128	4	-2.318	2.180	0.1126	0.8949	1.0000
450	0.8523	1.0207	0.8523	0.8130	0.8770	3.435	4	-0.334	2.180	0.1126	0.8523	0.9524
600	0.7918	0.9482	0.7918	0.7190	0.8770	10.240	4				0.7918	0.8848
750	0.5343	0.6398	0.5343	0.4650	0.5870	10.858	4				0.5343	0.5970
900	0.1225	0.1467	0.1225	0.0710	0.2240	56.606	4				0.1225	0.1369
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94129	0.805	-0.9034	2.12682
Bartlett's Test indicates equal variances (p = 0.06)	5.56001	9.21035		

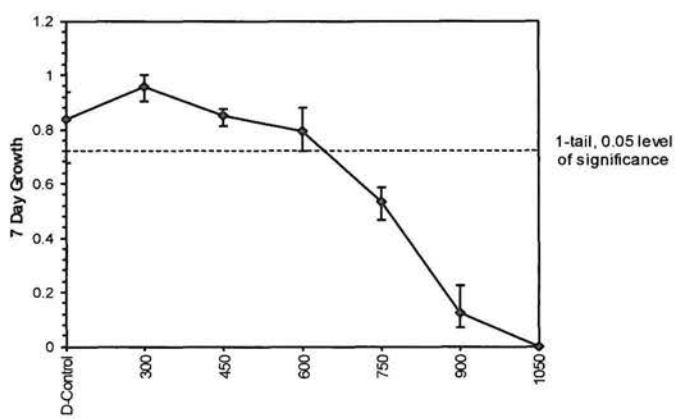
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	>450			0.11262	0.13487	0.01676	0.00534	0.09234	2, 9
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95%CL(Exp)		Skew
IC05	455.25	62.44	322.96	702.32	0.6472
IC10	566.19	53.71	372.50	672.81	-0.3397
IC15	618.12	30.54	489.62	675.60	-1.0534
IC20	644.18	21.10	564.36	702.53	-0.6454
IC25	670.25	18.25	609.87	727.60	-0.1524
IC40	748.44	13.21	706.75	782.11	-0.2986
IC50	781.63	9.32	753.70	810.68	0.0258



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **132**

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.)	7.97	7.78	7.81	7.67	7.78	7.74
	Dissolved oxygen (mg/L)	8.3	7.9	8.3	7.7	8.3	7.7
	Conductivity (µmhos/cm)	307		306		305	
	Alkalinity (mg $CaCO_3$/L)	60				63	
	Hardness (mg $CaCO_3$/L)	84			09.15.24	86	
	Temperature (°C)	24.8	24.9	24.8	24.8	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.94	7.75	7.90	7.65	7.86	7.71
	Dissolved oxygen (mg/L)	8.4	7.6	8.2	7.4	8.3	7.5
	Conductivity (µmhos/cm)	840		834		828	
	Temperature (°C)	24.9	24.6	24.8	24.7	24.8	24.8
450 mg KCl/L	pH (S.U.)	7.95	7.75	7.90	7.66	7.88	7.71
	Dissolved oxygen (mg/L)	8.4	7.6	8.2	7.4	8.3	7.5
	Conductivity (µmhos/cm)	1100		1080		1070	
	Temperature (°C)	24.7	24.6	24.9	24.7	24.7	24.7
600 mg KCl/L	pH (S.U.)	7.95	7.75	7.91	7.66	7.89	7.71
	Dissolved oxygen (mg/L)	8.4	7.6	8.2	7.4	8.3	7.5
	Conductivity (µmhos/cm)	1360		1340		1330	
	Temperature (°C)	24.7	24.8	24.8	24.7	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.96	7.76	7.92	7.67	7.90	7.70
	Dissolved oxygen (mg/L)	8.4	7.6	8.2	7.5	8.3	7.3
	Conductivity (µmhos/cm)	1620		1610		1600	
	Temperature (°C)	24.8	24.8	24.8	24.6	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.97	7.77	7.92	7.67	7.91	7.70
	Dissolved oxygen (mg/L)	8.4	7.7	8.2	7.4	8.3	7.4
	Conductivity (µmhos/cm)	1870		1860		1840	
	Temperature (°C)	24.8	24.7	24.9	24.6	24.9	25.0
1050 mg KCl/L	pH (S.U.)	7.97	7.77	7.93	7.68	7.92	7.71
	Dissolved oxygen (mg/L)	8.4	7.8	8.2	7.7	8.3	7.6
	Conductivity (µmhos/cm)	2130		2130		2110	
	Temperature (°C)	24.9	24.7	24.9	24.6	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **132**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.78	7.74	BSL 09-15-25 ~~7.78~~ (7.83)	7.59	7.84	7.60	7.83	7.38
	Dissolved oxygen (mg/L)	8.3	7.9	8.3	7.2	8.5	7.2	8.3	5.7
	Conductivity (µmhos/cm)	303		298		289		304	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)		XL 09-15-25 XL	88				XL 09-15-25 XL	
	Temperature (°C)	24.6	24.4	24.7	24.7	24.8	24.8	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.87	7.76	7.86	7.60	7.92	7.61	7.94	7.38
	Dissolved oxygen (mg/L)	8.3	7.9	8.3	7.2	8.5	7.4	8.2	5.6
	Conductivity (µmhos/cm)	834		793		804		834	
	Temperature (°C)	24.6	24.6	24.7	24.6	24.9	24.6	24.7	24.7
450 mg KCl/L	pH (S.U.)	7.89	7.76	7.93	7.60	8.00	7.64	7.96	7.39
	Dissolved oxygen (mg/L)	8.3	7.8	8.4	7.1	8.3	7.4	8.2	5.8
	Conductivity (µmhos/cm)	1080		1060		1070		1080	
	Temperature (°C)	24.7	24.4	24.8	24.7	25.0	24.6	24.8	24.9
600 mg KCl/L	pH (S.U.)	7.89	7.76	7.95	7.61	8.02	7.66	7.97	7.40
	Dissolved oxygen (mg/L)	8.3	7.7	8.4	7.1	8.4	7.3	8.3	5.9
	Conductivity (µmhos/cm)	1330		1320		1320		1330	
	Temperature (°C)	24.6	24.4	24.6	24.7	24.8	24.8	24.8	24.9
750 mg KCl/L	pH (S.U.)	7.91	7.75	7.96	7.65	8.02	7.67	7.99	7.40
	Dissolved oxygen (mg/L)	8.3	7.7	8.3	7.1	8.4	7.3	8.3	5.6
	Conductivity (µmhos/cm)	1580		1550		1520		1570	
	Temperature (°C)	24.7	24.4	24.6	24.8	24.8	24.5	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.91	7.79	7.98	7.72	8.03	7.73	7.99	7.42
	Dissolved oxygen (mg/L)	8.3	7.9	8.4	7.5	8.4	7.6	8.3	6.1
	Conductivity (µmhos/cm)	1830		1810		1800		1840	
	Temperature (°C)	24.6	24.7	24.7	24.5	25.0	24.7	24.7	24.6
1050 mg KCl/L	pH (S.U.)	7.92	7.81	7.99	7.71	8.04	7.77	8.00	7.45
	Dissolved oxygen (mg/L)	8.3	8.0	8.4	7.6	8.4	7.7	8.3	6.4
	Conductivity (µmhos/cm)	2090		2060		2030		2100	
	Temperature (°C)	24.6	24.6	24.7	24.8	25.0	24.7	24.7	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final