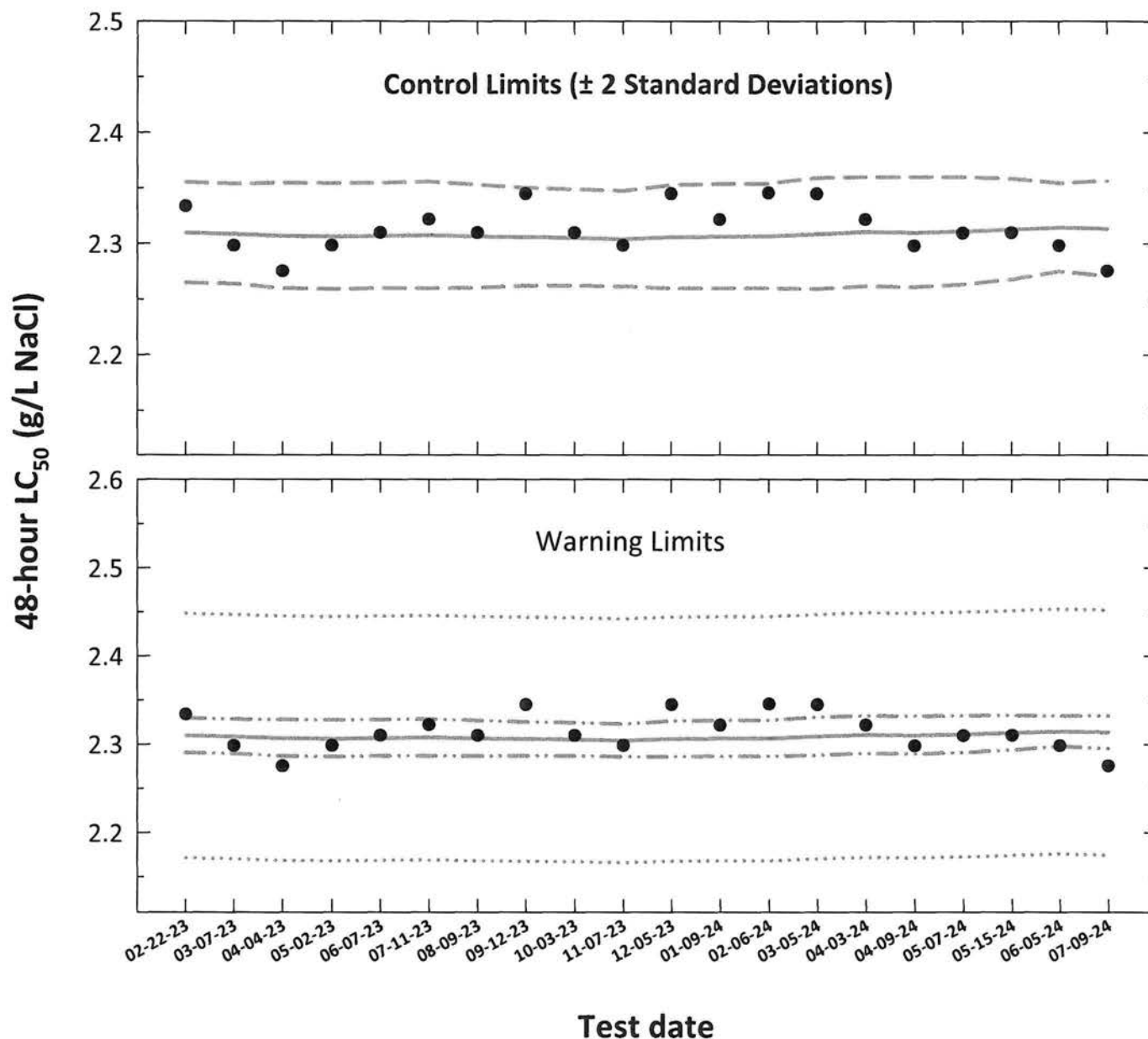


Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



- **48-hour LC₅₀** = 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 LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.10}$ converted to anti-logarithmic values,
S_{A.10} = 10th percentile of CVs reported nationally by USEPA)

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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	02-22-23	2.3339	0.3681	0.3636	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296
2	03-07-23	2.2984	0.3614	0.3634	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284
3	04-04-23	2.2753	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278
4	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272
5	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278
6	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286
7	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268
8	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251
9	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243
10	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229
11	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263
12	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271
13	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272
14	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307
15	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321
16	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317
17	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323
18	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327
19	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319
20	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322

Note: 48-hour LC₅₀ = 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 LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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 LC₅₀ Whole Effluent Toxicity Test, Species: Ceriodaphnia dubia
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 412

Dilution Preparation:

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

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

Stock solution INSS #: 2294

Chemical Analyses:

		Hours		
		0	24	48
Control, MHSW	Analyst	KD	KD	KD
	pH (S.U.)	7.37	7.44	7.49
	Dissolved oxygen (mg/L)	7.7	8.0	7.8
	Conductivity (µmhos/cm)	302		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	84		
	Temperature (°C)	25.1	25.2	25.2
1750 mg/L	pH (S.U.)	7.46	7.33	7.41
	Dissolved oxygen (mg/L)	7.7	8.0	7.7
	Conductivity (µmhos/cm)	3440		
	Temperature (°C)	25.0	25.1	25.0
2000 mg/L	pH (S.U.)	7.51	7.37	7.45
	Dissolved oxygen (mg/L)	7.7	8.0	7.7
	Conductivity (µmhos/cm)	3940		
	Temperature (°C)	25.0	25.0	25.0
2250 mg/L	pH (S.U.)	7.54	7.41	7.49
	Dissolved oxygen (mg/L)	7.7	8.0	7.7
	Conductivity (µmhos/cm)	4310		
	Temperature (°C)	25.2	25.0	25.0
2500 mg/L	pH (S.U.)	7.56	7.41	7.49
	Dissolved oxygen (mg/L)	7.8	8.0	7.8
	Conductivity (µmhos/cm)	4840		
	Temperature (°C)	25.2	25.2	25.2
2750 mg/L	pH (S.U.)	7.58	7.41	7.54
	Dissolved oxygen (mg/L)	7.8	8.0	7.9
	Conductivity (µmhos/cm)	5130		
	Temperature (°C)	25.1	25.2	25.2

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O 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	150604685

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 412

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-04-24	0515	H	0758	H	2B4	BLUE	07-01-24
24	07-10-24			0755	H			
48 Termination	07-11-24			0755	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):	07-01-24 D
Age:	< 24-hours old
Date and time organisms were born between:	07-08-24 1450 TO 07-09-24 0515
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.59
	Temperature (°C): 24.9

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 ^{1d}	4 ^{1d}	5	5	4 ^{1d}	2 ^{3d}	3 ^{2d}	3 ^{2d}
48 Termination	3 ^{2d}	2 ^{3d}	2 ^{3d}	2 ^{3d}	0 ^{4d}	0 ^{4d}	2 ^{3d}	1 ^{4d}	0 ^{4d}	0 ^{2d}	0 ^{3d}	0 ^{3d}
Mean Survival	45%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2203.8
Upper 95% confidence limit (mg NaCl/L)	2345.1
48-hour LC ₅₀ (mg NaCl/L)	2275.5

Comments:

Acute Daphnid Test-48 Hr Survival

Start Date: 7/9/2024 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 7/11/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.4000	0.4000	0.4000
2500	0.0000	0.0000	0.4000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root						N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.4500	0.4500	0.7351	0.6847	0.8861	13.697	4	10.00	10.00	11	20
*2500	0.1500	0.1500	0.3998	0.2255	0.6847	55.174	4	10.00	10.00	17	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

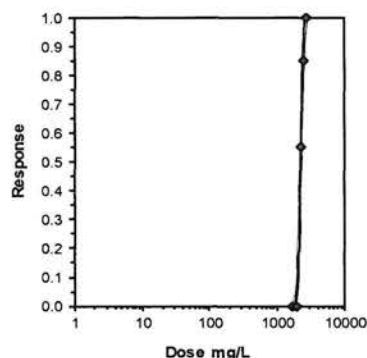
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7765	0.868	1.03546	3.99287

Equality of variance cannot be confirmed

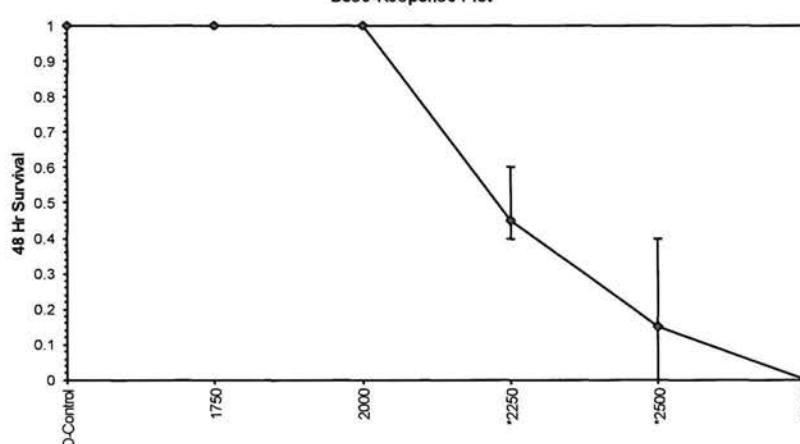
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						Mu	Sigma	Iter
				Control	Chi-Sq	Critical	P-value					
Slope	32.3602	6.05281	20.4967 44.2237	0	2.60823	7.81472	0.45605	3.35708	0.0309			6
Intercept	-103.64	20.3367	-143.5 -63.776									

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	1928.38	1735.07 2032.02
EC05	3.355	2024.2	1869.04 2110.02
EC10	3.718	2077.21	1943.32 2154.27
EC15	3.964	2113.75	1994.19 2185.65
EC20	4.158	2143.26	2034.79 2211.76
EC25	4.326	2168.9	2069.53 2235.21
EC40	4.747	2234.88	2155.12 2300.32
EC50	5.000	2275.53	2203.84 2345.14
EC60	5.253	2316.92	2249.53 2395.23
EC75	5.674	2387.4	2318.73 2490.34
EC80	5.842	2415.96	2344.34 2531.76
EC85	6.036	2449.69	2373.27 2582.28
EC90	6.282	2492.79	2408.71 2648.92
EC95	6.645	2558.07	2460.08 2753.24
EC99	7.326	2685.17	2555.38 2964.81



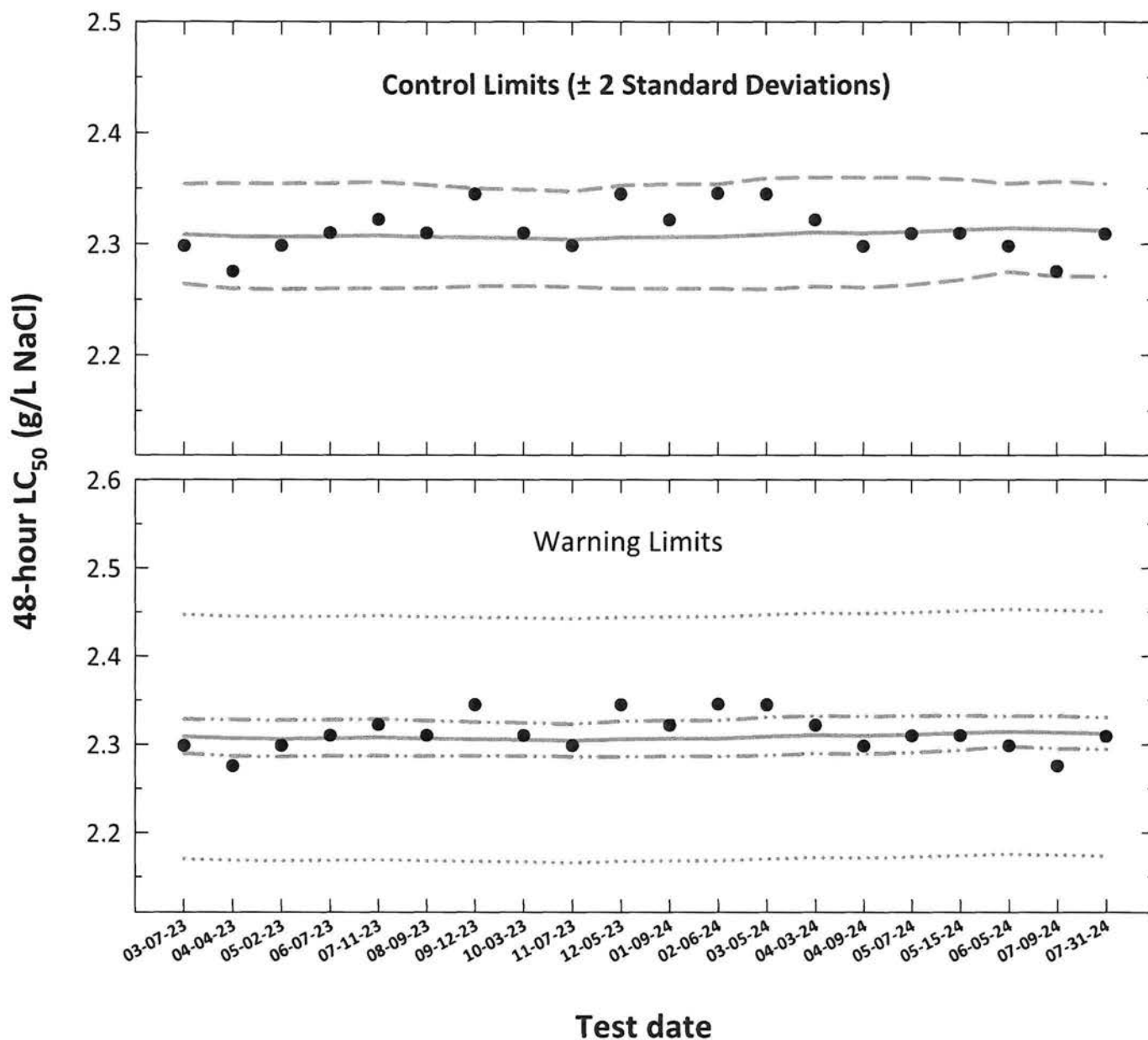
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = 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 LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- · · · · **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic LC₅₀ ± S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

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		CT	Anti-logarithmic Values (g/L NaCl)						
			48-hour LC ₅₀	CT		S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}		
1	03-07-23	2.2984	0.3614	0.3634	0.0042	2.3087	2.2641	2.3542	2.2894	2.3284	2.1702	2.4472
2	04-04-23	2.2753	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
3	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272	2.1680	2.4448
4	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
5	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
6	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
7	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
8	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
9	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
10	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
11	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
12	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
13	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
14	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
15	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
16	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
17	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
18	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
19	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
20	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510

Note: 48-hour LC₅₀ = 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 ToxCal).

CT = Central tendency of the LC₅₀ values.

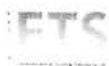
S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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 LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant TestCdNaClAC # 413

Dilution Preparation:

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

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

Stock solution INSS #: 2294

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.46	7.34	7.75
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	290		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	94		
	Temperature (°C)	24.9	25.2	25.3
1750 mg/L	pH (S.U.)	7.40	7.40	7.65
	Dissolved oxygen (mg/L)	7.7	7.8	7.6
	Conductivity (µmhos/cm)	3260		
	Temperature (°C)	25.2	25.1	25.0
2000 mg/L	pH (S.U.)	7.60	7.42	7.64
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	3750		
	Temperature (°C)	25.0	25.1	25.0
2250 mg/L	pH (S.U.)	7.62	7.46	7.70
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	4120		
	Temperature (°C)	25.0	24.9	25.0
2500 mg/L	pH (S.U.)	7.62	7.49	7.70
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	4530		
	Temperature (°C)	24.8	24.9	25.2
2750 mg/L	pH (S.U.)	7.63	7.44	7.70
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	5000		
	Temperature (°C)	25.2	25.3	25.2

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 1 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	1306046

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 413

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-31-24	0500	JK	0710	JK	2B3	RED	07-31-24
24	08-01-24			0711	JK			
48 Termination	08-02-24			0655	JK			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	07-23-24 A-D
Age:	< 24-hours old
Date and time organisms were born between:	07-30-24 1245 TO 07-31-24 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.59
	Temperature (°C): 21.5

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	5	5	4 nd	4 nd
48 Termination	2 nd	2 nd	3 rd	2 nd	2 nd	2 nd	3 rd	2 nd	0 th	0 th	0 th	0 th
Mean Survival	45%				30%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	2230.7
Upper 95% confidence limit (mg NaCl/L)	2386.0
48-hour LC ₅₀ (mg NaCl/L)	2309.1

Comments:

Test
Reviewed by:
JK

Acute Daphnid Test-48 Hr Survival

Start Date: 7/31/2024 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 8/2/2024 Lab ID: ETS-Envr. Testing Sol. Sample Type: NACL-Sodium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.4000	0.6000	0.4000
2500	0.4000	0.4000	0.0000	0.4000
2750	0.0000	0.0000	0.0000	0.0000

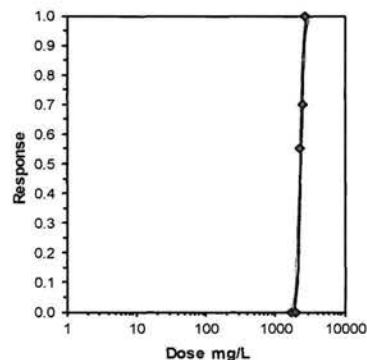
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.4500	0.4500	0.7351	0.6847	0.8861	13.697	4	10.00	10.00	11	20
*2500	0.3000	0.3000	0.5699	0.2255	0.6847	40.287	4	10.00	10.00	14	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.70402	0.868	-1.9668	7.55793

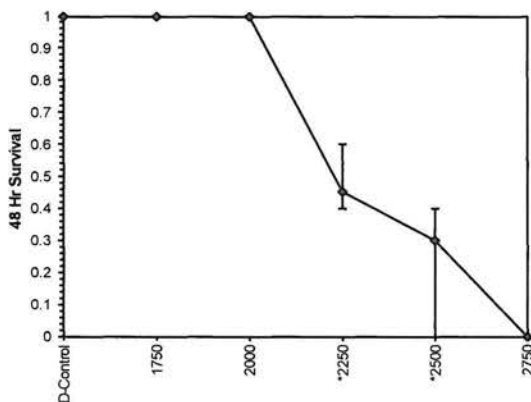
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	26.8159	4.68383	17.6356	35.9962	0	5.89653	7.81472	0.11675	3.36344	0.03729	7
Intercept	-85.194	15.7682	-116.1	-54.288							
TSCR						10					

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1890.97	1686.99	2006.63
EC05	3.355	2004.93	1839.65	2100.97
EC10	3.718	2068.46	1925.18	2154.64
EC15	3.964	2112.46	1984.13	2192.73
EC20	4.158	2148.09	2031.41	2224.42
EC25	4.326	2179.14	2072.04	2252.88
EC40	4.747	2259.38	2172.85	2331.77
EC50	5.000	2309.07	2230.74	2385.99
EC60	5.253	2359.85	2285.44	2446.54
EC75	5.674	2446.75	2369.24	2561.49
EC80	5.842	2482.12	2400.59	2611.59
EC85	6.036	2523.99	2436.22	2672.78
EC90	6.282	2577.68	2480.16	2753.65
EC95	6.645	2659.35	2544.39	2880.7
EC99	7.326	2819.62	2664.89	3140.35



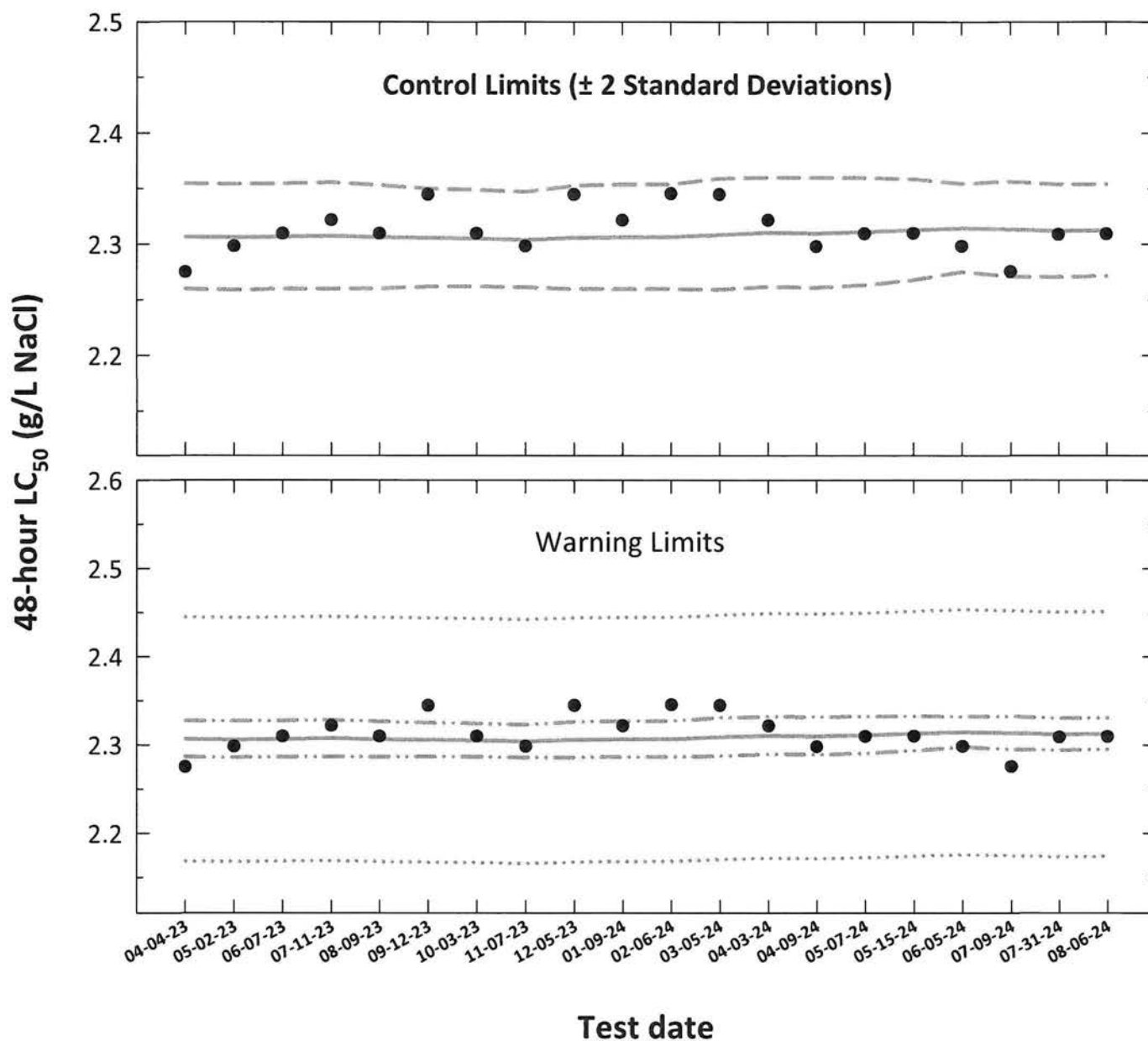
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = 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 LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

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		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	04-04-23	2.2753	0.3570	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
2	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272	2.1680	2.4448
3	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
4	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
5	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
6	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
7	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
8	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
9	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
10	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
11	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
12	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
13	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
14	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
15	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
16	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
17	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
18	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
19	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510
20	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308	2.1740	2.4516

Note: 48-hour LC₅₀ = 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 LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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 LC₅₀ Whole Effluent Toxicity Test, Species: Ceriodaphnia dubia
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 414

Dilution Preparation:

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

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

Stock solution INSS #: 2294

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	<u>7.67</u>	<u>7.37</u>	<u>7.35</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>8.0</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>292</u>		
	Alkalinity (mg/L CaCO ₃)	<u>61</u>		
	Hardness (mg/L CaCO ₃)	<u>84</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.2</u>	<u>25.2</u>
1750 mg/L	pH (S.U.)	<u>7.65</u>	<u>7.36</u>	<u>7.32</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.9</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>3410</u>		
	Temperature (°C)	<u>25.1</u>	<u>24.8</u>	<u>25.0</u>
2000 mg/L	pH (S.U.)	<u>7.64</u>	<u>7.36</u>	<u>7.32</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>7.9</u>	<u>7.9</u>
	Conductivity (µmhos/cm)	<u>3820</u>		
	Temperature (°C)	<u>24.9</u>	<u>25.1</u>	<u>25.0</u>
2250 mg/L	pH (S.U.)	<u>7.62</u>	<u>7.35</u>	<u>7.31</u>
	Dissolved oxygen (mg/L)	<u>7.8</u>	<u>8.0</u>	<u>8.0</u>
	Conductivity (µmhos/cm)	<u>4200</u>		
	Temperature (°C)	<u>24.8</u>	<u>24.7</u>	<u>24.7</u>
2500 mg/L	pH (S.U.)	<u>7.62</u>	<u>7.34</u>	<u>7.32</u>
	Dissolved oxygen (mg/L)	<u>7.9</u>	<u>8.0</u>	<u>8.0</u>
	Conductivity (µmhos/cm)	<u>4710</u>		
	Temperature (°C)	<u>24.8</u>	<u>24.9</u>	<u>25.2</u>
2750 mg/L	pH (S.U.)	<u>7.64</u>	<u>7.34</u>	<u>7.32</u>
	Dissolved oxygen (mg/L)	<u>8.0</u>	<u>8.0</u>	<u>8.0</u>
	Conductivity (µmhos/cm)	<u>5780</u>		
	Temperature (°C)	<u>25.0</u>	<u>25.1</u>	<u>25.0</u>

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	<u>13066165</u>

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 414

Hours	Date	Feeding		Test Initiation or Termination		Location (incubator/shelf)	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	08-06-24	0630	JL	0914	JL	2B2	BLACK	07-31-24A
24	08-07-24			0912	JL			
48 Termination	08-08-24			0915	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	07-30-24 D
Age:	< 24-hours old
Date and time organisms were born between:	08-05-24 1220 to 08-06-24 0630
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.75
	Temperature (°C): 25.0

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	4 ^u	5	5	5	4 ^u	4 ^u	4 ^u	4 ^u	3 ^u	2 ^u	4 ^u	3 ^u
48 Termination	2 ^u	3 ^u	3 ^u	2 ^u	1 ^u	1 ^u	1 ^u	2 ^u	0 ^u	0 ^u	0 ^u	0 ^u
Mean Survival	50%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2239.8
Upper 95% confidence limit (mg NaCl/L)	2383.6
48-hour LC ₅₀ (mg NaCl/L)	2309.6

Comments:



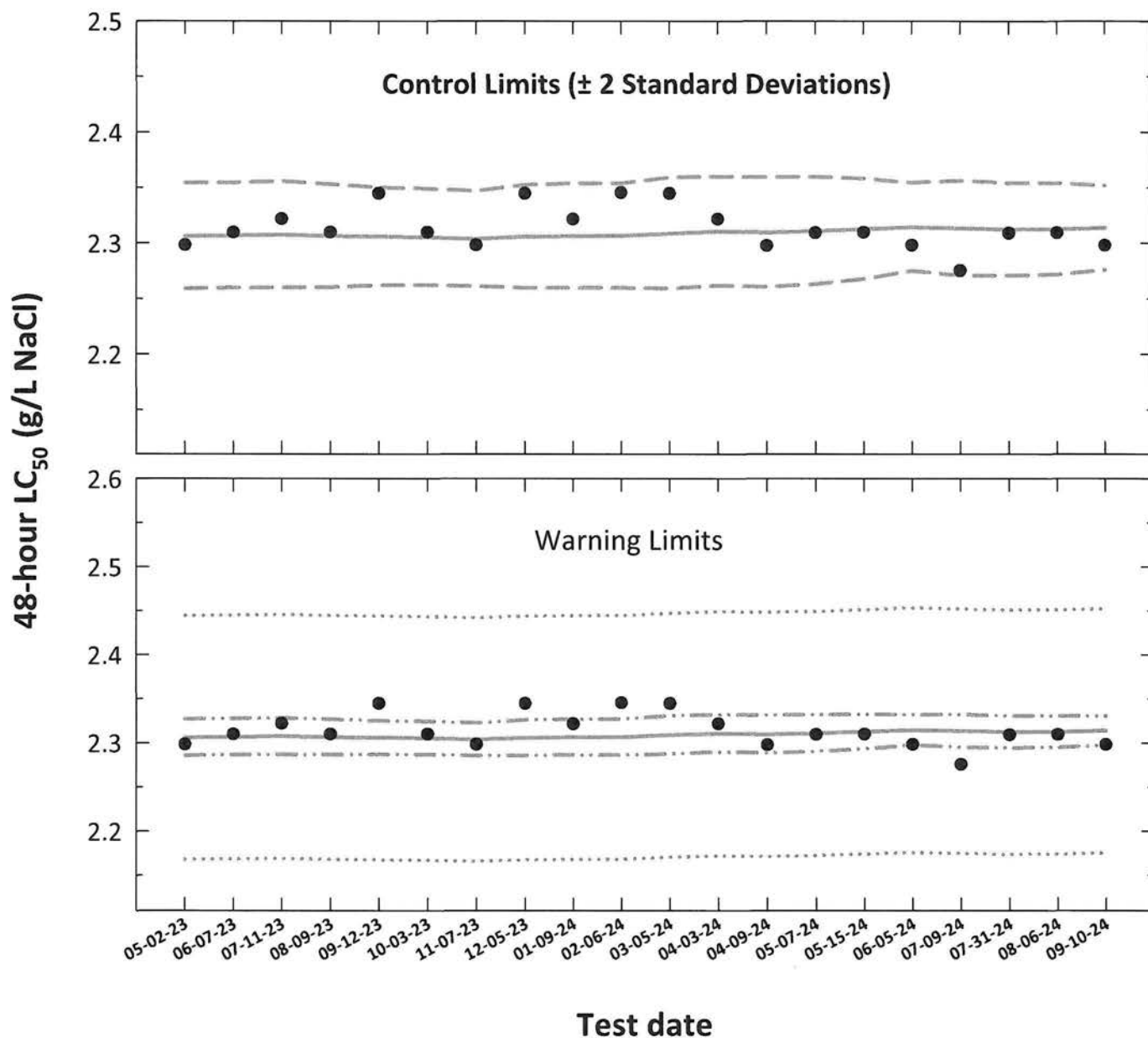
Acute Daphnid Test-48 Hr Survival												
Start Date:	8/6/2024		Test ID:	CdNaClAC			Sample ID:	REF-Ref Toxicant				
End Date:	8/8/2024		Lab ID:	ETS-Envir. Testing Sol.			Sample Type:	NACL-Sodium chloride				
Sample Date:			Protocol:	ACUTE-EPA-821-R-02-012			Test Species:	CD-Ceriodaphnia dubia				
Comments:												
Conc-mg/L	1	2	3	4								
D-Control	1.0000	1.0000	1.0000	1.0000								
1750	1.0000	1.0000	1.0000	1.0000								
2000	1.0000	1.0000	1.0000	1.0000								
2250	0.4000	0.6000	0.6000	0.4000								
2500	0.2000	0.2000	0.2000	0.4000								
2750	0.0000	0.0000	0.0000	0.0000								
Transform: Arcsin Square Root								Rank	1-Tailed	Number	Total	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number	
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20	
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20	
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20	
*2250	0.5000	0.5000	0.7854	0.6847	0.8861	14.802	4	10.00	10.00	10	20	
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20	
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20	
Auxiliary Tests								Statistic	Critical	Skew	Kurt	
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)								0.82032	0.868	0.91483	1.68855	
Equality of variance cannot be confirmed												
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU						
Steel's Many-One Rank Test			2000	2250	2121.32							
Treatments vs D-Control												
Maximum Likelihood-Probit												
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter	
Slope	28.8917	5.16275	18.7727	39.0106	0	3.64582	7.81472	0.30234	3.36353	0.03461	6	
Intercept	-92.178	17.3806	-126.24	-58.112								
TSCR												
Point	Probits	mg/L	95% Fiducial Limits									
EC01	2.674	1918.7	1718.88	2029.87								
EC05	3.355	2025.8	1864.5	2117.99								
EC10	3.718	2085.31	1945.73	2168.05								
EC15	3.964	2126.45	2001.56	2203.56								
EC20	4.158	2159.72	2046.25	2233.09								
EC25	4.326	2188.68	2084.59	2259.6								
EC40	4.747	2263.39	2179.46	2333.09								
EC50	5.000	2309.55	2233.77	2383.59								
EC60	5.253	2356.66	2284.96	2439.95								
EC75	5.674	2437.1	2363.07	2546.91								
EC80	5.842	2469.78	2392.17	2593.49								
EC85	6.036	2508.42	2425.18	2650.35								
EC90	6.282	2557.91	2465.79	2725.42								
EC95	6.645	2633.05	2524.95	2843.16								
EC99	7.326	2780.02	2635.45	3082.98								

Dose-Response Plot

Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = 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 LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.10}$ converted to anti-logarithmic values,
 $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

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	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV
1	05-02-23	2.2985	0.3614	0.3629	0.0045	2.3064	2.2593	2.3545	2.2860	2.3272
2	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278
3	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286
4	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268
5	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251
6	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243
7	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229
8	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263
9	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271
10	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272
11	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307
12	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321
13	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317
14	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323
15	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327
16	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319
17	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322
18	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304
19	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308
20	09-10-24	2.2984	0.3614	0.3644	0.0036	2.3140	2.2763	2.3522	2.2977	2.3305

Note: 48-hour LC₅₀ = 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 ToxCal).
CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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 LC₅₀ Whole Effluent Toxicity Test, Species: Ceriodaphnia dubia
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 415

Dilution Preparation:

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

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

Stock solution INSS #: 1320

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.35	7.37	7.39
	Dissolved oxygen (mg/L)	7.7	7.0	7.0
	Conductivity (µmhos/cm)	298		
	Alkalinity (mg/L CaCO ₃)	64		
	Hardness (mg/L CaCO ₃)	87		
	Temperature (°C)	25.3	25.2	25.3
1750 mg/L	pH (S.U.)	7.54	7.50	7.55
	Dissolved oxygen (mg/L)	7.9	7.8	7.6
	Conductivity (µmhos/cm)	3320		
	Temperature (°C)	25.1	25.4	25.0
2000 mg/L	pH (S.U.)	7.50	7.51	7.56
	Dissolved oxygen (mg/L)	7.9	7.8	7.6
	Conductivity (µmhos/cm)	3000		
	Temperature (°C)	25.3	25.1	25.0
2250 mg/L	pH (S.U.)	7.50	7.53	7.57
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	4230		
	Temperature (°C)	25.3	25.1	25.2
2500 mg/L	pH (S.U.)	7.58	7.54	7.60
	Dissolved oxygen (mg/L)	8.0	7.9	7.8
	Conductivity (µmhos/cm)	4640		
	Temperature (°C)	25.0	25.1	25.2
2750 mg/L	pH (S.U.)	7.58	7.55	7.61
	Dissolved oxygen (mg/L)	8.0	7.9	7.9
	Conductivity (µmhos/cm)	5130		
	Temperature (°C)	25.3	25.3	25.2

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O 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	13064685

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 415

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-10-24	0430	JL	0630	JL	2B3	BLNK	09-04-4A
24	09-11-24			0627	JL			
48 Termination	09-12-24			0625	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	09-03-24 D
Age:	< 24-hours old
Date and time organisms were born between:	09-09-24 1433 to 09-10-24 0430
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.82
	Temperature (°C): 25.0°C

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 ^d	4 ^d	5	5	3 ^d	3 ^d	2 ^d	4 ^d
48 Termination	3 ^{2d}	1 ^{4d}	4 ^{1d}	2 ^{3d}	2 ^{2d}	0 ^{4d}	1 ^{4d}	1 ^{4d}	0 ^{2d}	0 ^{3d}	0 ^{2d}	0 ^{4d}
Mean Survival	50%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2221.7
Upper 95% confidence limit (mg NaCl/L)	2370.1
48-hour LC ₅₀ (mg NaCl/L)	2298.4

Comments:



Start Date:	9/10/2024	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/12/2024	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	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7854	0.4636	1.1071	35.048	4	10.00	10.00	10	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Kurt

2.8664

ChV

2121.32

1000

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	30.6709	5.60227	19.6905 41.6514	0	2.7074	7.81472	0.43897	3.36142	0.0326	6

Point	Probits	mg/L	95% Fiducial Limits
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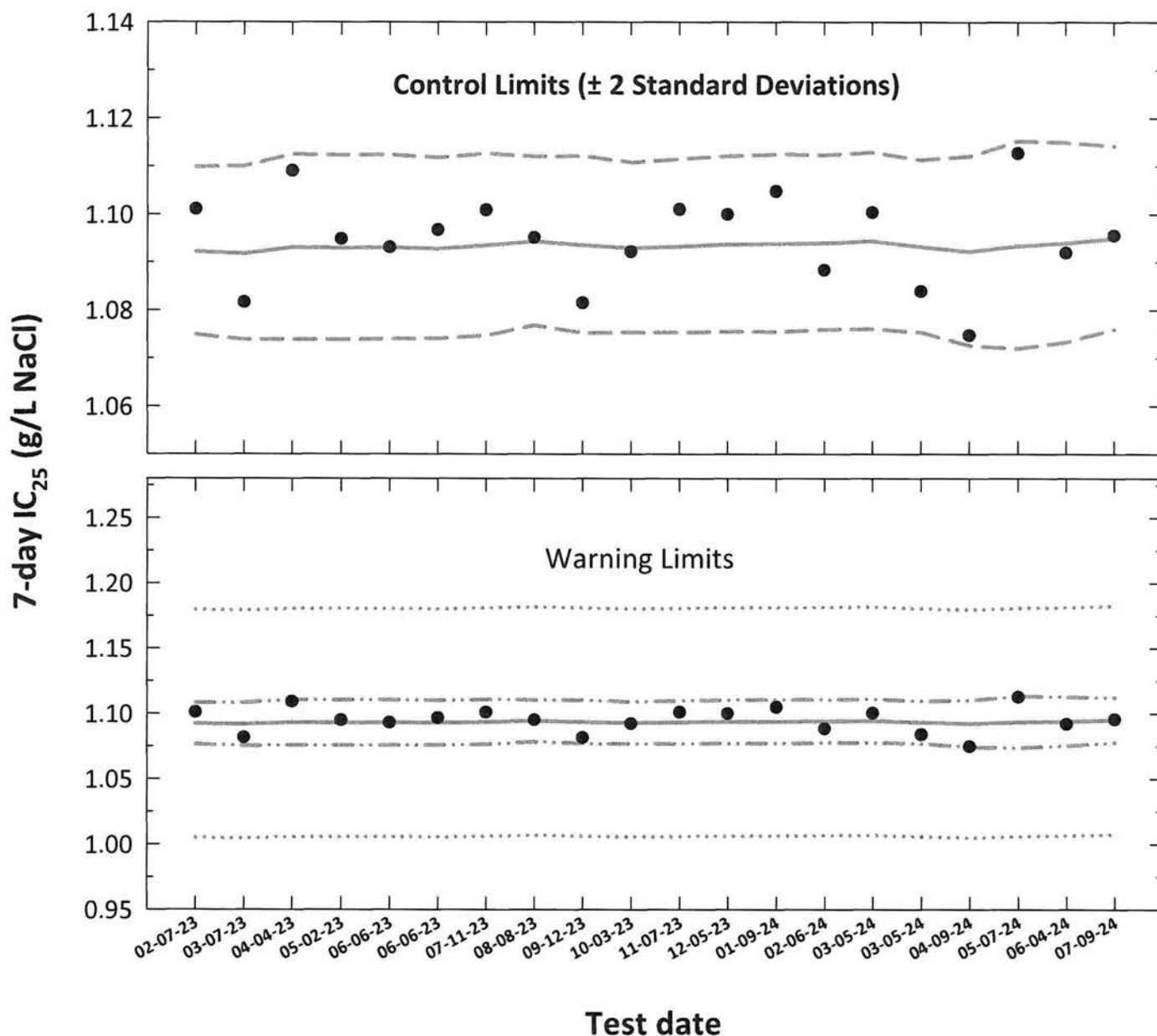
Dose (mg/L)	Response
1	0.00
10	0.00
100	0.00
1,000	0.00
1,500	0.50
2,000	0.80
2,500	1.00
10,000	1.00

Time After Irradiation (hr)	48 Hr Survival (Mean)	48 Hr Survival (SE)
Control	1.0	0.0
1750	1.0	0.0
2000	1.0	0.0
*2250	0.5	0.3
*2500	0.2	0.2
2750	0.0	0.0

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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A,10}$ converted to anti-logarithmic values,
 $S_{A,10} = 10^{\text{th}}$ percentile of CVs reported nationally by USEPA)

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	
1	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
2	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
3	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
4	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
5	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
6	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
7	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
8	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
9	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
10	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
11	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
12	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
13	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCal).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

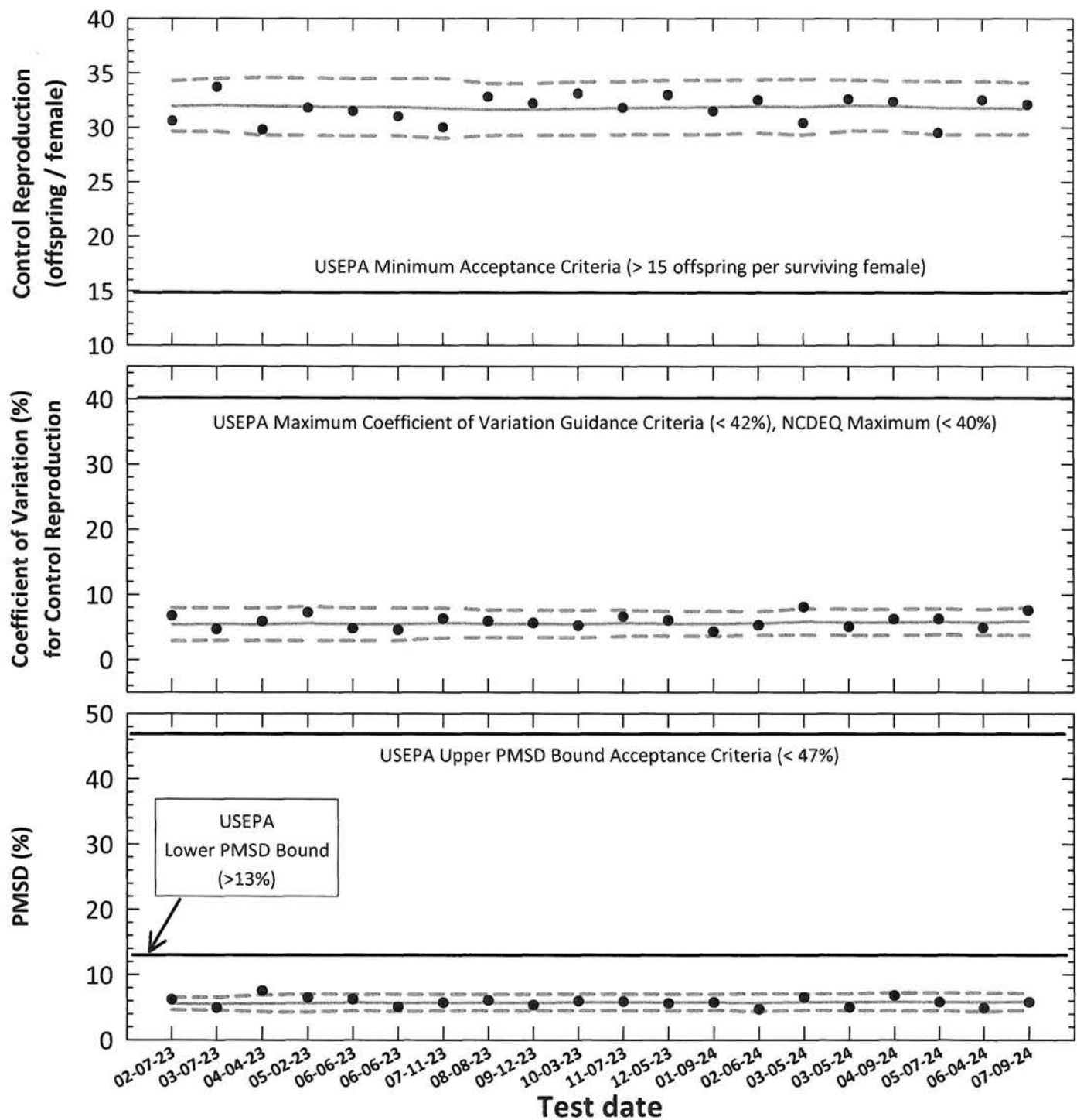
S_{A,10} = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA (S_{A,10} = 0.08).

CV = Coefficient of variation.

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 $\pm$ 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination					Control Reproduction			Control Reproduction CV			Test PMSD		
		Control Survival (%)	Control Reproduction		Test	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (offspring/female)	CV (%)											
1	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5
2	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
3	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9
4	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0
5	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0
6	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0
7	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
8	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
9	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
10	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
11	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
12	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
13	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)

Species: *Ceriodaphnia dubia*

CdNaClCR #: 300

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS 2294				
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:

Test randomization and location:

Organism age:	< 24-hours old										Randomizing template color:	Red
Date and times organisms were born between:	07-09-24 0515 TO 0800										Incubator number and shelf location:	2 B1
Culture board:	07-02-24 A											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	1	2	3	5	11	13	14	18	20	24		
Transfer vessel information:	pH (S.U.): 7.59 Temperature (°C): 24.9											
Average transfer volume (mL):	< 0.25 mL											

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches		MHSW batch used	Analyst
			<i>Selenastrum</i>	YWT		
0	07-09-24	0812	07-02-24	07-02-24	07-01-24 B	JL
1	07-10-24	0615	↓	↓	↓	JL
2	07-11-24	0612	↓	↓	07-08-24 A	JL
3	07-12-24	0612	↓	↓	↓	JL
4	07-13-24	0831	↓	↓	07-08-24 B	JL
5	07-14-24	0615	↓	↓	↓	JL
6	07-15-24	0738	↓	↓	↓	JL
7	07-16-24	0615				JL

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O 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:	07.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	21400
% Adults having 3 rd Broods:	1007.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	07.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	32.1	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.5
% CV:	7.67.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1095.6

Species: Ceriodaphnia dubia

CdNaClCR #: 300

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	4	5	4	3	3	4	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	9	12	10	10	12	13	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	19	14	18	17	17	17	19	13
Total young produced		30	34	33	30	31	30	33	36	35	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:

% Mortality: 07
Mean Offspring/Female: 32.1

600 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:

% Mortality: 07
Mean Offspring/Female: 33.5
% Reduction from Control: -4.42

Species: Ceriodaphnia dubia

CdNaClCR #: 300

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	3	5	5	4	4	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	15	11	10	10	12	13	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	20	17	18	15	18	19	18	15	15
Total young produced		33	38	33	34	30	32	35	35	31	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	33.4
% Reduction from Control:	-4.07.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	3	3	4	3	5	3	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	13	11	11	10	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	19	19	15	16	14	17	13	18	19	16
Total young produced		33	33	31	30	29	30	30	33	32	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:	07.
Mean Offspring/Female:	31.2
% Reduction from Control:	2.87.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 300

1200 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	17.7
% Reduction from Control:	44.97

1400 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	2	1	3	1	2	2	2	2	2	1
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	2	0	3	1	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		2	1	3	3	2	5	3	2	2	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:	07.
Mean Offspring/Female:	2.4
% Reduction from Control:	92.57.

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	4	5	4	3	3	4	6	4	4	41
5	11	12	9	12	10	10	12	13	12	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	19	14	18	17	17	17	19	13	167
Total	30	34	33	30	31	30	33	36	35	29	321

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	5	4	4	3	5	4	4	4	6	43
5	13	11	13	12	13	12	11	12	12	12	121
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	18	17	18	15	19	19	17	16	171
Total	33	32	35	33	34	32	34	35	33	34	335

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	3	5	5	4	4	4	4	6	44
5	12	13	13	11	10	10	12	13	12	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	17	20	17	18	15	18	19	18	15	15	172
Total	33	38	33	34	30	32	35	35	31	33	334

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	3	3	4	3	5	3	3	5	37
5	10	10	13	11	11	10	12	12	10	10	109
6	0	0	0	0	0	0	0	0	0	0	0
7	19	19	15	16	14	17	13	18	19	16	166
Total	33	33	31	30	29	30	30	33	32	31	312

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	4	4	3	4	4	4	5	4	39
5	10	5	7	8	5	9	11	5	7	6	73
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	3	7	10	5	6	9	6	5	65
Total	18	18	14	19	18	18	21	18	18	15	177

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	1	2	2	2	2	2	1	18
5	0	0	0	2	0	3	1	0	0	0	6
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	2	1	3	3	2	5	3	2	2	1	24

***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: CdNaClR #300
Test dates: July 09-16, 2024

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	34	33	30	31	30	33	36	35	29	100	32.1	7.6	Not applicable
600	33	32	35	33	34	32	34	35	33	34	100	33.5	3.2	-4.4
800	33	38	33	34	30	32	35	35	31	33	100	33.4	6.8	-4.0
1000	33	33	31	30	29	30	30	33	32	31	100	31.2	4.7	2.8
1200	18	18	14	19	18	18	21	18	18	15	100	17.7	11.0	44.9
1400	2	1	3	3	2	5	3	2	2	1	100	2.4	48.9	97.5

Dunnett's MSD value: 1.846
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.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 7/9/2024 Test ID: CdNaCICR Sample ID: REF-Ref Toxicant
End Date: 7/16/2024 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	34.000	33.000	30.000	31.000	30.000	33.000	36.000	35.000	29.000
600	33.000	32.000	35.000	33.000	34.000	32.000	34.000	35.000	33.000	34.000
800	33.000	38.000	33.000	34.000	30.000	32.000	35.000	35.000	31.000	33.000
1000	33.000	33.000	31.000	30.000	29.000	30.000	30.000	33.000	32.000	31.000
1200	18.000	18.000	14.000	19.000	18.000	18.000	21.000	18.000	18.000	15.000
1400	2.000	1.000	3.000	3.000	2.000	5.000	3.000	2.000	2.000	1.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%	Critical			MSD	Mean	N-Mean	
D-Control	32.100	1.0000	32.100	29.000	36.000	7.553	10					33.000	1.0000
600	33.500	1.0436	33.500	32.000	35.000	3.224	10	-1.735	2.287	1.846		33.000	1.0000
800	33.400	1.0405	33.400	30.000	38.000	6.798	10	-1.611	2.287	1.846		33.000	1.0000
1000	31.200	0.9720	31.200	29.000	33.000	4.730	10	1.115	2.287	1.846		31.200	0.9455
*1200	17.700	0.5514	17.700	14.000	21.000	10.997	10	17.841	2.287	1.846		17.700	0.5364
*1400	2.400	0.0748	2.400	1.000	5.000	48.908	10	36.796	2.287	1.846		2.400	0.0727

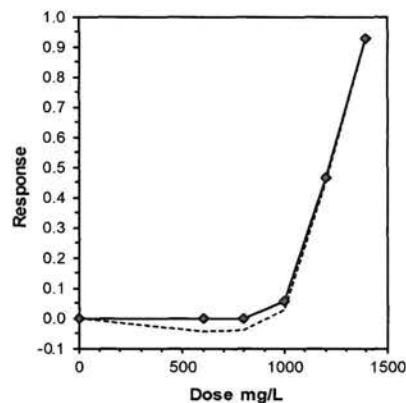
Auxiliary Tests

Kolmogorov D Test indicates normal distribution ($p > 0.01$) 0.67528 1.035 0.22276 0.21549
Bartlett's Test indicates equal variances ($p = 0.09$) 9.39794 15.0863

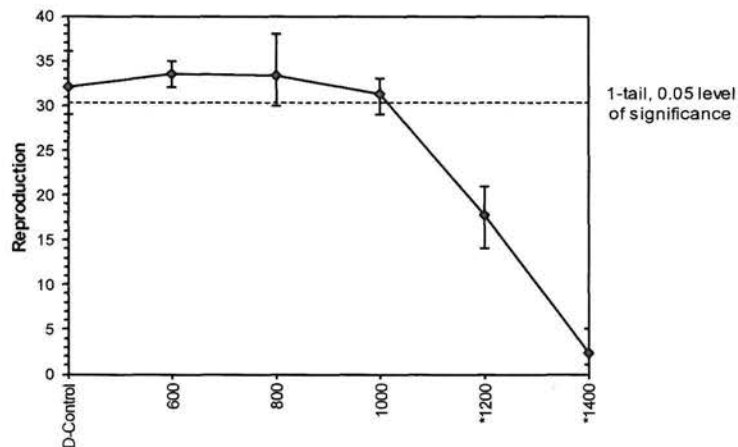
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.84567	0.0575	1591.39	3.25741	1.2E-43	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	983.333	37.5836	885.177	1009.55	-1.0302
IC10	1022.22	7.20018	1006.6	1034.36	0.1817
IC15	1046.67	6.72281	1033.58	1059.16	0.2854
IC20	1071.11	6.46394	1058.21	1083.88	0.3829
IC25	1095.56	6.44993	1082.39	1108.95	0.4451
IC40	1168.89	7.77534	1154.27	1183.68	0.3795
IC50	1215.69	7.10914	1200.96	1227.93	-0.0939



Dose-Response Plot



Species: Ceriodaphnia dubia

CdNaClCR #: 300

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	
		kd	kd n	kd n	kd n	kd n	n
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.37	7.47	7.41	7.45	7.32	7.74
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	8.0	7.7	7.9
	Conductivity (µmhos/cm)	302		304		306	
	Alkalinity (mg CaCO ₃ /L)	60				61	
	Hardness (mg CaCO ₃ /L)	84				84	
	Temperature (°C)	24.8	25.1	24.8	24.9	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.45	7.43	7.38	7.43	7.34	7.81
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	8.0	7.7	7.8
	Conductivity (µmhos/cm)	1450		1460		1420	
	Temperature (°C)	24.9	24.9	24.9	25.1	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.46	7.41	7.38	7.42	7.35	7.78
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	8.0	7.8	7.9
	Conductivity (µmhos/cm)	1800		1840		1790	
	Temperature (°C)	24.9	24.9	24.9	25.1	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.45	7.41	7.41	7.41	7.34	7.78
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	7.9	7.8	7.9
	Conductivity (µmhos/cm)	2170		2150		2150	
	Temperature (°C)	24.8	25.2	24.9	25.2	24.8	25.2
1200 mg NaCl/L	pH (S.U.)	7.47	7.40	7.38	7.40	7.35	7.78
	Dissolved oxygen (mg/L)	7.7	7.8	8.1	8.0	7.9	7.8
	Conductivity (µmhos/cm)	2540		2530		2500	
	Temperature (°C)	24.8	24.8	24.9	25.0	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.48	7.40	7.39	7.40	7.40	7.78
	Dissolved oxygen (mg/L)	7.8	7.9	8.1	8.0	7.9	7.8
	Conductivity (µmhos/cm)	2920		2940		2920	
	Temperature (°C)	24.8	25.0	24.9	25.2	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

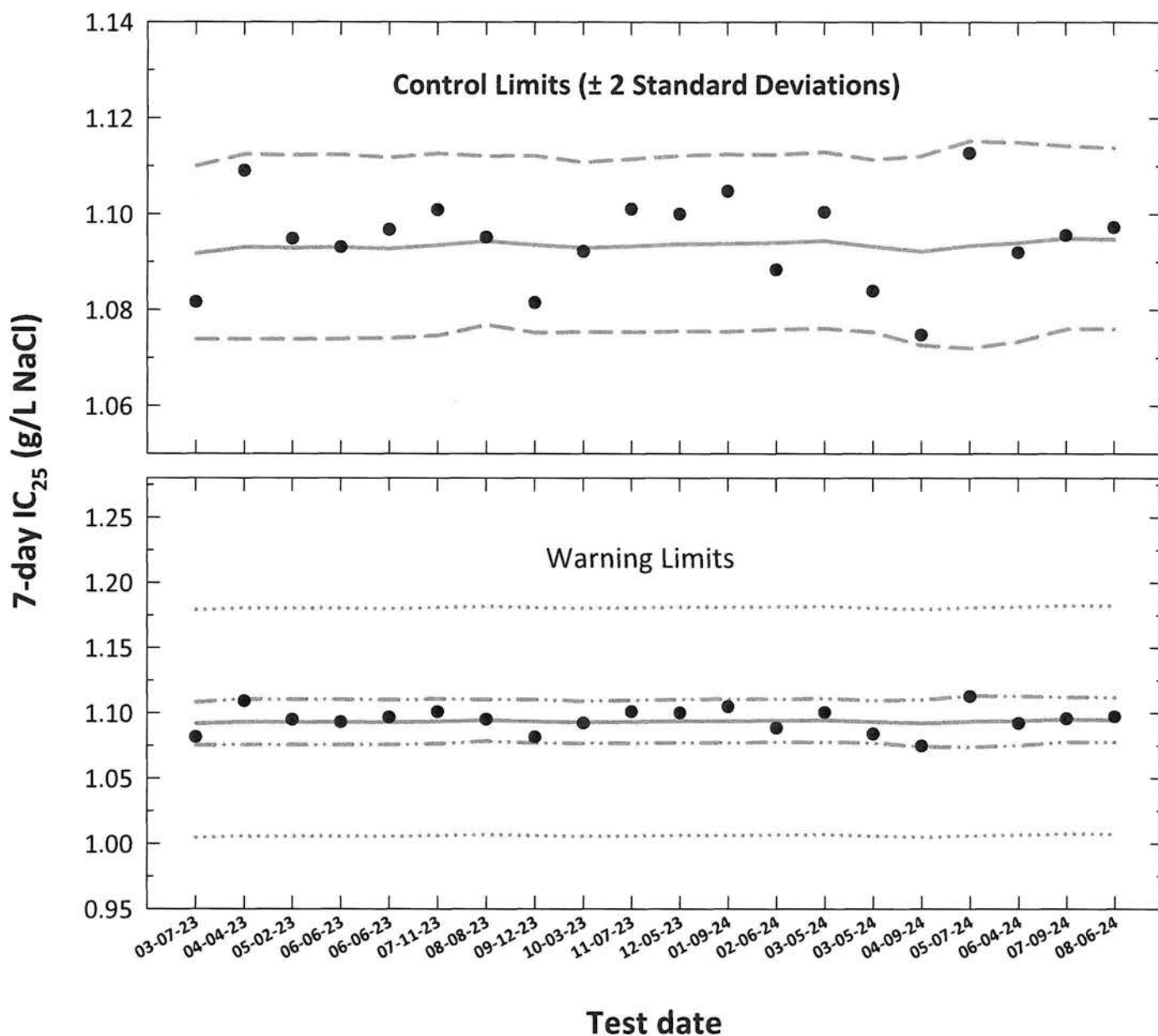
CdNaClCR #: 300

		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Analyst		W	BSL	BSL	BSL	BSL	W	W	W
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.04	7.58	7.55	7.57	7.66	7.55	7.44	7.57
	Dissolved oxygen (mg/L)	7.7	8.0	7.8	7.8	7.8	7.0	7.9	7.9
	Conductivity (µmhos/cm)	299		299		306		300	
	Alkalinity (mg CaCO ₃ /L)			61					
	Hardness (mg CaCO ₃ /L)			84					
	Temperature (°C)	24.7	25.1	24.8	24.9	24.8	25.1	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.06	7.57	7.45	7.52	7.38	7.54	7.54	7.56
	Dissolved oxygen (mg/L)	7.8	8.0	7.7	7.8	7.7	7.0	7.9	8.0
	Conductivity (µmhos/cm)	1370		1450		140		1450	
	Temperature (°C)	24.7	24.8	24.8	24.9	24.9	24.9	25.0	25.0
800 mg NaCl/L	pH (S.U.)	7.35	7.55	7.48	7.50	7.42	7.53	7.54	7.56
	Dissolved oxygen (mg/L)	7.8	8.0	7.7	7.8	7.8	7.9	7.9	8.0
	Conductivity (µmhos/cm)	1720		1820		1790		1840	
	Temperature (°C)	24.8	24.8	24.8	25.1	24.9	24.9	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.35	7.53	7.49	7.49	7.43	7.53	7.57	7.55
	Dissolved oxygen (mg/L)	7.8	8.0	7.8	7.9	7.9	7.9	8.0	8.0
	Conductivity (µmhos/cm)	2060		2180		2150		2210	
	Temperature (°C)	24.8	25.2	24.9	25.1	24.8	25.2	24.9	25.2
1200 mg NaCl/L	pH (S.U.)	7.04	7.51	7.49	7.47	7.43	7.52	7.56	7.55
	Dissolved oxygen (mg/L)	7.9	8.0	7.8	8.0	7.9	7.0	8.0	8.0
	Conductivity (µmhos/cm)	2430		2520		2500		2590	
	Temperature (°C)	24.9	25.0	24.9	25.1	24.8	25.2	24.8	25.1
1400 mg NaCl/L	pH (S.U.)	7.04	7.50	7.51	7.45	7.44	7.52	7.56	7.55
	Dissolved oxygen (mg/L)	7.9	8.0	7.9	8.0	7.9	7.0	8.0	7.9
	Conductivity (µmhos/cm)	1810		2820		2850		2990	
	Temperature (°C)	24.9	25.2	24.8	25.0	24.8	25.0	24.8	25.1
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC₂₅** = 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 IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ $\pm S_{A.10}$ converted to anti-logarithmic values,
S_{A.10} = 10th percentile of CVs reported nationally by USEPA)

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 CT - 2S CT + 2S		Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}		
1	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791
2	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
3	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
4	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
5	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
6	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
7	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
8	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
9	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
10	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
11	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
12	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCal).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

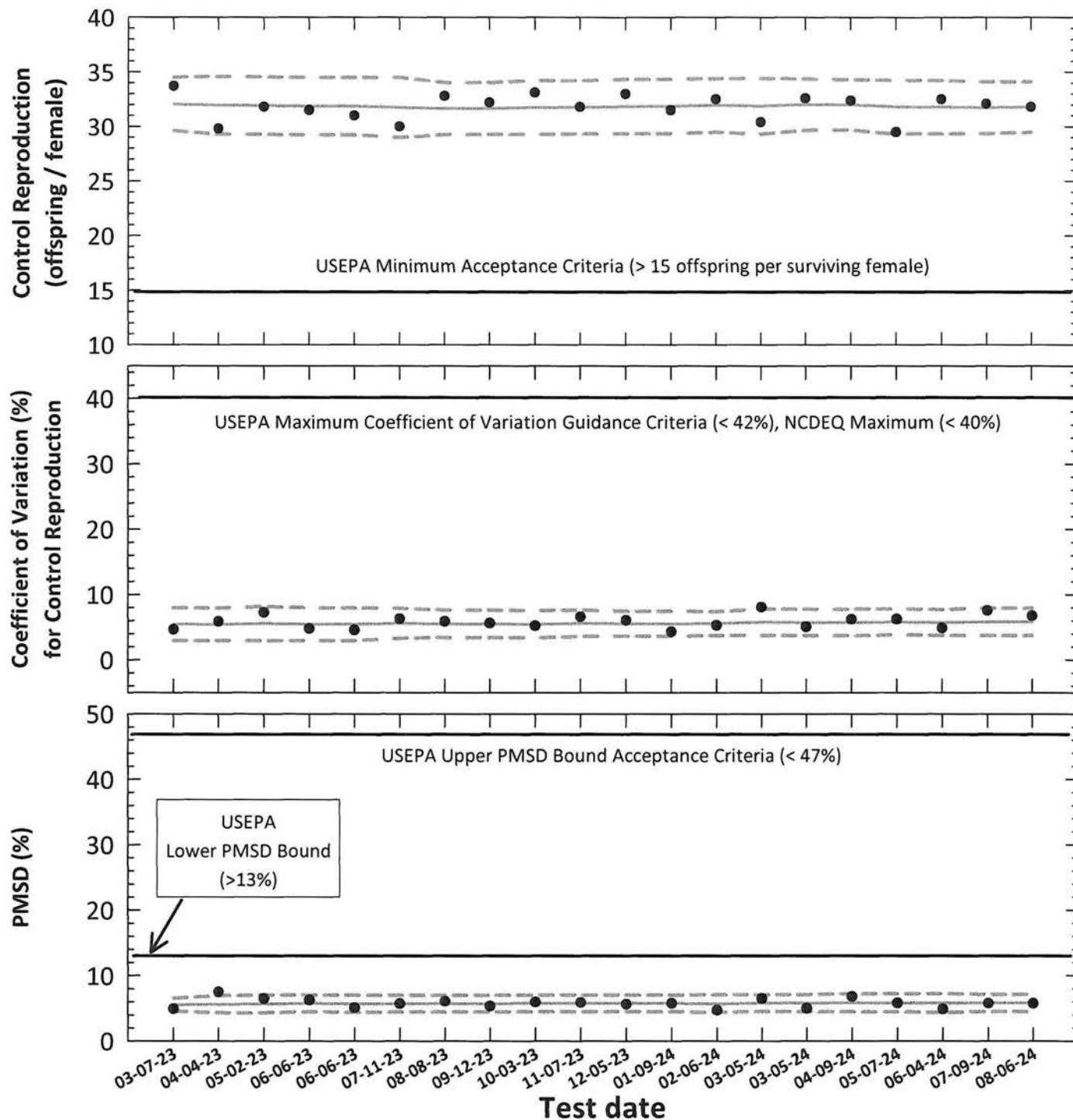
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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.

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 $\pm$ 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination					Control Reproduction			Control Reproduction CV			Test PMSD		
		Control Survival (%)	Control Reproduction		Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (offspring/female)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	4.5	6.5
2	04-04-23	100	29.8	5.9	2.241	7.5	32.0	29.3	34.6	5.4	2.9	7.9	5.6	4.3	6.9
3	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0
4	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0
5	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0
6	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
7	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
8	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
9	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
10	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
11	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
12	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)

Species: *Ceriodaphnia dubia*

CdNaClCR #: 301

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS 2294				
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:

Test randomization and location:

Organism age:	< 24-hours old										Randomizing template color:	Blue
Date and times organisms were born between:	08-06-24 0630 to 0850										Incubator number and shelf location:	2B1
Culture board:	01-30-24 A											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	3	4	7	8	12	18	20	21	22	23		
Transfer vessel information:	pH (S.U.): 7.73 Temperature (°C): 25.0											
Average transfer volume (mL):	< 0.25 mL											

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches <i>Selenastrum</i>	YWT	MHSW batch used	Analyst
0	08-06-24	0900	08-02-24	08-02-24	01-31-24A	H
1	08-07-24	0800			↓	H
2	08-08-24	0800			01-31-24D	H
3	08-09-24	0840			↓	H
4	08-10-24	0840			01-01-24A	H
5	08-11-24	0830			↓	H
6	08-12-24	0830			↓	H
7	08-13-24	0711				H

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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:	07.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	71400
% Adults having 3 rd Broods:	1007.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	07.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	31.8	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.5
% CV:	6.87.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1097.2

Species: Ceriodaphnia dubia

CdNaClCR #: 301

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	4	6	4	6	5	5	5	6	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	13	11	12	13	13	10	13	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	17	14	18	14	13	16	15	15	18
Total young produced		21	31	33	33	32	31	31	30	34	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd 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: 01.

Mean Offspring/Female: 31.8

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	4	6	5	4	5	5	5	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	12	13	13	12	10	12	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	17	15	19	16	19	18	14	18	16
Total young produced		31	33	33	37	33	36	33	31	35	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: 01.

Mean Offspring/Female: 33.6

% Reduction from Control: -5.77.

Species: Ceriodaphnia dubia

CdNaClCR #: 301

800 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	6	4	5	5	5	4	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	10	10	13	12	13	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	16	16	13	19	15	15	15	19	14
Total young produced		31	31	34	27	34	33	32	32	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:	07.
Mean Offspring/Female:	31.4
% Reduction from Control:	-0.37.

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	L	6	4	4	6	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	11	10	10	10	12	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	14	14	13	17	18	13	16	19	14
Total young produced		30	29	31	29	31	32	31	32	33	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:	07.
Mean Offspring/Female:	30.6
% Reduction from Control:	3.87.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 301

1200 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	5	5	4	4	4	3	3	3	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	7	10	9	11	7	8	12	5	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	5	5	6	3	6	4	5	9	6
Total young produced		17	17	20	19	18	17	15	20	17	21
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.1
% Reduction from Control:	43.17.

1400 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.7
% Reduction from Control:	94.77.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	1	2	3	4	5	6	7	8	9	10	Total
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	6	4	6	5	5	5	6	5	50
5	11	10	13	11	12	13	13	10	13	10	116
6	0	0	0	0	0	0	0	0	0	0	0
7	12	17	14	18	14	13	16	15	15	18	152
Total	27	31	33	33	32	31	34	30	34	33	318

1000 mg NaCl/L

Day	1	2	3	4	5	6	7	8	9	10	Total
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	6	6	4	4	6	6	4	4	49
5	12	11	11	10	10	10	12	10	10	10	106
6	0	0	0	0	0	0	0	0	0	0	0
7	13	14	14	13	17	18	13	16	19	14	151
Total	30	29	31	29	31	32	31	32	33	28	306

600 mg NaCl/L

Day	1	2	3	4	5	6	7	8	9	10	Total
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	6	4	6	5	4	5	5	5	5	6	51
5	11	12	12	13	13	12	10	12	12	12	119
6	0	0	0	0	0	0	0	0	0	0	0
7	14	17	15	19	16	19	18	14	18	16	166
Total	31	33	33	37	33	36	33	31	35	34	336

1200 mg NaCl/L

Day	1	2	3	4	5	6	7	8	9	10	Total
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	5	5	4	4	4	3	3	3	5	39
5	9	7	10	9	11	7	8	12	5	10	88
6	0	0	0	0	0	0	0	0	0	0	0
7	5	5	5	6	3	6	4	5	9	6	54
Total	17	17	20	19	18	17	15	20	17	21	181

800 mg NaCl/L

Day	1	2	3	4	5	6	7	8	9	10	Total
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	6	4	5	5	5	4	4	6	47
5	12	11	12	10	10	13	12	13	10	12	115
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	16	13	19	15	15	15	19	14	157
Total	31	31	34	27	34	33	32	32	33	32	319

1400 mg NaCl/L

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

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

Quality Control

Verification of Data Entry, Calculations, and Statistical Analyses

Test number: **CdNaClCR #301**
Test dates: **August 06-13, 2024**

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	27	31	33	33	32	31	34	30	34	33	100	31.8	6.8	Not applicable
600	31	33	33	37	33	36	33	31	35	34	100	33.6	5.8	-5.7
800	31	31	34	27	34	33	32	32	33	32	100	31.9	6.3	-0.3
1000	30	29	31	29	31	32	31	32	33	28	100	30.6	5.2	3.8
1200	17	17	20	19	18	17	15	20	17	21	100	18.1	10.2	43.1
1400	2	2	1	1	1	3	1	1	3	2	100	1.7	48.4	94.7

Dunnett's MSD value: 1.827 MSD = Minimum Significant Difference
PMSD: 5.7 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) = 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.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 8/6/2024 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
End Date: 8/13/2024 Lab ID: ETS-Envr. Testing Sol. Sample Type: NACL-Sodium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	27.000	31.000	33.000	33.000	32.000	31.000	34.000	30.000	34.000	33.000
600	31.000	33.000	33.000	37.000	33.000	36.000	33.000	31.000	35.000	34.000
800	31.000	31.000	34.000	27.000	34.000	33.000	32.000	32.000	33.000	32.000
1000	30.000	29.000	31.000	29.000	31.000	32.000	31.000	32.000	33.000	28.000
1200	17.000	17.000	20.000	19.000	18.000	17.000	15.000	20.000	17.000	21.000
1400	2.000	2.000	1.000	1.000	1.000	3.000	1.000	1.000	3.000	2.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed			Isotonic	
			Mean	Min	Max	CV%	N		Critical	MSD	Mean	N-Mean	
D-Control	31.800	1.0000	31.800	27.000	34.000	6.761	10					32.700	1.0000
600	33.600	1.0566	33.600	31.000	37.000	5.819	10	-2.253	2.287	1.827		32.700	1.0000
800	31.900	1.0031	31.900	27.000	34.000	6.347	10	-0.125	2.287	1.827		31.900	0.9755
1000	30.600	0.9623	30.600	28.000	33.000	5.156	10	1.502	2.287	1.827		30.600	0.9358
*1200	18.100	0.5692	18.100	15.000	21.000	10.237	10	17.150	2.287	1.827		18.100	0.5535
*1400	1.700	0.0535	1.700	1.000	3.000	48.428	10	37.680	2.287	1.827		1.700	0.0520

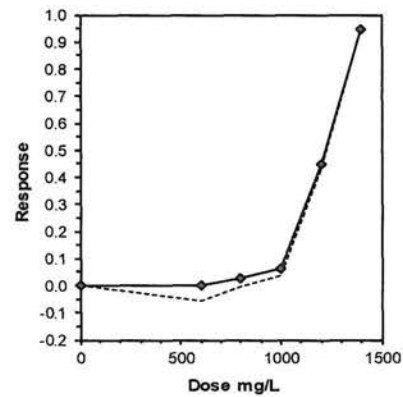
Auxiliary Tests

Kolmogorov D Test indicates normal distribution ($p > 0.01$)
Bartlett's Test indicates equal variances ($p = 0.16$)

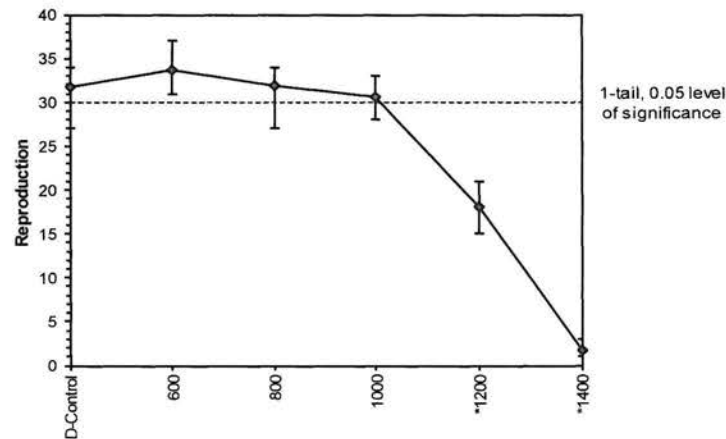
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.82668	0.05744	1577.58	3.19074	8.8E-44	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95%CL		Skew
IC05	928.462	79.8784	749.933	1010.35	-0.5879
IC10	1018.72	10.4457	994.693	1035.83	-1.0969
IC15	1044.88	8.46714	1028.12	1060.7	-0.1642
IC20	1071.04	7.91911	1057.04	1085.33	-0.0415
IC25	1097.2	7.6635	1083.47	1111.74	0.0763
IC40	1175.68	8.76142	1162.19	1194.92	0.2941
IC50	1221.34	6.35846	1209.28	1233.16	-0.0893



Dose-Response Plot



Species: *Ceriodaphnia dubia*

CdNaClCR #: 301

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					
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	N	N	N	N	N	N
	Dissolved oxygen (mg/L)	7.67	7.56	7.40	7.46	7.48	7.70
	Conductivity (µmhos/cm)	7.8	7.8	7.8	7.9	7.9	7.6
	Alkalinity (mg CaCO ₃ /L)	292		300		312	
	Hardness (mg CaCO ₃ /L)	61				61	
	Temperature (°C)	84				86	
600 mg NaCl/L	pH (S.U.)	24.8	25.1	24.9	25.2	24.8	25.2
	Dissolved oxygen (mg/L)	7.71	7.43	7.37	7.33	7.25	7.67
	Conductivity (µmhos/cm)	7.8	7.7	7.8	7.8	7.8	7.6
	Temperature (°C)	1410		1430		1480	
800 mg NaCl/L	pH (S.U.)	24.9	24.9	25.0	25.0	24.9	25.0
	Dissolved oxygen (mg/L)	7.71	7.43	7.37	7.31	7.24	7.67
	Conductivity (µmhos/cm)	7.8	7.7	7.8	7.9	7.8	7.6
	Temperature (°C)	1780		1800		1860	
1000 mg NaCl/L	pH (S.U.)	25.0	24.9	25.0	24.8	24.9	25.0
	Dissolved oxygen (mg/L)	7.71	7.42	7.37	7.32	7.26	7.65
	Conductivity (µmhos/cm)	7.8	7.7	7.7	7.9	7.8	7.7
	Temperature (°C)	2150		2160		2200	
1200 mg NaCl/L	pH (S.U.)	25.0	25.3	24.9	24.8	24.9	25.1
	Dissolved oxygen (mg/L)	7.71	7.42	7.37	7.32	7.28	7.64
	Conductivity (µmhos/cm)	7.8	7.7	7.7	7.9	7.8	7.8
	Temperature (°C)	2530		2530		2650	
1400 mg NaCl/L	pH (S.U.)	25.0	25.0	24.9	25.1	24.9	25.1
	Dissolved oxygen (mg/L)	7.71	7.39	7.37	7.31	7.29	7.65
	Conductivity (µmhos/cm)	7.9	7.7	7.7	7.9	7.8	7.6
	Temperature (°C)	3030		2980		3050	
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

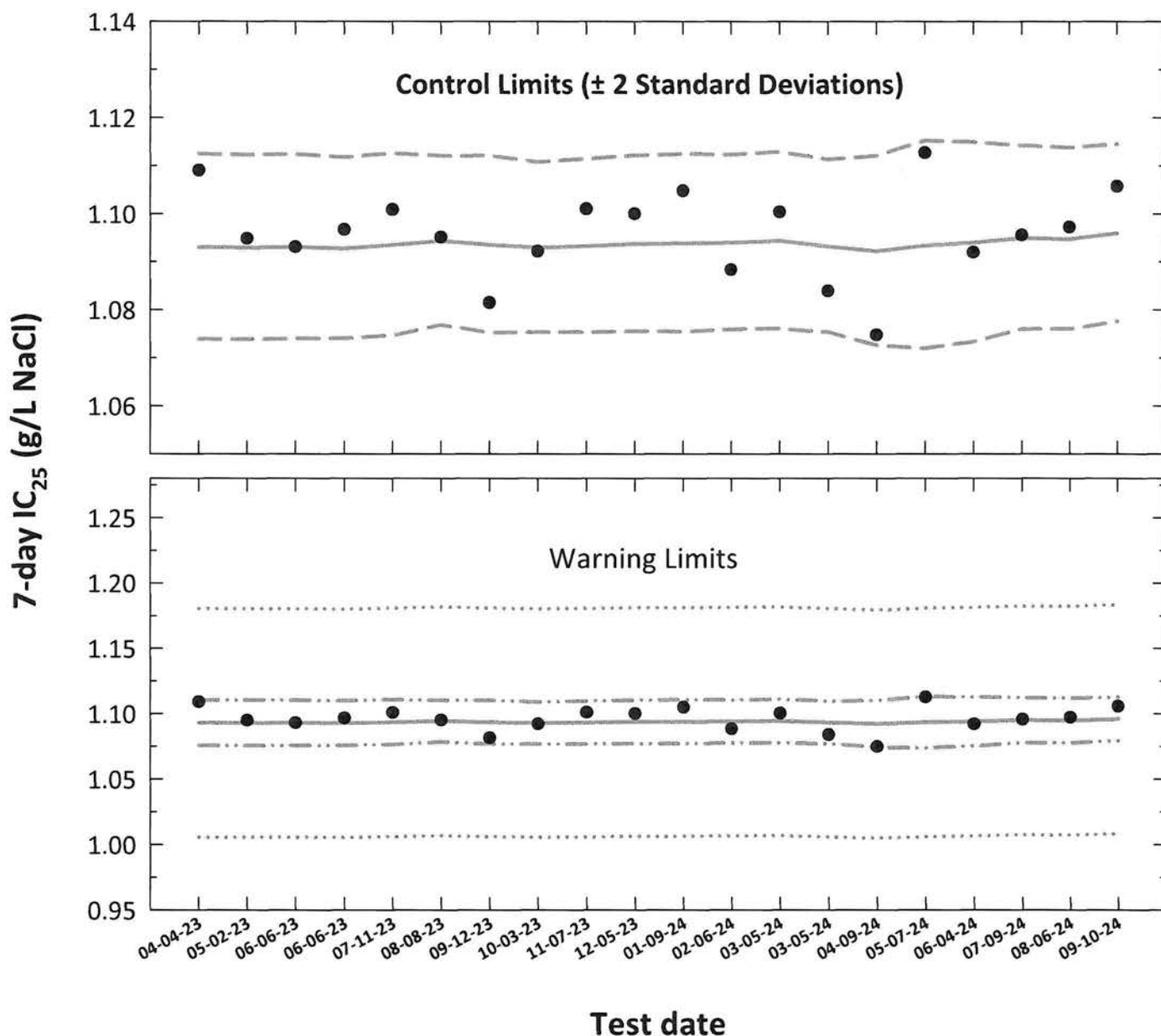
CdNaClCR #: 301

Concentration		Analyst	Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
			<i>N</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>BSL</i>	<i>N</i>	<i>N</i>	<i>K</i>
CONTROL, MHSW	Parameter									
	pH (S.U.)		<i>7.01</i>	<i>7.46</i>	<i>7.57</i>	<i>7.49</i>	<i>7.66</i>	<i>7.72</i>	<i>7.62</i>	<i>7.54</i>
	Dissolved oxygen (mg/L)		<i>7.8</i>	<i>8.0</i>	<i>7.9</i>	<i>7.7</i>	<i>7.7</i>	<i>7.8</i>	<i>7.9</i>	<i>7.9</i>
	Conductivity (µmhos/cm)		<i>294</i>		<i>292</i>		<i>297</i>		<i>315</i>	
	Alkalinity (mg CaCO ₃ /L)				<i>62</i>					
	Hardness (mg CaCO ₃ /L)				<i>88</i>					
	Temperature (°C)		<i>25.9</i>	<i>25.3</i>	<i>24.8</i>	<i>24.9</i>	<i>24.9</i>	<i>25.1</i>	<i>24.8</i>	<i>25.2</i>
600 mg NaCl/L	pH (S.U.)		<i>7.01</i>	<i>7.59</i>	<i>7.38</i>	<i>7.58</i>	<i>7.52</i>	<i>7.66</i>	<i>7.60</i>	<i>7.56</i>
	Dissolved oxygen (mg/L)		<i>7.8</i>	<i>8.0</i>	<i>7.9</i>	<i>7.8</i>	<i>7.7</i>	<i>7.8</i>	<i>7.9</i>	<i>7.9</i>
	Conductivity (µmhos/cm)		<i>1390</i>		<i>1400</i>		<i>1450</i>		<i>1450</i>	
	Temperature (°C)		<i>24.9</i>	<i>25.1</i>	<i>24.9</i>	<i>25.1</i>	<i>25.0</i>	<i>24.9</i>	<i>24.9</i>	<i>25.0</i>
800 mg NaCl/L	pH (S.U.)		<i>7.00</i>	<i>7.58</i>	<i>7.45</i>	<i>7.58</i>	<i>7.53</i>	<i>7.65</i>	<i>7.69</i>	<i>7.55</i>
	Dissolved oxygen (mg/L)		<i>7.7</i>	<i>8.0</i>	<i>7.9</i>	<i>7.8</i>	<i>7.8</i>	<i>7.8</i>	<i>7.9</i>	<i>7.9</i>
	Conductivity (µmhos/cm)		<i>1740</i>		<i>1790</i>		<i>1820</i>		<i>1840</i>	
	Temperature (°C)		<i>25.0</i>	<i>25.1</i>	<i>25.0</i>	<i>25.0</i>	<i>25.0</i>	<i>24.9</i>	<i>24.9</i>	<i>25.0</i>
1000 mg NaCl/L	pH (S.U.)		<i>7.00</i>	<i>7.56</i>	<i>7.45</i>	<i>7.56</i>	<i>7.55</i>	<i>7.63</i>	<i>7.69</i>	<i>7.55</i>
	Dissolved oxygen (mg/L)		<i>7.8</i>	<i>8.0</i>	<i>7.9</i>	<i>7.8</i>	<i>7.8</i>	<i>7.8</i>	<i>8.0</i>	<i>8.0</i>
	Conductivity (µmhos/cm)		<i>2120</i>		<i>2130</i>		<i>2210</i>		<i>2220</i>	
	Temperature (°C)		<i>25.0</i>	<i>25.2</i>	<i>25.0</i>	<i>24.8</i>	<i>24.9</i>	<i>25.2</i>	<i>24.9</i>	<i>25.3</i>
1200 mg NaCl/L	pH (S.U.)		<i>7.00</i>	<i>7.53</i>	<i>7.46</i>	<i>7.56</i>	<i>7.53</i>	<i>7.64</i>	<i>7.70</i>	<i>7.53</i>
	Dissolved oxygen (mg/L)		<i>7.8</i>	<i>8.0</i>	<i>8.0</i>	<i>7.8</i>	<i>7.8</i>	<i>7.8</i>	<i>9.0</i>	<i>8.0</i>
	Conductivity (µmhos/cm)		<i>2480</i>		<i>2500</i>		<i>2630</i>		<i>2610</i>	
	Temperature (°C)		<i>25.0</i>	<i>25.2</i>	<i>25.0</i>	<i>24.8</i>	<i>25.0</i>	<i>25.2</i>	<i>24.9</i>	<i>25.3</i>
1400 mg NaCl/L	pH (S.U.)		<i>7.79</i>	<i>7.51</i>	<i>7.51</i>	<i>7.55</i>	<i>7.54</i>	<i>7.66</i>	<i>7.70</i>	<i>7.53</i>
	Dissolved oxygen (mg/L)		<i>7.8</i>	<i>8.0</i>	<i>8.0</i>	<i>7.8</i>	<i>7.8</i>	<i>7.8</i>	<i>8.0</i>	<i>8.0</i>
	Conductivity (µmhos/cm)		<i>2900</i>		<i>2920</i>		<i>3000</i>		<i>3010</i>	
	Temperature (°C)		<i>25.0</i>	<i>25.2</i>	<i>25.0</i>	<i>24.8</i>	<i>25.1</i>	<i>25.0</i>	<i>25.0</i>	<i>25.0</i>
			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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values,
 $S_{A.10} = 10^{\text{th}}$ percentile of CVs reported nationally by USEPA)

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	
1	04-04-23	1.1090	0.0449	0.0386	0.0038	1.0930	1.0739	1.1125	1.0756	1.1105	1.0056	1.1805
2	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
3	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
4	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
5	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
6	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
7	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
8	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
9	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
10	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
11	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the sodium chloride concentration that would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCal).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

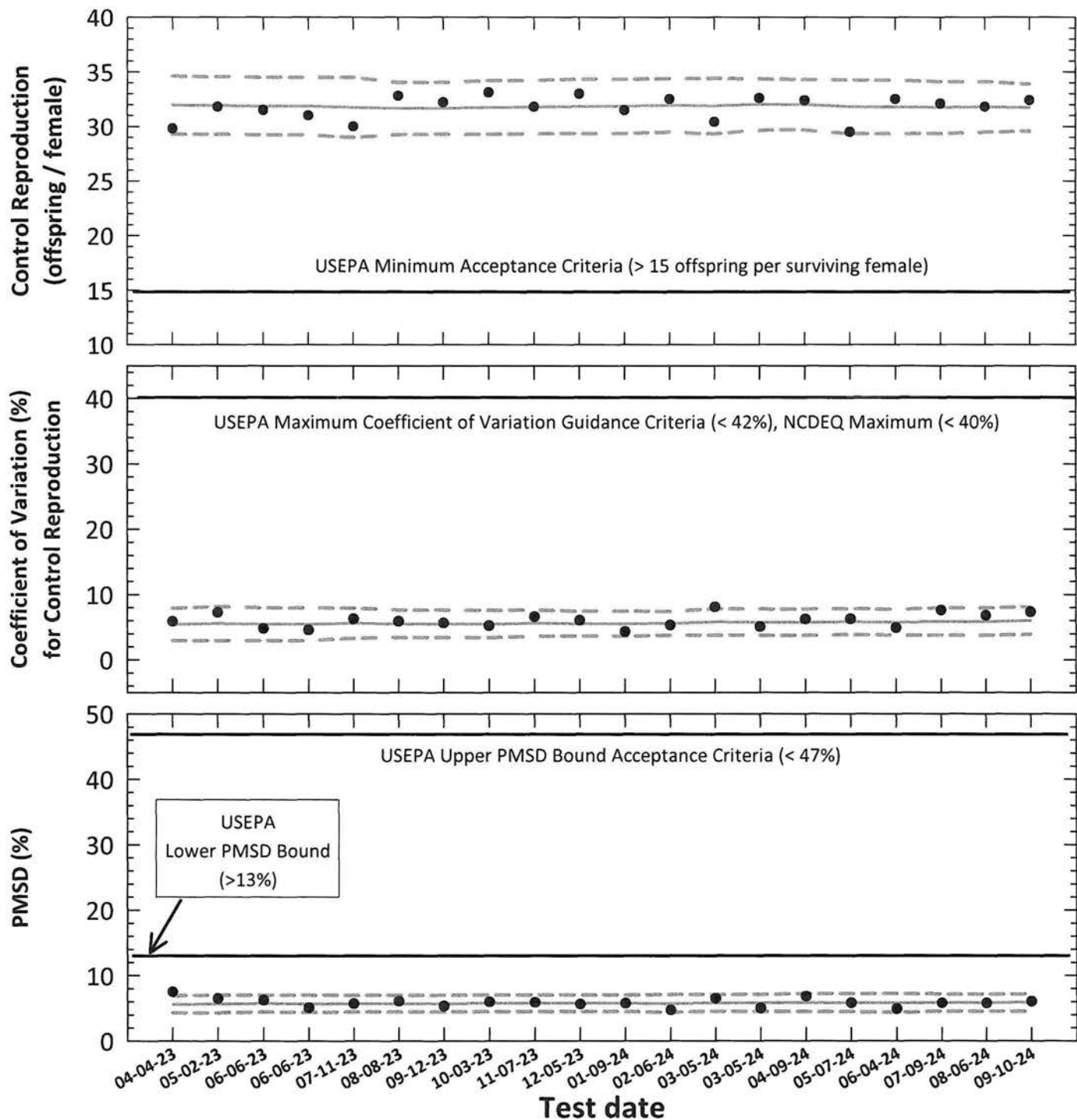
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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.

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 $\pm$ 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination				Control Reproduction		Control Reproduction		Control Reproduction CV		Test PMSD	
		Control Survival (%)	Control Reproduction		Test	CT	95% Confidence Interval CT - 2S CT + 2S	CT	95% Confidence Interval CT - 2S CT + 2S	CT	95% Confidence Interval CT - 2S CT + 2S	CT	95% Confidence Interval CT - 2S CT + 2S
			Mean (offspring/female)	CV (%)									
1	04-04-23	100	29.8	5.9	2.241	32.0	29.3	5.4	2.9	5.6	7.9	5.6	4.3
2	05-02-23	100	31.8	7.2	2.054	31.9	29.3	5.5	2.9	5.7	8.2	5.7	4.3
3	06-06-23	100	31.5	4.8	1.965	31.9	29.2	5.4	2.9	5.7	8.0	5.7	4.5
4	06-06-23	100	31.0	4.6	1.573	31.9	29.2	5.5	2.9	5.7	8.0	5.7	4.4
5	07-11-23	100	30.0	6.3	1.710	31.8	29.0	5.6	3.3	5.7	7.9	5.7	4.4
6	08-08-23	100	32.8	5.9	1.990	31.7	29.3	5.5	3.4	5.7	7.6	5.7	4.4
7	09-12-23	100	32.2	5.6	1.717	31.7	29.3	5.5	3.4	5.7	7.6	5.7	4.4
8	10-03-23	100	33.1	5.2	1.970	31.8	29.3	5.5	3.4	5.7	7.5	5.7	4.5
9	11-07-23	100	31.8	6.6	1.867	31.8	29.3	5.6	3.6	5.8	7.6	5.8	4.5
10	12-05-23	100	33.0	6.1	1.849	31.9	29.4	5.5	3.6	5.7	7.4	5.7	4.5
11	01-09-24	100	31.5	4.3	1.810	31.9	29.4	5.5	3.6	5.7	7.4	5.7	4.5
12	02-06-24	100	32.5	5.3	1.526	31.9	29.5	5.6	3.7	5.7	7.4	5.7	4.4
13	03-05-24	100	30.4	8.1	1.973	31.9	29.3	5.8	3.8	5.8	7.8	5.8	4.5
14	03-05-24	100	32.6	5.1	1.629	32.0	29.7	5.7	3.7	5.8	7.8	5.8	4.5
15	04-09-24	100	32.4	6.2	2.199	32.0	29.7	5.7	3.7	5.8	7.8	5.8	4.4
16	05-07-24	100	29.5	6.2	1.707	31.8	29.3	5.8	3.8	5.8	7.8	5.8	4.4
17	06-04-24	100	32.5	4.9	1.587	31.8	29.3	5.7	3.8	5.8	7.7	5.8	4.3
18	07-09-24	100	32.1	7.6	1.846	31.7	29.4	5.9	3.8	5.8	7.9	5.8	4.5
19	08-06-24	100	31.8	6.8	1.827	31.8	29.5	5.9	3.8	5.8	7.9	5.8	4.5
20	09-10-24	100	32.4	7.3	1.947	31.7	29.6	6.0	3.9	5.8	8.1	5.8	4.5

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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.

Sodium Chloride Chronic Reference Toxicant Test (EPA-821-R-02-013, Method 1002.0)

Species: *Ceriodaphnia dubia*

CdNaClCR #: 302

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS 2320					
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:	09-10-24 0430 TO 0800									
Culture board:	09-03-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	4	5	6	10	11	13	18	19	24
Transfer vessel information:	pH (S.U.): 7.82 Temperature (°C): 25.0°C									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches <i>Selenastrum</i>	YWT	MHSW batch used	Analyst
0	09-10-24	0813	09-06-24	09-06-24	09-04-24 A	jl
1	09-11-24	0745	↓	↓	↓	jl
2	09-12-24	0741	↓	↓	09-04-24 D	jl
3	09-13-24	0742	↓	↓	↓	jl
4	09-14-24	0842	↓	↓	09-09-24 B	jl
5	09-15-24	0840	↓	↓	↓	jl
6	09-16-24	0740	↓	↓	↓	jl
7	09-17-24	0617				jl

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O 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	1306646F5

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	07.	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	> 1400
% Adults having 3 rd Broods:	1007.	≥ 80%	NOEC (mg/L NaCl)	1000
% Mortality:	07.	≤ 20%	LOEC (mg/L NaCl)	1200
Mean Offspring/Female:	32.4	≥ 15.0 offspring/female	ChV (mg/L NaCl)	1095.5
% CV:	7.37.	< 40.0 %	IC ₂₅ (mg/L NaCl)	1105.7

Species: *Ceriodaphnia dubia*

CdNaClCR #: 302

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	4	5	4	4	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	11	11	11	10	11	12	10	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	14	17	19	18	15	17	19	19	15
Total young produced		30	36	33	34	34	29	32	37	33	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:

% Mortality:	07.
Mean Offspring/Female:	32.4

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	5	6	4	4	3	5	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	10	11	11	10	12	12	10	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	18	19	15	16	15	18	18	17
Total young produced		31	37	32	34	29	31	32	35	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:	07.
Mean Offspring/Female:	32.7
% Reduction from Control:	-0.97.

Species: Ceriodaphnia dubia

CdNaClCR #: 302

800 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	31.9
% Reduction from Control:	157.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	6	4	4	5	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	12	10	12	10	10	12	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	19	18	19	17	14	17	13	14	17
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		29	33	36	33	33	29	31	29	30	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	31.6
% Reduction from Control:	257.

Species: *Ceriodaphnia dubia*

CdNaClCR #: 302

1200 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	4	4	3	4	2	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	10	10	8	11	5	10	12	10	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	6	4	4	5	7	8	5	2	4	8
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		18	18	18	17	21	17	17	18	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:	18.0
% Reduction from Control:	44.4%

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	2	2	3	2	1	1	1	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	1	0	0	0	0	0	0	1	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
Total young produced		2	2	2	2	3	2	1	1	2	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:	2.0
% Reduction from Control:	93.8%

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	4	5	4	4	6	4	4	44
5	10	13	11	11	11	10	11	12	10	13	112
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	17	19	18	15	17	19	19	15	168
Total	30	30	33	34	34	29	32	37	33	32	324

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	6	4	4	5	4	4	4	4	43
5	10	10	12	10	12	10	10	12	12	12	110
6	0	0	0	0	0	0	0	0	0	0	0
7	15	19	18	19	17	14	17	13	14	17	163
Total	29	33	36	33	33	29	31	29	30	33	316

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	6	4	4	3	5	5	5	4	4	45
5	11	13	10	11	11	10	12	12	10	13	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	18	19	15	16	15	18	18	17	169
Total	31	37	32	34	29	31	32	35	32	34	327

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	4	4	3	4	2	4	4	4	36
5	9	10	10	8	11	5	10	12	10	6	91
6	0	0	0	0	0	0	0	0	0	0	0
7	6	4	4	5	7	8	5	2	4	8	53
Total	18	18	18	17	21	17	17	18	18	18	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	5	5	5	4	3	4	4	4	6	4	44
5	12	10	10	12	12	11	11	11	12	10	111
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	18	16	19	15	17	13	16	19	164
Total	32	31	33	32	34	30	32	28	34	33	319

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	2	2	3	2	1	1	1	3	18
5	0	0	0	0	0	0	0	0	0	0	0
6	0	1	0	0	0	0	0	0	1	0	2
7	0	0	0	0	0	0	0	0	0	0	0
Total	2	2	2	2	3	2	1	1	2	3	20

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

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

Test number: **CdNaClCR #302**
Test dates: **September 10-17, 2024**

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	30	33	34	34	29	32	37	33	32	100	32.4	7.3	Not applicable
600	31	37	32	34	29	31	32	35	32	34	100	32.7	7.1	-0.9
800	32	31	33	32	34	30	32	28	34	33	100	31.9	5.8	1.5
1000	29	33	36	33	33	29	31	29	30	33	100	31.6	7.5	2.5
1200	18	18	18	17	21	17	17	18	18	18	100	18.0	6.4	44.4
1400	2	2	2	2	3	2	1	1	2	3	100	2.0	33.3	93.8

Dunnett's MSD value: **1.947** MSD = Minimum Significant Difference
PMSD: **6.0** PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.

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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 9/10/2024 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
End Date: 9/17/2024 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	30.000	33.000	34.000	34.000	29.000	32.000	37.000	33.000	32.000
600	31.000	37.000	32.000	34.000	29.000	31.000	32.000	35.000	32.000	34.000
800	32.000	31.000	33.000	32.000	34.000	30.000	32.000	28.000	34.000	33.000
1000	29.000	33.000	36.000	33.000	33.000	29.000	31.000	29.000	30.000	33.000
1200	18.000	18.000	18.000	17.000	21.000	17.000	17.000	18.000	18.000	18.000
1400	2.000	2.000	2.000	2.000	3.000	2.000	1.000	1.000	2.000	3.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
D-Control	32.400	1.0000	32.400	29.000	37.000	7.304	10			32.550	1.0000
600	32.700	1.0093	32.700	29.000	37.000	7.070	10	108.00	75.00	32.550	1.0000
800	31.900	0.9846	31.900	28.000	34.000	5.809	10	100.00	75.00	31.900	0.9800
1000	31.600	0.9753	31.600	29.000	36.000	7.489	10	94.50	75.00	31.600	0.9708
*1200	18.000	0.5556	18.000	17.000	21.000	6.415	10	55.00	75.00	18.000	0.5530
*1400	2.000	0.0617	2.000	1.000	3.000	33.333	10	55.00	75.00	2.000	0.0614

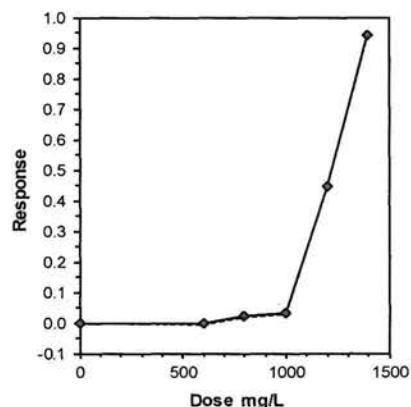
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution ($p > 0.01$)	1.00513	1.035	0.26346	0.5049
Bartlett's Test indicates unequal variances ($p = 5.88E-03$)	16.364	15.0863		

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

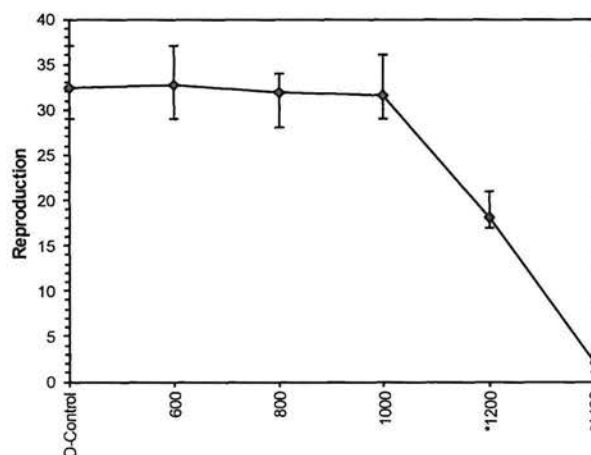
Treatments vs D-Control

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	1009.96	93.311	719.766 1022.95	-2.5346
IC10	1033.9	11.2597	1005.33 1045.91	-1.0407
IC15	1057.83	9.73901	1032.19 1069.24	-0.5890
IC20	1081.76	8.79896	1057.96 1092.75	-0.5443
IC25	1105.7	7.96272	1083.74 1116.55	-0.4675
IC40	1177.5	6.46636	1163.17 1188.98	0.0074
IC50	1221.56	4.81147	1211.46 1229.95	-0.0143



Dose-Response Plot

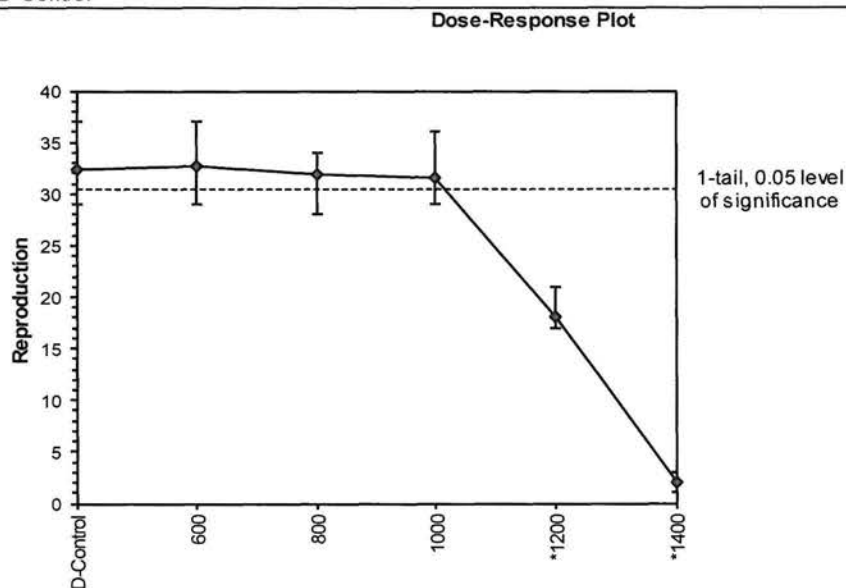




Statistical Analyses

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction												
Start Date:	9/10/2024	Test ID: CdNaClCR				Sample ID:		REF-Ref Toxicant				
End Date:	9/17/2024	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	30.000	33.000	34.000	34.000	29.000	32.000	37.000	33.000	32.000		
600	31.000	37.000	32.000	34.000	29.000	31.000	32.000	35.000	32.000	34.000		
800	32.000	31.000	33.000	32.000	34.000	30.000	32.000	28.000	34.000	33.000		
1000	29.000	33.000	36.000	33.000	33.000	29.000	31.000	29.000	30.000	33.000		
1200	18.000	18.000	18.000	17.000	21.000	17.000	17.000	18.000	18.000	18.000		
1400	2.000	2.000	2.000	2.000	3.000	2.000	1.000	1.000	2.000	3.000		
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed			
			Mean	Min	Max	CV%	N		Critical	MSD		
D-Control	32.400	1.0000	32.400	29.000	37.000	7.304	10					
600	32.700	1.0093	32.700	29.000	37.000	7.070	10	-0.352	2.287	1.947		
800	31.900	0.9846	31.900	28.000	34.000	5.809	10	0.587	2.287	1.947		
1000	31.600	0.9753	31.600	29.000	36.000	7.489	10	0.939	2.287	1.947		
*1200	18.000	0.5556	18.000	17.000	21.000	6.415	10	16.910	2.287	1.947		
*1400	2.000	0.0617	2.000	1.000	3.000	33.333	10	35.698	2.287	1.947		
Auxiliary Tests							Statistic	Critical	Skew	Kurt		
Kolmogorov D Test indicates normal distribution (p > 0.01)							1.00513	1.035	0.26346	0.5049		
Bartlett's Test indicates unequal variances (p = 5.88E-03)							16.364	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		1.94728	0.0601	1565.79	3.62593	3.1E-42	5, 54
Treatments vs D-Control												



Species: *Ceriodaphnia dubia*

CdNaClCR #: 302

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.

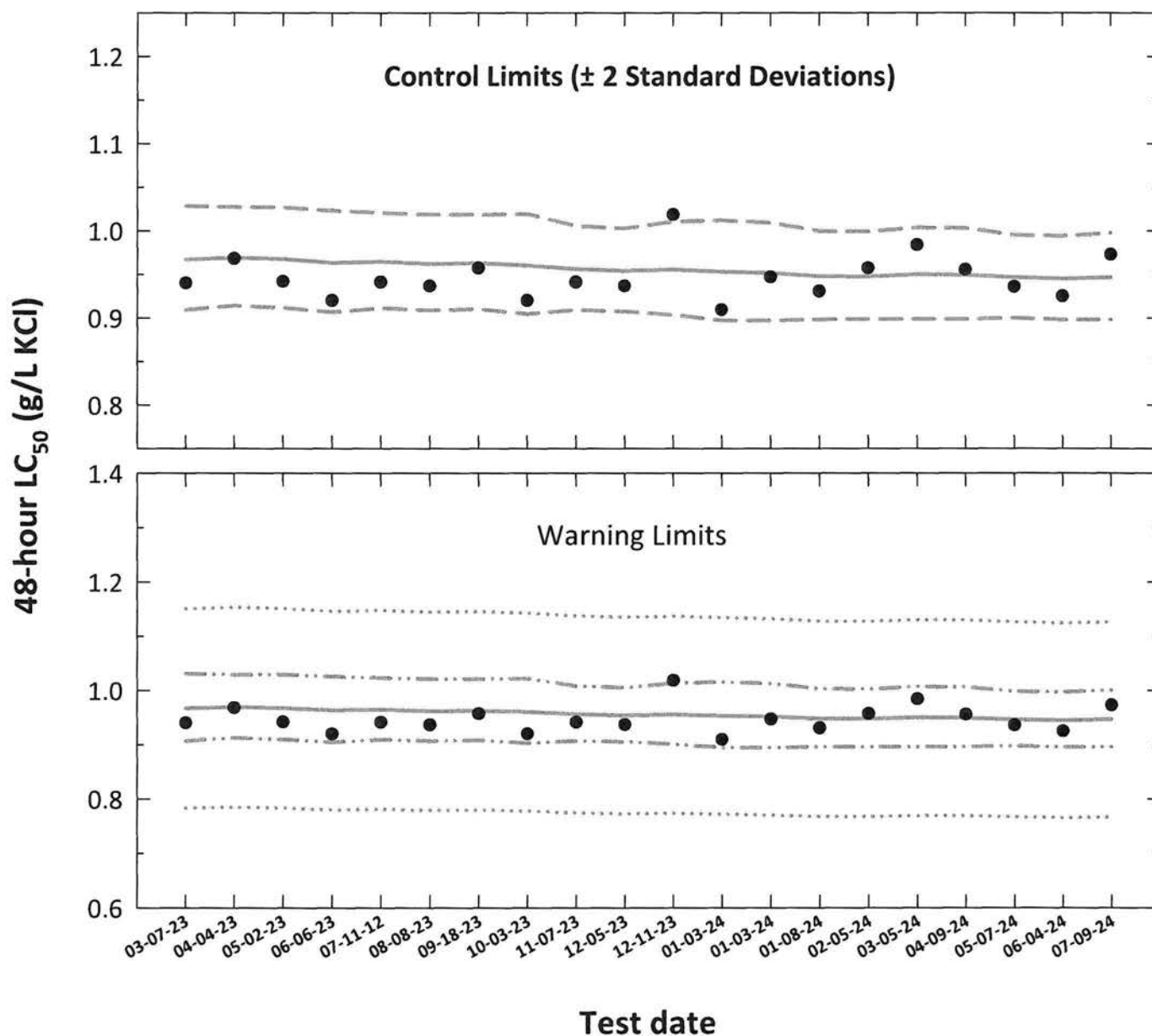
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		Analyst					
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	4.75	7.35	7.49	7.22	7.32	7.53
	Dissolved oxygen (mg/L)	7.7	7.0	7.0	7.0	7.0	7.0
	Conductivity (µmhos/cm)	290		330		318	
	Alkalinity (mg CaCO ₃ /L)	64				64	
	Hardness (mg CaCO ₃ /L)	87				89	
	Temperature (°C)	25.0	25.2	24.9	25.3	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.77	7.44	7.49	7.41	7.41	7.57
	Dissolved oxygen (mg/L)	7.7	7.0	7.0	7.0	7.0	7.7
	Conductivity (µmhos/cm)	1400		1490		1470	
	Temperature (°C)	25.1	25.0	25.0	25.2	25.0	25.0
800 mg NaCl/L	pH (S.U.)	7.77	7.46	7.50	7.43	7.45	7.57
	Dissolved oxygen (mg/L)	7.7	7.7	7.0	7.0	7.9	7.6
	Conductivity (µmhos/cm)	1730		1870		1830	
	Temperature (°C)	25.0	25.3	25.0	25.0	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.76	7.45	7.51	7.47	7.44	7.50
	Dissolved oxygen (mg/L)	7.0	7.7	7.0	7.0	7.0	7.6
	Conductivity (µmhos/cm)	2110		2240		2220	
	Temperature (°C)	25.0	25.1	25.0	25.0	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.74	7.48	7.51	7.47	7.50	7.50
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.0	7.0	7.6
	Conductivity (µmhos/cm)	2470		2610		2570	
	Temperature (°C)	25.0	25.3	25.0	25.3	25.0	24.9
1400 mg NaCl/L	pH (S.U.)	7.77	7.47	7.54	7.50	7.52	7.57
	Dissolved oxygen (mg/L)	0.0	7.0	7.9	7.0	7.0	7.6
	Conductivity (µmhos/cm)	2070		2980		2930	
	Temperature (°C)	25.0	25.2	25.0	25.1	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

CdNaClCR #: 302

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		KL	BSC	BSC	BSC	BSC	BM	BM	BM
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.42	7.32	7.43	7.41	7.41	7.31	7.31	7.39
	Dissolved oxygen (mg/L)	7.7	7.9	7.7	7.8	7.8	7.6 ^{7.9} 7.6	7.6	7.9
	Conductivity (µmhos/cm)	304		316		309		310	
	Alkalinity (mg CaCO ₃ /L)			64					
	Hardness (mg CaCO ₃ /L)			87					
	Temperature (°C)	24.9	25.2	24.9	25.3	24.9	25.2	24.8	26.4
600 mg NaCl/L	pH (S.U.)	7.63	7.56	7.47	7.62	7.63	7.60	7.65	7.60
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.9	7.8	7.8	7.7	8.0
	Conductivity (µmhos/cm)	1370		1430		1430		1420	
	Temperature (°C)	25.0	25.4	25.0	25.4	25.0	25.0	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.70	7.55	7.54	7.65	7.67	7.68	7.71	7.61
	Dissolved oxygen (mg/L)	7.7	7.9	7.9	7.9	7.9	7.8	7.7	8.0
	Conductivity (µmhos/cm)	1720		1800		1790		1830	
	Temperature (°C)	24.9	25.4	25.0	25.2	25.0	25.3	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.70	7.56	7.55	7.66	7.67	7.73	7.77 ^{7.77} 7.73	7.63
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.9	7.9	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2090		2160		2170		2190	
	Temperature (°C)	24.9	25.2	25.0	25.2	25.0	25.2	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.70	7.56	7.56	7.66	7.69	7.79	7.80	7.64
	Dissolved oxygen (mg/L)	7.6	8.0	8.0	7.9	8.0	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2430		2530		2510		2550	
	Temperature (°C)	24.9	25.2	25.0	25.2	24.9	25.3	25.0	25.3
1400 mg NaCl/L	pH (S.U.)	7.71	7.55	7.56	7.65	7.70	7.79	7.81	7.64
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	7.9	8.0	7.7	7.8	7.9
	Conductivity (µmhos/cm)	2800		2860		2910		2930	
	Temperature (°C)	25.0	25.2	24.9	25.2	24.9	25.3	24.9	25.3
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- **48-hour LC₅₀** = 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 LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.75}$ converted to anti-logarithmic values,
S_{A.75} = 75th percentile of CVs reported nationally by USEPA)

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	
1	03-07-23	0.9402	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308	0.7834	1.1509
2	04-04-23	0.9683	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293	0.7851	1.1535
3	05-02-23	0.9420	-0.0260	-0.0143	0.0129	0.9677	0.9117	1.0270	0.9098	1.0290	0.7838	1.1515
4	06-06-23	0.9201	-0.0362	-0.0162	0.0131	0.9633	0.9068	1.0233	0.9046	1.0256	0.7803	1.1463
5	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
6	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
7	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
8	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
9	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
10	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
11	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
12	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
13	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
14	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
15	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
16	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
17	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
18	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
19	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
20	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

Note: 48-hour LC₅₀ = 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 LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 166

Dilution Preparation:

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

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

Stock solution INSS #: 2288

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	KO	KO ✓	KO ✓
Control, MHSW	pH (S.U.)	7.37	7.42	7.26
	Dissolved oxygen (mg/L)	7.7	7.9	7.6
	Conductivity (µmhos/cm)	302		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	84		
	Temperature (°C)	24.6	24.8	24.4
500 mg/L	pH (S.U.)	7.74	7.43	7.30
	Dissolved oxygen (mg/L)	7.8	7.9	7.6
	Conductivity (µmhos/cm)	1200		
	Temperature (°C)	24.5	24.7	24.7
750 mg/L	pH (S.U.)	7.75	7.43	7.33
	Dissolved oxygen (mg/L)	7.8	7.9	7.5
	Conductivity (µmhos/cm)	1620		
	Temperature (°C)	24.5	24.7	24.7
1000 mg/L	pH (S.U.)	7.75	7.39	7.38
	Dissolved oxygen (mg/L)	7.8	8.0	7.6
	Conductivity (µmhos/cm)	2070		
	Temperature (°C)	24.7	24.9	24.7
1250 mg/L	pH (S.U.)	7.75	7.35	
	Dissolved oxygen (mg/L)	7.9	8.0	
	Conductivity (µmhos/cm)	2500		
	Temperature (°C)	24.7	24.6	
1500 mg/L	pH (S.U.)	7.74	7.33	
	Dissolved oxygen (mg/L)	7.9	8.0	
	Conductivity (µmhos/cm)	2980		
	Temperature (°C)	24.8	24.6	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by 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	130664685

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant TestPpKCIAC # 166

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	07-09-24	* 0510	J	0744	J	1C	Yellow	07-01-24B
24	07-10-24			0742	J			
48 Termination	07-11-24			0745	J			

*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:	06-26-24
Age (1 to 14 days old):	5 to 6 DAYS
Hatch date and times:	07-03-24 1147 TO 07-04-24 0659
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.83
	Temperature (°C): 24.0°C

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

Survival Data (number of living organisms):

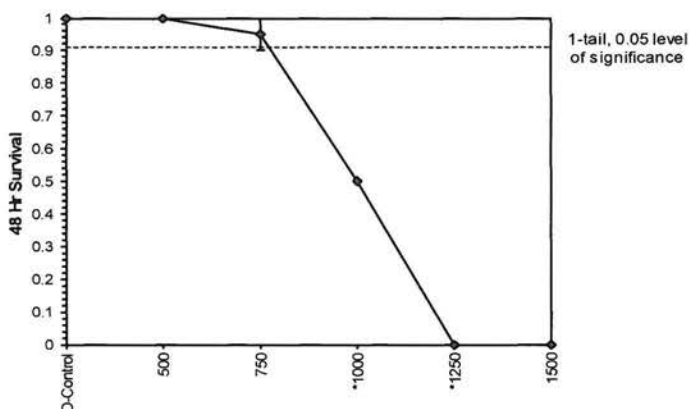
Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 ^d	5 ^{sd}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^u	10	5 ^{1d}	5	0	0	0	0
Mean Survival	1001.		1001.		957.		507.		07.		07.	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	905.3
Upper 95% confidence limit (mg KCl/L)	1035.5
48-hour LC ₅₀ (mg KCl/L)	972.9

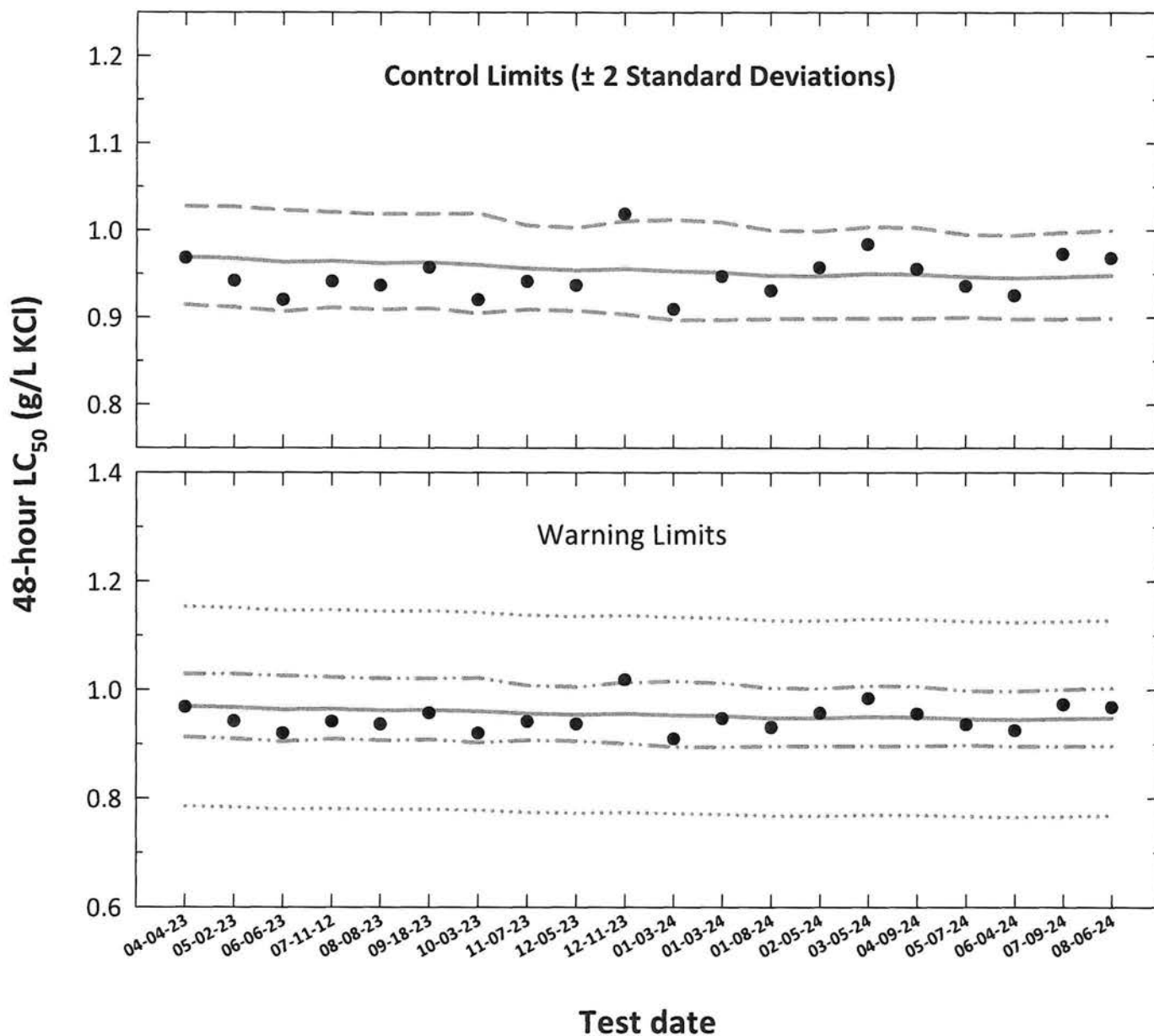
Comments:



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 $LC_{50} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $LC_{50} \pm 2$ coefficient 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)

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	04-04-23	0.9683	-0.0140	-0.0135	0.0126	0.9693	0.9145	1.0275	0.9127	1.0293	0.7851	1.1535
2	05-02-23	0.9420	-0.0260	-0.0143	0.0129	0.9677	0.9117	1.0270	0.9098	1.0290	0.7838	1.1515
3	06-06-23	0.9201	-0.0362	-0.0162	0.0131	0.9633	0.9068	1.0233	0.9046	1.0256	0.7803	1.1463
4	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
5	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
6	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
7	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
8	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
9	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
10	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
11	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
12	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
13	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 48-hour LC₅₀ = 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 LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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.



Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 167

Dilution Preparation:

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

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

Stock solution INSS #: 2296

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW	pH (S.U.)	7.67	7.50	7.59
	Dissolved oxygen (mg/L)	7.8	7.7	7.5
	Conductivity (µmhos/cm)	292		
	Alkalinity (mg/L CaCO ₃)	61		
	Hardness (mg/L CaCO ₃)	84		
	Temperature (°C)	24.7	24.6	24.3
500 mg/L	pH (S.U.)	7.78	7.46	7.50
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	Conductivity (µmhos/cm)	1110		
	Temperature (°C)	24.9	24.4	24.6
750 mg/L	pH (S.U.)	7.73	7.46	7.49
	Dissolved oxygen (mg/L)	7.8	7.7	7.5
	Conductivity (µmhos/cm)	1530		
	Temperature (°C)	24.7	24.7	24.7
1000 mg/L	pH (S.U.)	7.72	7.48	7.48
	Dissolved oxygen (mg/L)	7.8	7.7	7.4
	Conductivity (µmhos/cm)	1920		
	Temperature (°C)	24.7	24.4	24.5
1250 mg/L	pH (S.U.)	7.72	7.48	7.49
	Dissolved oxygen (mg/L)	7.8	7.7	7.4
	Conductivity (µmhos/cm)	2310		
	Temperature (°C)	24.8	24.4	24.5
1500 mg/L	pH (S.U.)	7.73	7.45	Flow
	Dissolved oxygen (mg/L)	8.0	7.7	
	Conductivity (µmhos/cm)	2770		
	Temperature (°C)	24.8	24.4	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-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	13066461

Acute LC₅₀ Whole Effluent Toxicity Test, Species: Pimephales promelas

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 167

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	08-06-24	0600	JL	0830	JL	1B	ORANGE	01-31-24A
24	08-07-24			0820	JL			
48 Termination	08-08-24			0822	JL			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	07-24-24
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	07-30-24 1326 to 07-31-24 0840
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.87
	Temperature (°C): 24.0 °C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	4d 6	4d 6	1d 3	3d 3	10d 0	10d 0
48 Termination	10	10	10	10	10	8	5	5	1	0	0	0
Mean Survival	100%		100%		90%		50%		5%		0%	

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

Statistics:

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

Comments:

Acute Fathead Minnow Test-24 Hr Survival															
Start Date:	8/6/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant										
End Date:	8/8/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride										
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas										
Comments:															
Conc-mg/L	1	2													
D-Control	1.0000	1.0000													
500	1.0000	1.0000													
750	1.0000	1.0000													
1000	0.6000	0.6000													
1250	0.1000	0.3000													
1500	0.0000	0.0000													
Transform: Arcsin Square Root															
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total 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	0.2324	0	20			
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2324	0	20			
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	6.449	2.850	0.2324	8	20			
*1250	0.2000	0.2000	0.4507	0.3218	0.5796	40.461	2	11.788	2.850	0.2324	16	20			
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20			
Auxiliary Tests															
Normality of the data set cannot be confirmed								Statistic	Critical	Skew	Kurt				
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		0.12039	0.12348	0.37918	0.00665	2.3E-04	4, 5			
Treatments vs D-Control															
Maximum Likelihood-Probit															
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter				
Slope	15.0382	2.88342	9.38669	20.6897	0	1.22962	7.81472	0.74591	3.02705	0.0665	5				
Intercept	-40.521	8.76493	-57.701	-23.342											
TSCR															
Point	Probits	mg/L	95% Fiducial Limits												
EC01	2.674	745.347	584.182	838.263											
EC05	3.355	827.325	687.559	908.154											
EC10	3.718	874.65	748.991	948.979											
EC15	3.964	908.101	792.826	978.399											
EC20	4.158	935.597	828.888	1003.15											
EC25	4.326	959.848	860.54	1025.6											
EC40	4.747	1023.78	941.81	1088.94											
EC50	5.000	1064.28	990.22	1133.63											
EC60	5.253	1106.37	1036.89	1184.97											
EC75	5.674	1180.07	1109.23	1287.18											
EC80	5.842	1210.65	1136.36	1333.6											
EC85	6.036	1247.31	1167.24	1391.72											
EC90	6.282	1295.01	1205.43	1470.73											
EC95	6.645	1369.09	1261.62	1599.59											
EC99	7.326	1519.67	1368.92	1879.75											

48 hr Survival

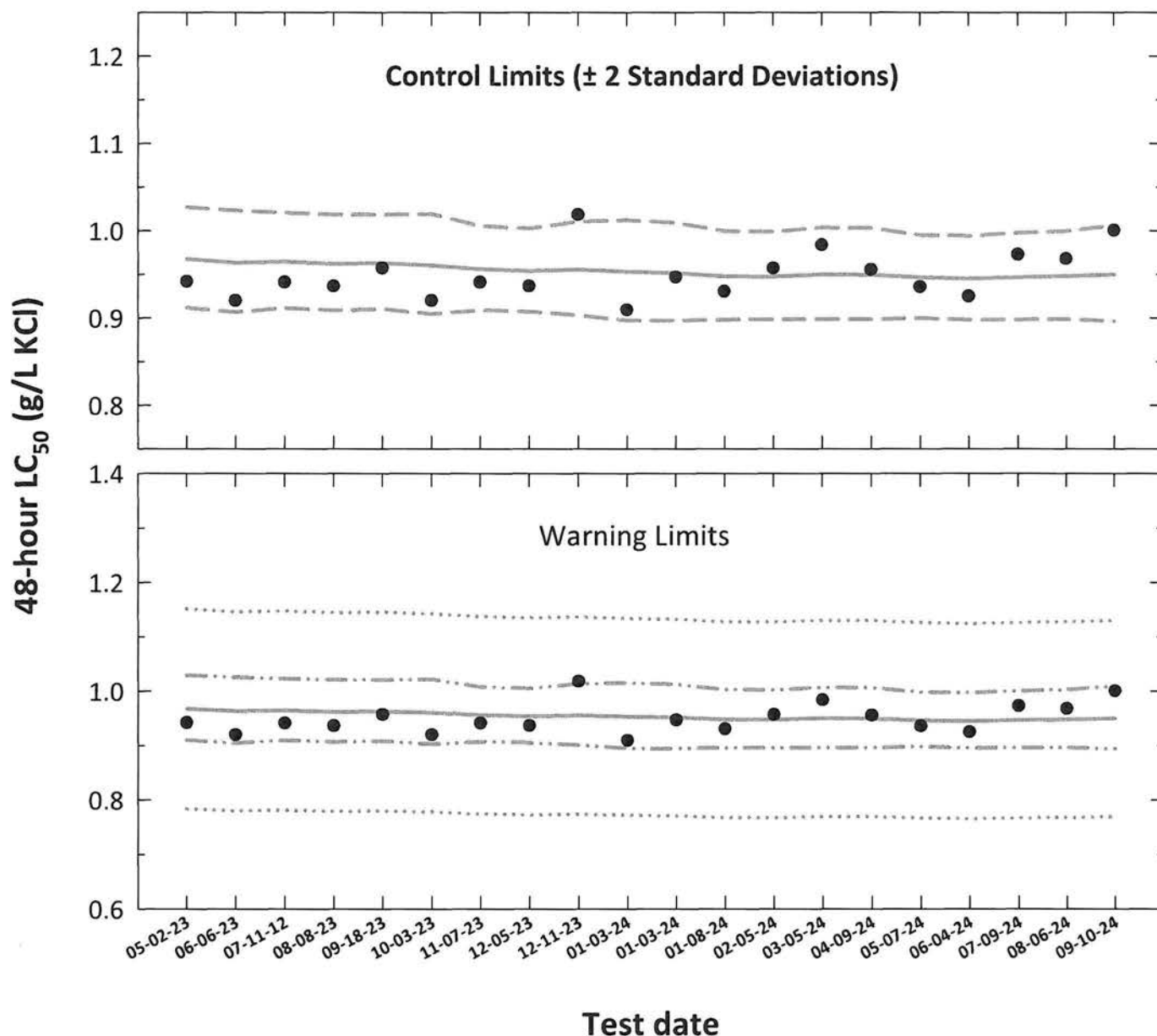
1-tail, 0.05 level of significance

Dose (mg/kg)	48 hr Survival
Control	1.0
500	1.0
750	0.9
1000	0.5
1250	0.05
1500	0.0

Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



- **48-hour LC₅₀** = 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 LC₅₀ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic LC₅₀ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic LC₅₀ $\pm S_{A.75}$ converted to anti-logarithmic values,
 $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

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 CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	05-02-23	0.9420	-0.0260	-0.0143	0.0129	0.9677	0.9117 1.0270	0.9098 1.0290	0.7838 1.1515
2	06-06-23	0.9201	-0.0362	-0.0162	0.0131	0.9633	0.9068 1.0233	0.9046 1.0256	0.7803 1.1463
3	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116 1.0208	0.9096 1.0229	0.7814 1.1479
4	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088 1.0185	0.9067 1.0208	0.7793 1.1449
5	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103 1.0185	0.9083 1.0207	0.7800 1.1459
6	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049 1.0194	0.9026 1.0219	0.7780 1.1429
7	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092 1.0055	0.9071 1.0078	0.7745 1.1378
8	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077 1.0028	0.9054 1.0052	0.7728 1.1353
9	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035 1.0107	0.9011 1.0133	0.7740 1.1372
10	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974 1.0124	0.8947 1.0153	0.7721 1.1343
11	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971 1.0093	0.8943 1.0123	0.7708 1.1323
12	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985 0.9999	0.8958 1.0027	0.7678 1.1279
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: 48-hour LC₅₀ = 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 ToxCal).

CT = Central tendency of the LC₅₀ values.

S = Standard deviation of the LC₅₀ values.

Control Limits = Mean logarithmic LC₅₀ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC₅₀ ± 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.

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 168

Dilution Preparation:

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

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

Stock solution INSS #: 2319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst			
Control, MHSW		<i>N</i>	<i>N</i>	<i>N</i>
	pH (S.U.)	7.75	7.40	7.38
	Dissolved oxygen (mg/L)	7.7	7.8	7.5
	Conductivity (µmhos/cm)	298		
	Alkalinity (mg/L CaCO ₃)	64		
	Hardness (mg/L CaCO ₃)	57		
	Temperature (°C)	25.2	25.4	25.1
500 mg/L	pH (S.U.)	7.71	7.53	7.43
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	25.0	25.2	25.4
750 mg/L	pH (S.U.)	7.70	7.56	7.56
	Dissolved oxygen (mg/L)	7.8	7.8	7.4
	Conductivity (µmhos/cm)	1570		
	Temperature (°C)	25.2	25.2	25.4
1000 mg/L	pH (S.U.)	7.69	7.59	7.59
	Dissolved oxygen (mg/L)	7.8	7.9	7.7
	Conductivity (µmhos/cm)	1990		
	Temperature (°C)	25.2	25.3	25.2
1250 mg/L	pH (S.U.)	7.70	7.59	7.59
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	2350		
	Temperature (°C)	25.2	25.5	25.3
1500 mg/L	pH (S.U.)	7.70	7.58	
	Dissolved oxygen (mg/L)	7.9	7.9	
	Conductivity (µmhos/cm)	2900		
	Temperature (°C)	25.0	25.2	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O 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	13061645

Acute LC₅₀ Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKCIAC # 168

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	09-10-24	* 0500	H	0700	H	1E	PINK	09-04-24A
24	09-11-24			0655	H			
48 Termination	09-12-24			0655	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
Spawning date:	08-28-24
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	09-04-24 1115 TO 09-05-24 0835
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.13
	Temperature (°C): 24.0°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^{ud}	7 ^{3d}	7 ^{3d}	4 ^{ud}	5 ^{5d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{ud}	9	3 ^{4d}	6 ^{1d}	2 ^{2d}	3 ^{2d}	0	0
Mean Survival	100%		100%		90%		45%		25%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	915.1
Upper 95% confidence limit (mg KCl/L)	1153.6
48-hour LC ₅₀ (mg KCl/L)	1059.4

1000.7

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date:	9/10/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	9/12/2024	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	0.7000	0.7000
1250	0.4000	0.5000
1500	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
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	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	6.948	2.850	0.1726	6	20
*1250	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	11.175	2.850	0.1726	11	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

Normality of the data set cannot be confirmed

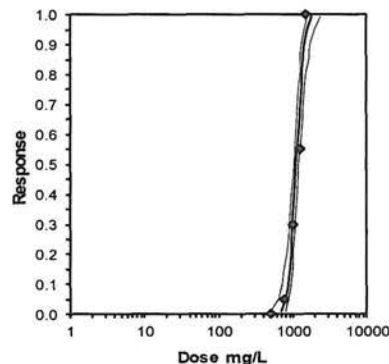
Equality of variance cannot be confirmed

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	0.18194	0.00367	3.3E-04	4, 5

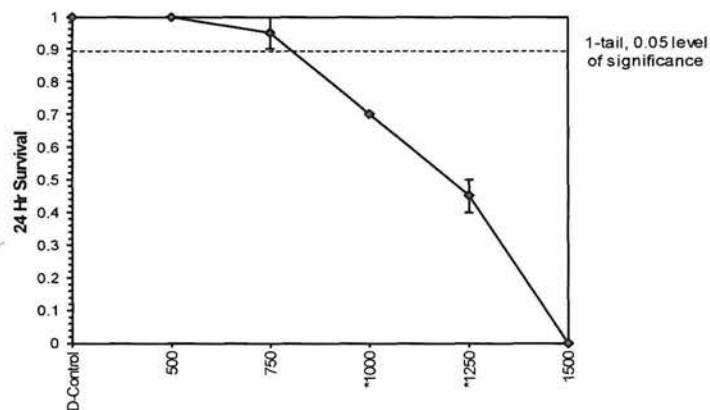
Treatments vs D-Control

Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	10.9533	2.02098	6.99214 14.9144	0	4.20529	7.81472	0.24013	3.05047	0.0913	4
Intercept	-28.413	6.18081	-40.527 -16.298							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	688.781	510.434	798.588
EC05	3.355	794.876	635.669	891.644
EC10	3.718	857.961	713.364	947.17
EC15	3.964	903.329	770.18	987.73
EC20	4.158	941.092	817.713	1022.24
EC25	4.326	974.744	859.961	1053.86
EC40	4.747	1064.98	970.155	1145.22
EC50	5.000	1123.23	1036.41	1211.77
EC60	5.253	1184.67	1100.42	1290.06
EC75	5.674	1294.34	1200.83	1449.3
EC80	5.842	1340.62	1239.28	1522.58
EC85	6.036	1396.67	1283.74	1615.08
EC90	6.282	1470.52	1339.78	1742.32
EC95	6.645	1587.23	1424.27	1953.83
EC99	7.326	1831.71	1591.33	2431.55



Dose-Response Plot



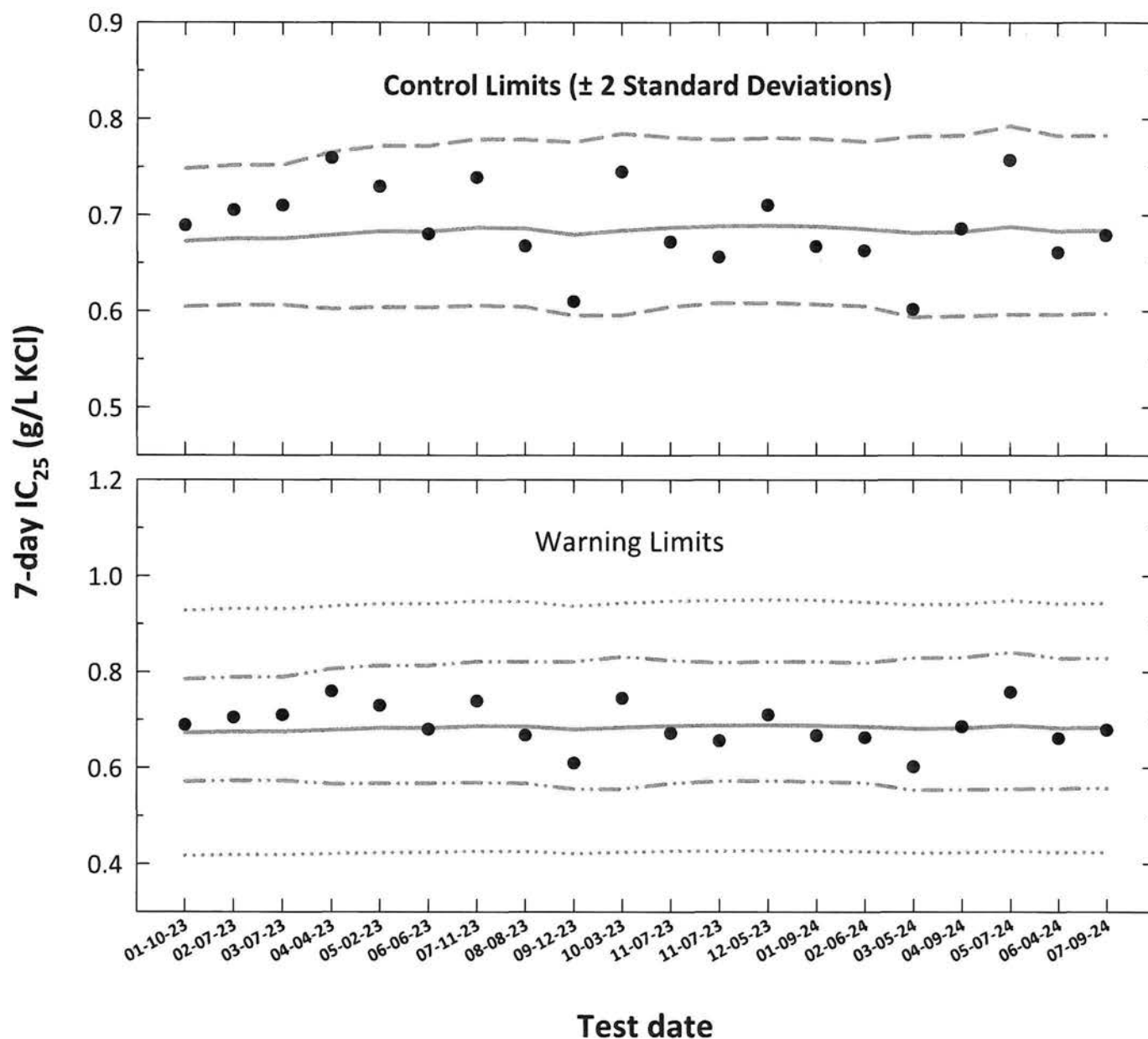
Start Date:	9/10/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	9/12/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%	N					
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	0.2953	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	1.573	2.850	0.2953	2	20
*1000	0.4500	0.4500	0.7329	0.5796	0.8861	29.567	2	6.555	2.850	0.2953	11	20
*1250	0.2500	0.2500	0.5216	0.4636	0.5796	15.723	2	8.593	2.850	0.2953	15	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Maximum Likelihood-Probit				
Parameter	Value	SE	95% Fiducial Limits	
Slope	10.2306	1.78774	6.72665 13.7346	
	Control	Chi-Sq	Critical	P-value
	0	2.13608	7.81472	0.54465
				3.00028
				0.09775
				4

log ₁₀ concentration	48 hr Survival	Significance
O-Control	1.0	
500	1.0	
750	0.9	
*1000	0.45	Significant
*1250	0.25	Significant
1500	0.0	

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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient 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)

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 CT - 2S CT + 2S
1	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047 0.7481
2	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064 0.7518
3	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063 0.7520
4	04-04-23	0.7592	-0.1196	-0.1680	0.0260	0.6792	0.6025 0.7656
5	05-02-23	0.7295	-0.1370	-0.1656	0.0266	0.6829	0.6042 0.7718
6	06-06-23	0.6800	-0.1675	-0.1657	0.0266	0.6828	0.6041 0.7717
7	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056 0.7789
8	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045 0.7784
9	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954 0.7757
10	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959 0.7846
11	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045 0.7806
12	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085 0.7786
13	12-05-23	0.7102	-0.1486	-0.1617	0.0269	0.6891	0.6088 0.7801
14	01-09-24	0.6671	-0.1758	-0.1624	0.0271	0.6880	0.6073 0.7795
15	02-06-24	0.6628	-0.1786	-0.1640	0.0270	0.6854	0.6052 0.7762
16	03-05-24	0.6019	-0.2205	-0.1666	0.0298	0.6814	0.5940 0.7817
17	04-09-24	0.6855	-0.1640	-0.1660	0.0298	0.6823	0.5949 0.7825
18	05-07-24	0.7569	-0.1210	-0.1627	0.0309	0.6876	0.5965 0.7927
19	06-04-24	0.6610	-0.1798	-0.1656	0.0295	0.6830	0.5963 0.7823
20	07-09-24	0.6789	-0.1682	-0.1650	0.0293	0.6839	0.5976 0.7826
						Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - $S_{A,75}$ CT + $S_{A,75}$
						0.5717 0.7848	0.4170 0.9282
						0.5733 0.7886	0.4186 0.9317
						0.5732 0.7889	0.4186 0.9318
						0.5663 0.8064	0.4211 0.9373
						0.5677 0.8131	0.4234 0.9424
						0.5676 0.8130	0.4233 0.9422
						0.5686 0.8208	0.4258 0.9478
						0.5673 0.8207	0.4253 0.9467
						0.5557 0.8211	0.4214 0.9378
						0.5552 0.8312	0.4239 0.9436
						0.5669 0.8232	0.4259 0.9479
						0.5724 0.8194	0.4268 0.9499
						0.5725 0.8211	0.4273 0.9510
						0.5707 0.8209	0.4266 0.9495
						0.5684 0.8179	0.4250 0.9459
						0.5531 0.8286	0.4225 0.9404
						0.5542 0.8292	0.4230 0.9416
						0.5551 0.8404	0.4263 0.9489
						0.5561 0.8283	0.4234 0.9425
						0.5577 0.8283	0.4240 0.9438

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.

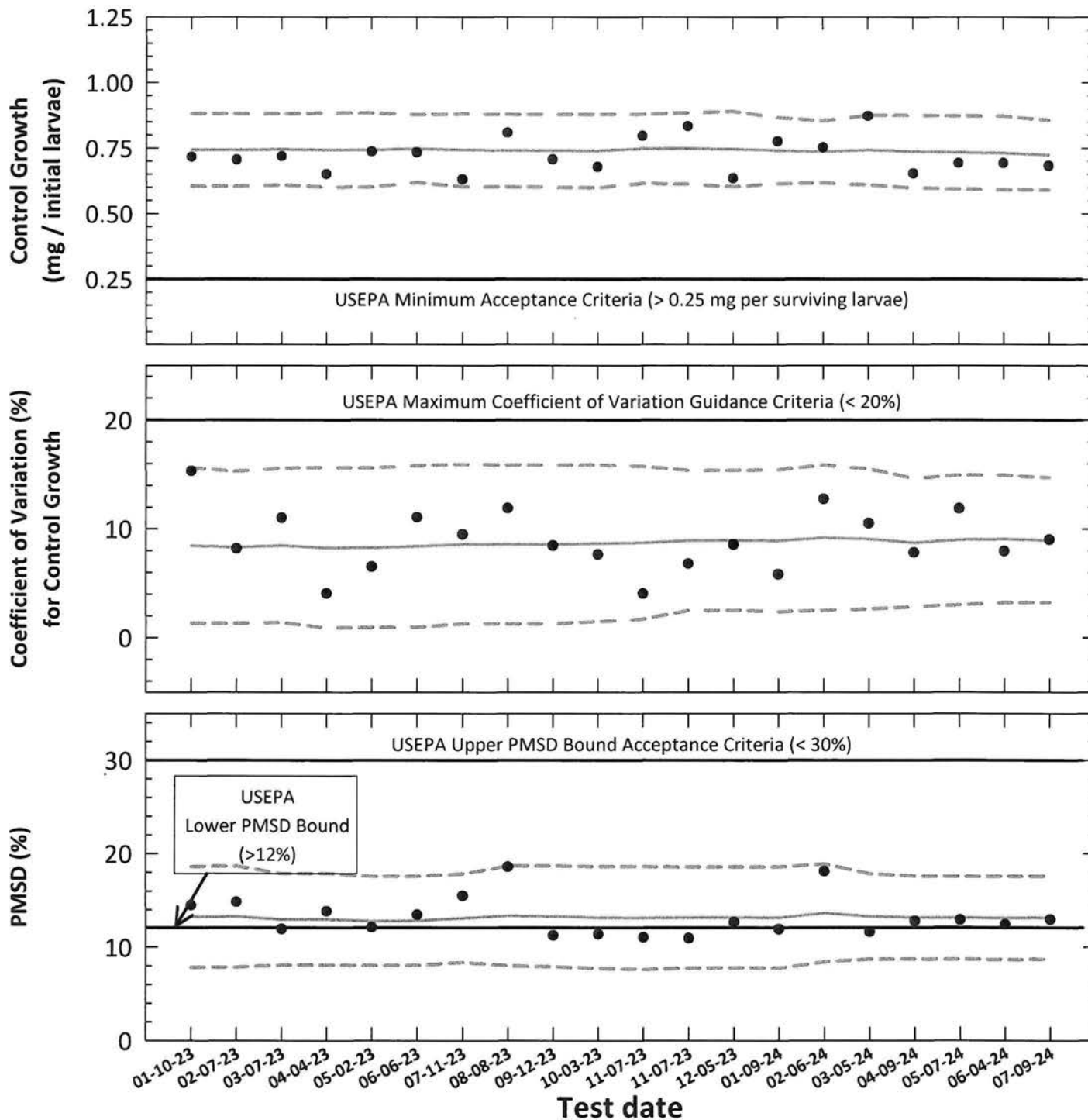
Warning Limits = Mean logarithmic $IC_{25} \pm 2CV$ or $S_{A,75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

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 $\pm$ 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination				Control Growth		Test		Control Growth		Control Growth CV		Test PMSD	
		Control Survival (%)	Control Growth		CV (%)	Mean (mg/initial larvae)	CT	MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	15.6	13.2	7.9	18.6
2	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3	7.9	18.7
3	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8
4	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9
5	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6
6	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6
7	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
8	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
9	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
10	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
11	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
12	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
13	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6
14	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
15	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
16	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
17	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
18	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
19	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.1	8.7	17.6
20	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.1	8.7	17.6

Note: Control Survival = USEPA minimum test acceptability criteria $\geq$ 80% survival.

Control Mean Growth = USEPA minimum test acceptability criteria $\geq$ 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.

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

Species: *Pimephales promelas*

PpKClCR Test Number: 117

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2288</u>				
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:	<u>Yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>07-01-24</u>	Artemia CHM number:	CHM1294
Hatch dates and times:	<u>07-01-14 1258 to 07-01-24 0600</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.83</u> S.U. Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>07-16-24 0600</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
		Date / Time out of oven:	<u>07-17-24 0600</u>
		*Final oven temperature:	<u>60°C</u>
		Total drying time:	<u>24-Hours</u>

*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	07-09-24	<u>0510</u>	<u>H</u>	<u>1410</u>	<u>H</u>	<u>0710</u>	<u>H</u>	<u>07-01-24B</u>
1	07-10-24	<u>0500</u>	<u>H</u>	<u>1100</u>	<u>H</u>	<u>0700</u>	<u>H</u>	<u>↓</u>
2	07-11-24	<u>0500</u>	<u>H</u>	<u>1215</u>	<u>H</u>	<u>0700</u>	<u>H</u>	<u>07-08-24 A</u>
3	07-12-24	<u>0530</u>	<u>H</u>	<u>1200</u>	<u>H</u>	<u>0730</u>	<u>H</u>	<u>↓</u>
4	07-13-24	<u>0600</u>	<u>H</u>	<u>1200</u>	<u>H</u>	<u>0800</u>	<u>H</u>	<u>07-08-24B</u>
5	07-14-24	<u>0600</u>	<u>H</u>	<u>1200</u>	<u>H</u>	<u>0800</u>	<u>H</u>	<u>↓</u>
6	07-15-24	<u>0500</u>	<u>H</u>	<u>1100</u>	<u>H</u>	<u>0705</u>	<u>H</u>	<u>↓</u>
7	07-16-24					<u>0522</u>	<u>H</u>	

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	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>825.3</u>
Average weight per initial larvae:	<u>0.683</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.683</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>750</u>
			ChV (mg/L KCl)	<u>670.8</u>
			IC ₂₅ (mg/L KCl)	<u>678.9</u>

Species: *Pimephales promelas*

PpKICR Test Number: 117

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 ^{sk}	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 ²⁶	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>fm</u> Date: <u>06-19-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>jl</u> Date: <u>07-18-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>jl</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>jl</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.683 0.923 -35.27 0.700 -2.67												

*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:

Species: *Pimephales promelas*

PpKCICR Test Number: 117

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}	8 ^{1d}	8 ^{2d}	7 ^{2d}	8 ^{2d}
2	10	10	10	10	9	9	9	9	8	6 ^{2d}	6 ^{1d}	6 ^{2d}
3	10	10	10	10	9	9	9	9	8	6	6	4 ^{2d}
4	10	10	10	10	9	9	9	9	6 ^{2d}	6	6	4
5	10	10	10	10	6 ^{3d}	6 ^{3d}	6 ^{3d}	7 ^{2d}	4 ^{2d}	4 ^{2d}	4 ^{2d}	4
6	10	10	10	10	6	6	6	7	4	4	4	4
7	10	10	10	10	6	6	5 ^{1d}	7	3 ^{1d}	3 ^{1d}	3 ^{1d}	4
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>EM</u> Date: <u>06-19-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>EM</u> Date: <u>07-18-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>EM</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>EM</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.780 -14.27 0.478 30.07 0.289 57.77												

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

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

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 117

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	5 ^{sd}	6 ^{sd}	5 ^{sd}	5 ^{sd}
2	5	6	5	5
3	5	6	5	5
4	5	3 ^{sd}	5	5
5	2 ^{sd}	3	2 ^{sd}	3 ^{sd}
6	2	3	2	3
7	2 ^{sd}	1 ^{sd}	1 ^{sd}	1 ^{sd}
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>BM</u> Date: <u>06-19-24</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>BM</u> Date: <u>07-18-24</u>				
C = Larvae weight (mg) = B - A Analyst: <u>BM</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>BM</u>				
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		

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

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

Comments:

***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: 117
Test dates: July 09-16, 2024

Concentration (mg/L KO)	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 (%)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.18	20.53	6.35	0.635			0.635			
	B	10	10	13.38	21.11	7.73	0.773	0.683	9.0	0.683	100.0	9.0	Not applicable
	C	10	10	13.30	19.90	6.60	0.660			0.660			
	D	10	10	14.67	21.29	6.62	0.662			0.662			
300	E	10	10	13.17	21.95	8.78	0.878	0.923	6.4	0.923	100.0	6.4	-35.2
	F	10	10	12.45	22.54	10.09	1.009			1.009			
	G	10	10	12.98	21.94	8.96	0.896			0.896			
	H	10	10	14.10	23.17	9.07	0.907			0.907			
450	I	10	10	13.49	20.37	6.88	0.688			0.688			
	J	10	10	14.34	21.67	7.33	0.733	0.700	4.1	0.700	100.0	4.1	-2.6
	K	10	10	14.08	20.75	6.67	0.667			0.667			
	L	10	10	13.50	20.62	7.12	0.712			0.712			
600	M	10	10	12.57	20.32	7.75	0.775			0.775			
	N	10	10	14.78	21.76	6.98	0.698	0.780	7.9	0.780	100.0	7.9	-14.2
	O	10	10	15.10	23.09	7.99	0.799			0.799			
	P	10	10	13.64	22.10	8.46	0.846			0.846			
750	Q	10	6	15.10	20.20	5.10	0.850	0.795	4.7	0.795	60.0	14.6	30.0
	R	10	6	14.12	18.84	4.72	0.787			0.472			
	S	10	5	13.05	16.88	3.83	0.766			0.383			
	T	10	7	12.98	18.43	5.45	0.779			0.545			
900	U	10	3	14.74	17.72	2.98	0.993			0.298			
	V	10	3	13.04	16.06	3.02	1.007	0.895	13.6	0.895	32.5	13.6	57.7
	W	10	3	13.47	15.79	2.32	0.773			0.232			
	X	10	4	12.31	15.54	3.23	0.808			0.323			
1050	Y	10	2	13.12	14.99	1.87	0.935	0.836	14.1	0.187	12.5	50.7	84.3
	Z	10	1	13.70	14.45	0.75	0.750			0.075			
	AA	10	1	13.16	13.88	0.72	0.720			0.072			
	BB	10	1	15.86	16.80	0.94	0.940			0.094			

Dunnett's MSD value: 0.0883
PMSD: 12.9

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. Lower PMSD bound determined by USEPA (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, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Larval Fish Growth and Survival Test-7 Day Survival											
Start Date:	7/9/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant						
End Date:	7/16/2024	Lab ID:	ETS-Envr. Testing Sol.	Sample Type:	KCL-Potassium chloride						
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas						
Comments:											
Conc-mg/L	1	2	3	4							
D-Control	1.0000	1.0000	1.0000	1.0000							
300	1.0000	1.0000	1.0000	1.0000							
450	1.0000	1.0000	1.0000	1.0000							
600	1.0000	1.0000	1.0000	1.0000							
750	0.6000	0.6000	0.5000	0.7000							
900	0.3000	0.3000	0.3000	0.4000							
1050	0.2000	0.1000	0.1000	0.1000							
Transform: Arcsin Square Root											
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.6000	0.6000	0.8872	0.7854	0.9912	9.469	4	10.00	10.00	16	40
*900	0.3250	0.3250	0.6059	0.5796	0.6847	8.671	4	10.00	10.00	27	40
*1050	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40
Auxiliary Tests								Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)								0.73587	0.896	0.93793	3.38451
Equality of variance cannot be confirmed											
Hypothesis Test (1-tail, 0.05)			NOEC	LOEC	ChV	TU					
Steel's Many-One Rank Test			600	750	670.82						
Treatments vs D-Control											
Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.5202	1.60657	9.37128	15.669	0	4.01469	9.48773	0.04042	2.91662	0.07987	5
Intercept	-31.516	4.69094	-40.711	-22.322							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	538.031	461.252	591.939							
EC05	3.355	609.873	543.657	656.304							
EC10	3.718	652.013	592.835	694.144							
EC15	3.964	682.079	628.048	721.424							
EC20	4.158	706.959	657.106	744.336							
EC25	4.326	729.026	682.684	765.043							
EC40	4.747	787.736	748.758	822.964							
EC50	5.000	825.307	788.575	863.143							
EC60	5.253	864.671	827.692	908.363							
EC75	5.674	934.303	891.171	995.37							
EC80	5.842	963.467	916.177	1033.87							
EC85	6.036	998.612	945.467	1081.49							
EC90	6.282	1044.66	982.8	1145.52							
EC95	6.645	1116.84	1039.64	1248.93							
EC99	7.326	1265.97	1152.87	1471.82							

Dose mg/L

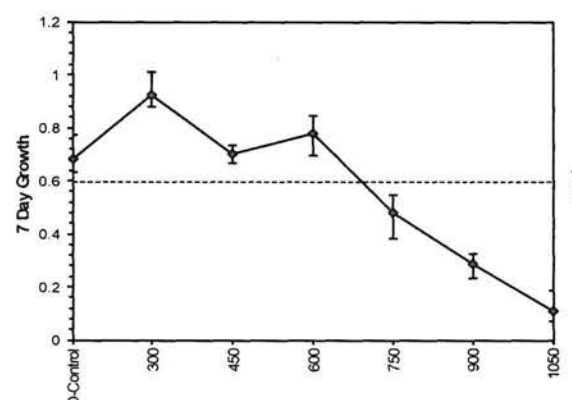
Dose-Response Plot

7 Day Survival

Larval Fish Growth and Survival Test-7 Day Growth													
Start Date:	7/9/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant								
End Date:	7/16/2024	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	0.6350	0.7730	0.6600	0.6620									
300	0.8780	1.0090	0.8960	0.9070									
450	0.6880	0.7330	0.6670	0.7120									
600	0.7750	0.6980	0.7990	0.8460									
750	0.5100	0.4720	0.3830	0.5450									
900	0.2980	0.3020	0.2320	0.3230									
1050	0.1870	0.0750	0.0720	0.0940									

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
D-Control	0.6825	1.0000	0.6825	0.6350	0.7730	9.021	4				0.8025	1.0000
300	0.9225	1.3516	0.9225	0.8780	1.0090	6.384	4	-6.222	2.290	0.0883	0.8025	1.0000
450	0.7000	1.0256	0.7000	0.6670	0.7330	4.096	4	-0.454	2.290	0.0883	0.7398	0.9218
600	0.7795	1.1421	0.7795	0.6980	0.8460	7.931	4	-2.515	2.290	0.0883	0.7398	0.9218
750	0.4775	0.6996	0.4775	0.3830	0.5450	14.596	4				0.4775	0.5950
900	0.2888	0.4231	0.2888	0.2320	0.3230	13.642	4				0.2888	0.3598
1050	0.1070	0.1568	0.1070	0.0720	0.1870	50.669	4				0.1070	0.1333

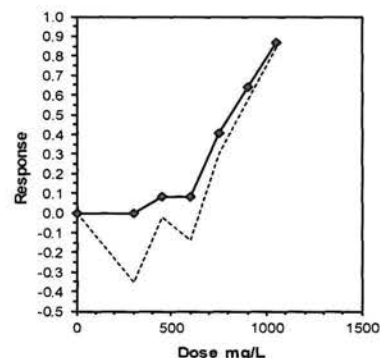
Auxiliary Tests					Statistic	Critical	Skew	Kurt					
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.93753	0.844	0.59748	-0.2051					
Bartlett's Test indicates equal variances (p = 0.64)					1.68851	11.3449							
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test				600	>600			0.08833	0.12942	0.04786	0.00298	1.7E-04	3, 12
Treatments vs D-Control													
Linear Interpolation (200 Resamples)													
Point	mg/L	SD	95%CL(Exp)		Skew								
IC05	395.92	83.66	335.72	738.75	1.4810								
IC10	610.01	77.27	300.53	645.13	-1.3457								
IC15	632.96	12.12	596.01	671.52	-0.1623								
IC20	655.91	12.27	619.25	696.01	-0.0339								
IC25	678.86	13.15	640.32	721.69	0.0321								
IC40	747.71	18.60	692.46	805.41	-0.0253								
IC50	810.60	19.50	726.19	855.68	-1.0030								



7 Day Growth

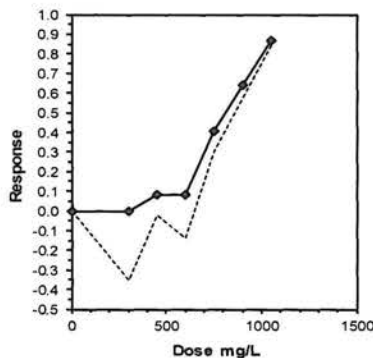
Dose mg/L

1-tail, 0.05 level of significance



Response

Dose mg/L



Species: *Pimephales promelas*

PpKCICR Test Number: 117

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.

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		KD	KD N	KD N	KD N	KD N	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.37	7.36	7.41	7.28	7.32	7.33
	Dissolved oxygen (mg/L)	7.7	7.6	8.0	7.7	7.7	7.3
	Conductivity (µmhos/cm)	302		304		306	
	Alkalinity (mg CaCO ₃ /L)	165				61	
	Hardness (mg CaCO ₃ /L)	84				84	
	Temperature (°C)	24.8	24.7	24.7	24.4	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.57	7.34	7.54	7.27	7.53	7.34
	Dissolved oxygen (mg/L)	7.7	7.6	8.0	7.7	7.9	7.2
	Conductivity (µmhos/cm)	856		876		846	
	Temperature (°C)	24.7	24.8	24.8	24.7	24.9	24.7
450 mg KCl/L	pH (S.U.)	7.57	7.35	7.54	7.29	7.54	7.31
	Dissolved oxygen (mg/L)	7.7	7.6	8.0	7.8	7.9	7.3
	Conductivity (µmhos/cm)	1120		1120		1100	
	Temperature (°C)	24.7	24.8	24.8	24.6	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.58	7.36	7.55	7.28	7.55	7.36
	Dissolved oxygen (mg/L)	7.8	7.6	8.0	7.8	7.9	7.3
	Conductivity (µmhos/cm)	1400		1380		1360	
	Temperature (°C)	24.5	24.5	24.8	24.6	24.8	24.7
750 mg KCl/L	pH (S.U.)	7.58	7.36	7.55	7.28	7.56	7.29
	Dissolved oxygen (mg/L)	7.8	7.6	8.1	7.8	8.0	7.2
	Conductivity (µmhos/cm)	1650		1650		1630	
	Temperature (°C)	24.8	24.8	24.9	24.6	24.8	24.8
900 mg KCl/L	pH (S.U.)	7.58	7.36	7.56	7.28	7.55	7.29
	Dissolved oxygen (mg/L)	7.8	7.5	8.1	7.7	8.0	7.12
	Conductivity (µmhos/cm)	1900		1950		1870	
	Temperature (°C)	24.5	24.7	24.9	24.8	25.0	24.5
1050 mg KCl/L	pH (S.U.)	7.58	7.38	7.56	7.37	7.57	7.36
	Dissolved oxygen (mg/L)	7.8	7.6	8.1	7.7	8.0	7.0
	Conductivity (µmhos/cm)	2180	2110	2190		2140	
	Temperature (°C)	24.6	24.7	25.0	24.5	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

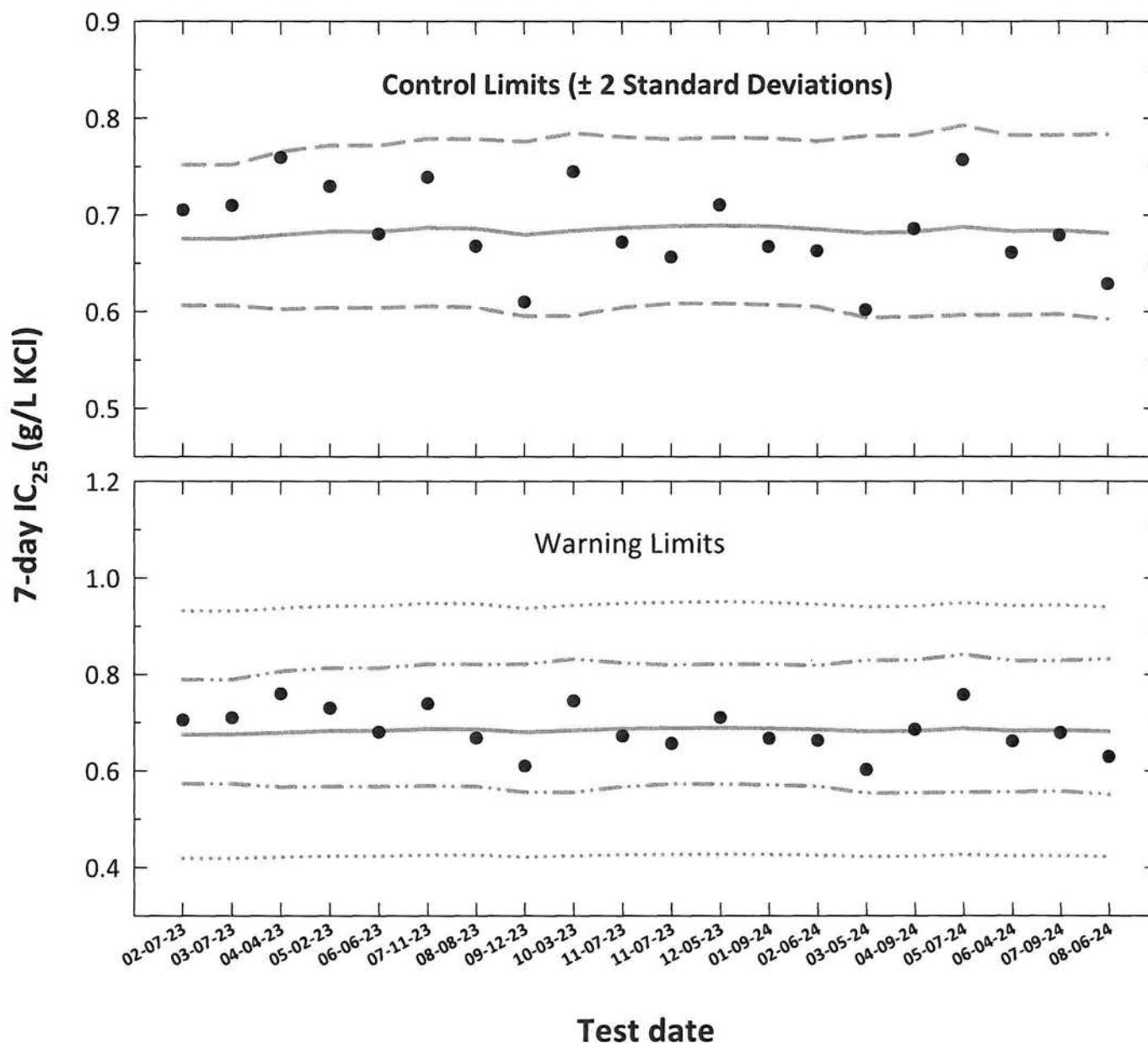
PpKICR Test Number: 117

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		Analyst							
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.20	7.11	7.55	7.00	7.46	7.22	7.44	7.63
	Dissolved oxygen (mg/L)	7.7	7.5	7.8	6.5	7.8	7.2	7.9	7.1
	Conductivity (µmhos/cm)	299		299		300		300	
	Alkalinity (mg CaCO ₃ /L)			61					
	Hardness (mg CaCO ₃ /L)			84					
	Temperature (°C)	24.8	24.6	24.8	25.1	24.8	24.7	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.21	7.20	7.44	7.11	7.59	7.26	7.55	7.57
	Dissolved oxygen (mg/L)	7.9	7.5	7.9	6.5	7.8	7.3	7.8	7.2
	Conductivity (µmhos/cm)	833		849		837		859	
	Temperature (°C)	24.9	24.8	24.9	24.8	24.9	24.9	24.7	24.6
450 mg KCl/L	pH (S.U.)	7.22	7.22	7.47	7.18	7.62	7.30	7.54	7.56
	Dissolved oxygen (mg/L)	7.9	7.4	7.9	6.5	7.9	7.3	7.8	7.2
	Conductivity (µmhos/cm)	1070		1110		1090		1100	
	Temperature (°C)	24.8	24.7	24.9	24.7	24.9	24.9	24.8	24.5
600 mg KCl/L	pH (S.U.)	7.22	7.20	7.49	7.25	7.43	7.27	7.58	7.56
	Dissolved oxygen (mg/L)	7.9	7.3	7.9	6.6	7.9	7.3	7.9	7.0
	Conductivity (µmhos/cm)	1320		1380		1340		1370	
	Temperature (°C)	24.8	24.6	25.0	24.7	25.0	24.6	24.7	24.8
750 mg KCl/L	pH (S.U.)	7.21	7.28	7.51	7.14	7.64	7.26	7.55	7.56
	Dissolved oxygen (mg/L)	8.0	7.6	7.9	6.7	8.0	7.3	7.9	7.0
	Conductivity (µmhos/cm)	1570		1630		1610		1620	
	Temperature (°C)	24.8	24.7	24.9	24.7	25.0	24.6	24.9	24.5
900 mg KCl/L	pH (S.U.)	7.20	7.23	7.51	7.17	7.64	7.26	7.57	7.54
	Dissolved oxygen (mg/L)	8.0	7.4	8.0	6.7	8.0	7.4	8.0	7.2
	Conductivity (µmhos/cm)	1830		1910		1910		1900	
	Temperature (°C)	24.7	24.7	24.9	24.9	25.0	24.9	24.9	24.5
1050 mg KCl/L	pH (S.U.)	7.23	7.26	7.53	7.22	7.65	7.31	7.59	7.54
	Dissolved oxygen (mg/L)	8.1	7.4	8.0	6.6	8.0	7.4	8.0	7.2
	Conductivity (µmhos/cm)	2110		2170		2140		2180	
	Temperature (°C)	24.7	24.7	24.9	24.9	25.0	24.8	24.8	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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . - . - **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient 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)

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 CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}
1	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064 0.7518	0.5733 0.7886	0.4186 0.9317
2	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063 0.7520	0.5732 0.7889	0.4186 0.9318
3	04-04-23	0.7592	-0.1196	-0.1680	0.0260	0.6792	0.6025 0.7656	0.5663 0.8064	0.4211 0.9373
4	05-02-23	0.7295	-0.1370	-0.1656	0.0266	0.6829	0.6042 0.7718	0.5677 0.8131	0.4234 0.9424
5	06-06-23	0.6800	-0.1675	-0.1657	0.0266	0.6828	0.6041 0.7717	0.5676 0.8130	0.4233 0.9422
6	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056 0.7789	0.5686 0.8208	0.4258 0.9478
7	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045 0.7784	0.5673 0.8207	0.4253 0.9467
8	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954 0.7757	0.5557 0.8211	0.4214 0.9378
9	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959 0.7846	0.5552 0.8312	0.4239 0.9436
10	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045 0.7806	0.5669 0.8232	0.4259 0.9479
11	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085 0.7786	0.5724 0.8194	0.4268 0.9499
12	12-05-23	0.7102	-0.1486	-0.1617	0.0269	0.6891	0.6088 0.7801	0.5725 0.8211	0.4273 0.9510
13	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
14	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
15	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
16	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
17	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
18	06-04-24	0.6610	-0.1798	-0.1656	0.0295	0.6830	0.5963 0.7823	0.5561 0.8283	0.4234 0.9425
19	07-09-24	0.6789	-0.1682	-0.1650	0.0293	0.6839	0.5976 0.7826	0.5577 0.8283	0.4240 0.9438
20	08-06-24	0.6287	-0.2015	-0.1667	0.0304	0.6813	0.5922 0.7837	0.5506 0.8316	0.4224 0.9402

Note: 7-day IC₂₅ = 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 IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

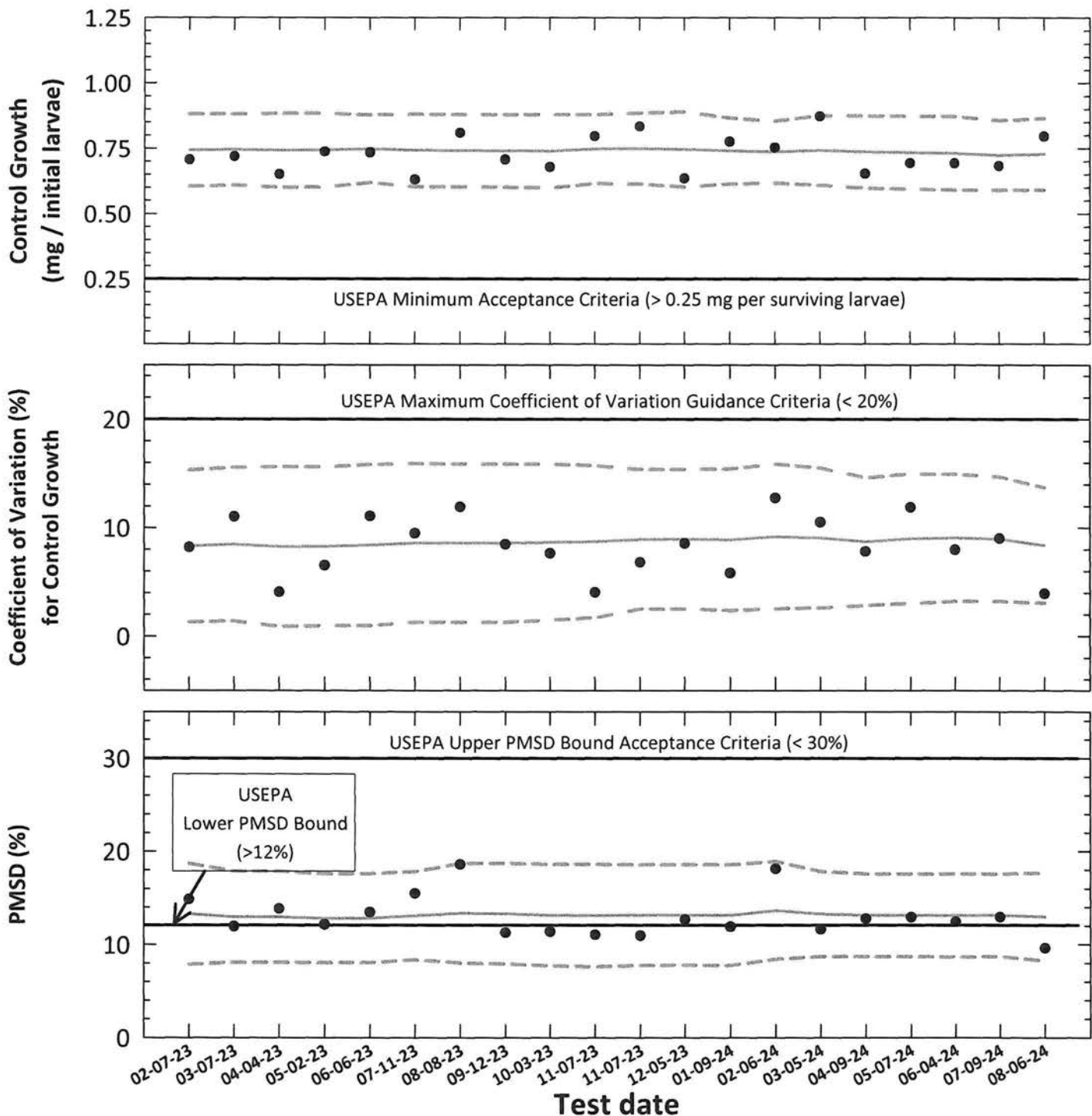
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

CV = Coefficient of variation.

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 $\pm$ 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD			
		Control Survival (%)	Control Growth		CV (%)	Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)			MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	15.3	13.3	7.9	18.7	
2	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8	
3	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9	
4	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6	
5	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6	
6	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8	
7	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7	
8	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7	
9	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6	
10	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6	
11	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6	
12	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6	
13	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	
14	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	
15	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	
16	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	
17	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	
18	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.1	8.7	17.6	
19	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.1	8.7	17.6	
20	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	8.3	17.7	

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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.

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

Species: *Pimephales promelas*

PpKICR Test Number: 118

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS <u>2298</u>				
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:	<u>Yellow</u>
Age:		< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:		<u>07-31-24</u>	Artemia CHM number:	CHM1294
Hatch dates and times:		<u>08-05-24 1230</u> to <u>08-06-24 0558</u>	Drying information for weight determination:	
Transfer vessel information:		pH = <u>7.87</u> Temperature = <u>24.0</u> °C	Date / Time in oven:	<u>08-13-24 0640</u>
Average transfer volume:		< 0.25 mL	*Initial oven temperature:	<u>60°C</u>
			Date / Time out of oven:	<u>08-14-24 0640</u>
			*Final oven temperature:	<u>60°C</u>
			Total drying time:	<u>24-Hours</u>

*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	08-06-24	0600	JL	1230	JL	0800	JL	07-31-24 A
1	08-07-24	0500	JL	1100	JL	0700	JL	↓
2	08-08-24	0500	JL	1100	JL	0700	JL	07-31-24 D
3	08-09-24	0600	JL	1200	JL	0800	JL	↓
4	08-10-24	0600	JL	1200	JL	0810	JL	08-06-24 A
5	08-11-24	0600	JL	1200	JL	0800	JL	↓
6	08-12-24	0600	JL	1200	JL	0800	JL	↓
7	08-13-24					0611	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	<u>130664685</u>

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>755.2</u>
Average weight per initial larvae:	<u>0.796</u>		NOEC (mg/L KCl)	<u>450</u>
Average weight per surviving larvae:	<u>0.796</u>	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	<u>600</u>
			ChV (mg/L KCl)	<u>519.6</u>
			IC ₂₅ (mg/L KCl)	<u>628.7</u>

Species: Pimephales promelas

PpKICR Test Number: 118

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: <u>Purple</u> Analyst: <u>EM</u> Date: <u>07-23-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>07-15-24 07-15-24 EM</u> Date: <u>07-15-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>EM</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>EM</u>												
Average weight per initial number of larvae (mg)	0.796				0.996				0.807			
Percent reduction from control (%)					-25.17				-1.37			

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

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

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 118

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	9 ^{id}	10	9 ^{id}	9 ^{id}	8 ^{id}	9 ^{id}	9 ^{id}
2	10	10	10	10	10	9	10	9	9	8	9	9
3	10	10	10	10	10	9	10	8 ^{id}	9	8	9	9
4	10	10	10	10	10	8 ^{id}	10	8	6 ^{id}	5 ^{id}	6 ^{id}	5 ^{id}
5	9 ^{id}	9 ^{id}	10	9 ^{id}	10	8	9 ^{id}	8	3 ^{id}	3 ^{id}	3 ^{id}	2 ^{id}
6	9	9	10	9	7 ^{id}	7 ^{id}	7 ^{id}	7 ^{id}	3	2 ^{id}	2 ^{id}	2
7	9	8 ^{id}	8 ^{id}	9	6 ^{id}	6 ^{id}	6 ^{id}	7	1 ^{id}	1 ^{id}	1 ^{id}	1 ^{id}
*A = Pan weight (mg) Tray color code: <u>purple</u> Analyst: <u>BM</u> Date: <u>07-23-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>BM</u> Date: <u>08-15-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>BM</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>BM</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.715 10.37. 0.494 38.07. 0.082 89.77.												

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

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

Comments:

Species: *Pimephales promelas*

PpKCICR Test Number: 118

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	7 ^{2d}	7 ^{3d}	5 ^{5d}	7 ^{3d}
2	6 ^{1d}	5 ^{2d}	5	6 ^{1d}
3	6	5	5	6
4	4 ^{2d}	5	4 ^{1d}	4 ^{2d}
5	2 ^{2d}	2 ^{3d}	2 ^{2d}	2 ^{2d}
6	1 ^{1d}	1 ^{1d}	1 ^{1d}	1 ^{1d}
7	0 ^{1d}	0 ^{1d}	1	1
*A = Pan weight (mg) Tray color code: <u>Black</u> Analyst: <u>PM</u> Date: <u>07-23-24</u>				
*B = Pan + Larvae weight (mg) Analyst: <u>PM</u> Date: <u>08-15-24</u>				
C = Larvae weight (mg) = B - A Analyst: <u>PM</u>				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>PM</u>				
Average weight per initial number of larvae (mg)	Percent reduction from control (%)			
	0.040		95.07.	

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

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

Comments:

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: 118
Test dates: August 06-13, 2024

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pin weight (mg)	B = Pin + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.79	22.64	7.85	0.785							
	B	10	10	15.07	22.72	7.65	0.765							
	C	10	10	12.93	21.32	8.39	0.839	0.796	3.9		100.0	0.796	3.9	Not applicable
	D	10	10	13.96	21.92	7.96	0.796							
300	E	10	10	14.16	24.53	10.37	1.037							
	F	10	10	13.82	23.27	9.45	0.945	0.996	5.4		100.0	0.996	5.4	-25.1
	G	10	10	13.92	24.40	10.48	1.048							
	H	10	10	14.13	23.68	9.55	0.955							
450	I	10	10	15.00	22.83	7.83	0.783							
	J	10	10	13.19	22.09	8.90	0.890	0.807	7.4		100.0	0.807	7.4	-1.3
	K	10	10	11.36	19.38	8.02	0.802							
	L	10	10	13.86	21.37	7.51	0.751							
600	M	10	9	14.48	21.12	6.64	0.738							
	N	10	8	12.91	19.45	6.54	0.818	0.840	11.4		85.0	0.715	14.7	10.3
	O	10	8	13.70	20.38	6.68	0.835							
	P	10	9	13.86	22.58	8.72	0.969							
750	Q	10	6	13.56	18.24	4.68	0.780							
	R	10	6	11.91	16.24	4.33	0.722	0.787	6.7		62.5	0.494	14.1	38.0
	S	10	6	14.98	19.77	4.79	0.798							
	T	10	7	14.96	20.90	5.94	0.849							
900	U	10	1	13.62	14.43	0.81	0.810							
	V	10	1	14.07	14.82	0.75	0.750	0.820	6.7		10.0	0.082	6.7	89.7
	W	10	1	12.50	13.34	0.84	0.840							
	X	10	1	14.96	15.84	0.88	0.880							
1050	Y	10	0	0.00	0.00	0.00	0.000							
	Z	10	0	0.00	0.00	0.00	0.000	0.790	26.9		5.0	0.040	119.6	95.0
	AA	10	1	11.42	12.36	0.94	0.940							
	BB	10	1	13.10	13.74	0.64	0.640							

Dunnett's MSD value: 0.0766
PMSD: 9.6

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. Lower PMSD bound determined by USEPA (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, 2001b). The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

Larval Fish Growth and Survival Test-7 Day Survival												
Start Date:	8/6/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant							
End Date:	8/13/2024	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	0.8000	0.8000	0.9000								
750	0.6000	0.6000	0.6000	0.7000								
900	0.1000	0.1000	0.1000	0.1000								
1050	0.0000	0.0000	0.1000	0.1000								

Transform: Arcsin Square Root												
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical	Number Resp	Total 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	0.8500	0.8500	1.1781	1.1071	1.2490	6.954	4	10.00	10.00	6	40	
*750	0.6250	0.6250	0.9123	0.8861	0.9912	5.759	4	10.00	10.00	15	40	
*900	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	36	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)					0.82735	0.896	0.18889	0.18104
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	12.4183	1.50152	9.47533	15.3613	0	4.43016	9.48773	0.35091	2.87808	0.08053	3
Intercept	-30.741	4.33976	-39.247	-22.235							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	490.624	421.994	540.242							
EC05	3.355	556.707	496.503	600.149							
EC10	3.718	595.501	540.958	635.346							
EC15	3.964	623.19	572.818	660.668							
EC20	4.158	646.112	599.153	681.873							
EC25	4.326	666.448	622.394	700.962							
EC40	4.747	720.576	682.924	753.789							
EC50	5.000	755.233	719.946	789.865							
EC60	5.253	791.556	756.773	830.074							
EC75	5.674	855.844	817.076	907.156							
EC80	5.842	882.782	840.859	941.326							
EC85	6.036	915.252	868.685	983.655							
EC90	6.282	957.81	904.095	1040.68							
EC95	6.645	1024.55	957.916	1132.91							
EC99	7.326	1162.55	1065	1331.87							

Larval Fish Growth and Survival Test-7 Day Growth

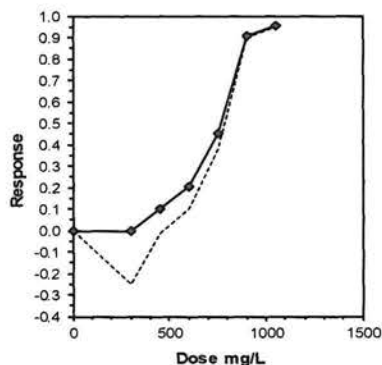
Start Date: 8/6/2024 Test ID: PpKICIR Sample ID: REF-Ref Toxicant
End Date: 8/13/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.7850	0.7650	0.8390	0.7960
300	1.0370	0.9450	1.0480	0.9550
450	0.7830	0.8900	0.8020	0.7510
600	0.6640	0.6540	0.6680	0.8720
750	0.4680	0.4330	0.4790	0.5940
900	0.0810	0.0750	0.0840	0.0880
1050	0.0000	0.0000	0.0940	0.0640

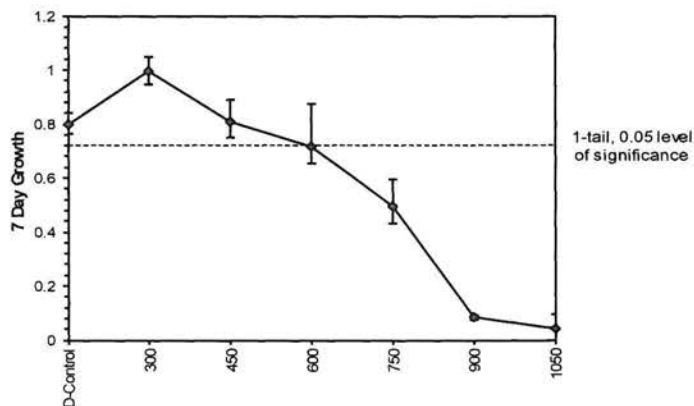
Conc-mg/L	Transform: Untransformed						N	1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%		t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7963	1.0000	0.7963	0.7650	0.8390	3.925	4				0.8963	1.0000
300	0.9963	1.2512	0.9963	0.9450	1.0480	5.395	4	-5.692	2.180	0.0766	0.8963	1.0000
450	0.8065	1.0129	0.8065	0.7510	0.8900	7.379	4	-0.292	2.180	0.0766	0.8065	0.8999
600	0.7145	0.8973	0.7145	0.6540	0.8720	14.719	4				0.7145	0.7972
750	0.4935	0.6198	0.4935	0.4330	0.5940	14.146	4				0.4935	0.5506
900	0.0820	0.1030	0.0820	0.0750	0.0880	6.680	4				0.0820	0.0915
1050	0.0395	0.0496	0.0395	0.0000	0.0940	119.561	4				0.0395	0.0441

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)				0.93093	0.805	0.53559	-0.8504
Bartlett's Test indicates equal variances ($p = 0.59$)				1.07003	9.21035		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnnett's Test				450	>450		
Treatments vs D-Control							
						MSDu	MSDp
						0.0766	0.0962
						MSB	MSE
						0.05074	0.00247
						F-Prob	df
						4.4E-04	2, 9

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	374.90	32.20	332.38	519.93	1.5116
IC10	449.79	43.21	364.76	641.17	1.1405
IC15	522.86	50.70	398.24	674.51	0.2285
IC20	595.92	38.99	457.84	667.50	-0.4148
IC25	628.72	28.35	533.95	682.72	-0.4052
IC40	719.97	17.87	670.34	776.56	0.1700
IC50	766.54	10.00	733.31	794.11	-0.1002



Dose-Response Plot



Species: *Pimephales promelas*

PpKCICR Test Number: 118

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.

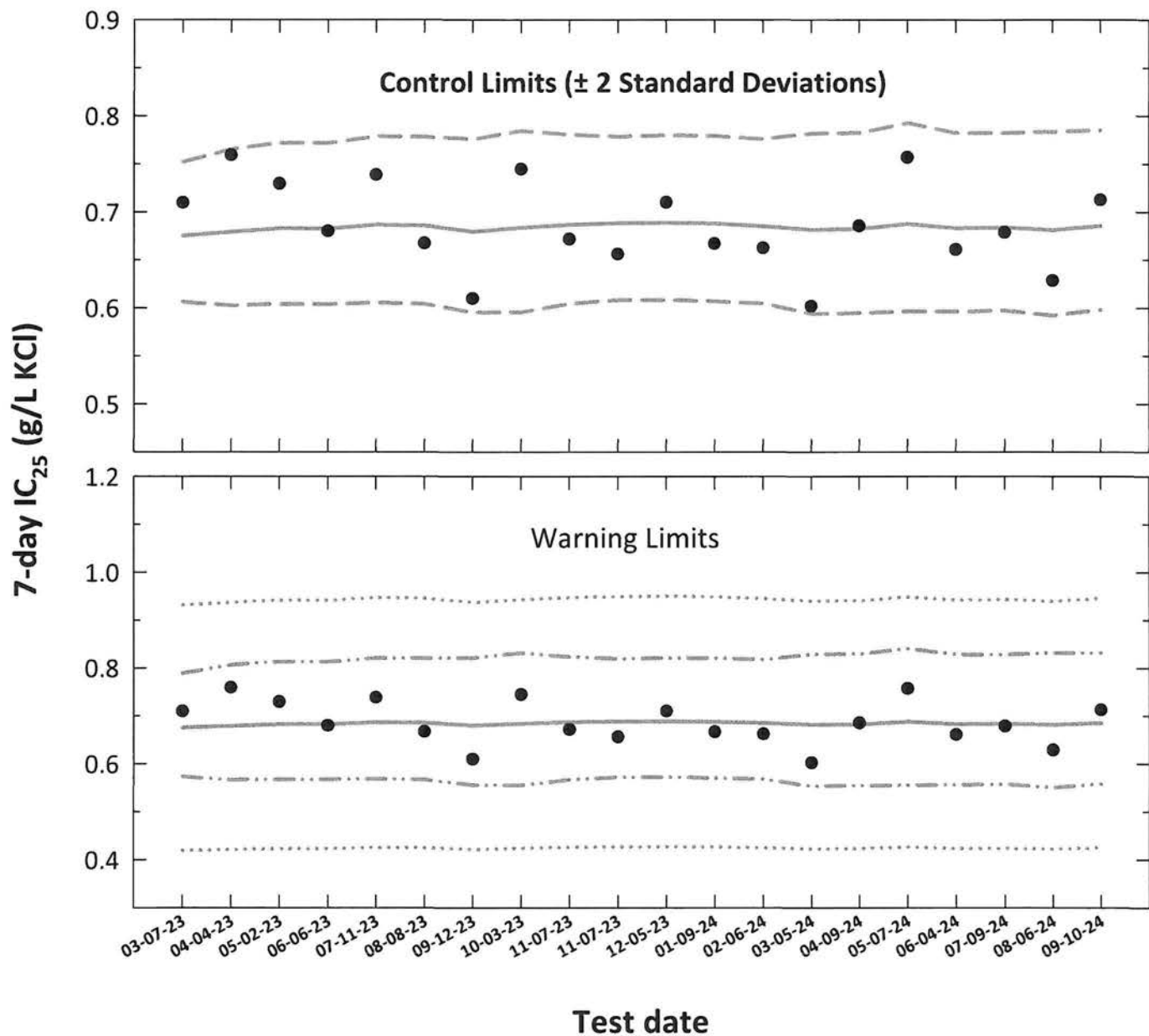
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		Analyst					
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.67	7.38	7.40	7.36	7.48	7.22
	Dissolved oxygen (mg/L)	7.8	7.9	7.8	7.7	7.9	5.6
	Conductivity (µmhos/cm)	292		300		312	
	Alkalinity (mg CaCO ₃ /L)	61				61	
	Hardness (mg CaCO ₃ /L)	84				86	
	Temperature (°C)	24.8	24.6	24.7	24.4	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.76	7.33	7.59	7.29	7.50	7.17
	Dissolved oxygen (mg/L)	8.0	7.9	7.7	7.6	7.8	5.6
	Conductivity (µmhos/cm)	816		815		853	
	Temperature (°C)	24.7	24.5	24.6	24.7	24.9	24.3
450 mg KCl/L	pH (S.U.)	7.72	7.33	7.59	7.29	7.50	7.13
	Dissolved oxygen (mg/L)	8.0	7.8	7.7	7.6	7.8	5.8
	Conductivity (µmhos/cm)	1040		1070		1120	
	Temperature (°C)	24.7	24.7	24.6	24.7	24.9	24.6
600 mg KCl/L	pH (S.U.)	7.71	7.32	7.56	7.28	7.50	7.11
	Dissolved oxygen (mg/L)	8.0	7.7	7.7	7.5	7.8	5.7
	Conductivity (µmhos/cm)	1300		1320		1370	
	Temperature (°C)	24.7	24.7	24.7	24.6	25.0	24.6
750 mg KCl/L	pH (S.U.)	7.71	7.34	7.56	7.29	7.49	7.09
	Dissolved oxygen (mg/L)	8.0	7.7	7.8	7.5	7.9	5.4
	Conductivity (µmhos/cm)	1540		1550		1620	
	Temperature (°C)	24.8	24.6	24.7	24.6	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.71	7.32	7.56	7.30	7.53	7.21
	Dissolved oxygen (mg/L)	8.0	7.6	7.8	7.5	7.9	5.6
	Conductivity (µmhos/cm)	1820		1810		1890	
	Temperature (°C)	24.8	24.6	24.7	24.4	24.8	24.7
1050 mg KCl/L	pH (S.U.)	7.72	7.33	7.56	7.34	7.53	7.15
	Dissolved oxygen (mg/L)	8.0	7.6	7.8	7.5	7.9	6.3
	Conductivity (µmhos/cm)	2130		2100		2180	
	Temperature (°C)	24.8	24.6	24.6	24.6	24.8	24.5
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

PpKCICR Test Number: 118

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		KL	BSL	BSL	BSL	BSL	KL	KL	KL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.01	7.23	7.54	7.10	7.66	7.36	7.62	7.35
	Dissolved oxygen (mg/L)	7.0	7.9	7.9	7.3	7.7	7.1	7.9	7.3
	Conductivity (µmhos/cm)	194		292		297		315	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			88					
	Temperature (°C)	24.6	24.4	24.7	24.5	24.7	25.1	24.6	24.7
300 mg KCl/L	pH (S.U.)	7.74	7.32	7.49	7.17	7.55	7.33	7.74	7.35
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	7.3	7.9	7.0	7.9	7.4
	Conductivity (µmhos/cm)	845		813		836		832	
	Temperature (°C)	24.6	24.7	24.8	24.8	24.8	24.7	24.9	24.5
450 mg KCl/L	pH (S.U.)	7.75	7.32	7.50	7.20	7.55	7.35	7.74	7.35
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	7.3	7.9	7.1	7.8	7.4
	Conductivity (µmhos/cm)	1050		1050		1090		1080	
	Temperature (°C)	24.6	24.8	24.8	24.6	24.7	24.7	24.9	24.5
600 mg KCl/L	pH (S.U.)	7.74	7.34	7.51	7.14	7.57	7.37	7.73	7.37
	Dissolved oxygen (mg/L)	7.0	7.8	8.0	7.2	7.9	7.1	7.9	7.3
	Conductivity (µmhos/cm)	1310		1290		1350		1330	
	Temperature (°C)	24.6	24.5	24.9	24.6	24.6	24.6	25.0	24.5
750 mg KCl/L	pH (S.U.)	7.74	7.39	7.54	7.22	7.58	7.37	7.73	7.36
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.1	8.0	7.1	8.0	7.3
	Conductivity (µmhos/cm)	1550		1540		1580		1580	
	Temperature (°C)	24.5	24.5	24.8	24.8	24.6	24.6	25.0	24.3
900 mg KCl/L	pH (S.U.)	7.73	7.33	7.54	7.26	7.59	7.38	7.73	7.36
	Dissolved oxygen (mg/L)	8.0	7.7	8.0	7.0	8.0	7.2	8.0	7.3
	Conductivity (µmhos/cm)	1800		1770		1860		1940	
	Temperature (°C)	24.5	24.6	24.9	24.7	24.7	24.6	25.0	24.6
1050 mg KCl/L	pH (S.U.)	7.74	7.37	7.59	7.17	7.62	7.39	7.73	7.36
	Dissolved oxygen (mg/L)	8.1	7.7	8.0	7.0	8.0	7.2	8.0	7.2
	Conductivity (µmhos/cm)	2070		2060		2130		2120	
	Temperature (°C)	24.5	24.6	24.9	24.7	24.6	24.9	24.9	24.4
		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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic $IC_{25} \pm 2$ coefficient 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)

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 CT - 2S CT + 2S
1	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063 0.7520
2	04-04-23	0.7592	-0.1196	-0.1680	0.0260	0.6792	0.6025 0.7656
3	05-02-23	0.7295	-0.1370	-0.1656	0.0266	0.6829	0.6042 0.7718
4	06-06-23	0.6800	-0.1675	-0.1657	0.0266	0.6828	0.6041 0.7717
5	07-11-23	0.7387	-0.1315	-0.1632	0.0273	0.6868	0.6056 0.7789
6	08-08-23	0.6677	-0.1754	-0.1637	0.0274	0.6860	0.6045 0.7784
7	09-12-23	0.6098	-0.2148	-0.1677	0.0287	0.6796	0.5954 0.7757
8	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959 0.7846
9	11-07-23	0.6718	-0.1728	-0.1631	0.0278	0.6869	0.6045 0.7806
10	11-07-23	0.6562	-0.1830	-0.1622	0.0268	0.6883	0.6085 0.7786
11	12-05-23	0.7102	-0.1486	-0.1617	0.0269	0.6891	0.6088 0.7801
12	01-09-24	0.6671	-0.1758	-0.1624	0.0271	0.6880	0.6073 0.7795
13	02-06-24	0.6628	-0.1786	-0.1640	0.0270	0.6854	0.6052 0.7762
14	03-05-24	0.6019	-0.2205	-0.1666	0.0298	0.6814	0.5940 0.7817
15	04-09-24	0.6855	-0.1640	-0.1660	0.0298	0.6823	0.5949 0.7825
16	05-07-24	0.7569	-0.1210	-0.1627	0.0309	0.6876	0.5965 0.7927
17	06-04-24	0.6610	-0.1798	-0.1656	0.0295	0.6830	0.5963 0.7823
18	07-09-24	0.6789	-0.1682	-0.1650	0.0293	0.6839	0.5976 0.7826
19	08-06-24	0.6287	-0.2015	-0.1667	0.0304	0.6813	0.5922 0.7837
20	09-10-24	0.7128	-0.1470	-0.1640	0.0295	0.6856	0.5983 0.7855
							Warning Limits CT - S _{A,75} CT + 2CV
							75th Percentile CV CT - S _{A,75} CT + S _{A,75}
1							0.4186 0.9318
2							0.4211 0.9373
3							0.4234 0.9424
4							0.4233 0.9422
5							0.4258 0.9478
6							0.4253 0.9467
7							0.4214 0.9378
8							0.4239 0.9436
9							0.4259 0.9479
10							0.4268 0.9499
11							0.4273 0.9510
12							0.4266 0.9495
13							0.4250 0.9459
14							0.4225 0.9404
15							0.4230 0.9416
16							0.4263 0.9489
17							0.4234 0.9425
18							0.4240 0.9438
19							0.4224 0.9402
20							0.4250 0.9461

Note: 7-day IC₂₅ = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCal).

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

Control Limits = Mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

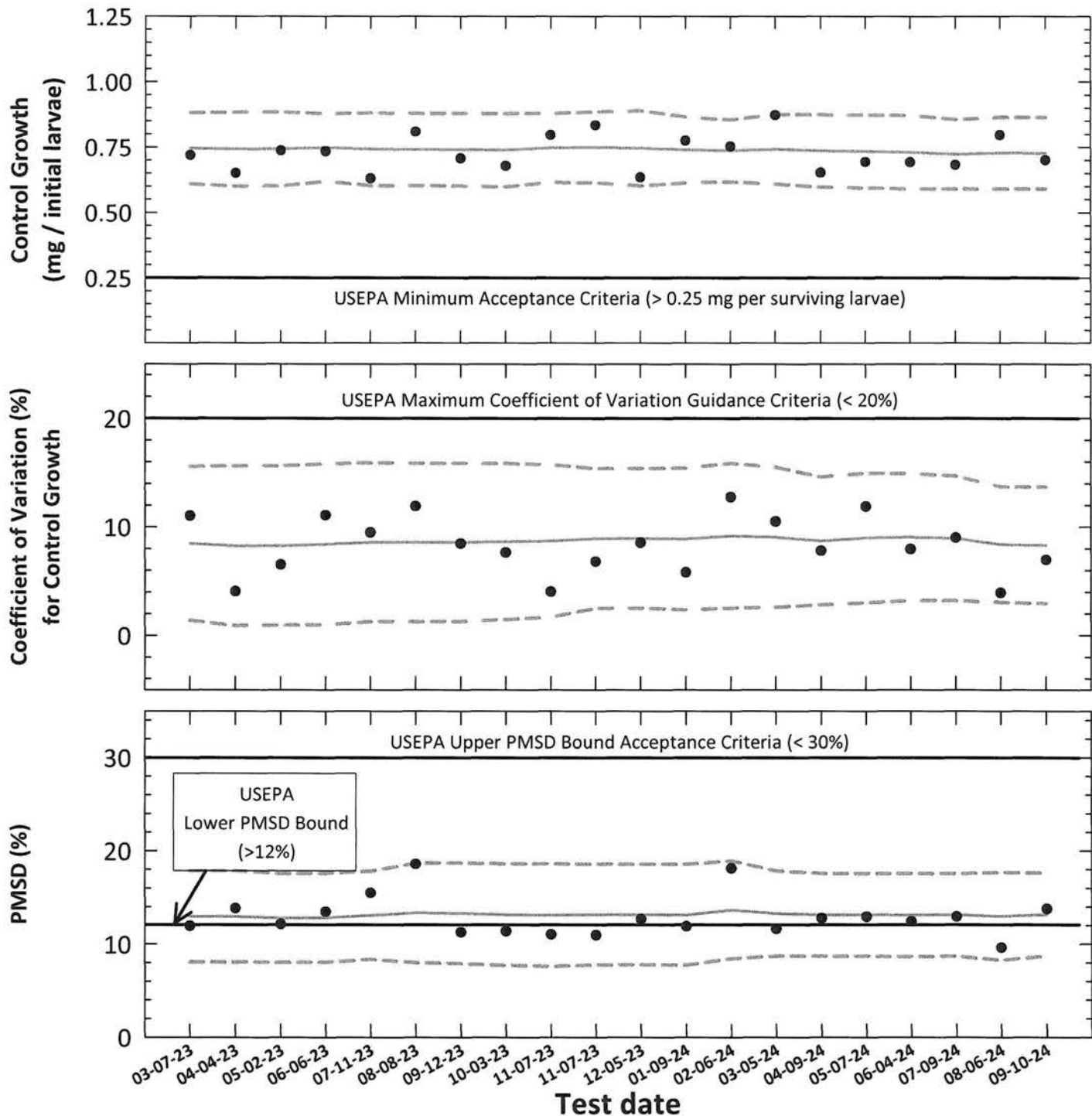
S_{A,75} = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA (S_{A,75} = 0.38).

CV = Coefficient of variation.

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 $\pm$ 2 Standard Deviations)

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

Test number	Test date	ToxCal Determination				Control Growth			Control Growth CV			Test PMSD		
		Control Survival (%)	Control Growth		Test	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)	CV (%)										
1	03-07-23	100	0.719	11.0	0.0858	0.745	0.609	0.881	8.5	1.4	15.6	13.0	8.1	17.8
2	04-04-23	100	0.651	4.1	0.0900	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9
3	05-02-23	100	0.737	6.5	0.0895	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6
4	06-06-23	100	0.734	11.1	0.0988	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6
5	07-11-23	100	0.630	9.5	0.0976	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
6	08-08-23	100	0.809	11.9	0.1504	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
7	09-12-23	100	0.707	8.5	0.0796	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
8	10-03-23	100	0.678	7.7	0.0772	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
9	11-07-23	100	0.797	4.0	0.0880	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
10	11-07-23	100	0.833	6.8	0.0913	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
11	12-05-23	100	0.635	8.6	0.0805	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6
12	01-09-24	100	0.776	5.8	0.0923	0.740	0.614	0.866	8.9	2.4	15.4	13.1	7.7	18.6
13	02-06-24	100	0.753	12.8	0.1364	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
14	03-05-24	100	0.873	10.5	0.1016	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
15	04-09-24	100	0.653	7.8	0.0834	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
16	05-07-24	100	0.693	11.9	0.0896	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
17	06-04-24	100	0.693	8.0	0.0862	0.732	0.591	0.872	9.1	3.2	14.9	13.1	8.7	17.6
18	07-09-24	100	0.683	9.0	0.0883	0.724	0.591	0.857	9.0	3.2	14.7	13.1	8.7	17.6
19	08-06-24	100	0.796	3.9	0.0766	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.3	17.7
20	09-10-24	100	0.701	7.0	0.0964	0.728	0.591	0.865	8.3	2.9	13.7	13.2	8.7	17.6

Note: Control Survival = USEPA minimum test acceptability criteria $\geq 80\%$ 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%.

Upper PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.

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

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

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

Species: *Pimephales promelas*

PpKCICR Test Number: **119**

Dilution preparation information:							Comments:
KCl Stock INSS number:			INSS 2319				
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:	7 B
Spawn date:	09-02-24	Artemia CHM number:	CHM1294
Hatch dates and times:	09-09-24 1355 TO 09-10-24 0405	Drying information for weight determination:	
Transfer vessel information:	pH = 8.13 S.U. Temperature = 24.0 °C	Date / Time in oven:	09-17-24 0605
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	60°C
		Date / Time out of oven:	09-18-24 0605
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	09-10-24	0500	<i>jl</i>	1430	<i>jl</i>	0711	<i>jl</i>	09-04-24 A
1	09-11-24	0500	<i>jl</i>	1100	<i>jl</i>	0700	<i>jl</i>	↓
2	09-12-24	0500	<i>jl</i>	1200	<i>jl</i>	0700	<i>jl</i>	09-04-24 D
3	09-13-24	0500	<i>jl</i>	1100	<i>jl</i>	0700	<i>jl</i>	↓
4	09-14-24	0600	<i>jl</i>	1200	<i>jl</i>	0800	<i>jl</i>	09-09-24 B
5	09-15-24	0600	<i>jl</i>	1200	<i>jl</i>	0800	<i>jl</i>	↓
6	09-16-24	0500	<i>jl</i>	1100	<i>jl</i>	0700	<i>jl</i>	↓
7	09-17-24					0518	<i>jl</i>	

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:	07	≤ 20%	7-day LC ₅₀ (mg/L KCl)	797.9
Average weight per initial larvae:	0.701		NOEC (mg/L KCl)	600
Average weight per surviving larvae:	0.701	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC ₂₅ (mg/L KCl)	712.8

Species: *Pimephales promelas*

PpKICR Test Number: 119

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: <u>Forest Green</u> Analyst: <u>BN</u> Date: <u>09-26-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>JL</u> Date: <u>09-19-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.701 0.776 -10.7% 0.699 0.27												

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

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

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 119

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}	5 ^{sk}	5 ^{sk}	5 ^{sk}	5 ^{sk}
2	10	10	10	10	9	9	8 ^{1d}	9	4 ^{1d}	5	5	5
3	10	10	10	10	9	9	7 ^{1d}	9	4	5	5	4 ^{1d}
4	10	10	10	10	9	8 ^{1d}	7	9	4	5	4 ^{1d}	4
5	10	9 ^{1d} 10 ^{1d}	10	10	8 ^{1d} 9 ^{1d}	8	7	9	2 ^{1d}	4 ^{1d}	3 ^{1d}	5 ^{1d}
6	10	9	10	10	8	8	7	7 ^{2d}	2	5 ^{1d}	2 ^{1d}	2 ^{1d}
7	10	9	10	10	8	7 ^{1d}	7	7	2	2 ^{1d}	2	1 ^{1d}
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>gm</u> Date: <u>08-26-24</u>												
*B = Pan + Larvae weight (mg) Analyst: <u>J</u> Date: <u>09-18-24</u>												
C = Larvae weight (mg) = B - A Analyst: <u>J</u>												
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>												
Average weight per initial number of larvae (mg) Percent reduction from control (%)												
0.690 1.57 0.509 27.47 0.154 78.07												

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

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

Comments:

Species: *Pimephales promelas*

PpKCICR Test Number: 119

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	4 ^{bd}	4 ^{bd}	4 ^{bd}	4 ^{bd}
2	4	4	4	4
3	4	4	4	4
4	3 ^{id}	2 ^{id}	3 ^{id}	4
5	1 ^{2d}	1 ^{1d}	1 ^{2d}	1 ^{3d}
6	1	0 ^{id}	0 ^{id}	1
7	0 ^{id}	0	0	0 ^{id}
*A = Pan weight (mg) Tray color code: <u>Forest Green</u> Analyst: <u>gm</u> Date: <u>08-26-24</u>				
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B - A Analyst: _____				
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>gm</u>				
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:

***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: 119
Test dates: September 10-17, 2024

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	15.37	21.96	6.59	0.659			0.659				
	B	10	10	14.01	21.31	7.30	0.730			0.730				
	C	10	10	11.67	19.21	7.54	0.754		7.0	0.754	100.0	0.701	7.0	Not applicable
	D	10	10	15.48	22.07	6.59	0.659			0.659				
300	E	10	10	14.88	21.67	6.79	0.679			0.679				
	F	10	10	14.70	22.74	8.04	0.804			0.804				
	G	10	10	11.53	20.15	8.62	0.862		10.0	0.862	100.0	0.776	10.0	-10.7
	H	10	10	14.29	21.87	7.58	0.758			0.758				
450	I	10	10	14.54	21.23	6.69	0.669			0.669				
	J	10	10	14.13	21.07	6.94	0.694			0.694				
	K	10	10	14.26	21.20	6.94	0.694		4.2	0.694	100.0	0.699	4.2	0.2
	L	10	10	13.44	20.84	7.40	0.740			0.740				
600	M	10	10	13.32	20.88	7.56	0.756			0.756				
	N	10	9	14.09	20.55	6.46	0.718			0.646				
	O	10	10	10.31	16.46	6.15	0.615		9.1	0.615	97.5	0.690	10.2	1.5
	P	10	10	12.36	19.80	7.44	0.744			0.744				
750	Q	10	8	13.97	18.82	4.85	0.606			0.485				
	R	10	7	12.71	17.65	4.94	0.706			0.494				
	S	10	7	15.03	20.11	5.08	0.726		10.4	0.508	72.5	0.509	5.4	27.4
	T	10	7	13.46	18.93	5.47	0.781			0.547				
900	U	10	2	13.54	15.01	1.47	0.735			0.147				
	V	10	2	11.90	13.61	1.71	0.855			0.171				
	W	10	2	13.67	15.73	2.06	1.030		14.0	0.206	17.5	0.154	30.8	78.0
	X	10	1	13.93	14.86	0.93	0.930			0.093				
1050	Y	10	0	0.00	0.00	0.00	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: 0.0964
PMSD: 13.8

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) = 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.

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 9/10/2024 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
End Date: 9/17/2024 Lab ID: ETS-Envr. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	0.9000	1.0000	1.0000
750	0.8000	0.7000	0.7000	0.7000
900	0.2000	0.2000	0.2000	0.1000
1050	0.0000	0.0000	0.0000	0.0000

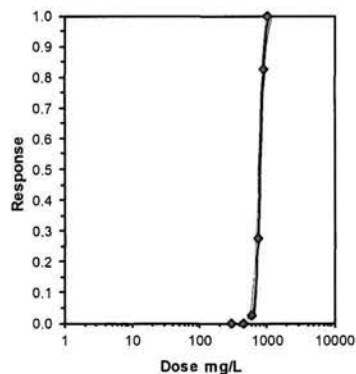
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				N	Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	40
*900	0.1750	0.1750	0.4282	0.3218	0.4636	16.570	4	10.00	10.00	33	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)	0.83255	0.884	-1.1518	2.78665
Equality of variance cannot be confirmed				

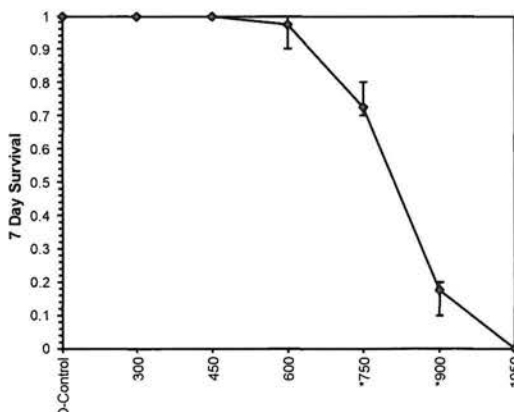
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

				Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.5884	2.49982	13.6888 23.488	0	1.5524	9.48773	0.81732	2.90193	0.0538	4

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	598.113	532.204 642.194
EC05	3.355	650.797	595.376 688.26
EC10	3.718	680.754	631.593 714.684
EC15	3.964	701.741	656.938 733.449
EC20	4.158	718.881	677.525 749.022
EC25	4.326	733.919	695.43 762.941
EC40	4.747	773.223	740.962 801.022
EC50	5.000	797.873	768.07 826.672
EC60	5.253	823.31	794.513 854.923
EC75	5.674	867.4	836.711 908.13
EC80	5.842	885.545	852.98 931.343
EC85	6.036	907.175	871.758 959.795
EC90	6.282	935.142	895.276 997.608
EC95	6.645	978.187	930.285 1057.57
EC99	7.326	1064.35	997.687 1182.3



Dose-Response Plot



Larval Fish Growth and Survival Test-7 Day Growth

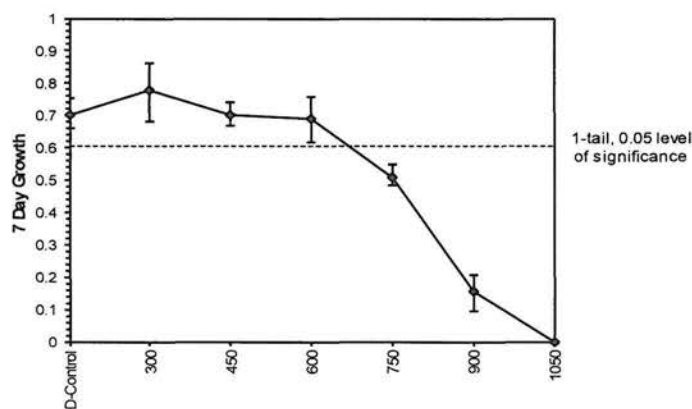
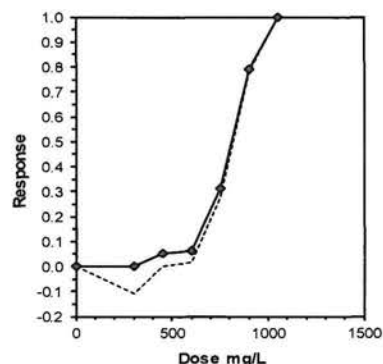
Start Date: 9/10/2024 Test ID: PpKCICR Sample ID: REF-Ref Toxicant
End Date: 9/17/2024 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: PP-Pimephales promelas
Comments:

Conc-mg/L	1	2	3	4
D-Control	0.6590	0.7300	0.7540	0.6590
300	0.6790	0.8040	0.8620	0.7580
450	0.6690	0.6940	0.6940	0.7400
600	0.7560	0.6460	0.6150	0.7440
750	0.4850	0.4940	0.5080	0.5470
900	0.1470	0.1710	0.2060	0.0930
1050	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Transform: Untransformed						N	1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%		t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7005	1.0000	0.7005	0.6590	0.7540	6.982	4				0.7381	1.0000
300	0.7758	1.1074	0.7758	0.6790	0.8620	9.961	4	-1.787	2.290	0.0964	0.7381	1.0000
450	0.6993	0.9982	0.6993	0.6690	0.7400	4.235	4	0.030	2.290	0.0964	0.6993	0.9473
600	0.6903	0.9854	0.6903	0.6150	0.7560	10.187	4	0.243	2.290	0.0964	0.6903	0.9351
750	0.5085	0.7259	0.5085	0.4850	0.5470	5.380	4				0.5085	0.6889
900	0.1543	0.2202	0.1543	0.0930	0.2060	30.781	4				0.1543	0.2090
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 ($p > 0.01$)				0.96531	0.844	-0.1234	-0.9219
Bartlett's Test indicates equal variances ($p = 0.48$)				2.46911	11.3449		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnett's Test				600	>600		
Treatments vs D-Control				0.09643	0.13765	0.00634	0.00355
				0.2031	3, 12		

Linear Interpolation (200 Resamples)				
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	442.40	94.81	335.36	734.02
IC10	621.41	52.67	360.82	669.84
IC15	651.87	24.21	569.22	695.40
IC20	682.32	19.02	609.57	721.65
IC25	712.78	16.45	650.55	749.57
IC40	777.79	6.25	756.63	795.37
IC50	809.04	5.75	789.39	825.47



Species: *Pimephales promelas*

PpKICR Test Number: 119

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.

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		U	EM N	EM N	EM N	EM N	U
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.75	7.20	7.49	7.13	7.32	7.16
	Dissolved oxygen (mg/L)	7.7	7.0	7.0	7.4	7.0	5.9
	Conductivity (µmhos/cm)	290		330		318	
	Alkalinity (mg CaCO ₃ /L)	64				64	
	Hardness (mg CaCO ₃ /L)	87				89	
	Temperature (°C)	24.9	25.2	24.7	24.9	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.00	7.28	7.38	7.26	7.43	7.14
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.4	7.7	5.9
	Conductivity (µmhos/cm)	830		889		874	
	Temperature (°C)	25.0	25.0	24.8	24.9	24.9	25.1
450 mg KCl/L	pH (S.U.)	7.70	7.34	7.43	7.25	7.48	7.21
	Dissolved oxygen (mg/L)	7.0	7.0	7.9	7.4	7.7	6.4
	Conductivity (µmhos/cm)	1070		1150		1140	
	Temperature (°C)	25.1	25.2	24.9	25.1	24.9	25.2
600 mg KCl/L	pH (S.U.)	7.70	7.40	7.48	7.32	7.51	7.23
	Dissolved oxygen (mg/L)	7.0	7.0	7.9	7.5	7.0	6.2
	Conductivity (µmhos/cm)	1340		1430		1410	
	Temperature (°C)	25.1	25.2	24.9	25.1	25.0	25.0
750 mg KCl/L	pH (S.U.)	7.70	7.43	7.52	7.32	7.53	7.20
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.5	7.0	6.3
	Conductivity (µmhos/cm)	1610		1690		1670	
	Temperature (°C)	24.9	24.9	24.8	25.2	25.0	25.0
900 mg KCl/L	pH (S.U.)	7.73	7.45	7.54	7.38	7.56	7.24
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.6	7.9	6.1
	Conductivity (µmhos/cm)	1890		1950		1930	
	Temperature (°C)	24.9	24.9	24.8	25.0	24.9	25.2
1050 mg KCl/L	pH (S.U.)	7.79	7.48	7.57	7.42	7.58	7.25
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.6	7.9	6.3
	Conductivity (µmhos/cm)	2130		2170		2150	
	Temperature (°C)	24.9	24.9	24.8	25.2	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

PpKCICR Test Number: **119**

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
		IL	BSL	BSL	BSL	BSL	BSL	BSL	BSL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.62	7.10	7.43	7.03	7.61	7.37	7.31	7.04
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.3	7.8	7.5	7.6	7.3
	Conductivity (µmhos/cm)	304		316		309		310	
	Alkalinity (mg CaCO ₃ /L)			64					
	Hardness (mg CaCO ₃ /L)			87					
	Temperature (°C)	24.8	25.0	24.8	25.3	24.8	24.9	24.8	25.1
300 mg KCl/L	pH (S.U.)	7.69	7.29	7.49	7.28	7.56	7.48	7.73	7.16
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.4	7.9	7.5	7.8	7.1
	Conductivity (µmhos/cm)	816		846		836		839	
	Temperature (°C)	24.9	24.9	24.9	25.0	24.9	25.1	24.9	24.9
450 mg KCl/L	pH (S.U.)	7.66	7.35	7.54	7.28	7.62	7.52	7.77	7.28
	Dissolved oxygen (mg/L)	7.8	7.6	7.9	7.4	7.9	7.5	7.7	7.4
	Conductivity (µmhos/cm)	1050		1090		1080		1090	
	Temperature (°C)	24.9	24.9	24.8	25.0	24.9	25.1	24.8	24.9
600 mg KCl/L	pH (S.U.)	7.65	7.36	7.55	7.27	7.64	7.59	7.81	7.36
	Dissolved oxygen (mg/L)	7.7	7.6	7.9	7.3	8.0	7.5	7.7	7.2
	Conductivity (µmhos/cm)	1300		1340		1320		1340	
	Temperature (°C)	24.8	25.1	24.8	25.0	25.0	25.3	24.8	24.9
750 mg KCl/L	pH (S.U.)	7.69	7.35	7.59	7.34	7.65	7.58	7.84	7.42
	Dissolved oxygen (mg/L)	7.7	7.6	7.9	7.2	8.0	7.5	7.8	7.2
	Conductivity (µmhos/cm)	1540		1540		1590		1590	
	Temperature (°C)	24.8	25.1	24.8	25.0	25.0	25.2	24.7	25.2
900 mg KCl/L	pH (S.U.)	7.72	7.40	7.60	7.34	7.66	7.61	7.86	7.46
	Dissolved oxygen (mg/L)	7.8	7.5	8.0	7.2	8.0	7.6	7.9	7.1
	Conductivity (µmhos/cm)	1790		1830		1830		1830	
	Temperature (°C)	24.7	25.1	25.0	25.1	25.0	25.2	24.7	25.0
1050 mg KCl/L	pH (S.U.)	7.74	7.46	7.65	7.42	7.70	7.65	7.88	7.44
	Dissolved oxygen (mg/L)	7.8	7.4	8.0	7.2	8.0	7.5	7.9	7.1
	Conductivity (µmhos/cm)	2020		2050		2070		2100	
	Temperature (°C)	24.9	24.8	24.8	25.1	25.0	25.2	24.7	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final