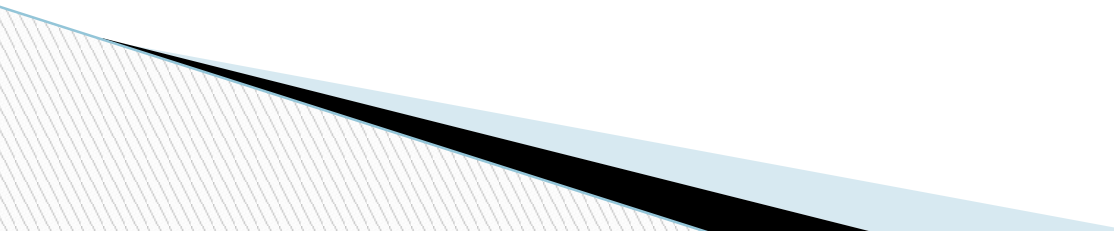


Summary of Acute & Chronic Whole Effluent Toxicity Testing Toxicity Identification Evaluations

September 28, 2021



Topics to be Covered

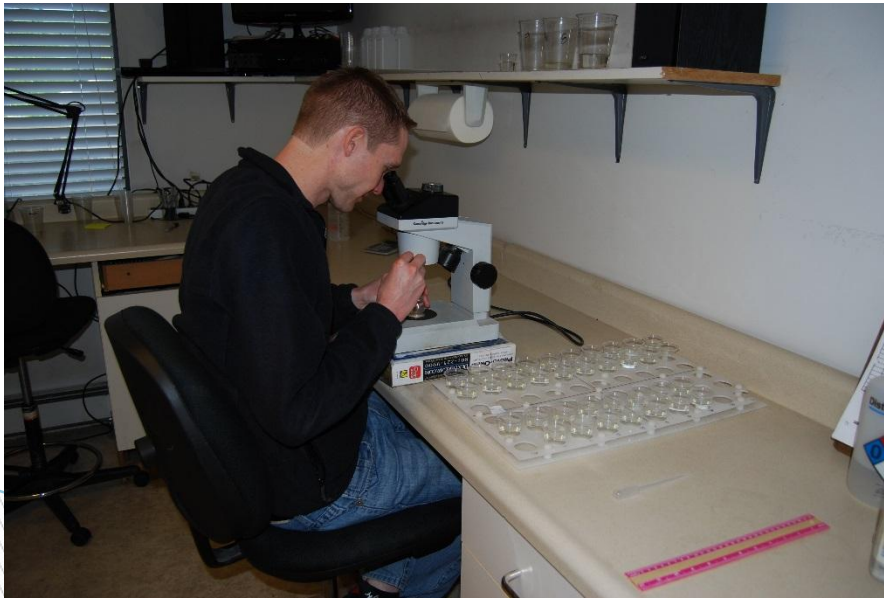
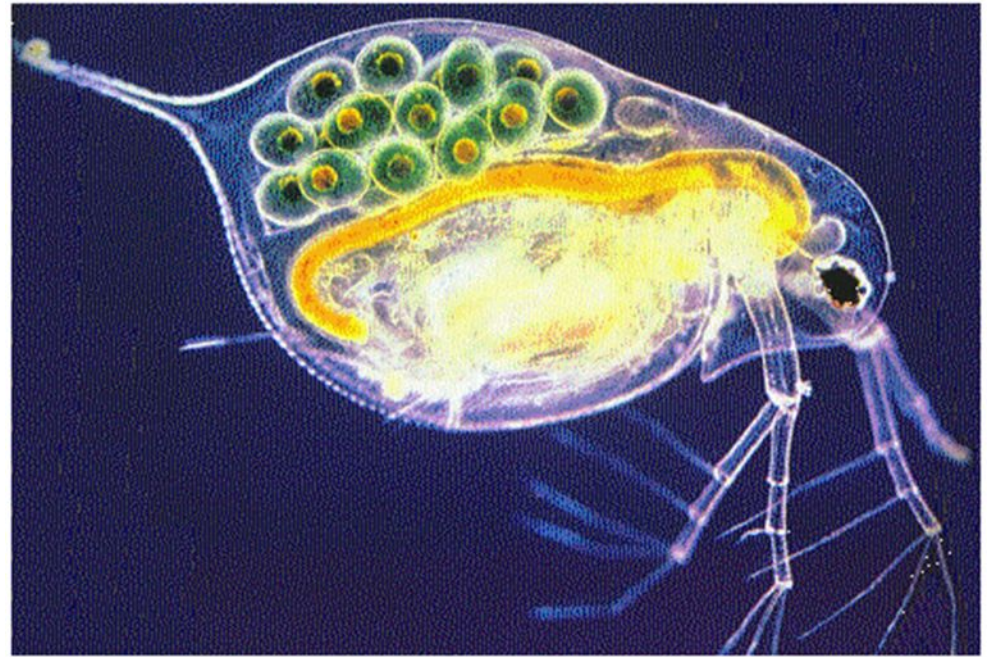
- Introduce the organisms
 - Terminology
 - Summary of Acute Tests
 - Summary of Chronic Tests
 - Basic statistical information in the report
 - What Happens when you fail
 - Toxicity Identification Evaluations
 - Pretreatment's role in toxicity reduction
- 

Whole Effluent Toxicity Testing

- ❑ Clean Water Act 1972 “no discharge of toxic compounds in toxic amounts”
- ❑ Developed by the EPA as a test to determine overall effect of effluent on the receiving water.
- ❑ Uses live organisms to test for presence of toxicity in effluents.
- ❑ Commonly used species are Fathead Minnows, Ceriodaphnia dubia and Daphnia Magna.
- ❑ If your Region requires plant species one commonly used is Selenastrum, a freshwater algae.



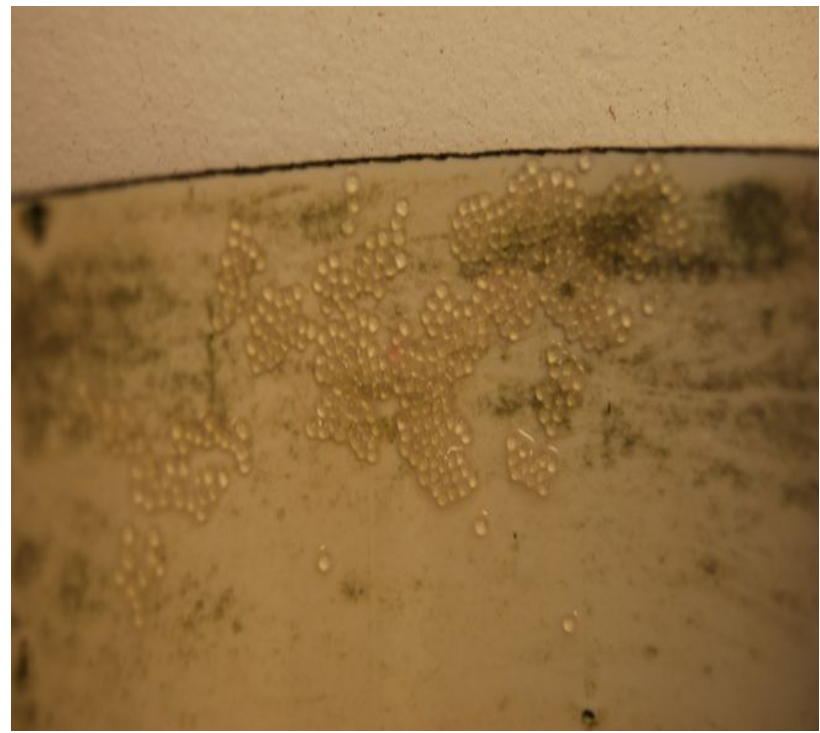
Ceriodaphnia dubia



Fathead Minnow ***Pimephales promelas***



Culturing



Terminology

▣ Acute

- LC50- Lethal Concentration 50%
 - Point estimate: the concentration at which 50% of the organisms will die

▣ Chronic

- IC25- Inhibitory Concentration 25% inhibition
 - Point estimate: the concentration at which growth or reproduction is 25% different from the control
- NOEC- No observable effect concentration
- LOEC- Lowest observable effect concentration
- MSDp- Mean Standard Deviation proportion
 - EPA set range for acceptable MSDp values for each species
 - MSDp range for Ceriodaphnia 13-47%
 - MSDp range for Fathead Minnows 12-30%

Acute Testing

Ceriodaphnia & Magna

- One sample collected
- <24 hours old
- 4 replicates
- 5 organisms
- Fed prior to testing
- 48 hours
- LC50- mortality
 - (if 10 out of 20 die in any dilution Fail)

Fathead Minnows

- One sample collected
- 1 – 14 days old
- 2 replicates
- 10 organisms
- Fed at 48 hours
- 96 hours
- LC50- mortality
 - (if 10 out of 20 die in any dilution Fail)

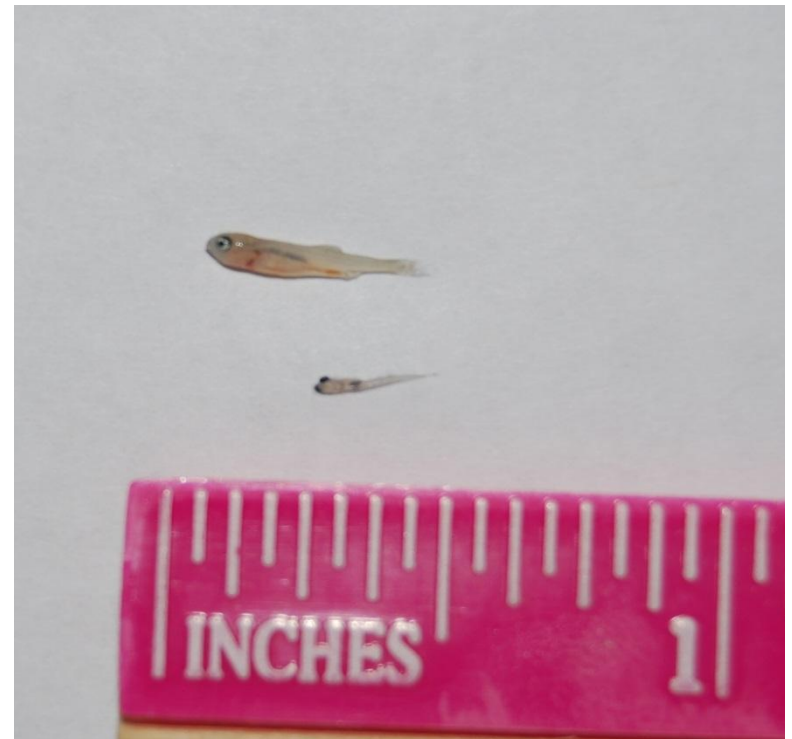
Acute & Chronic Endpoints

- Acute testing is looking for lethal effects
 - Did the organisms live or die
- Chronic testing looks at sub-lethal effects
 - The toxicity isn't enough to kill the organisms but does inhibit growth or reproduction.

Energy that would be spent on growth or reproduction is spent dealing with the environmental conditions.

Chronic Fathead Test

- Analysis requires three samples over 6 days
- Starts with 24 hour old organisms
- Test duration 7 days
- Fed twice a day newly hatched brine shrimp
- Looks at growth by weight
- “Statistically Different” from the control or IC25



Chronic Ceriodaphnia Test

- Start <24 hours old
- Grouped <8 hours in age
- Fed daily YTC and algae
- Reproduce by budding
- Test duration 6 – 8 days
- Ends when 60% or more of the control organisms have 3 broods
- Produce 15 to 50 young each
- Looks at reproduction
- “Statistically different” from control or IC25



Point Estimates

- 5 dilution test + Control
 - EPA's "Definitive Test"
- Used to estimate between dilutions
- Can set the desired Inhibitory Concentration
- Most commonly used IC25
 - (25% different from the control)
- Starting to see TUC
 - (Toxicity Unit for chronic test $100/IC_{25}$)

Hypothesis Test

- Can be used for 1 dilution tests or multiple dilution tests.
- Asks is each dilution significantly different from the control
- Based on dilutions used in the test
- NOEC, LOEC
- MSDp sets the limit of “significance” used to calculate difference from the control for that test
- EPA guidance documents set range for acceptable MSDp values for each species.
 - MSDp range for Ceriodaphnia 13-47%
 - MSDp range for Fathead Minnows 12-30%

Permit Showing IC25 Requirements

PART I
DISCHARGE PERMIT NO. UT0025721
WASTEWATER

Effluent Limitations					
Parameter	Yearly Maximum	Monthly Average	Maximum Weekly Average	Daily Minimum	Daily Maximum
BOD ₅ , mg/L	NA	25	35	NA	NA
BOD ₅ , Minimum % Removal	NA	85	NA	NA	NA
TSS, mg/L	NA	25	35	NA	NA
TSS, Minimum % Removal	NA	85	NA	NA	NA
E. Coli, no./100mL	NA	126	157	NA	NA
Total Dissolved Solids, mg/L	NA	NA	NA	NA	1200
Ammonia, mg/L	NA	NA	NA	NA	4.6
Oil & Grease, mg/L	NA	NA	NA	NA	10
pH, Standard Units	NA	NA	NA	6.5	9
WET, Acute Biomonitoring	NA	NA	NA	NA	Pass, LC50>100% effluent
WET, Chronic Biomonitoring	NA	NA	NA	NA	Pass, IC25>100% effluent

NA – Not Applicable

PART I
DISCHARGE PERMIT NO. UT0025721
WASTEWATER

initial sample was taken. The need for any additional samples, and/or a Toxicity Reduction Evaluation (TRE), see *Part I.C.4.f*, shall be determined by the Executive Secretary. If the second test shows no chronic toxicity, routine monitoring shall be resumed.

The chronic toxicity tests shall be conducted in general accordance with the procedures set out in the latest revision of *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, 4th Edition, (EPA 821/R-02-13), October 2002* as per 40 CFR 136.3(a) *TABLE 1A-LIST OF APPROVED BIOLOGICAL METHODS*, and the *Region VIII EPA NPDES Chronic Test Conditions - Static Renewal Whole Effluent Toxicity Test (August, 1997)*. Test species shall consist of Ceriodaphnia dubia and Pimephales promelas (fathead minnow).

Chronic toxicity occurs when the survival, growth, or reproduction for either test species, when exposed to a dilution of 100 percent effluent or lower, is significantly less (at 95% confidence level) than that of the control specimens. Dilutions of 100 percent only will be required, plus the control. If any of the acceptable control performance criteria are not met, the test shall be considered invalid. IC₂₅ is the inhibition concentration of toxicant (given in % effluent) that would cause a 25% reduction in mean young per female, or a 25% reduction in overall growth for the test population.

Quarterly test results shall be reported along with the Discharge Monitoring Report (DMR) submitted for the end of the reporting calendar quarter (e.g., biomonitoring results for the calendar quarter ending March 31 shall be reported with the DMR due April 28, with the remaining biomonitoring reports submitted with DMRs due each July 28, October 28, and January 28). All test results shall be reported along with the DMR submitted for that reporting period. The format for the report shall be consistent with the latest revision of the *Region VIII Guidance for Chronic Whole Effluent Reporting (August, 1997)* and shall include all the physical testing as specified.

Chronic Fathead Data

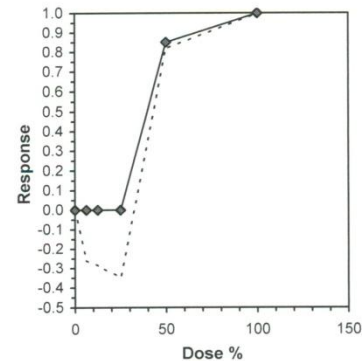
Larval Fish Growth and Survival Test-7 Day Growth					
Start Date:	12/7/2013 16:00	Test ID:	RT12-13cf	Sample ID:	Ref Tox 12-13 chronic fathead
End Date:	12/14/2013 18:25	Lab ID:	WET Inc	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2	3	4
D-Control	0.2790	0.2180	0.2980	0.3550
6.25	0.2730	0.3230	0.4660	0.3860
12.5	0.4280	0.3310	0.3480	0.3730
25	0.3050	0.3660	0.5280	0.3500
50	0.0280	0.0550	0.0510	0.0750
100	0.0000	0.0000	0.0000	0.0000

Conc-%	Transform: Untransformed						1-Tailed			Isotonic		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.2875	1.0000	0.2875	0.2180	0.3550	19.644	4				0.3517	1.0000
6.25	0.3620	1.2591	0.3620	0.2730	0.4660	23.021	4	-1.597	2.360	0.1101	0.3517	1.0000
12.5	0.3700	1.2870	0.3700	0.3310	0.4280	11.443	4	-1.768	2.360	0.1101	0.3517	1.0000
25	0.3873	1.3470	0.3873	0.3050	0.5280	25.132	4	-2.138	2.360	0.1101	0.3517	1.0000
*50	0.0523	0.1817	0.0523	0.0280	0.0750	36.893	4	5.042	2.360	0.1101	0.0523	0.1486
100	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 ($p > 0.05$)	0.94911	0.905	0.7661	0.62624						
Bartlett's Test indicates equal variances ($p = 0.17$)	6.39425	13.2767								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	25	50	35.3553	4	0.11011	0.38301	0.07756	0.00435	1.4E-05	4, 15

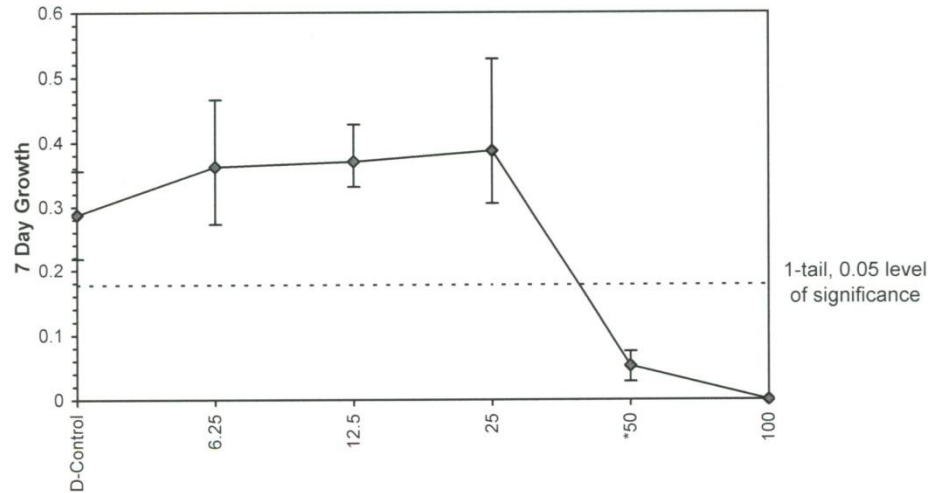
Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)	Skew	
IC05	26.468	1.786	15.949	26.594	-6.0789
IC10	27.936	1.240	24.946	28.189	-7.7455
IC15	29.404	0.833	26.659	29.783	-6.3462
IC20	30.872	0.671	28.343	31.378	-4.8337
IC25	32.341	0.639	29.991	32.972	-4.4945
IC40	36.745	0.576	34.683	37.756	-2.8411
IC50	39.681	0.566	37.776	40.945	-1.5731



Chronic Fathead Dose Response Plot

Larval Fish Growth and Survival Test-7 Day Growth					
Start Date:	12/7/2013 16:00	Test ID:	RT12-13cf	Sample ID:	Ref Tox 12-13 chronic fathead
End Date:	12/14/2013 18:25	Lab ID:	WET Inc	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:					

Dose-Response Plot



What Happens When I fail?

- Accelerated Testing
 - Acute testing. Up to 5 tests, one a week for up to 5 weeks. Okay if pass 2 in a row or 3 out of 5. Not okay if you fail 2 in a row or 3 out of 5.
 - Chronic Testing. Up to 5 tests, one every two weeks for up to ten weeks. Pass 2 in a row or 3 out of 5 then okay. Not okay if you fail 2 in a row or 3 out of 5.
- When you fail your permit will tell you how to proceed.
- Plant Review
- Written report to the regulatory authority.
 - Identify toxicant if known
 - Present a plan on how you will proceed



Plant Review



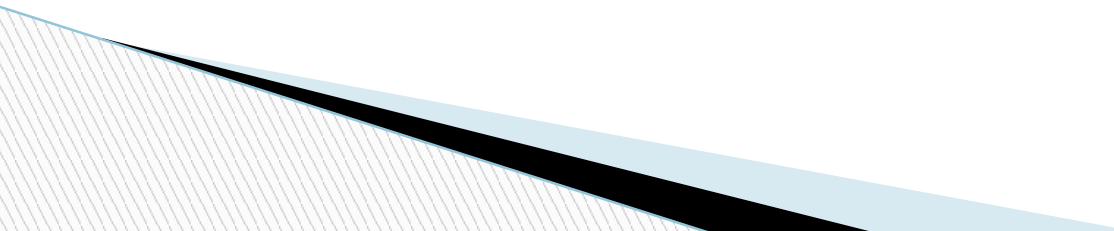
- Up to 70% of failures are due to problems at the treatment plant.
- Things to review:
 - How close is the plant to capacity
 - Changes in chemicals or chemical suppliers
 - Malfunctioning equipment
 - Housekeeping
 - Seasonal changes
 - Process problems
 - New construction
 - New personnel
 - Review of laboratory data for indications of changes from the norm
 - What does the WET laboratory think is the cause of toxicity

Toxicity Identification Evaluation

- EPA developed a procedure to help identify the specific toxicant.
- TIE uses the most sensitive species
- Includes 3 phases.
 - Phase 1- Toxicity characterization.
 - Phase 2- Toxicity identification.
 - Phase 3- Toxicity reduction.
 - Toxicity Characterization uses WET testing exclusively but to a lesser extent in other two phases.



Chronic Toxicity Characterization Tests

- Baseline Test
 - Aeration Test
 - Filtration Test
 - C-18 Column
 - C-18 Column Eluate
 - Graduated Test (pH6, pH7 and pH8)
 - EDTA Tests (4 & 8 mg/L)
 - Thiosulfate Tests (10 & 25 mg/L)
 - Add Biological treatment (UV, Cl₂, or Filtration)
- 

Toxicity Identification

- Focus on the family of chemicals from the Phase I Toxicity Characterization.
 - Metals screen (200.8 ICP/MS or lower).
 - Compare to literature values.
 - Anionic and/or Cationic resin removal of metals.
 - Spike specific metal(s) suspected into laboratory water and treated effluent (similar hardness to original sample).
 - Confirm accurate spikes by chemical analysis.
 - Is toxicity reproducible?
 - If so then good confidence that you have identified the culprit.
 - If not keep looking.

Cost

- Phase 1 Acute Test \$5,000 – \$10,000.
 - Difference in cost is how laboratory performs the test. EPA has left specifics about the procedure wide open. Such things as volumes, durations, replicates, are up to the preference of the lab.
- Phase 2 depends on the methods needed to identify and confirm the toxicant.
 - Metals are fairly inexpensive.
 - Organics can be very, very, very expensive.
- Phase 3 again depends on the cost of chemical laboratory analysis and how quickly the source is identified.
- Recommend getting a cost breakdown for WET Testing (price per test). What is included in the cost of Phase I. Is any preliminary testing requested.



Examples of Biological Toxicant

- Chronic Ceriodaphnia
 - Pseudomonas
 - Low nutrients and warmer temps
 - Post dechlorination contamination
- Acute & Chronic Fathead Minnow
 - Fungus & Mold
 - Incomplete disinfection
 - Post dechlorination contamination

Test ID	LC50	TU	Test ID	LC50	TU
Baseline	20	5	Ph6	>100	1
			Ph7	45	2.2
Aerated	20	5	Ph8	20	5
Aerated pH3	25	4			
Aerated pH11	10	10	EDTA 3 mg/L	25	4
			EDTA 6 mg/L	50	2
Filtered	20	5	EDTA 12 mg/L	>100	1
Filtered pH3	25	4			
Filtered pH11	10	10	Thiosulfate 5 mg/L	15	6.7
			Thiosulfate 10 mg/L	20	5
C-18	30	3.3	Thiosulfate 25 mg/L	25	4
C-18 pH3	25	4			
C-18 pH11	10	10			
C-18 eluate	>4X	No toxicity recovered			
C-18 eluate pH3	>4 X	No toxicity recovered			
C-18 eluate pH11	>4X	No toxicity recovered			

	IC25 %	TU	
Baseline Ceriodaphnia	25	4	
Aerated <input type="button" value="▼"/>	28 <input type="button" value="▼"/>	3.6 <input type="button" value="▼"/>	
Filtered	40	2.5	
C-18 Column	30	3.3	
C-18 Eluate	>4 X	No toxicity recovered	
PH 6.0	>100	1	
PH 7.0	75	1.3	
PH 8.0	25	4	
EDTA 3 mg/L	>100	1	
EDTA 8 mg/L	>100	1	
Thiosulfate 10 mg/L	25	4	
Thiosulfate 25 mg/L	30	1.3	
UV	25	4	
Cl2 1 mg/L	25	4	

Test ID	IC25 %	TU	
Baseline Ceriodaphnia	25	4	
Aerated <input type="button" value="▼"/>	28 <input type="button" value="▼"/>	3.6 <input type="button" value="▼"/>	
Filtered	40	2.5	
C-18 Column	30	3.3	
C-18 Eluate	>4 X	No toxicity recovered	
PH 6.0	40	2.5	
PH 7.0	75	1.3	
PH 8.0	25	4	
EDTA 3 mg/L	35	2.9	
EDTA 8 mg/L	40	2.5	
Thiosulfate 10 mg/L	25	4	
Thiosulfate 25 mg/L	30	3.3	
UV	>100	1	
Cl2 1 mg/L	>100	1	

Phase 3 Toxicity Reduction- Pretreatment Gets Involved

- Toxicant identified coming from outside the plant
 - Review Sig Cats
 - Who uses this or a similar chemical
 - How much volume do they discharge
 - Have you seen this chemical in previous testing at lower concentrations
 - Inspection
 - Sample trunk lines follow it up the pipe
 - Possible enforcement action
 - New permit

Questions

