Toxicity Investigations

Case Studies with Metal Mining Effluent

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Background – What Is A TRE?

- A Toxicity Reduction Evaluation (or TRE) is a site specific, step-wise diagnostic approach to resolving toxicity issues.
- TREs are used mostly for compliance purposes with rainbow trout and *Daphnia magna*
- Industries are trying to achieve a non-acutely lethal effluent

≤50% mortality in 100% effluent **= PASS**

>50% mortality in 100% effluent **= FAILURE**

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Background – What Is A TRE?

- Diagnostic approach, including:
  1. Information and Data Acquisition
  2. Evaluation of Remedial Actions to Optimize the Operation / Process
  3. Toxicity Identification Evaluation (TIE)
     - Phase 1 – Characterization
     - Phase 2 – Identification
     - Phase 3 – Confirmation
  4. Source Investigation (SI)
  5. Toxicity Treatability Evaluation (TTE)
  6. Confirmation of Removal of Toxicity

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Case Studies
A Tale of Four Effluents

- Toxicity investigations conducted at different times at **four different mining operations**
- Effluent characteristics and patterns of mortality were similar

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Characteristics	Site #1	Site #2	Site #3	Site #4
Operation	Cu & Zn	Ni & Cu	Ni & Cu	Zn & Pb
Species	Rbt and Dm	Rbt	Rbt and Dm	Rbt and Dm
Time to Mortality	72- to 96-h; often post 96-h	72- to 96-h in winter; earlier in summer	Variable, often within 24-h	72- to 96-h
Persistent?	No	Sometimes	No	Unknown
Consistent?	No	No	No	No
Magnitude	Mortality in 100%, but non-dose responses sometimes observed			
Timing	May; Aug to Oct	Increased mortality in summer	May to Oct	Mar to April
Total thiosalts	8 to 140 mg/L	300 to 600 mg/L	128 to 1700 mg/L	<10 to 1000 mg/L
Dominant Species	Thiosulfate	Thiosulfate	?; likely thiosulfate	Thiosulfate
Xanthates	<5 to 13 mg/L	0.1 to 2 mg/L	?, but xanthates are used	<5 to 24 mg/L

Case Studies
A Tale of Four Effluents

- For all 4 investigations, results from toxicity investigations suggested:
  - Multiple contaminants were responsible for toxicity
  - Metals, TDS, nitrite, nitrate, ammonia were NOT responsible
- In all 4 effluent samples – elevated thiosalts, but . . .
  - no correlation between total thiosalt concentration and toxicity
- So what was causing mortality?
  - **Direct toxicity** due to thiosalts (or individual thiosalt species)?
  - **Indirect toxicity** due to acidic generation?
  - Other **(secondary) toxicant(s)**?

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Direct Toxic Effects

- Thiosulfate LC50s
  - rainbow trout (96-hour) ~ 7,378 mg/L (AquaTox)
  - *Daphnia magna* (48-hour) ~1,012 mg (AquaTox)
  - *Daphnia magna* (48-hour) ~ 910 mg/L (US EPA, 1991)
- Trithionate LC50s
  - rainbow trout (96-hour) ~ ?
  - *Daphnia magna* (48-hour) ~1,400 mg/L (Schwartz et al., 2006)
- Tetrathionate LC50s
  - rainbow trout (96-hour) >800 mg/L (Schwartz et al., 2006)
  - *Daphnia magna* (48-hour) ~ 750 mg/L (Schwartz et al., 2006)

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Indirect Toxicity

- Thiosalts can undergo oxidation to produce sulfuric acid and a corresponding pH decline.
- Rainbow trout & *Daphnia magna*
  - prolonged exposure to **pH < 5** will probably cause extreme stress and very likely mortality
- We have often observed declines to as low as pH 3 in samples containing thiosalts.
- Tested other samples with similar thiosalt concentrations, but pH decline was not low enough to cause mortality.
- So what is causing toxicity?

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Indirect Toxicity

- Many factors can effect thiosalt oxidation (and acid generation):
  - Aeration (oxygen)
  - Light
  - Temperature (seasonality)
  - Bacteria (presence/absence)
- Why do we care?
  - these factors contribute to effluent variability and the expression of toxicity in an acute lethality test
  - effluents are not at equilibrium when thiosalts are present; toxicity can be a "moving target"
  - other possible contaminants could also be influenced if their toxicity is pH dependent

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Secondary Toxic Effects

- Detection of the secondary toxicants can be one of the most difficult aspects of any toxicity investigation
  - particularly complicated in the presence of thiosalts
  - pH decline caused by thiosalts may mask the presence of other toxicants
  - perhaps even causing other substances to become toxic
- Many TIE treatments that remove thiosalts also remove other toxicants.
- How do we identify secondary toxicants when thiosalts are present?
- Case Study

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Case Study

Summary of treatment effects on thiosalt degradation and rainbow trout mortality

TIE Treatment	0-h				% Trout Mortality @ 96-h
	Thiosulfate (mg/L)	Trithionate (mg/L)	Tetrathionate (mg/L)	Total Thiosalts (mg/L)	
Baseline	350	22	6.3	400	100
Anion Exchange	0.4	<2	<0.2	<10	0
XAD	350	21	4.8	310	0
SPE with C18 @ pH_i	350	24	3.8	323	0
Heat (boiling)	420	5.2	4.9	352	0
pH 3	350	25	9.6	296	0
Chlorination (5 mL/L)	0.4	<2	160	166	0
EDTA	350	22	14	318	0
H_2O_2 (0.5 mL/L) + 10 mg/L ferric sulfate	23	61	24	53	0

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Phase II TIE

- Treated the acutely lethal effluent sample using a C18 column (resin that removes relatively non-polar organics)
  - The treated effluent was non lethal
- Toxicant(s) is likely on the resin
  - Concentrate toxicant on C18 resin
- Extract toxicant from C18 resin using methanol (rinse the C18 column with 100% methanol)
  - Tested the 100% extract and found to be acutely lethal
  - Subjected this toxic fraction to GC/MS

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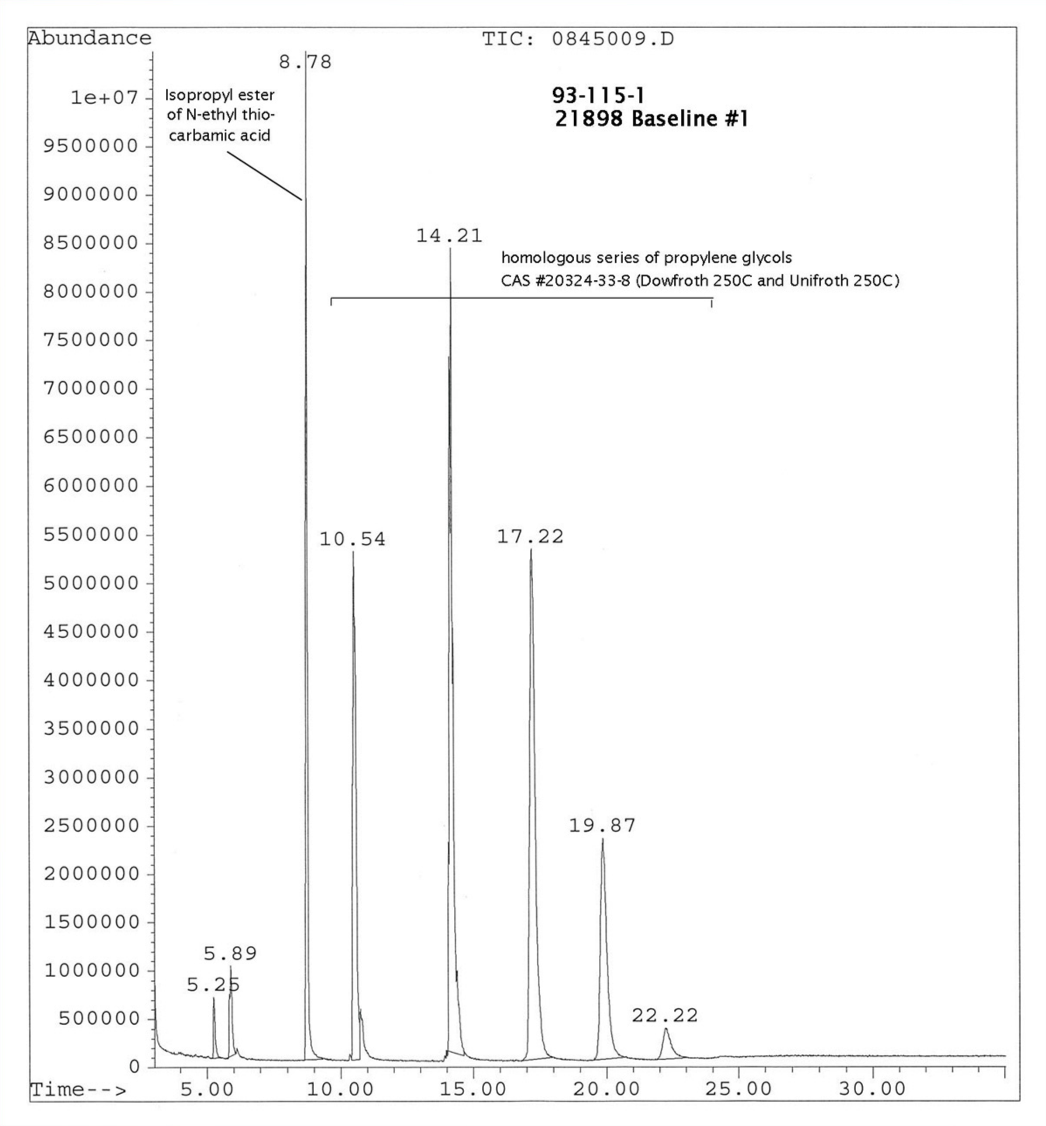
Phase II TIE – What Did We Find?



- Baseline sample:

1. 100% trout mortality
2. Total thiosalts = 400 mg/L
3. Isopropyl ester of N-ethyl thiocarbamic acid
4. A homologous series of propylene glycols

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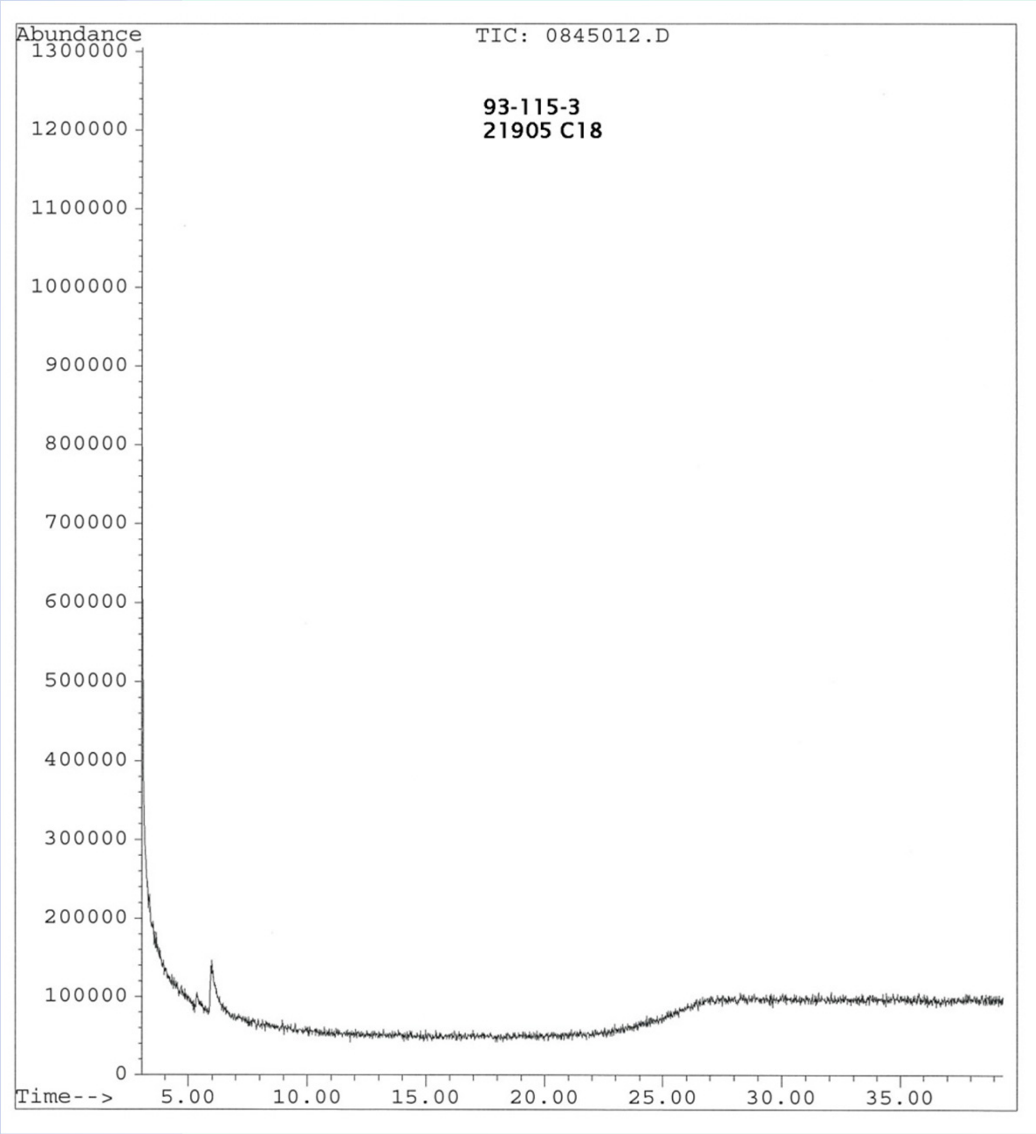
Phase II TIE – What Did We Find?



- Treatment with C18:

1. Eliminated toxicity
2. No effect on thiosalts
3. Removed the organics detected in Baseline

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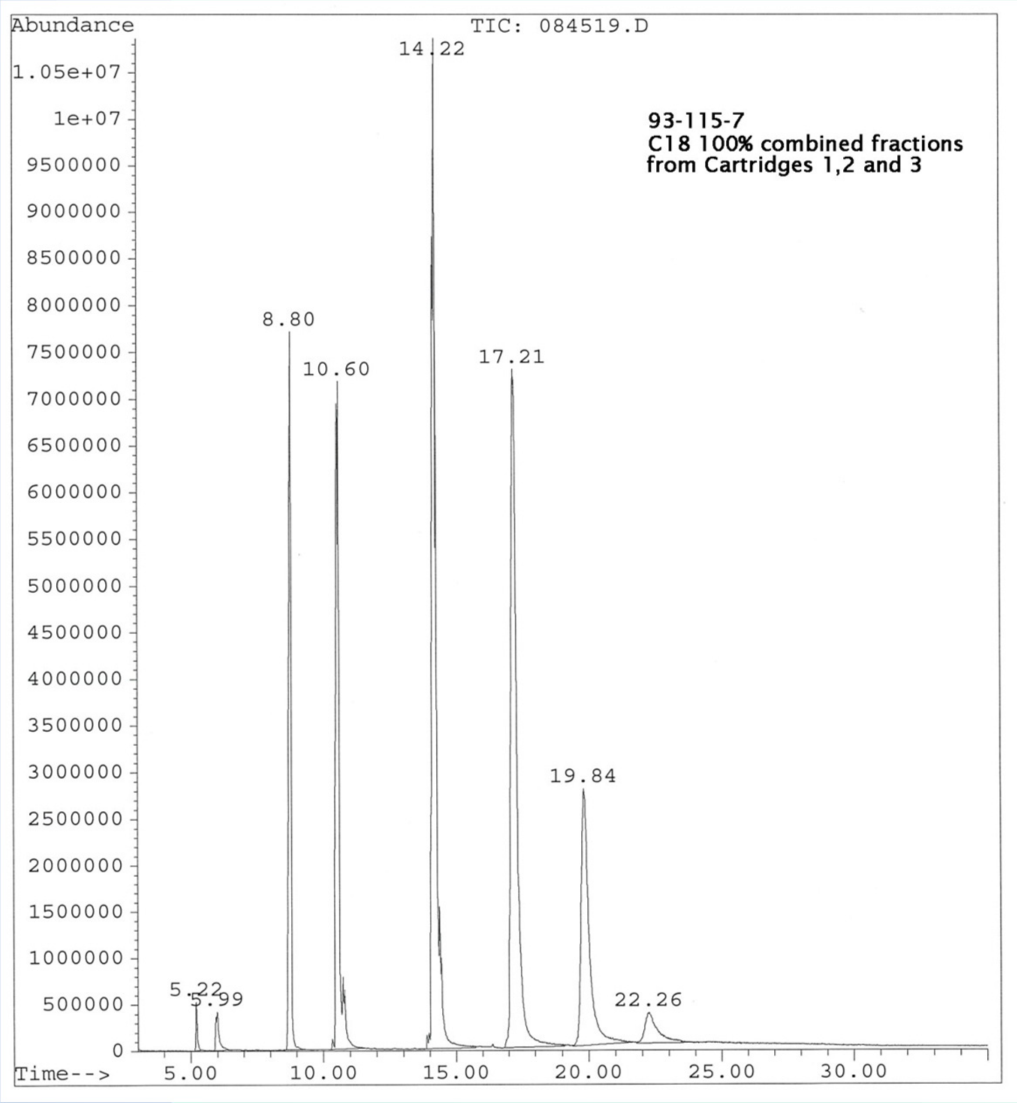
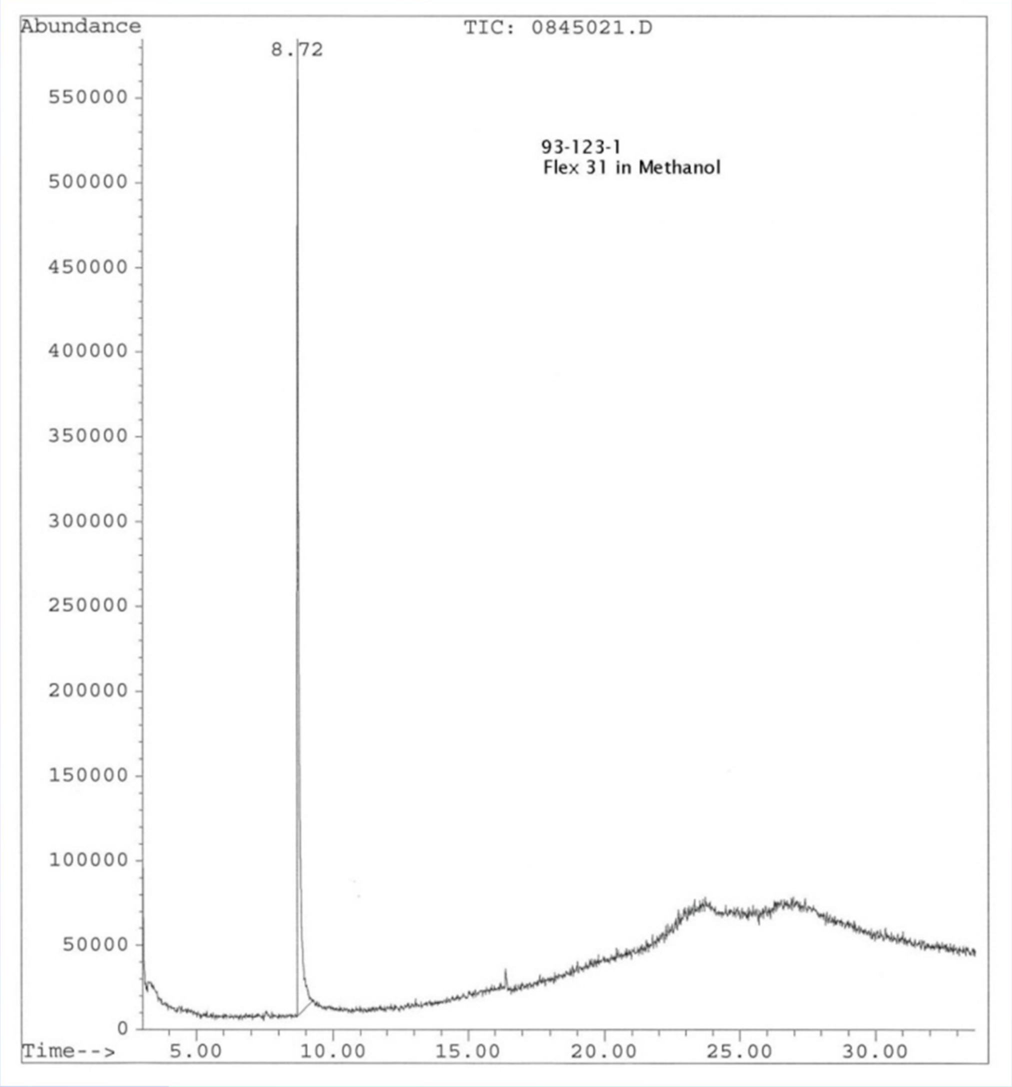
Phase II TIE – What Did We Find?



- 100% methanol extract:

1. Recovered toxicity
2. No effect on thiosalts
3. Recovered the organics detected in the baseline effluent

Phase II TIE – What Did We Find?



- Where are the chemicals coming from?
- Submitted various process chemicals for GC/MS analysis
- N-ethyl thiocarbamate found in the effluent samples was also present in a **xanthate** product

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Xanthates

- Used as collectors during the processing of sulphide ore by flotation.
- Hydrolysis is the principal factor in determining their fate in the environment, but this process is pH- and temperature-dependent.
- Under acidic conditions, it is unstable and rapidly hydrolyzes to: ethanol, carbon disulphide & caustic soda (Rao, 1971).

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Xanthates

- Examined available xanthate toxicity data
- More acutely lethal in effluent than lab dilution water
- Effluent samples also contained thiosalts

Product	Test Water	Rainbow trout 96-h LC50 (mg/L)
sodium isopropyl xanthate + additive	Effluent	1.1 - 3
	Dilution Water	> 300
potassium amyl xanthate	Effluent	<6.25
	Dilution Water	100 - 1000
potassium isobutyl	Effluent	?
	Dilution Water	?

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What Happens to Xanthates at Low pH?

- Spiked varying concentration of "*Flex*" (sodium isopropyl xanthate + additive) into lab dilution water
- Tested for toxicity to rainbow trout at three different pHs in clean lab water
  - pH 8, pH 6 and pH 5
- Measured (in selected exposure solutions) concentrations of:
  - Xanthate
  - Carbon disulfide
  - Sulphide
  - H_2S

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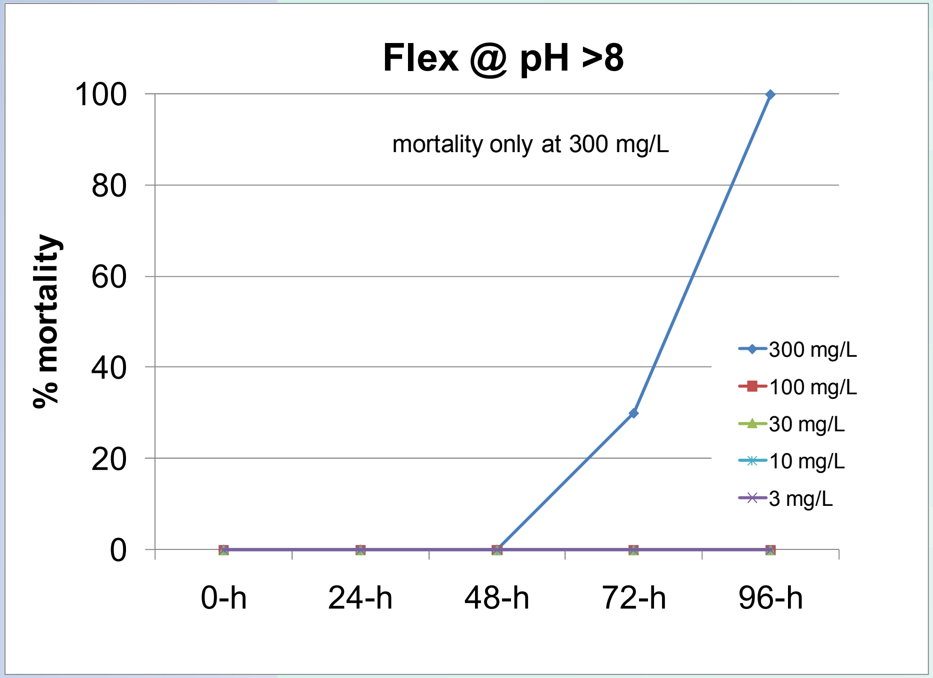
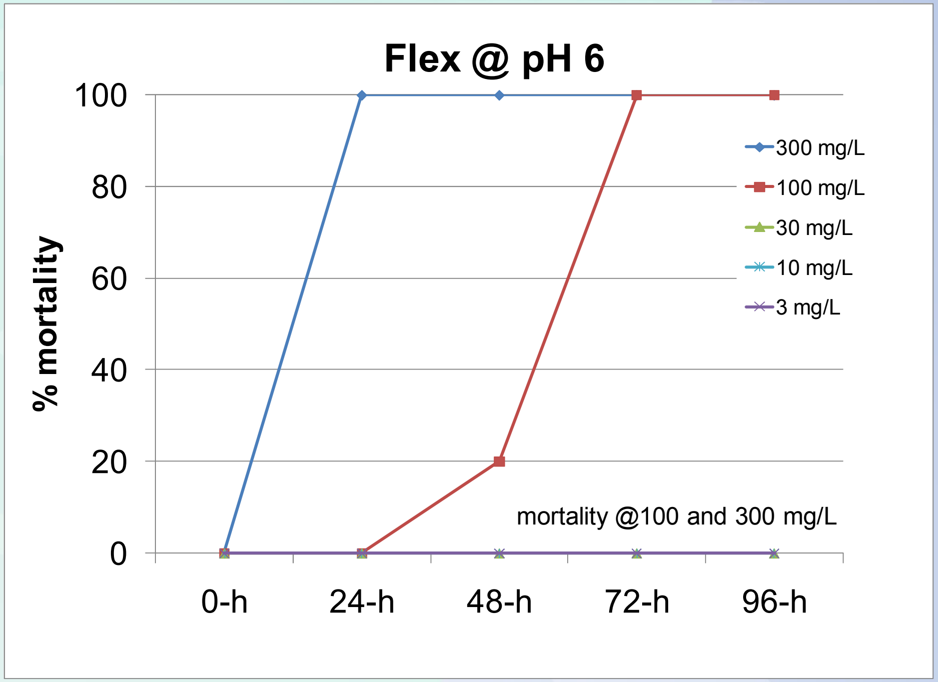
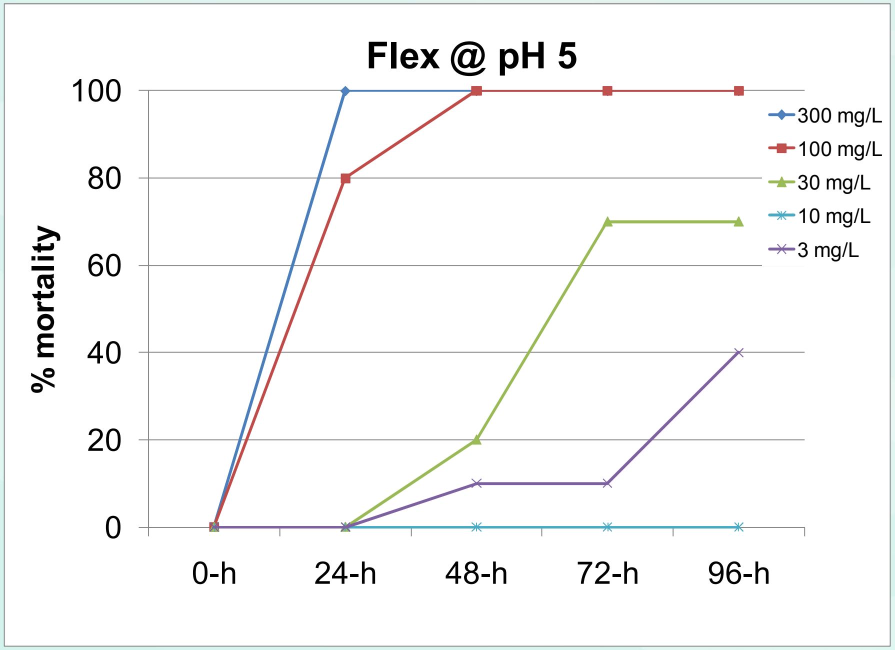

Flex @ pH >8
mortality only at 300 mg/L
% mortality
100
80
60
40
20
0
0-h
24-h
48-h
72-h
96-h
300 mg/L
100 mg/L
30 mg/L
10 mg/L
3 mg/L


Flex @ pH 6
% mortality
100
80
60
40
20
0
0-h
24-h
48-h
72-h
96-h
300 mg/L
100 mg/L
30 mg/L
10 mg/L
3 mg/L
mortality @100 and 300 mg/L


Flex @ pH 5
% mortality
100
80
60
40
20
0
0-h
24-h
48-h
72-h
96-h
300 mg/L
100 mg/L
30 mg/L
10 mg/L
3 mg/L

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What Happens to Xanthates at Low pH?

- Testing indicated that toxicity to Flex increased as pH decreased
- What happens to:
  - Xanthate?
  - Carbon disulfide?
  - Sulphide?
  - H_2S?

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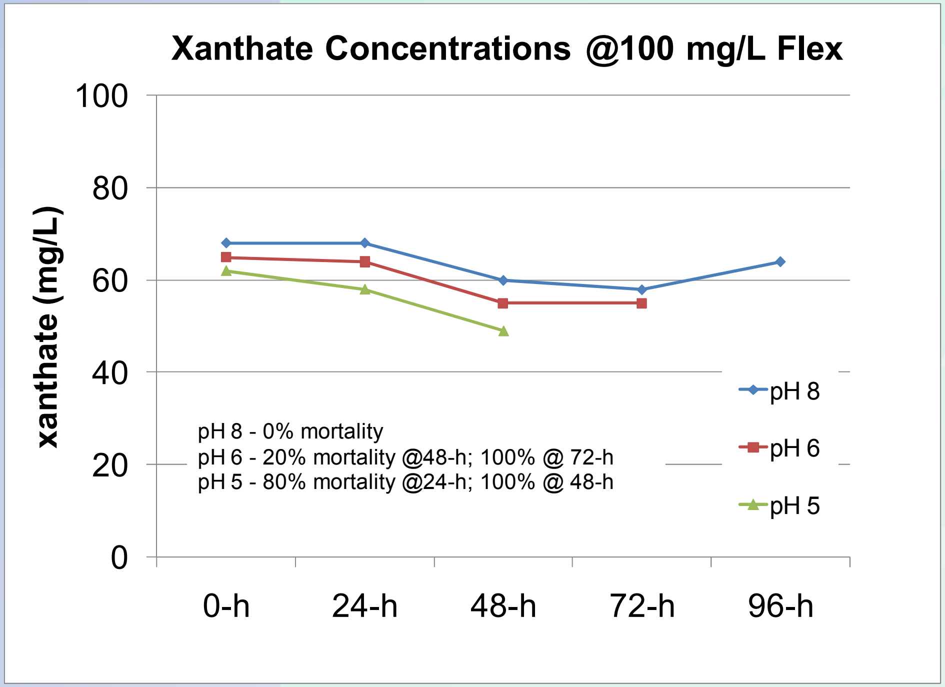
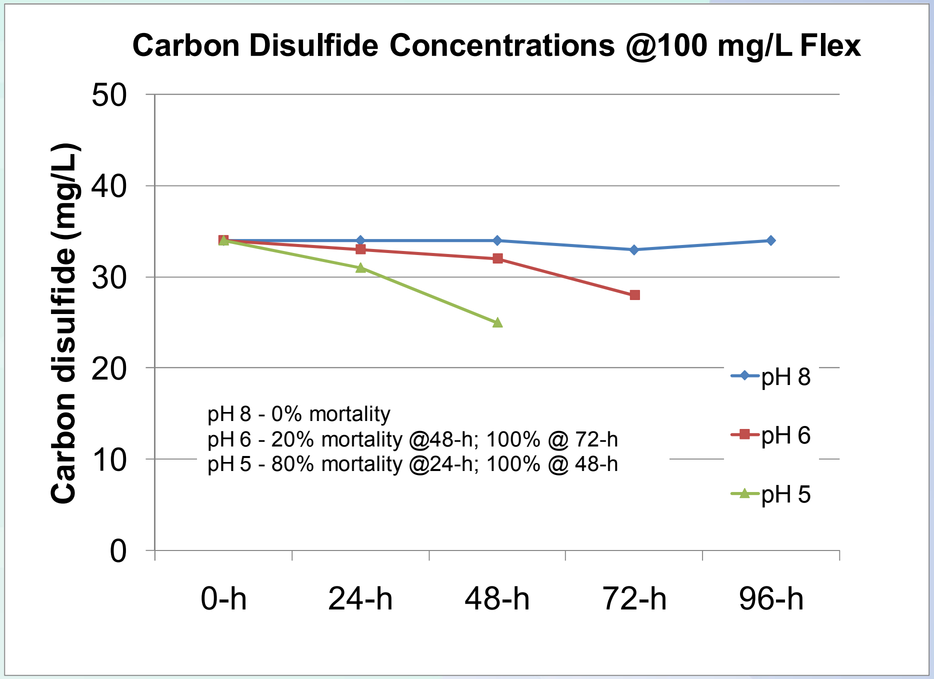
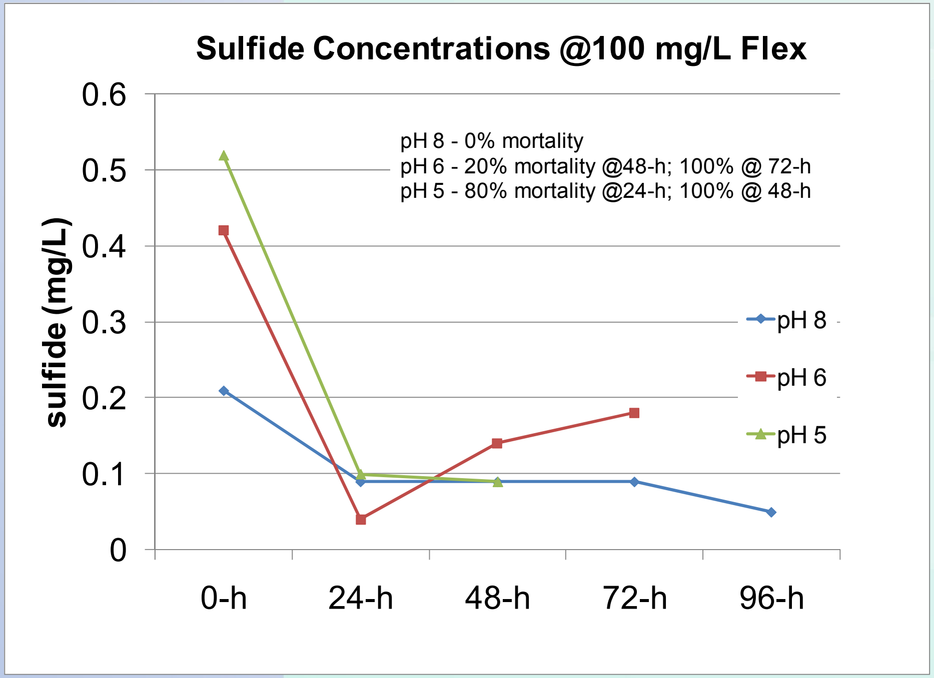


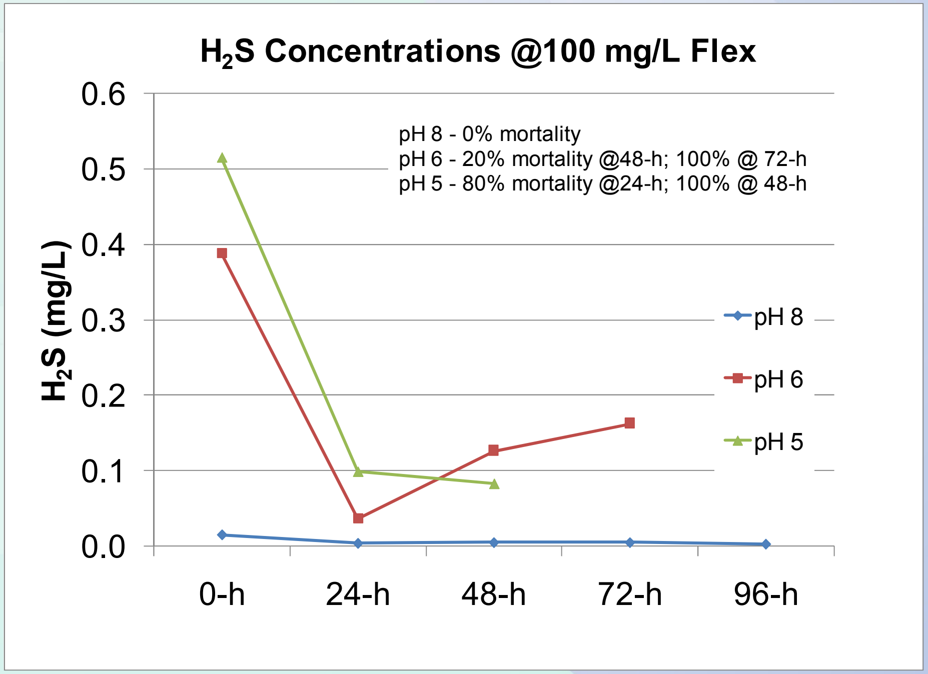
Xanthate Concentrations @100 mg/L Flex
xanthate (mg/L)
100
80
60
40
20
0
0-h
24-h
48-h
72-h
96-h
pH 8 - 0% mortality
pH 6 - 20% mortality @48-h; 100% @ 72-h
pH 5 - 80% mortality @24-h; 100% @ 48-h
pH 8
pH 6
pH 5



Carbon Disulfide Concentrations @100 mg/L Flex
Carbon disulfide (mg/L)
50
40
30
20
10
0
0-h
24-h
48-h
72-h
96-h
pH 8 - 0% mortality
pH 6 - 20% mortality @48-h; 100% @ 72-h
pH 5 - 80% mortality @24-h; 100% @ 48-h
pH 8
pH 6
pH 5



Sulfide Concentrations @100 mg/L Flex
sulfide (mg/L)
0.6
0.5
0.4
0.3
0.2
0.1
0
0-h
24-h
48-h
72-h
96-h
pH 8 - 0% mortality
pH 6 - 20% mortality @48-h; 100% @ 72-h
pH 5 - 80% mortality @24-h; 100% @ 48-h
pH 8
pH 6
pH 5



H_2S Concentrations @100 mg/L Flex
H_2S (mg/L)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0-h
24-h
48-h
72-h
96-h
pH 8 - 0% mortality
pH 6 - 20% mortality @48-h; 100% @ 72-h
pH 5 - 80% mortality @24-h; 100% @ 48-h
pH 8
pH 6
pH 5

What Happens to Xanthates at Low pH?

- Concentrations of total xanthate and carbon disulfide were generally lower at pH 5 than at pH 8
  - Yet mortality was greater at pH 5 than at pH 8
- Sulfide (S^{2-}) and H_2S concentrations increased with decreasing pH
- From the literature:
  - At pH 5 ~ 99% of S^{2-} exists as H_2S
  - At pH 6 ~ 93% of S^{2-} exists as H_2S
  - At pH 7 ~ 57% of S^{2-} exists as H_2S

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. . . And What About Thiosalts?

- Oxidation of thiosalts in the environment would be very slow if there was no bacterial oxidation (Silver and Dinardo,1981)
  - Thiobacillus (i.e., *T. thiooxidans* and *T. ferrooxidans*) oxidize thiosulfate to sulfate
- So what happens to thiosalts and effluent toxicity if we inhibit the bacteria?

Parameter	**Effluent ("As Is")**			**Effluent + Tetracycline**		
	Day 0	**Day 5**	**Day 8**	**Day 0**	**Day 5**	**Day 8**
Mortality (%)	0	20	100	0	0	0
pH	7.2	5.2	3.6	6.1	6.6	6.8
Thiosulfate	420	410	< 2	440	460	420
Trithionate	37	30	31	42	28	26
Tetrathionate	9.9	17	250	10	11	16

What Do We Think We Might or Might Not Know About Thiosalts, Xanthates & Acute Lethality?

- Effluents are not at equilibrium when thiosalts are present; toxicity can be a "moving target"
- Thiosalts on their own can cause toxicity
- Thiosalt oxidation results in acid generation
  - As pH decreases, secondary toxicants (like xanthates and H_2S) can result in mortality
- More research is still needed to understand mechanisms for thiosalts degradation and toxicity (e.g., bacterial role)
- Results help to explain the link between thiosalt oxidation, pH shift and the presence of one or more contaminants which may be pH-dependent in its expression of toxicity

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Thank You!

Questions?

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