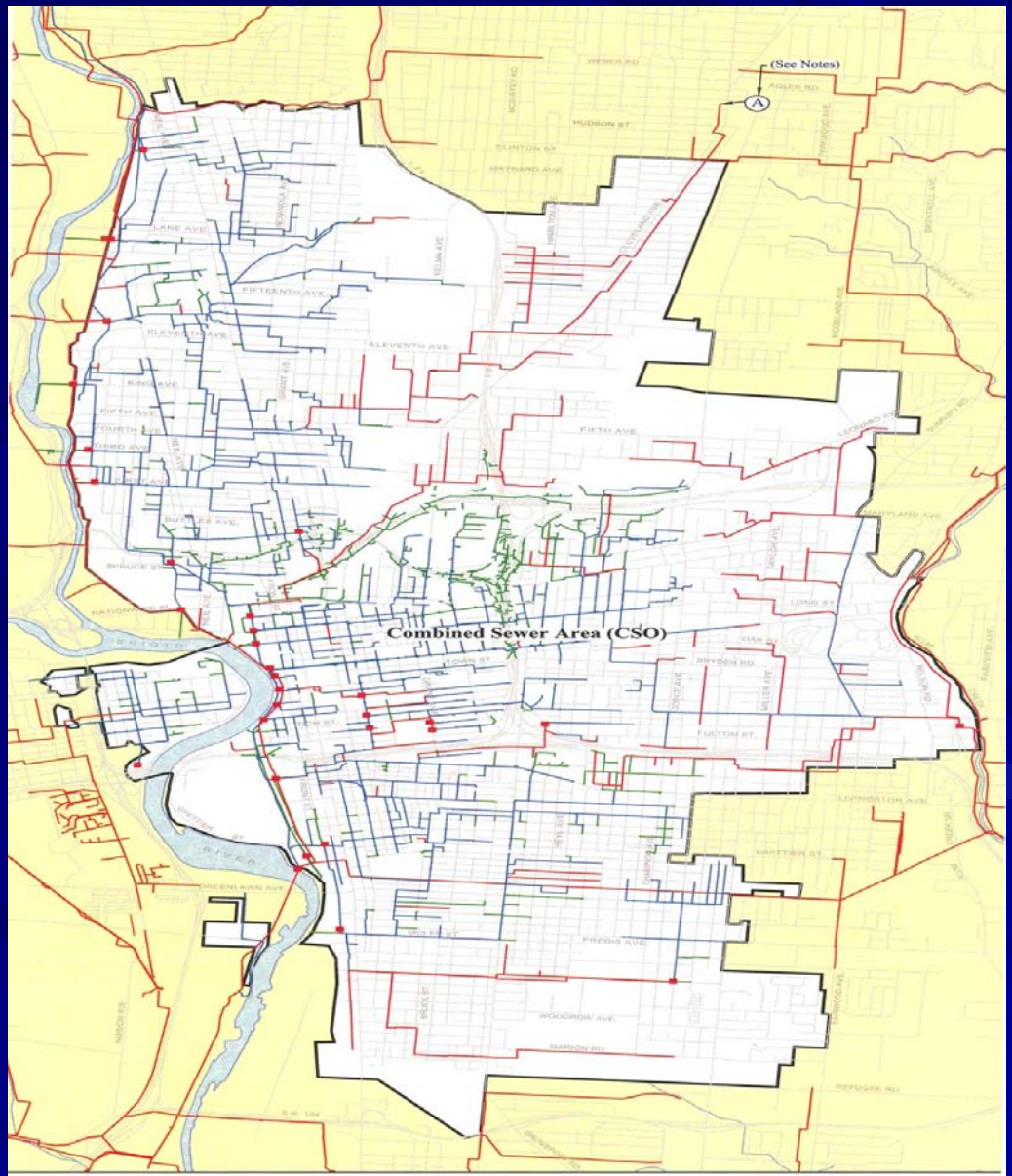
Toxicity Reduction Program for the City of Columbus Jackson Pike Wastewater Treatment Plant: A Success Story

By
Gary Hickman

Sewer/Watershed Tributary to Jackson Pike WWTP



4PF00000*JD

- September 1993 – OEPA issues new NPDES Permit requiring Whole Effluent Toxicity Monitoring beginning December 1993.
- Testing frequency:
  - 1/6 months (semi annual)
- Test organisms:
  - *Ceriodaphnia dubia* (water fleas)
  - *Pimephales promelas* (fathead minnows)

Toxicity Testing Requirements

- Reference: *Reporting and Testing Guidance for Biomonitoring Required by Ohio Environmental Protection Agency (*1991*)* and *July 1998 –Revision 1*
- Requires chronic toxicity testing as specified in section 2.
- Acute toxicity endpoints as described in section 2.H shall be derived from chronic tests.

Toxicity Endpoints

- Acute
  - Mortality 48 hours Ceriodaphnia dubia
  - Mortality 96 hours Pimephales promelas
- Chronic
  - Survival over 7 day test (both species)
  - Fish Growth (dry weight after test period)
  - Reproduction Ceriodaphnia dubia (total # of offspring – 3 broods)

*we'll revisit this slide again.

Definition of Terms

- LC 50 = Lethal Concentration in which 50% of the test organisms are dead.
- EC 50 = Effected Concentration in which 50% of the test organisms are either impaired or dead.
- NOEC = No Observable Effect Concentration
- LOEC = Lowest Observable Effect Concentration
- IC_{25} = Inhibition Concentration: A point estimate of the toxicant concentration that would cause a given percent reduction (25%) in a non-lethal biological measurement of the test organisms, such as reproduction or growth

Toxicity Calculations

- Toxicity Units (TU) =
  (100% / %Effluent concentration of toxic endpoint)

- Acute: TUa = 100/LC50 or 100/EC50

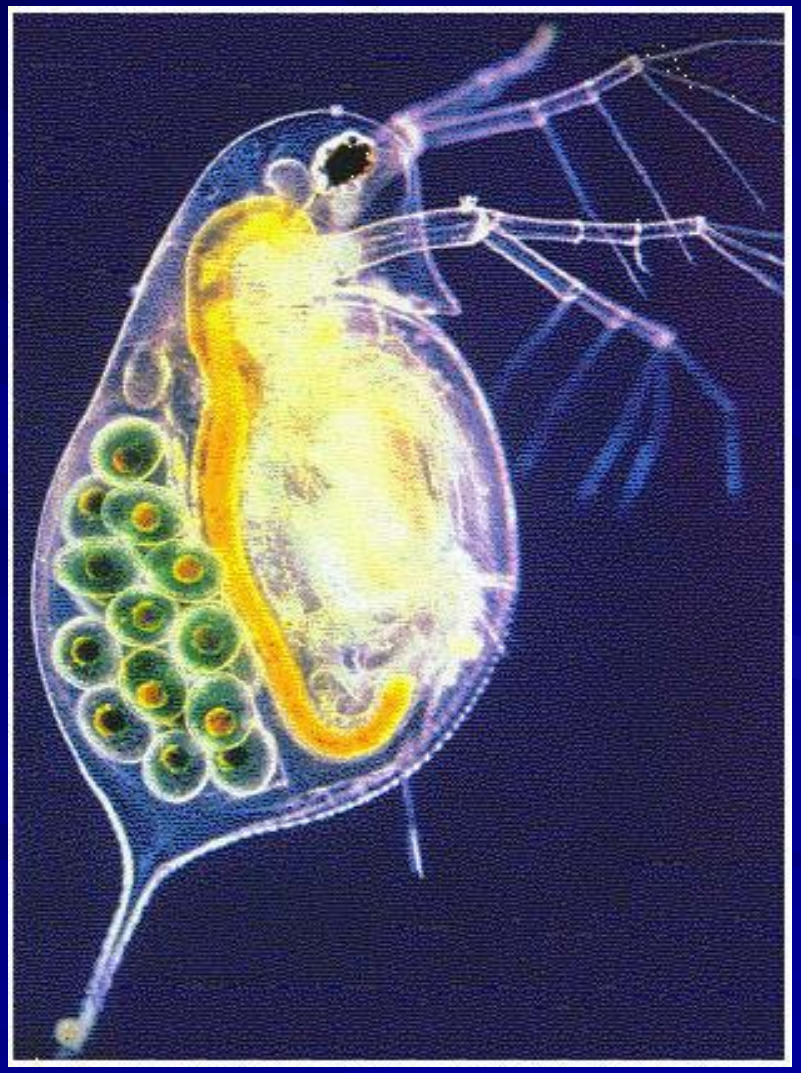
- Chronic: TUc = 100/sq. rt. of (NOEC x LOEC)
  or $100/IC_{25}$

All TU cannot be mathematically less than 1

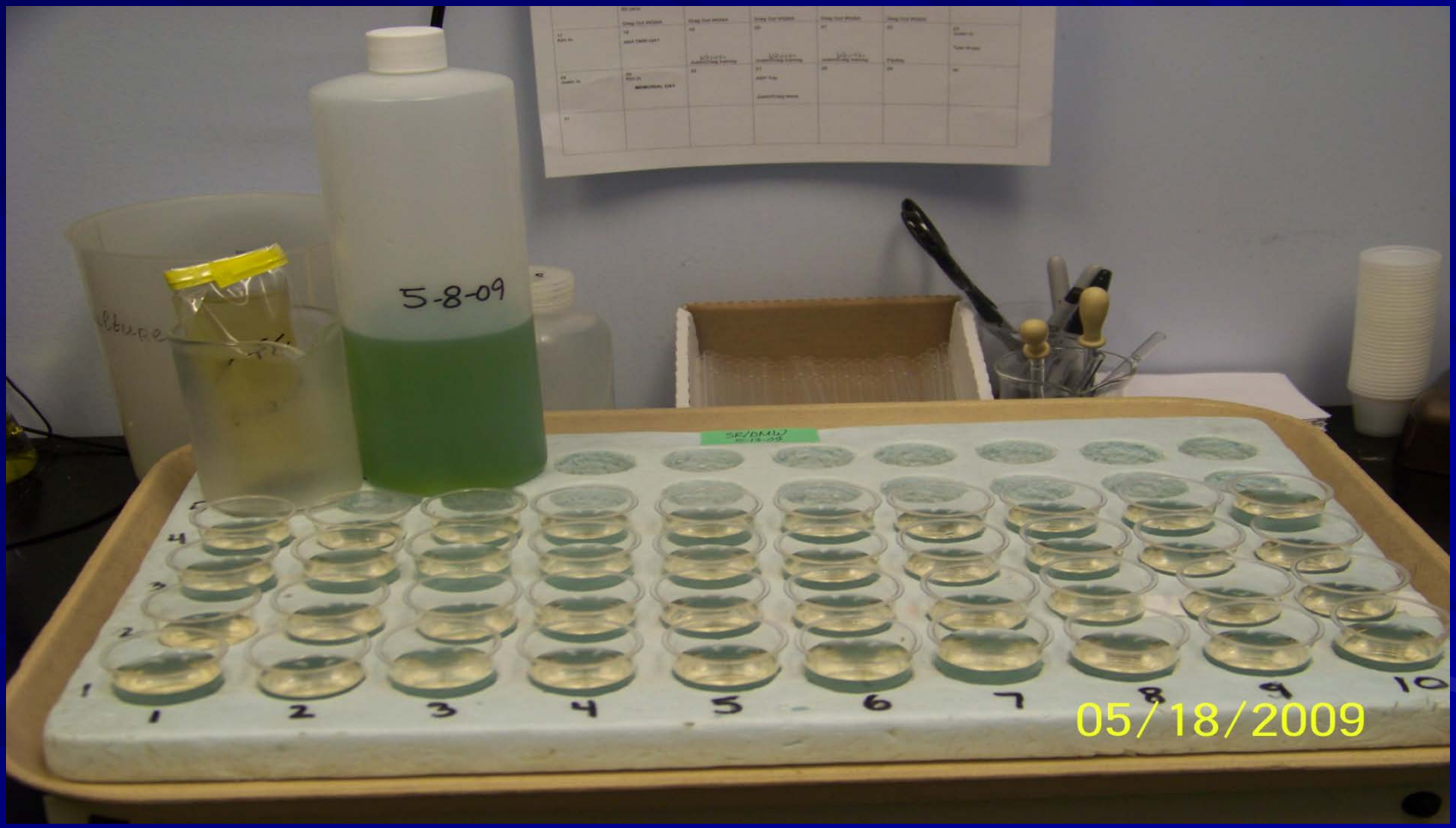
Testing Procedures

- *Ceriodaphnia dubia* (water fleas)
  - 10 replicate samples each containing 1 neonate daphnia.
  - Typical dilution series 6.25%, 12.5%, 25%, 50%, and 100% effluent
  - Upstream sample is used as the diluent and control water (unless toxic)
  - Moderately Hard Reconstituted Water is used as the (alternative) test control
  - Observing survival and reproduction each of the 7 days
  - Test Duration 7 days (only count first 3 broods).

Ceriodaphnia dubia



Ceriodaphnia Dubia Cultures



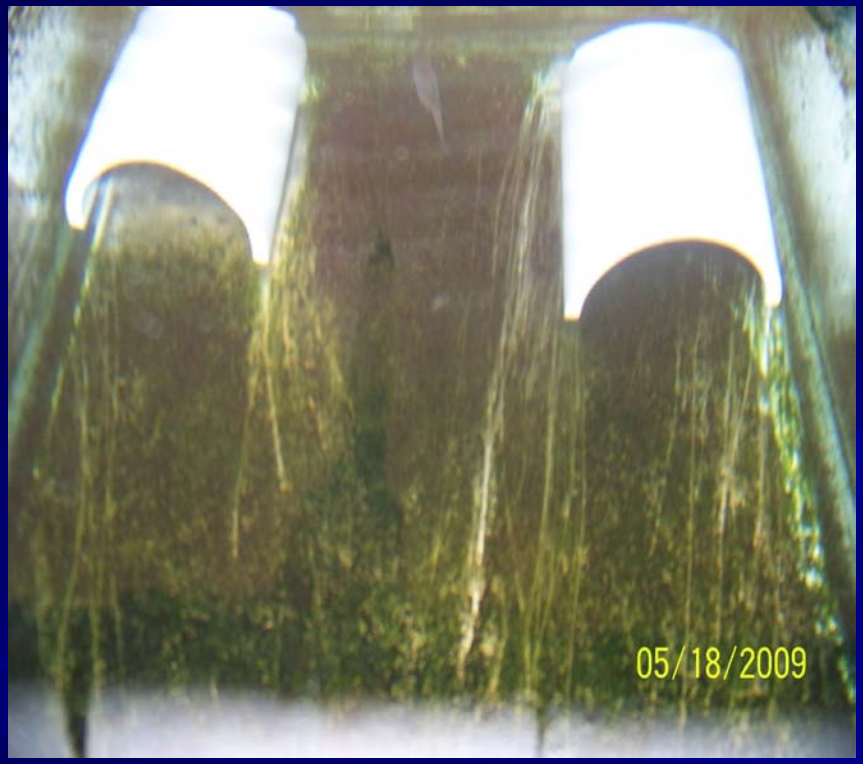
Testing Procedures

- *Pimephales promelas* (fathead minnows)
  - 3 replicates of 10 test organisms per dilution series
  - Typical dilution series 6.25%, 12.5%, 25%, 50%, and 100% effluent
  - Upstream sample is used as the diluent and test control water (unless toxic)
  - Moderately Hard Reconstituted Water is used as the (alternative) test control

  Looking for survival and growth
  - Test Duration 7 days

Pimephales promelas

Breeding Tank



Common Test Protocols

- Age of test organisms <24 hours old
  - Neonates age within 6 hrs of one another.*
- Multiple lineages of test organisms required*
- Light, photoperiod and temperature standardized
- Minimum Dissolved Oxygen Concentration 4.0 mg/l
- Feeding schedule standardized

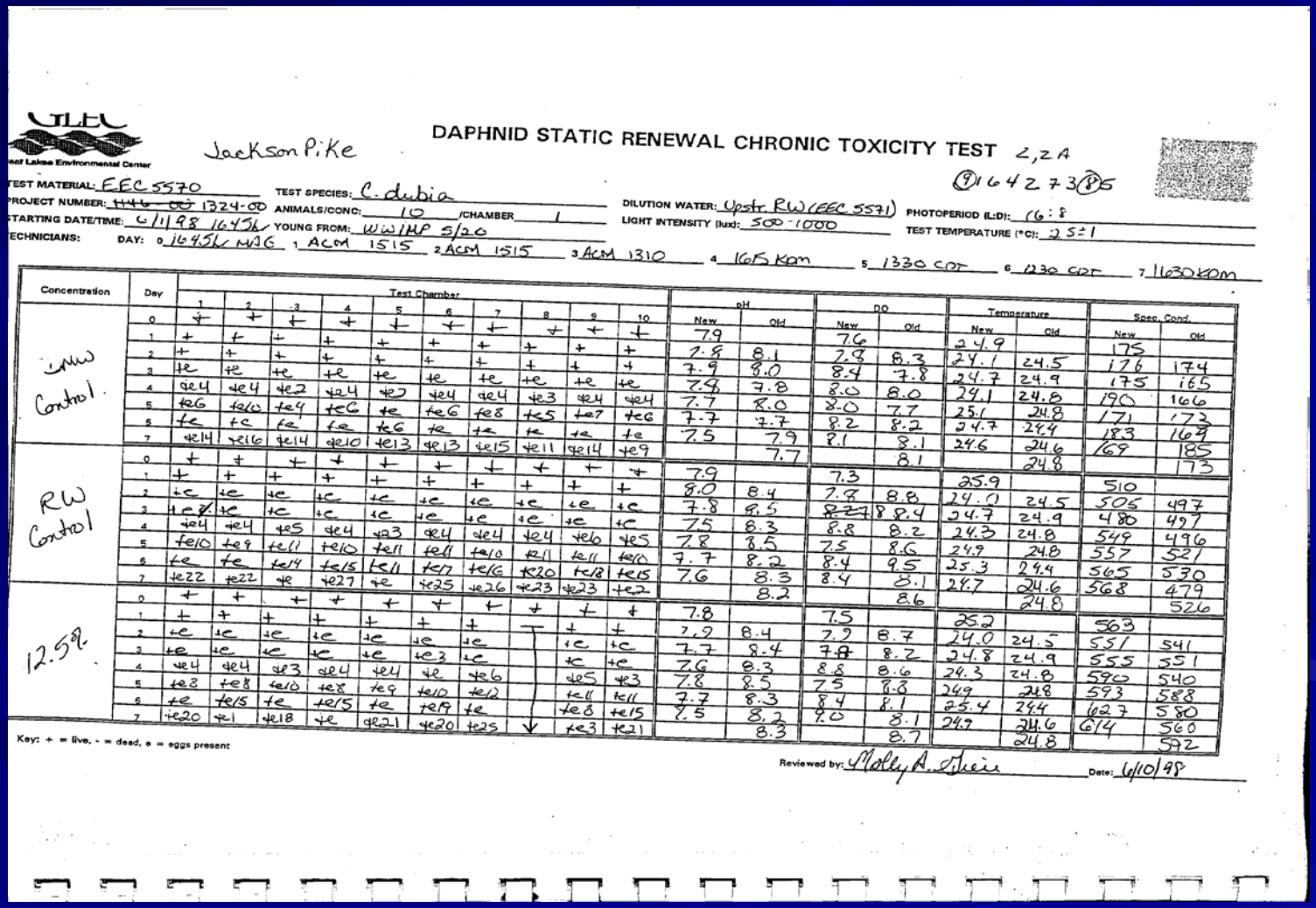
Testing Observations and Records

- Routine Chemical and Physical Determinations
  - DO, Temperature pH measurements at the beginning and end of each 24 hour period in at least one vessel for each test concentration and control
  - Temperature and pH are measured at the end of each 24 hour period
  - Temperature is measured continuously or in at least two locations and across a sufficient number of test vessels at the end of the 24 hour period to determine variation in temperature in the environmental chamber
  - pH is measured in the effluent sample daily
  - Conductivity, alkalinity and hardness are measured in each new sample; 100% effluent, receiving water, and control water
  - All measurements are recorded on the data sheet.

Testing Observations and Records

- Routine Biological Observations
  - Number of live fish larvae each day
  - Number of live adult Daphnia (first 3-4 days)
    - The number of off-spring per adult daphnia (last 3-4 days -3 broods)
  - Test duration 7 days
  - All observations are recorded on the data sheets.

Example of data Summary Sheet Daphnia



Great Lakes Environmental Center

Jackson Pike

DAPHNID STATIC RENEWAL CHRONIC TOXICITY TEST 2,2A

(9)16427385

TEST MATERIAL: EEC 5570 TEST SPECIES: C. dubia DILUTION WATER: Upstr. RW (EEC 5571) PHOTOPERIOD (L:D): 16:8

PROJECT NUMBER: ~~1446 00~~ 1324-00 ANIMALS/CONC: 10 /CHAMBER 1 LIGHT INTENSITY (lux): 500-1000 TEST TEMPERATURE (°C): 25±1

STARTING DATE/TIME: 6/1/98 1645h YOUNG FROM: WW IMP 5/20

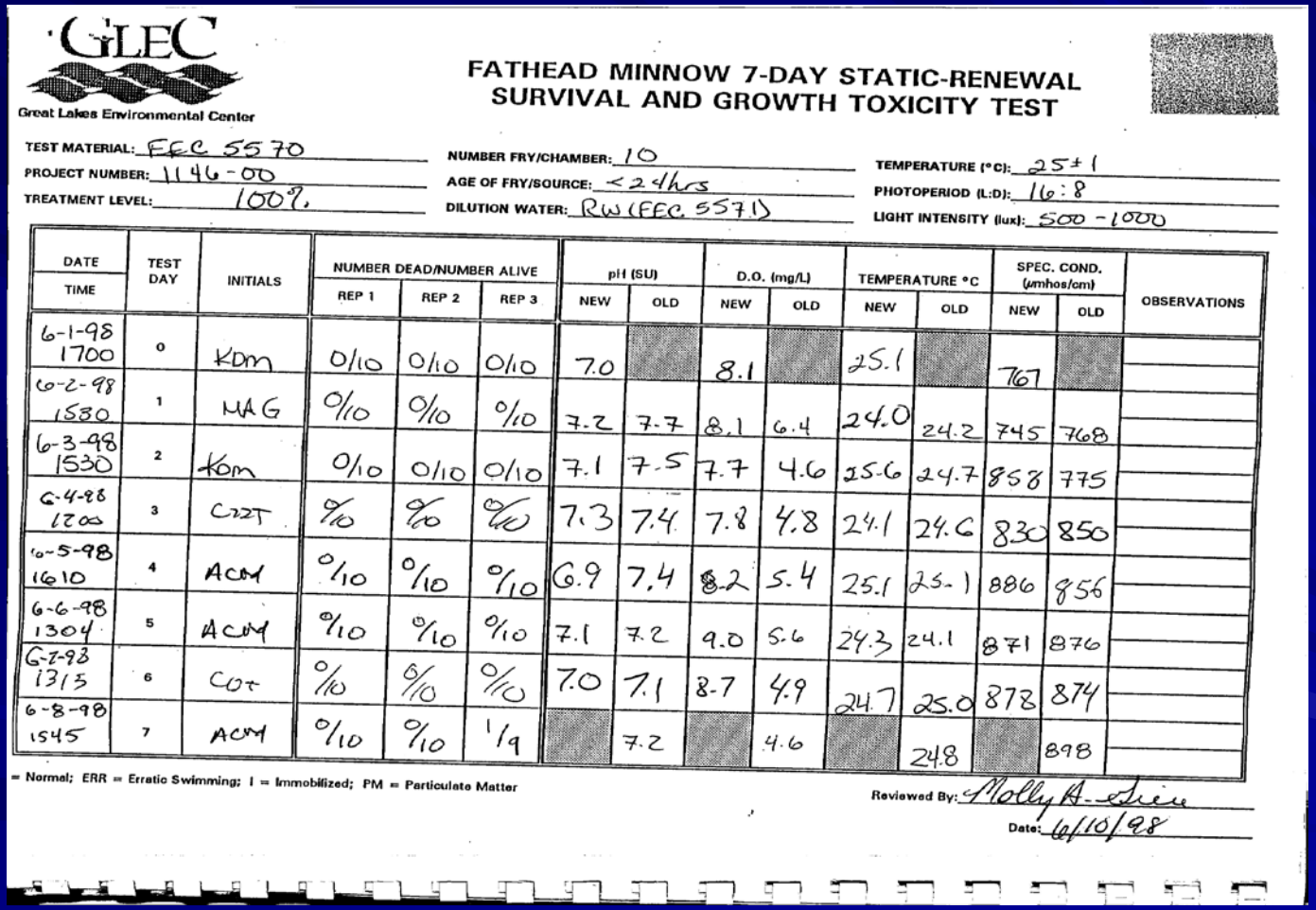
TECHNICIANS: DAY: 0 1645h MAG 1 ACM 1515 2 ACM 1515 3 ACM 1310 4 1605 KDM 5 1330 CDT 6 1230 CDT 7 1630 KDM

Concentration	Day	Test Chamber 1	2	3	4	5	6	7	8	9	10	pH New	pH Old	DO New	DO Old	Temperature New	Temperature Old	Spec. Cond. New	Spec. Cond. Old
WW Control	0	+	+	+	+	+	+	+	+	+	+	7.9		7.6		24.9		175	
	1	+	+	+	+	+	+	+	+	+	+	7.8	8.1	7.8	8.3	24.1	24.5	176	174
	2	+	+	+	+	+	+	+	+	+	+	7.9	8.0	8.4	7.8	24.7	24.9	175	165
	3	te	te	te	te	te	te	te	te	te	te	7.9	7.8	8.0	8.0	24.1	24.8	190	166
	4	te4	te4	te2	te4	te7	te4	te4	te3	te4	te4	7.7	8.0	8.0	7.7	25.1	24.8	171	173
	5	te6	te10	te4	te6	te	te6	te8	te5	te7	te6	7.7	7.7	8.2	8.2	24.7	24.4	183	169
	6	te	te	te	te	te6	te	te	te	te	te	7.5	7.9	8.1	8.1	24.6	24.6	169	185
	7	te14	te16	te14	te10	te13	te13	te15	te11	te14	te9		7.7		8.1		24.8		173
RW Control	0	+	+	+	+	+	+	+	+	+	+	7.9		7.3		25.9		510	
	1	+	+	+	+	+	+	+	+	+	+	8.0	8.4	7.8	8.8	24.1	24.5	505	497
	2	te	te	te	te	te	te	te	te	te	te	7.8	8.5	~~8.27~~	8.4	24.7	24.9	480	497
	3	te	te	te	te	te	te	te	te	te	te	7.5	8.3	8.8	8.2	24.3	24.8	549	496
	4	te4	te4	te5	te4	te3	te4	te4	te4	te6	te5	7.8	8.5	7.5	8.6	24.9	24.8	557	521
	5	te10	te9	te11	te10	te11	te11	te10	te11	te11	te10	7.7	8.2	8.4	9.5	25.3	24.4	565	530
	6	te	te	te14	te15	te11	te17	te16	te20	te18	te15	7.6	8.3	8.4	8.1	24.7	24.6	568	479
	7	te22	te22	te	te27	te	te25	te26	te23	te23	te2		8.2		8.6		24.8		526
12.5%	0	+	+	+	+	+	+	+	+	+	+	7.8		7.5		25.2		563	
	1	+	+	+	+	+	+	+	+	+	+	7.9	8.4	7.9	8.7	24.0	24.5	551	541
	2	te	te	te	te	te	te	te	+	te	te	7.7	8.4	7.8	8.2	24.8	24.9	555	551
	3	te	te	te	te	te	te3	te		te	te	7.6	8.3	8.8	8.6	24.3	24.8	590	540
	4	te4	te4	te3	te4	te4	te	te6		te5	te3	7.8	8.5	7.5	8.3	24.9	24.8	593	588
	5	te8	te8	te10	te8	te9	te10	te12		te11	te11	7.7	8.3	8.4	8.1	25.4	24.4	627	580
	6	te	te15	te	te15	te	te19	te		te8	te15	7.5	8.2	8.0	8.1	24.7	24.6	614	560
	7	te20	te1	te18	te	te21	te20	te25	↓	te3	te21		8.3		8.7		24.8		592

Key: + = live, - = dead, e = eggs present

Reviewed by: Molly A. Theis Date: 6/10/98

Example Data Summary Sheet Minnows



GLEC
Great Lakes Environmental Center

FATHEAD MINNOW 7-DAY STATIC-RENEWAL SURVIVAL AND GROWTH TOXICITY TEST

TEST MATERIAL: FEC 5570
PROJECT NUMBER: 1146-00
TREATMENT LEVEL: 100%

NUMBER FRY/CHAMBER: 10
AGE OF FRY/SOURCE: <24hrs
DILUTION WATER: RW (FEC 5571)

TEMPERATURE (°C): 25±1
PHOTOPERIOD (L:D): 16:8
LIGHT INTENSITY (lux): 500-1000

DATE / TIME	TEST DAY	INITIALS	NUMBER DEAD/NUMBER ALIVE REP 1	REP 2	REP 3	pH (SU) NEW	pH (SU) OLD	D.O. (mg/L) NEW	D.O. (mg/L) OLD	TEMPERATURE °C NEW	TEMPERATURE °C OLD	SPEC. COND. (µmhos/cm) NEW	SPEC. COND. (µmhos/cm) OLD	OBSERVATIONS
6-1-98 1700	0	KDM	0/10	0/10	0/10	7.0		8.1		25.1		767		
6-2-98 1530	1	MAG	0/10	0/10	0/10	7.2	7.7	8.1	6.4	24.0	24.2	745	768	
6-3-98 1530	2	KDM	0/10	0/10	0/10	7.1	7.5	7.7	4.6	25.6	24.7	858	775	
6-4-98 1200	3	CJZT	0/10	0/10	0/10	7.3	7.4	7.8	4.8	24.1	24.6	830	850	
6-5-98 1610	4	ACM	0/10	0/10	0/10	6.9	7.4	8.2	5.4	25.1	25.1	886	856	
6-6-98 1304	5	ACM	0/10	0/10	0/10	7.1	7.2	9.0	5.6	24.3	24.1	871	876	
6-7-98 1315	6	CDT	0/10	0/10	0/10	7.0	7.1	8.7	4.9	24.7	25.0	878	874	
6-8-98 1545	7	ACM	0/10	0/10	1/9		7.2		4.6		24.8		898	

= Normal; ERR = Erratic Swimming; I = Immobilized; PM = Particulate Matter

Reviewed By: Molly A. Shea
Date: 6/10/98

Data Summary Sheet - Daphnia

CHRONIC TOXICITY TEST RESULTS FOR *Ceriodaphnia dubia*

TABLE 4

Results of a 7-Day *Ceriodaphnia dubia* Survival and Reproduction Test Conducted 6/1/98 - 6/8/98 Using Effluent from Outfall 001.

Test Solutions	Cumulative Percent Mortality[a] (Cumulative Percent Adversely Affected)[a] Test Day							Number of Young Produced[a]	
	1	2	3	4	5	6	7	Total	Mean
Primary Control/ Dilution Water (RW)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	443	44.3
Secondary Control (DMW)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	228	22.8
12% Effluent	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	324	32.4*
25% Effluent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	285	28.5*
50% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
75% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
100% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
LC_{50} Values:	34.7	34.7	34.7	34.7	34.7	34.7	34.7	Calculated TUc Value for Survival: 2.8	
95% Confidence Limits LL	NA	NA	NA	NA	NA	NA	NA		
95% Confidence Limits UL	NA	NA	NA	NA	NA	NA	NA		
EC_{50} Values:	34.7	34.7	34.7	34.7	34.7	34.7	34.7	Calculated TUc Value for Reproduction: >8.3	
95% Confidence Limits LL	NA	NA	NA	NA	NA	NA	NA		
95% Confidence Limits UL	NA	NA	NA	NA	NA	NA	NA		
7-Day NOEC for Mortality: 25%		7-Day NOEC for Reproduction: <12%						Method(s) Used to Determine Values: Mortality - Fisher's Reproduction - Dunnett's	
7-Day LOEC for Mortality: 50%		7-Day LOEC for Reproduction: 12%							
Chronic Value for Mortality: 35.5%		Chronic Value for Reprod.: <12%							
IC_{25} = 14.7%									
a - indicate significant differences from the primary control with an * ($p < 0.05$).									

Data Summary Sheet - Minnows

CHRONIC TOXICITY TEST RESULTS FOR FATHEAD MINNOWS (*Pimephales promelas*)

TABLE 6

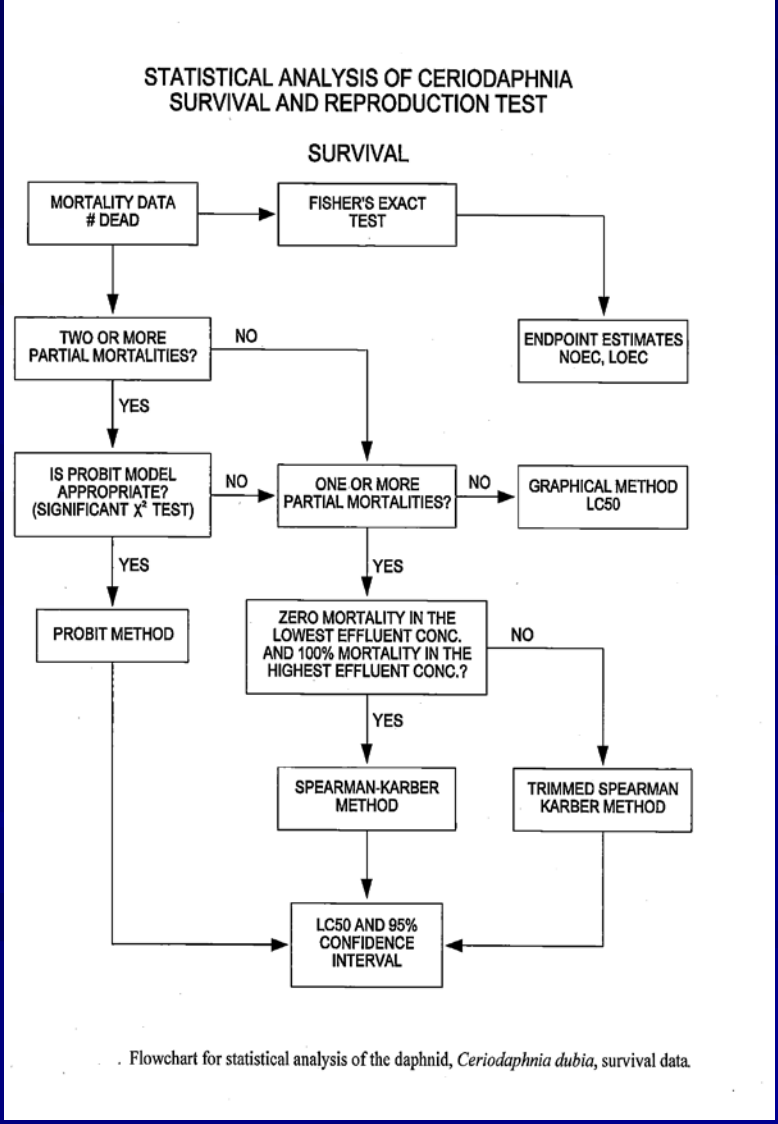
Results of a 7-Day *Pimephales promelas* Survival and Growth Test Conducted 6/1/98 - 6/8/98 Using Effluent from Outfall 001.

Test Solutions	Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)* Test Day							Dry Weight (per number initiated)*	
	1	2	3	4	5	6	7	Total	Mean
Primary Control/ Dilution Water (RW)	0 (0)	0 (0)	0 (3.3)	16.7 (16.7)	33.3 (33.3)	36.7 (36.7)	40[b] (40)	8.92	0.2973
Secondary Control (MH)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13.64	0.4547
12% Effluent	0 (0)	0 (0)	0 (0)	3.3 (3.3)	13.3 (13.3)	20 (20)	20 (20)	10.54	0.3513
25% Effluent	0 (0)	0 (0)	3.3 (3.3)	10 (10)	23.3 (23.3)	23.3 (23.3)	23.3 (23.3)	10.92	0.3640
50% Effluent	0 (0)	0 (0)	10 (10)	23.3 (23.3)	43.3 (43.3)	60 (60)	63.3 (63.3)	5.02	0.1673
75% Effluent	0 (0)	0 (0)	0 (0)	10 (10)	33.3 (33.3)	40 (40)	43.3 (43.3)	7.91	0.2637
100% Effluent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.3 (3.3)	13.51	0.4503
LC_{50} Values:	>100	>100	>100	>100	24.3	21.9	22.2	Calculated TUc Value for Survival: <1.0	
95% Confidence Limits LL	NA	NA	NA	NA	5.2	10.2	12.6		
95% Confidence Limits UL	NA	NA	NA	NA	114.0	47.2	39.0		
EC_{50} Values:	>100	>100	>100	>100	24.3	21.9	22.2	Calculated TUc Value for Growth: <1.0	
95% Confidence Limits LL	NA	NA	NA	NA	5.2	10.2	12.6		
95% Confidence Limits UL	NA	NA	NA	NA	114.0	47.2	39.0		

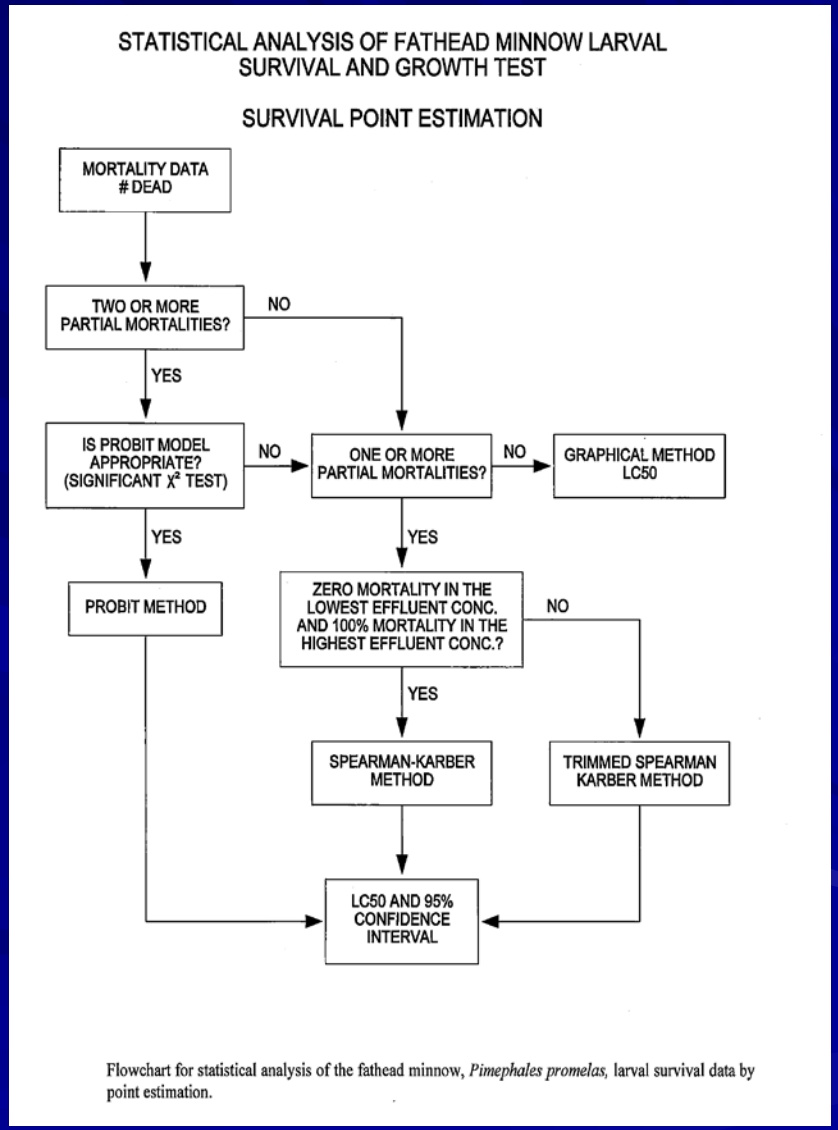
7-Day NOEC for Mortality: 100%	7-Day NOEC for Growth: 100%	Method(s) Used to Determine Values: Mortality - t-test Growth - t-test
7-Day LOEC for Mortality: >100%	7-Day LOEC for Growth: >100%	
Chronic Value for Mortality: >100%	Chronic Value for Growth: >100%	

a - indicate significant differences from the primary control with an * ($p < 0.05$).
b - secondary (laboratory) control used for statistical analysis due to toxicity in primary (receiving water) control.

Survival

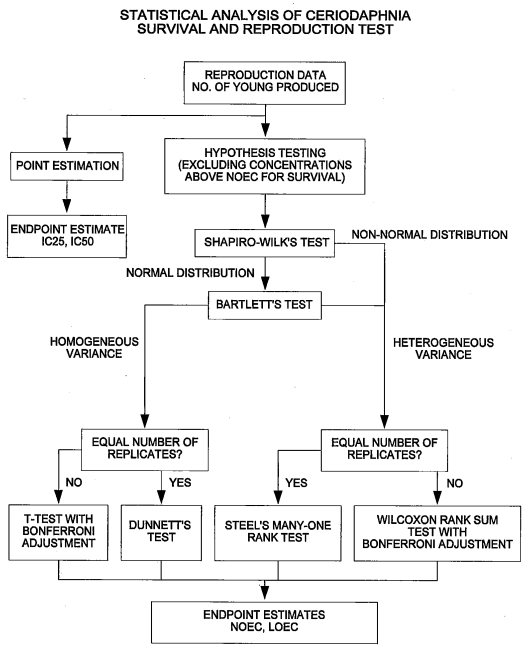


. Flowchart for statistical analysis of the daphnid, *Ceriodaphnia dubia*, survival data.

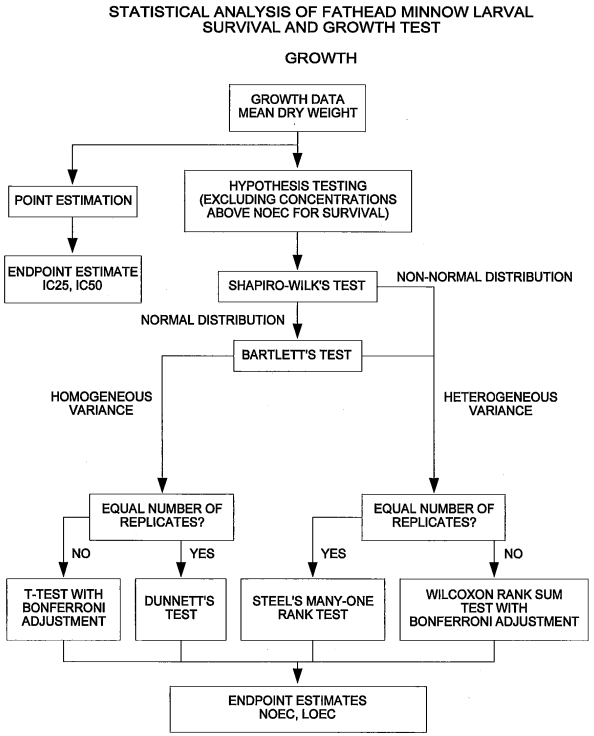


Flowchart for statistical analysis of the fathead minnow, *Pimephales promelas,* larval survival data by point estimation.

Reproduction/Growth



Flowchart for the statistical analysis of the daphnid, *Ceriodaphnia dubia*, reproduction data.



Flowchart for statistical analysis of fathead minnow, *Pimephales promelas,* larval growth data.

List of possible interferences

- Contaminants in dilution water, glassware, sample hardware and testing equipment
- Improper effluent sampling and handling
- Pathogenic and/or predatory organisms in the dilution water and effluent may affect test organism survival and confound test results
- The amount and type of natural food and nutrients in the effluent or dilution water may confound test results

WET Tests

- Based on all of the above, you could be wondering is this even a viable test to do?
- Yes it is.
- Scientific method.
- Enormous efforts to eliminate variability and provide readily defensible data
- Improvements made as scientists gain experience and endeavor to reduce test variability:
  - age and lineage of test species
  - Adjusting Dilution series used for testing certain effluents: 20%, 40%, 80%, 100% e.g.

What Happened

- OEPA raised concerns about the previous 10 years WET results in 2003 discussions on NPDES permit renewal.
- The City accelerated screening tests using 100% effluent, upstream, and downstream to see if a chronic toxicity issue could be identified.
  - Definitive testing as needed, if persistent toxicity was identified.
- Try to persuade the OEPA that at worst there was an intermittent toxicity issue.
- NPDES Permit 4PF00000*KD issued in 2004 with WET Limits and a compliance schedule.
  - Maximum TUc value of 1.8 for both species,
  - Quarterly testing,
  - Annual average limit of 1.0 TUc

Test Results Reported

TABLE 1. Results of Whole Effluent Toxicity Testing
Jackson Pike Wastewater Treatment Plant

Date	Ceriodaphnia dubia TUc	Pimephales promelas TUc
9-Dec-93	<1.0	<1.0
9-Jun-94	<1.0	<1.0
1-Dec-94	1.15	<1.0
11-Jun-95	1.15	<1.0
9-Dec-95	<1.0	<1.0
3-Jun-96	2.8	<1.0
7-Dec-96	>8.3	<1.0
8-Jun-97	<1.0	<1.0
7-Dec-97	1.6	5.8
1-Jun-98	>8.3	<1.0
6-Dec-98	1.2	>8.0
13-Jun-99	<1.0	1.2
5-Dec-99	<1.0	10.3
11-Jun-00	<1.0	<1.0
10-Dec-00	<1.0	<1.0
3-Jun-01	<1.0	<1.0
10-Dec-01	2.1	0
3-Jun-02	1.1	<1.0
2-Dec-02	1.1	<1.0
1-Jun-03	<1.0	<1.0
7-Dec-03	<1.0	<1.0
25-Jan-04	< 1.0*	< 1.0*
23-Feb-04	< 1.0*	< 1.0*
21-Mar-04	< 1.0*	< 1.0*
9-May-04	< 1.0*	< 1.0*
13-Jun-04	< 1.0	< 1.0
18-Jul-04	< 1.0*	< 1.0*
22-Aug-04	< 1.0*	< 1.0*
7-Nov-04	< 1.0	1.2
12-Dec-04	< 1.0	<1.0
6-Feb-05	< 1.0	< 1.0
1-May-05	< 1.0	<1.0
7-Aug-05	< 1.0	< 1.0
11-Sep-05	< 1.0	< 1.0
2-Oct-05	< 1.0	< 1.0
6-Nov-05	< 1.0	< 1.0
4-Dec-05	< 1.0	< 1.0
8-Jan-06	<1.0	<1.0
7-Feb-06	<1.0	<1.0

* Screening only no toxicity detected at 100%

Data Summary Sheet - Daphnia

CHRONIC TOXICITY TEST RESULTS FOR *Ceriodaphnia dubia*

TABLE 4

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	1	2	3	4	5	6	7	Total	Mean
Primary Control/ Dilution Water (RW)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	443	44.3
Secondary Control (DMW)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	228	22.8
12% Effluent	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	324	32.4*
25% Effluent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	285	28.5*
50% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
75% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
100% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
LC_{50} Values:	34.7	34.7	34.7	34.7	34.7	34.7	34.7	Calculated TUc Value for Survival: 2.8	
95% Confidence Limits LL	NA	NA	NA	NA	NA	NA	NA		
95% Confidence Limits UL	NA	NA	NA	NA	NA	NA	NA		
EC_{50} Values:	34.7	34.7	34.7	34.7	34.7	34.7	34.7	Calculated TUc Value for Reproduction: >8.3	
95% Confidence Limits LL	NA	NA	NA	NA	NA	NA	NA		
95% Confidence Limits UL	NA	NA	NA	NA	NA	NA	NA		
7-Day NOEC for Mortality: 25%		7-Day NOEC for Reproduction: <12%						Method(s) Used to Determine Values: Mortality - Fisher's Reproduction - Dunnett's	
7-Day LOEC for Mortality: 50%		7-Day LOEC for Reproduction: 12%							
Chronic Value for Mortality: 35.5%		Chronic Value for Reprod.: <12%							
IC_{25} = 14.7%									
a - indicate significant differences from the primary control with an * ($p < 0.05$).									

Data Summary Sheet - Minnows

CHRONIC TOXICITY TEST RESULTS FOR FATHEAD MINNOWS (*Pimephales promelas*)

TABLE 6

Results of a 7-Day *Pimephales promelas* Survival and Growth Test Conducted 6/1/98 - 6/8/98 Using Effluent from Outfall 001.

Test Solutions	Cumulative Percent Mortality[a] (Cumulative Percent Adversely Affected)[a] Test Day							Dry Weight (per number initiated)[a]	
	1	2	3	4	5	6	7	Total	Mean
Primary Control/ Dilution Water (RW)	0 (0)	0 (0)	0 (3.3)	16.7 (16.7)	33.3 (33.3)	36.7 (36.7)	40[b] (40)	8.92	0.2973
Secondary Control (MH)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13.64	0.4547
12% Effluent	0 (0)	0 (0)	0 (0)	3.3 (3.3)	13.3 (13.3)	20 (20)	20 (20)	10.54	0.3513
25% Effluent	0 (0)	0 (0)	3.3 (3.3)	10 (10)	23.3 (23.3)	23.3 (23.3)	23.3 (23.3)	10.92	0.3640
50% Effluent	0 (0)	0 (0)	10 (10)	23.3 (23.3)	43.3 (43.3)	60 (60)	63.3 (63.3)	5.02	0.1673
75% Effluent	0 (0)	0 (0)	0 (0)	10 (10)	33.3 (33.3)	40 (40)	43.3 (43.3)	7.91	0.2637
100% Effluent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.3 (3.3)	13.51	0.4503
LC_{50} Values:	>100	>100	>100	>100	24.3	21.9	22.2	Calculated TUc Value for Survival: <1.0	
95% Confidence Limits LL	NA	NA	NA	NA	5.2	10.2	12.6		
95% Confidence Limits UL	NA	NA	NA	NA	114.0	47.2	39.0		
EC_{50} Values:	>100	>100	>100	>100	24.3	21.9	22.2	Calculated TUc Value for Growth: <1.0	
95% Confidence Limits LL	NA	NA	NA	NA	5.2	10.2	12.6		
95% Confidence Limits UL	NA	NA	NA	NA	114.0	47.2	39.0		
7-Day NOEC for Mortality: 100%				7-Day NOEC for Growth: 100%				Method(s) Used to Determine Values: Mortality - t-test Growth - t-test	
7-Day LOEC for Mortality: >100%				7-Day LOEC for Growth: >100%					
Chronic Value for Mortality: >100%				Chronic Value for Growth: >100%					

a - indicate significant differences from the primary control with an * ($p < 0.05$).
b - secondary (laboratory) control used for statistical analysis due to toxicity in primary (receiving water) control.

Toxicity Endpoints (I'm Back)

- Acute
  - Mortality at 48 hours for Ceriodaphnia dubia
  - Mortality at 96 hours for Pimephales promelas
- Chronic
  - Survival over 7 day test (both species)
  - Fish Growth (dry weight after test period)
  - Reproduction Ceriodaphnia dubia (total # of offspring – 3 broods)

Points to Ponder

- Do we even have Chronic Toxicity? Or is it more likely Intermittent Acute Toxicity
  - Does the observed toxic effect occur with test initiation
  - Or is it observed after a subsequent static renewal interval?
- Do we have Intermittent Chronic Toxicity?
  - Is it related to the time of year the test was done (winter vs. summer)
  - Was it related to plant construction activities or start up of new processes, chemical addition, etc.
- Intermittent Dilution Water Toxicity – upstream control
- What if all three conditions occur

Data Summary Sheets

CHRONIC TOXICITY TEST RESULTS FOR *Ceriodaphnia dubia*

TABLE 4

Results of a 7-Day *Ceriodaphnia dubia* Survival and Reproduction Test Conducted 6/1/98 - 6/8/98 Using Effluent from Outfall 001.

Test Solutions	Cumulative Percent Mortality[a] (Cumulative Percent Adversely Affected)[a] Test Day							Number of Young Produced[a]	
	1	2	3	4	5	6	7	Total	Mean
Primary Control/ Dilution Water (RW)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	443	44.3
Secondary Control (DMW)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	228	22.8
12% Effluent	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	10 (10)	324	32.4*
25% Effluent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	285	28.5*
50% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
75% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
100% Effluent	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100* (100)	0	0.0
LC_{50} Values:	34.7	34.7	34.7	34.7	34.7	34.7	34.7	Calculated TUc Value for Survival: 2.8	
95% Confidence Limits LL	NA	NA	NA	NA	NA	NA	NA		
95% Confidence Limits UL	NA	NA	NA	NA	NA	NA	NA		
EC_{50} Values:	34.7	34.7	34.7	34.7	34.7	34.7	34.7	Calculated TUc Value for Reproduction: >8.3	
95% Confidence Limits LL	NA	NA	NA	NA	NA	NA	NA		
95% Confidence Limits UL	NA	NA	NA	NA	NA	NA	NA		
7-Day NOEC for Mortality: 25%	7-Day NOEC for Reproduction: <12%							Method(s) Used to Determine Values: Mortality - Fisher's Reproduction - Dunnett's	
7-Day LOEC for Mortality: 50%	7-Day LOEC for Reproduction: 12%								
Chronic Value for Mortality: 35.5%	Chronic Value for Reprod.: <12%								
IC_{25} = 14.7%									

a - indicate significant differences from the primary control with an * ($p < 0.05$).

CHRONIC TOXICITY TEST RESULTS FOR FATHEAD MINNOWS (*Pimephales promelas*)

TABLE 6

Results of a 7-Day *Pimephales promelas* Survival and Growth Test Conducted 6/1/98 - 6/8/98 Using Effluent from Outfall 001.

Test Solutions	Cumulative Percent Mortality[a] (Cumulative Percent Adversely Affected)[a] Test Day							Dry Weight (per number initiated)[a]	
	1	2	3	4	5	6	7	Total	Mean
Primary Control/ Dilution Water (RW)	0 (0)	0 (0)	0 (3.3)	16.7 (16.7)	33.3 (33.3)	36.7 (36.7)	40[b] (40)	8.92	0.2973
Secondary Control (MH)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13.64	0.4547
12% Effluent	0 (0)	0 (0)	0 (0)	3.3 (3.3)	13.3 (13.3)	20 (20)	20 (20)	10.54	0.3513
25% Effluent	0 (0)	0 (0)	3.3 (3.3)	10 (10)	23.3 (23.3)	23.3 (23.3)	23.3 (23.3)	10.92	0.3640
50% Effluent	0 (0)	0 (0)	10 (10)	23.3 (23.3)	43.3 (43.3)	60 (60)	63.3 (63.3)	5.02	0.1673
75% Effluent	0 (0)	0 (0)	0 (0)	10 (10)	33.3 (33.3)	40 (40)	43.3 (43.3)	7.91	0.2637
100% Effluent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.3 (3.3)	13.51	0.4503
LC_{50} Values:	>100	>100	>100	>100	24.3	21.9	22.2	Calculated TUc Value for Survival: <1.0	
95% Confidence Limits LL	NA	NA	NA	NA	5.2	10.2	12.6		
95% Confidence Limits UL	NA	NA	NA	NA	114.0	47.2	39.0		
EC_{50} Values:	>100	>100	>100	>100	24.3	21.9	22.2	Calculated TUc Value for Growth: <1.0	
95% Confidence Limits LL	NA	NA	NA	NA	5.2	10.2	12.6		
95% Confidence Limits UL	NA	NA	NA	NA	114.0	47.2	39.0		
7-Day NOEC for Mortality: 100%	7-Day NOEC for Growth: 100%							Method(s) Used to Determine Values: Mortality - t-test Growth - t-test	
7-Day LOEC for Mortality: >100%	7-Day LOEC for Growth: >100%								
Chronic Value for Mortality: >100%	Chronic Value for Growth: >100%								

a - indicate significant differences from the primary control with an * ($p < 0.05$).
b - secondary (laboratory) control used for statistical analysis due to toxicity in primary (receiving water) control.

Compliance Procedure

- What was required by the Permit
  - Initiate a Toxicity Reduction Evaluation (TRE) by December of 2005.
  - Submit a General Plan for “Toxicity” Reduction in March 2006.
  - Execute the General Plan.
  - Submit Annual Reports (September 2006, 2007, 2008, 2009)
  - Submit a Specific Plan for “Toxicity” reduction due January 2008.
  - Full Compliance by November 2009

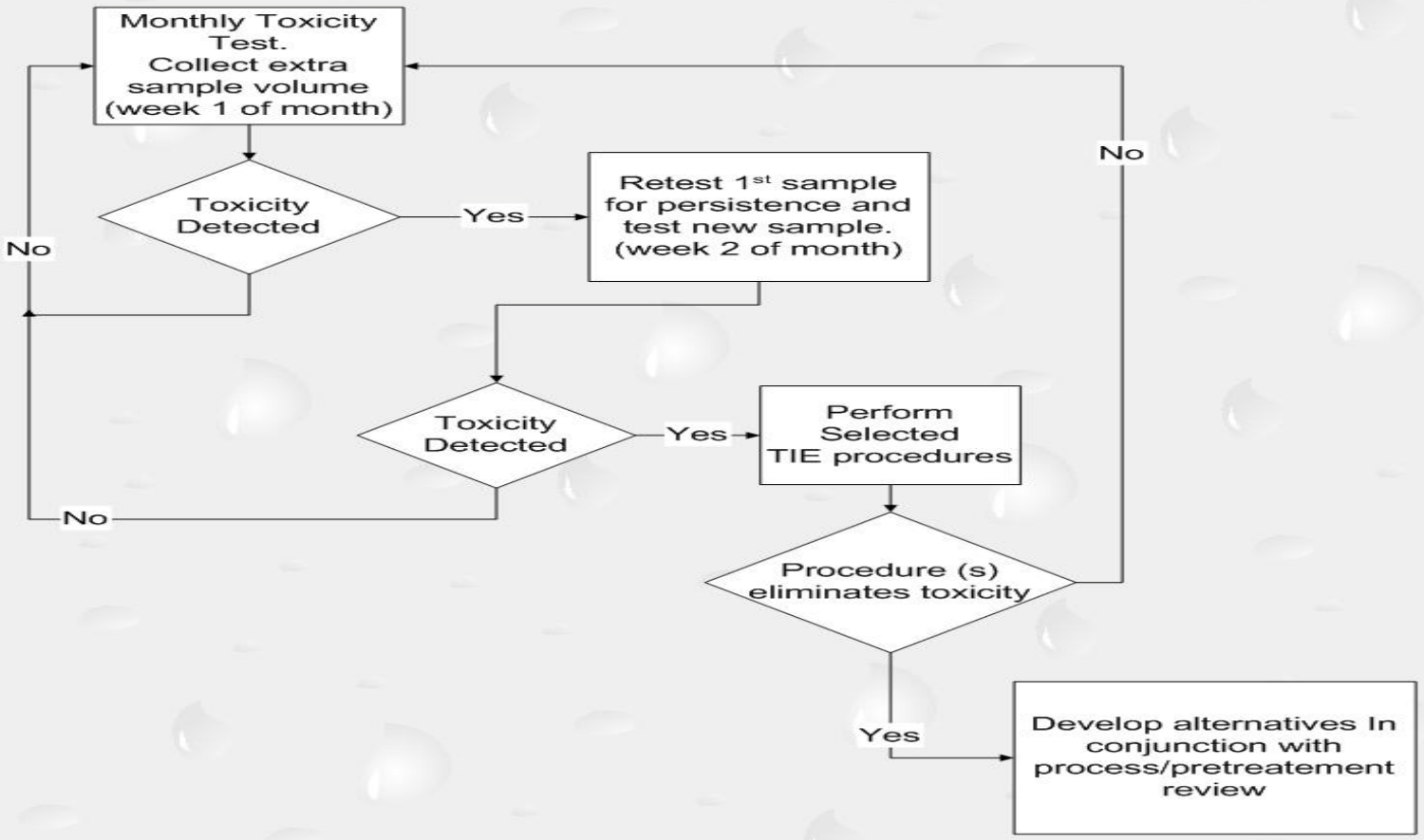
TRE - What We did

- Secured the services of a consultant to help us through the process.
- The TRE is a two stage process
- Toxicity Identification Evaluation (TIE) is a series of tests (on an effluent exhibiting toxic characteristics) to determine what is the physical, chemical, or biological property of the effluent causing toxicity.
- TRE is an engineering evaluation of plant processes to provide the treatment solution(s) necessary to remove the cause identified in the TIE.

General Plan Activities

- Review of Sampling and QA/QC protocols
- WET Laboratory review and selection
- Engineering review of Plant process to identify possible plant toxicity sources
  - Chemicals used in treatment processes
  - Recycle streams
  - Stormwater controls
- Review of Significant Industrial Users to Identify possible external sources of toxicity

TIE/TRE Process



What is Required for to Conduct a TIE?

- Persistent Effluent toxicity as a result of physical, chemical or biological constituents in the effluent.

TIE – Two Tier Approach

- Tier 1 – Approach involves manipulation of effluent samples:
  - Filtration
  - Aeration
  - Use of additives to chelate or reduce toxicants (EDTA)
  - Minor pH adjustments
  - Phase Separation techniques with C_{18} solid phase extraction (SPE) resin to remove possible organics
- Samples manipulated at the initial pH of the effluent (pH_i)

TIE – Two Tier Approach (Cont.)

- Tier 2 – Consists of performing the same manipulation steps on the effluent after adjusting samples to pH 3 and pH 10.
  - After manipulation completion readjust pH to effluent pH_i
- Compare the results with an unaltered effluent sample.

Results of Sampling

TABLE 2. Results of Whole Effluent Toxicity Testing Jackson Pike Wastewater Treatment Plant		
Date	Ceriodaphnia dubia TUc	Pimephales promelas TUc
Sept-06	< 1.0	< 1.0
Oct-06	< 1.0	< 1.0
Nov-06	< 1.0	< 1.0
Dec-06	< 1.0	< 1.0
Jan-07	< 1.0	< 1.0
Feb-07	< 1.0	< 1.0
Mar-07	< 1.0	< 1.0
Apr-07	< 1.0	< 1.0
May-07	< 1.0	< 1.0
June-07	< 1.0	< 1.0
09-July-07	1.2	< 1.0
09-July-07 Retest 7/17 *C. dubia*	< 1.0	NA
17-Jul-07	< 1.0	NA
Aug-07	< 1.0	< 1.0
Sep-07	< 1.0	< 1.0
Oct-07	< 1.0	< 1.0
04-Nov-07	< 1.0	< 1.0
25-Nov-07	< 1.0	< 1.0
Jan-08	< 1.0	< 1.0
Feb-08	< 1.0	< 1.0
Mar-08	< 1.0	< 1.0
Apr-08	< 1.0	< 1.0
May-08	< 1.0	< 1.0
Aug-08	< 1.0	< 1.0
Nov-08	< 1.0	< 1.0
Feb-09	< 1.0	< 1.0
May-09	< 1.0	< 1.0
Aug-09	< 1.0	< 1.0
Nov-10	< 1.0	< 1.0

Specific Plan

- Plant improvements required to attain compliance – Report due 01/01/2008
  - Demonstration that “Toxicity” meets limits
  - Plant modifications or process changes identified.
  - Pre-treatment Program changes required.
  - Demonstration that toxicity is not persistent, reproducible or identifiable.
  - Complete a PTI if required.
  - Schedule for construction.

2008 – 2010

- Continued monitoring Effluent using definitive testing on a quarterly basis as outlined in the permit.
- Formally requested removal of the limit from the permit - Part II, Paragraph Z.
- Met with Ohio EPA to discuss permit renewal.
- Successfully had WET limits removed from NPDES permit 4PF00000*OD (August 2010) and sampling frequency reduced to semi-annual.

Lessons Learned

- Process changes to accommodate various construction related activities can significantly impact stable operations, which in turn may influence the WET. (Ammonia – bleed through, start up shutdown of Sodium Hypochlorite/Bisulfite chemical system, change in polymer, salt on floors, etc.)
- Dilution Series may need to be adjusted to bracket the higher end of the scale: 20%, 40%, 60%, 80%, 100% to get more definitive results.
- Upstream Toxicity or the additive effects thereof, when combined with effluent, may need to be identified.
  - Combined Sewer discharges and other point and non-point source discharges may influence results
- Use of Moderately Hard Re-constituted Water for dilution water may be required if persistent upstream toxicity exists.

Lessons Learned

- Data Interpretation
  - The TU number should not be a stand alone value devoid of the context in which it was generated.

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