

Bioassays of the City of

Milton Wastewater Treatment Plant

Milton, Santa Rosa County, Florida

NPDES #FL0021903

Sampled 1/26, 28 & 30/98

July 1998

Biology Section

Division of Administrative & Technical Services

Comprehensive Quality Assurance Plan #870346G

Bioassays of the City of

Milton Wastewater Treatment Plant

Milton, Santa Rosa County, Florida

NPDES #FL0021903

Biology Section
Bureau of Laboratories
July 1998

Introduction

City of Milton Wastewater Treatment Plant, East Walker Street, Milton, Santa Rosa County, Florida, NPDES #FL0021903, tests performed on 27 January to 3 February, 1998.

This oxidation ditch/aeration basin wastewater treatment plant has a design flow of 2.5 MGD and an annual mean flow of 1.24 MGD. Wastewater is chlorinated for disinfection, then dechlorinated and aerated prior to being discharged into the Class III waters of the Blackwater River. Sludge is stabilized in two aerobic digestors and hauled to an approved private application field (facility summary provided by Glenn Butts, FDEP, Pensacola).

The toxicity tests discussed in this report were performed in accordance with methods described by Peltier, 1994, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 3rd Edition, EPA/600/4-91/002.

Results and Discussion

The FDEP Biology Section performed two seven-day chronic static-renewal definitive toxicity bioassays on samples of effluent from this facility. Three separate samples were collected over a five day period (following EPA guidelines) to ensure that there would be an adequate volume of sample for the duration of the test and to reduce the loss of possible toxicants due to volatilization or degradation. The samples were collected on January 26, 28, and 30, 1998, and were designated sample "A", sample "B", and sample "C", respectively. The species used for these tests were the waterflea, *Ceriodaphnia dubia,* and the bannerfin shiner, *Cyprinella leedsi*. Control performance was acceptable for both test species. The test results indicated that the effluent samples did not exhibit chronic toxicity to either test species (Table 1, *C. dubia* and Appendix A, *C. leedsi*). The NOEC (no observed effect concentration) was 100% sample in both tests (see Appendix B for statistical analyses). The IC_{20} (inhibition concentration at which there is a 20% decline in reproduction or growth) could not be calculated for either test due to the lack of effect. The mortality observed in the *Cyprinella leedsi* test concentrations was not significantly different (p=0.05) from the control mortality.

Total residual chlorine was not detected in any of the three samples. Total ammonia and unionized ammonia concentrations were, respectively, for sample "A", 0.230 mg/L and 0.006 mg/L; sample "B", 0.255 mg/L and 0.006 mg/L; and sample "C", 0.498mg/L and 0.013 mg/L. Further chemical analyses were performed on the sample and several metals were identified (Table 2). Lead, silver, and zinc exceeded their respective State of Florida Class III surface water criterion for freshwater (FAC 62-302.530) in one or more of the effluent samples (Table 3).

Conclusion

The samples of effluent collected from this facility on 26, 28, and 30 January, 1998, did not demonstrate acute or chronic toxicity to either test species. Several metal constituents of the samples exceeded State of Florida Class III surface water quality standards for freshwater.

Table 1. Data recorded during the chronic toxicity bioassay of samples of effluent from the City of Milton WWTP, Milton, Santa Rosa County, Florida, NPDES # FL0021903, tests performed on 27 January to 3 February, 1998.

Facility:	City of Milton WWTP	Test Beginning Date:	1/27/98	Time:	1400	Facility Type:	Wastewater Treatment Plant
NPDES #	FL0021903	Test Ending Date:	2/3/98	Time:	1100		
Location:	East Walker Street, Milton					Chlorination Type:	Dechlorinated
County:	Santa Rosa	Sample "A" Collection Date:	1/26/98	Time:	1150	Receiving Water:	Blackwater River
Contact/District:	Glenn Butts	Sample "B" Collection Date:	1/28/98	Time:	1500		
LIMS Sample #'s:	sample "A" 303836, sample "B" 304384, sample "C"304865	Sample "C" Collection Date:	1/30/98	Time:	1100		

Total Residual Cl2	mg/l
Sample "A" Field:	0.04
Sample "A" Lab:	<0.03
Sample "B" Field:	0.0
Sample "B" Lab:	<0.03
Sample "C" Field:	not measured
Sample "C" Lab:	<0.03

Ammonia	Total (mg/l)	Unionized (mg/l)
Diluent Batch #4:	<0.017	<0.017
Diluent Batch #5:	<0.017	<0.017
Sample "A":	0.230	0.006
Sample "B":	0.255	0.006
Sample "C":	0.498	0.004

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)	Salinity
Diluent Batch #4:	71	77	0
Diluent Batch #5:	60	81	0
Sample "A":	42	36	0
Sample "B":	40	38	0
Sample "C":	33	35	0

Diluent Batch Use	Days Used
Diluent Batch #4:	4
Diluent Batch #5:	3

N/A = not applicable

NOEC: 100%	LOEC:N/A	IC_{25} N/A
SURVIVAL		
Concentrations	# surviving adults	Young/adult
Control	9	19
6.25%	10	19.4
12.5%	9	18.5
25%	9	22.7
50%	10	21.8
100%	10	23.6

		pH	Temp	DO	Cond
Day 1	Control	8.2	23.9	8.0	205
New Sample "A"	6.25%	8.1			190
	12.5%	8.1			190
	25%	8.0			200
	50%	7.9			210
	100%	7.6	24.4	7.8	255
Day 2	Control	8.1	23.1	8.1	215
New Sample "A"	6.25%	8.2			185
	12.5%	8.2			185
	25%	8.2			185
	50%	8.1			205
	100%	7.6	23.7	7.7	235
Old Sample "A"	Control	8.1		7.8	
	6.25%	8.2		8.0	
	12.5%	8.2		8.0	
	25%	8.2		7.9	
	50%	8.2		7.9	
	100%	8.1		7.8	
Day 3	Control	8.0	23.1	7.9	161
New Sample "B"	6.25%	8.0			168
	12.5%	8.0			174
	25%	7.9			180
	50%	7.8			194
	100%	7.6	23.0	8.0	222
Old Sample 'A"	Control	8.1		7.8	
	6.25%	8.2		7.8	
	12.5%	8.2		7.8	
	25%	8.1		7.9	
	50%	8.1		7.9	
	100%	7.9		7.8	

continued

		pH	Temp	DO	Cond
Day 4	Control	8.0	23.6	7.9	160
New Sample "B"	6.25%	8.0			168
	12.5%	8.0			175
	25%	8.0			184
	50%	7.7			197
	100%	7.6	24.1	7.6	226
Old Sample "B"	Control	8.0		7.5	
	6.25%	8.1		7.6	
	12.5%	8.1		7.6	
	25%	8.0		7.7	
	50%	8.0		7.7	
	100%	7.8		7.6	
Day 5	Control	8.1	22.1	8.3	156
New Sample "C"	6.25%	8.1			165
	12.5%	8.1			170
	25%	8.0			178
	50%	7.8			191
	100%	7.2	22.2	8.2	225
Old Sample "B"	Control	8.1		7.6	
	6.25%	8.1		7.6	
	12.5%	8.1		7.6	
	25%	8.1		7.5	
	50%	8.0		7.5	
	100%	7.8		7.6	
Day 6	Control	8.1	22.7	7.6	158
New Sample "C"	6.25%	8.1			168
	12.5%	8.1			174
	25%	8.0			183
	50%	7.9			198
	100%	7.5	23.0	7.7	227
Old Sample "C"	Control	7.7		7.2	
	6.25%	7.7		7.3	
	12.5%	7.9		7.3	
	25%	8.0		7.3	
	50%	8.0		7.4	
	100%	8.0		7.5	

Table 1. Concluded. Data recorded during the chronic toxicity bioassay of samples of effluent from the City of Milton WWTP, Milton, Santa Rosa County, Florida, NPDES # FL0021903, tests performed on 27 January to 3 February, 1998.

Facility:	City of Milton WWTP	Test Beginning Date:	1/27/98	Time:	1400	Facility Type:	Wastewater Treatment
NPDES #	FL0021903	Test Ending Date:	2/3/98	Time:	1100		Plant
Location:	East Walker Street, Milton					Chlorination Type:	Dechlorinated
County:	Santa Rosa	Sample "A" Collection Date:	1/26/98	Time:	1150	Receiving Water:	Blackwater River
Contact/District:	Glenn Butts	Sample "B" Collection Date:	1/28/98	Time:	1500		
LIMS Sample #'s:	sample "A" 303836, sample "B" 304384, sample "C"304865	Sample "C" Collection Date:	1/30/98	Time:	1100	Page 2 of 2	

Total Residual Cl2	mg/l
Sample "A" Field:	0.04
Sample "A" Lab:	<0.03
Sample "B" Field:	0.0
Sample "B" Lab:	<0.03
Sample "C" Field:	not measured
Sample "C" Lab:	<0.03

Ammonia	Total (mg/l)	Unionized (mg/l)
Diluent Batch #4:	<0.017	<0.017
Diluent Batch #5:	<0.017	<0.017
Sample "A":	0.230	0.006
Sample "B":	0.255	0.006
Sample "C":	0.498	0.004

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)	Salinity
Diluent Batch #4:	71	77	0
Diluent Batch #5:	60	81	0
Sample "A":	42	36	0
Sample "B":	40	38	0
Sample "C":	33	35	0

Diluent Batch Use	Days Used
Diluent Batch #4:	4
Diluent Batch #5:	3

N/A = not applicable

NOEC: 100%	LOEC:N/A	IC_{25} N/A
	SURVIVAL	
Concentrations	# surviving adults	Young/adult
Control	9	19
6.25%	10	19.4
12.5%	9	18.5
25%	9	22.7
50%	10	21.8
100%	10	23.6

		pH	Temp	DO	Cond
Day 7	Control	8.0	23.5	8.0	163
New Sample "C"	6.25%	8.1			171
	12.5%	8.0			176
	25%	8.0			185
	50%	8.0			201
	100%	7.7	23.7	7.7	230
Old Sample "C"	Control	7.8		7.6	
	6.25%	7.8		7.6	
	12.5%	7.9		7.7	
	25%	8.0		7.7	
	50%	8.1		7.9	
	100%	8.2		7.9	

		pH	Temp	DO	Cond
Day 8	Control	8.1		7.5	
Old Sample "C"	6.25	8.1		7.6	
	12.5%	8.1		7.6	
	25%	8.1		7.6	
	50%	8.1		7.6	
	100%	8.1		7.6	

Table 2. Results of chemical analyses on the samples of effluent from the City of Milton WWTP sampled on January 26, 28, and 30, 1998.

Metals

Sample "A"

Aluminum	99	µg/L[J]
Cadmium	0.05	µg/L[I]
Calcium	11.7	mg/L[A]
Copper	2.5	µg/L[A]
Iron	92	µg/L[A]
Lead	0.70	µg/L[A]
Magnesium	1.19	mg/L[A]
Silver	0.31	µg/L[A]
Zinc	50	µg/L[A]

Sample "B"

Aluminum	79	µg/L
Cadmium	0.06	µg/L[I]
Calcium	11.7	mg/L
Copper	2.7	µg/L
Iron	81	µg/L
Lead	0.78	µg/L
Magnesium	1.23	mg/L
Silver	0.46	µg/L
Zinc	55	µg/L

Sample "C"

Aluminum	79	µg/L
Cadmium	0.07	µg/L[I]
Calcium	10.7	mg/L
Copper	2.7	µg/L
Iron	70	µg/L
Lead	0.88	µg/L
Magnesium	1.16	mg/L
Silver	0.89	µg/L
Zinc	54	µg/L

[A] Value reported is the mean of two or more determinations.

[I] Value reported is less than the minimum quantitation limit and greater than or equal to the minimum detection limit.

[J] Estimated value.

continued

Table 2. Concluded. Results of chemical analyses on the samples of effluent from the City of Milton WWTP sampled on January 26, 28, and 30, 1998.

Base, Neutral, & Acid Extractable Organics

None Detected in Samples "A", "B", or "C"

Pesticides

None Detected in Samples "A", "B", or "C"

Other Analyses

	Sample "A"		
Chloride		29	mg/L
	Sample "B"		
Chloride		29	mg/L
	Sample "C"		
Chloride		29	mg/L

Table 3. Lead, silver, and zinc concentrations detected in samples of effluent collected from the City of Milton WWTP with the respective State of Florida Class III freshwater surface water criteria for lead, silver. and zinc for each sample (FAC 62-302.530).

Sample	Hardness[A]	Ln(H)	Lead Standard [B]	Lead Conc.	Silver Standard	Silver Conc.	Zinc Standard [C]	Zinc Conc.
"A"	36	3.58	≤ 0.87	0.70	≤ 0.07	0.31[D]	≤ 44.6	50[D]
"B"	38	3.64	≤ 0.93	0.78	≤ 0.07	0.46[D]	≤ 46.69	55[D]
"C"	35	3.56	≤ 0.83	0.88[D]	≤ 0.07	0.89[D]	≤ 46.55	54[D]

All standards and concentrations are in µg/L.

[A] ppm as $CaCO_3$

[B] The Lead standard is derived from the formula $Pb \leq e^{(1.273[\ln H]-4.705)}$.

[C] The zinc standard is derived from the formula $Zn \leq e^{(0.8473[\ln H]+0.7614)}$.

[D] Exceeds standards.

Sample Source: Milton WWTP

NPDES Permit #: FL0021903 District: NWD

LIMS #: ~~3048~~ W Start Date: 1/27/98

Sample A 303836 TLH-1998-01-27-27 Sample C 304865 TLH-1998-02-02-04

Sample B 304384 TLH-1998-01-29-29

STATE OF FLORIDA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BIOLOGY LABORATORY CHRONIC BIOASSAY

Ceriodaphnia dubia BENCH SHEET - *CHEMISTRY*

		New Sample				Old Sample	
	Conc.	pH (su)	Temp (°C)	DO (mg/L)	Cond (µmhos/cm)	pH (su)	DO (mg/L)
Day 1	Control	8.2	23.9	8.0	205		
Sample A	6.25%	8.1			190		
Measured by DW	12.5%	8.1			190		
Recorded by FW	25%	8.0			200		
	50%	7.9			210		
	100%	7.6	~~24.4~~	7.4	255		Cond. Now ↑
Day 2	Control	8.1	23.1	8.1	7.8	8.1	215
Sample A	6.25%	8.2			8.0 did DO	8.2	185
Measured by DW	12.5%	8.2			8.0 DO	8.2	185
Recorded by FW	25%	8.2			7.9	8.2	185
	50%	8.1			7.9	8.2	205
	100%	7.4	23.7	7.7	7.8	8.1	235
Day 3	Control	8.0	23.1	7.9	161	8.1	7.8
Sample B	6.25%	8.0			168	8.2	7.8
Measured by MF	12.5%	8.0			174	8.2	7.8
Recorded by DW	25%	7.9			180	8.1	7.9
	50%	7.8			194	8.1	7.9
	100%	7.6	23.0	8.0	222	7.9	7.8
Day 4	Control	8.0	23.6	7.9	160	8.0	7.5
Sample B	6.25%	8.0			168	8.1	7.6
Measured by MF	12.5%	8.0			175	8.1	7.6
Recorded by MF	25%	8.0			184	8.0	7.7
	50%	7.7			197	8.0	7.7
	100%	7.2	24.1	7.6	226	7.8	7.6

		New Sample				Old Sample	
	Conc.	pH (su)	Temp (°C)	DO (mg/L)	Cond (µmhos/cm)	pH (su)	DO (mg/L)
Day 5	Control	8.1	22.1	8.3	156	8.1	7.6
Sample ~~C~~	6.25%	8.1			164	8.1	7.6
Measured by MF	12.5%	8.1			170	8.1	7.6
Recorded by MF	25%	8.0			178	8.1	7.5
	50%	7.9			191	8.0	7.5
	100%	7.2	22.2	8.2	225	7.8	7.6
Day 6	Control	8.1	22.7	7.6	158	7.7	7.2
Sample C	6.25%	8.1			168	7.7	7.3
Measured by MF	12.5%	8.1			174	7.9	7.3
Recorded by DC	25%	8.0			183	8.0	7.3
	50%	7.9			196	8.0	7.4
	100%	7.5	23.0	7.7	227	8.0	7.5
Day 7	Control	8.0	23.5	8.0	163	7.8	7.6
Sample C	6.25%	8.1			171	7.8	7.6
Measured by MF	12.5%	8.0			176	7.9	7.7
Recorded by MF	25%	8.0			185	8.0	7.7
	50%	8.0			201	8.1	7.9
	100%	7.7	23.7	7.7	230	8.2	7.7
Day 8	Control					8.1	7.5
	6.25%					8.1	7.6
	12.5%					8.1	7.6
Measured by MF	25%					8.1	7.6
Recorded by MF	50%					8.1	7.6
	100%					8.1	7.6

revised 9/15/93

Water-quality Parameters

	Field Cl2	Lab Cl2	Alkalinity	Hardness	NH3+NH4
Sample A:	0.04	<0.03	42	36	0.230
Method	—	DR-160	Hach	Hach	Orion
Measured by	Glenn Butts	JC	JC	JC	MF

Cl2 blank: <0.03 Ammonia meter slope: -56.1

Sample B:	0	<0.03	40	38	0.255
Method	—	DR 100	Hach	Hach	Orion
Measured by	Glenn Butts	DW	FW	FW	MF

Cl2 blank: <0.03 Ammonia meter slope: -54.0

Sample C:	—	<0.03	33	35	0.498
Method	—	DR100	Hach	Hach	Orion
Measured by	Not Measured	MF	FW	FW	MF

Cl2 blank: <0.03 Ammonia meter slope: -58.8

Diluent batch # 4 Days Used: 4

Diluent batch # 5 Days Used: 3

Remarks:

Day 2: Note New sample conductivity in old sample DO spaces and vice versa. DW 1/28/98

Investigators' Signatures

Marshall Faircloth

Reviewer

Sample Source: Milton WWTP
NPDES Permit #: FL0021903 District:: NWD
LIMS #: 303836 YCT Batch #: 5 Algae Batch #: 1

STATE OF FLORIDA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BIOLOGY LABORATORY CHRONIC BIOASSAY
Ceriodaphnia dubia BENCH SHEET - *REPRODUCTION*

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	# Young	# Adults	Young Adult
Control	1												10	
	2												10	
	3												10	
	4												10	
	5	2	1	6	6	2	2	3	4	1	4		10	
	6	8	8	0	0	8	5	0	0	5	10		10	
	7	0	X3	9	2	0	0	9	7	0	0		9	
	8	10	X0	9	10	10	7	8	13	10	8		9(10)	
	Total	20	12	24	18	20	14	20	24	16	22	190	÷10	19.0
6.25%	1												10	
	2												10	
	3												10	
	4												10	
	5	1	0	3	5	2	4	3	2	1	2		10	
	6	7	6	0	0	0	0	0	0	7	9		10	
	7	0	0	12	4	6	7	9	8	0	0		10	
	8	8	9	8	12	8	10	12	10	12	7		10	
	Total	16	15	23	21	16	21	24	20	20	18	194	÷10	19.4
12.5%	1												10	
	2												10	
	3												10	
	4												10	
	5	1	3	3	4	3	4	5	2	2	4		10	
	6	4	0	0	0	0	0	0	0	7	8		10	
	7	0	10	8	9	5	8	10	5	0	0		10	
	8	6	8	5	10	10	4X	9	12	8	9		9(10)	
	Total	11	21	16	22	18	16	24	19	17	21	185	÷10	18.5
25%	1												10	
	2												10	
	3												10	
	4										X		9	
	5	0	4	4	4	4	4	4	2	3			9	
	6	4	0	0	0	5	0	0	0	7			9	
	7	0	8	12	9	0	11	8	9	0			9	
	8	13	10	12	9	11	13	12	9	13			9 Ø/M	
	Total	17	22	28	22	20	28	24	20	23		204	÷9	22.7
50%	1												10	
	2												10	
	3												10	
	4												10	
	5	0	4	5	3	2	5	1	4	2	3		10	
	6	10	0	0	0	9	0	8	1	7	0		10	
	7	10	10	7	7	0	10	0	9	0	9		10	
	8	0	10	10	13	9	12	9	9	9	11		10	
	Total	20	24	22	23	20	27	18	23	18	23	218	÷10	21.8
100%	1												10	
	2												10	
	3												10	
	4												10	
	5	3	3	4	4	3	5	4	3	3	2		10	
	6	5	0	9	10	10	0	9	9	9	0		10	
	7	11	9	0	0	0	9	0	0	0	9		10	
	8	0	12	12	9	12	13	12	9	12	12		10	
	Total	19	24	25	23	25	27	25	21	24	23	236	÷10	23.6

Day	1	2	3	4	5	6	7	8
Counted by	MF	DW	MF	MF	MF	MF	MF	MF

Sample Collection
A: Date 0850 Time 1-26-98
B: Date 0830 Time 1-28-98
C: Date 0900 Time 1-30-98

Begin Test: Date 1-27-98 Time 1420
End Test: Date 2-03-98 Time 1400

Instrument Calibrations:

	Day 1	Day 5
pH	7.0@7.0 7.0@7.0	7.0@7.0 7.0@7.0
Temp	22.3@22.3	21.5@21.5
D.O.	8.1@25.2	8.2@25.4
Cond	1078@1413 99@1413 @25.4	1084@1413 985@1413 @25.7

	Day 2	Day 6
pH	7.0@7.0; 7.0@7.0	7.0@7.0; 7.0@7.0
Temp	21.9@21.9	23.7@23.7
D.O.	8.2@25.4°C	8.2@25.1°C
Cond	1081@1413; 1023@23.0 5882@1022; 9500@25675	1039@1022@24.4 9082@1005; 9925@1005

	Day 3	Day 7
pH	7.0@7.0; 7.0@7.0	7.0@7.0; 7.0@7.0
Temp	23.0@23.0	8.2@25.1°C
D.O.	8.2@25.3°C	23.1@23.1°C
Cond	1076@1032@24.4°C 9518@1025	1026@1032@25.1°C 9882@1025

	Day 4	Day 8
pH	7.0@7.0; 7.0@7.0	7.0@7.0; 7.0@7.0
Temp	21.4@21.4	23.3@22.9°C
D.O.	25.1@8.2	8.3@25.1°C
Cond	1032@1032@24.4	963@963@25.2
Meter #	9518@1025; 9440@1025	7698@1025 9925@1089

pH 094671
DO/Temp 29061
Cond 28149

DAY 5

Remarks:
pH 7.0@7.0; 7.0@7.0
Temp 21.5@21.5
D.O. 8.2@25.4°C
Cond 1010@1013.2
9770@1013
9300@9560@24.8°C

NOEC = 100%
IC_{20} cannot be calculated.

Investigators' Signatures

Reviewer

Appendix A:

Cyprinella leesdi test data and SRT data for the bioassays performed on samples of effluent collected from the City of Milton WWTP on January 26, 28, and 30, 1998.

Toxicant
~~Discharger~~: Milton inWTP Dates: 1/27/95

~~Location~~ species: Cyprinella leedsi Analyst: _____

No. Surviving Organisms

Conc:	Rep. No.	Day 1	2	3	4	5	6	7	Remarks
Control:		10	10	10	10	10	10	9 10	
CTC		10	10	10	10	10	10	10	
		10	10	9 10	9	9	9	8 10	
		10	10	10	9 10	9	9	7 20	
Conc:		10	10	10	9 10	9	8 10	8	
6.25%		10	10	10	8 20	8	8	8	
		10	10	10	10	10	10	8 20	
		10	10	10	10	10	10	10	
Conc:		10	10	10	9 10	9	7 20	6 10	
12.5%		10	10	10	10	9 10	9	8 10	
		10	10	10	10	10	9 10	9	
		10	10	10	10	10	9 10	8 10	
Conc:		10	10	10	7 30	7	7	7	
25%		10	~~10~~ 9 10	10	9 10	9	5 10	8	Day 2 = 10
		10	9 10	9	9	9	9	7 20	
		7 10	9	9	7 20	7	7	6 10	
Conc:		10	10	10	9 10	9	9	8 10	
50%		10	10	10	10	10	9 10	9	
		10	9 10	9	8 10	6 20	4 10 im	4 dd	
		10	10	10	10	10	10	8 20	
Conc:		10	9 10	8 10	7 10	7	7	6 10	
100%		10	10	10	7 30	7	7	~~8~~ 7 M	
		10	10	10	8 20	8	8	8	
		10	10	10	10	10	10	9 10	
Conc:									
Conc:									

Comments: Read and Initial by: GW FW FW MF MF FW JC

n
Mistake JC

Discharge: Milton Wastewater Treat. Test Date(s): 1/27/98 – 2/3/98 Drying Temperature (°C): 105
Species ~~Location~~: C. tredsj Weighing Date: Tare: 2/4/98 Initial: 2/5/98 Drying Time (h): 5 hr
Analyst: John Couch, Jr. / David Whiting

Conc:	Rep. No.	A Wgt. of tare ~~(mg)~~ g	B Dry wgt: tare and larvae ~~(mg)~~ g	B-A Total dry wgt of larvae ~~(mg)~~ g	C No. of original larvae ~~(mg)~~	(B-A)/C Mean dry wgt of larvae (mg)	Remarks
Control	A F1	0.02711	~~0.05370~~	0.00138	9	0.7866	0.03155 scale not zeroed properly on 2/5/98
	B F2	0.02987	0.03161	0.00177	10	0.7770	$\bar{x}$ = 0.7455
	C F3	0.02798	0.03164	0.00366	8	0.4575	
	D F4	0.02829	0.03118	0.00289	7	0.7125	
Conc:	A F5	0.02715	0.03075	0.00360	8	0.4500	
	B F6	0.02682	0.03049	0.00367	8	0.4587	
6.25%	C F7	0.02673	0.02943	0.00270	8	0.3375	
	D F8	0.02661	0.03096	0.00435	10	0.4350	
Conc:	A F9	0.02634	0.02874	0.00240	6	0.4000	
	B F10	0.02609	0.02893	0.00284	8	0.3550	
12.5%	C F11	0.02489	0.02897	0.00408	9	0.4533	
	D F12	0.02468	0.02846	0.00378	8	0.4725	
Conc:	A F13	0.02500	0.02666	0.00166	7	0.2371	
	B F14	0.02475	0.02748	0.00273	8	0.3412	
25.0%	C F15	0.02475	0.02846	0.00371	7	0.5300	
	D F16	0.02652	0.03048	0.00396	6	0.6600	
Conc:	A F17	0.02569	0.02927	0.00358	8	0.4475	
	B F18	0.02450	0.02853	0.00403	9	0.4477	
50.0%	C F19	0.02487	0.02646	0.00159	4	0.3975	
	D F20	0.02477	0.02885	0.00408	8	0.5100	
Conc:	A F21	0.02476	0.02849	0.00373	6	0.6216	
	B F22	0.02371	0.02721	0.00350	8	0.4375	$\bar{x}$ = 0.4799
100%	C F23	0.02463	0.02748	0.00285	8	0.3562	
	D F24	0.02468	0.02922	0.00454	9	0.5044	

	Tare	Bi, Wt + Tare
A1 after A5	0.02699	0.03156
A1 after A10	0.02696	0.03153
A1 after A15	0.02694	0.03156
A1 after A20	0.02703	0.03154

Toxicant: Milton WWTP	Test Organism: C. leedsi						Organism Batch # 7			
Test Number:	Date: 1/27/98			Hour of test: 8			Dilution H20 Batch #		Well Water	
	Before Renewal			Time: 14:30			After Renewal		Time: +5 No Time Recorded	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
CTL	A	8.1	24.9	8.1	245	B				
6.25%	A	8.0	25.0	8.1	250	B	7.9	25.0	7.1	250
12.50%	A	7.9	25.1	8.1	250	B	7.9	25.0	7.1	250
25.00%	A	7.8	25.1	8.1	245	B	7.9	25.0	6.9	250
50.00%	A	7.7	25.1	8.1	240	B				
100.00%	A	7.1	25.4	8.1	230	B				
Measured by/Recorded by		DW/FW	DW/FW	DW/FW	DW/FW					
Signatures	Comments									

Toxicant: Milton WWTP	Test Organism: C. reedsi						Organism Batch # 7			
Test Number:	Date: 1-28-98			Hour of test: 24			Dilution H20 Batch #		Well water	
	Before Renewal			Time: 11:30			After Renewal		Time: 12:00	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
CTL	B					B	7.8	25.5	7.6	255
6.25%	B	7.9	25.0	7.1	250	B	7.8	25.7	7.6	255
12.50%	B	7.9	25.0	7.1	250	B	7.8	25.7	7.7	250
25.00%	B	7.9	25.0	6.9	250	B	7.8	25.5	7.7	250
50.00%	B					B	7.8	25.4	7.7	245
100.00%	B					B	7.0	25.2	7.6	230
Measured by/Recorded by							DW/JC	DW/JC	DW/JC	DW/JC
Signatures	Comments									
John Couch Jr.	Pour out control + 12.5% before doing readings 1/28/98									

mt 5/12/98

Toxicant: Milton WWTP	Test Organism: C. leedsi						Organism Batch # 7			
Test Number:	Date: 1/29/98			Hour of test: 46			Dilution H20 Batch # Well water			
	Before Renewal			Time: 13:20			After Renewal		Time: 14:20	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
CTL	C	8.0	25.3	6.4	255	C	7.8	25.6	7.5	249
6.25%	C	8.0	26.3	6.4	261	C	7.9	25.9	7.5	260
12.50%	C	8.0	25.1	6.5	253	C	7.9	25.7	7.5	260
25.00%	C	8.0	25.0	6.5	247	C	7.8	25.4	7.5	260
50.00%	C	7.9	26.3	6.0	252	C	7.6	25.4	7.5	262
100.00%	C	7.7	25.1	6.0	238	C	7.2	24.8	7.5	241
Measured by/Recorded by		FW	FW	FW	FW		FW	FW	FW	FW
Signatures	Comments									

mf 5/12/98

Toxicant: Milton WWTP	Test Organism: C. leedsi						Organism Batch #	7		
Test Number:	Date: 1/30/98			Hour of test: ~~48:40~~ 72			Dilution H20 Batch #	Well water		
				Time written in hour space mfs 5/8/98						
	Before Renewal			Time: 10:10			After Renewal		Time: 11:40	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
CTL	D	8.1	25.5	6.8	2.57	D	7.8	25.6	7.6	250
6.25%	D	8.2	25.4	6.9	2.60	D	7.8	25.7	7.5	256
12.50%	D	8.1	25.1	6.7	2.61	D	7.8	25.7	7.5	256
25.00%	D	8.1	26.0	6.7	2.69	D	7.8	25.7	7.5	254
50.00%	D	7.9	27.2	6.4	2.79	D	7.7	25.5	7.5	251
100.00%	D	7.5	25.9	6.1	2.57	D	7.2	25.4	7.5	245
Measured by/Recorded by		JC/FW	JC	JC	~~FW~~ JC		FW/MF	FW/MF	FW/MF	FW/MF
Signatures: John Couch Jr, Fran Weindorf, Marshall Sanders	Comments									

10:40 cond X 100

mfs 5/12/98

Toxicant: Milton WWTP	Test Organism: C. reedsi						Organism Batch # 7			
Test Number:	Date: 1-31-98			Hour of test: 96			Dilution H20 Batch #		Well Water	
	Before Renewal			Time: 1435			After Renewal		Time: 1615	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
CTL	A	7.7	25.7	4.9	268	A	8.0	24.9	8.0	253
6.25%	A	7.7	25.7	4.6	265	A	7.9	25.3	7.8	256
12.50%	A	7.9	24.6	5.5	257	A	7.9	25.1	7.8	254
25.00%	A	7.7	25.5	4.7	261	A	7.8	24.9	7.7	253
50.00%	A	7.9	26.0	6.1	263	A	7.6	24.6	7.7	247
100.00%	A	7.5	26.0	4.5	259	A	7.0	24.2	7.8	241
Measured by/Recorded by	MF	MF	MF	MF	MF	MF	MF	MF	MF	MF
Signatures Marshall Faircloth	Comments			42X DO's MF 1/31/98						

MF 5/1/98

Toxicant: Milton WWTP	Test Organism: C. reedsi						Organism Batch # 7			
Test Number:	Date: 2-1-98			Hour of test: 120			Dilution H20 Batch #		Well Water	
	Before Renewal			Time: 0930			After Renewal		Time: 1015	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
-CTL	B	8.1	25.0	6.6 ~~6.5~~ A	255	B	7.8	25.4	7.7	254
6.25%	B	8.1	25.2	6.6	260	B	7.8	25.4	7.9	255
12.50%	B	8.1	25.9	6.5	264	B	7.9	25.5	7.8	256
25.00%	B	8.0	26.5	6.5	266	B	7.8	25.6	7.7	255
50.00%	B	7.8	26.4	6.7	261	B	7.7	25.1	7.6	249
100.00%	B	7.4	25.9	6.0	257	B	7.1	25.3	7.6	246
Measured by/Recorded by		MF/JC	MF/JC	MF/JC	MF/JC		MF/JC	MF/JC	MF/JC	MF/JC
Signatures Marshall Faircloth John Couch, Jr	Comments									

A Mistake JC

MF 5/12/98

Toxicant: Milton WWTP	Test Organism: C. reedsi						Organism Batch # 7			
Test Number:	Date: 5/2/98			Hour of test: 144			Dilution H20 Batch #		Well Water	
	Before Renewal			Time: 11:10			After Renewal		Time: 12:00	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
CTL	C	8.0	25.4	6.5	257	C	7.9	25.7	7.5	249
6.25%	C	8.0	24.9	6.5	257	C	7.9	26.0	7.7	257
12.50%	C	7.9	25.0	6.0	261	C	7.9	26.0	7.7	255
25.00%	C	7.8	27.4	6.0	274	C	7.8	26.7	7.6	257
50.00%	C	7.8	25.1	5.8	259	C	7.7	26.1	7.7	254
100.00%	C	7.5	25.0	5.8	256	C	7.2	25.9	7.7	250
Measured by/Recorded by		MF/FW	MF/FW	MF/FW	MF/FW	MF/FW	MF/FW	MF/FW	MF/FW	MF/FW
Signatures	Comments									

MF 5/12/98

Toxicant: milton micro P	Test Organism: Cyprinella leedsi						Organism Batch # 7			
Test Number:	Date: 2/3/98			Hour of test: 168			Dilution H20 Batch # well H2O			
	Before Renewal			Time: 1300			After Renewal		Time:	
Treatment	Rep	pH	Temp.C	D.O.	Cond.	Rep	pH	Temp.C	D.O.	Cond.
Control	D	8.0	25.5	6.5	255					
6.25%	D	8.0	25.6	6.6	255					
12.5%	D	7.9	25.7	6.1	255					
25%	D	7.9	25.6	5.8	260					
50%	D	7.7	27.0	5.5	270					
100%	D	7.5	25.8	5.7	255					
Measured by/Recorded by		MF/FW	MF/FW	MF/FW	MF					
Signatures Marshall Faircloth Tara Marshall	Comments									

MF 5/12/98

Appendix B:

Statistical analyses of test data for the bioassays performed on samples of effluent collected from the City of Milton WWTP on January 26, 28, and 30, 1998.

City of Milton WWTP C. dubia - reproduction
File: MilCds Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.953	14.278	22.538	14.278	3.953
OBSERVED	4	14	22	17	2

Calculated Chi-Square goodness of fit test statistic = 1.5026
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

City of Milton WWTP C. dubia - reproduction
File: MilCds Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 3.15
Closest, conservative, Table H statistic = 12.1 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 9
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 8.83
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

City of Milton WWTP C. dubia - reproduction
File: MilCds Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	224.659	44.932	4.099
Within (Error)	53	580.900	10.960	
Total	58	805.559		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

City of Milton WWTP C. dubia - reproduction
File: MilCds Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	19.000	19.000		
2	6.25	19.400	19.400	-0.270	
3	12.5	18.500	18.500	0.338	
4	25	22.667	22.667	-2.410	
5	50	21.800	21.800	-1.891	
6	100	23.600	23.600	-3.107	

Bonferroni T table value = 2.40 (1 Tailed Value, P=0.05, df=50,5)

City of Milton WWTP C. dubia - reproduction
File: MilCds Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	10			
2	6.25	10	3.559	18.7	-0.400
3	12.5	10	3.559	18.7	0.500
4	25	9	3.657	19.2	-3.667
5	50	10	3.559	18.7	-2.800
6	100	10	3.559	18.7	-4.600

City of Milton WWTP 1/26/98 C. leedsi
File: Mil198 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.507

W = 0.962

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

City of Milton WWTP 1/26/98 C. leedsi
File: Mil198 Transform: ARC SINE(SQUARE ROOT(Y))

Bartletts test for homogeneity of variance

Calculated B statistic = 2.59
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

City of Milton WWTP 1/26/98 C. leedsi
File: Mil198 Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.122	0.024	0.866
Within (Error)	18	0.507	0.028	
Total	23	0.629		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

City of Milton WWTP 1/26/98 C. leedsi
File: Mil198 Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	1.190	0.850		
2	6.25	1.183	0.850	0.055	
3	12.5	1.087	0.775	0.863	
4	25	0.994	0.700	1.651	
5	50	1.037	0.725	1.288	
6	100	1.087	0.775	0.863	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

City of Milton WWTP 1/26/98 C. leedsi
File: Mil198 Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	4			
2	6.25	4	0.244	28.8	0.000
3	12.5	4	0.244	28.8	0.075
4	25	4	0.244	28.8	0.150
5	50	4	0.244	28.8	0.125
6	100	4	0.244	28.8	0.075

City of Milton WWTP 1/26/98 C. leedsi-Growth
File: Mil198g Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.173

W = 0.969

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

City of Milton WWTP 1/26/98 C. leedsi-Growth
File: Mil198g Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 11.81
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

City of Milton WWTP 1/26/98 C. leedsi-Growth
File: Mil198g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.011	0.002	0.222
Within (Error)	18	0.173	0.010	
Total	23	0.183		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

City of Milton WWTP 1/26/98 C. leedsi-Growth
File: Mil198g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	0.458	0.458		
2	6.25	0.420	0.420	0.551	
3	12.5	0.420	0.420	0.553	
4	25	0.442	0.442	0.237	
5	50	0.451	0.451	0.113	
6	100	0.480	0.480	-0.310	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

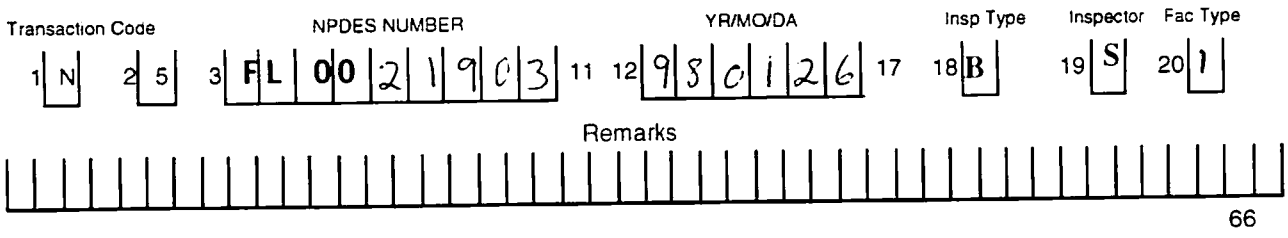
City of Milton WWTP 1/26/98 C. leedsi-Growth
File: Mil198g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	4			
2	6.25	4	0.167	36.4	0.038
3	12.5	4	0.167	36.4	0.038
4	25	4	0.167	36.4	0.016
5	50	4	0.167	36.4	0.008
6	100	4	0.167	36.4	-0.021

The Bioassay of the City of Milton WWTP effluent sampled on January 26, 28, and 30, 1998, NPDES #FL0021903.

Fill Out This Section For All Surface Water Discharger Inspections (CEI, CSI, CBI, PAI, XSI - RI Optional)



The Priority Pollutants Analysis for the Bioassay of the City of Milton WWTP effluent sampled on January 26, 28, and 30, 1998, NPDES #FL0021903.

Fill Out This Section For All Surface Water Discharger Inspections (CEI, CSI, CBI, PAI, XSI - RI Optional)

