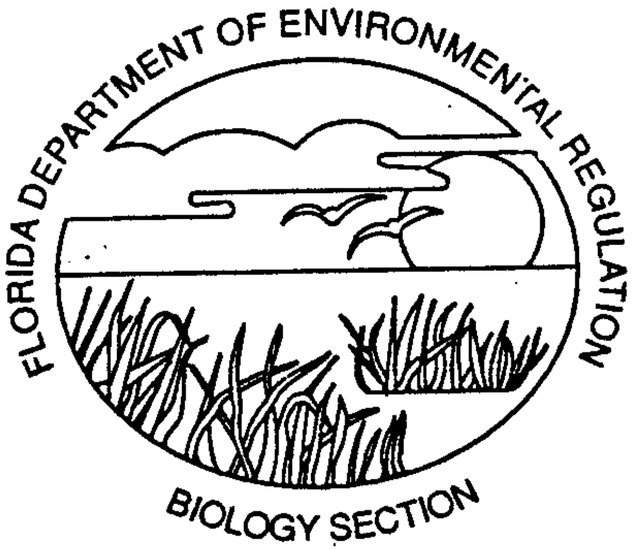


Bioassays of

City of Crestview Wastewater Treatment Plant

Crestview, Okaloosa County, Florida

NPDES #FL0027782

Sampled 8/1/88

January 10, 1989

Biology Section
Division of Administrative and Technical Services

Bioassays of

City of Crestview Wastewater Treatment Plant

Crestview, Okaloosa County, Florida

NPDES #FL0027782

Biology Section
Bureau of Laboratories
January 10, 1989

Executive Summary

City of Crestview Wastewater Treatment Plant, Crestview, Okaloosa County, Florida, NPDES #FL0027782 on 2 August to 4 August, 1988.

The sample of discharge from the Crestview WWTP was toxic to the bioassay test organism, *Ceriodaphnia dubia*, producing an LC_{50} of 39.6%. Three deaths were noted in the 100% sample tests with the bioassay test organism, *Daphnia pulex,* but an LC_{50} could not be calculated. Three random deaths occurred in the *Notropis leedsi* bioassay test. The sample adversely affected the Microtox® assay test organism, *Photobacterium phosphoreum*, producing an EC_{50} of 120.8%.

Chemical analyses of the sample detected in the pesticide extractables the pesticides, chlorpyrifos (0.2 µg/l), g-BHC (0.07 µg/l), and numerous unidentified components sensitive to electron capture (ECD) and nitrogen/phosphorus (NPD) detection. One unidentified compound was detected in the base neutral extractables analysis. The metals, arsenic (2.2 µg/l), silver (2.8 µg/l), and zinc (41.0 µg/l), were also detected in the sample. The sample contained no residual chlorine. The ammonia content of the sample was not determined.

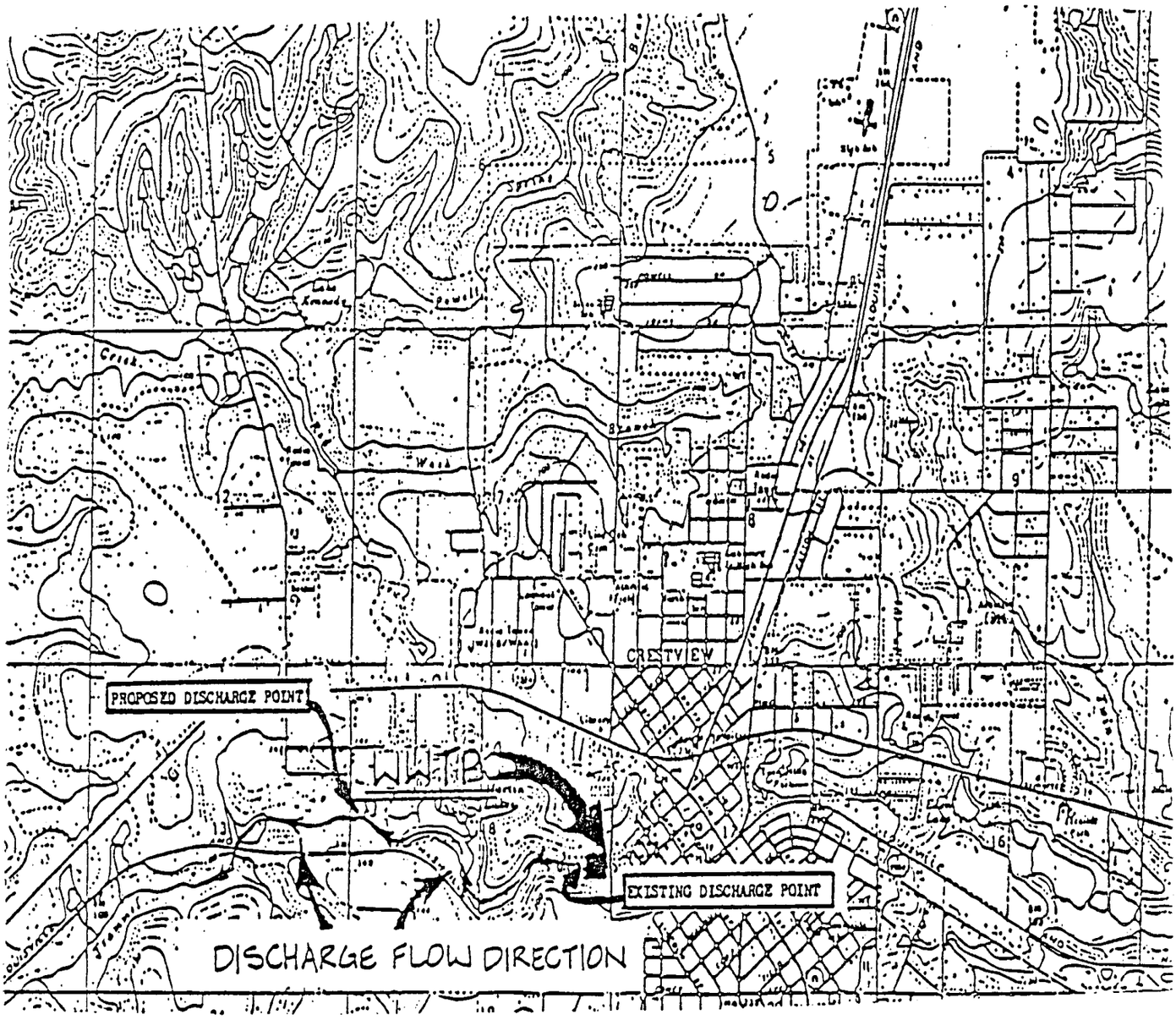
The chemical components detected in the sample, particularly the pesticide, chlorpyrifos, may have contributed to the observed mortality in the *C. dubia* and *D. pulex* tests and may have caused the adverse affect noted in the Microtox® assay.

Introduction

The Crestview Wastewater Treatment Plant is located at 351 North Lloyd Street, Crestview, Okaloosa County, Florida (Fig. 1). This 1.0 MGD design capacity municipal facility discharges an average of 0.88 MGD to Trammel Creek, which flows to the Yellow River. Treatment includes initial degritting, primary clarification, trickling filtration, final clarification, chlorination and dechlorination prior to discharge (Fig. 2).

In February, 1986, the Biology Section performed four static acute toxicity tests on a sample of discharge from the Crestview WWTP. The chlorinated sample produced an LC_{50} of < 10% in the *Daphnia pulex* test and an LC_{50} of 42.3% in the *Notropis leedsi* test. The dechlorinated sample tests produced an LC_{50} of 45.3% in the *D. pulex* test and several deaths in the *N. leedsi* test. Toxicity was attributed to the total residual chlorine (0.8 mg/l Hach) and to the pesticide, chlorpyrifos (0.39 µg/l).

In order to evaluate the existence and the extent of toxicity in the discharge from this facility, at the request of the FDER Northwest District Office in Pensacola, the Biology Section performed three static acute toxicity bioassays and one Microtox® assay on a sample of discharge from the facility from 2 August to 4 August, 1988. The results of those tests are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, L. A. Williams, and S. H. Wolfe.



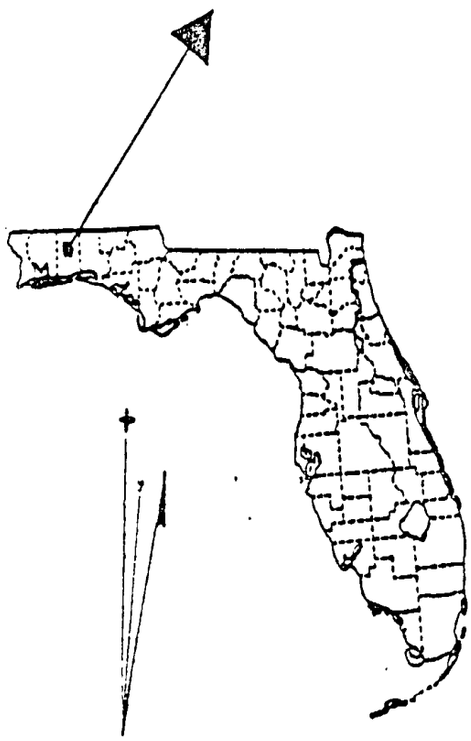


Figure 1. Portions of Okaloosa County, Florida, including the City of Crestview and the location of the sewage treatment plant

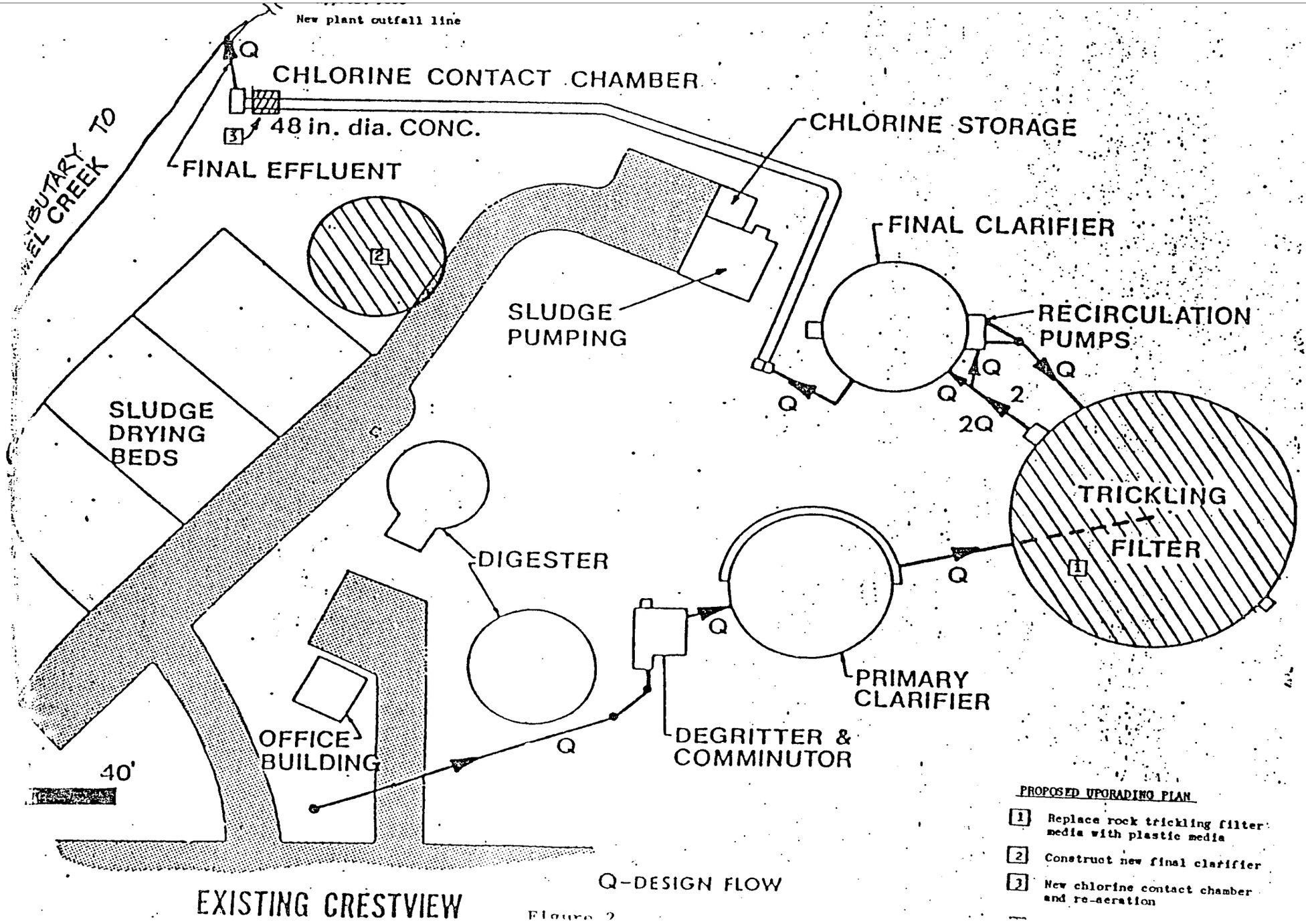

New plant outfall line
Q
CHLORINE CONTACT CHAMBER
48 in. dia. CONC.
3
FINAL EFFLUENT
TO
TRIBUTARY
EL CREEK
CHLORINE STORAGE
FINAL CLARIFIER
2
SLUDGE
PUMPING
RECIRCULATION
PUMPS
Q
Q
Q
2
2Q
SLUDGE
DRYING
BEDS
TRICKLING
FILTER
1
DIGESTER
Q
Q
PRIMARY
CLARIFIER
OFFICE
BUILDING
Q
DEGRITTER &
COMMINUTOR
40'
Q-DESIGN FLOW
EXISTING CRESTVIEW
PROPOSED UPGRADING PLAN
1 Replace rock trickling filter media with plastic media
2 Construct new final clarifier
3 New chlorine contact chamber and re-aeration

Figure 2

Methods and Materials

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include a species recommended by Peltier and Weber (1985) and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

1. History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea)—A breeding culture of *Daphnia pulex* was obtained from the EPA laboratory in Athens, Georgia, in June, 1987. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22 ±2 °C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Ceriodaphnia dubia (water flea)—A breeding culture of *Ceriodaphnia dubia* was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained in 2-1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26 ±2 °C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner)—Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22 ±2 °C under a 16:8 hour light-dark cycle and are fed Aquarian® flake food and frozen *Artemia* (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25 °C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched *Artemia* nauplii and finely crushed Aquarian® flake food. The fish used were 17 days old when the test commenced.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay.

Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox Reconstitution Solution).

2. Standard Reference Toxicity Assays

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

3. Test Methods

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Three separate 48 hour acute toxicity bioassays and one Microtox® assay were performed on a sample from the City of Crestview WWTP facility. The tests were performed from 1300 hours on 2 August, to 1300 hours on 4 August, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with $HN0_3$. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Ms. Toni Touart of the FDER Northwest District Office in Pensacola at 1300 hours on 1 August, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Test Temperature

Upon arrival of the iced (4 °C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20 ± 2 °C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Method

Freshwater for diluting the water samples being tested was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Four replicates consisting of 30 ml plastic vials containing 15 mls of the control and each test concentration were used in bioassays with the water flea, *Ceriodaphnia dubia*. Two replicates consisting of 250 ml flint glass jars containing 100 mls of the control and each test concentration were used with the water flea, *Daphnia pulex*. Adult *Ceriodaphnia* and *Daphnia* were separated using a nylon mesh sieve and placed into large culture dishes 18 hrs before the beginning of the test. Neonates produced during the intervening period (i.e., <18 hr old) were then captured by glass pipette, examined for injury, and transferred into the test chambers. Five (for *C. dubia*) or ten individuals (for *D. pulex*) were loaded per chamber, resulting in 20 per test concentration. The water fleas were not fed during these tests.

Two replicates of each concentration and a control were used for the *Notropis leedsi* (bannerfin shiner) bioassay. The fish were transferred individually or by twos using a pipette into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Five were loaded per chamber, resulting in 10 per concentration. Fish were not fed during this test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using Hach Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% reclaimed water concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated before use according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% reclaimed water test concentration at the beginning of the test. Total ammonia concentrations were determined using an Orion specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected for Microtox® analysis. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in a test range of 99%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

Results and Discussion

The sample of discharge from the City of Crestview WWTP was toxic to the bioassay test organism, *Ceriodaphnia dubia,* and adversely affected the Microtox® organism, *Photobacterium phoshoreum*, producing an LC_{50} of 39.6% (Table 4) and an EC_{50} of 120.8% (Table 5), respectively. Three deaths were noted in the 100% sample test with the bioassay organism, *Daphnia pulex*; however, an LC_{50} could not be calculated. Three random deaths were also noted in the *Notropis leedsi* bioassay (Table 2).

Chemical analyses were performed on the sample. In the pesticide extractables portion of the organics analyses, the pesticides, chlorpyrifos (0.2 µg/l), g-BHC (Lindane) (0.07 µg/l), and numerous unidentified components sensitive to electron capture (ECD) and nitrogen/phosphorus (NPD) detection. One unidentified compound was detected in the base neutral extractables organics analyses. The metals, arsenic (2.2 µg/l), silver (2.8 µg/l), and zinc (41.0 µg/l) were also detected in the sample (Table 6). The sample did not contain any residual chlorine (Hach method). The ammonia content of the sample was not determined.

It is thought that the chemical components of the sample, particularly the pesticides, contributed to the toxicity of the sample. LC_{50} values for chlorpyrifos have been reported as low as 0.11 µg/l for several macroinvertebrate crustaceans (USDI, 1980); thus, it is likely that chlorpyrifos was responsible for a significant portion of the noted toxicity.

In conclusion, the sample of discharge from the City of Crestview WWTP, was toxic on 1 August, 1988.

Biology Section Recommendations

The Biology Section recommends that the facility be required to begin a Toxicity Reduction Evaluation with the goal of meeting state water quality standards.

Table 1. Concentration Series and Volumes Used for These Static Bioassays.

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	15.0 ml	0.0 ml	15 ml
10.0%	13.5 ml	1.5 ml	15 ml
30.0%	10.5 ml	4.5 ml	15 ml
50.0%	7.5 ml	7.5 ml	15 ml
70.0%	4.5 ml	10.5 ml	15 ml
85.0%	2.25 ml	12.75 ml	15 ml
100.0%	0.0 ml	15.0 ml	15 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	15.0 ml	0.0ml	15 ml
100.0%	0.0 ml	15.0 ml	15 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	0 ml	500 ml
10.0%	450 ml	50 ml	500 ml
30.0%	350 ml	150 ml	500 ml
50.0%	250 ml	250 ml	500 ml
70.0%	150 ml	350 ml	500 ml
85.0%	75 ml	425 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during the 48 hour static acute bioassay of a sample of discharge from the City of Crestview WWTP, Crestview, Okaloosa County, Florida, NPDES #FL0027782, on 2 to 4 August, 1988.

Facility: **Crestview WWTP** NPDES # **Fl0027782** Facility Type: **Municipal WWTP**
Location: 351 N. Lloyd St. Crestview Contact/District: T. Touart
County: Okaloosa Test type: **definitive**
Sample Collection Date: **8/1/88** Time: 1300 # tests: 3 Receiving Water: unnamed trib. to Trammel Ck. to Yellow River
Test Beginning Date: 8/2/88 Time: 1300 Chlorination Type: **dechlorinated**
Test Ending Date: 8/4/88 Time: 1300

Total Residual Cl2	mg/l	Method
Field:	0.0	DR-100
Lab:	0.0	Hach
Lab:	-	-

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	-	-
Cond. Control Water (daphnids):	-	-
100% Sample:	-	-

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	122	112
Cond. Control Water (daphnids):	137	122
100% Sample:	60	89

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: - Binomial: 39.6% Moving Average: 36.1% Probit: 39.7%

Concentrations	SURVIVAL # at 0 hr	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	5	5	9.1	-	8.4	7.9	-	7.9	19.3	-	19.7	-	-
Control	5	5	5	9.1	-	8.4	7.9	-	7.9	-	-	-	-	-
Control	5	5	5	9.1	-	8.4	7.9	-	7.9	-	-	-	-	-
Control	5	5	5	9.1	-	8.4	7.9	-	7.9	-	-	-	205	196
10%	5	5	5	9.2	-	8.4	7.8	-	7.9	19.3	-	19.7	-	-
10%	5	5	5	9.2	-	8.5	7.8	-	7.9	-	-	-	-	-
10%	5	5	5	9.2	-	8.5	7.8	-	7.9	-	-	-	-	-
10%	5	5	5	9.2	-	8.5	7.8	-	7.9	-	-	-	238	256
30%	5	5	5	9.2	-	8.5	7.4	-	7.8	19.4	-	19.6	-	-
30%	5	5	5	9.2	-	8.5	7.4	-	7.8	-	-	-	-	-
30%	5	5	4	9.2	-	8.4	7.4	-	7.7	-	-	-	-	-
30%	5	5	4	9.2	-	8.4	7.4	-	7.7	-	-	-	269	284
50%	5	2	1	9.1	-	8.4	7.1	-	7.6	19.6	-	19.3	-	-
50%	5	1	1	9.1	-	8.4	7.1	-	7.6	-	-	-	-	-
50%	5	2	1	9.1	-	8.4	7.1	-	7.6	-	-	-	-	-
50%	5	1	0	9.1	-	8.4	7.1	-	7.6	-	-	-	310	323
70%	5	0	0	9.0	9.1	-	6.8	7.5	-	19.8	19.5	-	-	-
70%	5	0	0	9.0	9.2	-	6.8	7.5	-	-	-	-	-	-
70%	5	0	0	9.0	9.2	-	6.8	7.5	-	-	-	-	-	-
70%	5	0	0	8.9	9.3	-	6.8	7.6	-	-	-	-	348	-
85%	5	0	0	8.8	9.3	-	6.7	7.5	-	20.1	19.7	-	-	-
85%	5	0	0	8.8	9.3	-	6.7	7.5	-	-	-	-	-	-
85%	5	0	0	8.8	9.4	-	6.7	7.5	-	-	-	-	-	-
85%	5	0	0	8.8	9.4	-	6.7	7.5	-	-	-	-	375	-
100%	5	0	0	8.8	9.4	-	6.5	7.3	-	20.4	19.7	-	-	-
100%	5	0	0	8.8	9.4	-	6.5	7.3	-	-	-	-	-	-
100%	5	0	0	8.8	9.4	-	6.5	7.3	-	-	-	-	-	-
100%	5	0	0	8.8	9.4	-	6.5	7.3	-	-	-	-	410	-

Organism: ***Daphnia pulex*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

Concentrations	SURVIVAL # at 0 hr	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	10	10	10	9.1	-	8.3	8.0	-	7.9	19.8	-	19.3	-	-
Control	10	10	10	9.1	-	8.4	8.0	-	7.9	-	-	-	210	245
100%	10	10	8	8.2	-	8.4	6.4	-	7.3	21.4	-	19.2	-	-
100%	10	10	9	8.1	-	8.4	6.4	-	7.3	-	-	-	425	397

Table 2. Continued.

Facility:	**Crestview WWTP**		NPDES #	**FI0027782**	Facility Type: **Municipal WWTP**
Location:	351 N. Lloyd St. Crestview		Contact/District:	T. Touart	
County:	Okaloosa		Test type:	definitive	unnamed trib. to
Sample Collection Date:	**8/1/88**	Time: 1300	# tests:	3	Receiving Water: Trammel Ck. to
Test Beginning Date:	8/2/88	Time: 1300	Chlorination Type:	**dechlorinated**	Yellow River
Test Ending Date:	8/4/88	Time: 1300			**Page 2 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.0	DR-100
Lab:	0.0	Hach
Lab:	-	-

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	-	-
Cond. Control Water (daphnids):	-	-
100% Sample:	-	-

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	122	112
Cond. Control Water (daphnids):	137	122
100% Sample:	60	89

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Notropis leedsi*** Age: 17 days
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.8	9.1	8.3	7.6	7.7	7.8	21.0	18.9	19.0	-	-
Control	5	5	5	8.8	9.1	8.3	7.6	7.7	7.8	-	-	-	222	224
10%	5	5	5	8.8	9.1	8.3	7.4	7.6	7.7	21.1	18.7	18.9	-	-
10%	5	4	4	8.8	9.1	8.3	7.3	7.6	7.7	-	-	-	243	236
30%	5	5	5	8.8	9.0	8.3	6.9	7.4	7.6	21.4	18.6	18.8	-	-
30%	5	5	5	8.8	9.0	8.2	6.9	7.4	7.6	-	-	-	285	272
50%	5	5	5	8.5	8.9	8.4	6.7	7.2	7.5	22.0	18.6	18.8	-	-
50%	5	5	5	8.5	8.8	8.4	6.7	7.2	7.5	-	-	-	330	313
70%	5	5	5	8.2	8.4	8.3	6.5	7.0	7.3	22.6	18.6	18.8	-	-
70%	5	5	5	8.2	8.4	8.2	6.5	7.0	7.3	-	-	-	375	355
85%	5	4	4	8.1	8.4	8.1	6.4	6.9	7.1	23.1	18.6	18.7	-	-
85%	5	5	5	8.1	8.4	8.1	6.4	6.9	7.1	-	-	-	411	385
100%	5	5	5	8.0	8.1	7.9	6.3	6.7	6.9	23.4	18.8	18.7	-	-
100%	5	4	4	7.9	8.1	7.9	6.3	6.7	6.9	-	-	-	445	415

Table 3. **Microtox test results, Crestview WWTP, Okaloosa County, NPDES #FL0027782, on 2 August, 1988.**

Microtox Test — Culture Lot # M-606

% Sample	Final Reclaimed Water nonchlorinated	
	5-min	15-min
0	95	65
0	98	79
0	86	62
12.38	90	65
12.38	95	59
12.38	76	48
24.75	73	43
24.75	87	51
24.75	82	47
49.5	85	47
49.5	84	43
49.5	87	43
99	80	40
99	83	40
99	82	39

EC_{50} Nonchlorinated (15 minute, 15 °C) =120.8% sample

EC_{50} (15 minute, 15 °C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Table 4. LC_{50} Data for Bioassay

Organism	Method of Data Analysis	48 Hour LC_{50} (95% Confidence Intervals)
Chlorinated discharge		
Ceriodaphnia dubia	Binomial	39.59% (30.00% - 50.00%)
	Moving Average	36.12% (28.08% - 41.46%)
	Probit	39.65% (35.15% - 44.28%)
Daphnia pulex	Binomial	a
	Moving Average	a
	Probit	a
Notropis leedsi	Binomial	not toxic
	Moving Average	not toxic
	Probit	not toxic

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

Table 5. Results of chemical analyses on the sample of discharge from the City of Crestview WWTP, Crestview, Okaloosa County, Florida.

Organics

Acid Extractables	None Detected
Base Neutral Extractables	One unidentified compound
Pesticide Extractables	Chlorpyrifos (0.2µg/l)
	g-BHC (0.07µg/l)
	Numerous unidentified components sensitive to ECD and NPD

Metals

Arsenic	2.2 µg/l
Silver	2.8 µg/l
Zinc	41.0 µg/l

Literature Cited

Beckman Instruments, Inc. 1982. Microtox system operating manual.

Florida Department of Environmental Regulation. 1985. Quality assurance manual for performing acute toxicity tests (Second Edition). Biology Section, Tallahassee.

_____. 1986. Bioassays of the City of Crestview Sewage Treatment Plant, Okaloosa County, Florida, NPDES #FL0027782. Biology Section, Tallahassee. 17 p.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

U.S. Department of the Interior. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Fish and Wildlife Service/Resource Publication 137. Washington, D.C. 98 p.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly. Applicable portions of the F.A.C. are: 17-3.021(1), 17-3.021(4), 17-3.051(1)(d), 17-3.061(3)(p), 17-3.402(1), 17-4.244(4), and 17-4.244(6).

The particular restrictions applied to a given discharger depend upon the type of discharge, the type of water body which is impacted, and the type of mixing zone, if any, which has been permitted. The most stringent toxicity limitations which apply to the various combinations of these factors are listed below, specified in terms of the most dilute concentration of effluent which is allowed to kill 50% of the test organisms in 96 hours. Note that any listed concentration greater than 100% is extrapolated from toxicity curves determined from testing various effluent concentrations of 100% or less. Violations of the toxicity standards occur in the following conditions.

I. Discharges to Surface Water

   A. With no mixing zone, or with only a dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 2000\%$ at the point of discharge.

   B. With a non-dredge and fill mixing zone the discharge may not exhibit an $LC_{50} < 100\%$ at the point of discharge and may not exhibit an $LC_{50} < 2000\%$ at the edge of the mixing zone.

   C. With a non-dredge and fill mixing zone, and either discharging to the open ocean through multiport diffusers or meeting the provisions of 17-4.244(4)(b) the LC_{50} at the point of discharge cannot exceed 30% sample concentration.

II. Discharges to ground water may not exceed an LC_{50} of 300% at the point of contact with surface waters

A violation of these provisions occurs if toxicity is found at a more dilute concentration of the sampled discharge than allowed above.

MICROTOX™ Data Reduction Sheet

SOURCE: Crestview STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 120.8 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent

DATE ASSAYED: Aug 2 88 TIME: 9:15 Am

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE:

OPERATOR: Tabler Williams REAGENT VIAL LOT NO. M-606

REMARKS: no Rs'd

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	95	65	X	X	
B1		98	79			
C1		96	62			
A2	12.38	90	65	.029	.198	
B2		95	59			
C2		76	48			
A3	24.75	73	43	.194	.461	
B3		87	51			
C3		82	47			
A4	49.5	85	47	.129	.549	
B4		84	43			
C4		87	43			
A5	99	80	40	.180	.731	
B5		83	40			
C5		82	39			
EC_{50} (MIN, °C)				1843	120.8	

REVIEWED BY:
DATE:

Crestview WWTP
351 N. Lloyd St.
Toxic Smart INW
FL0027782

1 3

Control 004

8-1-88 1300
8-2-88 1300
8-3-88 1300

Test Organism: Ceriodaphnia dubia
Age/Life Stage: neonate

Solution	Test Concentration Number	Number of Organisms Surviving				Dissolved Oxygen (mg/l)				Temperature °C				Conductivity				Alkalinity/Hardness (mg/l as CaCO3)
		0	24	48	72	0	24	48	96	0	24	48	96	0	24	48	96	
Control	1/2	5/5	5/5	5/5		9.1/9.1	8.4/8.4			19.3	19.7							
Control	3/4	5/5	5/5	5/5		9.1/9.1	8.4/8.4							205		196		
10%	5/6	5/5	5/5	5/5		9.2/9.2	8.4/8.5			19.3	19.7							
10%	7/8	5/5	5/5	5/5		9.2/9.2	8.5/8.5							238		256		
30%	9/10	5/5	5/5	5/5		9.2/9.2	8.5/8.5			19.4	19.6							
30%	11/12	5/5	5/5	4/4		9.2/9.2	8.4/8.4							269		281		
50%	13/14	5/5	2/1	1/1		9.1/9.1	8.4/8.4			19.6	19.3							
50%	15/16	5/5	1/2	1/0		9.1/9.1	8.4/8.4							310		323		
70%	17/18	5/5	0/0	0/0		9.0/9.0	9.1/9.2			19.8	19.5							
70%	19/20	5/5	0/0	0/0		9.0/8.9	9.2/9.3							348				
85%	21/22	5/5	0/0	0/0		8.8/8.8	9.3/9.3			20.1	19.7							
85%	23/24	5/5	0/0	0/0		8.8/8.8	9.4/9.4							375				
100%	25/26	5/5	0/0	0/0		8.8/8.8	9.4/9.4			20.4	19.7							
100%	27/28	5/5	0/0	0/0		8.8/8.8	9.4/9.4							410				
Initial of Investigator		KML	SHW	mf		JMF	JMF	RBT		JMF	JMF	RBT		JMF	JMF	RBT		

Conductivity

Control Water: 137/122
100% Sample: 60/89
WCU WATER 122/112
Measured by: SHW

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour____

Control Water:
100% Sample:
Other:
Measured by:____

Signatures:
Steven H Wolfe
KM Lurding
Darrell Faircloth
Ruud Snyderburg

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7@7.9@9	9.0@21°C	1272 at 20.3 (1280)
24 Hours	7@7.9@9	9.2@20°C	
48 Hours	7@7.9@9	9.2@20°C	1165@20.1 (1275)
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 0.0 Method: DR-100
Lab: 0.0 Method: HACH
Lab:____ Method:____ Reviewed by:____

De-chlorinated:
Measurements by: KML

Date:____

Remarks: Conductivity meter check
100.0@100.0 1000@1000.0
10.00@10.0
1.01@1.0

Industry/Toxicant Crestview WWTP
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 2 of 3
Test Type: Static Renewal (Screening) Conventional
Other:

Sample Collection: Date 8-1-88 Time 1300
Test Beginning: Date 8-2-88 Time 1300
Test Ending: Date 8-3-88 Time 1300
Test Organism Daphnia pulex
Age/Life Stage neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	—	10	10	10			9.1		8.3			8.0		7.9			19.8		19.3							
Control	27	10	10	10			9.1		8.4			8.0		7.9								210		245		
100%	112	10	10	8			8.2		8.4			6.4		7.3			21.4		19.2							
100%	1	10	10	9			8.1		8.4			6.4		7.3								425		397		
Initial of Investigator		JMF	SHW	MF			JMF		ABH			JMF		RSH			JMF		JMF			JMF		RBH		

Alkalinity/Hardness (mg/l as $CaCO_3$)

Control Water:
100% Sample:
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour

Control Water:
100% Sample:
Other:
Measured by:

Signatures:
Steven H Wolfe
K M Lurding
Marshall Faircloth
Russell Hagedorn

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: Method:
Lab: Method:
Lab: Method:

Reviewed by:

Dechlorinated:
Measurements by:

Date:

Crestview WWTP

Test Number: 3 3

8-1-88 1300
8-2-88 1300
8-3-88 1300

Test Organism: Notropis leedsi
Age/Life Stage: 17 days

Conc. or Ctl	Beg. Container Number	Number of Fish Organisms Exposed 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96	Total Residual Chlorine (mg/l as CaCO3) 0	24	48	72	96
Control	99	5	5	5			8.8	9.1	8.3			7.6	7.7	7.8			21.0	18.9	19.0												
Control	5	5	5	5			8.8	9.1	8.3			7.6	7.7	7.8								222		221			Control Water:				
10%	120	5	5	5			8.8	9.1	8.3			7.4	7.6	7.7			21.1	18.7	18.9								100% Sample:				
10%	113	6	4	4			8.8	9.1	8.3			7.3	7.6	7.7								243		236			Other:				
30%	52	5	5	5			8.8	9.0	8.3			6.9	7.4	7.6			21.4	18.6	18.8								Measured by:				
30%	228	5	5	5			8.8	9.0	8.2			6.9	7.4	7.6								285		272			Ammonia unionized/total (mg/l as NH3/N) Hour 0				
50%	67	5	5	5			8.5	8.9	8.4			6.7	7.2	7.5			22.0	18.6	18.8								Control Water:				
50%	25	5	5	5			8.5	8.8	8.4			6.7	7.2	7.5								330		313			100% Sample:				
70%	2	5	5	5			8.2	8.4	8.3			6.5	7.0	7.3			22.6	18.6	18.8								Other:				
70%	38	5	5	5			8.2	8.4	8.2			6.5	7.0	7.3								375		355			Hour				
85%	10	5	4	4			8.1	8.4	8.1			6.4	6.9	7.1			23.1	18.6	18.7								Control Water:				
85%	—	5	5	5			8.1	8.4	8.1			6.4	6.9	7.1								411		385			100% Sample:				
100%	86	5	5	5			8.0	8.1	7.9			6.3	6.7	6.9			23.4	18.8	18.7								Other:				
100%	18	5	4	4			7.9	8.1	7.9			6.3	6.7	6.9								445		415			Measured by:				
Initial of Investigator		JMF	SHW	AF			JMF	JMF	RBF			JMF	JMF	RBF			JMF	JMF	JMF			JMF		RBF							

Signatures:
Steven H Wilfe
Al Lurding
Marshall Faribit
Russel Frydenborg

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: _____ Method: _____
Lab: _____ Method: _____
Lab: _____ Method: _____ Reviewed by: _____

De-chlorinated:
Measurements by: _____ Date: _____