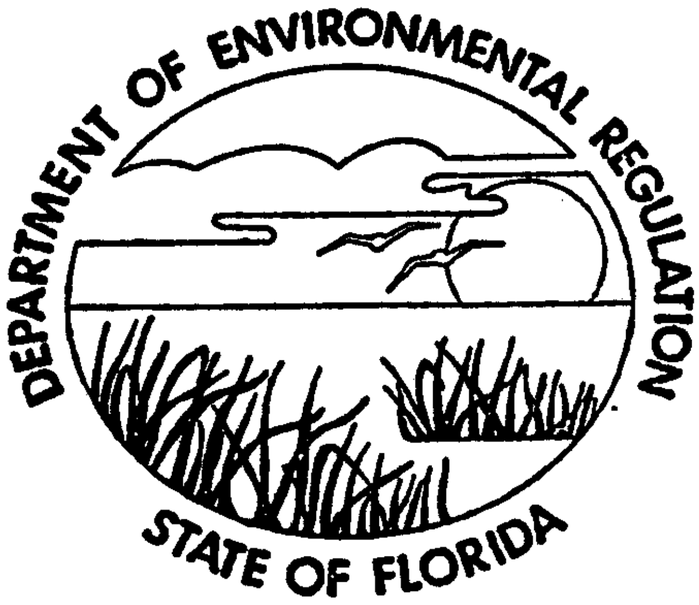


BIOASSAYS OF THE CITY OF CRESTVIEW

SEWAGE TREATMENT PLANT

OKALOOSA COUNTY, FLORIDA

NPDES #FL0027782

SAMPLED 2/3/86

JUNE 6, 1986

Biology Section

Division of Environmental Programs

BIOASSAYS OF THE CITY OF CRESTVIEW

SEWAGE TREATMENT PLANT

OKALOOSA COUNTY, FLORIDA

NPDES #FL0027782

Biology Section
Bureau of Laboratories and
Special Programs
June 6, 1986

EXECUTIVE SUMMARY

Crestview STP, Okaloosa County, NPDES #FL0027782, on 4 to 6 February, 1986.

The sample of final chlorinated effluent from this 1.0 MGD design capacity municipal waste treatment facility proved to be acutely toxic to the test organisms, Daphnia pulex and Notropis leedsi. The 48 hour static tests resulted in LC_{50}'s of < 10.0% and 42.3%, respectively. This toxicity can partly be attributed to the residual chlorine (0.8 mg/l) measured in the sample. This same effluent, after de-chlorination, was still acutely toxic to D. pulex (48 hour LC_{50} = 45.3%). Mortality was also noted in the N. leedsi test, but it was insufficient to calculate an LC_{50}. The extent of toxicity in the chlorinated effluent, and the presence of toxicity in the de-chlorinated effluent, indicates that the Crestview STP effluent contains toxic components in addition to chlorine. The ammonia measured in this effluent (7.3 mg/l total and 0.08 mg/l unionized) was insufficient to have contributed significantly to the toxicity.

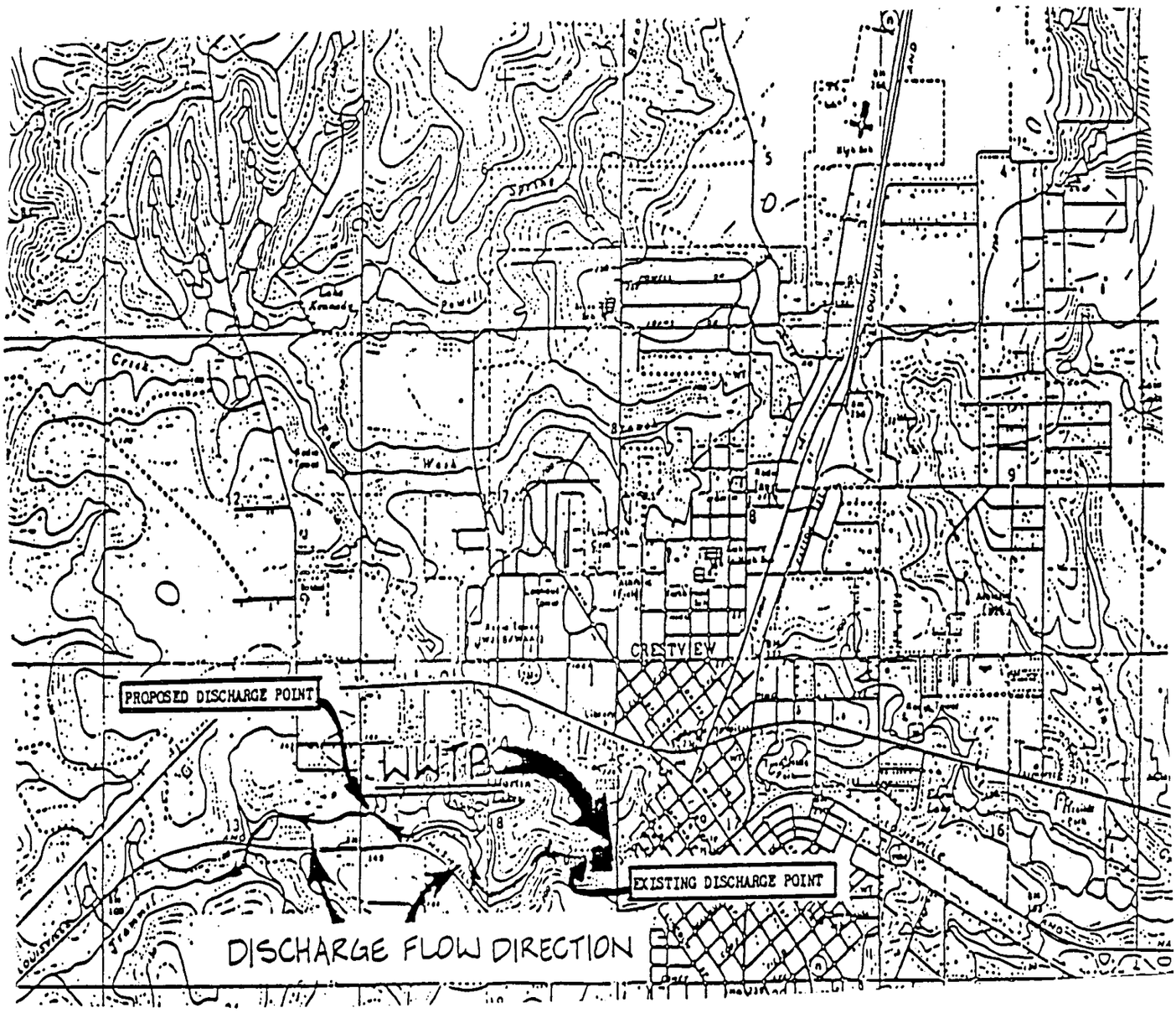
The results of chemical analyses indicated the presence of the pesticide chlorpyrifos (0.39 ug/l), and the metals silver and zinc in the Crestview effluent. Since reported LC_{50} values for chlorpyrifos for several macroinvertebrate crustaceans are as low as 0.11 ug/l, it is likely that this pesticide was responsible for a significant portion of the noted toxicity.

Due to the toxic nature of the Crestview STP effluent, we recommend that the facility undertake a toxicity identification and elimination plan, emphasizing on the elimination of the chloropyrifos, and demonstrate compliance with the total residual chlorine standard in the receiving waters. Additionally, a biomonitoring program should be incorporated into this facility's permit.

INTRODUCTION

The Crestview Sewage 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 clarifications, and chlorination prior to discharge (Fig. 2).

At the request of the Northwest District, the Biology Section performed static acute toxicity bioassays on a sample of this effluent, to evaluate the existence and extent of toxicity. Tests were performed from 4 to 6 February, 1986. The results are presented in this report. Those involved in performing the tests and in preparation of this report were: M. L. Roll and P. G. Weiland.



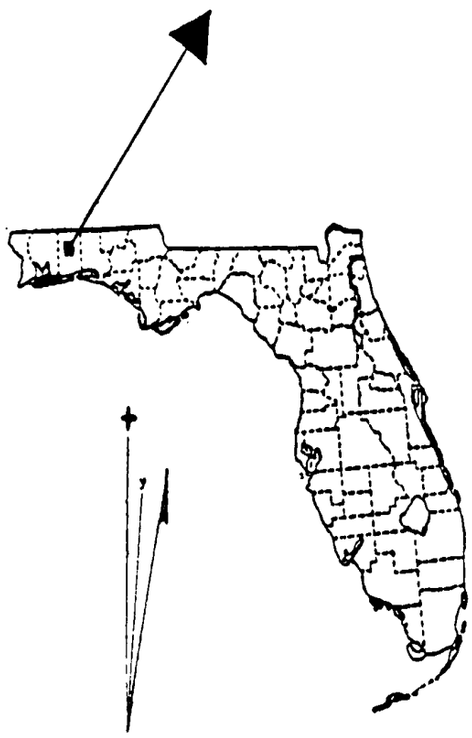


Figure 1. Portions of Okaloosa County, Florida, including the City of Crestview and the location of the sewage treatment plant

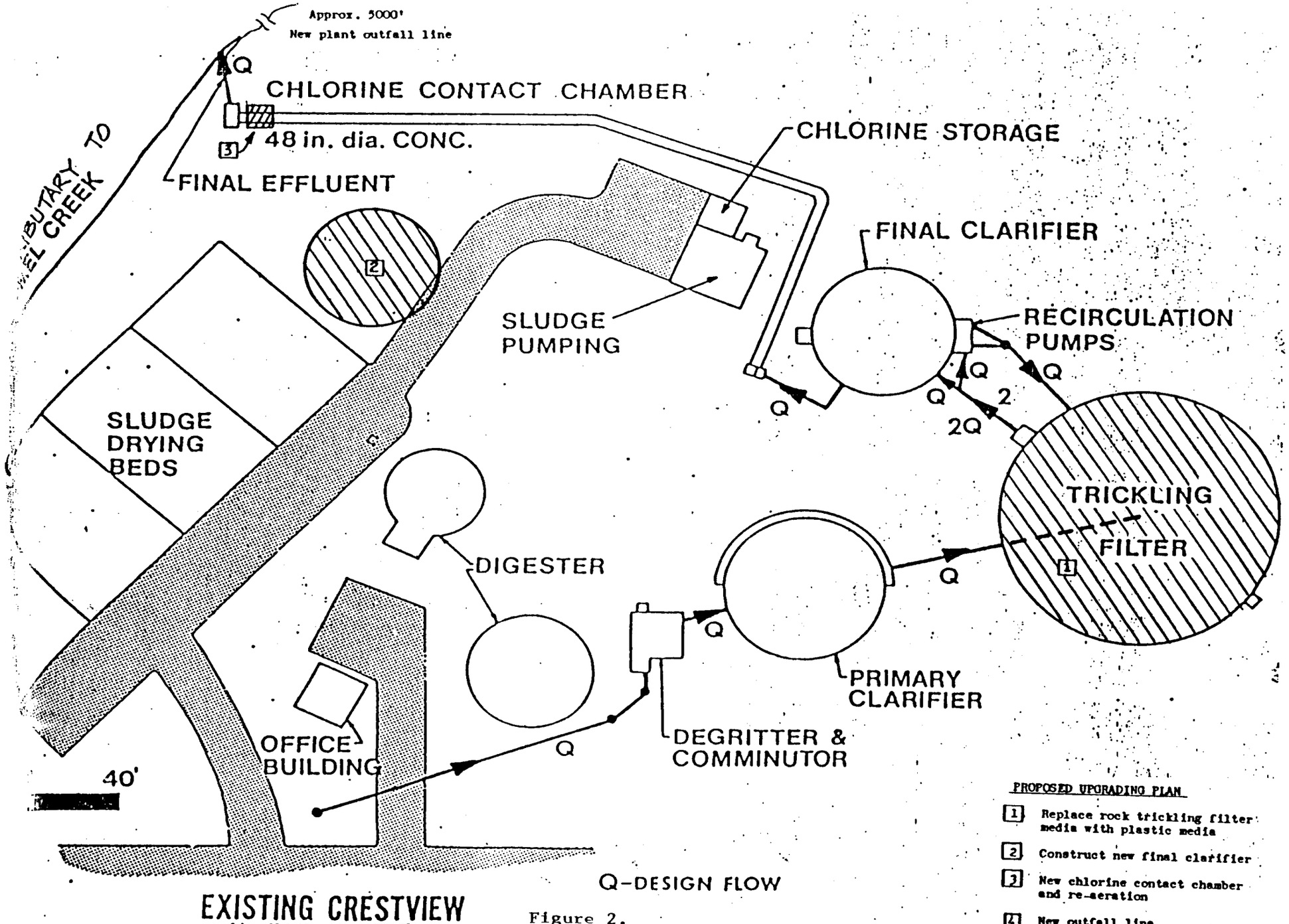

Approx. 5000'
New plant outfall line
Q
CHLORINE CONTACT CHAMBER
48 in. dia. CONC.
3
FINAL EFFLUENT
TO
TRIBUTARY
EL CREEK
CHLORINE STORAGE
FINAL CLARIFIER
2
SLUDGE
PUMPING
RECIRCULATION
PUMPS
Q
Q
Q
Q
2
2Q
SLUDGE
DRYING
BEDS
TRICKLING
FILTER
1
DIGESTER
Q
Q
PRIMARY
CLARIFIER
OFFICE
BUILDING
Q
DEGRITTER &
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
4 New outfall line

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 recommeded by Peltier and Weber (1985), the U.S. EPA (1978), 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.

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 March, 1979. 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.

Notorpis 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 30 and 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 cycle and are fed Tetra-Min® 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 liquified blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely curshed Tetra Min®. The fish used in this test were approximately 29 day old at the time of the test.

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.

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

A total of four separate 48 hour static acute toxicity bioassays were performed on samples of chlorinated and de-chlorinated effluents collected from the Crestview STP. The tests were performed from 1100 hours on 4 February, to 1120 hours on 6 February, 1986.

The samples were collected by Mr. Rick Bradburn of DER's Northwest District at 1210 hours on 3 Febraury, 1986, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for organic chemical analyses and for the bioassays were unpreserved. 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° $\pm$ 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 oranisms 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.

Aeration

Since the dissovled oxygen concentrations in certain individual test chambers had decreased to near 40% of saturation during the test, each of these affected test chambers and the controls were aerated. The exact amounts and levels of aeration are noted on the bioassay data forms.

Total Residual Chlorine

Since many wastewaters are chlorinated, especially those originating from sewage treatment facilities, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the wastewater. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated

wastewater in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025N soidium thiosulfate. The total residual chlorine concentrations of the tested effluents are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the wastewater.

Freshwater Tests

Freshwater for dilution of the effluent samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is an abbreviated version of that recommeded by APHA (1980).

Two replicates, of each concentration and a control, were used to test with the water flea, Daphnia pulex. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassays. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during these tests.

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% effluent 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 daily 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% effluent concentrations 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 through 5.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	--------	500 ml
10.0%	450 ml	50 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Crestview STP, Okaloosa County, NPDES #FL0027782, on 4 to 6 February, 1986.

Chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	
Control	10	10	10	8.6	8.9	8.8	7.5	7.8	7.8	21.6	21.5	21.3			
Control	10	9	9	8.6	8.9	8.8	7.5	7.8	7.8				209	215	
10.0%	10	5	0	8.5	8.9	8.7	7.5	7.8	7.8	21.5	21.4	21.2			
10.0%	10	6	1	8.5	8.9	8.7	7.5	7.8	7.8				222	228	
32.0%	10	0	0	8.5	8.8	-b	7.4	7.8	-b	21.5	21.3	-b			
32.0%	10	0	0	8.5	8.8	-	7.4	7.8	-				262	-b	
56.0%	10	0	0	8.4	8.7	-	7.3	7.8	-	21.5	21.2	-			
56.0%	10	0	0	8.4	8.7	-	7.3	7.8	-				310	-	
100.0%	10	0	0	8.5	8.3	-	7.2	7.7	-	21.6	21.2	-			
100.0%	10	0	0	8.5	8.3	-	7.2	7.7	-				390	-	

Control Water

NH_3(as NH_3)(mg/l): 0

Total ammonia (as N)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 100

Total Hardness (mg/l $CaCO_3$): 112

100% Effluent

NH_3(as NH_3)(mg/l): 0.06

Total ammonia (as N)(mg/l): 7.0

Total Alkalinity (mg/l $CaCO_3$): 124

Total Hardness (mg/l $CaCO_3$): 105

[a] Total Residual Chlorine: 0.8 mg/l (HACH DPD Method), 0.8 mg/l (Iodometric Method 1).

[b] Remaining parameters were not recorded since all organisms were dead by 24 hours.

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of the Crestview STP, Okaloosa County, NPDES #FL0027782, on 4 to 6 February, 1986.

Chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		
	0	24	48	0	24	48	0	24	48	0	24	48	0		48
Control	5	5	5	8.5	8.9	8.8	7.6	8.0	7.9	22.0	21.2	21.2			
Control	5	5	5	8.5	8.9	8.8	7.6	8.0	7.9				205		215
10.0%	5	5	5	8.5	6.7	7.4	7.5	7.6	7.6	21.9	21.1	21.1			
10.0%	5	4	4	8.5	6.7	7.4	7.5	7.6	7.6				230		238
32.0%	5	5	5	8.5	6.8	6.6	7.4	7.6	7.5	21.8	21.1	21.0			
32.0%	5	5	5	8.5	6.8	6.6	7.4	7.6	7.5				271		278
56.0%	5	2	0	8.5	5.8	3.3	7.4	7.5	7.3	21.7	21.0	21.0			
56.0%	5	0	0	8.5	6.0	-b	7.4	7.5	-b				330		337
100.0%	5	0	0	8.5	5.3	-	7.2	7.4	-	21.7	21.0	-b			
100.0%	5	0	0	8.5	4.8	-	7.2	7.4	-				402		-b

Control Water

NH_3(as NH_3)(mg/l): 0

Total ammonia (as N)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 100

Total Hardness (mg/l $CaCO_3$): 112

100% Effluent

NH_3(as NH_3)(mg/l): 0.06

Total ammonia (as N)(mg/l): 7.0

Total Alkalinity (mg/l $CaCO_3$): 124

Total Hardness (mg/l $CaCO_3$): 105

[a] Total Residual Chlorine: 0.8 mg/l (HACH DPD Method), 0.8 mg/l (Iodometric Method 1).

[b] Remaining parameters were not recorded since all organisms were deady by 24 hours.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Crestview STP, Okaloosa County, NPDES #FL0027782, on 4 to 6 February, 1986.

De-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	10	10	10	8.5	8.7	8.8	7.6	8.0	8.0	21.6	21.5	21.2		
Control	10	10	9	8.5	8.7	8.8	7.6	8.0	8.0				205	215
10.0%	10	10	9	8.5	8.7	8.6	7.6	8.0	7.9	21.6	21.4	21.2		
10.0%	10	10	10	8.5	8.7	8.6	7.6	8.0	7.9				230	233
32.0%	10	10	9	8.5	8.1	8.3	7.5	7.9	7.8	21.5	21.3	21.2		
32.0%	10	10	9	8.5	8.1	8.3	7.5	7.9	7.9				272	279
56.0%	10	10	3	8.4	7.4	7.6	7.4	7.9	7.7	21.6	21.4	21.2		
56.0%	10	10	4	8.4	7.4	7.6	7.4	7.8	7.7				320	329
100.0%	10	6	0	8.4	6.3	6.5	7.3	7.7	7.6	21.5	21.5	21.2		
100.0%	10	3	0	8.4	6.3	6.5	7.3	7.7	7.6				403	419

Control Water

NH_3(as NH_3)(mg/l): 0

Total ammonia (as N)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 100

Total Hardness (mg/l $CaCO_3$): 112

100% Effluent

NH_3(as NH_3)(mg/l): 0.08

Total ammonia (as N)(mg/l): 7.3

Total Alkalinity (mg/l $CaCO_3$): 130

Total Hardness (mg/l $CaCO_3$): 100

[a] Total Residual Chlorine: < 0.04 mg/l (HACH DPD Method).

Table 5. Data recorded during 48 hours, Notropis leedsi, static acute toxicity bioassay of the Crestview STP, Okaloosa County, NPDES #FL0027782, on 4 to 6 February, 1986.

De-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	
Control	5	5	5	8.5	8.9[b]	8.8	7.6	8.0	7.9	22.0	21.2	21.2			Control Water
Control	5	5	5	8.5	8.9[b]	8.8	7.6	8.0	7.9				205	215	NH_3(as NH_3)(mg/l) 0 — Total Alkalinity (mg/l $CaCO_3$) 100
10.0%	5	5	5	8.6	7.1	7.1	7.6	7.7	7.6	21.8	21.4	21.1			
10.0%	5	5	5	8.6	7.1	7.1	7.6	7.7	7.6				230	240	
32.0%	5	5	5	8.5	5.8	6.2	7.5	7.5	7.5	21.6	21.2	21.0			Total ammonia (as N)(mg/l) 0 — Total Hardness (mg/l $CaCO_3$) 112
32.0%	5	5	5	8.5	6.0	6.2	7.5	7.5	7.5				272	282	
56.0%	5	5	5	8.5	8.6[b]	8.5	7.4	8.1	8.0	21.5	21.1	21.0			
56.0%	5	5	5	8.5	8.6[b]	8.5	7.4	8.1	8.0				320	330	
100.0%	5	5	3	8.5	8.7[b]	8.7	7.2	8.0	8.0	21.3	21.3	21.0			
100.0%	5	5	5	8.5	8.7[b]	8.7	7.2	8.0	8.0				405	413	100% Effluent
															NH_3(as NH_3)(mg/l) 0.06 — Total Alkalinity (mg/l $CaCO_3$) 130
															Total ammonia (as N)(mg/l) 7.3 — Total Hardness (mg/l $CaCO_3$) 100

[a] Total Residual Chlorine: $\angle$ 0.04 mg/l (HACH DPD Method).

[b] These test chambers were aerated starting at hour 22 of the test since dissolved oxygen concentrations were approaching 40% of saturation.

Table 6. LC_{50} Data for Bioassays of the Crestview STP, NPDES #FL0027782, on 4 to 6 February, 1986.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Effluent			
Daphnia pulex	Binomial	10.8% (0 and 32)	< 10.0%, b
	Moving Average	a	
	Probit	a	
Notropis leedsi	Binomial	47.0% (32 and 100)	42.3% (32 and 56)
	Moving Average	46.7% (32 and 69)	a
	Probit	40.9% (0 and ∞)	a
De-chlorinated Effluent			
Daphnia pulex	Binomial	96.2% (56 and ∞)	48.7% (32 and 100)
	Moving Average	a	45.3% (37 and 56), c
	Probit	a	44.9% (0 and ∞)
Notropis leedsi	Binomial		a
	Moving Average	No Mortality	a
	Probit		a

[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.

[b] The LC_{50} is < 10.0% since more than half the organisms died in this lowest test concentration.

[c] This LC_{50} is reported in the text since it is the most statistically conservative value of the data set.

Table 7. Results of Chemical Analyses Performed on effluent from the Crestview STP, NPDES #FL0027782, collected on 3 February, 1986.

Organics	
Acid Extractables:	——[a]
Base Neutral Extractables:	——[a]
Pesticide Extractables:	Chlorpyrifos (0.39 ug/l)

Metals	
Arsenic	< 2 ug/l
Cadmium	< 0.2 ug/l
Chromium	N.D.
Copper	< 25 ug/l
Lead	-[b]
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	1.1 ug/l
Zinc	43 ug/l

[a] Analysis incomplete at the time of the report
[b] Lead analysis was invalidated
N.D. = None Detected

RESULTS AND DISCUSSION

The sample of final chlorinated effluent from the Crestview municipal waste treatment facility proved to be acutely toxic to the test organisms, Daphnia pulex and Notropis leedsi. The 48 hour static tests resulted in LC_{50}'s of < 10.0% and 42.3%, respectively (Table 6). This toxicity can partly be attributed to the residual chlorine (0.8 mg/l) measured in the sample. This same effluent, after de-chlorination, was still acutely toxic to D. pulex (48 hour LC_{50} = 45.3%). Mortality was also noted in the N. leedsi test (Table 5), but it was insufficient to calculate an LC_{50}. The extent of toxicity in the chlorinated effluent, and the presence of toxicity in the de-chlorinated effluent, indicates that the Crestview STP effluent contains toxic components in addition to chlorine. The ammonia measured in this effluent (7.3 mg/l total and 0.08 mg/l unionized) was insufficient to have contributed significantly to the toxicity.

The results of chemical analyses (Table 7) indicated the presence of the pesticide chlorpyrifos (0.39 ug/l), and the metals silver and zinc in the Crestview effluent. Since reported LC_{50} values for chlorpyrifos for several macroinvertebrate crustaceans are as low as 0.11 ug/l, it is likely that this pesticide was responsible for a significant portion of the noted toxicity (U.S. Dept. of Interior 1980).

Biology Section's Recommendation

Due to the toxic nature of the Crestview STP effluent, we recommend that the facility undertake a toxicity identification and elimination plan, emphasizing on the elimination of the chloropyrifos, and demonstrate compliance with the total residual chlorine standard in the receiving waters. Additionally, a biomonitoring program should be incorporated into this facility's permit.

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.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whetheras a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters [17-3.061] are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic [17-3.061(2)(p)]; that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters [17-3.051(1)(d)] applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 [17-4.244(4)]. At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

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