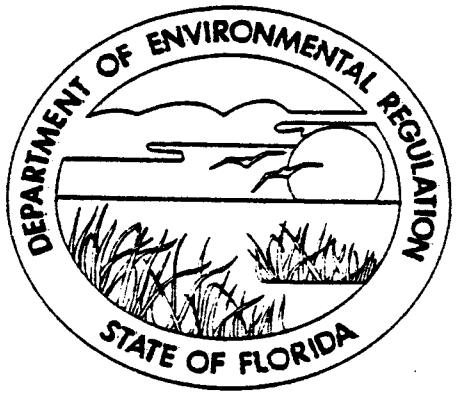


BIOASSAYS OF THE KANAPAHA ADVANCED WASTEWATER TREATMENT PLANT

GAINESVILLE, ALACHUA COUNTY, FLORIDA

NPDES #FL0032379

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Biology Section
Bureau of Water Analysis
May 22, 1984

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GAINESVILLE, ALACHUA COUNTY, FLORIDA

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EXECUTIVE SUMMARY

Kanapaha AWTP, Gainesville, NPDES #FL0032379, on 29 February to 2 March, 1984.

The sample of pre-chlorinated effluent from this municipal waste treatment facility, which disposes its final effluent through a deep well, was acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} of 96.2%. This same effluent was not acutely toxic to Notropis leedsi. Only random mortality of fish was noted during the 48 hour static test. The final chlorinated effluent was extremely toxic to D. pulex, producing a 48 hour LC_{50} of 1.9%. This toxicity was likely due to the significant concentration of total residual chlorine (2.94 mg/l) measured in that sample. The de-chlorinated effluent was lethal to a number of D. pulex, but the mortality was insufficient to calculate an LC_{50}. Ammonia was not detected in the effluents from this facility.

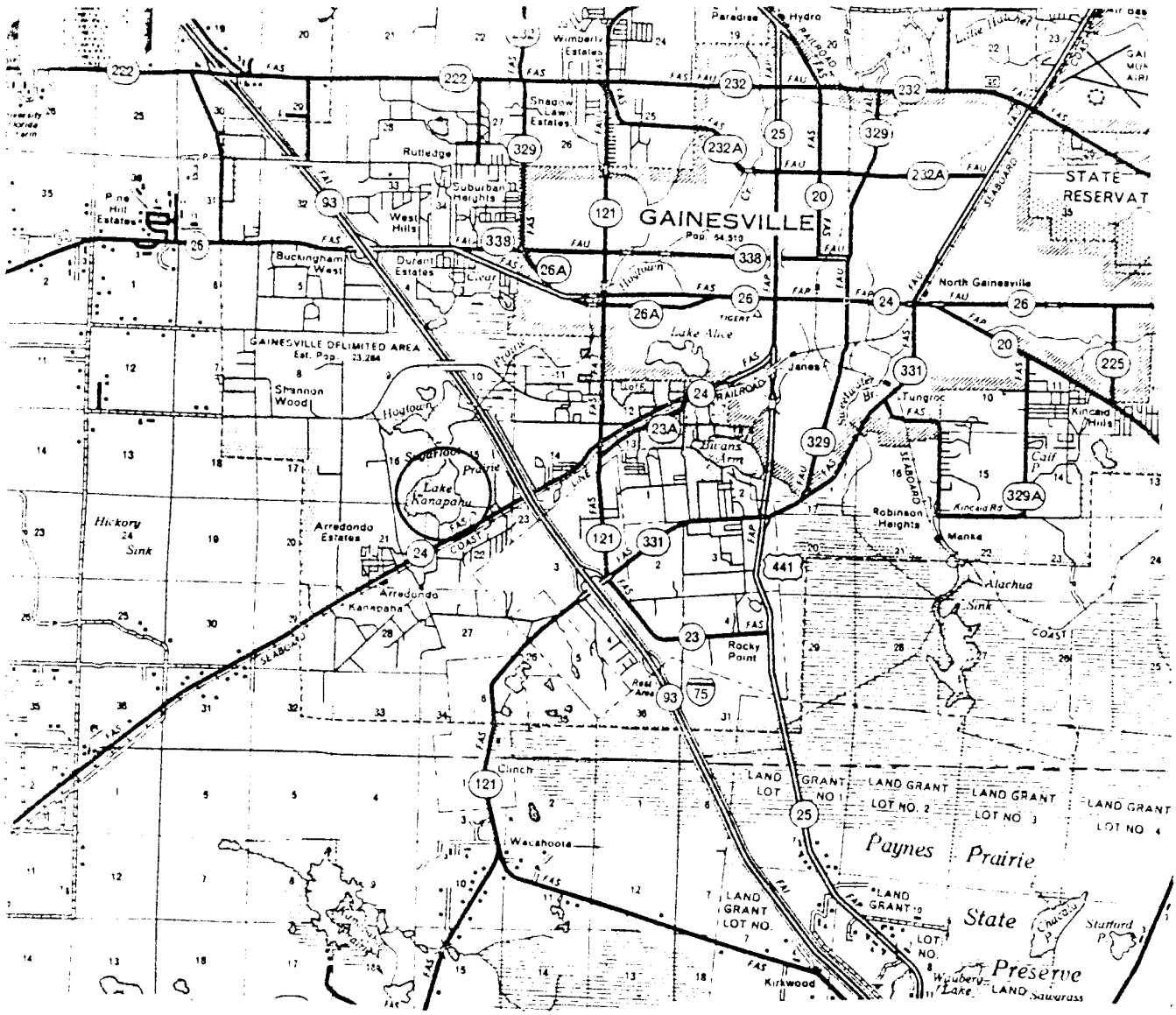
Chemical analyses of these effluents indicated the presence of several unidentified organic compounds in both the pre-chlorinated and final chlorinated effluents. Also present were small concentrations of mercury (0.29 µg/l) and zinc (12.9 µg/l). These chemical constituents may have contributed to the observed toxicity.

INTRODUCTION

The Kanapaha Advanced Wastewater Treatment Plant is located at 3901 SW 63rd Blvd. in Gainesville, Alachua County, Florida (Fig. 1). This facility has a design capacity of 7.0 MGD and treats an average of 5.9 MGD of domestic waste from a population of approximately 60,000. Included in this are approximately 10,000 GPD of industrial wastes. Treatment consists of grit removal, mechanical aeration, secondary clarification, denitrification, post aeration and chlorination. Discharge of the treated wastes is through deep well injection, but the facility does maintain an emergency outfall to Lake Kanapaha.

The DER Biology Section has previously evaluated the toxicity of effluent from this facility, by performing five acute toxicity bioassays using freshwater test organisms, in June, 1982. The results of those tests indicated that the pre-chlorinated and final post-chlorinated effluents were acutely toxic to Daphnia pulex, producing 48 hour LC_{50}'s of 96% and 10%, respectively (FDER 1982). This toxicity was attributed to the total residual chlorine in the chlorinated sample, the presence of the pesticide lindane, and to five other unidentified halogenated compounds. The de-chlorinated effluent was lethal to a number of D. pulex at that time, but an LC_{50} could not be calculated (FDER 1982).

To again evaluate the existence and extent of toxicity of this effluent to standard bioassay test organisms, the Biology Section performed four additional static acute toxicity bioassays on samples of pre-, post-, and de-chlorinated effluents on 29 February to 2 March, 1984. The results of these latest tests are presented in this report. Those involved in performing these tests and in preparation of this report were: P. D. Brooks, C. W. Dye, and M. L. Roll.



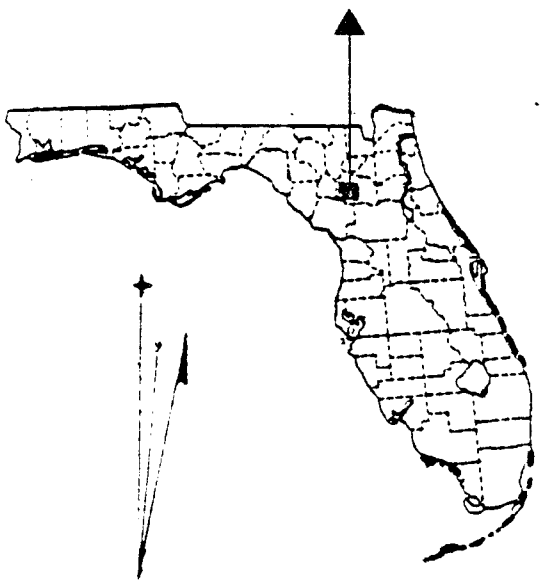


Figure 1. Portions of Alachua County, Florida, including Gainesville and study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include a species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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, and are kept at a constant temperature of 22° plus or minus 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. They are then refilled 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 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° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily. Breeding and egg hatching are encouraged at 25°C. The eggs are laid under a 1" thick slab of marble raised 3 mm above a removable horizontal pane of glass. Most of the eggs adhere to the glass and are easily removed to an aerated hatching tank. The narrow space between the marble slab and glass eliminates egg predation by adult fish. New hatching tanks are started weekly 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 Tetra Min®. The fish used in this test were approximately 20 days 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

Four separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the Kanapaha AWTP. The tests were performed from 1240 hours on 29 February to 1340 hours on 2 March, 1984.

The samples were collected by Mr. David Woodbridge of DER's Northeast District at 1545 hours on 28 February, 1984 using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. 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.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biology Section adopted the following test procedure:

Test #1. Pre-chlorinated Effluent (Tables 2 and 3). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination, and was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 4). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Table 5). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

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 that recommended by APHA (1980).

Two replicates, each with six concentrations 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, each containing 200 ml of the appropriate test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age of organisms (neonates). To accomplish this separation, 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 loaded 10 per chamber, resulting in 20 individuals per test concentration. Water fleas were not fed during these tests.

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

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 chamber 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 test 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, pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2 thru 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
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
18.0%	164.0 ml	36.0 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
18.0%	410 ml	90 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 Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 29 February to 2 March, 1984.

Test #1 Pre-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	8.8	9.6	8.7	7.9	8.1	8.2	111		141		19.9	18.3	19.3	0/0	-		
Control	10	10	10	8.8	9.6	9.0	7.9	8.1	8.2										189	192
5.6%	10	10	9	8.7	9.6	9.0	7.8	8.1	8.2					19.4	18.0	19.1				
5.6%	10	10	10	8.7	9.7	9.0	7.8	8.1	8.2										193	200
10.0%	10	10	10	8.7	9.7	9.2	7.8	8.1	8.2					19.5	17.9	19.0				
10.0%	10	10	10	8.7	9.7	9.2	7.8	8.1	8.2										200	202
18.0%	10	10	10	8.7	9.6	9.2	7.8	8.1	8.2					19.6	17.9	19.0				
18.0%	10	10	10	8.7	9.6	9.2	7.8	8.1	8.2										218	220
32.0%	10	10	10	8.6	9.6	9.2	7.7	8.1	8.2					19.4	18.0	19.0				
32.0%	10	10	10	8.6	9.6	9.2	7.7	8.1	8.2										239	245
56.0%	10	10	10	8.6	9.6	9.2	7.7	8.0	8.1					19.3	18.1	19.1				
56.0%	10	10	10	8.5	9.6	9.2	7.7	8.0	8.1										280	285
100.0%	10	8	5	8.4	9.6	9.1	7.4	7.9	8.0	80		138		19.4	18.3	19.4	0/.05	-		
100.0%	10	9	4	8.4	9.6	9.1	7.4	7.9	8.0										352	370

Remarks: [a] Total Residual Chlorine: None Detected

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of the Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 29 February to 2 March, 1984.

Test #1 Pre-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.5	9.5	8.6	7.6	8.0	8.1	111		141		19.6	17.9	18.8	0/0	-		
Control	5	4	4	8.5	9.6	8.6	7.6	8.0	8.1										188	183
10.0%	5	5	5	8.5	9.5	9.0	7.7	8.0	8.1					19.7	17.8	18.7				
10.0%	5	4	3	8.5	9.5	9.0	7.7	8.0	8.1										202	200
18.0%	5	5	5	8.5	9.5	9.0	7.7	8.0	8.1					19.7	17.7	18.6				
18.0%	5	4	4	8.5	9.5	9.0	7.7	8.0	8.1										218	213
32.0%	5	5	5	8.5	9.5	8.8	7.6	7.9	8.1					19.6	17.7	18.5				
32.0%	6[b]	6	6	8.5	9.5	8.8	7.6	7.9	8.1										240	240
56.0%	5	5	5	8.5	9.5	8.9	7.6	7.9	8.0					19.5	17.8	18.5				
56.0%	5	5	5	8.5	9.5	8.9	7.6	7.9	8.0										280	280
100.0%	5	5	5	8.4	9.4	8.8	7.4	7.8	7.9	80		138		19.4	18.0	18.7	0/.05	-		
100.0%	5	5	5	8.4	9.4	8.8	7.4	7.8	7.9										352	358

Remarks: [a] Total Residual Chlorine: None Detected

[b] Extra organism loaded by mistake, allowed to remain.

Table 4. Data recorded during 48 hour, *Daphnia pulex*, static acute toxicity bioassay of the Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 29 February to 2 March, 1984.

Test #2 Chlorinated Effluent [a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/1)			pH			Total Alakalinity (mg/1 as $CaCO_3$)		Total Hardness (mg/1 as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/1)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	9	8.5	9.4	9.1	7.7	8.1	8.2	111		141		19.7	18.4	19.3	0/0	-		
Control	10	10	10	8.6	9.5	9.1	7.7	8.1	8.2										181	192
5.6%	10	5	1	8.6	9.7	9.2	7.7	8.1	8.2					19.7	18.3	19.2				
5.6%	10	1	1	8.6	9.7	9.2	7.7	8.1	8.2										192	200
10.0%	10	3	1	8.6	9.7	9.2	7.7	8.1	8.2					19.7	18.2	19.1				
10.0%	10	0	0	8.6	9.8	9.2	7.7	8.1	8.2										200	198
18.0%	10	0	0	8.6	9.8	- [b]	7.6	8.1	- [b]					19.7	18.2	- [b]				
18.0%	10	0	0	8.6	9.8	-	7.6	8.1	-										212	- [b]
32.0%	10	0	0	8.6	9.9	-	7.5	8.0	-					19.7	18.3	-				
32.0%	10	0	0	8.6	9.9	-	7.5	8.0	-										239	-
56.0%	10	0	0	8.6	9.9	-	7.3	7.9	-					19.5	18.3	-				
56.0%	10	0	0	8.5	9.9	-	7.3	7.9	-										281	-
100.0%	10	0	0	8.4	9.9	-	7.0	7.7	-	63		135		19.6	18.4	-	0/0	-		
100.0%	10	0	0	8.4	9.9	-	7.0	7.7	-										353	-

Remarks: [a] Total Residual Chlorine: 2.25 mg/1 (HACH DPD Method), 2.94 mg/1 (Iodometric Method I)

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

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Kanapaha AWTP, Gainesville, Alachua County, Florida, NPDES #FL0032379, on 29 February to 2 March, 1984.

Test #3 De-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	8	8.5	9.9	9.2	7.5	8.1	8.3	111		141		19.4	17.8	18.8	0/0	-		
Control	10	10	9	8.5	9.9	9.2	7.6	8.1	8.3										180	191
5.6%	10	10	8	8.5	9.9	9.0	7.7	8.1	8.2					19.3	17.5	18.5				
5.6%	10	10	9	8.5	9.9	9.0	7.7	8.1	8.2										192	198
10.0%	10	10	9	8.6	10.0	9.1	7.7	8.1	8.2					19.4	17.5	18.5				
10.0%	10	10	10	8.6	10.0	9.1	7.7	8.1	8.2										200	205
18.0%	10	10	9	8.6	10.1	9.1	7.7	8.1	8.2					19.5	17.5	18.5				
18.0%	10	10	10	8.6	10.1	9.1	7.7	8.1	8.2										218	221
32.0%	10	10	10	8.6	10.1	9.1	7.6	8.1	8.2					19.3	17.5	18.4				
32.0%	10	10	10	8.6	10.1	9.1	7.6	8.1	8.2										244	250
56.0%	10	10	10	8.6	10.1	9.0	7.6	8.0	8.1					19.3	17.5	18.6				
56.0%	10	10	9	8.6	10.1	9.0	7.5	8.0	8.1										291	302
100.0%	10	10	8	8.6	10.0	8.8	7.3	7.8	8.0	63		135		19.6	18.0	18.9	0/0	-		
100.0%	10	9	8	8.6	10.0	8.8	7.3	7.8	8.0										377	390

Remarks: [a] Total Residual Chlorine: <0.06 mg/l (HACH DPD Method).

Table 6. LC_{50} Data for Bioassay of the Kanapaha AWTP, Gainesville, NPDES #FL0032379, on 29 February to 2 March, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: *D. pulex*	Binomial	a	96.2% (56 and∞),b
Pre-chlorinated	Moving Average	a	a
Sample	Probit	a	160.2% (0 and∞),b,c
Test #1: *N. leedsi*	Binomial	Random Mortality	Random Mortality
Pre-chlorinated	Moving Average		
Sample	Probit		
Test #2: *D. pulex*	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	4.2% (0.98 and 5.89),b	1.9% (0 and 4.3),b
Test #3: *D. pulex*	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	a	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] Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

[c] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

Table 7. Results of chemical analyses performed on effluents from the Kanapaha AWTP, Gainesville, NPDES #FL0032379, collected 28 February, 1984.

Organics

	Pre-chlorinated Effluent	Chlorinated Effluent
Acid Extractables:	N.D.	N.D.
Base Neutral Extractables:	Several Unidentified Compounds	Several Unidentified Compounds
Pesticide Extractables:	Several Unidentified Compounds	Several Unidentified Compounds

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	<0.8 µg/l[a]
Chromium	N.D.
Copper	N.D.
Lead	–[b]
Mercury	0.29 µg/l
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Thallium	N.D.
Zinc	12.9 µg/l

N.D. = None Detected
[a] Actual valve is known to be less than value given.
[b] Lead values invalidated

Results and Discussion

The sample of pre-chlorinated effluent from the Kanapaha AWTP proved to be acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} of 96.2% (Table 6). This same effluent was not acutely toxic to Notropis leedsi. Only random mortality of fish was noted during the 48 hour static test (Table 3). The final chlorinated effluent was extremely toxic to D. pulex, producing a 48 hour LC_{50} of 1.9% (Table 6). This toxicity can most likely be attributed to the total residual chlorine (2.94 mg/l) measured in that sample. The de-chlorinated effluent was lethal to a number of D. pulex at 48 hours, however, the mortality was insufficient to calculate an LC_{50}. The 96 hour LC_{50}'s for the acutely toxic effluents reported above would be less than or equal to their 48 hour values. Ammonia was not detected in any significant concentration in the effluents from this facility.

The results of these latest tests presented above were very similar to the results of the 1982 tests, in which 48 hour LC_{50}'s of 96% and 10% were calculated for D. pulex for the pre-chlorinated and final chlorinated effluents respectively (FDER 1982). This suggests that little or no improvements have been made at the facility since the earlier tests.

Since the toxicity of the pre-chlorinated effluent reported above could not be attributed to either ammonia or to total residual chlorine, other chemical components or characteristics may have been responsible. The DER Chemistry Section has analyzed samples of the Kanapaha effluents for certain organics and metals (Table 7). The results of these analyses indicated the presence of several unidentified organic compounds (base neutral and pesticide extractables) in both the pre-chlorinated and final chlorinated effluents. A significant condentration of mercury (0.29 µg/l) and a small concentration of zinc (12.9 µg/l) were also present. These chemcial constituents may have contributed to the observed toxicity.

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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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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