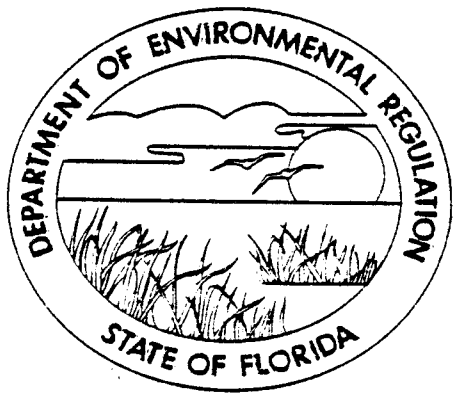
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BIOASSAYS OF THE JACKSONVILLE U.S. NAVAL AIR STATION

SEWAGE TREATMENT PLANT

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0021725

SAMPLED 8/21/83

Biology Section
Bureau of Water Analysis
September 14, 1983

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EXECUTIVE SUMMARY

Jacksonville N.A.S. STP, NPDES #FL0021725, on 22 to 24 August, 1983.

Samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from this U.S. government facility exhibited varying degrees of lethality to the test organisms, Daphnia pulex and Notropis leedsi. The pre-chlorinated effluent was lethal to a number of random D. pulex, but this test was invalidated at 24 hours due to excessive control mortality and no data are reported. The same effluent, however, was also lethal to a number of N. leedsi in the higher test concentrations. The chlorinated effluent was extremely toxic to D. pulex. All organisms were dead in every test concentration by hour 24 of the test (24 hour $LC_{50} < 5.6\%$). This toxicity can be attributed to the total residual chlorine (1.6 mg/l) measured in that sample. The de-chlorinated effluent was acutely toxic to D. pulex, producing a 24 hour LC_{50} value of 97.2%. Excessive control mortality invalidated the 48 hour test. This same effluent was lethal to a number of N. leedsi, but the mortality was apparently random. The small concentrations of total and unionized ammonia detected in these effluents could not have contributed significantly to the observed toxicity. Since the toxicity noted in the chlorinated sample was directly attributed to total residual chlorine, and the vitality of D. pulex used for these tests was suspect, chemical analyses of the effleunt were not performed.

INTRODUCTION

The Jacksonville U.S. Naval Air Station is a U.S. Government facility located in Jacksonville, Duval County, Florida (Figure 1). The associated sewage treatment plant treats waste from the air station, including domestic waste, water from industrial cooling systems, boiler wash, vehicle and equipment cleaning operations, and other miscellaneous sources. The plant has a design capacity of 3.0 MGD and has operated near this level, at 2.6 MGD. Discharge is to the St. Johns River (NPDES Outfall #001).

The NPDES permit for this facility contains discharge limits for many potentially toxic substances, including chromium, zinc, copper, cyanide and phenol. Additionally, this facility has received notices for exceeding its permit limitations and for the inadequate disposal of sludges and solids.

The Biology Section had previously performed bioassays on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this facility, in May, 1981. The results of those tests indicated that the pre-chlorinated and de-chlorinated effluents were not toxic to Daphnia pulex, while the chlorinated effluent was extremely toxic to Daphnia, with 24 and 48 hour LC_{50} values of <5.6% (FDER 1981). The toxicity was attributed to the presence of total residual chlorine.

Samples of pre-chlorinated and chlorinated effluents were retested, in August, 1982, using Daphnia pulex, Simocephalus serrulatus, and Heterandria formosa as test organisms. The effluents were not toxic at that time, only random mortalities were noted. The chlorinated effluent contained only a small amount of total residual chlorine, <0.05 mg/l (FDER 1982).

To further evaluate the existence and extent of toxicity of these effluents to the biota of the receiving waters, the Biology section performed five additional static acute toxicity bioassays on grab samples, on 22 to 24 August, 1983. The results of these latest tests are presented in this report. Those involved in performing these tests and preparing this report were: C. W. Dye, R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.

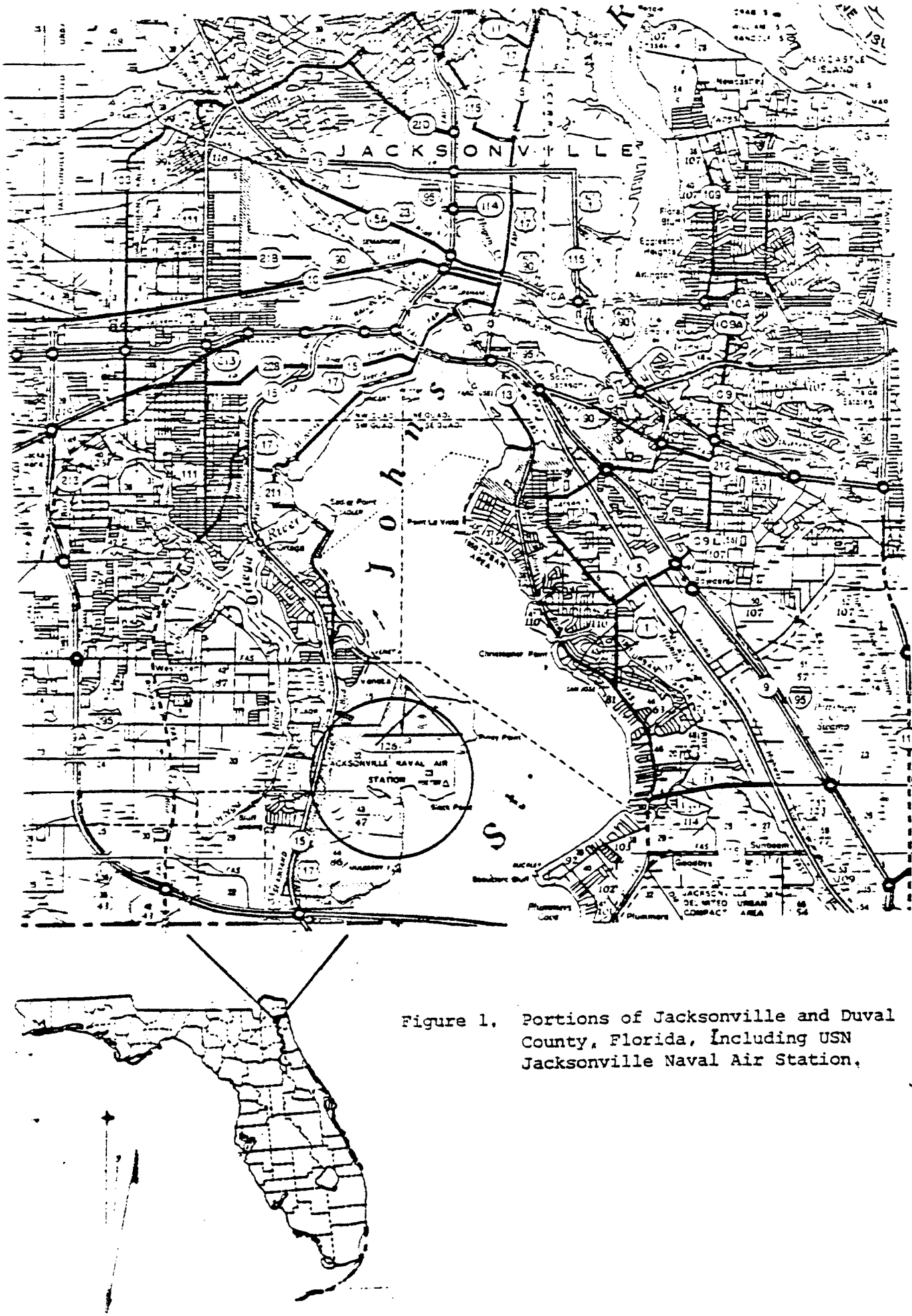


Figure 1. Portions of Jacksonville and Duval County, Florida, Including USN Jacksonville Naval Air Station.

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 these tests were approximately 5 days old at the time of the tests.

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

Five separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the Jacksonville N.A.S. STP. The tests were performed from 1530 hours on 22 August to 1530 hours on 24 August, 1983. The samples were collected by Ms. Teresa Rhodes of DER's Jacksonville office at approximately 0835 hours on 22 August, 1983, 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[a] (Table 2). 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 3). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Tables 4 and 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, 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 six concentrations 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.

[a] The Daphnia pulex pre-chlorinated effluent test was invalidated at 24 hours due to excessive control mortality, therefore, no data from this test are reported.

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 100% effluent concentrations. 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, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 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
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
5.6%	472 ml	28 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, Notropis leedsi, static acute toxicity bioassay of the Jacksonville Naval Air Station STP, Duval County, Florida, NPDES #FL0021725, on 22 to 24 August, 1983.

Test #1 - Pre-chlorinated Effluent[a]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (^oC)			NH_3/N (mg/l)		Conductivity (μmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.2	8.6	7.7	7.7	8.1	8.1					22.3	20.2	19.7	0/0	--		
Control	5	5	5	8.2	8.5	7.8	7.7	8.1	8.1										217	211
5.6%	5	5	5	8.2	8.6	7.9	7.7	8.0	8.1					22.2	20.0	19.8				
5.6%	5	5	5	8.2	8.6	8.0	7.7	8.1	8.1										231	218
10.0%	5	5	5	8.2	8.5	8.2	7.7	8.1	8.1					22.2	19.8	19.6				
10.0%	-[b]	-	-	-	-	-	-	-	-										-	-
18.0%	5	5	5	8.1	8.3	7.8	7.7	8.1	8.0					22.0	19.8	19.6				
18.0%	5	5	5	8.1	8.3	8.2	7.7	8.0	8.0										267	250
32.0%	5	5	5	7.9	8.1	8.2	7.6	8.0	8.0					21.8	19.7	19.2				
32.0%	5	5	4	7.9	8.2	8.2	7.6	8.0	8.0										303	288
56.0%	5	4	4	7.3	8.1	8.1	7.5	7.9	8.0					21.5	19.8	19.1				
56.0%	5	5	5	7.3	8.2	8.0	7.5	7.9	8.0										368	345
100.0%	5	5	4	6.1	8.2	8.1	7.3	7.9	7.9	65		82		21.2	19.8	19.2	.01/0.97	--		
100.0%	5	5	5	6.1	7.9	8.1	7.3	7.9	7.9										472	452

Remarks: [a] Total Residual Chlorine: None detected (HACH DPD Method).
[b] Jar not loaded by mistake, parameters not reported.

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Jacksonville Naval Air Station STP, Duval County, Florida, NPDES #FL0021725, on 22 to 24 August, 1983.

Test #2 - Chlorinated Effluent[a]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (µmhos)	
	0	24	48[b]	0	24	48[b]	0	24	48[b]	0	48	0	48	0	24	48[b]	0	48	0	48[b]
Control	10	10		8.2	8.6		7.8	8.3						22.1	20.3		0/0	-		
Control	10	9		8.2	8.6		7.8	8.3											212	
5.6%	10	0		8.2	8.7		7.7	8.3						22.2	20.2					
5.6%	10	0		8.2	8.7		7.7	8.2											231	
10.0%	10	0		8.2	8.7		7.7	8.2						22.1	20.2					
10.0%	10	0		8.2	8.7		7.7	8.2											242	
18.0%	10	0		8.2	8.5		7.6	8.2						22.1	20.3					
18.0%	10	0		8.2	8.5		7.6	8.2											263	
32.0%	10	0		7.9	8.5		7.5	8.1						21.8	20.3					
32.0%	10	0		7.9	8.5		7.5	8.1											302	
56.0%	10	0		7.5	8.6		7.4	8.1						21.7	20.3					
56.0%	10	0		7.5	8.6		7.4	8.1											367	
100.0%	10	0		6.3	8.7		7.3	8.0		62		98		21.5	20.2		.005/0.47	-		
100.0%	10	0		6.3	8.7		7.3	8.0											470	

Remarks:
[a] Total Residual Chlorine: 1.4 mg/l (HACH DPD Method), 1.6 mg/l (Iodometric Method I).
[b] No data are reported for the 48 hour test since all organisms were dead by hour 24.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Jacksonville Naval Air Station STP, Duval County, Florida, NPDES #FL0021725, on 22 to 24 August, 1983.

Test #3 - De-chlorinated Effluent[a]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (^oc)			NH_3/N (mg/l)		Conductivity (μmhos)	
	0	24	48[c]	0	24	48[c]	0	24	48[c]	0	48	0	48	0	24	48[c]	0	48	0	48[c]
Control	10	8	4	8.2	8.7		7.7	8.1						22.1	19.9		0/0	-		
Control	10	9	4	8.2	8.7		7.7	8.1											213	
5.6%	10	7		8.2	8.7		7.7	8.1						22.2	19.9					
5.6%	10	8		8.2	8.7		7.7	8.1											232	
10.0%	10	9		8.2	8.6		7.7	8.1						22.1	19.9					
10.0%	10	10		8.2	8.6		7.7	8.1											243	
18.0%	10	8		8.2	8.3		7.7	8.1						22.1	20.0					
18.0%	10	7		8.2	8.3		7.7	8.1											268	
32.0%	10	9		8.1	8.4		7.6	8.1						22.1	20.0					
32.0%	10	9		8.1	8.4		7.6	8.1											309	
56.0%	10	8		7.9	8.1		7.5	8.1						22.1	20.0					
56.0%	10	10		7.9	8.0		7.5	8.1											371	
100.0%	10	3		7.2	8.2		7.3	8.0		58		80		22.3	20.0		.007/0.63	-		
100.0%	11[b]	7		7.2	8.1		7.3	8.0											488	

Remarks: [a] Total Residual Chlorine: <0.04 mg/l (HACH DPD Method).
[b] Extra organism loaded by mistake, allowed to remain.
[c] No data for the 48 hour test are reported since excessive control mortality was observed.

Table 5. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of the Jacksonville Naval Air Station STP, Duval County, Florida, NPDES #FL0021725, on 22 to 24 August, 1983.

Test #3 - De-chlorinated Effluent[a]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (^oc)			NH_3/N (mg/l)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.2	8.4	7.8	7.7	8.0	8.2					22.0	19.8	19.3	0/0	-		
Control	5	5	5	8.2	8.6	7.9	7.7	8.0	8.2										217	205
5.6%	5	5	5	8.2	8.6	8.0	7.7	8.1	8.1					22.0	19.7	19.1				
5.6%	5	5	5	8.2	8.6	7.7	7.7	8.1	8.1										233	219
10.0%	5	5	5	8.2	8.6	7.8	7.7	8.1	8.1					22.1	19.7	19.1				
10.0%	5	5	4	8.2	8.6	7.8	7.7	8.1	8.1										249	232
18.0%	5	5	5	8.2	8.6	7.8	7.7	8.1	8.1					22.1	19.6	19.1				
18.0%	5	5	4	8.2	8.6	7.8	7.7	8.1	8.1										271	253
32.0%	5	4	4	8.1	8.6	7.8	7.6	8.0	8.0					22.0	19.6	19.1				
32.0%	5	5	4	8.1	8.6	7.9	7.6	8.0	8.0										310	292
56.0%	5	5	5	7.8	8.6	7.9	7.5	8.0	8.0					22.0	19.7	19.1				
56.0%	5	5	4	7.8	8.6	7.6	7.5	8.0	8.0										378	353
100.0%	5	5	5	7.1	8.5	7.5	7.3	7.9	7.9	58		80		22.1	19.9	19.2	.007/0.63	-		
100.0%	5	5	5	7.1	8.5	7.6	7.3	7.9	7.9										491	469

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

Table 6. LC_{50} Data for Bioassays of the Jacksonville N.A.S. STP, NPDES #FL0021725, on 22 to 24 August, 1983.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: D. pulex Pre-chlorinated Sample	Binomial Moving Average Probit	a	a
Test #1: N. leedsi Pre-chlorinated Sample	Binomial Moving Average Probit	b	b
Test #2: D. pulex Chlorinated Sample		<5.6 %, c	<5.6%, c
Test #3: D. pulex De-chlorinated Sample	Binomial Moving Average Probit	97.2% (56 and ∞), d 97.2% (80 and 175), d e	a
Test #3: N. leedsi De-chlorinated Sample	Binomial Moving Average Probit	b	b

[a] This test was invalidated due to excessive control mortality.

[b] 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.

[c] Since every organism was dead in every test concentration, the LC_{50} is <5.6%.

[d] 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.

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

RESULTS AND DISCUSSION

The samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the Jacksonville N.A.S. STP exhibited varying degrees of lethality to the test organisms, Daphnia pulex and Notropis leedsi. A number of D. pulex died in the pre-chlorinated effluent test, but the mortalities appeared to be random. Additionally, this test was invalidated at 24 hours due to excessive control mortality and no data are reported. This same effluent, however, was also lethal to a number of N. leedsi in the higher test concentrations (Table 2), but the mortality was insufficient to calculate an LC_{50} value (Table 6). The chlorinated effluent was extremely toxic to D. pulex, every organism in every test concentration had died by hour 24 of the test (Table 3). The LC_{50} for this effluent would, therefore, be <5.6% (Table 6). The toxicity noted can be attributed to the large concentration of total residual chlorine (1.6 mg/l) measured in that sample. The de-chlorinated effluent proved to be acutely toxic to D. pulex, producing a 24 hour LC_{50} value of 97.2% (Table 6). Excessive control mortality again resulted in the invalidation of the 48 hour test. This same effluent was lethal to a number of N. leedsi, but the mortality appeared to be random (Table 5). The small concentrations of total and unionized ammonia detected in these effluents could not have contributed significantly to the observed toxicity.

It is apparent from the results of these tests that the chlorinated effluent from this facility was extremely toxic to the test organism, primarily due to the presence of residual chlorine. Additionally, the acute toxicity exhibited by the de-chlorinated effluent to D. pulex (24 hour LC_{50} = 97.2%) suggests that some toxic property was associated with that effluent. The excessive control mortality noted above suggests that the D. pulex were stressed. This may have been due to a thorough cleaning and water exchange in all the D. pulex culture facilities three days prior to the tests. Since the toxicity noted in the chlorinated effluent test was attributed to residual chlorine, and the overall vitality of the D. pulex used for these tests was suspect, chemical analyses of the effluents were not requested.

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 discahrge. 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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