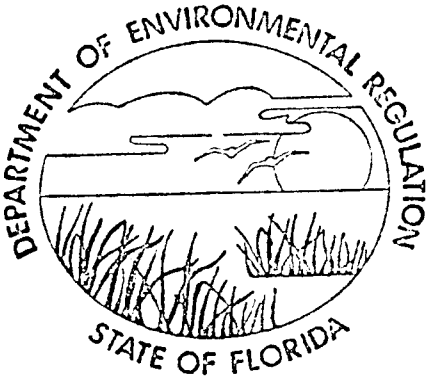


BIOASSAYS OF THE JACKSONVILLE

U. S. NAVAL AIR STATION

SEWAGE TREATMENT PLANT

NPDES #FL0000957

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MAY 11, 1988

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE JACKSONVILLE U. S. NAVAL AIR STATION SEWAGE TREATMENT PLANT NPDES #FL0000957

Biology Section
Bureau of Laboratories and
Special Programs
May 11, 1988

EXECUTIVE SUMMARY

Jacksonville NAS Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0000957, on 30 March to 1 April, 1988.

The final chlorinated sample of discharge from this U.S. Government facility was not acutely toxic to the water fleas Ceriodaphnia dubia or Daphnia pulex, but was acutely toxic to the fish, Notropis leedsi. Mortalities occurred in the 60% and 100% sample concentrations of the water fleas and in the 100% sample concentration of the fish. The approximate LC_{50} for the fish was 81.75%. The chlorinated sample was also toxic to the Microtox® test organism, Photobacterium phosphoreum, producing an EC_{50} of 50.6%. The toxicity of this sample can be attributed to the total residual chlorine (0.5 mg/l Hach DPD Method) and 0.8 mg/l Iodometric Method) measured in the sample. The sample after de-chlorination was not toxic to any of the test organisms.

Due to the non-toxic nature of the de-chlorinated sample, chemical analyses were not performed.

INTRODUCTION

The Jacksonville U. S. Naval Air Station is a U. S. Government facility located in Jacksonville, Duval County, Florida (Fig. 1). The associated sewage treatement 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 is currently operating near this level, 2.6 MGD. Discharge is to the St. Johns River (NPDES Outfall #001).

The Biology Section has previously performed bioassays on pre-chlorinated, chlorinated, and de-chlorinated samples from this facility in May, 1981 and August, 1983. The toxicity of these samples was attributed to total residual chlorine; although there was some mortality in the de-chlorinated portion of the August, 1983 sample (FDER 1987).

Grab samples were taken in August, 1982, from the STP discharge and in January, 1986 and January, 1987, from the STP discharge and from upstream of the industrial collection system (FDER 1987). The results of the August, 1982 bioassays indicated that the sample was non-toxic. The results of the January, 1986 and January, 1987 bioassays attributed toxicity to total residual chlorine. Some mortality was noted in the January, 1986 industrial sample, but an LC_{50} could not be calculated.

To again evaluate the existence and extent of toxicity of the facility discharge, the Biology Section performed bioassays and Microtox® assays from 30 March to 1 April, 1988, on a sample of the discharge. The results of these 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, and L. A. Williams.

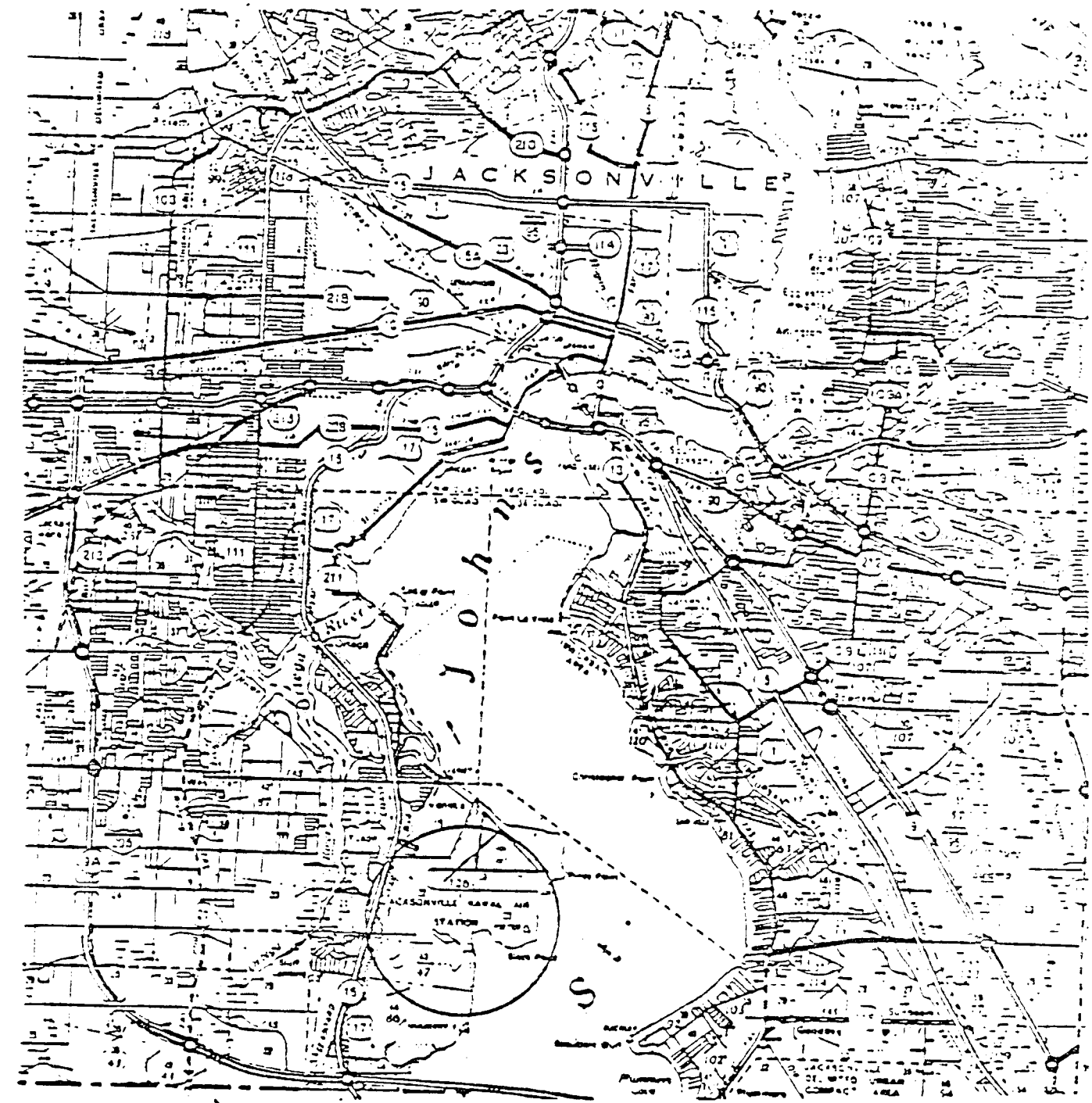


Figure 1. Portions of Jacksonville and Duval County, Florida, Including USN Jacksonville Naval Air Station.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier 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).

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 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-dark 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 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 27 days old at the time of the test.

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).

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.

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

Six separate 48 hour static acute bioassays and two Microtox® assays were performed on chlorinated and de-chlorinated samples of water from the Jacksonville NAS facility. The tests were performed from 1000 hours on 30 March, to 1000 hours on 1 April, 1988.

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 collected by Mr. Mark Sittig of FDER's Northeast District at 1020 hours on 29 March, 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° $\pm$ 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 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.

Total Residual Chlorine

Since many reclaimed waters 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 reclaimed water. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated reclaimed water in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested sample of reclaimed water 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 reclaimed water.

Freshwater Tests

Freshwater for dilution of the recovered water samples 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.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia 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) bioassay. 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% 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 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% reclaimed water test concentration at the beginning and end 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 and 3.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected in a small beaker. 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 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15°C was maintained throughout the assay. Ten microliters 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 4.

RESULTS AND DISCUSSION

The sample from the Jacksonville NAS facility proved to be toxic (LC_{50} = 81.75%) to the test organism Notropis leedsi in the chlorinated test. Mortalities occurred in the chlorinated Ceriodaphnia dubia and Daphnia pulex tests, but LC_{50}'s could not be calculated. The de-chlorinated tests were not toxic to any of the bioassay organisms. The conclusion that the total residual chlorine was responsible for the observed toxicity in this sample was supported by the Microtox® assays, which produced an EC_{50} of 50.6% in the chlorinated sample, but a non toxic EC_{50} > 100% in the de-chlorinated sample. These test results generally correspond to previous test results of this facility in that observed toxicity can be attributed to the total residual chlorine of the sample.

Due to the non-toxic nature of the de-chlorinated sample, chemical analyses were not performed.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
30.0%	35.0 ml	15.0 ml	50 ml
60.0%	20.0 ml	30.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
30.0%	140.0 ml	60.0 ml	200 ml
60.0%	80.0 ml	120.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
30.0%	350 ml	150 ml	500 ml
60.0%	200 ml	300 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassay of the Jacksonville NAS Sewage Treatment Plant, Chlorinated Discharge, Duval County, NPDES #FL0000957, on 30 March to 1 April, 1988.

Facility:	**Jacksonville NAS STP**			NPDES # **FL0000957**	Facility Type:	**Municipal**
Location:	Duval County			Contact/District: M. Sittig		
Sample Collection Date:	3/29/88	Time:	1020	Test type: Static Screening		
Test Beginning Date:	3/30/88	Time:	1000	# tests: 3	Receiving Water: St. Johns River	
Test Ending Date:	4/1/88	Time:	1000	Chlorination Type: chlorinated		

Parameter	
Total Residual Cl2:	0.5
Method:	Hach
By:	DAC
Salinity:	0.2

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0	0

Organism: ***Ceriodaphnia dubia*** Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	10	10	10	8.6	8.5	8.5	8	7.8	7.8	19.9	20.1	19.9		
Control	10	10	10	8.6	8.5	8.5	8	7.8	7.8					
30%	10	10	10	8.7	8.7	8.6	7.8	7.8	7.8	19.9	20	19.8		
30%	10	10	10	8.6	8.7	8.6	7.8	7.8	7.8					
60%	10	7	5	8.6	8.8	8.7	7.6	7.8	7.7	20	19.9	19.7		
60%	10	9	6	8.6	8.8	8.7	7.6	7.8	7.7					
100%	10	10	7	8.6	8.7	8.7	7	7.6	7.5	20.3	20	19.9		
100%	10	10	7	8.5	8.7	8.7	7	7.6	7.5					

	Control water	100% sample
Alkalinity:	130	55
Hardness:	128	250

Organism: ***Daphnia pulex*** Age/Life stage: Neonates
LC50: Binomial: Moving Average: Probit:

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	10	10	10	8.6	8.8	8.7	8.1	7.8	7.8	20.2	19.8	19.8		
Control	10	10	10	8.6	8.8	8.8	8.1	7.9	7.8				209	215
30%	10	10	10	8.7	8.8	8.7	7.8	7.8	7.7	20.5	19.5	19.8		
30%	10	10	10	8.7	8.8	8.7	7.8	7.8	7.7				340	340
60%	10	10	10	8.6	8.9	8.8	7.5	7.7	7.6	20.4	19.5	19.6		
60%	10	10	9	8.6	8.9	8.8	7.5	7.7	7.6				480	485
100%	10	10	10	8.5	9	8.7	7	7.5	7.5	20.4	19.6	19.7		
100%	10	9	9	8.5	9	8.6	7	7.5	7.5				700	700

	Control water	100% sample
Alkalinity:	130	55
Hardness:	128	250

Organism: ***Notropis leedsi*** Age/Life stage: 27 days old
LC50: Binomial: 81.75% Moving Average: Probit:
cl-toxicity

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.1	7.7	7.5	7.6	7.6	7.6	20.7	19.8	19.9		
Control	5	5	5	8.2	7.7	7.5	7.6	7.6	7.6				220	215
30%	5	5	5	8.2	7.8	7.7	7.4	7.5	7.4	20.8	19.7	19.9		
30%	5	5	5	8.2	7.9	7.7	7.4	7.5	7.4				355	350
60%	5	5	5	8.2	7.8	7.8	7.3	7.4	7.3	20.7	19.6	19.8		
60%	5	5	5	8.3	7.8	7.9	7.3	7.4	7.3				490	490
100%	5	1	1	8.3	8.4	6.1	7	7.3	6.9	20.6	19.6	19.8		
100%	5	0	0	8.3	8.4	5.5	7	7.3	6.9				700	700

	Control water	100% sample
Alkalinity:	118	55
Hardness:	125	250

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassays of Jacksonville NAS Sewage Treatment Plant, De-chlorinated Discharge, Duval County, NPDES #FL0000957, on 30 March to 1 April, 1988.

Facility:	Jacksonville NAS STP			NPDES # FL0000957		Facility Type:	Municipal
Location:	Duval County			Contact/District:	M. Sittig		
Sample Collection Date:	3/29/88	Time:	1020	Test type:	Static Screening		
Test Beginning Date:	3/30/88	Time:	1000	# tests:	3	Receiving Water:	St. Johns River
Test Ending Date:	4/1/88	Time:	1000	Chlorination Type:	de-chlorinated		

Parameter	
Total Residual Cl2:	0
Method:	Hach
By:	DAC
Salinity:	0.2

Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.00
Total NH3:	0	0

Organism: ***Ceriodaphnia dubia*** Age/Life stage: Neonates

LC50: Binomial: Moving Average: Probit:

non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	10	10	10	8.3	8.7	8.3	8.1	7.9	7.9	19.4	19.9	19.8		
Control	10	10	10	8.3	8.7	8.3	8.1	7.9	7.9					
30%	10	10	10	8.5	8.5	8.5	7.9	7.9	7.8	20	19.9	19.9		
30%	10	10	10	8.4	8.5	8.5	7.9	7.9	7.8					
60%	10	10	10	8.3	8.5	8.5	7.6	7.8	7.7	20	20	19.9		
60%	10	10	10	8.3	8.5	8.5	7.6	7.8	7.7					
100%	10	10	10	8.2	8.5	8.4	7.1	7.7	7.5	20.3	20.1	20		
100%	10	10	10	8.2	8.5	8.5	7.1	7.7	7.5					

	Control water	100% sample
Alkalinity:	130	55
Hardness:	128	250

Organism: ***Daphnia pulex*** Age/Life stage: Neonates

LC50: Binomial: Moving Average: Probit:

non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	10	10	10	8.4	8.5	8.5	8.2	7.9	7.8	20.6	19.8	19.8		
Control	10	10	10	8.5	8.2	8.6	8.2	7.9	7.8				212	210
30%	10	10	10	8.5	8.5	8.6	7.9	7.8	7.7	20.6	19.7	19.9		
30%	10	10	10	8.5	8.5	8.6	7.9	7.8	7.7				352	350
60%	10	10	10	8.5	8.5	8.7	7.5	7.7	7.6	20.6	19.7	19.8		
60%	10	10	10	8.4	8.7	8.6	7.5	7.7	7.6				495	490
100%	10	10	10	8.5	8.6	8.7	7	7.5	7.5	20.6	19.9	19.9		
100%	10	10	10	8.5	8.6	8.6	7	7.5	7.5				700	700

	Control water	100% sample
Alkalinity:	130	55
Hardness:	128	250

Organism: ***Notropis leedsi*** Age/Life stage: 27 days old

LC50: Binomial: Moving Average: Probit:

non-toxic

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hour	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	8.2	7.9	8	7.6	7.6	7.6	20.7	19.9	20.1		
Control	5	5	5	8.2	7.8	8.1	7.6	7.6	7.6				210	215
30%	5	5	5	8.2	7.7	7.7	7.4	7.4	7.4	20.6	19.8	19.9		
30%	5	5	5	8.2	7.6	7.7	7.4	7.4	7.4				355	355
60%	5	5	5	8.2	7.6	7.5	7.3	7.4	7.3	20.5	19.7	19.8		
60%	5	5	5	8.2	7.6	7.4	7.3	7.4	7.3				495	490
100%	5	5	5	8.2	7.7	7.9	7	7.3	7.2	20.3	19.8	19.8		
100%	5	5	5	8.2	7.9	8	7	7.3	7.2				700	710

	Control water	100% sample
Alkalinity:	118	55
Hardness:	125	250

Table 4. Microtox test results , Jacksonville NAS STP discharge , Duval County, NPDES #FL0000957, on 30 March , 1988.

Microtox Test Culture Lot # M-606

Final Reclaimed Water

% Sample	Chlorinated		Dechlorinated	
	5-min	15-min	5-min	15-min
0	70	46	51	35
0	66	44	53	37
0	63	42	58	41
12.38	60	36	55	37
12.38	65	40	55	36
12.38	61	36	53	36
24.75	53	30	53	35
24.75	55	32	55	35
24.75	50	28	59	38
49.5	43	21	51	31
49.5	41	21	49	28
49.5	53	28	53	32
99	28	13	48	27
99	28	13	36	19
99	30	14	40	22

EC50 Chlorinated (15 minute, 15 degrees C) = 50.6% sample
EC50 Dechlorinated (15 minute, 15 degree C) > 100%

EC50 (15 minute, 15 degrees C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

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cl toxic Not acutely toxic

BIOASSAY DATA FORM

Industry/Toxicant JAX NAS STP
Address Jacksonville Duval Co,
Contact/District NE Insitig
Effluent Serial Number 001
NPDES Permit Number FL0000957

Test Number: 1 of 3
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 3-29-88 Time 1020
Test Beginning: Date 3-30-88 Time 1000
Test Ending: Date 4-1-88 Time 1000
Test Organism Ceriodaphnia dubia
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					
Control	95	10	10	10			8.6	8.5	8.5			8.0	7.8	7.8			19.9	20.1	19.9							
Control	67	10	10	10			8.6	8.5	8.5			8.0	7.8	7.8												
30%	92	10	10	10			8.7	8.7	8.6			7.8	7.8	7.8			19.9	20.0	19.8							
30%	6	10	10	10			8.6	8.7	8.6			7.8	7.8	7.8												
60%	—	10	7	5			8.6	8.8	8.7			7.6	7.8	7.7			20.0	19.9	19.7							
60%	5	10	9	6			8.6	8.8	8.7			7.6	7.8	7.7												
100%	63	10	10	7			8.6	8.7	8.7			7.0	7.6	7.5			20.3	20.2	19.9							
100%	85	10	10	7			8.5	8.7	8.7			7.0	7.6	7.5												
Initial of Investigator		RBF	RBF	RBF			RBF	RBF	RBF			RBF	RBF	RBF			RBF	RBF	RBF			RBF				

Alkalinity/Hardness (mg/l as $CaCO_3$) A
Control Water: 130 | 1
100% Sample: 55 | 2
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0
100% Sample: 0
Other:

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

Signatures:
Marshall Faircloth
Rural Hydrology

Instrument Calibration:

	DO Meter:	pH Meter:	S-C-T Meter:
0 Hours	7.7 / 8.7	7.0 @ 21°C	7.5 @ 10; 27,030
24 Hours	7.7 / 8.7	8.5 @ 22°C	
48 Hours	7.7 / 8.7	7.0 @ 21°C	9.5 @ 10 27,030
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 0.5 Method: HACH
Lab: 0.3 Method: LCK2

Reviewed by:

Dechlorinated:
Measurements by: RBF

Date: 5/11/88

Remarks: Cond. 13000 @ 25°C 24th Inf
48th 12900 @ 19°C Inf

Industry/Toxicant: JAX NAS STP
Address: Jacksonville Duval Co
Contact/District: NE/M.S.; T.S.
Effluent Serial Number: 001
NPDES Permit Number: FL0000957

BIOASSAY DATA FORM

Test Number: 2 of 6
Test Type: (Static) Renewal, Screening (Conventional)
Other:

Sample Collection: Date 3-29-88 Time 1020
Test Beginning: Date 3-30-88 Time 1000
Test Ending: Date 4-1-88 Time 1000
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	68	10	10	10			8.6	8.8	8.7			8.1	7.8	7.8			20.2	19.8	19.8							
Control	65	10	10	10			8.6	8.8	8.8			8.1	7.9	7.8								209		215		
30%	43	10	10	10			8.7	8.8	8.7			7.8	7.8	7.7			20.5	19.5	19.8							
30%	55	10	10	10			8.7	8.8	8.7			7.8	7.8	7.7								340		340		
60%	26	10	10	10			8.6	8.9	8.8			7.5	7.7	7.6			20.4	19.5	19.6							
60%	50	10	10	9			8.6	8.9	8.8			7.5	7.7	7.6								480		485		
100%	7	10	10	10			8.5	9.0	8.7			7.0	7.5	7.5			20.4	19.6	19.7							
100%	32	10	9	9			8.5	9.0	8.6			7.0	7.5	7.5								700		700		
Initial of Investigator		MF	MF	MF			RBF	RBF	RBF			RBF	RBF	RBF			RBF	RBF	RBF			RBF		RBF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 130
100% Sample: 55
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water:
100% Sample:
Other:
Hour ____
Control Water:
100% Sample:
Other:
Measured by: MF

Signatures: Randall Faulk
Randall Fayhelberg

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: 1.3 Method: Hach
Lab: 0.5 Method: Hach
Lab: 0.8 Method: 1000

De-chlorinated:
Measurements by: RBF

Reviewed by:
Date: 5/11/88

Cl Toxic

BIOASSAY DATA FORM

Industry/Toxicant NAS JAX STP
Address Jacksonville Duval Co.
Contact/District NE Fl. (Sitts)
Effluent Serial Number 001
NPDES Permit Number FL 0000957

Test Number: 3 of 6
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 3/29/88 Time 1020
Test Beginning: Date 3/30/88 Time 1000
Test Ending: Date 4-1-88 Time 1000
Test Organism N. Cordi
Age/Life Stage 22 days

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	32	5	5	5			8.1	7.9	7.7	7.5		7.6	7.6	7.6			20.7	19.8	19.4							
Control	131	5	5	5			8.2	7.9	7.7	7.5		7.6	7.6	7.6								220		215		
30%	5	5	5	5			8.2	8.0	7.8	7.7		7.4	7.5	7.4			20.8	19.7	19.9							
30%	46	5	5	5			8.2	8.0	7.9	7.7		7.4	7.5	7.4								355		350		
60%	285	5	5	5			8.2	8.0	7.8	7.8		7.3	7.4	7.3			20.7	19.6	19.8							
60%	25	5	5	5			8.3	8.0	7.8	7.9		7.3	7.4	7.3								490		490		
100%	281	5	1	1			8.3	8.5	8.4	6.1		7.0	7.3	6.9			20.6	19.6	19.8							
100%	228	5	0	0			8.3	8.5	8.4	5.5		7.0	7.3	6.9								700		700		
Initial of Investigator		RBF	RBF	RBF SMF			RBF	RBF	RBF	RBF		RBF	RBF	RBF			RBF	RBF				RBF		RBF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 118 | 125
100% Sample: 55 | 250
Other:
Measured by: mf

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water:
100% Sample:
Other:

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

Sal 0.2

Signatures: Marshall Faubel, Russell Frydenborg

Instrument Calibrations:
pH Meter: DO Meter: SpC Meter:
0 Hours
24 Hours
48 Hours
72 Hours
96 Hours

Total Residual Chlorine:
First Daily: 1.3 Method: Hach
Lab: 0.5 Method: Hach
Lab: 0.8 Method: 1070

Residuals Measurements by: RBF

Reviewed by:
Date: 5/11/88

Industry/Toxicant NAS JAX STP
Address Jacksonville, Duval Co.
Contact/District M. SMITH/TNE
Effluent Serial Number 001
NPDES Permit Number FL0000953

de-d BIOASSAY DATA FORM

Test Number: 4 of 6
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 3-29-88 Time 1020
Test Beginning: Date 3-30-88 Time 1000
Test Ending: Date 4-1-88 Time 1020
Test Organism Ceriodaphnia dubia
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
Control	36	10	10	10	10		8.3	8.7	8.3			8.1	7.9	7.9			19.4	19.9	19.8		
Control	19	10	10	10	10		8.3	8.7	8.3			8.1	7.9	7.9			20.0				
30%	88	10	10	10	10		8.5	8.5	8.5			7.9	7.9	7.8			20.0	19.9	19.9		
30%	37	10	10	10	10		8.4	8.5	8.5			7.9	7.9	7.8							
60%	59	10	10	10	10		8.3	8.5	8.5			7.6	7.8	7.7			20.0	20.0	19.9		
60%	236	10	10	10	10		8.3	8.5	8.5			7.6	7.8	7.7							
100%	65	10	10	10	10		8.2	8.5	8.4			7.1	7.7	7.5			20.2	20.1	20.0		
100%	80	10	10	10	10		8.2	8.5	8.5			7.1	7.7	7.5							
Initial of Investigator		RBF					RBF	RBF	RBF			RBF	RBF	RBF			RBF	RBF	RBF		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water: 130 | 128
100% Sample: 55 | 25
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0
100% Sample: 0
Other: 0

Hour 0
Control Water:
100% Sample:
Other:
Measured by: MF

Signature: David Fajdetzky
Title: Biologist

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7.0 7.09	9.0 @ 21°C	28C 10, 27.2 @ 30
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Control - 130 @ 18°C

Total Residual Chlorine:
Field data: 1.3 Method: Hach
Lab: 0.5 Method: Hach
Lab: 0.8 Method: 1020 Reviewed by:

De-chlorinated:
Measurements by: RBF

Date: 5/11/88

Industry/Toxicant NAS JAX STP
Address Jacksonville, Duval Co.
Contact/District M. Sitts / NE
Effluent Serial Number 001
NPDES Permit Number FL0000957

da-d

BIOASSAY DATA FORM

Test Number: 5 of 6
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 3/29/88 Time 1020
Test Beginning: Date 3/30/88 Time 1000
Test Ending: Date 4/1/88 Time 1000
Test Organism DAPHNIA
Age/Life Stage NEO

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	COND 0	24	48	72	96
Control	8	10	10	10			8.4	8.5	8.5			8.2	7.9	7.8			20.6	19.8	19.8							
Control	119	10	10	10			8.5	8.2	8.6			8.2	7.9	7.8								212		210		
30%	23	10	10	10			8.5	8.5	8.6			7.9	7.8	7.7			20.6	19.7	19.9							
30%	28	10	10	10			8.5	8.5	8.6			7.9	7.8	7.7								352		350		
60%	27	10	10	10			8.5	8.5	8.7			7.5	7.7	7.6			20.6	19.7	19.8							
60%	138	10	10	10			8.4	8.7	8.8			7.5	7.7	7.6								495		490		
100%	39	10	10	10			8.5	8.6	8.7			7.0	7.5	7.5			20.6	19.9	19.9							
100%	14	10	10	10			8.5	8.6	8.6			7.0	7.5	7.5								700		700		
Initial of Investigator		MF	MF	RMF			RBF	RBF	RBF			RBF	RBF	RBF			RBF	RBF	RBF			RBF		RBF		

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

Control Water: 130 | 128
100% Sample: 55 | 250
Other:
Measured by: MF

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

Control Water: 0
100% Sample: 0
Other: 0

Hour ____

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

Signature: Marshall Fairchild
Russel Ingebrigtsen

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: 1.3 Method: Hach
Lab: 0.5 Method: Hach
Lab: 0.3 Method: DPD

Dechlorinated:
Measurements by: RBF

Reviewed by:
Date: 5/11/88

ele. cl

BIOASSAY DATA FORM

Industry/Toxicant: NAS JAX STP
Address: Jacksonville, Duval Co
Contact/District: N. SITE/NE
Effluent Serial Number: 001
NPDES Permit Number: FL0000353

Test Number: 6 of 6
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 3-29-88 Time 1020
Test Beginning: Date 3-30-88 Time 1000
Test Ending: Date 4-1-88 Time 1000
Test Organism: Mysids
Age/Life Stage: 27 days

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	Gavd. 0	24	48	72	96
Control	13	5	5	5			8.2	7.9	8.0			7.6	7.6	7.6			20.7	19.9	20.1							
Control	30	5	5	5			8.2	7.8	8.1			7.6	7.6	7.6								210		215		
30%	2	5	5	5			8.2	7.7	7.7			7.4	7.4	7.4			20.6	19.8	19.9							
30%	27	5	5	5			8.2	7.6	7.7			7.4	7.4	7.4								355		355		
60%	44	5	5	5			8.2	7.6	7.5			7.3	7.4	7.3			20.5	19.7	19.8							
60%	76	5	5	5			8.2	7.6	7.4			7.3	7.4	7.3								495		490		
100%	50	5	5	5			8.2	7.7	7.9			7.0	7.3	7.2			20.3	19.8	19.8							
100%	38	5	5	5			8.2	7.9	8.0			7.0	7.3	7.2								700		710		
Initial of Investigator		RBF	MF	RBF			RBF	RBF	RBF			RBF	RBF	RBF			RBF	RBF	RBF			RBF	~~RBF~~ MV	RBF		

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

Control Water: 118 | 125
100% Sample: 55 | 25
Other:
Measured by: MF

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

Control Water: 0
100% Sample: 0
Other: 0

Hour ____

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

Signatures:
Mark Fairchild
Rural Hydrology

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: 1.3 Method: Hach
Lab: 0.5 Method: Hach
Lab: 0.8 Method: ICDO

De-chlorinated:
Measurements by: RBF

Reviewed by:

Date: 5/11/88

080

MICROTOX™ Data Reduction Sheet

SOURCE: Jacksonville NAS

GRAPH CALC EC 50 (15 MIN, 15 °C) = >100 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent de Cl2

DATE ASSAYED: 3-30-88 TIME: 10:00 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: none

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

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	51	35			
B1		53	37			
C1		58	41			
A2	12.38	55	37	-.006	.037	
B2		55	36			
C2		53	36			
A3	24.75	55	35	-.041	.046	
B3		55	35			
C3		59	38			
A4	49.5	51	31	.059	.242	
B4		49	28			
C4		53	32			
A5	99	48	27	.306	.662	
B5		36	19			
C5		40	22			
EC_{50} (MIN, °C)				—	1268	

REVIEWED BY:

DATE: 5/11/88

C79

MICROTOX™ Data Reduction Sheet

SOURCE: Jacksonville NAS

GRAPH CALC EC 50 (15 MIN, 15 °C) = 50.6 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent Cl2

DATE ASSAYED: 3-30-88 TIME: 9:30 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: none

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

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	I5	I15	NOTES
A1	0	70	46	X	X	
B1		66	44			
C1		63	42			
A2	12.38	60	36	.070	.179	
B2		65	40			
C2		61	36			
A3	24.75	53	30	.259	.467	
B3		55	32			
C3		50	28			
A4	49.5	43	21	.453	.886	
B4		41	21			
C4		53	28			
A5	99	28	13	1.314	2.30	
B5		28	13			
C5		30	14			
EC_{50} (MIN, °C)				49.3	50.55	

REVIEWED BY:

DATE: 5/11/88