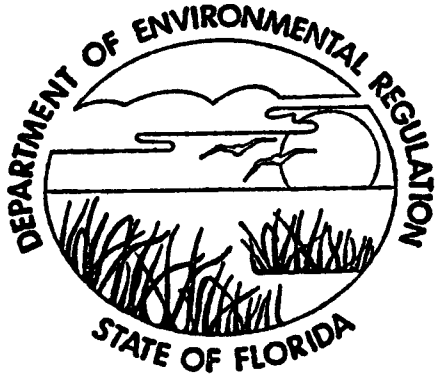



# BIOASSAYS OF AVONDALE

# SEWAGE TREATMENT PLANT

# PENSACOLA, ESCAMBIA COUNTY, FLORIDA

NPDES #FL0031801

SAMPLED 6/8/87

AUGUST 19, 1987

**Biology Section**
**Division of Environmental Programs**

# BIOASSAYS OF AVONDALE

# SEWAGE TREATMENT PLANT

# PENSACOLA, ESCAMBIA COUNTY, FLORIDA

# NPDES #FL0031801

Biology Section
Bureau of Laboratories and
Special Programs
August 19, 1987

## EXECUTIVE SUMMARY

Avondale STP, Pensacola, NPDES #FL0031801, on 9 to 11 June, 1987.

The Biology Section performed four static acute toxicity bioassays on samples of final chlorinated and de-chlorinated recovered waters from this domestic treatment facility, which discharges to Bayou Marcus Creek and eventually to Perdido Bay. The chlorinated sample proved to be immediately toxic to the test organisms, Ceriodaphnia dubia and Notropis leedsi, producing 24 hour $LC_{50}$'s of < 50%. This toxicity can mostly be attributed to the total residual chlorine (0.5 mg/l HACH, and 0.89 mg/l Iodometric) measured in the sample. The de-chlorinated sample, however, was also acutely toxic to C. dubia, producing a 24 hour $LC_{50}$ of 76.3% and a 48 hour value of < 50%. This same sample was not toxic to N. leedsi. The toxicity to C. dubia, therefore, suggests the presence of toxic components other than chlorine in the Avondale discharge.

The ammonia measured in these samples, 5.25 mg/l total and up to 0.084 mg/l unionized, probably contributed only slightly to the observed toxicity. The results of metal analyses indicated trace amounts of chromium, copper, and lead, and 12.0 ug/l of zinc. The most notable results of the organics analysis were the presence of the insecticides chlorpyrifos (0.38 ug/l), diazinon (0.91 ug/l), and endosulfan sulfate (< 0.03 ug/l). The presence of these pesticides in the recovered water sample can probably account for the observed toxicity to C. dubia in the de-chlorinated test.

## INTRODUCTION

The Avondale Sewage Treatment Plant is located west of the intersection of Bellview Road and Muldoon Road in Pensacola, Escambia County, Florida (Figures 1 and 2). The facility is owned and operated by the Escambia County Utilities and treats domestic wastes from the surrounding residential community. The design capacity of the plant is 1.0 MGD and treatment of waste includes screening, grit chamber, contact stabilization, clarification, polishing ponds, and final chlorination. Discharge is to an unnamed tributary to Bayou Marcus Creek, which flows a short distance to Perdido Bay. A schematic of the facility is presented in Figure 3 (data from NPDES file). Also noted in the file of this facility were various orders for the Avondale facility to stop discharging to surface waters.

At the request of the Northwest District, the Biology Section performed a series of static acute toxicity bioassays on samples of final chlorinated and de-chlorinated recovered waters from the Avondale facility in June, 1987. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: H. Cooper, J. M. Faircloth, and M. L. Roll.

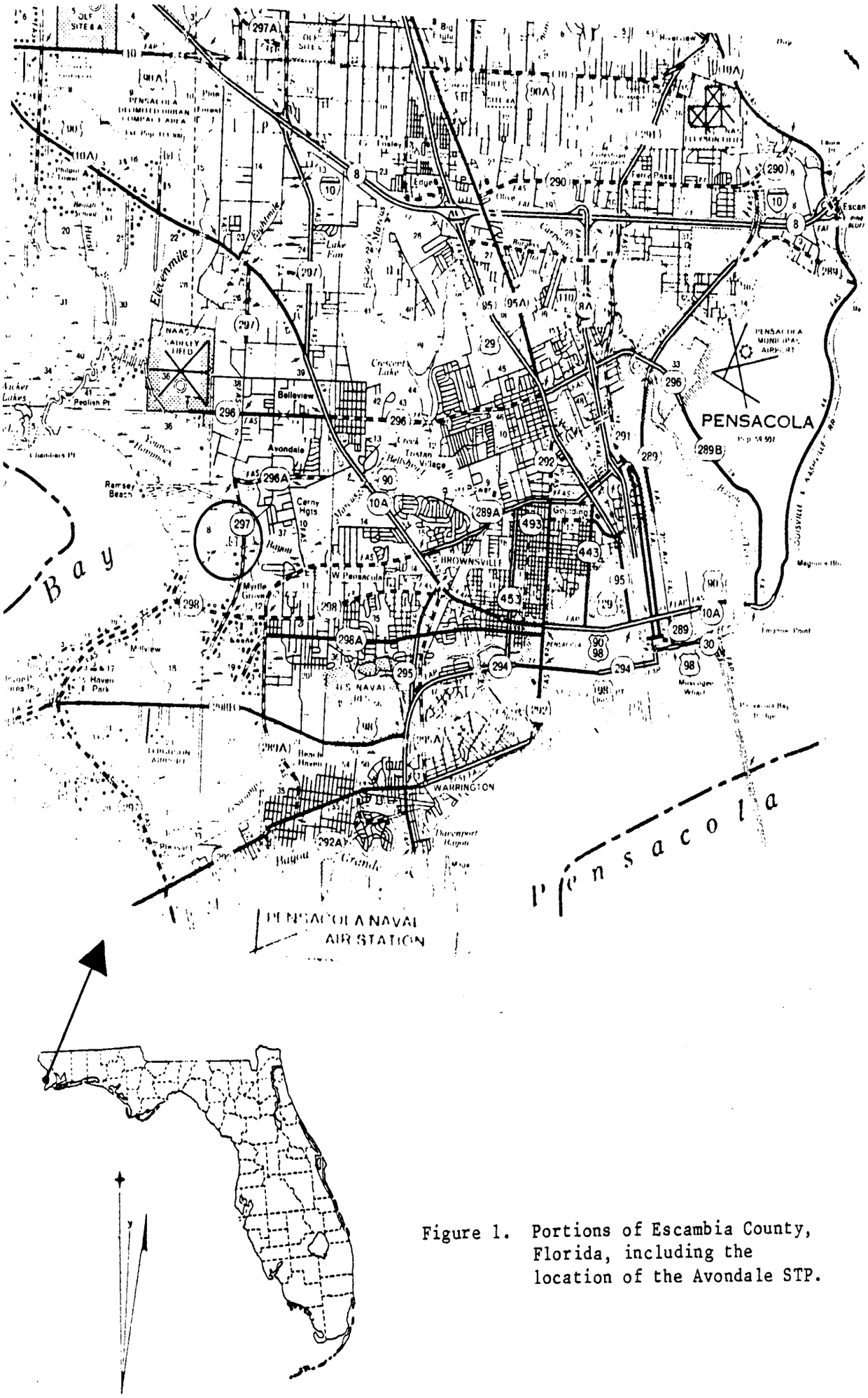



Figure 1. Portions of Escambia County, Florida, including the location of the Avondale STP.

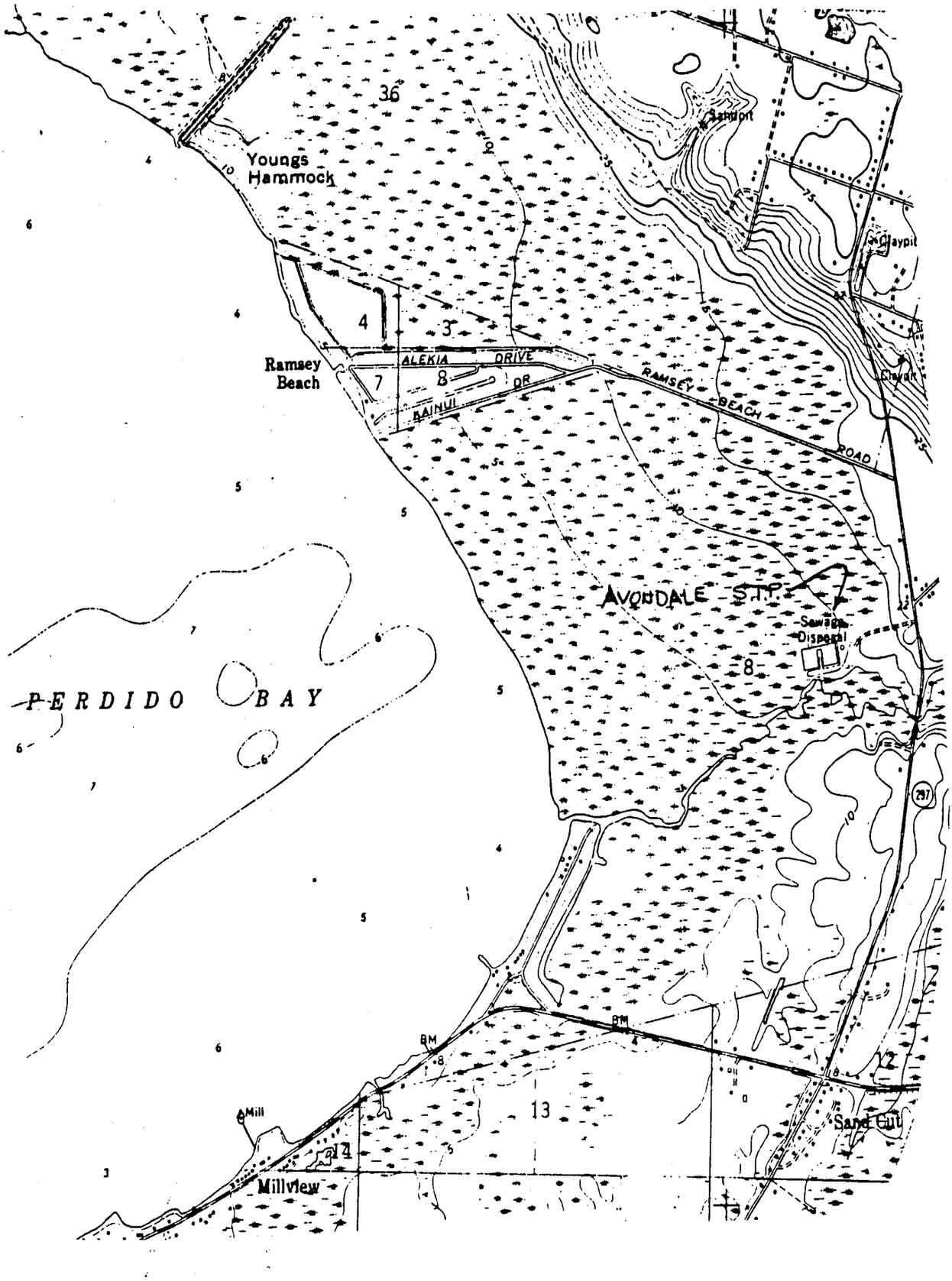



Figure 2. Location of Avondale STP and discharge point to Bayou Marcus Creek and Perdido Bay.

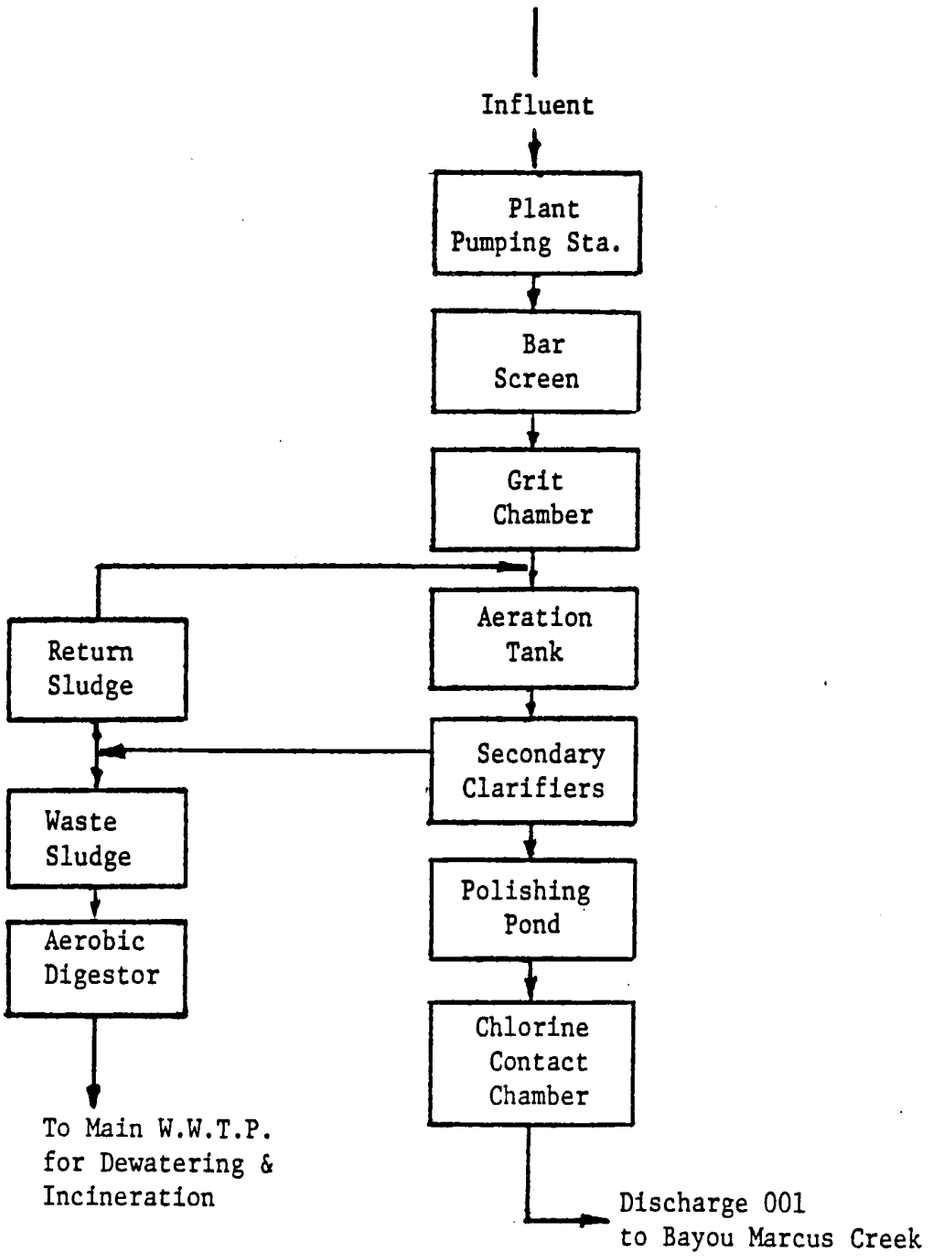



Figure 3. Schematic of Wastewater Flow
Avondale S.T.P.
Pensacola, Florida
Discharge Serial No. 001
1-11-83

## 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.

### History and Maintenance of Bioassay Organisms

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 approximately 26 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

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

Four separate 48 hour static acute toxicity screening bioassays (50% and 100% test concentrations only) were performed on samples of final chlorinated and de-chlorinated recovered waters from the Avondale STP facility. The tests were performed from 1030 hours on 9 June to 1030 hours on 11 June, 1987.

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. John Williams of DER's Northwest District at 1400 hours on 8 June, 1987, 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample 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 discharges 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 discharge. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated discharge 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 samples 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 discharge.

### Freshwater Tests

Freshwater for dilution of the 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 presented in Table 1.

Two replicates, of each concentration and a control, were used to test with the water flea, Ceriodaphnia dubia. Flint glass jars of 60 ml capacity were used as test chambers, and 50 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. 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% 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% test concentration at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2 and 3.

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 |
| 50.0% | 25.0 ml | 25.0 ml | 50 ml |
| 100.0% | ------- | 50.0 ml | 50 ml |

*Notropis leedsi* (bannerfin shiner)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Control | 500 ml | -------- | 500 ml |
| 50.0% | 250 ml | 250 ml | 500 ml |
| 100.0% | ------ | 500 ml | 500 ml |

Table 2. Data recorded during 48 hour, *Ceriodaphnia dubia* and *Notropis leedsi*, static acute toxicity bioassays of Avondale STP, Pensacola, Escambia County, Florida NPDES #FL0031801, on 9 to 11 June, 1987.

Chlorinated Sample[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 |
| *Ceriodaphnia dubia* | | | | | | | | | | | | | | |
| Control | 10 | 10 | -b | 8.4 | 8.3 | -b | 8.4 | 8.3 | -b | 19.2 | 19.9 | -b | | |
| Control | 10 | 10 | | 8.4 | 8.3 | | 8.4 | 8.3 | | | | | | |
| 50% | 10 | 0 | | 8.2 | 8.5 | | 7.8 | 8.3 | | 19.6 | 19.4 | | | |
| 50% | 10 | 0 | | 8.2 | 8.5 | | 7.8 | 8.3 | | | | | | |
| 100% | 10 | 0 | | 7.6 | 8.6 | | 7.5 | 8.3 | | 19.8 | 19.4 | | | |
| 100% | 10 | 0 | | 7.6 | 8.6 | | 7.5 | 8.2 | | | | | | |
| *Notropis leedsi* | | | | | | | | | | | | | | |
| Control | 5 | 5 | 5 | 8.2 | 7.8 | 7.1 | 7.9 | 7.8 | 7.7 | 20.9 | 19.7 | 19.6 | | |
| Control | 5 | 5 | 5 | 8.2 | 7.8 | 7.1 | 7.9 | 7.9 | 7.7 | | | | 202 | 200 |
| 50% | 5 | 1 | 1 | 8.0 | 8.2 | 5.9 | 7.6 | 7.8 | 7.5 | 20.7 | 19.6 | 19.7 | | |
| 50% | 5 | 0 | 0 | 8.0 | 8.2 | 5.9 | 7.6 | 7.8 | 7.5 | | | | 272 | 272 |
| 100% | 5 | 0 | 0 | 7.4 | 8.4 | -b | 7.4 | 7.7 | -b | 20.4 | 19.6 | -b | | |
| 100% | 5 | 0 | 0 | 7.4 | 8.4 | - | 7.4 | 7.7 | - | | | | 347 | -b |

Control Water

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

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

Total Alkalinity 113 (mg/l $CaCO_3$)

Total Hardness 120 (mg/l $CaCO_3$)

100% Sample

$NH_3$(as $NH_3$)(mg/l) 0.065 to 0.084

Total ammonia (as N)(mg/l) 5.25

Total Alkalinity 105 (mg/l $CaCO_3$)

Total Hardness 50 (mg/l $CaCO_3$)

[a] Total Residual Chlorine:
0.5 mg/l HACH DPD Method
0.89 mg/l Iodometric Method

[b] Test terminated and/or remaining parameters not recorded at 48 hours since all organisms were dead by 24 hours.

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia and Notropis leedsi, static acute toxicity bioassays of Avondale STP, Pensacola, Escambia County, Florida, NPDES #FL0031801, 9 to 11 June, 1987.

De-chlorinated Sample[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 |
| Ceriodaphnia dubia | | | | | | | | | | | | | | |
| Control | 10 | 10 | 10 | 8.4 | 8.2 | 8.1 | 8.4 | 8.2 | 8.1 | 20.1 | 19.8 | 19.9 | | |
| Control | 10 | 10 | 10 | 8.4 | 8.2 | 8.1 | 8.4 | 8.2 | 8.1 | | | | | |
| 50% | 10 | 8 | 0 | 8.2 | 8.3 | 8.3 | 7.8 | 8.0 | 7.9 | 19.7 | 19.9 | 20.0 | | |
| 50% | 10 | 8 | 0 | 8.2 | 8.3 | 8.3 | 7.8 | 8.0 | 7.9 | | | | | |
| 100% | 10 | 4 | 0 | 7.7 | 7.2 | 8.3 | 7.4 | 7.8 | 7.8 | 19.5 | 20.0 | 20.0 | | |
| 100% | 10 | 2 | 0 | 7.7 | 7.2 | 8.3 | 7.4 | 7.8 | 7.8 | | | | | |
| Notropis leedsi | | | | | | | | | | | | | | |
| Control | 5 | 5 | 5 | 8.2 | 7.8 | 7.1 | 7.9 | 7.8 | 7.7 | 20.9 | 19.7 | 19.6 | | |
| Control | 5 | 5 | 5 | 8.2 | 7.8 | 7.1 | 7.9 | 7.9 | 7.7 | | | | 202 | 200 |
| 50% | 5 | 5 | 5 | 7.7 | 7.4 | 7.7 | 7.6 | 7.7 | 7.7 | 20.3 | 19.8 | 19.7 | | |
| 50% | 5 | 5 | 5 | 7.7 | 7.4 | 7.7 | 7.6 | 7.7 | 7.7 | | | | 272 | 275 |
| 100% | 6[a] | 5 | 5 | 7.4 | 6.9 | 7.2 | 7.4 | 7.7 | 7.6 | 19.5 | 19.8 | 19.7 | | |
| 100% | 5 | 5 | 5 | 7.4 | 6.9 | 7.2 | 7.4 | 7.7 | 7.6 | | | | 350 | 352 |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

Control Water

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

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

Total Alkalinity 113 (mg/l $CaOO_3$)

Total Hardness 120 (mg/l $CaOO_3$)

100% Sample

$NH_3$(as $NH_3$)(mg/l) 0.061

Total ammonia (as N)(mg/l) 5.25

Total Alkalinity 105 (mg/l $CaOO_3$)

Total Hardness 50 (mg/l $CaOO_3$)

[a] Total Residual Chlorine: N.D. HACH

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

Table 4. $LC_{50}$ Data for Bioassays of Avondale STP, Pensacola, NPDES #FL0031801, on 9 to 11 June, 1987.

| Organism | Method of Data Analysis | 24 Hour $LC_{50}$ and 95% Confidence Intervals | 48 Hour $LC_{50}$ and 95% Confidence Intervals |
|---|---|---|---|
| Chlorinated Sample | | | |
| *Ceriodaphnia dubia* | Binomial<br>Moving Average<br>Probit | < 50%, a | < 50%, a |
| *Notropis leedsi* | Binomial<br>Moving Average<br>Probit | < 50%, a | < 50%, a |
| De-chlorinated Sample | | | |
| *C. dubia* | Binomial<br>Moving Average<br>Probit | 76.3% (50 and ∞)<br>76.3% (60.4 and 105.3), b<br>76.6% (59.8 and 104.9) | < 50%, a |
| *N. leedsi* | Binomial<br>Moving Average<br>Probit | c | c |

[a] The $LC_{50}$ is < 50% since all the organisms died in the smallest test concentration (50%).

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

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

Table 5. Results of chemical analyses performed on recovered water from Avondale STP, NPDES #FL0031801, collected 8 June, 1987.

## Organics

| | |
|---|---|
| Acid Extractables | N.D. |
| Base Neutral Extractables | trace amount of Petroleum Residue |
| Pesticide Extractables | 0.14 ug/l g-BHC |
| | < 0.03 ug/l[a] Endosulfan Sulfate |
| | 0.91 ug/l Diazinon |
| | 0.38 ug/l Chlorpyrifos |

## Metals

| | |
|---|---|
| Arsenic | N.D. |
| Cadmium | N.D. |
| Chromium | < 25.0 ug/l[a] |
| Copper | < 25.0 ug/l[a] |
| Lead | < 5.0 ug/l[a] |
| Mercury | N.D. |
| Nickel | N.D. |
| Selenium | N.D. |
| Silver | N.D. |
| Zinc | 12.0 ug/l |

N.D. - None Detected
[a] Actual value is known to be less than value given.

## RESULTS AND DISCUSSION

The sample of final chlorinated recovered water from the Avondale Sewage Treatment Plant proved to be acutely toxic to the test organisms, Ceriodaphnia dubia and Notropis leedsi, producing 24 hour $LC_{50}$'s of < 50% (Table 4). This toxicity can mostly be attributed to the total residual chlorine (0.5 mg/l) measured in the sample. The de-chlorinated sample, however, also was acutely toxic to C. dubia, producing a 24 hour $LC_{50}$ of 76.3% and a 48 hour value of < 50% (Table 4). This toxicity suggests the presence of other toxic components besides chlorine in the Avondale discharge. The de-chlorinated sample was not acutely toxic to N. leedsi.

The ammonia measured in the Avondale discharge, 5.25 mg/l total and up to 0.084 mg/l unionized, probably contributed only slightly to the observed toxicity. The results of chemical analyses (Table 5), indicated the presence of trace amounts of the metals chromium, copper, and lead, and 12.0 ug/l of zinc. Analysis for organic compounds revealed a trace amount of petroleum residue, as well as the insecticides g-BHC (0.14 ug/l), chlorpyrifos (0.38 ug/l), diazinon (0.91 ug/l), and endosulfan sulfate (< 0.03 ug/l). The presence of these pesticides, probably contributed to the observed toxicity to C. dubia in the de-chlorinated tests.

## 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 onetwentieth 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 discharges, representative of the 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, 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.

## LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

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

# BIOASSAY DATA FORM

Facility/Toxicant: Avondale STP
Address: Pensacola
NPDES Number: FL0031801
Outfall Number: 001

Test #: 1, 2, 3, 4
Type: Sewage
Organism: N. Laeda, C. Dubia

Sample Collected: Date 6-8-87 Time 1400
Test Beginning: Date 6-9-87 Time 1030
Test Ending: Date 6-11-87 Time 1030

| Test Concentration | Chamber Number | Live Organisms 0 | 24 | 48 | Dissolved Oxygen (mg/L) 0 | 24 | 48 | pH 0 | 24 | 48 | Temperature °C 0 | 24 | 48 | Conductivity 0 | 48 | Ammonia Total 0 | 48 | Ammonia Un-ionized 0 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 30 | 10 | 10 | - | 8.4 | 8.8 | 8.3 | 8.9 | 8.3 | - | 19.2 | 19.9 | - | | | 0 | | 0 | |
| Control | 93 | 10 | 10 | - | 8.4 | 8.8 | 8.3 | 8.4 | 8.3 | - | | | - | | | | | | |
| 50% | 75 | 10 | 0 | - | 8.2 | 8.3 | 8.5 | 7.8 | 8.3 | - | 19.6 | 19.5 | - | | | | | | |
| 50% | 21 | 10 | 0 | - | 8.2 | 8.3 | 8.5 | 7.8 | 8.3 | - | | | - | | | | | | |
| 100% | 4 | 10 | 0 | - | 7.6 | 8.3 | 8.6 | 7.5 | 8.3 | - | 19.3 | 19.4 | - | | | | | | |
| 100% | 95 | 10 | 0 | - | 7.6 | 8.2 | 8.6 | 7.5 | 8.2 | - | | | - | | | ↑ | | 084 | |
| Control | 51 | 5 | 5 | 5 | 8.2 | 7.8 | 7.8 | 7.9 | 7.8 | 7.7 | 20.9 | 19.7 | 19.6 | | | 5.75 | | | |
| Control | 37 | 5 | 5 | 5 | 8.2 | 7.9 | 7.8 | 7.9 | 7.9 | 7.7 | | | | 202 | 200 | 0 | | 0 | |
| 50% | 16 | 5 | 1 | 1 | 8.0 | 7.8 | 8.2 | 7.6 | 7.8 | 7.5 | 20.1 | 19.6 | 19.8 | | | | | | |
| 50% | 10 | 5 | 0 | 0 | 8.0 | 7.8 | 8.2 | 7.6 | 7.8 | 7.5 | | | | 272 | 272 | | | | |
| 100% | 65 | 5 | 0 | 0 | 7.4 | 7.7 | 8.4 | 7.4 | 7.7 | - | 20.4 | 19.6 | - | | | | | | |
| 100% | 31 | 5 | 0 | 0 | 7.4 | 7.7 | 8.4 | 7.4 | 7.7 | - | | | - | 347 | - | 5.25 | | 069 | |
| Control | 36 | 10 | 10 | 10 | 8.4 | 8.2 | 8.2 | 8.1 | 8.4 | 8.2 | 8.1 | 20.1 | 19.3 | 19.7 | | | | 0 | | 0 | |
| Control | 50 | 10 | 10 | 10 | 8.4 | 8.2 | 8.2 | 8.1 | 8.4 | 8.2 | 8.1 | | | | | | | | | |
| 50% | 20 | 10 | 8 | 0 | 8.2 | 8.0 | 8.3 | 8.3 | 7.8 | 8.0 | 7.9 | 19.7 | 19.9 | 20.0 | | | | | | |
| 50% | 80 | 10 | 8 | 0 | 8.2 | 8.0 | 8.3 | 8.3 | 7.8 | 8.0 | 7.9 | | | | | | | | | |
| 100% | 68 | 10 | 4 | 0 | 7.7 | 7.85 | 7.2 | 8.3 | 7.4 | 7.85 | 7.8 | 19.5 | 20.2 | 20.0 | | 5.25 | | 061 | |
| 100% | 85 | 10 | 2 | 0 | 7.7 | 7.85 | 7.2 | 8.3 | 7.4 | 7.85 | 7.8 | | | | | | | | | |
| 50% | 38 | 5 | 5 | 5 | 7.7 | 7.7 | 7.4 | 7.7 | 7.6 | 7.7 | 7.7 | 20.3 | 19.3 | 19.7 | | | | | | |
| 50% | 0 | 5 | 5 | 5 | 7.7 | 7.7 | 7.4 | 7.7 | 7.6 | 7.7 | 7.7 | | | | 272 | 275 | | | | |
| 100% | 55 | 6 | 5 | 5 | 7.4 | 7.7 | 6.9 | 7.3 | 7.4 | 7.7 | 7.6 | 19.5 | 19.8 | 19.2 | | | | | | |
| 100% | 58 | 5 | 5 | 5 | 7.4 | 7.7 | 6.9 | 7.2 | 7.4 | 7.7 | 7.6 | | | | 350 | 352 | 5.25 | | 061 | |
| | | MLR | MF | HC | MLR | MF | MLR | MLR | MLR | MF | MLR | MLR | MF | | MLR | MLR | MF | | MLR | |

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$): 123

Total Hardness (mg/l $CaCO_3$): 120

100% Sample

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

Total Hardness (mg/l $CaCO_3$): 50

Age of Test Organisms: N. laeda - 26 days

Height: ____

Instrument Calibrations:

| | pH Meter: | D.O. Meter: | S-C-T Meter: |
|---|---|---|---|
| 0 Hours | 707, 909 | 8.8 @ 22°C | 10.8 @ 10 27 @ 30 |
| 24 Hours | 707 409 | 9.0 @ 21°C | |
| 48 Hours | 207 269 | 9.1 @ 20.5°C | 10.8 @ 10 25 @ 30 |

Reviewed by: [signature]
Date: 8/17/87

$Cl_2$ = 0.5 mg/l HACH
0.89 mg/l Iodometric

$NH_3$-$(l)_2$ = N.D

C. Dubia
N. laedi
N. laedi
dec'd
C. Dubia
N. laedi