


# BIOASSAYS OF CHAMPION PAPER COMPANY

## KRAFT DIVISION

## CANTONMENT, ESCAMBIA COUNTY, FLORIDA

NPDES #FL0002526

SAMPLED 4/6/87

MAY 20, 1987

Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
May 20, 1987

## EXECUTIVE SUMMARY

Champion Paper Company, Cantonment, NPDES #FL0002526, 7 to 9 April, 1987

Three separate static acute toxicity bioassays were performed on a sample of effluent collected from Champion Paper Company's Outfall #001. The sample proved to be non-toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No mortality occurred in the 48 hour C. dubia and D. pulex tests, and mortality was insufficient to calculate an $LC_{50}$ in the N. leedsi test. The concentrations of total and unionized ammonia measured in this effluent (2.0 mg/l and 0.12 mg/l, respectively) were not high enough to have caused any toxicity. Because the sample was not acutely toxic, chemical analyses were not performed.

## INTRODUCTION

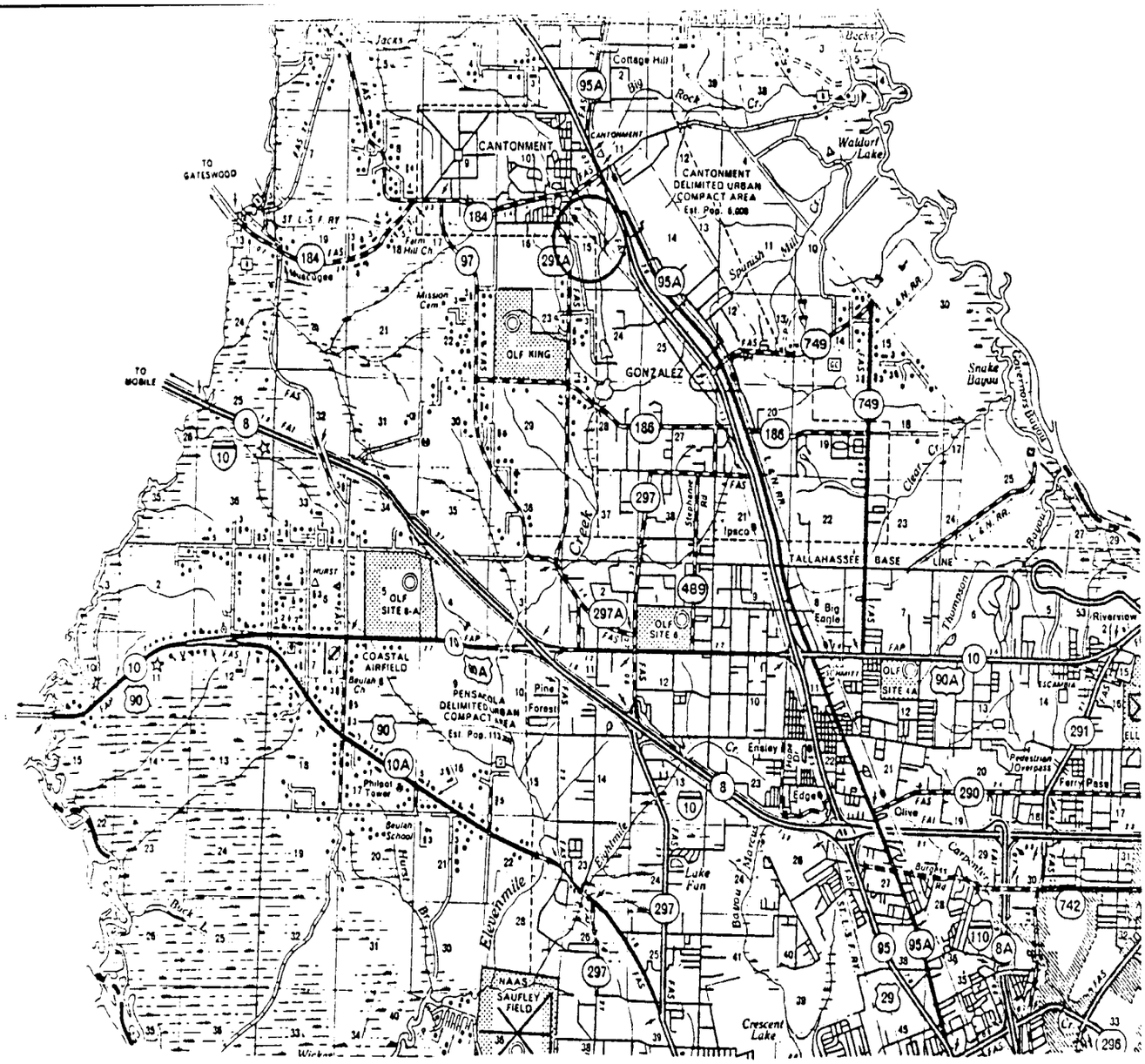
The Champion Paper Company, formerly the St. Regis Paper Company, is located at the intersection of U.S. Highway 29 and State Road 184 in Cantonment, Escambia County (Fig. 1). According to the NPDES permit (#FL00002526, issued in June 1979), this facility operates continuously, processing 1000 tons per day of pulpwood into paper products. Raw materials required for this operation include: pulpwood (pine or hardwood), salt cake, lime, caustic soda, chlorine, salt, sodium chlorate, rosin size, alum, sulfuric acid, and cornstarch.

Figure 2 depicts the water flow through the Champion Plant in Cantonment. The following compounds are associated with the paper manufacturing process and may be found in the wastewater stream: primaric acid, isoprimaric acid, sandaracopimaric acid, abietic acid, dehydroabietic acid, 3, 4, 5, trichloroquaiacol, 3, 4, 5, 6, tetrachloroquaiacol, mono-chloro-dehydroabietic acid (2 isomers), 9, 10, epoxystearic acid, hydrogen sulfide, methyl mercaptan, glycol inks, and sodium sulfide. Prior to discharge to the headwaters of Eleven Mile Creek, the wastewater is treated by a clarifier, a series of mechanically aerated lagoons and final settling in an additional lagoon. This system is designed for a 28 MGD wastewater flow. Champions Outfall #001 is located at State Road 186, approximately 2 miles from the plant site. Eleven Mile Creek eventually empties into the northern portion of Perdido Bay approximately 12 miles downstream of the plant outfall.

On 17 to 19 February, 1981, the Biology Section performed three separate static bioassays on an effluent sample from the Champion Paper Company, Outfall #001. The results of those tests indicated that the sample was not toxic to the test organisms, Daphnia pulex, Simocephalus serrulatus, and Pimephales promelas (FDER 1981).

To again determine the existence and extent of toxicity of this effluent, the Biology Section performed additional bioassays on samples of effluent collected from Champion Paper Company's Outfall #001 and from Eleven Mile Creek downstream of the outfall boil. The tests were performed from 20 to 23 August, 1985. The results of those tests indicated that both samples were acutely toxic to Notropis leedsi. Ammonia was believed to have been mostly responsible for this toxicity (FDER 1985a). As a result of this toxicity, the Northwest District requested that further bioassays be performed. A sample of effluent was collected from Champion's Outfall #001 on 28 April, 1986, and two 48 hour static bioassays were performed. At this time, the effluent was not toxic to the test organisms, Daphnia pulex, and Notropis leedsi (FDER 1986).

In April of 1987, the Biology Section received a sample collected from Champion's Outfall #001 and performed three static acute toxicity bioassays. Results 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 K. M. Lurding.



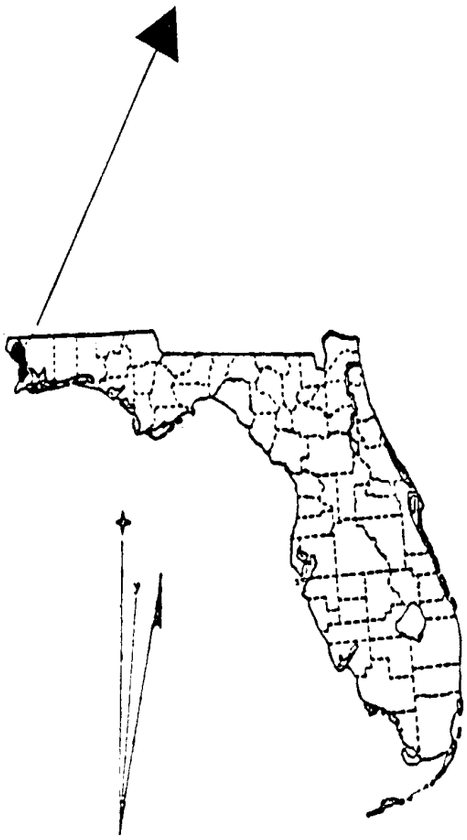



Figure 1. Portions of Escambia County, including the location of the Champion facility.

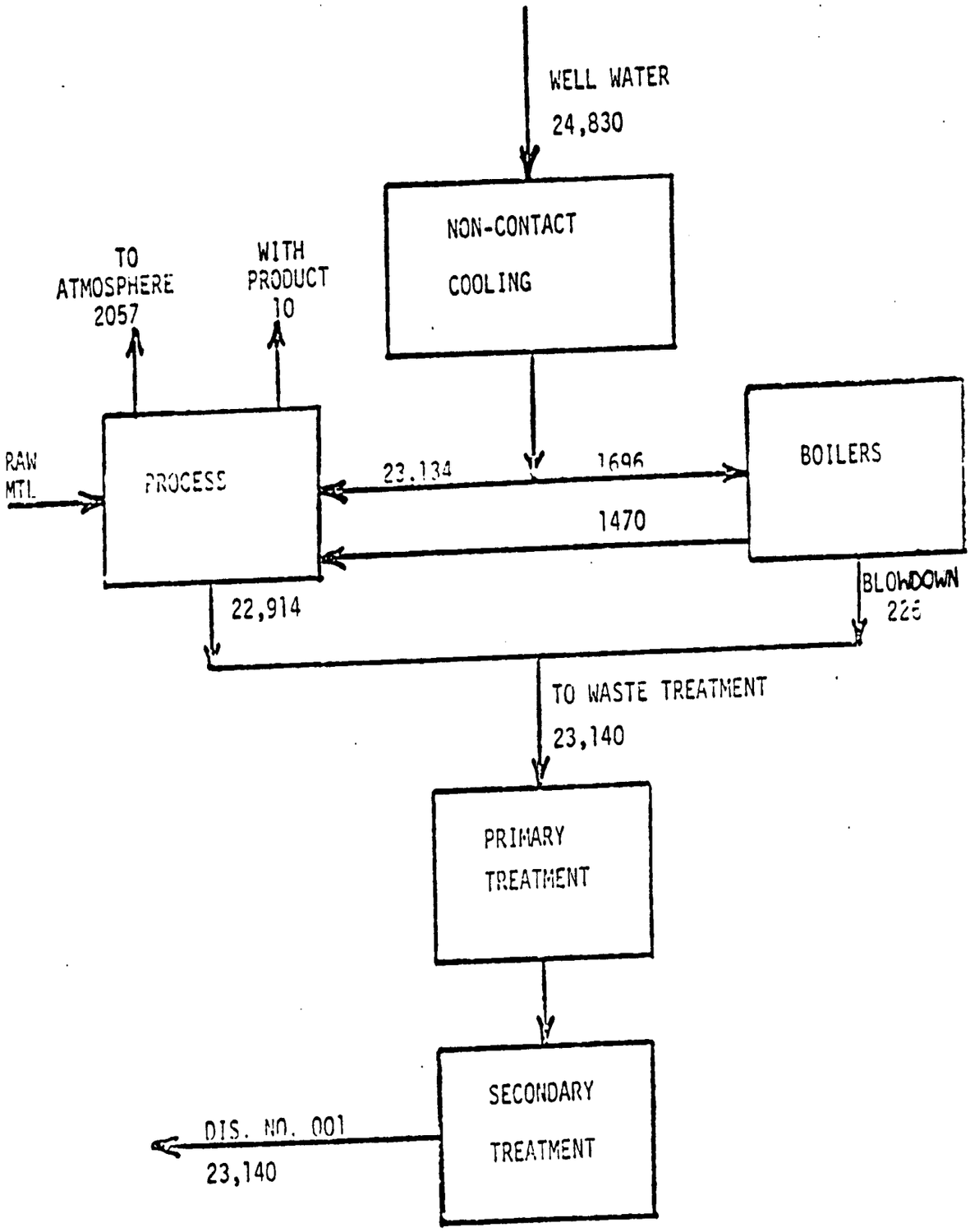

WELL WATER
24,830
NON-CONTACT
COOLING
TO
ATMOSPHERE
2057
WITH
PRODUCT
10
RAW
MTL
PROCESS
23,134
1696
1470
BOILERS
BLOWDOWN
22,914
TO WASTE TREATMENT
23,140
PRIMARY
TREATMENT
SECONDARY
TREATMENT
DIS. NO. 001
23,140
FLOW UNITS - 1000 GAL/DAY
SCHEMATIC OF WATER FLOW
ST. REGIS PAPER COMPANY
CANTONMENT
ESCAMBIA COUNTY
FLORIDA
JUNE 20, 1978

Figure 2.

## METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985b). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985b). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

### History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, in March, 1979. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

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 15 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 (1985b). 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

Three static acute toxicity bioassays were performed on a sample of effluent collected from Champion Paper Company's Outfall #001. The tests were conducted from 1100 hours on 7 April to 1100 hours on 9 April, 1987.

The sample was collected by Mr. Steven Baisden of DER's Northwest District Office at 1130 hours on 6 April, 1987, using standard wastewater sampling procedures. The sample was 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° ± 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.

### Freshwater Tests

Freshwater for dilution of the effluent samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study 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 this test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% effluent concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as $NH_3$, respectively), were recorded in the controls and the 100% effluent 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 Table 2.

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 |

*Daphnia pulex* (water flea)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Control | 200.0 ml | -------- | 200 ml |
| 50.0% | 100.0 ml | 100.0 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 |
| 50.0% | 250 ml | 250 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 bioassays of Champion Paper Company, NPDES #FL0002526, 7 to 9 April, 1987.

| 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 | 9.0 | 8.6 | 9.0 | 7.8 | 8.2 | 8.3 | 20.6 | 18.8 | 18.7 | | |
| Control | 10 | 10 | 10 | 9.0 | 8.6 | 9.0 | 7.8 | 8.2 | 8.3 | | | | | |
| 50% | 10 | 10 | 10 | 8.9 | 8.6 | 9.0 | 7.9 | 8.3 | 8.5 | 20.4 | 18.9 | 18.7 | | |
| 50% | 10 | 10 | 10 | 8.9 | 8.6 | 9.0 | 7.9 | 8.3 | 8.5 | | | | | |
| 100% | 10 | 10 | 10 | 8.9 | 8.5 | 9.0 | 8.1 | 8.6 | 8.7 | 20.3 | 18.8 | 18.8 | | |
| 100% | 10 | 10 | 10 | 8.9 | 8.5 | 8.9 | 8.1 | 8.6 | 8.7 | | | | | |
| *Daphnia pulex* | | | | | | | | | | | | | | |
| Control | 10 | 10 | 10 | 9.0 | 8.6 | 9.2 | 8.0 | 8.2 | 8.4 | 20.7 | 19.1 | 18.6 | | |
| Control | 10 | 10 | 10 | 9.0 | 8.5 | 9.2 | 8.0 | 8.2 | 8.4 | | | | 220 | 225 |
| 50% | 10 | 10 | 10 | 8.9 | 8.2 | 8.0 | 8.1 | 8.3 | 8.4 | 20.5 | 19.0 | 18.5 | | |
| 50% | 10 | 10 | 10 | 8.9 | 8.2 | 9.0 | 8.1 | 8.3 | 8.4 | | | | 1050 | 1050 |
| 100% | 10 | 10 | 10 | 8.9 | 7.8 | 8.4 | 8.1 | 8.4 | 8.5 | 20.1 | 18.9 | 18.6 | | |
| 100% | 10 | 10 | 10 | 8.9 | 7.8 | 8.3 | 8.1 | 8.4 | 8.5 | | | | 1850 | 1800 |
| *Notropis leedsi* | | | | | | | | | | | | | | |
| Control | 5 | 5 | 5 | 9.0 | 8.6 | 9.0 | 7.9 | 8.2 | 8.3 | 20.8 | 19.1 | 18.5 | | |
| Control | 5 | 5 | 5 | 9.0 | 8.6 | 9.0 | 7.9 | 8.2 | 8.3 | | | | 210 | 230 |
| 50% | 5 | 5 | 5 | 9.0 | 8.0 | 9.0 | 8.0 | 8.2 | 8.4 | 20.6 | 19.0 | 18.5 | | |
| 50% | 5 | 5 | 5 | 9.0 | 8.0 | 8.9 | 8.0 | 8.2 | 8.4 | | | | 1100 | 1100 |
| 100% | 5 | 5 | 5 | 9.0 | 7.3 | 8.1 | 8.1 | 8.3 | 8.4 | 20.2 | 19.0 | 18.5 | | |
| 100% | 5 | 5 | 5 | 9.0 | 7.4 | 8.0 | 8.1 | 8.3 | 8.4 | | | | 1900 | 1800 |

| Control Water | |
|---|---|
| $NH_3$(as $NH_3$)(mg/l) 0 | Total Alkalinity 117 (mg/l $CaCO_3$) |
| Total ammonia (as N)(mg/l) 0 | Total Hardness 121 (mg/l $CaCO_3$) |

| 100% Effluent | |
|---|---|
| $NH_3$(as $NH_3$)(mg/l) 0.12 | Total Alkalinity 300 (mg/l $CaCO_3$) |
| Total ammonia (as N)(mg/l) 2.0 | Total Hardness 269 (mg/l $CaCO_3$) |

## RESULTS AND DISCUSSION

The sample of effluent collected from Champion Paper Company's Outfall #001 was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No mortality occurred during the 48 hour C. dubia and D. pulex tests, and only one organism died in the N. leedsi bioassay (Table 2). The ammonia measured in this effluent (2.0 mg/l total and 0.12 mg/l unionized) was insufficient to have caused any toxicity. Because the sample was not acutely toxic, chemical analyses were not performed.

## LITERATURE CITED


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