BIOASSAYS OF [redacted], INCLUDING 96 HOUR FLOW-THROUGH,
96 HOUR STATIC RENEWAL, 48 HOUR RANGEFINDER AND
STANDARD REFERENCE TOXICANT
ITT RAYONIER, [redacted], NASSAU COUNTY, FLORIDA
NPDES #FL0000701

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Biological Section
Bureau of Water Analysis
January 20, 1982

# BIOASSAYS OF ITT RAYONIER, INCLUDING 96 HOUR FLOW-THROUGH, 96 HOUR STATIC RENEWAL, 48 HOUR RANGEFINDER AND STANDARD REFERENCE TOXICANT
## ITT RAYONIER, FERNANDINA BEACH, NASSAU COUNTY, FLORIDA
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## EXECUTIVE SUMMARY

ITT RAYONIER, FERNANDINA BEACH, NPDES #FL0000701

Four bioassays including a 48 hour static rangefinder (20 to 22 October), 96 hour flow-through (15 to 19 November), 96 hour static renewal (15 to 19 November) and a 48 hour Standard Reference Toxicant (15 to 17 November) were performed during testing of the ITT Rayonier paper mill located in Fernandina Beach, Florida. Results of the preliminary rangefinder bioassay indicated that effluent collected from the ITT Rayonier mill was acutely toxic to Mysidopsis bahia, with a calculated 48 hour $LC_{50}$ value of 74.8%. The results of the 96 hour flow-through bioassay, performed onsite at the ITT mill, also indicated the effluent was acutely toxic to the mysid shrimp, with 24 to 96 hour $LC_{50}$'s of 53.2% to 10.5%. The 96 hour $LC_{50}$ value obtained from the static renewal bioassay ($\bar{X}$=16%) was comparable to the value obtained from the flow-through. The results of the Standard Reference Toxicant Bioassay indicated that the mysid shrimp used for the onsite ITT Rayonier bioassays were not significantly different from shrimp used in previous bioassays in the Tallahassee laboratory with respect to their sensitivity to sodium lauryl sulfate.

# INTRODUCTION

The ITT Rayonier Pulp and Paper Mill is located on the Amelia River in Fernandina Beach, Nassau County, Florida (Fig. 1). The mill uses a sulfite process to produce approximately 509 tons of paper products per day. Operations are continuous, 24 hours a day, 7 days a week. Raw materials for this production include sulphuric and phosphoric acids, ammonia, caustic soda, wood, chlorine, sulfur and lime. Wastewater treatment includes primary clarification and mechanical aeration in a 35 acre lagoon. Sludge, containing primarily wood fibers, is either incinerated or disposed of in a landfill. An average of 15 MGD treated wastewater is discharged through a 850' pipe (the last 150' is a special diffuser-type pipe) to the bottom of the Amelia River. Discharge occurs only during the out-going tide.

The Biological Section had previously performed a 48 hour static acute toxicity bioassay on a grab sample of this discharge, on 16 to 18 June, 1981, using Mysidopsis bahia as the test organism. The bioassay was performed to evaluate the existence and extent of toxicity of the effluent to the biota of the receiving waters. The results of this test indicated that the effluent was toxic to the test organism, with 48 hour $LC_{50}$'s of 24.7% to 34.4% (FDER, 1981a). Since this facility has recently applied for a new NPDES permit, the St. Johns River Subdistrict requested further toxicity testing in support of the previous toxic results. Therefore, the Biological Section performed a definitive, onsite, 96 hour flow-through bioassay using our mobile bioassay trailer, at the ITT Rayonier plant in Fernandina Beach, on 15 to 19 November, 1981. The test organism used was Mysidopsis bahia. In addition to the flow-through bioassay, the results of a 96 hour static renewal bioassay and of a Standard Reference Toxicant bioassay, performed during the flow-through, are also presented. Additionally, a 48 hour static rangefinder bioassay was performed in the Tallahassee laboratory on 20 to 22 October, 1981, in preparation for the flow-through test. The results of that test are also presented in this report.




Figure 1. Portions of Nassau County, Florida, including Fernandina Beach and location of ITT Rayonier.

## METHODS AND MATERIALS

Test methods used to conduct these static, renewal and flow-through bioassays are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S. EPA (1978). The life history and maintenance procedures used to culture the organisms are detailed below.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under continuous lighting in 10 and 15 gallon all glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 1°C. Ten to fifteen day old (three-quarter adult size) mysids were used in these tests.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

## Test Methods

### 1. Preliminary Visit and Rangefinder Test

On October 19, 1981, Mr. Charles Biedermann and Mr. Martin Roll of DER's Biological Section visited the ITT Rayonier mill in Fernandina Beach to select a suitable location for the mobile bioassay trailer and to collect samples of the facility's effluent. Suitable dilution water from the Amelia River was also collected. The dilution water was collected from the Amelia River at Nassau Sound during an incoming tide, and therefore was believed to be free from effects of pollution. Mr. Lee Banks of DER's St. John's River Subdistrict, accompanied the Tallahassee personnel and provided assistance during the sampling. Effluent was collected to perform a static rangefinder test to determine the approximate concentration series to be used in the flow-through bioassay. The Amelia River water (flow-through diluent) was returned to the Tallahassee laboratory and used to acclimate the mysid shrimp to be used in the flow-through. This acclimation, (to salinities of 10 ppt, 20 ppt and 30 ppt) was performed gradually so as not to stress the organisms. No mortality of test organisms was noted during this period.

The 48 hour static rangefinder bioassay was performed from 1015 hours on 20 October to 1015 hours on 22 October, 1981. Amelia River water (salinity = 31 ppt), collected during the preliminary visit, was used as dilution water. For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (15 ppt to 29.5 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). An additional 100% effluent sample with its pH lowered with hydrochloric acid was tested, since the salinity adjustment raised the pH more than 0.2 units. This rise in pH may result in an increase of the toxic unionized portion of the total ammonia concentration, a significant amount of which was determined to be present in the effluent. A series of 4 control groups (Table 3) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, the salinity range, or the increase in pH. The control groups included: Amelia River water (salinity = 31 ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity=7.2 ppt), and sea salt adjusted well water, with its pH adjusted with hydrochloric acid to the original pH of the sample. Since sea salts increase the pH, thereby increasing the amount of toxic unionized ammonia, these additional tests were necessary to ensure that any mortality was not the result of an artificial rise in pH. The last control consisted of Amelia River water diluted with well water to the salinity of the 56% test concentration (15 ppt). The concentration series selected for this study (Table 1) is that recommended by the APHA (1980).

Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, 20 ppt, 25 ppt and 30 ppt salinity aquaria to culture dishes for observation and se-

lection of individuals for the test. Ten to fifteen day old (three-quarter adult size) mysids were used in this test. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The salinity of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and unionized fractions (expressed in mg/l as N and as $NH_3$, respectively), were recorded in a control and the 100% effluent test concentration at the beginning and end of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and a Corning® pH/millivolt meter. The unionized fraction was then determined 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.

96 Hour Flow Through Bioassay

DER's mobile bioassay laboratory was constructed following EPA design specifications (Peltier, 1978) and is equipped with two solenoid valve operated, flow-through, proportional dilutor systems. The mobile lab also has suitable working area for performing static tests concurrently with the flow-through tests.

The mobile laboratory was transported by commercial mover from its base in Tallahassee to the ITT Rayonier mill in Fernandina Beach, Florida, on November 13, 1981. The trailer was positioned adjacent to the wastewater lagoon and power hook-up was then accomplished by plant electricians.

During the flow-through test the effluent sampling pump was submerged below the water level in the sump at the end of a 50 foot dock into the wastewater lagoon and the effluent conveyed directly and continuously from the source to the proportional dilutor system. A 500 gallon Nalgene® tank was used as a reservoir for the diluent water which was obtained from a private dock on the Amelia River approximately 5 miles south of the ITT mill. To assure an adequate supply of diluent water in the reservoir throughout the test period, 200 gallons of fresh Amelia River water were conveyed, by truck, daily from the dock to the reservoir tank. The diluent sampling pump was submerged in the reservoir for a continuous flow to the dilutor system.

Calibration of the proportional dilutor was performed as soon as the pumps were connected and were determined to be operating properly. Additionally, calibration was verified at the end of the 96 hour test. These concentrations and volumes are presented in Table 1. Flow rates were adjusted to

operate the dilutor at approximately 7 cycles per hour, which provided for a complete volume change in the 8 liter flow-through aquaria approximately every 86 minutes. The dilutor system was operated for approximately 24 hours before the introduction of the test organisms at the beginning of the test at 11:30 A.M. on November 15, 1981. The mysid shrimp were transported to the site, under continuous aeration, in their pre-acclimated aquaria. No significant mortality was noted during the transportation.

Two replicates of six concentrations and a control were used for the flow-through test. To facilitate counting, the mysids were pipetted from their aquaria into retention chambers, which consisted of 10 cm glass petri dishes to which 2.5 cm high cylinders of Nitex® screen (243 micron mesh size) were attached by silicone cement. These Nitex® retention chambers allowed much easier counting of test organisms when lifted from the test aquaria, while not retarding water flow in the aquaria. Ten mysids were loaded per retention chamber per aquarium, resulting in 20 test organisms per test concentration. For each test concentration, the mysids were pre-acclimated to within 5 ppt salinity.

The number of live organisms, dissolved oxygen, pH, temperature and salinity were recorded at 0, 24, 48, 72 and 96 hours for each aquarium. Additionally, ammonia concentrations, including total and unionized fractions (expressed in mg/l as N and $NH_3$, respectively), in the control, mid and 72% test concentrations, were recorded daily. These values appear in Table 4. Organisms used in this test were fed Artemia nauplii daily, as recommended by Peltier (1978).

## 96 Hour Static Renewal Bioassay

Concurrently with the flow-through test, a 96 hour static renewal bioassay was performed onsite, in the bioassay trailer. This test consisted of transferring approximately 500 ml of each test concentration, taken directly from the flow-through dilutor system, into quart size glass jars. Five mysid shrimp were pipetted into Nitex® retention chambers (identical to the ones used in the flow-through aquaria) in each jar. Two replicates were used for each concentration, resulting in 10 test organisms per test concentration. At each 24 hour interval, the Nitex® chambers were removed and the contents of the jars discarded. Each jar was then immediately filled (500 ml) with the same test concentration as previously described. The mysid shrimp, in their Nitex® chambers, were then re-introduced into their appropriate jars. Since these renewals were performed at the same time that the physical-chemical parameters were being recorded in the flow-through bioassay, all daily parameters were identical in the 96 hour renewal bioassay. These values are reported in Table 5. The mysids used in this test were fed Artemia nauplii daily, as recommended by Peltier (1978).

## 48 Hour Standard Reference Toxicant Bioassay

During the first two days of the flow-through bioassay (15 to 17 November, 1981) a Standard Reference Toxicant bioassay was performed onsite, in the bioassay trailer. Sodium lauryl sulfate was used as the reference toxicant and

mysid shrimp were the test organisms. This test ensured that the sensitivities of the mysid shrimp used in these tests were not significantly different from sensitivities of shrimp used in previous tests in the Tallahassee laboratory. One gram of sodium lauryl sulfate was diluted with deionized water until a concentration of 100 ppm was achieved. Two replicates of four test concentrations and a control were used (Table 1), with Amelia River diluent as the dilution water. Dissolved oxygen, pH, temperature and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The salinity of each test concentration was recorded at the beginning and end of the test. All data recorded during this test are presented in Table 6. The mysids were fed Artemia nauplii daily, as recommended by Peltier (1978).

Table 1. Concentration Series and Volumes Used for these Bioassays

Mysidopsis bahia (mysid shrimp) used in all tests

1. 48 Hour Static Rangefinder Bioassay (20-22 October, 1981)

| Concentration | Dilution Water | Effluent | Total Volume |
|---|---|---|---|
| Controls | 500 ml | 0 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% | 0 ml | 500 ml | 500 ml |

2. Flow-Through Bioassay (15-19 November, 1981)

| Diluent[a] | | Effluent[a] | | Total Volume[a] | | Final Concentration[a] | |
|---|---|---|---|---|---|---|---|
| 0 Hours | 96 Hours | 0 Hours | 96 Hours | 0 Hours | 96 Hours | 0 Hours | 96 Hours |
| 905 ml | 911 ml | 0 ml | 0 ml | 905 ml | 911 ml | Control | Control |
| 859 ml | 867 ml | 53 ml | 53 ml | 912 ml | 920 ml | 5.8% | 5.8% |
| 907 ml | 908 ml | 99 ml | 100 ml | 1006 ml | 1008 ml | 9.8% | 9.9% |
| 656 ml | 660 ml | 134 ml | 144 ml | 790 ml | 804 ml | 17.0% | 17.9% |
| 610 ml | 616 ml | 295 ml | 299 ml | 905 ml | 915 ml | 32.6% | 32.7% |
| 371 ml | 375 ml | 474 ml | 475 ml | 845 ml | 850 ml | 56.1% | 55.9% |
| 251 ml | 258 ml | 660 ml | 660 ml | 911 ml | 918 ml | 72.4% | 71.9% |

3. 96 Hour Static Renewal Bioassay (15-19 November, 1981)

| Concentrations | Total Volume |
|---|---|
| Identical to Flow-Through Bioassay | 500 ml in each jar |

4. 48 Hour Standard Reference Toxicant Bioassay (15-17 November, 1981)

| Concentration | Dilution Water[b] | Toxicant[c] | Total Volume |
|---|---|---|---|
| Control | 500 ml | 0 ml | 500 ml |
| 5.6 ppm | 472 ml | 28 ml | 500 ml |
| 10.0 ppm | 450 ml | 50 ml | 500 ml |
| 18.0 ppm | 410 ml | 90 ml | 500 ml |
| 32.0 ppm | 340 ml | 160 ml | 500 ml |

a All values are the average of two or more replicates.
b The dilution water used was Amelia River diluent.
c The toxicant used was a 100 ppm solution of sodium lauryl sulfate in deionized water.

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of ITT Rayonier, Fernandina Beach, Nassau County, Florida, NPDES #FL0000701.

Rangefinder Bioassay, 20 to 22 October, 1981.

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/ℓ) | | | pH | | | Temperature (°C) | | | $NH_3/NH_3+NH_4$ (mg/ℓ) | | Salinity (o/oo) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 | 0 | 48 |
| 5.6% | 5 | 5 | 5 | 7.6 | 7.4 | 6.8 | 8.3 | 8.0 | 8.0 | 18.5 | 21.3 | 21.4 | | | | |
| 5.6% | 5 | 5 | 5 | 7.6 | 7.5 | 7.0 | 8.3 | 8.0 | 8.0 | | | | | | 29.5 | 31.0 |
| 10.0% | 5 | 5 | 5 | 7.3 | 7.3 | 6.8 | 8.2 | 8.0 | 8.0 | 18.5 | 21.2 | 21.6 | | | | |
| 10.0% | 5 | 5 | 5 | 7.3 | 7.2 | 6.8 | 8.2 | 8.0 | 8.0 | | | | | | 28.0 | 30.0 |
| 18.0% | 5 | 5 | 5 | 6.9 | 6.7 | 5.0 | 8.1 | 8.0 | 8.0 | 18.5 | 21.2 | 21.5 | | | | |
| 18.0% | 5 | 5 | 5 | 6.9 | 5.9 | 6.0 | 8.1 | 7.9 | 8.0 | | | | | | 26.0 | 27.0 |
| 32.0% | 5 | 5 | 5 | 5.8 | 6.4 | 5.5 | 8.0 | 7.9 | 7.9 | 18.5 | 21.4 | 21.6 | | | | |
| 32.0% | 5 | 5 | 5 | 5.8 | 6.2 | 5.6 | 8.0 | 7.9 | 7.9 | | | | | | 21.5 | 22.0 |
| 56.0% | 5(4)[a] | 4 | 4 | 3.2 | 6.0 | 6.4 | 7.7 | 7.9 | 8.0 | 18.8 | 21.6 | 21.7 | | | | |
| 56.0% | 5(4)[a] | 4 | 4 | 3.2 | 5.9 | 6.3 | 7.7 | 7.9 | 8.0 | | | | | | 15.0 | 15.0 |
| 100.0% | 5 | 0 | 0 | 1.3(6.2)[b] | 4.6 | -c | 7.7 | 7.8 | 8.2 | 19.7 | 21.7 | 21.8 | .55/29.4 | 2.21/33.6 | | |
| 100.0% | 5 | 0 | 0 | 1.6(6.3)[b] | 2.0 | -c | 7.7 | 7.8 | 8.1 | | | | | | 7.0 | 7.5 |
| 100.0% pH adjusted | 5 | 0 | 0 | 1.7(4.9)[b] | 1.7 | -c | 7.4 | 7.7 | 8.1 | 19.8 | 21.5 | 21.5 | .28/29.4 | 1.75/33.6 | | |
| 100.0% pH adjusted | 5 | 0 | 0 | 1.5(6.5)[b] | 1.9 | -c | 7.4 | 7.7 | 8.1 | | | | | | 7.0 | 7.5 |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |

Remarks:

a. Number in parentheses used in calculations since one organism was cannibalized between 0 and 24 hours.
b. These chambers were aerated prior to loading, number in parentheses represents dissolved oxygen when organisms were loaded.
c. Values not reported since all organisms were dead by hour 24.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of ITT Rayonier.

Control Series for Rangefinder Bioassay, 20 to 22 October, 1981.

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/ℓ) | | | pH | | | Temperature (°C) | | | $NH_3/NH_3+NH_4$ (mg/ℓ) | | Salinity (o/oo) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 | 0 | 48 |
| Amelia River Water | 5 | 5 | 5 | 7.4 | 6.2 | 6.5 | 8.3 | 8.0 | 7.9 | 19.0 | 21.4 | 21.4 | .02/.28 | .01/.22 | | |
| Amelia River Water | 5 | 5 | 5 | 7.5 | 6.7 | 6.8 | 8.3 | 8.0 | 7.9 | | | | | | 31.0 | 33.0 |
| Sea Salts Control | 5 | 5 | 5 | 8.3 | 7.4 | 7.2 | 8.8 | 8.3 | 8.1 | 21.8 | 21.4 | 21.7 | | | | |
| Seal Salts Control | 5 | 5 | 4 | 8.4 | 7.4 | 7.3 | 8.8 | 8.4 | 8.1 | | | | | | 7.2 | 7.5 |
| Sea Salts pH adjusted | 5 | 5 | 5 | 8.0 | 8.2 | 8.1 | 7.4 | 8.1 | 8.2 | 22.0 | 21.1 | 21.3 | | | | |
| Sea Salts pH adjusted | 5 | 5 | 5 | 8.0 | 7.9 | 8.3 | 7.4 | 8.1 | 8.2 | | | | | | 7.2 | 7.5 |
| Salinity Control | 5 | 5 | 5 | 8.0 | 6.6 | 6.8 | 8.2 | 8.0 | 8.0 | 20.1 | 21.6 | 21.8 | | | | |
| Salinity Control | 5 | 5 | 5 | 8.0 | 6.6 | 6.8 | 8.2 | 8.0 | 8.0 | | | | | | 15.0 | 15.0 |
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Remarks:

Table 4. Data recorded during 96 hour, Mysidopsis bahia, flow-through bioassay of ITT Rayonier, Fernandina Beach, Nassau County, Florida, NPDES #FL0000701, on 15 to 19 November, 1981.

| Conc. or % | Test Container Number | Number of Live Organisms | | | | | Dissolved Oxygen (mg/l) | | | | | pH | | | | | Temperature (°C) | | | | | Ammonia (as $NH_3$/N in mg/l) | | | | Salinity (ppt) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 |
| Control[c] | 1 | 10 | 10 | 10 | 10 | 10 | 7.7 | 7.7 | 8.3 | 8.0 | 7.6 | 7.9 | 8.0 | 7.9 | 8.0 | 8.0 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | .04 / 1.4 | | .05 / 1.7 | | .04 / .95 | 30.0 | 28.5 | 29.0 | 28.0 | 28.8 |
| Control[c] | 2 | 10 | 10 | 10 | 10 | 10 | 7.7 | 7.7 | 8.2 | 7.9 | 7.6 | 7.9 | 8.0 | 7.9 | 8.0 | 8.0 | 19.0 | 20.5 | 20.0 | 20.0 | 21.0 | | .11 / 2.8 | | .02 / .61 | | 29.5 | 28.5 | 29.0 | 27.5 | 28.3 |
| 5.8% | 3 | 10 | 10 | 10 | 10 | 9 | 7.7 | 7.5 | 7.7 | 7.3 | 7.1 | 7.9 | 7.9 | 7.9 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | | | | | | 28.3 | 27.3 | 28.0 | 26.5 | 27.0 |
| 5.8% | 4 | 10 | 10 | 10 | 10 | 10 | 7.7 | 7.5 | 7.6 | 7.3 | 7.5 | 7.9 | 7.9 | 7.8 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | | | | | | 28.5 | 27.3 | 28.0 | 26.5 | 27.0 |
| 9.8% | 5 | 10 | 10 | 10 | 10 | 7 | 7.5 | 7.2 | 6.9 | 6.3 | 5.6 | 7.9 | 7.8 | 7.8 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.0 | 21.2 | | | | | | 27.0 | 26.0 | 27.0 | 25.3 | 26.0 |
| 9.8% | 6 | 10 | 10 | 10 | 9 | 8 | 7.6 | 7.2 | 7.0 | 6.6 | 5.9 | 7.9 | 7.8 | 7.8 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.5 | 21.5 | | | | | | 27.5 | 26.0 | 27.0 | 25.3 | 26.0 |
| 17.0% | 7 | 10 | 10 | 10 | 10 | 0 | 7.3 | 6.6 | 6.4 | 5.6 | 5.1 | 7.8 | 7.8 | 7.7 | 7.8 | 7.7 | 19.5 | 21.0 | 20.0 | 20.0 | 21.2 | | | | | | 25.0 | 24.0 | 25.0 | 23.5 | 24.0 |
| 17.0% | 8 | 11[a] | 11 | 11 | 11 | 0 | 7.3 | 6.6 | 6.4 | 5.6 | 5.1 | 7.8 | 7.8 | 7.7 | 7.8 | 7.7 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | | | | | | 25.0 | 24.0 | 25.0 | 23.5 | 24.0 |
| 32.6% | 9 | 10 | 10 | 8 | 0 | 0 | 5.8 | 5.6 | 4.3 | 3.7 | 3.8 | 7.6 | 7.6 | 7.6 | 7.7 | 7.6 | 20.0 | 21.0 | 20.0 | 20.5 | 21.5 | .07 / 4.6 | | .03 / 2.1 | | .06 / 2.7 | 21.5 | 21.1 | 21.0 | 20.0 | 20.0 |
| 32.6% | 10 | 10 | 10 | 0 | 0 | 0 | 5.6 | 5.6 | 3.9 | 3.2 | 3.5 | 7.6 | 7.6 | 7.6 | 7.7 | 7.6 | 20.0 | 21.0 | 20.0 | 20.0 | 21.5 | | .15 / 8.8 | | .08 / 4.2 | | 21.0 | 20.8 | 20.5 | 19.8 | 20.0 |
| 56.1%[c] | 11 | 10 | 0 | 0 | 0 | 0 | 2.1 | 6.1 | 5.9 | 5.1 | 4.6 | 7.5 | 7.6 | 7.6 | 7.7 | 7.7 | 20.5 | 21.0 | 20.0 | 20.0 | 21.0 | | | | | | 15.0 | 14.0 | 14.0 | 13.5 | 14.0 |
| 56.1%[c] | 12 | 10 | 4 | 4 | 0 | 0 | 2.3 | 6.4 | 5.4 | 4.5 | 4.7 | 7.5 | 7.6 | 7.6 | 7.6 | 7.7 | 20.5 | 21.0 | 20.0 | 20.5 | 21.2 | | | | | | 15.0 | 14.3 | 14.3 | 14.0 | 14.0 |
| 72.4%[c] | 13 | 10 | 4 | 3 | 0 | 0 | 1.4 | 4.0 | 3.3 | 3.2 | 3.4 | 7.4 | 7.5 | 7.5 | 7.5 | 7.6 | 20.5 | 21.0 | 20.0 | 20.5 | 21.5 | .12 / 12.0 | | .15 / 12.0 | | .14 / 8.1 | 10.5 | 10.5 | 11.0 | 10.3 | 10.5 |
| 72.4%[c] | 14 | 10 | 2 | 2 | 0 | 0 | 1.4 | 4.1 | 5.2 | 4.2 | 2.8 | 7.4 | 7.5 | 7.5 | 7.5 | 7.6 | 21.0 | 21.0 | 20.5 | 21.0 | 21.8 | -b | | .14 / 10.4 | | | 10.0 | 9.5 | 9.5 | 9.0 | 9.5 |

Remarks:

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

b Probe membrane leaked while testing the 72.4% sample at 24 hours, therefore, erroneous value not reported. New membrane installed before hour 48.

c Each retention chamber, in the controls, 56.1% and 72.4% test concentrations, was aerated continuously throughout the test. The organisms were loaded immediately after the recording of parameters.

Table 5. Data recorded during 96 hour, Mysidopsis bahia, static renewal bioassay of ITT Rayonier, Fernandina Beach, Nassau County, Florida, NPDES #FL0000701, on 15 to 19 November, 1981.

| Conc. or % | Test Container Number | Number of Live Organisms | | | | | Dissolved Oxygen (mg/l) | | | | | pH | | | | | Temperature (°C) | | | | | Ammonia (as $NH_3$/N in mg/l) | | | | Salinity (ppt) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 |
| Control[c] | | 5 | 5 | 5 | 5 | 4 | 7.7 | 7.7 | 8.3 | 8.0 | 7.6 | 7.9 | 8.0 | 7.9 | 8.0 | 8.0 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | .04 / 1.4 | | .05 / 1.7 | | .04 / .95 | 30.0 | 28.5 | 29.0 | 28.0 | 28.8 |
| Control[c] | | 5 | 5 | 5 | 5 | 5 | 7.7 | 7.7 | 8.2 | 7.9 | 7.6 | 7.9 | 8.0 | 7.9 | 8.0 | 8.0 | 19.0 | 20.5 | 20.0 | 20.0 | 21.0 | | .11 / 2.8 | | .02 / .61 | | 29.5 | 28.5 | 29.0 | 27.5 | 28.3 |
| 5.8% | | 5 | 5 | 4(5)[a] | 4(5)[a] | 4(5)[a] | 7.7 | 7.5 | 7.7 | 7.3 | 7.1 | 7.9 | 7.9 | 7.9 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | | | | | | 28.3 | 27.3 | 28.0 | 26.5 | 27.0 |
| 5.8% | | 5 | 5 | 5 | 5 | 5 | 7.7 | 7.5 | 7.6 | 7.3 | 7.5 | 7.9 | 7.9 | 7.8 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | | | | | | 28.5 | 27.3 | 28.0 | 26.5 | 27.0 |
| 9.8% | | 5 | 5 | 5 | 5 | 4 | 7.5 | 7.2 | 6.9 | 6.3 | 5.6 | 7.9 | 7.8 | 7.8 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.0 | 21.2 | | | | | | 27.0 | 26.0 | 27.0 | 25.3 | 26.0 |
| 9.8% | | 5 | 5 | 5 | 5 | 5 | 7.6 | 7.2 | 7.0 | 6.6 | 5.9 | 7.9 | 7.8 | 7.8 | 7.9 | 7.8 | 19.5 | 21.0 | 20.0 | 20.5 | 21.5 | | | | | | 27.5 | 26.0 | 27.0 | 25.3 | 26.0 |
| 17.0% | | 5 | 5 | 5 | 5 | 0 | 7.3 | 6.6 | 6.4 | 5.6 | 5.1 | 7.8 | 7.8 | 7.7 | 7.8 | 7.7 | 19.5 | 21.0 | 20.0 | 20.0 | 21.2 | | | | | | 25.0 | 24.0 | 25.0 | 23.5 | 24.0 |
| 17.0% | | 5 | 5 | 5 | 5 | 0 | 7.3 | 6.6 | 6.4 | 5.6 | 5.1 | 7.8 | 7.8 | 7.7 | 7.8 | 7.7 | 19.5 | 21.0 | 20.0 | 20.0 | 21.5 | | | | | | 25.0 | 24.0 | 25.0 | 23.5 | 24.0 |
| 32.6% | | 5 | 5 | 4 | 3 | 0(1)[b] | 5.8 | 5.6 | 4.3 | 3.7 | 3.8 | 7.6 | 7.6 | 7.6 | 7.7 | 7.6 | 20.0 | 21.0 | 20.0 | 20.5 | 21.5 | .07 / 4.6 | | .03 / 2.1 | | .06 / 2.7 | 21.5 | 21.3 | 21.0 | 20.0 | 20.0 |
| 32.6% | | 5 | 5 | 5 | 4 | 0 | 5.6 | 5.6 | 3.9 | 3.2 | 3.5 | 7.6 | 7.6 | 7.6 | 7.7 | 7.6 | 20.0 | 21.0 | 20.0 | 20.0 | 21.5 | | .15 / 8.8 | | .08 / 4.2 | | 21.0 | 20.8 | 20.5 | 19.8 | 20.0 |
| 56.1%[c] | | 5 | 4 | 0 | 0 | 0 | 2.1 | 6.1 | 5.9 | 5.1 | 4.6 | 7.5 | 7.6 | 7.6 | 7.7 | 7.7 | 20.5 | 21.0 | 20.0 | 20.0 | 21.0 | | | | | | 15.0 | 14.0 | 14.0 | 13.5 | 14.0 |
| 56.1%[c] | | 5 | 5 | 4 | 4 | 4 | 2.3 | 6.4 | 5.4 | 4.5 | 4.7 | 7.5 | 7.6 | 7.6 | 7.6 | 7.7 | 20.5 | 21.0 | 20.0 | 20.5 | 21.2 | | | | | | 15.0 | 14.3 | 14.3 | 14.0 | 14.0 |
| 72.4%[c] | | 5 | 2 | 2 | 2 | 2 | 4.4 | 4.0 | 3.3 | 3.2 | 3.4 | 7.4 | 7.5 | 7.5 | 7.5 | 7.6 | 20.5 | 21.0 | 20.0 | 20.5 | 21.5 | .12 / 12.0 | | .15 / 12.0 | | .14 / 8.1 | 10.5 | 10.5 | 11.0 | 10.3 | 10.5 |
| 72.4%[c] | | 5 | 5 | 4 | 2 | 2 | 1.4 | 4.1 | 5.2 | 4.2 | 2.8 | 7.4 | 7.5 | 7.5 | 7.5 | 7.6 | 21.0 | 21.0 | 20.5 | 21.0 | 21.8 | -b | | .14 / 10.4 | | | 10.0 | 9.5 | 9.5 | 9.0 | 9.5 |

Remarks:

[a] Number in parentheses used in calculations since one organism was cannibalized between 24 and 48 hours.

[b] Number in parentheses used in calculations since one organism jumped out between 72 and 96 hours.

[c] Each retention chamber in the controls, 56.1% and 72.4% test concentrations were aerated continuously throughout the test.

Table 6. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of sodium lauryl sulfate (Standard Reference Toxicant), perfomed onsite at ITT Rayonier, Fernandina Beach, on 15 to 17 November, 1981.

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/ℓ) | | | pH | | | Temperature (°C) | | | $NH_3/NH_3+NH_4$ (mg/ℓ) | | Salinity (o/oo) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 | 0 | 24 | 48 |
| Control | 5 | 5 | 5 | 8.4 | 7.5 | 7.1 | 7.9 | 8.0 | 7.9 | | | | | | 29.0 | 29.0 | 27.5 |
| Control | 5 | 5 | 4(5)[a] | 8.4 | 7.6 | 7.1 | 7.9 | 8.0 | 7.9 | 19.0 | 19.0 | 17.0 | | | | | |
| 5.6 ppm | 5 | 5 | 5 | 8.6 | 6.7 | 7.1 | 7.9 | 7.9 | 7.9 | | | | | | 27.5 | 28.0 | 27.5 |
| 5.6 ppm | 5 | 5 | 3(5)[a] | 8.6 | 6.6 | 7.1 | 7.9 | 7.9 | 7.8 | 19.0 | 19.0 | 17.0 | | | | | |
| 10.0 ppm | 5 | 4 | 3 | 8.7 | 6.5 | 6.8 | 7.9 | 7.9 | 7.8 | | | | | | 26.5 | 26.5 | 25.5 |
| 10.0 ppm | 5 | 5 | 5 | 8.7 | 6.5 | 6.7 | 7.9 | 7.9 | 7.8 | 18.5 | 19.0 | 17.0 | | | | | |
| 18.0 ppm | 5 | 4 | 2 | 8.8 | 6.7 | 5.8 | 7.9 | 7.9 | 7.7 | | | | | | 24.0 | 24.3 | 23.3 |
| 18.0 ppm | 5 | 5 | 2 | 8.8 | 7.0 | 5.7 | 7.9 | 7.9 | 7.7 | 18.5 | 19.0 | 17.5 | | | | | |
| 32.0 ppm | 5 | 1 | 0 | 8.7 | 6.7 | 5.1 | 8.0 | 7.9 | 7.6 | | | | | | 20.5 | 21.0 | 20.0 |
| 32.0 ppm | 5 | 1 | 0 | 8.7 | 6.8 | 5.1 | 8.0 | 7.9 | 7.6 | 18.5 | 19.0 | 18.5 | | | | | |
| | | | | | | | | | | | | | | | | | |
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Remarks: [a] Numbers in parentheses used in calculations since organisms jumped out between 24 and 48 hours.

## RESULTS AND DISCUSSION

### Rangefinder Bioassay

The results of the 48 hour static rangefinder bioassay, performed on 20 to 22 October, 1981, indicated that the effluent was acutely toxic to the mysid shrimp. Both 24 and 48 hour $LC_{50}$'s were calculated to be 74.8% (Table 7). The cause of this toxicity, however, could not be attributed to a single factor. The high concentrations of total and unionized ammonia determined in this sample were near the concentrations reported to be toxic to certain freshwater fish (Thurston and Russo 1981). However, our experience with bioassay organisms suggest that mysid shrimp are less sensitive to ammonia concentrations than other aquatic organisms. Additionally, since all the mortality occurred in the 100% test concentrations, the low dissolved oxygen in the effluent may have stressed the organisms, even though the chambers were continuously aerated. Conversely, continuous aeration may result in erroneously reduced toxicity measurements since it may drive off volatile organic substances (Peltier 1978) and oxidize certain organics and reduced inorganic substances.

### Flow-Through Bioassay

The 96 hour flow-through bioassay, performed onsite at the ITT Rayonier mill in Fernandina Beach, on 15 to 19 November, 1981, resulted in acute toxicity to the mysid shrimp. Calculated $LC_{50}$ values from 24 to 96 hours were from 53.2% to 10.5% (Table 7). As in the rangefinder bioassay, the cause of this toxicity could not be attributed to a single factor, but may be due to the numerous chemical constituents of the effluent. Although unionized ammonia was not present in toxic concentrations, chemical analyses did indicate the presence of dimethyl disulfide and moderate amounts of chromium in the effluent (Table 8). Dimethyl disulfide is an extremely volatile waste product and may be toxic if present in sufficient concentration. Chromium, although present in sub lethal concentrations, may have acted synergistically with other components to contribute to the observed toxicity. Due to limitations on manpower and time, the only chemical analyses requested were for chloroform and the metals chromium and copper.

### Static Renewal Bioassay

The 96 hour static renewal bioassay, performed onsite during the flow-through bioassay, also resulted in acute toxicity to the mysid shrimp. Calculated $LC_{50}$ values from 24 to 96 hours ranged from 84.6% to 12.4% (Table 7). The $LC_{50}$ values for this static test paralleled the flow-through test.

### Standard Reference Toxicant Bioassay

The results of the Standard Reference Toxicant Bioassay, performed onsite during the flow-through bioassay, indicated that the mysid shrimp used in these tests exhibited comparable sensitivities to sodium lauryl sulfate as shrimp used in previous toxicity testing. The average 48 hour $LC_{50}$ value of 14.5 ppm

(Table 7) obtained in this test is comparable to the 15.0 ppm value obtained for mysid shrimp in recent Standard Reference Bioassays (FDER 1981b).

This indicates that the shrimp used in the ITT Rayonier flow-through and static renewal bioassays were not significantly different from shrimp used in previous bioassays in the Tallahassee laboratory, with respect to sensitivity to sodium lauryl sulfate.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from... components of discharges which...are acutely toxic." "Acute toxicity" is defined in Chapter 17-3.021(1) to mean the "the presence of one or more substances...in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 Hour $LC_{50}$)...". In the absence of a mixing zone, this 1/3 of the 96 hour $LC_{50}$ applies at the point of discharge. Chapter 17-4.244(4) states that "in no event shall the maximum concentration of wastes in the mixing zone exceed the amount lethal to 50% of the test organisms in 96 hours (96 hour $LC_{50}$) for a species significant to the indigenous aquatic community...". This criterion applies at the point of discharge if there is a mixing zone. The distinction between presence or absence of a mixing zone is not relevant here, however, since the testing procedure used does not involve effluent concentration. The 1/3 of the 96 hour $LC_{50}$ criterion is three times as stringent as the 96 hour $LC_{50}$ criterion, and therefore, in the absence of a mixing zone, the procedure used is very lenient. The procedure followed is also lenient in that testing is only done for 48 hours. The 96 hour $LC_{50}$ derived from this testing is therefore reported as "less than or equal to" the 48 hour measurement. The value found after 48 hours can never be more restrictive than the value found after 96 hours. It must be noted that all organisms 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.

Table 7. $LC_{50}$ Data for Bioassays of ITT Rayonier, Fernandina Beach, including Standard Reference Toxicant, 20 to 22 October and 15 to 19 November, 1981.

| Organism | Method of Data Analysis | 24 $LC_{50}$ and 95% Confidence Intervals | 48 Hour $LC_{50}$ and 95% Confidence Intervals | 96 Hour $LC_{50}$ and 95% Confidence Intervals |
|---|---|---|---|---|
| Mysidopsis bahia 48 Hour Range-finder 20-22 October, 1981 | Binomial | 74.8% (56 and 100) | 74.8% (56 and 100) | --- |
| | Moving Average | a | a | |
| | Probit | a | a | |
| Mysidopsis bahia 96 Hour Flow Through, 15-19 November, 1981 | Binomial | 47.4% (33 and ∞) | 30.2% (17 and 56) | 11.5% (10 and 17) |
| | Moving Average | 51.7% (44 and 63) | 36.6% (30 and 47) | 10.5% (9 and 12) |
| | Probit | 53.2% (46 and 60) | 38.1% (32 and 46) | 10.8% (9 and 13) |
| Mysidopsis bahia 96 Hour Static Renewal, 15-19 November, 1981 | Binomial | a | a | 12.4% (10 and ∞) |
| | Moving Average | a | a | 14.3% (8 and 22) |
| | Probit | 84.6% (68 and ∞),b | 65.8% (50 and 141),b | 20.5% (0 and ∞),c |
| Mysidopsis bahia Standard Reference Toxicant, 15-17 November, 1981 | Binomial | 25.2 ppm (18 and ∞) | 15.6 ppm (5.6 and 32) | --- |
| | Moving Average | 25.2 ppm (21 and 33) | 14.1 ppm (11 and 19) | --- |
| | Probit | 24.3 ppm (18 and 38) | 13.9 ppm (10 and 19) | --- |

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

b Due to the distribution of mortality, the $LC_{50}$ value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

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

Table 8. Results of Chemical Analyses Performed on Effluent and Diluent Used During Flow Through Bioassay of ITT Rayonier, Fernandina Beach, 15 to 19 November, 1981.

## Organics[a]

| Date | Location | Compound and Concentration |
|---|---|---|
| 15 November | 100% Effluent from Outfall Sump | Dimethyl Disulfide 11.1µg/l |
| 15 November | 100% Effluent for Bioassay Diluter | Dimethyl Disulfide 20.5µg/l |
| 15 November | Private Dock on Amelia River | None Detected |
| 15 November | Diluent from Bioassay Diluter | Dimethyl Disulfide <1µg/l |
| 19 November | 100% Effluent from Outfall Sump | Dimethyl Disulfide 14.6µg/l |
| 19 November | 100% Effluent from Bioassay Diluter | Dimethyl Disulfide 18.7µg/l |
| 19 November | Diluent from Bioassay Diluter | None Detected |

## Metals[b]

| Date | Location | Component | Concentration |
|---|---|---|---|
| 13 November | Field Blank | Copper | 26µg/l[c], |
| | | Chromium | Not Detected (50µg/l)[d] |
| 15 November | 100% Effluent from Outfall Sump | Copper | 53µg/l |
| | | Chromium | 100µg/l |
| 15 November | 100% Effluent from Bioassay Diluter | Copper | 61 µg/l |
| | | Chromium | 102µg/l |
| 15 November | Private Dock on Amelia River | Copper | 70 µg/l |
| | | Chromium | Not Detected |
| 15 November | Diluent from Bioassay Diluter | Copper | 72µg/l |
| | | Chroumium | Not Detected |
| 19 November | 100% Effluent from Outfall Sump | Copper | 58µg/l |
| | | Chromium | 129µg/l |
| 19 November | 100% Effluent from Bioassay Diluter | Copper | 54µg/l |
| | | Chromium | 105µg/l |
| 19 November | Diluent from Bioassay Diluter | Copper | 71µg/l |
| | | Chromium | Not Detected |

[a] Due to limitations on manpower and time, only analysis for the compound chloroform was requested, none of which was found in any sample tested. During this analysis, the compound dimethyl disulfide was detected.

[b] Only analyses for copper and chromium were requested.

[c] The significant concentration of copper in the field blank may make the results of this analysis questionable.

[d] The number in parentheses is the minimum detection limit.

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