


# BIOASSAYS OF TREESWEET CITRUS PRODUCTS, INCLUDING 96 HOUR FLOW THROUGH, 48 HOUR STATIC AND STANDARD REFERENCE TOXICANT ASSAYS

FORT PIERCE, ST. LUCIE COUNTY, FLORIDA

NPDES #FL0000841

SAMPLED 3/1/86

**FILE COPY**
**DO NOT REMOVE**

MAY 14, 1986

Biology Section
Division of Environmental Programs

# BIOASSAYS OF TREESWEET CITRUS PRODUCTS, INCLUDING 96 HOUR FLOW THROUGH, 48 HOUR STATIC AND STANDARD REFERENCE TOXICANT ASSAYS FORT PIERCE, ST. LUCIE COUNTY, FLORIDA NPDES #FL0000841

Biology Section
Bureau of Laboratories and
Special Programs
May 14, 1986

## EXECUTIVE SUMMARY

Treesweet, Ft. Pierce, NPDES #FL0000841, on 2 to 6 March, 1986

The Treesweet facility processes approximately 1120 tons of fruit per day in the production of canned citrus juice, concentrate, and other products. Weak wastewater, consisting of cooling water and process water conditioners, is collected in an aeration basin prior to discharge to the C-10 canal, which flows to Five Mile Creek. The results of an onsite 96 hour flow-through bioassay indicated that this wastewater, collected from the aeration basin, was not acutely toxic to Notropis leedsi. Additionally, the results of an onsite 48 hour static bioassay indicated that this wastewater was not acutely toxic to Daphnia pulex. Some difficulty was encountered with the use of Treesweet well water as diluent for the onsite tests, since this well water apparently caused some N. leedsi control mortality, and was unsuitable as diluent in the D. pulex tests. Both the D. pulex flow-through and reference toxicant tests using this diluent were invalidated. The successful D. pulex static test used Tallahassee well water as the diluent. The results of the N. leedsi Standard Reference Toxicant test (48 hour $LC_{50}$ = 3.9 ppm using sodium lauryl sulfate) indicated that the test organisms were viable and within the sensitivity ranges suggested by both the EPA and the DER.

Results of chemical analyses indicated the presence of several unidentified organic compounds and the metals cadmium and zinc in Treesweet's effluent. Additionally, organic analyses were performed on Treesweet's well water used as dilution water in the above tests. Results indicated the presence of several unidentified compounds and butyl benzyl phthalate.

## RECOMMENDATIONS

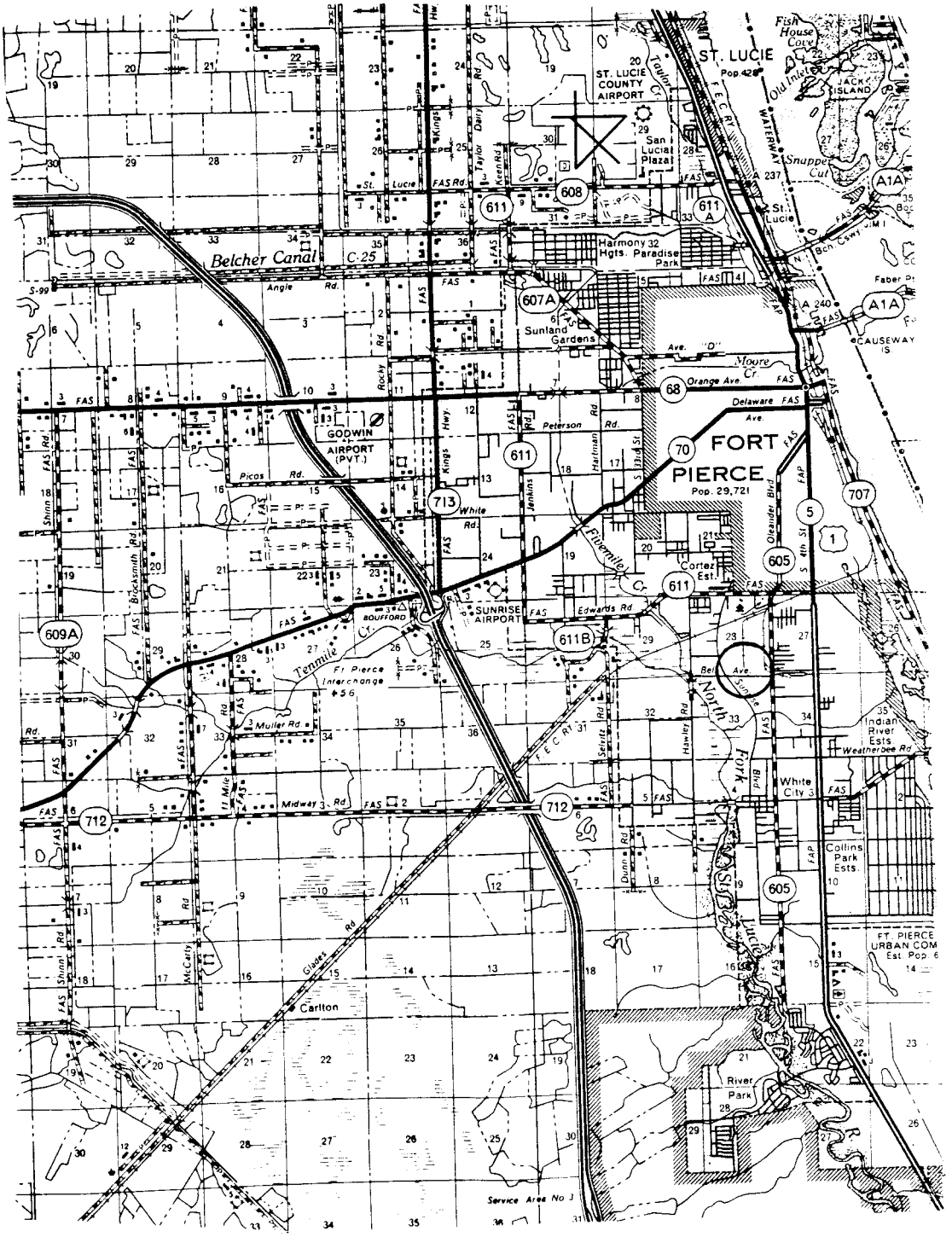
Based on the results of these tests, that is, the problems with Treesweet well water and the presence of chemical contaminants in both the wastewater and well water, we recommend the continuation of the district's efforts in the monitoring of groundwater and surface waters around this facility. Also, a yearly biomonitoring program, including both acute and chronic tests of the wastewater discharge, seems warranted.

## INTRODUCTION

The Treesweet citrus processing facility is located at 1000 Bell Avenue, Ft. Pierce, St. Lucie County, Florida (Fig. 1). The facility processes approximately 1120 tons of fruit per day in the production of canned citrus juice, frozen concentration, and by-products such as molasses and animal feed. Production is normally six days per week, year round. Strong wastewater from this operation is collected and disposed of via a lined sprayfield. Leachate from this sprayfield is pumped to a nearby orange grove for final dispersal. Weak wastewater, consisting of cooling water from the canning process and various process water regeneratives and conditioners, is collected in a 20,000 gallon aeration basin. Discharge from this basin is through a pipe approximately 1/4 mile distance to a riffle bed aerator on the C-10 canal, and is intermittant depending on volume. The C-10 canal flow a short distance to Five Mile Creek, a tributary of the north fork of the St. Lucie River. A diagram of the Treesweet facility and wastewater discharge is presented in Figure 2.

In January, 1984, the FDER Biology Section performed a series of screening bioassays on wastewater collected from Treesweet's final discharge to the C-10 canal. The wastewater was not acutely toxic to the test organisms, Daphnia pulex, Simocephalus serrulatus, or Notropis leedsi, at that time. In January, 1985, however, the U.S. EPA performed onsite 96 hour flow-through and 48 hour static bioassays on wastewater collected from the aeration basin. Several of these tests resulted in acute toxicity to the EPA's test organisms (U.S. EPA 1985). Toxicity may have been associated with the presence of residual chlorine in the wastewater.

To further evaluate the toxicity of Treesweet's wastewater the Southeast District requested the Biology Section perform onsite acute toxicity bioassays. These tests including 96 hours flow-throughs and a 48 hour static, were performed from 2 to 6 March, 1986. Wastewater was collected from the aeration basin. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.



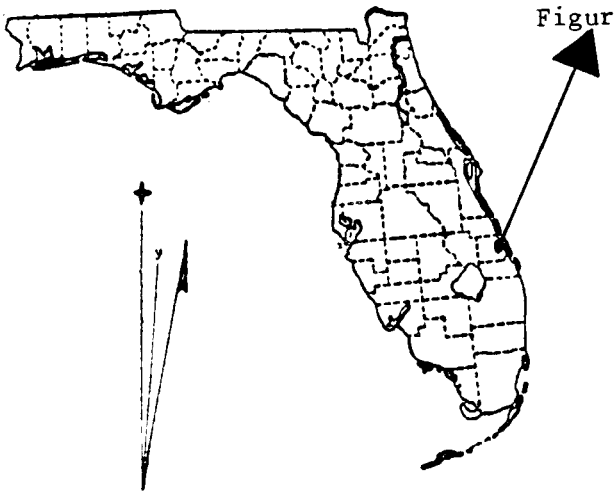



Figure 1. Portions of St. Lucie County, Florida, including Ft. Pierce and the location of the Treesweet facility.

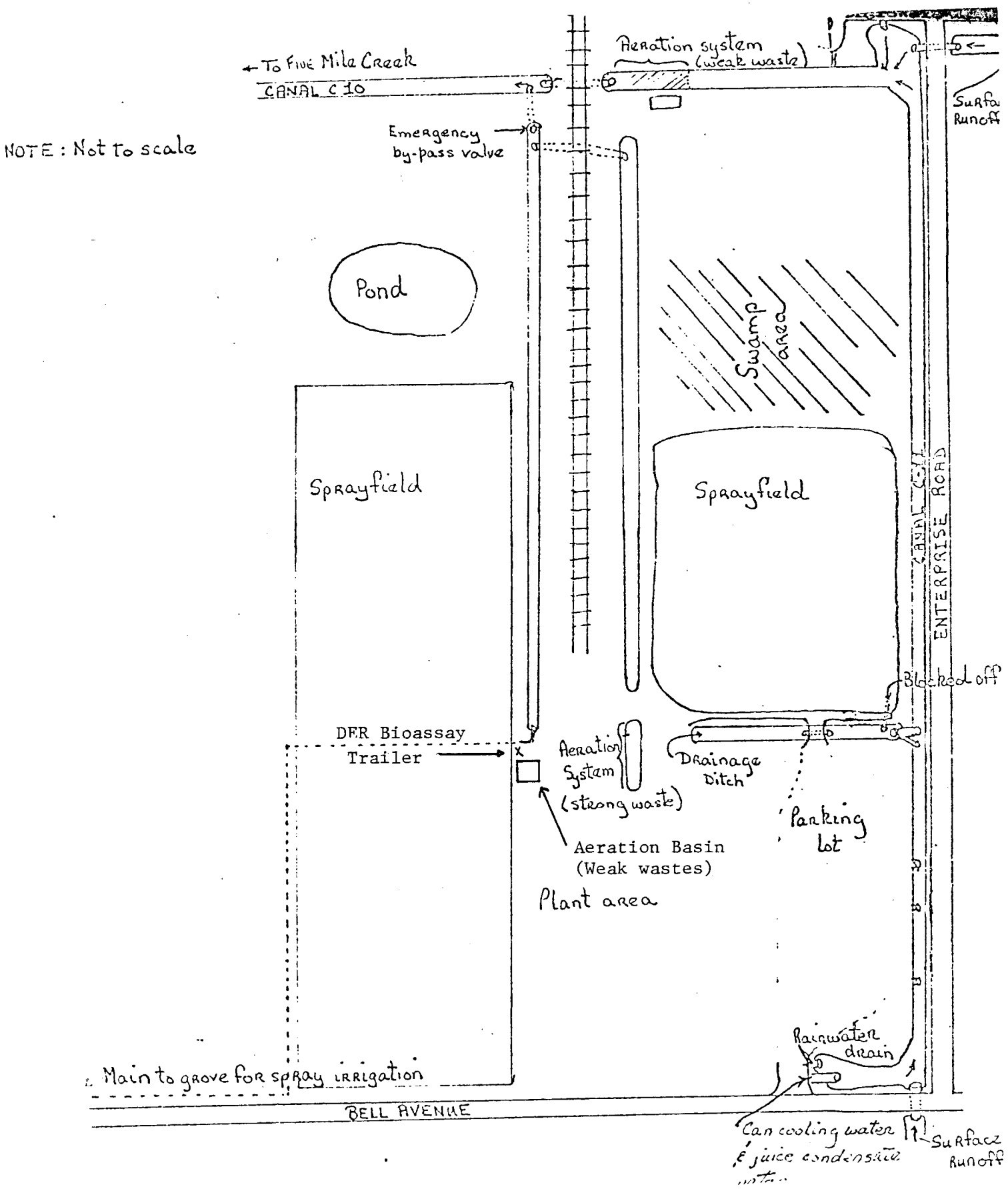



Figure 2. Treesweet's wastewater flow, including the location of DER's Bioassay Trailer.

## METHODS AND MATERIALS

Test methods used to conduct these flow-through and static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985a). The organisms selected for this study include a species rcommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985a). 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.

The D. pulex used in the Treesweet tests were obtained by siphoning individuals from each of our culturing aquaria into two 1 gallon glass jars, each containing well water obtained from the Treesweet facility during a previous inspection trip. However, there was considerable mortality of D. pulex during this acclimation period, leading us to suspect the quality of this water for use with this test organism. The jars were transported to the site by state vehicle and immediately transferred to the mobile bioassay lab, and therefore, did not undergo any significant temperature change or disturbance. The D. pulex were fed prior to transport.

Notorpis leedsi (bannerfin shiner) - Approximtely 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 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 N. leedsi used in the Treesweet tests began hatching in the Tallahassee lab on approximately February 20, 1986, in a 2 1/2 gallon all-glass aquarium. Water was replaced in this aquarium over a period of days with Treesweet well water (to be used as diluent in the on-site tests). Therefore, the fish were pre-acclimated to this dilution water. The fish were transported to the test site in this aquarium with constant aeration. The fish were fed daily with live, freshly hatched Artemia and were acclimated to 20°C in the mobile lab prior to the test. The fish did not undergo any significant temperature change or disturbance, nor was there any mortality observed prior to the test. Apparently, whatever characteristic of Treesweet's well water that affected D. pulex, did not affect N. leedsi. The fish were aproximtely 10 days old at the start 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. Additionally, on-site reference toxicant tests were performed in the mobile bioassy lab using Notropis leedsi and Daphnia pulex, to evaluate the sensitivity of these organisms after their transportation to the site and under the conditions in the mobile lab. The results of these tests would indicate whether the sensitivities of the organisms used in the on-site tests had changed significantly from that experienced in the Tallahassee lab or had deviated from the recommended EPA and DER sensitivity ranges.

## TEST METHODS

### Dilution Water

Water used to acclimate the freshwater organisms prior to the test, and as dilution water in the flow-through and reference toxicant tests, was obtained from a Treesweet well water hose near the plant's loading dock. This water was characterized by a pH of 7.0 to 7.5 standard units and a conductivity of 1010 to 1200 umhos. Approximately 250 gallons of this dilution water was conveyed daily in a 300 gallon Nalgene® tank to the test site. The residual chlorine present in this well water was completely neutralized daily by the addition of calculated amounts of sodium thiosulfate. We wish to thank Mr. John Lang of Treesweet for his personal assistance throughout the test.

This dilution water was, ultimately, completely unsuitable for use with Daphnia pulex. Both the flow-through and reference toxicant tests were invalidated at 24 hours due to excessive control mortality. A sample of this water was collected for organic chemical analysis. A 48 hour static test of Treesweet's wastewater, however, was completed using Tallahassee well water as the diluent.

## Pre-test Conditions and Preparations

DER's mobile bioassay laboratory was constructed following EPA design specifications (Peltier and Weber, 1985) 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 to Treesweet on February 28, 1986. The trailer was postitioned adjacent to the aeration basin (Fig. 2). Electric power for the mobile lab was supplied by Treesweet.

An effluent sampling pump was suspended in the basin at the opposite corner of the inffluent pipe. The pump was positioned approximately 0.5 meters beneath the surface and at least 0.5 meters off the bottom of the basin. Effluent was conveyed directly and continuously to the mobile bioassay lab via a 50' length of braided reinforced Tygon® hose. Dilution water was transferred to a 400 gallon Nalgene® tank positioned adjacent to the mobile lab. A similar sampling pump was submerged in this tank and dilution water was conveyed directly and continuously to the mobile lab via a 15' length of identical Tygon® hose. Upon entering the mobil lab, both effluent and dilution water sampling lines passed through a water bath. This water bath maintaned the temperature of the effluent and dilution water at 20°C $\pm$ 2°C.

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 again at the end of the 96 hour test. The concentrations and volumes produced by the flow-through dilutor system are presented in Table 1. Flow rates were adjusted to operate the dilutor at approximately 10 cycles per hour, which provided for approximately 15 complete volume changes in the 8 liter flow-through aquaria every 24 hours. The dilutor system was operated for approximately 20 hours before the introduction of the test organisms at the beginning of the test at 0730 hours on Sunday, March 2, 1986. The test was concluded at 0730 hours on Thursday, March 6, 1986.

## Flow-Through Methods and Parameters

Two replicates of six concentrations and a control were used for the *N. leedsi* and *D. pulex* flow-through tests. To facilitate counting, the organisms were pipetted from culture dishes into separate retention chambers, which consisted of glass petri dishes to which 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 organisms were loaded per retention chamber per aquarium, resulting in 20 test organisms per test concentration. As stated eariler, the *D. pulex* test was terminated at 24 hours due to excessive control mortality. No data from this are reported.

The number of live organisms, dissolved oxygen, pH, temperature and conductivity were recorded at 0, 24, 48, 72, and 96 hours for each aquarium. Ammonia concentratins, including total and unionized fractions (expressed in mg/l as N and $NH_3$, respectively), in each test concentration, were also recorded at the beginning of the test. Total alkalinity and total hardness were measured daily in the control and 100% concentrations. These values appear in Table 2.

Daphnia pulex Static Test

During flow-through test, a 48 hour static test was performed in the mobile trailer using the test organism, Daphnia pulex. The test was performed from 2 to 4 March, 1986. The D. pulex were tested, using Tallahassee well water as the diluent, to substantiate the results of the D. pulex flow-through test, since the suitability of Treesweet's well water was suspect using this organism.

Two replicates, each with six concentrations and a control, were used to test with water flea, Daphnia pulex. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Treesweet wastewater was obtained directly from the flow-through dilutor system. Daphnia were transferred from their holding jars to a large culture dish to select the proper size and age organisms. Juvenile Daphnia were chosen for this test. These organisms were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentrations. Water fleas were not fed during this test.

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 for the diluent. 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. These values were recorded on the bioassay data form and are presented in Table 3.

48 Hour Standard Reference Toxicant Bioassays

During the first two days of the flow-through bioassays (2 to 4 March, 1986) two Standard Reference Toxicant bioassays were performed on-site, in the bioassay trailer. Sodium lauryl sulfate was used as the reference toxicant, in a stock concentration of 100 ppm. This stock concentration was diluted in Treesweet dilution water to obtain a concentration series (Table 1) of from 1 to 10 ppm (the same series used in our monthly quality control test in the Tallahassee lab). Two replicates of each concentration was used, each containing either 10 Daphnia or 5 Notropis. The D. pulex test, however, was invalidated at 24 hours due to excessive control mortality, and no data are given.

The N. leedsi test ensured that the sensitivity of this organism used in the flow-through test was not significantly different from the sensitivity obtained in previous tests in the Tallahassee laboratory. 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 conductivity of each test concentration was recorded at the beginning and end of the test. All data recorded during this test are presented in Table 4. The organisms were not fed during the test.

Table 1. Diluent and Effluent Volumes, and Final Concentrations used in Flow-Through and Static Bioassays of Treesweet (2 to 6 March, 1986).

1. Flow-Through Bioassays (2 to 6 March, 1986), Notropis leedsi

| 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 |
| 933 ml | 890 ml | 0 ml | 0 ml | 933 ml | 890 ml | 0% | 0% |
| 892 ml | 900 ml | 53 ml | 52 ml | 943 ml | 952 ml | 5.6% | 5.4% |
| 941 ml | 944 ml | 105 ml | 105 ml | 1046 ml | 1049 ml | 10.0% | 10.0% |
| 740 ml | 741 ml | 156 ml | 154 ml | 896 ml | 895 ml | 17.4% | 17.2% |
| 634 ml | 635 ml | 311 ml | 310 ml | 945 ml | 945 ml | 32.9% | 32.0% |
| 369 ml | 373 ml | 460 ml | 459 ml | 829 ml | 832 ml | 55.4% | 55.1% |
| 0 ml | 0 ml | 960 ml | 960 ml | 960 ml | 960 ml | 100.0% | 100.0% |

2. 48 Hour Static Bioassay (2 to 4 March, 1986), Daphnia pulex.

| Concentration | Dilution Water[b] | Toxicant | Total Volume |
|---|---|---|---|
| Control | 200.0 ml | -------- | 200 ml |
| 5.6 ppm | 188.8 ml | 11.2 ml | 200 ml |
| 10.0 ppm | 180.0 ml | 20.0 ml | 200 ml |
| 18.0 ppm | 164.0 ml | 36.0 ml | 200 ml |
| 32.0 ppm | 136.0 ml | 64.0 ml | 200 ml |
| 56.0 ppm | 88.0 ml | 112.0 ml | 200 ml |
| 100.0 ppm | -------- | 200.0 ml | 200 ml |

3. Notropis leedsi Standard Reference Toxicant Test (2 to 4 March, 1986).

| Concentration | Dilution Water | Toxicant | Total Volume |
|---|---|---|---|
| Control | 500 ml | ----- | 500 ml |
| 1.0 ppm | 495 ml | 5 ml | 500 ml |
| 3.0 ppm | 485 ml | 15 ml | 500 ml |
| 7.0 ppm | 465 ml | 35 ml | 500 ml |
| 10.0 ppm | 450 ml | 50 ml | 500 ml |

Table 1. (Cont.)

4. *Daphnia pulex* Standard Reference Toxicant Test (2 to 4 March, 1986.

| Concentration | Dilution Water | Toxicant | Total Volume |
|---|---|---|---|
| Control | 200 ml | ----- | 200 ml |
| 1.0 ppm | 198 ml | 2 ml | 200 ml |
| 3.0 ppm | 194 ml | 6 ml | 200 ml |
| 7.0 ppm | 186 ml | 14 ml | 200 ml |
| 10.0 ppm | 180 ml | 20 ml | 200 ml |

[a] All values are the average of two or more replicates. Final concentrations are to nearest 0.1%. Reported test concentrations will be the average of the 0 and 96 hour values.

[b] Dilution water used was Tallahassee well water.

Table 2. Data recorded during 96 hour, Notropis leedsi, flow-through bioassay of Treesweet Products, Ft. Pierece, NPDES #FL0000841, on 2 to 6 March, 1986.

| Conc. or % | Test Container Number | Number of Live Organisms | | | | | Dissolved Oxygenb (mg/l) | | | | | pH | | | | | Total Alkalinity (mg/l as $CaCO_3$) | | | | | Total Hardness (mg/l as $CaCO_3$) | | | | | Temperature | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 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 | A | 10 | 9/10[a] | 10 | 10 | 10 | 9.2 | 9.4 | 8.3 | 6.9 | 6.7 | 7.3 | 7.0 | 7.1 | 7.5 | 7.1 | 260 | 280 | 290 | 290 | 285 | 340 | 360 | 370 | 350 | 340 | 20.0 | 19.5 | 20.2 | 20.6 | 19.5 |
| Control | B | 10 | 5/10[a] | 10 | 10 | 10 | 9.3 | 9.6 | 8.2 | 7.5 | 4.8 | 7.4 | 7.1 | 7.1 | 7.5 | 7.1 | 260 | | | | | 340 | | | | | 20.0 | 19.6 | 20.4 | 20.9 | 20.0 |
| 5.5% | A | 10 | 10 | 10 | 10 | 10 | 9.4 | 9.5 | 8.7 | 8.4 | 3.7 | 7.6 | 7.3 | 7.6 | 7.9 | 7.1 | | | | | | | | | | | 20.0 | 19.8 | 20.1 | 20.5 | 19.8 |
| 5.5% | B | 10 | 10 | 10 | 10 | 10 | 9.2 | 9.5 | 7.9 | 6.5 | 3.5 | 7.5 | 7.2 | 7.2 | 7.5 | 7.1 | | | | | | | | | | | 20.0 | 19.8 | 20.3 | 20.7 | 20.2 |
| 10.0% | A | 10 | 10 | 10 | 10 | 10 | 9.0 | 9.5 | 8.1 | 6.8 | 4.0 | 7.5 | 7.2 | 7.3 | 7.5 | 7.1 | | | | | | | | | | | 19.8 | 19.7 | 20.0 | 20.3 | 19.7 |
| 10.0% | B | 10 | 10 | 10 | 10 | 10 | 9.0 | 9.5 | 8.3 | 7.1 | 3.9 | 7.5 | 7.3 | 7.4 | 7.7 | 7.1 | | | | | | | | | | | 20.1 | 19.7 | 20.5 | 20.7 | 20.0 |
| 17.3% | A | 10 | 10 | 10 | 9 | 9 | 8.9 | 9.2 | 7.6 | 6.8 | 4.2 | 7.4 | 7.1 | 7.2 | 7.6 | 7.1 | | | | | | | | | | | 19.5 | 19.4 | 20.1 | 20.4 | 19.8 |
| 17.3% | B | 10 | 10 | 10 | 10 | 10 | 8.5 | 9.2 | 7.6 | 8.3 | 4.4 | 7.4 | 7.1 | 7.2 | 8.0 | 7.2 | | | | | | | | | | | 20.1 | 19.4 | 20.7 | 21.3 | 20.5 |
| 32.5% | A | 10 | 10 | 10 | 10 | 10 | 8.8 | 9.1 | 7.6 | 6.3 | 4.0 | 7.4 | 7.1 | 7.2 | 7.5 | 7.2 | | | | | | | | | | | 19.7 | 19.4 | 20.1 | 20.4 | 19.8 |
| 32.5% | B | 10 | 10 | 10 | 10 | 10 | 9.0 | 9.5 | 8.1 | 6.0 | 3.9 | 7.5 | 7.3 | 7.4 | 7.5 | 7.2 | | | | | | | | | | | 19.8 | 19.4 | 20.5 | 21.1 | 20.3 |
| 55.3% | A | 10 | 10 | 10 | 10 | 10 | 8.9 | 9.4 | 8.3 | 7.3 | 4.9 | 7.6 | 7.4 | 7.5 | 7.7 | 7.3 | | | | | | | | | | | 19.2 | 19.1 | 19.9 | 20.3 | 19.8 |
| 55.3% | B | 10 | 10 | 10 | 9 | 9 | 9.0 | 9.5 | 8.6 | 7.7 | 5.0 | 7.8 | 7.4 | 7.7 | 7.9 | 7.3 | | | | | | | | | | | 19.2 | 18.7 | 20.2 | 20.7 | 20.0 |
| 100.0% | A | 10 | 10 | 10 | 9 | 9 | 9.4 | 9.8 | 8.5 | 8.4 | 6.2 | 7.9 | 7.7 | 7.9 | 8.1 | 7.8 | 270 | 290 | 310 | 290 | 280 | 140 | 200 | 170 | 150 | 140 | 18.3 | 17.6 | 19.6 | 20.1 | 19.8 |
| 100.0% | B | 10 | 10 | 10 | 10 | 10 | 9.2 | 8.9 | 8.6 | 7.4 | 6.5 | 7.8 | 7.7 | 7.8 | 8.0 | 7.8 | 270 | | | | | 140 | | | | | 18.4 | 17.6 | 19.8 | 19.9 | 19.8 |

[a] The control mortality encountered here was probably due to a bad batch of diluent obtained the previous evening. Since mortality occurred only in the controls, the dead fish were replaced with live ones and the test was allowed to continue. Additionally, 10 more fish were added per chamber (not shown), resulting in a total of 40 control fish. No further mortality occurred for the remainder of the 96 hour test period.

[b] Each test chamber was continuously aerated via a portable air pump for the the entire 96 hour test period.

The total residual chlorine measured in the Treesweet wastewater and well water (diluent) ranged from 0 to trace amount (0.06 mg/l).

Table 2. Continued

| Conc. or % | Test Container Number | Conductivity (umhos) | | | | | Total Ammonia (mg/l as N) | | | | | Unionized Ammonia (mg/l as $NH_3$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 | 0 | 24 | 48 | 72 | 96 |
| Control | A | 1010 | 1200 | 1120 | 1040 | 1050 | 0 | | | | | 0 | | | | |
| Control | B | 1010 | 1200 | 1130 | 1050 | 1050 | | | | | | | | | | |
| 5.5% | A | 1020 | 1200 | 1130 | 1040 | 1070 | 0 | | | | | 0 | | | | |
| 5.5% | B | 1020 | 1200 | 1140 | 1060 | 1090 | | | | | | | | | | |
| 10.0% | A | 1020 | 1200 | 1140 | 1070 | 1090 | 0 | | | | | 0 | | | | |
| 10.0% | B | 1030 | 1200 | 1170 | 1090 | 1100 | | | | | | | | | | |
| 17.3% | A | 1030 | 1200 | 1160 | 1080 | 1100 | 0 | | | | | 0 | | | | |
| 17.3% | B | 1050 | 1200 | 1180 | 1095 | 1100 | | | | | | | | | | |
| 32.5% | A | 1060 | 1200 | 1170 | 1100 | 1100 | 0 | | | | | 0 | | | | |
| 32.5% | B | 1060 | 1200 | 1180 | 1100 | 1100 | | | | | | | | | | |
| 55.3% | A | 1070 | 1080 | 1170 | 1105 | 1110 | 0 | | | | | 0 | | | | |
| 55.3% | B | 1070 | 1050 | 1180 | 110 | 1120 | | | | | | | | | | |
| 100.0% | A | 1090 | 1100 | 1190 | 1140 | 1150 | 0 | | | | | 0 | | | | |
| 100.0% | B | 1090 | 1100 | 1180 | 1150 | 1150 | | | | | | | | | | |

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Treesweet, Ft. Pierce, NPDES #FL0000841, on 2 to 4 March, 1986.

| 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 |
| Control | 10 | 10 | 10 | 9.3 | 12.0 | 8.8 | 7.7 | 7.6 | 7.7 | 18.2 | 20.0 | 18.7 | | |
| Control | 10 | 10 | 10 | 9.3 | 12.0 | 8.8 | 7.7 | 7.6 | 7.7 | | | | 190 | 220 |
| 5.6% | 10 | 10 | 10 | 9.3 | 11.4 | 8.6 | 7.7 | 7.6 | 7.9 | 17.5 | 19.0 | 18.7 | | |
| 5.6% | 10 | 10 | 10 | 9.4 | 11.4 | 8.7 | 7.7 | 7.6 | 7.8 | | | | 226 | 245 |
| 10.0% | 10 | 10 | 10 | 9.4 | 11.4 | 8.5 | 7.7 | 7.6 | 7.8 | 17.7 | 19.2 | 18.7 | | |
| 10.0% | 10 | 10 | 10 | 9.4 | 11.5 | 8.6 | 7.7 | 7.6 | 7.9 | | | | 258 | 280 |
| 18.0% | 10 | 10 | 9 | 9.4 | 11.2 | 8.4 | 7.7 | 7.6 | 7.9 | 17.6 | 19.3 | 18.7 | | |
| 18.0% | 10 | 10 | 10 | 9.4 | 11.4 | 8.5 | 7.7 | 7.6 | 7.9 | | | | 319 | 345 |
| 32.0% | 10 | 7 | 7 | 9.3 | 11.0 | 8.3 | 7.7 | 7.7 | 7.9 | 17.6 | 19.3 | 18.8 | | |
| 32.0% | 10 | 9 | 9 | 9.3 | 11.0 | 8.4 | 7.7 | 7.7 | 7.9 | | | | 420 | 450 |
| 56.0% | 10 | 10 | 10 | 9.3 | 9.8 | 8.1 | 7.7 | 7.7 | 8.0 | 17.8 | 19.3 | 18.7 | | |
| 56.0% | 10 | 9 | 9 | 9.2 | 10.0 | 8.1 | 7.7 | 7.7 | 8.0 | | | | 690 | 700 |
| 100.0% | 10 | 9 | 9 | 8.6 | 7.1 | 7.8 | 7.7 | 7.7 | 8.0 | 18.0 | 19.5 | 18.7 | | |
| 100.0% | 10 | 10 | 10 | 8.5 | 7.5 | 7.8 | 7.7 | 7.7 | 8.0 | | | | 1000 | 1150 |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

Control Water

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

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

Total Alkalinity (mg/l $CaCO_3$): 110

Total Hardness (mg/l $CaCO_3$): 130

100% Effluent

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

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

Total Alkalinity (mg/l $CaCO_3$): 270

Total Hardness (mg/l $CaCO_3$): 140

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Sodium Lauryl Sulfate (Standard Reference Toxicant), performed during Treesween bioassay, 2 to 4 March, 1986.

| 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 | |
| Control | 5 | 5 | 5 | 9.3 | 11.2 | 6.3 | 7.1 | 7.5 | 7.8 | 17.5 | 20.2 | 20.0 | | | Control Water |
| Control | 5 | 5 | 5 | 9.3 | 10.8 | 6.3 | 7.1 | 7.6 | 7.8 | | | | 980 | 1100 | $NH_3$(as $NH_3$)(mg/l) / Total Alkalinity (mg/l $CaCO_3$) |
| 1.0 ppm | 5 | 5 | 5 | 9.2 | 11.4 | 7.4 | 7.2 | 7.6 | 7.9 | 16.7 | 19.7 | 20.0 | | | |
| 1.0 ppm | 5 | 5 | 5 | 9.2 | 11.4 | 7.4 | 7.2 | 7.6 | 7.9 | | | | 980 | 1050 | |
| 3.0 ppm | 5 | 5 | 4 | 9.2 | 11.2 | 7.0 | 7.2 | 7.6 | 7.9 | 16.3 | 19.1 | 20.0 | | | Total ammonia (as N)(mg/l) / Total Hardness (mg/l $CaCO_3$) |
| 3.0 ppm | 5 | 4 | 4 | 9.2 | 11.4 | 7.0 | 7.2 | 7.6 | 7.9 | | | | 920 | 1000 | |
| 7.0 ppm | 5 | 0 | 0 | 9.2 | 11.0 | -[a] | 7.2 | 7.6 | -a | 16.2 | 19.1 | -a | | | |
| 7.0 ppm | 5 | 0 | 0 | 9.2 | 11.0 | - | 7.2 | 7.6 | - | | | | 900 | -a | |
| 10.0 ppm | 5 | 0 | 0 | 9.3 | 11.2 | - | 7.2 | 7.7 | - | 16.9 | 19.1 | - | | | |
| 10.0 ppm | 5 | 0 | 0 | 9.3 | 11.2 | - | 7.2 | 7.7 | - | | | | 840 | - | 100% Effluent |
| | | | | | | | | | | | | | | | $NH_3$(as $NH_3$)(mg/l) / Total Alkalinity (mg/l $CaCO_3$) |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | Total ammonia (as N)(mg/l) / Total Hardness (mg/l $CaCO_3$) |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |

[a] Remaining parameters were not recorded since all organisms were dead by 24 hours.

Table 5. $LC_{50}$ Data for Bioassays Performed at Treesweet, Ft. Pierce, NPDES #FL0000841, 2 to 6 March, 1986.

| Organism | Method of Data Analysis | 24 Hour $LC_{50}$ and 95% Confidence Intervals | 48 Hour $LC_{50}$ and 95% Confidence Intervals | 96 Hour $LC_{50}$ and 95% Confidence Intervals |
|---|---|---|---|---|
| Flow-Through Tests | | | | |
| *Notropis leedsi* | Binomial | | | a |
| | Moving Average | No Mortality | No Mortality | a |
| | Probit | | | a |
| *Daphnia pulex* | Binomial | | | |
| | Moving Average | b | ____ | ____ |
| | Probit | | | |
| Static Test | | | | |
| *Daphnia pulex* | Binomial | a | a | |
| | Moving Average | a | a | |
| | Probit | a | a | |
| Reference Toxicant Tests | | | | |
| *Notropis leedsi* | Binomial | 4.2 ppm (3 and 7) | 3.9 ppm (1 and 7) | |
| | Moving Average | a | a | |
| | Probit | a | a | |
| *Daphnia pulex* | Binomial | | | |
| | Moving Average | b | ____ | |
| | Probit | | | |

[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] Test invalidated at 24 hours due to excessive control mortality.

Table 6. Results of Chemical Analyses Performed on Treesweet's Wastewater and Well Water, Collected 6 March, 1986.

## Organics

| | Treesweet Well Water | Effluent |
|---|---|---|
| Acid Extractables: | N.D. | N.D. |
| Base Neutral Extractables: | Several Unidentified compounds | Several unidentified compounds |
| Pesticide Extractables: | Butyl benzyl phthalate (5 ug/l) | N.D. |

## Metals

| | |
|---|---|
| Antimony | N.D. |
| Arsenic | N.D. |
| Beryllium | N.D. |
| Cadmium | 3 ug/l |
| Chromium | N.D. |
| Copper | < 25 ug/l |
| Lead | < 4 ug/l |
| Mercury | N.D. |
| Nickel | N.D. |
| Selenium | N.D. |
| Silver | < 0.1 ug/l |
| Thallium | N.D. |
| Zinc | 101 ug/l |

N.D. = None Detected

## RESULTS AND DISCUSSION

The results of an onsite 96 hour flow-through bioassay indicated that Treesweet's wastewater, collected from the aeration basin, was not acutely toxic to Notropis leedsi. Additionally, the results of an onsite 48 hour static bioassay indicated that this wastewater was not acutely toxic to Daphnia pulex. Some difficulty was encountered with the use of Treesweet well water as diluent for the onsite tests, since this well water apparently caused some N. leedsi control mortality (Table 2), and was completely unsuitable as diluent in the D. pulex tests. Both the D. pulex flow-through and reference toxicant tests using this diluent were invalidated. The above D. pulex static test used Tallahassee well water as the diluent.

The results of the N. leedsi Standard Reference Toxicant test (48 hour $LC_{50}$ = 3.9 ppm using sodium lauryl sulfate) indicated that the test organisms were viable and within the sensitivity ranges suggested by both the EPA and the DER.

Results of chemical analyses (Table 6) indicated the presence of several unidentified organic compounds and the metals cadmium and zinc in Treesweet's effluent. Additionally, organic analyses were performed on Treesweet's well water used as dilution water in the above tests. Results indicated the presence of several unidentified compounds and butyl benzyl phthalate. It was unlikely that these constituents were responsible for the problems encountered with this well water.

## RECOMMENDATION

Based on the results of these tests, that is, the problems with Treesweet well water and the presence of chemical contaminants in both the wastewater and well water, we recommend the continuation of the district's efforts in the monitoring of groundwater and surface waters around this facility. Also, a yearly biomonitoring program, including both acute and chronic tests of the wastewater discharge, seems warranted.

## 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.

U.S. Environmental Protection Agency. 1985. Treesweet Products, Fort Pierce, Florida. Lethality Tests. EPA, Athens, Georgia. 12 p.