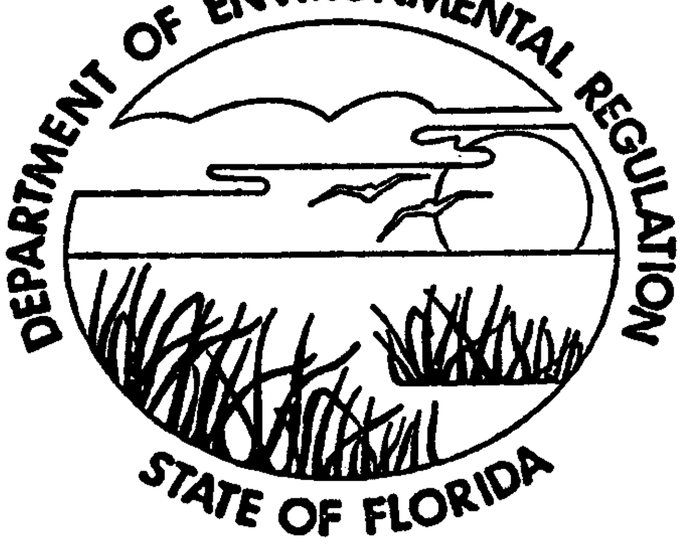



# BIOASSAYS OF GOLDKIST POULTRY

## SUWANNEE COUNTY, FLORIDA

NPDES #FL0001465

SAMPLED 3/24/86

MAY 28, 1986

## Biology Section
## Division of Environmental Programs

# BIOASSAYS OF GOLDKIST POULTRY

# SUWANNEE COUNTY, FLORIDA

# NPDES #FL0001465

Biology Section
Bureau of Laboratories and
Special Programs
May 28, 1986

## EXECUTIVE SUMMARY

Goldkist Poultry, Suwannee County, NPDES #FL0001465, on 25 to 27 March, 1986.

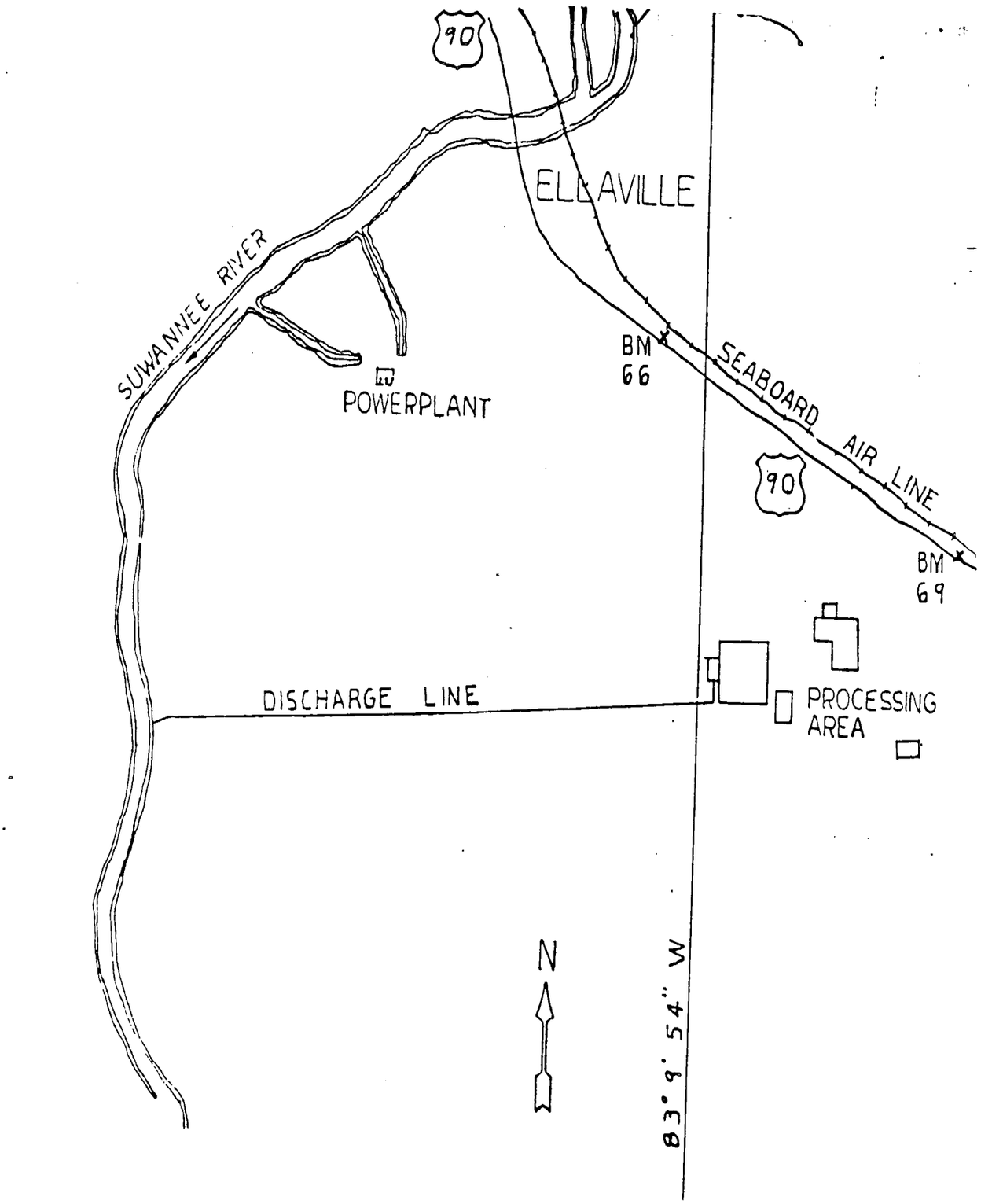
The sample of final chlorinated effluent from this large poultry slaughtering and packaging facility proved to be acutely toxic to Daphnia pulex, producing a 48 hour $LC_{50}$ of 21.3%. This same sample, however, was not acutely toxic to Notropis leedsi. Only 20% of the fish had died in the 100% effluent concentration by the end of the test, which was insufficient to calculate an $LC_{50}$. The toxicity to D. pulex can be attributed to the presence of residual chlorine (0.6 mg/l HACH) in the sample. The same effluent, after de-chlorination, was not toxic to these test organisms. Ammonia was not detected in this wastewater. Since the above toxicity was apparently due solely to the presence of residual chlorine, further chemical analyses were not requested.

Since the toxicity of Goldkist's effluent was due to chlorine it is recommended that this facility be required to consistently demonstrate compliance with the FAC criterion of a maximum chlorine residual of 0.02 mg/l in the receiving waters.

## INTRODUCTION

The Goldkist Poultry facility is located on U.S. Highway 90, 11 miles west of Live Oak, Suwannee County, Florida (Fig. 1). The facility slaughters and packs approximately 137,000 birds per day, totalling 650,750 pounds. By-products are processed into protein meal. Wastewater treatment for this operation includes both secondary and advanced systems, the latter primarily for nitrogenous waste removal. A schematic of wastewater origins and flow is presented in Figure 2. The final discharge of treated chlorinated wastewater, averaging 0.5 MGD, is through a pipe to the bottom of the Suwannee River.

To evaluate the aquatic toxicity potential of this Goldkist discharge, in cooperation with the U.S. EPA, the Biology Section performed four 48 hour static bioassays, on 25 to 27 March, 1986. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were M. L. Roll and P. G. Weiland.



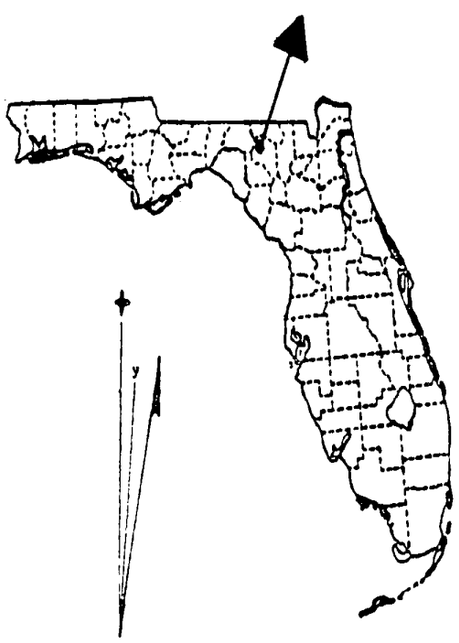



Figure 1. Portions of Suwannee County, Florida, including the location of the Goldkist poultry facility near the Suwannee River.

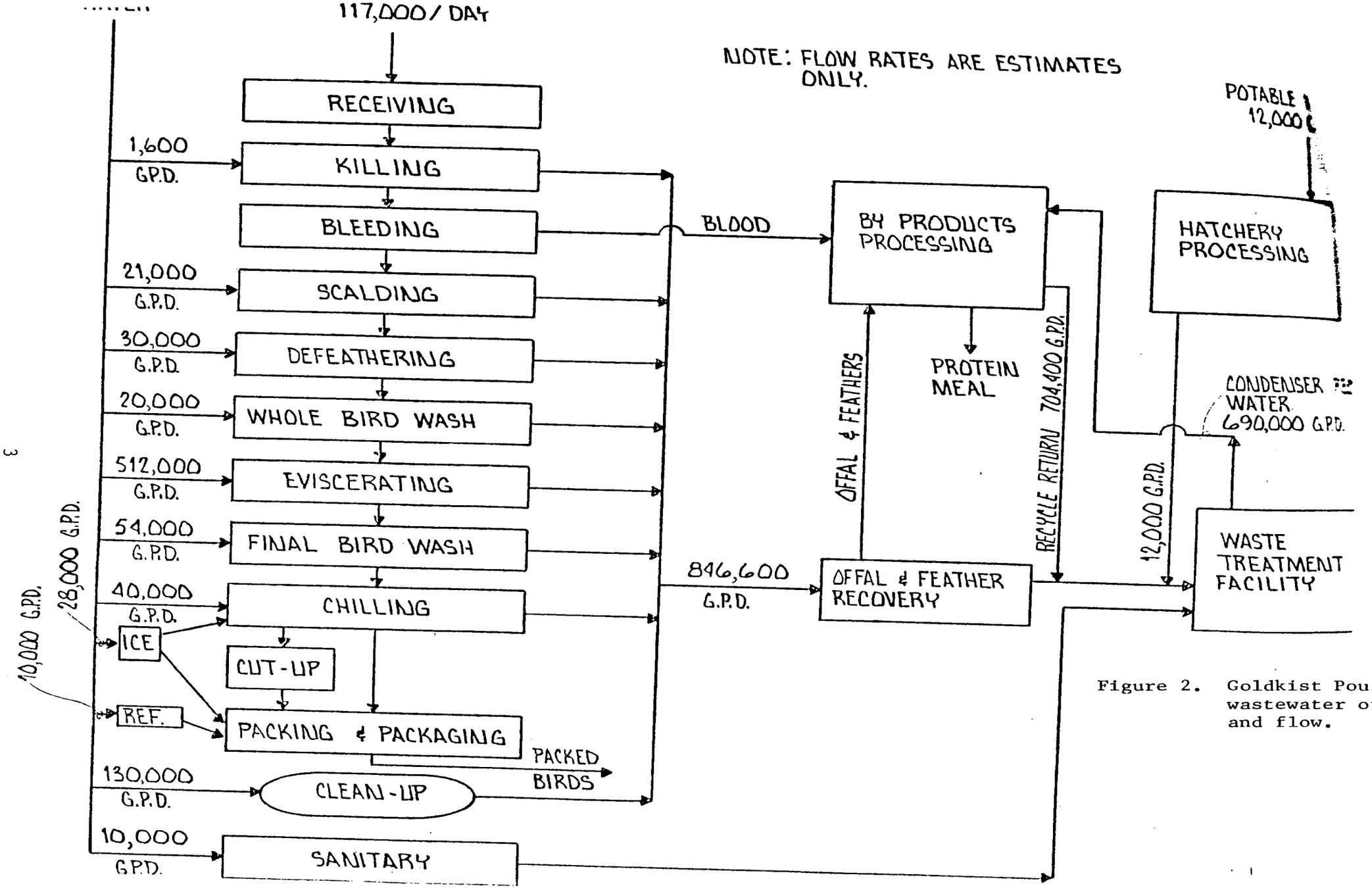



Figure 2. Goldkist Poultry's wastewater origins and flow.

## 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), the U.S. EPA (1978), 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

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.

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 cycle and are fed Tetra-Min® and frozen Artemia (brine shrmp) 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 liquified 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 14 to 20 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

A total of four seperate 48 hour static acute toxicity bioassays were performed on samples of Goldkist's final chlorinated effluent, and on effluent that had been de-chlorinated. The tests were performed from 1445 hours on 25 March to 1520 hours on 27 March, 1986.

The samples were collected by Mr. Dennis Murrin of DER's Northeast District at 1000 hours on 25 March, 1986, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Sampling for metal analyses were preserved with $HNO_3$. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

### Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperture range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassays protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

### Total Residual Chlorine

Since many wastewaters 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 the other components of the wastewater. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated wastewater 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 effluents 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 wastewater.

## Freshwater Tests

Freshwater for dilution of the effluent samples was obtained from the same DER deep well that is used to cuture the test organisms. The concentration series selected for this study is an abbreviated version of that recommended by APHA (1980).

Two replicates, of each concentration and a control, were used to test with the water flea, Daphnia pulex. Flint glass jars of 250 ml capacity were uses as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas 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) 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), temperture (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 conrols and the 100% effluent concentrations. 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 Tables 2 through 5.

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

*Daphnia pulex* (water flea)

| Concentration | Dilution Water | Effluent | Total Volume |
|---|---|---|---|
| Control | 200.0 ml | -------- | 200 ml |
| 10.0% | 180.0 ml | 20.0 ml | 200 ml |
| 32.0% | 136.0 ml | 64.0 ml | 200 ml |
| 56.0% | 88.0 ml | 112.0 ml | 200 ml |
| 100.0% | -------- | 200.0 ml | 200 ml |

*Notropis leedsi* (bannerfin shiner)

| Concentration | Dilution Water | Effluent | Total Volume |
|---|---|---|---|
| Control | 500 ml | -------- | 500 ml |
| 10.0% | 450 ml | 50 ml | 500 ml |
| 32.0% | 340 ml | 160 ml | 500 ml |
| 56.0% | 220 ml | 280 ml | 500 ml |
| 100.0% | ------ | 500 ml | 500 ml |

ble 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Goldkist Poultry, Suwannee County, NPDES #FL0001465, on 25 to 27 March, 1986.

Chlorinated Effluent[a]

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/1) | | | pH | | | Temperature (°C) | | | Conductivity (umhos) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 |
| Control | 10 | 10 | 9 | 8.5 | 8.2 | 9.0 | 7.8 | 7.8 | 7.9 | 20.5 | 19.9 | 20.8 | | |
| Control | 10 | 10 | 10 | 8.5 | 8.2 | 9.0 | 7.8 | 7.8 | 7.9 | | | | 202 | 222 |
| 10.0% | 10 | 10 | 10 | 8.5 | 8.3 | 9.0 | 7.8 | 7.9 | 7.9 | 20.7 | 19.6 | 20.7 | | |
| 10.0% | 10 | 10 | 10 | 8.5 | 8.3 | 9.0 | 7.8 | 7.9 | 7.9 | | | | 244 | 260 |
| 32.0% | 10 | 3 | 3 | 8.6 | 8.3 | 8.5 | 7.8 | 7.9 | 7.9 | 20.7 | 19.5 | 20.4 | | |
| 32.0% | 10 | 3 | 0 | 8.6 | 8.3 | 8.5 | 7.8 | 7.9 | 7.9 | | | | 338 | 352 |
| 56.0% | 10 | 0 | 0 | 8.9 | 8.4 | -b | 7.7 | 7.9 | -b | 20.9 | 19.5 | -b | | |
| 56.0% | 10 | 0 | 0 | 8.9 | 8.4 | - | 7.7 | 7.9 | - | | | | 440 | -b |
| 100.0% | 10 | 0 | 0 | 9.5 | 8.4 | - | 7.6 | 7.8 | - | 21.0 | 19.5 | - | | |
| 100.0% | 10 | 0 | 0 | 9.5 | 8.4 | - | 7.6 | 7.8 | - | | | | 700 | - |
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Control Water

$NH_3$(as $NH_3$)(mg/1) 0

Total ammonia (as N)(mg/1) 0

Total Alkalinity 113 (mg/1 $CaOO_3$)

Total Hardness 118 (mg/1 $CaOO_3$)

100% Effluent

$NH_3$(as $NH_3$)(mg/1) 0

Total ammonia (as N)(mg/1) 0

Total Alkalinity 84 (mg/1 $CaOO_3$)

Total Hardness 192 (mg/1 $CaOO_3$)

[a] Total Residual Chlorine: 0.6 mg/1 (HACH DPD Method) and 0.53 mg/1 (Iodometric Method I).

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

Table 3. Data recorded during 48 hour, *Notropis leedsi*, static acute toxicity bioassay of Goldkist Poultry, Suwannee County, NPDES #FL0001465, on 25 to 27 March, 1986.

Chlorinated Effluent[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 |
| Control | 5 | 5 | 5 | 8.6 | 8.2 | 8.7 | 7.6 | 7.8 | 7.9 | 21.0 | 19.5 | 20.4 | | |
| Control | 5 | 5 | 5 | 8.6 | 8.2 | 8.7 | 7.6 | 7.8 | 7.9 | | | | 205 | 217 |
| 10.0% | 5 | 5 | 5 | 8.7 | 8.1 | 8.9 | 7.6 | 7.8 | 7.9 | 21.1 | 19.3 | 20.1 | | |
| 10.0% | 5 | 5 | 5 | 8.7 | 8.1 | 8.9 | 7.6 | 7.8 | 7.9 | | | | 250 | 255 |
| 32.0% | 5 | 5 | 5 | 8.9 | 8.1 | 9.0 | 7.7 | 7.7 | 7.9 | 21.2 | 19.1 | 19.9 | | |
| 32.0% | 5 | 5 | 5 | 8.9 | 8.1 | 9.0 | 7.7 | 7.7 | 7.9 | | | | 342 | 352 |
| 56.0% | 5 | 5 | 5 | 9.1 | 8.2 | 9.1 | 7.7 | 7.8 | 7.8 | 21.2 | 19.0 | 19.8 | | |
| 56.0% | 5 | 5 | 5 | 9.1 | 8.2 | 9.1 | 7.7 | 7.8 | 7.8 | | | | 448 | 455 |
| 100.0% | 5 | 3 | 3 | 9.5 | 8.2 | 8.2 | 7.7 | 7.7 | 7.7 | 21.2 | 19.0 | 19.8 | | |
| 100.0% | 5 | 5 | 5 | 9.5 | 8.2 | 8.2 | 7.7 | 7.7 | 7.7 | | | | 710 | 710 |
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| Control Water | |
|---|---|
| $NH_3$(as $NH_3$)(mg/l) 0 | Total Alkalinity (mg/l $CaCO_3$) 113 |
| Total ammonia (as N)(mg/l) 0 | Total Hardness (mg/l $CaCO_3$) 118 |

| 100% Effluent | |
|---|---|
| $NH_3$(as $NH_3$)(mg/l) 0 | Total Alkalinity (mg/l $CaCO_3$) 84 |
| Total ammonia (as N)(mg/l) 0 | Total Hardness (mg/l $CaCO_3$) 192 |

[a] Total Residual Chlorine: 0.6 mg/l (HACH DPD Method) and 0.53 mg/l (Iodometric Method I).

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Goldkist Poultry, Suwannee County, NPDES #FL0001465, on 25 to 27 March, 1986.

De-chlorinated Effluent[a]

| Concentration or % | Number of Live Organisms | | | Dissolved Oxygen (mg/1) | | | pH | | | Temperature (°C) | | | Conductivity (umhos) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 24 | 48 | 0 | 48 |
| Control | 10 | 10 | 10 | 8.5 | 8.1 | 8.9 | 7.9 | 7.9 | 8.0 | 20.7 | 19.5 | 20.1 | | |
| Control | 10 | 10 | 10 | 8.5 | 8.1 | 8.9 | 7.9 | 7.9 | 8.0 | | | | 202 | 220 |
| 10.0% | 10 | 10 | 10 | 8.5 | 8.1 | 8.9 | 7.8 | 8.0 | 7.9 | 20.7 | 19.5 | 20.1 | | |
| 10.0% | 10 | 10 | 10 | 8.5 | 8.1 | 8.9 | 7.8 | 8.0 | 7.9 | | | | 248 | 262 |
| 32.0% | 10 | 10 | 9 | 8.7 | 8.0 | 8.9 | 7.8 | 7.9 | 7.9 | 20.7 | 19.5 | 20.2 | | |
| 32.0% | 10 | 10 | 10 | 8.7 | 8.0 | 8.9 | 7.8 | 7.9 | 7.9 | | | | 342 | 357 |
| 56.0% | 10 | 10 | 10 | 9.0 | 8.0 | 8.5 | 7.8 | 7.9 | 7.8 | 20.8 | 19.6 | 20.3 | | |
| 56.0% | 10 | 10 | 10 | 9.0 | 8.0 | 8.5 | 7.8 | 7.9 | 7.8 | | | | 442 | 460 |
| 100.0% | 10 | 10 | 10 | 9.5 | 8.0 | 8.2 | 7.7 | 7.8 | 7.7 | 20.7 | 19.5 | 20.3 | | |
| 100.0% | 10 | 10 | 10 | 9.5 | 8.0 | 8.2 | 7.7 | 7.8 | 7.7 | | | | 710 | 710 |
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**Control Water**

$NH_3$(as $NH_3$)(mg/1): 0

Total ammonia (as N)(mg/1): 0

Total Alkalinity (mg/1 $CaOO_3$): 113

Total Hardness (mg/1 $CaOO_3$): 118

**100% Effluent**

$NH_3$(as $NH_3$)(mg/1): 0

Total ammonia (as N)(mg/1): 0

Total Alkalinity (mg/1 $CaOO_3$): 84

Total Hardness (mg/1 $CaOO_3$): 195

[a] Total Residual Chlorine: < 0.02 mg/1 (HACH DPD Method).

Table 5. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Goldkist Poultry, Suwannee County, NPDES #FL0001465, on 25 to 27 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 | 8.6 | 8.2 | 8.7 | 7.6 | 7.8 | 7.9 | 21.0 | 19.0 | 20.4 | | |
| Control | 5 | 5 | 5 | 8.6 | 8.2 | 8.7 | 7.6 | 7.8 | 7.9 | | | | 205 | 217 |
| 10.0% | 5 | 5 | 5 | 8.7 | 8.0 | 8.9 | 7.7 | 7.8 | 7.9 | 20.9 | 19.0 | 19.7 | | |
| 10.0% | 5 | 5 | 5 | 8.7 | 8.0 | 8.9 | 7.7 | 7.9 | 7.9 | | | | 250 | 257 |
| 32.0% | 5 | 5 | 5 | 8.9 | 8.0 | 8.9 | 7.7 | 7.8 | 7.9 | 20.9 | 18.9 | 19.6 | | |
| 32.0% | 5 | 5 | 5 | 8.9 | 8.0 | 8.9 | 7.7 | 7.8 | 7.9 | | | | 348 | 352 |
| 56.0% | 5 | 5 | 5 | 9.1 | 7.9 | 8.7 | 7.7 | 7.8 | 7.8 | 20.8 | 19.0 | 19.6 | | |
| 56.0% | 5 | 5 | 5 | 9.1 | 7.9 | 8.7 | 7.7 | 7.8 | 7.8 | | | | 451 | 453 |
| 100.0% | 4[b] | 4 | 4 | 9.5 | 7.8 | 8.3 | 7.7 | 7.7 | 7.7 | 20.7 | 19.0 | 19.7 | | |
| 100.0% | 4[b] | 4 | 4 | 9.5 | 7.8 | 8.3 | 7.7 | 7.7 | 7.7 | | | | 710 | 710 |
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| Control Water | |
|---|---|
| $NH_3$(as $NH_3$)(mg/l) 0 | Total Alkalinity (mg/l $CaOO_3$) 113 |
| Total ammonia (as N)(mg/l) 0 | Total Hardness (mg/l $CaOO_3$) 118 |

| 100% Effluent | |
|---|---|
| $NH_3$(as $NH_3$)(mg/l) 0 | Total Alkalinity (mg/l $CaOO_3$) 84 |
| Total ammonia (as N)(mg/l) 0 | Total Hardness (mg/l $CaOO_3$) 195 |

[a] Total Residual Chlorine: < 0.02 mg/l (HACH DPD Method).

[b] Due to a shortage of fish, only four organisms were loaded into this chamber.

Table 6. $LC_{50}$ Data for Bioassays of Goldkist Poultry, Suwannee County, NPDES #FL0001465, on 25 to 27 March, 1986.

| Organism | Method of Data Analysis | 24 Hour $LC_{50}$ and 95% Confidence Intervals | 48 Hour $LC_{50}$ and 95% Confidence Intervals |
|---|---|---|---|
| Chlorinated Effluent | | | |
| *Daphnia pulex* | Binomial | 24.6% (10 and 56) | 21.3% (10 and 32) |
| | Moving Average | a | a |
| | Probit | a | a |
| *Notropis leedsi* | Binomial | a | a |
| | Moving Average | a | a |
| | Probit | a | a |
| De-chlorinated Effluent | | | |
| *D. pulex* | Binomial<br>Moving Average<br>Probit | No Mortality | Random Mortality |
| *N. leedsi* | Binomial<br>Moving Average<br>Probit | No Mortality | No Mortality |

[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.

## RESULTS AND DISCUSSION

The sample of final chlorinated effluent from the Goldkist Poultry slaughtering and packaging facility proved to be acutely toxic to Daphnia pulex, producing a 48 hour $LC_{50}$ of 21.3% (Table 6). This same sample, however, was not acutley toxic to Notropis leedsi. Only 20% of the fish had died in the 100% effluent concentrations by the end of the test, which was insufficient to calculate an $LC_{50}$. The toxicity to D. pulex can be attributed to the pressure of residual chlorine (0.6 mg/l HACH) in the sample. This same effluent, after de-chlorination, was not toxic to these test organisms. Only one random D. pulex and no N. leedsi died during the 48 hour static tests (Tables 4 and 5). Ammonia was not detected in this wastewater. Since the above toxicity was apparently due solely to the presence of residual chlorine, further chemical anlayses were not requested.

### Biology Section's Recommendation

Since the toxicity of Goldkist's effluent was due to chlorine it is recommended that this facility be required to consistently demonstrate compliance with the FAC criterion of a maximum chlorine residual of 0.02 mg/l in the receiving waters.

## 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 languge 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 one-twentieth 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 acutally is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whether as 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 orangisms, 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 effluent samples, representative of the effluent 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, effluent 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

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