

BIOASSAYS OF LAKELAND PACKING COMPANY

HIGHLAND CITY, POLK COUNTY, FLORIDA

NO NPDES PERMIT

FEBRUARY 6, 1986

Biology Section
Division of Environmental Programs

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Bureau of Laboratories and
Specical Programs
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EXECUTIVE SUMMARY

Lakeland Packing, Polk County, on 11 to 13 December, 1985.

The sample of wastewater collected from this citrus processing facility located in Highland City proved to be extremely toxic to the test organisms, Daphnia pulex and Notropis leedsi. A 24 hour LC_{50} of < 1.0% is given for D. pulex, since all organisms were dead in this concentration by 24 hours. Likewise, an 1 hour LC_{50} of 13.4% was calculated for N. leedsi. The sample had a pink color and a distinct odor of cleaning fluids.

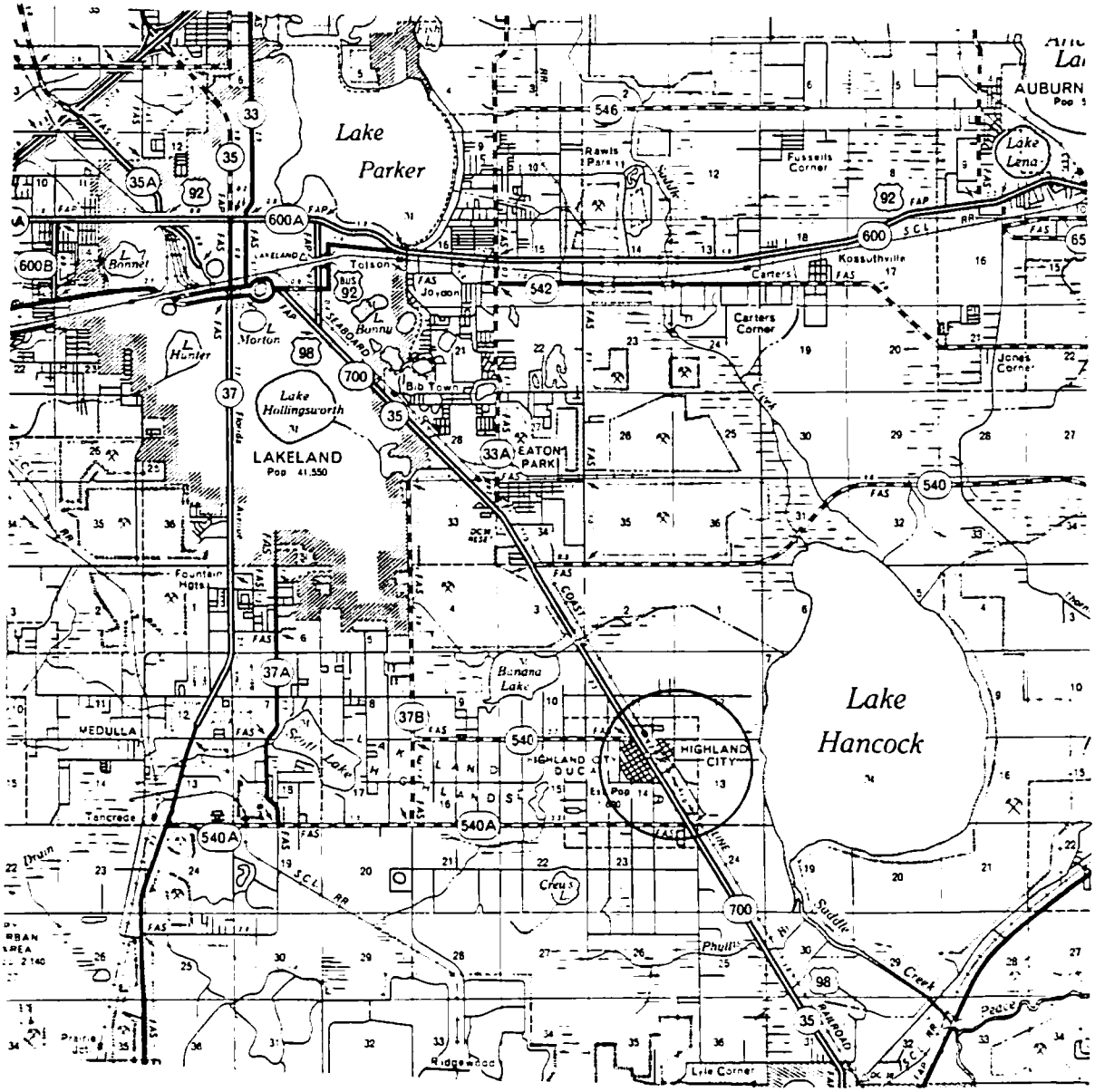
Chemical analyses indicated the presence of the metals arsenic (11 ug/l), copper (503 ug/l), and lead (50 ug/l) in this wastewater. Also, the organo-phosphorus insecticide ethion (64 ug/l), and several other large unidentified organic compounds were detected in this wastewater. The presence of ethion is of particular environmental importance due to the extreme toxicity of this compound. The toxicity of ethion to organisms such as Daphnia begins at approximately 3 ug/l.

We recommend that this facility and its wastewater be closely monitored so that serious environmental threats, such as to groundwater, do not occur.

INTRODUCTION

The Lakeland Packing Company is located in Highland City, just southeast of Lakeland, Polk County, Florida (Fig. 1). The facility processes various citrus fruits into juice and by-products. Wastewater from this operation is discharged from settling tanks into a ditch where it either evaporates or percolates into the groundwater.

At the request of the Southwest District, the Biology Section performed two static acute toxicity bioassays on this wastewater, to determine the existence and extent of toxicity. The sample, collected on 10 December, 1985, consisted of a pink liquid smelling strongly of cleaning fluids or solvents. The results of these bioassays are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.



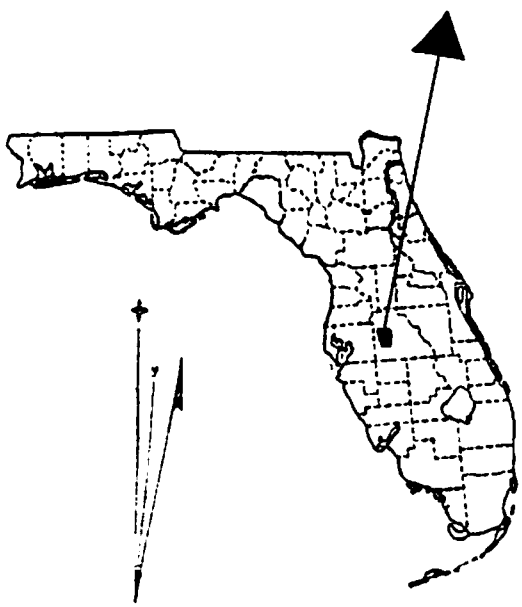


Figure 1. Portions of Polk County, Florida, including Highland City, the location of Lakeland Packing Company

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-dark cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were approximately three weeks 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

Two 48 hour static acute toxicity bioassays were performed on a sample of wastewater collected from Lakeland Packing Company. The tests were performed from 1030 hours on 11 December to 1030 hours on 13 Decmeber, 1985.

The sample was collected by Mr. David Demmi of DER's Southwest District at 1200 hours on 10 December, 1985, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were 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 temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Aeration

Since the sample to be used for bioassay analysis contained a dissolved oxygen concentration of less than 40% of saturation, the entire sample was aerated to approximately 6.0 mg/l. This was achieved using a single airstone connected to the laboratory compressed air supply.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In this particular case, the sample was adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely a result of aerating the sample. The pH of the freshwater sample was adjusted to $\pm$ 0.3 s.u. of the value measured upon arrival in the laboratory and prior to any adjustments. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH. This pH adjustment involved only a few tenths of a unit and required only a very small amount of 0.1N HCl.

Freshwater Tests

Freshwater for dilution of the effluent sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) 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 used 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 fed during this test.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% effluent concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentration at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2 and 3.

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
1.0%	198.0 ml	2.0 ml	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
18.0%	164.0 ml	36.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	--------	500 ml
1.0%	495 ml	5 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%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Lakeland Packing Company, Polk County, Florida, on 11 to 13 December, 1985.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48[a]	0	24	48[a]	0	24	48[a]	0	24	48[a]	0	48[a]
Control	10	10		8.4	8.0		7.9	8.1		21.9	22.5			
Control	10	9		8.4	8.0		7.9	8.1					205	
1.0%	10	0		8.4	7.3		7.8	8.0		22.1	22.8			
1.0%	10	0		8.4	7.3		7.8	8.0					215	
10.0%	10	0		8.2	4.2		7.7	7.7		21.9	22.7			
10.0%	10	0		8.2	4.2		7.7	7.7					239	
18.0%	10	0		8.0	2.6		7.7	7.6		21.8	22.4			
18.0%	10	0		8.0	2.6		7.7	7.6					268	

Control Water	
NH_3 (as NH_3)(mg/l) 0	Total Alkalinity (mg/l $CaCO_3$) 104
Total ammonia (as N)(mg/l) 0	Total Hardness (mg/l $CaCO_3$) 110

100% Effluent	
NH_3 (as NH_3)(mg/l) .003	Total Alkalinity (mg/l $CaCO_3$) 195
Total ammonia (as N)(mg/l) 0.29	Total Hardness (mg/l $CaCO_3$) 175

[a] 48 hour data were not recorded since all organisms were dead in every effluent concentration by 24 hours.

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Lakeland Packing Company, Polk County, Florida, on 11 to 13 December, 1985.

Concentration or %	Number of Live Organisms 0	1	24	Dissolved Oxygen (mg/l) 0	24	48[b]	pH 0	24	48[b]	Temperature (°C) 0	24	48[b]	Conductivity (umhos) 0	48[b]
Control	5	5	5	8.3	7.6		7.9	8.0		22.0	22.8			
Control	5	5	5	8.3	7.6		7.9	8.0					210	
1.0%	5	5	5	8.4	7.0		7.9	7.9		21.9	22.5			
1.0%	5	5	4	8.4	7.0		7.9	7.9					217	
10.0%	5	5	0	8.2	2.4[a]		7.7	7.5		21.9	22.3			
10.0%	5	5	0	8.2	2.4[a]		7.7	7.5					242	
18.0%	5	0	0	8.0	-[b]		7.6	-[b]		21.8	-[b]			
18.0%	5	0	0	8.0			7.6						270	
32.0%	5	0	0	7.6			7.5			21.8				
32.0%	5	0	0	7.6			7.5						317	
56.0%	5	0	0	6.9			7.4			21.6				
56.0%	5	0	0	6.9			7.4						390	
100.0%	5	0	0	5.5			7.2			21.4				
100.0%	5	0	0	5.5			7.2						520	

Control Water

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

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

Total Alkalinity (mg/l $CaCO_3$): 104

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

100% Effluent

NH_3(as NH_3)(mg/l): .002

Total ammonia (as N)(mg/l): 0.29

Total Alkalinity (mg/l $CaCO_3$): 195

Total Hardness (mg/l $CaCO_3$): 175

[a] Since these DO values were < 40% of saturation, the observed mortality in this test concentration was not used in an LC_{50} determination.

[b] Due to the rapid and extreme toxicity of this sample, the remaining 24 and 48 hour data were not recorded.

Table 4. LC_{50} Data for Bioassays of Lakeland Packing, Polk County, on 11 to 13 December, 1985.

Organism	Method of Data Analysis	1 Hour LC_{50} and 95% Confidence Intervals	24 Hour LC_{50} and 95% Confidence Intervals
Daphnia pulex			< 1.0%, a
Notropis leedsi	Binomial	13.4% (10 and 18)	3.1% (1 and 18)
	Moving Average	b	b
	Probit	b	b

[a] The LC_{50} is < 1.0% since all organisms were dead in this concentration at 24 hours.

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

Table 5. Resutls of Chemical Analyses Performed on Wastewater from Lakeland Packing, Polk County, collected 10 December, 1985.

Metals	
Arsenic	11 ug/l
Barium	N.D.
Cadmium	< 1.4 ug/l
Chromium	N.D.
Copper	503 ug/l
Lead	50 ug/l
Mercury	< 2.0 ug/l
Selenium	N.D.
Silver	N.D.
Organics	
Pesticide Extractables:	Ethion (64 ug/l) Several other large unidentified organic compounds

RESULTS AND DISCUSSION

The sample of wastewater collected from Lakeland Packing proved to be extremely toxic to the test organisms, Daphnia pulex and Notropis leedsi. A 24 hour LC_{50} < 1.0% is given for D. pulex, since all organisms were dead in this concentration by 24 hours (Table 4). Likewise, an 1 hour LC_{50} of 13.4% was calculated for N. leedsi (Table 4). The sample had a pink color and a distinct odor of cleaning fluids. The ammonia concentrations detected in this wastewater (0.29 mg/l total and 0.003 mg/l unionized) were insufficient to have caused any toxicity.

Chemical analyses (Table 5) indicated the presence of the metals arsenic (11 ug/l), copper (503 ug/l), and lead (50 ug/l) in this wastewater. Also, the organo-phosphorus insecticide ethion (64 ug/l), and several other large unidentified organic compounds were detected. Of the metals present in Lakeland Packing's wastewater, copper probably contributed somewhat to the observed toxicity, since this concentration is known to be acutely toxic to several aquatic organisms (U.S. EPA 1976). Also, the presence of ethion is of particular environmental importance due to the extreme toxicity of this compound. The toxicity of ethion to organisms such as Daphnia begins at approximately 3 ug/l (U.S. Department of the Interior, 1980).

BIOLOGY SECTION RECOMMENDATIONS

Since wastewater from Lakeland Packing is not discharged to surface waters, the FAC Chapter 17-3 criteria prohibiting toxic discharges may not be applicable. However, due to the extremely toxic nature of this wastewater, as demonstrated by these bioassays, we recommend close monitoring of this facility and its wastewater so that serious environmental threats, such as to groundwater, do not occur.

LITERATURE CITED

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