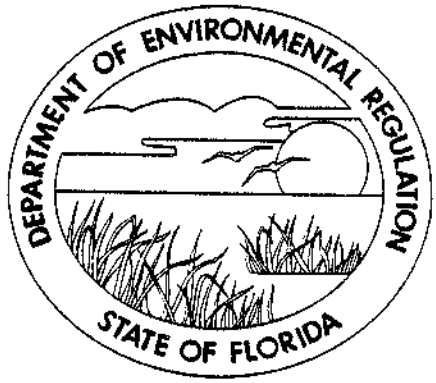


BIOASSAYS OF ALPHA CHEMICAL CORPORATION

POLK COUNTY, FLORIDA

NPDES #FL0029653

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Biological Section
Bureau of Water Analysis
July 22, 1981

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INTRODUCTION

The Alpha Chemical Corporation is a polyester resin facility located in Kathleen, Polk County, Florida (Fig. 1). The facility manufactures approximately 70 tons per day of synthetic resins. For this production, approximately 0.4 MGD of groundwater is used, the majority of which is non-contact cooling water and boiler-feed water. The wastewater is discharged (after cooling in two ponds) through a drainage ditch to Swampy Bog, which eventually flows to the Hillsborough River. Boiler wash and caustic cleaning waters are evaporated in a concrete lined pond. The remaining process water and solid/semi-solid wastes are contained in drums for off-site disposal.

On 12 to 14 May, 1981, the Biological Section performed static toxicity bioassays on a sample of Alpha Chemical Corporation's discharge to determine the existence and extent of toxicity to the biota of the receiving water. The results of these tests are presented in this report.

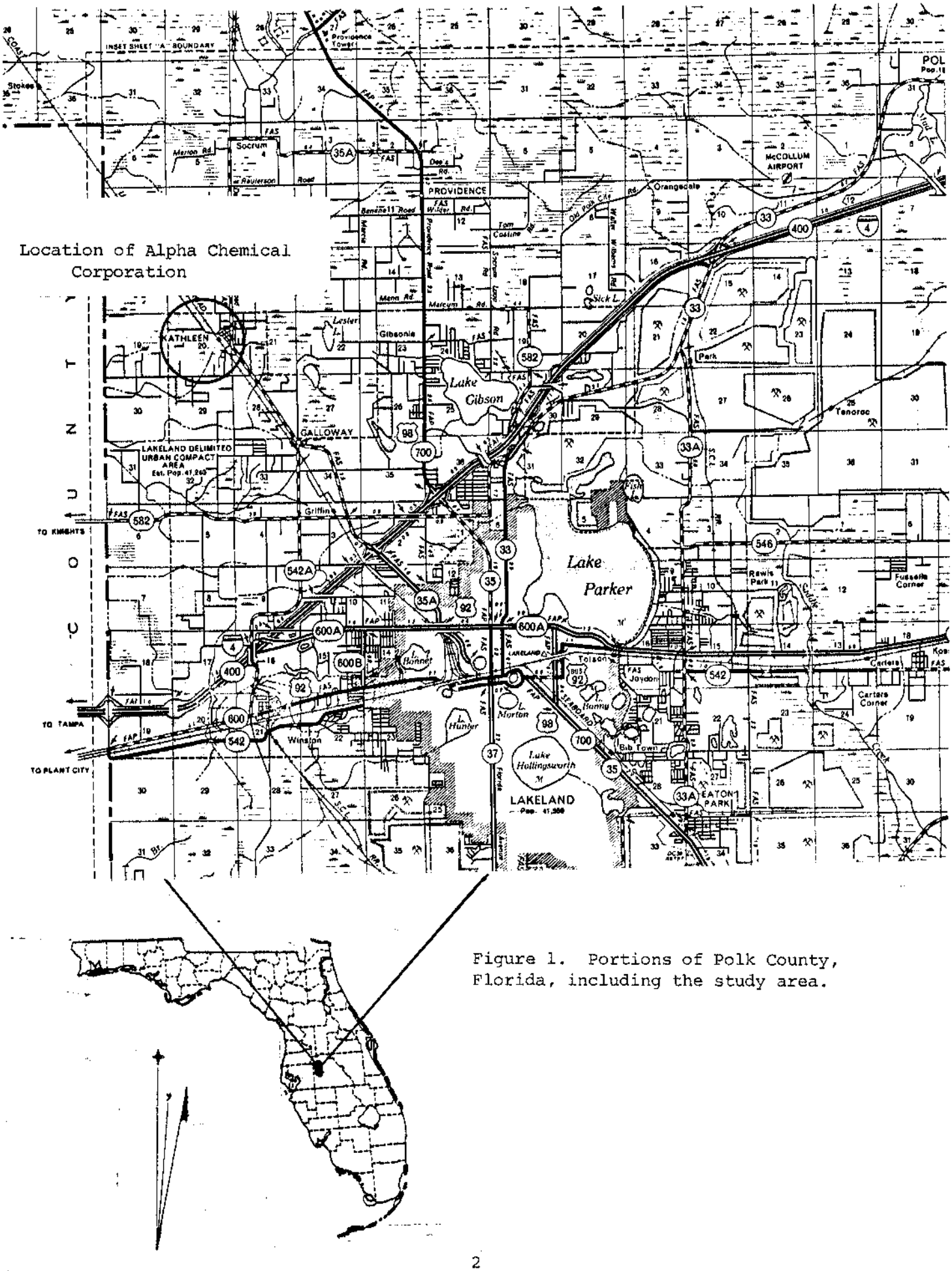


Figure 1. Portions of Polk County, Florida, including the study area.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. 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 2½ gallon all glass aquaria filled with unchlorinated DER well water, using a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of 22° ± 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. They are then refilled 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.

Simocephalus serrulatus (water flea) - An indigenous breeding population of S. serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of 22° ± 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio in May, 1979. The fish have been maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fish have been reared at a temperature of 22° ± 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills Trout Chow® and frozen Artemia (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were all four weeks old at the time of the test.

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

Three separate 48 hour static bioassays were conducted from 1100 hours on 12 May to 1100 hours on 14 May, 1981, on a grab sample of effluent from Outfall #001. The sample was collected by Mr. Matt McCann of DER's Southwest District[1] using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analysis were preserved with HNO_3. Samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

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 that recommended by APHA (1976).

Two replicates, each with six concentrations and a control, were used to test with Daphnia pulex and Simocephalus serrulatus. 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 and Simocephalus were transferred into large culture dishes through stainless steel screens to isolate the proper size and age organisms (neonates). These neonates were then transferred individually by glass pipette and were loaded 10 per chamber, resulting in 20 per test concentration.

Two replicates, of six concentrations and a control, were used for the fathead minnow bioassay. The fish were transferred individually or by twos, by net, into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Fathead minnows were loaded 5 per chamber resulting in 10 per concentration.

Dissolved oxygen, pH, temperature and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness were recorded at the beginning of the test for the controls and the 100% effluent concentration. Specific conductance was recorded for each test concentration at the beginning of the test. Additionally, ammonia concentrations[2], including total (expressed as mg/l as N) and unionized (expressed as mg/l as NH_3) fractions, were recorded at the beginning of the test for the controls and the 100% effluent concentrations. These values were recorded on the bioassay data forms and are presented in Tables 2, 3 and 4. Organisms were not fed during these tests.

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[1] The samples were collected at 1100 hours on 11 May, 1981.

[2] Total ammonia concentrations were determined using an Orion Model 95-10 specific ion probe. The unionized fraction was then calculated based on pH, temperature and salinity.

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

Water fleas (Daphnia pulex and Simocephalus serrulatus)

Concentration	Dilution Water	Effluent	Total Vol.
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100%	----	200 ml	200 ml

Fathead minnow (Pimephales promelas)

Concentration	Dilution Water	Effluent	Total Vol.
Control	500 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%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Alpha Chemical Corporation, Kathleen, Polk County, Florida, NPDES #FL0029653, Outfall #001, on 12 to 14 May, 1981.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	10	8.2	8.4	8.5	7.8	8.2	8.0	106		116		21.3	19.7	20.0	0/.08	—	
Control	10	10	10	8.2	8.5	8.7	7.8	8.2	8.1										202
5.6%	10	10	10	8.2	8.4	8.8	7.8	8.2	8.1					21.2	19.8	19.8			
5.6%	10	10	10	8.2	8.4	8.9	7.8	8.2	8.1										204
10%	10	10	10	8.2	8.4	8.8	7.8	8.2	8.1					21.2	19.8	19.8			
10%	10	10	9	8.2	8.4	8.9	7.8	8.1	8.1										206
18%	10	10	10	8.2	8.4	8.9	7.8	8.2	8.1					21.1	19.7	19.8			
18%	10	8	8	8.2	8.4	8.9	7.8	8.1	8.1										207
32%	10	10	10	8.1	8.4	8.8	7.8	8.1	8.1					20.9	19.8	19.7			
32%	10	10	10	8.1	8.5	8.9	7.8	8.1	8.1										200
56%	10	10	10	7.9	8.5	8.7	7.9	8.2	8.1					20.7	19.8	19.8			
56%	10	10	10	7.9	8.5	8.8	7.9	8.1	8.1										198
100%	10	10	10	7.3	8.5	8.7	7.9	8.1	8.1	101		96		20.6	20.0	20.0	.01/.14	—	
100%	10	10	9	7.3	8.5	8.7	7.9	8.1	8.1										198

Remarks:

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Alpha Chemical Corporation, Kathleen, Polk County, Florida, NPDES #FL0029653, Outfall #001 on 12 to 14 May, 1981.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	9	8.5	8.5	8.8	7.8	8.2	8.1	106		116		20.5	19.7	20.3	0/.08	—	
Control	10	10	8	8.5	8.6	8.8	7.8	8.2	8.1										202
5.6%	10	10	7	8.4	8.6	8.8	7.9	8.2	8.1					20.5	19.7	20.3			
5.6%	10	9	8	8.4	8.6	8.8	7.9	8.2	8.1										204
10%	10	10	5	8.3	8.6	8.9	7.9	8.2	8.1					20.6	19.7	20.4			
10%	10	10	7	8.3	8.6	8.9	7.9	8.2	8.1										206
18%	10	10	10	8.3	8.6	8.9	7.9	8.2	8.1					20.6	19.7	19.8			
18%	10	10	9	8.3	8.7	8.9	7.9	8.2	8.1										207
32%	10	10	7	8.1	8.6	8.9	7.9	8.2	8.1					20.6	19.5	19.7			
32%	10	10	6	8.1	8.6	8.9	7.9	8.2	8.1										200
56%	10	9	7	7.8	8.6	8.9	7.9	8.2	8.1					20.4	19.6	19.6			
56%	10	10	8	7.8	8.6	8.9	7.9	8.2	8.1										198
100%	10	10	8	6.7	8.6	8.9	7.9	8.2	8.1	101		96		20.1	19.8	19.7	.01/.14	—	
100%	10	10	7	6.7	8.5	8.8	7.9	8.2	8.1										198

Remarks:

Table 4. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of Alpha Chemical Corporation, Kathleen, Polk County, Florida, NPDES #FL0029653, Outfall #001 on 12 to 14 May, 1981.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	5	5	5	8.5	5.5	5.7	7.8	7.7	7.7	106		116		20.8	19.6	19.5	0/.08	—	
Control	5	5	5	8.5	6.0	6.7	7.8	7.7	7.7										210
5.6%	5	5	5	8.4	5.9	6.4	7.8	7.7	7.7					20.8	19.5	19.5			
5.6%	5	5	5	8.4	6.1	6.6	7.8	7.7	7.7										211
10%	5	5	5	8.3	6.1	6.9	7.9	7.7	7.7					20.7	19.5	19.5			
10%	5	5	5	8.3	6.2	6.6	7.9	7.8	7.7										209
18%	5	5	5	8.2	5.9	6.6	7.9	7.7	7.7					20.7	19.5	19.5			
18%	5	5	5	8.2	6.2	6.8	7.9	7.7	7.7										209
32%	5	5	5	8.0	5.6	6.6	7.9	7.7	7.7					20.5	19.5	19.5			
32%	5	5	5	8.0	6.2	6.6	7.9	7.7	7.7										207
56%	5	5	5	7.7	5.8	6.1	7.9	7.8	7.7					20.3	19.5	19.5			
56%	5	5	5	7.7	5.8	6.0	7.9	7.7	7.7										202
100%	5	5	5	6.8	5.1	5.7	7.9	7.7	7.7	101		96		20.0	19.7	19.6	.01/.14	—	
100%	5	5	5	6.7	6.1	6.6	7.9	7.7	7.7										199

Remarks: Average weight of fish: .099g

RESULTS AND DISCUSSION

This particular sample of effluent from Alpha Chemical Corporation was not acutely toxic to the organisms tested. The mortality observed in the Simocephalus serrulatus bioassay (Table 3) was random, and therefore was not attributed to toxicity of the sample.

The DER Chemistry Section has chemically characterized a sample of effluent from Alpha Chemical Corporation. The results of these analyses are presented in Table 5. Notable was the identification of di-n-butylphthalate (7.2 µg/l), a compound used as an insect repellent. Although this concentration is below the range reported to be acutely toxic to aquatic organisms, tests have shown that less than this amount can chronically affect (decreased fecundity) Daphnia pulex (U.S. EPA, 1976). No metals were found in significant amounts in this sample.

Table 5. Results of Chemical Analyses of Effluent from Alpha Chemical Corporation, NPDES #FL0029653 collected on 11 May 1981.

Organics

Acid Extractables	None Detected	
Base Neutral Extractables	Di-n-butylphthalate	7.2 µg/l
Pesticides	None Detected	

Metals

Antimony	< 200 µg/l
Arsenic	< 50 µg/l
Beryllium	< 10 µg/l
Cadmium	< 10 µg/l
Chromium	< 50 µg/l
Copper	< 25 µg/l
Lead	< 50 µg/l
Mercury	<0.2 µg/l
Nickel	< 50 µg/l
Selenium	< 10 µg/l
Silver	< 15 µg/l
Thallium	< 100 µg/l
Zinc	< 25 µg/l

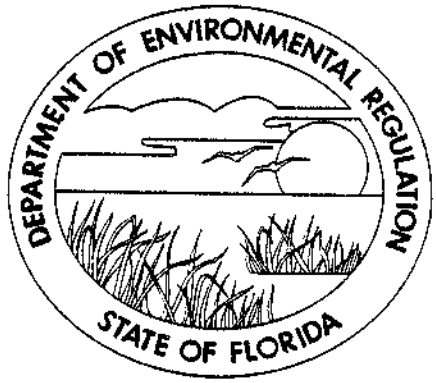
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