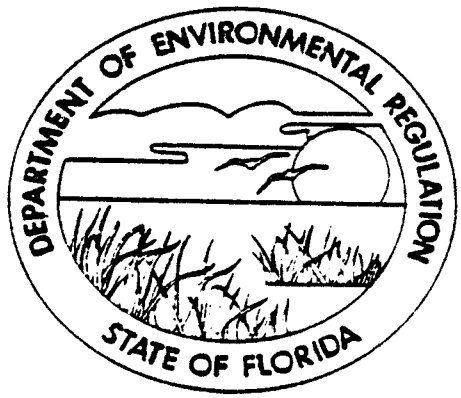


BIOASSAYS OF CHLORIDE INC., METALS DIVISION

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES DRAFT #FL0037923

SAMPLED 7/2/84

Biology Section
Bureau of Laboratories
September 26, 1984

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EXECUTIVE SUMMARY

Chloride Metals, Tampa, NPDES #FL0037923, on 3 to 5 July, 1984.

Wastewater sampled from Chloride Metals' Outfall #001 was acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} of 16.5%. Additional screening tests of this wastewater, using Notropis leedsi and Mysidopsis bahia, resulted in the death of all organisms within minutes of being introduced to the wastewater. This extreme toxicity was due mostly to the high pH (12.5 units) of the sample. However, the sample's high conductivity may also have contributed to the deaths of the freshwater test organisms, and the sample's low salinity may have contributed to the death of the saltwater test organism. Bioassays were also performed on a subsample of the wastewater in which the pH was lowered to a more habitable range (8.2 to 8.5 units). The D. pulex bioassay resulted in a 48 hour LC_{50} of 18.5%, almost identical to the high pH bioassay. This suggests that the high pH of the wastewater was not solely responsible for the observed toxicity. The N. leedsi bioassay resulted in a 48 hour LC_{50} of 18.9%, also suggesting that the high pH in the first tests was not the sole cause of toxicity. However, the high conductivity, again, may have contributed to this toxicity. An additional screening test of this pH adjusted wastewater, using M. bahia, resulted in the death of all organisms within minutes. The low salinity measured in this test may have contributed to this toxicity. In a similar M. bahia screening test, but with the addition of artificial sea salts, only 2 out of 10 shrimp died in 48 hours.

Chemical analyses for certain metals indicated the presence of significant concentrations of antimony (430 ug/l), arsenic (64.6 ug/l), cadmium (12.6 ug/l), lead (5500 ug/l), selenium (11 ug/l), silver (2.4 ug/l), and zinc (15 ug/l). The presence of these metals most likely contributed to the toxicity noted in these tests.

INTRODUCTION

Chloride Metals, Inc., is a secondary lead smelting operation located off U.S. Highway 41, in Tampa, Hillsborough County, Florida (Fig. 1). Lead is recovered from lead-acid storage batteries at a production rate of 48 tons per day (Chloride Inc., 1979).

The wastewater generated at this facility is currently retained on site in a series of percolation/evaporation ponds encompassing an area of 10,000 square feet (Chloride Inc., 1979). Potentially toxic components in the wastewater may include: sulfuric acid, arsenic, cadmium, chromium, copper, nickel, lead and antimony (U.S. EPA, 1980).

According to Chloride Inc. (1979), process wastewater will be treated by: clarification of acid insoluble material, neutralization of acid with sodium hydroxide (in a duplex system with pH recorder/controller), clarification of alkaline insoluble material, decanting, and filtration with a diatomaceous earth leaf filter plant. Waste sludge is recycled to the furnace feed with dry cake disposal to a landfill. The system is designed for 0.015 MGD wastewater and will have an average flow of 0.012 MGD. This treatment system should have been in complete routine operation by 15 March 1981 (FDER T.O.P. #29-34302 issued 24 October 1980).

Past site investigations by personnel from the Hillsborough County Environmental Protection Commission, DER's Southwest District Office and the U.S. EPA Surveillance and Analysis Division revealed that wastewater and leachate from Chloride Inc. was entering waters of the State through a series of drainage ditches (U.S. EPA, 1980). Leachate and wastewater contamination of surface waters may originate from three general sources:

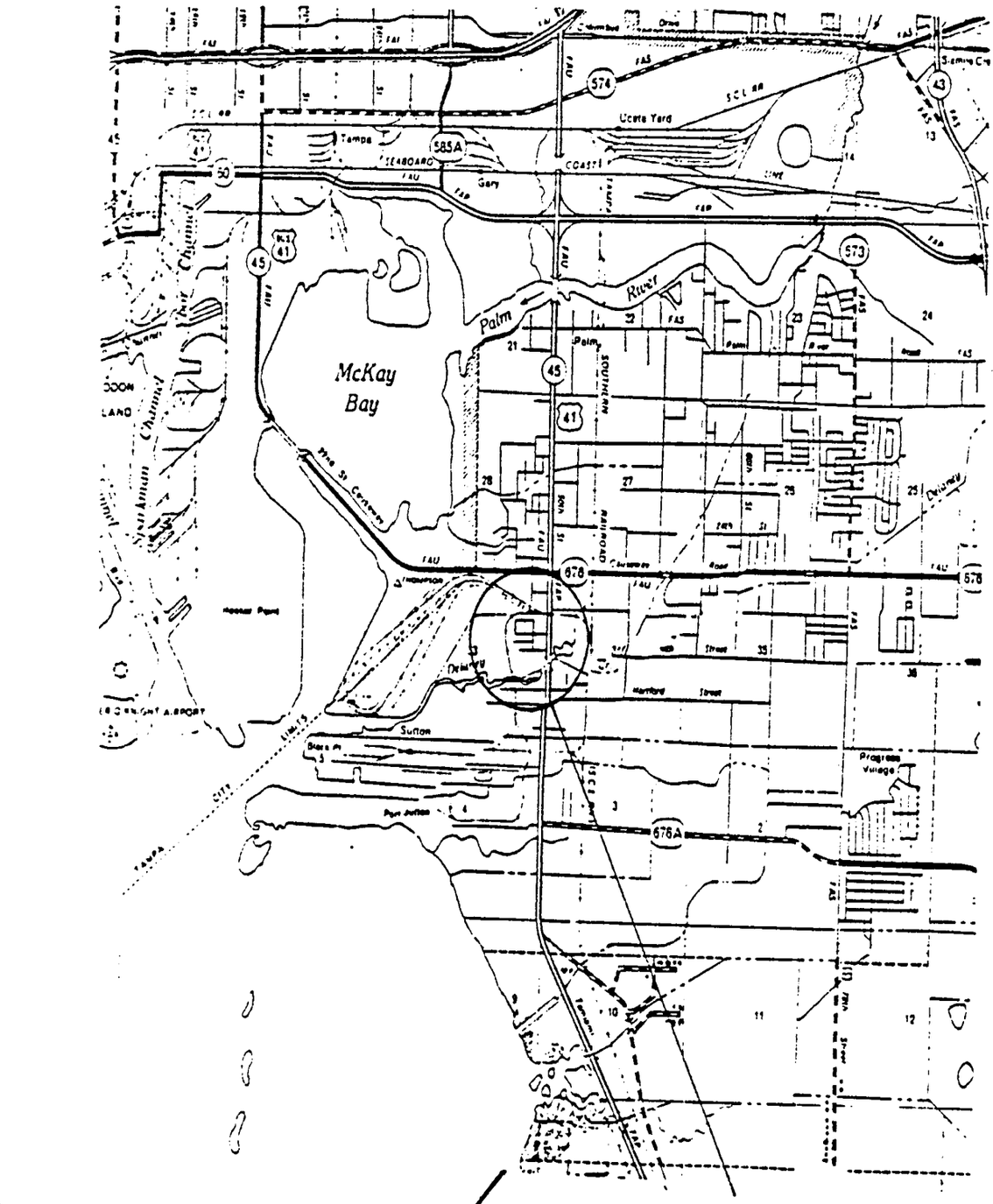
1. Contaminated storm water runoff entering drainage ditches around the battery slicing operation.

2. Leachate from the battery case disposal sites.

3. Leachate and direct wastewater discharge from the wastewater percolation evaporation pond.

The Biology Section had previously evaluated the existence and extent of the contaminants from this facility by obtaining water samples and performing static acute toxicity bioassays in November, 1980 and October, 1982. The samples were collected from a drainage ditch which received direct percolation/evaporation pond discharge as well as leachate runoff which surfaces and collects around the perimeter of this pond (Fig. 2). The wastewater was collected from a culvert as it left the Chloride Inc., property. The results of those tests indicated that the wastewater-leachate was acutely toxic (Table 1). Toxicity was attributed to the high concentrations of lead, cadmium, arsenic, and copper (FDER 1981, 1982).

To further evaluate the toxicity of wastewater from Chloride Metals, at the request of Mr. Sam Sahebzamani of DER's Tampa Office the Biology Section performed additional bioassays on a sample of actual discharge from the evaporation pond, Outfall #001, on 3 to 5 July, 1984. The results of these latest tests are presented in this report. Those involved in performing these tests and in preparation of this report were: C. W. Dye, M. Faircloth, R. B. Frydenborg, and M. L. Roll.



Location of Chloride Metals, Inc.

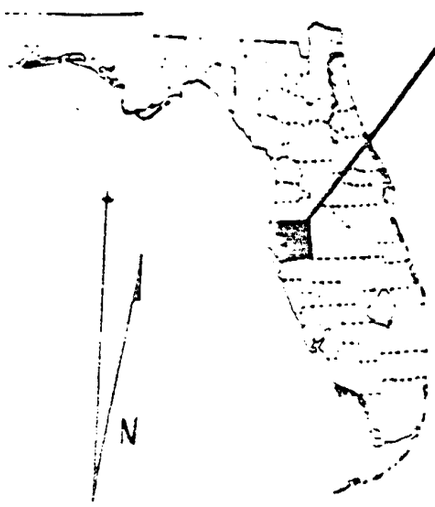


Figure 1. Portions of Hillsborough County including study area.

Table 1. Summary of bioassays performed on Chloride Metals, Inc., Tampa, Hillsborough County, Florida, from 1981 to 1984.

Date	48 hour LC_{50} or other effect	Organism	Comments
November 1980	49.5%	D. pulex	
November 1980	31.1%	S. serrulatus	
November 1980	52.6%	M. bahia	Salinity not adjusted (7 ppt)
November 1980	>100%	M. bahia	Salinity adjuste to 27 ppt
November 1980	a	P. promelas	
August 1982	a	H. formosa	
August 1982	52.5%	M. bahia	Salinity not adjusted (8.5 ppt)
July 1984	18.9%	N. leedsi	pH adjusted to 8.3
July 1984	16.5%	D. pulex	pH not adjusted (13.2)
July 1984	18.5%	D. pulex	pH adjusted to 8
July 1984	Complete mortality in 100% effluent	M. bahia	pH = 12.4 salinity = 7 ppt
July 1984	Complete mortality in 100% effluent	M. bahia	pH adjusted to 8.2; salinity = 4.9 ppt
July 1984	Partial mortality in 100% effluent	M. bahia	pH adjusted to 8.2; salinity adjusted to 13 ppt

[a] Due to an insufficient number of dead organisms, an LC_{50} could not be calculated.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include a species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). 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° plus or minus 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° plus or minus 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 fish used in this test were <3 weeks old at the time of the test.

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 a 16:8 hour light-dark cycle 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 and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° plus or minus 3°C. Adult or large juvenile mysids were used in this 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

Four separate 48 hour static acute toxicity bioassays were performed on two samples of wastewater from the Chloride Metals, Inc. percolation/evaporation pond outfall #001. The tests were performed from 1020 hours on 3 July to 1020 hours on 5 July, 1984. The samples were collected by Mr. Tim Neldner of DER's Southwest District Office at 1215 hours on 2 July, 1984 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 the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Since contaminants from Chloride Metals can be expected to impact organisms from both fresh and saline waters, tests were performed on a saltwater shrimp, Mysidopsis bahia and on two freshwater organisms, Notropis leedsi and Daphnia pulex. Due to an unusually high pH of the effluent (12.5), certain pH adjustments (lowering pH with 2N HCI) were instituted to differentiate between the toxicity resulting from high pH and the toxicity associated with some other component of the effluent.

Freshwater for dilution of the effluent samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is shown in Table 2.

Two separate bioassays were performed on D. pulex. One test consisted of effluents with no pH adjustment. The other D. pulex test was conducted on effluent that had been adjusted from the original pH of 12.5 to 8.5. Two replicates, each with three concentrations 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 not fed during these tests.

An N. leedsi bioassay was also conducted with the same pH adjusted effluent used for the D. pulex test. Two replicates, of three concentrations 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 these tests. Additionally, N. leedsi were exposed to two replicates of 100% non-pH adjusted effluent.

For M. bahia, a series of screening bioassays consisting of 100% effluent, 100% pH adjusted effluent, 100% pH and salinity effluent, and a sea salt control were conducted.

1. 100% Effluent Test - No adjustments or modifications were performed on the 100% effluent screening test. (pH = 12.4, salinity = 7.0 ppt).

2. 100% pH adjusted effluent - For the 100% pH adjusted effluent test, 2N HCI was added a drop at a time until a pH of 8.2 was achieved. This test ensured that any observed toxicity was a result of some factor

other than the exceedingly high pH of the effluent. However, after pH adjustment, the salinity of this test decreased to 4.9 ppt, slightly below the recommended M. bahia salinity range of 6235 ppt. However, M. bahia has been observed to survive for 72 hours at salinities of 2 to 3 ppt (U.S. EPA 1978), therefore, the results of this test are probably reliable, but some degree of uncertainty does exist.

3. 100% pH and salinity adjusted test - Even though the salinity of the effluent registered at 7 ppt, this value may have been caused by the high conductivity of the wastewater (9900 umhos) rather than reflecting a true salinity measurement. Therefore, artificial sea salts were added to achieve a 7 ppt increase in salinity from ambient, to ensure that the minimum salinity requirements of M. bahia were met. The pH in this salinity adjusted test was also lowered to 8.2.

4. Sea Salts Control - Well water, adjusted with artificial sea salts to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassays.

Two replicates, of each effluent and adjusted effluent screening test, and of the controls, were used to test with Mysidopsis bahia. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10 ppt and 15 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. 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 (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 freshwater tests for the controls and the 100% effluent concentration. The specific conductance or salinity (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 3 through 5.

Table 2. 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
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
100.0%	------	500 ml	500 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	-------	500 ml
10%	450 ml	50 ml	500 ml
50%	250 ml	250 ml	500 ml
100%	------	500 ml	500 ml

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Chloride Metals, Inc., Tampa, on 3 to 5 July, 1984.

pH unadjusted and adjusted tests

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
pH Unadjusted Test																				
Control	10	10	9	8.6	8.2	8.9	8.0	8.2	8.4	88		118		20.9	21.0	19.2	0/0	—		
Control	10	10	10	8.6	8.2	8.9	8.0	8.2	8.4										198	193
10%	10	9	9	8.4	8.2	8.9	10.4	9.5	9.2					20.9	21.0	19.3				
10%	10	8	7	8.4	8.2	8.9	10.4	9.5	9.2										850	820
50%	10	0	0	8.0	8.3	9.0	12.1	11.0	10.6					20.5	20.7	19.2				
50%	10	0	0	8.0	8.3	9.0	12.1	11.0	10.6										4350	3310
100%	10	0	0	7.5	8.3	9.0	12.4	11.7	10.9	1220		45		20.3	21.0	19.4	0/0	—		
100%	10	0	0	7.5	8.3	9.0	12.5	11.7	11.0										9000	6900
pH Adjusted Test																				
10%	10	10	9	8.3	8.1	8.7	8.2	8.2	8.2					21.0	20.6	19.1				
10%	10	9	7	8.3	8.1	8.7	8.2	8.2	8.2										1000	990
50%	10	1	1	8.1	8.3	8.2	8.5	8.5	8.3					21.0	20.5	19.0				
50%	10	2	1	8.1	8.3	8.5	8.5	8.5	8.3										3700	3530
100%	10	0	0	7.8	8.4	8.6	8.5	8.5	8.4					21.1	20.7	19.1	0/0	—		
100%	10	0	0	7.8	8.4	8.8	8.5	8.4	8.4										7000	7100

Remarks:

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Chloride Metals, Inc., Tampa, on 3 to 5 July, 1984.

pH adjusted test and screening test of unadjusted effluent

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alakalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/l)		Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	8.5	8.1	8.4	7.7	8.0	8.0	88	—	118	—	21.1	20.6	19.0	0/0	—		
Control	5	5	5	8.5	8.1	8.4	7.7	8.0	8.0										210	310
10% pH Adjusted	5	5	4	8.4	8.2	8.5	7.9	8.1	8.2					21.2	20.4	19.0				
10% pH Adjusted	5	5	5	8.4	8.2	8.5	7.9	8.1	8.2										1000	1030
50% pH Adjusted	5	5	0	8.2	8.2	7.8	8.1	8.2	8.1					21.2	20.5	19.1				
50% pH Adjusted	5	4	0	8.2	8.2	7.7	8.1	8.2	8.1										3750	3710
100% pH Adjusted	5	1	0	7.7	8.2	7.5	8.3	8.3	8.2					21.3	20.5	19.3				
100% pH Adjusted	5	3	1	7.7	8.2	7.5	8.3	8.3	8.2										7100	7100
100% Effluent	5	0	0	7.5	8.3	9.0	12.4	12.1	11.5	1220	—	45	—	19.9	20.7	19.4	0/0	—		
100% Effluent	5	0	0	7.5	8.3	9.0	12.4	12.1	11.5										9000	6900

Remarks:

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity screening bioassay of Chloride Metals, Inc., Tampa on 3 to 5 July, 1984.

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Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Sea Salts Control	5	5	5	8.4	7.7	6.3	8.4	8.3	7.9	21.5	21.0	19.2				
Sea Salts Control	5	5	5	8.4	7.7	6.3	8.4	8.3	7.9						6.7	6.8
Salinity and pH Adjusted Effluent	5	4	3	7.6	7.3	5.7	8.2	8.1	7.8	20.8	20.4	19.0				
Salinity and pH Adjusted Effluent	5	5	5	7.6	7.3	5.7	8.2	8.1	7.8						13.0	12.9
pH Adjusted Effluent	5	0	0	7.5	7.9	6.4	8.2	8.3	8.2	21.6	20.4	18.9				
pH Adjusted Effluent	5	0	0	7.5	7.9	6.7	8.2	8.2	8.1						4.9	4.9
100% Unadjusted Effluent	5	0	0	7.1	7.8	8.5	12.4	11.8	11.2	19.9	20.6	19.0				
100% Unadjusted Effluent	5	0	0	7.1	7.8	8.5	12.3	11.7	11.1						7.0	6.0

Remarks:

Table 6. LC_{50} Data for Bioassays of Chloride Metals, Inc., Tampa, on 3 to 5 July, 1984. NPDES #FL0037923.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	17.6% (10 and 50)	16.5% (10 and 50),d
	Moving Average	a	a
	Probit	a	a
D. pulex pH adjusted Effluent	Binomial	25.7% (10 and 50)	19.4% (10 and 50)
	Moving Average	25.9% (18.8 and 33.6)	19.4% (13.0 and 27.4)
	Probit	26.3% (18.1 and 35.7)	18.5% (12.5 and 26.2),d
N. leedsi pH Adjusted Effluent	Binomial	88.1% (50 and∞),b	18.9% (10 and 50),d
	Moving Average	88.1% (65.1 and 371.6),b	a
	Probit	89.2% (63.9 and 261.6),b	22.4% (0 and∞),c

[a] Due to the 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.

[d] This value is reported in the text since it is the most statistically conservative value of the data set.

Table 7. Results of Metal Analyses Performed on Wastewater from Chloride Metals, Collected 2 July, 1984.

Antimony	430 ug/l
Arsenic	64.6 ug/l
Beryllium	N.D.
Cadmium	12.6 ug/l
Chromium	N.D.
Copper	<25 ug/l[a]
Lead	5500 ug/l
Mercury	N.D.
Nickel	N.D.
Selenium	11 ug/l
Silver	2.4 ug/l
Thallium	N.D.
Zinc	15 ug/l

N.D. = None Detected
[a] = Actual value is known to be less than value given

RESULTS AND DISCUSSION

Wastewater sampled at Chloride Metals percolation/evaporation pond Outfall #001 was acutely toxic to all organisms tested. The 48 hour LC_{50}'s for non-pH adjusted effluent (pH approximately 12.5) and for pH adjusted effluent (pH approximately 8.0) were 16.5% and 18.5% respectively, using Daphnia pulex as the test organism (Table 6). This suggests that the unusually high pH of this sample was not solely responsible for the observed toxicity. The 48 hour LC_{50} for pH adjusted effluent for Notropis leedsi was 18.9%. This result is very similar to that of the water flea, indicating that this wastewater was equally toxic to both the freshwater vertebrate and invertebrate species tested. Unadjusted effluent produced death in all N. leedsi exposed to it within seconds.

A series of screening tests conducted on Mysidopsis bahia produced the following results:

100% effluent test - 100% mortality within seconds;
(pH = 12.4, salinity = 7.0)
100% pH adjusted effluent test - 100% mortality within seconds;
(pH = 8.2, salinity = 4.9)
100% pH and salinity adjusted effluent test - 20% mortality after 48 hours
(pH = 8.2, salinity = 13.0)

These M. bahia results suggest that the addition of artificial sea salts as well as the lowering of the pH had a partially mitigating effect on the wastewater's toxicity. Even though the salinity of the effluent registered at 7 ppt, this value was probably the result of the high conductivity of the wastewater (9900 umhos), rather than a true salinity. The addition of sea salts ensured that the minimum salinity requirements of M. bahia were met, and therefore, less toxicity was observed.

The results of metal analyses (Table 7), performed by DER's Chemistry Section, indicated the presence of antimony (430 ug/l), arsenic (64.6 ug/l), cadmium (12.6 ug/l), lead (5500 ug/l), selenium (11 ug/l), silver (2.4 ug/l), and zinc (15 ug/l). The presence of these metals, particularly the cadmium, silver and the large concentration of lead, most likely contributed to the toxicity noted in these tests.

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 language 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 actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existance of a mixing zone, whether as a specific part of the permit or based on the existance 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 organisms, 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.

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