BIOASSAYS OF AN ALUM DISCHARGE

FROM THE CITY OF TAMPA'S WATER TREATMENT PLANT

HILLSBOROUGH COUNTY, FLORIDA

NO NPDES PERMIT

SAMPLED 4/9/84

Biological Section
Bureau of Water Analysis
June 11, 1984

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EXECUTIVE SUMMARY

Tampa's Alum Discharge on 10 to 12 April 1984

A de-chlorinated sample of Alum supernatant from the City of Tampa's water treatment plant was not toxic at 24 hours to Mysidopsis bahia (saltwater test), or to Notropis leedsi and Daphnia pulex (freshwater tests). However, at 48 hours sufficient mortality of M. bahia and D. pulex was noted and LC_{50}'s of 79.6% and 314.2%, respectively, were calculated. No N. leedsi died during the 48 hour static tests. Results of metal analysis for aluminum and iron indicated the presence of these metals at 1990 ug/l and 551 ug/l respectively. The toxicity of this sample may be partially due to the high concentrations of aluminum.

INTRODUCTION

The city of Tampa's Alum Discharge consists of chlorinated alum supernatant from their water treatment plant. Besides chlorine, principal components of this supernatant are aluminum and iron (Mr. Don Moores, personal communication). This Alum supernatant is routinely discharged to the Hillsborough River just below the salinity barrier.

To evaluate the toxicity of this discharge to the biota of the receiving waters, the Southwest District office requested acute toxicity bioassays be performed. The Biology Section therefore performed one saltwater test using Mysidopsis bahia, and two freshwater screening tests using Daphnia pulex and Notropis leedsi as test organisms, on 10 to 12 April, 1984. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: P. D. Brooks, C. W. Dye, R. B. Frydenborg, and M. L. Roll.

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 three 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 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

One 48 hour saltwater definitive bioassay and two 48 hour freshwater screening bioassays were performed on a sample of Tampa's Alum Discharge. The tests were performed from 1020 hours on 10 April to 1020 hours on 12 April, 1984. The sample was collected by Ms. Phyllis Giwer, of DER's Southwest District at 1050 hours on 9 April, 1984, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. The sample for the bioassays was unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

To differentiate between the toxicity resulting from chlorine (0.2 mg/l) and the toxicity associated with some other component of the sample, the Biology Section decided to de-chlorinate the sample. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. The total residual chlorine concentration after de-chlorination was <0.04 mg/l (HACH), well below the lethal concentration.

Freshwater for dilution of the sample for the freshwater screening tests was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is presented in Table 1.

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 this test.

Two replicates, of three concentrations and a control, were used for the Notropis leedsi (bannerfin shiner) screening test. 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.

Saltwater for dilution of the sample for the Mysidopsis bahia definitive bioassay was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 23.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the sample, achieving a range of final salinities (10.5 ppt to 20.2 ppt). For the 100% effluent test concentration, freshly autoclaved artificial sea salts were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 4) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included:

1) Diluent Control - Turkey Point water (23 ppt). This represents the highest concentration of Turkey Point water used in the bioassay, and tests for toxicity in the diluent. This is also the highest salinity

used in the bioassay, and therefore tests the tolerance of the organisms to this salinity.

2) Sea Salts Control - Well water, adjusted with artificial sea salts to approximately 7 ppt salinity. This tests for toxicity of the artificial sea salts used in the bioassay, as well as for tolerance of the organisms to the lowest salinity used in the bioassay.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, and 20 ppt salinity aquaria to culture dishes for observation and selection of individuals for the test. 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 test for the controls and the 100% 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. These values were recorded on the bioassay data forms and are presented in Tables 2, 3 and 4.

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

Daphnia pulex (water flea) screening test

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

Notropis leedsi (bannerfin shiner) screening test

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

Mysidopsis bahia (mysid shrimp) definitive test

Concentration	Dilution Water	Effluent	Total Volume
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 screening test of Tampa Alum Discharge (de-chlorinated[a]), Hillsborough County, Florida, on 10 to 12 April, 1984.

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	10	10	10	8.5	8.6	9.0	7.8	8.2	8.2	88		133		20.9	20.8	18.6				
Control	10	10	10	8.5	8.6	9.0	7.8	8.2	8.2										172	187
10%	10	10	10	8.6	8.7	9.0	7.9	8.2	8.2					20.9	20.6	18.5				
10%	10	10	9	8.6	8.7	9.0	7.9	8.2	8.2										182	195
50%	10	10	10	8.7	8.8	9.1	8.4	8.2	8.1					21.2	20.7	18.6			212	
50%	10	10	10	8.7	8.7	9.1	8.4	8.2	8.1										215	230
100%	10	10	5	8.8	8.7	9.1	9.1	8.2	8.0					21.7	20.5	19.1			248	
100%	10	10	8	8.8	8.7	9.1	9.1	8.2	8.0	32		140							241	255

Remarks: [a]Total Residual Chlorine: <0.04 mg/l (HACH).

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute screening test of Tampa Alum Discharge (de-chlorinated[a]), Hillsborough County, Florida, on 10 to 12 April, 1984.

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	8.2	8.5	8.6	7.8	8.0	8.0	88		133		20.7	20.6	18.3				
Control	6[b]	6	6	8.2	8.5	8.6	7.8	8.0	8.0										179	192
10%	5	5	5	8.4	8.5	8.7	7.9	8.0	8.1					20.7	20.5	17.9				
10%	5	5	5	8.4	8.5	8.7	7.9	8.0	8.1										189	195
50%	5	5	5	8.5	8.5	8.5	8.4	8.1	8.0					21.0	20.3	17.8			340	
50%	5	5	5	8.5	8.5	8.5	8.4	8.1	8.0										340	335
100%	5	5	5	8.6	8.5	8.5	9.1	8.2	8.0					21.8	20.3	18.0			380	
100%	6[b]	6	6	8.6	8.5	8.5	9.1	8.2	8.0	32		140							298	298

Remarks: a Total residual chlorine: <0.04 mg/1 (HACH).

b Extra organism loaded by mistake, allowed to remain.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Tampa Alum Discharge (de-chlorinated[a]), Hillsborough County, Florida, on 10 to 12 April, 1984.

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
Diluent Control	5	5	5	8.6	6.7	7.5	8.2	7.9	7.8	19.9	20.5	18.5				
Diluent Control	5	5	5	8.6	6.8	7.5	8.2	7.9	7.8						22.8	22.0
Sea Salt Control	5	5	5	8.8	7.5	8.0	8.2	8.1	8.1	20.2	20.5	18.4				
Sea Salt Control	5	5	5	8.8	7.5	8.0	8.2	8.1	8.1						7.8	7.5
10%	5	5	5	8.4	6.7	7.3	8.2	7.9	7.8	20.0	20.6	18.4				
10%	5	5	5	8.4	6.7	7.3	8.2	7.9	7.8						20.2	19.5
18%	5	5	5	8.5	6.3	7.3	8.3	7.9	7.8	20.0	20.6	18.2				
18%	5	5	5	8.5	6.2	7.3	8.3	7.9	7.8						19.0	18.2
32%	5	5	5	8.7	6.0	7.3	8.4	7.9	7.8	20.2	20.6	18.3				
32%	5	5	5	8.7	6.0	7.3	8.4	7.9	7.8						16.0	15.2
56%	5	5	5	8.8	6.9	7.5	8.6	7.9	7.8	20.6	20.6	18.4				
56%	5	5	5	8.8	6.9	7.5	8.6	7.9	7.8						10.5	10.0
100%	5	5	0	8.9	7.1	7.6	9.1	8.3	7.9	20.8	20.7	18.7				
100%	5	5	1	8.9	7.2	7.6	9.1	8.3	7.9						7.5	7.0

Remarks: [a] Total residual chlorine 0.04 mg/l (HACH).

Table 5. LC_{50} Data for Bioassays of Tampa Alum Discharge, on 10 to 12 April 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia	Binomial		79.6% (56 and 100)
	Moving Average	No Mortality	a
	Probit		a
Notropis leedsi	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
Daphnia pulex	Binomial	No Mortality	a
	Moving Average		a
	Probit		314.2% (0 and ∞)b

[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] 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.

Table 6. Results of Chemical Analyses performed on a sample of Tampa's Alum Discharge, Collected 9 April, 1984.

Metals	
Aluminum	1990 ug/l
Iron	550 ug/l

RESULTS

The static acute bioassay of Tampa's Alum Discharge using Mysidopsis bahia demonstrated the toxicity of the sample, yielding an 48 hour LC_{50} of 79.6% (Table 5). Two screening tests performed on the sample had varying results. While no mortality was noted in the Notropis leedsi test, sufficient mortality was noted in the Daphnia pulex test at 48 hours to calculate an LC_{50} of 314.2% (Table 5). The 96 hour LC_{50}'s for these organisms would be less than or equal to their 48 hour values.

Analyses for aluminum and iron by the DER Chemistry Section indicated the presence of 1990 ug/l and 550 ug/l, respectively (Table 6). This high aluminum concentration may have been responsible for a portion of the toxicity observed in this sample.

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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