BIOASSAYS OF W. R. GRACE, INC., AGRICULTURAL CHEMICALS

BARTOW, POLK COUNTY, FLORIDA

NPDES #FL0001589

SAMPLED 7/28/87

SEPTEMBER 30, 1987

Biology Section
Division of Environmental Programs

BIOASSAYS OF W. R. GRACE, INC., AGRICULTURAL CHEMICALS

BARTOW, POLK COUNTY, FLORIDA

NPDES #FL0001589

Biology Section
Bureau of Laboratories and
Special Programs
September 30, 1987

EXECUTIVE SUMMARY

W. R. Grace, Inc., Agricultural Chemicals, Bartow, Polk County, Florida, NPDES #FL0001589, 29 to 31 July, 1987.

Three static acute toxicity bioassays were performed on a sample of effluent collected from W. R. Grace, Inc., NPDES Outfall #003. This agricultural chemical manufacturer discharges into a clay settling lagoon which overflows to the Peace River. Results indicated no toxicity to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No residual chlorine was measured in this sample, and the concentrations of total and unionized ammonia were insignificant (0.1 mg/l and 0.001 mg/l, respectively). Because the sample was not toxic, chemical analyses were not performed.

INTRODUCTION

W. R. Grace, Inc. is a manufacturer of fertilizers and other agricultural chemicals using mined phosphate rock as the chief raw material. NPDES Outfall #003 receives only stormwater runoff from several inactive mined and reclaimed areas. The water collects in an inactive clay settling pond of approximately 400 acres in size. The Bonny Lake Mine has been closed for nearly four years now. This 400 acre pond is under negotiation for sale to the International Minerals Corporation for use as a clay settling system for one of their nearby mines. The discharge ultimately flows to the Peace River.

To determine the extent and existence of toxicity the Southwest District requested bioassays to be performed. Bioassays were performed on samples collected from this facility in July, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, and M. L. Roll.



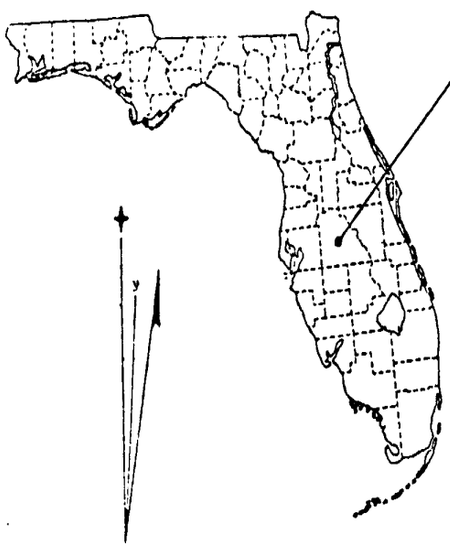


Figure 1. Portion of Polk County, Florida, including approximate location of NPDES Outfall #003 of the W. R. Grace Corporation Bonny Lake Mine.

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 species recommended by Peltier and Weber (1985) 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 June, 1987. 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.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained 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®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 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 26 days old at the time of the tests.

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

Three static acute toxicity bioassays were performed on a sample of effluent collected from Outfall #003 of W. R. Grace, Inc. The tests were performed from 1000 hours on 29 July to 1000 on 31 July, 1987.

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 collected by Mr. David Killingsworth of DER's Southwest District Office at 1040 hours on 28 July, 1987, using standard wastewater sampling procedures. 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 sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° ± 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.

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 is given in Table 1.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the appropriate test concentration was placed in each chamber. Ceriodaphnia

were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia 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) 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 Table 2.

RESULTS AND DISCUSSION

The sample of wastewater collected from W. R. Grace, Inc. was not toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No mortality of D. pulex or N. leedsi was noted during the 48 hour bioassays, and only one individual died in the C. dubia test (Table 2). No residual chlorine was measured in this sample, and the concentrations of total and unionized ammonia were insignificant (0.1 mg/l and 0.001 mg/l, respectively). Because the sample was not toxic, chemical analyses were not performed.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi, static acute toxicity bioassays of W. R. Grace, Inc., NPDES #FL0001589, 29 to 31 July, 1987.

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
Ceriodaphnia dubia														
Control	10	10	10	8.8	8.4	8.4	8.1	8.0	7.9	21.4	21.1	20.8		
Control	10	10	10	8.8	8.4	8.4	8.1	8.0	7.9					
50%	10	10	10	8.6	8.5	8.5	7.7	7.9	7.8	21.4	21.0	20.7		
50%	10	10	9	8.6	8.4	8.5	7.7	7.9	7.8					
100%	10	10	10	8.3	8.5	8.6	7.1	7.7	7.6	21.6	21.0	20.7		
100%	10	10	10	8.3	8.5	8.6	7.1	7.7	7.6					
Daphnia pulex														
Control	10	10	10	8.7	8.3	8.4	8.1	8.0	7.9	21.9	21.0	20.7		
Control	10	10	10	8.7	8.3	8.4	8.1	8.0	7.9				200	210
50%	10	10	10	8.5	8.3	8.5	7.7	7.9	7.7	22.0	20.8	20.5		
50%	10	10	10	8.5	8.4	8.5	7.7	7.9	7.7				295	290
100%	10	10	10	8.3	8.4	8.5	7.1	7.6	7.6	22.2	20.8	20.5		
100%	10	10	10	8.3	8.4	8.5	7.1	7.6	7.6				395	385
Notropis leedsi														
Control	5	5	5	8.5	7.4	7.7	7.7	7.7	7.7	22.2	21.1	20.7		
Control	5	5	5	8.5	7.5	7.7	7.7	7.7	7.7				210	215
50%	5	5	5	8.4	7.5	7.7	7.4	7.5	7.5	22.4	21.0	20.6		
50%	5	5	5	8.4	7.5	7.7	7.4	7.5	7.5				305	305
100%	5	5	5	8.3	7.5	7.5	7.1	7.3	7.3	22.4	20.9	20.5		
100%	5	5	5	8.3	7.5	7.5	7.1	7.3	7.3				400	395

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 110 (mg/l $CaCO_3$)

Total Hardness 119 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 0.001

Total ammonia (as N)(mg/l) 0.1

Total Alkalinity 47 (mg/l $CaCO_3$)

Total Hardness 166 (mg/l $CaCO_3$)

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1981. Bioassays of W. R. Grace, Inc., Agricultural Chemicals, Bartow, Polk County, Florida. NPDES #FL0001589. 9 p.

_____, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

Facility/Toxicant W.R. Grace — Test # 1,2,3 — Sample Collected: Date 7/28/87 Time 1040

Address St. Rd. 60 — Type Screen — Test Beginning: Date 7/29/87 Time 1000

NPDES Number FL0001571 — Organism C. dubia D. puley N. leedsi — Test Ending: Date 31 Jul 87 Time 1000

Outfall Number 003

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/l) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Conductivity 0	48	Ammonia Total 0	48	Ammonia Unionized 0	48
Control	—	10	10	10	8.6	8.4	8.4	8.1	8.0	7.9	21.4	21.1	20.8			0		0	
Control	83	10	10	10	8.6	8.4	8.4	8.1	8.0	7.9									
50%	36	10	10	10	8.6	8.5	8.5	7.7	7.9	7.8	21.4	21.0	20.7						
50%	4	10	10	9	8.6	8.4	8.5	7.7	7.9	7.8									
100%	75	10	10	10	8.3	8.5	8.6	7.1	7.7	7.6	21.6	21.0	20.7			.1		.001	
100%	45	10	10	10	8.3	8.5	8.6	7.1	7.7	7.6									
Control	64	10	10	10	8.7	8.3	8.4	8.1	8.0	7.9	21.9	21.0	20.7			0		0	
Control	8	10	10	10	8.7	8.3	8.4	8.1	8.0	7.9				200	210				
50%	78	10	10	10	8.5	8.3	8.5	7.7	7.9	7.7	22.0	20.8	20.5						
50%	55	10	10	10	8.5	8.4	8.5	7.7	7.9	7.7				295	310				
100%	151	10	10	10	8.3	8.4	8.5	7.1	7.6	7.6	22.2	20.8	20.5			.1		.001	
100%	27	10	10	10	8.3	8.4	8.5	7.1	7.6	7.6				395	385				
Control	31	5	5	5	8.5	7.4	7.7	7.7	7.7	7.7	22.2	21.1	20.7			0		0	
Control	30	5	5	5	8.5	7.5	7.7	7.7	7.7	7.7				210	215				
50%	2	5	5	5	8.4	7.5	7.7	7.4	7.5	7.5	22.4	21.0	20.6						
50%	56	5	5	5	8.4	7.5	7.7	7.4	7.5	7.5				305	305				
100%	62	5	5	5	8.3	7.5	7.5	7.1	7.3	7.3	22.4	20.9	20.9			.1		.001	
100%	46	5	5	5	8.3	7.5	7.5	7.1	7.3	7.3				400	375				
		MLR	JMF	MLR	MLR	JMF	MLR	MLR	JMF	MLR	MLR	JMF	MLR	MLR	RBF	MLR		KML	

C. dubia — D. puley — N. leedsi

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 110

Total Hardness (mg/l $CaCO_3$) 119

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 47

Total Hardness (mg/l $CaCO_3$) 166 KML

Age of Test Organisms Nothopis 26 days

Weight ——

Instrument Calibrations:

	pH Meter:	D.O. Meter:	S-C-T Meter:
0 Hours	7 at 7; 9 at 9 MLR	8.9 @ 21.5°C	10.5 at 10:27 at 30
24 Hours	7 at 7; 9 at 9	8.4 @ 22°C	10.2 at 10.26 at 30
48 Hours	7 at 7; 9 at 9	8.4 @ 21.5°C	

Cl_2: N.D. HACH KML

Reviewed by: [signature]

Date: 9/29/87