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

BARTOW, POLK COUNTY, FLORIDA

NPDES #FL0001589

SAMPLED 7/14/81

Biological Section
Bureau of Water Analysis
August 11, 1981

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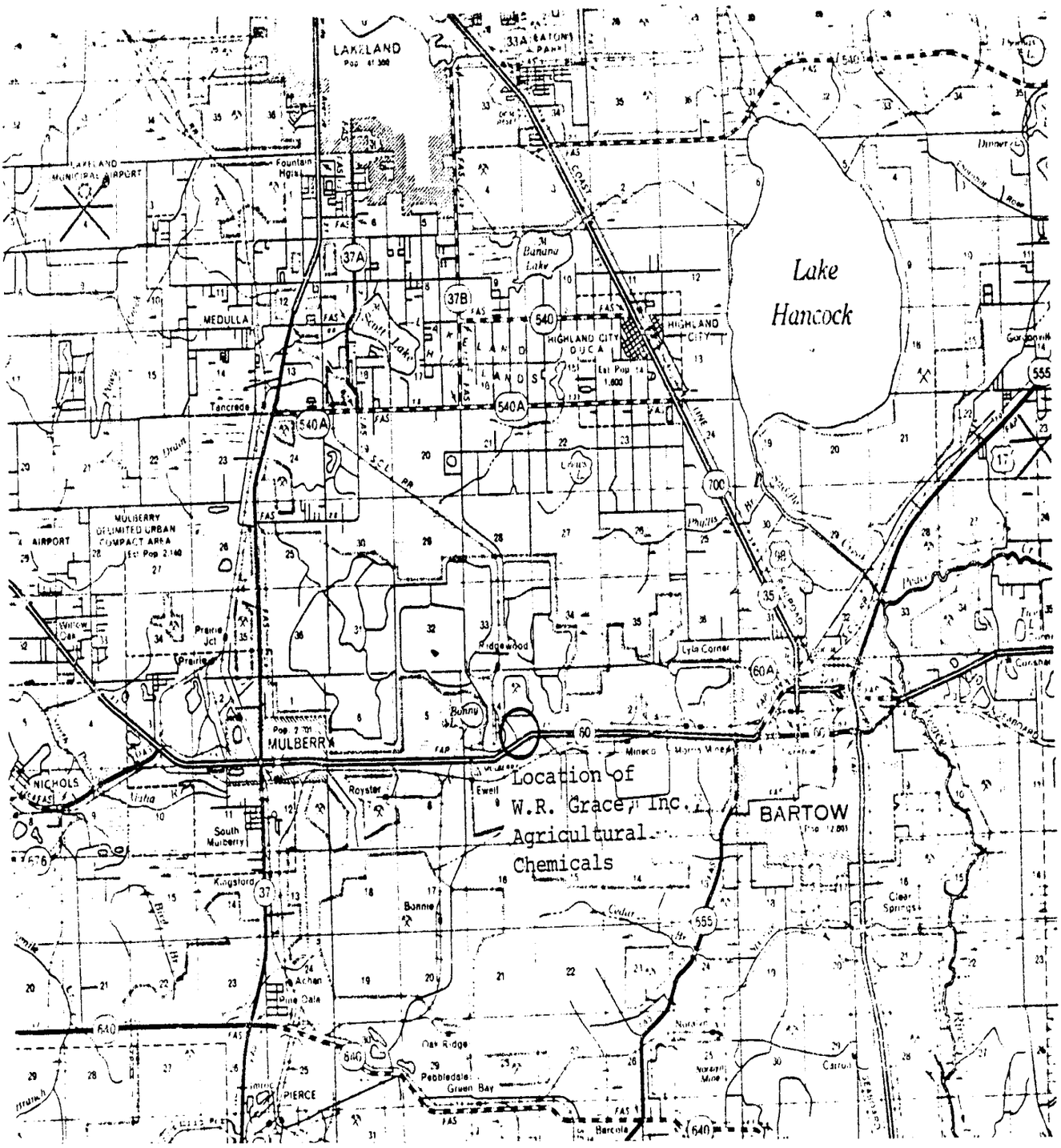
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INTRODUCTION

W. R. Grace, Inc. is a manufacturer of fertilizers and other agricultural chemicals using mined phosphate rock as the chief raw material. The plant is located north of State Road 60, between Bartow and Mulberry, Polk County, Florida (Fig. 1). There is no discharge of either process wastewater or of contact process cooling water. However, surface drainage and treated non-process wastewater is diverted to a 150 acre settling lagoon which overflows at Outfall #001 to Skinned Sappling Creek, a tributary of the Alafia River. The discharge averages 2.8 MGD.

According to the NPDES permit for this facility, the discharge may contain high levels of nutrients and other contaminants associated with phosphate rock mining. To evaluate the effects of this discharge on the biota of the receiving waters, the Biological Section performed static acute toxicity bioassays on a grab sample of effluent on 15 to 17 July, 1981. The results of these tests are presented in this report.



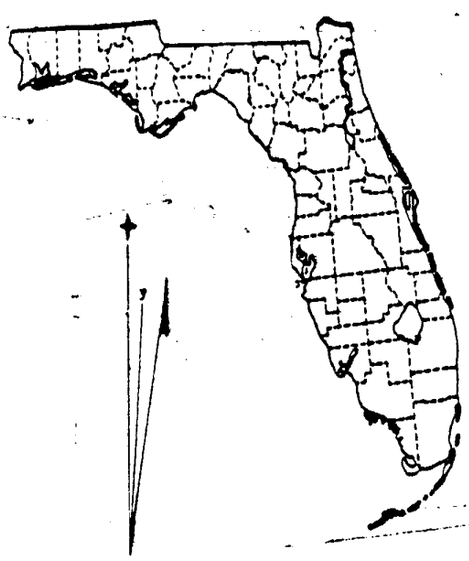


Figure 1. Portions of Polk County, Florida, including location of W. R. Grace, Inc.

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.

Heterandria formosa (least killifish) — Approximately 100 adult killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish have been maintained in 2½ to 5 gallon all glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The fish have been reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) twice daily. New born fish are initially maintained in the adult tanks, but are removed at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were one to ten days old.

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 0900 hours on 15 July, 1981, to 0900 hours on 17 July, 1981, on a grab sample of effluent from Outfall #001. The sample was collected by Mr. Matt McCann of DER's Southwest District at 0915 hours on 14 July, 1981, using standard wastewater sampling procedures. The unpreserved sample was 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 siphoned into large culture dishes through a stainless streel screen 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.

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 and end of the bioassays. Additionally, ammonia concentrations, including total (expressed in mg/l as N) and unionized (expressed in mg/l as NH_3) fractions, were recorded for the control water and the 100% effluent at the beginning of the test. Measurements of total ammonia were performed using an Orion specific probe. The unionized fraction was then calculated based on the pH, temperature and salinity. These values were recorded on the bioassay data forms and are presented in Tables 2 and 3. Organisms were not fed during these tests. Data recorded during the Daphnia pulex bioassay are not reported since the 24 and 48 hour tests had to be invalidated due to excessive (>20%) control mortality.

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

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

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

Heterandria formosa (least killifish)

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, *Simocephalus serrulatus*, static acute toxicity bioassay of W. R. Grace, Inc., Bartow, Polk County, Florida, NPDES #FL0001589, Outfall #001 on 15 to 17 July, 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[a]	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	7[b]	8.4	8.8	8.9	7.8	8.2	8.2	107		114		21.3	20.0	20.3	.01/.13	---		
Control	10	10	5[b]	8.4	8.8	8.9	7.8	8.2	8.2										200	211
5.6%	10	10	6	8.4	8.8	8.8	7.8	8.2	8.1					21.4	19.9	20.3				
5.6%	10	10	3[b]	8.4	8.8	8.8	7.8	8.2	8.1										226	228
10%	10	9	6	8.4	8.7	8.9	7.8	8.2	8.1					21.2	19.9	20.2				
10%	10	9	8	8.4	8.7	8.9	7.8	8.2	8.1										243	242
18%	10	10	8	8.4	8.7	8.9	7.7	8.1	8.1					21.0	19.8	20.2				
18%	10	10	10	8.4	8.7	8.9	7.7	8.1	8.1										285	272
32%	10	10	10	7.9	8.6	8.9	7.6	8.0	8.0					20.8	19.8	20.1				
32%	10	10	10	7.9	8.6	9.0	7.6	8.0	8.0										337	330
56%	10	10	10	7.2	8.5	8.9	7.4	7.9	8.0					20.5	19.8	20.2				
56%	10	8	8	7.2	8.5	8.7	7.4	7.9	8.0										428	430
100%	10	9	8	6.0	8.3	8.7	7.1	7.8	7.8	87		208		20.3	20.1	20.5	0/.39	---		
100%	10	10	9	5.9	8.3	8.9	7.1	7.8	7.8										640	550

Remarks:

[a] Test invalidated due to excessive control mortality.

[b] One body not found.

Table 3. Data recorded during 48 hour, Heterandria formosa, static acute toxicity bioassay of W. R. Grace, Inc., Bartow, Polk County, Florida, NPDES #FL0001589, Outfall #001 on 15 to 17 Juoy, 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	48
Control	5	5	5	8.5	8.3	8.5	7.7	8.1	8.1	107		114		21.4	19.6	19.8	.01/.13	---		
Control	5	5	5	8.5	8.3	8.6	7.7	8.1	8.1										200	195
5.6%	5	5	5	8.5	8.4	8.5	7.7	8.1	8.1					21.4	19.6	19.7				
5.6%	5	5	5	8.5	8.4	8.5	7.7	8.1	8.1										226	220
10%	5	5	5	8.4	8.4	8.6	7.7	8.0	8.1					21.4	19.5	19.6				
10%	5	5	5	8.4	8.4	8.6	7.7	8.0	8.1										243	235
18%	5	5	5	8.2	8.2	8.5	7.5	8.0	8.0					21.2	19.4	19.7				
18%	5	5	5	8.2	8.3	8.6	7.5	8.0	8.0										285	280
32%	5	5	5	7.9	8.3	8.7	7.5	7.9	8.0					21.0	19.4	19.7				
32%	5	5	5	7.9	8.3	7.7	7.5	7.9	8.0										337	330
56%	5	5	5	7.4	8.1	8.6	7.3	7.8	7.9					20.6	19.5	19.8				
56%	5	5	5	7.4	8.1	8.6	7.3	7.8	7.9										428	422
100%	5	5	5	5.9	7.9	8.6	7.0	7.7	7.8	87		208		20.4	19.7	19.9	0/.39	---		
100%	5	5	5	5.8	7.9	8.5	7.0	7.7	7.8										640	650

Remarks: Weight of each fish: 0.006 g (average of 10).

RESULTS AND DISCUSSION

This particular grab sample, collected from Outfall #001 of W. R. Grace, Inc., was not acutely toxic to the test organisms. Although a significant number of Simocephalus serrulatus died during the test (Table 2), the deaths occurred mostly in the lower test concentrations and therefore were not attributed to toxicity of the sample. Additionally, the 24 and 48 hour Daphnia tests (data not reported) and the 48 hour Simocephalus test were invalidated due to excessive control mortality. No Heterandria died during the test.

LITERATURE CITED

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