

BIOASSAYS OF THE DUBOSE OIL PRODUCTS

HAZARDOUS WASTE SAMPLE

ESCAMBIA COUNTY, FLORIDA

NO NPDES PERMIT

Biology Section
Bureau of Water Analysis
March 1, 1983

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EXECUTIVE SUMMARY

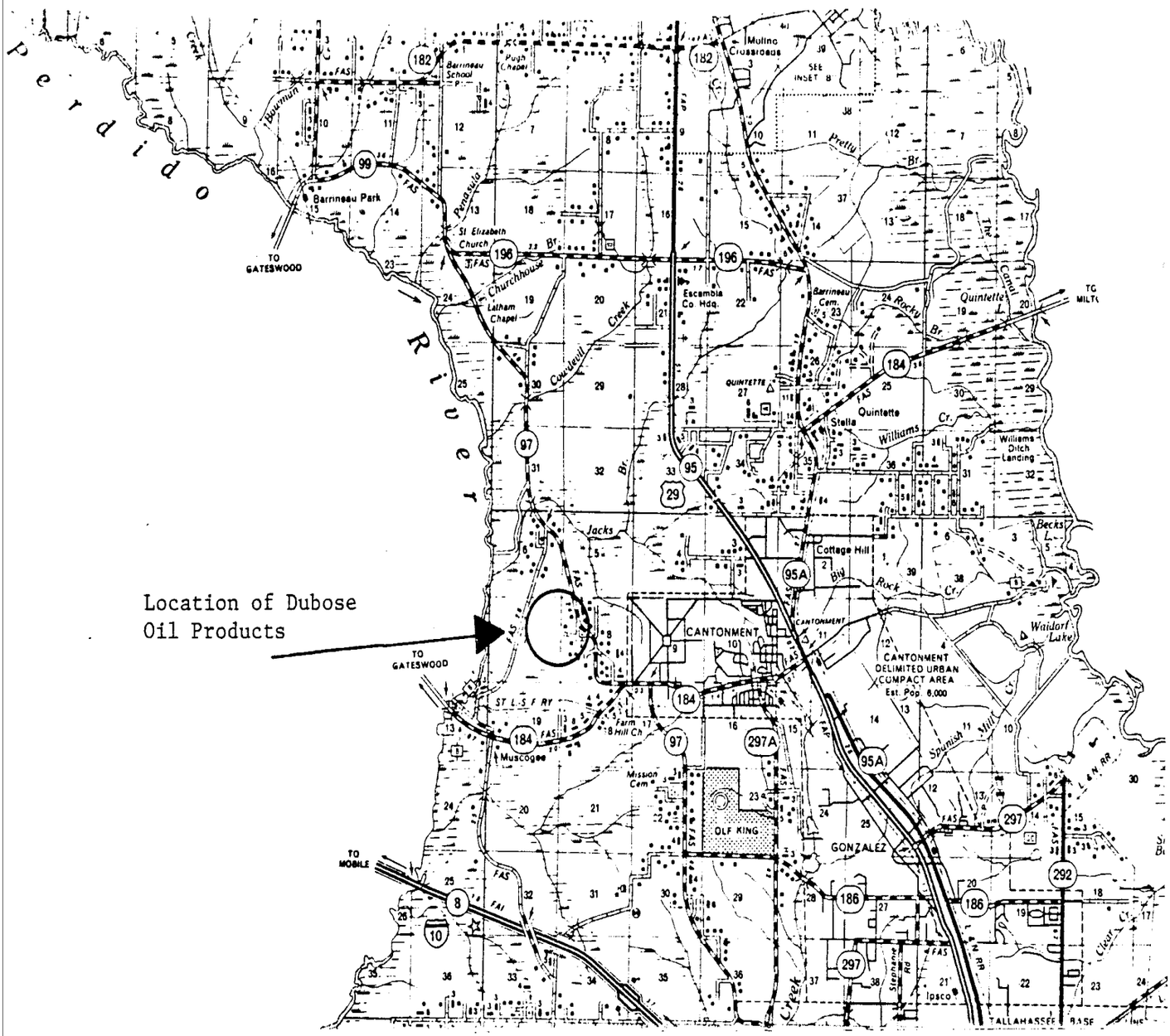
Dubose Oil Products, Escambia County, on 19 to 21 October, 1982.

The sample of waste material, collected from a discharge stream from the defunct Dubose Oil Products facility, was acutely toxic to the test organisms. The following 48 hour LC_{50} values were produced: Daphnia pulex - 72.0% (24 hour LC_{50}), Simocephalus serrulatus - 77.7%, and Heterandria formosa - 67.2%. The toxicity of the sample can probably be attributed to the large variety of organic chemicals present. Analyses of a sample of this material, collected during a previous site inspection, indicated the presence of at least 25 organic compounds, including derivatives of phenol, pentachlorophenol, benzene, chlorinated hydrocarbons, toluene, xylene and alcohols.

INTRODUCTION

The Dubose Oil Products facility is located near Cantonment, in Escambia County, Florida (Fig. 1). The facility ceased operation in late 1981. However, there is at least one wastewater pond that is contributing waste to a second pond at a lower elevation. Contaminated groundwater springs also apparently contribute to this second pond. The second pond overflows to form Jack's Branch, which flows to the Perdido River (Fig. 2).

Based on information from the Northwest District Hazardous Waste staff, the pond overflow may contain a large variety of organic chemicals and a large concentration of iron (Mike Clark, personal communication). To evaluate the toxicity of this pond overflow to aquatic organisms, the Hazardous Waste staff requested that a toxicity analysis be performed. Therefore, the Biology Section performed three 48 hour static acute toxicity bioassays on a sample of the material, on 19 to 21 October, 1982. The results of these tests are presented in this report.



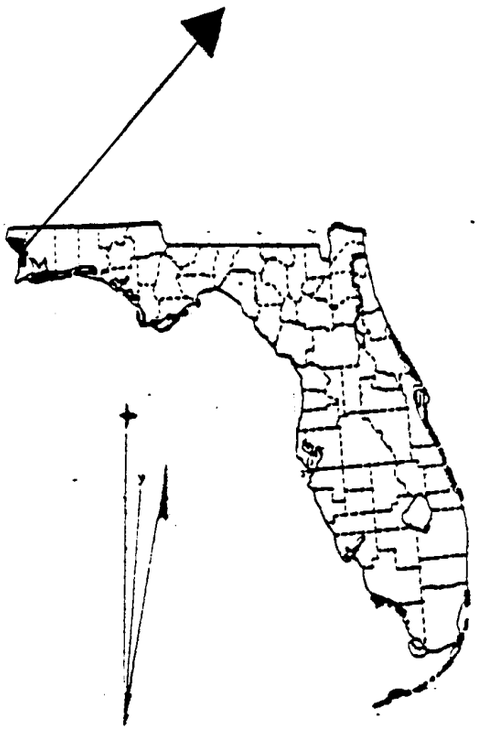


Figure 1. Portions of Escambia County, Florida, including study area.

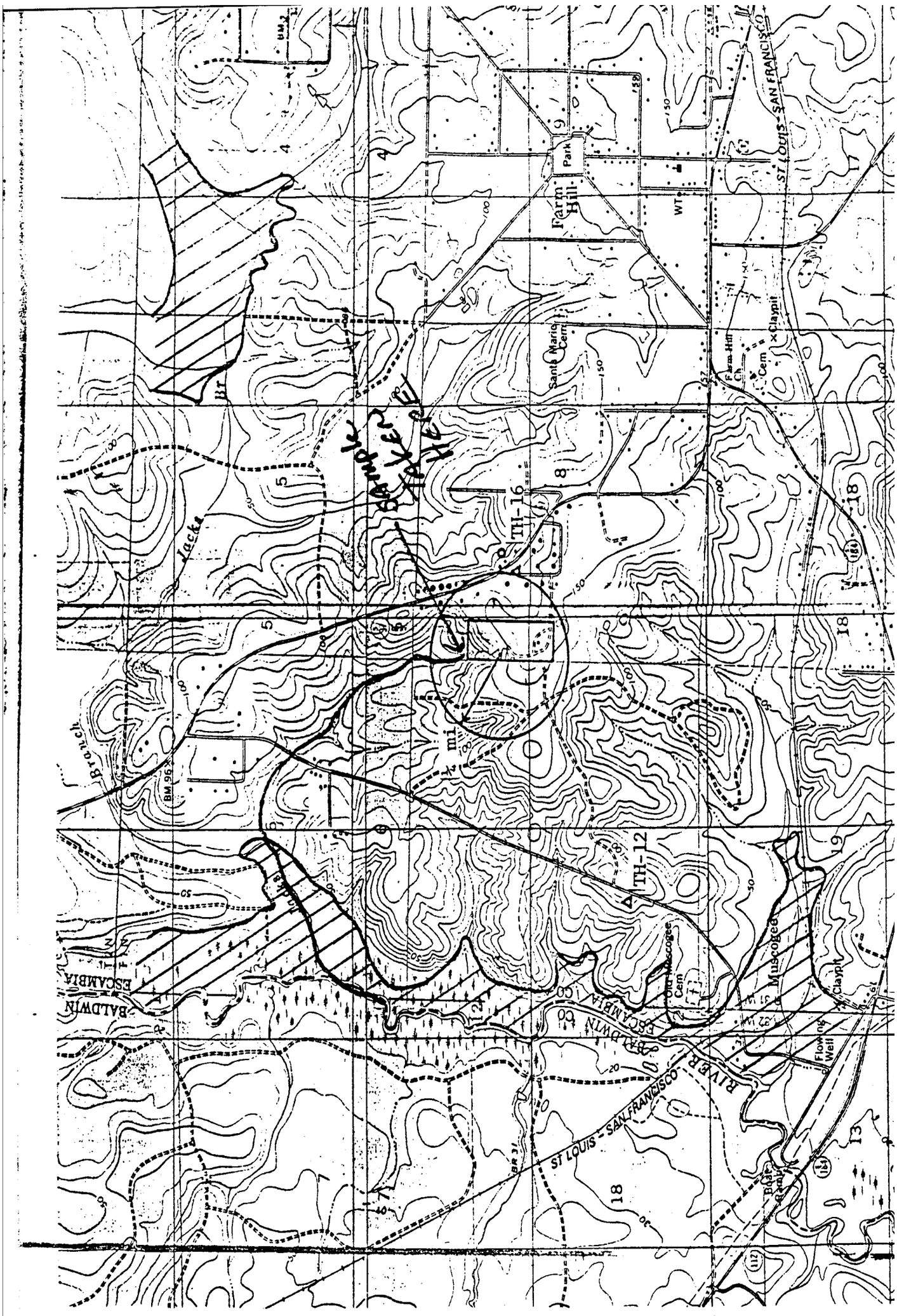


Figure 2. Location of Sampling Point

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include a 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. Two of these indigenous species were 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, and are kept at a constant temperature of 22° plus or minus 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. 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 Simocephalus serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half 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° plus or minus 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. 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.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 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 least killifish are reared at 22° plus or minus 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 1 to 3 weeks 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 acute toxicity bioassays were performed on a sample of Dubose Oil Products hazardous waste material collected as it overflowed from a pond. The tests were performed from 1000 hours on 19 October to 1015 hours on 21 October, 1982. The sample was collected by Mr. Mike Clark of DER's Northwest District at 1115 hours on 18 October, 1982, using standard wastewater sampling procedures. The sample was iced and transported to the DER laboratory in Tallahassee for analyses 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 (1980).

Two replicates, each with six concentrations and a control, were used to test with the water fleas, 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 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. Simocephalus neonates were separated from the adults visually in the culture dish prior to loading. 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 six concentrations and a control, were used for the Heterandria formosa (least killifish) 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.

Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. The specific conductance of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and unionized fractions (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 determined 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 2,3 and 4.

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

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

Concentration	Dilution Water	Effluent	Total Volume
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
100.0%	--	200 ml	200 ml

Heterandria formosa (least killifish)

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

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Dubose Oil Products, Hazardous Waste Sample, Escambia County, Florida, on 19 to 21 October, 1982.

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	8	3	7.9	8.9	8.3	7.6	8.1	8.2					21.0	20.3	19.8	.01/.25	--		
Control	10	10	10	7.9	8.9	8.5	7.6	8.1	8.2										200	230
5.6%	10	10	10	7.9	8.8	8.5	7.5	8.1	8.2					21.1	20.2	19.8				
5.6%	10	10	10	7.9	8.8	8.5	7.5	8.1	8.2										200	220
10.0%	10	10	10	7.8	8.6	8.5	7.4	8.1	8.2					21.0	20.2	19.7				
10.0%	10	10	10	7.8	8.6	8.5	7.4	8.0	8.2										200	225
18.0%	10	10	10	7.6	8.4	8.4	7.2	8.0	8.1					21.0	20.1	19.7				
18.0%	10	10	10	7.7	8.4	8.4	7.2	7.9	8.1										195	210
32.0%	10	10	10	7.8	7.6	8.2	7.0	7.9	8.0					20.9	20.3	19.7				
32.0%	10	10	9	7.8	7.6	8.2	7.0	7.9	8.0										185	195
56.0%	10	9	9	7.7	6.9	7.7	6.8	7.4	7.8					20.8	20.4	19.8				
56.0%	10	10	9	7.7	6.9	7.7	6.8	7.4	7.7										180	175
100.0%	10	0	0	8.2	5.2	-[b]	6.2	6.4	-[b]					20.5	20.7	--[b]	0/.50	--		
100.0%	10	0	0	8.2	5.1	-	6.2	6.3	-										175	150

Remarks:

[a] The 48 hour test is invalidated due to excessive control mortality (>20%).

[b] The remaining parameters were not recorded since all organisms were dead by hour 24.

Table 3. Data recorded during 48 hour, *Simocephalus serrulatus*, static acute toxicity bioassay of Dubose Oil Products, Hazardous Waste Sample, Escambia County, Florida, on 19 to 21 October, 1982.

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	10	10	8	7.9	9.0	8.6	7.6	8.1	8.2					21.1	20.1	19.5				
Control	10	10	10	7.9	9.0	8.6	7.6	8.1	8.2										200	220
5.6%	10	10	10	7.9	8.9	8.6	7.5	8.1	8.2					21.1	20.1	19.3				
5.6%	10	10	10	7.9	8.9	8.6	7.6	8.1	8.2										200	220
10.0%	10	10	10	7.8	8.5	8.6	7.4	8.1	8.1					21.0	20.0	19.2				
10.0%	10	10	10	7.8	8.5	8.6	7.4	8.0	8.1										200	215
18.0%	10	10	10	7.7	8.1	8.5	7.2	8.0	8.0					21.0	20.2	19.2				
18.0%	10	10	9(10)[a]	7.7	8.1	8.5	7.2	8.0	8.0										195	205
32.0%	10	9	9	7.8	7.6	8.3	7.0	7.9	7.9					20.9	20.3	19.3				
32.0%	10	10	10	7.8	7.6	8.3	7.0	7.8	7.9										185	195
56.0%	10	10	9	7.8	6.8	7.9	6.8	7.4	7.8					20.7	20.2	19.3				
56.0%	10	10	10	7.8	6.8	7.9	6.8	7.4	7.7										180	175
100.0%	10	1	0	8.3	4.9	7.1	6.2	6.3	6.7					20.5	20.2	--[b]	0/.5	--		
100.0%	10	0	0	8.3	4.9	6.9	6.2	6.2	6.7										175	150

Remarks:

[a] Number in parentheses used in calculations since one organism was not found.

[b] Last temperature was not recorded.

Table 4. Data recorded during 48 hour, Heterandria formosa,[a] static acute toxicity bioassay of Dubose Oil Company, Hazardous Waste Sample, Escambia County, Florida, on 19 to 21 October, 1982.

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	8.1	8.4	8.5	7.6	7.8	8.0					21.3	20.1	19.5	.01/.25	--		
Control	5	5	5	8.1	8.4	8.5	7.6	7.8	8.0										205	220
5.6%	5	5	5	8.0	7.6	7.9	7.5	7.8	7.9					21.2	20.0	19.3				
5.6%	5	5	5	8.0	7.6	7.9	7.5	7.8	7.9										205	215
10.0%	5	5	5	7.9	7.6	7.7	7.4	7.8	7.8					21.1	19.8	19.2				
10.0%	5	5	5	7.9	7.1	7.7	7.4	7.8	7.8										205	210
18.0%	5	5	5	7.9	6.7	7.0	7.2	7.6	7.8					21.0	19.8	19.2				
18.0%	5	5	5	7.9	7.4	7.5	7.2	7.7	7.8										200	205
32.0%	5	5	5	7.8	7.1	7.5	7.0	7.6	7.8					20.7	19.8	19.3				
32.0%	5	5	5	7.8	6.9	7.2	7.0	7.5	7.7										190	190
56.0%	5	5	4	7.8	5.7	6.2	6.8	7.1	7.5					20.4	19.8	19.3				
56.0%	5	4	4	7.8	5.7	6.0	6.8	7.1	7.5										170	175
100.0%	5	0	0	8.4	3.3	--[b]	6.2	6.2	--[b]					20.0	19.9	--[b]	0/.5	--		
100.0%	5	0	0	8.4	3.1	--	6.2	6.2	--										150	155

Remarks:

[a] Weight of fish: 10.7 mg each, average of 9 fish.

[b] Remaining parameters not recorded since all organisms were dead by hour 24.

Table 5. LC_{50} Data for Bioassays of Dubose Oil Products, Hazardous Waste Sample, on 19 to 21 October, 1982.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	72.0% (56 and 100)	b
	Moving Average	a	
	Probit	a	
S. serrulatus	Binomial	77.7% (56 and 100)	72.0% (56 and 100)
	Moving Average	a	69.4% (61 and 81)
	Probit	73.0% (0 and ∞), c	68.2% (0 and ∞), c
H. formosa	Binomial	70.4% (56 and 100)	67.2% (32 and 100)
	Moving Average	a	a
	Probit	a	a

[a] Due to the pattern of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

[b] This test was invalidated at 48 hours due to excessive control mortality (>20%).

[c] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

RESULTS AND DISCUSSION

The sample of waste material from Dubose Oil Products, collected as it overflowed from a pond to form Jack's Branch, was acutely toxic to the test organisms. The sample produced a 24 hour LC_{50} of 72.0% using Daphnia pulex, and although similar toxicity was noted at 48 hours, the test was invalidated due to excessive control mortality (Table 5). A similar 24 hour LC_{50} of 77.7% was calculated using Simocephalus serrulatus, and slightly lower LC_{50}'s of 69.4% and 72.0% were produced at 48 hours (Table 5). The species of fish tested, Heterandria formosa, appeared to be slightly more sensitive to the sample, since a 24 hour LC_{50} of 70.4% and a 48 hour value of 67.2% were calculated (Table 5). Almost all the mortality of these three species was noted within 24 hours.

The DER Chemistry Section has performed analyses on samples of this material from Dubose Oil Products, collected from the same location as was the bioassay, but at an earlier date. These results are presented in Table 6, along with results of analyses performed on samples from slightly different locations from this facility. At least 25 organic compounds were identified in the sample taken at the bioassay site (discharge stream off site), including derivatives of phenol, pentachlorophenol, benzene, chlorinated hydrocarbons, toluene, xylene and alcohols. The concentrations of these compounds varied from <5.0 µg/l for certain chlorinated hydrocarbon compounds to 815 µg/l for 2-butanone. Similar compounds, some at much greater concentrations, were present at the other sites. The toxicity of many of these compounds, particularly pentachlorophenol, is well documented. The toxicity observed in these bioassays, therefore, can probably be attributed to the presence of these organic compounds.

Table 6. Results of Chemical Analyses Performed on Samples of Waste Material from Dubose Oil Products, Escambia County, collected on 7 October, 1982.

Sample Location	Sample Location
Discharge Stream Off Site	Ditch on West Side of Property
Acid Extractables:	Acid Extractables:
Pentachlorophenol (285 µg/l)	Pentachlorophenol (91 µg/l)
Phenol (110 µg/l)	Phenol (154 µg/l)
Methyl phenol (94 µg/l)[a]	Methyl phenol (106 µg/l)[a]
3,4-Dimethyl phenol (43 µg/l)[a]	Ethyl phenol (121 µg/l)[a]
Base Neutral Extractables:	Base Neutral Extractables:
N.D.	Naphthalene (29 µg/l)
Pesticide Extractables:	Pesticide Extractables:
N.D.	N.D.
Volatile Organics:	
Benzene (12.0 µg/l)	
Chlorobenzene (<5 µg/l)	
1,1-Dichloroethane (38.4 µg/l)	
1,1-Dichloroethene (9.4 µg/l)	
trans-1,2-Dichloroethene (116 µg/l)	
Ethylbenzene (41.8 µg/l)	
Methylene chloride (< 2.5 µg/l)	
Tetrachloroethene (< 5 µg/l)	
1,1,1-Trichloroethane (145 µg/l)	
Trichloroethene (208 µg/l)	
Toluene (43.4 µg/l)	
Xylene (222 µg/l)	
n-Propylbenzene (<5 µg/l)	
Trimethylbenzene (44 µg/l)[b]	
2-Propanone (>500 µg/l)	
2-Propanol (> 15 µg/l)	
1,2-Dichloro-1,1,2-Trifluoroethane (10 µg/l)[b]	
2-Butanone (815 µg/l)[b]	
2-Butanol (86 µg/l)[b]	
4-Methyl-2-Pentanone (435 µg/l)[b]	
Ethyl-methyl-benzene (48 µg/l)[b]	

Table 6 (continued)

Sample Location

Small Pond On Site

Acid Extractables:
- Phenol (44 µg/l)
- Methyl phenol (91 µg/l)[a]
- Ethyl phenol (98 µg/l)[a]
- 4-Chlorophenol (123 µg/l)[a]
- 2,4,5-Trichlorophenol (46 µg/l)[a]

Base Neutral Extractables:
- N.D.

Pesticide Extractables:
- N.D.

Volatile Organics:
- Benzene (43.0 µg/l)
- Chlorobenzene (16.4 µg/l)
- 1,1-Dichloroethane (147 µg/l)
- 1,1-Dichloroethene (12.3 µg/l)
- trans-1,2-Dichloroethene (124 µg/l)
- Ethylbenzene (153 µg/l)
- Methylene chloride (<2.5 µg/l)
- Tetrachloroethene (<5 µg/l)
- 1,1,1-Trichloroethane (56 µg/l)
- Trichloroethene (87.4 µg/l)
- Toluene (19.7 µg/l)
- Xylene (548 µg/l)
- n-Propylbenzene (<5 µg/l)
- Trimethylbenzene (15 µg/l)[b]
- 2-Propanone (>300 µg/l)
- 2-Propanol (>15 µg/l)
- 1,2-Dichloro-1,1,2-Trifluoroethane (96 µg/l)[b]
- 2- Butanone (658 µg/l)[b]
- 2-Butanol (53 µg/l)[b]
- 4-Methyl-2-pentanone (530 µg/l)[b]
- Ethyl-methyl-benzene (58 µg/l)[b]

Sample Location

West End of Ditch Transecting Property

Volatile Organics:
- Benzene (36.4 µg/l)
- Chlorobenzene (18.5 µg/l)
- 1,1-Dichloroethane (295 µg/l)
- 1,1-Dichloroethene (59.4 µg/l)
- trans-1,2-Dichloroethene (840 µg/l)
- Ethylbenzene (3.39 µg/l)
- Methylene chloride (2.54 µg/l)
- Tetrachloroethene (52.3 µg/l)
- 1,1,1-Trichloroethane (1.36×10^3 µg/l)
- Trichloroethene (1.94×10^3 µg/l)
- Toluene (176 µg/l)
- Xylene (1.35×10^3 µg/l)
- n-Propylbenzene (17.6 µg/l)
- Trimethylbenzene (46 µg/l)[b]
- 2-Propanone (>500 µg/l)
- 2-Propanol (>50 µg/l)
- 1,2-Dichloro-1,1,2-Trifluoroethane (130 µg/l)[b]
- 2-Butanone (1100 µg/l)[b]
- 2-Butanol (176 µg/l)[b]
- 4-Methyl-2-Pentanone (720 µg/l)[b]
- Ethyl-methyl-benzene (127 µg/l)[b]

N.D. = None Detected

[a] Tentative identification, estimated concentration

[b] Estimated concentration

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 (3), 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 existence of a mixing zone, whether as a specific part of the permit or based on the existence 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)(o); 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 disharge, 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} standard. 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.

LITERATURE CITED

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