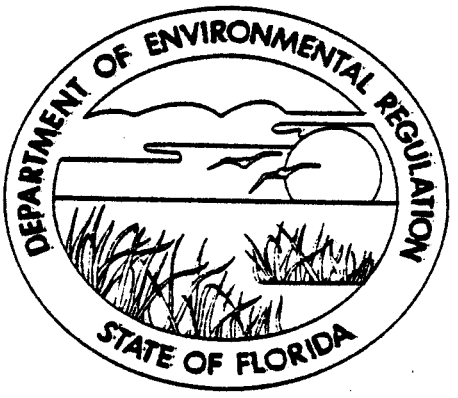


BIOASSAYS OF GROUNDWATER CONTAMINATION

TEXACO SERVICE STATION

S.R. 50 AND I-95, TITUSVILLE, BREVARD COUNTY

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Biology Section
Bureau of Water Analysis
May 14, 1984

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EXECUTIVE SUMMARY

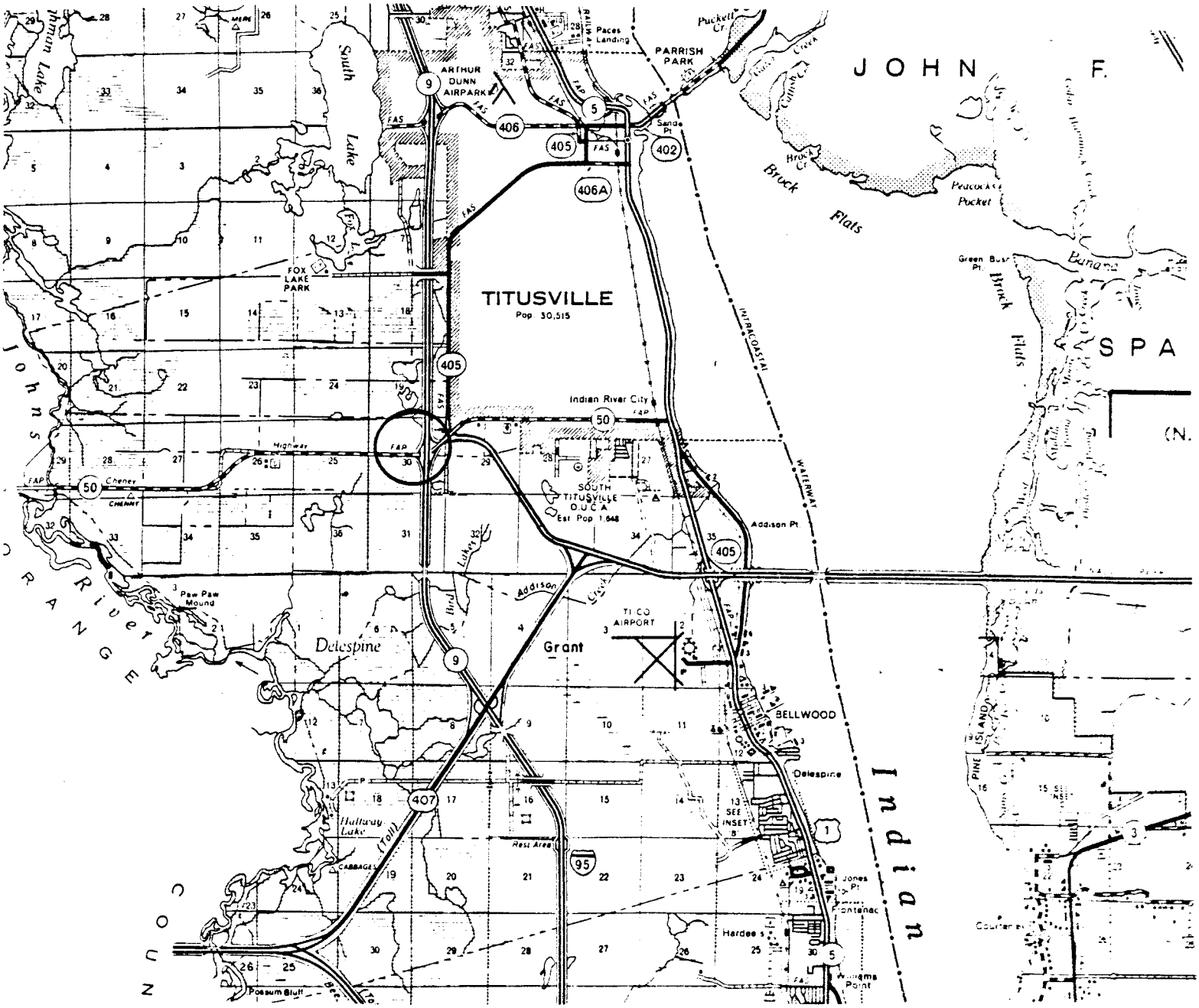
Texaco Service Station Groundwater Contamination, Brevard County, on 28 February to 2 March, 1984.

The sample of gasoline contaminated groundwater collected from a nearby shallow monitoring well was not lethal to any Heterandria formosa, but was lethal to a sufficient number of Mysidopsis bahia at 48 hours to produce an LC_{50} of 1222%. For comparison, this LC_{50} value of 1222% would be in violation of the chronic toxicity standard for surface water discharges stated in Chapter 17-3-3.021 of the F.A.C. Since the sample was collected from a relatively shallow well (3'), consideration must be given to the potential threat that this contaminated groundwater may pose to aquatic organisms. It was also noted that this bioassay sample was not collected from the most contaminated well, but from a distinctly less contaminated well closest to the surface waters of the St. Johns Wildlife Refuge (Dr. Alan Auwarter, personal communication).

INTRODUCTION

The Texaco Service Station is located at the intersection of S.R. 50 and I-95, near Titusville, Brevard County, Florida (Fig. 1). Apparently, the underground gasoline storage tanks at this facility have been leaking, thereby contaminating the shallow groundwater in the area. This gasoline leakage is a particularly serious threat because the groundwater of the area is known to flow westward, towards the aquatic systems of the St. Johns Wildlife Refuge, and may at tims be connected to this surface water.

Dr. Alan Auwarter of DER's Groundwater Section requested that acute toxicity bioassays be performed on this contaminated groundwater. Two bioassays were performed, on 29 February to 2 March, 1984, on a sample collected from a shallow monitoring well, to determine the groundwater's effect on aquatic organisms. 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 and M. L. Roll.



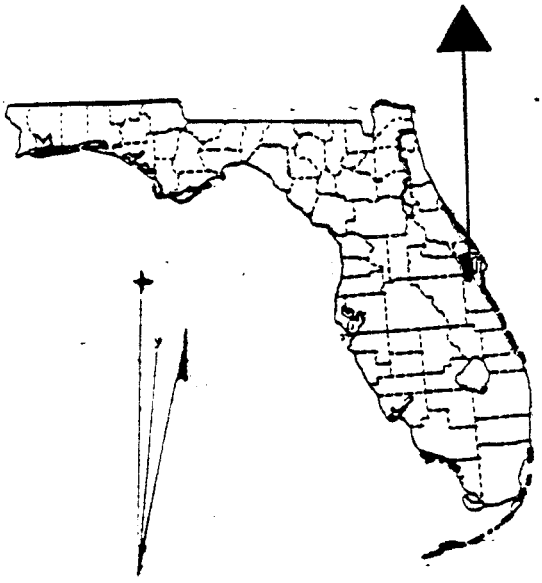


Figure 1. Portions of Brevard County, Florida, including Titusville and the location of the Texaco Service Station

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

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) and Tetra-Min® twice daily. New born fish are initially maintained in the adult tanks, but are transferred to separate aquaria at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 12 to 21 days old.

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 with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 22° plus or minus 2°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

Two separate 48 hour static acute toxicity bioassays were performed on a sample of groundwater collected from a monitoring well near the Texaco Service Station. The tests were performed from 1240 hours on 29 February to 1240 hours on 2 March, 1984.

The sample was collected by Mr. Ken Busen of DER's Groundwater Section at 1515 hours on 28 February, 1984, using standard wastewater sampling procedures. The sample was iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

1. Freshwater Test

Since the slightly saline (3.3 ppt) groundwater is said to flow towards a freshwater system, a freshwater fish (Heterandria formosa) known to tolerate this low salinity was selected as a test organism. Freshwater for dilution of the groundwater sample was obtained from the same DER deep well that is used to culture the test organism. The concentration series selected for this study is presented in Table 1.

Two replicates, of four 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.

2. Saltwater Test

Since the groundwater sample was of a low salinity (3.3 ppt), and the monitoring well was of close proximity to a saltwater marsh, the saltwater test organism Mysidopsis bahia was selected as the second test organism. Saltwater for dilution of the groundwater sample was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 22.0 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the sample, achieving a range of final salinities (13.5 ppt to 21.0 ppt). For the 100% test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 3) 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 (22.0 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 (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of 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 is presented in Table 1. 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 25 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 chamber at 0 hours, 24 hours, and 48 hours. 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 and 3.

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

Heterandria formosa (least killifish)

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Sample	Total Volume
Controls	500 ml	------	500 ml
10.0%	450 ml	50 ml	500 ml
25.0%	375 ml	125 ml	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Heterandria formosa, static acute toxicity bioassay of the Texaco Service Station Groundwater Contamination, Brevard County, Florida, on 29 February to 2 March, 1984.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/1)			pH			Total Alakalinity (mg/1 as $CaCO_3$)		Total Hardness (mg/1 as $CaCO_3$)		Temperature (°c)			NH_3/N (mg/1)		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	8.4	9.1	8.5	7.7	8.1	8.2					19.5	17.7	18.4				
Control	5	5	5	8.4	9.1	8.5	7.7	8.1	8.2										188	198
10.0%	5	5	5	8.3	9.4	8.3	7.6	8.0	8.1					19.5	17.4	18.1				
10.0%	5	5	5	8.3	9.4	8.3	7.6	8.0	8.1										730	720
25.0%	5	5	5	8.1	9.1	8.2	7.4	7.9	8.1					19.4	17.4	18.1				
25.0%	5	5	5	8.1	9.1	8.2	7.4	7.9	8.1										1520	1480
50.0%	5	5	5	7.6	9.1	8.2	7.3	7.9	8.2					19.2	17.5	18.3				
50.0%	5	5	5	7.6	9.0	8.2	7.3	7.9	8.2										2860	2730
100.0%	5	5	5	5.7	8.8	8.0	7.1	7.8	8.1					19.4	17.5	18.7				
100.0%	5	5	5	5.6	8.8	8.0	7.1	7.8	8.1										5010	5000

Remarks:

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of the Texaco Service Station Groundwater Contamination, Brevard County, Florida, on 29 February to 2 March, 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	24
Diluent Control	5	5	5	8.1	9.8	6.7	8.2	8.0	7.8	20.0	17.9	18.7				
Diluent Control	5	5	5	8.1	9.8	6.7	8.2	8.0	7.8						23.0	23.0
Sea Salts Control	5	5	5	8.4	9.8	7.6	8.2	8.2	8.1	20.0	17.7	18.5				
Sea Salts Control	5	5	5	8.4	9.8	7.6	8.2	8.2	8.1						7.3	7.2
10.0%	6[a]	6	6	8.0	9.8	6.5	7.9	7.9	7.8	20.0	17.8	18.6				
10.0%	5	5	5	8.0	9.8	6.5	7.9	7.9	7.8						21.0	21.0
25.0%	6[a]	5	4	7.9	9.6	6.7	7.5	7.9	7.9	19.8	18.1	18.8				
25.0%	5	5	5	7.8	9.6	6.7	7.5	7.9	7.9						18.5	18.1
50.0%	5(4)[b]	4	4	7.4	9.5	6.9	7.3	7.8	8.0	19.6	18.2	19.0				
50.0%	5	5	5	7.4	9.5	6.9	7.3	7.8	8.0						13.7	13.5
100.0%	5	5	5	7.0	8.7	6.9	7.3	7.9	8.2	19.3	18.3	19.3				
100.0%	5	4	3	6.8	8.7	6.9	7.3	7.9	8.2						7.2	7.0

Remarks: [a] Extra Organisms loaded by mistake, allowed to remain.

[b] Number in parentheses used for calculations since one organism jumped from the container between 0 and 24 hours.

Table 4. LC_{50} Data for Bioassays of the Texaco Service Station Groundwater Contamination on 29 February to 2 March, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
H. formosa	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
M. bahia	Binomial	a	a
	Moving Average	a	a
	Probit	a	1222% (119 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.

RESULTS AND DISCUSSION

The sample of gasoline contaminated groundwater collected from a monitoring well near the Texaco Service Station was not lethal to any Heterandria formosa, but was lethal to a sufficient number of Mysidopsis bahia at 48 hours to calculate an LC_{50} of 1222% (Table 4). This LC_{50}, however, must be viewed with some degree of uncertainity, since the mortality distribution was uneven (Table 3) and the 95% Confidence Intervals for the LC_{50} calculation were large (Table 4). For comparison, however, this LC_{50} value of 1222% would be in violation of the chronic toxicity standard stated in Chapter 17-3-3.021 of the F.A.C.

Since the sample was collected from a relatively shallow well (3') in close proximity to both fresh and saline waters, consideration must be given to the potential threat that this contaminated groundwater may pose to aquatic organisms. It was also noted that the sample collected for the bioassay analysis was not from the most contaminated well, but from a distinctly less contaminated well closest to the waters of the St. Johns Wildlife Refuge (Dr. Alan Auwarter, personal communication).

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1983. Quality Assurance Manual for Performing Acute Toxicity Tests. FDER. 22 p.

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Environmental Research Laboratory. Athens, Georgia. EPA-600/4-78-012 (Revised July 1978). 51 p.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.