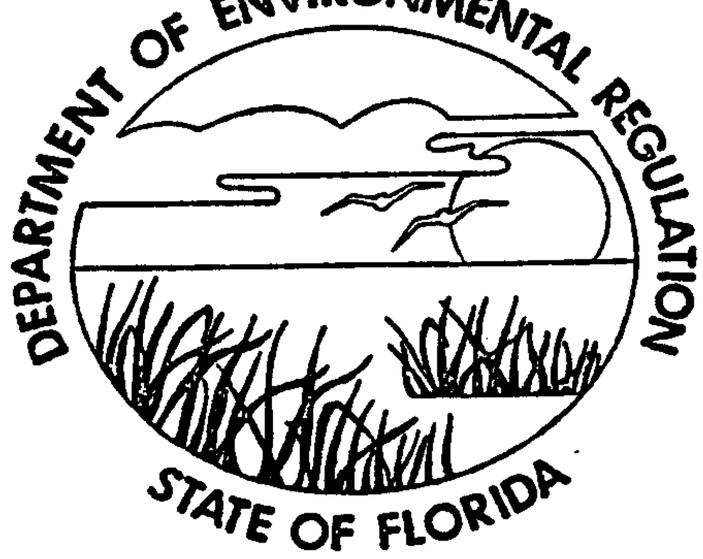



# BIOASSAYS OF BIG THREE INDUSTRIES

## PALM BEACH COUNTY, FLORIDA

## NO NPDES PERMIT

SAMPLED 6/2/86

JULY 31, 1986

Biology Section
Division of Environmental Programs

# BIOASSAYS OF BIG THREE INDUSTRIES

# PALM BEACH COUNTY, FLORIDA

# NO NPDES PERMIT

Biology Section
Bureau of Laboratories and
Special Programs
July 31, 1986

## EXECUTIVE SUMMARY

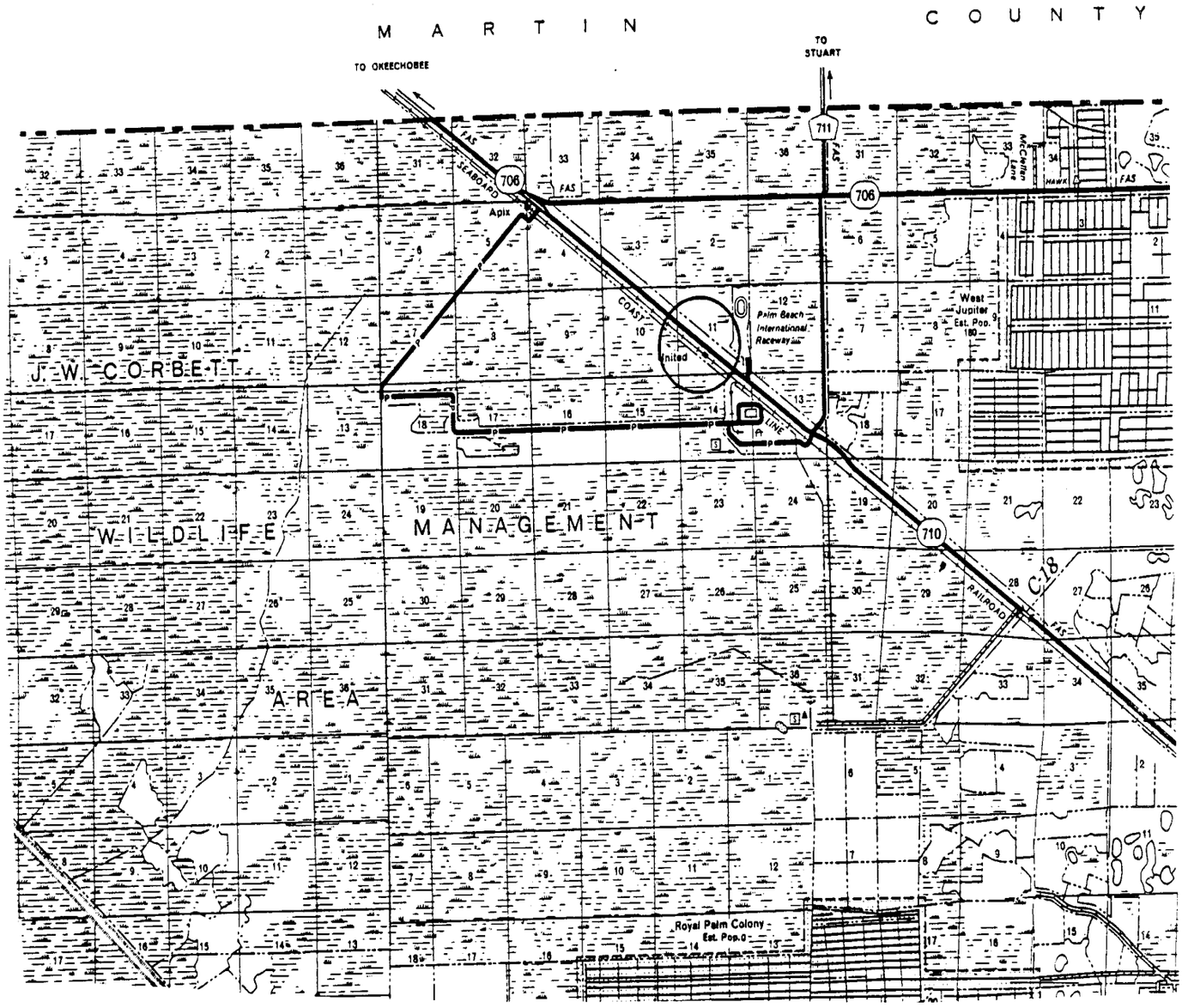
Big Three Industries, Palm Beach County, on 3 to 5 June, 1986.

Three 48 hour static acute toxicity bioassays were performed on a sample of cooling water effluent from Big Three Industries, a manufacturer of industrial gases. The tests indicated that the effluent was not acutely toxic to the test organisms, Daphnia pulex, Ceriodaphnia dubia, and Notropis leedsi. Some mortality was noted in the higher test concentrations, including 20% of the N. leedsi. A small amount of total residual chlorine (0.08 mg/l) was detected in the sample, which was apparently insufficient to have caused any significant toxicity. No ammonia was detected in the sample. Since the sample was not acutely toxic, additional chemical analyses were not performed.

## INTRODUCTION

Big Three Industries is located on the Beeline Highway (S.R. 710) near the Palm Beach Raceway, Palm Beach County, Florida (Fig. 1). The facility manufactures industrial gases such as nitrogen, using air as the source. Cooling water from this operation is discharged to a canal which empties into the C-18 canal.

To evaluate the toxic potential of this discharge, the Southeast District requested that acute toxicity bioassays be performed. Three such tests were performed on a sample of cooling water discharge from Big Three Industries, from 3 to 5 June, 1986. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: M. L. Roll and P. G. Weiland.



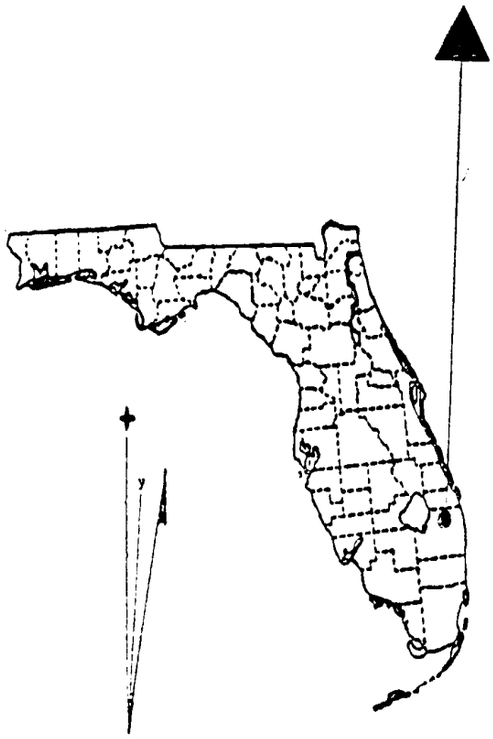



Figure 1. Portions of Palm Beach County, Florida, including the location of Big Three Industries.

## 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), the U.S. EPA (1978), 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 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° ± 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 cycle and are fed Tetra-Min® and frozen Artemia (brin 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 approximately 18 days old at the time of the 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

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 separate 48 hour static acute toxicity bioassays were performed on a sample of cooling water discharge from Big Three Industries' Outfall E. The tests were performed from 0930 hours on 3 June to 0930 hours on 5 June, 1986.

The sample was collected by Mr. William Thiel of DER's Southeast District at 1055 hours on 2 June, 1986, using standard wastewater sampling procedures. 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 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 samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 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 (Table 1) is an abbreviated version of that recommended by APHA (1980).

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 250 ml capacity were used as test chambers, and 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 also 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 Tables 2, 3, and 4.

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

*Ceriodaphnia dubia* (water flea)

*Daphnia pulex* (water flea)

| Concentration | Dilution Water | Effluent | Total Volume |
|---|---|---|---|
| Control | 200.0 ml | -------- | 200 ml |
| 10.0% | 180.0 ml | 20.0 ml | 200 ml |
| 32.0% | 136.0 ml | 64.0 ml | 200 ml |
| 56.0% | 88.0 ml | 112.0 ml | 200 ml |
| 100.0% | -------- | 200.0 ml | 200 ml |

*Notropis leedsi* (bannerfin shiner)

| Concentration | Dilution Water | Effluent | Total Volume |
|---|---|---|---|
| Control | 500 ml | -------- | 500 ml |
| 10.0% | 450 ml | 50 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 Big Three Industries, Palm Beach County, on 3 to 5 June, 1986.

| 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 | |
| Control | 10 | 10 | 10 | 8.3 | 8.2 | 8.7 | 8.1 | 8.0 | 8.0 | 22.0 | 19.4 | 19.6 | | | Control Water |
| Control | 10 | 10 | 10 | 8.3 | 8.2 | 8.7 | 8.1 | 8.0 | 8.0 | | | | 220 | 218 | $NH_3$(as $NH_3$)(mg/l) 0 — Total Alkalinity 110 (mg/l $CaOO_3$) |
| 10.0% | 10 | 10 | 10 | 8.3 | 8.3 | 8.7 | 8.0 | 8.0 | 7.9 | 21.9 | 19.3 | 19.5 | | | |
| 10.0% | 10 | 10 | 10 | 8.3 | 8.3 | 8.7 | 8.0 | 8.0 | 7.9 | | | | 340 | 328 | |
| 32.0% | 10 | 10 | 10 | 8.5 | 8.3 | 8.7 | 7.9 | 7.9 | 7.8 | 21.7 | 19.0 | 19.4 | | | Total ammonia (as N)(mg/l) 0 — Total Hardness 120 (mg/l $CaOO_3$) |
| 32.0% | 10 | 10 | 10 | 8.5 | 8.3 | 8.7 | 7.9 | 7.9 | 7.8 | | | | 680 | 640 | |
| 56.0% | 10 | 10 | 10 | 8.7 | 8.3 | 8.7 | 7.7 | 7.8 | 7.7 | 21.5 | 18.9 | 19.4 | | | |
| 56.0% | 10 | 10 | 9 | 8.7 | 8.3 | 8.7 | 7.7 | 7.8 | 7.7 | | | | 920 | 910 | |
| 100.0% | 10 | 10 | 10 | 8.9 | 8.4 | 8.8 | 7.2 | 7.3 | 7.2 | 21.3 | 19.0 | 19.5 | | | |
| 100.0% | 10 | 10 | 10 | 8.9 | 8.4 | 8.8 | 7.2 | 7.3 | 7.2 | | | | 1390 | 1380 | 100% Effluent |
| | | | | | | | | | | | | | | | $NH_3$(as $NH_3$)(mg/l) 0 — Total Alkalinity 20 (mg/l $CaOO_3$) |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | Total ammonia (as N)(mg/l) 0 — Total Hardness >500 (mg/l $CaOO_3$) |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |

Total Residual Chlorine: 0.08 mg/l (HACH DPD Method).

Table 3. Data recorded during 48 hour, Ceriodaphnia dubia, static acute toxicity bioassay of Big Three Industries, Palm Beach County, on 3 to 5 June, 1986.

| 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 |
| Control | 10 | 10 | 10 | 8.1 | 8.2 | 8.7 | 8.0 | 7.8 | 7.9 | 22.0 | 19.4 | 20.1 | | |
| Control | 10 | 10 | 10 | 8.1 | 8.2 | 8.7 | 8.0 | 7.8 | 7.9 | | | | 220 | 218 |
| 10.0% | 10 | 10 | 10 | 8.1 | 8.0 | 8.7 | 7.9 | 7.8 | 7.9 | 21.9 | 19.2 | 20.0 | | |
| 10.0% | 10 | 10 | 10 | 8.1 | 8.0 | 8.7 | 7.9 | 7.8 | 7.9 | | | | 340 | 328 |
| 32.0% | 10 | 10 | 10 | 8.3 | 8.1 | 8.8 | 7.8 | 7.8 | 7.8 | 21.7 | 19.2 | 19.8 | | |
| 32.0% | 10 | 10 | 10 | 8.3 | 8.1 | 8.8 | 7.8 | 7.8 | 7.8 | | | | 680 | 650 |
| 56.0% | 10 | 10 | 10 | 8.5 | 8.2 | 8.8 | 7.7 | 7.7 | 7.7 | 21.7 | 19.2 | 19.7 | | |
| 56.0% | 10 | 10 | 10 | 8.5 | 8.2 | 8.8 | 7.7 | 7.7 | 7.7 | | | | 920 | 910 |
| 100.0% | 10 | 10 | 9 | 8.9 | 8.3 | 8.8 | 7.2 | 7.4 | 7.3 | 21.5 | 19.2 | 19.7 | | |
| 100.0% | 10 | 10 | 10 | 8.9 | 8.2 | 8.8 | 7.2 | 7.4 | 7.3 | | | | 1390 | 1380 |
| | | | | | | | | | | | | | | |
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Control Water

$NH_3$(as $NH_3$)(mg/l): 0

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

Total Alkalinity (mg/l $CaCO_3$): 110

Total Hardness (mg/l $CaCO_3$): 120

100% Effluent

$NH_3$(as $NH_3$)(mg/l): 0

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

Total Alkalinity (mg/l $CaCO_3$): 20

Total Hardness (mg/l $CaCO_3$): >500

Total Residual Chlorine: 0.08 mg/l (HACH DPD Method).

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Big Three Industries, Palm Beach County, on 3 to 5 June, 1986.

| 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 |
| Control | 5 | 5 | 5 | 8.1 | 7.8 | 8.6 | 7.7 | 7.9 | 7.9 | 22.2 | 19.3 | 19.6 | | |
| Control | 5 | 5 | 5 | 8.1 | 7.8 | 8.6 | 7.7 | 7.9 | 7.9 | | | | 212 | 212 |
| 10.0% | 5 | 5 | 5 | 8.2 | 7.9 | 8.6 | 7.7 | 7.8 | 7.9 | 22.2 | 19.1 | 19.5 | | |
| 10.0% | 5 | 5 | 5 | 8.2 | 7.9 | 8.6 | 7.7 | 7.8 | 7.9 | | | | 338 | 325 |
| 32.0% | 5 | 5 | 5 | 8.4 | 7.9 | 8.7 | 7.6 | 7.8 | 7.8 | 22.0 | 19.0 | 19.4 | | |
| 32.0% | 5 | 5 | 5 | 8.4 | 7.9 | 8.7 | 7.6 | 7.8 | 7.8 | | | | 680 | 650 |
| 56.0% | 5 | 5 | 5 | 8.6 | 8.0 | 8.7 | 7.5 | 7.6 | 7.7 | 21.5 | 18.9 | 19.3 | | |
| 56.0% | 5 | 5 | 5 | 8.6 | 8.0 | 8.7 | 7.5 | 7.6 | 7.7 | | | | 920 | 920 |
| 100.0% | 5 | 5 | 5 | 8.9 | 8.1 | 8.7 | 7.2 | 7.2 | 7.2 | 21.2 | 18.9 | 19.2 | | |
| 100.0% | 5 | 5 | 3 | 8.9 | 8.1 | 8.7 | 7.2 | 7.2 | 7.2 | | | | 1395 | 1380 |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | |

Control Water

$NH_3$(as $NH_3$)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 110

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

Total Hardness (mg/l $CaCO_3$): 120

100% Effluent

$NH_3$(as $NH_3$)(mg/l): 0

Total Alkalinity (mg/l $CaCO_3$): 20

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

Total Hardness (mg/l $CaCO_3$): >500

Total Residual Chlorine: 0.08 mg/l (HACH DPD Method).

## RESULTS AND DISCUSSION

The sampling of cooling water effluent from Big Three Industries' Outfall E was not acutely toxic to the test organisms, *Daphnia pulex*, *Ceriodaphnia dubia*, and *Notropis leedsi*. Some mortality, however, was noted in the higher test concentrations, including 20% of the *N. leedsi* (Table 4). Total residual chlorine measured in the sample (0.08 mg/l) was apparently insufficient to have caused any significant mortality. No ammonia was detected in the sample. Since the sample was not acutely toxic, further chemical analyses were not performed.

## LITERATURE CITED

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