

DuPont HLR 61-94

Study Title

STATIC, ACUTE, 96-HOUR LC50 OF H-19704  
TO BLUEGILL SUNFISH, Lepomis macrochirus

Laboratory Project ID

Haskell Laboratory Report No. 61-94

Data Requirement

United States Environmental Protection Agency  
40 CFR 797.1400 Fish Acute Toxicity Test  
Toxic Substances Control Act

Author

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Study Completed On

26 August 1994

Performing Laboratory

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[ Medical Research Project ]

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

This study was conducted in compliance with EPA FIFRA (40 CFR 160) and EPA TSCA (40 CFR 792) Good Laboratory Practice Standards, OECD Principles of Good Laboratory Practice (C(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850) except for the deviations documented and explained below.

1. Stability and solubility of the test substance under test and storage conditions were not determined.

2. Tetramin® flake food was not tested for pesticide content.

The deviation(s) did not affect the integrity of the study.

Submitter: E. I. du Pont de Nemours and Company

Sponsor: DuPont Elastomers  
E. I. du Pont de Nemours and Company

Date

Study Director:

Keith B. Pierson  
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26 August 1994

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Registration Manager

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**GENERAL INFORMATION**

Material Tested: Octanoic acid, pentadecafluoro-, ammonium salt

Haskell Number: H-19704

Purity: [REDACTED]

Composition: [REDACTED]

Contaminants: None reported

CAS Registry Number: 3825-26-1

Sponsor: DuPont Elastomers  
E. I. du Pont de Nemours and Company  
Wilmington, Delaware 19898

Study Initiated/Completed: 13 August 1993 / 26 August 1994

In-Life Study Initiated/Completed: 23 August 1993 / 27 August 1993

**SUMMARY**

H-19704 exhibited low toxicity to bluegill sunfish, Lepomis macrochirus, in a 96-hour, unaerated, static test with nominal concentrations of 262, 328, 410, 512, 640, 800 and 1000 mg/L. The 96-hour LC50 based on nominal concentrations was 634 mg/L with a 95% fiducial interval of 567 to 725 mg/L.

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THIS REPORT IS APPROVED FOR ISSUE.

Keith B. Pierson  
Keith B. Pierson, Ph.D.  
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August 26 1994  
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QUALITY ASSURANCE DOCUMENTATION

(H-19704)

Dates of Inspection:

Conduct - 8/23/93

Records, Report(s) - 8/9-10/94

Date(s) Findings Reported to:

Study Director - 8/10/94

Management - 8/24/94

Reported by:

Donna R. Holt

Donna R. Holt

Quality Assurance Auditor

8/24/94  
Date

Company Sanitized. Does not contain TSCA CB!

## I. PURPOSE

The goal of this study was to assess the acute toxicity of H-19704 to juvenile bluegill sunfish, Lepomis macrochirus.

## II. RECORDS

All data and the final report will be maintained either at Haskell Laboratory for Toxicology and Industrial Medicine (Newark, DE) or be archived at the DuPont Records Management Center (Wilmington, DE).

## III. MATERIALS AND METHODS

### A. Test Substance

The test substance is described under General Information. Test solutions were prepared by weighing out the appropriate quantity of test substance, adding it to 10 liters of dilution water (see below) and stirring. The test substance appeared to be entirely in solution at study start. Stability and solubility of H-19704 in dilution water were not determined.

### B. Dilution Water

Water (Table 1) originated from the Haskell Laboratory well.

### C. Animal Culture

Bluegill sunfish, Lepomis macrochirus, were purchased from either Northeastern Biologists (Rhineback, NY) or Aquatic Research Organisms (Hampton, NH). Fish were acclimated in continuously-flowing, well water in 545-L circular, fiberglass tanks for 212 days at a mean temperature of 21.2°C (rangefinder) or for 32 days at a mean temperature of 22.6°C (definitive study) prior to study start. Fish were fed newly-hatched, live Artemia sp. (Aquarium Products, Glen Burnie, MD), frozen adult Artemia sp. (Golden Gate Brand, Kordon Division, Novalek, Inc., Hayward, CA) and/or TetraMin® flake food (TetraWerke, Germany) generally twice daily on weekdays and daily on weekends. Fish were treated with 2-3 parts per thousand salt (NaCl) on days 0-4 of the 212-day acclimation (rangefinder). Fish were treated with three handfuls of salt (NaCl) on day 0, 200 parts per million formalin for one hour on days 1-3 of the 32-day acclimation (definitive). Sickness, injury and abnormality were not present at study start. Animals were identified by labels on aquaria and test vessels.

### D. Test Methods [1]

Seven test concentrations and a control were used. Nominal test concentrations were 262, 328, 410, 512, 640, 800 and 1000 mg/L.

Glass aquaria [20 liter; 39.9 (length) x 19.8 (width) x 25.5 cm (height)]

containing 10 L of test solution (approximately 13.5-cm liquid depth) were employed. Positions of test chambers in the water bath used to maintain constant temperature were assigned using random numbers. Ten fish were added to each replicate using random numbers (two replicates per concentration; total 20 fish per concentration). Control fish ranged from 1.2 to 2.8 cm standard length (mean 2.1 cm) and 0.0451 to 0.524 g wet weight (mean 0.228 g). Control loading was 0.23 g/L at test conclusion. Test solutions ranged from 21.3 to 22.1°C (mean 21.7°C). Fish were not fed approximately 24 hours prior to nor during the test. A photoperiod of 16 hours light (312 to 344 Lux) versus 8 hours darkness was employed with 25 minutes of transitional light (<2.15 Lux) preceding and following the 16 hour light interval. Mortality and behavioral effects were noted daily.

Dissolved oxygen (YSI Model 54A, Yellow Springs Instruments, Yellow Springs, OH), pH (Corning Model 610A, Corning Scientific Instruments, Medfield, MA) and temperature (American Society of Testing Materials registered mercury thermometer) were measured in each replicate before addition of fish at test start, daily, at total fish mortality and/or at test end. Total alkalinity (Hach Chemical Company, Loveland, CO), EDTA hardness (Hach Chemical Company, Loveland, CO), and conductivity (YSI Model 33, Yellow Springs Instruments, Yellow Springs, OH) of the control water were measured before fish were added at test start. A continuously-recording thermometer in a water-control replicate was used to check temperature variations during the 96-hour test.

#### **E. Chemical Analysis**

Chemical analyses of the test substance concentrations in water were not performed because a suitable method was not available.

#### **F. Statistical Methods**

The 24-, 48-, 72- and 96-hour LC50s and their 95% fiducial intervals were calculated using probit analysis [2].

### **IV. RESULTS AND DISCUSSION**

Protocol amendments and/or SOP deviations appear in Appendix 1.

A rangefinder was conducted. Nominal test concentrations were 0, 0.1, 1, 10, 100 and 1000 mg/L. Mortality after 96 hours was 0, 0, 0, 0, 0 and 80%, respectively.

The definitive study was conducted using nominal H-19704 concentrations of 262, 328, 410, 512, 640, 800 and 1000 mg/L. We planned to measure H-19704 concentrations in test solutions, but the suggested analytical method failed. Preliminary research suggested it was not cost effective to develop a new method. Hence, nominal H-19704 concentrations are reported herein.

All chemical and physical parameters were within expected ranges (Tables 2-3).

The 96-hour LC50 (Tables 4 and 6) was 634 mg/L with a 95% fiducial interval of 567 to 725 mg/L based upon nominal concentrations. Behavioral observations are reported in Table 5. Fish metrics are reported in Table 7.

#### V. CONCLUSION

H-19704 exhibited low toxicity to juvenile bluegill sunfish, Lepomis macrochirus, in an unaerated, static test. The 96-hour LC50 was 634 mg/L (95% fiducial interval of 567 to 725 mg/L) based upon nominal test concentrations.

#### VI. LITERATURE CITED

1. 40 CFR 797.1400. Toxic Substances Control Act. Fish acute toxicity test.
2. Finney, D. J. 1971. Probit Analysis. 3rd Ed., Cambridge Univ. Press. 333 p.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water<sup>1</sup>

| Parameter                          | Concentration | Parameter             | Concentration   |
|------------------------------------|---------------|-----------------------|-----------------|
| BOD, mg/L                          | 3             | Lead, mg/L            | <0.1            |
| COD, mg/L                          | <7            | Magnesium, mg/L       | 3.8             |
| DOC, mg/L                          | 0.6           | MBAS/LAS, mg/L        | <0.02           |
| TOC <sup>2</sup> , mg/L            | 0.5           | Mercury, mg/L         | <0.00020        |
| Total Kjeldahl N, mg/L             | <0.2          | Nickel, mg/L          | <0.05           |
| Ammonia N, mg/L                    | <0.1          | Nitrite, mg/L         | <0.1            |
| Turbidity, NTU                     | <0.5          | Nitrate, mg/L         | 0.5             |
| Phenolics, mg/L                    | <0.005        | Ortho-Phosphate, mg/L | <1              |
| Color, apparent Co/Pt <sup>3</sup> | <5            | Potassium, mg/L       | 1.8             |
| Solids,                            |               | Selenium, mg/L        | <0.005          |
| total suspended, mg/L              | <7            | Silver, mg/L          | <0.02           |
| Aluminum, mg/L                     | <0.2          | Sodium, mg/L          | 8.7             |
| Antimony, mg/L                     | <0.2          | Sulfate, mg/L         | 5.2             |
| Arsenic, mg/L                      | <0.01         | Sulfide, mg/L         | <0.10           |
| Beryllium, mg/L                    | <0.01         | Zinc, mg/L            | <0.04           |
| Boron, mg/L                        | <0.04         |                       |                 |
| Bromide, mg/L                      | <0.5          | Volatile priority     |                 |
| Cadmium, mg/L                      | <0.01         | pollutants            | ND <sup>4</sup> |
| Calcium, mg/L                      | 24.5          | Acid extractable      |                 |
| Chloride, mg/L                     | 6.9           | priority pollutants   | ND <sup>5</sup> |
| Chromium, mg/L                     | <0.05         | Base/neutral          |                 |
| Cobalt, mg/L                       | <0.05         | priority pollutants   | ND <sup>6</sup> |
| Copper, mg/L                       | <0.02         | Pesticides/PCBs       | ND <sup>7</sup> |
| Cyanide, mg/L                      | <0.005        | Organophosphate       |                 |
| Iron, mg/L                         | <0.1          | pesticides, µg/L      | ND <sup>8</sup> |
| Fluoride, mg/L                     | 0.1           |                       |                 |

<sup>1</sup>Date of sample collection 12 May 1993 unless indicated otherwise, analyses performed at Lancaster Laboratories, Lancaster, Pennsylvania; <sup>2</sup>Date of sample collection 23 March 1993, analyses performed at Lancaster Laboratories, Lancaster, Pennsylvania; <sup>3</sup>Units based on cobalt/platinum reference; <sup>4</sup>None detected; level of detection ranged from < 5 to < 100 µg/L; <sup>5</sup>None detected; levels of detection ranged from < 10 to < 25 µg/L; <sup>6</sup>None detected; levels of detection ranged from < 10 to < 100 µg/L; <sup>7</sup>None detected; levels of detection ranged from < 0.01 to 4 µg/L; <sup>8</sup>None detected; levels of detection ranged from < 0.01 to < 0.1 µg/L.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 2

Water Chemistry of the Dilution Water at Test Start

| Total Alkalinity<br>(mg/L as CaCO <sub>3</sub> ) | EDTA Hardness<br>(mg/L as CaCO <sub>3</sub> ) | Conductivity<br>(umhos/cm) |
|--------------------------------------------------|-----------------------------------------------|----------------------------|
| 79                                               | 76                                            | 165                        |

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 3  
Physical and Chemical Characteristics of H-19704 [ ] Test Solutions

| Nominal<br>Concentration<br>(mg/L)   | Hours   |     |     |     |     |     |     |     |     |     |     |     |
|--------------------------------------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                                      | 0       |     |     | 24  |     |     | 48  |     |     | 72  |     |     |
|                                      | A       | B   |     | A   | B   |     | A   | B   |     | A   | B   |     |
| Dissolved Oxygen <sup>b</sup> (mg/L) | Control | 8.5 |     | 7.5 | 7.6 |     | 7.3 | 7.5 |     | 7.1 | 7.0 |     |
|                                      | 262     | 8.5 | 7.8 | 7.6 | 7.8 | 7.9 | 7.6 | 7.8 | 7.5 | 7.3 | 7.1 | 7.0 |
|                                      | 328     | 8.5 | 8.0 | 7.6 | 8.1 | 8.1 | 7.6 | 8.1 | 7.5 | 7.3 | 7.0 | 7.1 |
|                                      | 410     | 8.5 | 7.9 | 7.5 | 7.9 | 7.9 | 7.7 | 7.9 | 7.3 | 7.1 | 7.0 | 6.9 |
|                                      | 512     | 8.5 | 8.0 | 7.7 | 8.0 | 8.0 | 7.7 | 8.0 | 7.3 | 7.2 | 7.0 | 7.0 |
|                                      | 640     | 8.5 | 7.9 | 7.5 | 7.8 | 7.8 | 7.6 | 7.8 | 7.0 | 7.2 | 6.9 | 7.0 |
|                                      | 800     | 8.5 | 8.1 | 7.6 | 8.1 | 8.1 | 7.5 | 8.1 | 6.9 | 7.0 | 6.7 | 6.9 |
| pH                                   | 1000    | 8.5 | 8.2 | 7.7 | 8.1 | 8.1 | 7.6 | 8.2 | 7.0 | 7.3 | 6.7 | 6.8 |
|                                      | Control | 7.4 |     | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.3 |
|                                      | 262     | 7.3 | 7.4 | 7.4 | 7.4 | 7.4 | 7.3 | 7.4 | 7.3 | 7.4 | 7.3 | 7.4 |
|                                      | 328     | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.3 | 7.3 | 7.3 | 7.4 |
|                                      | 410     | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.3 | 7.3 | 7.3 | 7.3 |
|                                      | 512     | 7.2 | 7.1 | 7.3 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 |
|                                      | 640     | 6.9 | 6.9 | 7.2 | 7.3 | 7.3 | 7.2 | 7.3 | 7.2 | 7.2 | 7.2 | 7.2 |
| pH                                   | 800     | 6.9 | 7.3 | 7.2 | 7.3 | 7.3 | 7.2 | 7.3 | 7.1 | 7.3 | 7.3 | 7.2 |
|                                      | 1000    | 7.0 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.1 | 7.3 | 7.3 | 7.2 |

\*All fish had previously died so no reading was made.

<sup>b</sup>Theoretical saturation at 22°C was 8.8 mg/L.

Company Sanitized. Does not contain TSCA CBI

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 3 CONTINUED  
Physical and Chemical Characteristics of H-19704 [REDACTED] Test Solutions

| Nominal<br>Concentration<br>(mg/L) | Hours |      |  |      |      |  |      |      |  |      |      |  |
|------------------------------------|-------|------|--|------|------|--|------|------|--|------|------|--|
|                                    | 0     |      |  | 24   |      |  | 48   |      |  | 72   |      |  |
|                                    | A     | B    |  | A    | B    |  | A    | B    |  | A    | B    |  |
| Control                            | 22.1  | 22.1 |  | 21.6 | 21.7 |  | 21.8 | 21.9 |  | 21.6 | 21.5 |  |
| 262                                | 22.1  | 22.0 |  | 21.6 | 21.6 |  | 21.7 | 21.7 |  | 21.5 | 21.5 |  |
| 328                                | 22.0  | 22.1 |  | 21.6 | 21.6 |  | 21.7 | 21.7 |  | 21.4 | 21.4 |  |
| 410                                | 22.1  | 22.1 |  | 21.7 | 21.6 |  | 21.7 | 21.7 |  | 21.4 | 21.3 |  |
| 512                                | 22.1  | 22.0 |  | 21.7 | 21.6 |  | 21.7 | 21.7 |  | 21.4 | 21.4 |  |
| 640                                | 22.1  | 22.0 |  | 21.6 | 21.7 |  | 21.7 | 21.8 |  | 21.6 | 21.5 |  |
| 800                                | 22.1  | 22.0 |  | 21.6 | 21.6 |  | 21.7 | 21.7 |  | 21.6 | 21.4 |  |
| 1000                               | 22.0  | 22.0 |  | 21.6 | 21.6 |  | 21.8 | 21.8 |  | 21.5 | 21.4 |  |
|                                    |       |      |  |      |      |  |      |      |  | ---  | ---  |  |
|                                    |       |      |  |      |      |  |      |      |  | 21.6 | 21.4 |  |

\*All fish had previously died so no reading was made.

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Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 4

Mortality of Bluegill Sunfish, Lepomis macrochirus,  
Exposed to H-19704 [REDACTED] for 96 Hours  
in a Static, Un aerated Test

| Nominal<br>Concentrations<br>(mg/L) | Mortality in Percent |    |    |    |
|-------------------------------------|----------------------|----|----|----|
|                                     | Time (Hours)         |    |    |    |
|                                     | 24                   | 48 | 72 | 96 |
| Water Control                       |                      |    |    |    |
| A <sup>a</sup>                      | 0                    | 0  | 0  | 0  |
| B <sup>a</sup>                      | 0                    | 0  | 0  | 0  |
| Total                               | 0                    | 0  | 0  | 0  |
| 262                                 |                      |    |    |    |
| A                                   | 0                    | 0  | 0  | 0  |
| B                                   | 10                   | 10 | 10 | 10 |
| Total                               | 5                    | 5  | 5  | 5  |
| 328                                 |                      |    |    |    |
| A                                   | 10                   | 10 | 10 | 10 |
| B                                   | 10                   | 10 | 10 | 10 |
| Total                               | 10                   | 10 | 10 | 10 |
| 410                                 |                      |    |    |    |
| A                                   | 10                   | 10 | 10 | 10 |
| B                                   | 20                   | 20 | 20 | 20 |
| Total                               | 15                   | 15 | 15 | 15 |

<sup>a</sup>A and B represent replicates; each replicate contained 10 fish (total 20 fish per test concentration) at study start.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 4 CONTINUED

Mortality of Bluegill Sunfish, Lepomis macrochirus,  
Exposed to H-19704 ( ) for 96 Hours  
in a Static, Un aerated Test

| Nominal<br>Concentrations<br>(mg/L) | Mortality in Percent |     |     |     |
|-------------------------------------|----------------------|-----|-----|-----|
|                                     | Time (Hours)         |     |     |     |
|                                     | 24                   | 48  | 72  | 96  |
| 512                                 |                      |     |     |     |
| A <sup>a</sup>                      | 30                   | 30  | 30  | 30  |
| B <sup>a</sup>                      | 30                   | 30  | 30  | 30  |
| Total                               | 30                   | 30  | 30  | 30  |
| 640                                 |                      |     |     |     |
| A                                   | 40                   | 40  | 40  | 40  |
| B                                   | 40                   | 40  | 40  | 40  |
| Total                               | 40                   | 40  | 40  | 40  |
| 800                                 |                      |     |     |     |
| A                                   | 70                   | 70  | 70  | 70  |
| B                                   | 60                   | 60  | 60  | 60  |
| Total                               | 65                   | 65  | 65  | 65  |
| 1000                                |                      |     |     |     |
| A                                   | 90                   | 100 | 100 | 100 |
| B                                   | 90                   | 90  | 90  | 90  |
| Total                               | 90                   | 95  | 95  | 95  |

<sup>a</sup>A and B represent replicates; each replicate contained 10 fish (total 20 fish per test concentration) at study start.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 5

Behavior of Bluegill Sunfish, Lepomis macrochirus,  
Exposed to H-19704 [REDACTED] for 96 Hours  
in a Static, Un aerated Test

| Nominal<br>Concentrations<br>(mg/L) | Number Showing Behavior/Total Number of Fish Alive |                   |                   |                   |
|-------------------------------------|----------------------------------------------------|-------------------|-------------------|-------------------|
|                                     | Time (Hours)                                       |                   |                   |                   |
|                                     | 24                                                 | 48                | 72                | 96                |
| Water Control                       |                                                    |                   |                   |                   |
| A                                   | 0/10                                               | 0/10              | 0/10              | 0/10              |
| B                                   | 0/10                                               | 0/10              | 0/10              | 0/10              |
| 262                                 |                                                    |                   |                   |                   |
| A                                   | 0/10                                               | 0/10              | 0/10              | 0/10              |
| B                                   | 0/9                                                | 0/9               | 0/9               | 0/9               |
| 328                                 |                                                    |                   |                   |                   |
| A                                   | 0/9                                                | 0/9               | 0/9               | 0/9               |
| B                                   | 0/9                                                | 0/9               | 0/9               | 0/9               |
| 410                                 |                                                    |                   |                   |                   |
| A                                   | 0/9                                                | 0/9               | 0/9               | 0/9               |
| B                                   | 0/8                                                | 0/8               | 0/8               | 0/8               |
| 512                                 |                                                    |                   |                   |                   |
| A                                   | 0/7                                                | 0/7               | 0/7               | 0/7               |
| B                                   | 0/7                                                | 0/7               | 0/7               | 0/7               |
| 640                                 |                                                    |                   |                   |                   |
| A                                   | 0/6                                                | 0/6               | 0/6               | 0/6               |
| B                                   | 0/6                                                | 0/6               | 0/6               | 0/6               |
| 800                                 |                                                    |                   |                   |                   |
| A                                   | 3 <sup>a</sup> /3                                  | 3 <sup>a</sup> /3 | 3 <sup>a</sup> /3 | 3 <sup>a</sup> /3 |
| B                                   | 4 <sup>a</sup> /4                                  | 4 <sup>a</sup> /4 | 4 <sup>a</sup> /4 | 4 <sup>a</sup> /4 |
| 1000                                |                                                    |                   |                   |                   |
| A                                   | 1 <sup>a</sup> /1                                  | --- <sup>b</sup>  | --- <sup>b</sup>  | --- <sup>b</sup>  |
| B                                   | 1 <sup>a</sup> /1                                  | 1 <sup>a</sup> /1 | 1 <sup>a</sup> /1 | 1 <sup>a</sup> /1 |

<sup>a</sup>Lethargic.

<sup>b</sup>All fish had previously died.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 6

H-19704  Concentrations Causing Mortality to 50% (LC50) of  
Bluegill Sunfish, Lepomis macrochirus

| Time     | LC50<br>(mg/L <sup>a</sup> ) | 95% Fiducial Limits<br>(mg/L <sup>a</sup> ) |     | Slope | Y-Intercept |
|----------|------------------------------|---------------------------------------------|-----|-------|-------------|
| 24 Hours | 647                          | 575                                         | 746 | 4.83  | -13.6       |
| 48 Hours | 634                          | 567                                         | 725 | 5.09  | -14.3       |
| 72 Hours | 634                          | 567                                         | 725 | 5.09  | -14.3       |
| 96 Hours | 634                          | 567                                         | 725 | 5.09  | -14.3       |

<sup>a</sup>Based upon nominal concentrations.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

TABLE 7

Standard Lengths and Wet Weights of Control Fish  
At Study End

| Replicate | Number | Standard Length<br>(cm) | Wet Weight<br>(g) |
|-----------|--------|-------------------------|-------------------|
| A         | 1      | 2.3                     | 0.256             |
| A         | 2      | 2.1                     | 0.194             |
| A         | 3      | 2.1                     | 0.176             |
| A         | 4      | 1.9                     | 0.159             |
| A         | 5      | 2.3                     | 0.262             |
| A         | 6      | 2.3                     | 0.244             |
| A         | 7      | 1.2                     | 0.0451            |
| A         | 8      | 2.6                     | 0.415             |
| A         | 9      | 1.9                     | 0.183             |
| A         | 10     | 2.2                     | 0.224             |
| B         | 1      | 2.8                     | 0.524             |
| B         | 2      | 2.2                     | 0.221             |
| B         | 3      | 2.1                     | 0.226             |
| B         | 4      | 2.1                     | 0.202             |
| B         | 5      | 1.8                     | 0.142             |
| B         | 6      | 1.7                     | 0.100             |
| B         | 7      | 1.6                     | 0.103             |
| B         | 8      | 2.6                     | 0.456             |
| B         | 9      | 2.3                     | 0.269             |
| B         | 10     | 2.0                     | 0.153             |

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

APPENDIX 1

PROTOCOL AMENDMENTS AND SOP DEVIATIONS

PROTOCOL AMENDMENT NUMBER ONE

- a. The ratio between test concentrations is 0.8.
- b. Test concentrations should be assigned to test vessels using random numbers. (Note: Random numbers are also used to assign location of test vessels in water bath and to assign fish to test vessels.)
- c. Take samples for chemical analysis from each replicate before fish are added at test start and at test end. Samples should be refrigerated prior to analysis.
- d. Because of the small size of the bluegills, they will be starved for 24 (not 48) hours.
- e. TOC, COD and particulate analyses of the dilution water will not be performed.
- f. Purity and stock checks will not be performed.
- g. pH and DO readings should be made on all replicates prior to adding fish at study start, daily thereafter, at study end and/or at total mortality in any replicate.
- h. Dead fish in the controls do not need to be weighed and measured.
- i. Test is static (not static renewal).
- j. Measure lengths and weights of all surviving fish in both of the control replicates at study end.

Static, Acute, 96-Hour LC50 of H-19704 To  
Bluegill Sunfish, Lepomis macrochirus

APPENDIX 1 (CONTINUED)

PROTOCOL AMENDMENTS AND SOP DEVIATIONS CONTINUED

PROTOCOL AMENDMENT NUMBER TWO

We planned to measure H-19704 concentrations in each test solution. Samples for analysis were taken and the analyses were attempted. The analyses failed and preliminary research suggested that it was not cost effective to develop a new method. The samples were therefore discarded and nominal concentrations reported. Since the purpose of this study was to provide acute mortality data for designing a bioconcentration test, this procedure was considered acceptable.

In 1994 Haskell Laboratory adopted a new system for handling financial affairs. As a result, it was necessary to develop a new set of test codes for aquatic studies. Hence, the study code (226A) is incorrect. The correct new study code is 226.

PROTOCOL AMENDMENT NUMBER THREE

This study was performed under SOP AQ018-T-002 (not AQ018-T-001 as shown in the upper righthand corner of the Short-Term Study Protocol Sheet).

The current name of the sponsoring business is DuPont Elastomers (the previous name was Polymers for which Poly was the abbreviation which appears on the Short-Term Study Protocol Sheet).

SOP Deviations

- a. Test concentrations were not assigned to test chambers using random numbers.