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Study Title

STATIC, ACUTE, 96-HOUR LC50 OF [redacted]
TO FATHEAD MINNOWS (Pimephales promelas)

Data Requirement

United States Environmental Protection Agency
Environmental Effects Testing Guidelines
Subdivision EG-9

Author

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Study Completed On

September 4, 1991

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, Post Office Box 50
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Laboratory Project ID

Haskell Laboratory Report No. 395-91

[redacted]

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to United States Environmental Protection Agency's Good Laboratory Practice Regulations for TSCA (40 CFR 792). Areas of non-compliance are documented in the study records. No deviations existed that affected the validity of the study.

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

Sponsor: Du Pont Chemicals

Study Director: Kevin N. Baer 9/4/91
Mo/Day/Year

Kevin N. Baer, Ph.D.
Research Toxicologist
Environmental Sciences

QUALITY ASSURANCE DOCUMENTATION

STUDY: [redacted] H# 18,790

STATIC, ACUTE, 96-HOUR LC50 OF [redacted] TO FATHEAD MINNOWS (Pimephales promelas)

AUDITS:

Items Audited	Audit Dates
In-life Observations	5/15/91
Protocol, Records and Final Report	7/22,23/91

DATE FINDINGS REPORTED TO MANAGEMENT AND STUDY DIRECTOR: 7/23/91

Reported by: Joseph C. Hamill 8/28/91
Joseph C. Hamill
Senior Auditor
Quality Assurance
Date

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I. SUMMARY

[redacted] was not acutely toxic to juvenile, fathead minnows (Pimephales promelas) in a 96-hour, aerated, static test. Nominal test concentrations were 648, 1080, 1800, 3000, and 5000 mg/L. The 96-hour LC50 based on nominal concentrations was greater than 5000 mg/L, the highest concentration tested.

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THIS REPORT IS APPROVED FOR ISSUE.

Kevin N. Baer — 9/4/91
Kevin N. Baer, Ph.D.
Study Director
Mo/Day/Year

II. GENERAL INFORMATION

Material Tested: [redacted]

Medical Research Number: [redacted]

Haskell Number: 18,790

Haskell Laboratory Test Code: [redacted]

Other Codes: [redacted]

Synonyms: [redacted]

Purity: [redacted]

Composition: [redacted]

Contaminants: [redacted]

Sponsor: Du Pont Chemicals
E. I. du Pont de Nemours and Company
Wilmington, Delavare 19898

Submitted By: [redacted]
Du Pont Chemicals
Chambers Works
New Jersey

Study Schedule: Study Initiation Date: 5/14/91
Experimental Start Date: 5/15/91
Experimental Termination Date: 5/19/91
Study Completion Date: 9/4/91

Notebooks: [redacted]

Distribution: [redacted]

III. PURPOSE

The goal of this study was to assess the acute toxicity of [redacted] to juvenile, fathead minnows (*Pimephales promelas*).

IV. RECORDS

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

V. MATERIALS AND METHODS

A. Test Material

Source, composition, and purity of th test substance are described under General Information. The appropriate quantity of [redacted] was measured and dispensed into 4-L of dilution water (see below) to prepare each test concentration. Stability and solubility of [redacted] in dilution water were not determined.

B. Dilution Water

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

C. Animal Culture

Fathead minnows (Pimephales promelas) were reared in continuously flowing well water at Haskell Laboratory. Adult fish resided in 38 L aquaria at approximately 25°C and were fed frozen brine shrimp (Artemia sp., Golden Gate Brand, Korden Division, Novalek, Inc., Hayward, CA) generally twice daily, once on Saturday, and not on Sundays. Eggs were deposited on polyvinyl chloride tiles coated with sand. Tiles with eggs were aerated and incubated at approximately 25°C in a water bath. Larvae were reared in 22 L aquaria at approximately 22°C and were fed newly hatched Artemia generally twice daily on weekdays and daily on weekends. Juveniles were held in a 22 L aquaria at approximately 22°C and were fed newly hatched Artemia and/or frozen brine shrimp generally twice daily on weekdays and once on Saturday and Sundays. No treatment of the fish for diseases was required during the holding period. Sickness, injury, and abnormality were not observed. Animals were identified by labels on aquaria and test vessels.

D. Test Methods [1]

Five test concentrations and a water control were used. Nominal test concentrations were 648, 1080, 1800, 3000, and 5000 mg/L. The fathead minnows ranged from 1.5 to 2.4 cm in standard length (mean 1.9 cm) and 0.029 to 0.16 g in weight (mean 0.092 g). The fathead minnows were 85-86 days old at test start.

Glass aquaria (5-L) containing 4 L of test solution (approximately 12-cm liquid depth) were employed. Two replicates of each test concentration with ten fish per relicate were used. Fish were added to the test concentrations using random numbers and were not fed for 48 hours prior to the test nor during the test. Mean loading for controls at test conclusion was 0.23 g/L. Test solutions were held between 20.7 and 21.6°C (mean 21.2°C) and were unaerated, until at approximately 72 hours when low dissolved oxygen levels necessitated the use of gentle aeration (approximately 120 bubbles/minute). A photoperiod of 16 hours light (215 Lux) versus 8 hours darkness was employed.

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 stem thermometer) were measured in all test concentrations and the water control at the beginning of the test (before addition of fish) and daily thereafter. Total alkalinity (Hach kit, Hach Chemical Company, Loveland, CO), EDTA hardness (Hach kit, Hach Chemical Company, Loveland, CO), and conductivity (YSI Model 33, Yellow Springs Instrument, Yellow Springs, OH) of the dilution water were measured at the beginning of the test. Total organic carbon (Model 700 OI TOC analyzer) was measured in the dilution water at 48 hours.

E. Chemical Analysis

Chemical analysis of the test material was not conducted.

F. Statistical Methods

The 96-hour LC50 and its associated confidence interval could not be calculated because mortality was not observed at any test concentration.

VI. RESULTS AND DISCUSSION

A 96-hour rangefinding study was conducted using the following concentrations; water control, 1.0, 10, 100, and 1000 mg/L. Mortality was not observed at any test concentration.

Nominal [redacted] concentrations were 648, 1080, 1800, 3000, and 5000 mg/L. All test concentrations (excluding control) were cloudy throughout the testing period.

All chemical and physical parameters (Tables 2 and 3) were within expected ranges, except dissolved oxygen in replicate A of the 5000 mg/L test concentration at 72 hours. The dissolved oxygen value (5.3 mg/L) dropped slightly below 60% of the theoretical saturation value of 9.0 mg/L at 21°C. As a result, all test concentrations were gently aerated beginning at 72 hours (approximately 120 bubbles/minute). This apparent decrease in dissolved oxygen was not considered biologically significant based on the lack of observed mortality. No mortality or sublethal effects were seen in any test concentration (Table 4). The 96-hour LC50 based on nominal concentrations was greater than 5000 mg/L, the highest concentration tested.

VII. CONCLUSION

[redacted] was not acutely toxic to juvenile, fathead minnows (Pimephales promelas) in a 96-hour aerated, static, test. The 96-hour LC50 based on nominal concentrations was greater than 5000 mg/L, the highest concentration tested.

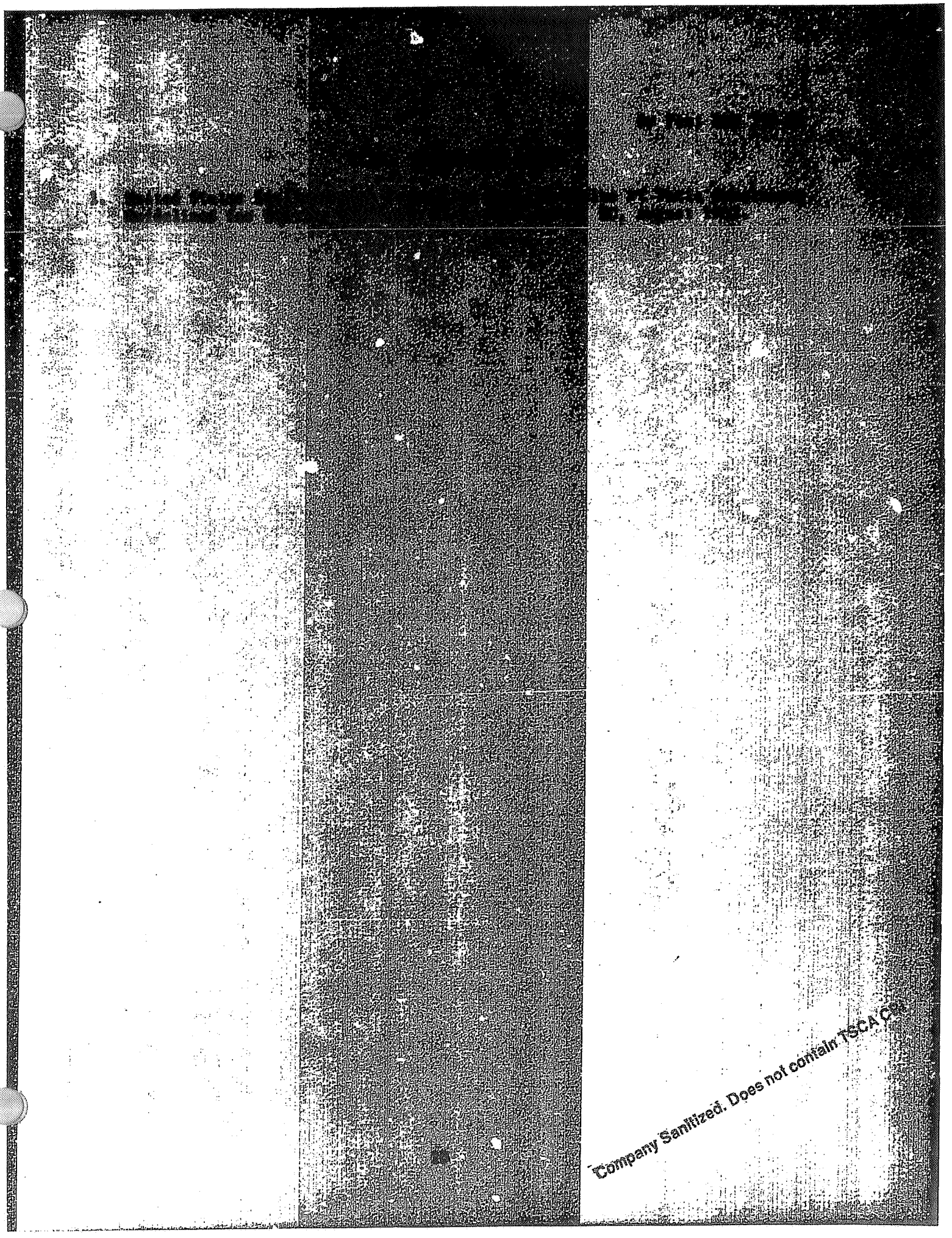
Company Sanitized. Does not contain TSCA CBI

TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water[1]

[redacted]

Parameter	Concentration	Parameter	Concentration
BOD[2], mg/L	<2	Lead[5], mg/L	<0.005
COD, mg/L	13	Magnesium, mg/L	3.5
DOC[3], mg/L	1.5	MBAS/LAS, mg/L	<0.05
TOC[4], mg/L	<0.5	Mercury, mg/L	<0.00020
Kjeldahl N, mg/L	0.2	Nickel, mg/L	<0.020
Ammonia N, mg/L	<0.05	Nitrite[6], mg/L	<0.1
Turbidity, NTU	<1.0	Nitrate[6], mg/L	1.6
Phenolics, mg/L	<0.050	Phosphate[6], mg/L	<0.1
Color, Co/Pt	<5	Potassium, mg/L	1.8
Solids		Selenium, mg/L	<0.005
total suspended, mg/L	<2	Silver, mg/L	<0.010
Aluminum, mg/L	<0.100	Sodium, mg/L	8.1
Antimony, mg/L	<0.060	Sulfate[6], mg/L	4.8
Arsenic, mg/L	<0.010	Sulfide, mg/L	<0.05
Beryllium, mg/L	<0.001	Thallium, mg/L	<0.01
Boron[5], mg/L	<0.020	Zinc[5], mg/L	<0.020
Cadmium, mg/L	<0.002	Volatile priority	
Calcium, mg/L	25.0	pollutants	ND[8]
Chloride[6], mg/L	6.3	Acid extractable	
Chromium, mg/L	<0.010	priority pollutants	ND[8]
Cobalt, mg/L	<0.020	Base/neutral	
Copper, mg/L	<0.010	priority pollutants	ND[8]
Cyanide, mg/L	<0.025	Pesticides/PCBs	ND[8]
Iron[6], mg/L	0.053	Organophosphate	
Fluoride[6], mg/L	<0.1	pesticides[7], µg/L	<0.50

[1]Date of analyses 17 October 1990 unless indicated otherwise, analyses performed at Environmental Testing and Certification Corporation, Edison, New Jersey; [2]Date of analyses 13 December 1990, analyses analysis performed at Environmental Testing and Certification Corporation, Edison, New Jersey; [3]Mean value; [4]Date of analyses 17 May 1991, performed at Du Pont Engineering Test Center, Newark, Delaware; [5]Below the Published Method Detection Limit (BMDL); [6]Date of analyses 9 November 1990, analyses performed at Du Pont Chemicals, Jackson Laboratory, Deepwater, New Jersey; [7]Date of analyses 23 October 1990, analyses performed at Hazleton Laboratories, Inc., Madison, WI; [8]None detected.

TABLE 2

Water Chemistry of the Dilution Water at Test Start

[redacted]

Total Alkalinity (mg/L as $CaCO_3$)	EDTA Hardness (mg/L as $CaCO_3$)	Conductivity (µmhos/cm)
82	78	160

[redacted] CBI

TABLE 3

Physical and Chemical Characteristics of [redacted] Test Solutions

Time (Hours)	Water Control A[a]	Water Control B[a]	Nominal Concentrations (mg/L) 648 A[a]	648 B[a]	1080 A[a]	1080 B[a]	1800 A[a]	1800 B[a]	3000 A[a]	3000 B[a]	5000 A[a]	5000 B[a]
Dissolved oxygen (mg/L)												
Start	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.7	8.7	8.5	8.6
24	7.9	7.7	7.7	7.4	7.4	7.6	7.2	7.7	7.6	7.5	7.8	7.7
48	7.7	7.7	7.2	7.3	7.1	6.9	6.4	7.1	6.6	6.4	6.7	6.0
72	7.6	7.5	7.2	7.3	6.9	6.8	6.7	6.8	5.9	6.2	5.3	5.5
96	8.2	8.0	7.7	7.6	7.3	7.2	7.6	6.9	6.3	6.7	6.1	6.7
pH												
Start	7.3	7.3	7.3	7.3	7.3	7.4	7.4	7.4	7.4	7.4	7.3	7.4
24	7.1	7.2	7.2	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
48	7.2	7.4	7.4	7.4	7.4	7.4	7.4	7.5	7.5	7.5	7.5	7.5
72	8.1	8.1	8.2	8.1	8.1	8.1	8.0	8.1	8.1	8.0	7.9	7.9
96	8.3	8.3	8.2	8.2	8.1	8.1	8.1	8.0	8.0	8.0	8.0	7.9

[a]A and B refer to replicates with ten fish each.

TABLE 4

Observed Mortality of Fathead Minnows Exposed to [redacted]
for 96 Hours in a Static, Aerated Test
[redacted]

Concentrations (mg/L)	Percent Cumulative Observed Mortality[a]							
	24 Hours		48 Hours		72 Hours		96 Hours	
	A[b]	B[b]	A[b]	B[b]	A[b]	B[b]	A[b]	B[b]
Water Control	0	0	0	0	0	0	0	0
648	0	0	0	0	0	0	0	0
1080	0	0	0	0	0	0	0	0
1800	0	0	0	0	0	0	0	0
3000	0	0	0	0	0	0	0	0
5000	0	0	0	0	0	0	0	0

[a]All fish appeared normal.

[b]A and B refer to replicates with ten fish each.