

TRADE SECRET

Study Title

STATIC, ACUTE, 96-HOUR LC50 OF H-19,517
TO FATHEAD MINNOWS, *Pimephales promelas*

Data Requirement

Organisation for Economic Co-Operation and Development (OECD)
Guideline for Testing Chemicals: 203

Author

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Study Completed On

November 6, 1992

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
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Laboratory Project ID

Haskell Laboratory Report No. 592-92

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to EPA FIFRA and TSCA Good Laboratory Practice Standards (40 CFR 160 and 40 CFR 792), OECD Principles of Good Laboratory Practice (C(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850) with the two exceptions noted below. Solubility and stability of the test substance in dilution water and under storage conditions were not determined. Concentrations of the test substance in dilution water were measured, but these measurements were not made in accordance with Good Laboratory Practice Standards. Protocol amendments and/or SOP deviations appear in Appendix I. Areas of noncompliance (if any) are also documented in the study records. No deviations existed that affected the scientific validity of the study.

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

Sponsor: Du Pont Chemicals
E. I. du Pont de Nemours and Company

STUDY DIRECTOR:

Keith B. Pierson
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6 Nov 1992
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GENERAL INFORMATION

Material Tested:

[REDACTED]

Synonyms:

[REDACTED]

[REDACTED]

Haskell Number:

H-19,517

Purity:

[REDACTED]

Composition:

[REDACTED]

Contaminants:

• Not supplied by sponsor

CAS Registry Number:

None available for this mixture

[REDACTED]

Sponsor:

Du Pont Chemicals
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898

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Du Pont HLR 592-92

Study Initiated/Completed: 7-17-92 / 11-6-92

In-Life Study
Initiated/Completed: 7-23-92 / 8-9-92

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STATIC, ACUTE, 96-HOUR LC50 OF H-19,517
TO FATHEAD MINNOWS, *Pimephales promelas*

SUMMARY

H-19,517 exhibited moderate toxicity in a 96-hour, unaerated, static, acute test using fathead minnows, *Pimephales promelas*. Nominal H-19,517 concentrations were 0, 0.409, 1.02, 2.59, 6.41, 16.0, 40.0, and 100 mg/L. H-19,517 measured concentrations at 96 hours were 0.00, 0.16, 0.55, 1.88, 4.03, 7.46, 15.78, and 48.11 mg/L. The 96-hour LC50 based on measured concentrations at 96 hours was 4.72 mg/L with a 95% confidence interval of 3.55 to 6.02 mg/L.

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THIS REPORT IS APPROVED FOR ISSUE.

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QUALITY ASSURANCE DOCUMENTATION

Dates of Inspection:

Conduct - 7/23/92; 8/5/92

Records, Report(s) - 10/27 - 30/92

Findings reported to:

Study Director - 10/30/92

Management - 11/6/92

Reported by:

Deborah A. Vick

Deborah A. Vick
Quality Assurance Auditor

11/6/92

Date

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I. PURPOSE

The goal of this study was to assess the acute toxicity of H-19,517 to fathead minnows, *Pimephales promelas*.

II. 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).

III. MATERIALS AND METHODS

A. Test Substance

The test substance is described under General Information. The test substance was added to dilution water (see below) and stirred for 30 minutes to create a stock solution. The stock solution was added to dilution water in appropriate volumes to make individual test solutions. Stability and solubility of H-19,517 in dilution water and under storage conditions 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 39 L aquaria at approximately 25°C and were fed frozen brine shrimp (*Artemia* sp., Golden Gate Brand, Kordon Division, Novalek, Inc., Hayward, CA) generally twice daily, once on Saturday and once on Sunday. 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 39 L aquaria at approximately 21°C and were fed newly hatched *Artemia* sp. (Aquarium Products, Glen Burnie, MD) generally twice daily on weekdays and daily on weekends. Juveniles were held either in 272-L circular, fiberglass tanks or in 39 L glass aquaria (23-cm depth; 50 (L) x 25 (W) x 31 cm (H)) at approximately 21°C and were fed newly hatched *Artemia* sp. and/or frozen brine shrimp generally twice daily on weekdays, once on Saturday and once on Sunday. Sickness, injury, and abnormality were not observed. Animals were identified by labels on aquaria and test vessels.

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D. Test Methods [1]

Seven test concentrations and a water control were used. Nominal concentrations were 0, 0.409, 1.02, 2.59, 6.41, 16.0, 40.0, and 100 mg/L. Control fish ranged from 1.7 to 2.4 cm standard length (mean 1.9 cm) and 0.11 to 0.25 g wet weight (mean 0.18 g).

Erlenmeyer glass flasks [4.4 liter; 21 (base) x 35 (height) cm] containing 4.4 L of test solution (35-cm depth) were employed as test chambers. Each flask was totally filled to minimize head space and was covered with Saran Wrap® to prevent loss of potentially volatile test substance components. The position of test chambers in the water bath was determined by use of random numbers. Ten fish were added to each replicate using random numbers (one replicate per concentration, total 10 fish per concentration). Control loading was 0.40 g/L at test conclusion. Test solutions ranged from 20.7 to 21.5°C (mean 21.0°C). Fish were not fed 27 hours prior to nor during the test. A photoperiod of 16 hours light (172 to 215 Lux) versus 8 hours darkness was employed with 25 minutes of transitional light (2 to 3 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) and pH (Corning Model 610A, Corning Scientific Instruments, Medfield, MA) were measured in the water control and all test concentrations before fish were added at the beginning of the test and daily thereafter. Temperature was measured (American Society of Testing Materials registered mercury thermometer) in the water control and all test concentrations before fish were added at the beginning of the test, daily thereafter, and at the end of the test. 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 Instruments, Yellow Springs, OH) of the dilution water were measured at the beginning of the test. A continuously-recording thermometer in a control vessel was used to check for temperature variation during the 96-hour test.

E. Chemical Analysis

Analyses were conducted after the test had ended at the Du Pont Jackson Laboratory at Chambers Works, Deepwater, NJ. Samples and standards were completely combusted in a hydrogen/oxygen Wickbold Torch. The resulting water was then analyzed for total fluoride concentration using an Orion fluoride ion selective electrode. Concentrations were then calculated from the standards using either a three or four point calibration curve.

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F. Statistical Analysis

The 24-, 48-, 72-, and 96-hour LC50s were calculated by using the moving average angle technique [2].

IV. RESULTS AND DISCUSSION

A rangefinding test with five fish per concentration (4.4 L Erlenmeyer flask filled to the top) was conducted with a water control and test concentrations of 0.1, 1, 10, 100, and 1000 mg/L. Mortalities were 0, 0, 0, 60, 100, and 100%, respectively.

Two definitive studies were conducted. Data from the first were discarded because of low dissolved oxygen concentrations, and data from the second are reported herein.

Nominal concentrations in the definitive test were 0, 0.409, 1.02, 2.59, 6.41, 16.0, 40.0, and 100 mg/L. Measured concentrations at 96 hours were 0.00, 0.16, 0.55, 1.88, 4.03, 7.46, 15.78 and 48.11 mg/L. The discrepancy between the measured and nominal concentrations (Table 2) may stem from the use of a stock solution in which the test substance was not totally dissolved. All chemical and physical parameters in the final definitive test (Tables 3 and 4) were within expected ranges with one exception. The dissolved oxygen concentration in the control and 4.03 mg/L test solutions on day 4 dropped to 58 and 56% of saturation, respectively. This observation was not of biological significance since fathead minnows can survive at much lower dissolved oxygen concentrations for extended periods. Behavioral observations appear in Table 6. The 96-hour LC50 based on measured concentrations at 96 hours was 4.72 mg/L with a 95% confidence interval of 3.55-6.02 mg/L (Tables 5 and 7).

V. CONCLUSION

H-19,517 was moderately toxic to fathead minnows, *Pimephales promelas*, in a 96-hour, static, unaerated test.

VI. LITERATURE CITED

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for Testing Chemicals: 203. 4 April 1984.
2. Peltier, W. H. and C. I. Weber, Eds. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. EPA/600/4-85/013. United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. Appendix E.

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TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water¹

Parameter	Concentration	Parameter	Concentration
BOD, mg/L	<2	Lead, mg/L	<0.075
COD, mg/L	<10	Magnesium, mg/L	3.7
DOC, mg/L	1.4	MBAS/LAS, mg/L	<0.05
TOC ² , mg/L	2.9	Mercury, mg/L	<0.00020
Total Kjeldahl N, mg/L	0.8	Nickel, mg/L	<0.020
Ammonia N, mg/L	<0.2	Nitrite ³ , mg/L	<0.1
Turbidity, NTU	<1.0	Nitrate ³ , mg/L	1.9
Phenolics, mg/L	<0.050	Phosphate ³ , mg/L	<0.1
Color, apparent Co/Pt ⁷	<5	Potassium, mg/L	1.7
Solids		Selenium, mg/L	<0.005
Total suspended, mg/L	<4	Silver, mg/L	<0.010
Aluminum, mg/L	<0.100	Sodium, mg/L	8.2
Antimony, mg/L	<0.060	Sulfate ³ , mg/L	5.0
Arsenic, mg/L	<0.010	Sulfide, mg/L	<0.10
Beryllium, mg/L	<0.001	Zinc, mg/L	<0.058
Boron ⁴ , mg/L	0.020	Volatile priority	
Cadmium ⁴ , mg/L	<0.002	pollutants	ND ⁶
Calcium, mg/L	24.3	Acid extractable	
Chloride ³ , mg/L	6.2	priority pollutants	ND ⁶
Chromium, mg/L	<0.010	Base/neutral	
Cobalt, mg/L	<0.020	priority pollutants	ND ⁶
Copper, mg/L	<0.010	Pesticides/PCBs	ND ⁶
Cyanide, mg/L	<0.025	Organophosphate	
Iron ³ , mg/L	0.001	pesticides ⁵ , µg/L	<0.50
Fluoride ³ , mg/L	<0.1		

¹Date of sample collection 26 May 1992 unless indicated otherwise. analyses performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ²Date of sample collection 1 July 1992, performed at Du Pont Engineering Test Center, Newark, Delaware; ³Date of collection 10 June 1992, analyses performed at Du Pont Chemicals, Jackson Laboratory, Deepwater, New Jersey; ⁴Below the Published Method Detection Limit (BMDL); ⁵Analyses performed at Hazleton Laboratories, Inc., Madison, WI; ⁶None detected; ⁷Units based on cobalt/platinum reference.

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TABLE 2

Measured Concentrations of H-19,517 in a
96-Hour, Static, Un aerated Test

Nominal Concentration (mg/L)	Measured Concentration (mg/L)	
	Start	End
Water Control	0.00	0.00
0.409	0.40	0.16
1.02	0.76	0.55
2.59	2.38	1.88
6.41	3.87	4.03
16.0	9.86	7.46 ^a
40.0	18.85	15.78 ^a
100	53.76	48.11 ^a

^aSamples taken at time of total mortality.

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TABLE 3

Water Chemistry of the Dilution Water at Test Start

Total Alkalinity (mg/L as CaCO ₃)	EDTA Hardness (mg/L as CaCO ₃)	Conductivity (μmhos/cm)
79	77	170

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TABLE 4

Physical and Chemical Characteristics of H-19,517
Test Solutions

Measured Concentration (mg/L)	Time				
	0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
<u>Dissolved Oxygen, mg/L</u>					
H ₂ O Control	8.4	7.0	6.4	6.0	5.2
0.16	8.4	7.0	6.4	6.1	6.0
0.55	8.4	7.2	6.7	6.3	5.7
1.88	8.4	7.2	6.6	6.3	5.4
4.03	8.5	7.4	6.9	6.4	5.0
7.46	8.5	7.7	a	a	a
15.78	8.4	8.0	a	a	a
48.11	8.4	8.0	a	a	a
<u>pH</u>					
H ₂ O Control	7.6	7.4	7.4	7.4	7.0
0.16	7.6	7.4	7.4	7.4	7.0
0.55	7.6	7.4	7.4	7.4	7.1
1.88	7.6	7.4	7.4	7.4	7.1
4.03	7.7	7.4	7.4	7.4	7.1
7.46	7.6	7.4	a	a	a
15.78	7.6	7.5	a	a	a
48.11	7.6	7.5	a	a	a

^aReading was not made due to total mortality.

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STATIC, ACUTE, 96-HOUR LC50 OF H-19,517
TO FATHEAD MINNOWS, *Pimephales promelas*

TABLE 4, Continued

Physical and Chemical Characteristics of H-19,517
Test Solutions

Measured Concentration (mg/L)	Time				
	0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
<u>Temperature, °C</u>					
H ₂ O Control	20.7	20.9	20.8	21.5	21.4
0.16	20.7	20.9	20.8	21.5	21.4
0.55	20.8	20.9	20.8	21.5	21.4
1.88	20.8	20.9	20.8	21.5	21.4
4.03	20.7	20.9	20.8	21.5	21.4
7.46	20.8	20.9	a	a	a
15.73	20.8	20.9	a	a	a
48.11	20.8	20.9	a	a	a

^aReading was not made due to total mortality.

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STATIC, ACUTE, 96-HOUR LC50 OF H-19,517
TO FATHEAD MINNOWS, *Pimephales promelas*

TABLE 5

Observed Mortality of Fathead Minnows, *Pimephales promelas*,
Exposed to H-19,517 for 96 Hours in a Static, Un aerated Test

Measured Concentration (mg/L)	Cumulative Mortality in Percent ^a			
	24 Hours	48 Hours	72 Hours	96 Hours
Water Control	0	0	0	0
0.16	0	0	0	0
0.55	0	0	0	0
1.88	0	0	0	0
4.03	0	20	20	20
7.46	100	100	100	100
15.78	100	100	100	100
48.11	100	100	100	100

^aThere were ten fish per test concentration at test start.

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STATIC, ACUTE, 96-HOUR LC50 OF H-19,517
TO FATHEAD MINNOWS, *Pimephales promelas*

TABLE 6

Observed Effects of H-19,517 on Fathead Minnows, *Pimephales promelas*,
for 96 Hours in a Static, Un aerated Test

Measured Concentrations (mg/L)	Number of Fish With Effect			
	24 Hours	48 Hours	72 Hours	96 Hours
Water Control	0/10	0/10	0/10	0/10
0.16	0/10	0/10	0/10	0/10
0.55	0/10	0/10	0/10	0/10
1.88	0/10	0/10	0/10	0/10
4.03	1 ^a /10 1 ^b /10	3 ^b /8	3 ^b /8	3 ^b /8
7.46	c	c	c	c
15.78	c	c	c	c
48.11	c	c	c	c

^aLying on bottom of the test vessels.

^bPartial loss of equilibrium.

^cTotal mortality.

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TABLE 7

H-19,517 Concentrations Causing Mortality to 50% (LC50) of
Fathead Minnows, *Pimephales promelas*, at Specific Time Intervals

Time	LC50 (mg/L ^a)	95% Confidence Limits (mg/L ^a)		Slope	Y-intercept
24 Hours	5.48	4.24	7.09	N/A ^b	N/A
48 Hours	4.72	3.55	6.02	N/A	N/A
72 Hours	4.72	3.55	6.02	N/A	N/A
96 Hours	4.72	3.55	6.02	N/A	N/A

^aBased upon measured concentrations at 96 hours.^bNot applicable. Slope and Y-intercept not calculated by the moving average angle technique

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APPENDIX 1

PROTOCOL AMENDMENTS and SOP DEVIATIONS

PROTOCOL AMENDMENT NUMBER ONE

- a. It is not necessary to weigh fish and determine test chamber loading prior to test start.
- b. Stability and solubility of the test substance in dilution water and under storage conditions will not be determined.
- c. Samples will be taken from each replicate at test start and end for chemical analysis of the test substance concentration.
- d. At test end surviving fish will be sacrificed according to aquatic SOP AQ029-P-002.
- e. Fish will be starved for 24 hours prior to test start.

PROTOCOL AMENDMENT NUMBER TWO

- a. The test substance concentrations were changed to facilitate the weighing process and will be: 0.409, 1.02, 2.59, 6.41, 16.0, 40.0, and 100 mg/L.

SOP DEVIATIONS

- a. Temperature of water bath was not recorded.
- b. Temperatures and times to equilibria of individual test containers were not recorded.
- c. Maintenance was not performed on balance L1642 when calibration was out of Standard Operating Procedure range.
- d. Which pH calibration buffers were used was not recorded during pH meter calibration on 7-25-92, 7-26-92, 7-27-92, and 8-5-92 through 8-9-92.
- e. Study Director not notified on 8-9-92 of low dissolved oxygen concentration in some test solution.

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