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Study Title

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

Data Requirement

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

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Study Completed On

2 July 1991

Performing Laboratory

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Laboratory Project ID

Haskell Laboratory Report No. 448-91

[redacted]

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to United States Environmental Protection Agency Good Laboratory Practice Regulations for FIFRA (40 CFR 160) and/or TSCA (40 CFR 792). Areas of non-compliance are documented in the study records. No deviations existed that significantly affected the validity of the study.

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

Sponsor: Du Pont Chemicals

Study Director: Keith B. Pierson — 24 June 1991

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QUALITY ASSURANCE DOCUMENTATION

STUDY: [redacted] H# 19,011 [redacted] STATIC, ACUTE 96-HOUR LC50 OF [redacted] TO FATHEAD MINNOWS (Pimephales promelas)

AUDITS:

Items Audited	Audit Dates
Protocol, conduct	6/14/91
Records, final report	6/27,28/91

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Reported by: James Mackay II 7/2/91
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I. SUMMARY

[redacted] was moderately toxic in a 96-hour, unaerated, static test using juvenile fathead minnows (Pimephales promelas). Nominal test concentrations were 7.8, 13, 22, 36, 60, and 100 mg/L. The 96-hour LC50 based on nominal concentrations was 19 mg/L with 95% fiducial limits of 15 to 25 mg/L.

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THIS REPORT IS APPROVED FOR ISSUE.

Keith B. Pierson — 2 JULY 1991
Keith B. Pierson, Ph.D.
Study Director
Day/Mo/Year

II. GENERAL INFORMATION

Material Tested:	[redacted]
Medical Research Number:	[redacted]
Haskell Number:	19,011
Haskell Laboratory Test Code:	[redacted]
CAS Registry Number:	[redacted]
Other Codes:	None
Synonyms:	None
Composition:	[redacted]
Purity:	Not supplied by sponsor
Sponsor:	Du Pont Chemicals E. I. du Pont de Nemours and Company Wilmington, Delavare 19898
Test Requested By:	[redacted] Du Pont Chemicals E. I. du Pont de Nemours and Company 1007 Market Street Wilmington, Delavare 19898
Study Schedule:	Study Initiation Date: 12 June 1991 Experimental Start Date: 14 June 1991 Experimental Termination Date: 18 June 1991 Study Completion Date: 2 July 1991
Notebooks:	[redacted]

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III. PURPOSE

The purpose 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 Materials

Source and composition of the test substance are described under General Information. Appropriate quantities of [redacted] were weighed and dissolved in dilution water (see below) in the test aquaria. 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, Kordon 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 sp. (Aquarium Products, Glen Burnie, MD) generally twice daily on weekdays and daily on weekends. Juveniles were held in 272-L circular, fiberglass tanks (49-cm depth; 84-cm diameter) at approximately 20°C and were fed newly hatched Artemia sp. and/or frozen brine shrimp generally twice daily on weekdays and once on Saturday and Sundays. Sickness, injury, and abnormality were not observed. Animals were identified by labels on aquaria and test vessels.

D. Test Methods [1]

Six test concentrations and a water control were used. Nominal concentrations were 7.8, 13, 22, 36, 60, and 100 mg/L. The control fish (Table 2) ranged from 1.0 to 1.6 cm in total length (mean 1.4 cm), 0.09 to 1.4 cm in standard length (mean 1.2), and 0.0061 to 0.023 g in weight (mean 0.015 g). The fathead minnows were 38 days old at test start.

Glass aquaria (23 [l] x 16 [w] x 14 cm [h]; 5-L) containing 4 L of test solution (12-cm depth) were employed. Ten fish were added to each replicate using random numbers (one replicate per concentration; total 10 fish per test concentration). Loading was 0.038 g/L. Test solutions ranged from 21.4 to 21.9°C (mean 21.7°C) and were unaerated. Fish were not fed 48 hours prior to nor during the test. A photoperiod of 16 hours light (409-452 lux) versus 8 hours darkness was employed. Observations were made 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 in all test concentrations before fish were added at the beginning of the test and daily thereafter. 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 dilution water were measured at the beginning of the test. Temperatures in the water control were taken daily with an American Society of Testing Materials registered mercury thermometer.

E. Chemical Analysis

Samples for chemical analysis were not taken at the request of the study sponsor.

F. Statistical Analysis

Median lethal concentrations and their associated 95% fiducial intervals were calculated using probit analysis [2].

VI. RESULTS AND DISCUSSION

A 96-hr rangefinder was conducted with five fish in 2-L test solution in a 2-L glass beaker at each test concentration. Total mortality occurred at 1000 and 100, 20% at 10, and 0% at 1.0 and 0.1 mg/L, respectively.

Nominal concentrations in the definitive test were 7.8, 13, 22, 36, 60, and 100 mg/L. All chemical and physical parameters (Tables 3 and 4) were within expected ranges. Behavioral observations are summarized in Table 5. The 24-, 48-, 72-, and 96-hour LC50s based on nominal concentrations and observed mortalities (Table 6) were 26 (21-31), 26 (21-31), 20 (16-26), and 19 (15-25) mg/L, respectively.

VII. CONCLUSION

[redacted] was moderately toxic in a 96-hour, unaerated, static test using juvenile fathead minnows (Pimephales promelas).

VIII. LITERATURE CITED

1. OECD Guideline for Testing of Chemicals: 203, 4 April 1984.

2. Finney, D. J. 1971. Probit Analysis. 3rd Ed. Cambridge Univ. Press. 333 p.

TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water[1]

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 24 July 1990, 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 Hazelton Laboratories, Inc., Madison, WI; [8]None detected.

TABLE 2

Lengths and Weights of Control Fathead Minnows (Pimephales promelas) at Test Conclusion

Number	Total Length (cm)	Standard Length (cm)	Wet Weight (g)
1	1.5	1.3	0.019
2	1.3	1.2	0.012
3	1.6	1.4	0.019
4	1.6	1.4	0.023
5	1.6	1.4	0.022
6	1.4	1.2	0.015
7	1.1	0.9	0.011
8	1.0	0.9	0.0061
9	1.2	1.0	0.0087
10	1.3	1.1	0.011

TABLE 3

Water Chemistry of Haskell Laboratory Dilution Water at Test Start

Total Alkalinity (mg/L as $CaCO_3$)	EDTA Hardness (mg/L as $CaCO_3$)	Conductivity (µmhos/cm)
72	83	170

TABLE 4
Physical and Chemical Characteristics of [redacted]
Haskell Number 19,011) Test Solutions

Nominal Concentrations (mg/L)	Time (hours)														
	pH					Dissolved Oxygen (mg/L)					Temperature (°C)				
	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Water Control	7.5	7.2	7.7	7.8	7.9	8.9	9.1	8.2	8.5	8.5	21.4	21.7	21.9	21.8	21.7
7.8	7.5	7.3	7.7	7.8	7.9	8.9	9.2	8.2	8.5	8.4	N[b]	N	N	N	N
13	7.5	7.4	7.7	7.8	7.9	8.8	9.2	7.8	8.5	8.5	N	N	N	N	N
22	7.5	7.5	7.7	7.8	7.9	8.8	9.4	8.0	8.5	8.5	N	N	N	N	N
36	7.5	7.5	7.8	7.8	D[a]	8.8	9.4	8.1	8.5	D	N	N	N	N	N
60	7.5	7.5	D	D	D	8.8	9.4	D	D	D	N	N	N	N	N
100	7.5	7.5	D	D	D	8.8	9.4	D	D	D	N	N	N	N	N

[a]D means all fish had previously died; [b]N means reading was not made.

TABLE 5

Behavior of Fathead Minnows (Pimephales promelas) Exposed to [redacted] Haskell Number 19,011) for 96 Hours in a Static, Unaerated Test

Nominal Concentrations (mg/L)	Number Affected/Total Number Alive			
	24 Hours	48 Hours	72 Hours	96 Hours
Water Control	0/10	0/10	0/10	0/10
7.8	1/10[a]	1/10[a]	0/9	0/9
13.	6/10[a]	3/10[a] 2/10[b]	1/9[c]	0/8
22.	3/7[a]	3/7[a]	1/6[d]	6/6[f]
36.	1/1[a]	1/1[a]	D[e]	D
60.	D	D	D	D
100.	D	D	D	D

[a]Lying on bottom; [b]at the surface; [c]at least one fish was at surface, at least one fish swam erratically, at least one fish was lying on bottom, at least one fish had partial loss of equilibrium, and at least one fish was hyperactive; [d]at least one fish was lying on bottom, at least one fish was hyperactive, at least one fish swam erratically, and at least one fish showed partial loss of equilibrium; [e]all fish had previously died so behavior could not be observed; and [f]hyperactive and showed partial loss of equilibrium.

TABLE 6

Mortality of Fathead Minnows (Pimephales promelas) Exposed to [redacted] Haskell Number 19,011) for 96 Hours in a Static, Unaerated Test

Nominal Concentrations (mg/L)	Cumulative Mortality in Percent			
	24 Hours	48 Hours	72 Hours	96 Hours
H_2O Control	0	0	0	0
7.8	0	0	10	10
13.	0	0	10	20
22.	30	30	40	40
36.	90	90	100	100
60.	100	100	100	100
100.	100	100	100	100

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