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Study Title

STATIC, ACUTE, 96-HOUR LC50 OF [REDACTED]
TO FATHEAD MINNOWS (PIMEPHALES PROMELAS)

Author

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Performing Laboratory

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[REDACTED]

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I. SUMMARY

[REDACTED] was moderately acutely toxic to juvenile, fathead minnows (*Pimephales promelas*) in a 96-hour, unaerated, static test using nominal test concentrations of 6.3, 13, 25, 50, and 100 mg/L. The 96-hour LC50 was 21 mg/L with a 95% confidence interval of 16 to 29 mg/L.

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II. GENERAL INFORMATION

Material Tested: [REDACTED]

Medical Research Number: [REDACTED]

Haskell Number: 18,920

Haskell Laboratory Test Code: [REDACTED]

CAS Registry Number: [REDACTED]

Other Codes: [REDACTED]

Synonyms: [REDACTED]

Composition: [REDACTED]

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

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Du Pont HLR 636-91

Submitted By: [REDACTED]
Du Pont Chemicals
Jackson Laboratory
Deepwater, NJ

Study Schedule: Study Initiation Date: 8/30/91
Experimental Start Date: 9/5/91
Experimental Termination Date: 9/9/91
Study Completion Date: 9/24/91

Notebooks: [REDACTED]

Distribution: [REDACTED]

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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 Materials

Source and composition of the 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 reside 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 concentrations were 6.3, 13, 25, 50, and 100 mg/L. The fathead minnows ranged from 1.3 to 2.0 cm in standard length (mean 1.5 cm) and 0.024 to 0.096 g in wet weight (mean 0.044 g). The fathead minnows were 51 days old at test start.

Glass beakers (4+-L) containing 4-L of test solution (approximately 13-cm liquid depth) were employed. One replicate of each test concentration with ten fish each 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 0.11 g/L. Test solutions were held between 21.0 and 21.8°C (mean 21.4°C) and were unaerated. A photoperiod of 16 hours light (129-183 Lux) versus 8 hours darkness was employed.

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 control and all test concentrations at the beginning of the test (before the addition of fish) and daily thereafter. Temperature (American Society of Testing Materials registered mercury stem thermometer) was measured in the dilution water control at the beginning of the test 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 Instruments, Yellow Springs, OH) of the dilution water were measured at test start.

E. Chemical Analysis

Chemical analysis of the test substance in the test solutions was not conducted.

F. Statistical Methods

The 96-hour LC50 and its associated confidence interval was calculated using a moving average-angle program [2].

VI. RESULTS AND DISCUSSION

Nominal [REDACTED] concentrations used in the definitive test were 6.3, 13, 25, 50, and 100 mg/L.

All chemical and physical parameters were within expected ranges (Table 2 & 3). The 96-hour LC50 was 21 mg/L with a 95% confidence interval of 16 to 29 mg/L.

VII. CONCLUSION

[REDACTED] was moderately acutely toxic to juvenile, fathead minnows (Pimephales promelas) in a 96-hour, unaerated, static test using nominal test concentrations of 6.3, 13, 25, 50, and 100 mg/L.

VIII. LITERATURE CITED

1. Zucker, E. 1985. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Fish. EPA-54019-85-006. United States Environmental Protection Agency, Office of Pesticide Programs, Washington, DC 20460.

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, OH.

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TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water¹

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

¹Date of analyses 17 October 1990 unless indicated otherwise, analyses performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ²Date of analyses 13 December 1990, analyses analysis performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ³Mean value; ⁴Date of analyses 24 July 1990, performed at Du Pont Engineering Test Center, Newark, Delaware; ⁵Below the Published Method Detection Limit (BMDL); ⁶Date of analyses 9 November 1990, analyses performed at Du Pont Chemicals, Jackson Laboratory, Deepwater, New Jersey; ⁷Date of analyses 23 October 1990, analyses performed at Hazelton Laboratories, Inc., Madison, WI; ⁸None detected.

TABLE 2

Water Chemistry of the Dilution Water at Test Start

Total Alkalinity (mg/L as CaCO₃)	EDTA Hardness (mg/L as CaCO₃)	Conductivity (μmhos/cm)
81	74	190

TABLE 3
Physical and Chemical Characteristics of [REDACTED]
Test Solutions [REDACTED]

Time (Hours)	Nominal Concentrations (mg/L)					
	Control	6.3	13	25	50	100
<u>pH</u>						
0	8.7	8.7	8.7	8.7	8.6	8.6
24	6.2	6.2	6.5	6.4	6.8	7.0
48	6.0	6.1	6.4	6.2	6.5	6.9
72	6.1	6.3	6.2	6.4	6.4	6.5
96	6.7	6.3	6.2	5.9	6.0	6.3
<u>Dissolved Oxygen</u>						
0	7.4	7.4	7.5	7.5	7.5	7.5
24	7.2	7.1	7.5	7.5	7.5	7.5
48	7.3	7.2	7.4	7.4	7.4	7.4
72	7.2	7.2	7.3	7.2	7.2	7.2
96	7.9	7.9	7.9	7.9	7.9	7.9

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TABLE 4

Observed Mortality of Fathead Minnows (*Pimephales promelas*) Exposed
to [REDACTED] for 96 Hours in Static, Un-aerated Test

Nominal Concentrations (mg/L)	Observed Mortality in Percent			
	Time (Hours)			
	24 Hours	48 Hours	72 Hours	96 Hours
Control	0	0	0	0
6.3	0	0	0	0
13	0	0	0	0
25	0 ^a	70 ^c	80 ^d	80 ^c
50	20 ^a	100	100	100
100	60 ^b	100	100	100

^aFive fish were at the surface, were dark in coloration, and had an accumulation of an unknown substance on their pectoral fins.

^bFour fish were at the surface, were dark in coloration, and had an accumulation of an unknown substance on their pectoral fins.

^cAll surviving fish were at the surface and had a dark coloration.

^dAll surviving fish exhibited dark coloration.

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