

DU PONT USE ONLY

Study Title

STATIC, ACUTE, 48- AND 64-HOUR EC50 OF [REDACTED] TO Daphnia magna

Data Requirement

Organisation for Economic Co-Operation and Development (OECD)
Guideline for Testing Chemicals: 202

Author

Kevin N. Baer, Ph.D.

Study Completed On

June 26, 1991

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, Post Office Box 50
Newark, Delaware 19714

Laboratory Project ID

Haskell Laboratory Report No. 457-91

[REDACTED]

Page 1 of 15

Company Sanitized. Does not contain TSCA CBI

Du Pont HLR 457-91

THIS PAGE RESERVED FOR SPECIFIC COUNTRY REQUIREMENTS

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to United States Environmental Protection Agency Good Laboratory Practice Regulations for FIFRA (40 CFR 160). 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:

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

6/24/91
Mo/Day/Yr

QUALITY ASSURANCE DOCUMENTATION

STATIC, ACUTE, 48- AND 64-HOUR EC50 OF [REDACTED] TO Daphnia Magna

STUDY: [REDACTED]

19,011

AUDITS:

<u>Items Audited</u>	<u>Audit Dates</u>
Protocol	6/19/91
Conduct	6/19/91
Records, Final Report	6/25/91

DATE FINDINGS REPORTED TO MANAGEMENT AND STUDY DIRECTOR: 6/25/91

Reported by: Kimberly B. Brebner
Kimberly B. Brebner
Quality Assurance Auditor

6/26/91
Date

Company Sanitized. Does not contain TSCA CBI

TABLE OF CONTENTS

	Page
Specific Country Requirements	2
Good Laboratory Practice Statement	3
Quality Assurance Documentation	4
Table of Contents	5
I. Summary	6
II. General Information	7
III. Purpose	9
IV. Records	9
V. Materials and Methods	10
A. Test Material	10
B. Dilution Water	10
C. Animal Culture	10
D. Test Methods	10
E. Chemical Analysis	11
F. Statistical Analysis	11
VI. Results and Discussion	11
VII. Conclusion	11
VIII. Literature Cited	11
IX. Tables	12
Table 1. Chemical Characteristics of Haskell Laboratory Well Water	12
Table 2. Water Chemistry of the Water Control at Test Start	13
Table 3. Physical and Chemical Characteristics of [REDACTED] Test Solutions	14
Table 4. Observed Immobility of <u>Daphnia magna</u> Exposed to [REDACTED] for 24, 48, and 64 Hours in a Static, Unaerated Test	15

I. SUMMARY

[REDACTED] was moderately toxic to Daphnia magna neonates (less than 24 hours old) in an unaerated, static, 48-hour test. Nominal test concentrations were 0.31, 0.63, 1.3, 2.5, 5.0, and 10 mg/L. The 48-hour EC50 was 8.3 with a 95% fiducial interval of 5.7 to 17 mg/L. Due to absence of 100% immobility in the high test concentration at 48 hours, the test was extended to 64 hours. As a result, immobility increased in all test concentrations and the 64-hour EC50 was 0.27 mg/L.

Work By:

Kevin N. Baer for WTB
W. Troy Baxter
Aquatic Technician

6/24/91
Mo/Day/Yr

Study Director:

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

6/24/91
Mo/Day/Yr

THIS REPORT IS APPROVED FOR ISSUE.

Kevin N. Baer
Kevin N. Baer, Ph.D.
Study Director

6/26/91
Mo/Day/Yr

II. GENERAL INFORMATION

Material Tested:

[REDACTED]

Medical Research Number:

[REDACTED]

Haskell Number:

19,011

CAS Registry Number:

[REDACTED]

[REDACTED]

Other Codes:

[REDACTED]

Synonyms:

[REDACTED]

Composition:

[REDACTED]

Purity:

[REDACTED]

Submitter's Notebook Number:

[REDACTED]

Sponsor:

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

Submitted By:

[REDACTED]

Du Pont Chemicals
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19803

Study Schedule:

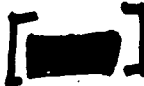
Study Initiation Date: 6-17-91
Experimental Start Date: 6-18-91
Experimental Termination Date: 6-21-91
Study Completion Date: 6-26-91

Notebooks:

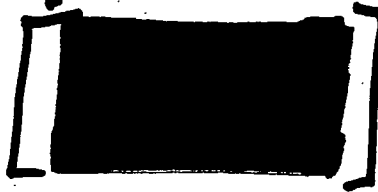
[REDACTED]

Du Pont HLR 457-91

Haskell Laboratory Cost Code:



Distribution:



III. PURPOSE

The goal of this study was to assess the acute toxicity of [REDACTED] to Daphnia magna neonates less than 24 hours old.

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 and composition of the test substance are described under General Information. A 10 mg/L stock solution was prepared in dilution water (see below) and used immediately. Stability and solubility of the test substance in dilution water were not determined.

B. Dilution Water

Water (Table 1) originated from the Haskell Laboratory well and flowed through aquaria containing fathead minnows, (Pimephales promelas), prior to use in this daphnid test.

C. Animal Culture

Parent Daphnia magna were reared at Haskell Laboratory in 2-L pyrex beakers (25 per beaker) which contained 1.8 L of filtered dilution water held at approximately 20°C. Daphnids were fed 25 mg/L (dry weight) of a mixture of frozen Purina trout chow and Fleischmann's yeast three times weekly during the water renewal process. Neonates were collected from approximately 29 day old parent daphnids for use in this test. Sickness, injury, and abnormality were not present. Daphnia magna were identified by labels on the beakers and test vessels.

D. Test Methods [1]

Six test concentrations and a dilution water control were used. Nominal test concentrations were 0.31, 0.63, 1.3, 2.5, 5.0, and 10 mg/L.

Pyrex beakers (250 mL; 7-cm diameter) containing 200 mL of test solution (6.8-cm depth) were used. Each replicate (four per concentration) contained five daphnids (1 daphnid/40 mL). Daphnids were added to the control and test beakers using random numbers. Test solutions were held between 20.0 and 20.6°C (mean 20.3°C) and were unaerated. Daphnids were not fed during this test. A photoperiod of 16 hours light 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 one replicate of the water control and all test concentrations before daphnids were added at the beginning, and at the end of the test (64 hours). 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 before daphnids were added at the beginning of the test. Temperature was measured using a registered American Society of Testing Materials mercury stem thermometer.

R. Chemical Analysis

Chemical analysis of the test substance was not conducted.

F. Statistical Analysis

The 48- and 64-hour EC50 was calculated using a probit method [2].

VI. RESULTS AND DISCUSSION

A rangefinding test was conducted using the following concentrations; 0.0 (control), 0.1, 1.0, 10, and 100 mg/L. Immobilities of 20, 0, 20, 100, and 100%, respectively, were observed.

All chemical and physical parameters in the definitive test (Tables 2 and 3) were within expected ranges.

The observed immobility at 24, 48, and 64 hours is presented in Table 4. Daphnids were considered immobile if they were unable to swim within 15 seconds after gentle prodding with a glass rod. All daphnids in the high test concentration (10 mg/L) were lying on the bottom of the test vessels at 48 hours. After testing these daphnids for immobility, 60% were considered to be immobilized. The remaining daphnids were clearly effected but were observed to briefly swim in the water column after gentle prodding and then return to the bottom of the test vessels. All mobile daphnids in the control and lower test concentrations appeared normal, i.e., actively swimming in the water column. Due to the lack of 100% immobilization in the high test concentration at 48 hours, the study was extended to 64 hours (overnight). As a result, daphnids in all test concentrations (excluding controls) exhibited immobility ranging from 40% in the low test concentration (0.31 mg/L) to 95% in the high test concentration (10 mg/L). Again, all control daphnids appeared normal. The 48-hour EC50 was 8.3 mg/L with a 95% fiducial interval of 5.7 to 17 mg/L. The 64-hour EC50 was 0.27 with an undefined lower 95% fiducial limit and 1.1 mg/L upper 95% fiducial limit.

VII. CONCLUSION

[REDACTED] was moderately toxic to Daphnia magna neonates (less than 24 hours old) in an unaerated, static, 48-hour test. The 48-hour EC50 was 8.3 mg/L, based on nominal concentrations and immobility.

VIII. LITERATURE CITED

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for Testing Chemicals: 202. 4 April 1984.
2. Finney, D. J. 1971. Probit Analysis. 3rd Ed., Cambridge University Press. 333 p.

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 Hazleton Laboratories, Inc., Madison, WI; ⁸None detected.

TABLE 2

Water Chemistry of the Water Control at Test Start

[REDACTED]

Total Alkalinity (mg/L as CaCO_3)	EDTA Hardness (mg/L as CaCO_3)	Conductivity ($\mu\text{mhos/cm}$)
82	75	170

TABLE 3

Physical and Chemical Characteristics of
Test Solutions

Nominal Concentrations (mg/L)							
Time (Hours)	Water Control	0.31	0.63	1.3	2.5	5.0	10
Dissolved Oxygen (mg/L)							
0	8.8	8.7	8.7	8.7	8.7	8.7	8.7
64	8.7	8.6	8.6	8.6	8.5	8.4	8.4
pH							
0	7.6	7.6	7.6	7.7	7.7	7.7	7.7
64	7.6	7.7	7.8	7.9	8.0	8.0	8.0

TABLE 4

Observed Immobility of *Daphnia magna* Exposed to [REDACTED]
for 24, 48, and 64 Hours in a Static, Un-aerated Test [REDACTED]

Nominal Concentrations (mg/L)	Percent Cumulative Observed Immobility											
	24 Hours				48 Hours				64 Hours			
	A ^a	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a
Water Control	0	0	0	0	0	0	0	0	0	0	0	0
0.31	0	0	0	0	0	0	0	0	20	60	60	20
0.63	0	0	0	0	0	0	20	0	100	60	60	80
1.3	0	0	0	0	0	0	0	0	80 ^b	60 ^b	100	60 ^b
2.5	0	0	20	0	0	20	20	20	60 ^b	100	100	100
5.0	0	20	20	20	20	40	20	40	20 ^b	60 ^b	100	100
10	20	20	40	40	40 ^b	60 ^b	60 ^b	80 ^b	80 ^b	100	100	100

^aA, B, C and D represent replicate test vessels containing five daphnids each.

^bAll "mobile" daphnids were lying on the bottom of the test vessels and were mobile only after gentle prodding with a glass rod (see Discussion for further comments).