

Du Pont HLO 235-91

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Study Title

Static Acute Toxicity of [REDACTED] to the
Freshwater Alga, *Selenastrum capricornutum*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

April 15, 1991

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 2130
One Lafayette Road
Hampton, New Hampshire 03842

Submitted To

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

Haskell Laboratory Project ID

Haskell Laboratory Outside Report No. 235-91
[REDACTED]

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Company Sanitized. Does not contain TSCA CB

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I. GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was performed in compliance with Good Laboratory Practices as defined in 40 CFR part 792, and presented in the Federal Register, Thursday, August 17, 1989 (Toxic Substances Control Act [TSCA]; Good Laboratory Practice Standards; Final Rule). Light intensity was not measured daily during the study. Neither the Study Director nor the sponsor are aware of any circumstances that would affect the integrity of this study.

 4/15/91

Timothy J. Ward
Study Director

II. QUALITY ASSURANCE STATEMENT

Submitted by: EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 2130
One Lafayette Road
Hampton, New Hampshire 03820

Certification: This study meets requirements of 40 CFR part 792. Data presented in this report were derived by methods and with materials identified in the section of the report entitled "Methods and Materials." The test was performed in accordance with EnviroSystems Protocol [REDACTED] and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual. The toxicity test was performed by Jeanne Magazu, Robert Boeri, and Timothy Ward.

All data transcribed from the raw data to the report were checked for accuracy, and all data were verified by Quality Assurance Auditors. Quality assurance audits were performed (and reported to management) on:

Aspect Audited	Audit Date	Reported to Study Director	Reported to Management
Protocol	7/ 5/90	7/ 5/90	--
In-life	9/25/90	9/25/90	10/ 2/90
Draft report	2/ 4/91	2/ 4/91	4/18/91
Final report	4/18/91	4/18/91	4/18/91

Stephanie Beck 4-18-91
Quality Assurance Representative

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V. SUMMARY

An acute toxicity test was conducted with [REDACTED] and the freshwater alga, *Selenastrum capricornutum*, to determine the effective concentrations (EC10, EC50, EC90). The test was conducted for E.I. duPont de Nemours and Company for 96 hours during September 21 to 25, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted according to the protocol developed for EnviroSystems Study Number [REDACTED]. The test substance was supplied by the sponsor.

The test was performed under static conditions with five concentrations of test substance and a dilution water control with 3 replicates per level. The test was conducted at a temperature of $24 \pm 2^\circ\text{C}$. The dilution water was sterile enriched media adjusted to a pH of 7.5. Nominal concentrations were: 0 (control), 315, 625, 1,250, 2,500, and 5,000 mg/L [REDACTED]. Cell counts were made daily with a haemocytometer. Nominal concentrations were used for all calculations. Water quality parameters were within acceptable limits throughout the test.

The test substance was not toxic to *Selenastrum capricornutum* at any tested concentration. Exposure of algae to the test substance for 96 hours resulted in a median effective concentration (EC50) of $>5,000 \text{ mg/L}$ [REDACTED] the highest tested concentration.

VI. RECORDS

All records from those portions of this study conducted at Resource Analysts, Inc. and the final report will be maintained at the EnviroSystems Division of Resource Analysts, Inc., 1 Lafayette Road, Hampton, New Hampshire.

VII. GENERAL INFORMATION

Material Tested:

Medical Research Number:

Haskell No.:

18,267

Synonyms:

Submitter's
Notebook Number:

Purity:

Contaminants:

Composition:

Sponsor:

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

Submitted by:

Du Pont Chemicals and Pigments
E.I du Pont de Nemours and Company
Jackson Laboratory
Deepwater, New Jersey 08023

Study Schedule:

Study Initiation Date: August 6, 1990
Experimental Start Date: September 21, 1990
Experimental Completion Date: September 25, 1990
Study Completion Date: April 15, 1991

**Haskell Laboratory
Test Code:**

Distribution:

[REDACTED]

VIII. METHODS AND MATERIALS

TEST SUBSTANCE:

[REDACTED] was delivered to EnviroSystems on June 18, 1990. It was contained in a glass bottle that was labelled with the following information: "H 18,267, 5-22-90, 412 grams gross". The test substance (a viscous, beige liquid) was shipped from E. I. duPont de Nemours and Company, Stine-Haskell Research Center, P.O. Box 30, Elkton Road, Newark, Delaware. Prior to use the test material was stored in the dark at 2-4°C. A reserve sample (approximately 1.2 grams) will be archived at EnviroSystems for a minimum of 10 years, and unused test substance is returned to the sponsor. A 5,000 mg/L stock solution was prepared by combining 5.0 g of test substance and dilution water and adjusting the mixture to 1,000 ml. The test substance was tested as received and assumed to have a purity of [REDACTED]

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was sterile enriched media (U.S. EPA, 1978; Resource Analysts, Inc. Standard Operating Procedure 01-02-1101) adjusted to a pH of 7.3. Results of chemical analysis of a representative sample of deionized water used to prepare the media are presented in Table 1.

TEST ORGANISM

Algae used for the test was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin and delivered to EnviroSystems on October 31, 1989. The culture was transferred to sterile enriched media identical to media used for this test and maintained for at least 14 days.

TOXICITY TESTING:

A screening test was conducted during August 9 to 13, 1990. Nominal concentrations of test substance were: 1, 10, 100, and 1,000 mg/L. After 96 hours the number of cells per ml was greater than the control value at 1,000 mg/L [REDACTED]

The definitive toxicity test was performed during September 21 to 25, 1990, according to the protocol developed for EnviroSystems Study [REDACTED]. The protocol is based on procedures of the U.S. Environmental Protection Agency (1985, 1987). The test was conducted at a target temperature of $24 \pm 2^\circ\text{C}$ with five concentrations of test material and a dilution water control. Nominal concentrations of [REDACTED] were: 0 (control), 315, 625, 1,250, 2,500, and 5,000 mg/L.

Table 1. Chemical characterization of a representative sample of deionized water used to prepare sterile enriched media used in the toxicity test with [REDACTED] and the freshwater alga *Selenastrum capricornutum*

Parameter	Unit of Measurement	Detection Limit	Measured Value
Total organic carbon	mg/L	1	
0 hour			ND
96 hour			11
Total suspended solids	mg/L	10	
0 hour			ND
96 hour			21
Ammonia	mg/L as N	0.2	ND
Aluminum	mg/L	0.025	ND
Arsenic	mg/L	0.01	ND
Boron	mg/L	0.1	ND
Cadmium	mg/L	0.001	ND
Chromium	mg/L	0.002	ND
Copper	mg/L	0.005	ND
Cobalt	mg/L	0.008	ND
Fluoride	mg/L	0.05	ND
Iron	mg/L	0.01	0.01
Lead	mg/L	0.005	ND
Mercury	mg/L	0.0003	ND
Nickel	mg/L	0.008	ND
Silver	mg/L	0.005	ND
Zinc	mg/L	0.005	ND
Organochlorine and Organophosphorus Pesticides and PCBs	ug/L	5.0	ND

- Notes: 1. ND = Not detected at or above the detection limit.
 2. Total organic carbon and total suspended solids determination was conducted on a sample of dilution water collected from a control test vessel during this toxicity test (96 hour values represent analysis of the test organism in the dilution water). All other chemical data are the result of analysis of a representative sample of deionized water collected during March, 1990 as part of routine bi-annual water quality analysis.

Algae was randomly and equally distributed among three replicates of each treatment at the rate of 10,000 cells/ml. The test was performed in 250 ml glass erlenmeyer flasks that contained 100 ml of test solution. Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm. The test vessels were placed in an incubator during the test (a random numbers table was used to select the location of each vessel). A 24 hour light and 0 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of $24 \mu\text{Es}^{-1}\text{m}^2$.

The number of algal cells/ml in each test vessel and the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells was determined visually by means of direct microscopic examination with a haemocytometer. Cell counts were made and recorded daily during the 96 hour test. Temperature of the incubator was measured and recorded daily (thermometer number 2211), and pH (Beckman model PHI 12 meter; instrument number PRL-4) was determined in each test vessel at the beginning and end of the test.

STATISTICAL METHODS:

Results of the toxicity test could not be interpreted by standard statistical techniques because cell growth at the highest tested concentration was greater than 50% of control growth. Determination of EC10, EC50, and EC90 values were performed by the author using percent of control growth data and the nominal concentrations of the test substance.

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IX. RESULTS

Water quality data generated by the acute toxicity test are presented in Appendix A. Water quality throughout the test was within acceptable limits. The range of values measured in test vessels during the test were: incubator temperature - 23.2 to 24.2°C, and pH - 7.5 to 9.0.

Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively. The 96 hour EC10, EC50, and EC90 values are >5,000 mg/L, the highest tested concentration. No effects (size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted.

Table 2. Cell growth data from the acute toxicity test with [REDACTED] and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration (mg/L)	rep.	Number of Cells/ml x 10 ³ (hour)				
		0	24	48	72	96
0 (control)	1	10	56	238	945	1,878
	2	10	61	212	1,070	1,929
	3	10	54	246	1,325	1,842
	mean	10	57	232	1,113	1,883
	% control	100	100	100	100	100
315	1	10	60	209	1,212	1,750
	2	10	49	227	938	1,825
	3	10	52	230	1,110	1,803
	mean	10	54	222	1,087	1,793
	% control	100	95	96	98	95
625	1	10	52	198	1,070	1,821
	2	10	48	212	1,083	1,800
	3	10	54	226	962	1,862
	mean	10	51	212	1,038	1,828
	% control	100	89	91	93	97
1,250	1	10	53	204	924	1,760
	2	10	51	221	981	1,740
	3	10	49	240	1,042	1,826
	mean	10	51	222	982	1,775
	% control	100	89	96	88	94
2,500	1	10	46	218	1,221	1,912
	2	10	57	212	989	1,716
	3	10	53	227	1,014	1,844
	mean	10	52	219	1,075	1,824
	% control	100	91	94	97	97
5,000	1	10	58	222	845	1,861
	2	10	47	241	910	1,840
	3	10	52	210	885	1,962
	mean	10	52	224	880	1,888
	% control	100	91	97	79	100

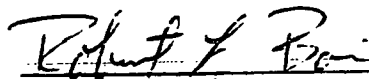
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X. PROTOCOL DEVIATIONS

No deviations were made from the protocol.

XI. SIGNATURE PAGE

 4/15/91
Timothy J. Ward
Study Director

 4-15-91
Robert L. Boeri
Coauthor

 4-15-91
Jeanne P. Magazo
Aquatic Toxicologist

XII. REFERENCES

U.S. EPA. 1978. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA-600/9-78-018. Environmental Research Laboratory, Corvallis, Oregon.

U.S. EPA. 1985. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Friday, September 27, 1985. Section 797.1050.

U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987. Section 797.1050.

U.S. EPA. 1989. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule. Federal Register, Thursday, August 17, 1989

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Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

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Table A.1. Temperature measured during toxicity test with [REDACTED] and the freshwater alga, *Selenastrum capricornutum*.

Hour of Exposure	Temperature of Incubator, °C
0	24.2
24	23.6
48	23.7
72	23.7
96	23.2

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Table A.2. pH values measured during toxicity test with [REDACTED] and the freshwater alga, *Selenastrum capricornutum*.

Nominal concentration (mg/L)	Replicate	pH	
		Initial	Final
0 (control)	1	7.5	8.3
	2	7.5	8.7
	3	7.5	8.7
315	1	7.5	8.9
	2	7.5	9.0
	3	7.5	8.9
625	1	7.5	9.0
	2	7.5	9.0
	3	7.5	9.0
1,250	1	7.5	9.0
	2	7.5	9.0
	3	7.5	9.0
2,500	1	7.5	9.0
	2	7.5	9.0
	3	7.5	9.0
5,000	1	7.5	9.0
	2	7.5	9.0
	3	7.5	9.0