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Study Title

Static Acute Toxicity of FM 3820
to the Daphnid, Daphnia magna

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Study Completed

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Performing Laboratory

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EnviroSystems Study Number 90171-3
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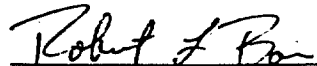
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I. GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to OECD Good Laboratory Practice Regulations. Neither the Study Director nor the sponsor are aware of any circumstances that would affect the integrity of this study.

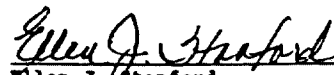
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II. QUALITY ASSURANCE STATEMENT

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Certification:

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 90171-3 and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual. The toxicity test was performed by Ellen Stanford, Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward. The original raw data and final report will be archived at Resource Analysts, Inc. for at least 10 years.

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 on

Audit Date	Reported to Study Director	Reported to Management
11/12/90	11/14/90	3/28/91
12/20/90	12/20/90	3/28/91
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V. SUMMARY

The acute toxicity of FM 3820 to the daphnid, Daphnia magna, is described in this final report. The test was conducted for 3M Company for 48 hours during December 18 to 20, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Ellen Stanford, Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward according to the protocol developed for EnviroSystems Study Number 90171-3.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a temperature of $20 \pm 1^\circ\text{C}$. The dilution water was filtered natural well water collected from wells at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of test substance were: 0 mg/L (control), 25 mg/L, 40 mg/L, 60 mg/L, 100 mg/L, and 150 mg/L. Nominal concentrations were used for all calculations.

Daphnids used in the test were produced from an in-house culture and were less than 24 hours old at the start of the test. After 48 hours of exposure the control daphnids had an average wet weight (blotted dry) of 0.0001 g, resulting in a loading rate of 0.002 g/L. All daphnids were in good condition at the beginning of the study. Exposure of daphnids to the test substance resulted in a 48 hour median effective concentration (EC50) of 49 mg/L FM 3820, with a 95% confidence interval of 43 - 56 mg/L.

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VI. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FM 3820 to the daphnid, a freshwater invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the test.

VII. METHODS AND MATERIALS

TEST SUBSTANCE:

FM 3820 (EnviroSystems Sample Number 2696E) was delivered to EnviroSystems on November 15, 1990. It was contained in a 250 mL plastic bottle that was labelled with the following information: "H 2864-6, FM 3820". The test substance was supplied by 3M Company, St. Paul, Minnesota. Prior to use it was stored in the dark at room temperature. A reserve sample will be archived at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was collected from wells at EnviroSystems in Hampton, New Hampshire. Water was stored in 500-gallon polyethylene tanks where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile daphnids employed as test organisms were from a single source and were identified using an appropriate taxonomic key. Daphnids used in the test were produced from an in-house culture and were less than 24 hours old at the start of the test. Control daphnids were weighed at the conclusion of the toxicity test. Prior to testing, daphnids were maintained in 100% dilution water under static conditions in 4 liter glass jars. During the 14 day acclimation daphnid cultures were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. During the acclimation period 24 hours prior to the test initiation the temperature was 19.0 - 19.8°C. Daphnids were fed yeast/trout chow and/or the freshwater alga Selenastrum capricornutum daily before the test.

Table 1: Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	7.8
Conductivity	umhos/cm	--	1200
Organochlorine pesticides	ug/L	2	ND
Organophosphorus pesticides	ug/L	5.0	ND
Polychlorinated biphenyls	ug/L	0.6	ND

Notes: 1. ND = Not detected above the reporting limit.
 2. The pH and conductivity values were determined in a sample of dilution water collected from a control test vessel at the beginning of the toxicity test. Additional chemical parameters were measured in dilution water during October, 1990 as part of routine biannual water quality analysis.

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TOXICITY TESTING:

A screening test with the test substance was conducted during December 5 to 7, 1990. Nominal concentrations of test substance were: 0.1, 1.0, 10, 100, and 1,000 mg/L. After 48 hours of exposure there was 0% survival at 1,000 mg/L, 60% survival at 100 mg/L, and 90% or greater survival at all other concentrations.

The definitive toxicity test was performed during December 18 to 20, 1990, according to EnviroSystems Test Protocol 90171-3 (Static, Acute Toxicity Test With FM 3820 And The Daphnid, Daphnia magna), which was signed by the Study Director on November 16, 1990. It is based on procedures of the OECD (1984).

The test was conducted at a target temperature of $20 \pm 1^\circ\text{C}$ with five concentrations of test substance and a dilution water control. A 1,000 mg/L stock solution was prepared by combining 1.0 g of test substance with dilution water and adjusting the final concentration to 1,000 ml without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L (control), 25 mg/L, 40 mg/L, 60 mg/L, 100 mg/L, and 150 mg/L FM 3820.

Twenty daphnids were randomly and equally distributed among four replicates of each treatment. The test was performed in 250 ml glass beakers that contained 200 ml of test solution (water depth was approximately 6 cm). Test vessels were randomly arranged in an incubator during the 48 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of $23 \text{ uEs}^{-1}\text{m}^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Daphnids were not fed during the test.

The number of surviving organisms and the occurrence of sublethal effects and immobilization (inability to swim within 15 seconds after gentle agitation) or other sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24 and 48 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model PHI 12 meter; instrument number PRL-4), conductivity (Labcomp SCT meter, instrument number PRL-9), and temperature (ASTM mercury thermometer) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The nominal concentration of test substance and the number of organisms killed or immobilized were used by the author to calculate the EC50 values.

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VIII. RESULTS

All test vessels appeared clear initially and remained free of insoluble material throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively. Ninety five percent survival occurred in the control exposure. Control daphnids had an average wet weight (blotted dry) of 0.0001 g at the end of the test. Loading rate during the toxicity test was approximately 0.0025 g/L. Exposure of daphnids to the reference toxicant sodium dodecyl sulfate resulted in a 48 hour median lethal concentration (LC50) of 15.6 mg/L.

The 24 and 48 hour EC50s for daphnids exposed to FM 3820 are presented in Table 3. Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 49 mg/L FM 3820, with a 95% confidence interval of 43 - 56 mg/L.

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Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive			Number Affected		
		0 hr	24 hr	48 hr	0hr	24hr	48hr
0 (control)	1	5	5	4	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
25	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	2
	4	5	5	5	0	0	0
40	1	5	5	3	0	0	0
	2	5	5	5	0	0	0
	3	5	5	3	0	0	1
	4	5	4	4	0	0	0
60	1	5	5	2	0	0	2
	2	5	5	4	0	0	0
	3	5	5	4	0	0	4
	4	5	5	5	0	0	3
100	1	5	5	0	0	0	-
	2	5	5	0	0	0	-
	3	5	5	0	0	0	-
	4	5	5	0	0	0	-
150	1	5	3	0	0	0	-
	2	5	5	0	0	1	-
	3	5	4	0	0	0	-
	4	5	4	0	0	0	-

Note: Affected daphnids were immobilized (unable to swim within 15 seconds after gentle agitation)

Table 3. Median effective concentrations (EC50s) from toxicity test

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	>150 mg/L	--	--
48 hours	49 mg/L	43 - 56 mg/L	Probit

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IX. REFERENCES

OECD. 1981. Decision of the Council Concerning the Mutual Acceptance of Data in the Assessment of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. C (81) 30 (Final).

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnid sp., Acute Immobilization Test and Reproduction Test. Adopted April 4, 1984.

Stephan, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

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Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

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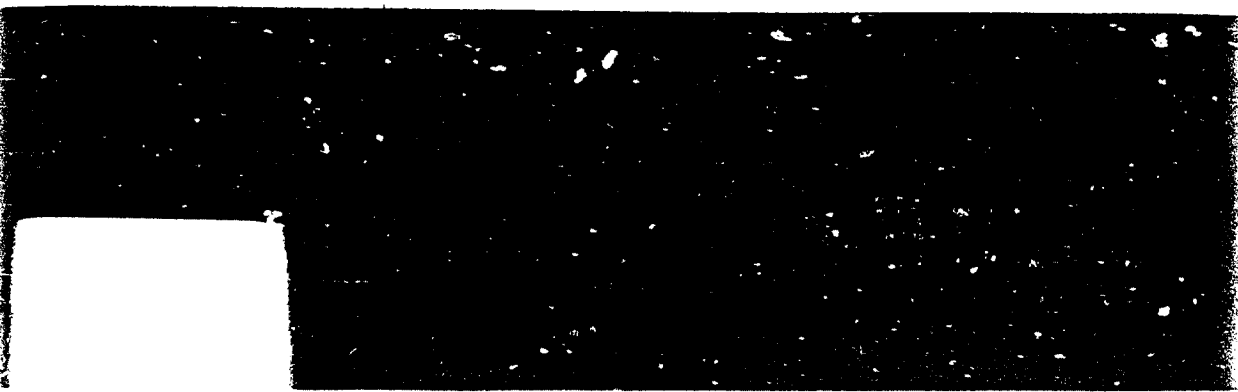


Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test

Nominal concentration (mg/L)	Rep.	Conductivity (umho/cm)			pH		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	1200	1300	1300	7.8	8.2	8.6
	2	1200	1300	1300	7.8	8.2	8.6
	3	1200	1300	1300	7.8	8.2	8.6
	4	1200	1300	1300	7.8	8.2	8.6
25	1	1200	1300	1300	7.8	8.2	8.6
	2	1200	1300	1300	7.8	8.2	8.6
	3	1200	1300	1300	7.8	8.2	8.6
	4	1200	1300	1300	7.8	8.2	8.6
40	1	1200	1300	1300	7.8	8.2	8.6
	2	1200	1300	1300	7.8	8.2	8.6
	3	1200	1300	1300	7.8	8.2	8.6
	4	1200	1300	1300	7.8	8.2	8.6
60	1	1200	1300	1300	7.8	8.2	8.6
	2	1200	1300	1300	7.8	8.2	8.6
	3	1200	1300	1300	7.8	8.2	8.6
	4	1200	1300	1300	7.8	8.2	8.6
100	1	1200	1300	1300	7.8	8.2	8.6
	2	1200	1300	1300	7.8	8.2	8.6
	3	1200	1300	1300	7.8	8.2	8.6
	4	1200	1300	1300	7.8	8.2	8.6
150	1	1200	1300	1300	7.8	8.3	8.6
	2	1200	1300	1300	7.8	8.3	8.6
	3	1200	1300	1300	7.8	8.3	8.6
	4	1200	1300	1300	7.8	8.3	8.6

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Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C)			Dissolved Oxygen (mg/L)		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	20.8	20.9	21.0	8.3	8.2	8.5
	2	20.8	21.0	21.0	8.3	8.2	8.5
	3	20.9	20.9	20.8	8.3	8.3	8.5
	4	20.9	20.9	20.9	8.3	8.2	8.5
25	1	20.8	20.8	20.9	8.2	8.2	8.5
	2	20.8	20.9	21.0	8.3	8.2	8.5
	3	20.7	20.8	21.0	8.3	8.1	8.5
	4	20.7	20.9	21.0	8.3	8.1	8.5
40	1	20.9	20.9	20.8	8.2	8.2	8.4
	2	20.8	20.8	20.6	8.2	8.2	8.3
	3	20.9	20.7	20.6	8.3	8.3	8.5
	4	20.9	20.8	20.9	8.3	8.2	8.5
60	1	20.7	20.8	20.9	8.3	8.1	8.0
	2	20.7	20.8	20.9	8.2	8.1	8.3
	3	20.9	20.9	20.9	8.2	8.2	8.3
	4	20.8	20.8	20.9	8.3	8.3	8.4
100	1	20.8	20.9	21.0	8.2	8.1	8.5
	2	20.7	20.8	20.9	8.3	8.1	8.4
	3	20.8	20.8	20.9	8.2	8.3	8.3
	4	20.8	20.9	21.0	8.2	8.2	8.4
150	1	20.9	20.8	20.9	8.3	8.1	8.5
	2	20.7	20.9	20.9	8.2	8.1	8.5
	3	20.8	20.9	20.9	8.2	8.2	8.4
	4	20.8	20.9	20.9	8.2	8.2	8.5

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