

MR 280349

October 26, 2004

8EHQ-1004-15958



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Office of Pollution, Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460
Attention: Section 8(e) Coordinator

COMPANY SANITIZED

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Re: **TSCA Section 8(e) Submissions**

Dear Sir/Madam:

3M Company ("3M") requests that EPA place the attached studies in the TSCA Section 8(e) docket. We have included a master index for these studies identifying the study title, test substance and CAS number. A Confidential Business Information (CBI) version of this index and the studies also is being submitted today pursuant to EPA procedures. 3M has not provided CBI substantiation with this submission, but would be willing to do so at the Agency's request.

3M has concluded that data in these studies may not be, strictly speaking, "corroborative" of previously reported or published information as defined in EPA's reporting guidance or otherwise potentially may warrant 8(e) submission based on EPA's reporting guidance.

3M appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.

Very truly yours,

Katherine E. Reed (974)

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Staff Vice President
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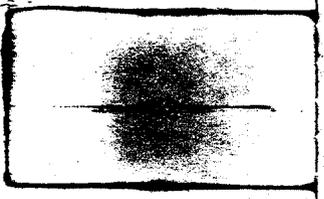
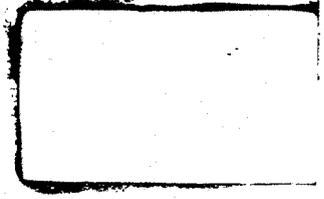
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Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
 (Confidential Business Information Redacted)

Title	Substance Information	CAS Information
CoCl ₂ 6H ₂ O as Co ²⁺ Toxicity to Microtox Reagent	Cobalt (as Co ²⁺ ion) (CoCl ₂ 6H ₂ O)	CAS 7791-13-1
Activated Sludge Respiration Inhibition Test on CoCl ₂ 6H ₂ O as Co ion	Cobalt (as Co ²⁺ ion) (CoCl ₂ 6H ₂ O)	CAS 7791-13-1
Acute Toxicity of CoCl ₂ 6H ₂ O as Co ion to <i>Daphnia magna</i> under Static Exposure Conditions	Cobalt (as Co ²⁺ ion) (CoCl ₂ 6H ₂ O)	CAS 7791-13-1
Acute Toxicity of CoCl ₂ 6H ₂ O as Co ion to Fathead Minnow under Static Exposure Conditions	Cobalt (as Co ²⁺ ion) (CoCl ₂ 6H ₂ O)	CAS 7791-13-1
Freshwater Algae Growth Inhibition Test	Cobalt (as Co ²⁺ ion) (CoCl ₂ 6H ₂ O)	CAS 7791-13-1
<i>Daphnia magna</i> 21-Day Chronic Reproduction Study	N-ethylperfluorooctane sulfonamideethanol	CAS 1691-99-2
Plant Growth Effects of []	[]	[]
Final Report (<i>Daphnia</i> and Microtox)	Monomethyl ether of hydroquinone	CAS 150-76-5
Microtox Test Results	2 Ethylhexyl Acrylate; Isooctyl Acrylate Monomer; 2-Methylbutyl acrylate; Methyl isoamyl acrylate; Isooctyl Acrylate	2 Ethylhexyl Acrylate (CAS 103-11-7); Isooctyl Acrylate Monomer (CAS 29590-42-9) 2-Methylbutyl acrylate (CAS 44914-03-6); Methyl isoamyl acrylate (CAS 18993-92-1); Isooctyl Acrylate (CAS 29590-42-9)
Phytotoxicity Test Results	[]	[]

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
 (Confidential Business Information Redacted)

Title	Substance Information	CAS Information
Plant Toxicity Comparison, Young Seedling Growth	[]	[]
<i>Ceriodaphnia dubia</i> Survival and Reproduction exposed to Opequon Creek Water Spiked with BETZ 1110 Polymer (November 4, 1987 sample) for seven days under static renewal conditions	BETZ 1110: Non-3M Product - Chemical composition not provided to 3M by manufacturer	MSDS provided by manufacturer states product is "not hazardous" and not "considered to be a carcinogen"
<i>Ceriodaphnia dubia</i> Survival and Reproduction exposed to Opequon Creek Water Spiked with Betz 1138 Polymer (November 4, 1987 sample) for seven days under static renewal conditions	BETZ 1138: Non-3M Product - Chemical composition not provided to 3M by manufacturer	MSDS provided by manufacturer states product is "not hazardous" and not "considered to be a carcinogen"
Toxicity of 1,6 - Hexanediol Diacrylate to <i>Daphnia magna</i>	1,6 Hexanediol diacrylate	CAS 13048-33-4
<i>Daphnia magna</i> Chronic Bioassay Under Static Renewal Conditions	Methyl isoamyl acrylate	CAS 18993-92-1
Estimating the Chronic Toxicity of Nalclear 7177 to <i>Ceriodaphnia</i> Survival and Reproduction Using Short-Term Tests	Nalclear 7177 wastewater treatment acrylamide/acrylate polymer - Chemical composition not provided to 3M by manufacturer	CAS Information not provided to 3M by manufacturer
Acute Toxicity of Isooctyl Acrylate to <i>Daphnia magna</i>	Isooctyl Acrylate Monomer	CAS 29590-42-9
Static Acute Toxicity of [] to the <i>Daphnid, Daphnia magna</i>	Tolyltriazole	CAS 29385-43-1
Static Acute Toxicity of [] to the <i>Alga, Selenastrum capricornutum</i>	Tolyltriazole	CAS 29385-43-1
Static Acute Toxicity of [] to the <i>Daphnid, Daphnia magna</i>	[]	[]
Static Acute Toxicity of [] to the Fathead Minnow, <i>Pimephales promelas</i>	[]	[]
Static Acute Toxicity of [] to the <i>Daphnid, Daphnia magna</i>	water; propylene-tetrafluoroethylene polymer; tert-butyl alcohol	water (7732-18-5); propylene-tetrafluoroethylene polymer (27029-05-6); tert-butyl alcohol (75-65-0)

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
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Title	Substance Information	CAS Information
Isocetyl acrylate: Fish, Acute Toxicity Test	Isocetyl Acrylate Monomer	CAS 29590-42-9
Isocetyl Acrylate: <i>Daphnia</i> sp. Acute Immobilization Test	Isocetyl Acrylate Monomer	CAS 29590-42-9
Isocetyl Acrylate: Alga, Growth Inhibition Test	Isocetyl Acrylate Monomer	CAS 29590-42-9
Isocetyl Acrylate: <i>Daphnia</i> sp. Reproduction Test	Isocetyl Acrylate Monomer	CAS 29590-42-9
Acute Toxicity of [] to the mysid, <i>Mysidopsis bahia</i>	[]	[]
Final Report (Microtox)	[]	[]
Determination of the Partition Coefficient (N-Octanol/Water) of T-5896 by High Performance Liquid Chromatography (HPLC)	N-methyl perfluorooctane sulfonamido ethanol; N-methyl perfluorooctane sulfonamide ethyl acrylate	N-methyl perfluorooctane sulfonamido ethanol (CAS 25268-77-3); N-methyl perfluorooctane sulfonamide ethyl acrylate (CAS 24448-09-7)
OECD Activated Sludge Respiration Inhibition Test Results	N-Dodecyltrimethylammonium chloride	CAS = 112-00-5
Final Report (Fish Acute Toxicity)	Mirataine CB (30% Cocamidopropyl betaine = Amides, coco, N-(3-(dimethylamino)propyl), alkylation products with chloroacetic acid, sodium salts, 70% Water and Inerts); Mirataine COB (30% Coco/Oleamidopropyl Betaine = 1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., inner salt)	Cocamidopropyl betaine (CAS 70851-07-9); Coco/Oleamidopropyl Betaine (CAS 61789-40-0)
A Flow-Through Life-Cycle Toxicity Test With the Saltwater Mysid (<i>Mysidopsis bahia</i>)	Perfluorooctane sulfonate	CAS 1763-23-1
Lithium: Alga, Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
An Early Life-Stage Toxicity Test With the Fathead Minnow (<i>Pimephales promelas</i>)	Perfluorooctane sulfonate	CAS 1763-23-1
Lithium: Fish, Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
Lithium: <i>Daphnia</i> , Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
Summary of Toxicity Testing on OSCI and OSF	Octane sulfonyl chloride and Octane sulfonyl fluoride	Octane sulfonyl fluoride (CAS 7795-95-1); Octane sulfonyl chloride (CAS 4063-63-5)
Toxicity to Microtox Test	Lauryldimethylamineoxide	CAS 1643-20-5

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004
(Confidential Business Information Redacted)

Title	Substance Information	CAS Information
Ecotoxicological Testing of CoCl ₂ ·6H ₂ O as Co ²⁺ ion (Seed Germination and Root Elongation)	Cobalt (as Co ²⁺ ion) (CoCl ₂ ·6H ₂ O)	CAS 7791-13-1

Study Title

Static Acute Toxicity of []
to the Daphnid, Daphnia magna

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

March 26, 1991

Performing Laboratory

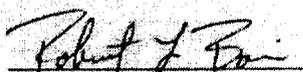
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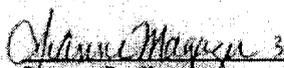
EnviroSystems Study Number 90169-3
Page 1 of 17

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.


Timothy J. Ford 3/24/91
Author and Study Director


Robert L. Boeri 3-27-91
Coauthor


Jeanne P. Maggini 3-27-91
Aquatic Toxicologist


Peter L. Kowalski 3-26-91
Aquatic Toxicologist


Ellen J. Stanford 3-26-91
Aquatic Toxicologist

II. QUALITY ASSURANCE STATEMENT

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

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 90169-3 and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual. The toxicity test was performed by Ellen Stanford, Jeanne Nagazu, 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/ 6/90	11/14/90	3/28/91
12/14/90	12/14/90	1/ 3/91
3/ 4/91	3/ 4/91	3/28/91
3/28/91	3/28/91	3/28/91

Stephanie Beck 3-28-91
Quality Assurance Representative

EnviroSystems Study Number 90169-3
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V. SUMMARY

The acute toxicity of [] to the daphnid, Daphnia magna, is described in this final report. The test was conducted for 3M Company for 48 hours during December 12 to 14, 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 90169-3.

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), 15 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 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 0.001 g, resulting in a loading rate of 0.02 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 19 mg/L [] with a 95% confidence interval of 12 - 25 mg/L.

VI. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of [redacted] 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:

[redacted] (EnviroSystems Sample Number 2694E) was delivered to EnviroSystems on November 15, 1990. It was contained in a 250 mL glass bottle that was labelled with the following information: [redacted] 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 acclimation daphnids 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 once or twice 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 collected during August, 1990 as part of routine biannual water quality analysis.

TOXICITY TESTING:

A screening test with the test substance was conducted during November 27 to 29, 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, 75% survival at 100 mg/L, and 100% survival at all other tested concentrations.

The definitive toxicity test was performed during December 12 to 14, 1990, according to EnviroSystems Test Protocol 90169-3 (Static, Acute Toxicity Test With [] 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), 15 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L []

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 $30 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. 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 37 meter; instrument number PRL-3), pH (Beckman model pH 12 meter; instrument number PRL-4), conductivity (Labcomp SCT meter, instrument number PRL-9), and temperature (ASTM mercury thermometer; thermometer number 2265) 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). Nominal concentrations of test substance and the number of daphnids killed or immobilized were used to calculate the EC50 value.

V. SUMMARY

The acute toxicity of [redacted] to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours during December 12 to 14, 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 90169-3.

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), 15 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 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 0.001 g, resulting in a loading rate of 0.02 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 19 mg/L [redacted] with a 95% confidence interval of 12 - 25 mg/L.

VIII. RESULTS

Test vessels containing [] 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 0.001 g at the end of the test. Loading rate during the toxicity test was approximately 0.02 g/L. Exposure of daphnids to the reference toxicant sodium dodecyl sulfate resulted in a 48 hour median lethal concentration (LC50) of 13.8 mg/L.

The 24 and 48 hour EC50s for daphnids exposed to [] are presented in Table 3. Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 19 mg/L RM 27513, with a 95% confidence interval of 12 - 25 mg/L.

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	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	4	0	0	0
	4	5	5	5	0	0	0
15	1	5	5	4	0	0	2
	2	5	4	4	0	0	0
	3	5	5	5	0	0	2
	4	5	5	4	0	1	0
25	1	5	5	2	0	4	2
	2	5	5	4	0	0	1
	3	5	5	4	0	0	1
	4	5	5	3	0	0	2
40	1	5	5	3	0	5	3
	2	5	5	4	0	0	4
	3	5	1	1	0	0	0
	4	5	1	1	0	0	0
60	1	5	3	1	0	1	1
	2	5	0	0	0	0	-
	3	5	5	3	0	2	0
	4	5	5	2	0	1	2
100	1	5	5	0	0	5	-
	2	5	2	0	0	0	-
	3	5	5	0	0	4	-
	4	5	4	1	0	1	1

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	34 mg/L	--	Binomial/nonlinear interpolation
48 hours	19 mg/L	12 - 25 mg/L	Probit

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.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

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

Nominal concentration (mg/L)	Rep.	Conductivity (uaho/cm)			pH		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	1200	1300	1300	7.8	8.3	8.4
	2	1200	1300	1300	7.8	8.3	8.4
	3	1200	1300	1300	7.8	8.3	8.4
	4	1200	1300	1300	7.8	8.3	8.4
15	1	1200	1300	1300	7.8	8.3	8.4
	2	1200	1300	1300	7.8	8.3	8.4
	3	1200	1300	1300	7.8	8.3	8.4
	4	1200	1300	1300	7.8	8.3	8.4
25	1	1200	1300	1300	7.8	8.2	8.4
	2	1200	1300	1300	7.8	8.2	8.4
	3	1200	1300	1300	7.8	8.2	8.4
	4	1200	1300	1300	7.8	8.2	8.4
40	1	1200	1300	1300	7.8	8.1	8.3
	2	1200	1300	1300	7.8	8.1	8.3
	3	1200	1300	1300	7.8	8.1	8.3
	4	1200	1300	1300	7.8	8.1	8.3
60	1	1200	1300	1300	7.7	8.2	8.3
	2	1200	1300	1300	7.7	8.2	8.3
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	2	1200	1300	1300	7.7	8.1	8.3
	3	1200	1300	1300	7.7	8.1	8.3
	4	1200	1300	1300	7.7	8.1	8.3

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	20.1	20.7	19.8	8.3	9.4	9.4
	2	20.2	20.2	19.8	8.3	9.4	9.4
	3	20.1	19.8	19.9	8.3	9.4	9.5
	4	20.1	19.8	20.0	8.3	9.4	9.4
15	1	20.2	20.4	19.7	8.3	9.4	9.5
	2	20.2	20.2	19.6	8.3	9.4	9.3
	3	20.1	19.4	19.2	8.3	9.4	9.4
	4	20.1	19.8	19.9	8.3	9.4	9.4
25	1	20.1	19.8	19.8	8.3	9.5	9.6
	2	20.0	20.0	19.8	8.3	9.5	9.6
	3	19.9	19.8	19.8	8.3	9.5	9.6
	4	19.8	20.0	19.8	8.3	9.5	9.6
40	1	19.8	19.8	19.3	8.3	9.3	9.6
	2	19.9	19.9	19.3	8.3	9.3	9.6
	3	20.0	20.2	19.2	8.3	9.3	9.6
	4	20.0	20.4	19.1	8.3	9.4	9.6
60	1	20.2	19.4	19.3	8.3	9.5	9.6
	2	20.2	19.6	19.3	8.3	9.5	9.5
	3	20.1	20.2	19.2	8.3	9.5	9.7
	4	20.1	20.4	19.2	8.3	9.5	9.6
100	1	20.1	20.4	19.0	8.3	9.5	9.2
	2	20.1	20.4	19.2	8.3	9.5	9.1
	3	20.0	20.4	19.7	8.3	9.5	9.2
	4	20.0	20.3	19.3	8.3	9.5	9.1

EnviroSystems Study Number 90169-3

RESOURCE ANALYSTS, INCORPORATED
ENVIROSYSTEMS DIVISION

PRODUCT REGISTRATION LABORATORY
AQUATIC TOXICOLOGY STUDY PROTOCOL

Static Acute Toxicity Test With []
And The Daphnid, *Daphnia magna*

Sponsor

JM Company
PO Box 33428
St. Paul, Minnesota 55133

Sponsor Approval of Protocol

Ausan A. Beach

Date 11/8/90

Testing Facility

EnviroSystems Division
Resource Analysts, Inc.
1 Lafayette Road
Hampton, NH 03842

Estimated Test Initiation Date: October, 1990

Estimated Test Completion Date: December, 1990

Study Director: Timothy J. Ward

Study Director Approval of Protocol

Timothy J. Ward

Date 11/14/90

THIS IS A CERTIFIED
TRUE COPY.
SIGNATURE CAB DATE 3/29/91
63 pages

EnviroSystems Study Number 90169-3

1.0 TITLE

Static Acute Toxicity Test With [] And The Daphnid, *Daphnia magna*

2.0 PURPOSE

To determine the 24 hour and 48 hour median effective concentrations (EC50s) of the test substance to the daphnid, when exposed for 48 hours under static conditions.

3.0 TEST MATERIAL

3.1 The test substance and related stability and purity data will be supplied by the sponsor. Test material will be stored at room temperature in the original shipping container unless alternate storage conditions are requested by the sponsor. Handling of the test material will be in accordance with information contained in the sponsor-supplied Material Safety Data Sheet and RAI Standard Operating Procedure 01-02-1106. A subsample of test substance (approximately 1 gram) will be removed from the original container and transferred to a glass vial for archiving. All unused test substance will be returned to the sponsor.

3.2 Calculations are based on nominal concentrations of the test substance. Test substance stock solutions are prepared in deionized or dilution water without the use of a solvent (carrier) if possible. If a solvent is required, dimethylformamide, triethylene glycol, or acetone will be used. The concentration of solvent will be limited to 100 mg/L.

4.0 TEST SPECIES

4.1 The selection of test species is determined by the sponsor. Healthy juveniles (free from known diseases) from a single source will be used to initiate the test. They will be obtained from an in-house culture with a known history and will be less than 24 hours old at the initiation of the test. The wet weight of control organisms will be determined at the end of the test.

4.2 Identification of the test animals shall be verified using appropriate taxonomic keys.

5.0 PRETEST OBSERVATIONS AND PROCEDURES

5.1 Pretest observation data concerning the source, handling procedures, receipt date, disease treatment (if any), health, feeding, and mortality of test animals will be recorded and reported.

5.2 Daphnids will be maintained under static or replacement conditions prior to test initiation in the same water and at the same photoperiod and temperature that will be used for testing. The animals will be fed at least once daily with a mixture of yeast, trout chow, and cerophyl and/or freshwater algae. Daphnid cultures will be maintained in dilution water at test conditions (temperature, hardness, and photoperiod) for at least 14 days prior to testing.

6.0 EXPOSURE CONDITIONS

6.1 The test will be conducted under static conditions, and test media will be supplied only at the beginning of the test.

6.2 Dilution water will be natural ground water. It will be free of measurable concentrations of pesticides. Water will be passed through a (20 micron filter and chemically characterized (at least twice yearly analysis at RAI) prior to use.

6.3 Water temperature will be $20 \pm 1^\circ\text{C}$.

6.4 Dissolved oxygen concentration should be above 70% saturation, but aeration will not be utilized.

6.5 Photoperiod will be automatically controlled and adjusted to 16 hours light and 8 hours dark.

6.6 The test vessels will be 250 ml beakers of that contain 200 ml of test media. The loading rate will be less than 0.5 g/L.

7.0 STUDY CONDUCT

7.1 The results of a range finding investigation and/or previous testing data will be used to select the concentration range for the definitive test. If a range finding test is conducted animals will be exposed to a series of concentrations of test substance plus a control and a solvent control (if required).

7.2 During the definitive test the animals will be exposed for 48 hours to at least five concentrations of test substance plus a control (dilution water without test substance) and a solvent control (if necessary) under static conditions. Test concentrations will be chosen to bracket the anticipated EC50.

7.3 At least 20 animals will be exposed to each treatment. The animals will be exposed in groups of 5 animals per vessel (4 replicates per concentration), each containing 200 ml of solution. The animals will be assigned to randomly positioned test vessels (a random numbers table will be used to choose the position of each test vessel), and they will not be fed.

7.4 Animals will be added to test media after test substance has been added and aeration will not be employed.

7.5 The highest tested concentration will be 1,000 mg/L.

7.6 A toxicity test shall also be conducted with a reference toxicant and the batch of daphnids used for the test.

7.7 Dissolved oxygen concentration, pH, temperature, and conductivity will be measured and recorded at the beginning of the test and daily thereafter in all test vessels, as long as living animals are present in those vessels.

7.8 Mortality and sublethal effects (immobilization) will be recorded at 24 hour intervals during the test. Dead animals will be removed.

7.9 Data Analysis and Statistical Methods

7.9.1 The EC50 values and their 95% confidence intervals will be calculated using mortality and/or immobilization data by the binomial, moving average or probit methods.

8.0 QUALITY ASSURANCE AND QUALITY CONTROL

8.1 The test will be repeated if any of the following conditions occur:

8.1.1 Control survival at the end of the test is less than 90%, or more than 10% of the control daphnids are stressed or immobilized, or any control animals are trapped at the surface.

8.1.2 Temperature in any test vessel is outside of the range of 19 - 21°C.

8.2 The test will be conducted according to OECD Good Laboratory Practice Standards.

8.3 The test will be audited by the Quality Assurance Unit.

8.4 The Study Director will be responsible for reviewing all data and for recording any changes to procedures outlined in this protocol in a protocol amendment. Protocol amendments will be approved by the sponsor.

8.5 Original data will be archived at Resource Analysts Inc. for at least 10 years or it will be transferred to the sponsor for archiving.

9.0 REPORT

9.1 An original report will be prepared after review of a draft report by the sponsor. The final report will be signed by the Study Director and staff that conducted the study and prepared the report, and by the Quality Assurance Unit Representative.

9.2 The final report will consist of at least the following information:

- 9.2.1 Title page, including the study title, data requirement, author, study completion date, testing facility, and sponsor.
- 9.2.2 Sponsor-supplied confidentiality claims.
- 9.2.3 Good Laboratory Practice Statement.
- 9.2.4 Certification of Good Laboratory Practices.
- 9.2.5 Table of Contents.
- 9.2.6 Summary of test procedures and results.
- 9.2.7 Introduction.
- 9.2.8 Methods and Materials, including a description of test methods, organisms, dilution water, and statistical techniques.
- 9.2.9 Results, including all data from range-finding and definitive tests, and EC50 values.
- 9.2.10 References.
- 9.2.11 Appendices, including all water quality data.

10.0 REFERENCES

- OECD. 1984. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test. Adopted 4 April 1984.
- U.S. EPA. 1986. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Monday, January 6, 1986. Section 797.1300.
- U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987. Section 797.1300.

SUMMARY OF TEST CONDITIONS

Acceptable Species:	<i>Daphnia magna</i>
Test Type:	Static
Test Duration:	48 Hours
Flow Rate:	Not Applicable
Dilution Water:	Natural Ground Water
Age/Size of Test Organisms:	Juveniles; Less Than 24 Hours Old
Acclimation Period:	Continuous
Temperature:	20 ± 1°C
Photoperiod:	16 Hours Light/8 Hours Dark
Light Intensity:	Ambient Laboratory Lighting
Number of Concentrations:	5 or More; less than 1,000 mg/L
Dilution Factor:	≤1.6
Number of Replicates:	4 or More
Number of Organisms/Replicate:	5
Test Vessel Size:	250 ml or larger
Test Vessel Volume:	200 ml or more
Solvent:	Dimethylformamide, Acetone, or Triethylene Glycol. Less than or equal to 100 mg/L (0.5 g/L)
Loading Rate:	None During Exposure
Feeding:	None
Aeration:	None
Effects Measured:	Death; Immobilization
Test Concentration Analysis:	None
Water Quality Measurements:	DO, pH, Temperature and Conductivity at 0, 24, and 48 Hours
Acceptability Criteria:	≤10% Control Mortality, Stress, or Immobilization Acceptable Temperature Range