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NOTOX REPORT

Study Title

Static Acute Toxicity of TerraZyme
to the Fathead Minnow, *Pimephales promelas*

Sponsor

Nature Plus, Inc. 555 Lordship
Boulevard Stratford, Connecticut
06497

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Study Completed
December, 1993

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I. SIGNATURE PAGE

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II. TABLE OF CONTENTS

<i>SECTION :</i>	<i>PAGE</i>
I. Signature Page	2
II. Table of Contents	3
III. Index of Tables	4
IV. Summary	5
V. Methods and Materials	6
VI. Results	9
VII. References	12

APPENDIX :

Appendix A. WaterQuality Data from Toxicity Test	13
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III. INDEX OF TABLES

Table 1.	Chemical Characterization of a representative a sample of dechlorinated tap water used as dilution water for the toxicity test with TerraZyme and the fathead minnow, Pimephales promelas	Page 7
Table 2.	Survival and sublethal effect data from the toxicity test with TerraZyme and the fathead minnow, Pimephales promelas	10
Table 3.	Median lethal concentrations (LC50s) from the toxicity test with TerraZyme and the fathead minnow, Pimephales promelas	11
Table A.1.	Conductivity, dissolved oxygen concentration, pH, and temperature measured during the toxicity test with TerraZyme and the fathead minnow, Pimephales promelas	13



IV. SUMMARY

The acute toxicity of TerraZyme to the fathead minnow, *Pimephales promelas*, is described in this report. The test was conducted for Nature Plus, Inc. for 96 hours from December 17 to 21, 1993 at T.R. Wilbury Laboratories in Marblehead, Massachusetts. It was conducted by Jeanne Magazu, Robert Boeri, and Timothy Ward according to U.S. EPA TSCA and FIFRA test guidelines and the T.R. Wilbury Standard Operating Procedures Manual.

The test was performed under static conditions with two concentrations of test substance and a dilution water control at a temperature of $22 \pm 1^\circ\text{C}$. The dilution water was filtered dechlorinated tap water collected at Marblehead, Massachusetts and adjusted to a hardness of 176 mg/L. Nominal concentrations of the test substance were: 0 (control), 100, and 1,000 mg/L. Nominal concentrations were used for all calculations.

Organisms used in the test were procured from a commercial supplier and acclimated to test conditions at T.R. Wilbury. All test organisms, were in good condition at the beginning of the study.

Exposure of test organisms to the test substance resulted in a 96 hour LC50 greater than 1,000 mg/L. The no observed effect concentration is 1,000 mg/L.

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V. METHODS AND MATERIALS

TEST SUBSTANCE:

Eco Care (T.R. Wilbury Sample Number 280) was delivered to T.R. Wilbury Laboratories on December 16, 1993. It was contained in a 1 gallon plastic jug that was labelled with the following information: "Eco care" 1002-250". The test substance (a clear liquid) was shipped from Nature Plus, Inc., 555 Lordship Boulevard, Stratford, Connecticut 06497. Prior to use the test material was stored in the dark at room temperature. Based on information supplied by the sponsor, the test substance is assumed to be 100% pure and stable under exposure conditions, information provided by the sponsor indicated that Eco Care and TerraZyme are the same. Unused test substance is returned to the sponsor.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was carbon filtered, dechlorinated tap water collected at T.R. Wilbury in Marblehead, Massachusetts. Water was adjusted to a hardness of 160-180 mg/L and stored in polyethylene tanks where it was aerated and recirculated through particle filters, activated carbon, and an ultraviolet sterilizer. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile fathead minnows employed as test organisms were procured from a commercial supplier and acclimated to test conditions at T.R. Wilbury. They were from a single source and were identified using an appropriate taxonomic key. The fish were maintained in 100% dilution water under flow through conditions. During acclimation test organisms were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Prior to the test the temperature of the culture ranged from 21 to 23°C. Test organisms were fed dry commercial fish food (Tetra min Staple Food) once daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed from December 17 to 21, 1993 according to U.S. EPA TSCA and FIFRA test guidelines and the T.R. Wilbury Standard Operating Procedures Manual. The test was conducted at 9 target temperature of $22 \pm 1^\circ\text{C}$ with two concentrations of test substance and a dilution water control. Appropriate volumes of the test substance were added directly to each treatment vessel to formulate test concentrations.

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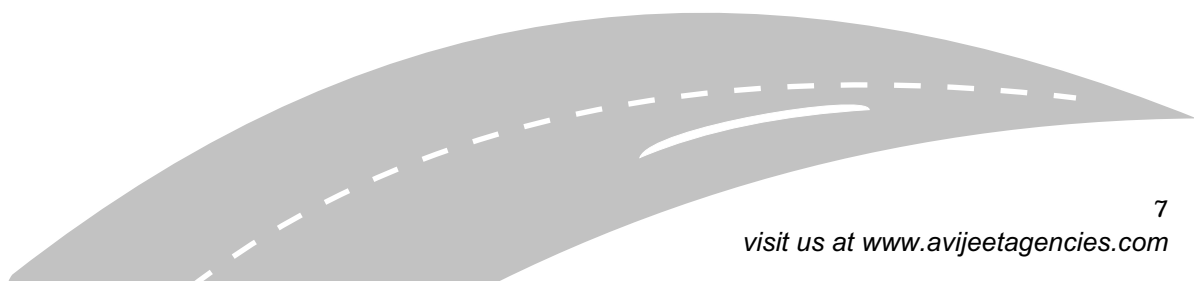
Table 1. Chemical Characterization of a representative sample of dechlorinated tap water used as dilution water for the toxicity test with TerraZyme and the fathead minnow, *Pimephales Promelas*.

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	8.2
Hardness	mg / L as CaCo3	--	176
Conductivity	μ mhos / cm	--	620
Organochlorine pesticides	Micrograms per liter	2.0	ND
Organophosphorus pesticides	Micrograms per liter	0.5	ND
Polychlorinated biphenyls	Micrograms per liter	1.0	ND

Note : pH, conductivity, and hardness were measured in dilution water from a control test vessel at the beginning of the toxicity test. Pesticide and PCD data was collected during March, 1993 as part of routing biannual water quality testing.

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ENVIRONMENT REPORTS





Ten test organisms were indiscriminately added to each of two replicates of each treatment. The test was performed in 20 liter glass aquaria that contained 15 liters of solution (media depth was approximately 18 cm). Test vessels were randomly arranged in a water bath during the 96 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 3 μEin . Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels.

The number of surviving organisms and the occurrence of sublethal effects (immobilization, loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number 1), pH (Beckman model pHI 12 meter; instrument number 96), conductivity (Cole Farmer meter; instrument number 3), and temperature (Beckman model pHI 12 meter; instrument number 96 with a pHT-1 probe) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity tests could not be interpreted by standard statistical techniques (Stephan, 1983) because greater than 50% survival occurred at all tested concentrations. The no observed effect concentration is the highest concentration of test substance that: allows at least 90% survival of exposed organisms *and* does not cause sublethal effects.

T.R. Wilbury I



VI. RESULTS

Test vessels were initially clear and remained clear throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and in Appendix A respectively. One hundred percent survival occurred in the control and at both concentrations of test substances.

The 24, 48, 72 and 96 hour LC50s for fathead minnows exposed to TerraZyme are presented in Table 3. The 96 hour LC50 is greater than 1,000 mg/L and the no observed effect concentration is 1,000 mg / L.



Table 2. Survival and sublethal effect data from the toxicity test with TerraZyme and the fathead minnow, *Pimephales promelas*.

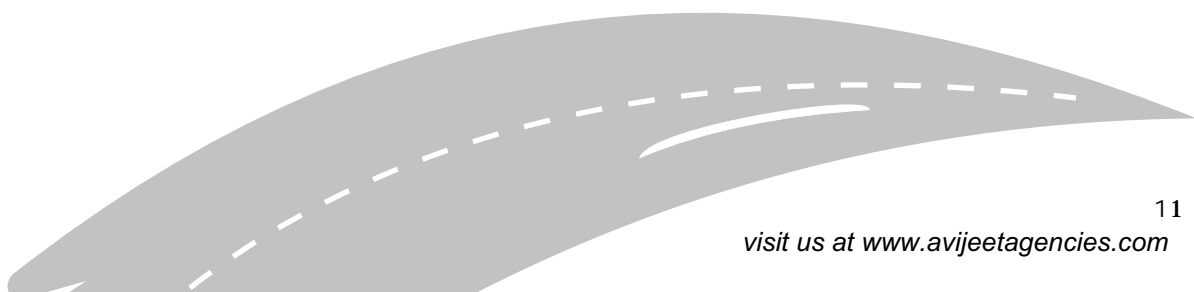
Nominal Concentration of Test Substance (mg/L)	Rep.	Number Alive					Number Affected				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0(Control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
100	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,000	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0



Table 3. Median lethal concentrations (Lc50s) from the toxicity test with TerraZyme and the fathead minnow, *Pimephales Promelas*.

Exposure Period	Lc 50	95 Percent confidence limit	Lc50 Calculation method
24 hours	> 1,000 mg/L	--	--
48 hours	> 1,00 mg/L	--	--
72 hours	> 1,000 mg/L	--	--
96 hours	> 1,000 mg/L	--	--

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VII . REFERENCES

Stephan, C.E. 1983. Computer program for calculation of Lc50 values. U.S. EPA. Duluth, MN. Personal Communication.

U.S. EPA. 1992. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules.

U.S. EPA. 1985. Standard Evaluation Procedure. Acute Toxicity Test for Freshwater Invertebrates. Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C. EPA 540/9-85-005.



Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A. 1. Conductivity, dissolved oxygen concentration, pH, and temperature measured during the toxicity test with Eco Care and the fathead minnow, *Pimephales promelas*.

Nominal Concentration of Test Substance (mg/L)	Rep.	Conductivity (umhos /cm)					pH				
		0 Hr	24 Hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0(Control)	1	620	600	600	590	590	8.2	8.1	7.8	7.7	8.0
	2	620	600	600	590	590	8.2	8.1	7.8	7.7	8.0
100	1	620	600	600	590	590	8.2	8.1	7.8	7.7	8.0
	2	620	600	600	590	590	8.2	8.1	7.8	7.7	8.0
1,000	1	620	600	600	590	590	8.2	8.1	7.8	7.7	8.0
	2	620	600	600	590	590	8.2	8.1	7.8	7.7	8.0

Nominal Concentration of Test Substance (mg/L)	Rep.	Dissolved Oxygen (mg/L)					Temperature (°C)				
		0 Hr	24 Hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 Hr	72 Hr	96 hr
0(Control)	1	8.6	7.8	6.8	5.8	6.2	22.0	21.9	21.2	21.5	21.8
	2	8.6	7.9	6.6	6.0	5.9	21.9	21.9	21.3	21.6	22.0
100	1	8.6	7.9	6.5	6.2	6.0	21.9	21.8	21.6	21.9	21.8
	2	8.6	7.9	6.5	6.0	6.0	21.7	21.9	21.4	22.0	22.0
1,000	1	8.6	7.8	6.2	5.9	6.0	21.7	21.9	21.2	21.9	21.9
	2	8.6	7.9	6.4	5.9	6.0	21.7	21.9	21.1	22.0	21.9

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