Study Title

Static Acute Toxicity of EcoCare™
to the Daphnid, *Daphnia magna*

Sponsor

Nature Plus, Inc.
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Stratford, Connecticut 06497

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Study Completed

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Testing Facility

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I. SIGNATURE PAGE

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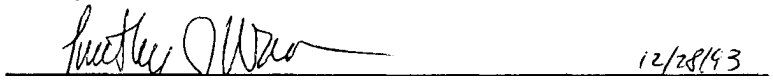

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IV. SUMMARY

The acute toxicity of EcoCare" to the daphnid, *Daphnia magna*, is described in this report. The test was conducted for Nature Plus, Inc. for 48 hours from December 22 to 24, 1993 at T.R. Wilbury Laboratories in Marblehead, Massachusetts. It was conducted by Jeanne Magazu 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 20 ± 2°C. The dilution water was filtered dechlorinated tap water collected at Marblehead, Massachusetts and adjusted to a hardness of 168 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 produced at T.R. Wilbury and were less than 24 hours old. All test organisms were in good condition at the beginning of the study.

Exposure of test organisms to the test substance resulted in a 48 hour LC50 greater than 1,000 mg/L. The no observed effect concentration is 1,000 mg/L.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

EcoCare™ (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: "EcoCare". 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.

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:

Daphnids employed as test organisms were less than 24 hours old at the start of the test. They were from a single source and were identified using an appropriate taxonomic key. They were produced at T.R. Wilbury. Prior to testing daphnid cultures were maintained in 100% dilution water under static 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. During the 14 days prior to the test the temperature of the culture ranged from 19 to 21°C. Test organisms were fed yeast and/or the freshwater algae *Selenastrum capricornutum* once daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed from December 22 to 24, 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 a target temperature of 20 ± 2°C with two concentrations of test substance and a dilution water control. Appropriate volumes of a 1,000 mg/L stock solution were added to each treatment vessel to formulate test concentrations. The 1,000 mg/L stock was prepared without the use of a solvent by the addition of 0.5 g test material to a 500 ml class A volumetric flask and bringing to volume with dilution water.

Table 1. Chemical characterization of a representative sample of dechlorinated tap water used as dilution water for the toxicity test with EcoCare™ and the daphnid, *Daphnia magna.*

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	8.3
Hardness	mg/L as CaC03	--	168
Conductivity	µmhos/cm	--	540
Organochlorine pesticides	micrograms per liter	2.0	ND
OrganophosphorUB pesticides	micrograms per liter	0.5	ND
Polychlorinated biphenyls	micrograms per liter	1.0	ND

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

Ten test organisms were indiscriminately added to each of two replicates of each treatment. The test was performed in approximately 250 ml glass beakers that contained 200 ml solution (media depth was approximately 6 cm). Test vessels were randomly arranged in an incubator during the 48 hour tests (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 7 µEin.

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, and 48 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 Parmer 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.

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 substance.

The 24 and 48 hour LC50s for daphnids exposed to EcoCare are presented in Table 3. The 48 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 EcoCare™ and the daphnid, *Daphnia magna.*

Nominal concentration of test substance	Rep.	Number of survivors			Number Affected		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 mg/L (control)	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
100 mg/L	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
1,000 mg/L	1	10	10	10	0	0	0
	2	10	10	10	0	0	0

Table 3. Median lethal concentrations (LC50s) from the toxicity test with EcoCare™ and the daphnid, *Daphnia magna*.

Exposure period	LC50	95 percent confidence limit	LC50 calculation method
24 hours	>1,000 mg/L	--	--
48 hours	>1,000 mg/L	--	--

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.l. Conductivity, dissolved oxygen concentration, pH, and temperature measured during the toxicity test with EcoCare™ and the daphnid, *Daphnia magna.*

Nominal concentration of test substance	Rep	Conductivity (µmhos/cm) 0 hr	24 hr	48 hr	Dissolved oxygen (mg/L) 0 hr	24 hr	48 hr
0 mg/L (control)	1	540	540	540	8.7	8.3	8.5
	2	540	540	540	8.7	8.3	8.5
100 mg/L	1	530	530	540	8.7	8.3	8.5
	2	530	530	540	8.7	8.3	8.5
1,000 mg/L	1	530	530	540	8.7	8.3	8.5
	2	530	530	540	8.7	8.3	8.5

Nominal concentration of test substance	Rep	Temperature (°C) 0 hr	24 hr	48 hr	pH 0 hr	24 hr	48 hr
0 mg/L (control)	1	19.8	20.7	20.1	8.3	8.2	8.1
	2	19.8	20.7	20.1	8.3	8.2	8.1
100 mg/L	1	19.8	20.6	20.1	8.3	8.2	8.1
	2	19.8	20.5	20.1	8.3	8.2	8.1
1,000 Mg/L	1	9.8	20.5	20.1	8.3	8.1	8.0
	2	19.8	20.5	20.1	8.3	8.1	8.0