

May 8, 1998

Mr. Dave Burgess
Elisha Technologies Co. L.L.C.
1961 Hirst Drive
P.O. Box 280
Moberly, MO 65270

Dear Dave:

The following is a summary of findings for the study "E-2000: A 96-hour static acute toxicity screening test with the saltwater mysid (*Mysidopsis bahia*)". This test was conducted by Wildlife International Ltd. for the Elisha Technologies Co. L.L.C. at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from May 4, 1998 to May 8, 1998. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 486A-105 in archives located on the Wildlife International Ltd. site.

The test substance was received at Wildlife International Ltd. on May 4, 1998 and was designated Wildlife International Ltd. test substance number 4455. The test substance was an off-white grease, identified as: E-2000. No additional information about this test substance was provided by the Sponsor. The test substance was stored at ambient room temperature.

Groups of saltwater mysids (*Mysidopsis bahia*) (<24 hours old) were exposed to three concentrations of the test substance, a negative (dilution water) control and a solvent control for 96 hours under static test conditions. One replicate test chamber was maintained in each treatment and control group, with 10 mysids in each test chamber for a total of 10 mysids per test concentration. Test chambers were 2-L glass beakers containing 1000 ml of test solution. Nominal test concentrations were negative control, solvent control, 1.0, 10, and 100 mg E-2000/L.

The solvent control and the 1.0, 10, and 100 mg E-2000/L test solutions were prepared by adding the appropriate amounts of test substance along with 0.50 ml of DMF to 1000 ml of filtered salt water. Test solutions were stirred vigorously approximately 30 minutes each using Teflon-coated magnetic stir bars and stir plates. Once mixing was complete, mysids were added to the test chambers. The solvent concentration in all of the treatment groups and the solvent control was 0.50 ml/L. All control test solutions appeared clear and colorless. Test solutions (1.0, 10, and 100 mg E-2000/L) appeared clear and colorless with particles of test substance on the surface and on sides of test chambers, increasing in intensity with increasing concentration.

Cool-white fluorescent light tubes were used to illuminate the test and were controlled with an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. Light intensity at test initiation was 285 lux at the surface of the water. The target test

temperature was $25 \pm 1^\circ\text{C}$. The test temperature, dissolved oxygen and pH were measured in each test chamber at the beginning and end of the test.

Observations of mortality and clinical signs of toxicity were made at approximately 3, 24, 48, 72, and 96 hours. Mortality data at 24, 48, 72, and 96 hours was used to estimate or calculate EC50 values. When possible, the EC50 values were calculated using the computer program of C.E. Stephan. The no-observed-effect-concentration (NOEC) was determined by visual interpretation of the mortality and clinical observation data.

Measurements of temperature ranged between 24.1 and 25.2°C throughout the test. Dissolved oxygen concentrations remained ≥ 5.5 mg/L (75 percent of saturation) and pH was 8.1 in all treatments and the control groups at the beginning and at the end of the test. At test initiation, the dilution water salinity was 20 parts per thousand (Table 1).

Observations of mortality and clinical signs of toxicity are presented in Table 2. Mysids in the negative and solvent controls, and all treatment groups appeared normal and healthy throughout the test. Any mortalities and clinical signs of toxicity in the treatment groups were considered to be incidental and not related to treatment. The 96 hour LC50 was determined to be > 100 mg E-2000/L and the NOEC was 100 mg E-2000/L (Table 3).

If you have any questions or require additional information, please let me know.

Sincerely,

Mark Mank
Study Director

Table 1

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Nominal Test Concentration (mg/L)	0 Hour			96 Hours		
	Temp ¹ (°C)	DO ² (mg/L)	pH	Temp (°C)	DO (mg/L)	pH
Sponsor:	Elisha Technologies Co. L.L.C.					
Test Substance:	E-2000					
Test Organism:	Saltwater mysid, <i>Mysidopsis bahia</i>					
Dilution Water:	Saltwater					
Negative Control	24.1	5.9	8.1	25.0	5.6	8.1
Solvent Control	24.2	5.9	8.1	25.1	5.6	8.1
1.0	24.1	5.9	8.1	25.2	5.5	8.1
10	24.1	5.9	8.1	25.1	5.5	8.1
100	24.1	5.9	8.1	25.1	5.5	8.1

¹ Temperature measured continuously during the test ranged from approximately 24.0 to 25.0°C.

² A dissolved oxygen concentration of 7.4 mg/L represents 100% saturation at 25°C in saltwater (20 ppt).

Table 2

Cumulative Percent Mortality and Treatment-Related Effects¹

Nominal Test Concentration (mg/L)	No. Exposed	0-24 Hours		24 Hours		48 Hours		72 Hours		96 Hours	
		No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects
Negative Control	10	0	10 AN	0	10AN	0	10 AN	0	10 AN	0	10 AN
Solvent Control	10	0	10 AN	0	10AN	0	10 AN	0	10 AN	0	10 AN
1.0	10	0	10 AN	0	10AN	0	10 AN	0	10 AN	0	10 AN
10	10	0	10 AN	0	10AN	0	10 AN	0	10 AN	0	10 AN
100	10	0	10 AN	0	10AN	0	10 AN	0	10 AN	0	10 AN

¹ Observed Effects: AN = Appears Normal

Table 3

EC50 Values

Time	EC50 (mg/L)	Lower 95% Confidence Limits (mg/L)	Upper 95% Confidence Limits (mg/L)	Statistical Method
96 Hours	>100	-- ¹	-- ¹	Probit

¹ Confidence limits could not be calculated with the mortality data obtained.