

**CHEM-CRETE  
PAVIX (CCC-100)**

48-Hour Acute Toxicity Test Report

*Ceriodaphnia dubia*  
*Pimephales promelas*

April 2004



**HUTHER AND ASSOCIATES, INC.**

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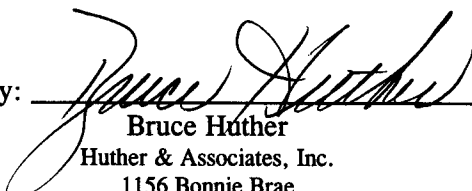
*Ceriodaphnia dubia*  
*Pimephales promelas*

April 2004

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48-HOUR LC50 PRODUCT REPORT

Client ..... Chem-Crete                      Project No: ..... CCPAV-1  
Sample ..... PaviX (CCC-100)                      Test Date ..... April 2004

**INTRODUCTION**

A product identified as PaviX (CCC-100) was delivered to Huther and Associates Inc. on April 2, 2004. Two acute toxicity tests were requested: a static acute 48-hour definitive toxicity test using *Ceriodaphnia dubia* (water flea) and a static acute 48-hour definitive toxicity test using *Pimephales promelas* (fathead minnow). Test procedures followed recommended methods contained in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition", EPA-821-R-02-012, October 2004.

*C. dubia* and *P. promelas* are two freshwater aquatic indicator organisms frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The tests are designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

**SAMPLE PREPARATION**

The product was initially prepared for definitive testing by adding wide concentrations of product to laboratory water (ranging tests). Based on the results of the ranging tests, definitive test solutions were prepared using a smaller range of concentrations.

The ranging test concentrations for both species were 5, 10, 25, 50, 100, and 200 ml/L. The definitive test concentrations for both species were 5, 10, 20, 30, 40, and 50 ml/L. The pH exceeded 9.0 in the 10 ml/L to 50 ml/L solutions. An aliquot of each solution was pH adjusted to 8.5 and also tested. Laboratory water/ control water was distilled, deionized water reconstituted with reagent grade chemicals to a hardness of 160 mg/L as CaCO<sub>3</sub> and a pH of 8.26.

**TEST DESIGN**  
*Ceriodaphnia dubia*

The 48-hour static, non-renewal, definitive *Ceriodaphnia dubia* test was conducted in 20 mL beakers containing 10 mL of test solution. The test was initiated April 5, 2004 following completion of the ranging test. Five *C. dubia* neonates, less than 24-hours old, were added to each of the four replicate beakers per concentration. Neonates were fed a concentration of *Selenastrum capricornutum* plus cerophyll extract while in holding prior to test initiation. The test was conducted for 48-hours during which survival was recorded daily.

A control of four replicate beakers containing five *C. dubia* neonates each in laboratory water was conducted concurrently with the test. The test was terminated on April 7, 2004. Survival data was statistically analyzed using the Trimmed Spearman-Kärber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

**RESULTS**  
*Ceriodaphnia dubia*

The following estimated LC50 value was determined for PaviX:

<b>48-Hour Definitive Test</b>				
Conc. (ml/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
5	20	20	0	100.0
10	20	20	0	100.0
20	20	1	19	5.0
30	20	0	20	0.0
40	20	0	20	0.0
50	20	0	20	0.0

Percent Spearman-Kärber Trim: 0.0%

**Estimated LC50 (ml/L): 7.32**

95% Upper C.L. (mg/L): 7.83

95% Lower C.L. (mg/L): 6.84

The following estimated LC50 value was determined for PaviX following pH adjustment to 8.5:

<b>48-Hour Definitive Test - pH Adjusted</b>				
Conc. (ml/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
10	20	20	0	100.0
20	20	0	20	0.0
30	20	0	20	0.0
40	20	0	20	0.0
50	20	0	20	0.0

Percent Spearman-Kärber Trim: 0.0%

**Estimated LC50 (ml/L): 14.14**

95% Upper C.L. (mg/L): N/A

95% Lower C.L. (mg/L): N/A

**TEST DESIGN**  
*Pimephales promelas*

The 48-hour static, non-renewal, definitive *Pimephales promelas* test was conducted in 250 mL beakers containing 200 mL of test solution. The test was initiated April 5, 2004 following completion of the rangefinding test. Ten *P. promelas* larvae were added to each of four replicate beakers per concentration. Larvae originated from laboratory cultures and were six days old at test initiation. Larvae were fed laboratory cultured *Artemia* nauplii during test exposure. The test was conducted for 48-hours during which survival was recorded daily.

A control of four replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. The test was terminated on April 7, 2004. Survival data was statistically analyzed using the Trimmed Spearman-Kärber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

**RESULTS**  
*Pimephales promelas*

The following estimated LC50 value was determined for PaviX:

<b>48-Hour Definitive Test</b>				
Conc. (ml/L)	# exposed	# alive	#dead	% survival
Control	40	39	1	97.5
5	40	40	0	100.0
10	40	32	8	80.0
20	40	31	9	77.5
30	40	7	33	17.5
40	40	0	40	0.0
50	40	0	40	0.0

Percent Spearman-Kärber Trim: 0.00%

**Estimated LC50 (ml/L): 20.29**

95% Upper C.L. (ml/L): 22.86

95% Lower C.L. (ml/L): 18.01

*Pimephales promelas*  
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The following estimated LC50 value was determined for PaviX following pH adjustment to 8.5:

<b>48-Hour Definitive Test</b>				
Conc. (ml/L)	# exposed	# alive	#dead	% survival
Control	40	40	0	100.0
10	40	38	2	95.0
20	40	27	13	67.5
30	40	15	25	37.5
40	40	21	19	52.5
50	40	10	30	25.0

Percent Spearman-Kärber Trim: 25.0%

**Estimated LC50 (ml/L): 29.80**

95% Upper C.L. (ml/L): 35.55

95% Lower C.L. (ml/L): 24.99

**DISCUSSION AND  
CONCLUSIONS**

*Ceriodaphnia dubia* were determined to be the more sensitive species to PaviX (CCC-100) with an estimated 48-hour LC50 concentration of 7.32 ml/L. The estimated 48-hour LC50 concentration to *Pimephales promelas* was 20.29 ml/L. Adjustment to pH 8.5 increased survival for both species.

Based on the recommended application rate and the probable dilution factor associated with a rainfall run-off event or drift during application, indications were that this product would not cause acute toxicity in the receiving body of water.