VISCO, INC.
SEAL-ONCE

48-Hour Acute Toxicity Test Report

Ceriodaphnia dubia

Pimephales promelas

June 2003

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TABLE OF CONTENTS

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48-HOUR LC50 PRODUCT REPORT

Client.....Visco, Inc. Project No.: DYN-3
Sample.....SEAL-ONCE Test Date: ..... June 2003

INTRODUCTION

A product identified as SEAL-ONCE (wood preservative) was delivered to Huther and Associates from Visco, Inc. on May 19, 2003. Two acute LC50 toxicity tests were requested: a static acute 48-hour definitive toxicity test using *Ceriodaphnia dubia* (opossum shrimp) and a static acute 48-hour definitive toxicity test using *Pimephales promelas*. Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition*", EPA/600/4-90/027F, August 1993.

SAMPLE PREPARATION

The product was prepared at a ratio of two parts water: one part product following the manufacturer's recommendation. The product went readily into solution. Two test concentrations were prepared by adding 0.037 mL of the two parts water: one part product (stock) to 1,000 mL laboratory water (0.0123 ml/L after dilution factor), and 0.370 mL of stock to 1,000 mL laboratory water (0.123 ml/L after dilution factor). Laboratory water and control water were distilled, deionized water reconstituted with reagent grade chemicals to a hardness of 140 mg/L as $CaCO_3$ and a pH of 8.1

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 June 18, 2003. Five *C. dubia* neonates, less than 24 hours old, were added to each of four replicate beakers per concentration. Neonates were fed a concentration of Selenastrum capricornutum plus cerophyll extract while in holding prior to test intiation. 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 June 20, 2003. Survival data was statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

RESULTS

Ceriodaphnia dubia ONCE:

The following estimated LC50 value was determined for SEAL-

48-Hour Definitive Test				
Conc. (ml/L)	# Exposed	# Alive	# Dead	% Survival
Control	20	20	0	100.0
0.0123	20	20	0	100.0
0.1230	20	5	10	50.0

Percent Spearman-Karber Trim:	50.0%
Estimated LC50 (ml/L):	**0.12**
95% Upper C.L. (ml/L):	N/A
95% Lower C.L. (ml/L):	N/A

TEST DESIGN

The 48-hour static, non-renewal, definitive *Pimephales promelas* test was conducted in 250 mL beakers containing 200 mL of test solution. Ten *P. Promelas* larvae were added to each of four replicate beakers per concentration. Larvae originated from laboratory cultures and were ten 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 June 18, 2003. Survival data was statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population.

RESULTS

The following estimated LC5O value was determined for SEAL-ONCE:

48-Hour Definitive Test				
Conc. (ml/L)	# Exposed	# Alive	# Dead	% Survival
Control	40	40	0	100.0
0.0123	40	40	0	100.0
0.1230	40	0	40	0.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (ml/L):	**0.04**
95% Upper C.L. (ml/L):	N/A
95% Lower C.L. (ml/L):	N/A

DISCUSSION

Pimephales promelas was determined to be the more sensitive species to SEAL-ONCE, with an estimated 48-hour LC50 concentration of 0.04 ml/L. The estimated 48-hour LC50 concentration to Ceriodaphnia dubia was 0.12 ml/L.