



May 31, 2001

Mr. Bill Gannon
Solidification Products International, Inc.
Post Office Box 423
North Haven, CT 06473

Re: Aquatic Toxicity Test Results
Sample ID: Filtered-Solidification Products
ERL Project Number: 08217-01

Dear Mr. Gannon:

This report provides you with the results of the toxicity tests performed at the Environmental Risk Limited (ERL) laboratory on a sample of synthetic laboratory control water that was passed through your filter. Toxicity tests were performed on the sample using two species of aquatic organisms according to the specifications you requested.

Sampling

US Mail transported your filter to the ERL laboratory. ERL personnel received the filter on May 21, 2001. The toxicity tests were initiated at 10:00 a.m. on May 22, 2001.

Aquatic Toxicity Analysis

Aquatic toxicity tests were performed on the samples using both *Ceriodaphnia dubia* (cerio) and *Pimephales promelas* (fathead minnow) as the standard test organisms. The test procedures followed the protocols described by the United States Environmental Protection Agency (USEPA) in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (EPA/600/4-90/027F). The complete aquatic toxicity test methodology is included in Attachment A. The Test Data Sheets and the Chain-of-Custody form are included in Attachment B.

The toxicity tests were performed using a Pass/Fail protocol, which consisted of four replicate chambers of filtered water and four replicate chambers of ERL laboratory synthetic soft water, reconstituted to 40 to 48 ppm as CaCO₃, as the controls. Both test organisms (*Cerio* and fathead minnows) were exposed to the same concentrations and test conditions. The results of the toxicity tests are presented in Table I.

Table I Results of Aquatic Toxicity Tests						
Sample I.D.	Test Date	Test Number	Species	Test Protocol	Test Duration	Percent Survival
Filtered Control	5/22/01	01-0556	<i>C. dubia</i>	Pass/Fail	48 hours	100%
Filtered Control	5/22/01	01-0557	<i>P. promelas</i>	Pass/Fail	48 hours	100%

Standard toxicant tests on both test species were run concurrently with the toxicity tests. Copper nitrate solution obtained from the CTDEP was used as the reference toxicant. Standard toxicant tests measure the sensitivity of the stock organisms to a standard reference toxicant and yield data that ensure the organisms are healthy and non-stressed. The data were in the acceptable range of LC50 values normally determined with these test species using copper nitrate as the standard toxicant. The results of the standard toxicant tests are presented in Table II.

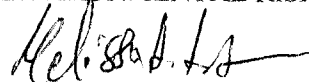
Table II Results of Standard Toxicant Tests					
Test Date	Reference Toxicant	Species	Test Protocol	Test Duration	LC50
5/21/01	CuNO ₃ #447-00	<i>C. dubia</i>	Std. Tox.	48 hours	4.401
5/21/01	CuNO ₃ #447-00	<i>P. promelas</i>	Std. Tox.	48 hours	34.260

Conclusions

The tests on the filtered sample resulted in 100% survival for both the *Ceriodaphnia* and the fathead minnow. If you have any questions concerning this report, please feel free to contact Susan Luchina or me at (860) 242-9933.

Sincerely,

ENVIRONMENTAL RISK LIMITED



Melissa A. Arthur
Environmental Technician

Attachment A

Aquatic Toxicity Test Methodology

ENVIRONMENTAL RISK LIMITED
REGION 2 - AQUATIC TOXICITY TEST METHODOLOGY

GENERAL INFORMATION

The aquatic toxicity tests conducted by the Environmental Risk Limited (ERL) laboratory are performed according to the United States Environmental Protection Agency (USEPA) methodology specified in "Methods of Measuring the Acute Toxicity of Effluent to Freshwater and Marine Organisms", August 1993 (EPA/600/4-90/027F). The organisms used for the evaluation of toxicity in freshwater are the invertebrate *Ceriodaphnia dubia* (*Ceriodaphnia*) and the vertebrate *Pimephales promelas* (fathead minnows). The tests are either static-acute, nonrenewal tests conducted for 48 hours or 7-day chronic, daily renewal tests. During the test procedures, the temperature is maintained between 24°C and 26°C. All effluents and dilution waters are brought within this temperature range before test initiation. The photoperiod is 16 hours of illumination for each 24 hour period. Dissolved oxygen, temperature and pH are measured and recorded at test initiation and every 24 hours thereafter. During chronic tests, these measurements are taken on the existing and renewal test solutions. The number of surviving organisms and daily reproduction of each female (for chronic *Ceriodaphnia* tests only) is also recorded every 24 hours. No aeration of the test vessels is necessary unless the dissolved oxygen concentration falls below 40% saturation.

MATERIALS

Test Organisms

Test organisms used by ERL are cultured in the ERL laboratory. The organisms were originally obtained from the EPA laboratory in Newton, Ohio. On occasion, when egg production is low or when a large number of tests has depleted the laboratory's supply, organisms are purchased from a reliable organism vendor. Acclimation procedures are initiated immediately upon receipt of the outside organisms and standard toxicant tests are performed to ensure quality.

The *Ceriodaphnia* used in the ERL laboratory for toxicity testing are neonates less than 24 hours old. The neonates are collected from actively reproducing cultures of females which have had at least three broods. The fathead minnows used in toxicity tests are hatched from eggs collected on a daily basis from breeder fish. The fish used in acute toxicity testing are 1 to 14 days old. The chronic tests are conducted using newly hatched larvae less than 24 hours old.

Dilution Water

The water used for effluent dilutions is reconstituted laboratory water made according to EPA protocol. The water is prepared by adding salts to deionized water to reach a desired hardness level. The hardness of this synthetic dilution water depends on permit specifications. Hardness is analyzed according to the EDTA method as given in "Standard Methods for The Examination of Water and Wastewater", 18th Edition. The water is prepared in a critically clean carboy and discarded if not used within 14 days. In some cases, receiving water collected upstream of the discharge is used as the diluent. When this occurs, an additional control consisting of reconstituted laboratory water is included.

METHODS

48-Hour Static Acute Tests

The definitive (LC50) tests are performed using five effluent dilutions and a control. The dilution series is generally 6.25%, 12.5%, 25%, 50% and 100%. This series may change to accommodate a permit specific effluent toxicity limit. All dilutions are prepared from lowest to highest effluent concentration utilizing the same series of glassware (graduated cylinders, mix beakers, etc.). Each of the six concentration levels is run in duplicate (A & B) for the fish test, with ten test organisms per replicate. The test vessels are 600 ml beakers with 450 ml of test solution per beaker. The *Ceriodaphnia* tests utilize four replicates per concentration. The test vessels are 30 ml beakers with 25 ml of test solution per beaker. Each of the four replicates contains five test organisms. All glassware, including that used for the dilution procedure, is critically cleaned with detergent, acid and acetone before finally rinsing several times with deionized water.

Dissolved oxygen, temperature, and pH are determined at test initiation and measured every 24 hours during the test. Conductivity, hardness, alkalinity and chlorine concentration are measured on the control water and the 100% effluent dilution prior to test initiation. Each day of the test, survival of the organisms is recorded. Death is considered to be the point when the organism is immobile and unresponsive when prodded. Any test in which the mean survival of the organisms in the control chambers is less than 90% is considered an invalid test and must be repeated.

The LC50 value is computed using four different statistical programs. The LC50 values used to determine compliance are those that yield the tightest 95% confidence interval for that particular test.

7-day Daily Renewal Chronic Tests

Chronic toxicity tests are performed using a series of five effluent dilutions and a control. The dilution series is generally 6.25%, 12.5%, 25%, 50% and 100% effluent although this may be altered to include the chronic no-observed effect concentration (C-NOEC) defined by the discharge permit. All dilutions are prepared from lowest to highest effluent concentration utilizing the same series of glassware (graduated cylinders, mix beakers etc.). Three 24-hour composite effluent samples are used during the tests. All glassware is critically cleaned with detergent, acid and acetone before finally rinsing several times with deionized water.

For the chronic fish toxicity test, each of the six concentrations is run in triplicate (A, B & C) with 10 test organisms per replicate. The test vessels are 600 ml beakers each containing 250 ml of test solution. Each replicate is fed 0.1 ml of newly hatched brine shrimp (*Artemia salina*) twice daily. The first feeding takes place at the beginning of the day, the second feeding is after test solution renewal. Renewal entails siphoning the previous days test solution and any uneaten brine shrimp, and replacing with freshly made test solution.

The *Ceriodaphnia dubia* chronic utilizes 10 replicates per concentration, each containing one test organism. The test vessels are 30 ml plastic beakers each with 25 ml of test solution. The daphnia are fed once per day at a rate of 0.1 ml YCT and 0.1 ml of the green algae *Selenastrum capricornutum*. Feeding takes place in conjunction with test renewal. Renewal is performed by gently transferring each *Ceriodaphnia* to a new test beaker with fresh test solution via a pipette.

Dissolved oxygen, temperature and pH are determined at test initiation and every 24 hours during the test. These measurements are taken on the existing and renewal test solutions. Conductivity measurements are made daily on each effluent concentration and the control. Hardness, alkalinity and chlorine concentration are measured on each of the three effluent samples and the dilution water. Each day of the test, reproduction of each *Ceriodaphnia* female is recorded as well as the survival of the test organisms (both *Ceriodaphnia* and fish). Death is considered to be the point when the organism is immobile and unresponsive when prodded. Any chronic fish test in which the mean survival of the organisms in the control chambers is less than 80% is considered an invalid test and must be repeated. A *Ceriodaphnia* test is considered valid when 80% or greater survival of the control organisms is achieved, 60% of the surviving control organisms have had a third brood, and the average young per female is 15.

For those modified chronic tests which require 48-hour LC50 results, the LC50 is computed using four different statistical programs. The LC50 values used to determine compliance are those that yield the tightest 95% confidence interval for that particular test. The chronic no-observed effect concentration (C-NOEC) is determined by a ANNOVA/DUNNETTS software package by D.L. Weiner, Computer Sciences Corp., Cincinnati, Ohio.

QUALITY ASSURANCE

EPA protocol is followed for sampling and handling of effluents, chemical analysis and aquatic toxicity testing. All laboratory meters and equipment are calibrated daily and maintained according to the manufacturers instructions. Illumination is maintained on a regular cycle at 16 hours of light and 8 hours of dark per 24 hour period by timers which are checked periodically. Ambient room temperature is recorded with an automatic temperature recorder and the charts are kept on file for at least two years.

The sensitivity and general health of the test species are monitored at least monthly and generally weekly by running mortality studies on the cultures using a standard toxicant. The standard toxicant used for acute reference testing is copper nitrate supplied by the Connecticut Department of Environmental Protection. The toxicant used in chronic reference testing is reagent grade sodium chloride purchased from a chemical supplier. The results from the standard toxicant test run during the same time period as the effluent toxicity test are included in the toxicity report and raw data is filed for use in tracking mortality data. In addition to these in-house reference tests, ERL also participates in the USEPA QA/QC program and various State QA/QC programs.

Attachment B

Raw Test Data and Chain-of-Custody Form

Toxicity Test Data Sheet

Facility Name: Solidification Products
 Dilution Water Source: ERL LAB
 Date Collected: N/A

Sample ID: _____
 Hardness: 56 46 44 ppm as CaCO₃
 Date Received: 5/21/01

Test Organism: Ceriodaphnia dubia
 Test Duration: 48 Hours

Age: 24 Hours/Days
 Beginning Date: 5/22/01 Time: 10:00am

Effluent Dilution (%)	Hour	Number of Organisms Surviving			Dissolved Oxygen (mg/L)			Temperature (°C)			pH (su)			Conductivity (µMHOS)			
		00	24	48	00	24	48	00	24	48	00	24	48	00	24	48	
100 A	5	5	5	5	7.9	7.2	7.7	25	25	25	7.2	7.4	7.6	167	171	175	
100 B	5	5	5	5	7.9	7.2	7.6	25	25	25	7.2	7.5	7.6	167	175	180	
100 C	5	5	5	5	7.9	7.2	7.6	25	25	25	7.2	7.6	7.6	167	172	180	
100 D	5	5	5	5	7.9	7.2	7.6	25	25	25	7.2	7.6	7.7	167	175	181	
CONTROL A	5	5	5	5	7.8	7.6	8.2	25	25	25	7.6	7.6	7.7	150	165	170	
CONTROL B	5	5	5	5	7.8	7.5	8.0	25	25	25	7.6	7.6	7.7	150	165	172	
CONTROL C	5	5	5	5	7.8	7.5	8.1	25	25	25	7.6	7.7	7.8	150	168	168	
CONTROL D	5	5	5	5	7.8	7.5	8.0	25	25	25	7.6	7.8	7.8	150	169	170	
MEAN SAMPLE SURVIVAL (%)										CONTROL SURVIVAL (%)				A	B	C	D
[(A+B+C+D)/4] X 10 = <u>100%</u>														<u>100%</u>	<u>100%</u>	<u>100%</u>	<u>100%</u>

Test Organism: Pimephales promelas
 Test Duration: 48 Hours

Age: 12 Hours/Days
 Beginning Date: 5/22/01 Time: 10:00am

Effluent Dilution (%)	Hour	Number of Organisms Surviving			Dissolved Oxygen (mg/L)			Temperature (°C)			pH (su)			Conductivity (µMHOS)			
		00	24	48	00	24	48	00	24	48	00	24	48	00	24	48	
100 A	5	5	5	5	7.9	7.2	7.7	25	25	25	7.2	7.4	7.6	167	171	175	
100 B	5	5	5	5	7.9	7.2	7.6	25	25	25	7.2	7.5	7.6	167	175	180	
100 C	5	5	5	5	7.9	7.2	7.6	25	25	25	7.2	7.6	7.6	167	172	180	
100 D	5	5	5	5	7.9	7.2	7.6	25	25	25	7.2	7.6	7.7	167	175	181	
CONTROL A	5	5	5	5	7.8	7.6	8.2	25	25	25	7.6	7.6	7.7	150	165	176	
CONTROL B	5	5	5	5	7.8	7.5	8.0	25	25	25	7.6	7.6	7.7	150	165	172	
CONTROL C	5	5	5	5	7.8	7.5	8.1	25	25	25	7.6	7.7	7.8	150	168	168	
CONTROL D	5	5	5	5	7.8	7.5	8.0	25	25	25	7.6	7.8	7.8	150	169	170	
MEAN SAMPLE SURVIVAL (%)										CONTROL SURVIVAL (%)				A	B	C	D
[(A+B+C+D)/4] X 10 = <u>100%</u>														<u>100%</u>	<u>100%</u>	<u>100%</u>	<u>100%</u>

Toxicity Test Data Sheet

Facility Name: Solidification Products
 Date Received: 5/21/01

Sample ID: _____
 Hardness: 46 ppm as CaCO₃

REFERENCE TOXICANT RESULTS				
SPECIES	DATE	REFERENCE TOXICANT	SOURCE	LC50
<i>Ceriodaphnia dubia</i>	5/21/01	CuNO ₃ # 447-00	CTDEP	4.401 ppb
<i>Pimephales promelas</i>	5/21/01	CuNO ₃ # 447-00	CTDEP	34.260 ppb

Ceriodaphnia dubia

Parameter	Hours	100% Test Sample	0% (Control)
		Initial (00)	Initial (00)
Alkalinity		50	30
Hardness		46	44
TRC		0.02	0.00

Pimephales promelas

Parameter	Hours	100% Test Sample	0% (Control)
		Initial (00)	Initial (00)
Alkalinity		50	30
Hardness		46	44
TRC		0.02	0.00

Laboratory Official: Susan Luchina Title: Lab Manager
 Signature: Susan L Date: 5/31/01