

**BIOASSAY REPORT**  
**ACUTE TOXICITY TESTS**

Conducted December 16 through 20, 2003

Prepared for  
Volatile Free, Inc.  
Brookfield, Wisconsin

Prepared by  
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## Summary

S-F Analytical Laboratories conducted acute toxicity tests on laboratory water exposed to a film of VFI205 polyurea coating provided by Volatile Free, Inc., Brookfield, Wisconsin. The bioassays were conducted from December 16 through 20, 2003, as part of noncompliance toxicity evaluation. *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows were used as the test organisms. The following is a summary of the test results:

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Test Media	Acute Toxicity as LC <sub>50</sub>		
	<i>Ceriodaphnia dubia</i>	<i>Daphnia magna</i>	Fathead Minnow
VFI205 polyurea coating exposed water	>100%	>100%	>100%

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The results of the tests show that:

- Laboratory control water data were acceptable in all bioassays.
- Because there was no toxicity recorded in the highest sample concentrations tested, the statistical analysis for determining the 48-hour and 96-hour LC<sub>50</sub> values could not be conducted. Therefore, the *Ceriodaphnia dubia* and *Daphnia magna* 48-hour LC<sub>50</sub> values for the VFI205 polyurea coating exposed water sample was greater than 100 percent and the fathead minnow 96-hour LC<sub>50</sub> for the VFI205 polyurea coating exposed water sample was greater than 100 percent.

## Introduction

This report presents the results of the laboratory acute toxicity tests conducted by S-F Analytical Laboratories on laboratory water exposed to a film of VFI205 polyurea coating, provided by Volatile Free, Inc., Brookfield, Wisconsin. The bioassays used *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows as the test organisms and were performed from December 16 through 20, 2003, as part of a noncompliance toxicity evaluation.

## Methods

All laboratory methods, including organism culture, sample handling, test procedures, and data analyses, were in accordance with the recommendations of the U.S. Environmental Protection Agency (EPA) [1] and the S-F Analytical Bioassay Laboratory Standard Operating Procedures.

## Sample Collection and Handling

A photocopy of the sample receipt form is included in Appendix B. One sample film of the VFI205 polyurea coating was used as follows:

Description	Sample No.	Date Received	Date Tested
VFI205 polyurea coating film	031239.01	12/11/03	12/16-20/03

The sample was provided by Volatile Free, Inc. and was delivered to the S-F Analytical Bioassay Laboratory. Upon arrival, the sample was logged in and stored at room temperature for later use.

## Test Organisms

All test organisms were cultured at the S-F Analytical Bioassay Laboratory.

## **Test Procedures**

### ***Sample Preparation***

Two 3.25" x 3.25" pieces of the film were cut and submersed in separate containers of S-F Bioassay laboratory control water and place on a shaker table (set at 100 oscillations per minute) for 24 hours. The size of the sample film per volume of water was calculated to simulate the area and volume ratio of the expected usage at a Minnesota fish hatchery. The exposure period and agitation on a shaker table was designed to allow potential contaminants to leach into the water. At the end of the exposure period, the pieces of film were removed from the test media and concentrations were prepared daily using laboratory control water as the diluent.

### ***Bioassays***

Bioassay test conditions are summarized in Tables 1 through 3.

### ***Physicochemical Monitoring***

Total alkalinity and hardness were measured in the laboratory control.

Dissolved oxygen (DO), pH, and conductivity were measured initially and daily thereafter in all test solution renewals. DO and pH were measured in one test chamber or composite of each 24-hour old test solution.

Bioassay incubator temperature was electronically monitored hourly by thermocouple and data logger and a 24-hour summary of mean values was recorded.

### ***Data Analysis***

When appropriate, an LC<sub>50</sub> (median lethal concentration) was calculated on each data set using a computer program.

Acute toxicity was defined according to the following EPA criteria:

- Less than 50 percent survival of test organisms at test termination (48 hours for *Ceriodaphnia dubia* and *Daphnia magna*; 96 hours for fathead minnows).

**Table 1**  
**Summary of Test Conditions for the**  
*Ceriodaphnia* **Acute Bioassay**  
**Conducted for Volatile Free, Inc.**  
**Brookfield, Wisconsin**  
**December 16 through 18, 2003**

1.	Test organism	<i>Ceriodaphnia dubia</i> (Crustacea: Cladocera)
2.	Test type	Static renewal
3.	Age of test organisms	Less than 24 hours
4.	Test chamber size	30 mL
5.	Test solution volume	25 mL
6.	Renewal of test solutions	Daily
7.	Number of replicate chambers per solution	4
8.	Number of test organisms per chamber	5
9.	Primary control/dilution water	Moderately hard reconstituted laboratory medium
10.	Test media	VFI205 polyurea coating exposed water
11.	Test concentrations	6.25, 12.5, 25, 50, and 100%
12.	Temperature	20 ± 1°C
13.	Feeding regime	None
14.	Aeration	None
15.	Test duration	48 hours
16.	Effects measured/Endpoint	Survival/LC <sub>50</sub>
17.	Test acceptability	90% or greater mean survival in the laboratory water control.

**Table 2**  
**Summary of Test Conditions for the**  
***Daphnia* Acute Bioassay**  
**Conducted for Volatile Free, Inc.**  
**Brookfield, Wisconsin**  
**December 16 through 18, 2003**

1.	Test organism	<i>Daphnia magna</i> (Crustacea: Cladocera)
2.	Test type	Static renewal
3.	Age of test organisms	Less than 24 hours
4.	Test chamber size	30 mL
5.	Test solution volume	25 mL
6.	Renewal of test solutions	Daily
7.	Number of replicate chambers per solution	4
8.	Number of test organisms per chamber	5
9.	Primary control/dilution water	Moderately hard reconstituted laboratory medium
10.	Test media	VFI205 polyurea coating exposed water
11.	Test concentrations	6.25, 12.5, 25, 50, and 100%
12.	Temperature	$20 \pm 1^{\circ}\text{C}$
13.	Feeding regime	None
14.	Aeration	None
15.	Test duration	48 hours
16.	Effects measured/Endpoint	Survival/LC <sub>50</sub>
17.	Test acceptability	90% or greater mean survival in the laboratory water control.

**Table 3**  
**Summary of Test Conditions for the**  
**Fathead Minnow Acute Bioassay**  
**Conducted for Volatile Free, Inc.**  
**Brookfield, Wisconsin**  
**December 16 through 20, 2003**

1.	Test organism	<i>Pimephales promelas</i> (Osteichthyes: Cyprinidae)
2.	Test type	Static renewal
3.	Age of test organisms	10 days old
4.	Test chamber size	250 mL
5.	Test solution volume	200 mL
6.	Renewal of test solutions	Daily
7.	Number of replicate chambers per solution	4
8.	Number of test organisms per chamber	5
9.	Primary control/dilution water	Moderately hard reconstituted laboratory medium
10.	Test media	VFI205 polyurea coating exposed water
11.	Test concentrations	6.25, 12.5, 25, 50, and 100%
12.	Temperature	20 ± 1°C
13.	Feeding regime	None
14.	Aeration	None, unless DO concentration falls below 40% saturation (then, continuous at a rate not exceeding 100 bubbles per minute)
15.	Test duration	96 hours
16.	Effects measured/Endpoint	Survival/LC <sub>50</sub>
17.	Test acceptability	90% or greater mean survival in the laboratory water control

## Quality Assurance

Part of the quality assurance and quality control (QA/QC) program at the S-F Analytical Bioassay Laboratory includes the performance of organisms concurrently tested in laboratory media. Tables 1 through 3 present the test acceptability criteria for laboratory control data. The results of the laboratory control tests are listed in Table 4.

In addition, other QA/QC procedures include performing monthly reference toxicant tests using reagent-grade sodium chloride. The results of reference toxicant tests conducted during the past 12 months on the appropriate test organisms are summarized in Appendix C.

## Results

Photocopies of laboratory data and computer printouts of the statistical analyses are found in Appendix A. There were no excursions from the protocols and all test conditions were within the limits required by the EPA. The results of the tests are summarized below.

### Acute Bioassays

Table 4 presents the results of the acute bioassays. Because no toxicity was recorded in the highest sample concentrations tested, the statistical analysis for determining the 48-hour and 96-hour  $LC_{50}$  values could not be conducted. Therefore, the *Ceriodaphnia dubia* and *Daphnia magna* 48-hour  $LC_{50}$  values for the VFI205 polyurea coating exposed water sample was greater than 100 percent and the fathead minnow 96-hour  $LC_{50}$  for the VFI205 polyurea coating exposed water sample was greater than 100 percent.

Laboratory control water data were acceptable in all tests.

### Physicochemical Data

All physicochemical parameters measured satisfied the bioassay requirements (see Appendix A).

**Table 4**  
**Summary of Results of Acute Bioassays**  
**Conducted for Volatile Free, Inc.**  
**Brookfield, Wisconsin**  
**December 4 through 8, 2000**

Test Media	<u>Mean Percent Survival</u>		
	<i>Ceriodaphnia dubia</i>	<i>Daphnia magna</i>	Fathead Minnow
Laboratory Control	100	100	100
<b>VFI205 polyurea coating exposed water</b>			
6.25%	100	100	90
12.5%	100	100	90
25%	100	100	90
50%	100	100	85
100%	100	100	85
LC <sub>50</sub>	>100%	>100%	>100%

### Conclusions

The results of the laboratory bioassays conducted December 16 through 20, 2003 on the VFI205 polyurea coating film, provided by Volatile Free, Inc., and exposed to laboratory water, show the following:

- Laboratory control water data were acceptable in all bioassays.
- Because there was no toxicity recorded in the highest sample concentrations tested, the statistical analysis for determining the 48-hour and 96-hour LC<sub>50</sub> values could not be conducted. Therefore, the *Ceriodaphnia dubia* and *Daphnia magna* 48-hour LC<sub>50</sub> values for the VFI205 polyurea coating exposed water sample was greater than 100 percent and the fathead minnow 96-hour LC<sub>50</sub> for the VFI205 polyurea coating exposed water sample was greater than 100 percent.

### Reference

1. Weber, C.I. (ed.). 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (Fourth Edition). EPA/600/4-90/027F. U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. 293 p.