


**Celsis Laboratory Group**

## R E P O R T O F A N A L Y S I S

ATTN: Satya Ganti Sarva Bio Remed 604 Quail Ridge Drive Plainsboro, NJ 08536	ID#: 834461  Report Date: 11/18/1999 Received : 11/05/1999 Page : 1 of 1
SAMPLE: SpillRemed (Marine)	
LOT# : 1 P.O.# : S. Ganti	
TEST : Beta Hemolytic Streptococci METHOD: USP 23 CLAIM : N/A LIMIT : Negative RESULT: Date Assayed: 11/09/1999  Negative -- Conforms	
TEST : Fecal Coliforms METHOD: USP 23 CLAIM : N/A LIMIT : <3 MPN/g RESULT: Date Assayed: 11/10/1999  <3 MPN/g -- Conforms	
TEST : S. aureus METHOD: USP 23 CLAIM : N/A LIMIT : Negative RESULT: Date Assayed: 11/09/1999  Negative -- Conforms	
TEST : Salmonella METHOD: USP 23 CLAIM : N/A LIMIT : Negative RESULT: Date Assayed: 11/09/1999  Negative -- Conforms	
TEST : Shigella METHOD: USP 23 CLAIM : N/A LIMIT : Negative RESULT: Date Assayed: 11/09/1999  Negative -- Conforms	

Celsis Laboratory Group

by: Julia 11/18/99

Unless alternate arrangements have been made, samples will be retained for 30 days and raw data for 7 years after report date.

New Jersey Division 165 Fieldcrest Avenue • Edison, New Jersey 08837 • 732 346-5100 • Fax 732 346-5115

Celsis Laboratory Group, a global network of contract laboratories represents that this is a confidential report which may be used when requested by physician and health officials, but is not to be used in any form of advertising without written permission.



SARVA BIO REMED CO. SPILLREMEDIATION (MARINE)  
NCP DISPERSANT TOXICITY TEST  
*Mysidopsis bahia & Menidia beryllina*

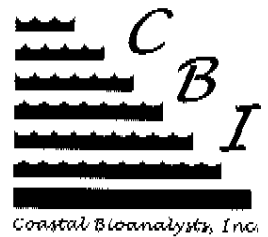
Submitted to:

Mr. Satya Ganti  
Sarva Bio Remed Co.  
604 Quail Ridge Drive  
Plainsboro, NJ 08536

By:

Coastal Bioanalysts, Inc.  
6400 Enterprise Court  
Gloucester, VA 23061  
(804) 694-8285

Sarva Bio Remed Co. SpillRemed (Marine)  
NCP Standard Dispersant Toxicity Test  
Test I.D.: SARV9901



## I. GENERAL METHODS

### Test Organisms

Mysids (*Mysidopsis bahia*) used in all tests were obtained from in-house cultures maintained at  $20 \pm 1$  g/kg salinity and  $25-26^{\circ}\text{C}$ . Mysids were 5 days old at the start of the tests. Silversides (*Menidia beryllina*) were obtained from a commercial hatchery (Aquatic Indicators Inc., St. Augustine, FL) and shipped by overnight carrier to the testing lab. Fish were 7 days old at the start of tests. Prior to use in tests animals were fed *Artemia* nauplii *ad libitum* daily.

### Dilution Water

Natural seawater, filtered through a 10 um mesh screen, was used for all toxicity tests. Water was collected prior to testing (11/9/99) from the Ware River, a tributary of Chesapeake Bay, at Roanes Wharf, VA. Seawater was collected into Nalgene carboys, transported to the laboratory, filtered, warmed to test temperature and gently aerated before use. Dilution water was 20 g/kg salinity and pH 7.9 on the day of use.

### Test Materials

EPA/API standard reference No. 2 fuel oil was obtained in sealed glass ampuls from Resource Technology Corporation, Laramie, WY. Ampuls of oil were stored in the dark at  $1-3^{\circ}\text{C}$  until used in tests. Ampuls were opened immediately prior to withdrawal of oil for preparing test solutions.

SpillRemed (Marine) was obtained in a 100 ml plastic jar from Sarva Bio Remed Co. Neat (i.e. 100%) solution was used to prepare both the 10:1 oil: product tests and the product-only toxicity tests.

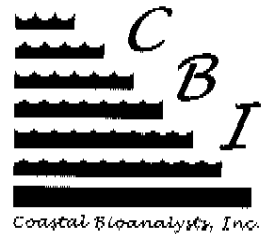
Sodium lauryl sulfate (sodium dodecyl sulfate; Lot No. 115H0348), SigmaUltra (>99%) grade was obtained from Sigma Chemical Co., St. Louis, MO.

### Preparation of Test Solutions

Preliminary range-finding tests were performed to determine appropriate test concentrations for the product-only and product + oil definitive tests with both species. Concentrations for oil-only and sodium dodecyl sulfate definitive tests were based on results of previous tests with these toxicants and species. Range-finding test methods were generally similar to those of definitive tests but used only 5 animals per concentration, a wider dilution factor and artificial seawater.

For the definitive mysid tests 550 ml of filtered seawater was blended with SpillRemed, oil or oil plus SpillRemed (10:1) at approximately 1000 rpm. For these solutions 550 ul of oil or 500 ul of oil and 50 ul

*Sarva Bio Remed Co. SpillRemed (Marine)*  
*NCP Standard Dispersant Toxicity Test*  
*Test I.D.: SARV9901*



of SpillRemed were added to the seawater using Hamilton (Reno, NV) "GASTIGHT" syringes. Appropriate volumes of the resulting 1000 ppm solutions were delivered to clean seawater to produce test concentrations of 50, 28, 16, 9, 5 and 2.8 ppm. Because of the low toxicity of the SpillRemed it was necessary to make up a higher concentration stock solution (10000 ppm; 5.5 ml in 550 ml seawater) to allow preparation on test solutions at 1000, 560, 320, 180 and 100 ppm concentrations. Solutions for the reference toxicant test were by dissolving 18 mg/l sodium lauryl sulfate in seawater and preparing dilutions of 18, 10.1, 5.8, 3.2 and 1.8 mg/l.

Solutions for the *Menidia* tests were made up similarly to the mysid solutions except that individual test dilutions were mixed in 3.5-L glass jars on a Thermolyne (Dubuque, IA) "Bigger Bill" orbital shaker table. Jars were capped and shaken at ca. 155 rpm (2 cm orbital stroke diameter) for 5 minutes. Test concentrations consisted of 50, 28, 16, 9 and 5 ppm oil or oil plus SpillRemed (10:1). For the SpillRemed-only a dilution series of 1000, 560, 320, 180 and 100 ppm was used based on the results of the preliminary range-finding test. The same concentrations of sodium lauryl sulfate were used as for the mysid reference test.

### Test Methods

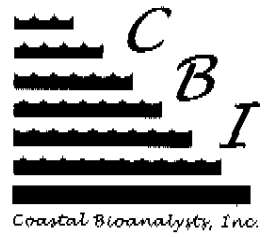
Test methods generally followed those described in EPA/600/4-90/027F. All treatments were tested using two replicates of 10 mysids or fish each (20 total animals per treatment). Test duration was 48 h for mysids and 96 h for fish. Test chambers consisted of 1-l borosilicate glass beakers each containing 1000 ml of solution. Test chambers were arranged in a randomized-block design and provided with ambient laboratory fluorescent illumination and a 16:8 L:D photoperiod. Test temperature was maintained at  $25 \pm 1^{\circ}\text{C}$ . Animals were fed *Artemia* nauplii at a rate of ca. 50 nauplii/animal/day.

Each day temperature, salinity, pH and dissolved oxygen concentration were measured in one replicate of each treatment in which surviving animals occurred in the preceding 24-h period. The number of surviving animals were counted and observations regarding unusual behavior, etc noted daily. Death was defined as lack of movement (spasms, movement of appendages, etc.) following gentle prodding with a pasteur pipet.

### Data Analysis

LC50s were determined using the EPA Trimmed Spearman-Kärber or probit program. The correct model used for calculation of the LC50 was selected based on the distribution of the data and model requirements.

*Sarva Bio Remed Co. SpillRemed (Marine)*  
*NCP Standard Dispersant Toxicity Test*  
*Test I.D.: SARV9901*



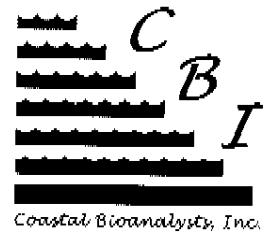
## II. RESULTS

### A. Overall Summary Results

Table 1. LC50 Comparisons (ppm)

Test	48- LC50 <i>M. bahia</i>	96-h LC50 <i>M. beryllina</i>
SpillRemed	343.8	392.3
No. 2 Oil	17.4	40.5
Oil + SpillRemed (10:1 v:v)	22.4	37.4

Sarva Bio Remed Co. SpillRemed (Marine)  
 NCP Standard Dispersant Toxicity Test  
 Test I.D.: SARV9901



## B. SpillRemed Test Results

Table 2. Summary Test Results  
 (Mean (%) Organism Survival)

Concentration (ppm)	<i>M. bahia</i>		Concentration (ppm)	<i>M. beryllina</i>			
	24-h	48-h		24-h	48-h	72-h	96-h
0	100	100	0	100	100	100	100
100	100	100	100	100	100	100	100
180	100	95	180	100	100	95	95
320	100	45	320	95	95	65	60
560	85	15	560	35	30	30	30
1000	50	5	1000	20	5	5	5

48-h LC50: 343.8 ppm  
 95% Conf. Limits: 285.6-413.9

96-h LC50: 392.3 ppm  
 95% Conf. Limits: 325.1-475.2