

Shell Oil Company



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Office of Toxic Substances
U.S. Environmental Protection Agency
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Washington, D.C. 20460
ATTN: 8(e) Coordinator

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Dear Sir:

SUBJECT: AQUATIC TOXICITY OF NEODOL® 23-6.5 (CAS# 66455-14-9) IN
SELENASTRUM CAPRICORNUTUM

The subject TSCA 8(e) submission was filed by Shell Oil Company April 7, 1994 and preliminary data transmissions were provided. The complete report (attached) is now available and is provided as supplemental information to the original TSCA 8(e) filing.

This supplemental report is filed to provide information EPA may find useful. In no way is it intended as a waiver of any rights or privileges belonging to Shell Oil Company as the reporting corporation, its agents or employees. The reporting corporation, its agents and employees, reserve the right to object to this report's use or admissibility in any subsequent judicial or administrative proceeding against the corporation, its agents or employees.

This report has been compiled based on information available as of the date of filing. The corporation, its agents and employees reserve the right to supplement the data contained in this report, and to revise and amend any conclusions drawn therefrom.

This report contains no confidential business information.

The following person should be contacted if you have questions or a need for discussion.

11/16/94

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Very truly yours,



R. N. Shulman, General Manager
Health, Safety, and Environment
Shell Oil Company

THG/sjh

Attachments

NEODOL® 23-6.5: A 96-HOUR TOXICITY TEST WITH
THE FRESHWATER ALGA (*Selenastrum capricornutum*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-119

TITLE 40 OF THE CODE OF FEDERAL REGULATIONS
PART 797, SECTION 1050

OECD GUIDELINE 201

AUTHORS:

Susan G. Thompson
James P. Swigert, Ph.D.

Contains No CBI

STUDY INITIATION DATE: March 10, 1994

STUDY COMPLETION DATE: September 13, 1994

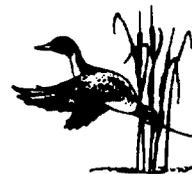
Submitted to

Shell Development Company
Westhollow Technology Center
3333 Highway Six South
Houston, Texas 77082



WILDLIFE INTERNATIONAL LTD.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600



GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Shell Development Company

TITLE: NEODOL® 23-6.5: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-119

STUDY COMPLETION DATE: September 13, 1994

This study was conducted to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, in 40 CFR Part 792, 17 August 1989; and OECD, ISBN 92-84-12367-9, Paris 1982.

STUDY DIRECTOR:

Susan G. Thompson
Susan G. Thompson
Senior Aquatic Biologist

DATE: 9/13/94

REPORT APPROVED BY:

James P. Swigert
James P. Swigert, Ph.D.
Manager, Aquatic Toxicology Laboratory

DATE: 9/13/94

Sponsor

DATE: _____

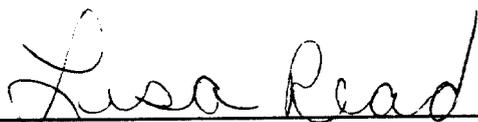
Applicant/Submitter

DATE: _____

QUALITY ASSURANCE STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989, and OECD, ISBN 92-84-12367-9, Paris 1982. The dates of all audits and inspections and the date that any findings were reported to the Study Director/Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO STUDY DIRECTOR/MANAGEMENT
Protocol Review	March 11, 1994	March 14, 1994
Test substance preparation, and pH measurements	June 13, 1994	June 13, 1994
Data and Draft Report	July 20 and 21, 1994	July 21, 1994
Second Draft Report	September 8, 1994	September 8, 1994
Final Report	September 13, 1994	September 13, 1994



Lisa Read
Quality Assurance Representative

DATE

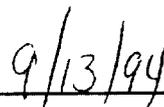


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SUMMARY

SPONSOR:	Shell Development Company
PRINCIPAL CONTACT:	Ms. Diana C.L. Wong
LOCATION OF STUDY RAW DATA AND FINAL REPORT:	Shell Development Company Westhollow Technology Center 3333 Highway Six South Houston, Texas 77082

Certain laboratory specific records are retained by the conducting facility, with copies filed in the study records.

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	109A-119
TEST SUBSTANCE:	NEODOL® 23-6.5
STUDY:	NEODOL® 23-6.5: A 96-Hour Toxicity Test with the Freshwater alga (<i>Selenastrum capricornutum</i>)
NOMINAL CONCENTRATIONS:	Negative Control; 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg NEODOL® 23-6.5/L
0-HOUR MEASURED CONCENTRATIONS:	Negative Control; 0.24, 0.42, 0.99, 1.7, 3.7 and 7.7 mg NEODOL® 23-6.5/L
TEST DATES:	Experimental Start - June 13, 1994 96-Hour Exposure Termination - June 17, 1994 Recovery Phase Termination - June 26, 1994 Experimental Termination - July 1, 1994
LENGTH OF TEST:	96 Hours with a 9-Day Recovery Phase

TEST ORGANISM:	<i>Selenastrum capricornutum</i>
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. Cultures Easton, Maryland 21601

96-HOUR IC10:	0.50 mg NEODOL® 23-6.5/L
95% CONFIDENCE LIMITS:	< 0.0 and 0.66 mg NEODOL® 23-6.5/L
96-HOUR IC50:	1.2 mg NEODOL® 23-6.5/L
95% CONFIDENCE LIMITS:	0.96 and 1.4 mg NEODOL® 23-6.5/L
96-HOUR IC90:	3.0 mg NEODOL® 23-6.5/L
95% CONFIDENCE LIMITS:	2.5 and 3.3 mg NEODOL® 23-6.5/L
STATISTICALLY-DEFINED NO OBSERVED EFFECT CONCENTRATIONS:	0.42 mg NEODOL® 23-6.5/L

INTRODUCTION

This study was conducted by Wildlife International Ltd. for Shell Development Company at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from June 13, 1994 to June 26, 1994. A certified copy of the final report and raw data generated at Wildlife International Ltd. are filed under Project Number 109A-119 in archives located at Wildlife International Ltd. The original raw data generated at Wildlife International Ltd. and the final report were archived at Wildlife International and then transferred to Shell Development Company.

OBJECTIVE

The objective of this study was to evaluate the acute toxicity of NEODOL® 23-6.5 to the freshwater green alga, *Selenastrum capricornutum*, during a 96-hour exposure period under static conditions.

EXPERIMENTAL DESIGN

The green alga, *Selenastrum capricornutum*, was exposed to a geometric series of six test concentrations and a negative control under static conditions for 96 hours. Three replicate test chambers were maintained for each treatment and control group. Nominal test concentrations were selected in consultation with the Sponsor and were based upon the results of a range finding test. Nominal test concentrations selected were 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg NEODOL® 23-6.5/L. Measured concentrations were determined from samples of test solution collected from each treatment and control group at test initiation and 96 hours. The samples were analyzed by Battelle Ocean Sciences, Duxbury, Massachusetts.

At test initiation, an inoculum of algal cells was prepared from the stock culture at a concentration of approximately 1.0×10^6 cells/mL. The concentration of algal cells in the inoculum was verified and 1.0 mL was added to each test chamber to achieve a nominal

concentration of approximately 1.0×10^4 cells/mL. Samples were collected from each replicate test chamber at approximate 24-hour intervals during the 96-hour portion of the test to determine cell densities. Cell densities were measured for each replicate and were used to calculate percent inhibition values relative to the control over the 96-hour exposure period. IC10, IC50 and IC90 values were also calculated, if possible, based on cell densities for each 24-hour interval. The no observed effect concentration (NOEC) was determined based upon statistical analysis of the cell densities.

No visible evidence of algal growth was observed in the 4.0 and 8.0 mg NEODOL® 23-6.5/L treatment groups after 96 hours. Therefore, aliquots of the test solution were diluted to a concentration of the test substance that theoretically would not inhibit growth, and were monitored for a period of nine days to determine whether the effect upon algal growth was reversible (i.e., algistatic or algicidal). Samples were collected every 3 days during the recovery phase to determine cell densities.

MATERIALS AND METHODS

Test methods were based on procedures outlined in Title 40 of the Code of Federal Regulations, Part 797, Section 1050, *Algal Acute Toxicity Test* (1); *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (2); OECD Guideline 201, *Alga, Growth Inhibition Test* (3). The test methods are also based on, but may not explicitly follow, the procedures of Shell Research Limited/Sittingbourne Research Centre.

Test Substance

The test substance was received from Shell Development Company on February 17, 1994 and was assigned Wildlife International Ltd. Identification Number WIL-2831 upon receipt. The test substance was a white viscous liquid, identified on the label as: WRC TOX SAMPLE NO.

1201; MSDS NO. 5,680-10; STORAGE COND. AMBIENT; COMPOSITION NEODOL 23-6.5; HAZARDS see accompanying safety sheet for hazard information; DATE DISPENSED 14 FEB 1994; RESPONSIBLE INDIVIDUAL H.C. SMITH/EC-644; EXPIR. DATE Feb 1995; COMPOSITION NEODOL 23-6.5 LR 20944-175; DATE REC 8/13/92; LOCATION C2342; NAME BAGNAS/EVETTS. Test substance characterization provided by the Sponsor indicated a purity of 100% and that NEODOL® 23-6.5 was soluble in water (Material Safety Data Sheet). The test substance was stored at ambient room temperature in a clear glass bottle.

Test Solution Preparation

The test substance was melted by heating in a water bath at approximately 60°C until clear and colorless. The test substance was stirred with a glass rod to ensure homogeneity and was allowed to cool to ambient room temperature prior to use. A primary stock solution was prepared by dissolving the test substance in freshwater algal medium. The concentration of the stock was 0.010 g NEODOL® 23-6.5/mL. In order to further aid in the dissolution of the test substance, the primary stock was sonicated and mixed until the test substance completely dissolved. Aliquots of the primary stock solution were diluted to one liter with culture medium to prepare the 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg NEODOL® 23-6.5/L nominal test concentrations. All treatment groups were clear and colorless, with no signs of precipitation upon preparation. The primary stock concentration and the resultant test concentrations were prepared on a total product basis (i.e., the test concentrations were not corrected for test substance purity). All glassware was serially rinsed with reverse osmosis water, 100% isopropanol, and reverse osmosis water prior to use.

At 96 hours, treatment groups that exhibited no visual evidence of algal growth were diluted with culture medium to concentrations of the test substance that theoretically would not inhibit growth. One-half milliliter aliquots of test solution were removed from each replicate flask, were pooled by treatment and then diluted to 100 mL with culture medium. This provided one flask per treatment for observations of growth recovery. A negative control was prepared

by diluting one-half milliliter of solution from one negative control replicate to 100 mL with culture medium.

Test Organism

The freshwater green alga, *Selenastrum capricornutum*, was selected as the test species for this study. The species is representative of an important group of freshwater algae, and was selected for use in the test based upon a past history of use, and ease of culturing in the laboratory. Original algal cultures were obtained from UTEX-The Culture Collection of Algae at the University of Texas at Austin and have been maintained in culture medium at Wildlife International Ltd. *Selenastrum capricornutum* cells used in the test were obtained from Wildlife International Ltd. cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. The negative control organisms were expected to exhibit exponential growth over the 96-hour exposure period. Exponential growth phase, defined as the period of growth where the algal cells are dividing at a constant rate, is indicated by the linear section of the growth curve (Figure 1).

Culture Medium

The algal cells were cultured and tested in freshwater algal medium with vitamins (2). Stock nutrient solutions were prepared by adding reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water (Appendix I). The pH of the medium was adjusted to 7.5 ± 0.1 using 10% HCl and the medium was sterilized by filtration (0.2 μm) prior to use.

Test Apparatus

Test chambers were sterile 250-mL Erlenmeyer flasks labelled with the project number, concentration and replicate, and containing 100 mL of test or control medium. The test chambers were serially rinsed with reverse osmosis water, 100% isopropanol, reverse osmosis

water and the corresponding test solution prior to use. The test chambers were plugged with gauze-wrapped cotton stoppers and were indiscriminately positioned in an environmental chamber designed to maintain the desired temperature throughout the test. The test chambers were shaken continuously at approximately 100 rpm on a mechanical shaker. The shaker was checked daily to ensure the proper setting.

Environmental Conditions

Test chambers were held in an environmental chamber at a temperature of $24 \pm 2^{\circ}\text{C}$. The temperature in the environmental chamber was recorded twice daily during the test using a calibrated, hand-held mercury thermometer.

The algae were held under continuous lighting throughout the test. The light source consisted of cool white fluorescent tubes. The target light intensity was approximately 4300 lux. Light intensity was measured at the four corners and the middle of the shaker table daily during the test.

The pH of the medium prepared for each treatment and control group was measured at test initiation using a Fisher Accumet Model 915 pH meter. At 96 hours, pH was measured for each individual replicate of the treatment and control groups.

Algal Growth Measurements

Test medium samples were collected from each replicate of the treatment and control groups for determination of algal cell densities. Samples were collected at approximate 24-hour intervals during the 96-hour exposure. Samples were held at approximately 4°C until cell counts could be performed. Cell counts were conducted using a hemacytometer and microscope. Each sample was diluted using an electrolyte solution (Isoton[®]), as needed, to maintain counting accuracy. A small amount of each sample was placed onto a hemacytometer and 10 grids were counted. The mean number of cells per grid was calculated and this value was used to calculate

the cell density of the sample. Using this technique, the minimum quantifiable cell density was 1,000 cells/mL. Percent growth inhibition values were calculated based on mean cell densities.

Statistical Analyses

Cell densities, mean cell densities and percent inhibition values were calculated using "Lotus 1-2-3 Release 3" (4), while statistical analyses were conducted using "ICPIN Version 2.0" (5) and "TOXSTAT Release 3.2" (6). Percent growth inhibition was calculated for each treatment group as the percent reduction in cell density relative to the mean cell density in the control replicates. The following formula was used:

$$\text{Percent Inhibition} = \frac{\text{Mean Cell Density}_{\text{Control}} - \text{Mean Cell Density}_{\text{Treatment}}}{\text{Mean Cell Density}_{\text{Control}}} \times 100$$

Cell densities were analyzed statistically using the computer program ICPIN[®] (5) to estimate the IC10, IC50 and IC90 values (i.e., the theoretical test concentrations that would produce a 10%, 50%, or 90% reduction in cell density, respectively) and 95% confidence limits for the 96-hour test period. This program was designed to calculate the IC values and 95% confidence limits by linear interpolation. Cell densities were evaluated for normality and homogeneity of variances and the treatment groups were compared to the control using Dunnett's test (6). Results of those statistical analyses were used to determine the no observed effect concentration (NOEC).

Analytical Chemistry

Samples of test medium were collected from each treatment and control group at the beginning and end of the test to measure concentrations of the test substance. A sample of the primary stock solution was also collected at test initiation. Samples of test medium collected at 0 hours were taken from the liter of each test medium prepared at test initiation. Samples collected at 96 hours consisted of the composited test medium from each of the three replicates in each respective treatment and control group. The samples were collected in glass bottles with

Teflon-lined caps and were preserved by adding 1% formalin to each sample (e.g., 5.0 mL formalin:500 mL sample). The 96-hour samples were centrifuged to remove algal cells prior to preserving with formalin. The samples were stored at ambient room temperature until shipped to Battelle Ocean Sciences for analysis.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Analytical measurements were performed to verify exposure concentrations of NEODOL® 23-6.5 in the test medium. Results of those analyses are presented in Table 1 and in the Analytical Chemistry Report (Appendix II). Nominal concentrations selected for use in this study were 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg NEODOL® 23-6.5/L. Samples collected at 0 hours showed measured values of 0.24, 0.42, 0.99, 1.7, 3.7 and 7.7 mg NEODOL® 23-6.5/L, which represent 96, 84, 99, 85, 93 and 96% of nominal, respectively. Measured values for samples collected at 96 hours were 0.14, 0.18, 0.19, 0.17, 2.2 and 5.2 mg NEODOL® 23-6.5/L representing 56, 36, 19, 8.5, 55 and 65% of nominal, respectively and 58, 43, 19, 10, 59 and 68% of the 0-hour concentrations, respectively. Since several of the 96-hour concentrations fell greater than 70% of the initial concentrations, all test results are presented based on the 0-hour measured concentrations.

Environmental Measurements

Measurements of temperature and light intensity are presented in Table 2, while measurements of pH are shown in Table 3. The temperatures ranged from 23.1 to 24.4°C and were within the limits established for this test ($24 \pm 2^\circ\text{C}$). The average light intensity surrounding the shaker ranged from 3956 to 4264 lux over the testing period. Measurements of pH ranged from 7.4 to 7.6 at test initiation and from 7.7 to 9.6 at 96 hours.

Cell Density Analyses

The effects of NEODOL® 23-6.5 upon *Selenastrum capricornutum* were determined by measuring differences in cell densities at the end of the 96-hour exposure period. Mean cell densities were used to calculate growth inhibition values for each 24-hour period. Mean cell densities are shown in Table 4 and are illustrated graphically in Figure 1, while cell densities for each individual replicate are presented in Appendix III. Percent inhibition values are given in Table 4. IC10, IC50 and IC90 values and 95% confidence limits for each 24-hour interval, calculated using cell densities presented in Appendix III, are given in Table 5.

Changes in cell density indicated that exponential growth occurred in the negative control replicates (Figure 1). There were no statistically significant ($p > 0.05$) reductions in cell density at 0.24 and 0.42 mg NEODOL® 23-6.5/L when compared to the negative control group. Statistically significant ($p < 0.05$) reductions in cell density were measured in the 0.99, 1.7, 3.7 and 7.7 mg NEODOL® 23-6.5/L treatments compared to the negative control group. Growth inhibition values for those treatments were 40, 71, 100 and 100%, respectively.

Visual and Microscopic Observations

After 96 hours, clumping, flocculation or adherence of the algae to the test flask were not visually evident in any of the treatment groups. There were no noticeable changes in cell color, size, or morphology in any of the treatment groups when compared to the negative control.

Reversibility of Growth Inhibition

Cell densities for the recovery period are presented in Table 6 and are illustrated graphically in Figure 2. Aliquots of the 3.7 and 7.7 mg NEODOL® 23-6.5/L test solutions were diluted with algal medium to a concentration of the test substance expected to have no effect on growth (≤ 0.12 mg NEODOL® 23-6.5/L). Those groups were monitored to determine if the observed effects of the test substance upon algal growth were algistatic or algicidal. Algal cells

in the 3.7 and 7.7 mg NEODOL® 23-6.5/L treatments did not resume normal growth compared to the negative control replicates after nine days during the recovery period.

CONCLUSIONS

The IC10 value for *Selenastrum capricornutum* exposed to NEODOL® 23-6.5 for 96 hours was calculated based on 0-hour measured concentrations and was determined to be 0.50 mg NEODOL® 23-6.5/L. The 95% confidence limits were < 0.0 and 0.66 mg NEODOL® 23-6.5/L. The 96-hour IC50 value was 1.2 mg NEODOL® 23-6.5/L with 95% confidence limits of 0.96 and 1.4 mg NEODOL® 23-6.5/L. The 96-hour IC90 value was 3.0 mg NEODOL® 23-6.5/L with 95% confidence limits of 2.5 and 3.3 mg NEODOL® 23-6.5/L. The statistically-defined 96-hour no observed effect concentration was 0.42 mg NEODOL® 23-6.5/L. Since the algal cells in the 3.7 and 7.7 mg NEODOL® 23-6.5/L treatments did not resume normal growth after nine days during the recovery period, the effects upon algal growth were considered to be algicidal, rather than algistatic.

REFERENCES

- 1 Title 40 of the Code of Federal Regulations, Part 797, Section 1050, *Algal Acute Toxicity Test*. July, 1992.
- 2 Horning, W.B. and C.I. Weber. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, EPA 600/4-85/014, December 1985.
- 3 OECD Guidelines for Testing of Chemicals. Guideline 201, *Alga, Growth Inhibition Test*. C(81)30(Final). Adopted 7 June 1984.
- 4 Lotus Development Corporation, Lotus 1-2-3 Release 3. Copyright 1989.
- 5 Norberg-King, T.J. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0)*. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota, June 1993.
- 6 Gulley, D.D. "TOXSTAT Release 3.2," The University of Wyoming, July, 1990.

Table 1
Summary of Analytical Chemistry Data

Sponsor:	Shell Development Company	
Test Substance:	NEODOL® 23-6.5	
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>	
Dilution Water:	Freshwater Algal Medium with Vitamins	
Nominal Concentration (mg NEODOL® 23-6.5/L)	Sampling Time (Hours)	Measured ¹ Concentration (mg NEODOL® 23-6.5/L)
Negative Control	0 ²	ND
	96 ³	0.29
0.25	0	0.24
	96	0.14
0.50	0	0.42
	96	0.18
1.0	0	0.99
	96	0.19
2.0	0	1.7
	96	0.17
4.0	0	3.7
	96	2.2
8.0	0	7.7
	96	5.2

¹ ND = Not Detected.
² 0-hour samples were collected from the single batch of test solution prepared at test initiation to provide each of the three replicates per treatment.
³ 96-hour samples were composites of the solution remaining in each of the three individual replicates per treatment pooled by concentration.

Table 2

Temperature and Light Intensity Measurements

Sponsor:	Shell Development Company		
Test Substance:	NEODOL® 23-6.5		
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>		
Dilution Water:	Freshwater Algal Medium with Vitamins		
	Temperature °C		Mean Light Intensity ¹ (Lux)
Time (Days)	Measurement 1	Measurement 2	
0	23.1	23.2	4090
1	23.3	23.4	4094
2	23.5	23.4	4154
3	23.2	23.2	4042
4	23.3	23.3	4042
5	23.3	23.4	4102
6	23.4	23.3	4068
7	23.4	23.3	4044
8	23.3	23.4	4096
9	23.3	23.3	4264
10	23.4	23.7	4110
11	23.7	23.7	4054
12	23.6	24.4	3956
13	23.4	23.3	4174

¹ Average of five measurements taken surrounding the test flasks at test solution level.

Table 3
pH Measurements

Sponsor:		Shell Development Company			
Test Substance:		NEODOL® 23-6.5			
Test Organism:		Freshwater Alga, <i>Selenastrum capricornutum</i>			
Dilution Water:		Freshwater Algal Medium with Vitamins			
pH Measurements					
0-Hour Measured Concentration (mg NEODOL® 23-6.5/L)	0 Hours ¹	Rep.	96 Hours ²		
			A	B	C
Negative Control	7.4		8.9	9.6	9.3
0.24	7.6		8.9	9.3	9.4
0.42	7.6		9.2	9.4	9.6
0.99	7.6		9.0	8.7	8.8
1.7	7.6		8.2	7.9	8.3
3.7	7.6		7.7	7.7	7.7
7.7	7.6		7.7	7.7	7.7

¹ 0-hour samples were collected from the 1000-mL batch of test solution prepared at test initiation to provide solution for each of the three replicates per treatment and control group.

² 96-hour samples were taken from test solution remaining in each of the three replicates per treatment.

Table 4

Mean Cell Densities and Percent Inhibition for each 24-Hour Interval During the Test

Sponsor: Test Substance: Test Organism: Dilution Water:	24 Hours		48 Hours		72 Hours		96 Hours	
	Cell Density	Percent Inhibition	Cell Density	Percent Inhibition	Cell Density	Percent Inhibition	Cell Density	Percent Inhibition
Shell Development Company NEODOL® 23-6.5 Freshwater Alga, <i>Selenastrum capricornutum</i> Freshwater Algal Medium with Vitamins								
0-Hour Measured Concentration (mg NEODOL® 23-6.5/L)								
Negative Control	42,000	--	141,000	--	957,000	--	2,287,000	--
0.24	27,000	36	108,000	23	830,000	13	2,203,000	3.7
0.42	26,000	38	128,000	9.2	763,000	20	2,173,000	5.0
0.99	15,000	64	59,000	58	408,000	57	1,377,000 ¹	40
1.7	12,000	71	30,000	79	97,000	90	670,000 ¹	71
3.7	3,000	93	4,000	97	3,000	100	3,000 ¹	100
7.7	8,000	81	9,000	94	6,000	99	6,000 ¹	100

¹ Statistically significant ($p < 0.05$) compared to the negative control.

Table 5

IC10, IC50 and IC90 Values Over the 96-Hour Exposure Period

Sponsor: Shell Development Company		95% Confidence Limits		95% Confidence Limits		95% Confidence Limits	
Test Substance: NEODOL® 23-6.5		IC10	IC50	IC90	IC90	IC90	IC90
Test Organism: Freshwater Alga, <i>Selenastrum capricornutum</i>		(mg NEODOL® 23-6.5/L)	(mg NEODOL® 23-6.5/L)	(mg NEODOL® 23-6.5/L)	(mg NEODOL® 23-6.5/L)	(mg NEODOL® 23-6.5/L)	(mg NEODOL® 23-6.5/L)
Dilution Water: Freshwater Algal Medium with Vitamins							
Time							
24 Hours	< 0.24 ²	-- ²	0.67	< 0.0 ¹ and 1.1	> 7.7 ²	-- ²	
48 Hours	< 0.24 ²	-- ²	0.88	0.65 and 1.1	3.1	2.4 and 3.5	
72 Hours	< 0.24 ²	-- ²	0.88	0.53 and 1.3	1.7	1.6 and 2.9	
96 Hours	0.50	< 0.0 ¹ and 0.66	1.2	0.96 and 1.4	3.0	2.5 and 3.3	

¹ Statistical program computed a lower confidence limit less than zero.² The growth response data did not facilitate the calculation of an IC value and 95% confidence limits. Therefore, the IC values were determined by visual interpretation of the percent inhibition data.

Table 6

Cell Densities for the Recovery Period

Sponsor:	Shell Development Company		
Test Substance:	NEODOL® 23-6.5		
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>		
Dilution Water:	Freshwater Algal Medium with Vitamins		
Nominal ¹ Concentration (mg NEODOL® 23-6.5/L)	Cell Densities (Cells/mL) ²		
	Day 3	Day 6	Day 9
Negative Control	1,520,000	4,440,000	5,300,000
3.7	2,000	1,000	2,000
7.7	< MQD ³	< MQD ³	8,000
¹ The treatments were diluted to a concentration of the test substance that theoretically would not inhibit growth (≤ 0.12 mg NEODOL® 23-6.5/L).			
² Due to the method used to prepare recovery phase test solutions, initial cell densities were not equivalent throughout the treatments.			
³ The minimum quantifiable cell density (MQD) using a hemacytometer was established as 1,000 cells/mL.			

Figure 1. Algal Growth, Expressed in Cell Density, Over the 96-Hour Exposure.

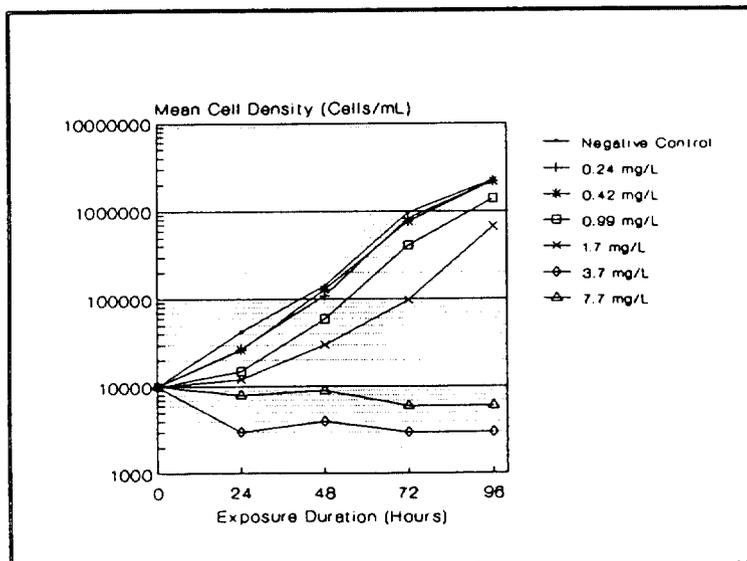
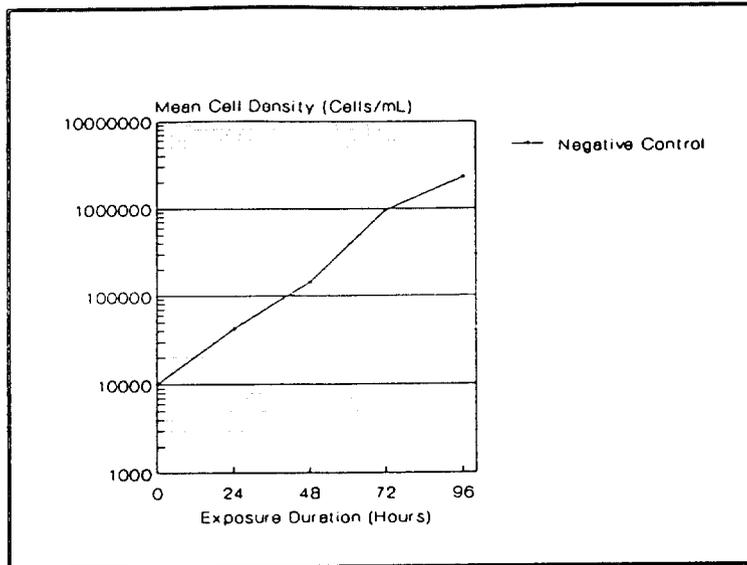
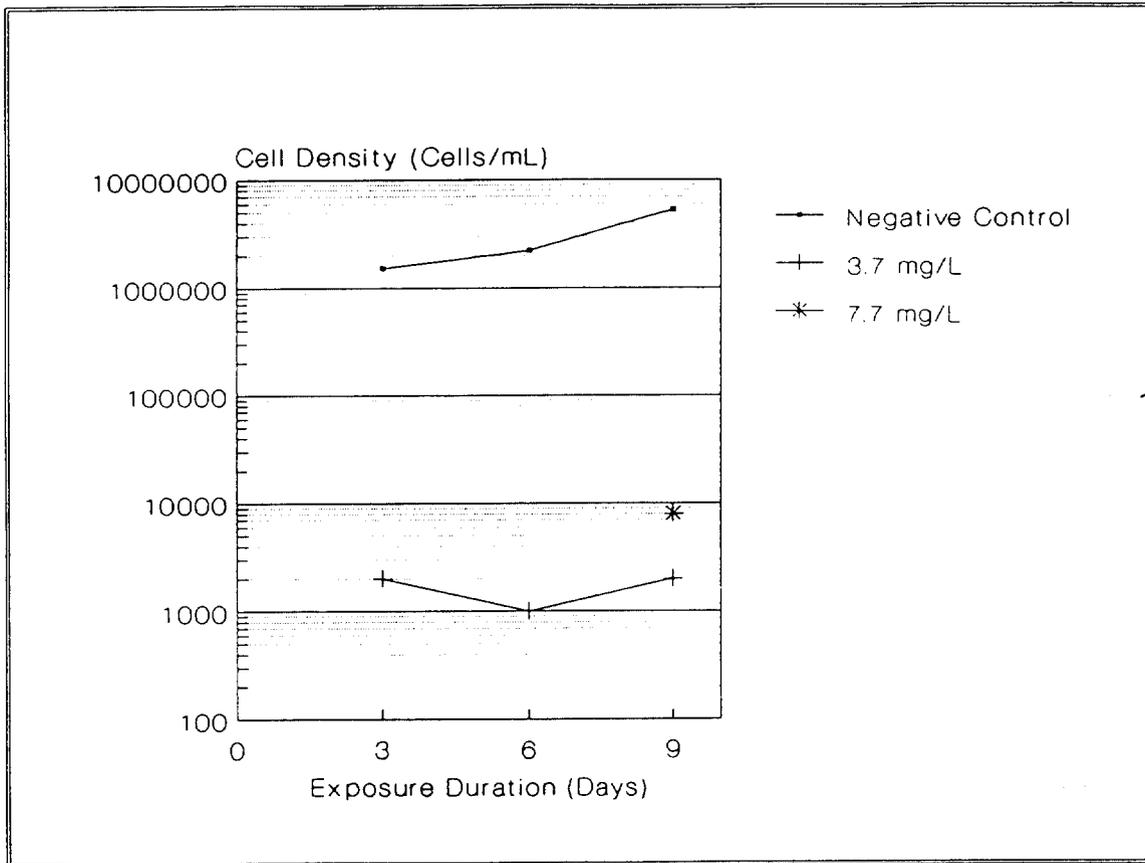


Figure 2. Algal Growth, Expressed in Cell Density, Over the Recovery Period.



APPENDIX I

Freshwater Algal Medium with Vitamins

Sponsor:	Shell Development Company
Test Substance:	NEODOL® 23-6.5
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>
Dilution Water:	Freshwater Algal Medium with Vitamins

Compound	Nominal Concentration	
MgCl ₂ •6H ₂ O	12.16	mg/L
CaCl ₂ •2H ₂ O	4.40	mg/L
H ₃ B ₃	0.1856	mg/L
MnCl ₂ •4H ₂ O	0.416	mg/L
ZnCl ₂	3.28	µg/L
FeCl ₃ •6H ₂ O	0.1598	mg/L
CoCl ₂ •6H ₂ O	1.428	µg/L
Na ₂ MoO ₄ •2H ₂ O	7.26	µg/L
CuCl ₂ •2H ₂ O	0.012	µg/L
Na ₂ EDTA•2H ₂ O	0.300	mg/L
NaNO ₃	25.50	mg/L
MgSO ₄ •7H ₂ O	14.70	mg/L
K ₂ HPO ₄	1.044	mg/L
NaHCO ₃	15.0	mg/L
Thiamine hydrochloride	0.25	mg/L
Biotin	0.05	µg/L
B ₁₂	0.0005	mg/L

¹ The pH of the medium was adjusted to 7.5 ± 0.1 using 0.1N NaOH or 10% HCl, as necessary.

APPENDIX II

Measurement of NEODOL® 23-6.5 in Dilute Aqueous Samples

in Support of

Aquatic Toxicity Testing with

Selenastrum capricornutum

FINAL DATA REPORT

Study Title

Measurement of Neodol® 23-6.5 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum*

Data Requirements

United States Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA),
Good Laboratory Practice Standards (40 CFR, Part 792)

Submitted To

Shell Development Company
Westhollow Research Center
Houston, TX 77251-1380

Performing Laboratory

Battelle Ocean Sciences
397 Washington Street
Duxbury, MA 02332

Author

Gregory S. Durell

Study Initiation Date

March 10, 1994

Study Completion Date

July 14, 1994

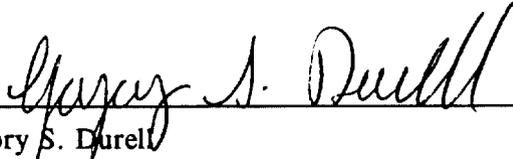
Battelle Study Number

SD-930126

SIGNATURE PAGE

for SD-930126

Measurement of Neodol® 23-6.5 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum*



Gregory S. Durell
Analytical Chemistry Task Leader
Battelle Ocean Sciences

08/11/94

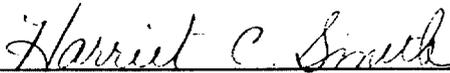
Date



Allen D. Uhler
Chemistry Department Manager
Battelle Ocean Sciences

8/11/94

Date



Harriet C. Smith
Project Monitor
Shell Development Company

August 31, 1994

Date

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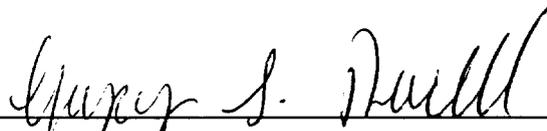
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COMPLIANCE STATEMENT

for SD-930126

Measurement of Neodol® 23-6.5 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum*

The work conducted at Battelle through Battelle Proposal/Agreement No. 882-H-0641 "Analysis of Non-ionic Surfactants in Water Samples by HPLC and ELSD" was performed in compliance with United States Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards (40 CFR, Part 792), August 17, 1989.



Gregory S. Durell
Analytical Chemistry Task Leader
Battelle Ocean Sciences

08/11/94

Date

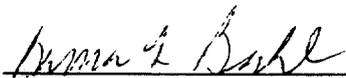
QUALITY ASSURANCE STATEMENT

for SD-930126

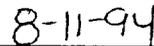
**Measurement of Neodol® 23-6.5 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum***

In accordance with Good Laboratory Practice Standards (40 CFR, Part 792) dated August 17, 1989, this study has been monitored by Battelle Ocean Science's Quality Assurance Unit. Study audit dates and dates when the results were reported to the Study Director and management are listed in the following table.

To the best of my knowledge, the analyses reported here accurately represent the data generated during this study.



Rosanna L. Buhl
Quality Assurance Coordinator
Battelle Ocean Sciences



Date

QUALITY ASSURANCE AUDITS

Conducted for SD-930126

**Measurement of Neodol® 23-6.5 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum***

Audit Type	Audit Date	Date of Report to Analytical Task Leader	Date of Report to Study Director	Date of Report to Management
Initiation	2-28-94	NA ¹	NA	NA
	3-28-94 •	NA	NA	NA
Lab Inspection	6-16-94	7-11-94	8-11-94	8-11-94
	6-27-94	7-11-94	8-11-94	8-11-94
	6-30-94	7-11-94	8-11-94	8-11-94
Data Package	8-2-94/8-3-94	8-3-94	8-11-94	8-11-94
Report Review	8-2-94/8-3-94	8-3-94	8-11-94	8-4-94

¹ NA: Not applicable. No issues noted and no report prepared.

STUDY PARTICIPANTS

SD-930126

**Measurement of Neodol® 23-6.5 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum***

Mr. Gregory S. Durell	Analytical Chemistry Task Leader; HPLC Analysts
Mr. Richard Restucci	Laboratory Technician; HPLC Analyst (in training)
Mr. Lyle Roberts	Laboratory Technician (in training)

1.0 INTRODUCTION

The work reported in this document is a component of aquatic toxicological testing that has been requested and initiated by the Sponsor of this study. The toxicological testing was conducted by Wildlife International. Battelle Ocean Sciences was responsible for providing chemical analytical support to the toxicological testing by determining concentrations of the Test Substance in samples received from Wildlife International. The results of these chemical analyses are presented in this Final Data Report.

The objective of the work reported in this document was to perform chemical analysis of aqueous samples and primary stock solutions, for the determination of concentrations of alcohol ethoxylate surfactants using the analytical method titled *Analysis of Alcohol Ethoxylate Surfactants Using Solid Phase Extraction (SPE) Cartridges and High Performance Liquid Chromatography with Evaporative Light Scattering Detection (HPLC/ELSD) in Dilute Aqueous Solutions*. This analytical procedure was approved by the Analytical Chemistry Task Leader on February 25, 1994, and by the Sponsor's Project Monitor on March 7, 1994.

1.1 Test Substance Identification

The Test Substance analyzed in this study was an alcohol ethoxylate (AE) surfactant (Neodol® 23-6.5). The Test Substance was provided by the Sponsor (Shell Development Company). The Sponsor was also responsible for providing Battelle with the lot number, analysis, purity, stability, storage requirements, and all other relevant chemical and physical characterization data for the Test Substance. The Test System and Test Substance identity and characterization information, and other relevant test information for this study, as provided by the Sponsor, is summarized below.

Test System:	<i>Selenastrum capricornutum</i>
Test Substance:	Neodol® 23-6.5
Test Substance CAS#:	66455-14-9
Test Substance Lot#:	60G8120 1201 (WRC Tox Sample Number)
Test Substance Purity:	100%

Test Substance Composition:	A C ₁₂ -C ₁₃ alcohol ethoxylate with an average of 6.5 moles of ethylene oxide per mole of alcohol. Traces of free ethylene oxide (less than or equal to 6 ppm) may be present in the neat Test Substance.
Test Substance Analysis:	The percent purity data is based on process knowledge, and research and development prior to manufacture of the Test Substance used. In addition, the compound was characterized by the Sponsor prior to toxicological testing using the following analytical tests: hydroxyl number, percent water, cloud point, ethylene oxide distribution, carbon number distribution, and percent weight polyethylene glycol. The data from these analyses will be maintained by Shell Development Company's Westhollow Research Center (WRC) in the WRC Analytical Special Collection of Files.
Test Substance Solubility:	Completely soluble in water. May form gel.
Test Substance Stability:	Stable. An expiration date of one year (March 1995) was assigned to the Test Substance by the Sponsor before providing the material to Battelle.
Test Substance Storage Requirements:	Cool, dry place.

2.0 MATERIALS AND METHODS

2.1 Analytical Method Description

The analytical procedure used was developed for the determination of total alcohol ethoxylate surfactants in aqueous samples. The method involves an extraction step to isolate the surfactant from a water sample and a high performance liquid chromatography (HPLC) analytical procedure to quantify the nonionic surfactant concentration. The results are reported as total alcohol ethoxylate surfactant concentrations. Concentrations of the surfactant were also determined in primary stock solutions received from the toxicology testing laboratory.

In order to analyze alcohol ethoxylate (AE) surfactants in aqueous matrices at low levels the surfactant must first be isolated from the water, concentrated, and analyzed using an appropriate

method of detection to quantitate the amount of surfactant originally present in the aqueous sample. The method used employs solid phase extraction (SPE) with a C_8 reverse phase cartridge for isolating the analyte and concentrating the sample. After elution of the surfactant from the SPE cartridge with methyl and isopropyl alcohol it is analyzed using an HPLC procedure (octyl HPLC-column separation and methanol/water mobile phase gradient) that employs an evaporative light scattering detector (ELSD) for analyte detection and quantification. This method quantifies total AE surfactant without distinguishing between the various individual AEs or carbon chain distributions.

The primary stock solution surfactant concentration was determined by simply diluting the sample to the appropriate concentration using methanol and analyzing it by HPLC/ELSD. No extraction step was needed.

Formaldehyde testing was performed on, at least, one in ten samples to verify that the toxicological testing laboratory had preserved the samples prior to shipping them to Battelle. A commercially available formaldehyde test kit was used for the semi-quantitative determination of the presence of formaldehyde. The procedure is a colorimetric, wet-chemistry, method that involves the addition of a color forming reagent to the water sample that has been adjusted to be an alkaline solution. The intensity of the color that is formed is directly proportional to the formaldehyde concentration, and approximate formaldehyde concentrations are determined in parts per million (ppm).

The analytical procedure is described in detail in the document entitled *Analysis of Alcohol Ethoxylate Surfactants Using Solid Phase Extraction (SPE) Cartridges and High Performance Liquid Chromatography with Evaporative Light Scattering Detection (HPLC/ELSD) in Dilute Aqueous Solutions*, which was developed specifically for these analyses. This Test Substance specific document was prepared by Battelle and approved by the Sponsor on March 7, 1994. The analytical procedure document, and associated study-specific analytical information, is included in Battelle's data-package for this study. This data-package will be provided to the Sponsor and a copy maintained by Battelle Ocean Sciences.

2.2 Laboratory Quality Control

The water samples were processed in analytical batches of no more than 20 test samples. Each batch of test samples included four laboratory quality control (QC) samples: one procedural blank (PB), one matrix spike (MS), one matrix spike duplicate (MSD), and one blank spike (BS). The procedural blank (which consists of Milli-Q laboratory water carried through all steps and treated as other samples) sample was used to ensure that there were no significant levels of laboratory contamination. The matrix spike (test sample spiked with the target analyte), matrix spike duplicate, and blank spike (1% formalin in Milli-Q water spiked with the target analyte) samples were used to demonstrate

laboratory accuracy and precision; these QC samples were also carried through all sample processing procedures and treated as the rest of the samples.

A portion of a non-fortified (control) test sample was used to prepare the matrix spike samples because it contained no background analyte levels yet had a sample matrix that was representative of the test samples. The blank spike was processed to determine if the accuracy/recovery of the analyte was affected by the sample matrix.

Each sequence of samples analyzed by the HPLC/ELSD instrument was initiated with a seven-point multilevel calibration. Test samples followed the initial calibration in the analysis sequence, and a calibration check standard was analyzed at least every 12 samples to verify the validity of the calibration.

Summarized below are the QC data quality objectives that applied for this study.

Data Quality Objectives

QC Analysis	Criteria Objective
Blank spike analyte recovery	70%-120%
Matrix spike analyte recovery	70%-120%
Matrix spike/spike duplicate precision	≤ 30% RPD
Procedural blank	< 1 × limit of quantification (LOQ)
Instrument multilevel calibration	Correlation coefficient > 0.995
Instrument calibration check	15% RPD in determined and actual standard concentration

2.3 Calculations

Sample Concentration Calculations

An external standard method of calibration and quantification was used. Sample extract concentrations were determined by applying the multilevel quadratic calibration equation using a chromatography data system on which the analytical data were acquired during the instrumental analysis. A seven-point calibration curve which bracketed the expected concentration range of

exposure samples was generated at the initiation of the HPLC analysis. Calibration standard concentrations were approximately 21, 41, 83, 124, 166, 207, and 270 $\mu\text{g}/\text{mL}$. Original water sample concentrations were subsequently determined by applying the water extraction volume (WEV) and pre-injection volume (PIV) information. The PIV of the PB sample, controls, 250 and 500 parts per billion (ppb) nominal concentration samples was 500 μL . For the 1,000 and 2,000 ppb nominal concentration samples, and the BS, MS, and MSD samples, the PIV was 1.00 mL, and it was 5.00 mL for the samples with nominal concentrations of 4,000 and 8,000 ppb. Analyte concentrations of the original water samples were determined in ppb. Analyte concentration of the primary stock solution samples were determined in parts per million (ppm).

$$\text{Determined Water Sample Concentration (ppb)} = \text{EC} \times \text{PIV} \times (1 / \text{WEV}) \times 1000$$

$$\text{Primary Stock Solution Concentration (ppm)} = \text{EC} \times \text{DIL VOL}_1 \times (1 / \text{DIL VOL}_2)$$

EC = Extract (HPLC sample) concentration ($\mu\text{g}/\text{mL} = \text{ppm}$)

PIV = Pre-injection volume (mL)

WEV = Water extraction volume (mL)

DIL VOL₁ = Final volume of diluted Primary Stock subsample (mL)

DIL VOL₂ = Volume of Primary Stock subsample taken for the dilution (mL)

Quality Control Sample Calculations

Two separate calculations were performed on the Quality Control (QC) sample data. Percent recoveries were determined for the blank spike and matrix spike samples, and the relative percent difference (%RPD) between the two percent recovery values was determined for the matrix spike/duplicate sample pair.

$$\begin{aligned} \% \text{ Recovery} &= \text{WC}_D \times (1 / \text{WC}_S) \times 100\% = \\ &(\text{Determined concentration} / \text{Expected concentration}) \times 100\% \end{aligned}$$

$$\begin{aligned} \% \text{RPD} &= [\% \text{REC}_{\text{MS}} - \% \text{REC}_{\text{MSD}}] \times (2 / (\% \text{REC}_{\text{MS}} + \% \text{REC}_{\text{MSD}})) \times 100\% = \\ &(\text{Difference between MS and MSD recovery} / \text{Average of MS and MSD recovery}) \times 100\% \end{aligned}$$

WC_D = Determined water sample concentration (ppb) — calculated as shown above

WC_S = Spiked water sample concentration (ppb) — prepared concentration

%REC_{MS} = Percent recovery of the matrix spike sample

%REC_{MSD} = Percent recovery of the matrix spike duplicate sample

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) was determined for the analyses. The target analyte had to provide a detector response of a minimum 3:1 signal:noise ratio to be identified and considered detected in the analyses.

The LOD was calculated by using the peak height equivalent to a signal:noise ratio of 3:1 in samples as the signal, comparing it to the peak height of the analyte in the low-level calibration standard to convert the signal to a sample extract concentration (EC), and applying the water extraction volume (WEV) and the pre-injection volume (PIV), as shown previously. The LOD was determined using two samples with the lowest nominal concentration and averaging the values obtained in the two determinations.

The LOQ was determined as the water sample concentration equivalent to a final extract concentration that is the same as the low calibration standard. The LOQ was determined using the PIVs and WEVs used for the samples with the lowest nominal concentration.

$$\text{LOD (ppb)} = H_{3x} \times (C_{LS}/H_{LS}) \times \text{PIV} \times (1/\text{WEV}) \times 1000$$

$$\text{LOQ (ppb)} = C_{LS} \times \text{PIV} \times (1/\text{WEV}) \times 1000$$

H_{3x} = Peak height equivalent to 3× the noise in the sample

H_{LS} = Peak height of analyte in the low-level calibration standard

C_{LS} = Concentration of analyte in the low-level calibration standard ($\mu\text{g/mL} = \text{ppm}$)

PIV = Pre-injection volume (mL)

WEV = Water extraction volume (mL)

3.0 RESULTS

3.1 Analytical Results — Toxicological Test Samples

The results of the chemical analyses of the samples received from the toxicological testing laboratory are presented in Table 1. The analyses of the test samples were performed in one analytical batch containing both the t=0 hr and t= 96 hr samples. Table 1 also presents the data for the Primary Stock Solution analysis.

The measured analyte concentrations in the test samples that had been fortified with the Test Substance ranged from 144 ppb (for sample NG70, a sample with a nominal concentration of 250

Table 1. Neodol® 23-6.5 Concentrations in Samples Received from the Toxicological Testing Laboratory

Battelle Sample ID	Test Sample Time/Type	Nominal Conc. (ppb)	Measured Conc. (ppb)
Batch #1			
NG61	t=0	0	ND
NG62	t=0	250	244
NG63	t=0	500	416
NG64	t=0	1,000	990
NG65	t=0	2,000	1714
NG66	t=0	4,000	3703
NG67	t=0	8,000	7730
NG69	t=96	0	286
NG70	t=96	250	144
NG71	t=96	500	181
NG72	t=96	1,000	185
NG73	t=96	2,000	171
NG74	t=96	4,000	2163
NG75	t=96	8,000	5173
Primary Stock Solution		(ppm)	(ppm)
NG68	t=0, stock	10,000	11,160

ND: Not detected; <LOD.

LOD (limit of detection) = 116 ppb.

ppb) to 7,730 ppb (for sample NG67, a sample with a nominal concentration of 8,000 ppb). The measured concentrations were between 9 percent (sample NG73) and 99 percent (sample NG64) of the nominal concentration. On an average, the concentrations in the t=96 hour samples are lower than the concentrations in the t=0 samples, suggesting that there may be a some loss of the analyte with time.

Some interference with the Neodol® 23-6.5 signal/peaks was evident in the HPLC/ELSD chromatograms of samples NG69, NG70, and NG71, probably contributing to the measured concentration of these sample. A significant amount of analyte was reported for one of the two toxicological control samples; the equivalent of 286 ppb was measured in sample NG69, the t=96 hr control. However, this is possibly the result of matrix interference and not actual surfactant concentrations; the three samples with the observed matrix interference were all t=96 hr samples, were concentrated to the lowest PIV, and these sample extracts were visibly green. It is likely that the matrix, and not surfactant, is contributing the majority, and possibly all, of the concentrations reported for these three samples.

The concentration measured for the Primary Stock Solution was slightly higher than the expected concentration, with measured concentration of 11,160 ppm; a sample which had a nominal/expected concentration of 10,000 ppm. The measured Primary Stock Solution concentration was 12 percent higher than the expected concentration.

3.2 Analytical Results — Quality Control Samples

All quality control objectives were met for this work. The seven-point multi-level instrument calibration used had a correlation coefficient of 0.999463 for the quadratic equation, and the continuing calibration check analyses ranged from 4.5 to 11.9 relative percent difference between the determined and actual standard concentration.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined as described in Section 2.3, and were as follows.

Limit of Detection and Limit of Quantitation

Limit of Detection (LOD)	116 ppb
Limit of Quantitation (LOQ)	104 ppb

Table 2. Laboratory Quality Control Sample Analysis Results

Battelle Sample ID	QC Sample Type	Concentration		Recovery (%)
		Expected (ppb)	Determine (ppb)	
Batch #1				
NH87PB	Procedural Blank	ND	ND	ND
NH88BS	Blank Spike	1,037	989	95.3
NH89MS	Matrix Spike	1,037	1,056	101.9
NH90MSD	Matrix Spike Duplicate	1,037	1,008	97.2
MS/MSD %RPD:				4.6

ND: Not detected; <LOD.

LOD (limit of detection) = 116 ppb.

The concentrations for all samples with anticipated analyte concentrations (i.e., all samples except the laboratory procedural blanks and toxicological test control samples) had measurable levels of analyte and determined to be above the LOD and the LOQ.

The results of the laboratory quality control (QC) sample analyses are presented in Table 2. The target analyte was not detected in the procedural blank sample. The analyte recovery in the blank spike (BS) sample was 95%. The analyte recovery in the two matrix spike (MS/MSD) samples were 102% and 97%, and these data suggest that there were no significant matrix effects on the analytical procedure. Acceptable precision was observed for the analytical batch. The relative percent differences in the measured analyte recoveries for the MS/MSD duplicate analysis was 5%.

The QC data indicate that the laboratory analysis was in control for this work. There were no identified circumstances or occurrences during the conduct of this work that may have affected the quality or integrity of the data.

4.0 ARCHIVING OF DATA

Study records that will be maintained by Battelle include, but are not limited to:

- Verified copies of all raw data and documentation records
- Verified copy of the signed and approved Analytical Chemistry Method, and associated amendments and deviations
- All correspondence, memos, or notes pertaining to the study
- Copy of the signed Final Data Report
- Test Substance records, including receipt and inventory, and physical and chemical characterization data, as supplied by the Sponsor

All project files, including verified copies of the raw data and the Final Data Report, will be archived by Battelle after the submission of this Final Data Report. The Battelle Quality Assurance Unit manages the limited-access data archival. Additionally, a small amount of Test Substance will be archived by Battelle.

APPENDIX A

Deviations to Analytical Method

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930126

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: August 10, 1994

Subject: Miscellaneous Deviations to Analytical Method

- The temperature recorded for Refrigerator #2, where standards and samples were stored, ranged from 1 to 10°C for March through June. Standards were stored in this refrigerator since March, and water samples and/or extracts were stored in this refrigerator in May and June. Unextracted water samples were to be stored at approximately 4°C, as indicated in the Analytical Procedure Document. Although this temperature range is larger than what may be considered covered by "approximately 4°C", it is not expected to have impacted the integrity of the samples or results, because of the stability of the test substance.
- The samples selected for formaldehyde testing were not selected using a true, statistically valid, randomization process — random selection is specified in the Analytical Procedure Document. The random procedure was not used because it may result in several samples from the same test day being selected while other test days may not be selected for testing the formaldehyde content. It was considered more important to get good test/sampling day coverage of the different test days, and this change in selection procedure is not expected to have impacted the integrity of the samples or representativeness of the results.
- The temperature for Refrigerator #2 was recorded twice, not three times as it should be, during the week of May 29, 1994.
- The Study-Specific Information memo for this study was not provided to the study personnel prior to the start of any study activity (sample receipt), as indicated in the Analytical Procedure Document. However, the memo was provided to the study personnel prior to the start of any laboratory sample processing activities, and this is the information covered in the memo.
- Samples with PIVs of 0.5 and 1.0 mL were brought to the PIV the day before they were analyzed by HPLC. The Analytical Procedure Document specifies that for samples ≤ 1.0 mL the PIV should be adjusted on the day of analysis. However, the samples were stored refrigerated after the PIV was adjusted, and the storage time was no more than one day, and this is not expected to have impacted the PIV or results.

Approved: Gregory S. DurellDate: 08/11/94

APPENDIX III
Cell Densities for Each Replicate Over the 96-Hour Exposure

Sponsor: Shell Development Company		Cell Densities (Cells/mL) ¹					
Test Substance: NEODOL® 23-6.5		Replicate		24 Hours	48 Hours	72 Hours	96 Hours
Test Organism: Freshwater Alga, <i>Selenastrum capricornutum</i>							
Dilution Water: Freshwater Algal Medium with Vitamins							
0-Hour Measured Concentration (mg NEODOL® 23-6.5/L)							
Negative Control		A	B	C			
		37,000	36,000	54,000	140,000	1,200,000	2,250,000
					143,000	930,000	2,120,000
					140,000	740,000	2,490,000
0.24		A	B	C			
		37,000	24,000	21,000	117,000	920,000	2,280,000
					76,000	790,000	2,300,000
					131,000	780,000	2,030,000
0.42		A	B	C			
		31,000	29,000	19,000	124,000	810,000	2,270,000
					110,000	670,000	2,100,000
					150,000	810,000	2,150,000
0.99		A	B	C			
		14,000	12,000	18,000	44,000	460,000	1,480,000
					78,000	460,000	1,370,000
					55,000	305,000	1,280,000
1.7		A	B	C			
		11,000	10,000	14,000	26,000	123,000	740,000
					23,000	72,000	450,000
					41,000	95,000	820,000
3.7		A	B	C			
		3,000	< MQD ^{2,3}	6,000	5,000	4,000	4,000
					5,000	3,000	1,000
					2,000	1,000	4,000
7.7		A	B	C			
		4,000	11,000	10,000	5,000	5,000	3,000
					9,000	6,000	6,000
					13,000	7,000	10,000

¹ The initial cell density of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 10,000 cells/mL at test initiation (Day 0).

² The minimum quantifiable cell density (MQD) using a hemacytometer was established as 1,000 cells/mL.

³ A zero was used for further calculations and statistical analyses where cell density is reported as < MQD.

APPENDIX IV

Statistical Analyses

APPENDIX IV

109A-119 96-hour Cell Density
 File: a:109A-119 Transform: SQUARE ROOT(Y)

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	5	9	0

Calculated Chi-Square goodness of fit test statistic = 7.6969
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

109A-119 96-hour Cell Density
 File: a:109A-119 Transform: SQUARE ROOT(Y)

Bartlett's test for homogeneity of variance

Calculated B statistic = 8.92
 Table Chi-square value = 16.81 (alpha = 0.01)
 Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
 Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

APPENDIX IV

TITLE: 109A-119 96-hour Cell Density

FILE: a:109A-119

TRANSFORM: SQUARE ROOT(Y)

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	2250000.0000	1500.0000
1	Control	2	2120000.0000	1456.0220
1	Control	3	2490000.0000	1577.9734
2	0.24	1	2280000.0000	1509.9669
2	0.24	2	2300000.0000	1516.5751
2	0.24	3	2030000.0000	1424.7807
3	0.42	1	2270000.0000	1506.6519
3	0.42	2	2100000.0000	1449.1377
3	0.42	3	2150000.0000	1466.2878
4	0.99	1	1480000.0000	1216.5525
4	0.99	2	1370000.0000	1170.4700
4	0.99	3	1280000.0000	1131.3708
5	1.7	1	740000.0000	860.2325
5	1.7	2	450000.0000	670.8204
5	1.7	3	820000.0000	905.5385
6	3.7	1	4000.0000	63.2456
6	3.7	2	1000.0000	31.6228
6	3.7	3	4000.0000	63.2456
7	7.7	1	3000.0000	54.7723
7	7.7	2	6000.0000	77.4597
7	7.7	3	10000.0000	100.0000

APPENDIX IV

109A-119 96-hour Cell Density
 File: a:109A-119 Transform: SQUARE ROOT(Y)

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	3	1456.022	1577.973	1511.332
2	0.24	3	1424.781	1516.575	1483.774
3	0.42	3	1449.138	1506.652	1474.026
4	0.99	3	1131.371	1216.553	1172.798
5	1.7	3	670.820	905.539	812.197
6	3.7	3	31.623	63.246	52.705
7	7.7	3	54.772	100.000	77.411

109A-119 96-hour Cell Density
 File: a:109A-119 Transform: SQUARE ROOT(Y)

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	3814.343	61.760	35.657
2	0.24	2621.095	51.197	29.558
3	0.42	871.879	29.528	17.048
4	0.99	1818.043	42.639	24.617
5	1.7	15503.697	124.514	71.888
6	3.7	333.333	18.257	10.541
7	7.7	511.389	22.614	13.056

APPENDIX IV

109A-119 96-hour Cell Density
File: a:109A-119 Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	7527519.790	1254586.632	344.751
Within (Error)	14	50947.560	3639.111	
Total	20	7578467.350		

Critical F value = 2.85 (0.05,6,14)

Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

APPENDIX IV

109A-119 96-hour Cell Density

File: a:109A-119

Transform: SQUARE ROOT(Y)

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	1511.332	2286666.667		
2	0.24	1483.774	2203333.333	0.559	
3	0.42	1474.026	2173333.333	0.757	
4	0.99	1172.798	1376666.667	6.873	*
5	1.7	812.197	670000.000	14.194	*
6	3.7	52.705	3000.000	29.614	*
7	7.7	77.411	6333.333	29.112	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

109A-119 96-hour Cell Density

File: a:109A-119

Transform: SQUARE ROOT(Y)

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	3			
2	0.24	3	361142.091	15.8	83333.333
3	0.42	3	361142.091	15.8	113333.333
4	0.99	3	361142.091	15.8	910000.000
5	1.7	3	361142.091	15.8	1616666.667
6	3.7	3	361142.091	15.8	2283666.667
7	7.7	3	361142.091	15.8	2280333.333

APPENDIX IV

Conc. Tested 0.0 0.24 0.42 0.99 1.7 3.7 7.7

 Response 1 3700037000310001400011000 3000 4000
 Response 2 3600024000290001200010000 011000
 Response 3 5400021000190001800014000 600010000

*** Inhibition Concentration Percentage Estimate ***
 Toxicant/Effluent: 109A-119
 Test Start Date: Test Ending Date:
 Test Species:
 Test Duration: 24 hours
 DATA FILE: 109a119.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	42333.333	10115.994	42333.333
2	3	0.240	27333.333	8504.901	27333.333
3	3	0.420	26333.333	6429.101	26333.333
4	3	0.990	14666.667	3055.050	14666.667
5	3	1.700	11666.667	2081.666	11666.667
6	3	3.700	3000.000	3000.000	5666.667
7	3	7.700	8333.333	3785.939	5666.667

The Linear Interpolation Estimate: 0.0677 Entered P Value: 10

Number of Resamplings: 80 (LC_{0.24}) ST_{91/6/94}
 The Bootstrap Estimates Mean: 0.0866 Standard Deviation: 0.0495
 Original Confidence Limits: Lower: 0.0458 Upper: 0.2476
 Expanded Confidence Limits: Lower: 0.0217 Upper: 0.4454
 Resampling time in Seconds: 2.25 Random_Seed: 2039662482

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APPENDIX IV

```

Conc. Tested  0.0 0.24 0.42 0.99  1.7  3.7  7.7
-----
Response  1 3700037000310001400011000 3000 4000
Response  2 3600024000290001200010000      011000
Response  3 5400021000190001800014000 600010000
-----

```

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: 109A-119
Test Start Date: Test Ending Date:
Test Species:
Test Duration: 24 hours
DATA FILE: 109a119.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	42333.333	10115.994	42333.333
2	3	0.240	27333.333	8504.901	27333.333
3	3	0.420	26333.333	6429.101	26333.333
4	3	0.990	14666.667	3055.050	14666.667
5	3	1.700	11666.667	2081.666	11666.667
6	3	3.700	3000.000	3000.000	5666.667
7	3	7.700	8333.333	3785.939	5666.667

The Linear Interpolation Estimate: 0.6724 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 0.6304 Standard Deviation: 0.1787
Original Confidence Limits: Lower: 0.2342 Upper: 0.8981
Expanded Confidence Limits: Lower: -0.2478 Upper: 1.1463
Resampling time in Seconds: 2.25 Random_Seed: -1127672702

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APPENDIX IV

Conc. ID	1	2	3	4	5	6	7
Conc. Tested	0.0	0.24	0.42	0.99	1.7	3.7	7.7
Response 1	37000	37000	31000	14000	11000	3000	4000
Response 2	36000	24000	29000	12000	10000	011000	
Response 3	54000	21000	19000	18000	14000	6000	10000

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119

Test Start Date: Test Ending Date:

Test Species:

Test Duration: 24 hours *IC90*

DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	42333.333	10115.994	42333.333
2	3	0.240	27333.333	8504.901	27333.333
3	3	0.420	26333.333	6429.101	26333.333
4	3	0.990	14666.667	3055.050	14666.667
5	3	1.700	11666.667	2081.666	11666.667
6	3	3.700	3000.000	3000.000	5666.667
7	3	7.700	8333.333	3785.939	5666.667

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 10% of the control response mean.

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APPENDIX IV

```

Conc. Tested  0.0 0.24 0.42 0.99  1.7  3.7  7.7
-----
Response  1 1400001170001240004400026000 5000 5000
Response  2 143000760001100007800023000 5000 9000
Response  3 1400001310001500005500041000 200013000
-----

```

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119
 Test Start Date: Test Ending Date:
 Test Species:
 Test Duration: 48 hours
 DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	141000.000	1732.051	141000.000
2	3	0.240	108000.000	28583.212	118000.000
3	3	0.420	128000.000	20297.783	118000.000
4	3	0.990	59000.000	17349.352	59000.000
5	3	1.700	30000.000	9643.651	30000.000
6	3	3.700	4000.000	1732.051	6500.000
7	3	7.700	9000.000	4000.000	6500.000

The Linear Interpolation Estimate: 0.1471 Entered P Value: 10

Number of Resamplings: 80 (LC0.24) 5/16/94
 The Bootstrap Estimates Mean: 0.2139 Standard Deviation: 0.1170
 Original Confidence Limits: Lower: 0.0905 Upper: 0.4591
 Expanded Confidence Limits: Lower: 0.0282 Upper: 0.8023
 Resampling time in Seconds: 2.20 Random_Seed: -1226515870

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APPENDIX IV

Conc. Tested 0.0 0.24 0.42 0.99 1.7 3.7 7.7

 Response 1 1400001170001240004400026000 5000 5000
 Response 2 143000760001100007800023000 5000 9000
 Response 3 1400001310001500005500041000 200013000

*** Inhibition Concentration Percentage Estimate ***
 Toxicant/Effluent: 109A-119
 Test Start Date: Test Ending Date:
 Test Species:
 Test Duration: 48 hours
 DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	141000.000	1732.051	141000.000
2	3	0.240	108000.000	28583.212	118000.000
3	3	0.420	128000.000	20297.783	118000.000
4	3	0.990	59000.000	17349.352	59000.000
5	3	1.700	30000.000	9643.651	30000.000
6	3	3.700	4000.000	1732.051	6500.000
7	3	7.700	9000.000	4000.000	6500.000

 The Linear Interpolation Estimate: 0.8789 Entered P Value: 50

Number of Resamplings: 80
 The Bootstrap Estimates Mean: 0.8815 Standard Deviation: 0.0594
 Original Confidence Limits: Lower: 0.7680 Upper: 0.9882
 Expanded Confidence Limits: Lower: 0.6461 Upper: 1.1084
 Resampling time in Seconds: 2.26 Random_Seed: -1534569646

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APPENDIX IV

```

Conc. Tested  0.0 0.24 0.42 0.99  1.7  3.7  7.7
-----
Response  1 1400001170001240004400026000 5000 5000
Response  2 143000760001100007800023000 5000 9000
Response  3 1400001310001500005500041000 200013000
-----

```

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119

Test Start Date: Test Ending Date:

Test Species:

Test Duration: 48 hours

DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	141000.000	1732.051	141000.000
2	3	0.240	108000.000	28583.212	118000.000
3	3	0.420	128000.000	20297.783	118000.000
4	3	0.990	59000.000	17349.352	59000.000
5	3	1.700	30000.000	9643.651	30000.000
6	3	3.700	4000.000	1732.051	6500.000
7	3	7.700	9000.000	4000.000	6500.000

The Linear Interpolation Estimate: 3.0532 Entered P Value: 90

Number of Resamplings: 80

The Bootstrap Estimates Mean: 3.0516 Standard Deviation: 0.1351

Original Confidence Limits: Lower: 2.7560 Upper: 3.2728

Expanded Confidence Limits: Lower: 2.4291 Upper: 3.5144

Resampling time in Seconds: 2.25 Random_Seed: -221368254

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APPENDIX IV

Conc. Tested 0.0 0.24 0.42 0.99 1.7 3.7 7.7

 Response 1 1200000920000810000460000123000 4000 5000

Response 2 93000079000067000046000072000 3000 6000

Response 3 74000078000081000030500095000 1000 7000

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119

Test Start Date: Test Ending Date:

Test Species:

Test Duration: 72 hours

DATA FILE: 109a119.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	956666.667	231156.513	956666.667
2	3	0.240	830000.000	78102.497	830000.000
3	3	0.420	763333.333	80829.038	763333.333
4	3	0.990	408333.333	89489.292	408333.333
5	3	1.700	96666.667	25540.817	96666.667
6	3	3.700	2666.667	1527.525	4333.333
7	3	7.700	6000.000	1000.000	4333.333

 The Linear Interpolation Estimate: 0.8776 Entered P Value: 50

Number of Resamplings: 80

The Bootstrap Estimates Mean: 0.8881 Standard Deviation: 0.1026

Original Confidence Limits: Lower: 0.7120 Upper: 1.0849

Expanded Confidence Limits: Lower: 0.5299 Upper: 1.3129

Resampling time in Seconds: 2.19 Random_Seed: 1121606066

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APPENDIX IV

Conc. Tested 0.0 0.24 0.42 0.99 1.7 3.7 7.7

 Response 1 1200000920000810000460000123000 4000 5000
 Response 2 93000079000067000046000072000 3000 6000
 Response 3 74000078000081000030500095000 1000 7000

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119

Test Start Date: Test Ending Date:

Test Species:

Test Duration: 72 hours

DATA FILE: 109a119.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	956666.667	231156.513	956666.667
2	3	0.240	830000.000	78102.497	830000.000
3	3	0.420	763333.333	80829.038	763333.333
4	3	0.990	408333.333	89489.292	408333.333
5	3	1.700	96666.667	25540.817	96666.667
6	3	3.700	2666.667	1527.525	4333.333
7	3	7.700	6000.000	1000.000	4333.333

 The Linear Interpolation Estimate: 1.7217 Entered P Value: 90

Number of Resamplings: 80

The Bootstrap Estimates Mean: 1.8098 Standard Deviation: 0.1902

Original Confidence Limits: Lower: 1.6416 Upper: 2.2676

Expanded Confidence Limits: Lower: 1.5536 Upper: 2.8682

Resampling time in Seconds: 2.25 Random_Seed: 1233313442

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APPENDIX IV

```

Conc. Tested  0.0 0.24 0.42 0.99  1.7  3.7  7.7
-----
Response  1  2250000228000022700001480000740000 4000 3000
Response  2  2120000230000021000001370000450000 1000 6000
Response  3  2490000203000021500001280000820000 400010000
-----

```

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119

Test Start Date: Test Ending Date:

Test Species:

Test Duration: 96 hours

DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	2286666.667	187705.443	2286666.667
2	3	0.240	2203333.333	150443.788	2203333.333
3	3	0.420	2173333.333	87368.949	2173333.333
4	3	0.990	1376666.667	100166.528	1376666.667
5	3	1.700	670000.000	194679.223	670000.000
6	3	3.700	3000.000	1732.051	4666.667
7	3	7.700	6333.333	3511.885	4666.667

The Linear Interpolation Estimate: 0.5025 Entered P Value: 10

Number of Resamplings: 80

The Bootstrap Estimates Mean: 0.4691 Standard Deviation: 0.0849

Original Confidence Limits: Lower: 0.2041 Upper: 0.5784

Expanded Confidence Limits: Lower: -0.1241 Upper: 0.6619

Resampling time in Seconds: 2.25 Random_Seed: -1222326926

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APPENDIX IV

Conc. Tested 0.0 0.24 0.42 0.99 1.7 3.7 7.7

Response 1 2250000228000022700001480000740000 4000 3000

Response 2 2120000230000021000001370000450000 1000 6000

Response 3 2490000203000021500001280000820000 400010000

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: 109A-119

Test Start Date: Test Ending Date:

Test Species:

Test Duration: 96 hours

DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	2286666.667	187705.443	2286666.667
2	3	0.240	2203333.333	150443.788	2203333.333
3	3	0.420	2173333.333	87368.949	2173333.333
4	3	0.990	1376666.667	100166.528	1376666.667
5	3	1.700	670000.000	194679.223	670000.000
6	3	3.700	3000.000	1732.051	4666.667
7	3	7.700	6333.333	3511.885	4666.667

The Linear Interpolation Estimate: 1.2244 Entered P Value: 50

Number of Resamplings: 80

The Bootstrap Estimates Mean: 1.2191 Standard Deviation: 0.0633

Original Confidence Limits: Lower: 1.0977 Upper: 1.3317

Expanded Confidence Limits: Lower: 0.9583 Upper: 1.4497

Resampling time in Seconds: 2.26 Random_Seed: -1226515870

APPENDIX IV

Conc. Tested 0.0 0.24 0.42 0.99 1.7 3.7 7.7

 Response 1 2250000228000022700001480000740000 4000 3000
 Response 2 2120000230000021000001370000450000 1000 6000
 Response 3 2490000203000021500001280000820000 400010000

*** Inhibition Concentration Percentage Estimate ***
 Toxicant/Effluent: 109A-119
 Test Start Date: Test Ending Date:
 Test Species:
 Test Duration: 96 hours
 DATA FILE: 109a1191.icp

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	2286666.667	187705.443	2286666.667
2	3	0.240	2203333.333	150443.788	2203333.333
3	3	0.420	2173333.333	87368.949	2173333.333
4	3	0.990	1376666.667	100166.528	1376666.667
5	3	1.700	670000.000	194679.223	670000.000
6	3	3.700	3000.000	1732.051	4666.667
7	3	7.700	6333.333	3511.885	4666.667

 The Linear Interpolation Estimate: 3.0267 Entered P Value: 90

Number of Resamplings: 80
 The Bootstrap Estimates Mean: 3.0277 Standard Deviation: 0.1091
 Original Confidence Limits: Lower: 2.7984 Upper: 3.1564
 Expanded Confidence Limits: Lower: 2.5473 Upper: 3.2991
 Resampling time in Seconds: 2.25 Random_Seed: -1534569646

APPENDIX V

Changes to Protocol

The study was conducted in accordance with the approved Protocol with the following changes:

1. The proposed experimental start date, experimental termination date and test concentrations were added to the protocol by amendment.
2. The protocol was amended to clarify the procedure for monitoring algal growth.
3. The protocol was amended to clarify recovery phase procedures.
4. The "Records to be Maintained" section of the protocol was amended to indicate that observations of the test organism may not be recorded daily.
5. Several light intensity measurements over the test period were slightly outside of the range desired for the test.
6. Samples were not collected for the determination of cell densities at recovery phase initiation.

In the opinion of the Study Director, the above changes in the approved protocol did not adversely affect the results of this study.

APPENDIX VI

Personnel Involved in the Study

The following key personnel were involved in the conduct or management of this study:

Aquatic Toxicology Laboratory:

1. James P. Swigert, Ph.D., Manager, Aquatic Toxicology
2. Susan G. Thompson, Senior Aquatic Biologist
3. Cynthia Roberts, Senior Aquatic Biologist
4. Kristen G. MacGregor, Aquatic Biologist

Analytical Chemistry Laboratory

1. Gregory Durell, Chemist, Battelle Ocean Sciences