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Dear Sir:

SUBJECT: ACUTE TOXICITY OF DOBANOL 91-8 TO THE FATHEAD MINNOW,
DAPHNIDS, AND SELENASTRUM CAPRICORNUTUM

The subject TSCA 8(e) submission was filed by Shell Oil Company on November 17, 1993, and preliminary data transmissions were provided. The complete reports (attached) are now available and are provided as supplemental information to the original TSCA 8(e).

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.

8/1/94

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

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Very truly yours,



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

THG/sjh

Attachments

**DOBANOL[®] 91-8 - ACUTE TOXICITY TO
FATHEAD MINNOW (*Pimephales promelas*)
UNDER STATIC RENEWAL CONDITIONS**

TSCA TEST GUIDELINES § 797.1400

Submitted to:

**Shell Development Company
P.O. Box 1380
Houston, Texas 77251**

SLI Report #93-11-5036

Sponsor Protocol/Project No.: WRC TOX No. 1227

SLI Study #777.0993.6105.101

Study Director: Maura K. Collins

**Springborn Laboratories, Inc.
Environmental Sciences Division
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Wareham, Massachusetts 02571**

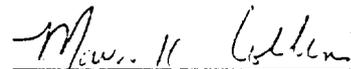
11 February 1994

FINAL REPORT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for "Dobanol[®] 91-8 - Acute Toxicity to Fathead Minnow (*Pimephales promelas*) Under Static Renewal Conditions" were produced and compiled in accordance with all pertinent TSCA Good Laboratory Practice regulations with the following exception: routine water contaminant screening analyses for pesticides, PCBs and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, Pennsylvania. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Stability, characterization, and verification of the test material identity and maintenance of records on the test material are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test material will be sent to the Study Sponsor. Archival of a sample of the test material is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

 2-11-94Maura K. Collins
Study Director

Date

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SUMMARY**Dobanol[®] 91-8 - Acute Toxicity to Fathead Minnow
(*Pimephales promelas*) Under Static Renewal Conditions**

SPONSOR: Shell Development Company
P.O. Box 1380
Houston, Texas 77251

PROTOCOL TITLE: "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with Fathead Minnows Following TSCA Test Guidelines § 797.1400," Springborn Protocol #072993/TSCA/SHELL/FM-SR and Protocol Amendments #1 and #2 dated 29 September 1993 and 13 January 1994, respectively.

REPORT NUMBER: 93-11-5036

**SPONSOR PROTOCOL/
PROJECT NO.:** WRC TOX No. 1227

STUDY NUMBER: 777.0993.6105.101

TEST MATERIAL: Dobanol[®] 91-8, CAS #68439-46-3, Lot #97002310, WRC TOX. Sample #1227, PSIS #7075

DATE RECEIVED: 2 September 1993

DESCRIPTION: A clear liquid reported by the Study Sponsor to contain 100% active ingredient.

**EXPERIMENTAL
START DATE:** 18 October 1993

**EXPERIMENTAL
TERMINATION DATE:** 22 October 1993

TEST ORGANISM:	<i>Pimephales promelas</i> Total length: Mean = 37 mm; range = 29 to 42 mm; N = 30 Wet weight: Mean = 0.51 g; range = 0.26 to 0.71 g; N = 30 Source: Springborn Laboratories culture facility
DILUTION WATER:	reconstituted well water (2 batches used during definitive testing) pH: 7.4 (both) Specific conductivity: 220 and 230 μ mhos/cm, respectively Total hardness as CaCO ₃ : 58 and 61 mg/L, respectively Total alkalinity as CaCO ₃ : 37 and 36 mg/L, respectively
TEST CONDITIONS:	96-hour duration, 21 to 22 °C, illumination of 430 lux (40 footcandles), photoperiod of 16 hours light and 8 hours dark
NOMINAL TEST CONCENTRATIONS:	1.3, 2.5, 5.1, 10 and 20 mg/L
MEAN MEASURED CONCENTRATIONS:	0.98, 2.2, 3.9, 8.5 and 17 mg/L
RESULTS:	The 96-hour LC50 value was estimated by nonlinear interpolation to be 11 mg/L (95% confidence interval calculated by binomial probability of 8.5 to 17 mg/L). The 96-hour No-Observed-Effect Concentration (NOEC) was determined to be 3.9 mg/L.

1.0 INTRODUCTION

The purpose of this study was to estimate the acute toxicity (LC50) of Dobanol® 91-8 to fathead minnow (*Pimephales promelas*) under static renewal conditions. The LC50 is defined as the concentration of test material in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from release of the test material into aquatic environments. The study was initiated on 10 September 1993, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the 96-hour definitive test was conducted from 18 to 22 October 1993 at the Environmental Sciences Division of Springborn Laboratories, Inc. (SLI), in Wareham, Massachusetts. All original raw data and the final report produced during this study are stored at Shell Development Company.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this acute toxicity study followed those described in the Springborn protocol entitled "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with Fathead Minnows Following TSCA Test Guidelines § 797.1400," Springborn Protocol #072993/TSCA/SHELL/FM-SR and Protocol Amendments #1 and #2 dated 29 September 1993 and 13 January 1994, respectively (Appendix I). The method described in this protocol meet or exceed the standard procedures described in the U.S. EPA Toxic Substances Control Act (TSCA) Test Guidelines § 797.1400 (U.S. EPA, 1992) and meet the primary technical objectives of the Shell Research Limited/Sittingbourne Research Center guidelines (SOP No. 81, Edition No. 8).

2.2 Test Material

A sample of the Dobanol® 91-8 (CAS #68439-46-3, Lot #97002310, WRC TOX. Sample #1227, PSIS #7075, expiration date September 1994), a clear liquid, reported by the Study Sponsor to contain 100% active ingredient, was received from Shell Westhollow Research Center, Houston, Texas, on 2 September 1993 (Certificate of Analysis, Appendix II). Upon receipt at

Springborn, the test material was stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Nominal and measured test concentrations are expressed as milligrams of Dobanol[®] 91-8 per liter of solution and are reported as mg/L.

2.3 Test Organisms

The fathead minnow (Springborn Lot #93A71) used in this toxicity test were obtained from laboratory cultures maintained at Springborn. The culture water was "soft" water and was drawn from a 100-meter deep bedrock well into a concrete reservoir where it was aerated and supplemented with well water supplied by the Town of Wareham, Massachusetts. Prior to testing, the fish were held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. Other parameters monitored in the holding tank were a pH of 6.4 to 7.1, dissolved oxygen concentration with a range of 78 to 81% of saturation and conductivity with a range of 100 to 120 micromhos per centimeter ($\mu\text{mhos/cm}$). This water had total hardness and alkalinity ranges as calcium carbonate (CaCO_3) of 24 to 28 mg/L and 18 to 20 mg/L, respectively. (SLI Weekly Record of Fish Holding Water Characteristics, Vol. 5 and the SLI Gravity Feed Tank Water Quality Analysis Logbook, Vol. 8). Test fish were maintained under these conditions for a minimum of 14 days. The temperature range in the holding tank was 22 to 23 °C during this period. The fish were fed a dry commercial pelleted food, *ad libitum*, daily except during the 48-hours prior to testing. Representative samples of the food source were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix III). Food sources were considered to be of acceptable quality since the total concentration of pesticides measured was less than 0.3 mg/kg (ASTM, 1985). No mortality was observed among the test fish population during the 48-hour period prior to test initiation (SLI Daily Record of Fish Holding Conditions). A representative sample (N = 30) of fish from the test population had a mean total length of 37 mm (range 29 to 42 mm) and a mean wet weight of 0.51 g (range 0.26 to 0.71 g) (SLI Fish Length and Weight Log, Vol. 10).

2.4 Reference Test

A copper nitrate reference test was conducted with the test organism population from 16 to 20 November 1993. The resulting 96-hour LC50 was calculated by probit analysis to be

190 $\mu\text{g/L}$ (95% confidence interval of 150 to 230 $\mu\text{g/L}$) (SLI *Pimephales promelas* Copper Nitrate Reference Log, Vol. III). In addition to the above data, culture records document the ability of this population of *Pimephales promelas* to successfully and actively feed, grow and reproduce over a period of several generations. Based on the results of the reference test and the successful culture of *Pimephales promelas*, it was established that this population was suitable for testing.

2.5 Test Dilution Water

Two separate batches of dilution water were prepared for the study by reconstituting "soft" laboratory water (e.g., a mixture of unadulterated well water and untreated town well water) with various reagents to a specified hardness. The dilution water was from the same source as the culture water described above. The two batches of water had a total hardness as CaCO_3 of 58 and 61 mg/L, respectively; a total alkalinity as CaCO_3 of 37 and 36, respectively; a pH of 7.4 and 7.4, respectively; and a specific conductivity of 220 and 230 $\mu\text{mhos/cm}$, respectively (SLI Statics Modified GFT Water Quality Log Book, Vol. II). Representative samples of the dilution water source were analyzed for the presence of metals, pesticides and PCBs (Appendix IV). None of these compounds were detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with ASTM Standard Practice (ASTM, 1980). In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentration. Based on these analyses, the TOC concentration of the dilution water source ranged from 0.81 to 1.4 mg/L for the months of April to September 1993 (SLI TOC Master Log). In addition, TOC concentration and total suspended solids (TSS) analyses were conducted at Springborn on the batch of dilution water used during this study. The TOC concentration of the hard reconstituted water, Batch #2 and #3, ranged from 1.4 to 2.9 mg/L for the month of October. The Total Suspended Solids (TSS) for these batches were 9.0 and 9.8 mg/L, respectively for the month of October. Several species of daphnids (a representative freshwater organism generally recognized to be sensitive to chemical challenges) maintained in water from the same source as the dilution water utilized in this study have successfully survived and reproduced over several generations. The performance of the daphnid cultures, in

combination with the previously mentioned analyses, confirms the acceptability of this dilution water.

2.6 Test Conditions

Test vessels were positioned in stratified random order in a waterbath designed to maintain test solution temperatures at 22 ± 2 °C. Test solutions were not aerated. The photoperiod during testing was the same as that provided in the fathead minnow culture area. Light at an intensity of 430 lux (40 footcandles) was provided at the solutions' surface. The culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Sudden transitions from light to dark and vice versa were avoided.

2.7 Test Concentrations

Selection of nominal Dobanol[®] 91-8 concentrations for the 96-hour definitive static renewal toxicity test with *Pimephales promelas* was based on toxicity information developed at Springborn through preliminary testing. The nominal concentrations chosen were 1.3, 2.5, 5.1, 10 and 20 mg /L.

2.8 Test Procedure

The static renewal toxicity test was conducted in 18.9-L glass aquaria which contained 15 L of test solution. The exposure solution in each test vessel had a depth of 18.4 cm and a surface area of 819 cm². Duplicate test aquaria were established for each treatment level and control. Prior to test initiation, a 20 mg/mL stock solution was prepared by heating the glass bottle of test material in a glass container to a temperature ranging from 50 to 60 °C, until the test material was observed to be completely melted. Forty grams (40.00 g) of test material was then removed using a glass pipet, and was diluted with 2 L of distilled, deionized water. The stock solution was observed to be clear and colorless with no sign of undissolved test material.

Replicate treatment level solutions with nominal concentrations of 1.3, 2.5, 5.1, 10 and 20 mg/L were prepared by diluting the appropriate amount of the stock solution with 15 L of dilution water. A 50% dilution factor was used to calculate the nominal test concentrations to

ensure that both LC50 and NOEC values were achieved. The exposure solutions were stirred for 30 seconds with a TAMCO Model 700 laboratory stirrer. The test solutions were observed to be clear and colorless with no sign of undissolved test material. One set of control vessels was also established which contained the same dilution water and was maintained under the same conditions as the test vessels but contained no Dobanol[®] 91-8. A duplicate set of exposure vessels was established to prepare renewal solutions. Test solutions were renewed at 24, 48 and 72 hours of exposure following the procedure mentioned above.

Approximately 30 minutes after the test solutions were prepared, fathead minnow were impartially added to each test vessel (10 fathead minnow per replicate, 20 fathead minnow per treatment level and control). Fathead minnow were added to the test vessels no more than two at a time until all vessels contained two fish. This procedure was repeated until all replicate test vessels contained ten fish. Dead fathead minnow were removed from the test vessels at each observation interval. At each renewal period, the fathead minnow were carefully transferred from the aged test solutions into their respective freshly prepared test solutions using a modified fine-mesh dip net. Fathead minnow were not fed during the study.

2.9 Test Monitoring

All aquaria were examined after 0, 24, 48, 72 and 96 hours of exposure as follows: mortalities were recorded, dead fish were removed, and observations of the fish for sublethal effects (e.g. loss of equilibrium) and the physical characteristics of the test solutions were recorded. Dissolved oxygen concentration, temperature and pH were measured in all exposure solutions at test initiation and at each 24-hour interval. Water quality parameters were recorded in both the aged and freshly prepared test solutions at 24, 48 and 72 hours of exposure. Total hardness, total alkalinity, acidity and specific conductance were measured at 0-hour in each replicate of the control and test solutions. In addition, the temperature of the surrounding water in the waterbath was continuously monitored throughout the exposure period.

Total hardness concentration presented in this report was measured by the EDTA titrimetric method and total alkalinity concentration was determined by potentiometric titration to

an endpoint of pH 4.5 (APHA *et al.*, 1985). Specific conductivity was measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe. A Jenco Model 601A pH meter and combination electrode was used to measure pH. Dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe. Daily temperature was measured with a Brooklyn alcohol thermometer. Continuous temperature monitoring was performed using a Fisher Scientific Min/Max thermometer.

2.10 Analytical Measurements

During the definitive exposure period, water samples were removed from each replicate solution of each treatment level and the control at 0, 24, 72 and 96 hours. A composite of the water samples (replicates A and B) for each treatment level and control were analyzed for Dobanol[®] 91-8 concentration. Sample containers were approximately 800-mL borosilicate glass bottles with Teflon[®]-lined screw caps. Samples analyzed at the 0- and 72-hour sampling intervals were removed from the freshly prepared exposure solutions. Samples analyzed at 24 and 96 hours were removed from the aged exposure solutions. In addition, a sample of the primary stock solution used to formulate the exposure solutions was collected for analysis at 0, 24 and 72 hours. Each exposure solution sample was collected from the approximate midpoint of the test vessel with a volumetric pipet. Sample containers were completely filled to minimize headspace. On the day of collection, all samples were preserved with 10% formalin and delivered to Battelle Ocean Sciences, Duxbury, Massachusetts for analysis. Samples were analyzed in accordance with methods described in Battelle Ocean Sciences Study #SD-930116 (Appendix V). All of the glassware used in testing and sample collection was thoroughly washed with sequential rinses of a 10% solution of nitric acid, acetone, distilled-deionized water, isopropanol and distilled-deionized water.

2.11 Determination of LC50 and NOEC

The measured concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 is defined as the concentration of the test material in dilution water which caused mortality of 50% of the test organism population at the

stated time interval. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program, modified from the program of C. Stephan (Peltier et al, 1985), was used to calculate the LC50 values and 95% confidence intervals.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce mortality of between 0 and 100% in the test population. The selection of reported LC50 values and 95% confidence intervals was based upon an examination of the database and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship, the number of concentrations causing partial responses, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there was no toxicant-related mortality or physical and behavioral abnormalities (e.g., lethargy, loss of equilibrium), with respect to the control organisms.

3.0 RESULTS

3.1 Preliminary Test

Prior to initiating the definitive study, a preliminary range-finding test was conducted at Springborn from 23 to 27 September 1993 at nominal concentrations of 0.64, 1.6, 8.0, 20 and 50 mg/L. One exposure vessel was established for each treatment level and control. At 24 hours of exposure, 100% mortality was observed in the two highest test concentrations (20 and 50 mg/L). At test termination, sublethal effects (e.g. complete loss of equilibrium, darkened pigmentation) were observed among all of the fish exposed to the 8 mg/L treatment level. No mortality or sublethal effects were observed in the remaining treatment levels (0.64 and 1.6 mg/L) or the control. Based on these results, the definitive study was conducted at a nominal concentration range that was calculated with a 50% dilution factor in an effort to obtain data

sufficient to establish both an LC50 and a NOEC value. The nominal concentrations of 1.3, 2.5, 5.1, 10 and 20 mg/L were selected for the definitive study with Dobanol[®] 91-8.

3.2 Definitive Test

3.2.1 Evaluation of Test Conditions - The measurements of the water quality parameters (i.e., pH, dissolved oxygen concentration, temperature) recorded during the definitive study are presented in Tables 1 and 2. Analysis of the control and test solutions at test initiation established a total hardness (as CaCO₃) ranging from 56 to 62 mg/L, a total alkalinity (as CaCO₃) ranging from 32 to 36 mg/L and specific conductivity ranging from 210 to 220 μmhos/cm. Throughout the exposure period, the pH and dissolved oxygen saturation for the control and test solutions ranged from 7.2 to 7.6 and 69 to 102%, respectively. These results demonstrate that the water quality parameters measured were unaffected by the concentrations of Dobanol[®] 91-8 tested and remained within acceptable ranges for the survival of fathead minnow. Daily temperature monitoring of the test solutions and continuous temperature monitoring of the water in the surrounding waterbath established that the temperature in the test solutions ranged from 21 to 22 °C throughout the exposure period.

3.2.2 Analytical Results - The results of the analysis of the primary stock solution and the test solutions for Dobanol[®] 91-8 concentrations are summarized in Table 3. Results of the analysis of the primary stock solution (20,000 mg/L) used to formulate the test solutions indicate the concentration of Dobanol[®] 91-8 was 96% of nominal concentration. Measured concentrations for all treatment levels were consistent between sampling intervals. Mean measured concentrations averaged 82% of nominal and defined the treatment levels as 0.98, 2.2, 3.9, 8.5 and 17 mg/L. Analytical results are presented in Battelle Ocean Sciences Study #SD-930116 (Appendix V).

3.2.3 Biological Results - The concentrations tested, the corresponding cumulative percent mortality and the observations made during the definitive exposure are presented in Table 4. All exposure solutions were observed to be clear and colorless and contained no visible signs of undissolved test material. Following 24 hours of exposure, 100% mortality was observed

among fathead minnow exposed to the highest concentration tested (17 mg/L). At test termination (96 hours of exposure), 10% mortality was observed among fathead minnow exposed to the 8.5 mg/L test concentration. In addition, all of the surviving fathead minnow exposed to this treatment level exhibited a complete loss of equilibrium. No mortality or sublethal effects were observed among fathead minnow exposed to the remaining test concentrations (0.98, 2.2 and 3.9 mg/L) or the control. The 24-, 48-, 72- and 96-hour concentration-response (mortality) curves for this study are presented in Figures 1, 2, 3 and 4, respectively. Table 5 summarizes the 24-, 48-, 72- and 96-hour LC50 values and corresponding 95% confidence intervals, and presents the No-Observed-Effect Concentration (NOEC) through 96 hours. Based on mean measured concentrations of Dobanol[®] 91-8, the 96-hour LC50 value was estimated by nonlinear interpolation to be 11 mg/L with a corresponding 95% confidence interval calculated by binomial probability of 8.5 to 17 mg/L. The 96-hour NOEC for this study was 3.9 mg/L.

PROTOCOL DEVIATION

The study protocol states that the water samples will be collected in 500-mL glass containers which are completely filled to minimize headspace, and that the water samples will be preserved with formalin (i.e., 5 mL formalin/500 mL sample). During this study, sample containers were of approximately 800 mL in volume which were completely filled to minimize headspace, and the water samples were inadvertently preserved with 5 mL formalin at a concentration of 10%.

Although test solution samples were not prepared in accordance with the study protocol, measured concentrations established during the 96-hour exposure period averaged 82% of the nominal fortified concentrations. Based on these data, it is our opinion that the sample preparation procedures utilized during this study did not negatively impact the samples.

It is our opinion that this deviation did not affect the results of this study.

SPRINGBORN LABORATORIES, INC.

 2-11-94

Maura K. Collins
Study Director

date

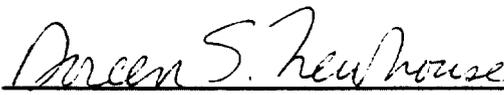
QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "Dobanol[®] 91-8 - Acute Toxicity To Fathead minnow (*Pimephales promelas*) Under Static Renewal Conditions" were inspected by the Quality Assurance Unit (QAU) at Springborn Laboratories Inc., Environmental Sciences Division to determine adherence with the study protocol and laboratory standard operating procedures. In addition, inspection of certain phases of the in-life portion of the study was performed. Dates of study inspections, dates reported to the Study Director and to Management are listed below.

Based on these inspections, it was determined that this report accurately reflects the raw data collected during this study.

<u>Inspection Date</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
10/22/93	10/22/93	10/22/93
11/3/93	11/4/93	11/5/93
1/13/94	1/14/94	1/14/94
1/19/94	1/19/94	1/28/94
2/10/94	2/10/94	2/11/94

SPRINGBORN LABORATORIES, INC.


11 Feb 1994

 Doreen S. Newhouse Date
 Supervisor
 Quality Assurance Unit

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TABLES

Table 1. The pH, dissolved oxygen concentration and temperature measurements recorded during the 96-hour static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

Nominal Concentration (mg/L)	0-Hour		24-Hour ^a		48-Hour ^a		72-Hour ^a		96-Hour	
	A	B	A	B	A	B	A	B	A	B
pH										
Control	7.3	7.3	7.4/7.4	7.3/7.4	7.4/7.5	7.4/7.4	7.5/7.5	7.5/7.5	7.5	7.5
1.3	7.3	7.4	7.4/7.3	7.3/7.5	7.4/7.5	7.4/7.4	7.5/7.5	7.5/7.5	7.5	7.5
2.5	7.3	7.4	7.4/7.4	7.4/7.4	7.4/7.5	7.4/7.4	7.5/7.6	7.4/7.5	7.5	7.4
5.1	7.4	7.4	7.4/7.4	7.2/7.4	7.4/7.5	7.4/7.4	7.6/7.5	7.4/7.5	7.5	7.4
10	7.4	7.4	7.3/7.3	7.3/7.4	7.4/7.4	7.4/7.4	7.5/7.5	7.5/7.5	7.4	7.4
20	7.4	7.4	7.3/7.3	7.4/7.3	7.4/7.4	7.4/7.4	7.5/7.5	7.5/7.5	7.5	7.5
Dissolved Oxygen, mg/L (% saturation)										
Control	8.6 (98)	8.4 (96)	6.4/8.6 (73/98)	6.2/8.4 (71/96)	6.2/8.6 (71/98)	6.5/8.7 (74/99)	6.9/8.6 (79/98)	6.6/8.7 (75/99)	6.6 (75)	6.7 (77)
1.3	8.6 (98)	8.5 (97)	6.2/8.6 (71/98)	6.0/8.5 (69/97)	6.0/8.6 (69/98)	6.5/8.6 (74/98)	6.8/8.7 (78/99)	6.8/8.8 (78/101)	6.8 (78)	6.6 (75)
2.5	8.5 (97)	8.6 (98)	6.4/8.7 (73/99)	6.2/8.5 (71/97)	6.2/8.7 (71/99)	6.5/8.6 (74/98)	6.8/8.8 (78/101)	6.9/8.8 (79/101)	6.6 (75)	7.2 (82)
5.1	8.6 (98)	8.4 (96)	6.6/8.6 (75/98)	6.4/8.4 (73/96)	6.4/8.6 (73/98)	6.6/8.7 (75/99)	7.2/8.7 (82/99)	7.4/8.9 (85/102)	7.1 (81)	6.9 (79)
10	8.5 (97)	8.4 (96)	7.2/8.6 (82/98)	6.8/8.6 (78/98)	7.6/8.6 (87/98)	7.6/8.7 (87/99)	7.6/8.9 (87/102)	7.6/8.7 (87/99)	7.8 (89)	8.2 (94)
20	8.4 (96)	8.5 (97)	8.0/8.5 (91/97)	7.6/8.6 (87/98)	8.6/8.8 (98/101)	8.4/8.9 (96/102)	7.8/8.9 (89/102)	7.9/8.8 (90/101)	8.2 (94)	7.9 (90)
Temperature (°C)^b										
	22		22/22		22/22		22/22		22	

^a Exposure solutions were renewed at this interval. Measurements are presented as aged/freshly prepared.
^b Values presented represent the daily temperatures measured (Brooklyn alcohol thermometer) in all test concentrations and the control at the stated time interval. Continuous temperature monitoring (Fisher Scientific Min-Max thermometer) of the surrounding water in the waterbath established a temperature range of 21 to 22 °C throughout the exposure period.

Table 2. Total hardness, total alkalinity and specific conductance measured at 0-hour in the test solutions during the 96-hour static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

Nominal Concentration (mg/L)		Total Hardness (mg/L as CaCO ₃)	Total Alkalinity (mg/L as CaCO ₃)	Specific Conductance (μmhos/cm)	Acidity (mg/L as CaCO ₃)
Control	A	62	34	220	21
	B	60	36	220	22
1.3	A	58	36	220	25
	B	60	36	220	27
2.5	A	62	36	220	25
	B	60	34	220	27
5.1	A	60	32	220	26
	B	56	36	220	28
10	A	62	34	220	28
	B	56	32	210	26
20	A	60	34	220	26
	B	62	36	220	27

Table 3. Concentrations of Dobanol[®] 91-8 measured in the exposure solutions during the 96-hour toxicity test with *Pimephales promelas*.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)					% Nominal ^{cd}
	0-Hour ^a	24-Hour ^b	72-Hour ^a	96-Hour ^b	Mean ^c	
Control	ND ^e	ND	ND	ND	NA ^f	NA
1.3	1.1	0.94	0.97	0.92	0.98	75
2.5	2.3	2.1	2.2	2.1	2.2	86
5.1	4.3	3.8	3.8	3.7	3.9	76
10	8.5	8.5	8.7	8.3	8.5	85
20	18	17	17	17	17	87
Stock Solution ^g (20,000)	19,000	19,000	19,000	NA	19,000	96

^a Samples analyzed at this interval were removed from the freshly prepared exposure solutions.

^b Samples analyzed at this interval were removed from the aged exposure solutions.

^c Calculated values are based on actual analytical results and not on rounded values (two significant figures) presented in this table.

^d Mean % of Nominal = 82% (calculation excludes stock solution)

^e ND = Not detected; less than the limit of detection (LOD)

^f NA = Not Applicable

^g Nominal concentration of stock solution is presented in parentheses.

Table 4. Concentrations tested, corresponding cumulative percent and number of mortalities and observations made during the 96-hour static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

Mean Measured Concentration (mg/L)	Cumulative Percent Mortality ^a											
	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
0.98	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
2.2	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
3.9	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
8.5	0 (0)	0 (0)	0 ^b	20 (2)	0 (0)	10 ^b	20 (2)	0 (0)	10 ^b	20 (2)	0 (0)	10 ^b
17	100 (10)	100 (10)	100	100 (10)	100 (10)	100	100 (10)	100 (10)	100	100 (10)	100 (10)	100

^a The actual number of dead fathead minnow is presented in parentheses.

^b All of the surviving fish exhibited a complete loss of equilibrium.

Table 5. The LC50 values, corresponding 95% confidence intervals and No Observed Effect Concentration (NOEC) established during the 96-hour static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

	LC50 (mg/L)	95% Confidence Interval	
		Lower (mg/L)	Upper (mg/L)
24-Hour ^a	12	8.5	17
48-Hour ^a	11	8.5	17
72-Hour ^a	11	8.5	17
96-Hour ^a	11	8.5	17

NOEC through 96 hours = 3.9 mg/L

^a LC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.

FIGURES

Figure 1. The 24-hour concentration-response (mortality) curve for the static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

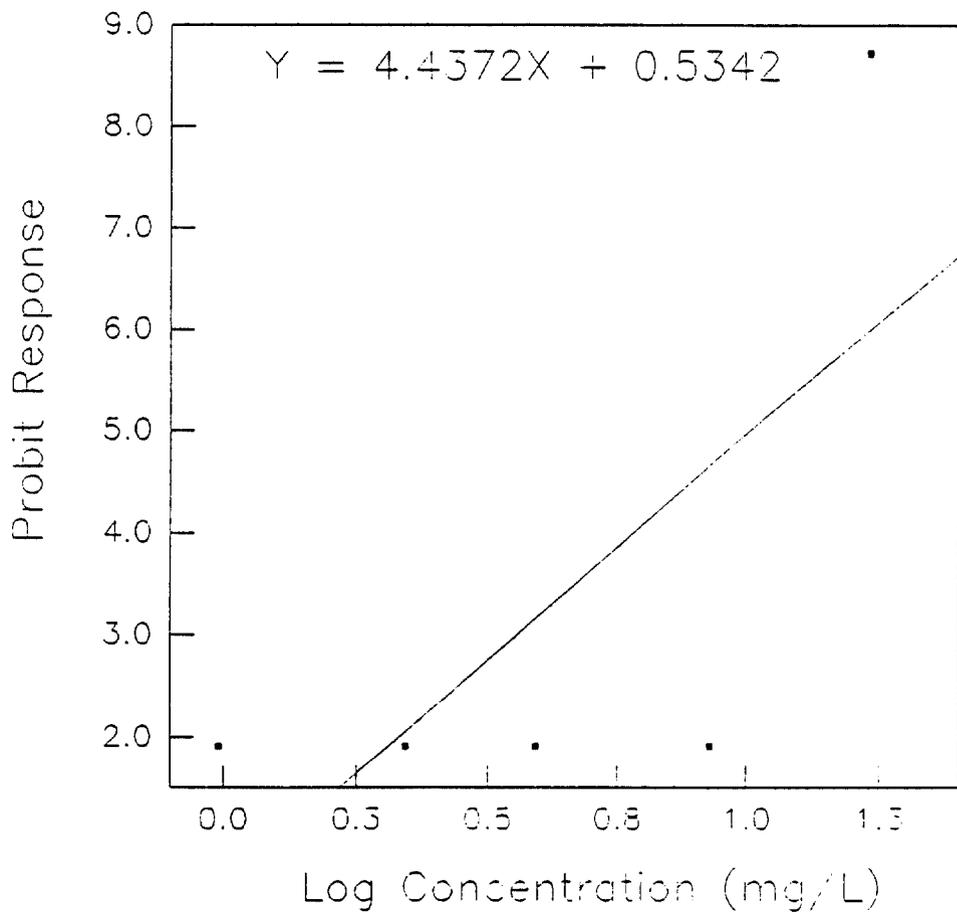


Figure 2. The 48-hour concentration-response (mortality) curve for the static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

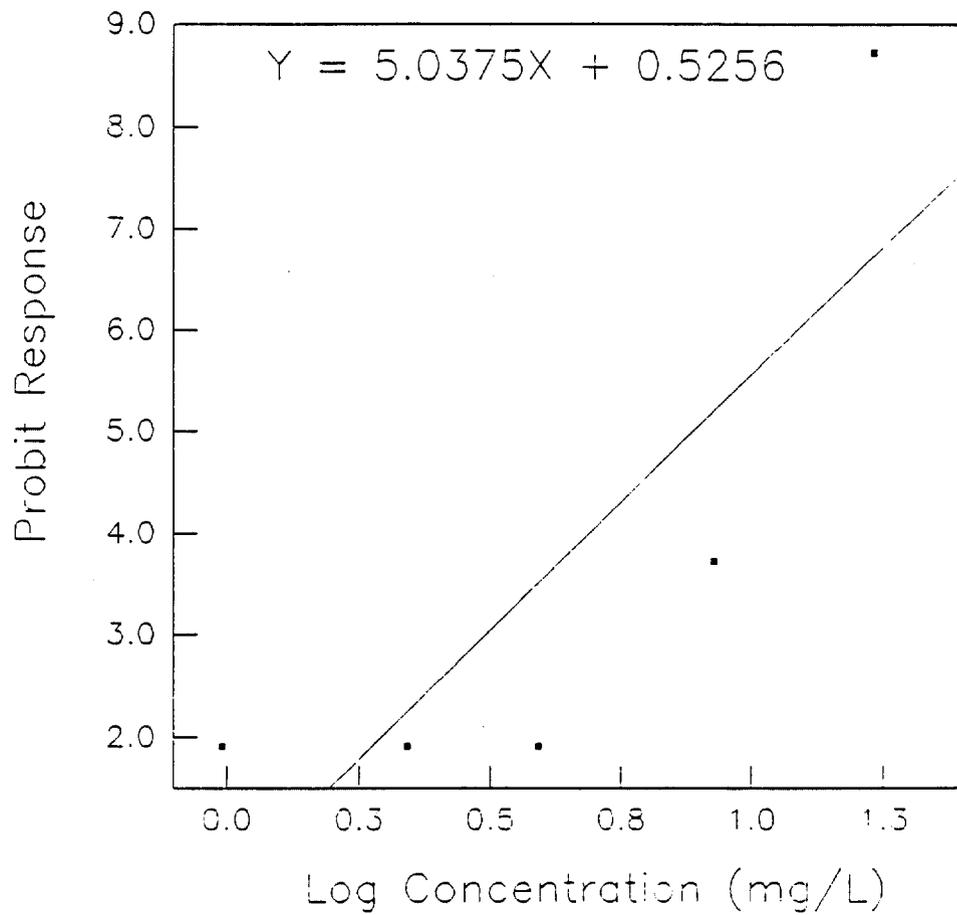


Figure 3. The 72-hour concentration-response (mortality) curve for the static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.

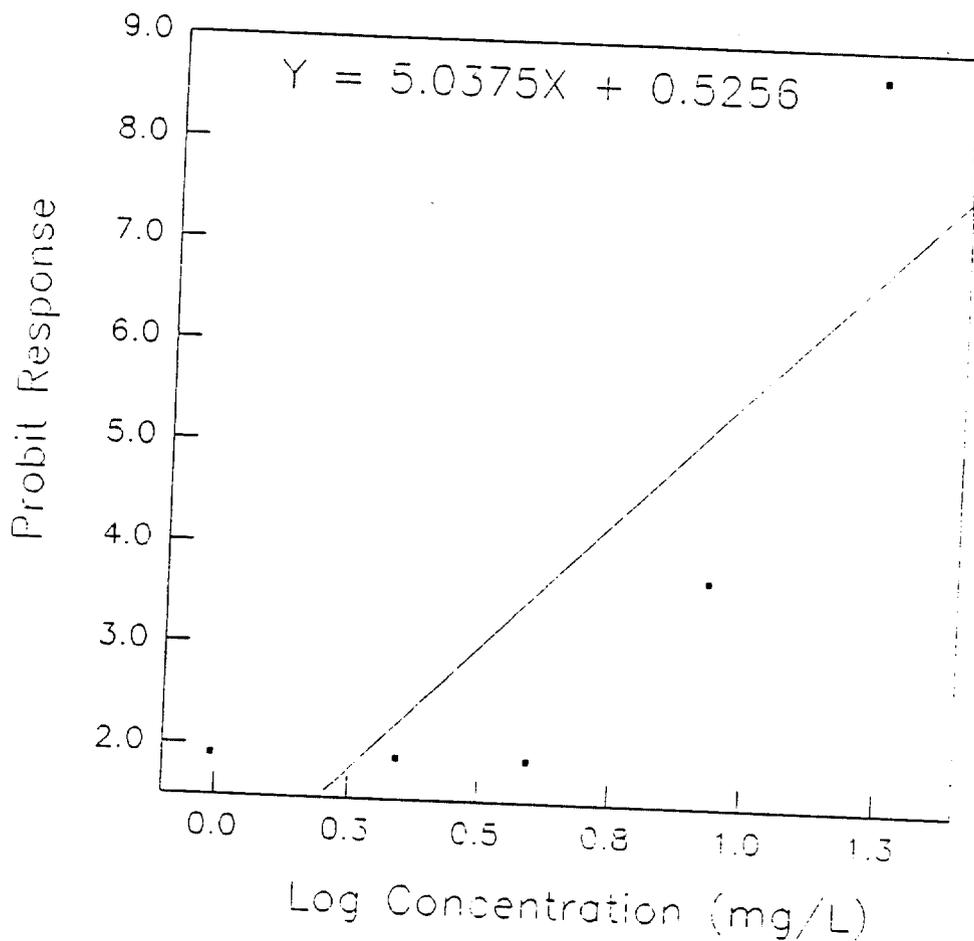
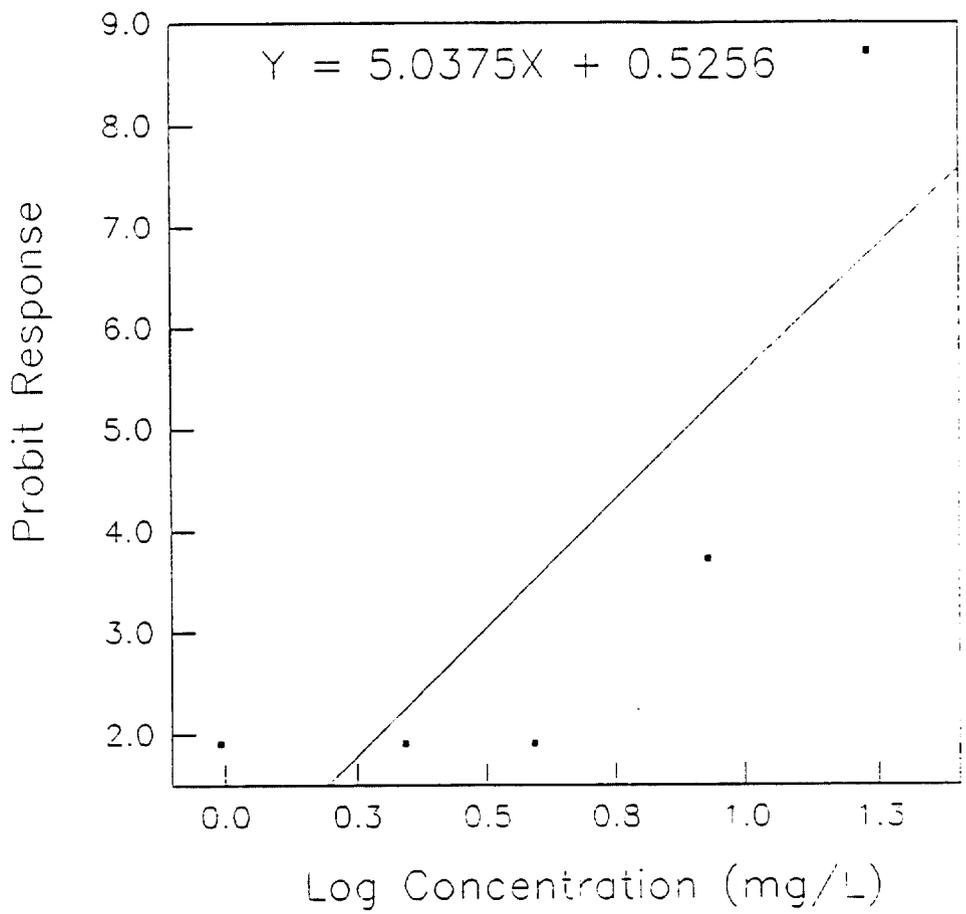


Figure 4. The 96-hour concentration-response (mortality) curve for the static renewal exposure of fathead minnow (*Pimephales promelas*) to Dobanol[®] 91-8.



SIGNATURES AND APPROVAL

SUBMITTED BY:

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

PREPARED BY:

Maura K. Collins 2/11/94
Maura K. Collins Date
Study Director

Donald C. Surprenant for R.H.D. 2/11/94
Robin H. Dyer Date
Principal Investigator

Susan P. Shepherd 11 Feb 94
Susan P. Shepherd Date
Coordinator, Data Management
and Reporting Unit

APPROVED BY:

Donald C. Surprenant 2/14/94
Donald C. Surprenant Date
Program Manager
Environmental Toxicology

Doreen S. Newhouse 11 Feb 94
Doreen S. Newhouse Date
Supervisor
Quality Assurance Unit

This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

4.0 APPENDIX I - STUDY PROTOCOL

Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with Fathead Minnows Following TSCA Test Guidelines § 797.1400

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: Shell Development Company
 Address: P.O. Box 1380, Houston, Texas
 Phone: (713) 493-8040

Sponsor Protocol/Project No.: WKC Tox No. 1227

Test Substance: Doban 91-8

Purity: 100% CAS# or LOT#: 68439-46-3

Additional Comments and/or Modifications:

Diana Conway 8/31/93
 Sponsor Approval Date

TO BE COMPLETED BY SPRINGBORN LABORATORIES PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc., 790 Main St., Wareham, MA 02571

Study Director: Maura K Collins SLI Study No.: 777.0993.6105.1a1

Test Concentrations: 20, 10, 5.1, 2.5 and 1.3 mg/L plus Controls

Solvent Used: Distilled deionized water CAS# or LOT#: NA

Proposed Schedule: (Start) 18 October 1993 (Completion) 22 October 1993

Proposed Draft Report Date:

Maura K Collins 9.10.93
 Study Director Date

Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with Fathead Minnows Following TSCA Test Guidelines § 797.1400.

1.0 INTRODUCTION

The purpose of this test is to determine the acute lethal effects of an alcohol ethoxylate surfactant on fathead minnows under static renewal conditions. Test results will be reported as the 24-, 48-, 72- and 96-hour LC50 values (the median concentration which will kill 50% of the number of fish exposed) with 95% confidence limits. The No-Observed-Effect Concentration (NOEC) will also be reported. The test procedures performed during the biological portions of this study will meet or exceed the standard procedures described in the U.S. EPA Toxic Substance Control Act (TSCA) Test Guidelines § 797.1400 (U.S. EPA, 1985) and will meet the primary technical objectives of Shell Research Limited/Sittingbourne Research Centre guidelines (SOP No. 81, Edition No.8).

2.0 MATERIALS AND METHODS

- 2.1.1. **Justification for Test System.** Characteristics which make this test organism suitable for acute toxicity testing are their ease of culturing and handling, their sensitivity to a variety of chemical substances, and the extensive data base for this common freshwater fish species.
- 2.1.2. **Species.** Juvenile fathead minnow, *Pimephales promelas*, will be used to conduct the static acute toxicity test. The fish will be of approximately the same size and age, i.e., the length of the largest fish will not exceed the length of the smallest fish by more than two-fold. Fish will weigh less than 2.0 grams at the initiation of the study. Very young (not actively feeding), sexually mature, spawning and/or recently spent fish will not be used.
- 2.1.3. **Origin and Acclimation.** The fish will be obtained from in-house cultures. Fish will be gradually acclimated to the test conditions, and will be held for at least an additional 14 days in the dilution water prior to testing. They will be held a minimum of 48 hours at the required test temperature, during which time total mortality must not exceed three percent, or the fish will not be used.
- 2.1.4. **Feeding.** The fish will be fed a commercial pelleted food at least once daily prior to the test, but will not be fed during the final 48 hours before the test, nor during the 96-hour toxicity test. Periodic analyses of representative samples of the food will be conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the fish.

- 2.1.5. **Handling.** Fine-mesh dip nets will be used to transfer the fish from the culture vessel to the test chambers at test initiation, taking care to minimize possible stress due to handling. Fish that are damaged or dropped during transfer will be not used.
- 2.1.6. **Loading.** Fish biomass to solution ratio ("loading") will not exceed 0.5 grams per liter.
- 2.1.7. **Reference Tests.** In an effort to monitor the general health of the test organism culture, reference tests will be conducted, under static conditions, with *Pimephales promelas* using copper nitrate as the reference toxicant. The results of these tests will be evaluated based on nominal concentrations. The reference tests will be conducted using the same population of fish within 30 days of the definitive exposure (i.e., either 30 days prior to or 30 days following the definitive exposure).

2.2 PHYSICAL SYSTEM.

- 2.2.1. **Test Containers.** The test chambers used in the static acute bioassay will be 19-L clear glass vessels which will be chemically clean. Each test vessel will contain approximately 15 liters of test medium. This size will be adequate to meet the maximum allowable loading requirements (see above).
- 2.2.2. **Glassware Preparation.** All glassware used in testing will be thoroughly washed with detergent and rinsed with tap water. This will be followed by sequential rinsing with a 10% solution of nitric acid, acetone, distilled deionized water, isopropanol and finally distilled deionized water.
- 2.2.3. **Dilution Water.** Dilution water will consist of unadulterated water from a 100-meter bedrock well mixed in varying proportions with untreated town well water, and will be characterized as soft water with a typical total hardness of 50 - 70 mg/L as CaCO₃, and alkalinity of 20 - 35 mg/L CaCO₃. The pH range will be 6.0 to 8.5, and the specific conductance will be 80 to 150 micromhos/cm. The well water will be fortified based on the formulation for soft water presented in "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians" (USEPA, 1975). Total hardness and alkalinity will be determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 1985).

The dilution water will be prepared in 500-L batches. New batches of dilution water will be prepared when the previous batch is exhausted or when a water quality parameter (total hardness, alkalinity, etc.) has varied from the normal ranges. The dilution water will be aerated with an air pump and air stones to bring the pH and dissolved gases into equilibrium with the atmosphere. Fiberglass containers will be used to hold the dilution water. Total hardness, total alkalinity, pH and specific conductance of the dilution water will be monitored prior to use to assure that these parameters are within the normal acceptable ranges. Total organic carbon (TOC) will be monitored approximately once per month. Periodic analyses of representative samples of dilution water will be conducted to ensure the absence of potential toxicants, including pesticides, PCBs, unionized ammonia, residual chlorine and selected toxic metals, at concentrations which may be

harmful to the fish.

- 2.2.4. Replication and Control of Bias.** Two replicates will be included with each test concentration and control. Test aquaria will be positioned inside a water bath by stratified random design, and labeled by replicate and concentration (or control). Each replicate test vessel will contain ten individuals, i.e., 20 fish will be used per concentration or control(s). Fish will be added impartially to the test vessels by adding no more than two fish to each vessel until all vessels contain two fish. This procedure will be repeated until each test vessel contains ten fish. Test organisms will be added to the exposure solutions within 30 minutes of preparation.

2.3 CHEMICAL SYSTEM.

- 2.3.1. Test Material.** Upon arrival at Springborn Laboratories, Inc., the external packaging of the test material will be inspected for damage. The packaging will be removed and the primary storage container will also be inspected for leakage or damage. The sample identity and percent active ingredient will be recorded and, unless different arrangements will be made with the study sponsor, the material will be stored in the dark at approximately 20°C until used.
- 2.3.2. Toxicant Concentration Selection.** Toxicant concentrations for the acute toxicity test will be selected based on information provided by the Sponsor and obtained from a 96-hour preliminary range-finding study exposing fathead minnow to the test material. The preliminary test will consist of five widely spaced concentrations, usually of 15-L volume, each containing at least ten test fish. The range of concentrations selected for the definitive test is intended to include effect levels (> 50% mortality) and at least one no-effect level, but due to the nature of some test materials, one or both levels may not be observed. A geometric series of five concentrations and one control will be used for each definitive test after consultation with the Sponsor. A dilution factor of at least 60% will be used.
- 2.3.3. Stock Preparation.** The surfactant will be heated in a glass container at a temperature of 50 - 60°C until completely melted. The melted test material will be stirred to ensure homogeneity. A glass pipet will be used to transfer the material for weighing. The test material will be weighed on an analytical balance for which a calibration log will be maintained. The stock solution will be mixed for several minutes. A Chemical Usage Log will also be maintained in which the amount, the date, the intended use and the user's initials are recorded each time test material is used. The primary stock will be prepared in distilled deionized water. The final concentration of the primary stock solution will be 1 - 2% active surfactant. A new primary stock solution will be prepared for each renewal of test solutions. Secondary stocks, if necessary, may be prepared in either distilled deionized water or in dilution water (if the volume displacement of dilution water during test solution preparation will be greater than 1%).
- 2.3.4. Measurement of Exposure Solution and Stock Solution Concentration.** Samples from each test chamber of each concentration and control(s) will be removed at test initiation

(new solutions) and test termination (old solutions). In addition, a set of samples will also be removed at 24 hours (old solutions) and at 72 hours (new solutions). Replicate solutions at each test concentration will be pooled. All primary stock solutions prepared during the course of the test will also be sampled.

2.3.5. Sampling. Water samples (approximately 500-mL) will be taken from a point approximately midway between the surface, bottom and sides of each vessel. All samples will be collected in 500 mL borosilicate glass containers with Teflon®-lined caps which have been serially rinsed with deionized water, isopropanol and deionized water as specified in Section 2.2.2. Sampling containers will be completely filled to minimize head space. Samples will be preserved with 1% Formalin (i.e., 5 mL formalin/500 mL sample) and shipped within 24 hours to Battelle Ocean Sciences, Duxbury, Massachusetts.

2.3.6. Analytical Chemistry. Analyses of analytical samples will be conducted by Battelle Ocean Sciences, Duxbury, Massachusetts, using a Shell Development Company analytical method entitled "Analysis of Alcohol Ethoxylate Surfactants Using Solid Phase Extraction (SPE) and HPLC/ELSD (Evaporative Light Scattering Detection) in Dilute Aqueous Solutions".

2.4 EXPERIMENTAL CONDITIONS.

2.4.1. Measurement of Water Quality Variables. At test initiation, total hardness, alkalinity, acidity, TOC, TSS and specific conductivity will be measured and recorded in each replicate vessel in each test concentration and control. Temperature, pH and dissolved oxygen concentration will be recorded daily at each replicate of each concentration and control. Measurements will be recorded for the aged and for the freshly prepared solutions on renewal days.

2.4.2. Photoperiod. A combination of fluorescent bulbs will be used to illuminate the aquaria, providing a wide spectrum that simulates natural sunlight. Light intensity at the water surface will be 30 to 100 footcandles. An 8-hour dark and 16-hour light photoperiod will be maintained during the test. There will be a transition period between light and dark.

2.4.3. Dissolved Oxygen. Total dissolved oxygen will exceed 90% of saturation (i.e., 7.9 mg/L at 22 °C) at the initiation of the test, and will not be allowed to drop below 60% of saturation (i.e., 5.3 mg/L at 22 °C) for the remainder of the test. Should the dissolved oxygen fall below 60% of saturation, appropriate action will be taken after consultation with the Study Sponsor.

2.4.4. Temperature. Water temperature of the test solutions will be maintained at $22 \pm 2^\circ\text{C}$ by maintaining the aquaria in a waterbath.

2.4.5. pH. The pH of the control solutions will be maintained in a range of 6.0 to 8.5.

2.4.6. Biological Data. At 0, 24, 48, 72, and 96 hours during exposure, observations of stress,

abnormal behavioral activity and mortality will be made. Dead fish will be removed from exposure solutions twice daily. In addition, prior to test initiation and whenever test organisms are observed, characteristics of the test solutions will also be observed and recorded, e.g., precipitated materials, cloudiness, etc.

- 2.4.7. **Renewal Scheme.** Test solutions will be prepared at 0, 24, 48, and 72 hours of exposure. Approximately 80 - 90% of the aged exposure solutions will be replaced at 24, 48, and 72 hours of exposure. Test organisms will be carefully transferred to the freshly prepared solutions.
- 2.4.8. **Initiation and Test Duration.** The study will be initiated when all test organisms have been impartially added to the exposure solutions. The study will be terminated following 96 hours of exposure at which time, mortality of the control organisms will not exceed 10% or the test will be considered unacceptable.

3.0 DATA EVALUATION

Mortality data derived from the acute test will be used to statistically estimate a median lethal concentration (LC50) and its 95% confidence interval after each 24-hour interval of exposure. The LC50 is the estimated mean measured concentration of the test material in dilution water which produces 50% mortality in the test fish population at the stated times of exposure.

The computer program utilized estimates LC50 values using one of three statistical methods: probit analysis, moving average method, or binomial probability. The method selected will be determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.). An LC50 value cannot be calculated if the mortality data derived is insufficient according to any of the three statistical methods. The probit method provides values of the slope, and includes 95% confidence intervals as well as appropriate statistical tests to evaluate goodness-of-fit.

Following 96 hours of exposure, data obtained on organism survival will be evaluated to establish the No-Observed-Effect Concentration (NOEC). This level is defined as the highest test concentration at and below which there were no toxicant-related mortalities or physical and behavioral abnormalities (e.g., lethargy).

The concentration-response data generated during this test will be provided to the Study Sponsor in Lotus® format.

4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and

Springborn Laboratories Protocol #: 072993/TSCA/SHELL/FM-SR

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documentation generated at Springborn Laboratories as a result of the study.

5.0 REPORTING

The raw data generated at Springborn Laboratories and final draft of the report will be reviewed by the Quality Assurance Unit and Study Director. All measurements (e.g. water quality) will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will initially be submitted to the Study Sponsor for review. Upon acceptance by the Sponsor, three (3) copies of the final report will be submitted. All reports include, but are not limited to, the following information.

- * Springborn Laboratories, Inc., report and project numbers.
- * Laboratory and site, the dates of testing and personnel involved in the study, e.g., Quality Assurance Unit, Program Coordinator (if applicable), Study Director, Principal Investigator.
- * All information pertaining to the test material which appears on the sample bottle, e.g., its source, percent active ingredient, physical properties, Sponsor's test material I.D., and sample number (if applicable).
- * Characterization and origin of the dilution water.
- * Scientific name of the test organisms, source, and culturing information.
- * Range-finding study results.
- * The 96-hour LC50 value with the 95% confidence limits and control performance of applicable copper nitrate reference test.
- * Test container volume, dilution water volume, number of replicates used per concentration, and number of organisms tested per treatment.
- * Definition of criteria used to determine the sublethal effects, and general observations on non-quantifiable effects.
- * Description of exposure system and stock preparation.
- * Test temperatures, dissolved oxygen concentration, and pH; photoperiod and light intensity; and specific conductance, total alkalinity and total hardness measured.
- * Description of, or reference to, chemical and statistical procedures applied.
- * Percentage of mortality observed in the controls and in each treatment level at each observation period, in tabular form.

- * The 24-, 48-, 72- and 96-hour LC50's with 95 percent confidence limits, and the No-Observed-Effect Concentration (NOEC), when possible. All calculations will be based on mean measured concentrations.
- * Graph of the concentration response curve at each observation period for which an LC50 is calculated. Mean measured concentrations will be used to establish the concentration-response curve.
- * Deviations from the protocol not addressed in protocol amendments together with a discussion of the impact on the study, signed by the Study Director.
- * Good Laboratory Practice (GLP) compliance statement (for the biological portion of the study) signed by the Study Director.
- * Dates of Quality Assurance reviews, signed by the QA Unit.
- * Location of raw data and final report.

6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the impact of the change on results of the study, if any. If necessary, amendments initially may be in the form of verbal authorization, followed by Springborn's written documentation of amendment. In such a case, the effective date of the amendment will be the date of verbal authorization.

7.0 SPECIAL PROVISIONS

GOOD LABORATORY PRACTICES (GLP): All test procedures, documentation, records, and reports related to the biological portion of this study will comply with the U. S. Environmental Protection Agency's Good Laboratory Practices as promulgated under the Toxic Substance Control Act (FEDERAL REGISTER, Part III, 17 August, 1989)

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final test report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

ARCHIVAL: All raw data and the final report will be archived at Shell Development Company unless different arrangements are made.

8.0 REFERENCES

- APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, Washington, DC. 2168 pp.
- U.S. EPA. 1975. *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians*. Ecological Research Series (EPA-660/3-75-009). 61 pp.
- U.S. Environmental Protection Agency. 1989. *Toxic Substances Control; Good Laboratory Practice Standards; Final Rule*. (40 CFR, Part 792) Federal Register, Part III, 48(230):53922-53944, August 17, 1989.
- U.S. Environmental Protection Agency. 1985. *Toxic Substances Control Act Test Guidelines*. Federal Register 50(188):39252-39516, September 27, 1985. Amended May 20, 1987, July 1, 1991, and July 1, 1992.

Springborn Laboratories, Inc.

Environmental Sciences Division

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PROTOCOL AMENDMENT**AMENDMENT #:** 1**DATE:** 29 September 1993**PROTOCOL TITLE:** "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with Fathead Minnows Following TSCA Test Guidelines § 797.1400."**STUDY SPONSOR:** Shell Development Company**TEST SUBSTANCE:** Dobanol 91-8**SLI STUDY #:** 777.0993.6105.101**SPONSOR PROTOCOL/PROJECT NO.:** WRC TOX NO.: 1227**AMENDMENT(S):****Amendment (Section 2.2.3)**

The protocol states that the dilution water is characterized as soft water with a typical total hardness of 50 - 70 mg/L as CaCO₃, an alkalinity of 20 - 35 mg/L as CaCO₃. The pH range will be 6.0 - 8.5 and the specific conductance will be 80 - 150 microhos/cm.

The amended alkalinity range will be 25-45 mg/L as CaCO₃ and the specific conductance range will be 150-250 microhos/cm.

Reason for change:

The alkalinity and conductivity ranges originally stated in the protocol were not consistent with the desired total hardness range (i.e., 50 - 70 mg/L as CaCO₃) for this study.

Amendment (Section 2.2.3)

The protocol states that total hardness, total alkalinity, pH and specific conductance of the dilution water will be monitored prior to use to assure that these parameters are within normal acceptable ranges.

Amended, the total hardness, total alkalinity, acidity, pH, TOC, TSS and specific conductance of the dilution water will be monitored prior to use.

Springborn Laboratories Protocol #: 072993/TSCA/SHELL/FM-SRPage 1 of 3


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Reason for change:

The additional dilution water parameters measured (i.e., acidity, TOC and TSS) are included in order to meet the requirements for dilution water as stated in TSCA Guideline 797.1400.

Amendment (Section 2.2.3)

The protocol states that periodic analyses of dilution water are conducted to ensure the absence of potential toxicants, including pesticides, PCB's, unionized ammonia, residual chlorine and selected toxic metals which may be harmful to fish.

Amended, the periodic analyses will be conducted on the dilution water source.

Reason for change:

The dilution water used in this study is prepared by the addition of various salts to standard laboratory dilution water in order to achieve specified dilution water quality. Periodic analyses are conducted on the standard laboratory dilution water used as the source water for this study.

Amendment (Section 2.4.1)

The protocol states at test initiation total hardness, alkalinity, acidity, TOC, TSS and specific conductivity will be measured and recorded in each replicate vessel in each test concentration and control.

Amended, at test initiation total hardness, alkalinity, acidity and specific conductivity will be measured in each replicate vessel of each concentration and control.

Reason for change:

The TOC and TSS of the test solutions is not required by the TSCA Guideline. These parameters are measured in the dilution water prior to use to ensure they are within normal acceptable ranges.

Amendment (Section 2.4.7)

The protocol states that approximately 80 - 90% of the aged solutions will be replaced at 24, 48 and 72-hours of exposure and that test organisms will be carefully transferred to the freshly prepared solutions.

Amended, the test organisms will be carefully transferred to the freshly prepared solutions.

Reason for change:

The protocol is contradictory. Transferral of the fish to freshly prepared solutions does not require partial replacement of the aged solutions. Replacement of 80 - 90% of the aged solution was originally included in the protocol as a means of avoiding the transfer of fish. Transfer and excessive handling of fish during renewal is considered detrimental. However, Springborn Laboratories, Inc., will utilize a procedure to transfer the fish which minimizes physical trauma. Therefore, test solution replacement of 100% of the solution will be possible.

Approval Signatures:	<u>Maura K Collins</u>	<u>9-29-93</u>
	Maura K. Collins SLI Study Director	Date
	<u>Diana C. L. Wong</u>	<u>10-14-93</u>
	Diana C. L. Wong Sponsor Study Monitor	Date

Springborn Laboratories, Inc.
Environmental Sciences Division
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PROTOCOL AMENDMENT

AMENDMENT #: 2

DATE: 13 January 1994

PROTOCOL TITLE: "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with Fathead Minnows Following TSCA Test Guidelines § 797.1400."

STUDY SPONSOR: Shell Development Company

TEST SUBSTANCE: Dobanol 91-8

SLI STUDY #: 777.0993.6105.101

SPONSOR PROTOCOL/PROJECT NO.: WRC TOX NO.: 1227

AMENDMENT(S):

Amendment (Section 2.3.2)

The study protocol specifies that a dilution factor of at least 60% will be used. Per Sponsor's request, the dilution factor utilized during this study was changed to 50%.

Reason for change:

This revision was made in an effort to achieve a No-Observed-Effect Concentration (NOEC) and a LC50.

Approval Signatures: Maura K. Collins 1/13/94
Maura K. Collins Date
SLI Study Director

Diana C. L. Wong 1-25-94
Diana C. L. Wong Date
Sponsor Study Monitor

Springborn Laboratories Protocol #: 072993/TSCA/SHELL/FM-SR

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Springborn
LABORATORIES

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5.0 APPENDIX II - CERTIFICATE OF ANALYSIS

Shell Development Company
A Division of Shell Oil Company



Westhollow Research Center
P. O. Box 1380
Houston, TX 77251-1380

September 10, 1993

Pamela M. Lincoln
Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

Dear Ms. Lincoln:

The missing information, pertaining to the test substance Dobanol 91-8, that you requested of me in your letter dated 2 September, 1993 is as follows:

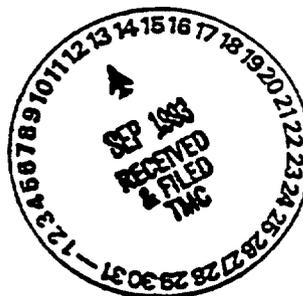
Lot Number:	97002310
% Active Ingredient:	100%
Net Amount Shipped	678 g
Molecular Weight	avg 510 g/mol

The certificate of analysis for this test substance is attached.

If you have any questions then please do not hesitate to contact me at the above address or by phone at (713) 493-7955.

Sincerely,

Carolyn A. Matula



09/18/93 14:39 WESTHOLLOW EC BLDG - SP15082958187
Shell Chemicals

NO. 736 082

Shell Chemicals U.K. Limited

CARRINGTON WORKS LIVERPOOL MANCHESTER M21 4AJ
TELEPHONE 061-778 3000 TELEDC 867381 FAX 061-779 3814

Certificate of Analysis

Order reference 061 776 3456

Deterol 41-8

Your ref

Our Ref HTE/88

Tank 291 SRO 15418

Date 24.08.92

Hydroxyl Value mg KOH/g	110.2
1% Cloud Point E	31.3
Pey % m/m	0.25
Water % m/m	0.05
Free Alcohol % wt	1.4
+ 1 ED	1.0
+ 2 ED	1.2
+ 3 ED	2.4
+ 4 ED	3.5
+ 5 ED	5.1
+ 6 ED	6.6
+ 7 ED	8
+ 8 ED	9
+ 9 ED	9.8
+ 10 ED	9.6
+ 11 ED	9.2
+ 12 ED	8.2
+ 13 ED	6.4
+ 14 ED	5.4
+ 15 ED	4.1
+ 16 ED	3.1
+ 17 ED	2.2
+ 18 ED	1.7
+ 19 ED	0.4
+ 20 ED	0.5
+ 21 ED	0.2
Average ED	3.2



Average ED



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Metulio



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Joe Martin

6.0 APPENDIX III - CULTURE FOOD ANALYSIS

Zeigler Brothers, Inc. Salmon Starter Feed Sample*		
Date Submitted: 11/13/92 Date Reported: 12/1/92		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen I;II;III	Result as Received	
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0.01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Hectachlor	< 0.01 mg/kg	0.01
Aldrin	< 0.01 mg/kg	0.01
Hectachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0.01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg/kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	< 0.01 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCBs	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.05 mg/kg	0.05
Endosulfan I	< 0.01 mg/kg	0.01
Endosulfan II	< 0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
Chlorpyrifos	< 0.01 mg/kg	0.01
* Analyzed by Lancaster Laboratories, Inc.		

Zeigler Brothers Inc. Salmon Starter Feed Sample*		
Date Submitted:11/13/92 Date Reported:12/1/92		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Arsenic	2.1 ppm	0.1
Cadmium	0.4 ppm	0.1
Copper	2.1 mg/100 g	0.2
Lead	0.4 ppm	0.2
Mercury	0.10 ppm	0.02
Zinc	29.4 mg/100 g	0.2
Selenium (fluorometric)	1.6 ppm	0.1
* Analyzed by Lancaster Laboratories, Inc.		

7.0 APPENDIX IV - DILUTION WATER ANALYSIS

Well ¹ Water Sample*		
Date Collected: 7/29/93 Date Reported: 9/17/93		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.3 µg/l	0.3
Toxaphene	< 4 µg/l	4
PCBs	< 1 µg/l	1
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
¹ Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		

Well ¹ Water Sample*		
Date Collected: 8/9/93 Date Reported: 8/26/93		
Analysis	Result As Received	Limit of Quantitation
Mercury	< 0.00020 mg/l	0.00020
Arsenic	< 0.20 mg/l	0.20
Selenium	< 0.20 mg/l	0.2
Boron	< 0.040 mg/l	0.04
Thallium	< 0.30 mg/l	0.3
Aluminum	< 0.20 mg/l	0.2
Antimony	< 0.20 mg/l	0.2
Barium	< 0.10 mg/l	0.1
Beryllium	< 0.010 mg/l	0.01
Cadmium	< 0.010 mg/l	0.01
Calcium	7.71 mg/l	0.2
Chromium	< 0.050 mg/l	0.05
Cobalt	< 0.050 mg/l	0.05
Copper	< 0.020 mg/l	0.02
Iron	< 0.10 mg/l	0.1
Lead	< 0.10 mg/l	0.1
Magnesium	2.31 mg/l	0.1
Manganese	< 0.010 mg/l	0.01
Molybdenum	< 0.10 mg/l	0.1
Nickel	< 0.050 mg/l	0.05
Potassium	1.07 mg/l	0.5
Silver	< 0.020 mg/l	0.02
Sodium	14.0 mg/l	0.4
Titanium	< 0.010 mg/l	0.01
Vanadium	< 0.010 mg/l	0.01
Zinc	< 0.040 mg/l	0.04
Total Organic Carbon ***	< 1. mg/L	1.
¹ Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		
*** Represents "non-purgeable TOC"		

8.0 APPENDIX V - ANALYTICAL METHODOLOGY

FINAL DATA REPORT

Study Title

Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with Fathead Minnow

Data Requirements

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

Submitted To

Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

Performing Laboratory

Battelle Ocean Sciences
397 Washington Street
Duxbury, MA 02332

Author

Gregory S. Durell

Study Initiation Date

September 10, 1993

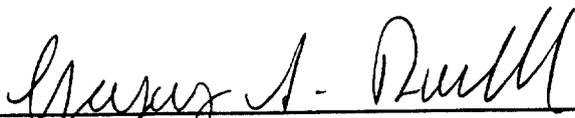
Study Completion Date

November 23, 1993

Battelle Study Number

SD-930116

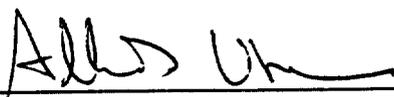
SIGNATURE PAGE



Gregory S. Durrell
Analytical Chemistry Task Leader
Battelle Ocean Sciences

01/13/94

Date



Allen D. Uhler
Chemistry Department Manager
Battelle Ocean Sciences

1/13/94

Date



Carolyn A. Matula
Project Monitor
Shell Development Company

1 February, 1994

Date

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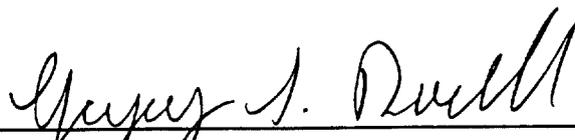
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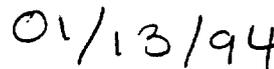
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COMPLIANCE STATEMENT

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



Date

QUALITY ASSURANCE STATEMENT

for

**Measurement of Dobanol[®] 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with Fathead Minnow**

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

Rosanna L. Buhl
Quality Assurance Coordinator
Battelle Ocean Sciences

1-13-94

Date

QUALITY ASSURANCE AUDITS

Conducted for SD-930116

**Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with Fathead Minnow**

Audit Type	Audit Date	Date of Report to Analytical Task Leader	Date of Report to Study Director	Date of Report to Management
Initiation	09/13/93	09/15/93	09/22/93	09/15/93
Lab Inspection	11/09/93 11/10/93	01/03/94	01/13/94	01/13/94
Data Package	12/28-31/93	12/31/93	01/13/94	01/13/94
Report Review	12/28-31/93	12/31/93	01/13/94	12/31/93

STUDY PARTICIPANTS

SD-930116

**Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with Fathead Minnow**

Mr. Gregory S. Durell	Analytical Chemistry Task Leader
Ms. Lynn A. Lariviere	HPLC Analyst; Sample Custodian
Mr. Richard Restucci	Laboratory Technician

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 Springborn Laboratories, Inc. 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 Springborn Laboratories. 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 August 27, 1993, and by the Sponsor's Project Monitor on August 31, 1993.

1.1 Test Substance Identification

The Test Substance analyzed in this study was an alcohol ethoxylate (AE) surfactant (Dobanol 91-8®). 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:	Fathead Minnow
Test Substance:	Dobanol® 91-8
Test Substance CAS#:	68439-46-3
Test Substance Lot#:	LR21662-54
Test Substance Purity:	100%

Test Substance Composition:	A C ₉ -C ₁₁ alcohol ethoxylate with an average of 8 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.
Test Substance Solubility:	Completely soluble in water. May form gel.
Test Substance Stability:	Stable. A stability and holding time of two years from receipt at Battelle was assigned to the Test Substance.
Test Substance Storage Requirements:	Ambient temperature or lower.

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 C₈ 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.

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 August 31, 1993. 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 (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 five-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 five-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 31, 64, 103, 168, and 258 $\mu\text{g/mL}$. Original water sample concentrations were subsequently determined by applying the water extraction volume (WEV) and pre-injection volume (PIV) information. The PIV of the QC samples, controls, and 1,300 and 2,500 parts per billion (ppb) nominal concentration samples was 1.00 mL, and it was 5.00 mL for the samples with nominal concentrations of 5,100, 10,000, and 20,000 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 ($\mu\text{g/mL} = \text{ppm}$)

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.

$$\% \text{ Recovery} = \text{WC}_D \times (1 / \text{WC}_S) \times 100\%$$

$$\% \text{RPD} = [\% \text{REC}_{\text{MS}} - \% \text{REC}_{\text{MSD}}] \times (2 / (\% \text{REC}_{\text{MS}} + \% \text{REC}_{\text{MSD}})) \times 100\%$$

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 considered detected.

The water sample equivalent LOD was calculated by comparing the peak height equivalent to a signal:noise ratio of 3:1 in the sample to the peak height of the analyte in the low-level calibration standard, 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 concentrations.

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

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

H_{3x} = Peak height equivalent to $3 \times$ 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 two analytical batches, the first batch containing the $t=0$ hr and $t=24$ hr samples and the second batch containing the $t=72$ hr and $t=96$ hr samples. Table 1 also presents the data for the Primary Stock Solution analyses.

The measured analyte concentrations in the test samples that had been fortified with the Test Substance ranged from 922 ppb (for sample NB62, a sample with a nominal concentration of 1,300 ppb) to 17,720 ppb (for sample NB45, a sample with a nominal concentration of 20,000 ppb). The measured concentrations were between 71 percent (sample NB62) and 91 percent (sample NB42) of the nominal concentration.

The concentrations measured for the Primary Stock Solutions were slightly lower than the expected concentrations, with measured concentrations ranging from 18,980 to 19,380 ppm for the three samples, all of which had nominal/expected concentrations of 20,000 ppm. The measured Primary Stock Solution concentrations were between 3 and 5 percent lower than the expected concentration.

Table 1. Dobanol® 91-8 Concentrations in Samples Received from the Toxicological Testing Laboratory

Battelle Sample ID	Test Sample Time/Type	Nominal Conc. (ppb)	Measured Conc. (ppb)
Batch #1			
NB40	t=0	0	ND
NB41	t=0	1,300	1,081
NB42	t=0	2,500	2,274
NB43	t=0	5,100	4,271
NB44	t=0	10,000	8,514
NB45	t=0	20,000	17,720
NB47	t=24, old	0	ND
NB48	t=24, old	1,300	936
NB49	t=24, old	2,500	2,095
NB50	t=24, old	5,100	3,820
NB51	t=24, old	10,000	8,468
NB52	t=24, old	20,000	17,280
Batch #2			
NB54	t=72, new	0	ND
NB55	t=72, new	1,300	970
NB56	t=72, new	2,500	2,212
NB57	t=72, new	5,100	3,790
NB58	t=72, new	10,000	8,650
NB59	t=72, new	20,000	17,430
NB61	t=96	0	ND
NB62	t=96	1,300	922
NB63	t=96	2,500	2,059
NB64	t=96	5,100	3,703
NB65	t=96	10,000	8,320
NB66	t=96	20,000	17,350
Primary Stock Solution		(ppm)	(ppm)
NC03	t=0, stock	20,000	19,380
NC04	t=24, stock	20,000	18,980
	t=48, stock	NA	NA
NC05	t=72, stock	20,000	19,090

ND: Not detected; <LOD.

NA: Not applicable. No t=48 hr Primary Stock Solution was submitted for analysis.

3.2 Analytical Results — Quality Control Samples

All quality control objectives were met for this work. The five-point multi-level instrument calibration used had a correlation coefficient of 0.999836 for the quadratic equation generated, and the continuing calibration check analyses ranged from 2.0 to 6.5 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)	192 ppb
Limit of Quantitation (LOQ)	618 ppb

Sample concentrations above the LOD were reported for this study. Sample concentrations below the LOD were reported as “ND” (not detected). 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 either of the two procedural blank samples. The analyte recovery in the blank spike (BS) samples were 92% and 98% for analytical batches #1 and #2, respectively. The analyte recovery in the four matrix spike (MS/MSD) samples ranged from 91% to 95%, and these data suggest that there were no significant matrix effects on the analytical procedure. Acceptable precision was observed for both analytical batches. The relative percent differences in the measured analyte recoveries for the MS/MSD duplicate analyses were 2% and 1% for analytical batches #1 and #2, respectively.

Table 2. Laboratory Quality Control Sample Analysis Results

Battelle Sample ID	QC Sample Type	Recovery (%)
Batch #1		
NB38-PB	Procedural Blank	ND
NB39-BS	Blank Spike	92.0
NB72-MS	Matrix Spike	92.8
NB73-MSD	Matrix Spike Duplicate	91.1
	MS/MSD %RPD:	1.8
Batch #2		
NB74-PB	Procedural Blank	ND
NB75-BS	Blank Spike	98.1
NA98-MS	Matrix Spike	94.4
NA99-MSD	Matrix Spike Duplicate	95.3
	MS/MSD %RPD:	0.9

ND: Not detected; <LOD.

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
- Verified 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-930116

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Purity of Nitrogen Gas for ELSD Detector

The purity of the liquid nitrogen used for the ELSD detector is guaranteed by the supplier (Liquid Carbonic) to be a minimum of 99.998%, not 99.999% as specified in the Analytical Procedure Document. The typical purity, as specified by the supplier, is 99.999%.

Approved: Gregory S. Durell

Date: 12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930116

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Return and Archival of Test Substance

Only a small amount of Test Substance was received by Battelle for this work (in a vial of approximately 4 mL volume) and the unused material at the end of the study will be kept and archived at Battelle, not returned to the toxicological testing laboratory.

Approved: Gregory S. Durell

Date: 12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930116

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 20, 1993

Subject: Calculations of Original Water Sample Concentrations

The original water sample concentrations were calculated in spreadsheet tables by applying the water extraction volumes (WEV) and pre-injection volumes (PIV) to the sample extract concentrations determined using the chromatography software in the Hewlett-Packard LAS data system. These calculations are described in the Study Report. The Analytical Procedure Document specified that the original water sample concentrations be determined directly in the H-P LAS datasystem by entering the WEV and PIV data there.

Analyte concentrations of the original water samples were determined in parts per billion (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 ($\mu\text{g}/\text{mL}$ = 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)

Approved: Gregory S. Durell

Date: 12/20/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 2

Project Title: Gap-Filling Project

Study Number: SD-930116

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: January 7, 1994

Subject: Miscellaneous Deviations to Analytical Method

Concentrator tubes were not rinsed and the rinsate transferred to a 4 mL vial for storage on the day sample processing was completed, as indicated in the Analytical Procedure Document and associated sample preparation check list. Instead the procedure was to store the securely sealed concentrator tube refrigerated until HPLC analysis was completed and then rinse and transfer any remaining sample in the concentrator tube and HPLC vial to a common vial of suitable size (sometimes 4 mL and sometimes larger). This deviation has no impact on the integrity of the samples because proper storage was maintained.

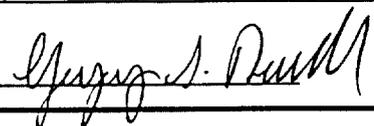
The Test Substance was stored refrigerated and not at room temperature as specified in the Analytical Procedure Document. This deviation has no impact on the integrity of the material or results, because suitable storage conditions were maintained.

There were a few instances of incomplete temperature monitoring of Refrigerator #2 (where water samples are stored pre-extraction and extracts stored until archival) during the performance of this work. Specifically, during the third week of November the monitoring/logging was missed three times, during the fourth week of November it was missed one time, and during the first week of December it was missed one time. However, all sample extraction had been completed by these dates, and only sample extracts (in methanol) were stored in the refrigerator. Sample extracts can be stored at, or below, room temperature. This deviation would not impact the integrity of the samples or the quality of the data.

One of the Control samples (NB61) was not tested for formaldehyde content. The Analytical Procedure Document specified that all control samples be checked for formaldehyde. However, at least one in ten samples were tested, as specified in the Document.

Page 2 of 2

Not all of the 7 concentration levels listed in the Study-Specific were analyzed by HPLC at the beginning of the analytical sequence. These 7 standards had been analyzed by HPLC prior to the analysis of samples for this study, and the approximate response was therefore known. A total of 5 standards were analyzed and a 5-point calibration generated, in accordance with the Analytical Procedure Document.

Approved:  Date: 04/07/94

88940000057

Page 1 of 80

**DOBANOL® 91-8 - ACUTE TOXICITY TO
DAPHNIDS (*Daphnia magna*) UNDER STATIC
RENEWAL CONDITIONS**

Contains No CBI

TSCA TEST GUIDELINES § 797.1300

Submitted to:

**Shell Development Company
P.O. Box 1380
Houston, Texas 77251**

SLI Report #93-11-5011

Sponsor Protocol/Project No.: WRC TOX No. 1227

SLI Study #777.0993.6104.110

Study Director: Maura K. Collins

**Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571**

14 February 1994

FINAL REPORT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for "Dobanol[®] 91-8 - Acute Toxicity to Daphnids (*Daphnia magna*) Under Static Renewal Conditions" were produced and compiled in accordance with all pertinent TSCA Good Laboratory Practice regulations with the following exception: routine water contaminant screening analyses for pesticides, PCBs and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, Pennsylvania. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Stability, characterization, and verification of the test material identity and maintenance of records on the test material are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test material will be sent to the Study Sponsor. Archival of a sample of the test material is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES

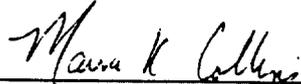

Maura K. Collins 2.14.94
Study Director Date

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SUMMARY**Dobanol[®] 91-8 - Acute Toxicity to Daphnids
(*Daphnia magna*) Under Static Renewal Conditions**

SPONSOR: Shell Development Corporation
P.O. Box 1380
Houston, Texas 77251

PROTOCOL TITLE: "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with *Daphnia magna*, Following TSCA Test Guidelines § 797.1300," Springborn Protocol #072993/TSCA/SHELL/DM-SR and Protocol Amendment #1 dated 29 September 1993.

REPORT NUMBER: 93-11-5011

STUDY NUMBER: 777.0993.6104.110

TEST MATERIAL: Dobanol[®] 91-8, CAS #68439-46-3, Lot #97002310, WRC TOX. Sample #1227, PSIS #7075

DATE RECEIVED: 2 September 1993

DESCRIPTION: A clear liquid reported by the Study Sponsor to contain 100% active ingredient.

**EXPERIMENTAL
START DATE:** 19 October 1993

**EXPERIMENTAL
TERMINATION DATE:** 21 October 1993

SPECIES: *Daphnia magna*

Age: ≤ 24 hrs

Source: Springborn Laboratories culture facility

LABORATORY**DILUTION WATER:**

Fortified well water

pH: 8.0

Specific conductivity: 500 μ mhos/cmTotal hardness as CaCO₃: 160 mg/LTotal alkalinity as CaCO₃: 110 mg/L**TEST CONDITIONS:**

48-hour duration, 19 to 20 °C, illumination at 70 footcandles (750 lux), photoperiod of 16 hours light and 8 hours dark

NOMINAL TEST**CONCENTRATIONS:**

1.3, 2.5, 5.0, 10 and 20 mg/L

MEAN MEASURED**CONCENTRATIONS:**

1.1, 2.1, 4.4, 9.0 and 18 mg/L

RESULTS:

Based on mean measured concentrations, the 48-hour EC50 value was estimated by nonlinear interpolation to be 12 mg/L (95% confidence interval calculated by binomial probability to 18 mg/L). The 48-hour No-Observed-Effect Concentration (NOEC) was determined to be 4.4 mg/L.

1.0 INTRODUCTION

The purpose of this study was to estimate the acute toxicity (EC50) of Dobanol[®] 91-8 to daphnids (*Daphnia magna*) under static renewal conditions. The EC50 is defined as the concentration of test material in dilution water which causes immobilization of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from release of the test material into aquatic environments. The study was initiated on 10 September 1993, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the 48-hour definitive test was conducted from 19 to 21 October 1993 at the Environmental Sciences Division of Springborn Laboratories, Inc. (SLI), in Wareham, Massachusetts. All original raw data and the final report produced during this study are stored at Shell Development Company.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this acute toxicity study followed those described in the Springborn protocol entitled "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with *Daphnia magna*, Following TSCA Test Guidelines § 797.1300," Springborn Protocol #072993/TSCA/SHELL/DM-SR and Protocol Amendment #1 dated 29 September 1993 (Appendix I). The method described in this protocol meet or exceed the standard procedures described in the U.S. EPA Toxic Substances Control Act (TSCA) Test Guidelines § 797.1300 (U.S. EPA, 1992) and meet the primary technical objectives of the Shell Research Limited/Sittingbourne Research Center guidelines (SBT SOP No. 167, Edition No. 9).

2.2 Test Material

A sample of the Dobanol[®] 91-8 (CAS #68439-46-3, Lot #97002310, WRC TOX. Sample #1227, PSIS #7075, expiration date September 1994), a clear liquid reported by the Study Sponsor to contain 100% active ingredient, was received from Shell Westhollow Research Center, Houston, Texas, on 2 September 1993. Upon receipt at Springborn, the test material was stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Test concentrations are

expressed as milligrams of Dobanol[®] 91-8 per liter of solution and are reported as mg/L.

2.3 Test Organisms

The *Daphnia magna* used in this toxicity test were obtained from laboratory cultures maintained at Springborn. The culture water was prepared by fortifying well water based on the formula for hard water (ASTM, 1980) and filtering it through an Amberlite XAD-7 resin column to remove any potential organic contaminants. This water had total hardness and alkalinity ranges as calcium carbonate (CaCO₃) of 160 to 180 mg/L and 110 to 130 mg/L, respectively, a pH range of 7.9 to 8.3, a temperature of 20 ± 2 °C, a dissolved oxygen concentration of greater than 60% of saturation and a specific conductivity range of 400 to 600 micromhos per centimeter (μmhos/cm) (SLI Invertebrate Water Quality Log Book, Vol. 14).

The daphnid culture area received a daily regulated photoperiod of 16 hours of light and 8 hours of darkness. Light at an intensity of 30 to 100 footcandles (320 to 1100 lux) at the surface of the culture solutions was provided by Durotest Vitalite[®] fluorescent bulbs. The ambient air temperature in the culture area was controlled in order to maintain the culture solution temperature at 20 ± 2 °C. Daphnids were fed a combination of a trout food (Ziegler Brothers[®] Salmon Starter #1) suspension and a unicellular green algae (*Ankistrodesmus falcatus*) once daily. The food solution contained 5.0 mg/mL trout food and approximately 4 x 10⁷ cells/mL of algae. Routine analyses were conducted on representative samples of the food sources for the presence of metals, pesticides and PCBs (Appendix III). Food sources were considered to be of acceptable quality since the total concentration of pesticides measured was less than 0.3 mg/kg (ASTM, 1985) (SLI Invertebrate culture log for *Daphnia magna*, Vol. 9).

2.4 Reference Test

A copper nitrate reference test was conducted with the test organism population from 6 to 8 October 1993. The resulting 48-hour LC50 was calculated by moving average angle analysis to be 46 μg/L (95% confidence interval of 39 to 54 μg/L) (SLI *Daphnia magna* Copper Nitrate Reference Log, Vol. II). In addition to the above data, culture records document the ability of this population of *Daphnia magna* to successfully and actively feed, grow and reproduce over a

period of several generations. Based on the results of the reference test and the successful culture of *Daphnia magna*, it was established that this population was suitable for testing.

2.5 Test Dilution Water

The dilution water used during this study was from the same source as the culture water described above and had a total hardness and total alkalinity (as CaCO₃) of 160 mg/L and 110 mg/L, respectively, a pH of 8.0 and a specific conductivity of 500 μ mhos/cm (SLI IWQ Log Book, Vol. 14). Representative samples of the dilution water source were analyzed for the presence of metals, pesticides and PCBs (Appendix IV). None of these compounds were detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with ASTM Standard Practice (ASTM, 1980). In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentration. Based on these analyses, the TOC concentration of the dilution water source ranged from 0.81 to 1.4 mg/L for the months of April to September 1993 (SLI TOC Master Log). In addition, TOC concentration and total suspended solids (TSS) analyses were conducted at Springborn on the batch of dilution water used during this study. The TOC concentration of the batch of hard reconstituted water, Batch #1688, ranged from 0.38 to 0.42 mg/L for the month of October. The TSS for this batch was 6.4 mg/L for the month of October. Several species of daphnids are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over several generations. The success of the cultured daphnids, in combination with the previously mentioned analyses, confirms the acceptability of this dilution water for bioassays.

2.6 Test Conditions

Test vessels were positioned in stratified random order in a waterbath designed to maintain test solution temperatures at 20 ± 2 °C. Test solutions were not aerated. The test area was illuminated with Dura-Test Vita Lite and General Electric Coolwhite fluorescent bulbs at an intensity of 750 lux. The photoperiod during testing was the same as that provided in the daphnid culture area. The culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Sudden transitions from light to dark and vice versa were avoided.

2.7 Test Concentrations

Selection of nominal Dobanol[®] 91-8 concentrations for the 48-hour definitive toxicity test with *Daphnia magna* was based on toxicity information developed at Springborn through preliminary testing. The nominal concentrations selected were 1.3, 2.5, 5.0, 10 and 20 mg/L.

2.8 Test Procedures

The static renewal toxicity test was conducted in 250-mL glass beakers which contained 200 mL of test solution. The exposure solution in each test vessel had a depth of 6.2 cm and a surface area of 33 cm². Four replicates were maintained for each test concentration and control. A 20 mg/mL stock solution was prepared by heating the glass bottle of test material in a 1000-mL Pyrex beaker to a temperature ranging from 50 to 60 °C. Forty grams (40.000 g) of test material was then removed using a glass pipet, and was diluted in 2 L of dilution water in a 2-L volumetric flask. This stock solution was stirred for approximately 10 to 15 minutes to ensure that the test material completely dissolved.

Test solutions with nominal concentrations of 1.3, 2.5, 5.0, 10 and 20 mg/L were prepared by diluting the appropriate amount of the stock solution with 2000 mL of dilution water. Two thousand milliliters (2000 mL) of solution were prepared for each concentration in order to accommodate analytical sampling and to provide sufficient volume for water quality analyses. The exposure solutions were stirred for 30 seconds with a magnetic laboratory stir plate and a Teflon[®]-coated stir bar. The test solutions were observed to be clear and colorless with no sign of undissolved test material. Replicate sterile 250-mL glass beakers, four per treatment level and control, were conditioned prior to use. Two hundred milliliters (200 mL) of the appropriate test solution was then placed in each of four replicate flasks. Following this procedure, all exposure solutions were observed to be clear and colorless and contained no visible signs of undissolved test material. One set of control vessels was also established which contained the same dilution water and was maintained under the same conditions as the test vessels but contained no Dobanol[®] 91-8. A duplicate set of exposure vessels was established to prepare renewal solutions. Test solutions were renewed at 24 hours of exposure following the procedure mentioned above.

The test was initiated when daphnids were added to each test vessel (5 daphnids per replicate, 20 daphnids per treatment level and control). Daphnids were added to the test vessels one at a time until all test vessels contained one daphnid. This procedure was repeated until all replicate test vessels contained 5 daphnids. At the renewal period, the daphnids were carefully transferred one at a time from the old test solutions into their respective new test solutions using a wide bore pipet. Daphnids that were observed to be immobilized at the time of renewal were not transferred into the new test solutions. Daphnids were not fed during the study.

2.9 Test Monitoring

The number of immobilized daphnids in each replicate test vessel was recorded at 24 and 48 hours of exposure. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 hours. The pH, dissolved oxygen concentration and temperature were measured in each test concentration and the control at 0, 24 and 48 hours of exposure. Water quality measurements performed at 24 hours were made in both the old and new test solutions when applicable (i.e., less than 100% immobilization). Total hardness, total alkalinity, acidity and specific conductivity were measured at 0-hour in each replicate of the control and test solutions. In addition, the temperature of the surrounding water in the waterbath was continuously monitored throughout the exposure period using a minimum/maximum thermometer.

Total hardness concentration presented in this report was measured by the EDTA titrimetric method and total alkalinity concentration was determined by potentiometric titration to an endpoint of pH 4.5 (APHA *et al.*, 1985). Specific conductivity was measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe. A Jenco Model 601A pH meter and combination electrode was used to measure pH. Dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe. Daily temperature was measured with a Brooklyn alcohol thermometer. Continuous temperature monitoring was performed using a Fisher Scientific Min/Max thermometer.

2.10 Analytical Measurements

During the definitive exposure period, water samples were removed from the four replicate solutions of the treatment level and the control at 0, 24 and 48 hours for the analysis of Dobanol[®] 91-8 concentration. Samples analyzed at 0 hour were removed from the excess test solution remaining in the volumetric flasks prior to division into the test vessels. At the 24-hour interval, both old and new test solutions were analyzed for Dobanol[®] 91-8 concentration. Samples removed from the old solutions were composited (replicates A, B, C and D) for each treatment level and control. New solution samples were removed from excess test solution remaining in the volumetric flasks prior to division into the test vessels. Samples analyzed at 48 hours were removed from the composited test solution (replicates A, B, C and D) for each treatment level and the control after biological observations and water quality measurements were taken. Each exposure solution sample was collected from the approximate midpoint of the test vessel with a volumetric pipet. Samples were collected in 800-mL borosilicate glass containers with Teflon[®]-lined caps. Containers were completely filled to minimize head space and were preserved with 10% formalin. Within 24 hours of preparation, the samples were shipped to Battelle Ocean Sciences, Duxbury, Massachusetts, for analysis using the Shell Development Company analytical method entitled "Analysis of Alcohol Ethoxylate Surfactants Using Solid Phase Extraction (SPE) Cartridges and HPLC/ELSD (Evaporative Light Scattering Detection) in Dilute Aqueous Solutions". This method is described in Battelle Ocean Sciences Study #SD-930115 (Appendix V). All of the glassware used in testing and sample collection was thoroughly washed with detergent and rinsed with tapwater, and then washed with sequential rinses of a 10% solution of nitric acid, acetone, distilled-deionized water, isopropanol and distilled-deionized water.

2.11 Determination of EC50 and NOEC

The mean measured concentrations tested and the corresponding biological-response data (immobilization) derived from the toxicity test were used to estimate 24- and 48-hour median effect concentrations (EC50) and 95% confidence intervals. The EC50 is defined as the concentration of the test material in dilution water which caused immobilization of 50% of the test organism population at the stated time interval. If at least one test concentration caused immobilization of greater than or equal to 50% of the test population, then a computer program,

modified from the program of C. Stephan (Peltier *et al*, 1985), was used to calculate the EC50 values and 95% confidence intervals.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce immobilization of between 0 and 100% in the test population. The selection of reported EC50 values and 95% confidence intervals was based upon an examination of the database and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship, the number of concentrations causing partial responses, and the span of responses bracketing the EC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The No-Observed-Effect Concentration (NOEC) during the 48-hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there were no toxicant-related immobilization or physical and behavioral abnormalities (e.g., lethargy, flared carapace), with respect to the control.

3.0 RESULTS

3.1 Preliminary Test

Prior to initiating the definitive study, a preliminary range-finding test was conducted at Springborn from 23 to 25 September 1993 at nominal concentrations of 0.64, 1.6, 8.0, 20 and 50 mg/L. At 24 hours of exposure, 100% immobilization was observed in the two highest test concentrations (20 and 50 mg/L). At test termination, no immobilization was observed in the remaining test concentrations (0.64 to 8.0 mg/L). Based on the results of this range-finding test, nominal concentrations of 1.3, 2.5, 5.0, 10 and 20 mg/L were selected for the definitive study with Dobanol[®] 91-8.

3.2 Definitive Test

3.2.1 Evaluation of Test Conditions - The measurements of the water quality parameters (i.e., pH, dissolved oxygen concentration, temperature) recorded during the definitive

study are presented in Table 1a. Total hardness, total alkalinity and specific conductivity recorded during the definitive study are presented in Table 1b. Analysis of the control and test solutions at test initiation established a total hardness (as CaCO₃) ranging from 160 to 180 mg/L, a total alkalinity (as CaCO₃) of 110 mg/L, a specific conductivity of 500 μ mhos/cm and an acidity (as CaCO₃) ranging from 4 to 6 mg/L. Throughout the exposure period, the pH and dissolved oxygen saturation for the control and test solutions ranged from 8.1 to 8.2 and 90 to 97%, respectively. These results demonstrate that the water quality parameters measured were unaffected by the concentrations of Dobanol[®] 91-8 tested and remained within acceptable ranges for the survival of daphnids. Daily temperature monitoring of the test solutions and continuous temperature monitoring of the water in the surrounding waterbath established that the temperature in the test solutions ranged from 19 to 20 °C throughout the exposure period.

3.2.2 Analytical Results - The results of the analyses of the primary stock solutions and the exposure solutions for Dobanol[®] 91-8 concentration during the exposure period are presented in Table 2. Results of the analyses of the primary stock solutions (20,000 mg/L) used to formulate the test solutions established an average concentration of Dobanol[®] 91-8 of 93% of the nominal concentration. Measured concentrations for all treatment levels were consistent between sampling intervals. The mean measured concentrations averaged 87% of nominal and defined the test concentrations as 1.1, 2.1, 4.4, 9.0 and 18 mg/L. Analytical results are presented in Battelle Ocean Sciences Study #SD-930115 (Appendix V).

3.2.3 Biological Results - The mean measured concentrations tested, the corresponding cumulative percent immobilization and the observations made during the definitive exposure are presented in Table 3. All exposure solutions were observed to be clear and colorless and contained no visible signs of undissolved test material. Following 24 hours of exposure, 80% immobilization was observed among daphnids exposed to the highest concentration tested (18 mg/L). In addition, all of the mobile daphnids exposed to this treatment level were observed to be lethargic and at the surface of the test solution. Several daphnids exposed to the 9.0 mg/L treatment level were observed to be lethargic. At test termination (48 hours of exposure), immobilization of 100% was observed among daphnids exposed to the

18 mg/L test concentration. Immobilization of 5% was observed among daphnids exposed to the 9.0 mg/L test concentration. In addition, several of the daphnids were observed to be lethargic, and one was observed on the surface of the test solution. No immobilization or sublethal effects were observed among daphnids exposed to the remaining test concentrations (1.1, 2.1 and 4.4 mg/L). The 24-hour concentration-response (immobilization) curve for this study is presented in Figure 1. The 48-hour concentration-response curve is presented in Figure 2.

Table 4 summarizes the 24- and 48-hour EC50 values and corresponding 95% confidence intervals, and presents the No-Observed-Effect Concentration (NOEC) through 48 hours. Based on mean measured concentrations of Dobanol[®] 91-8, the 48-hour EC50 value was estimated by nonlinear interpolation to be 12 mg/L with a corresponding 95% confidence interval calculated by binomial probability of 9.0 to 18 mg/L. The NOEC for this study was 4.4 mg/L.

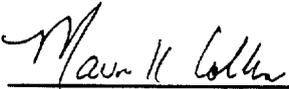
PROTOCOL DEVIATIONS

The study protocol states that the water samples will be collected in 500-mL glass containers which are completely filled to minimize headspace, and that the water samples will be preserved with 5 mL of a 1% formalin solution (i.e., 5 mL formalin/500 mL sample). During this study, sample containers were of approximately 800 mL in volume which were completely filled to minimize headspace, and the water samples were inadvertently preserved with 5 mL formalin at a concentration of 10%.

Although test solution samples were not prepared in accordance with the study protocol, measured concentrations established during the 48-hour exposure period averaged 87% of the nominal fortified concentrations. Based on this data, it is our opinion that the sample preparation procedures utilized during this study did not negatively impact the samples.

It is our opinion that this deviation did not affect the results of this study.

SPRINGBORN LABORATORIES, INC.

 2.14.94

Maura K. Collins
Study Director

Date

QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "Dobanol® 91-8 - Acute Toxicity To Daphnids (*Daphnia magna*) Under Static Renewal Conditions" were inspected by the Quality Assurance Unit (QAU) at Springborn Laboratories Inc., Environmental Sciences Division to determine adherence with the study protocol and laboratory standard operating procedures. In addition, inspection of certain phases of the in-life portion of the study was performed. Dates of study inspections, dates reported to the Study Director and to Management are listed below.

Based on these inspections, it was determined that this report accurately reflects the raw data collected during this study.

<u>Inspection Date</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
10/20/93	10/20/93	10/22/93
1/6/94	1/6/94	1/14/94
1/21/94	1/21/94	1/28/94
1/25/94	1/25/94	1/28/94
1/27/94	1/27/94	1/28/94
2/10/94	2/10/94	2/11/94
2/14/94	2/14/94	2/14/94

SPRINGBORN LABORATORIES, INC.

Doreen S. Newhouse 14 Feb 94
 Doreen S. Newhouse Date
 Supervisor
 Quality Assurance Unit

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TABLES

Table 1a. The pH, dissolved oxygen concentration and temperature measurements recorded during the 48-hour static exposure of daphnids (*Daphnia magna*) to Dobanol[®] 91-8.

Nominal Concentration (mg/L)	0-Hour				24-Hour ^a				48-Hour			
	A	B	C	D	A	B	C	D	A	B	C	D
pH												
Control	8.1	8.1	8.1	8.1	8.1/8.1	8.1/8.1	8.1/8.1	8.1/8.1	8.1	8.1	8.1	8.1
1.3	8.1	8.1	8.1	8.1	8.1/8.2	8.1/8.2	8.1/8.2	8.1/8.2	8.2	8.2	8.2	8.2
2.5	8.1	8.1	8.1	8.1	8.1/8.2	8.1/8.2	8.1/8.2	8.1/8.1	8.2	8.2	8.2	8.2
5.0	8.1	8.1	8.1	8.1	8.1/8.2	8.1/8.1	8.1/8.2	8.1/8.2	8.2	8.2	8.2	8.2
10	8.1	8.1	8.1	8.1	8.1/8.2	8.1/8.2	8.1/8.2	8.1/8.2	8.2	8.2	8.2	8.2
20	8.1	8.1	8.1	8.1	8.2/8.2	8.2/8.2	8.2/8.2	8.2/8.2	8.2	8.2	8.2	8.2
Dissolved Oxygen, mg/L (% saturation)												
Control	8.7 (95)	8.7 (95)	8.7 (95)	8.7 (95)	8.5/8.6 (93/94)	8.5/8.5 (93/93)	8.5/8.6 (93/94)	8.5/8.6 (93/94)	8.2 (90)	8.2 (90)	8.3 (91)	8.4 (92)
1.3	8.7 (95)	8.7 (95)	8.7 (95)	8.7 (95)	8.6/8.7 (94/95)	8.5/8.6 (93/94)	8.5/8.6 (93/94)	8.5/8.6 (93/94)	8.3 (91)	8.3 (91)	8.3 (91)	8.3 (91)
2.5	8.8 (97)	8.7 (95)	8.8 (97)	8.7 (95)	8.6/8.7 (94/95)	8.6/8.7 (94/95)	8.5/8.7 (93/95)	8.5/8.6 (93/94)	8.3 (91)	8.3 (91)	8.3 (91)	8.3 (91)
5.0	8.7 (95)	8.7 (95)	8.7 (95)	8.7 (95)	8.6/8.6 (94/94)	8.5/8.6 (93/94)	8.5/8.7 (93/95)	8.5/8.7 (93/95)	8.3 (91)	8.3 (91)	8.3 (91)	8.3 (91)
10	8.8 (97)	8.7 (95)	8.7 (95)	8.7 (95)	8.6/8.6 (94/94)	8.5/8.6 (93/94)	8.5/8.7 (93/95)	8.6/8.6 (94/94)	8.3 (91)	8.4 (92)	8.3 (91)	8.3 (91)
20	8.7 (95)	8.7 (95)	8.7 (95)	8.7 (95)	8.7/8.6 (95/94)	8.7/8.7 (95/95)	8.7/8.7 (95/95)	8.7/8.7 (95/95)	8.3 (91)	8.4 (92)	8.4 (92)	8.3 (91)
Temperature (°C)^b												
20				20/20				20				

^a Exposure solutions were renewed at the 24-hour interval. Measurements are presented as Old/New.
^b Values presented represent the daily temperatures measured (Brooklyn alcohol thermometer) in all test concentrations and the control at the stated time interval. Continuous temperature monitoring (Fisher Scientific Min-Max thermometer) of the surrounding water in the waterbath established a temperature range of 19 to 20 °C throughout the exposure period.

Table 2. Concentrations of Dobanol[®] 91-8 measured in the replicate test solutions during the 48-hour static renewal exposure of daphnids (*Daphnia magna*).

Nominal Concentration (mg/L)	0-Hour Measured Concentration (mg/L)	24-Hour Measured Concentration (mg/L) ^a	48-Hour Measured Concentration (mg/L)	Mean Measured Concentration (mg/L) ^{bc}
Control	ND ^d	ND/ND	ND	NA ^e
1.3	1.2	1.1/1.1	0.82	1.1 (0.16)
2.5	2.2	2.2/2.2	1.8	2.1 (0.20)
5.0	4.8	5.3/3.7	3.7	4.4 (0.79)
10	9.4	9.3/8.8	8.6	9.0 (0.40)
20	19	18/19	18	18 (0.58)

^a Exposure solutions were renewed at the 24-hour interval. Measurements are presented as Old/New.

^b Mean measured concentrations were calculated using the actual unrounded analytical results and not the rounded (two significant figures) values presented in this table.

^c Standard deviation is presented in parentheses.

^d ND = Not detectable; below the limit of detection.

^e NA = Not Applicable

Table 3. Mean measured concentrations tested, corresponding cumulative percent and number of immobilized organisms and observations made during the 48-hour static acute exposure of daphnids (*Daphnia magna*) to Dobanol[®] 91-8.

Mean Measured Concentration (mg/L)	Cumulative Percent of Immobilized Organisms ^a									
	24-Hour					48-Hour				
	A	B	C	D	Mean	A	B	C	D	Mean
Control	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
1.1	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
2.1	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
4.4	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
9.0	0 ^b (0)	0 ^c (0)	0 ^b (0)	0 ^b (0)	0	0 ^b (0)	20 ^d (1)	0 ^e (0)	0 ^f (0)	5
18	80 ^g (4)	100 (5)	80 ^g (4)	60 ^g (3)	80	100 (5)	100 (5)	100 (5)	100 (5)	100

- ^a The actual number of immobilized daphnids is presented in parentheses.
^b Two of the mobile daphnids were lethargic.
^c Several of the mobile daphnids were lethargic.
^d All of the mobile daphnids were lethargic.
^e One of the mobile daphnids was lethargic.
^f One of the mobile daphnids was lethargic and on the surface of the solution.
^g All of the mobile daphnids were lethargic and on the surface of the solution.

Table 4. The EC50 values, corresponding 95% confidence intervals and No Observed Effect Concentration (NOEC) established during the 48-hour static renewal exposure of daphnids (*Daphnia magna*) to Dobanol[®] 91-8.

	EC50 (mg/L)	95% Confidence Interval	
		Lower (mg/L)	Upper (mg/L)
24-Hour ^a	15	9.0	18
48-Hour ^a	12	9.0	18

NOEC through 48 hours = 4.4 mg/L

^a EC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.

FIGURES

Figure 1. The 24-hour concentration-response (immobilization) curve for the static renewal exposure of daphnids (*Daphnia magna*) to Dobanol[®] 91-8.

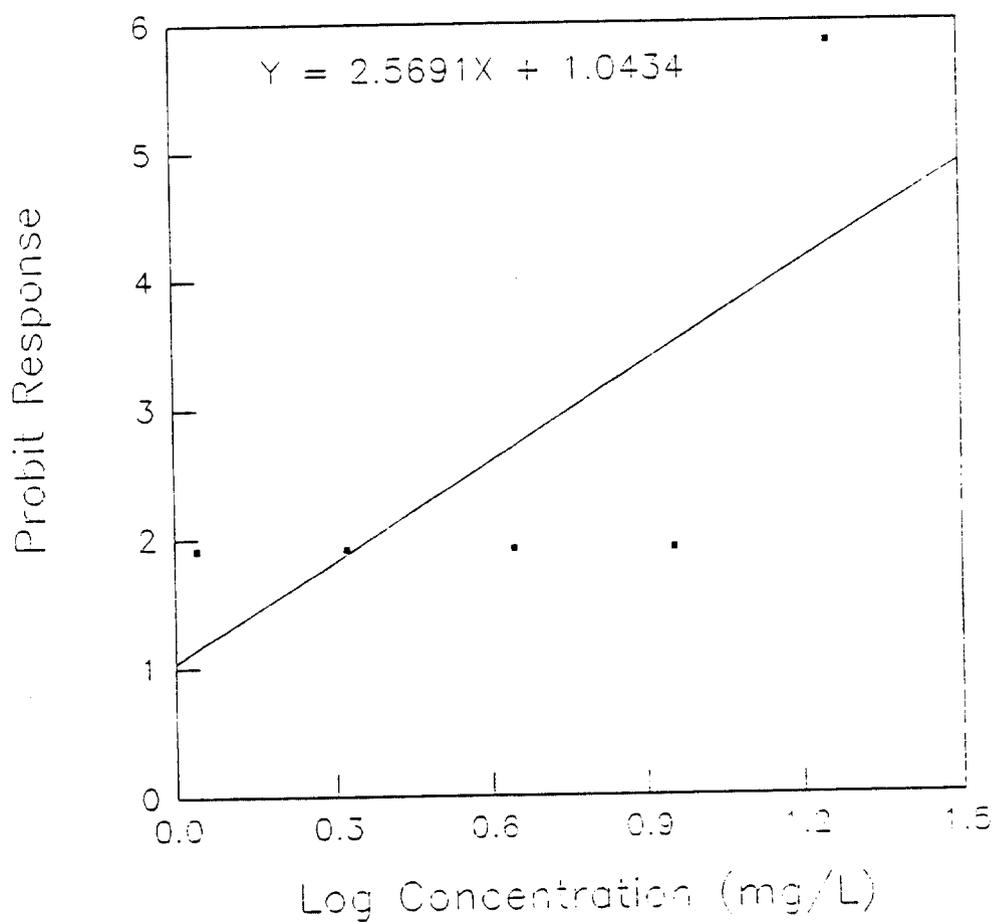
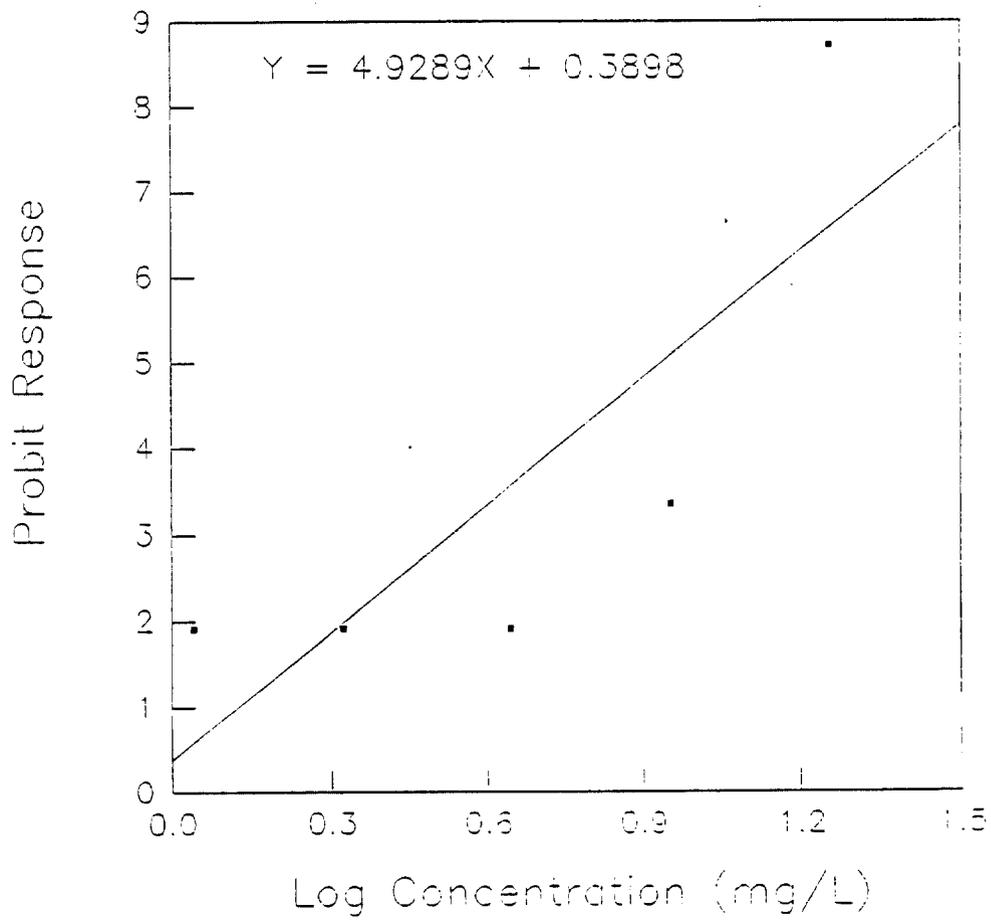


Figure 2. The 48-hour concentration-response (immobilization) curve for the static renewal exposure of daphnids (*Daphnia magna*) to Dobanol[®] 91-8.



SIGNATURES AND APPROVAL

SUBMITTED BY:

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Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

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Maura K. Collins Date
Study Director

James J O'Brien 2-14-94
James J. O'Brien Date
Principal Investigator

Susan P Shepherd 14 Feb 94
Susan P. Shepherd Date
Coordinator, Data Management
and Reporting Unit

APPROVED BY:

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Donald C. Surprenant Date
Program Manager
Environmental Toxicology

Doreen S Newhouse 14 Feb 94
Doreen S. Newhouse Date
Supervisor
Quality Assurance Unit

This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

4.0 APPENDIX I - STUDY PROTOCOL

Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with *Daphnia magna*, Following TSCA Test Guidelines § 797.1300

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: Shell Development Company

Address: P.O. Box 1380, Houston, Texas

Phone: (713) 493-8042

Sponsor Protocol/Project No.: WRC Tox No. 1227

Test Substance: Dobanol 91-8

Purity: 100% CAS# or LOT#: 468439-463

Additional Comments and/or Modifications:

Orval Jwoy

8/31/93

Sponsor Approval

Date

TO BE COMPLETED BY SPRINGBORN LABORATORIES PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc., 790 Main St, Wareham, MA 02571

Study Director: M. K. Collins SLI Study No.: 777.0993.6104.110

Test Concentrations: 20, 10, 5.0, 2.5 and 1.3 mg/L plus control

Solvent Used: distilled deionized water CAS# or LOT#: NA

Proposed Schedule: (Start) 19 October 1993 (Completion) 21 October 1993

Proposed Draft Report Date:

© 26 1-4 84

M. K. Collins

9.10.93

Study Director

Date

Springborn Laboratories Protocol #: 072993/TSCA/SHELL/DM-SR

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Springborn

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Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with *Daphnia magna*, Following TSCA Test Guidelines § 797.1300

1.0 INTRODUCTION

The purpose of this test is to determine the acute effects of an alcohol ethoxylate surfactant on the water flea, *Daphnia magna*, under static renewal conditions. Test results will be reported as 24- and 48-h EC50 values, (the median concentration that will immobilize 50% of the number of daphnids exposed) with 95% confidence limits. The No-Observed-Effect Concentration (NOEC) will also be reported. The test procedures performed during the biological portions of this study will meet or exceed the standard procedures described in the U.S. EPA Toxic Substances Control Act (TSCA) Test Guidelines § 797.1300 (U.S. EPA, 1985) and will meet the primary technical objectives of the Shell Research Limited/Sittingbourne Research Centre guidelines (SBT SOP No. 167, Edition No. 9).

2.0 MATERIALS AND METHODS

2.1 TEST ORGANISMS.

- 2.1.1. **Justification for Test System.** Characteristics which make this test organism suitable for acute toxicity testing are their ease of culturing and handling, their sensitivity to a variety of chemical substances, and the extensive data base for this common freshwater invertebrate species.
- 2.1.2. **Species.** The daphnid crustacean (water flea), *Daphnia magna*, will be the species used in this test. Test organisms will be \leq 24 hours old at the initiation of the test. Daphnids will be obtained by removing all immature daphnids from the culture vessel, thus isolating sexually mature daphnids 24 hours prior to initiating the test. All organisms will originate from the same culture population. Young produced by these organisms will be subsequently pipetted into the test beakers. Young for testing will not be taken from cultures where adults contain ephippia.
- 2.1.3. **Origin and Acclimation.** *D. magna* will be obtained from cultures maintained at Springborn Laboratories, Inc. Daphnids will be cultured in 2-L glass vessels containing 1 L of water. Water used to culture the daphnids will be prepared in the same manner and will have the same characteristics as described for dilution water. Culture water will be maintained at the required test temperature ($20 \pm 2^\circ\text{C}$). Each culture vessel will be cleaned once weekly. Young will not be used if more than 20% of the culture stock die within the 48 hours preceeding the test.
- 2.1.4. **Feeding.** While being maintained in culture prior to the test, organisms will be fed daily a combination of a trout food (Ziegler Brothers® Salmon Starter #1) suspension and a unicellular green algae, *Ankistrodesmus falcatus*. The food solution will be prepared to

contain 5 mg/mL trout food and approximately 4×10^7 cells/mL of algae. An aliquot of 0.5 mL of the trout food suspension and 2 mL of algae will be manually introduced to each culture vessel once daily. Daphnids will not be fed during the 48-hour exposure period. Periodic analyses of representative samples of the food will be conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the daphnids.

2.1.5. **Handling.** Wide-bore pipets will be used to transfer the daphnids, taking care to minimize possible stress due to handling. Daphnids that are damaged or dropped during transfer will not be used.

2.1.6. **Reference Tests.** In an effort to monitor the general health of the test organism culture, reference tests will be conducted, under static conditions, with *Daphnia magna* using copper nitrate as the reference toxicant. The results of these tests will be evaluated based on nominal concentrations. The reference tests will be conducted using young from the same culture population within 30 days of the definitive exposure (i.e., either 30 days prior to or 30 days following the definitive exposure).

2.2 PHYSICAL SYSTEM.

2.2.1. **Test Containers.** The test chambers used in the static acute bioassay will be 250-mL glass beakers which are chemically clean. Each beaker will contain 200 mL of the test solution or the control dilution water. The test vessels will be loosely covered.

2.2.2. **Glassware Preparation.** All glassware used in testing will be thoroughly washed with detergent and rinsed with tap water. This will be followed by sequential rinsing with a 10% solution of nitric acid, acetone, distilled deionized water, isopropanol and finally distilled deionized water.

2.2.3. **Dilution Water.** Dilution water will consist of hard fortified well water with a total hardness of 160 to 180 mg/L as CaCO_3 . Hard water will be used since *D. magna* are generally found in hard water habitats in the natural environment. The well water (total hardness about 30 mg/L as CaCO_3) will be fortified according to the formulation for hard water presented in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975). Dilution water will be filtered through an amberlite XAD-7 resin column (30 cm long and 1.6 cm wide) and an activated carbon bed. This filtration will effectively remove any potential organic contaminants from the water.

Quality of the dilution water used to conduct daphnid acute tests will be judged by the ability of the daphnid cultures to survive and reproduce in the water free of stress. The dilution water will be prepared in 1,900-L batches. New batches of dilution water will be prepared when the previous batch is exhausted, when a water quality parameter (total hardness, alkalinity, etc.) has varied from the normal range, or after two weeks of holding. The dilution water will be aerated with an air pump and air stones to bring the pH and dissolved gases into equilibrium with the atmosphere. Fiberglass containers will be used

to hold the dilution water. Total hardness, total alkalinity, pH and specific conductance of the dilution water will be monitored prior to use to assure that these parameters are within the normal acceptable ranges. Total organic carbon (TOC) will be monitored approximately once per month. Periodic analyses of representative samples of dilution water source will be conducted to ensure the absence of potential toxicants, including pesticides, PCBs, unionized ammonia, residual chlorine and selected toxic metals, at concentrations which may be harmful to the daphnids.

- 2.2.4. Replication and Control of Bias.** Four replicates will be included with each test concentration and control. Test beakers will be labeled by replicate letter and concentration (or control), and will be positioned in stratified random order. The daphnids will be impartially added to the test vessels by adding one daphnid to the first beaker, one to the next beaker and so forth until each beaker contains one test organism. This procedure will be repeated until each beaker contains five daphnids. Test organisms will be added to the exposure solutions within 30 minutes of preparation.

2.3 CHEMICAL SYSTEM.

- 2.3.1. Test Material.** Upon arrival at Springborn Laboratories, Inc., the external packaging of the test material will be inspected for damage. The packaging will be removed and the primary storage container will also be inspected for leakage or damage. The sample identity will be recorded and the material will be stored in the dark at approximately 20°C until used, unless specified differently by the test Sponsor.
- 2.3.2. Toxicant Concentration Selection.** Toxicant concentrations for the acute toxicity test will be selected based on information provided by the Sponsor and obtained from a 48-hour preliminary range-finding study exposing *D. magna* to the test material. The range-finding study will consist of five widely-spaced concentrations, usually of 1.0-L volume, each containing ten daphnids. The range of concentrations selected for the definitive test is intended to include both 100% effect and no-effect levels, but due to the nature of some compounds, one or both levels may not be observed. Five concentrations and one control will be used for each definitive test, each concentration consisting of twenty test daphnids after consultation with the Sponsor. A dilution ratio of 1.5 to 2.0 will be used.
- 2.3.3. Stock Preparation.** The surfactant will be heated in a glass container at a temperature of 50 - 60°C until completely melted. The melted test material will be stirred to ensure homogeneity. A glass pipet will be used to transfer the material for weighing. The test material will be weighed on an analytical balance for which a calibration log is maintained. The stock solution will be mixed for several minutes. A Chemical Usage Log will also be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test material is used. The primary stock will be prepared in distilled deionized water. The final concentration of the primary stock solution will be 1 - 2% active surfactant. A new primary stock solution will be prepared for each renewal of test solutions. Secondary stocks, if necessary, may be prepared in either distilled deionized water or in dilution water (if the volume displacement of dilution water during preparation of the test solutions will be greater than 1%).

- 2.3.4. **Measurement of Exposure Solution and Stock Solution Concentration.** Samples from each test chamber of each concentration and control(s) will be removed at test initiation (new solutions), midterm (old and new solutions) and test termination (old solutions). Replicate solutions at each test concentration will be pooled. All primary stock solutions prepared during the test will also be sampled.
- 2.3.5. **Sampling.** Water samples (approximately 500-mL) will be taken from a point approximately midway between the surface, bottom and sides of each vessel. All samples will be collected in 500 mL borosilicate glass containers with Teflon®-lined caps which have been serially rinsed with deionized water, isopropanol and deionized water as specified in Section 2.2.2. Sampling containers will be completely filled to minimize head space. Samples will be preserved with 1% formalin (i.e., 5 mL formalin/500 mL sample) and shipped within 24 hours to Battelle Ocean Sciences, Duxbury, Massachusetts.
- 2.3.6. **Analytical Chemistry.** Analyses of analytical samples will be conducted by Battelle Ocean Sciences, Duxbury, Massachusetts, using a Shell Development Company analytical method entitled "Analysis of Alcohol Ethoxylate Surfactants Using Solid Phase Extraction (SPE) and HPLC/ELSD (Evaporative Light Scattering Detection) in Dilute Aqueous Solutions".

2.4 EXPERIMENTAL CONDITIONS.

- 2.4.1. **Measurement of Water Quality Variables.** At test initiation, total hardness, alkalinity, acidity, TOC, TSS and specific conductance will be measured and recorded in each replicate vessel in each test concentration and control. Temperature, pH and dissolved oxygen will be recorded daily in each replicate of each concentration and control. Measurements will be recorded for the aged and for the freshly prepared solutions on renewal days.
- 2.4.2. **Photoperiod.** The tests will be illuminated at a light intensity of 30 - 100 footcandles using a combination of fluorescent bulbs. A 16-hour light, 8-hour dark photoperiod will be maintained with an automatic timer. There will be a transition period between light and dark.
- 2.4.3. **Dissolved Oxygen.** Total dissolved oxygen will not be allowed to drop below 60% or exceed 105% of saturation for the duration of the test. Should the dissolved oxygen fall below 60% of saturation, appropriate action will be taken after consultation with the Study Sponsor.
- 2.4.4. **Temperature.** Water temperature of the test solutions will be maintained at $20 \pm 2^\circ\text{C}$ by conducting the test in a waterbath.
- 2.4.5. **pH.** The pH of the control solutions will be maintained in a range of 6.0 to 8.5.

- 2.4.6. Biological Data. The number of immobilized daphnids in each test vessel will be recorded after 24 and 48 hours of test initiation. Immobilization is defined as the lack of movement by the test organisms except for minor activity of the appendages. In addition, prior to test initiation and whenever test organisms are observed, characteristics of the test solutions will also be observed and recorded, e.g., precipitated materials, cloudiness, etc.
- 2.4.7. Renewal Scheme. Test solutions will be prepared at 0 and 24 hours of exposure. Daphnids will be carefully transferred to the freshly prepared solutions using a wide-bore pipet.
- 2.4.8. Initiation and Test Duration. The study will be initiated when all test organisms have been impartially added to the exposure solutions. The study will be terminated following 48 hours of exposure at which time mortality of the control organisms will not exceed 10% or the test will be considered unacceptable.

3.0 DATA EVALUATION

Test results derived from the acute test will be used to statistically estimate a median effective concentration (EC50) and its 95% confidence interval after 24 and 48 hours of exposure. The EC50 is the estimated mean measured concentration of the test material in dilution water which produces 50% immobility in the test populations of daphnids at the stated times of exposure.

The computer program utilized estimates EC50 values using three statistical methods: probit analysis, moving average method, and binomial probability. The method selected and reported will be determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.). An EC50 value cannot be calculated if the data derived is insufficient according to any of the three statistical methods. The probit method provides values of the slope, including 95% confidence intervals, as well as appropriate statistical tests to evaluate goodness-of-fit.

Following 48 hours of exposure, data obtained on organism survival will be evaluated to establish the No-Observed-Effect Concentration (NOEC). This level is defined as the highest test concentration at and below which there were no toxicant-related mortalities or physical and behavioral abnormalities (e.g., lethargy).

The concentration-response data generated during this test will be provided to the Study Sponsor in Lotus® format.

4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include but will not be limited to correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated at Springborn Laboratories as a result of the study.

5.0 REPORTING

The raw data generated at Springborn Laboratories and final draft of the report will be reviewed by the Quality Assurance Unit and Study Director. All measurements (e.g. water quality) will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will initially be submitted to the study Sponsor for review. Upon acceptance by the Sponsor, three (3) copies of the final report will be submitted. All reports include, but will not be limited to, the following information:

- * Springborn Laboratories, Inc., report and project numbers.
- * Laboratory and site, the dates of testing and personnel involved in the study, e.g., Quality Assurance Unit, Program Coordinator (if applicable), Study Director, Principal Investigator.
- * All information pertaining to the test material which appears on the sample bottle, e.g., its source, percent active ingredient, physical properties, Sponsor's test material I.D., and sample number (if applicable).
- * Characterization and origin of the dilution water.
- * Scientific name of the test organisms, source, and culturing information.
- * Range-finding study results.
- * The 48-hour EC50 value with the 95% confidence limits and control performance of applicable copper nitrate reference test.
- * Test container volume, dilution water volume, number of replicates used per concentration, and number of daphnids used per treatment.
- * Definition of criteria used to determine the sublethal effects, and general observations on non-quantifiable effects.
- * Description of exposure system and stock preparation.
- * Test temperatures, dissolved oxygen concentration, and pH; photoperiod and light intensity; and specific conductance, total alkalinity and total hardness measured.

- * Description of, or reference to, chemical and statistical procedures applied.
- * Percentage of daphnids that were immobilized in the controls and in each treatment at each observation period, in tabular form.
- * The 24- and 48-hour EC50's with 95 percent confidence limits, and the No-Observed-Effect Concentration (NOEC), when possible. All calculations will be based on mean measured concentrations.
- * Graph of the concentration-response curve at each observation period for which an EC50 is calculated. Mean measured concentrations will be used to establish the concentration-response curve.
- * Deviations from the protocol not addressed in protocol amendments, together with a discussion of the impact on the study, signed by the Study Director.
- * Good Laboratory Practice (GLP) compliance statement (for the biological portion of this study) signed by the Study Director.
- * Dates of Quality Assurance reviews, signed by the QA Unit.
- * Location of raw data and final report.

6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the impact of the change on results of the study, if any. If necessary, amendments initially may be in the form of verbal authorization, followed by Springborn's written documentation of the amendment. In such a case, the effective date of the amendment will be the date of verbal authorization.

7.0 SPECIAL PROVISIONS

GOOD LABORATORY PRACTICES (GLP): All test procedures, documentation, records, and reports related to the biological portion of this study will comply with the U. S. Environmental Protection Agency's Good Laboratory Practices as promulgated under the Toxic Substance Control Act (FEDERAL REGISTER, Part IV, 17 August, 1989)

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final test report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

Springborn Laboratories Protocol #: 072993/TSCA/SHELL/DM-SR

Page 8 of 9

ARCHIVAL: All raw data and the final report will be archived at Shell Development Company unless different arrangements are made.

8.0 REFERENCES

- APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, Washington, DC. 2168 pp.
- U.S. EPA. 1975. *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians*. Ecological Research Series (EPA-660/3-75-009). 61 pp.
- U.S. Environmental Protection Agency. 1989. *Toxic Substances Control; Good Laboratory Practice Standards; Final Rule*. (40 CFR, Part 792) Federal Register, Part III, 48(230):53922-53944, August 17, 1989.
- U.S. Environmental Protection Agency. 1985. *Toxic Substances Control Act Test Guidelines*. Federal Register 50(188):39252-39516, September 27, 1985. Amended May 20, 1987, July 1, 1991 and July 1, 1992.

Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

PROTOCOL AMENDMENT**AMENDMENT #:** 1**DATE:** 29 September 1993**PROTOCOL TITLE:** "Alcohol Ethoxylate Surfactants: Protocol for Conducting an Acute Toxicity Test, Under Static Renewal Conditions, with *Daphnia magna*, Following TSCA Test Guidelines § 797.1300."**STUDY SPONSOR:** Shell Development Company**TEST SUBSTANCE:** Dobanol 91-8**SLI STUDY #:** 777.0993.6104.110**SPONSOR PROTOCOL/PROJECT NO.:** WRC TOX NO.: 1227**AMENDMENT(S):****Amendment (Section 2.2.3)**

The protocol states that total hardness, total alkalinity, pH and specific conductance of the dilution water will be monitored prior to use to assure that these parameters are within normal acceptable ranges.

Amended, the total hardness, total alkalinity, acidity, pH, TOC, TSS and specific conductance of the dilution water will be monitored prior to use.

Reason for change:

The additional dilution water parameters measured (i.e., acidity, TOC and TSS) are included in order to meet the requirements for dilution water as stated in TSCA Guideline 797.1300.

Amendment (Section 2.3.4)

The protocol states that samples will be taken from each test chamber of each concentration and control(s). Replicate solutions at each test concentration will be pooled.

Amended, the samples will be taken for the new solutions by removing the appropriate aliquot from each test solution prior to splitting the solution into replicate chambers. Samples for the old solutions will be taken as originally stated in the protocol.

Springborn Laboratories Protocol #: 072993/TSCA/SHELL/DM-SRPage 1 of 2
 **Springborn**
LABORATORIES

LETTERS AND REPORTS: Springborn Laboratories, Inc. letters and reports are issued for the exclusive use of the clients to whom they are addressed. No quotations from reports or use of the Springborn Laboratories, Inc. name is permitted except as expressly authorized in writing. Letters and reports apply only to the specific materials, products or processes tested, examined or surveyed and are not necessarily indicative of the quality of laboratory services or similar materials, products or processes. The liability of Springborn Laboratories, Inc. with respect to services rendered shall be limited to the amount of the consideration paid for such services and not include any consequential damages.

Reason for change:

The amended method presented is a clarification of the intended procedure.

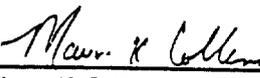
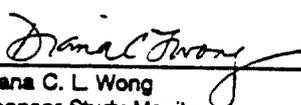
Amendment (Section 2.4.1)

The protocol states at test initiation total hardness, alkalinity, acidity, TOC, TSS and specific conductance will be measured and recorded in each replicate vessel in each test concentration and control.

Amended, at test initiation total hardness, alkalinity, acidity and specific conductance will be measured in each replicate vessel of each concentration and control.

Reason for change:

The TOC and TSS of the test solutions is not required by the TSCA Guideline. These parameters are measured in the dilution water prior to use to ensure they are within normal acceptable ranges.

Approval Signatures:		<u>9-29-93</u>
	Maura K. Collins SLI Study Director	Date
		<u>10-14-93</u>
	Diana C. L. Wong Sponsor Study Monitor	Date

5.0 APPENDIX II - CERTIFICATE OF ANALYSIS

Shell Development Company

A Division of Shell Oil Company



Westhollow Research Center
P. O. Box 1390
Houston, TX 77251-1390

September 10, 1993

Pamela M. Lincoln
Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

Dear Ms. Lincoln:

The missing information, pertaining to the test substance Dobanol 91-8, that you requested of me in your letter dated 2 September, 1993 is as follows:

Lot Number:	97002310
% Active Ingredient:	100%
Net Amount Shipped	878 g
Molecular Weight	avg 510 g/mol

The certificate of analysis for this test substance is attached.

If you have any questions then please do not hesitate to contact me at the above address or by phone at (713) 493-7955.

Sincerely,

A handwritten signature in cursive script that reads "Carolyn A. Matula".

Carolyn A. Matula



Shell Chemicals

Shell Chemicals U.K. Limited

CARRINGTON WORKS LIVERPOOL MANCHESTER M31 4AJ
 TELEPHONE: 081-776 3000 TELEEX: 887301 FAX: 081-776 3815

Certificate of Analysis

Dolanol 41-8

Tank 241 SRO 15418

Order reference: 061 776 3456

Your ref

Our Ref: MTS/88

Date: 24.08.92

Hydroxy Value mg KOH/g	110.2
1% Ac Cloud Point E	31.3
Pey % m/m	0.25
Water % m/m	0.05
Free Alcohol % wt	1.4
+ 1 ED	1.0
+ 2 ED	1.2
+ 3 ED	2.4
+ 4 ED	3.5
+ 5 ED	5.1
+ 6 ED	6.6
+ 7 ED	8
+ 8 ED	9
+ 9 ED	9.8
+ 10 ED	9.6
+ 11 ED	9.2
+ 12 ED	8.2
+ 13 ED	6.4
+ 14 ED	5.4
+ 15 ED	4.1
+ 16 ED	3.1
+ 17 ED	2.2
+ 18 ED	1.7
+ 19 ED	0.4
+ 20 ED	0.5
+ 21 ED	0.2
	3.2



Coverage ED

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See MTS/88

6.0 APPENDIX III - CULTURE FOOD ANALYSIS

Zeigler Brothers Inc. Salmon Starter Feed Sample*		
Date Submitted:11/13/92 Date Reported:12/1/92		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Arsenic	2.1 ppm	0.1
Cadmium	0.4 ppm	0.1
Copper	2.1 mg/100g	0.2
Lead	0.4 ppm	0.2
Mercury	0.10 ppm	0.02
Zinc	29.4 mg/100g	0.2
Selenium (fluorometric)	1.6 ppm	0.1

* Analyzed by Lancaster Laboratories, Inc.

Ankistrodesmus Grab Sample*		
Date Collected: 7/28/93 Date Reported: 9/6/93		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Sodium	26. mg/l	10.
* Analyzed by Lancaster Laboratories, Inc.		

7.0 APPENDIX IV - DILUTION WATER ANALYSIS

Well ¹ Water Sample*		
Date Collected: 7/29/93 Date Reported: 9/17/93		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.3 µg/l	0.3
Toxaphene	< 4. µg/l	4.
PCBs	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
¹ Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		

Well ¹ Water Sample*		
Date Collected: 8/9/93 Date Reported: 8/26/93		
Analysis	Result As Received	Limit of Quantitation
Mercury	< 0.00020 mg/l	0.00020
Arsenic	< 0.20 mg/l	0.20
Selenium	< 0.20 mg/l	0.2
Boron	< 0.040 mg/l	0.04
Thallium	< 0.30 mg/l	0.3
Aluminum	< 0.20 mg/l	0.2
Antimony	< 0.20 mg/l	0.2
Barium	< 0.10 mg/l	0.1
Beryllium	< 0.010 mg/l	0.01
Cadmium	< 0.010 mg/l	0.01
Calcium	7.71 mg/l	0.2
Chromium	< 0.050 mg/l	0.05
Cobalt	< 0.050 mg/l	0.05
Copper	< 0.020 mg/l	0.02
Iron	< 0.10 mg/l	0.1
Lead	< 0.10 mg/l	0.1
Magnesium	2.31 mg/l	0.1
Manganese	< 0.010 mg/l	0.01
Molybdenum	< 0.10 mg/l	0.1
Nickel	< 0.050 mg/l	0.05
Potassium	1.07 mg/l	0.5
Silver	< 0.020 mg/l	0.02
Sodium	14.0 mg/l	0.4
Titanium	< 0.010 mg/l	0.01
Vanadium	< 0.010 mg/l	0.01
Zinc	< 0.040 mg/l	0.04
Total Organic Carbon ***	< 1. mg/L	1.
¹ Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		
*** Represents "non-purgeable TOC"		

FINAL DATA REPORT**Study Title**

Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Daphnia magna*

ge

Data Requirements

15

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

17

Submitted To

Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

Performing Laboratory

Battelle Ocean Sciences
397 Washington Street
Duxbury, MA 02332

Author

Gregory S. Durell

Study Initiation Date

September 10, 1993

Study Completion Date

November 19, 1993

Battelle Study Number

SD-930115

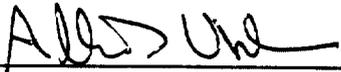
SIGNATURE PAGE



Gregory S. Durell
Analytical Chemistry Task Leader
Battelle Ocean Sciences

02/13/94

Date



Allen D. Uhler
Chemistry Department Manager
Battelle Ocean Sciences

1/13/94

Date



Carolyn A. Matula
Project Monitor
Shell Development Company

1 February, 1994

Date

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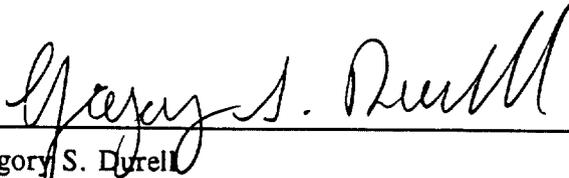
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COMPLIANCE STATEMENT

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. Durrell
Analytical Chemistry Task Leader
Battelle Ocean Sciences

04/13/94

Date

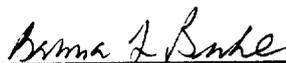
QUALITY ASSURANCE STATEMENT

for

**Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Daphnia magna***

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

1-13-94

Date

QUALITY ASSURANCE AUDITS

Conducted for SD-930115

**Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Daphnia magna***

Audit Type	Audit Date	Date of Report to Analytical Task Leader	Date of Report to Study Director	Date of Report to Management
Initiation	09/13/93	09/15/93	09/22/93	09/15/93
Lab Inspection	11/09/93 11/10/93	01/03/94	01/13/94	01/13/94
Data Package	12/28-31/93	12/31/93	01/13/94	01/13/94
Report Review	12/28-31/93	12/31/93	01/13/94	12/31/93

STUDY PARTICIPANTS

SD-930115

**Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Daphnia magna***

Mr. Gregory S. Durell	Analytical Chemistry Task Leader
Ms. Lynn A. Lariviere	HPLC Analyst; Sample Custodian
Mr. Richard Restucci	Laboratory Technician

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 Springborn Laboratories, Inc. 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 Springborn Laboratories. 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 August 27, 1993, and by the Sponsor's Project Monitor on August 31, 1993.

1.1 Test Substance Identification

The Test Substance analyzed in this study was an alcohol ethoxylate (AE) surfactant (Dobanol 91-8®). 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>Daphnia magna</i>
Test Substance:	Dobanol® 91-8
Test Substance CAS#:	68439-46-3
Test Substance Lot#:	LR21662-54
Test Substance Purity:	100%

Test Substance Composition:	A C ₉ -C ₁₁ alcohol ethoxylate with an average of 8 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.
Test Substance Solubility:	Completely soluble in water. May form gel.
Test Substance Stability:	Stable. A stability and holding time of two years from receipt at Battelle was assigned to the Test Substance.
Test Substance Storage Requirements:	Ambient temperature or lower.

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 C₈ 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.

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 August 31, 1993. 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 (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 five-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 five-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 31, 64, 103, 168, and 258 $\mu\text{g/mL}$. Original water sample concentrations were subsequently determined by applying the water extraction volume (WEV) and pre-injection volume (PIV) information. The PIV of the QC samples, controls, and 1,300 and 2,500 parts per billion (ppb) nominal concentration samples was 1.00 mL, and it was 5.00 mL for the samples with nominal concentrations of 5,000, 10,000, and 20,000 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.

$$\% \text{ Recovery} = \text{WC}_D \times (1 / \text{WC}_S) \times 100\%$$

$$\% \text{RPD} = [\% \text{REC}_{\text{MS}} - \% \text{REC}_{\text{MSD}}] \times (2 / (\% \text{REC}_{\text{MS}} + \% \text{REC}_{\text{MSD}})) \times 100\%$$

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 considered detected.

The water sample equivalent LOD was calculated by comparing the peak height equivalent to a signal:noise ratio of 3:1 in the sample to the peak height of the analyte in the low-level calibration standard, 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 concentrations.

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

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

H_{3x} = Peak height equivalent to $3 \times$ 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 two analytical batches, the first batch containing the $t=0$ hr and $t=24$ hr (old) samples and the second batch containing the $t=24$ hr (new) and $t=48$ hr samples. Table 1 also presents the data for the Primary Stock Solution analyses.

The measured analyte concentrations in the test samples that had been fortified with the Test Substance ranged from 817 ppb (for sample NB23, a sample with a nominal concentration of 1,300 ppb) to 19,140 ppb (for sample NB06, a sample with a nominal concentration of 20,000 ppb). The measured concentrations were between 63 percent (sample NB23) and 106 percent (sample NB11) of the nominal concentration, with the majority of the samples having a measured concentration ranging from approximately 70 to approximately 95 percent of the nominal concentration. Some interference with the Dobanol® 91-8 signal/peaks was evident in the HPLC/ELSD chromatogram of sample NB11, probably contributing a small amount to the measured concentration of this sample. On an average, the concentrations in the $t=48$ hour samples are slightly lower than the other, suggesting that there may be a slight loss of the analyte with time.

Table 1. Dobanol® 91-8 Concentrations in Samples Received from the Toxicological Testing Laboratory

Battelle Sample ID	Test Sample Time/Type	Nominal Conc. (ppb)	Measured Conc. (ppb)
Batch #1			
NB01	t=0	0	ND
NB02	t=0	1,300	1,168
NB03	t=0	2,500	2,211
NB04	t=0	5,000	4,795
NB05	t=0	10,000	9,416
NB06	t=0	20,000	19,140
NB08	t=24, old	0	ND
NB09	t=24, old	1,300	1,136
NB10	t=24, old	2,500	2,243
NB11	t=24, old	5,000	5,282
NB12	t=24, old	10,000	9,313
NB13	t=24, old	20,000	18,480
Batch #2			
NB19	t=24, new	0	ND
NB18	t=24, new	1,300	1,077
NB17	t=24, new	2,500	2,174
NB16	t=24, new	5,000	3,708
NB14	t=24, new	10,000	8,804
NB15	t=24, new	20,000	18,600
NB22	t=48, old	0	ND
NB23	t=48, old	1,300	817
NB24	t=48, old	2,500	1,823
NB25	t=48, old	5,000	3,707
NB26	t=48, old	10,000	8,582
NB27	t=48, old	20,000	17,730
Primary Stock Solution		(ppm)	(ppm)
NC01	t=0, stock	20,000	18,320
NC02	t=24, stock	20,000	18,820

ND: Not detected; <LOD.

The concentrations measured for the Primary Stock Solutions were slightly lower than the expected concentrations, with measured concentrations ranging from 18,320 to 18,820 ppm for the two samples, both of which had nominal/expected concentrations of 20,000 ppm. The measured Primary Stock Solution concentrations were between 6 and 8 percent lower than the expected concentration.

3.2 Analytical Results — Quality Control Samples

All quality control objectives were met for this work. The five-point multi-level instrument calibration used had a correlation coefficient of 0.999983 for the quadratic equation generated, and the continuing calibration check analyses ranged from 2.0 to 5.2 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)	125 ppb
Limit of Quantitation (LOQ)	618 ppb

Sample concentrations above the LOD were reported for this study. Sample concentrations below the LOD were reported as “ND” (not detected). 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 either of the two procedural blank samples. The analyte recovery in the blank spike (BS) samples were 98% and 93% for analytical batches #1 and #2, respectively. The analyte recovery in the four matrix spike (MS/MSD) samples ranged from 90% to 94%, and these data suggest that there were no significant matrix effects on the analytical procedure.

Table 2. Laboratory Quality Control Sample Analysis Results

Battelle Sample ID	QC Sample Type	Recovery (%)
Batch #1		
NB30-PB	Procedural Blank	ND
NB31-BS	Blank Spike	97.6
NB32-MS	Matrix Spike	94.0
NB33-MSD	Matrix Spike Duplicate	91.5
	MS/MSD %RPD:	2.6
Batch #2		
NB34-PB	Procedural Blank	ND
NB35-BS	Blank Spike	93.3
NB36-MS	Matrix Spike	89.8
NB37-MSD	Matrix Spike Duplicate	93.2
	MS/MSD %RPD:	3.7

ND: Not detected; <LOD.

Acceptable precision was observed for both analytical batches. The relative percent differences in the measured analyte recoveries for the MS/MSD duplicate analyses were 3% and 4% for analytical batches #1 and #2, respectively.

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
- Verified 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-930115

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Provision of Test Substance

The Test Substance for this study was provided by the Sponsor, not the toxicological testing laboratory as specified in the Analytical Procedure Document. The Sponsor communicated that the Test Substance provided to Battelle and the toxicological testing laboratory came from the same lot/source.

Approved: Gregory S. Durell

Date: 12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930115

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X **No**

Entered by: Gregory S. Durell

Date: January 7, 1994

Subject: Sample Receipt and Shipment from the Toxicological Testing Laboratory

The samples shipped by the toxicological testing laboratory to Battelle for analysis were received in coolers or boxes with no ice packs and were at ambient temperature. They were not shipped in coolers with ice packs as specified in the Analytical Procedure Document. This issue was communicated to the Sponsor by telephone who verbally approved Battelle to proceed with the work on the samples received. The lack of refrigeration while in transit should not impact the integrity of these samples.

Approved:

Gregory S. Durell

Date:

01/07/94

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930115

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Return and Archival of Test Substance

Only a small amount of Test Substance was received by Battelle for this work (in a vial of approximately 4 mL volume) and the unused material at the end of the study will be kept and archived at Battelle, not returned to the toxicological testing laboratory.

Approved: Gregory S. Durell

Date: 12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930115

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 20, 1993

Subject: Calculations of Original Water Sample Concentrations

The original water sample concentrations were calculated in spreadsheet tables by applying the water extraction volumes (WEV) and pre-injection volumes (PIV) to the sample extract concentrations determined using the chromatography software in the Hewlett-Packard LAS data system. These calculations are described in the Study Report. The Analytical Procedure Document specified that the original water sample concentrations be determined directly in the H-P LAS datasystem by entering the WEV and PIV data there.

Analyte concentrations of the original water samples were determined in parts per billion (ppb). Analyte concentration of the primary stock solution samples were determined in parts per million (ppm).

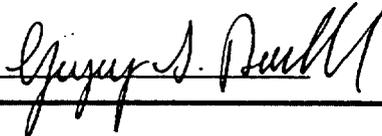
$$\text{Determined Water Sample Concentration (ppb)} = \text{EC} \times \text{PIV} \times (1/\text{WEV}) \times 1000$$

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

EC = Extract (HPLC sample) concentration ($\mu\text{g}/\text{mL}$ = 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)Approved: Date: 12/20/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 2

Project Title: Gap-Filling Project

Study Number: SD-930115

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: January 7, 1994

Subject: Miscellaneous Deviations to Analytical Method

Sample extracts were stored in sealed vials/tubes until HPLC analysis could be performed, and not transferred to HPLC vials on the day the sample processing was completed, as specified in the Analytical Procedure Document. The PIVs were not always adjusted on the day that sample extraction was completed or that samples were submitted for HPLC analysis. The PIV was carefully visually inspected for each sample prior to transfer for instrumental analysis. These deviations have no impact on the integrity of the samples or results, because of the stability of the test substance and because proper storage was maintained.

Concentrator tubes were not rinsed and the rinsate transferred to a 4 mL vial for storage on the day sample processing was completed, as indicated in the Analytical Procedure Document and associated sample preparation check list. Instead the procedure was to store the securely sealed concentrator tube refrigerated until HPLC analysis was completed and then rinse and transfer any remaining sample in the concentrator tube and HPLC vial to a common vial of suitable size (sometimes 4 mL and sometimes larger). This deviation has no impact on the integrity of the samples because proper storage was maintained.

The Test Substance was stored refrigerated and not at room temperature as specified in the Analytical Procedure Document. This deviations has no impact on the integrity of the material or results, because suitable storage conditions were maintained.

Page 2 of 2

There were a few instances of incomplete temperature monitoring of Refrigerator #2 during the performance of this work. Specifically, during the third week of November the monitoring/logging was missed three times, during the fourth week of November it was missed one time, and during the first week of December it was missed one time. However, all sample extraction had been completed by these dates, and only sample extracts (in methanol) were stored in the refrigerator. Sample extracts can be stored at, or below, room temperature. This deviation would not impact the integrity of the samples or the quality of the data.

Approved: *Gregory J. Duell*

Date: 01/07/94

**DOBANOL[®] 91-8 - TOXICITY TO THE
FRESHWATER GREEN ALGA, *Selenastrum
capricornutum***

TSCA TEST GUIDELINE § 797.1050

Submitted to:

**Shell Development Company
P.O. Box 1380
Houston, Texas 77251**

SLI Report #93-12-5062

Sponsor Protocol/Project No.: WRC TOX No. 1227

SLI Study #777.0993.6106.430

Study Director: James R. Hoberg

**Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571**

11 February 1994

FINAL REPORT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for "**Dobanol[®] 91-8 - Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum***" were produced and compiled in accordance with all pertinent TSCA Good Laboratory Practice Regulations with the following exception: routine water contaminant screening analyses for pesticides, PCBs and metals were conducted using standard U.S. TSCA procedures by Lancaster Laboratories, Lancaster, Pennsylvania. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Stability, characterization, and verification of the test material identity and maintenance of records on the test material are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test material will be sent to the Study Sponsor. Archival of a sample of the test material is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

 2/11/94
James R. Hoberg Date
Study Director

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SUMMARY**Dobanol[®] 91-8 - Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum***

SPONSOR: Shell Development Company
P.O. Box 1380
Houston, Texas 77251

PROTOCOL TITLE: "Alcohol Ethoxylate Surfactants: Protocol for Conducting a 96-Hour Toxicity Test with the Freshwater Green Alga, *Selenastrum capricornutum*, Following TSCA Test Guideline § 797.1050," Springborn Protocol #072993/TSCA/SHELL/SEL and Protocol Amendment #1 dated 21 October 1993.

REPORT NUMBER: 93-12-5062

STUDY NUMBER: 777.0993.6106.430

TEST MATERIAL: Dobanol[®] 91-8, CAS #68439-46-3, Lot #97002310, WRC TOX. Sample #1227, PSIS #7075

DATE RECEIVED: 2 September 1993

DESCRIPTION: A clear liquid reported by the Study Sponsor to contain 100% active ingredient.

EXPERIMENTAL START DATE: 18 October 1993

EXPERIMENTAL TERMINATION DATE: 22 October 1993

TEST ORGANISM: *Selenastrum capricornutum*, inoculum - 3 days since previous transfer, source - Springborn culture

DILUTION WATER: Algal Assay Procedure (AAP) medium

TEST CONDITIONS: 96 hour duration, 24 to 25 °C, continuous illumination at 3500 to 5100 lux (325 to 475 footcandles), shaking at 100 rpm

NOMINAL TEST CONCENTRATIONS: 0.080, 0.16, 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L

**MEAN MEASURED
CONCENTRATIONS:**

0.074, 0.15, 0.24, 0.51, 0.89, 1.9, 4.4 and 9.1 mg/L

EFFECT CRITERION:

Inhibition of cell density relative to the control

RESULTS:

Based on mean measured concentrations, the 96-hour EC50 value was calculated to be 0.35 mg/L (95% confidence of 0.089 to 1.3 mg/L).

The 96-hour No-Observed-Effect Concentration (NOEC) was determined to be 0.074 mg/L.

1.0 INTRODUCTION

The objective of this study was to determine the effect of Dobanol[®] 91-8 on the growth of the freshwater green alga *Selenastrum capricornutum*. The results are based on mean measured concentrations and are reported as the 96-hour No-Observed-Effect Concentration (NOEC) and EC10, EC50 and EC90 values (i.e., the concentrations of test material that reduce culture density by 10, 50 and 90%, respectively, as compared with the control). The study was initiated on 8 September 1993, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the 96-hour definitive test was conducted from 18 to 22 October 1993 at the Environmental Sciences Division of Springborn Laboratories, Inc. (SLI), in Wareham, Massachusetts. All original raw data and the final report produced during this study are stored at Shell Development Company.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this acute toxicity study followed those described in the Springborn protocol entitled "Alcohol Ethoxylate Surfactants: Protocol for Conducting a 96-Hour Toxicity Test with the Freshwater Green Alga, *Selenastrum capricornutum*, Following TSCA Test Guideline § 797.1050," Springborn Protocol #072993/TSCA/SHELL/SEL and Protocol Amendment #1 dated 21 October 1993 (Appendix I). The method described in this protocol meet or exceed the standard procedures described in the U.S. EPA Toxic Substance Control Act (TSCA) Test Guidelines § 797.1050 (U.S. EPA, 1985) as amended in the Federal Register on 20 May 1987 (U.S. EPA, 1987) and meet the primary technical objectives of The Shell Research Limited/Sittingbourne Research Center guidelines (SBT SOP No. 169, Edition No. 8).

2.2 Test Material

A sample of the Dobanol[®] 91-8 (CAS #68439-46-3, Lot #97002310, WRC TOX. Sample #1227, PSIS #7075, expiration date September 1994), a clear liquid, reported by the Study Sponsor to contain 100% active ingredient, was received from Shell Westhollow Research Center, Houston, Texas, on 2 September 1993 (Certificate of Analysis, Appendix II). Upon receipt at

Springborn, the test material was stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Test concentrations are expressed as milligrams of Dobanol® 91-8 per liter of solution and are reported as mg/L.

2.3 Test Organism

The alga used in this toxicity test was the freshwater green alga *Selenastrum capricornutum*, strain 1648, Class Chlorophyceae. The alga was originally obtained from the Carolina Biological Supply Company, Burlington, North Carolina, and was maintained in stock culture at Springborn.

The culture medium used was Algal Assay Procedure (AAP) medium prepared with sterile, deionized water. The components used to formulate AAP medium are presented in Table 1. Representative samples of the dilution water source used in the preparation of the culture medium were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix III). None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed in agreement with U.S. EPA guidelines. In addition, a representative sample of AAP medium is analyzed monthly for total organic carbon (TOC) concentration. The TOC concentration was 1.2 mg/L for the month of October 1993 (SLI TOC and TSS Master Log, 1993).

The pH of the culture medium was adjusted to $\text{pH } 7.5 \pm 0.1$ with either 0.10 N hydrochloric acid or 0.10 N sodium hydroxide. Stock cultures were grown in 125-mL glass flasks containing 50 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange.

The stock cultures were maintained within the following conditions: a shaking rate of 100 ± 10 rpm, a temperature of 24 ± 2 °C and continuous illumination at the surface of the medium at an approximate light intensity of 3200 to 5400 lux (300 to 500 footcandles) for a minimum of three days prior to test initiation (SLI Algae Conditions Daily Log, 1993). Temperature was controlled using an environmental chamber. Lighting was supplied by Duro-

Test, Inc. Vita-Lite® fluorescent lights. Culture flasks were agitated continuously on an orbital shaker.

Stock cultures were transferred to fresh medium approximately twice weekly. The inoculum used to initiate the toxicity test with Dobanol® 91-8 was taken from a stock culture that had been transferred to fresh medium three days before testing.

2.4 Reference Test

A copper nitrate reference test was conducted with the test organism culture from 25 to 29 October 1993. The resulting 96-hour EC50 based on measured test concentrations was calculated to be 0.028 mg/L (95% confidence interval of 0.018 to 0.042 mg/L). Based on the results of the reference test and the successful culture of *Selenastrum capricornutum*, it was established that this culture was suitable for testing.

2.5 Test Dilution Water

The AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium. Several liters of AAP medium were prepared using deionized water, autoclaved and equilibrated to test temperature. The pH of this medium was 7.6 and required no adjustment prior to use.

2.6 Test Concentrations

Based on the results of a preliminary test conducted from 4 to 8 October 1993, nominal test concentrations of 0.080, 0.16, 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L were selected for the definitive exposure.

2.7 Preparation of Test Solution

Prior to preparation of the stock solution, the test material was heated to a temperature range of 50 to 60 °C and stirred with a glass rod to ensure homogeneity. Forty grams (40.000 g) of test material was then removed using a glass pipet, and was diluted in 2000 mL of distilled, deionized water to create a 20 mg/mL primary stock solution. This primary stock solution was

stirred for 15 minutes to ensure that the test material completely dissolved, at which time the solution was observed to be clear and colorless. The highest test solution, 10 mg/L, was then prepared by diluting 0.50 mL of the 20 mg/mL primary stock solution to a volume of 1000 mL with AAP medium. Test solutions with nominal concentrations of 0.080, 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L were prepared by diluting the appropriate volume of either the highest test solution or primary stock solution with AAP medium to a volume of 1000 mL. Additional untreated AAP medium was prepared and designated the control. All of the test solutions were observed to be clear and colorless with no sign of undissolved test material present.

Replicate sterile 250-mL Erlenmeyer flasks, three per treatment level and control, were conditioned by rinsing with the appropriate test solution. One hundred mL of the appropriate test solution was then placed in each replicate flask. Three control flasks which contained AAP medium were maintained under the same conditions as the treatment level flasks but contained no Dobanol[®] 91-8. All test vessels were fitted with stainless steel caps which permitted gas exchange.

3.0 TEST PROCEDURES

3.1 Test Initiation

Approximately thirty minutes after the test solutions were prepared and added to the test flasks, 0.87 mL of an inoculum of *Selenastrum capricornutum* cells at an approximate density of 115×10^4 cells/mL was aseptically introduced into each flask. This inoculum provided the required cell density of approximately 1.0×10^4 cells/mL.

3.2 Test Monitoring

3.2.1 Algal Growth. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer (Neubauer Improved) and an Olympus compound microscope. One sample was taken from each flask for counting. One or more hemacytometer fields, each 0.1 x 0.1 cm in surface area and 0.01 cm deep and containing 0.0001 mL of culture, were examined for each sample until at least 400 algal cells or four fields were counted. Observations of the health of the cells were made and recorded at each 24-hour interval.

3.2.2 Recovery. At test termination, since no test concentration completely inhibited algal growth, a 0.40 mL sample was removed from the composite of the three replicates of the highest test concentration (10 mg/L) which most severely inhibited algal growth. This sample was then diluted with fresh AAP medium to prepare a subculture with a nominal test concentration equal to the highest test concentration (0.080 mg/L) in which no growth inhibition was observed. This subculture was used to determine if the effects of the test material on the algae were algistatic (in which case cells would resume growth in the subculture) or algicidal (in which case no growth would occur in the subculture). The subculture was incubated under test conditions for four days (i.e., until growth was observed, as determined by cell counts taken every other day).

3.2.3 Test Conditions. The test was conducted in an environmental chamber designed to maintain the following test conditions: a temperature of 24 ± 2 °C, continuous lighting with a light intensity within the range of 3200 to 5400 lux (300 to 500 footcandles) and a shaking table rate of 100 ± 10 rpm.

Temperature was measured continuously with a Taylor Thermometer Company, Inc. minimum/maximum thermometer located in a flask of water adjacent to the test flasks in the environmental chamber. The shaking rate of the orbital shakers was recorded daily. The light intensity of the test area was measured with a General Electric Type 214 light meter at 0 hour and each 24-hour interval of the exposure period. Light intensity was measured in footcandles and converted to lux based on the equivalency of 1 footcandle = 10.76 lux. Test flasks were randomly placed on the shaking table at test initiation based upon computer-generated random numbers. Following each observation interval, the test vessels were returned to the initial random positions established at test initiation.

Water quality parameters (pH and conductivity) were measured prior to test initiation and at the termination of the 96-hour exposure period. Measurements at 0-hour were conducted on the test solution remaining in the 1000-mL volumetric flasks after the individual test flasks had been filled. At test termination, after cell counts were completed, pH measurements were taken in each replicate test solution. The three replicates for each test concentration and the control

were then individually composited and a portion of each composite solution was transferred to a 100-mL beaker for conductivity measurements. Test solution pH was measured with a LaMotte Model HA pH meter, and conductivity was measured with a Yellow Springs Instrument Model #33 salinity-conductivity-temperature meter.

3.2.4 Chemical Analysis. At test initiation (0 hour) and test termination (96 hours), a single sample from each test solution and the control was analyzed for Dobanol[®] 91-8 concentration. Sample containers were approximately 800 mL borosilicate glass bottles with teflon[®]-lined screw caps. Samples (300 mL) collected at 0 hour were removed from the excess test solution remaining in the 1000-mL volumetric flasks after the test vessels were filled. Additionally, a sample of the primary stock solution used to formulate the test solutions was collected for analysis at test initiation. Samples collected at 96 hours were removed from the composited test solution (replicates A, B and C) for each treatment level and the control after cell counts and water quality measurements were taken. At test termination, the test solutions were centrifuged at 1500 rpm for 20 minutes to remove algal cells from the test solutions prior to analysis. Approximately 275 mL of the supernatant of each composited solution was poured into the sample containers. On the day of collection, all samples were preserved with 0.1% formalin and delivered to Battelle Ocean Sciences, Duxbury, Massachusetts for analysis. Samples were analyzed in accordance with methods described in Battelle Ocean Sciences Study #SD-930117 (Appendix IV). All of the glassware used in testing and sample collection was thoroughly washed with sequential rinses of a 10% solution of nitric acid, acetone, distilled-deionized water, isopropanol and distilled-deionized water.

4.0 STATISTICAL ANALYSIS

The cell density of each culture at each 24-hour interval was calculated by dividing the number of cells counted by the total volume of culture examined. A mean and standard deviation was calculated for the cell density of each treatment level and the control.

The highest test concentration that caused no statistical adverse effect on cell density, the No-Observed-Effect Concentration (NOEC), was determined using Williams' Test (Williams, 1971, 1972). The data were first checked for normality using the Shapiro-Wilks Test (Weber *et al.*, 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). All statistical determinations were made at the 95% level of certainty, except in the case of Bartlett's and Shapiro-Wilks Tests, where a level of 99% certainty was applied.

EC10, EC50 and EC90 values (the concentration of test material which reduced cell densities by 10, 50 and 90%, respectively) were calculated based on cell density after 24, 48, 72, and 96 hours of exposure. The EC10, EC50 and EC90 values and their 95% confidence limits were determined by linear regression of response (percent reduction of cell density as compared with the control) vs. mean measured exposure concentration over the range of test concentrations where a clear exposure-response relationship was observed. Four linear regressions were estimated based on (a) untransformed data, (b) untransformed response vs. logarithm-transformed concentration, (c) probit-transformed response vs. untransformed concentration, and (d) probit-transformed response vs. logarithm-transformed concentration. The regression that best fit the data was selected based on the highest coefficient of determination (r^2). This regression equation was then applied to estimate the EC values and their 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981).

5.0 RESULTS AND DISCUSSION

5.1 Preliminary Testing

A preliminary range-finding exposure was conducted at Springborn from 4 to 8 October 1993 at nominal concentrations of 0.010, 0.10, 1.0, 10 and 100 mg/L. Duplicate exposure vessels were established for each concentration and control. Following 96 hours of exposure, cell densities in the treatment levels (0.010 to 100 mg/L) averaged 261, 219, 123, 7.6 and 0 x 10⁴ cells/mL, respectively. The control solution averaged 262 x 10⁴ cells/mL. Based on these results, nominal concentrations of 0.080, 0.16, 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L were selected for the definitive study.

5.2 Definitive Testing

5.2.1 Evaluation of Test Conditions - Conductivity, pH, temperature and light intensity measurements recorded during the test are presented in Table 2. Conductivity of the exposure solutions ranged from 70 to 90 μ mhos/cm throughout the exposure period. The pH of the exposure solutions ranged from 7.2 to 7.6 at test initiation, increasing to 7.7 to 8.5 at test termination. This pH change is common in static algal cultures due to photosynthesis by the algae. Continuous temperature monitoring established that the temperature ranged from 24 to 25 °C throughout the study period. The shaking rate was maintained throughout the exposure at a constant rate of 100 rpm. Light intensity of the test area ranged from 3500 to 5100 lux (325 to 475 footcandles).

5.2.2 Analytical Results - The results of the analysis of the primary stock solution and the test solutions for Dobanol[®] 91-8 concentrations are summarized in Table 3. Results of the analysis of the primary stock solution (20,000 mg/L) used to formulate the test solutions indicate the concentration of Dobanol[®] 91-8 was 99.6% of nominal concentration. Measured concentrations for all treatment levels were consistent between sampling intervals. Mean measured concentrations averaged 84% of nominal and defined the treatment levels as 0.074, 0.15, 0.24, 0.51, 0.89, 1.9, 4.4 and 9.1 mg/L. Analytical results are presented in Battelle Ocean Sciences Study #SD-930117 (Appendix IV).

5.2.3 Biological Results - Cell densities determined at each observation interval are presented in Table 4. At test termination, cell fragments and bloated cells were observed in the six highest treatment levels (0.24, 0.51, 0.89, 1.9, 4.4 and 9.1 mg/L). Cells exposed to the remaining treatment levels (0.074 and 0.15 mg/L) and the control were observed to be normal throughout the exposure. At test termination, cell densities followed the established concentration gradient, decreasing with increasing test concentration. At test termination, cell density in the 0.15, 0.24, 0.51, 0.89, 1.9, 4.4 and 9.1 mg/L treatment levels averaged 62, 48, 35, 25, 20, 10 and 2×10^4 cells/mL, respectively, which were significantly reduced compared to the cell density of the control (101×10^4 cells/mL). Cell density at the 0.074 mg/L treatment level was

97×10^4 cells/mL, which was comparable to the control. Based on these results, the 96-hour No-Observed-Effect Concentration (NOEC) for cell density was determined to be 0.074 mg/L.

Since no test concentration completely inhibited algal growth, a subsample of (0.40 mL) was removed from a composite of the highest test concentration (10 mg/L, nominal) which most severely inhibited algal growth to determine algistatic/algicidal effects. This subsample was diluted to 50 mL with fresh AAP medium to yield a nominal concentration of 0.080 mg/L and resulted in an estimated cell density of 0.014×10^4 cells/mL. After two days, 13×10^4 cells/mL were observed and after four days, 77×10^4 cells/mL were observed. The growth of *S. capricornutum* after transfer to fresh medium indicates that Dobanol[®] 91-8 had an algistatic, rather than an algicidal, effect at the 10 mg/L nominal test concentration.

Table 5 presents the EC10, EC50 and EC90 values and their corresponding 95% confidence limits. The 96-hour EC50 value for Dobanol[®] 91-8, based on cell density, was calculated to be 0.35 mg/L (95% confidence limits of 0.089 and 1.3 mg/L).

QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "Dobanol[®] 91-8 - Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*" were inspected by the Quality Assurance Unit (QAU) at Springborn Laboratories Inc., Environmental Sciences Division to determine adherence with the study protocol and laboratory standard operating procedures. In addition, inspection of certain phases of the in-life portion of the study was performed. Dates of study inspections, dates reported to the Study Director and to Management are listed below.

Based on these inspections, it was determined that this report accurately reflects the raw data collected during this study.

<u>Inspection Date</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
10/27/93	10/27/93	11/5/93
11/17/93	11/17/93	11/19/93
1/6/94	1/6/94	1/14/94
1/10/94	1/11/94	1/14/94
1/11/94	1/11/94	1/14/94
2/11/94	2/11/94	2/11/94

SPRINGBORN LABORATORIES, INC.

Doreen S. Newhouse 11 Feb 94

Doreen S. Newhouse
Supervisor, Quality Assurance Unit

Date

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TABLES

Table 1. Composition of algal growth medium (AAP medium) used in this study.

Compound	Concentration
NaNO ₃	25.5 mg/L
MgCl ₂ · 6H ₂ O	12.16 mg/L
CaCl ₂ · 2H ₂ O	4.41 mg/L
MgSO ₄ · 7H ₂ O	14.7 mg/L
K ₂ HPO ₄ · 3H ₂ O	1.368 mg/L
NaHCO ₃	15.0 mg/L
H ₃ BO ₃	185.5 µg/L
Na ₂ SeO ₄ ^a	1.88 µg/L
MnCl ₂ · 4H ₂ O	415.4 µg/L
ZnCl ₂	3.270 µg/L
CoCl ₂ · 6H ₂ O	1.43 µg/L
CuCl ₂ · 2H ₂ O	0.012 µg/L
Na ₂ MoO ₄ · 2H ₂ O	7.26 µg/L
FeCl ₃ · 6H ₂ O	159.8 µg/L
Na ₂ EDTA · 2H ₂ O	300.0 µg/L

pH was adjusted to 7.5 ± 0.1 with 0.1 N NaOH or 0.1 N HCl

^a Additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.

Source: Miller, W.E., J.C. Greene and T. Shiroyama. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA 600/9-78-018. U.S. Environmental Protection Agency, Washington, D.C.

Table 2. Conductivity, pH, temperature and light intensity measured during the 96-hour exposure of *Selenastrum capricornutum* to Dobanol[®] 91-8.

Nominal Concentration (mg/L)	pH				Conductivity (μ mhos/cm)	
	0-Hour	96-Hour			0-Hour	96-Hour
		A	B	C		
Control	7.6	8.3	8.2	8.2	80	70
0.080	7.6	8.3	8.0	8.0	80	70
0.16	7.4	7.7	7.7	7.9	80	80
0.31	7.3	7.9	7.8	7.7	80	80
0.63	7.2	8.5	8.4	7.9	90	80
1.3	7.2	8.5	8.0	8.0	90	80
2.5	7.2	8.0	8.4	7.8	90	80
5.0	7.2	7.8	8.0	8.2	90	80
10	7.2	8.0	7.9	7.8	90	80

Minimum/Maximum Temperature (°C)

24-hour	48-hour	72-hour	96-hour
25/25	25/25	24/25	25/25

Light Intensity^a

	0-hour	24-hour	48-hour	72-hour	96-hour
footcandles:	325-475	325-475	325-475	325-475	325-475
lux:	3500-5100	3500-5100	3500-5100	3500-5100	3500-5100

^a Range represents measurements on both shaking tables used.

Table 3. Concentrations of Dobanol[®] 91-8 measured in the exposure solutions during the 96-hour toxicity test with *Selenastrum capricornutum*.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)			
	0-Hour	96-Hour ^a	Mean ^b	% Nominal ^{bc}
Control	ND ^d	0.021	NA ^e	NA
0.080	0.063	0.086	0.074	93
0.16	0.16	0.15	0.15	94
0.31	0.27	0.20	0.24	77
0.63	0.62	0.41	0.51	82
1.3	1.2	0.56	0.89	68
2.5	2.2	1.7	1.9	78
5.0	4.8	4.1	4.4	89
10	9.8	8.5	9.1	91
Stock Solution ^f (20,000)	20,000	NA	NA	99.6

^a Samples were centrifuged for 20 minutes at 1500 rpm to remove algal cells from test solutions prior to analysis.

^b Calculated values are based on actual analytical results and not on rounded values (two significant figures) presented in this table.

^c Mean % of Nominal = 84%

^d ND = Not detected; less than the limit of detection (LOD)

^e NA = Not Applicable

^f Nominal concentration of stock solution is presented in parentheses.

Table 4. Cell density ($\times 10^4$ cells/mL) of *Selenastrum capricornutum* after 24, 48, 72 and 96 hours of exposure to Dobanol[®] 91-8.

Mean Measured Concentration (mg/L)		Cell Density ($\times 10^4$ cells/mL)				96-Hour % Inhibition
		Observation Interval (Hours)				
		24	48	72	96	
Control	A	3	21	58	91	NA
	B	4	21	71	109	
	C	3	17	66	103	
	Mean(SD) ^a	3(1)	20(2)	65(6)	101(9)	
0.074	A	4	12	43	89	12
	B	3	17	78	95	6
	C	4	12	68	107	-6
	Mean(SD) ^a	3(1)	14(3)	63(18)	97(9)	4
0.15	A	1	6	34	61	40
	B	2	12	53	64	37
	C	2	14	45	60	40
	Mean(SD) ^a	2(1)	10(4)	44(10)	62(2) ^b	39
0.24	A	2	7	34	42	59
	B	2	6	44	49	51
	C	4	12	45	54	47
	Mean(SD) ^a	2(1)	8(3) ^c	41(6) ^c	48(6) ^{bcd}	52
0.51	A	1	6	22	26	75
	B	3	4	20	43	57
	C	2	6	25	38	63
	Mean(SD) ^a	2(1)	5(1) ^{cd}	22(3) ^{cd}	35(9) ^{bcd}	65
0.89	A	2	6	17	18	82
	B	2	7	19	25	75
	C	2	6	17	32	69
	Mean(SD) ^a	2(<1)	6(1) ^{cd}	18(1) ^{cd}	25(7) ^{bcd}	75

^a Mean and standard deviation (SD) are calculated from original raw data, not from the rounded values (two significant figures) presented in this table.

^b Significantly different when compared to control data, according to William's Test.

^c Cell fragments were observed.

^d Bloated cells were observed.

Table 4. Continued. Cell density ($\times 10^4$ cells/mL) of *Selenastrum capricornutum* after 24, 48, 72 and 96 hours of exposure to Dobanol[®] 91-8.

Mean Measured Concentration (mg/L)		Cell Density ($\times 10^4$ cells/mL)				96-Hour % Inhibition
		Observation Interval (Hours)				
		24	48	72	96	
1.9	A	1	3	12	24	77
	B	2	2	13	18	82
	C	2	4	14	19	81
	Mean(SD) ^a	1(<1) ^c	3(1) ^{cd}	13(1) ^{cd}	20(3) ^{bcd}	80
4.4	A	1	1	7	9	91
	B	1	2	9	10	90
	C	2	1	8	11	89
	Mean(SD) ^a	1(1) ^c	1(<1) ^{cd}	8(1) ^{cd}	10(1) ^{bcd}	90
9.1	A	<1	<1	2	1	99
	B	1	1	1	2	98
	C	1	1	1	2	98
	Mean(SD)	1(<1) ^{cd}	1(<1) ^{cd}	2(1) ^{cd}	2(1) ^{bcd}	98

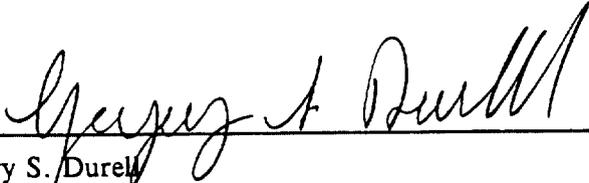
^a Mean and standard deviation (SD) are calculated from original raw data, not from the rounded values (two significant figures) presented in this table.

^b Significantly different when compared to control data, according to William's Test.

^c Cell fragments were observed.

^d Bloated cells were observed.

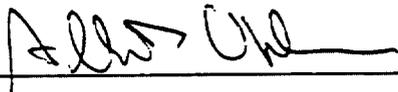
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Analytical Chemistry Task Leader
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01/13/94

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1/13/94

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Carolyn A. Matula
Project Monitor
Shell Development Company

1 February, 1994

Date

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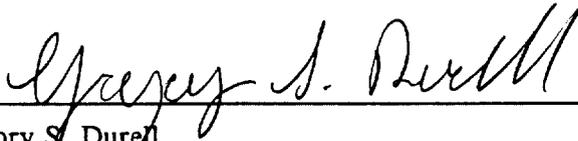
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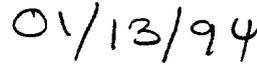
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COMPLIANCE STATEMENT

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



Date

QUALITY ASSURANCE STATEMENT

for

**Measurement of Dobanol® 91-8 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

Rosanna L. Buhl
Quality Assurance Coordinator
Battelle Ocean Sciences

1-13-94

Date

QUALITY ASSURANCE AUDITS

Conducted for SD-930117

**Measurement of Dobanol® 91-8 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	09/13/93	09/15/93	09/22/93	09/15/93
Lab Inspection	11/09/93 11/10/93	01/03/94	01/13/94	01/13/94
Data Package	12/28-31/93	12/31/93	01/13/94	01/13/94
Report Review	12/28-31/93	12/31/93	01/13/94	12/31/93

Table 5. EC10, EC50 and EC90 values for Dobanol[®] 91-8 calculated from results (cell density) of the 96-hour toxicity test with *Selenastrum capricornutum*.

X = Mean Measured Concentration (mg/L).
Y = Percent Inhibition (in cell density, compared with control)

24-Hour Results	EC10	EC50	EC90
EC value (mg/L):	0.059	0.95	> 15
95% Confidence Limits:	0.0010 - 1.3	0.034 - 29	0.69 - 1000
Regression Equation:	Y = 51 + 33 Log(X)		
r ² :	0.56		
N:	24		
Concentration Range ^a :	0.074 - 9.1 mg/L		
48-Hour Results	EC10	EC50	EC90
EC value (mg/L):	0.013	0.19	2.6
95% Confidence Limits:	0.0029 - 0.050	0.050 - 0.65	0.75 - 9.8
Regression Equation:	Probit (Y) = 5.8 + 1.1 Log(X)		
r ² :	0.89		
N:	24		
Concentration Range ^a :	0.074 - 9.1 mg/L		
72-Hour Results	EC10	EC50	EC90
EC value (mg/L):	0.051	0.44	3.8
95% Confidence Limits:	0.0087 - 0.24	0.090 - 2.1	0.82 - 20
Regression Equation:	Y = 65 + 43 Log(X)		
r ² :	0.84		
N:	24		
Concentration Range ^a :	0.074 - 9.1 mg/L		

^a Exposure-response relationship was judged to be linear over this concentration range; values for this concentration range were included in the linear regression.

Table 5. Continued. EC10, EC50 and EC90 values for Dobanol[®] 91-8 calculated from results (cell density) of the 96-hour toxicity test with *Selenastrum capricornutum*.

X = Mean Measured Concentration (mg/L).
 Y = Percent Inhibition (in cell density, compared with control)

96-Hour Results	EC10	EC50	EC90
EC value (mg/L):	0.035	0.35	3.5
95% Confidence Limits:	0.0075 - 0.14	0.089 - 1.3	0.93 - 15
Regression Equation:	Y = 68 + 40 Log(X)		
r ² :	0.87		
N:	24		
Concentration Range ^a :	0.074 - 9.1 mg/L		

^a Exposure-response relationship was judged to be linear over this concentration range; values for this concentration range were included in the linear regression.

SIGNATURES AND APPROVAL

SUBMITTED BY: Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

PREPARED BY:

James R. Hoberg 2/11/94
James R. Hoberg Date
Study Director

Carlene Hardy 2/11/94
Carlene Hardy Date
Principal Investigator

Susan P. Shepherd 11 Feb 94
Susan P. Shepherd Date
Coordinator, Data Management
and Reporting Unit

APPROVED BY:

Donald C. Surprenant 2/11/94
Donald C. Surprenant Date
Program Manager
Environmental Toxicology

Doreen S. Newhouse 11 Feb 94
Doreen S. Newhouse Date
Supervisor
Quality Assurance Unit

This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

6.0 APPENDIX I - STUDY PROTOCOL

Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-3107

TEST PROTOCOL

PROTOCOL TITLE: Alcohol Ethoxylate Surfactants: Protocol for Conducting a 96-Hour Toxicity Test with the Freshwater Green Alga, *Selenastrum capricornutum*, Following TSCA Guideline § 797.1050

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: Shell Development Company

Address: P.O. Box 1380 Houston, Texas

Phone: (713) 493-8040

Sponsor Study No.: WRC Tox No. 49-1227 (Amudala)

Test Substance: Dobanol 91-8 2 8/31/93 web work #

Purity: 100% CAS# or LOT#: 68439-46-3

Additional Comments and/or Modifications:

Diana C. Wong 8/31/93
Sponsor Approval Date

TO BE COMPLETED BY SLI PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc., 790 Main St., Wareham MA 02571

Study Director: J. Hoberg SLI Study No.: 777.0993.6106.430

Test Concentrations: To be supplied by amendment.

Solvent Used: Dist. deionized CAS# or LOT#: water

Proposed Schedule: (Start) 13 Sept. 1993 (Completion) 30 Sept. 1993

Proposed Draft Report Date: 30 October 1993

James R. Hoberg 9/8/93
Study Director Date

Alcohol Ethoxylate Surfactants: Protocol for Conducting a 96-Hour Toxicity Test with
the Freshwater Green Alga, *Selenastrum capricornutum*, Following TSCA
Guideline § 797.1050

1.0 INTRODUCTION

The purpose of this test is to determine the effects of an alcohol ethoxylate surfactant on the growth of *Selenastrum capricornutum* under static conditions. The results of this study will be reported as the 96-hour EC10, EC50 and EC90 values; i.e., the concentrations of test substance that reduce culture density by 10, 50 and 90%, respectively, as compared with the control. The test procedures performed during the biological portions of the study will meet or exceed the standard procedures described in the U.S. EPA Toxic Substance Control Act (TSCA) Test Guidelines 797.1050 (U.S. EPA 1985) as amended in the Federal Register on 20 May 1987 (U.S. EPA 1987) and will meet the primary technical objectives of The Shell Research Limited/Sittingbourne Research Centre guidelines (SBT SOP No. 169, Edition No. 8).

2.0 MATERIALS AND METHODS

2.1 TEST ORGANISM.

- 2.1.1. **Justification for Test System.** Characteristics which make this test organism suitable for acute toxicity testing are their ease of culturing and handling, their sensitivity to a variety of chemical substances, and the extensive data base for this common freshwater algal species.
- 2.1.2. **Species.** *Selenastrum capricornutum* will be the alga used in this test. The particular strain and supplier of the test species will be identified in the final report.
- 2.1.3. **Source.** Culture conditions will be similar to the testing conditions (i.e., 24 ± 2 °C, continuous lighting at 3200 - 5400 lux (300 - 500 footcandles) and agitation rate at 100 ± 10 rpms). Cultures will be maintained in an environmental chamber and transfers will be made regularly into fresh medium to provide two- to seven-day-old cultures for test inoculations. Cultures used to inoculate the test solutions will be in logarithmic phase growth. Cultures will be maintained under the above conditions for at least the period of time from the last transfer.
- 2.1.4. **Reference Test.** In an effort to monitor the general health of the test organism culture, reference test will be conducted with *S. capricornutum* using copper nitrate as the toxicant. Results of this test will be evaluated based on measured test concentrations. A reference test will be conducted within 30 days of the definitive test (i.e., either 30 days prior to or 30 days following the definitive exposure).

2.2 PHYSICAL SYSTEM.

- 2.2.1. **Test Containers.** Test vessels will be 250-mL Erlenmeyer flasks containing 100 mL of test solution and will be covered with stainless steel caps which permit gas exchange. Flasks and caps will be autoclaved before use.
- 2.2.2. **Glassware Preparation.** All glassware used in testing will be thoroughly washed with detergent and rinsed with tap water. This will be followed by sequential rinsing with a 10% solution of nitric acid, acetone, distilled deionized water, isopropanol and finally distilled deionized water. The cleaned glassware will be stored in closed cabinets.
- 2.2.3. **Dilution Water.** Stock solutions used in the preparation of algal growth medium will be prepared by adding appropriate amounts of nutrients to sterile, deionized water. The stocks solutions will be stored in amber glass bottles in the dark at approximately 4°C to minimize photochemical changes, and will be renewed every six months. The test medium, Algal Assay Procedure (AAP) medium (Table I), will be prepared by adding appropriate volumes of stock solutions to sterile, deionized water. Culture medium will contain $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (300 $\mu\text{g/L}$), however, $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ will be excluded from the medium used in the toxicity and reference tests to avoid chelation. The medium will be allowed to equilibrate to test temperature before use. Each batch of medium will be adjusted to $\text{pH } 7.5 \pm 0.1$ with dilute hydrochloric acid or sodium hydroxide before use. Periodic analysis of representative samples of dilution water source will be conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the test organism. In addition, a sample of AAP medium will be analyzed monthly for total organic carbon (TOC) content.
- 2.2.4. **Replication and Control of Bias.** Three replicate flasks will be established for each treatment and control. Test flasks will be randomly positioned based on computer-generated random numbers, on an orbital shaker table in an environmentally-controlled chamber. Exposure flasks will be labelled by replicate and concentration or control.

2.3 CHEMICAL SYSTEM.

- 2.3.1. **Test Substance.** Upon arrival at Springborn Laboratories, Inc., the external packaging of the test substance will be inspected for damage. The packaging will be removed and the primary storage container will also be inspected for leakage or damage. The sample identity will be recorded and the material will be stored in the dark at approximately 20°C until used, unless specified differently by the test Sponsor.
- 2.3.2. **Toxicant Concentration Selection.** A 96-hour preliminary test will generally be conducted using five widely spaced test concentrations beginning with 1000 mg/L or the water saturation concentration. If less than 50% reduction in cell density occurs at 1000 mg/L or the water saturation concentration, a single concentration definitive test will be conducted at that maximum test concentration. If greater than or equal to 50% reduction in cell density is observed at 1000 mg/L or the water saturation

concentration, then five or more definitive test concentrations will be used. The definitive test concentrations will be selected in consultation with the Sponsor. A control, untreated algal growth medium, will be included in the test. Except for the control, the nominal concentration of test substance in each treatment will be approximately 50% of the next higher one. Definitive test concentrations will be selected to produce a sufficient response to determine an EC50. Additionally, a No-Observed-Effect Concentration (NOEC) will be defined.

- 2.3.3. **Stock Solution Preparation.** A primary stock solution at a final concentration of 1 - 2% active surfactant will be formulated on the day of test initiation in distilled deionized water. The surfactant will be heated to 50 - 60°C in a glass container until completely melted. The material will be stirred to ensure homogeneity and a glass pipet will be used to transfer the liquified material for weighing. The test substance will be weighed on an analytical balance for which a calibration log will be maintained. The stock solution will be mixed for several minutes. A Chemical Usage Log will also be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used. Secondary stocks, if necessary, may be prepared in distilled water or AAP media (if volume displacement of dilution water during test solution preparation will be greater than 1%).
- 2.3.4. **Test Solution Preparation.** Test vessels will be conditioned by rinsing with the appropriate test solution. Appropriate volumes of the test solutions will then be placed into the flasks.
- 2.3.5. **Sampling.** Samples (approximately 300-mL) will be collected from each test solution at test initiation and termination for analyses of test substance concentration. Additionally, the primary stock solution will be analyzed. Samples removed at test initiation will be collected from the freshly prepared exposure solutions before the algae is added. Test termination samples will be a composite of the three replicate solutions of each treatment level and control. The analytical samples collected at test termination will be centrifuged prior to preservation to remove algal cells. The supernatant of each centrifuged sample will be transferred to a borosilicate glass container and covered with a Teflon®-lined cap. All samples will be preserved with 1% formalin (i.e., 3 mL formalin/300 mL sample in a 500 mL bottle) and shipped within 24 hours to Battelle Ocean Sciences, Duxbury, Massachusetts for analysis. All glassware used during the sampling process will be prepared as described in Section 2.2.2.
- 2.3.6. **Analytical Chemistry.** Analyses of analytical samples will be conducted by Battelle Ocean Science, Duxbury, Massachusetts, using a Shell Development Company analytical method entitled "Analysis of Alcohol Ethoxylate Surfactants Using Solid Phase Extraction (SPE) and HPLC/ELSD (Evaporative Light Scattering Detection) in Dilute Aqueous Solutions".

2.4 EXPERIMENTAL CONDITIONS

- 2.4.1. **Inoculation.** Algae will be transferred aseptically from the stock cultures to each test vessel within 30 minutes of test solution preparation to provide an initial culture density of approximately 1.0×10^4 cells/mL.
- 2.4.2. **Monitoring.** Culture density in each test vessel will be monitored at 24, 48, 72 and 96 hours after the start of the test. Algal density will be determined by cell counts using a Neubauer Improved hemacytometer and a compound microscope. One sample will be taken from each test vessel, and one count will be made on each sample. One or more hemacytometer fields, each 0.1 X 0.1 cm in surface area and 0.01 cm deep, containing 0.0001 mL of culture, will be counted for each sample until at least 400 cells or four fields are counted.
- At the time of each culture density determination, visual observations will be made of any unusual appearances of the algal cells including cell size, shape, color, occurrence of flocculation, adherence to glass walls, and/or aggregation.
- 2.4.3. **Measurement of Water Quality Variables.** Conductivity and pH in each test concentration will be measured at the start and finish of the test. At test initiation, water quality variables will be measured in the test solution remaining in the preparation vessels subsequent to filling the individual test flasks. At test termination, pH is measured in each replicate test and control solution. Conductivity will be measured at test termination in a composite solution of the three replicate solutions for each test concentration and control.
- 2.4.4. **Photoperiod.** The tests will be continuously illuminated at a light intensity of 3200 - 5400 lux (300 - 500 footcandles) using Duro-Test, Inc., Vita Lite® fluorescent bulbs. Light intensity of the test area will be measured daily.
- 2.4.5. **Temperature.** The water temperature of the test solutions will be maintained at 24 ± 2 °C by controlling the air temperature within the environmental chamber. Test solution temperature will be continuously monitored with a minimum/maximum thermometer in an additional vessel containing water placed adjacent to the test vessels.
- 2.4.6. **Agitation.** The agitation rate of the orbital shaker will be monitored daily and maintained at 100 ± 10 rpms.
- 2.4.7. **Test Duration.** The test will be initiated when all test and control solutions have been inoculated with algae. The test will be terminated following the 96-hour observation interval.
- 2.4.8. **Acceptance Criterion.** The control cultures must be in log phase growth throughout the 96-hour exposure period or the test will be considered unacceptable.

- 2.4.9. Evaluation of Algicidal/Algistatic Effects. At test termination the following procedure is used to differentiate between algistatic and algicidal effects. A sample of known volume of the culture is taken from the composite of the three replicate solutions of each test concentration which completely inhibited algal growth. If algal growth is not completely inhibited at any test concentration, the highest test concentration that inhibited growth is used. Sufficient fresh algal growth medium is added to dilute the test chemical to a concentration that does not affect growth. This subculture is incubated under test conditions for up to 9 days, during which time, it is examined microscopically every other day to determine whether growth has resumed. As soon as growth is observed (i.e., 10X) the subculture test is discontinued.

3.0 DATA EVALUATION

Cell densities will be calculated as means and standard deviations for each group of control and exposure replicates.

For a single concentration test, cell densities in the single treatment concentration will be compared with cell densities in the control, using a t-Test. Cell density in the treatment group will also be expressed in terms of percent reduction or stimulation from the control.

Data from a five concentration test will be subjected to Williams' Test (Williams 1971, 1972) to determine the No-Observed-Effect Concentration (NOEC). The NOEC is defined as the highest test concentration which causes no significant reduction in cell density when compared to the control data. Williams' Test will be preceded by Shapiro-Wilk's and Bartlett's Tests which test for normality and homogeneity of the data set. If necessary, replicate values will be transformed using square root, arcsine square root or log conversion procedures. If either Shapiro-Wilk's or Bartlett's Tests continue to fail after these conversions, Dunn's Test, a non-parametric procedure, will be used to establish the NOEC. All comparisons will be made at 95% level of certainty ($P \leq 0.05$).

For each observation interval, EC10, EC50 and EC90 values (the concentrations of test substance which reduced culture density by 10, 50 and 90%, respectively) and 95% confidence limits will be determined by linear regression of response (percent reduction of culture density, as compared with control) vs. mean measured exposure concentration. Four linear regression curves will be computed based on (a) untransformed data, (b) untransformed response vs. logarithm-transformed concentration, (c) probit-transformed response vs. untransformed concentration, and (d) probit-transformed response vs. logarithm-transformed concentration. The regression line which provides the best fit of the untransformed or transformed data will be selected based on the highest coefficient of determination, i.e., r^2 . This regression equation will then be applied to calculate the EC10, EC50 and EC90 values and their 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981). A computer program developed and validated at SLI will be used to assist in these computations. The concentration-response data generated during this test will be provided to the Study Sponsor in Lotus® format.

4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

5.0 REPORTING

The raw data generated at Springborn Laboratories, Inc., and draft and final report will be reviewed by the SLI Quality Assurance Unit and Study Director. All measurements (e.g., water quality) will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will initially be submitted to the study Sponsor for review. Upon acceptance by the Sponsor, three (3) copies of the final report will be submitted. All reports include, but will not be limited to, the following information:

- * Springborn Laboratories, Inc., report and project numbers.
- * Laboratory and site, the dates of testing and personnel involved in the study, e.g., Quality Assurance Unit, Program Coordinator (if applicable), Study Director.
- * All information pertaining to the test substance which appears on the sample bottle, e.g., its source, percent active ingredient, physical properties, Sponsor's test substance I.D., and sample number.
- * Scientific name of the test organism, strain, source, and culturing information.
- * Results (i.e., 96-hour EC50 value and 95% confidence limits) of applicable copper nitrate reference test.
- * A copy of the periodic analysis of the dilution water source for concentrations of toxic metals and pesticides.
- * Range-finding study results.
- * Test container volume, test solution volume, and inoculum culture density.
- * Description of exposure solution preparation scheme.
- * Description of test conditions.
- * Criteria for determination of toxic effects and general observations on nonquantifiable effects.
- * A table of culture density measurements for each 24-hour interval.

- * Data on test temperatures, specific conductivity, pH, illumination and agitation.
- * The EC10, EC50 and EC90 values and 95% confidence limits for 24, 48, 72 and 96 hours of exposure, when possible, and calculation methods used. All calculations will be based on mean measured concentrations.
- * Deviations from the protocol not addressed in protocol amendments, together with a discussion of the impact on the study, signed by the Study Director.
- * Good Laboratory Practice (GLP) compliance statement (for the biological portion of the study) signed by the Study Director.
- * Dates of Quality Assurance reviews, signed by the QA Unit.
- * Location of raw data and final report.

6.0 PROTOCOL CHANGES

All amendments to the approved protocol will be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. All deviations will be documented by the Study Director. Protocol amendments and deviations will include the reasons for the change and the impact of the change on results of the study, if any. Amendments should be authorized by the Sponsor's contact or the Study Monitor in advance of initiation of definitive test. If necessary, amendments initially may be in the form of verbal authorization, followed by Springborn's written documentation of the amendment. In such a case, the effective date of the amendment will be the date of verbal authorization.

7.0 SPECIAL PROVISIONS

GOOD LABORATORY PRACTICES (GLP): All test procedures, documentation, records, and reports related to the biological portion of this study will comply with the U. S. Environmental Protection Agency's Good Laboratory Practices as promulgated under the Toxic Substance Control Act (FEDERAL REGISTER, Part IV, 17 August, 1989)

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final report, the test substance will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

DATA ARCHIVAL: All raw data and the final report will be archived at Shell Development Company unless different arrangements are made.

8.0 REFERENCES

- Miller W.E., J.C. Green and T. Shiroyama. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA 600/9-78-018. U.S. Environmental Protection Agency, Washington, D.C.
- Sokal, R.R., and F.J. Rohlf. 1981. *Biometry, 2nd Ed.* W.H. Freeman and Co., New York. 859 pp.
- U.S. Environmental Protection Agency. 1985. *Toxic Substances Control Act Test Guidelines*. Federal Register 50(188):39252-39516, September 27, 1985. Amended May 20, 1987, July 1, 1991 and July 1, 1992.
- U.S. Environmental Protection Agency 1989. *Toxic Substances Control; Good Laboratory Practice Standards; Final Rule*. (40 CFR, Part 792). Federal Register, Part III, 48(230):53922-53944, August 17, 1989.
- Williams, D.A. 1971. A test for differences between treatment means when survival dose levels are compared with a zero dose control. *Biometrics* 27: 103-117.
- Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28: 519-531.

TABLE I
COMPOSITION OF ALGAL ASSAY
PROCEDURE (AAP) MEDIUM

Compound	Final Concentration
NaNO ₃	25.5 mg/L
MgCl ₂ · 6H ₂ O	12.16 mg/L
CaCl ₂ · 2H ₂ O	4.41 mg/L
MgSO ₄ · 7H ₂ O	14.7 mg/L
K ₂ HPO ₄	1.044 mg/L
NaHCO ₃	15.0 mg/L
H ₃ BO ₃	185.52 µg/L
MnCl ₂ · 4H ₂ O	415.4 µg/L
ZnCl ₂	3.270 µg/L
CoCl ₂ · 6H ₂ O	1.43 µg/L
CuCl ₂ · 2H ₂ O	0.012 µg/L
Na ₂ MoO ₄ · 2H ₂ O	7.26 µg/L
FeCl ₃ · 6H ₂ O	159.8 µg/L
Na ₂ SiO ₃ · 9H ₂ O ^a	20 mg/L
Na ₂ EDTA · 2H ₂ O ^c	300 µg/L
Na ₂ SeO ₄ ^b	1.88 µg/L

^a Na₂SiO₃ is included in medium for the diatom *Navicula pelliculosa* only.

^b Additional nutrient required, personal communication, Dr. R.R.L. Guillard, June 1991.

^c Culture medium contains 300 µg/L Na₂ EDTA · 2H₂O. Test medium does not contain Na₂ EDTA · 2H₂O.

SOURCE: Miller W.E., J.C. Greene and T. Shiroyama. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA 600/9-78-018. U.S. Environmental Protection Agency, Washington, DC.

Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

PROTOCOL AMENDMENT

AMENDMENT #: 1

DATE: 21 October 1993

PROTOCOL TITLE: Alcohol Ethoxylate Surfactants: Protocol for Conducting a 96-Hour Toxicity Test with the Freshwater Green Alga, *Selenastrum capricornutum*, Following TSCA Guideline 797-1050

SPECIES: *Selenastrum capricornutum*

STUDY SPONSOR: Shell Development Company

TEST MATERIAL: Dobanol 91-8

SLI STUDY NO: 777.0993.6106.430

AMENDMENT(S):

1. The definitive test will be conducted at nominal concentrations of 10, 5.0, 2.5, 1.3, 0.63, 0.31, 0.16 and 0.080 mg/L.
2. The protocol states that the test medium used for the toxicity test and the reference test will not contain Na₂ EDTA 2H₂O. At the Sponsor's request, Na₂ EDTA 2H₂O will be added to the medium used for each test at a concentration of 300 µg/L.

Approval Signatures:

*James R. Hoberg*James R. Hoberg
SLI Study Director

10/21/93

Date

*Diana C. L. Wong*Diana C. L. Wong
Sponsor Contact

11/11/93

Date

Protocol #072993/TSCA/SHELL/SEL

NOV 1993


 Springborn
LABORATORIES

7.0 APPENDIX II - CERTIFICATE OF ANALYSIS

Shell Development Company
A Division of Shell Oil Company



Woodrow Research Center
P. O. Box 1380
Houston, TX 77251-1380

September 10, 1993

Pamela M. Lincoln
Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

Dear Ms. Lincoln:

The missing information, pertaining to the test substance Ocbanc1 91-8, that you requested of me in your letter dated 2 September, 1993 is as follows:

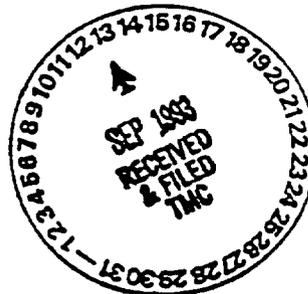
Lot Number:	97002310
% Active Ingredient:	100%
Net Amount Shipped	678 g
Molecular Weight	avg 510 g/mol

The certificate of analysis for this test substance is attached.

If you have any questions then please do not hesitate to contact me at the above address or by phone at (713) 493-7955.

Sincerely,

Carolyn A. Matula
Carolyn A. Matula



09/18/93 14:39 WESTHOLLOW EC BLDG - 9915082958107
Shell Chemicals

NO. 756 002

Shell Chemicals U.K. Limited

CARRINGTON WORKS URWISTON MANCHESTER M21 4AJ
TELEPHONE: 061-776 3000 TELEXC: 607301 FAX: 061-776 3616

Certificate of Analysis

Order reference 061 776 3456

Dolanol 41-8

Your ref

Our Ref MTE/88

Tank 241 SRO 15418

Date 24.08.92

Hydroxyl Value mg KOH/g	110.2
1% Ac Cloud Point E	31.3
Pet % m/m	0.25
Water % m/m	0.05

Free Alcohol	% wt	1.4
+ 1 ED		1.0
+ 2 ED		1.2
+ 3 ED		2.4
+ 4 ED		3.5
+ 5 ED		5.1
+ 6 ED		6.6
+ 7 ED		8
+ 8 ED		9
+ 9 ED		9.8
+ 10 ED		9.6
+ 11 ED		9.2
+ 12 ED		8.2
+ 13 ED		6.4
+ 14 ED		5.4
+ 15 ED		4.1
+ 16 ED		3.1
+ 17 ED		2.2
+ 18 ED		1.7
+ 19 ED		0.4
+ 20 ED		0.5
+ 21 ED		0.2
Average ED		3.2



Average ED

Metals



A Company Committed to Responsible Care

7741704 contains the essential details of the Certificate. It is available to 05 6730680 8082
Registered in England No. 407204. Registered Office: Haverthorpe House, Chester Business Park, Chester CH4 5CA

See Martin

8.0 APPENDIX III - DILUTION WATER ANALYSIS

Well ¹ Water Sample*		
Date Collected: 7/29/93 Date Reported: 9/17/93		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.3 µg/l	0.3
Toxaphene	< 4. µg/l	4.
PCBs	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03

¹ Well water supplemented by Town of Wareham water

* Analyzed by Lancaster Laboratories, Inc.

Well ¹ Water Sample*		
Date Collected: 8/9/93** Date Reported: 8/26/93		
Analysis	Result As Received	Limit of Quantitation
Mercury	< 0.00020 mg/l	0.00020
Arsenic	< 0.20 mg/l	0.20
Selenium	< 0.20 mg/l	0.2
Boron	< 0.040 mg/l	0.04
Thallium	< 0.30 mg/l	0.3
Aluminum	< 0.20 mg/l	0.2
Antimony	< 0.20 mg/l	0.2
Barium	< 0.10 mg/l	0.1
Beryllium	< 0.010 mg/l	0.01
Cadmium	< 0.010 mg/l	0.01
Calcium	7.71 mg/l	0.2
Chromium	< 0.050 mg/l	0.05
Cobalt	< 0.050 mg/l	0.05
Copper	< 0.020 mg/l	0.02
Iron	< 0.10 mg/l	0.1
Lead	< 0.10 mg/l	0.1
Magnesium	2.31 mg/l	0.1
Manganese	< 0.010 mg/l	0.01
Molybdenum	< 0.10 mg/l	0.1
Nickel	< 0.050 mg/l	0.05
Potassium	1.07 mg/l	0.5
Silver	< 0.020 mg/l	0.02
Sodium	14.0 mg/l	0.4
Titanium	< 0.010 mg/l	0.01
Vanadium	< 0.010 mg/l	0.01
Zinc	< 0.040 mg/l	0.04
Total Organic Carbon ***	< 1. mg/L	1.
¹ Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		
** The original sample for this analysis (collected 7/29/93) was damaged during transport. Resampled 8/9/93.		
*** Represents "non-purgeable TOC"		

9.0 APPENDIX IV - ANALYTICAL METHODOLOGY

FINAL DATA REPORT

Study Title

Measurement of Dobanol® 91-8 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

Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

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Battelle Study Number

SD-930117

STUDY PARTICIPANTS

SD-930117

**Measurement of Dobanol® 91-8 in Dilute Aqueous Samples in Support of
Aquatic Toxicity Testing with *Selenastrum capricornutum***

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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 Springborn Laboratories, Inc. 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 Springborn Laboratories. 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 August 27, 1993, and by the Sponsor's Project Monitor on August 31, 1993.

1.1 Test Substance Identification

The Test Substance analyzed in this study was an alcohol ethoxylate (AE) surfactant (Dobanol 91-8®). 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:	Dobanol® 91-8
Test Substance CAS#:	68439-46-3
Test Substance Lot#:	LR21662-54
Test Substance Purity:	100%

Test Substance Composition:	A C ₉ -C ₁₁ alcohol ethoxylate with an average of 8 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.
Test Substance Solubility:	Completely soluble in water. May form gel.
Test Substance Stability:	Stable. A stability and holding time of two years from receipt at Battelle was assigned to the Test Substance.
Test Substance Storage Requirements:	Ambient temperature or lower.

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 C₈ 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.

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 August 31, 1993. 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 (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 five-point multilevel calibration. Test samples followed the initial calibration in the analysis sequence, and a calibration check standard was analyzed at least every 12-14 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 five-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 31, 64, 103, 168, and 258 $\mu\text{g/mL}$. 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, and 80 and 160 parts per billion (ppb) nominal concentration samples was 250 μL . For the 310, 630, and 1,300 ppb nominal concentration samples the PIV was 500 μL , and it was 2.00 mL for the BS, MS, and MSD samples and samples with nominal concentrations of 2,500, 5,000, and 10,000 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.

$$\% \text{ Recovery} = \text{WC}_D \times (1 / \text{WC}_S) \times 100\%$$

$$\% \text{RPD} = [\% \text{REC}_{\text{MS}} - \% \text{REC}_{\text{MSD}}] \times (2 / (\% \text{REC}_{\text{MS}} + \% \text{REC}_{\text{MSD}})) \times 100\%$$

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 considered detected.

The water sample equivalent LOD was calculated by comparing the peak height equivalent to a signal:noise ratio of 3:1 in the sample to the peak height of the analyte in the low-level calibration standard, 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 concentrations.

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

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

H_{3x} = Peak height equivalent to $3 \times$ 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 was performed in one analytical batch. Table 1 also presents the data for the Primary Stock Solution analyses.

The measured analyte concentrations in the test samples that had been fortified with the Test Substance ranged from 62.8 ppb (for sample NB81, a sample with a nominal concentration of 80 ppb) to 9,766 ppb (for sample NB88, a sample with a nominal concentration of 10,000 ppb). The measured concentrations were between 43 percent (sample NB95) and 107 percent (sample NB91) of the nominal concentration, with the majority of the samples having a measured concentration ranging from approximately 65 to approximately 100 percent of the nominal concentration. On an average, the concentrations in the $t=96$ hour samples were slightly lower than in the $t=0$ hr samples, suggesting that there may be a slight loss of the analyte with time.

A small amount of the analyte was detected in one of the two toxicological control samples; 21.4 ppb was measured in sample NB90, the $t=96$ hr control. The signal/peak that was responsible for the reported concentration did not resemble a Dobanol® 91-8 signal, and was probably not due to Dobanol® 91-8 contamination. However, it is reported because it would interfere with a Dobanol® 91-8 signal. The concentrations reported for samples NB91 and NB92 most likely have contribution

Table 1. Dobanol® 91-8 Concentrations in Samples Received from the Toxicological Testing Laboratory

Battelle Sample ID	Test Sample Time/Type	Nominal Conc. (ppb)	Measured Conc. (ppb)
Batch #1			
NB80	t=0	0	ND
NB81	t=0	80	62.8
NB82	t=0	160	155
NB83	t=0	310	274
NB84	t=0	630	617
NB85	t=0	1,300	1,221
NB86	t=0	2,500	2,243
NB87	t=0	5,000	4,822
NB88	t=0	10,000	9,766
NB90	t=96	0	21.4
NB91	t=96	80	86.0
NB92	t=96	160	146
NB93	t=96	310	203
NB94	t=96	630	410
NB95	t=96	1,300	558
NB96	t=96	2,500	1,650
NB97	t=96	5,000	4,077
NB98	t=96	10,000	8,450
Primary Stock Solution		(ppm)	(ppm)
NC06	t=0, stock	20,000	19,920

ND: Not detected; <LOD.

from this interference, the magnitude of which is probably comparable to the concentration reported for NB90. The sample extracts from samples NB90, NB91, and NB92 were bright green in color, and the chromatograms showed significant amounts of non-Dobanol® 91-8 sample components. These three samples were the t=96 hr samples that had the greatest concentration factor in the sample preparation/processing (a factor of 1000), and the rest of the samples were concentrated much less (factors of 25 to 200 times) and had no evidence of significant matrix interference or other matrix constituents.

The concentration measured for the Primary Stock Solution was only slightly lower than the expected concentration, with a measured concentration of 19,920 ppm, as compared with a nominal/expected concentrations of 20,000 ppm. The measured Primary Stock Solution concentration was within 1 percent of the expected concentration.

3.2 Analytical Results — Quality Control Samples

All quality control objectives were met for this work. The five-point multi-level instrument calibration used had a correlation coefficient of 0.999836 for the quadratic equation generated, and the continuing calibration check analyses ranged from 1.9 to 3.9 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)	10.0 ppb
Limit of Quantitation (LOQ)	30.9 ppb

Sample concentrations above the LOD were reported for this study. Sample concentrations below the LOD were reported as "ND" (not detected). 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. One sample (NB90, the t=96 hr control) had a detectable (i.e., above the LOD) amount of target analyte which was determined to be below the LOQ — 21.4 ppb detected versus an LOQ of 30.9 ppb.

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 97%. The analyte recovery in the two matrix spike (MS/MSD) samples were 91% and 93%, and these data suggest that there were no significant matrix effects on the analytical procedure. Acceptable precision was observed for the analysis. The relative percent difference in the measured analyte recoveries for the MS/MSD duplicates was 2%.

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
- Verified 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.

Table 2. Laboratory Quality Control Sample Analysis Results

Battelle Sample ID	QC Sample Type	Recovery (%)
Batch #1		
NB76-PB	Procedural Blank	ND
NB77-BS	Blank Spike	97.3
NB78-MS	Matrix Spike	91.1
NB79-MSD	Matrix Spike Duplicate	93.4
	MS/MSD %RPD:	2.4

ND: Not detected; <LOD.

APPENDIX A

Deviations to Analytical Method

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930117

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Provision of Test Substance

The Test Substance for this study was provided by the Sponsor, not the toxicological testing laboratory as specified in the Analytical Procedure Document. The Sponsor communicated that the Test Substance provided to Battelle and the toxicological testing laboratory came from the same lot/source.

Approved:

Gregory S. Durell

Date:

12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930117

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Purity of Nitrogen Gas for ELSD Detector

The purity of the liquid nitrogen used for the ELSD detector is guaranteed by the supplier (Liquid Carbonic) to be a minimum of 99.998%, not 99.999% as specified in the Analytical Procedure Document. The typical purity, as specified by the supplier, is 99.999%.

Approved:

Gregory S. Durell

Date:

12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930117

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No _____

Entered by: Gregory S. Durell

Date: January 7, 1994

Subject: Sample Receipt and Shipment from the Toxicological Testing Laboratory

The samples shipped by the toxicological testing laboratory to Battelle for analysis were received in coolers or boxes with no ice packs and were at ambient temperature. They were not shipped in coolers with ice packs as specified in the Analytical Procedure Document. This issue was communicated to the Sponsor by telephone who verbally approved Battelle to proceed with the work on the samples received. The lack of refrigeration while in transit should not impact the integrity of these samples.

Approved: _____

Gregory S. Durell

Date: _____

01/07/94

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930117

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 6, 1993

Subject: Return and Archival of Test Substance

Only a small amount of Test Substance was received by Battelle for this work (in a vial of approximately 4 mL volume) and the unused material at the end of the study will be kept and archived at Battelle, not returned to the toxicological testing laboratory.

Approved:

Gregory S. Durell

Date:

12/06/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 1

Project Title: Gap-Filling Project

Study Number: SD-930117

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: December 20, 1993

Subject: Calculations of Original Water Sample Concentrations

The original water sample concentrations were calculated in spreadsheet tables by applying the water extraction volumes (WEV) and pre-injection volumes (PIV) to the sample extract concentrations determined using the chromatography software in the Hewlett-Packard LAS data system. These calculations are described in the Study Report. The Analytical Procedure Document specified that the original water sample concentrations be determined directly in the H-P LAS datasystem by entering the WEV and PIV data there.

Analyte concentrations of the original water samples were determined in parts per billion (ppb). Analyte concentration of the primary stock solution samples were determined in parts per million (ppm).

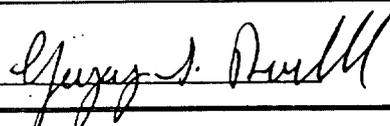
$$\text{Determined Water Sample Concentration (ppb)} = \text{EC} \times \text{PIV} \times (1/\text{WEV}) \times 1000$$

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

EC = Extract (HPLC sample) concentration ($\mu\text{g}/\text{mL}$ = 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)Approved: Date: 12/20/93

BATTELLE OCEAN SCIENCES
Miscellaneous Documentation Form

Page 1 of 2

Project Title: Gap-Filling Project

Study Number: SD-930117

Battelle Project Number: N8328-0002

This is a Deviation to Analytical Method:

Yes X No

Entered by: Gregory S. Durell

Date: January 7, 1994

Subject: Miscellaneous Deviations to Analytical Method

Sample extracts were stored in sealed vials/tubes until HPLC analysis could be performed, and not transferred to HPLC vials on the day the sample processing was completed, as specified in the Analytical Procedure Document. The PIVs were not always adjusted on the day that sample extraction was completed or that samples were submitted for HPLC analysis. The PIV was carefully visually inspected for each sample prior to transfer for instrumental analysis. These deviations have no impact on the integrity of the samples or results, because of the stability of the test substance and because proper storage was maintained.

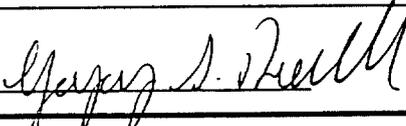
Concentrator tubes were not rinsed and the rinsate transferred to a 4 mL vial for storage on the day sample processing was completed, as indicated in the Analytical Procedure Document and associated sample preparation check list. Instead the procedure was to store the securely sealed concentrator tube refrigerated until HPLC analysis was completed and then rinse and transfer any remaining sample in the concentrator tube and HPLC vial to a common vial of suitable size (sometimes 4 mL and sometimes larger). This deviation has no impact on the integrity of the samples because proper storage was maintained.

The Test Substance was stored refrigerated and not at room temperature as specified in the Analytical Procedure Document. This deviation has no impact on the integrity of the material or results, because suitable storage conditions were maintained.

Not all of the 7 concentration levels listed in the Study-Specific were analyzed by HPLC at the beginning of the analytical sequence. These 7 standards had been analyzed by HPLC prior to the analysis of samples for this study, and the approximate response was therefore known. A total of 5 standards were analyzed and a 5-point calibration generated, in accordance with the Analytical Procedure Document.

There were a few instances of incomplete temperature monitoring of Refrigerator #2 (where water samples are stored pre-extraction and extracts stored until archival) during the performance of this work. Specifically, during the third week of November the monitoring/logging was missed three times, during the fourth week of November it was missed one time, and during the first week of December it was missed one time. However, all sample extraction had been completed by these dates, and only sample extracts (in methanol) were stored in the refrigerator. Sample extracts can be stored at, or below, room temperature. This deviation would not impact the integrity of the samples or the quality of the data.

There was one instance in the HPLC analysis sequence when there were 14 samples between two calibration check standards. The Analytical Procedure Document specifies that calibration check standards be analyzed no less frequently than every 12 samples. However, this deviation is not expected to have any impact on the quality of the data because all calibration checks in the sequence (including before and after the set of 14 samples) consistently easily met the calibration criteria.

Approved:  Date: 01/07/00
