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March 26, 2007

**Contains No CBI**

TSCA Confidential Business Information Center (7407M)  
EPA East – Room 6428  
Attn: TSCA Section 8(e) Coordinator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, NW  
Washington, DC 20460-0001

C



Re: Supplemental Submission to 8EHQ-06-16440 TSCA Section 8(e) Notification of Substantial Risk: *In Vitro* Mammalian Chromosome Aberration Test

Dear TSCA Section 8(e) Coordinator:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act (TSCA), as interpreted in the Statement of Interpretation and Enforcement Policy (68 Federal Register 33129; June 3, 2003) and other Agency guidance, the Silicones Environmental, Health and Safety Council of North America (SEHSC)<sup>1</sup>, on behalf of its member companies, submits the following information as a supplemental submission to our initial TSCA Section 8(e) notification on April 20, 2006, 8EHQ-06-16440. The submission was based on the preliminary findings that dimethylsilanediol induced chromosomal aberrations in the *In Vitro* Mammalian Chromosomal Aberration Test (OECD 472) when tested at 1250 – 5000 ug/ml. It was determined later that this concentration range (13.5 – 54 mM) exceeds that specified in the guideline for this material (10 mM). As such the test was repeated evaluating DMSD at 10 mM and below. No clastogenicity was demonstrated at these levels. *In vivo* testing to assess the mutagenic potential of DMSD is not planned given the negative findings in the repeat testing. This supplemental submission summarizes the findings from the repeated study at the lower dose levels.

Neither SEHSC, nor any member company, has made a determination at this time that any significant risk of injury to human health or the environment is presented by these findings.

**Chemical Substance**

1066-42-8 Dimethylsilanediol



**Finalized Study**

*In Vitro* Mammalian Chromosome Aberration Test. BioReliance study number: AB21RP.331.BTL. (This study was conducted in compliance with the testing guidelines of the ICH (1996 and 1997) and the OECD (1998).)

<sup>1</sup> SEHSC is a not-for-profit trade association whose mission is to promote the safe use of silicones through product stewardship and environmental, health and safety research. The Council is comprised of North American silicone chemical producers and importers.

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### Summary

The test article, dimethylsilanediol (DMSD), was tested in an *in vitro* chromosome aberration assay using Chinese hamster ovary (CHO) cells, in both the absence and presence of an Aroclor-induced S9 activation system. A preliminary toxicity test was performed to establish the dose range for the chromosome aberrations assay. The dosing solution concentrations were adjusted to compensate for the purity of 99.0%, however, the actual purity of the test article was 98.1%.

Water was the solvent of choice based on the solubility of the test article and compatibility with the target cells. The test article was soluble in water at a concentration of approximately 50 mg/ml, the maximum concentration tested for solubility.

In the preliminary toxicity assay, the maximum dose tested was 5000 µg/ml (54 mM). The test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.

Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in all three exposure groups. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 625 to 5000 µg/ml for all three exposure groups. The molecular weight was inadvertently not provided to the laboratory. Upon review of the results of the initial chromosome aberration assay, it was discovered that the test article was tested at an excessive concentration of 54 mM (5000 µg/ml). The chromosome aberration test was repeated at dose levels of 230, 460, and 920 µg/ml in the non-activated 4 and 20-hour treatment groups and in the S9-activated 4-hour treatment group. In the repeat chromosome aberration assay, the cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours after treatment initiation. The test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period. All three dose levels tested were selected for microscopic analysis in all three treatment groups. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ( $p > 0.05$ , Fisher's exact test).

Based on findings from the repeat assay at dose levels up and including the guideline maximum, Dimethylsilanediol (DMSD) was negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the non-activated and the S9-activated test systems.

**Summary Tables**

**Analysis of CHO cells treated with DMSD in the  
 Absence of Exogenous Metabolic Activation**

Treatment µg/ml	Treatment Time	Mean Mitiotic Index	Cells Scored		Aberrations per Cell (mean ± SD)	Cells with Aberrations	
			Numerical	Structural		Numerical,%	Structural,%
Water	4	8.6	200	200	0.010 ± 0.100	4.5	1.0
DMSD							
230	4	8.2	200	200	0.010 ± 0.100	3.5	1.0
460	4	9.1	200	200	0.000 ± 0.000	0.0	0.0
920	4	9.0	200	200	0.005 ± 0.071	0.0	0.5
MMC <sup>(1)</sup>							
0.2	4	6.5	200	100	0.620 ± 1.813	0.0	20.0**

Treatment µg/ml	Treatment Time	Mean Mitiotic Index	Cells Scored		Aberrations per Cell (mean ± SD)	Cells with Aberrations	
			Numerical	Structural		Numerical,%	Structural,%
Water	20	9.7	200	200	0.005 ± 0.071	0.5	0.5
DMSD							
230	20	8.3	200	200	0.005 ± 0.071	0.5	0.5
460	20	8.2	200	200	0.005 ± 0.071	2.5	0.5
920	20	8.5	200	200	0.025 ± 0.291	1.5	1.0
MMC <sup>(1)</sup>							
0.1	20	5.4	200	100	0.480 ± 1.514	0.0	21.0**

<sup>(1)</sup> Mitomycin C, CAS number 50-07-7

\*\* p ≤ 0.01, Fisher's Exact Test

**Analysis of CHO cells treated with DMSD in the  
 Presence of Exogenous Metabolic Activation**

Treatment µg/ml	Treatment Time	Mean Mitiotic Index	Cells Scored		Aberrations per Cell (mean ± SD)	Cells with Aberrations	
			Numerical	Structural		Numerical,%	Structural,%
Water	4	8.6	200	200	0.005 ± 0.071	4.5	0.5
DMSD							
230	4	8.4	200	200	0.005 ± 0.071	0.0	0.5
460	4	8.2	200	200	0.000 ± 0.000	3.0	0.0
920	4	8.5	200	200	0.010 ± 0.100	0.0	1.0
CP <sup>(2)</sup>							
10	4	4.0	200	100	0.480 ± 1.514	0.0	24.0**

<sup>(2)</sup> Cyclophosphamide, CAS number 6055-19-2

\*\* p ≤ 0.01, Fisher's Exact Test

**Discussion**

Based on findings from the repeat assay at dose levels up and including the guideline maximum, Dimethylsilanediol (DMSD) was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the non-activated and the S9-activated test systems.

**Actions**

Previously proposed *In vivo* testing to assess the mutagenic potential of DMSD is not planned given the negative findings in the repeat testing.

A copy of the report is attached. If you have any questions concerning this study, please contact me at (703) 788-6570, [rmanning@sehsc.com](mailto:rmanning@sehsc.com), or at the address provided herein.

Sincerely,



Reo Menning  
 Executive Director

**FINAL REPORT**

**Study Title**

***In Vitro* Mammalian Chromosome Aberration Test**

**Test Article**

Dimethylsilanediol (DMSD)

**Authors**

Ramadevi Gudi, Ph.D.  
Meena Rao, B.S.

**Study Completion Date**

15 February 2007

**Testing Facility**

BioReliance  
9630 Medical Center Drive  
Rockville, Maryland 20850

**BioReliance Study Number**

AB21RP.331.BTL

**Sponsor**

Silicones Environmental Health and Safety Council (SEHSC)  
SEHSC, Suite 500, 2325 Dulles Corner Blvd.  
Herndon, VA 20171

STATEMENT OF COMPLIANCE

Study No. AB21RP.331.BTL was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the US EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice in all material aspects with the following exception:

Analyses to determine the uniformity, concentration or stability of the test article dosing preparations were not performed by the testing facility or the Sponsor.

Ramadevi Gudi  
Ramadevi Gudi, Ph.D.  
Study Director

15 Feb 2007  
Date

Valentine O. Wagner, III  
BioReliance Study Management

15 Feb 2007  
Date

# Quality Assurance Statement

**Study Title:** *In Vitro* Mammalian Chromosome Aberration Test

**Study Number:** AB21RP.331.BTL

**Study Director:** Ramadevi Gudi, Ph.D.

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**Inspect On:** 08-Mar-06 - 08-Mar-06 To Study Dir 08-Mar-06 To Mgmt 08-Mar-06  
**Phase:** Evaluation of Cytotoxicity

**Inspect On:** 30-Mar-06 - 31-Mar-06 To Study Dir 31-Mar-06 To Mgmt 31-Mar-06  
**Phase:** Draft Report and Data Audit

**Inspect On:** 29-Aug-06 - 29-Aug-06 To Study Dir 29-Aug-06 To Mgmt 31-Aug-06  
**Phase:** Revised Draft Report and Data Audit(B2)

**Inspect On:** 14-Feb-07 - 14-Feb-07 To Study Dir 15-Feb-07 To Mgmt 15-Feb-07  
**Phase:** Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.



Michael Tekin, B.S.

QUALITY ASSURANCE

  
DATE

***In Vitro* Mammalian Chromosome Aberration Test**

**STUDY INFORMATION**

Sponsor: **Silicones Environmental Health and Safety Council  
(SEHSC)  
SEHSC, Suite 500, 2325 Dulles Corner Blvd.  
Herndon, VA 20171**

Sponsor Representative: **Sharon Mudgett  
Dow Corning Corporation  
2200 West Salzburg Rd., Mail # C03101  
Midland, MI 48686**

Testing Facility: **BioReliance  
9630 Medical Center Drive  
Rockville, Maryland 20850**

Study Director: **Ramadevi Gudi, Ph.D.**

BioReliance Study No.: **AB21RP.331.BTL**

Test Article I.D.: **Dimethylsilanediol (DMSD)**

Test Article Lot Number: **20444-42 (Sample 1 used in preliminary toxicity  
assay)  
20444-102 (Sample 2 used in chromosome aberration  
assay)**

Test Article CAS Number: **1066-42-8**

Test Article Purity: **99.0 ± 0.04 area % (Sample 1)  
98.1 ± 0.18 area % (Sample 2)**

Test Article Description: **White crystalline powder**

Storage Conditions: **Room temperature, stored in the dark/desiccate  
Store loosely capped over P<sub>2</sub>O<sub>5</sub> (Sample 1)  
-60 °C or colder, in the dark/desiccate (Sample 2)**

Test Article Receipt/Login Date: **21 February 2006 (Sample 1)  
01 August 2006 / 02 August 2006 (Sample 2)**

Study Initiation: **23 February 2006**

Experimental Start Date: **28 February 2006**

Experimental Completion Date: **20 August 2006**

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## SUMMARY

The test article, Dimethylsilanediol (DMSD), was tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced S9 activation system. A preliminary toxicity test was performed to establish the dose range for the chromosome aberration assay. The chromosome aberration assay was used to evaluate the clastogenic potential of the test article. The dosing solution concentrations were adjusted to compensate for the purity of 99.0%, however, the actual purity of the test article was 98.1%.

Water was the solvent of choice based on the solubility of the test article and compatibility with the target cells. The test article was soluble in water at a concentration of approximately 50 mg/mL, the maximum concentration tested for solubility.

In the preliminary toxicity assay, the maximum dose tested was 5000 µg/mL. The test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.

Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose levels in all three exposure groups. Based on these findings, the dose levels chosen for the chromosome aberration assay ranged from 625 to 5000 µg/mL for all three exposure groups. The molecular weight was inadvertently not provided to the laboratory. Upon review of the results of the initial chromosome aberration assay, it was discovered that the test article was tested at an excessive concentration of 54 mM (5000 µg/mL). Once the correct molecular weight (92) was provided by the Sponsor, the definitive chromosome aberration test was repeated at dose levels 230, 460 and 920 µg/mL in the non-activated 4 and 20-hour treatment groups and in the S9-activated 4-hour treatment group. After the completion of the repeat assay, it was determined the findings noted in the initial assay were due to extremely high doses. The results of the initial chromosome assay are included in Appendix IV of this report.

In the repeat chromosome aberration assay, the cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours after treatment initiation. The test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.

All three dose levels tested were selected for microscopic analysis in all three treatment groups.

The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ( $p > 0.05$ , Fisher's Exact test).

The results of the assay are summarized in the following table:

Treatment Time	Recovery Time	Harvest Time	S9	Toxicity* at highest dose scored (920 µg/mL)	Mitotic Index Reduction **	LED <sup>1</sup> for Structural Aberrations µg/mL	LED <sup>1</sup> for Numerical Aberrations µg/mL
4 hr	16 hr	20 hr	-	15%	None	None	None
20 hr	0 hr	20 hr	-	3%	12%	None	None
4 hr	16 hr	20 hr	+	4%	1%	None	None

\* cell growth inhibition

\*\* relative to solvent control at high dose evaluated for chromosome aberrations

<sup>1</sup>LED = lowest effective dose

Based on the findings from the repeat assay at the appropriate dose levels, Dimethylsilanediol (DMSD) was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the non-activated and the S9-activated test systems.

## PURPOSE

The purpose of this study was to evaluate the clastogenic potential of a test article based upon its ability to induce chromosome aberrations in CHO cells. Copies of the study protocol and amendments are included in Appendix I.

The study was conducted in compliance with the testing guidelines of the ICH (1996 and 1997) and OECD (1998).

## CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, Dimethylsilanediol (DMSD), was received by BioReliance on 01 August 2006. The test article was characterized by the Sponsor as a translucent white solid that should be stored in a -60 to -80°C freezer in presence of desiccant.

Upon receipt, the test article was described as a white crystalline powder. The test article was stored at -60°C or colder, in the dark with desiccant. The Sponsor has determined the identity, strength, purity, composition or other characteristics to define the test article. A copy of the Characterization of Dimethylsilanediol (Lot Number 20444-102) Technical Report is included in Appendix II. Based on the expiration date in the Technical Report, Dimethylsilanediol (Lot Number 20444-102) was considered to be stable for the purpose of this study through 12 September 2006. A copy of the Characterization of Dimethylsilanediol (Lot Number 20444-42) Technical Report is included in Appendix IV. Based on the expiration date in the Technical Report, Dimethylsilanediol (Lot Number 20444-42) was considered to be stable for the purpose of this study through 14 April 2006.

The solvent used to deliver Dimethylsilanediol (DMSD) to the test system was sterile water (CAS No.: 7732-18-5) obtained from Invitrogen. The dosing solutions were adjusted to compensate for a test article purity of 99.0%. Test article dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light.

Mitomycin C (MMC; CAS No.: 50-07-7), was obtained from Sigma Chemical Company and was dissolved and diluted in sterile distilled water to stock concentrations of 1 and 2 µg/mL for use as the positive control in the non-activated test system. Cyclophosphamide (CP; CAS No.: 6055-19-2), was obtained from Sigma Chemical Company and was dissolved and diluted in sterile distilled water to stock concentrations of 0.1 and 0.2 mg/mL for use as the positive control in the S9-activated test system. For each positive control, one dose with sufficient scorable metaphase cells was selected for analysis. The solvent for the test article was used as the solvent control at the same concentration as that found in the test article-treated groups.

The solvent and positive control articles have been characterized as per the Certificates of Analysis on file with the testing facility. The stability of the solvent and positive control articles and their mixtures was demonstrated by acceptable results that met the criteria for a valid test.

## MATERIALS AND METHODS

### Test System

Chinese hamster ovary (CHO-K<sub>1</sub>) cells (repository number CCL 61) were obtained from American Type Culture Collection, Manassas, VA. In order to assure the karyotypic stability of the cell line, working cell stocks were not used beyond passage 20. The frozen lot of cells was tested using the Hoechst staining procedure and found to be free of mycoplasma contamination.

This cell line has an average cell cycle time of 10-14 hours with a modal chromosome number of 20. The use of CHO cells has been demonstrated to be an effective method of detection of chemical clastogens (Preston et al., 1981).

### Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 (Lot No. 1916 for the preliminary and initial chromosome aberration assays and Lot No. 1958 for the repeat chromosome aberration assay) was obtained from Moltox. Each bulk preparation of S9 was assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz( $\alpha$ )anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 was thawed and mixed with a cofactor pool to contain 2 mM magnesium chloride, 6 mM potassium chloride, 1 mM glucose-6-phosphate, 1 mM nicotinamide adenine dinucleotide phosphate (NADP) and 20  $\mu$ L S9 per milliliter medium (McCoy's 5A serum-free medium supplemented with 100 units penicillin/mL, 100  $\mu$ g streptomycin/mL, and 2 mM L-glutamine).

### Solubility Test

A solubility test was conducted to select the solvent using purified water permitting preparation of the highest soluble stock concentration, up to 50 mg/mL.

### Preliminary Toxicity Assay

The preliminary toxicity assay was performed for the purpose of selecting dose levels for the chromosome aberration assay and consisted of an evaluation of test article effect on cell growth. CHO cells were seeded for each treatment condition at approximately  $5 \times 10^5$  cells/25 cm<sup>2</sup> flask and were incubated at 37 $\pm$ 1°C in a humidified atmosphere of 5 $\pm$ 1% CO<sub>2</sub> in air for 16-24 hours. Treatment was carried out by refeeding the flasks with 4.5 mL complete medium (McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 100 units penicillin/mL, 100  $\mu$ g streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or S9 reaction mixture (3.5 mL serum-free medium plus 1 mL of S9/cofactor pool) for the activated study, to which was added 500  $\mu$ L dosing solution of test article in solvent or solvent alone. The osmolality of the solvent and of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape.

The cells were exposed to solvent alone and to nine concentrations of the test article for 4 hours in both the presence and absence of S9 activation, and for 20 hours continuously in the absence of S9 activation. The cells were incubated at  $37\pm 1^\circ\text{C}$  in a humidified atmosphere of  $5\pm 1\%$   $\text{CO}_2$  in air. At completion of the 4-hour exposure period, the treatment medium was removed, the cells washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS), refed with 5 mL complete medium and returned to the incubator for a total of 20 hours from the initiation of treatment. At 20 hours after the initiation of treatment the cells were harvested by trypsinization and counted using a Coulter counter. The presence of test article precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control.

### **Chromosome Aberration Assay**

The chromosome aberration assay was performed using standard procedures (Evans, 1976), by exposing duplicate cultures of CHO cells to the test article as well as positive and solvent controls. For the chromosome aberration assays CHO cells were seeded at approximately  $5 \times 10^5$  cells/25  $\text{cm}^2$  flask and were incubated at  $37\pm 1^\circ\text{C}$  in a humidified atmosphere of  $5\pm 1\%$   $\text{CO}_2$  in air for 16-24 hours. Treatment was carried out by refeeding duplicate flasks with 4.5 mL complete medium (McCoy's 5A medium supplemented with 10% FBS, 100 units penicillin/mL and 100  $\mu\text{g}$  streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or 4.5 mL S9 reaction mixture for the S9-activated study, to which was added 500  $\mu\text{L}$  of dosing solution of test or control article in solvent or solvent alone. The osmolality of the solvent and of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape.

In the non-activated study, the cells were exposed to the test article for 4 hours or continuously for 20 hours up to the cell harvest at  $37\pm 1^\circ\text{C}$  in a humidified atmosphere of  $5\pm 1\%$   $\text{CO}_2$  in air (Swierenga et al., 1991). In the 4-hour exposure group, after the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid<sup>®</sup> was added to duplicate flasks for each treatment condition at a final concentration of 0.1  $\mu\text{g}/\text{mL}$  and the flasks returned to the incubator until cell collection.

In the S9-activated study, the cells were exposed for 4 hours at  $37\pm 1^\circ\text{C}$  in a humidified atmosphere of  $5\pm 1\%$   $\text{CO}_2$  in air (Swierenga et al., 1991). After the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid<sup>®</sup> was added to duplicate flasks for each treatment condition at a final concentration of 0.1  $\mu\text{g}/\text{mL}$  and the flasks were returned to the incubator until cell collection.

A concurrent toxicity test was conducted in both the non-activated and the S9-activated test systems. After cell harvest an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter. The presence of test article precipitate was assessed using the

unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control.

### **Collection of Metaphase Cells**

Two hours after the addition of Colcemid<sup>®</sup>, metaphase cells were harvested for both the non-activated and S9-activated studies by trypsinization. Cells were collected approximately 20 hours after initiation of treatment (Galloway et al., 1994). The cells were collected by centrifugation at approximately 800 rpm for 5 minutes. The cell pellet was resuspended in 2-4 mL 0.075 M potassium chloride (KCl) and allowed to stand at room temperature for 4-8 minutes. The cells were collected by centrifugation, the supernatant aspirated and the cells fixed with two washes of approximately 2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v). The cells were stored overnight or longer in fixative at approximately 2-8°C.

### **Slide Preparation**

To prepare slides, the fixed cells in 15 mL centrifuge tubes were centrifuged for 5 minutes at about 800 rpm. The supernatant was aspirated leaving approximately 0.2 mL above the cell pellet, and 1 mL cold fresh Carnoy's fixative was added to each tube. The cells were collected by centrifugation and the supernatant was aspirated, leaving 0.1 to 0.3 mL fixative above the cell pellet (volume dependent upon the cell number). An aliquot of the cell suspension was dropped from an appropriate distance by means of a Pasteur pipet on clean microscope slides and allowed to air dry at room temperature. If necessary, the following techniques may have been used to achieve optimum spreading of metaphases: dip slides in cold water or ice, use of a slide warmer or additional centrifugation steps, as needed. Each slide was identified by the study number, dose level, treatment condition, replicate tube designation and harvest date. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

### **Selection of Dose Levels for Analysis**

In the initial chromosome aberration assay, due to absence of both test article precipitation in the treatment medium and at least 50% toxicity, the highest dose level evaluated for chromosome aberrations was 5000 µg/mL in all harvests. Two additional lower dose levels were included in the evaluation. In the repeat chromosome aberration assay, all three dose levels tested were included for microscopic evaluation.

### **Evaluation of Metaphase Cells**

To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Slides were coded using random numbers by an individual not involved with the scoring process. Metaphase cells with 20±2 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations (Scott et al., 1990). The number of metaphase spreads that were

examined and scored per duplicate flask was reduced when the percentage of aberrant cells reached a statistically significant level before 100 cells were scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells ( $\geq 10$  aberrations) were also recorded. Chromatid gaps (an aligned achromatic region in one chromatid, the size of which is equal to or smaller than the width of the chromatid) and isochromatid gaps (an aligned, achromatic region in both chromatids, the size of which is equal to or smaller than the width of the chromatids) were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using a calibrated microscope stage. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored.

### **Controls**

MMC was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2  $\mu\text{g/mL}$ . CP was used as the positive control in the S9-activated study at final concentrations of 10 and 20  $\mu\text{g/mL}$ . For both positive controls, one dose level exhibiting a sufficient number of scorable metaphase cells was selected for analysis. The solvent for the test article was used as the solvent control at the same concentration as that found in the test article-treated groups.

### **Evaluation of Test Results**

The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell were calculated and reported for each treatment group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell.

Statistical analysis of the percent aberrant cells was performed using the Fisher's Exact test. Fisher's Exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's Exact test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

All conclusions were based on sound scientific basis; however, as a guide to interpretation of the data, the test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ( $p \leq 0.05$ ). However, values that are statistically significant but do

not exceed the range of historical solvent controls may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

#### **Criteria for a Valid Test**

The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ( $p \leq 0.05$ , Fisher's Exact test) relative to the solvent control. The Historical Control Data is included in Appendix III.

#### **Deviations**

No known deviations from the protocol or assay method SOPs occurred during the conduct of this study.

#### **Archives**

All raw data, the protocol and amendments, and all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years. Raw data, the protocol and reports generated at other facilities other than BioReliance will be archived per the contractual arrangements between that facility and the Sponsor. Following finalization of the report, residual test article will be disposed. Unless otherwise indicated, the slides will be discarded after the finalization of the report.

## **RESULTS AND DISCUSSION**

#### **Solubility Test**

Water was the solvent of choice based on the solubility of the test article and compatibility with the target cells. The test article was soluble in water at a concentration of approximately 50 mg/mL, the maximum concentration tested for solubility.

#### **Preliminary Toxicity Assay**

Dose levels for the chromosome aberration assay were selected following a preliminary toxicity test. CHO cells were exposed to solvent alone and to nine concentrations of test article ranging from 50 to 5000  $\mu\text{g/mL}$  in the absence and presence of an S9 reaction mixture. The test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period. The osmolality in treatment medium of the highest concentration tested, 5000  $\mu\text{g/mL}$ , was 287 mmol/kg. The osmolality of the solvent (water) in

treatment medium was 270 mmol/kg. The osmolality of the test article concentration in treatment medium is acceptable because it did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was 7.0.

The results of the evaluation of cell growth inhibition are presented in Tables 1-3. Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in all three exposure groups. Based upon the results of the toxicity study, the dose levels ranging from 625 to 5000  $\mu\text{g/mL}$  were selected for the initial chromosome aberration assay. However, upon review of the results of that assay, it was discovered that those dose levels were not appropriate based on molecular weight. A summary discussing the initial chromosome aberration assay can be found in Appendix IV.

### **Chromosome Aberration Assay**

Upon receipt of the molecular weight information, the dose levels tested in the chromosome aberration assay were 230, 460 and 920  $\mu\text{g/mL}$ . For this chromosome aberration assay, the test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period. The osmolality in treatment medium of the highest concentration tested, 920  $\mu\text{g/mL}$ , was 275 mmol/kg. The osmolality of the solvent (water) in treatment medium was 280 mmol/kg. The osmolality of the test article concentration in treatment medium is acceptable because it did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was 7.0.

Toxicity of Dimethylsilanediol (DMSD) (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hours in the absence of S9 activation was 15% at 920  $\mu\text{g/mL}$ , the highest test concentration evaluated for chromosome aberrations (Table 4). The activity of Dimethylsilanediol (DMSD) in the induction of chromosome aberrations is presented by treatment flask in Table 5 and summarized by group in Table 10. The mitotic index at the highest dose level evaluated for chromosome aberrations, 920  $\mu\text{g/mL}$ , was not reduced relative to the solvent control. The dose levels selected for microscopic analysis were 230, 460 and 920  $\mu\text{g/mL}$ . The percentage of cells with structural aberrations in the non-activated 4-hour exposure group was not significantly increased above that of the solvent control at any dose level ( $p > 0.01$ , Fisher's Exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (20.0%) was statistically significant.

Toxicity of Dimethylsilanediol (DMSD) (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hours in the presence of S9 activation was 4% at 920  $\mu\text{g/mL}$ , the highest test concentration evaluated for chromosome aberrations (Table 6). The activity of Dimethylsilanediol (DMSD) in the induction of chromosome aberrations is presented by treatment flask in Table 7 and summarized by group in Table 10. The mitotic index at the highest dose level evaluated for chromosome aberrations, 920  $\mu\text{g/mL}$ , was 1% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 230, 460 and 920  $\mu\text{g/mL}$ . The percentage of cells with structural aberrations in the S9-activated 4-hour exposure group was not significantly increased above that of the solvent control at any dose

level ( $p > 0.01$ , Fisher's Exact test). The percentage of structurally damaged cells in the CP (positive control) treatment group (24.0%) was statistically significant.

Toxicity of Dimethylsilanediol (DMSD) (cell growth inhibition relative to the solvent control) in CHO cells when treated for 20 hours in the absence of S9 activation was 3% at 920  $\mu\text{g/mL}$ , the highest test concentration evaluated for chromosome aberrations (Table 8). The activity of Dimethylsilanediol (DMSD) in the induction of chromosome aberrations is presented by treatment flask in Table 9 and summarized by group in Table 10. The mitotic index at the highest dose level evaluated for chromosome aberrations, 920  $\mu\text{g/mL}$ , was 12% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 230, 460 and 920  $\mu\text{g/mL}$ . The percentage of cells with structural aberrations in the non-activated 20-hour exposure group was not significantly increased above that of the solvent control at any dose level ( $p > 0.01$ , Fisher's Exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (21.0%) was statistically significant.

### CONCLUSION

The positive and solvent controls fulfilled the requirements for a valid test.

Based on the findings of the assay conducted at the appropriate dose levels, Dimethylsilanediol (DMSD) was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the non-activated and the S9-activated test systems.

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TABLE 1  
 PRELIMINARY TOXICITY TEST USING Dimethylsilanediol (DMSD)  
 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment μg/mL	Cell Count (x10 <sup>6</sup> )	Cell Viability (%)	Viable Cells/ Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.72	99%	1.70	100%	
Dimethylsilanediol (DMSD)					
50	1.84	99%	1.82	107%	-7%
350	1.90	98%	1.86	110%	-10%
700	1.88	100%	1.88	111%	-11%
1000	1.80	99%	1.78	105%	-5%
1500	1.95	98%	1.91	113%	-13%
2000	1.81	99%	1.80	106%	-6%
2500	1.76	100%	1.76	104%	-4%
3500	1.94	98%	1.91	112%	-12%
5000	1.77	99%	1.75	103%	-3%

**Treatment:** CHO cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Viable Cells/Flask** = cell count x % viable cells

**Cell Growth Index** = (cells per flask treated group/cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

TABLE 2  
 PRELIMINARY TOXICITY TEST USING Dimethylsilanediol (DMSD)  
 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment μg/mL	Cell Count (x10 <sup>6</sup> )	Cell Viability (%)	Viable Cells/ Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.81	100%	1.81	100%	
Dimethylsilanediol (DMSD)					
50	1.78	98%	1.75	97%	3%
350	1.72	98%	1.69	93%	7%
700	1.55	99%	1.54	85%	15%
1000	1.70	99%	1.68	93%	7%
1500	1.91	99%	1.89	104%	-4%
2000	1.78	97%	1.73	96%	4%
2500	1.82	99%	1.80	99%	1%
3500	1.89	98%	1.85	102%	-2%
5000	1.93	98%	1.89	105%	-5%

**Treatment:** CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Viable Cells/Flask** = cell count x % viable cells

**Cell Growth Index** = (cells per flask treated group/cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

**TABLE 3**  
**PRELIMINARY TOXICITY TEST USING Dimethylsilanediol (DMSD)**  
**IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION**

**20-HOUR CONTINUOUS TREATMENT**

Treatment μg/mL	Cell Count (x10 <sup>6</sup> )	Cell Viability (%)	Viable Cells/ Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.94	99%	1.92	100%	
Dimethylsilanediol (DMSD)					
50	1.94	100%	1.94	101%	-1%
350	1.90	98%	1.87	97%	3%
700	1.98	97%	1.92	100%	0%
1000	2.09	99%	2.07	108%	-8%
1500	1.93	98%	1.89	98%	2%
2000	1.74	98%	1.71	89%	11%
2500	1.75	100%	1.75	91%	9%
3500	1.82	99%	1.80	94%	6%
5000	1.84	98%	1.80	94%	6%

**Treatment:** CHO cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Viable Cells/Flask** = cell count x % viable cells

**Cell Growth Index** = (cells per flask treated group/cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

TABLE 4  
 CONCURRENT TOXICITY TEST USING Dimethylsilanediol (DMSD)  
 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment µg/mL	Flask	Cell Count Averages (x10 <sup>6</sup> )	Cell Viability (%)	Mean Cells per Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	1.85	98%	1.84	100%	
	B	1.87	100%			
Dimethylsilanediol (DMSD) 230	A	1.65	99%	1.63	89%	11%
	B	1.64	99%			
460	A	1.74	98%	1.69	92%	8%
	B	1.69	99%			
920	A	1.47	97%	1.56	85%	15%
	B	1.71	98%			
MMC, 0.1	A	1.32	99%	1.39	76%	24%
	B	1.51	98%			
MMC, 0.2	A	1.33	100%	1.36	74%	26%
	B	1.43	97%			

**Treatment:** CHO cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Mean Viable Cells/Flask** = cell count x % viable cells, reported as mean of Flasks A and B.

**Cell Growth Index** = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

TABLE 5  
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Dimethylsilanediol (DMSD)  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment ( $\mu\text{g/mL}$ )	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations				Severely Damaged Cells	Average Aberrations Per Cell		
			Numerical	Structural	Numerical	Structural	Gaps		Chromatid				Chromosome	
							Br	Ex	Br	Dic			Br	Ring
Water	A	9.0	100	100	4	1	0	1	0	0	0	0	0	0.010
	B	8.2	100	100	5	1	0	1	0	0	0	0	0	0.010
Dimethylsilanediol (DMSD) 230	A	8.0	100	100	3	0	0	0	0	0	0	0	0	0.000
	B	8.4	100	100	4	2	0	1	1	0	0	0	0	0.020
460	A	9.2	100	100	0	0	0	0	0	0	0	0	0	0.000
	B	9.0	100	100	0	0	0	0	0	0	0	0	0	0.000
920	A	8.8	100	100	0	1	0	0	0	0	0	1	0	0.010
	B	9.2	100	100	0	0	0	0	0	0	0	0	0	0.000
MMC, 0.2	A	6.2	100	50	0	20	0	9	5	0	0	0	0	0.280
	B	6.8	100	50	0	20	0	2	8	0	0	0	0	0.200

Treatment: CHO cells were treated for 4 hours at  $37 \pm 1^\circ\text{C}$  in the absence of an exogenous source of metabolic activation.

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 6  
 CONCURRENT TOXICITY TEST USING Dimethylsilanediol (DMSD)  
 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment µg/mL	Flask	Cell Count Averages (x10 <sup>6</sup> )	Cell Viability (%)	Mean Cells per Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	1.57	99%	1.57	100%	
	B	1.62	97%			
Dimethylsilanediol (DMSD) 230	A	1.63	96%	1.60	102%	-2%
	B	1.66	98%			
460	A	1.70	99%	1.71	109%	-9%
	B	1.75	99%			
920	A	1.47	97%	1.50	96%	4%
	B	1.61	98%			
CP, 10	A	1.25	98%	1.24	79%	21%
	B	1.28	99%			
CP, 20	A	1.19	98%	1.22	78%	22%
	B	1.31	98%			

**Treatment:** CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Mean Viable Cells/Flask** = cell count x % viable cells, reported as mean of Flasks A and B.

**Cell Growth Index** = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

TABLE 7  
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Dimethylsilanediol (DMSD)  
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment ( $\mu\text{g}/\text{mL}$ )	Flask Index	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations					Severely Damaged Cells	Average Aberrations Per Cell		
			Numerical	Structural	Numerical	Structural	Gaps		Chromatid					Chromosome	
							Br	Ex	Br	Dic	Ring				
Water	A	9.2	100	100	6	1	0	1	0	0	0	0	0	0.010	
	B	8.0	100	100	3	0	0	0	0	0	0	0	0	0.000	
Dimethylsilanediol (DMSD) 230	A	8.2	100	100	0	1	0	0	0	0	0	0	1	0.010	
	B	8.6	100	100	0	0	0	0	0	0	0	0	0	0.000	
460	A	8.0	100	100	3	0	0	0	0	0	0	0	0	0.000	
	B	8.4	100	100	3	0	0	0	0	0	0	0	0	0.000	
920	A	8.8	100	100	0	1	0	0	1	0	0	0	0	0.010	
	B	8.2	100	100	0	1	0	0	1	0	0	0	0	0.010	
CP, 10	A	4.2	100	50	0	24	0	2	12	0	2	0	1	0.520	
	B	3.8	100	50	0	24	0	11	10	0	1	0	0	0.440	

Treatment: CHO cells were treated for 4 hours at  $37 \pm 1^\circ\text{C}$  in the presence of an exogenous source of metabolic activation.

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 8  
 CONCURRENT TOXICITY TEST USING Dimethylsilanediol (DMSD)  
 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20-HOUR CONTINUOUS TREATMENT

Treatment µg/mL	Flask	Cell Count Averages (x10 <sup>6</sup> )	Cell Viability (%)	Mean Cells per Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	1.60	98%	1.50	100%	
	B	1.43	100%			
Dimethylsilanediol (DMSD) 230	A	1.67	99%	1.66	111%	-11%
	B	1.71	97%			
460	A	1.63	98%	1.61	107%	-7%
	B	1.65	98%			
920	A	1.48	98%	1.46	97%	3%
	B	1.50	97%			
MMC, 0.1	A	1.78	99%	1.61	107%	-7%
	B	1.48	98%			
MMC, 0.2	A	1.45	100%	1.55	103%	-3%
	B	1.66	99%			

**Treatment:** CHO cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Mean Viable Cells/Flask** = cell count x % viable cells, reported as mean of Flasks A and B.

**Cell Growth Index** = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

TABLE 9  
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Dimethylsilanediol (DMSD)  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment ( $\mu\text{g/mL}$ )	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations			Severely Damaged Cells	Average Aberrations Per Cell		
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid	Chromosome				
							Br	Ex	Br	Dic	Ring		
Water	A	10.0	100	100	1	1	0	1	0	0	0	0	0.010
	B	9.4	100	100	0	0	0	0	0	0	0	0	0.000
Dimethylsilanediol (DMSD) 230	A	8.0	100	100	1	0	0	0	0	0	0	0	0.000
	B	8.6	100	100	0	1	0	1	0	0	0	0	0.010
460	A	8.4	100	100	0	0	0	0	0	0	0	0	0.000
	B	8.0	100	100	5	1	0	1	0	0	0	0	0.010
920	A	8.4	100	100	3	1	0	4	0	0	0	0	0.040
	B	8.6	100	100	0	1	0	0	0	0	1	0	0.010
MMC, 0.1	A	5.0	100	50	0	22	0	6	5	0	0	2	0.660
	B	5.8	100	50	0	20	0	10	4	0	0	1	0.300

Treatment: CHO cells were treated for 20 hours at  $37\pm 1^\circ\text{C}$  in the absence of an exogenous source of metabolic activation.

Mitotic index = number mitotic figures x 100/500 cells counted.

% Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 10  
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water	-S9	4	8.6	200	200	0.010	±0.100	4.5	1.0
Dimethylsilanediol (DMSD)									
230	-S9	4	8.2	200	200	0.010	±0.100	3.5	1.0
460	-S9	4	9.1	200	200	0.000	±0.000	0.0	0.0
920	-S9	4	9.0	200	200	0.005	±0.071	0.0	0.5
MMC, 0.2	-S9	4	6.5	200	100	0.240	±0.571	0.0	20.0**
Water	+S9	4	8.6	200	200	0.005	±0.071	4.5	0.5
Dimethylsilanediol (DMSD)									
230	+S9	4	8.4	200	200	0.005	±0.071	0.0	0.5
460	+S9	4	8.2	200	200	0.000	±0.000	3.0	0.0
920	+S9	4	8.5	200	200	0.010	±0.100	0.0	1.0
CP, 10'	+S9	4	4.0	200	100	0.480	±1.314	0.0	24.0**
Water	-S9	20	9.7	200	200	0.005	±0.071	0.5	0.5
Dimethylsilanediol (DMSD)									
230	-S9	20	8.3	200	200	0.005	±0.071	0.5	0.5
460	-S9	20	8.2	200	200	0.005	±0.071	2.5	0.5
920	-S9	20	8.5	200	200	0.025	±0.291	1.5	1.0
MMC, 0.1	-S9	20	5.4	200	100	0.480	±1.514	0.0	21.0**

**Treatment:** Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

**Aberrations per Cell:** Severely damaged cells were counted as 10 aberrations.

**Percent Aberrant Cells:** \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; using Fisher's Exact test.

**APPENDIX I**

**Study Protocol and Amendments**

QA #42  
21 MAR 2006  
APPROVED

PROTOCOL AMENDMENT 1

Sponsor: Silicones Environmental Health and Safety Council (SEHSC)

Test Article I.D.: Dimethylsilanediol (DMSD)

BioReliance Study No.: AB21RP.331.BTL

Protocol Title: *In Vitro* Mammalian Chromosome Aberration Test

1. LOCATION: Page 1, §2.3, Sponsor Representative and §2.4, Sponsor Address

AMENDMENT: Amend the sections to:

Sharon Mudgett  
 Dow Corning Corporation  
 2200 West Salzburg Rd., Mail # C02101  
 Midland, MI 48686  
 989-496-6901  
 Sharon.mudgett@dowcorning.com

REASON FOR THE AMENDMENT: To indicate a change of Sponsor Representative.

APPROVALS:

Ramadan Cadi  
 BioReliance Study Director

17 Mar 2006  
 Date

[Signature]  
 BioReliance Study Management

17 MAR 2006  
 Date

[Signature]  
 Sponsor Representative

22/03/2006  
 Date

**PROTOCOL AMENDMENT 2**

Sponsor: **Silicones Environmental Health and Safety Council (SEHSC)**

Test Article I.D.: **Dimethylsilanediol (DMSD)**

BioReliance Study No.: **AB21RP.331.BTL**

Protocol Title: ***In Vitro* Mammalian Chromosome Aberration Test**

---

1. **LOCATION:** Page 3, Section 7.0 Experimental Design and Methodology

**AMENDMENT:** Add the following at the end of the paragraph, "The definitive chromosome aberration test will be repeated at the following dose levels and the treatment conditions: 230, 460 and 920 µg/mL in the non-activated 4 hour and 20 hour treatment groups and the S9-activated 4 hour treatment groups. The dose level of 920 µg/mL (10 mM) was selected based on molecular weight of 92 as indicated by the Sponsor.

**REASON FOR THE AMENDMENT:** The initial chromosome aberration test was tested in an excessive of 54 mM concentration (5000 µg/mL). This repeat study is designed to investigate if the positive finding was due to extremely high dose.

**APPROVALS:**

Ramadevi Audi  
BioReliance Study Director

02 Aug 2006  
Date

Leann Augerson  
BioReliance Study Management

02 Aug 2006  
Date

Saron L. Mudgett  
Sponsor Representative

07-24-06  
Date

QA  
21/02/2006  
APPROVED

Received by RA/OA 23 Feb 2006

BioReliance Study Number: AB21RP.331.BTL

*In Vitro* Mammalian Chromosome Aberration Test

1.0 PURPOSE

The purpose of this study is to evaluate the clastogenic potential of a test article based upon its ability to induce chromosome aberrations in Chinese hamster ovary (CHO) cells.

2.0 SPONSOR

- 2.1 Sponsor Name: Silicones Environmental Health and Safety Council (SEHSC)
- 2.2 Address: SEHSC, Suite 500, 2325 Dulles Corner Blvd., Herndon, VA 20171
- 2.3 Representative: Larry Segal, Ph.D.  
GE Advanced Materials  
Haven 1053 Nieuwe Weg 1
- 2.4 Address: Zwigndrecht B2070, Belgium  
Phone: 32 03 2502652  
Fax: 32 03 2502950  
Email: larry.segal@ge.com

3.0 TEST AND CONTROL ARTICLES

- 3.1 Test Article Name: Dimethylsilanediol (DMSD)  
Lot Number: 20444-42  
Physical Description: translucent white solid  
Chemical Characterization: The test article was characterized prior to experimental start. The results of the characterization showed that the test article met the physical description, purity, and composition specifications. A Certification of Analysis for the test article will be included in the report
- Handling Precautions: See MDMS and other information provided by the Sponsor
- Stability: Stable, according to the information provided by the sample submitter
- Purity: 99.0 ± 0.04 area percent purity, as determined by the characterization study
- Storage Conditions: Room temperature, according to the information provided by the sample submitter
- Solubility: See information supplied by the Sponsor

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Expiration Date: 17 April 2006

3.2 Controls: Solvent: Test Article Solvent (or Vehicle)  
Positive: Mitomycin C (MMC)  
Cyclophosphamide (CP)

3.3 Characterization and Stability of the Test Article and Test Article Mixtures

BioReliance will not perform analysis of the test article or dosing solutions unless details of the analysis are provided below. The Sponsor will be directly responsible for determination and documentation of the analytical purity, composition and stability of the test article, and the stability and strength of the test article in the solvent (or vehicle).

3.4 Test Article Retention Sample

Since the in-life portion of this study is less than four weeks in duration, BioReliance will not retain a reserve sample of the test article.

3.5 Residual Test Article and Dosing Preparations

Dosing preparations, excluding those saved for concentration or homogeneity analysis (if any), will be disposed of following administration to the test system. Following finalization of the report, residual test article will be discarded unless the Sponsor indicates the shipping conditions for return of the test article.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1 Name: Toxicology Testing Facility  
BioReliance

4.2 Address: 9630 Medical Center Drive  
Rockville, MD 20850

4.3 Study Director: Ramadevi Gudi, Ph.D.  
Phone: 301-610-2169  
Fax: 301-738-2362  
E-mail: rgudi@bioreliance.com

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 03 Mar 2006

5.2 Proposed Experimental Completion Date: 24 Mar 2006

5.3 Proposed Report Date: 17 April 2006

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## 6.0 TEST SYSTEM

The CHO-K<sub>1</sub> cell line is a proline auxotroph with a modal chromosome number of 20 and a population doubling time of 10-14 hours. CHO-K<sub>1</sub> cells were obtained from the American Type Culture Collection (repository number CCL 61), Manassas, VA. The stability of the modal chromosome number of the cell line is routinely checked and the cell line is routinely tested and determined to be free from mycoplasma contamination. This system has been demonstrated to be sensitive to the clastogenic activity of a variety of chemicals (Preston et al., 1981).

## 7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The chromosome aberration assay will be conducted using standard procedures (Evans, 1976), by exposing cultures of CHO cells to a minimum of four concentrations of the test article as well as to positive and solvent controls. In the non-activated test system, treatment will be for 4 hours and for 20 hours; in the S9 activated test system, exposure will be for 4 hours (Swierenga et al., 1991). To ensure evaluation of first division metaphase cells the dividing cells will be arrested in metaphase and harvested for microscopic evaluation of chromosome aberrations at approximately 20 hours (1.5 normal cell cycles) after the initiation of treatment (Galloway et al., 1994). The clastogenic potential of the test article will be measured by its ability to increase structural chromosome aberrations in a dose-responsive manner when compared to the solvent control group. In the event of a positive response in the 4 hour non-activated study, the prolonged exposure non-activated study may not be scored. The test article will also be assessed for its ability to induce numerical chromosome aberrations.

### 7.1 Solubility Determination

Unless the Sponsor has indicated the test article vehicle a solubility determination will be conducted to determine the maximum soluble concentration or workable suspension as indicated below. Vehicles compatible with this test system, in order of preference, include but are not limited to deionized water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5) and acetone (CAS 67-64-1). The vehicle of choice will be the solvent, selected in order of preference, which permits preparation of the highest workable or soluble stock concentration, up to 50 mg/mL for aqueous vehicles and up to 500 mg/mL for organic vehicles. Based on the molecular weight of the test article, the solvents to be tested and the dose to be achieved in the assay, alternate stock concentrations may be tested, as needed.

### 7.2 Preliminary Toxicity Test for Selection of Dose Levels

Selection of the dose levels for the cytogenetics assay will be based upon post-treatment toxicity (cell growth inhibition relative to the solvent control) and solubility of the test article. CHO cells will be exposed to solvent alone and to at least nine concentrations of test article. The highest concentration tested will be

5 mg/ml or 10 mM whichever is lower for freely soluble test articles, or the maximum concentration resulting in a workable suspension for poorly soluble test articles not to exceed 5 mg/ml. The pH will be measured at the highest test article treatment condition and will be adjusted, if necessary, in order to maintain a neutral pH in the treatment medium. The osmolality of the highest dose level, lowest precipitating dose level (where applicable) and the highest soluble dose level (where applicable) in treatment medium will also be measured. Cells seeded 16-24 hours earlier will be exposed for 4 hours in the absence and presence of S9 and for 20 hours in the absence of S9. Just prior to trypsinization the cell cultures will be visually inspected for the extent of monolayer confluency relative to the solvent control. Twenty hours after treatment initiation the cells will be harvested by trypsinization and counted using an automatic cell counter and the cell viability will be assessed using trypan blue dye exclusion. The cell counts and percent viability will be used to determine cell growth inhibition relative to the solvent control.

Whenever possible, the high dose to evaluate chromosome aberrations will be selected to give at least 50% toxicity (cell growth inhibition relative to the solvent control) irrespective of solubility but not to exceed 5 mg/ml or 10 mM. At least two additional dose levels, demonstrating minimal or no toxicity, will be included. In the event the test article cannot be dissolved at a high enough concentration in an appropriate solvent to be toxic, then the highest dose to be tested in the chromosome aberration assay will be the concentration resulting in minimum precipitation in test medium. Precipitation will be determined by direct visual inspection. In the event the test article demonstrates a dose-responsive increase in toxicity at concentrations that exceed solubility in treatment medium, then the highest dose to be tested will be the maximum concentration that results in at least 50% toxicity. In the event that neither cytotoxicity nor insolubility is observed in the preliminary test, the highest dose in the chromosome aberration assay will be 5 mg/ml or 10 mM whichever is lower. If the osmolality of the treatment medium is considered excessive, the Sponsor will be consulted. The doses levels once determined for the definitive chromosome aberration assay will be documented in the raw data and report

### 7.3 Frequency and Route of Administration

Target cells will be treated for 4 hours in the absence and presence of S9, and for 20 hours in the absence of S9, by incorporation of the test article-solvent mixture into the treatment medium. This technique has been demonstrated to be an effective method of detection of chemical clastogens in this test system (Evans, 1976).

If the Sponsor is aware of specific metabolic requirements, then this information will be utilized in the preparation of the study design. Verification of a clear positive response is not required. Negative results will not be confirmed when justification can be provided. Equivocal results may be confirmed, upon consultation with the Sponsor, and may employ a modification of the study design. This guidance is based on the OECD Guideline 473 (1997) and ICH Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (1996).

#### 7.4 Activation System

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The S9 will be prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The S9 will be batch prepared and stored frozen at approximately -70°C until used. Each batch preparation of S9 will be assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 will be thawed and mixed with cofactors to contain 2 mM magnesium chloride (MgCl<sub>2</sub>), 6 mM potassium chloride (KCl), 1mM glucose-6-phosphate, 1 mM nicotinamide adenine dinucleotide phosphate (NADP) and 20 µl S9 per ml serum free medium.

#### 7.5 Controls

##### 7.5.1 Solvent (or Vehicle) Control

The solvent for the test article will be used as the solvent control. For solvents other than water, physiological buffer, or medium, the final concentration in treatment medium will not exceed 1%.

##### 7.5.2 Positive Controls

Mitomycin C will be used at a concentration within 0.05-0.3 µg/ml as the positive control in the non-activated study. Cyclophosphamide will be used at a concentration within 10-50 µg/ml as the positive control in the S9-activated study.

#### 7.6 Preparation of Target Cells

Exponentially growing CHO-K<sub>1</sub> cells will be seeded in complete medium (McCoy's 5A medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 units penicillin/ml and 100 µg streptomycin/ml) for each treatment condition at approximately 5 x 10<sup>5</sup> cells/25 cm<sup>2</sup> flask. The flasks will be incubated at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO<sub>2</sub> in air for 16-24 hours.

#### 7.7 Identification of Test System

Using a permanent marking pen, the treatment flasks will be identified by the BioReliance study number and a code system to designate the treatment condition and test phase.

#### 7.8 Treatment of Target Cells

Unless otherwise instructed by the Sponsor, test article dosing solutions will be prepared immediately prior to use. All test article dosing will be at room temperature under yellow light. Treatment will be carried out in duplicate by refeeding the flasks with 5 ml complete medium for the non-activated exposure or 5 ml S9 reaction mixture (4 mL culture medium + 1 mL of S9 cofactor pool) for the S9-activated exposure, to which will be added 50 µl of dosing solution of test or control article in solvent or solvent alone. Larger volumes of dosing solution may be used if water, physiological buffer, or medium is used as the solvent.

In the non-activated study, the cells will be treated for 4 hours and for 20 hours; in the S9-activated study the cells will be treated for 4 hours. Treatment will be carried out at  $37 \pm 1^\circ\text{C}$  in a humidified atmosphere of  $5 \pm 1\%$   $\text{CO}_2$  in air. After the 4 hour treatment period in the non-activated and the S9-activated studies, the treatment medium will be aspirated, the cells washed with phosphate buffered saline, refed with complete medium and returned to the incubator.

A concurrent toxicity test to determine cell growth inhibition relative to the solvent control will be conducted in both the non-activated and the S9-activated studies.

#### 7.9 Collection of Metaphase Cells

Cells will be collected approximately 20 hours after initiation of treatment. This post-treatment harvest time represents approximately 1.5 normal cell cycles and was selected to ensure that the cells are analyzed in the first division metaphase after initiation of treatment. Two hours prior to cell harvest, Colcemid® will be added to the cultures at a final concentration of 0.1 µg/ml.

Cells will be harvested by trypsinization, collected by centrifugation and an aliquot will be removed for counting using an automatic cell counter and trypan blue dye exclusion. The remainder of the cells will be swollen with 0.075M KCl, washed with two consecutive changes of fixative (methanol:glacial acetic acid, 3:1 v/v), capped and may be stored overnight or longer at approximately 2-8°C. The cell counts and percent viability will be used to determine cell growth inhibition relative to the solvent control (% toxicity). To prepare slides, the cells will be collected by centrifugation and resuspended in fresh fixative. The suspension of fixed cells will be applied to glass microscope slides and air-dried. The slides will be identified by the experiment number, treatment condition and date. The slides will be stained with Giemsa and permanently mounted.

#### 7.10 Scoring for Metaphase Aberrations

To ensure that a sufficient number of metaphase cells are present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) be determined and recorded for each coded treatment group selected for scoring chromosome aberrations. Slides will be coded using random numbers by an individual not

involved with the scoring process. In the event of a positive response in the 4 hour non-activated study, the prolonged exposure non-activated study may not be scored. Metaphase cells with  $20 \pm 2$  centromeres will be examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads from each dose level (100 per duplicate flask) will be examined and scored for chromatid-type and chromosome-type aberrations (Scott et al., 1990). The number of metaphase spreads that will be examined and scored per duplicate flask may be reduced if the percentage of aberrant cells reaches a significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure will be scored as a break (chromatid or chromosome). Fragments observed with an exchange figure will not be scored as an aberration but will be considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells ( $\geq 10$  aberrations) will also be recorded. Chromatid and isochromatid gaps will be recorded but not included in the analysis. The XY coordinates for each cell with a structural aberration will be recorded using a calibrated microscope stage. The percent polyploid and endoreduplicated cells will be evaluated per 100 cells for each dose level analyzed for structural aberrations.

Unless otherwise indicated, the slides will be discarded after the finalization of the report.

## 8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

### 8.1 Solvent Control

The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control.

### 8.2 Positive Control

The percentage of cells with aberrations must be statistically increased ( $p \leq 0.05$ , Fisher's exact test) relative to the solvent control.

## 9.0 EVALUATION OF TEST RESULTS

The toxic effects of treatment are based upon cell growth inhibition relative to the solvent control and will be presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell will be calculated and reported for each treatment group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or

more aberrations or in the frequency of structural aberrations per cell. Statistical analysis of the percentage of aberrant cells will be performed using the Fisher's exact test. The Fisher's test will be used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test article dose level, the Cochran-Armitage test will be used to measure dose-responsiveness. All conclusions will be based on sound scientific basis; however, as a guide to interpretation of the data, the test article will be considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ( $p \leq 0.05$ ). However, values that are statistically significant but do not exceed the range of historic negative or solvent controls may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

#### 10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. Unless alternate arrangements are made, the report will be initially issued as a QA-audited draft. After receipt of the Sponsor's comments a final report will be issued. The report will include:

Results presented will include, but not be limited to:

- Test substance: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.
- Solvent/Vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- Source of cells, karyotype features (modal chromosome number) and suitability of the cell type used, absence of mycoplasma, cell cycle length, passage number.
- Test conditions: composition of medium; CO<sub>2</sub> concentration; incubation time; cell seeding density; solvent and solvent selection rationale; concentration of test article and concentration selection rationale; composition and acceptability criteria for the metabolic activation (S9) system; duration of treatment; duration of treatment with and concentration of Colcemid®; type of metabolic activation system used; positive and solvent controls; methods of slide preparation; number of cell cultures; criteria for scoring aberrations and criteria for considering studies positive, negative.
- Results: description of precipitation; pH and osmolality of the treatment medium; cell growth inhibition relative to the solvent control; mitotic index and number of metaphases analyzed; type and number of aberration (structural and numerical) given separately for each treated and control culture; concentration-response relationship; statistical analysis; historical control data.

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- Discussion
- Appendices: Historical Control Data (negative and positive controls with ranges, means and standard deviations), copy of protocol and any amendment, and, if provided by the Sponsor, copies of the analyses that characterized the test article, its stability and the stability and strength of the dosing preparations.
- Statement of Compliance
- Quality Assurance Statement

If an electronic copy of the protocol, the report or another study document is provided by BioReliance, the executed paper document is considered the official master document. If there is a discrepancy between an electronic copy and the corresponding master document, the master document will be considered the official document.

#### 11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years. Raw data, the protocol and reports generated at facilities other than BioReliance will be archived per the contractual arrangements between that facility and the Sponsor.

#### 12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline 473 (*In Vitro* Mammalian Chromosome Aberration Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998 and with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996 and 1997).

Portion of this study performed at BioReliance will be conducted in compliance with the provisions of the US FDA GLP Regulations 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice, as applicable to the product being tested. An in-process phase, the raw data, and report(s) will be inspected per the Standard Operating Procedures (SOPs) of BioReliance by the Quality Assurance Unit of BioReliance for compliance with GLPs, the SOPs of BioReliance and the study protocol. At least one, study-specific, in-process inspection will be performed for this study. A signed QA statement will be included in the final report. This statement will list the study-specific phases inspected, the dates of each inspection, and the dates the results of each inspection were reported to the Study Director and the Study Director's

management. In addition, a signed GLP compliance statement will be included in the final report. This statement will cite the GLP guideline(s) with which the study is compliant and any exceptions to this compliance, if applicable, including the omission of characterization or stability analyses of the test article or its mixtures.

Raw data, the protocol and reports generated at facilities other than BioReliance will or will not be QA audited per the contractual arrangements between that facility and the Sponsor.

### 13.0 REFERENCES

Evans, H.J. (1976) Cytological methods for detecting chemical mutagens, in: A. Hollaender (Ed.), *Chemical Mutagens, Principles and Methods for their Detection*, vol. 4. Plenum Press, New York, NY.

Galloway, S.M., M.J. Aardema, M. Ishidate Jr., J.L. Ivett, D.J. Kirkland, T. Morita, P. Mosesso and T. Sofuni (1994) Report from working group on in vitro tests for chromosomal aberrations, *Mutation Research* 312(3):241-261.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. *Federal Register* 61:18198-18202, April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. *Federal Register* 62:16026-16030, November 21, 1997.

OECD Guideline for the Testing of Chemicals, Guideline 473 (*In Vitro* Mammalian Chromosome Aberration Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998.

Preston, R.J., W. Au, M.A. Bender, J.G. Brewen, A.V. Carrano, J.A. Heddle, A.F. McFee, S. Wolff and J.S. Wassom (1981) Mammalian in vivo and in vitro cytogenetic assays: a report of the Gene-Tox Program, *Mutation Research*, 87:143-188.

Scott, D., N.D. Danford, B.J. Dean and D.J. Kirkland. 1990. Metaphase Chromosome Aberration Assays In Vitro. In: *Basic Mutagenicity Tests: UKEMS Recommended Procedures*. D.J Kirkland (ed). Cambridge University Press, New York, NY.

Swierenga S.H.H., J.A. Heddle, E.A. Sigal, J.P.W. Gilman, R.L. Brillinger, G.R. Douglas and E.R. Nestmann (1991) Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese lung and human lymphocyte cultures, *Mutation*

Research 246:301-322.

14.0 APPROVAL

  
\_\_\_\_\_  
Larry Segal, Ph.D.  
Sponsor Representative

22-02-2006  
Date

  
\_\_\_\_\_  
BioReliance Study Director

23 Feb 2006  
Date

  
\_\_\_\_\_  
BioReliance Study Management

23 Feb 2006  
Date

**APPENDIX II**

**Characterization of Dimethylsilanediol  
(Lot Number 20444-102)  
Technical Report**

**DOW CORNING CORPORATION  
HEALTH & ENVIRONMENTAL SCIENCES  
TECHNICAL REPORT**

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**Report Number:** 2006-I0000-56510

**Title:** Characterization of Dimethylsilanediol  
(Lot Number 20444-102)

**HES Study Number:** 10266-102

**Test Article:** Dimethylsilanediol

**Study Director:** Debra Stutts

**Sponsor:** Silicones Environmental, Health and Safety Council (SEHSC)  
2325 Dulles Corner Boulevard, Suite 500  
Herndon, VA 20171

**HES Group Manager:** Roy A. Campbell  
Health and Environmental Sciences (HES)  
Chemistry and Environmental Fate

**Testing Facility:** Health and Environmental Sciences  
Dow Corning Corporation  
2200 W. Salzburg Road  
Auburn, MI 48611

**Study Completion Date:** 3 October 2006

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**ABSTRACT**

Dimethylsilanediol was characterized according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792. Characterization included physical description, area percent purity by GC/FID and confirmation of identity by GC-MS.

The physical appearance of the test article was observed to be a translucent white solid. The GC/FID area percent purity for dimethylsilanediol was  $98.1 \pm 0.18$  based on the average of 3 replicate injections. GC-MS supports the identification of the test article as dimethylsilanediol. This test article meets the specification for physical description and identification as defined in the Product Data Management (PDM) document.

**GLP COMPLIANCE STATEMENT**

This study was conducted in accordance with EPA (TSCA) Good Laboratory Practices 40 CFR Part 792.

*Debra Stutts*  
Debra Stutts, B. S.  
Senior Analytical Chemist  
Study Director

3 Oct 06  
Date

*Roy A Campbell*  
Roy A. Campbell, B. S.  
Health and Environmental Sciences  
HES Group Manager – Chemistry and Environmental Fate

03 Oct - 2006  
Date

### QUALITY ASSURANCE STATEMENT

Title: Characterization of Dimethylsilanediol  
(Lot Number 20444-102)

Study Number: 10266-102

This study has been audited by the Dow Corning Corporation Health and Environmental Sciences Quality Assurance Unit according to approved Standard Operating Procedures to assure that the raw data are accurately reflected within this final report. The following are the inspection dates and the dates inspection findings were reported.

<u>Dates of Inspection</u>	<u>Phase Inspected</u>	<u>Findings Reported to Study Director and Management</u>
12 Jul 06	Draft Protocol Review	12 Jul 06
27 Jul 06	Physical Description	27 Jul 06
25 Aug 06	Draft Final Report and Associated Raw Data Review	30 Aug 06



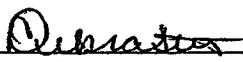
Lisa T. Drottar, B.A.  
Quality Assurance Team Leader  
Dow Corning Corporation  
Health & Environmental Sciences

02 Oct 2006

Date

APPROVAL SIGNATURES

This report consists of pages 1 through 15.

  
\_\_\_\_\_  
Debra Stutts, B. S.  
Senior Analytical Chemist  
Study Director

30 Oct 06  
Date

  
\_\_\_\_\_  
Roy A. Campbell, B. S.  
Health and Environmental Sciences  
HES Group Manager – Chemistry and Environmental Fate

03-Oct-2006  
Date

**STUDY INFORMATION**

Study Initiation Date:	26 Jul 2006
Experimental Start Date:	27 Jul 2006
Experimental Termination Date:	27 Jul 2006
Study Completion Date:	3 October 2006

**A. Study Purpose**

The objective of this study was to characterize the test article, dimethylsilanediol according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792.

**B. Test Article / Substance / Item**

**Identification:** Dimethylsilanediol (supplied as Dow Corning ® XX-1240 Dev Sample)

**Lot Number:** 20444-102

**Expiration Date:** 9/12/06

**Source:** Dow Corning Corporation, Auburn, MI 48611

**CAS Number:** 1066-42-8

**Physical Description:** Translucent white solid, as determined by this study

**Stability:** Stable

**Purity:** 98.1± 0.18 %, as determined by this study

**Solubility:** Soluble in acetone, diethyl ether, water

**Storage Conditions:** In freezer in presence of dessicant, refer to information provided by sample submitter

**Archive:** A reserve sample was not retained for this study.

**C. Labeling**

The original test article container for this study was labeled with the following information: study number, name of test article, lot number, container accession number, concentration, storage conditions, expiration date, initials of the individual distributing the test article and date the test article was dispensed into the container.

**D. Route of Exposure and Justification**

As the test article is the test system, this is not applicable.

**E. Experimental Procedures****1. Physical Description**

The physical appearance of the test article was determined by visual examination.

**2. Sample Preparation**

Approximately 8 mg of test article was dissolved in approximately 1 mL diethyl ether for GC/FID analysis. Approximately 34 mg of test article was dissolved in approximately 1 mL diethyl ether for GC-MS analysis.

**3. GC/FID Analysis**

A Hewlett Packard (HP) 6890 GC with flame ionization detector and HP 7683B autosampler was used to determine an area percent purity of dimethylsilanediol, based on the average of 3 replicate injections (1.0 µL). Prior to analyzing dimethylsilanediol, the oven temperature was increased to approximately 280 °C for 80 minutes to prevent carry over by heating the column to burn off any residual material left from previous analyses. Once a stable baseline was observed, the GC performance was considered acceptable for use. The GC/FID performance was verified by conducting triplicate 1 µL injections of an Agilent checkout sample using the autosampler. Hewlett Packard Chemstation revision A.10.02 software was used for data acquisition and analysis. A solvent blank (ethyl

ether) was injected (1  $\mu$ L injection volume) prior to the test article solution analysis. The actual instrument parameters are shown in Table I. The GC area percent results were calculated as the ratio of dimethylsilanediol peak area divided by the sum of all peak areas attributable to the test article.

**Table I. GC/FID Instrument Parameters**

Parameter	Setting
Oven Temperature Program	70 °C (hold 2 min) to 280 °C at 25 °C/min (hold 9.6 min)
Inlet Temperature	250 °C
Detector Temperature	280 °C
Capillary Column	HP-5 (5% PhenylMethyl Siloxane), 0.25 mm inner diameter 30 m length, 1.0 $\mu$ m film thickness
Mode	Constant pressure, He carrier
Initial Flow	2.2 mL/min
Split Ratio	15:1

#### 4. GC-MS Analysis

An HP 5890A Series II GC with an HP 5970B mass selective detector (MSD) was used to verify the identity of the major component in the test article solution as dimethylsilanediol by manual injection (1.0  $\mu$ L). The standard autotune procedure was conducted prior to analyzing the test article. The standard autotune is an automated tuning program that uses perfluorotributylamine (PFTBA) to optimize the MS for general-purpose electron impact ionization (EI) operation. The GC oven temperature was increased to 280 °C for 225 min to prevent carry over by burning off residual material remaining from previous analyses. HP ChemStation version B.02.05 software and HP MS ChemStation version C.03.00 software were used for data acquisition and analysis. An ethyl ether solvent blank was injected (1.0  $\mu$ L) prior to the test article solution analysis. The mass spectrum for the major component in the total ion chromatogram (TIC) for the test article analysis was library searched and a reasonable match for dimethylsilanediol was not obtained. The actual instrument parameters used are shown in Table II.

**Table II. GC-MS Instrument Parameters**

Parameter	Setting
Oven Temperature Program	50 °C (hold 3 min) to 210 °C at 20°C/min (hold 10 min)
Inlet Temperature	250 °C
Capillary Column	HP-5MS (Crosslinked 5%-phenyl-methylsilicone), 0.25 mm inner diameter, 30 m length, 0.25 $\mu$ m film thickness
Mode	Constant flow, He carrier 7.3 psi at 70 °C, vacuum compensation on
Split Ratio	20.0:1
Mass Scan Range	30-500 atomic mass units

#### F. Data Analysis

Calculation of average and standard deviation for area percent purity was performed.

#### G. Deviations

There were no circumstances that affected the quality or integrity of the data.

## H. Results and Discussion

### 1. Physical Description

The physical appearance of the test article was observed to be a translucent white solid, which is consistent with the physical form and color in the PDM<sup>2</sup> (Physical Properties Section).

### 2. GC/FID Analysis

The GC/FID area percent purity for dimethylsilanediol was  $98.1 \pm 0.18\%$  based on the average of 3 replicate injections. The results are summarized in Table III. A representative GC/FID chromatogram of the test article and a solvent blank is shown in Figures 1 and 2, respectively. The GC/FID performance verification passed the acceptance criteria.

### 3. GC-MS Analysis

The major peak in the total ion chromatogram (TIC) eluted at a retention time of 2.138 minutes and is shown in Figure 3. A small molecular ion ( $m/z$  92) is present in the mass spectrum shown in Figure 4. The base peak ( $m/z$  77) corresponds to  $\text{CH}_3\text{Si}(\text{OH})_2^+$  and is formed by the loss of a methyl group from the parent molecule ( $M = 92$ ). The fragmentation pattern is consistent with that expected for dimethylsilanediol. The GC-MS fragmentation pattern of the most abundant impurity, eluting at 4.69 minutes, is consistent with that expected for dihydroxytetramethyldisiloxane (dimerdiol), displaying a prominent peak at  $m/z$  151 which corresponds to the loss of a methyl group from the parent molecule ( $M = 166$ ). The base peak ( $m/z$  133) corresponds to the ion formed from loss of a methyl group and water from the parent.

## I. Conclusions

Dimethylsilanediol was characterized according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792. The physical appearance of the test article was observed to be a translucent white solid. The GC/FID area percent purity for dimethylsilanediol was  $98.1 \pm 0.18\%$  based on the average of 3 replicate injections. GC-MS supports the identification of the test article as dimethylsilanediol. This test article meets the specification for physical description and identification as defined in the PDM<sup>2</sup>.

## J. Archive

All raw data were archived at the same location as the protocol, study authorization documentation and final report; Dow Corning Corporation, Health and Environmental Sciences, 2200 W. Salzburg Road, Auburn, MI 48611.

## K. References

1. Moore, J. A. In *The Analytical Chemistry of Silicones*; A. L. Smith, ed.; Mass Spectrometry, Volume 112, Chapter 13; John Wiley & Sons, Inc.; New York, NY; 1991.
2. Product Data Management (PDM), unpublished document of Dow Corning Corporation.
3. Dow Corning Material Safety Data Sheet (MSDS), Version 1.0, Revision Date 1/20/2006.

Table III. GC/FID Purity Results for Dimethylsilanediol

Replicate	Peak #	Retention Time (min)	Area %	Peak Assignment
1	1	1.533		Impurity in diethylether
	2	2.076	98.29	Dimethylsilanediol
	3	3.331		Impurity in diethylether
	4	4.140	1.25	Dimerdiol
	5	5.688	0.19	Not identified
	6	6.844	0.11	Not identified
	7	7.867	0.07	Not identified
	8	8.833	0.05	Not identified
	9	9.698	0.04	Not identified
2	1	1.536		Impurity in diethylether
	2	2.079	98.09	Dimethylsilanediol
	3	3.333		Impurity in diethylether
	4	4.142	1.42	Dimerdiol
	5	5.689	0.21	Not identified
	6	6.844	0.11	Not identified
	7	7.869	0.07	Not identified
	8	8.833	0.05	Not identified
	9	9.698	0.04	Not identified
3	1	1.535		Impurity in diethylether
	2	2.078	97.94	Dimethylsilanediol
	3	3.332		Impurity in diethylether
	4	4.142	1.53	Dimerdiol
	5	5.688	0.23	Not identified
	6	6.844	0.12	Not identified
	7	7.868	0.07	Not identified
	8	8.834	0.05	Not identified
	9	9.698	0.04	Not identified
	10	10.463	0.03	Not identified
Mean (n=3)			98.1	
Standard Deviation			0.18	
RSD (%)			0.18	

Figure 1. GC/FID Chromatogram of Dimethylsilanediol in Diethyl Ether

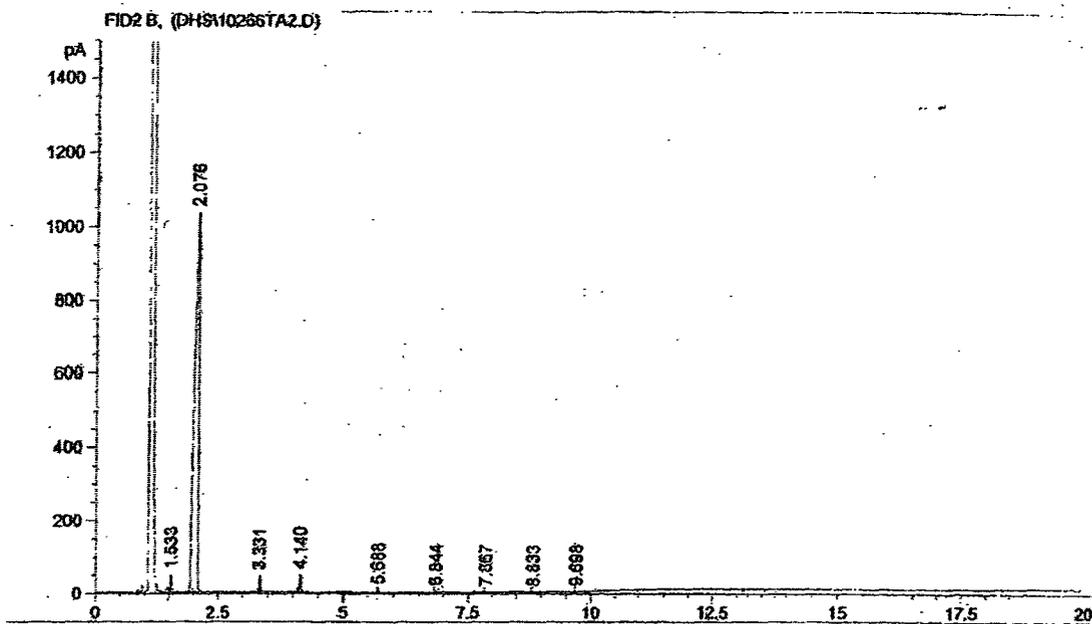


Figure 2. GC/FID Chromatogram of Diethyl Ether

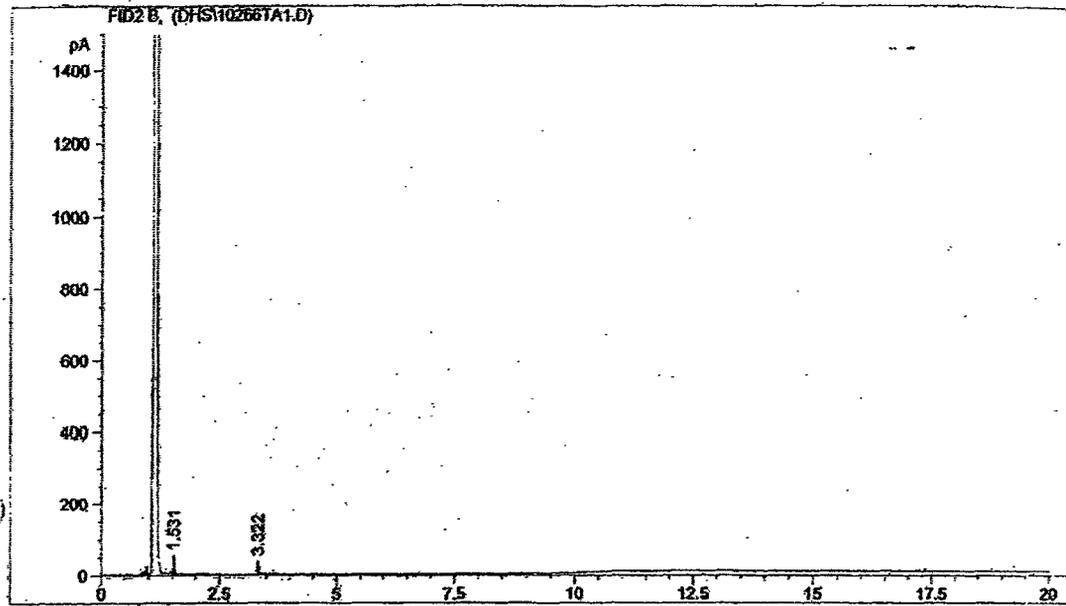


Figure 3. Total Ion Chromatogram from GC-MS Analysis of Dimethylsilanediol in Diethyl Ether

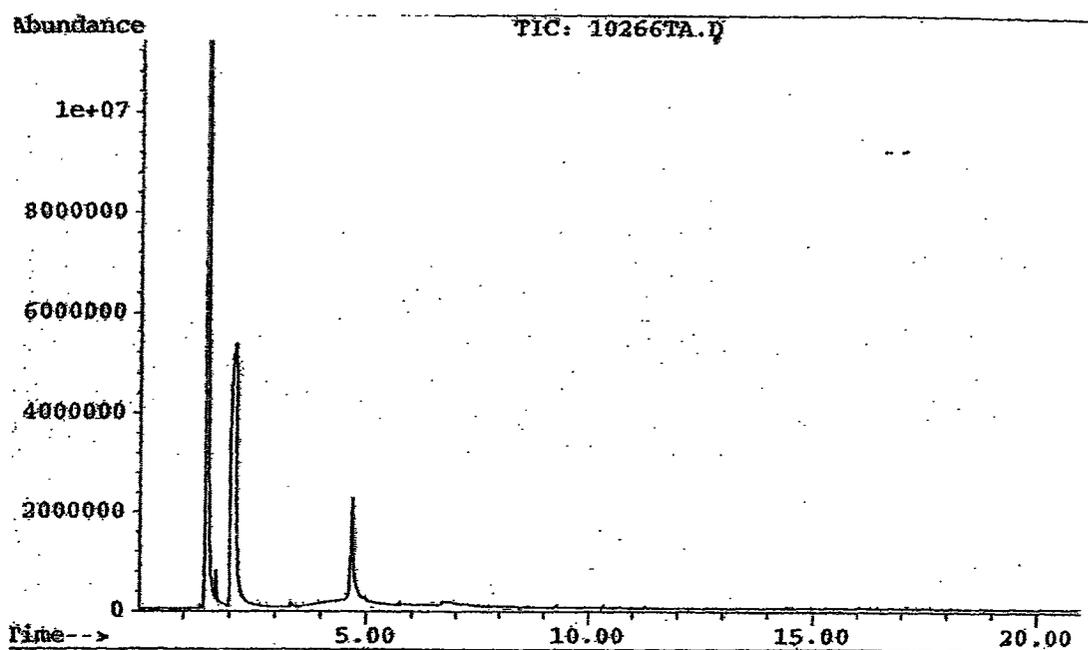
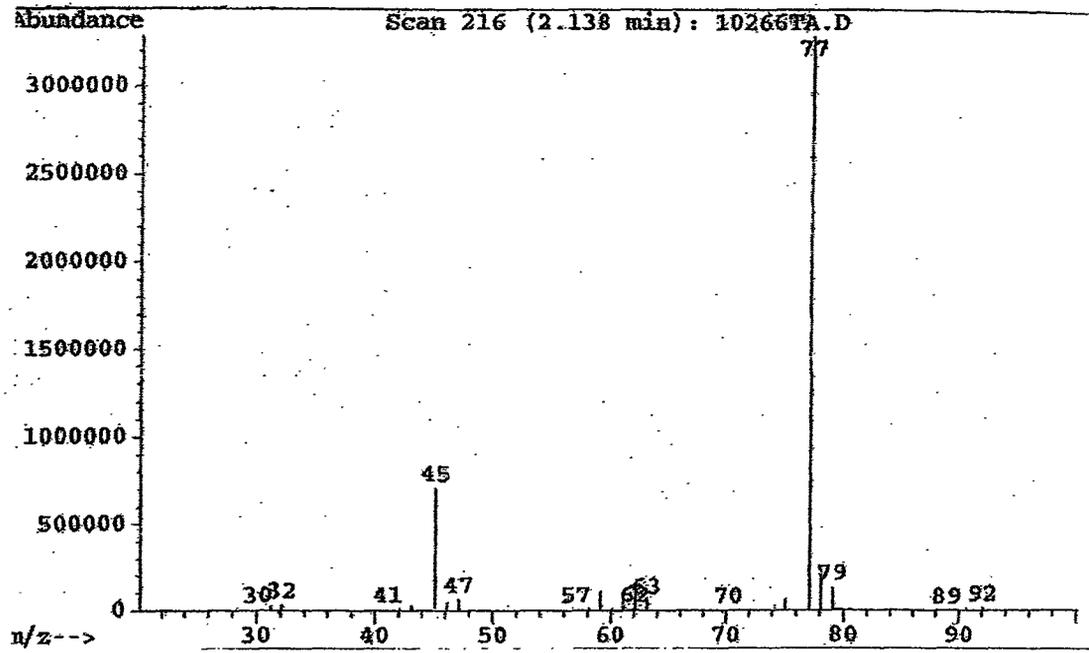


Figure 4. Mass Spectrum of Dimethylsilanediol



**APPENDIX III**

**Historical Control Data**

IN VITRO MAMMALIAN CYTOGENETIC TEST USING  
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES  
STRUCTURAL ABERRATIONS

2002-2004

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control <sup>2</sup> (%)
Mean	0.7	19.1
±SD <sup>1</sup>	1.0	7.4
Range	0.0-5.0	8.0-56.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control <sup>3</sup> (%)
Mean	0.9	22
±SD <sup>1</sup>	1.2	11.0
Range	0.0-5.0	8.0-70.0

<sup>1</sup> SD = standard deviation.

<sup>2</sup> Positive control for non-activated studies, Mitomycin C (MMC, 0.1-0.2 µg/mL).

<sup>3</sup> Positive control for S9-activated studies, cyclophosphamide (CP, 10-20 µg/mL).

IN VITRO MAMMALIAN CYTOGENETIC TEST USING  
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES  
COMBINED NUMERICAL ABERRATIONS  
(POLYPLOID AND ENDOREDUCATED CELLS)  
2002-2004

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control <sup>2</sup> (%)
Mean	1.7	2.0
±SD <sup>1</sup>	1.5	1.7
Range	0.0-7.5	0.0-8.5

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control <sup>3</sup> (%)
Mean	2.3	2.1
±SD <sup>1</sup>	2.0	1.8
Range	0.0-10.0	0.0-6.5

1

SD = standard deviation.

2

Positive control for non-activated studies, Mitomycin C (MMC, 0.1-0.2 µg/mL).

3

Positive control for S9-activated studies, cyclophosphamide (CP, 10-20 µg/mL).

**APPENDIX IV**

**Summary of Initial Assay and Results**

***In Vitro* Mammalian Chromosome Aberration Test  
(Initial Assay)**

**STUDY INFORMATION**

**Sponsor:** Silicones Environmental Health and Safety  
Council (SEHSC)  
SEHSC, Suite 500, 2325 Dulles Corner Blvd.  
Herndon, VA 20171

**Sponsor Representative:** Sharon Mudgett  
Dow Corning Corporation  
2200 West Salzburg Rd., Mail # C03101  
Midland, MI 48686

**Testing Facility:** BioReliance  
9630 Medical Center Drive  
Rockville, Maryland 20850

**Study Director:** Ramadevi Gudi, Ph.D.

**BioReliance Study No.:** AB21RP.331.BTL

**Test Article I.D.:** Dimethylsilanediol (DMSD)

**Test Article Lot Number:** 20444-42

**Test Article CAS Number:** 1066-42-8

**Test Article Purity:** 99.0 ± 0.04 area % (provided by Sponsor)

**Test Article Description:** White powder

**Storage Conditions:** Room temperature, stored in the dark/desiccate  
Store loosely capped over P<sub>2</sub>O<sub>5</sub> (Sample 1)

**Test Article Receipt/Login Date:** 21 February 2006 (Sample 1)

**Study Initiation:** 23 February 2006

**Experimental Start Date:** 28 February 2006

**Experimental Completion Date:** 20 August 2006

## SUMMARY

The test article, Dimethylsilanediol (DMSD), was tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced S-9 activation system. A preliminary toxicity test was performed to establish the dose range for the chromosome aberration assay. In the absence both of test article precipitation in the treatment medium and at least 50% toxicity; and the molecular weight inadvertently not being provided to the laboratory, the highest dose level evaluated for chromosome aberrations was 5000 µg/mL in all harvests. Two additional lower dose levels were included in the evaluation. As mentioned within the body of the report, when it was discovered that inappropriate dose levels were inadvertently selected, the assay was rerun at the appropriate dose levels.

In this assay, the cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours after treatment initiation. The test article was soluble in water and in the treatment medium at all dose levels tested at the beginning and conclusion of the treatment period.

In this assay, only the non-activated and S9-activated 4-hour exposure groups were evaluated for chromosome aberrations.

## CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, Dimethylsilanediol (DMSD), was received by BioReliance on 21 February 2006 and was assigned the code number AB21RP. Expiration date of 17 April 2006 for Sample 1 was provided. Upon receipt, Sample 1 of the test article was described as a white powder. Sample 1 was stored at room temperature, in the dark with desiccant, loosely capped over P<sub>2</sub>O<sub>5</sub>. The Sponsor has determined the identity, strength, purity, composition or other characteristics to define the test article. A copy of the Characterization of Dimethylsilanediol (Lot Number 20444-42) Technical Report is included at the end of this section. Based on the expiration date in the Technical Report, Dimethylsilanediol (Lot Number 20444-42) was considered to be stable for the purpose of this study through 17 April 2006.

The solvents used and control articles were the same as those used for the repeat assay. Information about stability and characterization of the solvents and positive control articles can be found in the main text of the report.

## MATERIALS AND METHODS

The materials and methods used for this assay are the same as those used in the repeat assay.

### Evaluation of Test Results

The results of this assay are presented in tables within this appendix; however, since the dose levels selected in this assay were not appropriate based on molecular weight information, no further evaluation of these results will be conducted or reported.

APPENDIX IV - Table 1

CONCURRENT TOXICITY TEST USING Dimethylsilanediol (DMSD)  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD - INITIAL ASSAY

Treatment µg/mL	Flask	Cell Count Averages (x10 <sup>6</sup> )	Cell Viability (%)	Mean Cells per Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	1.63	100%	1.64	100%	
	B	1.65	100%			
Dimethylsilanediol (DMSD) 625	A	1.70	100%	1.65	100%	0%
	B	1.61	99%			
1250	A	1.79	100%	1.76	107%	-7%
	B	1.74	100%			
2500	A	1.62	99%	1.63	99%	1%
	B	1.66	100%			
5000	A	1.42	99%	1.44	87%	13%
	B	1.48	99%			
MMC, 0.1	A	1.75	98%	1.64	100%	0%
	B	1.58	99%			
MMC, 0.2	A	1.29	99%	1.37	84%	16%
	B	1.51	97%			

**Treatment:** CHO cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Mean Viable Cells/Flask** = cell count x % viable cells, reported as mean of Flasks A and B.

**Cell Growth Index** = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

APPENDIX IV - Table 2

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Dimethylsilanediol (DMSD)  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD - INITIAL ASSAY

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations				Severely Damaged Cells	Average Aberrations Per Cell		
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid	Chromosome					
								Br	Ex	Br	Dic	Ring		
Water	A	10.2	100	100	3	1	0	1	0	0	0	0	0	0.010
	B	9.8	100	100	4	4	0	2	1	0	1	0	0	0.040
Dimethylsilanediol (DMSD) 1250	A	8.8	100	100	2	5	0	2	2	1	0	1	0	0.060
	B	9.2	100	100	5	5	0	3	1	0	1	0	0	0.050
2500	A	9.2	100	50	6	14	0	6	1	0	0	0	0	0.140
	B	8.2	100	100	8	7	0	4	0	0	2	0	1	0.160
5000	A	8.8	100	50	0	22	0	5	4	0	4	0	0	0.260
	B	8.8	100	50	0	18	0	5	1	0	2	1	0	0.180
MMC, 0.2	A	6.0	100	50	1	24	0	5	7	5	0	1	0	0.360
	B	7.0	100	50	0	24	0	5	4	1	2	2	3	0.880

Treatment: CHO cells were treated for 4 hours at 37±1°C in the absence of an exogenous source of metabolic activation. An additional dose level of 625 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination.

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

APPENDIX IV - Table 3

CONCURRENT TOXICITY TEST USING Dimethylsilanediol (DMSD)  
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD - INITIAL ASSAY

Treatment µg/mL	Flask	Cell Count Averages (x10 <sup>6</sup> )	Cell Viability (%)	Mean Cells per Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	1.22	99%	1.21	100%	
	B	1.24	98%			
Dimethylsilanediol (DMSD) 625	A	1.34	97%	1.28	106%	-6%
	B	1.29	98%			
1250	A	1.23	98%	1.20	99%	1%
	B	1.21	98%			
2500	A	1.21	100%	1.22	101%	-1%
	B	1.24	100%			
5000	A	1.13	99%	1.07	88%	12%
	B	1.01	100%			
CP, 10	A	0.88	98%	0.87	72%	28%
	B	0.91	97%			
CP, 20	A	0.87	99%	0.84	69%	31%
	B	0.83	98%			

**Treatment:** CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Mean Viable Cells/Flask** = cell count x % viable cells, reported as mean of Flasks A and B.

**Cell Growth Index** = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

APPENDIX IV - Table 4

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH Dimethylsilanediol (DMSD)  
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD - INITIAL ASSAY

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations				Severely Damaged Cells	Average Aberrations Per Cell		
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid	Chromosome					
							Br	Ex	Br	Dic	Ring			
Water	A	11.0	100	100	1	2	0	1	0	0	1	0	0	0.020
	B	10.2	100	100	0	1	0	0	0	0	1	0	0	0.010
Dimethylsilanediol (DMSD) 1250	A	8.0	100	100	2	13	0	6	8	1	1	1	1	0.270
	B	8.4	100	100	2	10	0	1	6	1	2	2	0	0.120
2500	A	7.8	100	50	1	20	0	7	8	0	0	1	1	0.520
	B	8.0	100	50	3	22	0	5	7	3	0	0	0	0.300
5000	A	8.4	100	100	1	15	0	5	11	2	0	1	1	0.290
	B	8.6	100	100	0	11	0	4	4	1	3	2	0	0.140
CP, 10	A	6.0	100	25	1	56	0	9	12	1	1	0	5	2.920
	B	5.8	100	25	0	52	0	8	10	0	0	1	7	3.560

Treatment: CHO cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation. An additional dose level of 625 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination.

Mitotic index = number mitotic figures x 100/500 cells counted.

%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

APPENDIX IV - Table 5

CONCURRENT TOXICITY TEST USING Dimethylsilanediol (DMSD)  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20-HOUR CONTINUOUS TREATMENT - INITIAL ASSAY

Treatment µg/mL	Flask	Cell Count Averages (x10 <sup>6</sup> )	Cell Viability (%)	Mean Cells per Flask (x10 <sup>6</sup> )	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	1.61	100%	1.60	100%	
	B	1.63	97%			
Dimethylsilanediol (DMSD) 625	A	1.83	98%	1.73	108%	-8%
	B	1.70	98%			
1250	A	1.63	97%	1.65	103%	-3%
	B	1.77	97%			
2500	A	1.44	97%	1.45	90%	10%
	B	1.53	98%			
5000	A	1.43	100%	1.43	90%	10%
	B	1.47	97%			
MMC, 0.1	A	1.64	98%	1.52	95%	5%
	B	1.45	99%			
MMC, 0.2	A	1.28	99%	1.28	80%	20%
	B	1.30	99%			

**Treatment:** CHO cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.

**Cell Viability:** determined by trypan blue dye exclusion.

**Mean Viable Cells/Flask** = cell count x % viable cells, reported as mean of Flasks A and B.

**Cell Growth Index** = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

**Cell Growth Inhibition** = 100% - % cell growth index; not calculated for negative controls.

APPENDIX IV - Table 6

SUMMARY - INITIAL ASSAY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water	-S9	4	10.0	200	200	0.025	±0.157	3.5	2.5
Dimethylsilanediol (DMSD)									
1250	-S9	4	9.0	200	200	0.055	±0.250	3.5	5.0
2500	-S9	4	8.7	200	150	0.153	±0.857	7.0	9.3**
5000	-S9	4	8.8	200	100	0.220	±0.462	0.0	20.0**
MMC, 0.2	-S9	4	6.5	200	100	0.620	±1.813	0.5	24.0**
Water	+S9	4	10.6	200	200	0.015	±0.122	0.5	1.5
Dimethylsilanediol (DMSD)									
1250	+S9	4	8.2	200	200	0.195	±0.831	2.0	11.5**
2500	+S9	4	7.9	200	100	0.410	±1.223	2.0	21.0**
5000	+S9	4	8.5	200	200	0.215	±0.850	0.5	13.0**
CP, 10	+S9	4	5.9	200	50	3.240	±4.099	0.5	54.0**

**Treatment:** Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

**Aberrations per Cell:** Severely damaged cells were counted as 10 aberrations.

**Percent Aberrant Cells:** \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; using Fisher's Exact test.

**Characterization of Dimethylsilanediol  
(Lot Number 20444-42)  
Technical Report**

**DOW CORNING CORPORATION  
HEALTH & ENVIRONMENTAL SCIENCES  
TECHNICAL REPORT**

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**Report Number:** 2006-I0000-56101

**Title:** Characterization of Dimethylsilanediol  
(Lot Number 20444-42)

**HES Study Number:** 10179-102

**Test Article:** Dimethylsilanediol

**Study Director:** Debra Stutts

**Sponsor:** Silicones Environmental, Health and Safety Council (SEHSC)  
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**Study Completion Date:** 25 April 2006

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### ABSTRACT

The test article, dimethylsilanediol (Lot Number 20444-42) was characterized in accordance with EPA (TSCA) Good Laboratory Practices 40 CFR Part 792.

The physical appearance of the test article was observed to be a translucent white solid. The GC-MS fragmentation pattern is consistent with that expected for dimethylsilanediol. The area percent purity was determined to be  $99.0 \pm 0.04$  by GC/FID. The GC-MS fragmentation pattern of the most abundant impurity, present at 0.5%, is consistent with that expected for dihydroxytetramethyldisiloxane (dimerdiol). This test article meets the specification for physical description and identification as defined in the PDM.

**GLP COMPLIANCE STATEMENT**

This study was conducted in accordance with EPA (TSCA) Good Laboratory Practices 40 CFR Part 792.

  
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Debra Stutts, B. S.  
Senior Analytical Chemist  
Study Director

5 May 2006  
Date

  
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Roy A. Campbell, B. S.  
Health and Environmental Sciences  
HES Group Manager - Chemistry

25-Apr-2006  
Date

Title: Characterization of Dimethylsilanediol  
(Lot Number 20444-42)

Study Number: 10179-102

This study has been audited by the Dow Corning Corporation Health and Environmental Sciences Quality Assurance Unit according to approved Standard Operating Procedures to assure that the raw data are accurately reflected within this final report. The following are the inspection dates and the dates inspection findings were reported.

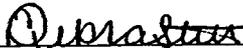
<u>Dates of Inspection</u>	<u>Phase Inspected</u>	<u>Findings Reported to Study Director and Management</u>
11 Feb 06	Draft Protocol Review	13 Feb 06
14 Feb 06	GC-FID Analysis	14 Feb 06
20 Feb 06	GC-FID Analysis	20 Feb 06
20-24 and 27-30 Mar 06	Draft Final Report and Associated Raw Data Review	05 Apr 06

  
\_\_\_\_\_  
Lisa T. Drott, B.A.  
Quality Assurance Team Leader  
Dow Corning Corporation  
Health & Environmental Sciences

19 Apr 2006  
Date

APPROVAL SIGNATURES

This report consists of pages 1 through 14.

  
\_\_\_\_\_  
Debra Stutts, B. S.  
Senior Analytical Chemist  
Study Director

5 May 06  
Date

  
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Health and Environmental Sciences  
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25-Apr-2006  
Date

**STUDY INFORMATION**

Study Initiation Date:	13 Feb 2006
Experimental Start Date:	14 Feb 2006
Experimental Termination Date:	20 Feb 2006
Study Completion Date:	25 April 2006

**A. Study Purpose**

The objective of this study was to characterize the test article, dimethylsilanediol according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792.

**B. Test Article / Substance / Item**

**Identification:** Dimethylsilanediol, supplied as Dow Corning ® XX-1240 Dev Sample

**Lot Number:** 20444-42

**Expiration Date:** 17 April 2006

**Source:** Dow Corning Corporation, Auburn, Michigan 48611

**CAS Number:** 1066-42-8

**Physical Description:** translucent white solid, as determined by this study

**Stability:** 8 weeks, refer to information provided by the sample submitter

**Purity:** 99.0 ± 0.04 area % purity, as determined by this study

**Solubility:** Acetone, diethyl ether

**Storage Conditions:** Room Temperature in presence of desiccant

**Archive:** A reserve sample was not retained for this study

**C. Labeling**

The original test article container for this study was labeled with the following information: study number, name of test article, lot number, container accession number, concentration, storage conditions, expiration date, initials of the individual distributing the test article and date the test article was dispensed into the container.

**D. Route of Exposure and Justification**

As the test article is the test system, this is not applicable.

**E. Experimental Procedures****1. Physical Description**

The physical appearance of the test article was determined by visual examination.

**2. Sample Preparation**

The test article was dissolved in approximately 1 mL anhydrous ethyl ether.

**3. GC/FID Analysis**

A Hewlett Packard (HP) 6890 GC with flame ionization detector and HP 7683B autosampler was used to determine an area percent purity of dimethylsilanediol, based on the average of 3 replicate injections (2.0 µL). A solvent blank (ether) was injected (2 µL injection volume) prior to the test article solution analysis. The actual instrument parameters are shown in Table I. The GC area percent results were calculated as the ratio of dimethylsilanediol peak area divided by the sum of all peak areas, excluding those attributed to the solvent. Prior to analyzing dimethylsilanediol, the oven temperature was increased to approximately 280 °C for 30 minutes to prevent carry over by heating the column to burn off any residual material left from previous analyses. Once a stable baseline was observed, the GC performance was considered acceptable for use. The GC/FID performance was verified by conducting triplicate 1 µL injections of an Agilent

checkout sample using the autosampler. Hewlett Packard Chemstation revision A.10.02 software was used for data acquisition and analysis.

Table I. GC/FID Instrument Parameters

Parameter	Setting
Oven Temperature Program	70 °C (hold 2 min) to 280 °C (hold 5 min) at 25 °C/min
Inlet Temperature	250 °C
Detector Temperature	280 °C
Capillary Column	HP-5 (5% Phenyl Methyl Siloxane), 0.25 mm inner diameter 30 m length, 1.0 µm film thickness
Mode	Constant pressure, He carrier
Initial Flow	1.5 mL/min
Split Ratio	40:1

#### 4. GC-MS Analysis

An HP 5890A Series II GC with an HP 5970B mass selective detector (MSD) was used to verify the identity of the major component in the test article solution as dimethylsilanediol by manual injection (<1.0µL). A diethylether solvent blank was injected (<1.0 µL) prior to the test article solution analysis. The standard autotune procedure was conducted prior to analyzing the test article. The standard autotune is an automated tuning program that uses perfluorotributylamine (PFTBA) to optimize the MS for general-purpose electron impact ionization (EI) operation. The GC oven temperature was increased to 280 °C for 60 minutes to prevent carry over by burning off residual material remaining from previous analyses. HP ChemStation version B.02.05 software and HP MS ChemStation version C.03.00 software were used for data acquisition and analysis. The mass spectrum for the major component in the total ion chromatogram (TIC) for the test article analysis was library searched and a reasonable match for dimethylsilanediol was not obtained. The actual instrument parameters used are shown in Table II.

Table II. GC-MS Instrument Parameters

Parameter	Setting
Oven Temperature Program	70 °C (hold 2 min) to 280 °C at 20 °C/min
Inlet Temperature	250 °C
Capillary Column	HP-5MS (Low Bleed 5%-Diphenyl-95%-dimethylsiloxane copolymer), 0.25 mm inner diameter, 30 m length, 0.25 µm film thickness
Mode	Constant flow, He carrier 2.2 psi at 70 °C, vacuum compensation on
Mass Scan Range	15-500 atomic mass units

#### F. Data Analysis

Calculation of average, standard deviation and percent relative standard deviation for GC/FID was performed.

**G. Deviations**

In the GC/FID analysis of dimethylsilanediol, a HP7683B autosampler was used rather than the HP7673B as specified in the protocol. This did not affect the quality or integrity of the data.

**H. Results and Discussion****1. Physical Description**

The physical appearance of the test article was observed to be a translucent white solid, which is consistent with the physical form and color in the PDM (Physical Properties Section).

**2. GC/FID Analysis**

The GC/FID area percent purity for test article name was  $99.0 \pm 0.04$  based on the average of 3 replicate injections. The results are summarized in Table III. Representative GC/FID chromatograms of the diethyl ether blank and the test article is shown in Figures 1 and 2, respectively. The GC/FID performance verification passed the acceptance criteria described in protocol.

**3. GC-MS Analysis**

The major peak in the total ion chromatogram (TIC) eluted at a retention time of 1.686 minutes and is shown in Figure 3. The molecular ion ( $m/z$  92) is present in the mass spectrum shown in Figure 4. The base peak ( $m/z$  77) corresponds to  $\text{CH}_3\text{Si}(\text{OH})_2^+$  and is formed by the loss of a methyl group from the parent molecule ( $M = 92$ ). The fragmentation pattern is consistent with that expected for dimethylsilanediol. The GC-MS fragmentation pattern of the most abundant impurity, eluting at 2.9 minutes, is consistent with that expected for dihydroxytetramethyldisiloxane (dimerdiol), displaying a prominent peak at  $m/z$  151 which corresponds to the loss of a methyl group from the parent molecule ( $M = 168$ ). The base peak ( $m/z$  133) corresponds to the ion formed from loss of a methyl group and water from the parent.

**I. Conclusions**

Dimethylsilanediol was characterized according to EPA (TSCA) Good Laboratory Practices 40 CFR Part 792. The physical appearance of the test article was observed to be a translucent white solid. The GC/MS fragmentation pattern is consistent with that expected for dimethylsilanediol. The area percent purity was determined to be  $99.0 \pm 0.04$  by GC/FID. The GC-MS fragmentation pattern of the most abundant impurity, present at 0.5%, is consistent with that expected for dihydroxytetramethyldisiloxane (dimerdiol). This test article meets the specification for physical description and identification as defined in the PDM.

**J. Archive**

All raw data were archived at the same location as the protocol, amendments, study authorization documentation and final report; Dow Corning Corporation, Health and Environmental Sciences, 2200 W. Salzburg Road, Auburn, MI 48611.

**K. References**

1. Product Data Management (PDM), unpublished document of Dow Corning.

Table III. GC/FID Purity Results for Dimethylsilanediol

Replicate	Peak #	Retention Time (min)	Area %	Peak Assignment
1	1	~1.5	Not integrated	Diethyl Ether
	2	1.923	Not reported	Impurity in Diethyl Ether
	3	2.006	Not reported	Impurity in Diethyl Ether
	4	2.545	98.94	Dimethylsilanediol
	5	3.927	0.1333	Not identified
	6	4.718	0.5331	dimerdiol
	7	6.297	0.2345	Not identified
	8	7.488	0.1582	Not identified
2	1	~1.5	Not integrated	Diethyl Ether
	2	1.924	Not reported	Impurity in Diethyl Ether
	3	2.007	Not reported	Impurity in Diethyl Ether
	4	2.198	Not reported	Impurity in Diethyl Ether
	5	2.545	98.97	Dimethylsilanediol
	6	3.928	0.1359	Not identified
	7	4.718	0.5119	dimerdiol
	8	6.298	0.2245	Not identified
	9	7.489	0.1587	Not identified
3	1	~1.5	Not integrated	Diethyl Ether
	2	1.923	Not reported	Impurity in Diethyl Ether
	3	2.006	Not reported	Impurity in Diethyl Ether
	4	2.197	Not reported	Impurity in Diethyl Ether
	5	2.545	99.02	Dimethylsilanediol
	6	3.928	0.1413	Not identified
	7	4.718	0.4689	dimerdiol
	8	6.298	0.2131	Not identified
	9	7.489	0.1557	Not identified
Mean (n=3)			99.0	
Standard Deviation			0.04	
RSD (%)			0.04	

Figure 1. GC/FID Chromatogram of Diethyl Ether

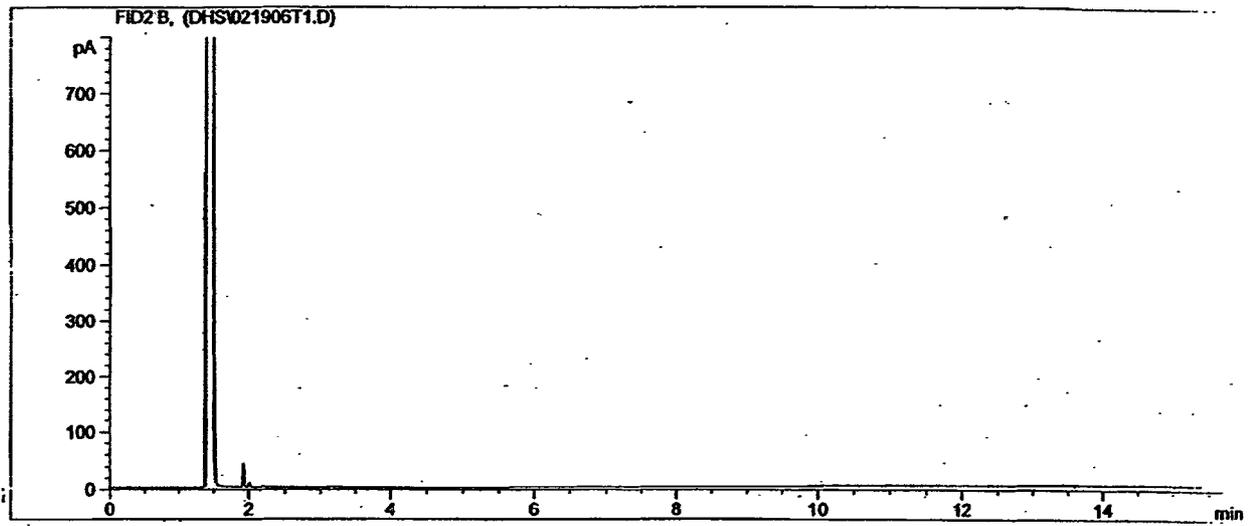


Figure 2. GC/FID Chromatogram of Dimethylsilanediol in Diethyl Ether

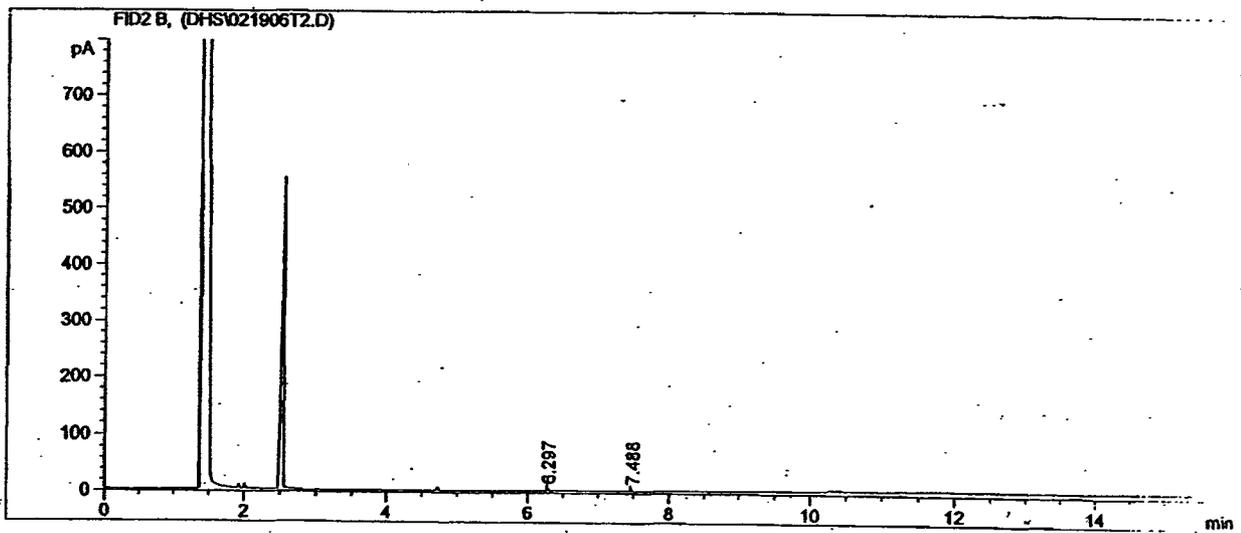


Figure 3. Total Ion Chromatogram from GC/MS Analysis of Dimethylsilanediol in Diethyl Ether

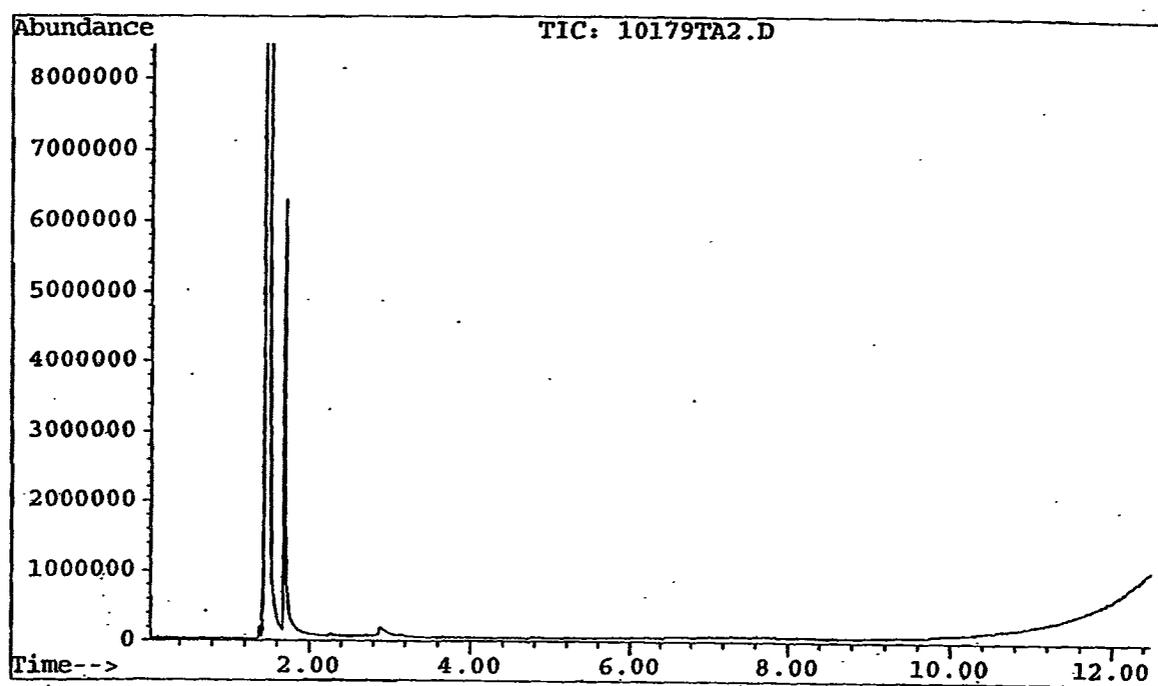


Figure 4. Mass Spectrum of Dimethylsilanediol

