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DOW CORNING

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8EHQ-0198-13569

Confidential No CBI

January 8, 1998

TSCA Document Processing Center (7407)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Attn: TSCA Section 8(e) Coordinator
401 M Street S.W.
Washington, D.C. 20460

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Re: Supplemental Submission to 8EHQ-0196-13559
TSCA Section 8(e) Notification of Substantial Risk
Octamethylcyclotetrasiloxane (OMCTS, D_4)

Dear Sir:

8EHQ-96-13559

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 (40 FR 11110, March 16, 1978), Dow Corning is submitting a recently completed immunological evaluation, comprising 14 studies, as a supplemental submission to our Notification of Substantial Risk of January 17, 1996 (8EHQ-0196-13559) and ensuing supplemental submission of May 13, 1996 (8EHQ-0596-13569).

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These studies was conducted as part of our siloxane research program, a thirty million dollar voluntary health effects research program on six siloxane materials which has been reviewed with EPA's Office of Pollution Prevention and Toxics on several occasions and which was the subject of Memorandum of Understanding between Dow Corning and EPA which was signed April 9, 1996.

Chemical Substance:

541-02-6	Decamethylcyclopentasiloxane, 4 wt.%
541-05-9	Hexamethylcyclotrisiloxane, 0.1 wt.%
556-67-2	Octamethylcyclotetrasiloxane, 96 wt.%
	in DOW CORNING® 244 Fluid (octamethylcyclotetrasiloxane)

Manufacturer:

Dow Corning Corporation
2200 West Salzburg Road
Midland, Michigan 486867-0994

89980000108

Dow Corning Corporation
Midland, Michigan 48686-0994
Phone: (517) 496-4000

While Dow Corning will continue to provide EPA with information which meets the spirit and intent of TSCA Section 8(e), it is clear that our initial notification of January 17, 1996 was premature.

Actions:

A copy of this final report also is being provided to EPA under TSCA Section 8(d) (health and safety data reporting).

For purposes of supplemental reporting under TSCA Section 8(e), the general PROPRIETARY designation on the attached health and safety report is waived by Dow Corning.

Dow Corning will inform EPA of any pertinent information that may be developed concerning this material.

If you have any questions concerning this study, please contact me at the address provided herein. If you require further general information regarding this submission, please contact Dr. Rhys G. Daniels, Regulatory Compliance Specialist, Regulatory Compliance Department, at 517-496-4222 or at the address provided herein.

Sincerely,

Michael P. Hill
Americas Vice President and Corporate Director
Health and Environmental Sciences
(517) 496-4057

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Recently Completed Study

IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRA-SILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Dow Corning Corporation
1997-I0000-41338
December 29, 1997

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Executive Summary:

As a supplemental submission to our earlier TSCA Section 8(e) notification of January 17, 1996 and ensuing supplemental submission of May 13, 1996, Dow Corning is submitting the final report for an immunological evaluation of octamethylcyclotetrasiloxane (OMCTS, D_4) in male and female Fischer 344 rats. Based on the now complete data, these studies show that OMCTS is not toxic to the immune system of Fischer 344 rats under the study conditions employed and should not be classified as an immunotoxic agent.

Background:

In a letter dated January 17, 1996, Dow Corning Corporation provided EPA with a notification of substantial risk under TSCA Section 8(e) reporting preliminary results from immunotoxicology studies on OMCTS in male and female Fischer 344 rats, highlighting changes in humoral activity, the number and percentages of subpopulations of lymphocytes in the spleen, splenic phagocytosis, and hematological parameters. As was reported in our supplemental notification of May 13, 1996, and is confirmed in the final report which we now are submitting as a final supplemental submission, our outside investigators have repeated the surface marker and immunotoxicological studies on OMCTS. Based on the collective data sets, they have concluded that OMCTS, under the laboratory conditions employed, did not produce an immunotoxic response in the Fischer 344 rat and should not be classified as an immunotoxic agent.

Details of Study Findings:

Groups of male and female Fischer 344 rats (10 per group) were administered OMCTS via oral gavage daily for 28 days at doses of 10, 30, 100 and 300 mg/kg in corn oil. Biological parameters were measured one day after the last exposure and immune cells were derived from blood and spleen. Blood for hematological studies and serum for antibody titers were collected by cardiac puncture.

Test animals showed a normal increase in body weight, with body weight change equivalent to the control group. There was a slight but dose-dependent decrease

in the erythroid elements in male and female rats. Liver weights were increased and thymus weights decreased as a function of OMCTS dose. These organ weight changes were more pronounced in female rats.

Humoral activity, as measured by the IgM response to the T-dependent antigen sheep erythrocytes (sRBC), was not altered by OMCTS exposure in male rats as measured by the hemolytic antibody plaque assay (AFC) and serum antibody titers. In female rats, there was a dose-dependent increase in the IgM response when assessed by the AFC response but not with the antibody titers.

There were no changes in cell-mediated immunity as measured by the one-way mixed lymphocyte (MLR) response. There also were no changes in Natural Killer (NK) cell activity.

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Macrophage function as measured by the vascular clearance and phagocytic uptake of ^{51}Cr sRBC into the liver spleen and lungs showed some slight OMCTS-associated changes. These changes are considered to be related to liver size and, thus, liver blood volume associated with OMCTS.

Phenotyping of lymphocytes derived from the blood and spleen showed no biologically significant alterations associated with OMCTS exposure.

In separate studies performed as amendments to the protocol, OMCTS was delivered in 4 different vehicles. Liver weight was used an indicator of systemic exposure. OMCTS produced the greatest increase in liver weight when administered in corn oil, followed by 10% emulphor and a commercially available antigas/antiacid preparation . No increase in liver weight was observed when OMCTS was administered in a 35 cs polydimethylsiloxane vehicle.

Overall, these studies show that OMCTS, when administered in corn oil at doses between 10 and 300 mg/kg, does not cause immune suppression in male and female Fischer 344 rats.

Conclusions:

Following repeat studies, the interim immunotoxic observations on OMCTS submitted to EPA in our initial notification of January 17, 1996, clearly are not reproducible. As was indicated in our supplemental submission of May 13, 1996, based on the now complete data set, our investigators have concluded that OMCTS, under the laboratory conditions employed, did not produce an immunotoxic response in the Fischer 344 rat and should not be classified as an immunotoxic agent.

DOW CORNING CORPORATION
HEALTH & ENVIRONMENTAL SCIENCES
TECHNICAL REPORT

MEDICAL COLLEGE OF VIRGINIA
VIRGINIA COMMONWEALTH UNIVERSITY
DEPT. OF PHARMACOLOGY & TOXICOLOGY
AND PROGRAM OF BIOMEDICAL ENGINEERING
RICHMOND, VA 23298-0613

Report No: 1997-i0000-41338

Title: Immunological Evaluation of Octamethylcyclotetrasiloxane (D4) Using a Twenty-Eight Day Exposure in Male and Female Fischer 344 Rats

Study No.: Dow Corning File Number 6389

External Testing Facility No.: D4-28-1R-PO

Test Article: Octamethylcyclotetrasiloxane

Study Director: Albert E. Munson, Ph.D.
Professor of Pharmacology & Toxicology

Author: Albert E. Munson, Ph.D.
Professor of Pharmacology & Toxicology

Sponsor: Dow Corning Corporation

Sponsor Representative: Paal C. Klykken, Ph.D.
Associate Scientist

Testing Facility: Medical College of Virginia
Virginia Commonwealth University
Department of Pharmacology & Toxicology
and Program of Biomedical Engineering
Richmond, VA 23298-0613

Study Completion Date:

Security Statement: Dow Corning Proprietary information. Distribution outside the Corporation must be approved by the U.S. Area Vice President, Director of HES. When no longer needed, this report should be disposed of by shredding, burning or depositing in a file paper container.

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ABSTRACT

The studies presented in this report consist of the investigations related to Protocol D4-28-1R-PO (Dow Corning Study No. 8389). This research is part of the overall immunotoxicological Assessment of Siloxanes as established in the Research Agreement of March 15, 1994 between Dow Corning Corporation and the Foundation for Immunotoxicology.

The purpose of this study is to determine the potential effects of octamethylcyclotetrasiloxane (D4) on the immune system. The in-life phase for the studies was initiated April 19, 1994. The protocol was amended on May 16, 1995 to include repeat studies on those biological parameters which showed significant differences between D4-exposed groups and the control group. Two additional studies were included in the protocol via a note to file #11, dated April 12, 1996. These studies address the serum antibody response to sRBC on day 4 and day 6.

This report encompasses 14 studies and consists of the development of a rat-based model for immunological assessment of test articles, the concurrent validation/evaluation of D4 for any potential immunological events, and studies directed at ascertaining if there was any vehicle dependence on dose. The departure from the classical mouse-based immunological assessment was prompted by the study sponsor in order to limit the mouse to rat extrapolations and to facilitate the comparison of other toxicological data generated for this compound in rats.

In these D4 studies male and female Fischer 344 rats (10 per group) were used as the experimental animals. The route of administration was by oral gavage and the duration of exposure was daily for 28 days. Doses of D4 used were 10, 30, 100 and 300 mg/kg and were administered in corn oil. The biological parameters were measured one day after the last exposure and immune cells were derived from blood and spleen. Blood for hematological studies and serum for antibody titers were collected by cardiac puncture.

Rats, administered D4, showed a normal increase in body weight, with body weight change equivalent to the vehicle control group. There was a slight, but dose-dependent decrease in the erythroid elements in male and female rats. Liver weights were increased and thymus weights decreased as a function of D4 dose. These organ weight changes were more pronounced in the female.

Humoral immunity, as measured by the IgM response to the T-dependent antigen sheep erythrocytes (sRBC), was not altered by D4 exposure in male rats as measured by the hemolytic antibody plaque assay (AFC) and serum antibody titers. In female rats there was a

dose-dependent increase in the IgM response to sRBC when assessed by the AFC response but not with the antibody titers.

There were no changes in cell-mediated immunity as measured by the one-way mixed lymphocyte (MLR) response. There were also no changes in Natural Killer (NK) cell activity.

Macrophage function as measured by the vascular clearance and phagocytic uptake of ^{51}Cr sRBC into the liver, spleen and lungs showed some slight D4-associated changes. These changes are considered to be related to liver size and, thus, liver blood volume associated with D4.

Phenotyping of lymphocytes derived from the blood and spleen showed no biologically significant alterations associated with D4 exposure.

In separate studies performed as amendments to the protocol, D4 was delivered in 4 different vehicles. Liver weight was used as an indicator of systemic exposure. D4 produced the greatest increase in liver weight when administered in corn oil, followed by 10% emulphor and Maalox. No increase in liver weight was noted when D4 was administered in a 35 cs polydimethylsiloxane (PDMS) vehicle.

Overall, these studies show that D4, when administered in corn oil at doses between 10 and 300 mg/kg, does not cause immune suppression in male and female Fischer 344 rats.

GLP COMPLIANCE STATEMENT

This study was conducted in compliance with USEPA TSCA Good Laboratory Practice Regulations (40-Code of Federal Regulation, Part 792) and in accordance with agreements between the sponsor's representative and the study director at the Medical College of Virginia/Virginia Commonwealth University.

Name: Albert E Munson Date: 12/29/97

Albert E. Munson

Degree: Ph.D.

Title: Professor of Pharmacology & Toxicology

Study Director

1. Neither the Test Article (D4), the D4 mixtures, nor the control materials were analyzed in accordance with GLPs. The best available practices were used.

2. The Positive Control materials were not retained or archived because of their chemical instability.

QUALITY ASSURANCE STATEMENT

Test Article: Octamethylcyclotetrasiloxane

Report Title: Immunological Evaluation of Octamethylcyclotetrasiloxane (D4) Using a Twenty-Eight Day Exposure in Male and Female Fischer 344 Rats

Protocol Title: Immunological Evaluation of Octamethylcyclotetrasiloxane (D4) Using a Twenty-Eight Day Exposure in Male and Female Fischer 344 Rats

Protocol No.: D4-28-1R-PO

The final report for the indicated protocol has been reviewed by the Quality Assurance Unit of the Medical College of Virginia in accordance with the Good Laboratory Practice Regulations as set forth in 40-CFR 792.35 (b) (Federal Register, Vol. 54: 34043). It has been found that the described and authorized methods and standard operating procedures were conducted in compliance with the Good Laboratory Practice Regulations as stated in the compliance statement. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and management.

Inspection/Audits were performed and reported on the following dates:

Performed	Reported	Activity
March 24, 1994	March 24, 1994	Protocol Evaluation
May 5, 1994	May 9, 1994	Food Consumption Records
May 6, 1994	May 6, 1996	Dosing Rats
May 13, 1994	May 16, 1994	Injection of sRBC
May 17, 1994	May 17, 1994	Audit AFC Assay
June 7, 1994	June 7, 1994	Dosing Rats
June 16, 1994	June 17, 1994	Food Consumption Records
June 30, 1994	June 30, 1994	Audit RES Assay
July 19, 1994	July 19, 1994	Weighing Rats
July 19, 1994	July 19, 1994	Dosing Rats
July 28, 1994	July 28, 1994	Dosing Rats
August 5, 1994	August 5, 1994	Dosing Rats and Positive Control
September 27, 1994	September 27, 1994	Dosing Rats
September 29, 1994	September 29, 1994	Recording Food Consumption
October 14, 1994	October 14, 1994	Dosing Rats
November 4, 1994	November 8, 1994	Dosing Rats
November 10, 1994	November 10, 1994	Recording Food Consumption
November 11, 1994	November 11, 1994	Dosing Rats
December 1, 1994	December 1, 1994	Audit NK, Leuco. Proliferation Splenic Karyotyping

(continued on next page)

Quality Assurance Statement, continued

Performed	Reported	Activity
February 6, 1995	February 6, 1995	Dosing Rats
March 1, 1995	March 1, 1995	Assay
August 1, 1995	August 1, 1995	Weighing Rats
August 2, 1995	August 2, 1995	Dosing Rats
August 2, 1995	August 2, 1995	Recording Food Consumption
August 8, 1995	August 9, 1995	Dosing Rats
August 9, 1995	August 9, 1995	Weighing Rats
August 30, 1995	August 30, 1995	Assay
September 12, 1995	September 12, 1995	Weighing Rats
September 26, 1995	September 26, 1995	Dosing Rats
October 6, 1995	October 6, 1995	Dosing Rats
October 11, 1995	October 11, 1995	Assay
December 4, 1995	December 4, 1995	Dosing Rats
December 7, 1995	December 7, 1995	Weighing Rats
December 12, 1995	December 12, 1995	Dosing Rats
December 13, 1995	December 13, 1995	Assay
December 27 1995	December 27 1995	Chromate sRBC
December 28, 1995	December 28, 1995	RES Assay
December 28, 1995	December 28, 1995	Randomize Rats
January 5, 1996	January 5, 1996	Weighing Rats
January 11, 1996	January 11, 1996	Dosing Rats
January 12, 1996	January 12, 1996	Weighing Rats
January 25, 1996	January 25, 1996	Assay
February 8, 1996	February 8, 1996	Assay
February 23, 1996	February 23, 1996	Dosing Rats
March 6, 1996	March 8, 1996	Dosing Rats
March 22, 1996	March 22, 1996	Assay
March 26, 1996	March 26, 1996	Assay
October 8, 1996	October 8, 1996	Data audit
December 22, 1997	December 22, 1997	Final report audit

Approved and submitted by: Aileen Reingle
Quality Assurance Manager

22 December 1997
Date

APPROVAL SIGNATURES

This report consists of pages 1 through 286 including Tables 1 through 65, Figures 1 through 4, Charts 1 through 32, and Appendices A through I.

Name Albert E Munson Date 12/29/97

Study Director Albert E. Munson

Degree: Ph.D.

Title: Professor of Pharmacology & Toxicology

Name Paal C. Klykken Date 12/26/97

Sponsor Representative Paal C. Klykken

Degree: Ph.D.

Title: Associate Scientist

STUDY INFORMATION

Study Initiation Date:	March 24, 1994
Experimental Start Date:	April 19, 1994
Experimental Termination Date:	March 26, 1996
Study Completion Date:	TBD
Study Director:	Albert E. Munson, Ph.D., Professor of Pharmacology & Toxicology
Sponsor:	Dow Corning Corporation
Sponsor Representative:	Paal C. Klykken, Ph.D., Associate Scientist
Study Personnel:	Albert E. Munson, Ph.D., Study Director J. Ann McCay Munson, B.S., Study Coordinator H. Leon Pringle, Ph.D., Quality Assurance Manager Kimber L. White, Jr., Ph.D., Immunology Deborah Musgrove, B.S., Laboratory Specialist Senior Ronnetta D. Brown, B.S., Laboratory Specialist Senior Leon Butterworth, B.S., Laboratory Specialist Patricia C. Gerber, LVT, B.S., Laboratory Specialist Daniel W. David, M.S., Research Associate Shirley S. Griffey, Laboratory Technician Senior Susan Wilson, DVM, Ph.D. B. Jean Meade, DVM, Ph.D. Nancy L. Smith, LVT, Division of Animal Resources Rebecca E. Perry, LVT, Division of Animal Resources Randolph H. Davis, Division of Animal Resources Lennie H. Venable, Division of Animal Resources C. L. Jones, Division of Animal Resources

In-Life Study Dates:	
Study 1	19 Apr 94 to 17 May 94
Study 2	2 June 94 to 30 June 94
Study 3	12 July 94 to 9 Aug 94
Study 4	22 Sept 94 to 20 Oct 94
Study 5	3 Nov 94 to 1 Dec 94
Study 6	1 Feb 95 to 1 Mar 95
Study 7	2 Aug 95 to 30 Aug 95
Study 8	12 Sept 95 to 11 Oct 95
Study 9	5 Jan 96 to 2 Feb 96
Study 9/Recovery	5 Jan 96 to 16 Feb 96
Study 10	15 Nov 95 to 14 Dec 95
Study 10/Recovery	15 Nov 95 to 28 Dec 95
Study 11	28 Dec 95 to 25 Jan 96
Study 11/Recovery	28 Dec 95 to 8 Feb 96
Study 12	14 Nov 95 to 13 Dec 95
Study 12/Recovery	14 Nov 95 to 27 Dec 95
Study 13	23 Feb 96 to 22 Mar 96
Study 14	27 Feb 96 to 26 Mar 96

INTRODUCTION

This report encompasses 14 studies and consists of the development of a rat-based model for immunological assessment of test articles, the concurrent validation/evaluation of D4 for any potential immunological events, and studies directed at ascertaining if there was any vehicle dependence on dose. The departure from the classical mouse-based immunological assessment was prompted by the study sponsor in order to limit the mouse to rat extrapolations and to facilitate the comparison of other toxicological data generated for this compound in rats. The studies reported herein are related to Protocol D4-28-1R-PO (Dow Corning Study No. 8389). This research is part of the overall Immunotoxicological Assessment of Siloxanes as established in the Research Agreement of March 15, 1994 between Dow Corning Corporation and the Foundation for Immunotoxicology.

The purpose of these studies was to determine the potential effects of octamethylcyclotetrasiloxane (D4) on the immune system. Male and female Fischer 344 rats (10 per group) were used as the experimental animals. The route of administration was by oral gavage and the duration of exposure was daily for 28 days. Doses of D4 used were 10, 30, 100 and 300 mg/kg and were administered in corn oil. The biological parameters were measured one day after the last exposure and immune cells were derived from blood and spleen. Blood for hematological studies and serum for antibody titers were collected by cardiac puncture. Food consumption, organ and body weight measurements were also included as indicators of general health.

METHODS OF PROCEDURE

EXPERIMENTAL DESIGN

SPECIES SELECTION. Male and female Fischer 344 rats were used in these studies. The Fischer 344 rat is currently the rat strain of choice in conducting immunological studies and was also requested to be used by the sponsor. The major components of the immune system in the rat and man are the same; agents that perturb the immune system in man perturb the immune system in the rat in a similar manner. The rat has previously been found to be sensitive to the effects of various environmental chemicals on the immune system.

SPECIES MAINTENANCE. Male and female Fischer 344 rats were purchased from Charles River Breeding Laboratories, Raleigh, NC or Kingston, NY. The rats were hepatitis and Sendai viruses free. Animals arrived at 6-8 weeks of age (120 to 150 g). Upon arrival the rats were quarantined for at least 5-7 days prior to commencement of exposure. All rats were

randomized, using a computer-generated randomization procedure and a prestudy weight obtained. Rats were individually identified by tail markings and cage cards in accordance with standard operating procedures. Rats were housed 1 per cage in wire hanging cages and maintained on Agway Rat and Rat Ration (NIH 07 required diet) and water *ad libitum* from fresh tap water. The cages were placed on the rack so that the individual dose groups were ordered from top to bottom. NIH 07 required diet was suitable to preclude contamination that might interfere with the outcome of the study. Tap water analysis records are on file with the Division of Animal Resources. Animals were housed in Sanger Rooms B2-035 A-C and 6-053A where the temperature was maintained between 64.4-78.8°F and the relative humidity between 40-70%. The light/dark cycle was maintained at 12-hour intervals. Rat pans were cleaned and sanitized two times per week.

TEST GROUPS. Following randomization, animals were assigned to either standard toxicological studies or immunological studies. The vehicle and experimental groups consisted of 10 animals and the positive control groups consisted of 6 animals. Within each study, animals were assigned to one of the following groups shown in Chart 1 - Experimental Design.

Chart 1
Experimental Design

Group	Designation	Exposure Group	mg/kg
1	VH	Vehicle	0
2	G1	D4	10
3	G2	D4	30
4	G3	D4	100
5	G4	D4	300
6	PC1	CPS	25
7	PC2	Anti Asialo GM1	1:10
8	PC3	MVE	50

TEST ARTICLE ADMINISTRATION

TEST ARTICLE. Octamethylcyclotetrasiloxane (D4) (M.W. 296.62; CAS Number 000556672; Lot No. LL121629 and MCV Chemical No. 11-10-93-1 for Studies 1-5; Lot No. LL044530 and MCV Chemical No. 24-1-95-1 for Study 6; and Lot No. LL084732 and MCV Chemical No. 29-6-95-1 for Studies 7-14) was received at the Medical College of Virginia/Virginia Commonwealth University on October 11, 1993, January 24, 1995, and June 29, 1995, respectively. Data relating to the purity and character of the three test article lots range from 97.5 to 99.7% as provided in Appendix G. Samples (the 3 lots) are archived with the sponsor.

D4's stability was determined by the sponsor (Appendix I). The study material was stored at room temperature in a glass bottle. D4 was prepared daily as a solution in corn oil by w/v. In an amendment to the study, other vehicles were used for specific studies. The solutions were prepared daily in amber vials and were stored at room temperature for disposal. The concentrations and stability of the solutions were determined by the sponsor as seen below in Chart 2.

Chart 2

Summary of Analysis of Dosing Solutions

	Sample	Expected Concentration mg/ml	Concentration/Day (mg/ml)
1. Corn Oil Mazola	Day 21	60	56.23 ± 1.73
	Day 28	60	58.62 ± 0.13
2. 35 cs PDMS (X9-6370 Fluid)	Day 21	60	59.67 ± 0.89
	Day 28	60	56.82 ± 0.39
3. Maalox TC	Day 21	60	56.69 ± 1.84
	Day 28	60	54.99 ± 1.94
4. 10% Aqueous Emulphor	Day 21	60	51.07 ± 2.65
	Day 28	60	52.14 ± 1.93

Data are expressed as the mean ± SD derived from measurements of 3 samples per timepoint. A complete analytical report is provided in Appendix I.

1. Stability of D4 dosing solutions in corn oil was established over a 37 day period.
2. Stability of D4 dosing solutions in 35 cs PDMS fluid was not addressed since the test material and vehicle are both polydimethylsiloxane.
3. Stability of D4 dosing solutions in Maalox TC was measured over a 23 day period.
4. Redispersing of D4 in 10% Aqueous Emulphor was performed within 2 hours of administration.

VEHICLE CONTROL. Corn oil was the vehicle control. Vehicle control animals received 0.05 ml/10 grams of body weight by gavage. Vehicle control animals were dosed at the same time as the exposure groups. A listing of the lot numbers for all vehicles for each study is provided in Chart 3.

POSITIVE CONTROL NUMBER 1. Cyclophosphamide (CPS; Sigma Chemical Co., Lot No. 72H0088) was given as a positive control. The positive control animals received 25 mg/kg of CPS on days 25 through 28 by intraperitoneal (i.p.) injection. CPS was prepared in phosphate buffered saline (PBS; Sigma Chemical Co., Lot No. 024H-8933 and 055H-8901) by w/v at concentrations of 5 mg/ml and stored at -20°F. Positive control animals received 0.05 ml/10 grams of body weight. This translates into 1 ml/200 gram rat.

Chart 3

Lot Numbers for Vehicles Used for Each Study

Lot No.	Study No.
Mazola Corn Oil*	
Expiration: 6-2-95	1,2
Expiration: 7-27-95	3
Expiration: 11-29-95A	4
Expiration: 2-14-96A	5
Expiration: 4-2-96	6
Expiration: 11-01-96	7.1,8.1
Expiration: 2-26-97	9,11
Expiration: 1-1-97	10,12
Expiration: 1-19-97A	13,14
Maalox	
98462	7.2, 8.2
10% Aqueous Emulphor	
SPZH021180/G896274	7.3, 8.3
35 cs PDMS Fluid	
LL099608	7.4, 8.4

*Expiration Date = Lot Number

POSITIVE CONTROL NUMBER 2. Anti Asialo GM1 (Rabbit) antibody (AAGM1; Wako BioProducts, Lot No. PTJ7681) was given as a positive control in the Natural Killer (NK) cell assay. The positive control animals received 1.0 ml of a 1:10 dilution by v/v of the antibody on day 28 by intravenous injection. Anti Asialo GM1 was diluted in sterile distilled water. Positive control animals received 0.05 ml/10 grams of body weight.

POSITIVE CONTROL NUMBER 3. Maleic vinyl ether (MVE; XA, Lot No. 124-188) was used as a positive control for modulation of the functional activity of reticuloendothelial system (RES). The stock solution of MVE was prepared in phosphate buffered saline (Sigma Chemical Co., Lot No. 024H-8933 and 055H-8901) by w/v in a concentration of 5.0 mg/ml at a pH between 6.8-7.2. During study, the dose of MVE was decreased and the concentration was 2.5 mg/ml in PBS. MVE was injected intravenously 24 hours prior to evaluation of the RES. Positive control animals received 0.05 ml/10 grams of body weight. This dose markedly suppressed hepatic phagocytic function which was manifested in a decrease in blood clearance and a decrease in liver uptake of the test particle (^{51}Cr erythrocytes). Consistent with its usage as a positive control for a functional assay, MVE was not analyzed for concentration. A blockade of the RES was used as an indicator of adequate dose of the drug.

ROUTE OF ADMINISTRATION. D4 was administered by gastric intubations using a 16 gauge gavage tube. Animals received 0.05 ml/10 grams of body weight.

DURATION OF EXPOSURE. All animals received the test article daily for 28 consecutive days. On day 29, one day after the last exposure, the animals were evaluated for the indicated immunological or toxicological parameters.

VARIABLES ASSESSED

The variables which were investigated are presented in two categories - standard toxicology and immunology. Each category has several components.

STANDARD TOXICOLOGY STUDIES

Observations. Animals were observed at the time of dosing and daily throughout the experimental period for pharmacotoxicological signs. Any remarkable effects were recorded in the protocol log sheet present in the exposure room in accordance with the SOP.

Body Weights. Animals were weighed twenty-four hours after arrival and prior to exposure on day 1. They were also weighed on days 8, 15 and 22 of the exposure period and on day 29, the day of sacrifice. In the recovery studies, the body weights were continued on day 36 and day 43.

Food Consumption. Food consumption was measured two times a week during the experimental period.

Terminal Body and Organ Weights. Liver weight was measured on each study and provided a non-immunological D4 effect that was used for comparison across studies. Other organ weights were measured for Studies 5 and 6. On day 29, one day after the last exposure to the test article, rats were weighed and sacrificed using CO_2 anesthesia and cervical dislocation. Animals were examined for gross pathology and the thymus, liver and spleen were removed, cleaned of connective tissue and weighed.

Hematology. Selected hematological parameters were assessed. On day 29, one day after the last exposure to the test article, blood was collected by a cardiac puncture into EDTA tubes. A blood smear was prepared at the time of blood collection and, after dehydration with methanol, stained with Wright-Giemsa. The following parameters were assessed: erythrocyte and leukocyte number, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocytes and platelets. Leukocytes were differentiated into lymphocytes, neutrophils and eosinophils. Total lymphocytes, neutrophils and eosinophils were calculated by multiplying the total leukocyte count by the percent of each cell type which was determined by the differential count.

IMMUNOLOGY STUDIES

Splenocyte Phenotyping. Rats were exposed to the test article for 28 days. On day 29, spleens were teased between two microscope slides into single cell preparations and seeded at $1x10^6$ cells per well into a 96-well microtiter plate. Analysis of each spleen preparation required 4 wells. Each well contained spleen cells and 100 µl of the appropriate monoclonal antibody conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). The specific monoclonal antibodies are: W3/13 conjugated to FITC to enumerate T cells, W3/25 conjugated to FITC to enumerate $CD4^+$ cells, OX8 conjugated to PE to enumerate $CD8^+$ cells and OX4 conjugated to FITC to enumerate B cells. Following the initial staining with antibody and washing with staining buffer, 100 µl of a propidium iodide (PI) solution was added to each well as a viability stain.

Peripheral Blood Phenotyping. Blood was collected by cardiac puncture into heparinized tubes for processing and the same procedure for staining was used as described for the splenocytes.

Acquisition of Data: The cell preparation in the control well was run on the flow cytometer and viewed as a dot plot of forward scatter (FSC) on the X axis, and side scatter (90 degree; SSC) on the Y axis in the linear mode. The majority of the red cell population and debris were eliminated electronically by increasing the threshold setting on FSC. Non-viable cells were eliminated by setting a live gate on FL3 (red fluorescence which in this case is PI). Data for all samples are then acquired in list mode with no other gating. Photomultiplier tubes (PMT) for fluorescence operate in the log mode. Cells stained with FITC are detected by the FL1 PMT (green fluorescence) and cells stained with PE are detected by the FL2 PMT (orange fluorescence). Five thousand cells are counted for each sample.

Analysis of Data: Following acquisition, the list data for each sample are recalled from the computer memory. The remainder of debris and red cells is eliminated from analysis by electronic gating of a FSC by SSC dot plot. The percentage of T cells and B cells in the gated population is calculated from histograms of fluorescence intensity vs cell number.

The number of T and B lymphocytes in the spleens and peripheral blood of treated rats provides important data for the interpretation of functional studies. The methodology for analyzing B and T cell populations and T cell subsets ($CD4^+$ and $CD8^+$) includes the use of a Becton Dickinson FACScan Flow Cytometer, monoclonal antibodies conjugated to FITC or PE, and the DNA specific fluorescent stain PI. This assay is one of the Tier II assays used by the NTP[1].

In addition, the number of natural killer cells ($CD56^+$) was enumerated. The monoclonal antibody for the NK cell in the rat has not been extensively used and validation among laboratories has not been done.

Spleen IgM Antibody Response to the T-dependent Antigen, sRBC. The primary IgM response to sheep erythrocytes (sRBC) was enumerated using a modified hemolytic plaque assay of Jerne[2]. Rats receiving the appropriate exposure were sensitized with $2x10^6$ sRBC i.v. on day 25 of exposure. On day 29, one day after the last exposure to D4, animals were sacrificed, and spleen cells were prepared by mincing the spleen between two frosted microscope slides and diluted to 6 ml. An aliquot of cells was added to a test tube containing guinea pig complement, sheep erythrocytes and warm agar. After thoroughly mixing, the test tube mixture was plated in a petri dish, covered with a microscope cover slip, and incubated at 37°C for 3 hours. Cell counts were performed on the 6 ml sample and the number of cells/spleen, AFC/spleen and AFC/10^6 spleen cells determined. The plaques which developed were counted using a Bellco plaque viewer. A plaque results from the lysis of sheep erythrocytes. It is elicited as a result of the interaction of complement and antibodies directed against sheep erythrocytes which are produced in response to the i.v. sensitization. Each plaque is generated from a single IgM antibody-producing B cell and, thus, the number of antibody-forming cells (AFC) present in the whole spleen can be calculated. The data are expressed as specific activity (AFC/10^6 spleen cells) and total spleen activity (AFC per spleen).

As background, sheep erythrocytes are a T-dependent antigen and, thus, T cells, B cells and macrophages are required to function properly in order to obtain an AFC response. If a xenobiotic affects any of these cell-types to a significant degree, an altered response will be observed. As a result, the T-dependent IgM response to sRBC is one of the most sensitive immunotoxicological assays currently in use. A significant modulation in the IgM AFC response, when appropriately compared to untreated controls, indicates that the test agent is capable of modifying the humoral immune response in the whole animal and, thus, has the potential for immunotoxicity. This assay is one of the Tier 1 assays used by the NTP[3].

Serum IgM Antibody Titers to the T-dependent Antigen, sRBC as measured by ELISA. An ELISA assay system, developed in our laboratory[4], was used to determine the serum titers of the primary IgM response to sheep erythrocytes (sRBC) in the same animals evaluated with the hemolytic plaque assay. Serum was obtained from blood collected by cardiac puncture under CO_2 anesthesia.

Mixed Leukocyte Response to Long Evans and Brown Norway Spleen Cells. The mixed leukocyte response (MLR) is an *in vitro* assay which analyzes the ability of spleen T cells from xenobiotic-exposed rats to recognize allogeneic cells as "non-self" and proliferate in response to the presence of the allogeneic cells. Male and female Fischer 344 rats were exposed *in vivo* to D4 for 28 days. On day 29, the rats were sacrificed and spleen cells prepared under sterile conditions by mincing the spleens between two frosted microscope slides. Spleen cells were counted and adjusted to $1x10^6$/ml. Spleen cells were added to each well of a U-bottom microtiter plate at a concentration of $1x10^5$ cells per well. Brown Norway or Long Evans spleen cells were used as the allogeneic cell (stimulator cell) for the Fischer (responder) rats. Stimulator cells were treated with Mitomycin C (Sigma Chemical Co., Lot No. 24H2504 and 114H2507) to render them unable to proliferate; the ratio of stimulators to responders has previously been optimized at 2:1. The cells were cultured for 5 days, during the last 18 hours in the presence of 1 µCi ^{3}H-thymidine. The cells were collected with a cell harvester and counted in a LKB liquid scintillation counter, and the data were collected directly onto computer diskettes for analysis. The incorporation of ^{3}H-thymidine into the proliferating cells was used as the endpoint of the assay, and the data are expressed as cpm/culture.

Peripheral Blood Leukocytes. Blood was collected by cardiac puncture into heparinized tubes for processing and the same procedure for MLR was used as described for the splenocytes.

The mixed leukocyte response (MLR) has proven to be a sensitive assay for detecting effects on cell-mediated immunity. This may be due to the complex nature of the MLR, in which recognition of the allogeneic cells and proliferation are necessary. While this assay is well established and reproducible in the mouse, at times, the same assay in the rat does not always work; that is, the responder cells do not proliferate. When this occurs, the assay is invalidated. A possible reason that this assay does not work every time is due to an increased number of suppressor cells in the rat. Several other cell-mediated immunity assays are in developmental stages. A significant change in the MLR, when appropriately compared to untreated controls, indicates that the test agent is capable of modulating recognition and/or proliferation in

response to stimulation with allogeneic cells. Agents which produce such effects have the potential for modifying the immune system.

Clearance of sRBC by the Reticuloendothelial System (RES). The assay was performed as described by White *et al.*[5] Rats were exposed to the test article on day 1-28, and on day 29 the functional activity of the fixed macrophages was evaluated. The activity of the fixed macrophages of the reticuloendothelial system was determined by injecting 5 µl/gram body weight of ^{51}Cr-labeled sheep erythrocytes. The concentration of ^{51}Cr-labeled sheep erythrocytes was 20% hematocrit which is equal to approximately $5x10^8$ sheep erythrocytes/ml. The clearance of the labeled sheep erythrocytes from the blood was determined over the first 30 minutes by taking 10 µl of blood at 3, 6, 9, 12, 15 and 30 minutes after ^{51}Cr-labeled sheep erythrocytes injection. The radioactivity in the blood was used to calculate the phagocytic index and determine the half-life. After 60 minutes, the animals were sacrificed by carbon dioxide and decapitation, and the distribution of the labeled sheep erythrocytes into the major organs of the reticuloendothelial system was determined. Uptake into the liver, spleen, lungs, thymus and kidneys were determined by radioassay and the data are expressed as percent uptake in the total organ and per mg.

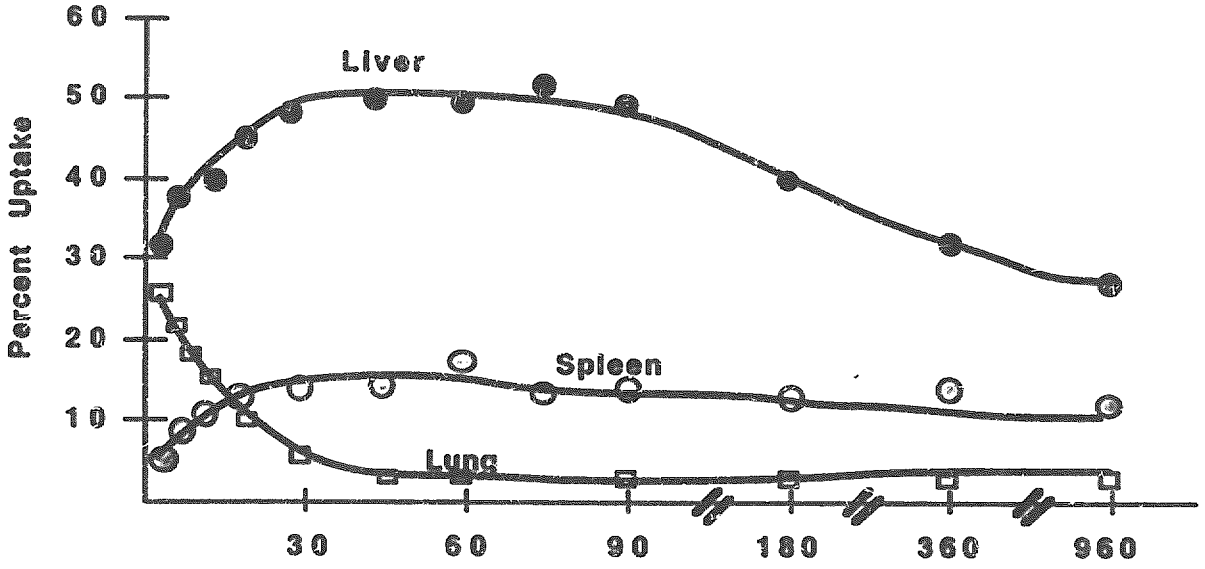
Organ distribution (phagocytosis), as a function of time after the injections of ^{51}Cr-labeled sheep erythrocytes, is different for the different organs. Figure 1 below illustrates the time course distribution. There is a rapid uptake of ^{51}Cr-labeled sheep erythrocytes into the liver and spleen with a plateau occurring in 30 minutes. Distribution into the lung is more complex. The first phase of localization into the lung is the accumulation of ^{51}Cr-labeled sheep erythrocytes based on first pass action, i.e. the vascular bed of the lung is the first site which sees the bolus of cells. Thus, there is an early large accumulation, up to 30%, of ^{51}Cr-labeled sheep erythrocytes, followed by a clearance from the lung over the first 30 minutes after injections. The actual phagocytosis of ^{51}Cr-labeled sheep erythrocytes of the lung macrophages is less than 1% which can be measured 60 minutes after injection.

As background, the fixed macrophages of reticuloendothelial system provide a first line of defense for blood born particulates including pathogens and non-pathogens. These macrophages line the liver endothelium (Kupffer cells), the spleen, the lymph nodes (reticular cells), the lung (interstitial macrophages) and other organs such as the thymus and bone marrow. One of the main tasks of this system is to maintain a sterile blood supply and to initiate the acquired immune response. The cells of the reticuloendothelial system are also involved in removing and reprocessing old erythrocytes, leukocytes and platelets. They have also been shown to be

involved in metabolism of substances such as steroids. A decreased function of the fixed cells of the reticuloendothelial system can decrease host resistance to microbes. Thus, decreases in reticuloendothelial function related to drug treatment represent an adverse effect.

Figure 1

Time Course for Uptake of ^{51}Cr Sheep Erythrocytes



Natural Killer (NK) Cell Activity. Rats were exposed daily for 28 days. Twenty-four hours after the last exposure, the body weights were recorded and the spleens were removed and weighed. Single cell suspensions from individual spleens were prepared aseptically. Following centrifugation, the pellet was resuspended in RPMI (Roswell Park Memorial Institute) 1640 supplemented with 10% fetal calf serum. The cell suspension was adjusted to three concentrations, 10^7, $5x10^6$ and $2.5x10^6$ cells/ml, to obtain effector-to-target ratios of 100:1, 50:1 and 25:1, respectively. In the male study the effector-to-target ratios were expanded to include 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1. The NK cell was the effector cell and the YAC-1 was the target cell. The YAC-1 cells were obtained from American Type Culture Collection (ATCC TIB 160). YAC-1 is a mouse lymphoma which was introduced by inoculation of the Moloney leukemia virus (MLV) into a newborn A/Sn mouse. This cell line is sensitive to the cytotoxic activity of "naturally" occurring killer cells in rats (NK cell). Therefore, YAC-1 cells are often used as target cells in NK assays. The YAC-1 cells which are maintained in a stock culture at MCV/VCU were adjusted to a concentration of 10^7 cells/ml. These cells were labeled by incubation with 1000 µCi of ^{51}Cr in a volume of 1.0 ml for 90 minutes in a 37°C water bath with frequent agitation. Following the incubation, the cells were washed three times in balanced salt solution (BSS) plus 25mM HEPES, counted, and adjusted to 10^6 nucleated

cells/ml. The maximum release was determined by adding 0.1 ml of the 10^5 YAC-1 cells/ml and 0.1 ml 10% Triton X to each of 12 replicate wells on a 96-well microtiter plate. The targets were 0.1 ml of the 10^5 YAC-1 cells/ml added to each well. The effector cells were added (0.1 ml) to each of four replicate wells of target cells at each effector concentration. The spontaneous release was determined by adding 0.1 ml of medium to 12 replicate cultures containing the targets. The plates were incubated for 4 hours at 37°C at 5% CO_2. Following the incubation, the plates were centrifuged at 250 x g for 10 minutes, and 0.1 ml of the supernatant was removed from each well and radioassayed on LKB Gamma counter. The mean (± SE) percent cytotoxicity at each effector concentration was determined for each treatment group and compared to the respective values for the vehicle rats.

Peripheral Blood Leukocytes. Blood was collected by cardiac puncture into heparinized tubes for processing and the same procedure for the NK was used as described for the splenocytes.

Natural killer cells are large granular lymphocytes which are shown to possess anti-tumor activity without prior acquisition of information. That is, NK cell activity is an innate activity which requires no prior exposure to the pathogen. This is in contrast to the acquired immune response which requires sensitization and time to develop the ability to bring about the desired action. In the last few years, the NK cells have also been shown to possess activity against virally infected cells as well as have activity against other pathogens. The NK cells reside in the lymphoid tissue and also in the lung and liver. The origin of the NK cells is believed to be the bone marrow. Decrease in NK cell activity as a result of drug treatment is considered an adverse effect.

DATA HANDLING AND STATISTICAL ANALYSIS

DATA COLLECTION. Data were collected onto customized data forms established by the MCV Immunotoxicology Program and entered from the forms into the computer. When appropriate, modifications of the data, such as determining the change in body weight, were computed. The data were then verified and formatted as necessary. Individual study values are provided in archived study records. During this study, Data Entry, Modification and Statistics Programs Version 6.0 were used in conducting statistical analyses. P values of 0.05 or less were considered statistically significant and were indicated with a single asterisk (*) as compared to the vehicle control group. A double asterisk (**) was used to indicate a $p < 0.01$ as compared to the vehicle control group. In the text, the word significant indicates that the response was statistically significant at $p < 0.05$ or less.

ANALYSIS OF DATA. The data were analyzed as follows. The Bartlett's Test for homogeneity[6] was used to select the type of analysis to be conducted. Homogeneous data were analyzed using a one-way analysis of variance[7], and the Dunnett's t test[8] was used to determine differences between the experimental and the control group. For non-homogeneous data, a non-parametric analysis of variance was used[9], and differences between the control group and the experimental groups were determined by the Gehan-Wilcoxon Rank Test[10]. The Jonckheere's Test[11] was used to test for dose-related trends across the vehicle and treatment groups. The positive control data were evaluated using the Student's t Test[12] and compared to the vehicle control. In the recovery studies, the data for 300 mg/kg dose of D4 were evaluated using the Student's t Test and compared to the vehicle control. In the vehicle studies, the Naive group was included as an additional experimental group in Studies 7.4 and 8.4.

DEVIATIONS

Complete documentation of the deviations are presented in Appendix C. There were 18 deviations, none of which affected the outcome of the study. The following deviations occurred during the studies for protocol D4-28-1R-PO.

1. The description of a positive control article was omitted from the protocol. The following paragraph describes the positive control used for the reticuloendothelial.

   POSITIVE CONTROL Number PC3: MVE (XA 124-188) will be used as a positive control for modulation of the functional activity of reticuloendothelial system (RES). MVE will be prepared in phosphate buffered saline in a concentration of 10.0 mg/ml (50 mg/kg) at a pH between 6.8-7.2. MVE will be injected intravenously 24 hours prior to evaluation of the RES. This dose will markedly suppress hepatic phagocytic function which will be manifested in a decrease in blood clearance and a decrease in liver uptake of the test particle (sheep erythrocytes). MVE will not be analyzed for concentration. A blockade of the RES will be used as an indicator of adequate dose of the drug.

2. Study 1 - Week 2 has a questionable data value. The delta weight of the intermediate bottle, value 6.86, is not congruent with the other weeks. It appears that one day 1.26 mls may not have been removed during the first week. All log records indicate that the rats were administered the appropriate amounts of D4 daily for 28 days.

3. The NK assay in Study 5 using the peripheral blood samples will not be reported. There was an insufficient cell number for many samples which gave inconsistent results. This assay could be addressed in a repeat study.

4. Liver weights were obtained from all the D4-28-1R-PO studies as a consistent parameter to measure from each study.

5. Additional D4 was added to the same intermediate bottle for Studies 2-6. This activity was pointed out by the Quality Assurance site visitors to be inappropriate. The procedure did not appear to preclude contamination of the test article.

6. During Studies 1-6, the humidity and temperature in the animal room (B2-035A) were observed to be outside the range of (40-70%) and (64.4-78.8°F) indicated in the protocol. The dates and the recorded values are indicated below.

Deviations from Temperature & Humidity Ranges

Date	Humidity (%)	Temp (°F)	Date	Humidity (%)	Temp (°F)
April 22, 1994	72%		June 30, 1994	72%	
April 25, 1994	84%		July 12, 1994	80%	
April 26, 1994	80%		July 13, 1994	84%	
April 27, 1994	90%		July 14, 1994	80%	
April 28, 1994	96%		July 15, 1994	80%	
April 29, 1994	94%		July 16, 1994	84%	
April 30, 1994	90%		July 17, 1994	80%	
May 1, 1994	80%		July 18, 1994	82%	
May 6, 1994	100%		July 19, 1994	84%	
May 7, 1994	76%		July 20, 1994	100%	
May 10, 1994	82%		July 21, 1994	84%	
May 12, 1994	76%		July 22, 1994	82%	
May 13, 1994	76%		July 23, 1994	82%	
May 14, 1994	72%		July 24, 1994	92%	
May 15, 1994	79%		July 25, 1994	100%	
May 16, 1994	100%		July 26, 1994	72%	
June 2, 1994	100%		July 27, 1994	74%	
June 3, 1994	88%		July 28, 1994	80%	
June 4, 1994	88%		July 29, 1994	72%	
June 5, 1994	100%		July 31, 1994	80%	
June 7, 1994	92%		August 1, 1994	80%	
June 8, 1994	90%		August 2, 1994	76%	
June 9, 1994	74%		August 3, 1994	76%	
June 10, 1994	100%		August 4, 1994	78%	
June 11, 1994	78%		October 11, 1994	38%	
June 12, 1994	89%		October 17, 1994	37%	
June 13, 1994	100%		November 3, 1994	36%	
June 14, 1994	94%		November 7, 1994	36%	
June 15, 1994	88%		November 8, 1994	34%	
June 16, 1994	100%	80°	November 9, 1994	30%	
June 19, 1994	78%		November 11, 1994	30%	
June 20, 1994	80%		November 12, 1994	28%	
June 21, 1994	78%		November 13, 1994	32%	
June 22, 1994	80%		November 19, 1994	36%	
June 23, 1994	82%		November 23, 1994	24%	
June 24, 1994	80%		November 24, 1994	25%	
June 25, 1994	78%		November 25, 1994	25%	
June 26, 1994	72%		November 26, 1994	28%	
June 27, 1994	78%		November 27, 1994	30%	
June 29, 1994	90%		November 29, 1994	34%	

Date	Humidity (%)	Temp (°F)	Date	Humidity (%)	Temp (°F)
November 30, 1994	36%		February 8, 1995	36%	
February 1, 1995	22%		February 9, 1995	34%	
February 2, 1995	26%		February 10, 1995	36%	
February 3, 1995	22%		February 13, 1995	38%	
February 4, 1995	25%		February 14, 1995	37%	
February 5, 1995	18%		February 15, 1995	40%	
February 6, 1995	32%		February 17, 1995	38%	
February 7, 1995	33%		February 28, 1995	73%	

It is the opinion of the Study Director that the differences in humidity and slight difference in temperature did not affect the results of the study.

7. The dosage volume in Study 9 was not adjusted on Day 8. The volume adjustment was calculated from the new mean body weights and changed on Day 9. The delay was due to a shortage of personnel on an inclement weather day and the desire to keep the exposure time consistent from day to day.

8. The day for weighing Study 10 was delayed by one day from 22 Nov 95 to 23 Nov 95, due to the holiday schedule.

9. During Studies 7-14, the humidity and temperature in the animal rooms (B2-035A, B2-035C, and 6-053A) were observed to be outside the range of (40-70%) and (64.4-78.8°F) indicated in the protocol. The dates and the recorded values are indicated below.

Deviations from Humidity & Temperature Ranges

Date	Humidity (%)	Temp (°F)	Date	Humidity (%)	Temp (°F)
Room 6-053A	None	None	January 11, 1996	20%	
Room B2-035A			January 12, 1996	28%	
November 10, 1995	34 %		January 13, 1996	25%	
November 13, 1995	36%		January 14, 1996	25%	
November 15, 1995	38%		January 15, 1996	26%	
November 16, 1995	33%		January 16, 1996	28%	
November 17, 1995	36%		January 17, 1996	38%	
November 18, 1995	34%		January 20, 1996	28%	
November 20, 1995	38%		January 21, 1996	26%	
November 22, 1995	30%		January 22, 1996	26%	
November 23, 1995	31%		January 23, 1996	27%	
November 25, 1995	33%		January 24, 1996	30%	
November 26, 1995	34%		January 26, 1996	27%	
November 29, 1995	38%		February 2, 1996	26%	
November 30, 1995	34%		February 5, 1996	18%	
December 5, 1995	36%		February 6, 1996	21%	
December 7, 1995	36%		February 7, 1996	26%	
December 8, 1995	30%		February 8, 1996	37%	
December 9, 1995	38%		**Room B2-035C**		
December 10, 1995	30%		November 15, 1995	36%	
December 11, 1995	30%		November 16, 1995	32%	
December 12, 1995	25%		November 17, 1995	30%	
December 13, 1995	28%		November 18, 1995	32%	
December 18, 1995	30.5%		November 20, 1995	33%	
December 20, 1995	32%		November 21, 1995	37%	
December 21, 1995	32%		November 22, 1995	26%	
December 24, 1995	28%		November 23, 1995	28%	
December 27, 1995	27%		November 25, 1995	30%	
December 28, 1995	27%		November 26, 1995	30%	
December 29, 1995	30%		November 29, 1995	33%	
December 30, 1995	30%		November 30, 1995	30%	
December 31, 1995	32%		December 1, 1995	36%	
January 3, 1996	38%		December 2, 1995	31%	
January 4, 1996	28%		December 3, 1995	34%	
January 5, 1996	30%		December 5, 1995	35%	
January 6, 1996	27%		December 7, 1995	36%	
January 7, 1996	29%		December 8, 1995	30%	
January 8, 1996	30%		December 9, 1995	36%	
January 9, 1996	26%		December 10, 1995	30%	
January 10, 1996	32%		December 11, 1995	30%	

Deviations from Humidity & Temperature Ranges

Date	Humidity (%)	Temp (°F)	Date	Humidity (%)	Temp (°F)
December 12, 1995	28%		February 1, 1996	20%	
December 18, 1995	30%		February 2, 1996	26%	
December 19, 1995	37%		February 5, 1996	18%	
December 20, 1995	30%		February 6, 1996	20%	
December 21, 1995	30%		February 7, 1996	26%	
December 24, 1995	29%		February 8, 1996	35%	
December 27, 1995	28%		February 11, 1996	34%	
December 28, 1995	26%		February 12, 1996	29%	
December 29, 1995	30%		February 13, 1996	26%	
January 3, 1996	38%		February 14, 1996	34%	
January 4, 1996	36%		February 15, 1996	34%	
January 5, 1996	34%		February 16, 1996	33%	
January 6, 1996	34%		February 19, 1996	31%	
January 7, 1996	34%		February 25, 1996	30%	
January 8, 1996	34%		February 29, 1996	30%	
January 9, 1996	28%		March 1, 1996	29%	
January 10, 1996	30%		March 2, 1996	30%	
January 11, 1996	25%		March 3, 1996	30%	
January 12, 1996	30%		March 4, 1996	24%	
January 13, 1996	28%		March 5, 1996	37%	
January 14, 1996	30%		March 8, 1996	29%	
January 15, 1996	32%		March 9, 1996	26%	
January 16, 1996	28%		March 10, 1996	28%	
January 17, 1996	37%		March 11, 1996	30%	
January 20, 1996	28%		March 12, 1996	28%	
January 21, 1996	24%		March 13, 1996	36%	
January 22, 1996	25%		**Room B2-035B**		
January 23, 1996	31%		December 20, 1995	27%	
January 28, 1996	24%		December 21, 1995	28%	
January 29, 1996	32%		December 24, 1995	24%	
January 30, 1996	28%		December 27, 1995	24%	
January 31, 1996	32%				

It is the opinion of the Study Director that the differences in humidity did not affect the results of the study.

10. The Study Director changed the dose of the positive control (MVE) in Study 10 from 50 mg/kg to 25 mg/kg. In Study 4, the 50 mg/kg dose caused a RES blockade greater than was previously seen in rats. There was concern that this group of rats was more sensitive to the effects of MVE and thus the dose was decreased. The 25 mg/kg dose was sufficient to produce the hepatic blockade but did not block splenic phagocytosis. Thus, in the future studies (Study 11), the 50 mg/kg dose was used.

11. The Quality Assurance Officer noted a typing error in the protocol on page 3 in the table of the third column heading. The units for the dose read ml/kg and it should read mg/kg.

12. The Quality Assurance Officer noted a discrepancy in the molecular weight of octamethylcyclotetrasiloxane in the protocol compared to the chemical preparation forms. The protocol should read 296.6 as the correct molecular weight, verified by the Merck Index.

13. The calculations for D4 dosing solutions were based on the correct density of 0.9558 for Studies 1-6. However, a new lot of D4 was received for Studies 7-14. Inadvertently, the

density for these calculations was made using 0.9900. The expected concentration was 60 mg/ml; however, when the density 0.9900 was used for the dosing solution calculations of D4 rather than 0.9558, the calculated concentration was of 57 mg/ml. The trend of D4 effects is still a reality and in the total report the doses are represented by 10, 30, 100, and 300 mg/kg rather than the subtle change in concentration which occurred in studies 7-14.

14. A discrepancy was noted in the protocol under the Positive Control Number 2. The administration of anti asialo GM1 should read by the intravenous injection, not the intraperitoneal injection.

15. The following items represent minor corrections to the protocol and had no impact on the integrity or results of the study.

a. The Positive Control Number 1: days of administration were stated to be on days 24 through 28 by i.p. injection should have read on days 25 though 28 by i.p. injection. The studies all reflect day 25.

PARAMETERS TO BE MONITORED:

b. The body weight of animals in the recovery studies (Amendments 4-7) had additional weigh days of 36 and 43.

c. Under hematology, platelet counts were included in the evaluation.

d. Under the Mixed Leukocyte Response, the stimulation index was not included in the evaluation and not deemed to be pertinent information.

e. Under the Natural Killer Cell Assay, additional effector-to-target cell ratios were added after Study 5 to evaluate the results by using lytic unit calculations (100:1, 50:1 and 25:1 to 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1). These new ratios would be used provided the cell count was sufficient.

STATISTICAL ANALYSES:

f. In the recovery studies, the 300 mg/kg dose group was evaluated using the Student's t Test and compared to the vehicle control group.

g. In the vehicle studies, the data are evaluated the same as stated in the protocol with the Naive group included as one of the dose groups.

16. Two items in Study 5 represent deviations to the protocol in the splenic and peripheral blood phenotyping and percent reticulocytes. These deviations had no impact on the integrity or results of the study.

a. The monoclonal antibodies used were OX12 (B) instead of OX4 and OX19 (T) instead of W3/13 because of commercial availability and desired specificity. Both antibodies were verified for specificity prior to including them in the study.

b. The percent reticulocytes were inadvertently not included in the hematology evaluation. These data were generated in a repeat Study 12.

17. This deviation is a deviation to Amendments 2 and 3 of the protocol relating to the day samples would be prepared and sent to Dow Corning Corporation for concentration and stability analysis. These deviations had no impact on the integrity or results of the study.

    The days of analysis for Studies 7 and 8 were day 21 and 28 rather than day 1 and 15 as stated in the Protocol Amendments 2 and 3.

18. Studies in which food consumption was performed are clarified.

    Food consumption weights were performed on Studies 1 through 8.4. In Studies 9 through 14, food consumption was not continued. No additional information could be gained for the additional data. No impact on the integrity or results of the study occurred.

    It is the opinion of the Study Director that the deviations which occured in this protocol did not affect the integrity or the results of these studies.

RESULTS AND DISCUSSION

This report encompasses 14 studies which were carried out in completing the protocol and amendments. The assays performed in each of the studies are listed in Chart 4 - Concomitant Assays. The results section of the report has three components. The first component consists of the results of the standard toxicology studies including repeats. The second component provides the immunology results with the associated repeat and recovery studies. The third component encompasses the results of the vehicle studies.

In this report, a summary chart has been prepared for each assay. The data for each of the parameters of the assays have been presented as percent change compared to the vehicle controls. The mean values of the experimental groups that are statistically significant from the appropriate Vehicle Control groups are denoted with a single asterisk (*) when $p <$ than 0.05 and a double asterisk (**) when $p < 0.01$.

Chart 4
Concomitant Assays

Study No.	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
1 Female	IgM AFC Response	Serum Titers	Liver Wgts Spleen Wgts	Food Consumption	Body Wgts
2 Male	PFS	Organ Wgts	Body Wgts	Food Consumption	
3 Male	IgM AFC Response	Serum Titers	Liver Wgts Spleen Wgts	Food Consumption	Body Wgts
4 Female	PFS	Organ Wgts	Body Wgts	Food Consumption	
5 Female	Phenotyping/ MLR	NK	Hematology	Food Consumption	Organ Wgts/ Body Wgts
6 Male	Phenotyping/ MLR	NK	Hematology	Food Consumption	Organ Wgts/ Body Wgts
7	Role of the vehicle on D4-induced hepatomegaly in female Fischer 344 Rat Food Consumption				
8	Role of the vehicle on D4-induced hepatomegaly in male Fischer 344 Rat Food Consumption				
9 Female	IgM AFC Response Repeat&Recovery	Serum Titers	Liver Wgts Spleen Wgts	Body Wgts	
10 Female	PFS Repeat&Recovery	Organ Wgts	Body Wgts		
11 Male	PFS Repeat&Recovery	Organ Wgts	Body Wgts		
12 Female	Phenotyping Repeat&Recovery	Hematology	Organ Wgts	Body Wgts	
13 Female	IgM AFC Response Repeat Day 4	Serum Titers	Liver Wgts* Spleen Wgts	Body Wgts	
14 Female	IgM AFC Response Day 6	Serum Titers	Liver Wgts Spleen Wgts	Body Wgts	

*Not taken

STANDARD TOXICOLOGICAL STUDIES

OBSERVATIONS. This report covers male and female rats treated with D4 daily for 28 days. There were no overt signs of toxicity related to D4 treatment. All animals gained weight over the experimental period and showed no abnormal excretions from any orifices. Except for animals treated with D4 in 35 cs PDMS fluid (Studies 7 and 8), the fur was normal. The 35 cs PDMS fluid caused some anal leakage which was transferred to the fur. In Chart 5 are the animals which died while on study.

Chart 5
Animals Lost on Study

Study No.	Parameter	Animal No.	Day of Death	Cause of Death
3	AFC	VHM119	22	Gavage Error
6	NK	D4M331	3	Gavage Error
6	NK	D4M327	9	Gavage Error
7.2	Liver Wgts.	G5MF477	29	Gavage Error
8.4	Liver Wgts.	G6PM910	17	Gavage Error
8.4	Liver Wgts.	G5PM904	24	Gavage Error
9	AFC	G3F966	26	Gavage Error
10	PFS	G4F1080	19	Gavage Error

BODY WEIGHT AND BODY WEIGHT CHANGES. The body weight data sets from each of the individual studies are shown in Appendix E - Tables 1-24.

Male.

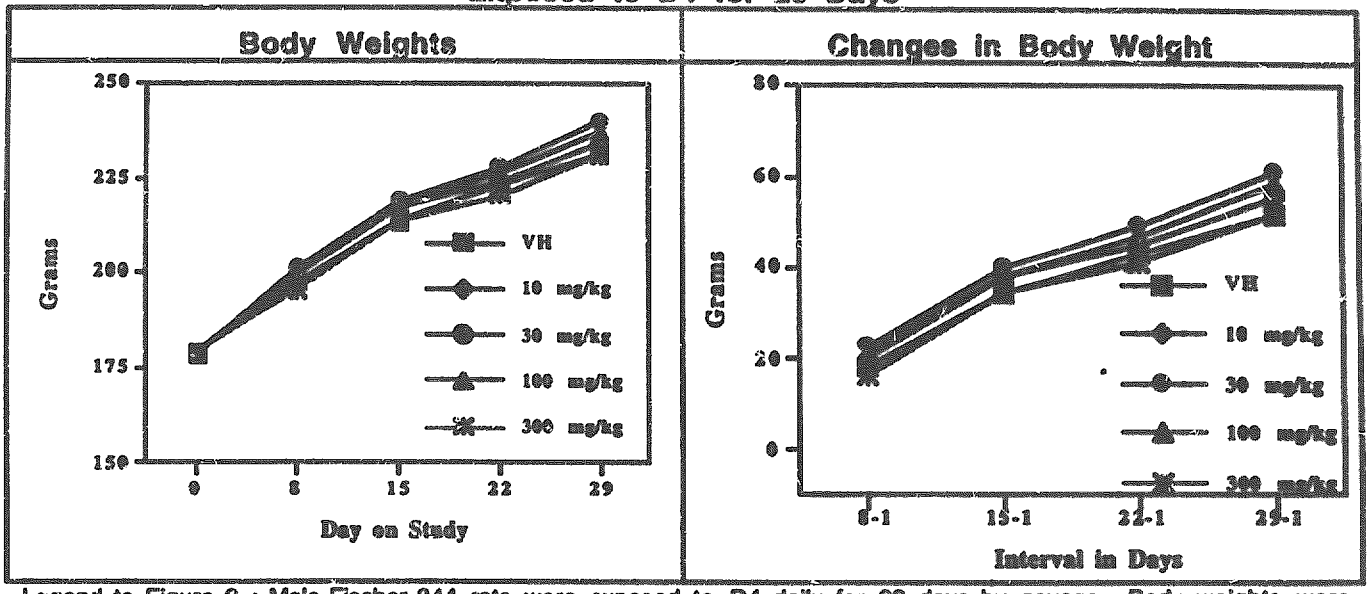
The combined body weight and change in body weight data for Studies 2, 3 and 6 are shown in Appendix D - Table 1 and Figure 2. No biologically significant changes in body weight or body weight changes were associated with D4 exposure. A statistically significant 4% increase in body weight of rats treated with 30 mg/kg occurred on day 29 of the experimental period. With respect to body weight change, rats treated with 10, 30 and 100 mg/kg showed an increase of 11, 25 and 16%, respectively, during the first week on study. Rats treated with 300 mg/kg D4 showed a 9% decrease in body weight during the same period. However, there were no statistical differences in body weight change at the other intervals.

Female.

The combined body weight and change in body weight data for Studies 1, 4 and 5 are shown in Appendix D - Table 2 and Figure 3. Chart 6 tabulates the percent change for each group at each time interval as compared to the vehicle control group. The only statistically significant D4-associated change in body weight was a 3% increase in rats receiving 300 mg/kg for 28 days.

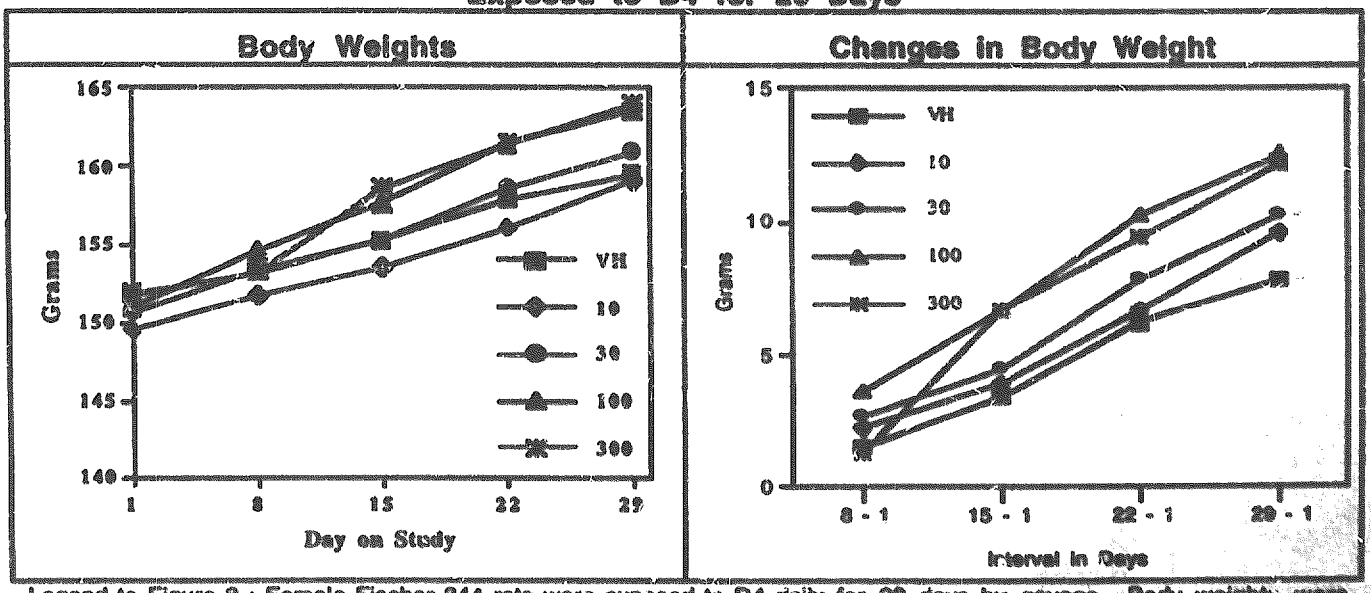
However, based on weight change, there was a significant increase at all dose levels at all time periods except during the first week in rats exposed to 300 mg/kg. At this time period, there was a 15% decrease in body weight gain.

Figure 2
Body Weights (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to D4 for 28 Days



Legend to Figure 2 : Male Fischer 344 rats were exposed to D4 daily for 28 days by gavage. Body weights were measured on the indicated day. Each point of the graph represents 30 animals per group except for the 300 mg/kg group which had 29 animals on day 8 and 28 animals on days 15, 22 and 29; the vehicle group had 29 animals on days 22 and 29.

Figure 3
Body Weights (g) and Body Weight Changes (g) In Female Fischer 344 Rats Exposed to D4 for 28 Days



Legend to Figure 3 : Female Fischer 344 rats were exposed to D4 daily for 28 days by gavage. Body weights were measured on the indicated day. Each point of the graph represents 30 animals per group.

Chart 6
Percent Alterations in Body Weights (g) and Body Weight Changes (g) for Female Fischer 344 Rat Exposed to D4

		Percent Alteration			
Organ	Day/Interval	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Body Wgt	29	NS	NS	NS	3 *
Body Wgt Change	Day 8 -Day 1	46**	74**	135**	-15**
Body Wgt Change	Day 15-Day 1	14**	30**	92**	93**
Body Wgt Change	Day 22-Day 1	8**	26**	66**	52**
Body Wgt Change	Day 29-Day 1	23**	31**	60**	57**

NS = Not Significant

FOOD CONSUMPTION. Food consumption was measured twice a week. The data are provided in Appendix F - Tables 1-14. Food consumption was not remarkably affected. Early in the exposure period, food consumption was decreased in animals exposed to 300 mg/kg but increased later in the exposure period.

TERMINAL BODY AND ORGAN WEIGHTS. Presented in Appendix D - Tables 3-11 are terminal body weights and selected organ weights of the D4-exposed rats.

Male.

The data for body and organ weights in male rats exposed to D4 are shown in Appendix D - Tables 3 and 4. Charts 7 and 8 summarize the percent alterations of the organ weights. The data are presented both as percent changes in absolute weight and percent changes in organ/body weight ratios. Overall, there was a dose-dependent decrease in the thymus weight that reached 27% at the 300 mg/kg dose. There was dose-dependent increase in liver weight which was more pronounced in the female rats than in the male rats. Spleen weight was not significantly affected.

Chart 7
Comparison of Percent Alteration in Absolute Organ Weights (mg) in Male Fischer 344 Rats Exposed to D4
Study 3

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	-0.6	0.6	3.1	-0.6	-37.0**
Thymus	-7.1	-16.2*	-13.9	-25.6**	-64.1**
Liver	5.7	13.5	18.4**	51.1**	8.3

Chart 7a
Comparison of Percent Alteration in Organ Weights (mg) as Percent of Body Weight in Male Fischer 344 Rats Exposed to D4
Study 3

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	1.00	-1.5	2.2	3.1	-36.6
Thymus	-6.9	-17.9*	-14.7	-22.8*	-63.9*
Liver	6.1	11.3	17.5*	56.9*	9.1

Chart 8
Comparison of Percent Alteration in Absolute Organ Weights (mg) in Male Fischer 344 Rats Exposed to D4
Study 6

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	2.6	7.2	7.0	3.3	-35.9**
Thymus	-14.0	-10.8	-12.4	-27.4	-77.7**
Liver	12.9	16.1**	24.8**	45.6**	6.4

Chart 8a
Comparison of Percent Alteration in Organ Weights (mg) as Percent of Body Weight in Male Fischer 344 Rats Exposed to D4
Study 6

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	-1.5	2.0	1.5	4.5	-37.5**
Thymus	-16.1	-14.6	-16.1	-27.0**	-78.1**
Liver	8.0**	10.6**	20.1**	47.0**	5.0*

Female.

The data for body and organ weights in female rats exposed to D4 are shown in Appendix D - Tables 5-11. Charts 9-12 summarize the percent alterations of the organ weights. In the female, the greatest increase in liver weight was 88% in rats exposed to 300 mg/kg. In the recovery study (Charts 10b and 11b and Appendix D - Tables 8 and 10), the liver weight did not return to the level of the vehicle control group. Spleen weight was not significantly affected.

Chart 9
Comparison of Percent Alteration in Absolute Organ Weights (mg) in Female Fischer 344 Rats Exposed to D4
Study 5

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	-1.7	0.8	5.2	4.4	-31.1**
Thymus	-5.3	-4.8	2.1	-17.0**	-62.2**
Liver	10.2**	15.4**	44.3**	87.9**	5.8

Chart 9a
Comparison of Percent Alteration in Organ Weights (mg) as Percent of Body Weight in Female Fischer 344 Rats Exposed to D4
Study 5

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	-2.2	-0.4	2.2	0.4	-30.2**
Thymus	-4.2	-5.0	-1.7	-18.5**	-62.2**
Liver	10.1**	14.2**	40.1**	81.7**	7.3*

Chart 10
Comparison of Percent Alteration in Absolute Organ Weights (mg) in Female Fischer 344 Rats Exposed to D4
Study 9

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	2.5	2.7	5.4	6.4	-39.8**
Thymus	ND	ND	ND	ND	ND
Liver	4.5	15.8**	30.5**	78.3**	-5.8

ND = Not Done

Chart 10a
Comparison of Percent Alteration in Organ Weights (mg) as Percent of Body Weight in Female Fischer 344 Rats Exposed to D4
Study 9

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	2.7	1.6	5.1	3.1	-34.5**
Thymus	ND	ND	ND	ND	ND
Liver	5.7	14.3**	31.4**	74.3**	2.9

ND = Not Done

Chart 10b
Comparison of Percent Alteration in Absolute Organ Weights (mg) in Female Fischer 344 Rats Exposed to D4
Study 9/Recovery

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	ND	ND	ND	-2.6	-40.5**
Thymus	ND	ND	ND	ND	ND
Liver	ND	ND	ND	20.8**	-1.5

ND = Not Done

Chart 10c
Comparison of Percent Alteration in Organ Weights (mg) as Percent of Body Weight in Female Fischer 344 Rats Exposed to D4
Study 9/Recovery

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	ND	ND	ND	-0.4	-37.3**
Thymus	ND	ND	ND	ND	ND
Liver	ND	ND	ND	23.5**	5.9

ND = Not Done

Chart 11
Comparison of Percent Alteration in Absolute Organ Weights (mg) in Female Fischer 344 Rats Exposed to D4
Study 12

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	-0.3	0.8	-1.0	-2.4	-35.9**
Thymus	ND	ND	ND	ND	ND
Liver	1.7	9.9**	28.0**	64.1**	-4.0

ND = Not Done

Chart 11a
Comparison of Percent Alteration in Organ Weights (mg) as Percent of Body Weight in Female Fischer 344 Rats Exposed to D4
Study 12

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	-0.8	1.2	-3.7	-1.7	-33.9**
Thymus	ND	ND	ND	ND	ND
Liver	1.9	10.9**	25.3**	66.3**	0.0

ND = Not Done

Chart 11b
Comparison of Percent Alteration in Absolute Organ Weights (mg)
in Female Fischer 344 Rats Exposed to D4
Study 12/Recovery

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	ND	ND	ND	-3.4	-36.4**
Thymus	ND	ND	ND	-2.4	-58.6**
Liver	ND	ND	ND	7.6**	-6.1

ND = Not Done

Chart 11c
Comparison of Percent Alteration in Organ Weights (mg) as
Percent of Body Weight in Female Fischer 344 Rats Exposed to D4
Study 12/Recovery

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	ND	ND	ND	-0.8	-33.7**
Thymus	ND	ND	ND	0.0	-57.4**
Liver	ND	ND	ND	10.7**	-1.4

ND = Not Done

Chart 12
Comparison of Percent Alteration in Absolute Organ Weights (mg)
in Female Fischer 344 Rats Exposed to D4
Study 14

	Percent Alteration				
Organ	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen	2.1	-2.9	-0.8	-0.8	ND
Thymus	ND	ND	ND	ND	ND
Liver	11.0	11.4	30.3**	76.7**	ND

ND = Not Done

HEMATOLOGY. Hematological evaluation was conducted on male and female Fischer 344 rats in Studies 5, 6 and 12. Blood was collected by cardiac puncture on day 29, one day after the last exposure. Hematological values are shown in Appendix D - Tables 12-22.

Male.

The data for hematological parameters in male rats exposed to D4 are shown in Appendix D - Tables 12-14 and Chart 13 summarizes the D4-induced changes in male rats. There is a dose-dependent decrease in erythrocyte number, hemoglobin and hematocrit. The decreases in these

erythrocyte elements are related to an increase in the reticulocytes number and in the MCV and MCH. These changes are consistent with a depletion of erythrocytes in the periphery as compared to a decrease in bone marrow synthesis. There is a slight decrease in leukocyte number which is not associated with a specific cell type, i.e. neutrophils or lymphocytes. Platelet number is slightly elevated in male rats exposed to 30 mg/kg and 100 mg/kg doses but not 300 mg/kg.

Chart 13
Comparison of Percent Alteration in Hematological Parameters
in Male Fischer 344 Rats Exposed to D4
Study 6

	Percent Alteration				
Hematological Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Erythrocytes	-1.7	-3.1	-5.9**	-12.6**	-3.7
Reticulocytes	-0.7	8.9	1.0	28.5	-95.7**
Hemoglobin	0.0	0.0	-1.2	-8.1**	-1.2
Hematocrit	-0.5	-2.1	-3.7	-10.1**	-5.0*
MCV	1.3*	1.3*	2.3**	3.1**	-1.3*
MCH	2.1**	3.6**	5.2**	4.7**	2.6**
MCHC	0.5	2.2**	2.4**	1.9	4.1**
Leukocytes	-8.7	-13.6*	-3.9	-17.8**	-86.0**
Platelets	6.7	6.9*	7.8*	-1.3	-41.1**

Female.

The data for hematological parameters in female rats exposed to D4 are shown in Appendix D - Tables 15-22. Charts 14 and 15 summarize the D4-induced changes in female rats. Because the reticulocytes were not enumerated in Study 5, a second study (Study 12) was conducted. The D4-associated anemia, coupled with the increase in MCV and reticulocytosis, suggests that the erythrocyte was targeted by D4 either by a direct or indirect mechanism. The hypothesis that the decrease in erythrocytes was caused by increased phagocytosis by the Kupffer cells is supported in part by the increase in liver weight and coupled with an increase in blood volume (See RES section). The D4-associated effects on the erythroid elements returned to the level of the vehicle control group in 14 days (Chart 15a and Appendix D - Tables 20 and 21). There were no significant changes in leukocyte number and a slight decrease in platelets. From these 3 hematological studies, we conclude that the leukocytes and platelets were not targeted by D4.

Chart 14
Comparison of Percent Alteration in Hematological Parameters in Female Fischer 344 Rats Exposed to D4
Study 5

	Percent Alteration				
Hematological Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Erythrocytes	-4.2*	-6.1**	-10.0**	-9.3**	-12.6**
Reticulocytes	ND	ND	ND	ND	ND
Hemoglobin	-1.9	-3.7*	-6.8**	-7.5**	-9.9*
Hematocrit	-3.9*	-5.3**	-8.3**	-7.6**	-12.2**
MCV	0.2	0.7	1.8**	1.8**	0.4
MCH	2.0**	2.5**	3.5**	1.5*	3.0**
MCHC	1.6**	1.9**	1.6**	-0.3	2.7**
Leukocytes	-9.6	8.8	3.7	1.5	-69.1**
Platelets	0.9	-0.2	-6.9	-9.4**	-43.3**

ND = Not Done

Chart 15
Comparison of Percent Alteration in Hematological Parameters in Female Fischer 344 Rats Exposed to D4
Study 12

	Percent Alteration				
Hematological Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Erythrocytes	1.0	-2.6	-4.5**	-6.6**	-6.1**
Reticulocytes	-8.3	18.3*	13.5	71.9**	-94.5**
Hemoglobin	1.4	0.0	-2.7	-6.1**	-4.8**
Hematocrit	1.0	-2.1	-3.1*	-4.9**	-6.7**
MCV	-0.2	0.6	1.3**	1.7**	-0.6
MCH	0.5	3.0	2.5	1.0	1.5
MCHC	0.3	1.8	0.5	-1.0	1.8
Leukocytes	19.7	-4.7	-1.3	-0.9	-74.6**
Platelets	4.1	1.7	2.1	-5.5	-39.1**

Chart 15a
Comparison of Percent Alteration in Hematological Parameters in Female Fischer 344 Rats Exposed to D4
Study 12/Recovery

	Percent Alteration				
Hematological Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Erythrocytes	ND	ND	ND	-2.4*	-14.4**
Reticulocytes	ND	ND	ND	-4.4	-96.5**
Hemoglobin	ND	ND	ND	-1.3	-8.7**
Hematocrit	ND	ND	ND	-2.3*	-14.5**
MCV	ND	ND	ND	0.2	-0.2
MCH	ND	ND	ND	1.0	7.1
MCHC	ND	ND	ND	0.8	7.2
Leukocytes	ND	ND	ND	-1.1	-84.4**
Platelets	ND	ND	ND	5.2	-41.3**

ND = Not Done

SUMMARY OF STANDARD TOXICOLOGY STUDIES. D4 was administered by oral gavage in doses between 10 and 300 mg/kg to male and female Fischer 344 rats daily for 28 consecutive days. Biological actions associated with indicators of general systemic changes include: (i) slight and variable increases in food consumption in males and females (300 mg/kg), (ii) decrease in erythrocyte elements in both males (100-300 mg/kg) and females (10-300 mg/kg), (iii) increases in liver weight in both males (30-300 mg/kg) and females (10-300 mg/kg), and (iv) decreases in thymus weight in males and females (300 mg/kg).

IMMUNOLOGY STUDIES

Indicators of humoral, cell-mediated and innate immunity of immune competent cells of the peripheral blood, spleen and liver were assessed in rats exposed to D4 for 28 days. Lymphocyte phenotyping, IgM antibody response to sRBC (T-dependent antigen), MLR, RES and NK functions were used to assess the immune system.

T CELL AND T SUBSETS AND B CELL ENUMERATION.

The percent and number of lymphocytes of the spleen and peripheral blood in rats were measured in order to determine if D4 had a direct effect on cell viability or body distribution. Rat were exposed to D4 for 28 days, and, on day 29, spleens were removed and spleen cell suspensions prepared, and surface marker analysis for T lymphocytes $CD3^+$, $CD4^+$ and $CD8^+$ subsets, and B lymphocytes performed. At the time the studies were performed, the best

commercial available immunological reagents were used, but for the rat these reagents were not as well characterized as for the mouse. At the time of the repeat studies, newer, less cross-reactive reagents were utilized.

Male.

The data for lymphocyte phenotyping in male rats exposed to D4 are shown in Appendix D - Tables 23-26. These data indicate that there were no changes in lymphocyte phenotypes in the peripheral blood or spleen of the D4-exposed male rats when compared to the vehicle control group.

Female.

The data for lymphocyte phenotyping in female rats exposed to D4 are shown in Appendix D - Tables 27-37. Chart 16 summarizes the significant percent alterations in splenocyte surface marker differentials in absolute values in D4-exposed rats. This study was the first performed in the rat using the first monoclonals available and considered the best available at the time. The changes observed in the D4-exposed groups showed no consistent pattern (Appendix D - Tables 27-29) and, because of the lack of an historical control base, a repeat study was conducted before interpretation was made.

Chart 16
Comparison of Percent Alteration in Splenocyte Surface Marker Differentials in Female Fischer 344 Rats Exposed to D4 (Absolute Values)
Study 5

		Percent Alteration				
Parameter	Cell Type	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
$OX12^+$	B cells	-18.1	-15.5	-21.1*	-25.1**	-89.8**
$OX19^+$	T cells	10.7	5.8	1.5	-10.7	-67.6**
$W3/25^+$	CD4	1.7	-6.7	-21.7*	-26.1**	-77.2**
$OX8^+$	CD8	-10.2	-16.0	-25.1*	-36.7**	-61.0**

The repeat study (Study 12) also included a 14-day recovery component (Study 12/Recovery). The results are present in Appendix D - Tables 30-37. A new monoclonal reagent, i.e. $OX33^+$, was used as the B cell marker in the repeat study. Chart 17 shows the percent changes compared to the vehicle control group. The decrease in absolute lymphocyte values are directly related to a decrease in spleen cellularity. When the data are presented as percentage of each subset, the only statistically significant effect is a 8.1 percent decrease for rats exposed to 300 mg/kg D4 in the $OX33^+$ cells (B cells). In light of the dose and the marked increase in liver

weight, this change is not considered biologically relevant. The female rats which were allowed a 14-day rest period after the cessation of exposure showed a 14.3% decrease in the absolute number of W3/25$^+$ cells (CD4$^+$) and 12.1% decrease in OX8$^+$ (CD8$^+$) cells lymphocyte subsets in the spleen. These decreases can partially be explained by a decrease in spleen cellularity. When calculated on a percentage basis of total splenic lymphocytes, only the W3/25$^+$ (CD4$^+$) cells showed a slight decrease (10.4%). These T cell populations changes are considered minor and do not reflect the change seen in the animals studied at the end of exposure period (Compare Appendix D - Tables 30 and 31 to Tables 35 and 36). Thus, this is either a delayed alteration due to D4 or represents variability between experiments. In either case, it is not considered biologically relevant without some collaborating data showing functional or other changes. Of note again is that the livers of these animals are undergoing rapid changes and these minor changes may be some secondary reflection of this activity. In all cases, the positive control produced the expected marked changes in spleen cell number and lymphocyte subsets.

Chart 17
Comparison of Percent Alteration in Splenocyte Surface Marker Differentials in Female Fischer 344 Rats Exposed to D4 (Absolute Values)
Study 12

		Percent Alteration				
Parameter	Cell Type	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
OX33$^+$	B cells	-4.3	-0.4	-12.2**	-16.4**	-94.0**
OX19$^+$	T cells	-5.1	-0.7	-14.6**	-13.1**	-61.9**
W3/25$^+$	CD4	-2.6	-4.5	-11.5	-9.2	-68.9**
OX8$^+$	CD8	-3.8	-2.8	-15.5	-10.1	-37.3**
Spleen Cells (x 10^7)		-3.1	2.1	-10.5*	-9.7*	-67.0**

In summary, the studies in female and male rats exposed to D4 show no biologically significant changes in lymphocyte numbers or percentages in spleen and peripheral blood.

SPLEEN IGM ANTIBODY RESPONSE TO THE T-DEPENDENT ANTIGEN, SRBC. The T-dependent IgM antibody response to sRBC was used as an indicator of humoral immune status. Two types of endpoints were used. The spleen IgM response is the measurement of the number of B lymphocyte/plasma cells that produce antibody to sRBC. The serum IgM antibody titer measures the concentrations of antibody derived from all the antibody producing cells (spleen, lymph nodes, bone marrow). Measurement of the serum antibody titer by an ELISA method is a newer approach of assessing humoral immunity. The humoral immune response was measured in Studies 1, 3, 9, 13 and 14.

Rats were exposed to D4 for 28 days and were immunized with sRBC on day 25 of the experimental period. The spleen IgM antibody-forming cell (AFC) response was measured one day after the last exposure which was day 4 after antigen. Day 4 after antigen is the peak day for the spleen IgM response. Results are presented as the AFC/10^6 spleen cells (specific activity) and as AFC/spleen x 10^3 (total spleen activity) and antibody titers (Appendix D - Tables 38-49).

Male.

Chart 18 summarizes the percent alterations in D4-induced changes in the spleen antibody-forming cell response in male rats (Appendix D - Tables 38 and 39). There were no statistical changes in the spleen antibody response to sRBC, whether expressed as specific activity (IgM AFC/10^6 spleen cells) or as the total number of antibody-forming cells per spleen (IgM AFC/spleen x 10^3). CPS, administration to male rats, produced the expected abolishment of the immune response. The elevation in the IgM AFC response is noteworthy, although not statistically significant in a dose-dependent manner. However, there is no immune suppression at these doses.

Chart 18
Comparison of Percent Alteration in AFC Response to Sheep Erythrocytes in Male Fischer 344 Rats Exposed to D4
Study 3

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen Wgt	-0.6	0.6	3.1	-0.6	-37.0**
Spleen Cell Number	-4.7	0.1	1.8	-7.6	-70.1**
IgM AFC/10^6 Spleen Cells	10.6	34.1**	23.6	23.8	-99.9**
IgM AFC/Spleen x 10^3	5.1	34.1**	25.6	13.6	-100.0**

Chart 19 summarizes the percent alterations in D4-induced changes in the serum antibody titers to sheep erythrocytes. Note again that the mid dosed (30 and 100 mg/kg) animals have elevated titers, but they are not statistically significant.

Chart 19
Comparison of Percent Alteration in Serum Titers to Sheep Erythrocytes in Male Fischer 344 Rats Exposed to D4
Study 3

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Serum Titers	-2.5	32.9	7.6	-10.1	-79.7**

Female.

The data for the IgM antibody response to sRBC in female rats exposed to D4 are shown in Appendix D - Tables 40-49. Charts 20-27 summarize the percent changes as compared to the vehicle controls.

Chart 20 summarizes the percent alterations in D4-induced changes in the spleen antibody-forming cell response in female rats from Study 1. There were no statistical changes in the spleen antibody response to sRBC, whether expressed as specific activity (IgM AFC/10^6 spleen cells) or as the total number of antibody-forming cells per spleen (IgM AFC/spleen x10^3). CPS administration to female rats produced the expected abolishment of the immune response. There was more variability in this study than expected and a repeat study was performed (Study 9).

Chart 20
Comparison of Percent Alteration in AFC Response to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Study 1

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen Wgt	1.3	-0.5	6.6	-0.3	-38.2**
Spleen Cell Number	0.3	-7.2	-1.8	-1.5	-66.8**
IgM AFC/10^6 Spleen Cells	-25.0	10.6	-21.2	-8.5	-91.7**
IgM AFC/Spleen x 10^3	-25.2	3.0	-22.2	-12.9	-97.9**

Chart 21 summarizes the percent alterations in D4-induced changes in the serum antibody titers to sheep erythrocytes. The decrease in the antibody titer was not expected based on the results of the spleen antibody response. The need for a repeat study was also reinforced by the fact that this was the first rat titer study and that day 4 is not the peak day of response for serum titer.

Chart 21
Comparison of Percent Alteration in Serum Titers to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Study 1

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Serum Titers	-23.6	-19.8	-21.7	-41.0**	-86.8**

The data for the repeat study on female rats exposed to D4 are shown in Appendix D - Tables 42-45 and the percent changes summarized in Charts 22 and 23. Included in this study was a 14-day recovery phase. There was no suppression of the immune response but a dose-dependent increase which was statistically significant at the 100 or 300 mg/kg D4 dose levels. CPS produced the expected marked suppression of this immune response.

Chart 22
Comparison of Percent Alteration in AFC Response to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Study 9

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen Wgt	0.7	1.2	3.6	4.6	-40.8**
Spleen Cell Number	-5.1	-9.5	-2.0	-3.1	-76.7**
IgM AFC/10^6 Spleen Cells	27.1	0.2	59.4**	49.6**	-98.5**
IgM AFC/Spleen x 10^9	20.1	-10.2	55.5**	42.8*	-99.8**

The increased IgM response to sRBC was also apparent when the serum antibody response was measured (Charts 22 and 23 and Appendix D - Tables 43 and 45). In contrast to the spleen derived data, the increase in the serum antibody titer was not statistically significant when compared to the vehicle control group.

Chart 23
Comparison of Percent Alteration in Serum Titers to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Study 9

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Serum Titers	-6.5	-0.6	14.9	39.6	-89.6*

The results of the recovery study are present in Appendix D - Tables 44 and 45 and Charts 23a and 23b. Fourteen days after the last D4 administration, there remained a 39.1 and 33.8% elevation in the spleen antibody-forming cell response in rats exposed to 300 mg/kg. The elevation was not manifested in the serum.

Chart 23a
Comparison of Percent Alteration in AFC Response to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Study 9/Recovery

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Spleen Wgt	ND	ND	ND	-2.6	-40.5**
Spleen Cell Number	ND	ND	ND	-4.1	-72.2**
IgM AFC/10^6 Spleen Cells	ND	ND	ND	39.1**	-98.4**
IgM AFC/Spleen x 10^3	ND	ND	ND	33.8**	-99.5**

ND = Not Done

Chart 23b
Comparison of Percent Alteration in Serum Titers to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Study 9/Recovery

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Serum Titers	ND	ND	ND	-14.2	-93.9**

ND = Not Done

We hypothesized that the difference between the spleen antibody-forming cell response and the serum antibody titers was due to the difference in peak day of response to the two parameters. The peak response for the spleen antibody-forming cells is 4 days after antigen administration and 6 days for the serum antibody titer. The titers were measured on day 4 in order not to use an additional group of animals. In the studies reported by Temple *et al.*[4], it appeared that a immunosuppressive response would be identified prior to the peak day. The data are consistent with selecting day 4 for immune suppression. However, elevation in the antibody response may require the full complement of days in order for the antibody to traffic from the lymphoid tissue to the blood. In order to determine if the peak day of the individual response was the determining factor, a study was conducted to determine the IgM antibody response at both day 4 and day 6. Studies 13 and 14 were performed and the results are presented in Appendix D - Tables 46-49 and Charts 24-27. The increase in the spleen antibody response repeated both on day 4 and on day 6 (Charts 24 and 26 and Appendix D - Tables 46 and 48). However, consistent with the first set of observations, there was no increase in the antibody response as measured by antibody titers (Charts 25 and 27 and Appendix D - Tables 47 and 49).

Chart 24
Comparison of Percent Alteration in AFC Response to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Day 4 Response
Study 13

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
IgM AFC/10^6 Spleen Cells	-14.7	3.7	35.1	55.6**	ND
IgM AFC/Spleen x 10^3	-15.8	7.5	33.2	45.0*	ND

ND = Not Done

Chart 25
Comparison of Percent Alteration in Serum Titers to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Day 4 Response
Study 13

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Serum Titers	-7.6	-9.2	5.9	-14.8	ND

ND = Not Done

Chart 26
Comparison of Percent Alteration in AFC Response to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Day 6 Response
Study 14

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
IgM AFC/10^6 Spleen Cells	40.1	34.7	47.7	98.6**	ND
IgM AFC/Spleen x 10^3	47.1	27.9	51.5	95.6**	ND

ND = Not Done

Chart 27
Comparison of Percent Alteration in Serum Titers to Sheep Erythrocytes in Female Fischer 344 Rats Exposed to D4
Day 6 Response
Study 14

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	CPS 25 mg/kg
Serum Titers	-35.0	-30.1	-40.8	-10.7	ND

ND = Not Done

We conclude from the studies on the IgM antibody response that D4, in the dose range tested, does not produce a humoral immune suppression. The elevation in the spleen IgM antibody response is not reflected in the serum titers.

MIXED LEUKOCYTE RESPONSE TO LONG EVANS & BROWN NORWAY SPLEEN CELLS. The one-way mixed leukocyte response (MLR) is a functional method for assessing cell-mediated immunity. As indicated in the protocol, the appropriate matching of the stimulator and responder cells is needed to obtain a proliferative response. Thus, Long Evans or Brown Norway spleen cells were used as the allogeneic stimulator cell for the Fischer 344 responder lymphocyte. Responder cells were obtained from both the spleen and peripheral blood of the Fischer 344 rat. On day 29, one day following the last exposure to D4, animals were sacrificed, spleens were removed, single cell suspensions prepared, and the response to allogeneic lymphocytes assessed in the one-way mixed leukocyte reaction. These MLR data were derived from Studies 5 and 6. The data are shown in Appendix D - Tables 50-53. Although the data are presented in the tables for both allogeneic stimulators cells, the best response was obtained using the Brown Norway spleen cells. In all cases, there were no D4-associated changes in the MLR response for either the peripheral blood or spleen cells. The positive control CPS produced the expected marked suppression of the MLR response.

CLEARANCE OF SRBC BY THE RETICULOENDOTHELIAL SYSTEM (RES). The functional activity of the RES was measured on day 29, one day after the last exposure to D4, by determining the vascular clearance rate of ^{51}Cr sRBC and the uptake of the ^{51}Cr sRBC into the liver, spleen, lungs and thymus. Kidney localization of ^{51}Cr sRBC was also determined as a non-phagocytizing organ. Changes in uptake in this organ reflects other factors (e.g., changes in blood in the tissue, hemolysis of the ^{51}Cr sRBC). The results are derived from Studies 2, 4, 10 and 11 and are presented in Appendix D - Tables 54-59. Maleic vinyl ether (MVE) was used as a positive control in this assay to suppress the vascular clearance rate and liver uptake of ^{51}Cr sRBC.

Male.

The RES activity data of male rats exposed to D4 are shown in Appendix D - Table 54 and the percent alteration from the vehicle control group provided in Chart 28. Since the liver and spleen are the major organs phagocytizing the ^{51}Cr sRBC, often there is a correlation between clearance rate and organ uptake of ^{51}Cr sRBC. Often, if the liver has increased phagocytic activity, the uptake in the spleen, lungs and thymus is decreased due to availability of the phagocytizing particle.

Chart 28
Comparison of Percent Alteration in RES Activity in in Male Fischer 344 Rats Exposed to D4
Study 2

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	MVE 50 mg/kg
Half-Life	-8.5	-26.9	-22.3	-27.7	490.0**
Liver Uptake (%)	4.1	-2.5	5.7	10.6	-87.8**
Liver Uptake(cpm/mg)	4.7	-9.4	-11.6	-25.4**	-88.8**
Spleen Uptake (%)	0.0	18.3	-1.4	-35.9	-64.1**
Spleen Uptake (cpm/mg)	1.0	12.2	-4.9	-34.7	-67.6**
Lung Uptake (%)	13.6	13.6	8.5	-18.6	447.5**
Lung Uptake (cpm/mg)	3.3	0.0	-16.7	-30.0	305.7**
Thymus Uptake (%)	-10.7	21.4	-14.3	-42.9**	296.4*
Thymus Uptake (cpm/mg)	-2.0	30.6	-8.2	-28.6**	267.3**
Kidney Uptake (%)	7.1	-17.9	-10.7	-50.0**	-57.1**
Kidney Uptake (cpm/mg)	14.1	-15.3	-9.4	-49.4**	-56.5**

The dose-dependent decrease (8.5%-27.7%) in the vascular half-life is reflected as an increase in hepatic uptake (up to 10.6%). In turn, the increased hepatic uptake as a percent of the injected material may be more a reflection of the increase in liver weight which may allow more blood to be left in the liver. Liver uptake, based on liver weight (cpm/mg), is decreased dose dependently reaching 25.4% in rats exposed to 300 mg/kg. Since the Kupffer cell is responsible for the liver uptake, the decrease in uptake based on liver weight indicates that the liver weight increase is not associated with these phagocytic cells and is caused by an increase in parenchymal cell size or cell number. Although there was no statistical decrease in spleen uptake, there was a 34.7% decrease in the 300 mg/kg dose group. This may be a reflection of liver uptake. A similar decrease in organ uptake was seen in the lungs, thymus, and non-phagocytizing kidney. Since the kidney reflects blood left in the organ or free ^{51}Cr from lysed cells, the dose-dependent decrease in kidney uptake confirms that the decreases noted in the spleen, lungs and thymus uptake are a reflection of the increase in liver weight. Because of these changes, a repeat study coupled with a recovery study was performed. These data are shown in Appendix D - Tables 55 and 56 and summarized in Charts 29 and 29a.

Chart 29
Comparison of Percent Alteration in RES Activity in in Male Fischer 344 Rats Exposed to D4
Study 11

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	MVE 50 mg/kg
Half-Life	-12.9	11.7	-33.6**	-6.3	64.5**
Liver Uptake (%)	1.8	2.4	16.6	32.2**	-79.5**
Liver Uptake(cpm/mg)	2.0	-2.5	5.4	-5.4	-79.3**
Spleen Uptake (%)	-16.3	-12.8	0.0	-27.9	-58.1*
Spleen Uptake (cpm/mg)	-14.5	-13.0	-1.7	-25.8	-60.3**
Lung Uptake (%)	-18.9	-12.6	-20.0	-31.6**	287.4**
Lung Uptake (cpm/mg)	-13.0	-8.7	-15.2	-28.3**	265.2**
Thymus Uptake (%)	-2.5	2.5	-12.5	-27.5	67.5**
Thymus Uptake (cpm/mg)	-9.6	-4.3	-14.9	-23.4	79.8**
Kidney Uptake (%)	0.0	3.8	-3.8	-15.4*	-7.7
Kidney Uptake (cpm/mg)	3.8	2.5	-5.0	-16.3*	-10.6

This repeat study shows the same trends as the previous study. The vascular half-life of the vehicle control group was at the limits of the historical control range that we have seen in rats. The dose-dependent decrease in half-life of ^{51}Cr sRBC is interpreted as being caused by the increase in liver uptake. The vascular half-life of the rats exposed to 300 mg/kg had a higher variability than the other rats in the study. However, the trend analysis indicated dose dependency and the liver uptake confirms this trend. Liver uptake was increased by as much as 32.2% when expressed as percent of injected material. There were similar decreases in uptake by the spleen, lungs, thymus and kidney, all indicative of the liver effect which was described above.

The data for the 14-day recovery study are shown in Appendix D - Table 56 and summarized in Chart 29a. Recovery was not complete for the liver effects when compared to the vehicle control group but all other parameters had returned to control values.

Chart 29a
Comparison of Percent Alteration in RES Activity in
in Male Fischer 344 Rats Exposed to D4
Study 11/Recovery

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	MVE 50 mg/kg
Half-Life	ND	ND	ND	1.7	123.2*
Liver Uptake (%)	ND	ND	ND	32.0**	-72.6**
Liver Uptake(cpm/mg)	ND	ND	ND	11.7*	-73.1**
Spleen Uptake (%)	ND	ND	ND	20.8	-77.8**
Spleen Uptake (cpm/mg)	ND	ND	ND	22.1	-75.1**
Lung Uptake (%)	ND	ND	ND	-18.2	[illegible]5**
Lung Uptake (cpm/mg)	ND	ND	ND	-14.0	153.0**
Thymus Uptake (%)	ND	ND	ND	-9.8	115.7*
Thymus Uptake (cpm/mg)	ND	ND	ND	-11.1	111.1**
Kidney Uptake (%)	ND	ND	ND	-11.5	3.8
Kidney Uptake (cpm/mg)	ND	ND	ND	-12.8*	-4.5

ND = Not Done

Overall, the D4 changes in RES activity of male rats are interpreted to be associated with the increase in liver weight, and the increase in liver weight was not due to an increase in phagocytic cell number. The liver changes did not completely recover in 14 days.

Female.

Two RES studies and one recovery study were conducted on female rats under this protocol and amendments. The results from Study 4 are shown in Appendix D - Table 57 and summarized in Chart 30. The vascular half-life of ^{51}Cr sRBC was decreased and reached statistical significance in rats exposed to 300 mg/kg. This decreased clearance rate was not associated with an alteration in liver uptake, but was associated with a decrease in splenic uptake. There were no significant changes in the uptake of the other organs. Because of the discrepancy between the vascular clearance and liver uptake, a repeat study with a recovery component (Study 10) was conducted and the data are presented in Appendix D - Tables 58 and 59 and Charts 31 and 31a.

Chart 30
Comparison of Percent Alteration in RES Activity in in Female Fischer 344 Rats Exposed to D4
Study 4

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	MVE 50 mg/kg
Half-Life	17.6	42.0	48.7	100.0	579.0*
Liver Uptake (%)	-7.4	-4.8	-0.2	-0.2	-82.8**
Liver Uptake(cpm/mg)	-11.7	-18.4**	-29.2**	-43.8**	-84.7**
Spleen Uptake (%)	-3.6	-11.7	-41.4	-48.6*	-79.3**
Spleen Uptake (cpm/mg)	-1.7	-9.3	-37.1	-43.6*	-75.9**
Lung Uptake (%)	16.7	16.7	0.0	-33.3	900.0**
Lung Uptake (cpm/mg)	2.6	7.7	-5.1	-25.6	635.9**
Thymus Uptake (%)	0.0	-4.5	-4.5	-18.2	113.6**
Thymus Uptake (cpm/mg)	1.8	1.8	10.9	0.0	256.4**
Kidney Uptake (%)	5.4	5.4	13.5	-16.2	-43.2**
Kidney Uptake (cpm/mg)	1.1	2.2	5.9	-19.4	-52.7**

Chart 31
Comparison of Percent Alteration in RES Activity in in Female Fischer 344 Rats Exposed to D4
Study 10

Parameter	Percent Alteration				
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	MVE 25 mg/kg
Half-Life	24.2	40.1	-4.4	41.9	109.7*
Liver Uptake (%)	3.4	9.4	21.8	15.7	-69.0**
Liver Uptake(cpm/mg)	4.7	6.7	2.6	-26.9*	-69.4**
Spleen Uptake (%)	-16.5	-22.0	-19.3	-52.3**	4.6
Spleen Uptake (cpm/mg)	-16.3	-19.2	-16.4	-48.3	-7.1
Lung Uptake (%)	-10.0	-10.0	-10.0	10.0	270.0**
Lung Uptake (cpm/mg)	-6.3	-12.5	-12.5	9.4	240.6**
Thymus Uptake (%)	-5.3	-24.6	-21.1	-10.5	43.9*
Thymus Uptake (cpm/mg)	-8.2	-17.8	-11.0	8.2	64.4**
Kidney Uptake (%)	-3.8	-1.9	-5.7	-7.5	-35.8*
Kidney Uptake (cpm/mg)	0.0	2.4	-4.0	-7.9	-41.3**

In this study there was again an increase in vascular clearance rate but not as large as in Study 4. The values for the half-life and liver uptake, as represented by the percent of injected sRBC, were not statistically significant from the vehicle control group. The decrease in spleen uptake also repeated suggesting a more direct action on the spleen, i.e., this may not be a function of the increase in liver size but may be related to cellular makeup of the spleen. Although the lymphocyte populations were not altered (See phenotyping section), the spleen is a hematopoietic organ and the change in erythrocyte elements may be related to this effect.

In the 14-day recovery study (Appendix D - Table 59 and summarized in Chart 31a), there were no statistically significant effects seen when the D4-exposed animals were compared to the vehicle control group. Although not statistically significant, the decrease in splenic phagocytosis was 24% and indicates that the effect has not completely returned to normal.

Chart 31a
Comparison of Percent Alteration in RES Activity in in Female Fischer 344 Rats Exposed to D4
Study 10/Recovery

	Percent Alteration				
Parameter	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	MVE 25 mg/kg
Half-Life	ND	ND	ND	-6.0	38.7*
Liver Uptake (%)	ND	ND	ND	13.1	-70.4**
Liver Uptake(cpm/mg)	ND	ND	ND	-2.5	-73.9**
Spleen Uptake (%)	ND	ND	ND	-24.0	173.0**
Spleen Uptake (cpm/mg)	ND	ND	ND	-21.8	104.6**
Lung Uptake (%)	ND	ND	ND	-25.0*	200.0**
Lung Uptake (cpm/mg)	ND	ND	ND	-22.9*	162.9**
Thymus Uptake (%)	ND	ND	ND	-5.6	69.4**
Thymus Uptake (cpm/mg)	ND	ND	ND	0.0	99.1**
Kidney Uptake (%)	ND	ND	ND	0.0	-4.9
Kidney Uptake (cpm/mg)	ND	ND	ND	-2.4	-16.9**

ND = Not Done

To summarize the RES activity of female rats exposed to D4, the effects on liver phagocytosis are not seen as is in the male, and there is a decrease in splenic phagocytosis.

NATURAL KILLER (NK) CELL ACTIVITY. On day 29, one day following the last exposure to D4, animals were sacrificed, spleens were removed, single cell suspensions prepared, and NK cell activity assessed. Six ratios of effector-to-target cells were used in Study 6 (male rats) and 3 ratios used in Study 5 (female rats). The data are shown in Appendix D - Tables 60-63. There were no D4-associated changes in the NK cell activity when the cells were derived from either the peripheral blood or spleen.

SUMMARY OF IMMUNOLOGY STUDIES. Indicators of alterations in the immune system include: (i) increase in spleen antibody-forming cell response to sRBC (100-300 mg/kg) in female rats with no change in serum titers to the antigen; (ii) slight increase in RES activity (30-300 mg/kg) in the male rats with the liver representing the target; (iii) a decrease in RES activity (100-300 mg/kg) in female rats with the spleen representing the target; and (iv) no

biologically significant changes in lymphocyte subsets in the spleen or peripheral blood. Cellular immunity, as measured by the MLR, was not altered by D4 exposure. Similarly, NK activity was not affected by D4 administration.

COMPARISON OF VEHICLES

Studies were conducted under amendments to the protocol to assess the role of the vehicle to elicit a D4 biological response. Body weight and body weight changes are presented in Appendix E - Tables 7-14. Food consumption data are provided in Appendix F - Tables 7-14. The results are presented in Appendix D - Tables 64 and 65 and are best summarized in Figures 4a and 4b. In both male and female rats, D4 produced a dose-dependent increase in liver weight with the greatest response occurring when D4 was administered in corn oil. A dose-dependent increase in liver weight was also seen when D4 was administered in 10% Emulphor and Maalox. In male and female rats, there was no increase in liver weight when D4 was administered in 35 cs PDMS. Thus, the potency of D4 was associated with the vehicle with the order of vehicles being corn oil > 10% Emulphor > Maalox > 35 cs PDMS fluid.

Figure 4
Liver Weights (mg) in Male and Female Fischer 344 Rats
Exposed to D4 for 28 Days
Comparison of Vehicle Studies

Figure 4a
Males

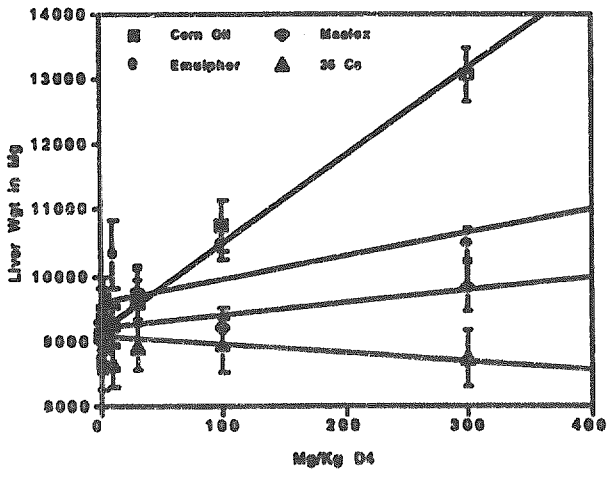
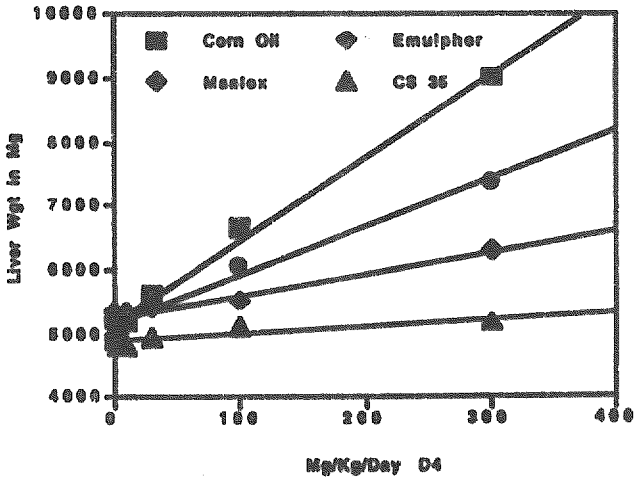


Figure 4b
Females



CONCLUSIONS

D4 was administered by oral gavage in doses between 10 and 300 mg/kg to male and female Fischer 344 rats daily for 28 consecutive days. Biological actions associated with indicators of general systemic changes include: (i) slight and variable increases in food consumption in males and females (300 mg/kg); (ii) decreases in erythrocyte elements in both males (100-300 mg/kg) and females (10 -300 mg/kg); (iii) increases in liver weight in both males (30-300 mg/kg) and females (10 -300 mg/kg); and (iv) decreases in thymus weight in males and females (300 mg/kg). Indicators of alterations in the immune system include: (i) increases in spleen antibody-forming cell response to sRBC (100-300 mg/kg) in female rats with no change in serum titers to the antigen; (ii) slight increases in RES activity (30-300 mg/kg) in the males with the liver representing the target; (iii) a decrease in RES activity (100-300 mg/kg) in females with the spleen representing the target; and (iv) no biologically significant changes in lymphocyte subsets in the spleen or peripheral blood. Cellular immunity as measured by the MLR was not altered by D4 exposure. Similarly, NK activity was not affected by D4 administration.

D4, administered in corn oil, produced the most marked effect on liver weight. Potency, with respect to D4 elicitation of increased liver weight, of the remaining vehicles was 10% Emulphor > Maalox > 35 cs PDMS. When PDMS was the carrier, no increase in liver weight was observed. The role of the vehicle is important in the risk assessment of D4 administered by the oral route, as discussed above.

ARCHIVE

All data and records pertaining to this study will be submitted to the sponsor at the time of the final report. Upon completion of this study, the report and raw data for this study will be maintained in the archives of Dow Corning Corporation, Midland, Michigan. Records which were maintained for this protocol are shown below in Chart 32.

Chart 32
Records Maintained In Archives

Protocol	Preparation and Handling of Test Article Records
Study Sheets	Food Lot Records
Log Sheets	Animal Quarantine Report Forms
Room Activity Sheets	Authorized Signatures and Initial Forms
Necropsy Forms	Correspondence
Curriculum Vitae for the Key Personnel	Final Report

REFERENCES

1. Luster, M.I., Munson, A.E., Thomas, P., Holsapple, M.P., Fenters, J., White, K.L., Jr., Lauer, L.D., Germolec, D.R., Rosenthal, G.J. and Dean, J.H. (1988). Development of a Testing Battery to Assess Chemical-Induced Immunotoxicity: National Toxicology Program Guidelines for Immunotoxicity Evaluation in Mice. *Fundam. Appl. Toxicol.*, 10:2-19.

2. Jerne, N.K., Henry, C., Nordin, A.A., Fun, H., Koros, M.C., and Lefkovits, I. (1974). Plaque Forming Cells: Methodology and Theory. *Transpl. Rev.*, 18:130-191.

3. Luster, M.I., Portier, C., Pait, D.G., White, K.L., Jr., Gennings, C., Munson, A.E. and Rosenthal, G.J. (1992). Risk Assessment in Immunotoxicity: I. Sensitivity and Predictability of Immune Tests. *Fundam. Appl. Toxicol.*, 18:200-210.

4. Temple, L., Kawabata, T.T., Munson, A.E. and White, K.L., Jr. (1993). Comparison of ELISA and Plaque-Forming Cell Assay for Measuring the Humoral Immune Response to SRBC in Animals Treated with Benzo(a)pyrene or Cyclophosphamide. *Fund. Appl. Toxicol.*, 21:412-419.

5. White, K.L.,Jr., Krasula, R.W., Munson, A.E., and Holsapple, M.P. (1986). Effects of hydroxyethylstarch, (HespanR) a plasma expander, on the functional activity of the reticuloendothelial system. *Drug Chem. Toxicol.*, 9:305-322.

6. Bartlett, M.S. (1937). Sub-Sampling for Attributes. *J. Roy. Stat. Soc. Suppl.*, 4:131-135.

7. Kruskall W.H. and Wallis, W.A. (1952). Use of Ranks in One-Criterion Variance Analysis. *J. Amer. Stat. Assoc.*, 47:583-621.

8. Dunnett, C.W. (1955). A Multiple Comparison Procedure for Comparing Several Treatments with a Control. *J. Amer. Stat. Assoc.*, 50:1096 -1121.

9. Wilson, K.V. (1956). A Distribution Free Test of Analysis of Variance Hypothesis. *Psychol. Bull.*, 53:96-101.

10. Gehan-Wilcoxon Test (1975): In. *Survival Distribution: Reliability Applications in the Biomedical Sciences*, ed. by A.J. Gross and V.A. Clark. John Wiley & Sons, New York, pp. 120-123.

11. Jonckheere's Test (1975). Non-Parametric Statistics Methods. M. Hollander and D.A. Wolfe, eds. John Wiley & Sons, New York, p. 120-123.

12. Sokal, R.R. and Rohlf, F.J. (1981). *Biometry*. Freeman, San Francisco, p. 226-231.

APPENDIX A

PROTOCOL D4-28-1R-PO

IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

PURPOSE: The purpose of this study is to determine the potential effects of Octamethylcyclotetrasiloxane (D4) on the immune system.

PROTOCOL IDENTIFIER: D4-28-1R-PO

DATE PROTOCOL PREPARED: September 26, 1993

DATE PROTOCOL REVISED: February 7, 1994

PROPOSED START DATE: March 24, 1994

PROPOSED DATE OF COMPLETION: August 15, 1994

PROPOSED DATE OF PROTOCOL REPORT: September 25, 1994

SPONSOR:
Dow Corning Corporation
2200 W. Salzburg Rd.
Midland, Michigan 48686-0994

SPONSOR'S REPRESENTATIVE:
Paal C. Klykken, Ph.D.
Dow Corning Corporation
2200 W. Salzburg Rd. CO3101
Midland, Michigan 48686-0994
Telephone (517) 496-6112
FAX: (517) 496-8783

TESTING FACILITY:
Medical College of Virginia,
Virginia Commonwealth University
Department of Pharmacology and Toxicology
and Program of Biomedical Engineering
Richmond, Virginia 23298

PERSONNEL RESPONSIBILITIES:

Study Director: Albert E. Munson, Ph.D.
Immunology: Kimber L. White, Jr., Ph.D.
Immunology Research Assistant: J. Ann McCay, B.S.
Quality Assurance: H. Leon Pringle, Ph.D.

PROTOCOL APPROVAL:

Study Director: Albert E. Munson
Date: March 24, 1994

Quality Assurance: H. Leon Pringle
Date: 3/30/94

Sponsor: Paal C. Klykken
Date: 3/31/94

STUDY GUIDELINES: The study will be conducted in compliance with EPA Good Laboratory Practice Regulations (40 CFR 792 Part A), and in accordance with agreements between the sponsor's representative and the study director at the Medical College of Virginia/Virginia Commonwealth University.

TEST ARTICLE: Octamethylcyclotetrasiloxane (D4); Mol. Wt. 215.66, CAS Number 000556672, was obtained from Dow Corning Corporation, Lot # LL121629. Data relating to the purity of the test article is provided by the Dow Corning. Date received at MCV: 11 Oct 93 MCV Chemical #11-10-93-1.

TEST ARTICLE STABILITY: The study material stability will be determined by the Sponsor.

TEST ARTICLE PREPARATION: D4 will be prepared daily as a solution in corn oil. The solutions will be prepared in amber glass vials and will be stored at room temperature. The concentrations of the solutions will be determined by the Sponsor.

DOSES TO BE ADMINISTERED: The doses to be administered will be 300, 100, 30, and 10 mg/kg as indicated by the Sponsor.

ROUTE OF ADMINISTRATION: The test article will be administered by gastric intubation using an 16 gauge gavage tube. Animals will receive 0.05 ml/10 gram body weight.

VEHICLE CONTROL: Corn Oil will be used as the vehicle control. Vehicle control animals will receive 0.05 ml/10 gram body weight. Vehicle animals will be dosed at the same time as the treatment groups.

POSITIVE CONTROL Number 1: Cyclophosphamide (Sigma Chemical Co.) will be given as a positive control. The positive control animals will receive 25 mg/kg of cyclophosphamide (CPS) on days 24 through 28 by i.p. injection. CPS will be prepared in phosphate buffered saline at concentration of 5 mg/ml and stored at -20°F(SOP/TAP/026). Positive control animals will receive 0.05 ml/10 grams of body weight.

POSITIVE CONTROL Number 2: Anti Asialo GM1 (Rabbit) antibody (Wako BioProducts) will be given as a positive control in the natural killer cell assay. The positive control animals will receive 1.0 ml of a 1:10 dilution of the antibody on day 28 by intraperitoneal injection. Anti Asialo GM1 will be diluted in sterile distilled water.

STUDY DURATION: All animals will receive the test article for 28 days. On day 29, animals will be evaluated for the appropriate immunological and toxicological parameters.

SPECIES SELECTION: Male and Female Fischer 344 rats will be used in this study. The Fischer 344 strain will be used at the sponsor's request. The major components of the immune system in the rat and man are the same. Agents that perturb the immune system in man perturb the immune system in the rat in a similar manner. The rat has previously been found to be sensitive to the effects of various environmental chemicals on the immune system.

TEST SYSTEM: Male and Female Fischer 344 rats will be purchased from Charles River Breeding Laboratories. The rats will be ordered hepatitis and Sendai free. Animals will arrive at approximately 6-8 weeks of age or between 120 and 150 grams depending on sex. Upon

arrival the rats will be quarantined for at least 5-7 days prior to commencement of treatment. All rats will be randomized using an Apple computer generated randomization procedure (SOP/CSA/010) and a pre- study weight obtained. Rats will be individually identified by tail marking and cage card in accordance with standard operating procedures (SOP/IDN/014,010). Rats will be housed 1 animal per cage in wire hanging cages and maintained on Agway Rat and Rat Ration (NIH 07), NIH required diet, and tap water ad libitum from a fresh water tap. The cages will be placed on the rack so that the individual dose groups are not all at one level, i.e., the cages will be ordered from top to bottom. NIH 07 required diet is suitable to preclude contamination that might interfere with the outcome of the study and tap water analysis records are on file with the Division of Animal Resources. Animals will be housed in the Sanger Room B2-035, where the temperature will be maintained at 18-26°C and the relative humidity between 40 and 70%. The light/dark cycle will be maintained on 12-hour intervals. Rat pans will be cleaned and sanitized twice per week.

EXPERIMENTAL DESIGN: Following randomization, animals will be assigned to one of the following treatment groups. The treatment groups and their designations are shown in the table below.

Group	Treatment	Dose (ml/kg)	Designation
1	Vehicle	0	VH
2	D4	10	D1
3	D4	30	D2
4	D4	100	D3
5	D4	300	D4
6	PC-1	25 mg/kg	PC1
7	PC-2	Anti Asialo GM1 1:10	PC2

The vehicle and experimental groups will consist of 10 animals and the positive control groups will consist of 6 animals.

PARAMETERS TO BE MONITORED:

1. General

   a. Body Weight. Animals will be weighed twenty-four hours after arrival, and prior to treatment, day 8, 15, 22 of the treatment period and on the day of sacrifice (SOP/TOX/002).

   b. Observations. Animals will be observed at the time of dosing for any pharmacotoxicological signs (SOP/TOX/028). Any remarkable effects will be recorded on the study log sheet, present in the exposure room, in accordance with SOP/TOX/028.

   c. Food consumption will be measured two times a week.

   d. Terminal body weights and organ weights (liver, spleen and thymus) from the RES study and one other study not injected with s-RBC's.

   e. Hematology (erythrocytes, leukocytes, hematocrit, hemoglobin, MCV, MCH, MCHC, and reticulocytes) (SOP/HEM/004, 006, 010, 012, 038)

f. Leukocyte differentials (SOP/HEM/014)

2. Immunology Section

a. Spleen IgM antibody response to a T-dependent antigen, sheep erythrocytes sRBC)(day 4 response) (SOP/PFC/006, 012). Serum antibody levels to sRBC will be measured in the same animals (SOP/ELA/008).

As background, sheep erythrocytes (sRBC) are a T-dependent antigen and thus, T cells, B cells and macrophages are required to function properly in order to obtain an antibody forming cell (AFC) response. If the test article affects any of these cell types to a significant degree, an altered response will be observed. As a result, the T-dependent IgM response to sRBC is one of the most sensitive immunotoxicological assays currently in use. A significant modulation in the IgM AFC response, when appropriately compared to untreated controls, indicates that the test agent is capable of modifying the humoral immune response in the whole animal and, thus, has the potential for immunotoxicity. This assay is one of the Tier I assays used by the NTP[1].

The primary IgM response to sheep erythrocytes will be measured using a modified hemolytic plaque assay of Jerne[2]. Rats will be exposed to the test article for 28 days and on day 25 they will be injected with sheep erythrocytes. On day 29 (peak day of IgM response), animals will be sacrificed, spleen cells will be prepared by mincing the spleen between two frosted microscope slides and diluted to a final volume of 6 ml. An aliquot of cells will be added to a test tube containing guinea pig complement, sRBC and warm agar. After thoroughly mixing, the test tube mixture will be plated in a petri dish, covered with a microscope cover slip, and incubated at 37°C for 3 hours. Cell counts will be performed on the 6 ml sample and the number of cells/spleen, AFC/spleen, and AFC/10^6 spleen cells determined. The plaques which developed will be counted using a Bellco plaque viewer. A plaque results from the lysis of sRBC and is elicited as a result of the interaction of complement and antibodies directed against sheep erythrocytes which are produced in response to the intravenous sensitization. Each plaque is generated from a single IgM antibody-producing B cell and, thus, the number of antibody forming cells (AFC) present in the whole spleen can be calculated. The data will be expressed as specific activity (AFC/10^6 spleen cells) and AFC/spleen.

b. Mixed leukocyte response (SOP/MLR/006)

The mixed leukocyte response (MLR) has proven to be a sensitive assay for detecting effects on cell-mediated immunity. This may be due to the complex nature of the MLR, in which recognition of the allogeneic cells and proliferation are necessary. While this assay is well established and reproducible in the mouse, at times, the same assay in the rat does not always work; that is, the responder cells do not proliferate. When this occurs, the assay in invalidated. A possible reason that this assay does not work every time is due an increased number of suppresser cells in the rat. Several other cell-mediated immunity assays are in developmental stages. A significant change in the MLR, when appropriately compared to untreated controls, indicates that the test agent is capable of modulating recognition and/or proliferation in response to stimulation with allogeneic cells. Agents which produce such effects have the potential for modifying the immune system.

The MLR is an *in vitro* assay which analyzes the ability of T cells from exposed rats to recognize allogeneic cells as "non-self" and proliferate in response to the presence of the allogeneic cells. Female Fischer rats are exposed *in vivo* to the test article for 28 days. On day 29, the rats will be sacrificed and spleen and peripheral blood cells will be prepared under sterile conditions. Spleens are minced between two frosted microscope slides. Peripheral blood lymphocytes are isolated by hypotonic shock of erythrocytes. Spleen or blood lymphocytes are counted and adjusted to $1x10^6$/ml. Spleen cells are added to each well of a U-bottom microtiter plate at a concentration of $1x10^5$ cells per well. Wistar or Long Evans spleen cells are used as the allogeneic cell (stimulator cell) for the Fischer (responder) rats. Stimulator cells are treated with Mitomycin C to render them unable to proliferate and the ratio of stimulators to responders has previously been optimized at 2:1. The cells are cultured for 5 days, during the last 18 hours in the presence of 1 µCi ^{3}H-thymidine. The cells are collected with a cell harvester and counted in an LKB liquid scintillation counter; data are collected directly onto computer diskettes for analysis. The incorporation of ^{3}H-thymidine into the proliferating cells is used as the endpoint of the assay, and the data are expressed as cpm/culture. The data are also expressed as a stimulation index which is the cpm of the responder cells in the presence of the stimulator cells, divided by the cpm of the responder cells in the absence of the stimulator cells.

c. Natural killer assay. (SOP/CYT/010)

Natural killer cells are large granular lymphocytes which are shown to possess anti-tumor activity without prior acquisition of information. That is, natural killer cell activity is an innate activity which requires no prior exposure to the pathogen. This is in contrast to the acquired immune response which requires sensitization and time to develop the ability to bring about the desired action. In the last few years, the natural killer cells have also been shown to possess activity against virally infected cells as well as have activity against other pathogens. The natural killer cells reside in the lymphoid tissue and also in the lung and liver. The origin of the natural killer cells is believed to be the bone marrow. Decrease in natural killer cell activity as a result of drug treatment is considered an adverse effect.

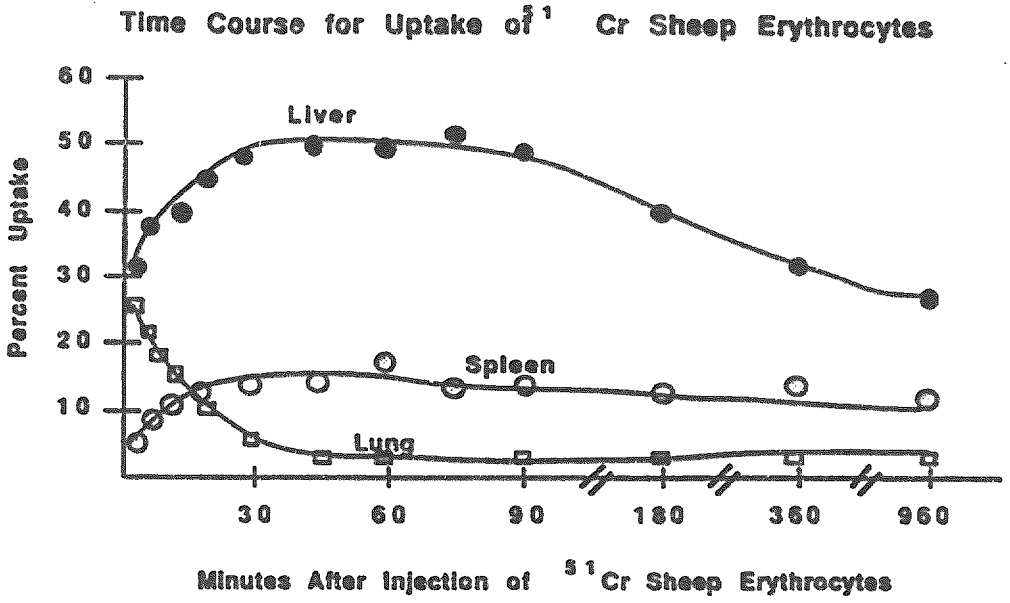
Rats will be exposed to the test article for 28 days. On day 29, spleens and peripheral blood will be obtained as describe above. Following centrifugation, the pellet will be resuspended in RPMI (Roswell Park Memorial Institute) 1640 medium, supplemented with 10% fetal calf serum. The cell suspension will be adjusted to three concentrations, 10^7, $5x10^6$, and $2.5x10^6$ cells/ml, to obtain effector-to-target ratios of 100:1, 50:1, and 25:1. The natural killer cell will be the effector cell and the YAC-1 will be the target cell. The YAC-1 cells were obtained from American Type Culture Collection (ATCC TIB 160). YAC-1 is a rat lymphoma which was introduced by inoculation of the Moloney leukemia virus (MLV) into a newborn A/Sn rat. This cell line is sensitive to the cytotoxic activity of "naturally" occurring killer cells in rats (natural killer cell). Therefore, YAC-1 cells are often used as target cells in natural killer assays. The YAC-1 cells which are maintained in a stock culture at MCV/VCU, will be adjusted to a concentration of 10^7 cells/ml. These cells will be labeled by incubation with 1000 µCi of ^{51}Cr in a volume of 1.0 ml for 90 minutes in a 37°C water bath with frequent agitation. Following the incubation, the cells will be washed three times in balanced salt solution (BSS) plus 25mM HEPES, counted, and adjusted to 10^5 nucleated cells/ml. The maximum release will be determined by adding 0.1 ml of the 10^5 YAC-1 cells/ml and 0.1 ml 10% Triton X to each of 12 replicate wells on a 96-well microtiter plate. The targets will be 0.1 ml of the 10^5 YAC-1 cells/ml added to each well. The effector

cells will be added (0.1 ml) to each of four replicate wells of target cells at each effector concentration. The spontaneous release will be determined by adding 0.1 ml of medium to 12 replicate cultures containing the targets. The plates will be incubated for 4 hours at 37°C at 5% CO_2. Following the incubation, the plates will be centrifuged at 250 x g for 10 minutes, and 0.1 ml of the supernatant will be removed from each well and counted. The mean (± SE) percent cytotoxicity at each effector concentration will be determined for each treatment group and compared to the respective values for the vehicle rats.

d. Clearance of sRBC by reticuloendothelial system (SOP/RES/004)

As background, the fixed macrophages of reticuloendothelial system provide a first line of defense for blood born particulates including pathogens and non-pathogens. These macrophages line the liver endothelium (Kupffer cells), the spleen, the lymph nodes (reticular cells), the lung (interstitial macrophages), and other organs such as the thymus and bone marrow. One of the main tasks of this system is to maintain a sterile blood supply and to initiate the acquired immune response. The cells of the reticuloendothelial system are also involved in removing and reprocessing old erythrocytes, leukocytes, and platelets. They have also been shown to be involved in metabolism of substances such as steroids. A decreased function of the fixed cells of the reticuloendothelial system can decrease host resistance to microbes. Thus, decreases in reticuloendothelial function related to drug treatment represent an adverse effect.

The assay will be performed as described by Munson et al[9]. Rats will be treated with the test article on day 1-28 and on day 29 the functional activity of the fixed macrophages will be evaluated. The activity of the fixed macrophages of the reticuloendothelial system will be determined by injecting 5 µl/gram body weight of ^{51}Cr-labeled sheep erythrocytes. The concentration of ^{51}Cr-labeled sheep erythrocytes will be 20% hematocrit which is equal to approximately $5x10^9$ sheep erythrocytes/ml. The clearance of the labeled sheep erythrocytes from the blood will be determined over the first 30 minutes by taking 10 µl of blood at 3, 6, 9, 12, 15, and 30 minutes after ^{51}Cr-labeled sheep erythrocytes injection. The radioactivity in the blood will be used to calculate the phagocytic index and determine the half-life. After 60 minutes, the animals will be sacrificed by carbon dioxide and decapitation, and the distribution of the labeled sheep erythrocytes into the major organs of the reticuloendothelial system will be determined. Uptake into the liver, spleen, lungs, thymus and kidneys will be determined by radioassay and the data will be expressed as percent uptake in the total organ and per mg. Organ distribution (phagocytosis), as a function of time after the injections of ^{51}Cr-labeled sheep erythrocytes, is different for the different organs. The Figure below illustrates the time course distribution. There is a rapid uptake of ^{51}Cr-labeled sheep erythrocytes into the liver and spleen with a plateau occurring in 30 minutes. Distribution into the lung is more complex. The first phase of localization into the lung is the accumulation of ^{51}Cr-labeled sheep erythrocytes based on first pass action, i.e. the vascular bed of the lung is the first site which sees the bolus of cells. Thus, there is an early large accumulation, up to 30%, of ^{51}Cr-labeled sheep erythrocytes, followed by a clearance from the lung over the first 30 minutes after injections. The actual phagocytosis of ^{51}Cr-labeled sheep erythrocytes of the lung macrophages is less than 1% which can be measured 60 minutes after injection.



e. Splenocyte Phenotyping (SOP/CST/012,014)

The number of T and B lymphocytes in the spleens and peripheral blood of treated rats provides important data for the interpretation of functional studies. The methodology for analyzing B and T cell populations and T cell subsets ($CD4^+$ and $CD8^+$) includes the use of a Becton Dickinson FACScan Flow Cytometer, monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE), and the DNA specific fluorescent stain propidium iodide (PI). This assay is one of the Tier II assays used by the NTP[1].

In addition, the number of Natural Killer Cells (CD56) will be enumerated. The monoclonal antibody for the Natural Killer cell in the rat has not been extensively used and validation among laboratories has not been done. However, CD 56 cells will be assessed in human studies and comparison to NK cells in the rat is of value.

Rats will be exposed to the test article for 28 days. On day 29, spleens will be teased between two microscope slides into single cell preparations and seeded at 1×10^6 cells per well into a 96 well microtiter plate. Analysis of each spleen preparation will require 4 wells. Each well will contain spleen cells and 100 µl of the appropriate monoclonal antibody conjugated to FITC or PE. The specific monoclonal antibodies are: W3/13 conjugated to FITC to enumerate T cells, W3/25 conjugated to FITC to enumerate CD4+ cells, OX8 conjugated to PE to enumerate CD8+ cells and OX4 conjugated to FITC to enumerate B cells. Following the initial staining with antibody and washing with staining buffer, 100 µl of a PI solution will be added to each well as a viability stain.

Acquisition of Data: The cell preparation in the control well is run on the flow cytometer and viewed as a dot plot of forward scatter (FSC), on the X axis, and side scatter (90 degree; SSC), on the Y axis in the linear mode. The majority of the red cell population and debris is eliminated electronically by increasing the threshold setting on FSC. Non-viable cells are eliminated by setting a live gate on FL3 (red

fluorescence which in this case is PI). Data for all samples are then acquired in list mode with no other gating. Photomultiplier tubes (PMT) for fluorescence operate in the log mode. Cells stained with FITC are detected by the FL1 PMT (green fluorescence) and cells stained with PE are detected by the FL2 PMT (orange fluorescence). Five thousand cells are counted for each sample.

Analysis of Data: Following acquisition the list data for each sample are recalled from the computer memory. The remainder of debris and red cells is eliminated from analysis by electronic gating of a FSC by SSC dot plot. The percentage of T cells and B cells in the gated population is calculated from histograms of fluorescence intensity vs cell number.

STATISTICAL ANALYSES: The data obtained in this study will be analyzed in accordance with standard operating procedure SOP/CSA/006. Data will be first tested for homogeneity of variances using the Bartlett's Chi Square Test[3]. Homogeneous data will be evaluated by a parametric one-way analysis of variance[4]. When significant differences occur, the treatment groups will be compared to the vehicle control group using the Dunnett's t Test[5]. Non-homogeneous data will be evaluated using a non-parametric analysis of variance[4]. When significant differences occur, the treatment groups will be compared to vehicle control using the Gehan-Wilcoxon Test[6] when appropriate. The Jonckheere's Test[7] will be used to test for dose related trend across the vehicle and treatment groups. The positive control data will be evaluated using the Student's t Test[8] and compared to the vehicle control.

REPORT: The report will contain all procedures and/or references to published procedures used during the study. Methods used for statistical analysis will also be included.

DATA AND RECORD RETENTION: All data and records pertaining to this study will be submitted to the sponsor at the time of the final report. Upon completion of this study, the report and raw data for this study will be maintained in the archives of Dow Corning Corporation, Midland, Michigan. Records to be maintained for this protocol will include:

Study Sheets
Log Sheets
Room Activity Sheets
Necropsy Forms(if needed)
Preparation and Handling of Test Article Records
Food Lot Records
Animal Quarantine Report Forms
Authorized Signatures and Initial Forms

PERSONNEL: Curriculum vitae for the key personnel involved in this study will become part of the permanent records for this study.

SAFETY: The appropriate safety precautions for the use of chemicals are described in the Chemical Waste Management Program and in the guidelines established by MCV/VCU's Chemical Safety Committee.

REFERENCES:

1. Luster, M.I., A.E. Munson, P. Thomas, M.P. Holsapple, J. Fenters, K.L. White, Jr., L.D. Lauer, and J.H. Dean. 1988. Development of a testing battery to assess chemical-induced immunotoxicity. *Fund. Appl. Toxicol. 10*:2-19.

2. Jerne, N.K., C. Henry, A.A. Nordin, H. Fun, M.C. Koros, and I. Lefkovits. 1974. Plague forming cells: Methodology and theory. *Transpl. Rev. 18*:130-191.

3. Bartlett, M.S. 1937. Sub-sampling for attributes. *J. Roy. Stat. Soc. Suppl.* 4:131-135.

4. Kruskal, W.H., and W.A. Wallis. 1952. Use of ranks in one-criterion variance analysis. *J. Amer. Stat. Assoc. 47*:583-621.

5. Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Stat. Assoc. 50*:1096-1121.

6. Gross, A.J., and V.A. Clark. 1975. Gehan-Wilcoxon Test. In *Survival Distribution: Reliability Applications in Biomedical Sciences*. A.J. Gross and V.A. Clark, eds. John Wiley and Sons, New York, P.120-123.

7. Hollander, M. and D.A. Wolfe, 1975. Jonckheere's Test:Non-Parametric Statistics Methods, eds M. Hollander and D.A. Wolfe, John Wiley and Sons, New York, P.124-129.

8. Sokal, R.R. and F.J. Rohlf, 1981 *Biometry*. Freeman, San Francisco, P.226-231.

9. White, K.L.,Jr., Krasula, R.W., Munson, A.E., and Holsapple, M.P. 1986 Effects of hydroxyethylstarch, (HespanR) a plasma expander, on the fucntional activity of the reticuloendothelial system.*Drug Chem. Toxicol.* 9:305-322.

APPENDIX B

PROTOCOL AMENDMENTS

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRA-SILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 1

Amendment: Change of Date for Proposed Date of Completion and Proposed Date of Report.

Protocol states:

PROPOSED DATE OF COMPLETION: August 15, 1994

PROPOSED DATE OF PROTOCOL REPORT: September 25, 1994

Amended to:

PROPOSED DATE OF COMPLETION: August 15, 1995

PROPOSED DATE OF PROTOCOL REPORT: September 25, 1995

Reason for Addition: An error was made in the year for the these dates. The year should have been 1995 instead of 1994.

Albert E Munson — May 16, 1995
Albert E. Munson, Ph.D. — Date
Study Director

Paal C Klykken — 5/19/95
Paal C. Klykken, Ph.D — Date
Sponsor's Representative

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 2

Amendment: Comparison of Vehicles for D4 in Female Rat Gavage Studies

Study Number: This study will be referred to as Study 7 in the Protocol D4-28-1R-PO.

Reason for Addition: There is a question of relevance for the use of corn oil as a vehicle for D4 in the toxicology studies where the route of administration is by gavage. Corn oil is not the vehicle in which D4 is commercially used. Thus, this amendment to the protocol provides for comparison of 4 vehicles. The vehicles will be a) Corn Oil b) Maalox, c) Emulphor, and d) 35 Centistoke PDMS Fluid. The experimental groups are shown in the table below. The animal identification numbers are presented in each cell of the table.

Experimental Design

Dose D4 mg/kg	Corn Oil Animal ID	Maalox Animal ID	Emulphor Animal ID	PDMS Fluid Animal ID
0	349 - 358	419 - 428	489 - 498	559 - 568
1	359 - 368	429 - 438	499 - 508	569 - 578
3	369 - 378	439 - 448	509 - 518	579 - 588
10	379 - 388	449 - 458	519 - 528	589 - 598
30	389 - 398	459 - 468	529 - 538	599 - 608
100	399 - 408	469 - 478	539 - 548	609 - 618
300	409 - 418	479 - 488	549 - 558	619 - 628
Naive	629 - 638			

NUMBER OF ANIMALS: Two hundred ninety female Fischer 344 rats will be required for this study. Three hundred animals will be ordered. After randomization to group and assurance of homogeneity, 10 animals will be maintained as naive (untreated) to compare the potential effects of the vehicle on liver weights. Ten animals will be used per group. Ten animals per group should be sufficient to determine a no effect level.

EXPOSURE: Rats will be gavaged daily for 28 consecutive days. Rats will be weighed weekly. On day 29, the rats will be weighed and sacrificed with carbon dioxide. The livers will be excised, cleared of connective tissue and weighed. Liver weights will be presented as total weight and as a percent of body weight.

VEHICLE #1: Corn Oil will be used as the vehicle #1. Mazola Corn Oil will be obtained from a grocery store and one lot used for this study. Corn Oil control animals will receive

0.05 ml/10 grams of body weight by gavage. Vehicle animals will be dosed at the same time as the treatment groups.

VEHICLE #2: Maalox Therapeutic Concentrate (High Potency Antacid) will be used as Vehicle #2. Maalox will be purchased from a pharmacy in 335 ml bottles. Maalox control animals will receive 0.05 ml/10 grams of body weight by gavage. Vehicle animals will be dosed at the same time as the treatment groups.

VEHICLE #3: Emulphor EL 620 will be used as Vehicle #3. Emulphor (CAS 00000075-21-8) was a gift of Rhone-Poulenc, Inc. Personal Care, 1101 Pittman St. West Point, GA. Emulphor will be prepared as a 10% solution in distilled water. Vehicle animals will be dosed at the same time as the treatment groups. Emulphor control animals will receive 0.05 ml/10 grams of body weight by gavage.

VEHICLE #4: PDMS 35 Centistoke Fluid will be used as Vehicle #4. PDMS Fluid will be provided Dow Corning Incorporated. The Lot # is LL099608, the Product # is X9-6370 and the CAS 63148-62-9. Expiration Date: To be provided by Dow Corning Incorporated. PDMS 35 Centistoke fluid will be use in the native state. Vehicle animals will be dosed at the same time as the treatment groups. PDMS fluid control animals will receive 0.05 ml/10 grams of body weight by gavage.

Assurance of Concentration and Stability of D4 in Vehicles. On Day 1 and 15 of the experimental period, 50 ml of D4 at each concentration in each vehicle will be prepared and submitted to Dow Corning Corporation for determination of D4 concentration.

PROPOSED IN LIFE START DATE: To Be Determined

PROPOSED IN LIFE DATE OF COMPLETION: To Be Determined

STATISTICAL ANALYSIS: Dose response will be analyzed using a linear regression model. Test for parallelism of the dose response curve, estimation of the ED50 for liver weight and no effect levels will be determined.

Albert E. Munson, Ph.D.
Study Director
May 16, 1995
Date

Paal C. Klykken, Ph.D.
Sponsor's Representative
5/19/95
Date

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 3

Amendment: Comparison of Vehicles for D4 in Male Rat Gavage Studies

Study Number: This will be referred to as Study 8 in the Protocol D4-28-1R-PO.

Reason for Addition: There is a question of relevance for the use of corn oil as a vehicle for D4 in the toxicology studies where the route of administration is by gavage. Corn oil is not the vehicle in which D4 is commercially used. Thus, this amendment to the protocol provides for comparison of 4 vehicles. The vehicles will be a) Corn Oil b) Maalox, c) Emulphor, and d) 35 Centistoke PDMS Fluid. The experimental groups are shown in the table below. The animal identification numbers are presented in each cell of the table.

Experimental Design

Dose D4 mg/kg	Corn Oil Animal ID	Maalox Animal ID	Emulphor Animal ID	PDMS Fluid Animal ID
0	639 - 648	709 - 718	779 - 788	849 - 858
1	649 - 658	719 - 728	789 - 798	859 - 868
3	659 - 668	729 - 738	799 - 808	869 - 878
10	669 - 678	739 - 748	809 - 818	879 - 888
30	679 - 688	749 - 758	819 - 828	889 - 898
100	689 - 698	759 - 768	829 - 838	899 - 908
300	699 - 708	769 - 778	839 - 848	909 - 918
Naive	919 - 928			

NUMBER OF ANIMALS: Two hundred ninety male Fischer 344 rats will be required for this study. Three hundred animals will be ordered. After randomization to group and assurance of homogeneity, 10 animals will be maintained as naive (untreated) to compare the potential effects of the vehicle on liver weights. Ten animals will be used per group. Ten animals per group should be sufficient to determine a no effect level.

EXPOSURE: Rats will be gavaged daily for 28 consecutive days. Rats will be weighed weekly. On day 29, the rats will be weighed and sacrificed with carbon dioxide. The livers will be excised, cleared of connective tissue and weighed. Liver weights will be presented as total weight and as a percent of body weight.

VEHICLE #1: Corn Oil will be used as the vehicle #1. Mazola Corn Oil will be obtained from a grocery store and one lot used for this study. Corn Oil control animals will

receive 0.05 ml/10 grams of body weight by gavage. Vehicle animals will be dosed at the same time as the treatment groups.

VEHICLE #2: Maalox Therapeutic Concentrate (High Potency Antacid) will be used as Vehicle #2. Maalox will be purchased from a pharmacy in 335 ml bottles. Maalox control animals will receive 0.05 ml/10 grams of body weight by gavage and will be dosed at the same time as the treatment groups.

VEHICLE #3: Emulphor EL 620 will be used as Vehicle #3. Emulphor (CAS 00000075-21-8) was a gift of Rhone-Poulenc, Inc. Personal Care 1101 Pittman St. West Point, GA. Emulphor will be prepared as a 10% solution in distilled water. Emulphor control animals will receive 0.05 ml/10 grams of body weight by gavage and will be dosed at the same time as the treatment groups.

VEHICLE #4: PDMS 35 Centistoke Fluid will be used as Vehicle #4. PDMS Fluid will be provided Dow Corning Incorporated. The Lot # is LL099608, the Product # is X9-6370 and the CAS 63148-62-9. Expiration Date: To be provided by Dow Corning Incorporated. PDMS 35 Centistoke fluid will be use in the native state. PDMS fluid control animals will receive 0.05 ml/10 grams of body weight by gavage and will be dosed at the same time as the treatment groups.

Assurance of Concentration and Stability of D4 in Vehicles. On Day 1 and 15 of the experimental period, 50 ml of D4 at each concentration in each vehicle will be prepared and submitted to Dow Corning Corporation for determination of D4 concentration.

PROPOSED IN LIFE START DATE: To Be Determined

PROPOSED IN LIFE DATE OF COMPLETION: To Be Determined

STATISTICAL ANALYSIS: Dose response will be analyzed using a linear regression model. Test for parallelism of the dose response curve, estimation of the ED50 for liver weight and no effect levels will be determined.

Albert E. Munson, Ph.D. — May 16, 1995
Study Director — Date

Paal C. Klykken, Ph.D. — 5/19/95
Sponsor's Representative — Date

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 4

Amendment:

1) Repeat of Study #1 (T-dependent Antibody response to the antigen, s-RBC) using the spleen Antibody Forming Cell (AFC), serum ELISA Antibody titer and liver weights in female rats.

2) Perform a 14 recovery study on female rats administered D4 in corn oil. The variables to be monitored in the recovery study will be spleen AFC, the ELISA antibody titer, spleen and liver weights.

Study Number: This study will be referred to as Study 9 in the Protocol D4-28-1R-PO.

Reason for Change: The results of Study #1 (females) showed a dose dependent decrease in the serum ELISA titer to s-RBC with statistically significant suppression at the 100 and 300 mg/kg doses. This suppression was not seen in study 3 with the male rats. These results need to be confirmed or refuted in a repeat study and a recovery study added.

The general experimental design is shown in the table below.

Group	Treatment	Dose (mg/kg)	n	Designation	Parameter 1 Monitored	Parameter 2 Monitored
1	Vehicle	0	10	VH	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
2	D4	10	10	G1	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
3	D4	30	10	G2	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
4	D4	100	10	G3	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
5	D4	300	10	G4	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
6	PC-1	25 mg/kg CPS	6	PC1	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
7	Vehicle Recovery	0	10	VH	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
8	D4 Recovery	300	10	G4	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt
9	PC-1	25 mg/kg CPS	6	PC1	AFC, Serum ELISA AB Titer	Liver Wgt Spleen Wgt

CPS = Cyclophosphamide n = number of animals

Albert E. Munson, Ph.D.
Study Director
May 16, 1995
Date

Paal C. Klykken, Ph.D.
Sponsor's Representative
5/19/95
Date

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLO-TETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 5

Amendment:

1) Repeat of Study #4: Female Fischer 344 rats: D4 RES Function and Organ Weights (SOP/RES/004).

2) Perform a 14 day recovery study on female rats administered D4 in corn oil. The parameters to be monitored in the recover study will be RES function and organ weights.

Study Number: This will be referred to as Study 10 in the Protocol D4-28-1R-PO.

Reason for Change: The changes in the spleen phagocytosis and vascular clearance rates need to be confirmed or refuted. In addition, 4 of the control animals died at the time of assay due to a malfunction of a heating pad. Although the other 6 control animals may be sufficient for interpretation, the increase in spleen phagocytosis with a concomitant decrease in vascular clearance is difficult to reconcile. Thus, a repeat study is warranted.

Experimental Design

Group	Treatment	Dose (mg/kg)	n	Designation	Parameter 1 Monitored	Parameter 2 Monitored
1	Vehicle	0	10	VH	RES	Organ Wgts
2	D4	10	10	G1	RES	Organ Wgts
3	D4	30	10	G2	RES	Organ Wgts
4	D4	100	10	G3	RES	Organ Wgts
5	D4	300	10	G4	RES	Organ Wgts
6	PC-3	50 mg/kg MVE	6	PC3	RES	Organ Wgts
7	Vehicle Recovery	0	10	VH	RES	Organ Wgts
8	D4 Recovery	300	10	G4	RES	Organ Wgts
9	PC-3 Recovery	50 mg/kg MVE	6	PC3	RES	Organ Wgts

MVE = maleic vinyl ether n= number of rats per group

Albert E. Munson, Ph.D.
Study Director

May 16, 1995
Date

Paal C. Klykken, Ph.D
Sponsor's Representative

5/19/95
Date

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLO-TETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 6

Amendment:

1) Repeat of Study #2: Male Fischer 344 rats: D4 RES Function and Organ Weights (SOP/RES/004).

2) Perform a 14 day recovery study on male rats administered D4 in corn oil. The parameters to be monitored in the recover study will be RES function and organ weights.

Study Number: This will be referred to as Study 11 in the Protocol D4-28-1R-PO.

Reason for Change: A repeat on the RES assay for males rats will be performed because of the different results seen in the sexes as it relates to vascular clearance rates. The vascular clearance rates in females were decreased dose dependently and slightly increased in the males. The recovery study is being performed to determine reversal of the splenic phagocytosis suppression.

Experimental Design

Group	Treatment	Dose (mg/kg)	n	Designation	Parameter 1 Monitored	Parameter 2 Monitored
1	Vehicle	0	10	VH	RES	Organ Wgts
2	D4	10	10	G1	RES	Organ Wgts
3	D4	30	10	G2	RES	Organ Wgts
4	D4	100	10	G3	RES	Organ Wgts
5	D4	300	10	G4	RES	Organ Wgts
6	PC-3	25 mg/kg MVE	6	PC3	RES	Organ Wgts
7	Vehicle Recovery	0	10	VH	RES	Organ Wgts
8	D4 Recovery	300	10	G4	RES	Organ Wgts
9	PC-3 Recovery	25 mg/kg MVE	6	PC3	RES	Organ Wgts

MVE = maleic vinyl ether n= number of rats per group

Albert E. Munson May 16, 1995

Albert E. Munson, Ph.D. Date
Study Director

Paal C. Klykken 5/19/95

Paal C. Klykken, Ph.D Date
Sponsor's Representative

Protocol Amendment

Date of Amendment: May 16, 1995

Title of Protocol: IMMUNOLOGICAL EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) USING A TWENTY-EIGHT DAY EXPOSURE IN MALE AND FEMALE FISCHER 344 RATS

Protocol Identifier: D4-28-1R-PO

Amendment Number: 7

Amendment:

1) Partial repeat of Study #5: Female Fischer 344 rats: (Lymphocyte Phenotyping of Spleen and Blood; Hematology).

2) Perform a 14 recovery study on female rats administered D4 in corn oil. The parameters to be monitored in the recover study will be spleen and blood phenotyping, hematology and liver weights.

Study Number: This study will be referred to as Study 12 in the Protocol D4-28-1R-PO.

Reason for Change: The results of Study #5 showed a dose dependent decrease in the total number and percent of spleen CD4 (T Helper) and CD8 (T cytotoxic) lymphocytes. This decrease was not seen for the peripheral blood. These results need to be confirmed or refuted in a repeat study. Hematology studies are being repeated because the reticulocyte counts were not performed in Study 5.

The general experimental design is shown in the table below.

Group	Treatment	Dose (mg/kg)	n	Designation	Parameter 1 Monitored	Parameter 2 Monitored	Parameter 3 Monitored
1	Vehicle	0	10	VH	Phenotyping	Hematology	Liver Wgt
2	D4	10	10	G1	Phenotyping	Hematology	Liver Wgt
3	D4	30	10	G2	Phenotyping	Hematology	Liver Wgt
4	D4	100	10	G3	Phenotyping	Hematology	Liver Wgt
5	D4	300	10	G4	Phenotyping	Hematology	Liver Wgt
6	PC-1	25 mg/kg CPS	6	PC1	Phenotyping	Hematology	
7	Vehicle Recovery	0	10	VH	Phenotyping	Hematology	Liver Wgt
8	D4 Recovery	300	10	G4	Phenotyping	Hematology	Liver Wgt
9	PC-1 Recovery	25 mg/kg CPS	6	PC1	Phenotyping	Hematology	Liver Wgt

CPS = Cyclophosphamide n = number animals

Albert E. Munson, Ph.D.
Study Director
May 16, 1995
Date

Paal C. Klykken, Ph.D.
Sponsor's Representative
5/19/95
Date

APPENDIX C

PROTOCOL DEVIATIONS

Deviation Number: 1

Protocol Number: D4-28-1R-PO

Date of Deviation: June 2, 1994

Protocol Deviation: The description of a positive control article was omitted from the protocol. The following paragraph describes the positive control used for the reticuloendothelial system.

POSITIVE CONTROL Number PC3: MVE (XA 124-188) will be used as a positive control for modulation of the functional activity of reticuloendothelial system (RES). MVE will be prepared in phosphate buffered saline in a concentration of 10.0 mg/ml at a pH between 6.8-7.2. MVE will be injected intravenously 24 hours prior to evaluation of the RES. This dose will markedly suppress hepatic phagocytic function which will be manifested in a decrease in blood clearance and a decrease in liver uptake of the test particle (sheep erythrocytes). MVE will not be analyzed for concentration. A blockade of the RES will be used as an indicator of adequate dose of the drug.

Acknowledgment of Protocol Deviation

Albert E Munson — 6/2/94

A. E. Munson, Ph.D.
Study Director

Date

Deviation Number: 2

Protocol Number: D4-28-1R-PO

Date of Deviation: May 3, 1994

Protocol Deviation: Study 1-week 2 has a questionable data value. The delta weight of the intermediate bottle, value 6.86, is not congruent with the other weeks. It appears that one day 1.26 mls may not have been removed during the first week. All log records indicate that the rats were administered the appropriate amounts of D4 daily for 28 days.

Acknowledgment of Protocol Deviation

Albert E Munson 5/3/94

A. E. Munson, Ph.D.
Study Director

Date

Deviation Number: 3

Protocol Number: D4-28-1R-PO

Date of Deviation: December 3, 1994

Protocol Deviation: The natural killer assay in study 5 using the peripheral blood samples will not be reported. There was an insufficient cell number for many samples which gave inconsistent results. This assay could be addressed in a repeat study.

Acknowledgment of Protocol Deviation

Robert E Munson

A. E. Munson, Ph.D.
Study Director

12/3/94
Date

Date of Deviation: May 17, 1994

Deviation Number: 4

Protocol Number: D4-28-1R-PO

Protocol Deviation: Liver weights were obtained from all the D4-28-1R-PO studies as a consistent parameter to measure from each studies.

Acknowledgment of Protocol Deviation

Robert E Munson 5/30/94

A. E. Munson, Ph.D. Date
Study Director

Date of Deviation: May 5, 1995

Deviation Number: 5

Protocol Number: D4-28-1R-PO

Protocol Deviation: Additional D4 was added to the same intermediate bottle for studies 2-6. This activity was pointed out by the Quality Assurance site visitors to be inappropriate. The procedure did not appear to preclude contamination of the test article.

Acknowledgment of Protocol Deviation

Albert E Munson — 5/25/95

A. E. Munson, Ph.D.
Study Director

Date

Deviation Number: 6

Protocol Number: D4-28-1R-PO

Date of Deviation: February 28, 1995

Protocol Deviation: During studies 1-6, the humidity and temperature in the animal room (B2-035A) were observed to be outside the range of (40-70%) and (64.4-78.8 °F) indicated in the protocol. The dates and the recorded values are indicated below.

Date	Humidity (%)	Temp (°F)
April 22, 1994	72%	
April 25, 1994	84 %	
April 26, 1994	80 %	
April 27, 1994	90%	
April 28, 1994	96%	
April 29, 1994	94%	
April 30, 1994	90%	
May 1, 1994	80%	
May 6, 1994	100%	
May 7, 1994	76%	
May 10, 1994	82%	
May 12, 1994	76%	
May 13, 1994	76%	
May 14, 1994	72%	
May 15, 1994	79%	
May 16, 1994	100%	
June 2,1994	100%	
June 3,1994	88%	
June 4,1994	88%	
June 5,1994	100%	
June 7,1994	92%	
June 8,1994	90%	
June 9,1994	74%	
June 10,1994	100%	
June 11,1994	78%	
June 12,1994	89%	
June 13,1994	100%	
June 14,1994	94%	
June 15,1994	88%	
June 16,1994	100%	80°
June 19,1994	78%	
June 20,1994	80%	
June 21,1994	78%	
June 22,1994	80%	
June 23,1994	82%	
June 24,1994	80%	
June 25,1994	78%	
June 26,1994	72%	
June 27,1994	78%	
June 29,1994	90%	

Date	Humidity (%)	Temp (°F)
June 30,1994	72%	
July 12,1994	80%	
July 13,1994	84%	
July 14,1994	80%	
July 15,1994	80%	
July 16,1994	84%	
July 17,1994	80%	
July 18,1994	82%	
July 19,1994	84%	
July 20,1994	100%	
July 21,1994	84%	
July 22,1994	82%	
July 23,1994	82%	
July 24,1994	92%	
July 25,1994	100%	
July 26,1994	72%	
July 27,1994	74%	
July 28,1994	80%	
July 29,1994	72%	
July 31,1994	80%	
August 1,1994	80%	
August 2,1994	76%	
August 3,1994	76%	
August 4,1994	78%	
October 11, 1994	38%	
October 17, 1994	37%	
November 3, 1994	36%	
November 7, 1994	36%	
November 8, 1994	34%	
November 9, 1994	30%	
November 11, 1994	30%	
November 12, 1994	28%	
November 13, 1994	32%	
November 19, 1994	36%	
November 23, 1994	24%	
November 24, 1994	25%	
November 25, 1994	25%	
November 26, 1994	28%	
November 27, 1994	30%	
November 29, 1994	34%	

Date	Humidity (%)	Temp (°F)	Date	Humidity (%)	Temp (°F)
November 30, 1994	36%		February 8, 1995	36%	
February 1, 1995	22%		February 9, 1995	34%	
February 2, 1995	26%		February 10, 1995	36%	
February 3, 1995	22%		February 13, 1995	38%	
February 4, 1995	25%		February 14, 1995	37%	
February 5, 1995	18%		February 15, 1995	40%	
February 6, 1995	32%		February 17, 1995	38%	
February 7, 1995	33%		February 28, 1995	73%	

It is the opinion of the Study Director that the differences in humidity and slight difference in temperature did not affect the results of the study.

Acknowledgment of Protocol Deviation

Albert E. Munson — March 5, 1996

A. E. Munson, Ph.D.
Study Director

Date

Date of Deviation: January 13, 1996

Deviation Number: 7

Protocol Number: D4-28-1R-PO

Protocol Deviation: The dosage volume in study 9 was not adjusted on Day 8. The volume adjustment was calculated from the new mean body weights and changed on Day 9. The delay was due to a shortage of personnel on an inclement weather day and the desire to keep the exposure time consistent from day to day.

Acknowledgment of Protocol Deviation

Robert E Munson — 2/1/96

A. E. Munson, Ph.D.
Study Director

Date

Date of Deviation: November 23, 1995

Deviation Number: 8

Protocol Number: D4-28-1R-PO

Protocol Deviation: The day for weighing study #10 was delayed by one day from 22 Nov 95 to 23 Nov 95, due to the holiday schedule.

Acknowledgment of Protocol Deviation

Robert S. Munson 12/1/95

A. E. Munson, Ph.D.
Study Director

Date

Deviation Number: 9

Protocol Number: D4-28-1R-PO

Date of Deviation: March 27, 1996

Protocol Deviation: During studies 7-14, the humidity and temperature in the animal room (B2-035A), (B2-035C), and (6-053A) were observed to be outside the range of (40-70%) and (64.4-78.8 °F) indicated in the protocol. The dates and the recorded values are indicated below.

Date	Humidity (%)	Temp (°F)
Room 6-053A	None	None
Room B2-035A		
November 10, 1995	34 %	
November 13, 1995	36%	
November 15, 1995	38%	
November 16, 1995	33%	
November 17, 1995	36%	
November 18, 1995	34%	
November 20, 1995	38%	
November 22, 1995	30%	
November 23, 1995	31%	
November 25, 1995	33%	
November 26, 1995	34%	
November 29, 1995	36%	
November 30, 1995	34%	
November 16, 1995	100%	
December 5, 1995	36%	
December 7, 1995	36%	
December 8, 1995	30%	
December 9, 1995	38%	
December 10, 1995	30%	
December 11, 1995	30%	
December 12, 1995	25%	
December 13, 1995	28%	
December 18, 1995	30.5%	
December 20, 1995	32%	
December 21, 1995	32%	
December 24, 1995	28%	
December 27, 1995	27%	
December 28, 1995	27%	
December 29, 1995	30%	
December 30, 1995	30%	
December 31, 1995	32%	
January 3, 1996	38%	
January 4, 1996	28%	
January 5, 1996	30%	
January 6, 1996	27%	
January 7, 1996	29%	
January 8, 1996	30%	
January 9, 1996	26%	
January 10, 1996	32%	
January 11, 1996	20%	
January 12, 1996	29%	
January 13, 1996	25%	
January 14, 1996	25%	
January 15, 1996	26%	
January 16, 1996	28%	
January 17, 1996	38%	
January 20, 1996	28%	
January 21, 1996	26%	
January 22, 1996	26%	
January 23, 1996	27%	
January 24, 1996	30%	
January 26, 1996	27%	
Febuary 2, 1996	26%	
Febuary 5, 1996	18%	
Febuary 6, 1996	21%	
Febuary 7, 1996	26%	
Febuary 8, 1996	37%	
Room B2-035C		
November 15, 1995	36%	
November 16, 1995	32%	
November 17, 1995	30%	
November 18, 1995	32%	
November 20, 1995	33%	
November 21, 1995	37%	
November 22, 1995	26%	
November 23, 1995	28%	
November 25, 1995	30%	
November 26, 1995	30%	
November 29, 1995	33%	
November 30, 1995	30%	
December 1, 1995	36%	
December 2, 1995	31%	
December 3, 1995	34%	
December 5, 1995	35%	
December 7, 1995	36%	
December 8, 1995	30%	
December 9, 1995	36%	
December 10, 1995	30%	

Date	Humidity (%)	Temp (°F)	Date	Humidity (%)	Temp (°F)
December 11, 1995	30%		January 29, 1996	32	
December 12, 1995	28%		January 30, 1996	28	
December 18, 1995	30%		January 31, 1996	32	
December 19, 1995	37%		February 1, 1996	20	
December 20, 1995	30%		Febsuary 2, 1996	26	
December 21, 1995	30%		February 5, 1996	18	
December 24, 1995	29%		February 6, 1996	20	
December 27, 1995	28%		February 7, 1996	26	
December 28, 1995	26%		February 8, 1996	35	
December 29, 1995	30%		February 11, 1996	34	
January 3, 1996	38%		February 12, 1996	29	
January 4, 1996	36		February 13, 1996	26	
January 5, 1996	34		February 14, 1996	34	
January 6, 1996	34		February 15, 1996	34	
January 7, 1996	34		February 16, 1996	33	
January 8, 1996	34		February 19, 1996	31	
January 9, 1996	28%		February 25, 1996	30%	
January 10, 1996	30%		February 29, 1995	30%	
January 11, 1996	25%		March 1, 1996	29%	
January 12, 1996	30%		March 2, 1996	30%	
January 13, 1996	28%		March 3, 1996	30%	
January 14, 1996	30%		March 4, 1996	24%	
January 15, 1996	32%		March 5, 1996	37%	
January 16, 1996	28%		March 8, 1996	29%	
January 17, 1996	37		March 9, 1996	26%	
January 20, 1996	28		March 10, 1996	28%	
January 21, 1996	24		March 11, 1996	30%	
January 22, 1996	25		March 12, 1996	28%	
January 23, 1996	31		March 13, 1996	30%	
January 28, 1996	24				

It is the opinion of the Study Director that the differences in humidity did not affect the results of the study.

Acknowledgment of Protocol Deviation

Albert E Munson March 28, 1996

A. E. Munson, Ph.D. Date
Study Director

Deviation Number: 10

Protocol Number: D4-28-1R-PO

Date of Deviation: December 10, 1995

Protocol Deviation: The Study Director changed the dose of the positive control (MVE) in study 10 from 50 mg/kg to 25 mg/kg. In study number 4, the 50 mg/kg dose caused a RES blockade greater than was previously seen in rats. There was concern that this group of rats were more sensitive to the effects of MVE and thus the dose was decreased. The 25 mg/kg dose was sufficient to produce the hepatic blockade but did not block splenic phagocytosis. Thus, in the future studies (study 11), the 50 mg/kg dose was used.

Acknowledgment of Protocol Deviation

Robert E Munson 12/15/95

A. E. Munson, Ph.D. Date
Study Director

Deviation Number: 11

Protocol Number: D4-28-1R-PO

Date of Deviation: October 30, 1996

Protocol Deviation: The Quality Assurance Officer noted a typing error in the protocol on page 3 in the table of the third column heading. The units for the dose read ml/kg and it should read mg/kg.

Acknowledgment of Protocol Deviation

A. E. Munson, Ph.D.
Study Director

11/7/96
Date

Deviation Number: 12

Protocol Number: D4-28-1R-PO

Date of Deviation: October 30, 1996

Protocol Deviation: The Quality Assurance Officer noted a discrepancy in the molecular weight of Octamethylcyclotetrasiloxane in the protocol compared to the chemical preparation forms. The protocol should read 296.6 as the correct molecular weight, verified by the Merck Index.

Acknowledgment of Protocol Deviation

Albert E. Munson 11/7/96

A. E. Munson, Ph.D.
Study Director

Date

Deviation Number: 13

Protocol Number: D4-28-1R-PO

Date of Deviation: October 30, 1996

Protocol Deviation: The calculations for D4 dosing solutions were based on the correct density of 0.9558 for studies 1-6. However, a new lot of D4 was received for study 7-14. Inadvertently the density for these calculations was made using 0.9900. The expected concentration was 60 mg/ml, however when the density, 0.9900, was used for the dosing solution calculations of D4 rather than 0. 9558 the calculated concentration was of 57 mg/ml. The trend of D4 effects is still a reality and in the total report the doses are represented by 10, 30, 100, and 300 mg/kg rather than the subtle change in concentration which occurred in studies 7-14.

Acknowledgment of Protocol Deviation

Robert E Munson
A. E. Munson, Ph.D.
Study Director

11/15/96
Date

Deviation Number: 14

Protocol Number: D4-28-1R-PO

Date of Deviation: May 13, 1997

Protocol Deviation: A discrepancy was noted in the protocol under the Positive Control Number 2. The administration of anti asialo GM1 should read, by the intravenous injection not the intraperitoneal injection.

Acknowledgment of Protocol Deviation

Robert E Munson
A. E. Munson, Ph.D.
Study Director

5/13/97
Date

Deviation Number: 15

Protocol Number: D4-28-1R-PO

Date of Deviation: March 24, 1994

Protocol Deviation: The following items represent minor corrections to the protocol and had no impact on the integrity or results of the study.

a. The Positive Control Number 1: days of administration were stated to be on days 24 through 28 by i.p. injection should have read on days 25 though 28 by i.p. injection. The studies all reflect day 25.

PARAMETERS TO BE MONITORED:

b. The body weight of animals in the recovery studies (Amendments 4-7) had additional weigh days of 36 and 43.

c. Under hematology, platelet counts were included in the evaluation.

d. Under the Mixed Leukocyte Response, the stimulation index was not included in the evaluation and not deemed to be pertinent information.

e. Under the Natural Killer Cell Assay, additional effector-to-target cell ratios were added after Study 5 to evaluate the results by using lytic unit calculations (100:1, 50:1 and 25:1 to 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1). These new ratios would be used provided the cell count was sufficient.

STATISTICAL ANALYSES:

f. In the recovery studies, the 300 mg/kg dose group was evaluated using the Student's t Test and compared to the vehicle control group.

g. In the vehicle studies, the data are evaluated the same as stated in the protocol with the Naive group included as one of the dose groups.

Acknowledgment of Protocol Deviation

Albert E Munson — March 28, 1994

A.E. Munson, Ph.D.
Study Director

Date

Deviation Number: 16

Protocol Number: D4-28-1R-PO

Date of Deviation: December 1, 1994

Protocol Deviation: Two items in Study 5 represent deviations to the protocol in the splenic and peripheral blood phenotyping and percent reticulocytes. These deviations had no impact on the integrity or results of the study.

a. The monoclonal antibodies used were OX12 (B) instead of OX4 and OX19 (T) instead of W3/13 because of commercial availability and desired specificity. Both antibodies were verified for specificity prior to including them in the study.

b. The percent reticulocytes were inadvertently not included in the hematology evaluation. These data were generated in the repeat Study 12.

Acknowledgment of Protocol Deviation

Albert E Munson — 12/5/94

A.E. Munson, Ph.D.
Study Director

Date

Deviation Number: 17

Protocol Number: D4-28-1R-PO

Date of Deviation: May 16, 1995

Protocol Deviation: This deviation is a deviation to Amendments 2 and 3 of the protocol relating to the day samples would be prepared and sent to Dow Corning Corporation for concentration and stability analysis. These deviations had no impact on the integrity or results of the study.

The days of analysis for Studies 7 and 8 were day 21 and 28 rather than day 1 and 15 as stated in the Protocol Amendments 2 and 3.

Acknowledgment of Protocol Deviation

Albert E. Munson — May 18, 1995

A.E. Munson, Ph.D.
Study Director

Date

Deviation Number: 18

Protocol Number: D4-28-1R-PO

Date of Deviation: January 5, 1996

Protocol Deviation: Studies in which food consumption was performed are clarified.

Food consumption weights were performed on Studies 1 through 8.4. In Studies 9 through 14, food consumption was not continued. No additional information could be gained for the additional data. No impact on the integrity or results of the study occurred.

Acknowledgment of Protocol Deviation

Robert E. Munson
A.E. Munson, Ph.D.
Study Director

January 10, 1996
Date

APPENDIX D

DATA ASSAY TABLES

Appendix D - Table 1

Combined Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Studies 2, 3 and 6

Parameter	Vehicle (30)	Octamethylcyclotetrasiloxane (mg/kg) 10 (30)	30 (30)	100 (30)	300 (30)	H/NH	Trend Analysis
			Body Weight				
Day 1	179.5 ± 1.5	179.6 ± 1.5	179.1 ± 1.7	179.1 ± 1.6	179.4 ± 1.9	H	NS
Day 8	197.6 ± 1.1	199.5 ± 1.8	201.7 ± 1.5	200.1 ± 1.4	195.9 ± 1.4[a]	H	NS
Day 15	213.7 ± 1.3	217.7 ± 2.8	218.9 ± 2.2	216.9 ± 1.8	213.9 ± 1.9[b]	NH	NS
Day 22	222.9 ± 1.7[a]	224.2 ± 2.3	228.3 ± 2.2	226.0 ± 2.1	220.5 ± 2.0[b]	H	NS
Day 29	231.6 ± 2.0[a]	234.9 ± 2.9	240.1 ± 2.7*	237.3 ± 2.2	231.2 ± 2.1[b]	H	NS
			Body Weight Changes				
Day 8-Day 1	18.05 ± 1.18	19.95 ± 1.91**	22.58 ± 1.22**	20.96 ± 1.44**	16.47 ± 1.05**[a]	NH	NS
Day 15-Day 1	34.11 ± 1.25	38.11 ± 2.33	39.83 ± 1.68	37.75 ± 1.53	34.28 ± 1.22[b]	NH	NS
Day 22-Day 1	43.06 ± 1.61[a]	44.66 ± 2.13	49.15 ± 1.65	46.82 ± 1.80	40.93 ± 1.42[b]	H	NS
Day 29-Day 1	51.76 ± 2.08[a]	55.36 ± 2.90	60.98 ± 2.33	58.16 ± 2.34	51.64 ± 1.57[b]	NH	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 29 animals; [b]N = 28 animals

Appendix D - Table 2

Combined Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Studies 1, 4 and 5

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				H/NH	Trend Analysis
		10	30	100	300		
	(30)	(30)	(30)	(30)	(30)		
Body Weight							
Day 1	151.7 ± 1.2	149.5 ± 1.1	150.7 ± 0.9	151.0 ± 1.1	151.9 ± 0.8	H	NS
Day 8	153.2 ± 1.1	151.7 ± 0.9	153.4 ± 1.0	154.6 ± 1.2	153.2 ± 1.0	H	NS
Day 15	155.2 ± 1.1	153.5 ± 1.0	155.2 ± 0.9	157.7 ± 1.4	158.6 ± 0.9	H	p < 0.01
Day 22	157.9 ± 1.1	156.2 ± 1.1	158.5 ± 1.0	161.4 ± 1.4	161.4 ± 1.2	H	p < 0.01
Day 29	159.5 ± 1.2	159.1 ± 1.0	160.9 ± 1.2	163.5 ± 1.5	164.1 ± 1.5*	H	p < 0.01
Body Weight Changes							
Day 8-Day 1	1.54 ± 0.41	2.25 ± 0.56**	2.68 ± 0.36**	3.62 ± 0.62**	1.31 ± 0.56**	NH	NS
Day 15-Day 1	3.48 ± 0.53	3.97 ± 0.59**	4.51 ± 0.38**	6.68 ± 0.77**	6.72 ± 0.60**	NH	p < 0.01
Day 22-Day 1	6.22 ± 0.60	6.73 ± 0.80**	7.81 ± 0.53**	10.33 ± 0.79**	9.45 ± 0.92**	NH	p < 0.01
Day 29-Day 1	7.81 ± 0.71	9.61 ± 0.73**	10.26 ± 0.76**	12.53 ± 1.03**	12.23 ± 1.34**	NH	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 3

Body Weight (g) and Organ Weights (mg) in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 3

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(9)	(10)	(10)	(10)	(10)	(6)		
Body Wt (g)	232.5 ± 4.5	231.8 ± 5.0	237.3 ± 4.9	234.5 ± 5.3	224.0 ± 3.6	231.0 ± 4.0	H	NS
Liver (mg)	9376 ± 430	9913 ± 391	10641 ± 373	11104 ± 356**	14165 ± 231**	10153 ± 314	H	p < 0.01
Spleen (mg)	511 ± 9	508 ± 13	514 ± 9	527 ± 6	508 ± 10	322 ± 5**	H	NS
Thymus (mg)	309 ± 17	287 ± 13	259 ± 7*	266 ± 10	230 ± 17**	111 ± 14**	H	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 4

Body Weight (g) and Organ Weights (mg) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 6

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	AAGM1	H/NH	Trend
		10	30	100	300	25 mg/kg	1:10		Analysis
	(10)	(10)	(10)	(10)	(8)	(6)	(6)		
Body Wt (g)	229.6 ± 3.6	238.9 ± 6.1	240.9 ± 3.8*	238.6 ± 2.4	227.6 ± 2.7	232.5 ± 4.0	239.9 ± 3.9	NH	NS
Liver (mg)	9721 ± 226	10972 ± 493	11290 ± 256**	12127 ± 160**	14149 ± 181**	10344 ± 323	10317 ± 406	NH	p < 0.01
% Body Wt	4.23 ± 0.05	4.57 ± 0.09**	4.68 ± 0.05**	5.08 ± 0.04**	6.22 ± 0.07**	4.44 ± 0.08*	4.29 ± 0.10	H	p < 0.01
Spleen (mg)	457 ± 8	469 ± 12	490 ± 8	489 ± 7	472 ± 9	293 ± 7**	512 ± 11**	H	NS
% Body Wt	0.200 ± 0.003	0.197 ± 0.003	0.204 ± 0.002	0.203 ± 0.003	0.209 ± 0.004	0.125 ± 0.002**	0.212 ± 0.005*	H	NS
Thymus (mg)	314 ± 24	270 ± 14	280 ± 14	275 ± 10	228 ± 11	70 ± 6**	266 ± 8	NH	p < 0.01
% Body Wt	0.137 ± 0.009	0.115 ± 0.005	0.117 ± 0.006	0.115 ± 0.003	0.100 ± 0.004**	0.030 ± 0.003**	0.112 ± 0.005	NH	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 5

Body Weight (g) and Organ Weight (mg) in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 1

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(10)	(10)	(10)	(10)	(6)		
Body Wt (g)	157.3 ± 1.7	159.1 ± 1.7	157.9 ± 1.1	160.9 ± 3.8	158.6 ± 1.8	154.3 ± 2.0	NH	NS
Liver (mg)	5200 ± 89	5570 ± 114*	6195 ± 73**	7358 ± 243**	9674 ± 147**	5625 ± 126*	NH	p < 0.01
% Body Wt	3.31 ± 0.04	3.50 ± 0.05	3.92 ± 0.04**	4.57 ± 0.07**	6.10 ± 0.07**	3.64 ± 0.04**	H	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 6

Body Weight (g) and Organ Weights (mg) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 5

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	AAGM1 1:10 (6)	H/NH	Trend Analysis
Body Wt (g)	156.6 ± 1.5	156.9 ± 1.4	158.3 ± 1.6	161.6 ± 1.7	162.3 ± 1.8	154.9 ± 2.3	156.4 ± 3.8	H	p < 0.01
Liver (mg)	4968 ± 102	5473 ± 96**	5734 ± 104**	7169 ± 90**	9336 ± 117**	5257 ± 105	5335 ± 232	H	p < 0.01
% Body Wt	3.17 ± 0.05	3.49 ± 0.06**	3.62 ± 0.05**	4.44 ± 0.03**	5.76 ± 0.05**	3.40 ± 0.07*	3.41 ± 0.08*	H	p < 0.01
Spleen (mg)	363 ± 7	357 ± 5	366 ± 9	382 ± 8	379 ± 9	250 ± 10**	373 ± 16	H	p < 0.05
% Body Wt	0.232 ± 0.004	0.227 ± 0.003	0.231 ± 0.006	0.237 ± 0.005	0.233 ± 0.005	0.162 ± 0.004**	0.238 ± 0.008	H	NS
Thymus (mg)	188 ± 7	178 ± 8	179 ± 6	192 ± 7	156 ± 8**	71 ± 8**	180 ± 14	H	p < 0.05
% Body Wt	0.119 ± 0.005	0.114 ± 0.005	0.113 ± 0.004	0.117 ± 0.005	0.097 ± 0.004**	0.045 ± 0.004**	0.117 ± 0.007	H	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 7

Body Weight (g) and Organ Weights (mg) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 9

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(10)	(10)	(9)	(10)	(6)		
Body Wgt (g)	159.2 ± 1.6	159.3 ± 2.4	161.0 ± 2.6	159.2 ± 2.2	164.4 ± 2.5	147.9 ± 2.5**	H	NS
Liver (mg)	5581 ± 148	5833 ± 165	6462 ± 223**	7283 ± 138**	9951 ± 196**	5260 ± 171	H	p < 0.01
% Body Wgt	3.5 ± 0.1	3.7 ± 0.1	4.0 ± 0.1**	4.6 ± 0.1**	6.1 ± 0.1**	3.6 ± 0.1	H	p < 0.01
Spleen (mg)	407 ± 9	417 ± 9	418 ± 12	429 ± 7	433 ± 9	245 ± 5**	H	NS
% Body Wgt	0.255 ± 0.005	0.262 ± 0.003	0.259 ± 0.004	0.268 ± 0.005	0.263 ± 0.003	0.167 ± 0.004**	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 8

Body Weight (g) and Organ Weights (mg) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 9

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	CPS 25 mg/kg (6)
Body Wgt (g)	167.2 ± 3.1	163.8 ± 2.5	158.6 ± 4.6
Liver (mg)	5766 ± 213	6964 ± 177**	5682 ± 236
% Body Wgt	3.4 ± 0.1	4.2 ± 0.1**	3.6 ± 0.1
Spleen (mg)	425 ± 6	414 ± 8	253 ± 7**
% Body Wgt	0.255 ± 0.003	0.254 ± 0.004	0.160 ± 0.005**

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 39-42. On day 43 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and the positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 9

Body Weight (g) and Organ Weights (mg) in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 12

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(10)	(10)	(10)	(10)	(6)		
Body Wgt (g)	158.6 ± 1.2	157.7 ± 3.0	157.4 ± 2.3	161.9 ± 4.0	156.5 ± 3.7	152.0 ± 4.6	NH	NS
Liver (mg)	5693 ± 163	5790 ± 175	6260 ± 84**	7292 ± 197**	9345 ± 315**	5455 ± 253	NH	p < 0.01
% Body Wgt	3.59 ± 0.10	3.66 ± 0.05	3.98 ± 0.04**	4.50 ± 0.05**	5.97 ± 0.14**	3.59 ± 0.10	NH	p < 0.01
Spleen (mg)	382 ± 9	381 ± 11	385 ± 9	378 ± 10	373 ± 9	245 ± 7**	H	NS
% Body Wgt	0.242 ± 0.005	0.240 ± 0.004	0.245 ± 0.003	0.233 ± 0.004	0.238 ± 0.003	0.160 ± 0.004**	H	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 10

Body Weight (g) and Organ Weights (mg) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 12

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	CPS 25 mg/kg (6)
Body Wt (g)	161.8 ± 2.8	157.3 ± 3.2	154.2 ± 4.4
Liver (mg)	5914 ± 153	6366 ± 161	5551 ± 134
% Body Wt	3.65 ± 0.05	4.04 ± 0.04**	3.60 ± 0.05
Spleen (mg)	409 ± 8	395 ± 14	260 ± 8**
% Body Wt	0.252 ± 0.002	0.250 ± 0.006	0.167 ± 0.002**
Thymus (mg)	210 ± 7	205 ± 12	87 ± 8**
% Body Wt	0.129 ± 0.003	0.129 ± 0.006	0.055 ± 0.005**

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 39-42. On day 43 after the last eposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 11

Body Weight (g) and Organ Weights (mg) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 14

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				H/NH	Trend Analysis
		10	30	100	300		
	(10)	(10)	(10)	(10)	(10)		
Body Wgt (g)	158.1 ± 3.6	161.3 ± 3.0	158.7 ± 3.4	158.9 ± 3.4	158.3 ± 2.7	H	NS
Liver (mg)	5101 ± 254	5663 ± 186	5680 ± 187	6645 ± 233**	9016 ± 222**	H	p < 0.01
% Body Wgt	3.2 ± 0.1	3.5 ± 0.1	3.6 ± 0.1*	4.2 ± 0.1**	5.7 ± 0.1**	H	p < 0.01
Spleen (mg)	379 ± 13	387 ± 11	368 ± 11	376 ± 10	376 ± 9	H	NS
% Body Wgt	0.239 ± 0.007	0.239 ± 0.004	0.233 ± 0.003	0.238 ± 0.004	0.237 ± 0.003	H	p < 0.05

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 12

Hematology in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 6

Exposure	Erythrocytes (10^6/mm^3)	Hemoglobin (g/dl)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelets (10^5/mm^3)	Leukocytes (10^3/mm^3)
Vehicle	8.33 ± 0.12 (10)	16.1 ± 0.2 (10)	43.6 ± 0.6 (10)	52.3 ± 0.2 (10)	19.3 ± 0.1 (10)	36.9 ± 0.1 (10)	667 ± 26 (10)	8.95 ± 0.33 (10)
Octamethylcyclotetrasiloxane								
10 mg/kg	8.19 ± 0.06 (9)	16.1 ± 0.1 (9)	43.4 ± 0.3 (9)	53.0 ± 0.2* (9)	19.7 ± 0.1** (9)	37.1 ± 0.1 (9)	712 ± 11 (9)	8.17 ± 0.41 (9)
30 mg/kg	8.07 ± 0.08 (10)	16.1 ± 0.2 (10)	42.7 ± 0.4 (10)	53.0 ± 0.2* (10)	20.0 ± 0.1** (10)	37.7 ± 0.2** (10)	713 ± 8* (10)	7.73 ± 0.31* (10)
100 mg/kg	7.84 ± 0.06** (10)	15.9 ± 0.1 (10)	42.0 ± 0.3 (10)	53.5 ± 0.1** (10)	20.3 ± 0.1** (10)	37.8 ± 0.1** (10)	719 ± 8* (10)	8.60 ± 0.36 (10)
300 mg/kg	7.28 ± 0.12** (8)	14.8 ± 0.3** (8)	39.2 ± 0.5** (8)	53.9 ± 0.2** (8)	20.2 ± 0.1** (8)	37.6 ± 0.3 (8)	658 ± 14 (8)	7.36 ± 0.20** (8)
Cyclophosphamide 25 mg/kg	8.02 ± 0.09 (6)	15.9 ± 0.2 (6)	41.4 ± 0.4* (6)	51.6 ± 0.1* (6)	19.8 ± 0.1** (6)	38.4 ± 0.1** (6)	393 ± 11** (6)	1.25 ± 0.10** (6)
Anti Asialo GM1 1:10	8.39 ± 0.06 (6)	16.3 ± 0.2 (6)	44.0 ± 0.4 (6)	52.4 ± 0.2 (6)	19.5 ± 0.1 (6)	37.1 ± 0.1 (6)	629 ± 13 (6)	4.03 ± 0.43** (6)
H/NH	H	H	H	H	H	NH	NH	H
Trend Analysis	p < 0.01	p < 0.01	p < 0.01	p < 0.01	p < 0.01	p < 0.01	NS	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, blood was obtained by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.
NS = Not Significant

Appendix D - Table 13

Reticulocyte Counts in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 6

Exposure	Reticulocytes %
Vehicle	3.02 ± 0.21 (9)
Octamethylcyclotetrasiloxane	
10 mg/kg	3.00 ± 0.10 (10)
30 mg/kg	3.29 ± 0.19 (10)
100 mg/kg	3.05 ± 0.13 (10)
300 mg/kg	3.88 ± 0.66 (8)
Cyclophosphamide	
25 mg/kg	0.13 ± 0.02** (6)
Anti Asialo GM1	
1:10	2.50 ± 0.09 (6)
H/NH	NH
Trend Analysis	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, blood was drawn by cardiac puncture and reticulocytes enumerated on a Becton Dickinson FACSCAN. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 14

Leukocyte Cell Differential Blood Counts in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 6

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	AAGM1	H/NH	Trend
		10	30	100	300	25 mg/kg	1:10		Analysis
	(10)	(10)	(10)	(10)	(8)	(6)	(6)		
Leukocytes ($10^3/mm^3$)	8.95 ± 0.33	7.77 ± 0.56	7.73 ± 0.31	8.60 ± 0.36	7.36 ± 0.20*	1.25 ± 0.10**	4.03 ± 0.43**	H	p < 0.05
% Lymphocytes	85 ± 2	85 ± 1	88 ± 0	84 ± 1	86 ± 2	94 ± 1**	85 ± 3	NH	NS
% Neutrophils	15 ± 2	15 ± 1	12 ± 0	15 ± 1	14 ± 2	6 ± 1**	13 ± 2	NH	NS
% Eosinophils	0.90 ± 0.28	0.80 ± 0.33	0.60 ± 0.16	1.10 ± 0.38	0.25 ± 0.16	0.33 ± 0.33	2.00 ± 0.58	NH	p < 0.01
				per mm^3					
Lymphocytes	7572 ± 347	6620 ± 517	6790 ± 278	7223 ± 337	6306 ± 257	1171 ± 99**	3449 ± 394**	H	p < 0.05
Neutrophils	1306 ± 138	1100 ± 76	909 ± 36**	1279 ± 80	1040 ± 142	76 ± 13**	511 ± 93**	NH	NS
Eosinophils	78 ± 24	50 ± 17	31 ± 13	98 ± 33	18 ± 12	4 ± 4	74 ± 20	NH	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, blood was drawn by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 15

Hematology in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 5

Exposure	Erythrocytes (10^6/mm^3)	Hemoglobin (g/dl)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelets (10^5/mm^3)	Leukocytes (10^3/mm^3)
Vehicle	8.03 ± 0.10 (10)	16.1 ± 0.2 (10)	43.5 ± 0.5 (10)	54.2 ± 0.1 (10)	20.1 ± 0.1 (10)	37.1 ± 0.1 (10)	644.00 ± 11.21 (10)	7.51 ± 0.73 (10)
Octamethylcyclotetrasiloxane								
10 mg/kg	7.69 ± 0.07* (9)	15.8 ± 0.1 (9)	41.8 ± 0.3* (9)	54.3 ± 0.1 (9)	20.5 ± 0.1** (9)	37.7 ± 0.1** (9)	649.67 ± 10.47 (9)	6.79 ± 0.61 (9)
30 mg/kg	7.54 ± 0.09** (7)	15.5 ± 0.2* (7)	41.2 ± 0.5** (7)	54.6 ± 0.1 (7)	20.6 ± 0.1** (7)	37.8 ± 0.1** (7)	642.86 ± 15.88 (7)	8.17 ± 0.50 (7)
100 mg/kg	7.23 ± 0.06** (10)	15.0 ± 0.1** (10)	39.9 ± 0.3** (10)	55.2 ± 0.1** (10)	20.8 ± 0.1** (10)	37.7 ± 0.2** (10)	599.30 ± 33.49 (10)	7.79 ± 0.46 (10)
300 mg/kg	7.38 ± 0.10** (9)	14.9 ± 0.2** (9)	40.3 ± 0.5** (9)	55.2 ± 0.1** (9)	20.4 ± 0.1* (9)	37.0 ± 0.2 (9)	583.44 ± 13.75** (9)	7.62 ± 0.50 (9)
Cyclophosphamide 25 mg/kg	7.02 ± 0.40** (6)	14.5 ± 0.8* (6)	38.2 ± 2.2** (6)	54.4 ± 0.2 (6)	20.7 ± 0.1** (6)	38.1 ± 0.3** (6)	365.33 ± 26.91** (6)	2.32 ± 0.32** (6)
Anti Asialo GM1 1:10	8.06 ± 0.14 (5)	16.5 ± 0.2 (5)	44.1 ± 0.7 (5)	54.7 ± 0.3* (5)	20.5 ± 0.1* (5)	37.4 ± 0.1 (5)	614.20 ± 15.65 (5)	4.02 ± 0.25** (5)
H/NH	H	H	H	H	H	H	NH	H
Trend Analysis	p < 0.01	p < 0.01	p < 0.01	p < 0.01	p < 0.01	NS	p < 0.01	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, after the last exposure, blood was obtained by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.
NS = Not Significant

Appendix D - Table 16

Leukocyte Cell Differential Blood Counts in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 5

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	AAGM1	H/NH	Trend
		10	30	100	300	25 mg/kg	1:10		Analysis
	(10)	(9)	(7)	(10)	(9)	(6)	(5)		
Leukocytes (10^3/mm^3)	7.51 ± 0.75	6.79 ± 0.61	8.17 ± 0.50	7.79 ± 0.46	7.62 ± 0.50	2.32 ± 0.32**	4.02 ± 0.25**	H	NS
% Lymphocytes	87 ± 1	84 ± 1	88 ± 1	86 ± 1	87 ± 1	93 ± 2*	84 ± 2	H	NS
% Neutrophils	12 ± 1	15 ± 1	11 ± 1	13 ± 1	12 ± 1	6 ± 1**	15 ± 2	H	NS
% Eosinophils	1.30 ± 0.40	1.67 ± 0.33	0.71 ± 0.36	0.90 ± 0.28	1.11 ± 0.35	1.00 ± 0.63	1.40 ± 0.75	H	$p < 0.01$
				per mm^3					
Lymphocytes	6304 ± 635	5666 ± 519	7223 ± 465	6731 ± 416	6624 ± 442	2155 ± 310**	3372 ± 238**	H	NS
Neutrophils	904 ± 134	1017 ± 133	893 ± 79	989 ± 55	916 ± 97	141 ± 28**	585 ± 70	H	NS
Eosinophils	102 ± 34	105 ± 21	52 ± 27	71 ± 20	82 ± 25	21 ± 13	63 ± 35	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, blood was drawn by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 17

Hematology in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 12

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg) 10	30	100	300	CPS 25 mg/kg	H/NH	Trend Analysis
Erythrocytes ($10^6/mm^3$)	7.27 ± 0.05 (8)	7.34 ± 0.06 (10)	7.08 ± 0.05 (10)	6.96 ± 0.05** (10)	6.79 ± 0.04** (10)	6.83 ± 0.09** (6)	H	p < 0.01
Hemoglobin (g/dl)	14.7 ± 0.1 (8)	14.9 ± 0.2 (10)	14.7 ± 0.2 (10)	14.3 ± 0.1 (10)	13.8 ± 0.1** (10)	14.0 ± 0.1** (6)	H	p < 0.01
Hematocrit (%)	38.6 ± 0.3 (8)	39.0 ± 0.4 (10)	37.8 ± 0.2 (10)	37.4 ± 0.3* (10)	36.7 ± 0.2** (10)	36.0 ± 0.5** (6)	H	p < 0.01
MCV (fl)	53.1 ± 0.1 (8)	53.0 ± 0.1 (10)	53.4 ± 0.2 (10)	53.8 ± 0.2** (10)	54.0 ± 0.1** (10)	52.8 ± 0.2 (6)	H	p < 0.01
MCH (pg)	20.2 ± 0.1 (8)	20.3 ± 0.1 (10)	20.8 ± 0.3 (10)	20.7 ± 0.1 (10)	20.4 ± 0.1 (10)	20.5 ± 0.2 (6)	NH	NS
MCHC (g/dl)	38.2 ± 0.2 (8)	38.3 ± 0.1 (10)	38.9 ± 0.5 (10)	38.4 ± 0.2 (10)	37.8 ± 0.1 (10)	38.9 ± 0.2 (6)	NH	p < 0.05
Platelets ($10^5/mm^3$)	716.13 ± 17.31 (8)	745.70 ± 12.39 (10)	728.10 ± 11.64 (10)	731.00 ± 12.98 (10)	676.50 ± 13.53 (10)	436.00 ± 8.70** (6)	H	p < 0.05
Leukocytes ($10^3/mm^3$)	6.38 ± 0.46 (8)	7.64 ± 0.38 (10)	6.08 ± 0.37 (10)	6.30 ± 0.45 (10)	6.32 ± 0.54 (10)	1.62 ± 0.16** (6)	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, blood was obtained by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.
NS = Not Significant

Appendix D - Table 18

Reticulocyte Counts in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 12

Exposure	Reticulocytes %
Vehicle	3.27 ± 0.16 (10)
Octamethylcyclotetrasiloxane	
10 mg/kg	3.00 ± 0.16 (10)
30 mg/kg	3.87 ± 0.15* (10)
100 mg/kg	3.71 ± 0.38 (10)
300 mg/kg	5.62 ± 0.41** (10)
Cyclophosphamide	
25 mg/kg	0.18 ± 0.01** (6)
H/NH	NH
Trend Analysis	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, blood was drawn by cardiac puncture and reticulocytes enumerated on a Becton Dickinson FACSCAN. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 19

Leukocyte Cell Differential Blood Counts in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 12

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(8)	(10)	(10)	(10)	(10)	(6)		
Leukocytes (10^3/mm^3)	6.38 ± 0.46	7.64 ± 0.38	6.08 ± 0.37	6.30 ± 0.45	6.32 ± 0.54	1.62 ± 0.16**	H	NS
% Lymphocytes	87 ± 3	88 ± 1	88 ± 2	84 ± 1	87 ± 1	93 ± 2	H	NS
% Neutrophils	13 ± 3	12 ± 1	11 ± 2	15 ± 1	12 ± 1	5 ± 2*	NH	NS
% Eosinophils	0.50 ± 0.27	0.80 ± 0.33	0.50 ± 0.17	0.30 ± 0.15	1.00 ± 0.39	2.33 ± 0.67*	NH	p < 0.1
				per mm^3				
Lymphocytes	5576 ± 495	6676 ± 314	5374 ± 350	5294 ± 348	5544 ± 517	1493 ± 144**	H	NS
Neutrophils	768 ± 136	900 ± 99	673 ± 114	985 ± 117	716 ± 71	86 ± 30**	H	NS
Eosinophils	32 ± 18	64 ± 27	33 ± 11	20 ± 10	60 ± 26	38 ± 13	NH	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, blood was drawn by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 20

Hematology in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 12

Parameter	Vehicle	Octamethylcyclotetrasiloxane 300 mg/kg	CFS 25 mg/kg
Erythrocytes (10^6/mm^3)	7.50 ± 0.05 (10)	7.32 ± 0.07* (10)	6.42 ± 0.17** (6)
Hemoglobin (g/dl)	14.9 ± 0.1 (10)	14.7 ± 0.1 (10)	13.6 ± 0.2** (6)
Hematocrit (%)	39.9 ± 0.2 (10)	39.0 ± 0.3* (10)	34.1 ± 1.0** (6)
MCV (fl)	53.2 ± 0.2 (10)	53.3 ± 0.2 (10)	53.1 ± 0.1 (6)
MCH (pg)	19.8 ± 0.1 (10)	20.0 ± 0.1 (10)	21.2 ± 0.9 (6)
MCHC (g/dl)	37.3 ± 0.1 (10)	37.6 ± 0.1 (10)	40.0 ± 1.7 (6)
Platelets (10^5/mm^3)	751.70 ± 20.97 (10)	790.60 ± 12.80 (10)	441.50 ± 22.13** (6)
Leukocytes (10^3/mm^3)	6.30 ± 0.39 (10)	6.23 ± 0.56 (10)	0.98 ± 0.09** (6)

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 39-42. On day 43, blood was obtained by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.
MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

Appendix D - Table 21

Reticulocyte Counts in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 12

Exposure	Reticulocytes %
Vehicle	5.93 ± 0.33 (10)
Octamethylcyclotetrasiloxane 300 mg/kg	5.67 ± 0.28 (10)
Cyclophosphamide 25 mg/kg	0.21 ± 0.02** (6)

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 39-42. On day 43, blood was drawn by cardiac puncture and reticulocytes enumerated on a Becton Dickinson FACSCAN. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 22

Leukocyte Cell Differential Blood Counts in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 12

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	CPS 25 mg/kg (6)
Leukocytes ($10^3/mm^3$)	6.30 ± 0.39	6.23 ± 0.56	0.98 ± 0.09**
% Lymphocytes	87 ± 2	86 ± 2	97 ± 1**
% Neutrophils	12 ± 2	13 ± 2	3 ± 1**
% Eosinophils	0.50 ± 0.34	0.70 ± 0.21	0.00 ± 0.00
		per mm^3	
Lymphocytes	5524 ± 407	5372 ± 521	951 ± 85**
Neutrophils	751 ± 108	815 ± 108	33 ± 9**
Eosinophils	25 ± 17	43 ± 13	0 ± 0

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, CPS, i.p. on days 39-42. On day 43, blood was drawn by cardiac puncture and hematological parameters evaluated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 23

Splenocyte Surface Marker Differential in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Absolute Values)

Study 6

Exposure	Spleen Cells ($x10^7$)	OX12+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	41.48 ± 1.34 (10)	195.6 ± 7.0 (10)	178.8 ± 6.8 (10)	107.9 ± 3.5 (10)	67.8 ± 3.1 (10)
Octamethylcyclotetrasiloxane					
10 mg/kg	40.06 ± 1.65 (10)	191.8 ± 8.9 (10)	176.9 ± 7.9 (10)	110.8 ± 5.1 (10)	64.3 ± 3.5 (10)
30 mg/kg	39.44 ± 1.56 (10)	184.4 ± 8.3 (10)	168.9 ± 6.7 (10)	102.6 ± 4.2 (10)	63.7 ± 2.8 (10)
100 mg/kg	40.66 ± 1.07 (10)	186.8 ± 5.8 (10)	173.1 ± 6.1 (10)	114.3 ± 4.2 (10)	59.0 ± 2.0 (10)
300 mg/kg	38.66 ± 1.41 (8)	173.7 ± 5.7 (8)	163.8 ± 4.9 (8)	106.6 ± 4.2 (8)	58.0 ± 2.2 (8)
Cyclophosphamide	9.90 ± 0.14** (6)	13.5 ± 0.7** (6)	49.5 ± 1.4** (6)	22.6 ± 0.7** (6)	31.0 ± 1.7** (6)
H/NH	H	H	H	H	H
Trend Analysis	NS	$p < 0.05$	NS	NS	$p < 0.01$

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values are expressed as the absolute number per spleen x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 24

Peripheral Blood Surface Marker Differential in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Percent Values)

Study 6

Exposure	OX12* (B cells)	OX19* (T cells)	W3/25* (CD4)	OX8* (CD8)
Vehicle	26.1 ± 0.6 (10)	41.7 ± 1.5 (10)	28.6 ± 0.8 (10)	22.6 ± 0.7 (10)
Octamethylcyclotetrasiloxane				
10 mg/kg	25.6 ± 0.4 (9)	41.9 ± 1.4 (9)	29.5 ± 0.8 (9)	23.5 ± 0.8 (9)
30 mg/kg	25.5 ± 0.8 (10)	45.4 ± 0.9 (10)	31.6 ± 0.4** (10)	23.6 ± 0.3 (10)
100 mg/kg	25.3 ± 0.8 (10)	44.2 ± 1.3 (10)	30.5 ± 0.5 (10)	22.5 ± 0.5 (10)
300 mg/kg	22.9 ± 0.7* (8)	47.2 ± 1.3* (8)	33.9 ± 0.5** (8)	22.8 ± 0.6 (8)
Cyclophosphamide				
25 mg/kg	8.1 ± 1.0** (6)	59.4 ± 2.2** (6)	36.2 ± 1.2** (6)	40.8 ± 2.3** (6)
H/NH	H	H	H	NH
Trend Analysis	p < 0.01	p < 0.01	p < 0.01	NS

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 25

Peripheral Blood Surface Marker Differential in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Absolute Values)

Study 6

Exposure	Peripheral Blood WBC/ml x 10^6	OX12$^+$ (B cells)	OX19$^+$ (T cells)	W3/25$^+$ (CD4)	OX8$^+$ (CD8)
Vehicle	5.11 ± 0.13 (10)	1.33 ± 0.04 (10)	2.14 ± 0.11 (10)	1.46 ± 0.06 (10)	1.16 ± 0.05 (10)
Octamethylcyclotetrasiloxane					
10 mg/kg	4.51 ± 0.29 (9)	1.16 ± 0.08 (9)	1.90 ± 0.16 (9)	1.34 ± 0.11 (9)	1.07 ± 0.09 (9)
30 mg/kg	4.17 ± 0.55 (10)	1.06 ± 0.14 (10)	1.88 ± 0.24 (10)	1.32 ± 0.18 (10)	1.00 ± 0.14 (10)
100 mg/kg	4.65 ± 0.28* (10)	1.17 ± 0.07* (10)	2.05 ± 0.13 (10)	1.41 ± 0.08 (10)	1.04 ± 0.07 (10)
300 mg/kg	3.19 ± 0.35** (8)	0.73 ± 0.08** (8)	1.50 ± 0.15 (8)	1.08 ± 0.12 (8)	0.73 ± 0.08** (8)
Cyclophosphamide					
25 mg/kg	0.46 ± 0.09** (6)	0.04 ± 0.01** (6)	0.27 ± 0.05** (6)	0.16 ± 0.03** (6)	0.19 ± 0.04** (6)
H/NH	NH	NH	H	NH	NH
Trend Analysis	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.01$

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values are expressed as the absolute number per ml of whole blood x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 26

Splenocyte Surface Marker Differential in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Percent Values)

Study 6

Exposure	OX12+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	47.1 ± 0.6 (10)	43.1 ± 0.7 (10)	26.0 ± 0.5 (10)	16.3 ± 0.4 (10)
Octamethylcyclotetrasiloxane				
10 mg/kg	47.8 ± 0.8 (10)	44.2 ± 0.7 (10)	27.7 ± 0.6 (10)	16.0 ± 0.4 (10)
30 mg/kg	46.7 ± 0.7 (10)	42.9 ± 0.8 (10)	26.0 ± 0.5 (10)	16.2 ± 0.4 (10)
100 mg/kg	45.9 ± 0.4 (10)	42.5 ± 0.8 (10)	28.1 ± 0.7 (10)	14.5 ± 0.3** (10)
300 mg/kg	45.0 ± 0.7 (8)	42.6 ± 1.1 (8)	27.6 ± 0.7 (8)	15.0 ± 0.3 (8)
Cyclophosphamide				
25 mg/kg	13.6 ± 0.7** (6)	50.0 ± 1.2** (6)	22.8 ± 0.7** (6)	31.3 ± 1.5** (6)
H/NH	H	H	H	NH
Trend Analysis	$p < 0.01$	NS	NS	$p < 0.01$

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 27

Splenocyte Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Absolute Values)

Study 5

Exposure	Spleen Cells ($x10^7$)	$OX12^+$ (B cells)	$OX19^+$ (T cells)	$W3/25^+$ (CD4)	$OX8^+$ (CD8)
Vehicle	29.95 ± 1.45 (10)	129.5 ± 7.8 (10)	120.1 ± 9.3 (4)	80.8 ± 5.2 (9)	48.2 ± 4.7 (9)
Octamethylcyclotetrasiloxane					
10 mg/kg	31.02 ± 1.23 (10)	106.0 ± 6.0 (10)	133.0 ± 6.1 (10)	82.2 ± 5.7 (9)	43.3 ± 3.2 (9)
30 mg/kg	30.61 ± 0.72 (10)	109.4 ± 5.7 (10)	127.1 ± 6.9 (10)	75.4 ± 3.7 (10)	40.5 ± 2.1 (10)
100 mg/kg	30.17 ± 1.16 (10)	102.2 ± 7.0* (10)	121.9 ± 5.0 (10)	63.3 ± 3.7* (10)	36.1 ± 1.7* (10)
300 mg/kg	29.39 ± 1.45 (10)	97.0 ± 6.2** (10)	107.3 ± 5.7 (10)	59.7 ± 4.8** (10)	30.5 ± 2.0** (10)
Cyclophosphamide					
25 mg/kg	10.42 ± 0.23** (6)	13.2 ± 1.2** (6)	38.9 ± 2.0** (6)	18.4 ± 1.0** (6)	18.8 ± 0.8** (6)
H/NH	H	H	H	H	NH
Trend Analysis	NS	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values are expressed as the absolute number per spleen x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 28

Peripheral Blood Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Percent Values)

Study 5

Exposure	OX12+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	24.9 ± 0.8 (10)	53.9 ± 1.5 (10)	42.5 ± 1.0 (10)	26.6 ± 0.9 (10)
Octamethylcyclotetrasiloxane				
10 mg/kg	22.4 ± 0.8 (10)	55.0 ± 2.1 (10)	42.1 ± 1.4 (10)	26.4 ± 0.6 (10)
30 mg/kg	20.1 ± 2.0 (10)	57.9 ± 2.8 (10)	40.8 ± 2.1 (10)	28.6 ± 1.4 (10)
100 mg/kg	21.4 ± 1.1 (10)	59.7 ± 1.5 (10)	40.6 ± 0.8 (10)	27.1 ± 0.8 (10)
300 mg/kg	23.5 ± 1.7 (10)	58.3 ± 2.1 (10)	39.0 ± 1.0 (10)	28.5 ± 0.9 (10)
Cyclophosphamide				
25 mg/kg	8.1 ± 1.2** (6)	61.0 ± 8.9 (6)	32.5 ± 4.4* (6)	42.8 ± 6.6** (6)
H/NH	NH	H	NH	H
Trend Analysis	NS	p < 0.01	p < 0.05	p < 0.05

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 29

Splenocyte Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Percent Values)

Study 5

Exposure	OX12+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	43.4 ± 2.0 (10)	41.7 ± 1.5 (4)	27.1 ± 1.0 (9)	15.8 ± 0.8 (9)
Octamethylcyclotetrasiloxane				
10 mg/kg	34.1 ± 1.2** (10)	42.9 ± 1.2 (10)	26.7 ± 2.1 (9)	13.8 ± 0.8 (9)
30 mg/kg	35.8 ± 1.7** (10)	41.3 ± 1.4 (10)	24.6 ± 0.9 (10)	13.2 ± 0.4* (10)
100 mg/kg	33.7 ± 1.7** (10)	40.4 ± 0.4 (10)	21.1 ± 1.2** (10)	12.0 ± 0.4** (10)
300 mg/kg	33.1 ± 1.4** (10)	36.6 ± 0.7** (10)	20.1 ± 1.0** (10)	10.4 ± 0.4** (10)
Cyclophosphamide				
25 mg/kg	12.7 ± 1.3** (6)	37.3 ± 1.6 (6)	17.6 ± 0.7** (6)	18.0 ± 0.5 (6)
H/NH	H	NH	H	H
Trend Analysis	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 30

Splenocyte Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Absolute Values)

Study 12

Exposure	Spleen Cells ($x10^7$)	$OX33^+$ (B cells)	$OX19^+$ (T cells)	$W3/25^+$ (CD4)	$OX8^+$ (CD8)
Vehicle	40.79 ± 1.08 (10)	180.8 ± 4.7 (9)	217.2 ± 6.8 (9)	126.2 ± 4.7 (9)	61.2 ± 2.1 (9)
Octamethylcyclotetrasiloxane					
10 mg/kg	39.52 ± 0.93 (10)	173.0 ± 3.6 (9)	206.1 ± 3.9 (9)	122.9 ± 2.2 (9)	58.9 ± 2.5 (9)
30 mg/kg	41.64 ± 1.38 (10)	181.6 ± 6.7 (10)	215.6 ± 8.1 (10)	120.5 ± 5.4 (10)	59.5 ± 3.2 (10)
100 mg/kg	36.52 ± 0.84* (10)	158.8 ± 4.8** (10)	185.4 ± 4.3** (10)	111.7 ± 3.8 (10)	51.7 ± 2.2 (10)
300 mg/kg	36.82 ± 0.78* (10)	151.1 ± 3.3** (10)	188.8 ± 4.3** (10)	114.6 ± 2.8 (10)	55.0 ± 2.1 (10)
Cyclophosphamide					
25 mg/kg	13.48 ± 0.48** (6)	10.9 ± 0.8** (6)	82.7 ± 3.6** (6)	39.3 ± 1.9** (6)	38.4 ± 1.3** (6)
H/NH	H	H	H	H	H
Trend Analysis	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values are expressed as the absolute number per spleen x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 31

Splenocyte Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Percent Values)

Study 12

Exposure	OX33+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	44.7 ± 0.7 (9)	53.7 ± 1.0 (9)	31.2 ± 0.8 (9)	15.2 ± 0.5 (9)
Octamethylcyclotetrasiloxane				
10 mg/kg	44.4 ± 0.8 (9)	53.0 ± 1.1 (9)	31.6 ± 0.7 (9)	15.1 ± 0.5 (9)
30 mg/kg	43.6 ± 0.6 (10)	51.7 ± 0.6 (10)	28.9 ± 0.7* (10)	14.2 ± 0.5 (10)
100 mg/kg	43.4 ± 0.5 (10)	50.8 ± 0.7 (10)	30.5 ± 0.5 (10)	14.2 ± 0.5 (10)
300 mg/kg	41.1 ± 0.5** (10)	51.3 ± 0.5 (10)	31.1 ± 0.5 (10)	14.9 ± 0.3 (10)
Cyclophosphamide				
25 mg/kg	8.0 ± 0.4** (6)	61.4 ± 1.6** (6)	29.1 ± 0.6 (6)	28.6 ± 0.9** (6)
H/NH	H	NH	H	H
Trend Analysis	p < 0.01	p < 0.01	NS	NS

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 32

Peripheral Blood Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Absolute Values)

Study 12

Exposure	Peripheral Blood WBC/ml x 10^6	OX33+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	4.44 ± 0.34 (10)	0.74 ± 0.08 (10)	2.4 ± 0.2 (10)	1.6 ± 0.1 (10)	0.9 ± 0.1 (10)
Octamethylcyclotetrasiloxane					
10 mg/kg	5.51 ± 0.17 (10)	0.93 ± 0.05 (10)	3.0 ± 0.1* (10)	2.2 ± 0.1* (10)	1.2 ± 0.1 (10)
30 mg/kg	4.13 ± 0.31 (10)	0.69 ± 0.06 (10)	2.3 ± 0.2 (10)	1.7 ± 0.1 (10)	0.9 ± 0.1 (10)
100 mg/kg	4.03 ± 0.38 (10)	0.67 ± 0.06 (10)	2.2 ± 0.2 (10)	1.6 ± 0.1 (10)	0.8 ± 0.1 (10)
300 mg/kg	3.94 ± 0.37 (10)	0.69 ± 0.08 (10)	2.2 ± 0.2 (10)	1.6 ± 0.1 (10)	0.8 ± 0.1 (10)
Cyclophosphamide					
25 mg/kg	0.43 ± 0.04** (6)	0.00 ± 0.00** (6)	0.25 ± 0.04** (6)	0.18 ± 0.02** (6)	0.12 ± 0.03** (6)
H/NH	H	H	H	H	H
Trend Analysis	$p < 0.01$	$p < 0.05$	$p < 0.05$	NS	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values are expressed as the absolute number per ml of whole blood x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 33

Peripheral Blood Surface Marker Differential in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Percent Values)

Study 12

Exposure	OX33+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	16.5 ± 0.9 (10)	53.8 ± 2.2 (10)	37.6 ± 1.0 (10)	20.6 ± 1.2 (10)
Octamethylcyclotetrasiloxane				
10 mg/kg	17.0 ± 0.7 (10)	54.5 ± 0.9 (10)	39.2 ± 0.6 (10)	21.1 ± 0.6 (10)
30 mg/kg	16.9 ± 0.7 (10)	55.3 ± 0.9 (10)	40.2 ± 0.6 (10)	21.5 ± 0.6 (10)
100 mg/kg	16.7 ± 0.6 (10)	54.7 ± 1.2 (10)	40.3 ± 0.7 (10)	20.5 ± 0.3 (10)
300 mg/kg	17.0 ± 0.6 (10)	54.8 ± 1.6 (10)	40.3 ± 0.9 (10)	19.5 ± 0.8 (10)
Cyclophosphamide				
25 mg/kg	1.9 ± 0.3** (6)	58.0 ± 5.3 (6)	40.6 ± 3.0 (6)	27.2 ± 3.8 (6)
H/NH	H	NH	H	NH
Trend Analysis	NS	NS	$p < 0.05$	NS

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 29, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 34

Peripheral Blood Surface Marker Differential in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study (Absolute Values)

Study 12

Exposure	Peripheral Blood WBC/ml x 10^6	OX33$^+$ (B cells)	OX19$^+$ (T cells)	W3/25$^+$ (CD4)	OX8$^+$ (CD8)
Vehicle	4.07 ± 0.28 (10)	0.7 ± 0.1 (10)	2.1 ± 0.1 (10)	1.6 ± 0.1 (10)	0.9 ± 0.1 (10)
Octomethylcyclotetrasiloxane 300 mg/kg	3.06 ± 0.38* (10)	0.5 ± 0.1 (10)	1.6 ± 0.2 (10)	1.2 ± 0.2 (10)	0.7 ± 0.1 (10)
Cyclophosphamide 25 mg/kg	0.50 ± 0.20** (6)	0.0 ± 0.0** (6)	0.2 ± 0.1** (6)	0.2 ± 0.1** (6)	0.1 ± 0.0** (6)

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 39-42. On day 43, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values are expressed as the absolute number per ml of whole blood x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and the positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 35

Splenocyte Surface Marker Differential in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study (Absolute Values)

Study 12

Exposure	Spleen Cells ($x10^7$)	$OX33^+$ (B cells)	$OX19^+$ (T cells)	$W3/25^+$ (CD4)	$OX8^+$ (CD8)
Vehicle	41.40 ± 1.07 (10)	180.1 ± 6.4 (10)	221.3 ± 7.5 (10)	143.1 ± 3.4 (10)	75.5 ± 3.4 (10)
Octamethylcyclotetrasiloxane 300 mg/kg	39.46 ± 1.07 (10)	171.9 ± 4.9 (10)	204.2 ± 6.1 (10)	122.6 ± 4.4** (10)	66.4 ± 2.2* (10)
Cyclophosphamide 25 mg/kg	12.32 ± 0.58** (6)	9.1 ± 1.1** (6)	78.7 ± 4.8** (6)	40.1 ± 3.2** (6)	41.3 ± 3.1** (6)

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 39-42. On day 43, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values are expressed as the absolute number per spleen x 10^6. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 36

Splenocyte Surface Marker Differential in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study (Percent Values)

Study 12

Exposure	OX33+ (B cells)	OX19+ (T cells)	W3/25+ (CD4)	OX8+ (CD8)
Vehicle	43.4 ± 0.7 (10)	53.4 ± 0.8 (10)	34.6 ± 0.4 (10)	18.2 ± 0.6 (10)
Octamethylcyclotetrasiloxane 300 mg/kg	43.6 ± 0.7 (10)	51.8 ± 0.9 (10)	31.0 ± 0.5** (10)	16.8 ± 0.4 (10)
Cyclophosphamide 25 mg/kg	7.3 ± 0.6** (6)	63.7 ± 1.4** (6)	32.3 ± 1.4 (6)	33.3 ± 1.1** (6)

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 39-42. On day 43, the rats were sacrificed, spleens removed, and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 37

Peripheral Blood Surface Marker Differential in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study (Percent Values)

Study 12

Exposure	OX33* (B cells)	OX19* (T cells)	W3/25* (CD4)	OX8* (CD8)
Vehicle	15.8 ± 0.6 (10)	52.0 ± 1.3 (10)	39.5 ± 0.7 (10)	21.5 ± 0.6 (10)
Octamethylcyclotetrasiloxane 300 mg/kg	16.1 ± 0.5 (10)	53.8 ± 1.0 (10)	40.4 ± 0.7 (10)	21.6 ± 0.5 (10)
Cyclophosphamide 25 mg/kg	2.6 ± 0.4** (6)	63.0 ± 7.7 (6)	44.7 ± 3.6 (6)	27.5 ± 2.9* (6)

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 39-42. On day 43, the rats were sacrificed, peripheral blood drawn, lysed and T & B cells and subsets were enumerated. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 38

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes
in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
Day 4 Response

Study 3

Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x 10^3$)
Vehicle	232.5 ± 4.5 (9)	511 ± 9 (9)	46.63 ± 1.35 (9)	1053 ± 63 (9)	493 ± 36 (9)
Octamethylcyclotetrasiloxane					
10 mg/kg	231.8 ± 5.0 (10)	508 ± 13 (10)	44.46 ± 1.02 (10)	1165 ± 60 (10)	518 ± 31 (10)
30 mg/kg	237.3 ± 4.9 (10)	514 ± 9 (10)	46.68 ± 1.37 (10)	1412 ± 77** (10)	661 ± 43** (10)
100 mg/kg	234.5 ± 5.3 (10)	527 ± 6 (10)	47.47 ± 0.62 (10)	1302 ± 75 (10)	619 ± 37 (10)
300 mg/kg	224.0 ± 3.6 (10)	508 ± 10 (10)	43.07 ± 1.01 (10)	1304 ± 78 (10)	560 ± 33 (10)
Cyclophosphamide					
25 mg/kg	231.0 ± 4.0 (6)	322 ± 5** (6)	13.95 ± 0.76** (6)	1.0 ± 0.20** (6)	0.1 ± 0.03** (6)
H/NH	H	H	H	H	H
Trend Analysis	NS	NS	NS	$p < 0.01$	NS

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 25, the rats were immunized (iv) with $2x10^8$ sRBC. On day 29, spleen cells were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 39

Serum IgM Titers in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 3

Exposure	Serum Titers (OD) 0.5
Vehicle	79 ± 10 (9)
Octamethylcyclotetrasiloxane	
10 mg/kg	77 ± 10 (10)
30 mg/kg	105 ± 14 (10)
100 mg/kg	85 ± 13 (10)
300 mg/kg	71 ± 9 (10)
Cyclophosphamide	
25 mg/kg	16 ± 0** (6)
H/NH	NH
Trend Analysis	NS

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 25, the rats were immunized (iv) with $2x10^6$ sRBC and serum obtained through cardiac puncture on day 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 40

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes
in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
Day 4 Response

Study 1

Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Vehicle	157.3 ± 1.7 (10)	395 ± 9 (10)	33.90 ± 0.62 (10)	2047 ± 214 (10)	698 ± 78 (10)
Octamethylcyclotetrasiloxane					
10 mg/kg	159.1 ± 1.7 (10)	400 ± 5 (10)	34.00 ± 0.60 (10)	1536 ± 104 (10)	522 ± 36 (10)
30 mg/kg	157.9 ± 1.1 (10)	393 ± 7 (10)	31.46 ± 0.79 (10)	2264 ± 210 (10)	719 ± 76 (10)
100 mg/kg	160.9 ± 3.8 (10)	421 ± 9 (10)	33.30 ± 1.45 (10)	1613 ± 61 (10)	543 ± 40 (10)
300 mg/kg	158.5 ± 1.8 (10)	394 ± 9 (10)	33.38 ± 1.38 (10)	1874 ± 280 (8)	608 ± 73 (8)
Cyclophosphamide					
25 mg/kg	154.3 ± 2.0 (6)	244 ± 5** (6)	11.25 ± 0.86** (6)	169 ± 159** (6)	15 ± 14** (6)
H/NH	NH	H	NH	NH	H
Trend Analysis	NS	NS	NS	NS	NS

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 25, the rats were immunized (iv) with $2x10^8$ sRBC. On day 29, spleen cells were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 41

Serum IgM Titers in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 1

Exposure	Serum Titers (OD) 0.5
Vehicle	212 ± 18 (9)
Octamethylcyclotetrasiloxane	
10 mg/kg	162 ± 13 (9)
30 mg/kg	170 ± 12 (8)
100 mg/kg	166 ± 12 (8)
300 mg/kg	125 ± 17** (6)
Cyclophosphamide	
25 mg/kg	28 ± 7** (6)
H/NH	H
Trend Analysis	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 25, the rats were immunized (iv) with $2x10^6$ sRBC and serum obtained through cardiac puncture on day 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 42

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes
in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
Day 4 Response

Study 9

Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x 10^3$)
Vehicle	160.1 ± 1.5 (9)	414 ± 7 (9)	38.65 ± 1.17 (9)	1030 ± 78 (9)	402 ± 38 (9)
Octamethylcyclotetrasiloxane					
10 mg/kg	159.3 ± 2.4 (10)	417 ± 9 (10)	36.68 ± 0.73 (10)	1309 ± 90 (10)	483 ± 41 (10)
30 mg/kg	161.4 ± 2.9 (9)	419 ± 13 (9)	34.96 ± 0.83 (9)	1032 ± 71 (9)	361 ± 27 (9)
100 mg/kg	159.2 ± 2.2 (9)	429 ± 7 (9)	37.86 ± 0.80 (9)	1642 ± 133** (9)	625 ± 58** (9)
300 mg/kg	164.4 ± 2.5 (10)	433 ± 9 (10)	37.46 ± 1.15 (10)	1541 ± 85** (10)	574 ± 30* (10)
Cyclophosphamide					
25 mg/kg	147.9 ± 2.5** (6)	245 ± 5** (6)	8.99 ± 0.35** (6)	15 ± 3** (6)	1 ± 0** (6)
H/NH	H	H	H	H	N
Trend Analysis	NS	$p < 0.05$	NS	$p < 0.01$	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 25, the rats were immunized (iv) with $2x10^8$ sRBC. On day 29, spleens were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 43

Serum IgM Titers in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 9

Exposure	Serum Titers (OD) 0.5
Vehicle	154 ± 42 (10)
Octamethylcyclotetrasiloxane	
10 mg/kg	164 ± 34 (10)
30 mg/kg	153 ± 35 (10)
100 mg/kg	177 ± 35 (9)
300 mg/kg	215 ± 34 (10)
Cyclophosphamide	
25 mg/kg	16 ± 0* (6)
H/NH	NH
Trend Analysis	$p < 0.05$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. On day 25, the rats were immunized (iv) with $2x10^6$ sRBC and serum obtained through cardiac puncture on day 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 44

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study
Day 4 Response

Study 9

Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x 10^3$)
Vehicle	167.2 ± 3.1 (10)	425 ± 6 (10)	35.92 ± 0.82 (10)	1100 ± 68 (10)	394 ± 24 (10)
Octamethylcyclotetrasiloxane 300 mg/kg	163.8 ± 2.5 (10)	414 ± 8 (10)	34.43 ± 0.81 (10)	1530 ± 104** (10)	527 ± 38** (10)
Cyclophosphamide 25 mg/kg	158.6 ± 4.6 (6)	253 ± 7** (6)	10.00 ± 0.34** (6)	18 ± 3** (6)	2 ± 0** (6)

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage for 28 days or the positive control, cyclophosphamide, i.p. on days 39-42. On day 39, the rats were immunized (iv) with $2x10^8$ sRBC. On day 43, spleens were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 45

Serum IgM Titers in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 9

Exposure	Serum Titers (OD) 0.5
Vehicle	261 ± 46 (9)
Octamethylcyclotetrasiloxane 300 mg/kg	224 ± 36 (10)
Cyclophosphamide 25 mg/kg	16 ± 0** (6)

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 39-42. On day 39, the rats were immunized (i.v.) with $2x10^8$ sRBC and serum obtained through cardiac puncture on day 43. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and the positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 46

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
Day 4 Response

Study 13

Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x 10^3$)
Vehicle	158.7 ± 2.6 (10)	394 ± 6 (10)	34.33 ± 0.56 (10)	1152 ± 132 (10)	398 ± 49 (10)
Octamethylcyclotetrasiloxane					
10 mg/kg	157.1 ± 2.5 (10)	381 ± 9 (10)	34.31 ± 1.22 (10)	983 ± 75 (10)	335 ± 24 (10)
30 mg/kg	158.5 ± 2.1 (10)	407 ± 12 (10)	35.40 ± 1.05 (10)	1195 ± 103 (10)	428 ± 46 (10)
100 mg/kg	162.0 ± 1.6 (10)	425 ± 8 (10)	34.01 ± 0.89 (10)	1556 ± 119 (10)	530 ± 45 (10)
300 mg/kg	164.5 ± 3.4 (10)	418 ± 12 (10)	32.13 ± 1.20 (10)	1792 ± 156** (10)	577 ± 51* (10)
H/NH	H	H	H	H	H
Trend Analysis	$p < 0.05$	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage for 28 days. On day 25, the rats were immunized (iv) with $2x10^8$ sRBC. On day 29, spleens were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 47

Serum IgM Titers in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days Day 4 Response

Study 13

Exposure	Serum Titers (OD) 0.5	Serum Titers Log_2
Vehicle	304 ± 35 (10)	8.16 ± 0.17 (10)
Octamethylcyclotetrasiloxane		
10 mg/kg	281 ± 34 (10)	8.00 ± 0.22 (10)
30 mg/kg	276 ± 31 (10)	8.02 ± 0.18 (10)
100 mg/kg	322 ± 52 (9)	8.18 ± 0.24 (9)
300 mg/kg	259 ± 36 (10)	7.88 ± 0.21 (10)
H/NH	H	H
Trend Analysis	NS	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. On day 25, the rats were immunized (iv) with $2x10^8$ sRBC and serum obtained through cardiac puncture on day 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 48

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
Day 6 Response

Study 14

Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Vehicle	158.1 ± 3.6 (10)	379 ± 13 (10)	30.73 ± 1.25 (10)	222 ± 36 (10)	68 ± 12 (10)
Octamethylcyclotetrasiloxane					
10 mg/kg	161.3 ± 3.0 (10)	387 ± 11 (10)	32.26 ± 0.96 (10)	311 ± 27 (10)	100 ± 9 (10)
30 mg/kg	158.7 ± 3.4 (10)	368 ± 11 (10)	29.83 ± 1.12 (10)	299 ± 33 (10)	87 ± 8 (10)
100 mg/kg	158.9 ± 3.4 (10)	376 ± 10 (10)	31.22 ± 1.12 (10)	328 ± 28 (10)	103 ± 10 (10)
300 mg/kg	158.3 ± 2.7 (10)	376 ± 9 (10)	30.13 ± 0.63 (10)	441 ± 30** (10)	133 ± 9** (10)
H/NH	H	H	H	H	H
Trend Analysis	NS	NS	NS	$p < 0.01$	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage for 28 days. On day 23, the rats were immunized (iv) with $2x10^8$ sRBC. On day 29, spleens were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 49

Serum IgM Titers in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days Day 6 Response

Study 14

Exposure	Serum Titers (OD) 0.5	Serum Titers Log_2
Vehicle	569 ± 100 (10)	8.92 ± 0.29 (10)
Octamethylcyclotetrasiloxane		
10 mg/kg	370 ± 59 (9)	8.39 ± 0.23 (9)
30 mg/kg	398 ± 54 (10)	8.53 ± 0.18 (10)
100 mg/kg	337 ± 49 (10)	8.12 ± 0.36 (10)
300 mg/kg	508 ± 40 (9)	8.96 ± 0.11 (9)
H/NH	H	NH
Trend Analysis	NS	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. On day 23, the rats were immunized (iv) with $2x10^8$ sRBC and serum obtained through cardiac puncture on day 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 50

Mixed Leukocyte Response in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Spleen Lymphocytes)

Study 6

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(10)	(10)	(10)	(8)	(6)		
				CPM				
Responders	2864 ± 126	3560 ± 238*	3660 ± 120**	3365 ± 192	3207 ± 99	3459 ± 254*	NH	NS
Responders + Long Evans Stimulators	6136 ± 687	4795 ± 1146	4210 ± 436	3448 ± 872	3640 ± 326	3613 ± 530*	NH	$p < 0.01$
Responders + Brown Norway Stimulators	6198 ± 949	5551 ± 600	5897 ± 1103	6836 ± 1723	7177 ± 941	5765 ± 789	NH	NS
Spleen Cells x 10^7	41.5 ± 1.3	40.1 ± 1.7	39.4 ± 1.6	40.7 ± 1.1	38.7 ± 1.4	9.9 ± 0.1**	H	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, spleens were prepared into a single cell suspension and incubated alone or with stimulator cells from Long Evans or Brown Norway rats for 5 days in Costar U-bottom microtiter plates. The stimulator spleen cells were treated with 40 µg/ml mitomycin C for 45 minutes, washed 4 times, and added to the assay plates at a ratio 2.0 stimulators:1 responder cell or 1.0×10^6 cells/well:5×10^5 cells/well (0.1 ml ± 0.1 ml). Prior to harvest, the cells were pulsed with ^{3}H-thymidine for 18-24 hours. Media used for this experiment was RPMI 1640 supplemented with 10% FCS and 5×10^{-5} M 2-mercaptoethanol. The data are presented as mean ± SE from 4 replicate cultures. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 51

Mixed Leukocyte Response in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Peripheral Blood Leukocytes)

Study 6

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(9)	(10)	(10)	(8)	(6)		
				CPM				
Responders	632 ± 96	794 ± 32	920 ± 52	672 ± 47	1244 ± 216	762 ± 92	NH	$p < 0.05$
Responders + Long Evans Stimulators	6976 ± 2612	3702 ± 391	3703 ± 853	4484 ± 955	5231 ± 943	860 ± 52	NH	NS
Responders + Brown Norway Stimulators	18587 ± 1739	15321 ± 2271	12947 ± 1368	16046 ± 3190	17316 ± 1303	2261 ± 584**	NH	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, rats were anesthetized by CO_2 and blood obtained by cardiac puncture. Responder cells were incubated alone or with stimulator cells from Long Evans or Brown Norway rats for 6 days in Costar U-bottom microtite. plates. The stimulator spleen cells were treated with 40 µg/ml mitomycin C for 45 minutes, washed 4 times, and added to the assay plates at a ratio 2.5 stimulators:1 responder cell or 2.5×10^5 cells/well:1×10^5 cells/well (0.1 ml ± 0.1 ml). Prior to harvest, the cells were pulsed with ^{3}H-thymidine for 18-24 hours. Media used for this experiment was RPMI 1640 supplemented with 10% FCS and 5×10^{-5} M 2-mercaptoethanol. The data are presented as mean ± SE from 4 replicate cultures. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 52

Mixed Leukocyte Response in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Spleen Lymphocytes)

Study 5

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(9)	(10)	(10)	(10)	(6)		
				CPM				
Responders	2309 ± 78	2659 ± 159	2451 ± 108	2567 ± 154	2259 ± 105	625 ± 54**	H	NS
Responders + Long Evans Stimulators	5250 ± 1106	3046 ± 574	3418 ± 665	2902 ± 801	3183 ± 666	665 ± 26**	H	NS
Responders + Brown Norway Stimulators	6771 ± 1276	6291 ± 1122	8311 ± 1013	5912 ± 1184	6797 ± 1130	576 ± 51**	H	NS
Spleen Cells x 10^7	29.9 ± 1.5	31.3 ± 1.3	30.6 ± 0.7	30.2 ± 1.2	29.4 ± 1.5	10.4 ± 0.2**	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, spleens were prepared into a single cell suspension and incubated alone or with stimulator cells from Long Evans or Brown Norway rats for 5 days in Costar U-bottom microtiter plates. The stimulator spleen cells were treated with 40 µg/ml mitomycin C for 45 minutes, washed 4 times, and added to the assay plates at a ratio 2 stimulators:1 responder cell or $1x10^6$ cells/well:$5x10^5$ cells/well (0.1 ml ± 0.1 ml). Prior to harvest, the cells were pulsed with ^3H-thymidine for 18-24 hours. Media used for this experiment was RPMI 1640 supplemented with 10% FCS and $5x10^{-5}$M 2-mercaptoethanol. The data are presented as mean ± SE from 4 replicate cultures. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 53

Mixed Leukocyte Response in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Peripheral Blood Leukocytes)

Study 5

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(8)	(8)	(9)	(9)	(10)	(6)		
				CPM				
Responders	889 ± 172	932 ± 137	1146 ± 98	1430 ± 252	1399 ± 147	703 ± 116	H	p < 0.01
Responders + Long Evans Stimulators	6402 ± 1556	4292 ± 1257	9000 ± 3117	4866 ± 1802	4580 ± 1047	935 ± 58*	NH	NS
Responders + Brown Norway Stimulators	18791 ± 2305	16515 ± 2371	18700 ± 2063	14478 ± 2558	18077 ± 1039	922 ± 108**	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. On day 29, rats were anesthetized by CO_2 and blood obtained by cardiac puncture. Responder cells were incubated alone or with stimulator cells from Long Evans or Brown Norway rats for 6 days in Costar U-bottom microtiter plates. The stimulator spleen cells were treated with 40 µg/ml mitomycin C for 45 minutes, washed 4 times, and added to the assay plates at a ratio 2.5 stimulators:1 responder cell or 2.5 x 10^5 cells/well:1 x 10^5 cells/well (0.1 ml ± 0.1 ml). Prior to harvest, the cells were pulsed with ^{3}H-thymidine for 18-24 hours. Media used for this experiment was RPMI 1640 supplemented with 10% FCS and 5 x 10^{-5}M 2-mercaptoethanol. The data are presented as mean ± SE from 4 replicate cultures. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
Cells recovered from 3-5 mls whole blood lysed in NH_4Cl lysing buffer.
NS = Not Significant

Appendix D - Table 54

Functional Activity of the Reticuloendothelial System in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 2

Parameter	Vehicle (9)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Half-Life	13.0 ± 1.9	11.9 ± 0.7	9.5 ± 0.7*	10.1 ± 0.5	9.4 ± 0.5**	76.7 ± 13.2**	NH	p < 0.01
Body Wgt (g)	234.1 ± 2.4	234.1 ± 3.9	242.0 ± 5.3	238.8 ± 3.3	241.4 ± 1.5	235.7 ± 4.4	NH	p < 0.05
Liver Wgt (mg)	8621 ± 196	8606 ± 303	9603 ± 297*	10532 ± 303**	13128 ± 196**	9128 ± 336	H	p < 0.01
% Uptake	43.6 ± 1.6	45.4 ± 0.9	42.5 ± 1.3	46.1 ± 1.0	48.2 ± 1.7	5.3 ± 0.5**	H	p < 0.05
cpm/mg	276 ± 11	289 ± 10	250 ± 9	244 ± 7	206 ± 8**	31 ± 2**	H	p < 0.01
Spleen Wgt (mg)	460 ± 6	457 ± 8	495 ± 11*	486 ± 8	462 ± 5	489 ± 25	H	NS
% Uptake	14.2 ± 1.3	14.2 ± 1.6	16.8 ± 2.0	14.0 ± 1.4	9.1 ± 1.1	5.1 ± 1.5**	H	p < 0.05
cpm/mg	1673 ± 153	1690 ± 187	1877 ± 186	1591 ± 155	1092 ± 127	542 ± 143**	H	p < 0.05
Lung Wgt (mg)	1074 ± 26	1151 ± 35	1260 ± 52*	1438 ± 47**	1307 ± 46**	1446 ± 89**	H	p < 0.01
% Uptake	0.59 ± 0.05	0.67 ± 0.08	0.67 ± 0.09	0.64 ± 0.05	0.48 ± 0.04	3.23 ± 0.31**	H	NS
cpm/mg	30 ± 2	31 ± 3	30 ± 4	25 ± 2	21 ± 2	122 ± 8**	H	p < 0.01
Thymus Wgt (mg)	304 ± 18	289 ± 8	292 ± 15	291 ± 7	251 ± 11*	313 ± 26	H	p < 0.01
% Uptake	0.028 ± 0.003	0.025 ± 0.001	0.034 ± 0.005	0.024 ± 0.002	0.016 ± 0.001**	0.111 ± 0.037*	NH	p < 0.01
cpm/mg	4.9 ± 0.4	4.8 ± 0.2	6.4 ± 0.8	4.5 ± 0.4	3.5 ± 0.2**	18.0 ± 4.5**	NH	p < 0.01
Kidney Wgt (mg)	1781 ± 18	1674 ± 28**	1809 ± 51	1811 ± 21	1818 ± 34	1834 ± 28	NH	p < 0.05
% Uptake	2.8 ± 0.3	3.0 ± 0.1	2.3 ± 0.3	2.5 ± 0.2	1.4 ± 0.1**	1.2 ± 0.1**	H	p < 0.01
cpm/mg	85 ± 8	97 ± 4	72 ± 9	77 ± 6	43 ± 4**	37 ± 4**	NH	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. On day 29, the mice were evaluated for phagocytic activity by measuring the vascular clearance rate and organ distribution of ^{51}Cr sRBC. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 55

Functional Activity of the Reticuloendothelial System in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 11

Parameter	Vehicle (9)	Octamethylcyclotetrasiloxane (mg/kg) 10 (8)	30 (8)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Half-Life	25.6 ± 2.7	22.3 ± 2.3	28.6 ± 3.0	17.0 ± 0.8**	24.0 ± 4.1	42.1 ± 1.8**	NH	p < 0.05
Body Wgt (g)	245.8 ± 4.1	241.4 ± 5.6	239.3 ± 3.0	234.9 ± 3.1	224.6 ± 3.4**	240.6 ± 2.5	H	p < 0.01
Liver Wgt (mg)	9926 ± 227	9734 ± 238	10149 ± 209	10456 ± 242	12615 ± 190**	9624 ± 322	H	p < 0.01
% Uptake	33.2 ± 2.3	33.8 ± 1.6	34.0 ± 2.1	38.7 ± 1.3	43.9 ± 2.0**	6.8 ± 0.5**	H	p < 0.01
cpm/mg	203 ± 16	207 ± 11	198 ± 12	214 ± 7	192 ± 6	42 ± 2**	H	NS
Spleen Wgt (mg)	476 ± 9	460 ± 7	469 ± 7	459 ± 5	423 ± 18	484 ± 10	NH	p < 0.01
% Uptake	8.6 ± 1.3	7.2 ± 0.6	7.5 ± 0.9	8.6 ± 1.4	6.2 ± 0.6	3.6 ± 0.9*	NH	NS
cpm/mg	1081 ± 152	924 ± 67	940 ± 101	1063 ± 167	802 ± 57	429 ± 100**	NH	NS
Lung Wgt (mg)	1244 ± 61	1139 ± 25	1165 ± 37	1136 ± 17	1099 ± 38	1308 ± 61	NH	p < 0.05
% Uptake	0.95 ± 0.06	0.77 ± 0.04	0.83 ± 0.06	0.76 ± 0.05	0.65 ± 0.06**	3.68 ± 0.33**	H	p < 0.01
cpm/mg	46 ± 2	40 ± 2	42 ± 3	39 ± 2	33 ± 2**	168 ± 17**	H	p < 0.01
Thymus Wgt (mg)	262 ± 9	267 ± 17	265 ± 12	250 ± 14	215 ± 12*	243 ± 13	H	p < 0.01
% Uptake	0.040 ± 0.002[a]	0.039 ± 0.005	0.041 ± 0.005	0.035 ± 0.003	0.029 ± 0.003	0.067 ± 0.007**	H	p < 0.01
cpm/mg	9.4 ± 0.5[a]	8.5 ± 0.7	9.0 ± 1.0	8.0 ± 0.3	7.2 ± 0.6	16.9 ± 2.6**	H	p < 0.01
Kidney Wgt (mg)	1961 ± 44	1871 ± 30	1927 ± 50	1921 ± 40	1821 ± 22	2000 ± 35	H	p < 0.05
% Uptake	5.2 ± 0.2	5.2 ± 0.1	5.4 ± 0.2	5.0 ± 0.2	4.4 ± 0.3*	4.8 ± 0.3	H	p < 0.01
cpm/mg	160 ± 5	166 ± 6	164 ± 7	152 ± 7	134 ± 8*	143 ± 9	H	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. On day 29, the rats were evaluated for phagocytic activity by measuring the vascular clearance rate and organ distribution of ^{51}Cr sRBC. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 8 animals

Appendix D - Table 56

Functional Activity of the Reticuloendothelial System in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 11

Parameter	Vehicle (9)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	MVE 50 mg/kg (4)
Half-Life	23.7 ± 3.0	24.1 ± 2.5	52.9 ± 13.7*
Body Wgt (g)	263.4 ± 4.7	256.9 ± 4.3	250.5 ± 5.7
Liver Wgt (mg)	10107 ± 290	11658 ± 392**	9950 ± 362
% Uptake	28.1 ± 1.4	37.1 ± 1.7**	7.7 ± 1.1**
cpm/mg	145 ± 6	162 ± 5*	39 ± 5**
Spleen Wgt (mg)	540 ± 14	532 ± 13	459 ± 19**
% Uptake	7.2 ± 1.1	8.7 ± 0.9	1.6 ± 0.3**
cpm/mg	684 ± 99	835 ± 85	170 ± 33**
Lung Wgt (mg)	1343 ± 60	1289 ± 26	1457 ± 142
% Uptake	1.1 ± 0.1	0.9 ± 0.1	3.8 ± 0.7**
cpm/mg	43 ± 5	37 ± 2	126 ± 17**
Thymus Wgt (mg)	290 ± 10	292 ± 7	259 ± 54
% Uptake	0.051 ± 0.004	0.046 ± 0.004	0.110 ± 0.032*
cpm/mg	9 ± 1	8 ± 1	19 ± 4**
Kidney Wgt (mg)	2031 ± 38	2028 ± 44	2131 ± 13
% Uptake	5.2 ± 0.2	4.6 ± 0.2	5.4 ± 2.0
cpm/mg	133 ± 6	116 ± 5*	127 ± 48

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 42. On day 43, the rats were evaluated for phagocytic activity by measuring the vascular clearance rate and organ distribution of ^{51}Cr sRBC. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 57

Functional Activity of the Reticuloendothelial System in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 4

Parameter	Vehicle (6)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Half-Life	11.9 ± 0.9	14.0 ± 1.6	16.9 ± 3.0	17.7 ± 2.5	23.8 ± 6.4[a]	80.8 ± 28.3*	NH	NS
Body Wgt (g)	163.2 ± 2.5	161.3 ± 1.9	166.6 ± 2.1	168.2 ± 1.5	173.1 ± 1.7**	152.4 ± 1.7**	H	p < 0.01
Liver Wgt (mg)	5047 ± 180	5226 ± 136	6012 ± 136**	7284 ± 117**	9147 ± 316**	5271 ± 107	NH	p < 0.01
% Uptake	51.7 ± 1.8	47.9 ± 1.7	49.2 ± 1.6	51.6 ± 2.4	51.6 ± 3.0	8.9 ± 1.2**	H	p < 0.05
cpm/mg	555 ± 25	490 ± 19	453 ± 19**	393 ± 16**	312 ± 19**	85 ± 12**	H	p < 0.01
Spleen Wgt (mg)	372 ± 14	359 ± 7	372 ± 6	363 ± 9	367 ± 5	291 ± 11**	H	NS
% Uptake	11.1 ± 1.6	10.7 ± 1.4	9.8 ± 1.3	6.5 ± 0.7	5.7 ± 0.9*	2.3 ± 0.4**	H	p < 0.01
cpm/mg	1592 ± [illegible]	1565 ± 189	1444 ± 186	1001 ± 108	898 ± 152*	384 ± 60**	H	p < 0.01
Lung Wgt (mg)	886 ± 35	914 ± 46	914 ± 21	893 ± 16	882 ± 20	1071 ± 95	NH	NS
% Uptake	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	6.0 ± 0.6**	H	p < 0.01
cpm/mg	39 ± 7	40 ± 3	42 ± 3	37 ± 6	29 ± 5	287 ± 29**	H	p < 0.05
Thymus Wgt (mg)	214 ± 10	205 ± 9	211 ± 10	196 ± 5	189 ± 10	120 ± 5**	H	p < 0.05
% Uptake	0.022 ± 0.002	0.022 ± 0.002	0.021 ± 0.001	0.021 ± 0.003	0.018 ± 0.003	0.047 ± 0.006**	H	p < 0.05
cpm/mg	5.5 ± 0.5	5.6 ± 0.4	5.6 ± 0.4	6.1 ± 1.0	5.5 ± 0.6	19.6 ± 2.0**	NH	NS
Kidney Wgt (mg)	1083 ± 38	1103 ± 18	1133 ± 20	1174 ± 24	1172 ± 25	1177 ± 18	H	p < 0.01
% Uptake	3.7 ± 0.1	3.9 ± 0.2	3.9 ± 0.2	4.2 ± 0.3	3.1 ± 0.4	2.1 ± 0.5**	NH	NS
cpm/mg	186 ± 6	188 ± 7	190 ± 9	197 ± 13	150 ± 21	88 ± 20**	NH	NS

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. On day 29, the mice were evaluated for phagocytic activity by measuring the vascular clearance rate and organ distribution of ^{51}Cr sRBC. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

NS = Not Significant; [a]N = 9 animals

Appendix D - Table 58

Functional Activity of the Reticuloendothelial System in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 10

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				MVE	H/NH	Trend
		10	30	100	300	25 mg/kg		Analysis
	(10)	(10)	(10)	(10)	(10)	(4)		
Half-Life	22.7 ± 2.9	28.2 ± 3.5	31.8 ± 8.2	21.7 ± 2.4	32.2 ± 6.6[b]	47.6 ± 14.5*	NH	NS
Body Wgt (g)	163.3 ± 2.9	162.2 ± 1.9	161.4 ± 2.5	163.3 ± 2.5	160.1 ± 3.2	151.3 ± 2.7*	H	NS
Liver Wgt (mg)	5857 ± 181	5793 ± 162	5920 ± 165	6926 ± 129**	9121 ± 170**	5422 ± 149	H	p < 0.01
% Uptake	38.1 ± 2.9	39.4 ± 3.3	41.7 ± 2.9	46.4 ± 1.9	44.1 ± 4.2	11.8 ± 2.3**	H	p < 0.05
cpm/mg	193 ± 15	202 ± 18	206 ± 14	198 ± 7	141 ± 14*	59 ± 11**	H	p < 0.01
Spleen Wgt (mg)	381 ± 10	384 ± 13	366 ± 5	377 ± 7	345 ± 12*	391 ± 21	H	p < 0.01
% Uptake	10.9 ± 1.4	9.1 ± 1.4	8.5 ± 1.1	8.8 ± 1.0	5.2 ± 1.3**	11.4 ± 2.9	H	p < 0.01
cpm/mg	834 ± 93	698 ± 100	674 ± 91	697 ± 85	431 ± 107	775 ± 189	H	p < 0.01
Lung Wgt (mg)	908 ± 24	881 ± 32	903 ± 22	908 ± 29	875 ± 21	930 ± 24	H	NS
% Uptake	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1[a]	0.9 ± 0.1	1.1 ± 0.1	3.7 ± 0.8**	H	NS
cpm/mg	32 ± 2	30 ± 3	28 ± 3[a]	28 ± 2	35 ± 5	109 ± 22**	H	NS
Thymus Wgt (mg)	235 ± 15	236 ± 7	212 ± 10	205 ± 7	183 ± 11**	188 ± 15	H	p < 0.01
% Uptake	0.057 ± 0.004	0.056 ± 0.008	0.043 ± 0.003	0.045 ± 0.004	0.051 ± 0.009	0.082 ± 0.014*	NH	p < 0.05
cpm/mg	7.3 ± 0.7	6.7 ± 0.9	6.0 ± 0.4	6.5 ± 0.5	7.9 ± 1.1	12.0 ± 1.7**	NH	NS
Kidney Wgt (mg)	1235 ± 28	1185 ± 22	1175 ± 26	1227 ± 25	1211 ± 34	1265 ± 29	H	NS
% Uptake	5.3 ± 0.4	5.1 ± 0.3	5.2 ± 0.2	5.0 ± 0.2	4.9 ± 0.3	3.4 ± 0.3*	H	NS
cpm/mg	126 ± 7	126 ± 7	129 ± 6	121 ± 4	116 ± 6	76 ± 6**	H	NS

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. On day 29, the rats were evaluated for phagocytic activity by measuring the vascular clearance rate and organ distribution of ^{51}Cr sRBC. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals; [b]N = 8 animals

Appendix D - Table 59

Functional Activity of the Reticuloendothelial System in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 10

Parameter	Vehicle (9)	Octamethylcyclotetrasiloxane 300 mg/kg (9)	MVE 25 mg/kg (6)
Half-Life	21.7 ± 1.7	20.4 ± 1.9	30.1 ± 2.5*
Body Wgt (g)	168.9 ± 3.9	164.1 ± 4.8	161.2 ± 2.1
Liver Wgt (mg)	5571 ± 191	6274 ± 277	5987 ± 94
% Uptake	35.8 ± 1.4	40.5 ± 4.0	10.6 ± 0.6**
cpm/mg	241 ± 9	235 ± 23	63 ± 3**
Spleen Wgt (mg)	395 ± 13	374 ± 14	498 ± 15**
% Uptake	10.0 ± 0.8	7.6 ± 1.0	27.3 ± 2.7**
cpm/mg	945 ± 72	739 ± 90	1933 ± 137**
Lung Wgt (mg)	883 ± 60	808 ± 24	931 ± 29
% Uptake	0.8 ± 0.1	0.6 ± 0.1*	2.4 ± 0.3**
cpm/mg	35 ± 2	27 ± 2*	92 ± 10**
Thymus Wgt (mg)	206 ± 8	191 ± 12	169 ± 12*
% Uptake	0.036 ± 0.002	0.034 ± 0.004	0.061 ± 0.004**
cpm/mg	6.6 ± 0.4	6.6 ± 0.7	13.0 ± 0.3**
Kidney Wgt (mg)	1232 ± 32	1236 ± 21	1352 ± 30*
% Uptake	4.1 ± 0.1	4.1 ± 0.3	3.9 ± 0.2
cpm/mg	124 ± 2	121 ± 7	103 ± 5**

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 42. On day 43, the rats were evaluated for phagocytic activity by measuring the vascular clearance rate and organ distribution of ^{51}Cr sRBC. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix D - Table 60

Natural Killer Cell Activity in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Spleen Cells)

Study 6

Exposure	Effector/Target Ratio						LU/ 10^7 Cells	LU/Spleen	Spleen Wt (mg)
	6.25/1	12.5/1	25/1	50/1	100/1	200/1			
Vehicle	1.0 ± 0.1 (10)	2.4 ± 0.2 (10)	4.1 ± 0.2 (10)	7.2 ± 0.3 (10)	12.9 ± 0.8 (10)	19.2 ± 0.6 (10)	14 ± 1 (10)	292 ± 23 (10)	457 ± 8 (10)
Octamethylcyclotetrasiloxane									
10 mg/kg	1.1 ± 0.1 (10)	1.8 ± 0.2* (10)	3.4 ± 0.2 (10)	6.5 ± 0.3 (10)	11.3 ± 0.3 (10)	16.9 ± 0.4* (10)	12 ± 1* (10)	235 ± 14 (10)	469 ± 12 (10)
30 mg/kg	1.3 ± 0.2 (10)	2.3 ± 0.2 (10)	4.1 ± 0.2 (10)	7.2 ± 0.2 (10)	12.0 ± 0.5 (10)	18.1 ± 0.7 (10)	13 ± 1 (10)	251 ± 15 (10)	490 ± 8 (10)
100 mg/kg	1.5 ± 0.1 (10)	2.4 ± 0.1 (10)	3.8 ± 0.2 (10)	6.8 ± 0.3 (10)	11.4 ± 0.4 (10)	19.2 ± 0.6 (10)	12 ± 1 (10)	241 ± 5 (10)	489 ± 7 (10)
300 mg/kg	2.5 ± 0.3** (8)	3.6 ± 0.4* (8)	5.4 ± 0.5** (8)	8.4 ± 0.7 (8)	12.9 ± 0.7 (8)	17.1 ± 0.9 (8)	16 ± 2 (8)	311 ± 37 (8)	472 ± 9 (8)
Anti Asialo GM1 1:10	1.5 ± 0.1* (6)	1.6 ± 0.2** (6)	2.2 ± 0.2** (6)	3.0 ± 0.4** (6)	4.0 ± 0.4** (6)	4.2 ± 0.4** (6)	0 ± 0** (6)	1 ± 0** (6)	512 ± 11** (6)
H/NH	H	NH	H	NH	H	H	NH	NH	H
Trend Analysis	p < 0.01	p < 0.01	p < 0.05	NS	NS	NS	NS	NS	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, anti asialo GM1, was administered i.v. on day 26. On day 29, after the last exposure, spleen cells were prepared into single cell suspensions and were assayed 4 hours later for natural killer cell activity using ^{51}Cr-labeled YAC cells as the target. The effector-to-target ratio was 200/1, 100/1, 50/1, 25/1, 12.5/1 and 6.25/1. Spontaneous release over the 4-hour incubation period was 6.6% of maximum release. Lytic Unit (LU) is defined as the number of splenocytes required to kill 10% of maximum release. The values represent the mean ± SE derived from the number of rats indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 61

Natural Killer Cell Activity in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days
(Peripheral Blood Leukocytes)

Study 6

Exposure	Effector/Target Ratio 3.125/1	6.25/1	12.5/1	25/1	50/1	100/1	LU/ 10^7 Cells	LU/ml of Blood
Vehicle	-0.5 ± 0.2 (10)	0.5 ± 0.2 (10)	1.9 ± 0.4 (10)	5.0 ± 0.7 (10)	10.5 ± 1.3 (10)	15.0 ± 1.5 (10)	21 ± 3 (10)	11 ± 1 (10)
Octamethylcyclotetrasiloxane								
10 mg/kg	0.3 ± 0.3 (8)	1.2 ± 0.6 (8)	2.9 ± 0.8 (8)	6.7 ± 1.4 (8)	13.2 ± 2.1 (8)	18.1 ± 2.3 (8)	28 ± 7 (8)	12 ± 3 (8)
30 mg/kg	-0.1 ± 0.2 (10)	0.8 ± 0.3 (10)	2.7 ± 0.5 (10)	6.0 ± 0.8 (10)	11.2 ± 1.0 (10)	15.3 ± 1.6 (10)	22 ± 3 (10)	10 ± 2 (10)
100 mg/kg	-0.2 ± 0.4 (10)	1.0 ± 0.4 (10)	3.1 ± 1.1 (10)	6.2 ± 1.6 (10)	12.2 ± 1.9 (10)	18.8 ± 2.1 (10)	27 ± 7 (10)	12 ± 3 (10)
300 mg/kg	0.3 ± 0.3 (8)	1.2 ± 0.3 (8)	2.4 ± 0.5 (8)	5.8 ± 0.9 (8)	11.0 ± 0.9 (8)	16.4 ± 0.7 (8)	25 ± 3 (8)	8 ± 1 (8)
Anti Asialo GM1 1:10	0.4 ± 0.5 (5)	1.3 ± 0.7 (5)	2.5 ± 1.0 (5)	4.8 ± 1.8 (5)	8.5 ± 2.7 (5)	8.6 ± 2.9* (5)	16 ± 6 (5)	2 ± 1** (5)
H/NH	H	H	NH	H	H	NH	NH	NH
Trend Analysis	NS	NS	NS	NS	NS	NS	NS	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, after the last exposure, whole blood was collected into EDTA tubes. The red blood cells were lysed. Natural killer cell activity was assayed using ^{51}Cr-labeled YAC cells as the target. The effector-to-target ratio was 100/1, 50/1, 25/1, 12.5/1, 6.25/1 and 3.125/1. Spontaneous release over the 4-hour incubation period was 11.3% of maximum release. Results are expressed as percent lysis of target cells and lytic units per 10^7 cell and ml of blood. Lytic Unit (LU) is defined as the number of splenocytes required to kill 10% of the target cells. The values represent the mean ± SE derived from the number of rats indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 62

Natural Killer Cell Activity in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days (Peripheral Blood Leukocytes)

Study 5

Exposure	Effector/Target Ratio 100/1	50/1	25/1
Vehicle	15.9 ± 1.8 (9)	13.0 ± 1.3 (9)	9.2 ± 0.8 (9)
Octamethylcyclotetrasiloxane			
10 mg/kg	16.3 ± 1.6 (8)	13.6 ± 1.1 (8)	10.2 ± 0.7 (8)
30 mg/kg	24.7 ± 2.7* (7)	21.1 ± 2.0** (7)	15.2 ± 1.2** (7)
100 mg/kg	17.9 ± 1.9 (9)	16.7 ± 1.6 (9)	12.0 ± 1.2 (9)
300 mg/kg	26.8 ± 2.5** (9)	20.4 ± 1.5** (9)	13.9 ± 1.9* (9)
Anti Asialo GM1 1:10	7.6 ± 1.2* (3)	7.1 ± 0.6* (3)	3.6 ± 0.6** (3)
H/NH	H	H	NH
Trend Analysis	p < 0.01	p < 0.01	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, after the last exposure, whole blood was collected by cardiac puncture into EDTA tubes. The red blood cells were lysed. Natural killer cell activity was assayed using ^{51}Cr-labeled YAC cells as the target. The effector-to-target ratio was 100/1, 50/1 and 25/1. Spontaneous release over the 4-hour incubation period was 8.0% of maximum release. Results are expressed as percent lysis of target cells. The values represent the mean ± SE derived from the number of rats indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix D - Table 63

Natural Killer Cell Activity in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days (Spleen Cells)

Study 5

Exposure	Effector/Target Ratio 100/1	50/1	25/1
Vehicle	25.0 ± 1.3 (10)	15.5 ± 0.9 (10)	10.2 ± 0.5 (10)
Octamethylcyclotetrasiloxane			
10 mg/kg	22.6 ± 1.8 (10)	13.7 ± 1.1 (10)	8.9 ± 0.9 (10)
30 mg/kg	29.8 ± 1.2 (10)	17.7 ± 0.7 (10)	12.9 ± 0.5* (10)
100 mg/kg	24.9 ± 1.1 (10)	15.8 ± 0.6 (10)	11.4 ± 0.5 (10)
300 mg/kg	21.3 ± 1.5 (10)	12.9 ± 1.1 (10)	10.2 ± 0.8 (10)
Anti Asialo GM1 1:10	1.7 ± 0.1** (6)	0.7 ± 0.1** (6)	0.6 ± 0.1** (6)
H/NH	H	H	H
Trend Analysis	NS	NS	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, anti asialo GM1, was administered i.v. on day 28. On day 29, after the last exposure, spleen cells were prepared into single cell suspensions and were assayed 4 hours later for natural killer cell activity using ^{51}Cr-labeled YAC cells as the target. The effector-to-target ratio was 100/1, 50/1 and 25/1. Spontaneous release over the 4-hour incubation period was 5.4% of maximum release. The values represent the mean ± SE derived from the number of rats indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk, while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix D - Table 64

Body Weights (g) and Liver Weights (mg) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Studies 8.1, 8.2, 8.3 and 8.4

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)						Naive	H/NH	Trend Analysis
		1	3	10	30	100	300			
	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)		
Study 8.1	Corn Oil									
Body Wt (g)	228.5 ± 3.1	224.0 ± 3.1	235.7 ± 4.2	229.4 ± 5.2	223.1 ± 5.9	231.2 ± 3.6	226.1 ± 4.6		H	NS
Liver (mg)	9097 ± 288	8620 ± 183	9665 ± 227	9254 ± 360	9613 ± 465	10764 ± 201**	13080 ± 261**		H	p < 0.01
% Body Wt	4.0 ± 0.1	3.8 ± 0.0	4.1 ± 0.1	4.0 ± 0.1	4.3 ± 0.1*	4.7 ± 0.0**	5.8 ± 0.0**		NH	p < 0.01
Study 8.2	Maalox									
Body Wt (g)	225.0 ± 4.5	223.8 ± 6.0	226.2 ± 3.7	227.4 ± 4.2	229.5 ± 5.7	221.9 ± 3.6	224.1 ± 4.5		H	NS
Liver (mg)	9288 ± 251	9004 ± 391	9106 ± 285	9252 ± 277	9760 ± 420	9208 ± 284	9840 ± 398		H	NS
% Body Wt	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.4 ± 0.1		H	p < 0.01
Study 8.3	Emulphor									
Body Wt (g)	219.9 ± 4.7	219.1 ± 4.1	224.1 ± 4.1	233.9 ± 6.2	225.1 ± 5.0	233.0 ± 3.2	220.1 ± 4.5		H	NS
Liver (mg)	9134 ± 342	9241 ± 353	9457 ± 266	10345 ± 513	9678 ± 432	10473 ± 210*	10497 ± 248*		H	p < 0.01
% Body Wt	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.5 ± 0.1*	4.8 ± 0.1**		H	p < 0.01
Study 8.4	35 cs PDMS Fluid									
Body Wt (g)	220.9 ± 4.8	228.7 ± 4.7	228.1 ± 5.2	221.0 ± 4.3	225.1 ± 3.9	218.1 ± 4.9[a]	220.1 ± 6.0[a]	230.5 ± 4.8	H	NS
Liver (mg)	9206 ± 330	9454 ± 360	9183 ± 393	8648 ± 322	8915 ± 365	8937 ± 391[a]	8747 ± 414[a]	8935 ± 296	H	NS
% Body Wt	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	4.1 ± 0.1[a]	4.0 ± 0.1[a]	3.9 ± 0.1	H	NS

Male Fischer 344 rats were administered either naive (nothing), vehicle 8.1 (corn oil), vehicle 8.2 (Maalox), vehicle 8.3 (emulphor), vehicle 8.4 (35 cs PDMS fluid) or octamethylcyclotetrasiloxane by gavage daily for 28 days. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix D - Table 65

Body Weights (g) and Liver Weights (mg) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Studies 7.1, 7.2, 7.3 and 7.4

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)						Naive	H/NH	Trend Analysis
		1	3	10	30	100	300			
	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)		
Study 7.1	Corn Oil									
Body Wt (g)	157.3 ± 2.5	158.1 ± 2.9	157.1 ± 2.8	156.4 ± 2.3	159.9 ± 1.6	157.4 ± 3.5	157.6 ± 1.3		H	NS
Liver (mg)	5268 ± 203	5249 ± 140	5073 ± 188	5201 ± 127	5624 ± 136	6672 ± 223**	9037 ± 92**		H	p < 0.01
% Body Wt	3.3 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	3.5 ± 0.1	4.2 ± 0.1**	5.7 ± 0.0**		H	p < 0.01
Study 7.2	Maalox									
Body Wt (g)	159.6 ± 1.6	157.2 ± 2.7	153.4 ± 1.6	158.7 ± 1.9	158.3 ± 2.6	157.5 ± 2.1[a]	157.5 ± 1.7		H	NS
Liver (mg)	5337 ± 78	5168 ± 140	5074 ± 112	5342 ± 147	5441 ± 124	5527 ± 126[a]	6302 ± 134**		H	p < 0.01
% Body Wt	3.3 ± 0.0	3.3 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.4 ± 0.0	3.5 ± 0.1[a]	4.0 ± 0.1**		H	p < 0.01
Study 7.3	Emulphor									
Body Wt (g)	159.8 ± 2.1	153.3 ± 2.6	153.8 ± 1.7	155.4 ± 1.7	159.9 ± 2.3	161.0 ± 1.9	162.0 ± 2.3		H	p < 0.05
Liver (mg)	5289 ± 126	4902 ± 171	5202 ± 226	5215 ± 167	5418 ± 156	6060 ± 172*	7399 ± 147**		H	p < 0.01
% Body Wt	3.3 ± 0.0	3.2 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.8 ± 0.1**	4.6 ± 0.1**		H	p < 0.01
Study 7.4	35 cs PDMS Fluid									
Body Wt (g)	155.8 ± 3.0	157.0 ± 2.2	153.5 ± 2.0	157.2 ± 3.1	155.6 ± 3.6	157.7 ± 2.0	160.1 ± 2.6	159.0 ± 3.4	H	NS
Liver (mg)	4914 ± 176	4931 ± 100	4792 ± 154	4809 ± 192	4933 ± 195	5135 ± 179	5178 ± 142	5470 ± 176	H	NS
% Body Wt	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.4 ± 0.1*	H	NS

Female Fischer 344 rats were administered either naive (nothing), vehicle 7.1 (corn oil), vehicle 7.2 (Maalox), vehicle 7.3 (emulphor), vehicle 7.4 (35 cs PDMS fluid) or octamethylcyclotetrasiloxane by gavage daily for 28 days. On day 29 after the last exposure, the rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

APPENDIX E

BODY WEIGHT DATA TABLES

Appendix E - Table 1

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 1

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				Cyclophosphamide	H/NH	Trend Analysis
		10	30	100	300	25 mg/kg		
	(10)	(10)	(10)	(10)	(10)	(6)		
				Body Weight				
Day 1	150.6 ± 1.6	151.0 ± 1.8	150.2 ± 1.6	151.0 ± 1.7	150.1 ± 1.1	149.7 ± 2.1	H	NS
Day 8	151.4 ± 1.8	152.8 ± 1.7	152.1 ± 1.7	154.8 ± 2.8	149.9 ± 1.4	153.4 ± 2.5	H	NS
Day 15	154.4 ± 1.8	155.2 ± 1.7	155.3 ± 1.4	157.6 ± 3.3	156.0 ± 1.4	155.8 ± 2.4	H	NS
Day 22	156.2 ± 1.6	158.3 ± 1.5	157.4 ± 1.4	160.5 ± 3.2	158.5 ± 1.9	158.1 ± 1.5	NH	NS
Day 29	157.3 ± 1.7	159.1 ± 1.7	157.9 ± 1.1	160.9 ± 3.8	158.6 ± 1.8	154.3 ± 2.0	NH	NS
				Body Weight Changes				
Day 8-Day 1	0.77 ± 0.82	1.82 ± 1.05	1.89 ± 0.69	3.75 ± 1.46	-0.11 ± 1.14	3.65 ± 1.04*	H	NS
Day 15-Day 1	3.82 ± 0.80	4.24 ± 1.18	5.13 ± 0.44	6.59 ± 1.95	5.94 ± 1.42	6.07 ± 1.53	NH	NS
Day 22-Day 1	5.63 ± 0.81	7.37 ± 1.34	7.26 ± 0.84	9.51 ± 1.93	8.45 ± 2.14	8.42 ± 1.27	NH	NS
Day 29-Day 1	6.66 ± 1.04	8.15 ± 1.33	7.74 ± 1.14	9.90 ± 2.40	8.54 ± 1.95	4.58 ± 0.93	H	NS

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 2

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 2

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Body Weight								
Day 1	188.3 ± 1.9	188.5 ± 1.5	188.6 ± 2.5	188.2 ± 1.6	191.2 ± 1.5	186.1 ± 2.5	H	NS
Day 8	199.4 ± 1.5	199.3 ± 4.0	204.4 ± 3.1	201.8 ± 1.4	202.6 ± 1.8	206.8 ± 3.0*	NH	NS
Day 15	217.0 ± 1.2	224.6 ± 6.2	221.9 ± 4.5	221.2 ± 1.8	223.3 ± 1.6	228.4 ± 3.8**	NH	$p < 0.05$
Day 22	226.9 ± 1.7	228.1 ± 3.3	233.5 ± 4.2	231.9 ± 2.5	230.7 ± 1.5	241.6 ± 3.7**	NH	NS
Day 29	232.8 ± 2.4	234.1 ± 3.9	242.0 ± 5.3	238.8 ± 3.3	241.4 ± 1.5	235.7 ± 4.4	NH	$p < 0.05$
Body Weight Changes								
Day 8-Day 1	11.09 ± 1.64	10.83 ± 3.83	15.83 ± 1.45	13.53 ± 1.83	11.41 ± 1.29	20.75 ± 1.57**	NH	NS
Day 15-Day 1	28.69 ± 1.83	36.11 ± 5.68	33.29 ± 2.78	32.98 ± 2.35	32.06 ± 1.71	42.35 ± 2.81**	NH	NS
Day 22-Day 1	38.63 ± 2.28	39.59 ± 3.12	44.93 ± 2.79	43.70 ± 2.93	39.47 ± 1.50	55.52 ± 3.07**	H	NS
Day 29-Day 1	44.57 ± 2.98	45.65 ± 3.64	53.47 ± 4.23	50.51 ± 3.81	50.15 ± 1.65	49.63 ± 3.45	H	$p < 0.05$

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 3

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 3

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	Cyclophosphamide 25 mg/kg (6)	H/NH	Trend Analysis
				Body Weight				
Day 1	174.9 ± 1.5	172.6 ± 1.8	173.1 ± 2.1	173.9 ± 2.3	170.7 ± 2.3	173.1 ± 2.2	H	NS
Day 8	197.0 ± 1.7	197.4 ± 2.2	200.0 ± 2.7	198.0 ± 3.6	191.1 ± 2.4	199.7 ± 2.5	H	NS
Day 15	211.9 ± 2.6	210.6 ± 3.1	215.4 ± 3.9	213.0 ± 4.3	205.7 ± 3.0	215.9 ± 3.8	H	NS
Day 22	224.6 ± 3.7[a]	221.0 ± 4.0	225.7 ± 4.0	222.8 ± 4.9	213.9 ± 3.1	227.6 ± 3.6	H	$p < 0.05$
Day 29	232.5 ± 4.5[a]	231.8 ± 5.0	237.3 ± 4.9	236.5 ± 5.3	224.0 ± 3.6	231.0 ± 4.0	H	NS
				Body Weight Changes				
Day 8-Day 1	22.05 ± 1.05	24.80 ± 1.69	26.91 ± 1.07**	24.10 ± 2.60	20.37 ± 1.03	26.58 ± 1.78*	NH	NS
Day 15-Day 1	37.00 ± 2.14	38.06 ± 2.65	42.28 ± 2.36	39.10 ± 3.31	35.04 ± 2.10	42.83 ± 3.35	H	NS
Day 22-Day 1	49.21 ± 3.23[a]	48.42 ± 3.86	52.57 ± 2.66	48.86 ± 4.44	43.24 ± 2.46	54.53 ± 3.34	H	NS
Day 29-Day 1	57.12 ± 4.18[a]	59.25 ± 4.93	64.21 ± 3.46	60.61 ± 5.08	53.32 ± 3.17	57.92 ± 3.92	H	NS

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix E - Table 4

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 4

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Body Weight								
Day 1	157.0 ± 1.7	151.6 ± 1.5	153.7 ± 1.5	154.8 ± 1.3	155.2 ± 0.8	152.2 ± 2.5	H	NS
Day 8	158.2 ± 1.4	152.4 ± 1.3*	156.6 ± 1.6	157.0 ± 1.3	157.0 ± 1.3	155.1 ± 2.2	H	NS
Day 15	159.8 ± 1.6	154.4 ± 1.4*	157.9 ± 1.6	161.1 ± 1.3	162.8 ± 1.2	156.5 ± 2.2	H	$p < 0.05$
Day 22	162.2 ± 1.9	157.2 ± 1.5	161.6 ± 1.8	164.6 ± 1.5	166.5 ± 1.8	158.8 ± 2.5	H	$p < 0.01$
Day 29	164.6 ± 2.1	161.3 ± 1.9	166.6 ± 2.1	168.2 ± 1.5	171.6 ± 2.1	152.4 ± 1.7**	H	$p < 0.01$
Body Weight Changes								
Day 8-Day 1	1.21 ± 0.61	0.85 ± 0.75	2.82 ± 0.30	2.20 ± 0.83	1.80 ± 0.73	2.95 ± 0.80	H	NS
Day 15-Day 1	2.73 ± 1.09	2.87 ± 0.77	4.17 ± 0.50	6.36 ± 0.99*	7.60 ± 0.86**	4.32 ± 0.93	H	$p < 0.01$
Day 22-Day 1	5.16 ± 1.32	5.66 ± 1.09	7.84 ± 0.74	9.78 ± 0.95*	11.33 ± 1.51**	6.62 ± 1.34	H	$p < 0.01$
Day 29-Day 1	7.60 ± 1.43	9.73 ± 1.14	12.90 ± 1.09*	13.40 ± 1.30*	16.41 ± 2.00**	0.23 ± 2.35*	H	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 5

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 5

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				CPS	AAGM1	H/NH	Trend
		10	30	100	300	25 mg/kg	1:10		Analysis
	(10)	(10)	(10)	(10)	(10)	(6)	(6)		
Body Weight									
Day 1	147.4 ± 1.6	146.0 ± 1.9	148.2 ± 1.0	147.3 ± 2.0	150.6 ± 1.6	148.6 ± 1.1	146.0 ± 2.8	H	NS
Day 8	150.1 ± 1.7	150.0 ± 1.5	151.5 ± 1.4	152.2 ± 1.8	152.8 ± 1.6	153.7 ± 0.8	150.0 ± 3.2	H	NS
Day 15	151.3 ± 1.6	150.8 ± 1.8	152.4 ± 1.5	154.4 ± 2.1	157.2 ± 1.5	155.1 ± 1.1	151.2 ± 3.4	H	$p < 0.01$
Day 22	155.3 ± 1.7	153.1 ± 2.3	156.5 ± 1.5	159.0 ± 2.0	159.1 ± 1.5	158.8 ± 2.1	155.7 ± 3.6	H	$p < 0.05$
Day 29	156.6 ± 1.5	156.9 ± 1.4	158.3 ± 1.6	161.6 ± 1.7	162.3 ± 1.8	154.9 ± 2.3	156.4 ± 3.8	H	$p < 0.01$
Body Weight Changes									
Day 8-Day 1	2.65 ± 0.58	4.08 ± 0.85	3.32 ± 0.76	4.90 ± 0.75	2.25 ± 0.49	5.08 ± 0.65*	4.05 ± 0.84	H	NS
Day 15-Day 1	3.90 ± 0.88	4.80 ± 1.05	4.22 ± 0.92	7.08 ± 0.98	6.62 ± 0.75	6.50 ± 0.84	5.22 ± 1.26	H	$p < 0.01$
Day 22-Day 1	7.86 ± 0.82	7.16 ± 1.71	8.32 ± 1.18	11.70 ± 1.07	8.58 ± 0.89	10.17 ± 1.46	9.75 ± 1.40	H	NS
Day 29-Day 1	9.17 ± 1.15	10.94 ± 1.26	10.15 ± 1.30	14.28 ± 1.26*	11.73 ± 1.09	6.35 ± 1.98	10.45 ± 1.71	H	$p < 0.05$

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 6

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 6

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	AAGM1 1:10 (6)	H/NH	Trend Analysis
				Body Weight					
Day 1	175.5 ± 1.7	177.7 ± 1.3	175.7 ± 1.4	175.3 ± 1.3	176.2 ± 1.3	178.7 ± 2.2	174.2 ± 2.0	H	NS
Day 8	196.5 ± 2.5	201.9 ± 2.9	200.7 ± 2.0	200.6 ± 1.8	194.0 ± 1.2[a]	205.5 ± 3.4*	201.6 ± 1.9	H	NS
Day 15	212.1 ± 2.7	217.8 ± 4.4	219.6 ± 3.0	216.5 ± 2.5	212.4 ± 1.6[b]	223.7 ± 3.8*	220.5 ± 2.9	H	NS
Day 22	217.4 ± 3.0	223.6 ± 4.7	225.6 ± 3.1	223.2 ± 2.3	216.1 ± 2.4[b]	230.1 ± 4.3*	226.7 ± 3.2	H	NS
Day 29	229.6 ± 3.6	238.9 ± 6.1	240.9 ± 3.8*	238.6 ± 2.4	227.6 ± 2.7[b]	232.5 ± 4.0	239.9 ± 3.9	NH	NS
				Body Weight Changes					
Day 8-Day 1	21.02 ± 1.20	24.22 ± 1.93	24.99 ± 1.84	25.26 ± 0.84	17.76 ± 1.74[a]	26.85 ± 1.70*	27.67 ± 1.03**	H	NS
Day 15-Day 1	36.64 ± 1.53	40.15 ± 3.52	43.91 ± 2.61	41.16 ± 1.42	36.11 ± 2.58[b]	45.08 ± 2.35**	46.33 ± 1.90**	NH	NS
Day 22-Day 1	41.96 ± 1.95	45.96 ± 3.83	49.95 ± 2.86	47.91 ± 1.23	39.88 ± 3.50[b]	51.43 ± 3.71*	52.53 ± 2.06**	NH	NS
Day 29-Day 1	54.13 ± 2.61	61.18 ± 5.27	65.24 ± 3.63	63.34 ± 1.55	51.39 ± 3.41[b]	53.87 ± 3.41	65.73 ± 4.23*	NH	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals; [b]N = 8 animals

Appendix E - Table 7

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.1

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
				Body Weight					
Day 0	147.4 ± 1.8	147.0 ± 2.0	147.7 ± 2.3	146.6 ± 1.9	147.8 ± 1.7	146.9 ± 1.8	147.1 ± 2.0	H	NS
Day 8	151.3 ± 2.0	150.8 ± 2.2	150.7 ± 2.2	148.8 ± 1.9	150.8 ± 1.7	151.1 ± 2.5	151.1 ± 1.8	H	NS
Day 15	152.2 ± 2.1	152.7 ± 2.0	152.6 ± 2.1	150.5 ± 2.2	153.7 ± 1.7	153.5 ± 2.6	154.1 ± 1.1	H	NS
Day 22	153.4 ± 2.1	154.9 ± 2.2	153.8 ± 2.5	153.4 ± 2.5	156.0 ± 1.9	154.4 ± 2.7	154.8 ± 1.0	H	NS
Day 29	157.3 ± 2.5	158.1 ± 2.5	157.1 ± 2.8	156.4 ± 2.3	159.9 ± 1.6	157.4 ± 3.5	157.6 ± 1.3	H	NS
				Body Weight Changes					
Day 8-Day 0	3.85 ± 0.54	3.81 ± 0.78	3.02 ± 1.52	2.22 ± 1.05	3.01 ± 1.02	4.15 ± 1.04	3.97 ± 1.23	H	NS
Day 15-Day 0	4.80 ± 0.67	5.70 ± 1.24	4.95 ± 1.47	3.85 ± 1.19	5.95 ± 1.15	6.62 ± 1.32	7.00 ± 1.50	H	NS
Day 22-Day 0	5.98 ± 0.91	7.83 ± 1.55	6.14 ± 1.89	6.82 ± 1.57	8.25 ± 1.71	7.54 ± 1.53	7.68 ± 1.64	H	NS
Day 29-Day 0	9.84 ± 1.20	11.11 ± 1.76	9.38 ± 2.12	9.80 ± 1.32	12.15 ± 1.91	10.48 ± 2.35	10.48 ± 1.94	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 8

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.2

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
				Body Weight					
Day 0	147.5 ± 2.3	147.4 ± 2.0	147.5 ± 2.1	147.0 ± 1.7	146.8 ± 2.0	146.7 ± 1.8	147.5 ± 2.1	H	NS
Day 8	153.4 ± 2.3	150.0 ± 2.0	149.3 ± 1.8	151.4 ± 1.9	150.0 ± 2.3	150.0 ± 1.7	149.9 ± 1.8	H	NS
Day 15	153.8 ± 1.9	151.9 ± 2.2	149.4 ± 1.8	153.5 ± 2.0	151.0 ± 2.0	150.4 ± 1.8	150.2 ± 1.6	H	NS
Day 22	155.4 ± 1.4	154.2 ± 2.1	151.4 ± 1.4	156.2 ± 1.9	154.4 ± 2.5	153.0 ± 2.1	153.9 ± 1.4	H	NS
Day 29	159.6 ± 1.6	157.2 ± 2.7	153.4 ± 1.6	158.7 ± 1.9	158.3 ± 2.6	157.5 ± 2.1[a]	157.5 ± 1.7	H	NS
				Body Weight Changes					
Day 8-Day 0	5.98 ± 0.97	2.56 ± 1.23	1.80 ± 1.29	4.32 ± 0.87	3.16 ± 1.27	3.34 ± 0.83	2.42 ± 1.55	H	NS
Day 15-Day 0	6.29 ± 1.54	4.46 ± 1.53	1.92 ± 1.33	6.50 ± 1.26	4.21 ± 1.68	3.70 ± 1.87	2.69 ± 1.49	H	NS
Day 22-Day 0	7.96 ± 1.38	6.81 ± 1.45	3.93 ± 1.56	9.15 ± 1.60	7.61 ± 1.87	6.25 ± 2.13	6.45 ± 1.81	H	NS
Day 29-Day 0	12.10 ± 1.47	9.80 ± 1.77	5.94 ± 1.67	11.65 ± 1.57	11.44 ± 2.21	10.90 ± 1.96[a]	10.04 ± 2.03	H	NS

Female Fischer 344 rats were administered vehicle (Maalox) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix E - Table 9

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.3

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
				Body Weight					
Day 0	147.6 ± 2.3	147.4 ± 1.6	147.0 ± 1.6	147.2 ± 1.9	147.6 ± 1.8	148.2 ± 2.1	146.9 ± 2.0	H	NS
Day 8	150.0 ± 2.5	147.4 ± 1.6	149.9 ± 1.3	150.5 ± 1.8	151.2 ± 2.1	152.5 ± 1.9	152.8 ± 2.2	H	NS
Day 15	151.6 ± 2.5	148.2 ± 1.8	150.5 ± 1.7	150.3 ± 2.1	152.8 ± 2.1	154.7 ± 1.6	156.7 ± 2.0	H	$p < 0.01$
Day 22	154.9 ± 2.4	149.4 ± 1.8	152.9 ± 1.7	153.0 ± 1.7	156.3 ± 2.5	157.6 ± 1.5	159.0 ± 2.4	H	$p < 0.01$
Day 29	159.8 ± 2.1	153.3 ± 2.6	153.8 ± 1.7	155.4 ± 1.7	159.9 ± 2.3	161.0 ± 1.9	162.0 ± 2.3	H	$p < 0.05$
				Body Weight Changes					
Day 8-Day 0	2.37 ± 1.29	-0.01 ± 0.74	2.84 ± 0.98	3.29 ± 1.06	3.62 ± 1.37	4.35 ± 1.33	5.87 ± 0.64	H	$p < 0.01$
Day 15-Day 0	3.97 ± 1.81	0.33 ± 1.19	3.50 ± 1.22	3.09 ± 1.10	5.22 ± 1.78	6.52 ± 1.13	9.77 ± 1.23*	H	$p < 0.01$
Day 22-Day 0	7.28 ± 1.65	2.08 ± 1.42	5.90 ± 1.74	5.76 ± 1.03	8.70 ± 1.90	9.42 ± 1.20	12.03 ± 1.24	H	$p < 0.01$
Day 29-Day 0	12.14 ± 1.55	5.95 ± 1.94	6.81 ± 1.72	8.19 ± 1.39	12.38 ± 1.94	12.78 ± 1.52	15.07 ± 1.68	H	$p < 0.01$

Female Fischer 344 rats were administered vehicle (emulphor) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 10

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.4

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	Naive (10)	H/NH	Trend Analysis
					Body Weight					
Day 0	147.3 ± 2.4	146.9 ± 1.8	146.8 ± 2.0	147.6 ± 1.9	147.4 ± 1.9	147.8 ± 1.6	147.5 ± 2.1	146.7 ± 1.7	H	NS
Day 8	146.9 ± 3.0	151.3 ± 1.7	147.0 ± 2.0	149.7 ± 1.9	150.4 ± 2.5	151.9 ± 1.9	153.6 ± 2.2	151.0 ± 2.1	H	p < 0.05
Day 15	149.7 ± 3.0	152.7 ± 1.6	148.2 ± 2.0	151.2 ± 2.6	152.4 ± 3.0	153.7 ± 2.0	155.6 ± 2.0	153.3 ± 2.0	H	p < 0.05
Day 22	153.7 ± 2.6	155.1 ± 2.0	151.2 ± 1.9	154.1 ± 2.8	153.6 ± 3.2	155.5 ± 1.9	158.0 ± 2.5	157.2 ± 2.6	H	NS
Day 29	155.8 ± 3.0	157.0 ± 2.2	153.5 ± 2.0	157.2 ± 3.1	155.6 ± 3.6	157.7 ± 2.0	160.1 ± 2.6	159.0 ± 2.4	H	NS
					Body Weight Changes					
Day 8-Day 0	-0.48 ± 2.38	4.45 ± 0.69	0.27 ± 0.82	2.08 ± 1.54	3.03 ± 1.11	4.11 ± 1.20	6.12 ± 0.95	4.30 ± 1.10	NH	p < 0.01
Day 15-Day 0	2.32 ± 1.76	5.87 ± 0.86	1.46 ± 1.72	3.66 ± 2.32	5.03 ± 1.47	5.89 ± 1.54	8.06 ± 1.19	6.59 ± 1.21	H	p < 0.05
Day 22-Day 0	6.36 ± 1.64	8.23 ± 0.68	4.46 ± 1.56	6.49 ± 2.35	6.23 ± 1.70	7.73 ± 1.59	10.49 ± 1.37	10.51 ± 1.54	H	NS
Day 29-Day 0	8.42 ± 1.85	10.14 ± 1.01	6.73 ± 1.56	9.62 ± 2.63	8.26 ± 2.13	9.91 ± 1.63	12.63 ± 1.77	12.29 ± 1.54	H	NS

Female Fischer 344 rats were administered nothing (naive), vehicle (35 cs PDMS fluid) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 11

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.1

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Body Weight									
Day 0	173.9 ± 2.0	173.3 ± 2.7	173.9 ± 2.4	173.4 ± 2.6	172.6 ± 2.0	174.0 ± 2.5	174.9 ± 2.3	H	NS
Day 8	196.6 ± 2.1	192.3 ± 2.5	196.5 ± 3.0	194.5 ± 3.0	193.6 ± 3.0	193.5 ± 3.6	191.4 ± 2.7	H	NS
Day 15	209.7 ± 2.8	206.2 ± 3.0	213.7 ± 3.4	208.8 ± 3.6	206.2 ± 3.8	209.9 ± 4.1	206.4 ± 3.3	H	NS
Day 22	221.2 ± 3.2	217.6 ± 2.9	228.2 ± 3.9	221.1 ± 4.3	216.4 ± 4.9	222.7 ± 4.2	219.6 ± 3.9	H	NS
Day 29	228.5 ± 3.1	224.0 ± 3.1	235.7 ± 4.2	229.4 ± 5.2	223.1 ± 5.9	231.2 ± 3.6	226.1 ± 4.6	H	NS
Body Weight Changes									
Day 8-Day 0	23.75 ± 1.62	18.99 ± 0.64	22.62 ± 1.33	21.16 ± 1.70	21.00 ± 1.85	19.46 ± 2.18	16.54 ± 2.11	NH	$p < 0.05$
Day 15-Day 0	35.85 ± 3.15	32.89 ± 1.34	39.81 ± 1.74	35.48 ± 2.47	33.64 ± 2.72	35.92 ± 2.99	31.56 ± 3.18	H	NS
Day 22-Day 0	47.26 ± 2.39	44.30 ± 2.08	54.27 ± 2.55	47.73 ± 3.36	43.82 ± 3.92	48.65 ± 3.13	44.73 ± 4.21	H	NS
Day 29-Day 0	54.65 ± 2.19	50.70 ± 2.16	61.78 ± 3.12	56.02 ± 4.16	50.46 ± 4.94	57.17 ± 2.58	51.25 ± 5.12	NH	NS

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 12

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.2

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Body Weight									
Day 0	173.5 ± 2.6	174.6 ± 2.3	175.0 ± 2.4	173.1 ± 1.9	173.5 ± 2.4	173.7 ± 1.9	174.5 ± 2.0	H	NS
Day 8	190.3 ± 3.6	193.2 ± 3.7	192.3 ± 2.6	191.8 ± 3.0	191.7 ± 3.5	191.4 ± 2.9	194.5 ± 2.8	H	NS
Day 15	202.6 ± 3.7	206.1 ± 5.0	205.9 ± 2.8	205.7 ± 3.5	205.6 ± 4.5	203.7 ± 3.1	207.1 ± 3.2	H	NS
Day 22	215.6 ± 4.2	217.2 ± 5.5	218.2 ± 3.7	216.7 ± 3.9	221.0 ± 5.2	216.9 ± 3.3	217.9 ± 4.3	H	NS
Day 29	225.0 ± 4.5	223.8 ± 6.0	226.2 ± 3.7	227.4 ± 4.2	229.5 ± 5.7	221.9 ± 3.6	224.1 ± 4.5	H	NS
Body Weight Changes									
Day 8-Day 0	16.51 ± 1.55	18.63 ± 2.10	18.31 ± 1.06	18.64 ± 2.07	18.20 ± 2.33	17.68 ± 1.52	19.96 ± 1.98	H	NS
Day 15-Day 0	29.09 ± 2.21	31.52 ± 3.60	30.84 ± 1.71	32.59 ± 2.88	33.08 ± 3.45	30.03 ± 1.75	33.52 ± 2.78	H	NS
Day 22-Day 0	42.10 ± 3.85	42.53 ± 4.48	43.21 ± 2.59	45.60 ± 3.38	47.45 ± 4.06	41.15 ± 2.64	43.35 ± 4.01	H	NS
Day 29-Day 0	51.44 ± 3.18	49.19 ± 4.85	51.14 ± 2.42	54.29 ± 3.65	55.97 ± 4.45	48.14 ± 2.18	49.58 ± 4.33	H	NS

Male Fischer 344 rats were administered vehicle (Maalox) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 13

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.3

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Body Weight									
Day 0	172.9 ± 2.3	173.7 ± 2.4	173.6 ± 2.3	173.8 ± 2.2	173.9 ± 2.5	173.5 ± 2.3	173.4 ± 2.0	H	NS
Day 8	190.3 ± 3.3	191.3 ± 2.8	192.6 ± 2.3	197.1 ± 3.9	193.1 ± 3.3	193.9 ± 3.3	191.4 ± 2.8	H	NS
Day 15	200.8 ± 4.2	202.5 ± 3.3	203.3 ± 2.9	210.8 ± 4.9	206.8 ± 4.0	209.6 ± 3.4	203.2 ± 3.4	H	NS
Day 22	211.7 ± 4.7	211.9 ± 3.6	216.2 ± 3.3	224.8 ± 5.5	217.1 ± 4.5	224.6 ± 3.5	212.9 ± 3.9	H	NS
Day 29	219.9 ± 4.7	219.1 ± 4.1	224.1 ± 4.1	233.9 ± 6.2	225.1 ± 5.0	233.0 ± 3.2	220.1 ± 4.5	H	NS
Body Weight Changes									
Day 8-Day 0	17.36 ± 2.11	17.62 ± 0.73	19.01 ± 1.31	23.24 ± 2.04	19.14 ± 2.10	20.00 ± 2.37	18.07 ± 1.77	H	NS
Day 15-Day 0	27.92 ± 3.09	28.78 ± 1.69	29.78 ± 1.73	36.99 ± 3.21	30.85 ± 3.06	35.70 ± 2.64	29.86 ± 2.44	H	NS
Day 22-Day 0	38.81 ± 3.71	38.15 ± 2.18	42.61 ± 2.26	50.99 ± 3.86*	43.11 ± 3.82	50.71 ± 2.63*	39.51 ± 2.98	H	NS
Day 29-Day 0	47.00 ± 3.87	45.40 ± 2.76	50.56 ± 2.98	60.01 ± 4.62	51.12 ± 4.51	59.08 ± 2.47	46.69 ± 3.60	H	NS

Male Fischer 344 rats were administered vehicle (emulphor) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 14

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.4

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	Naive (10)	H/NH	Trend Analysis
Body Weight										
Day 0	173.4 ± 2.3	173.4 ± 2.2	174.4 ± 2.5	173.9 ± 2.2	172.7 ± 2.6	172.9 ± 2.3	173.5 ± 2.2	172.7 ± 2.7	H	NS
Day 8	190.9 ± 2.5	192.8 ± 3.4	198.1 ± 3.2	189.6 ± 2.3	190.7 ± 2.2	189.7 ± 2.8	190.3 ± 3.7	197.9 ± 3.1	H	NS
Day 15	201.6 ± 2.9	204.6 ± 4.1	208.7 ± 3.4	199.1 ± 2.7	201.7 ± 3.2	200.1 ± 3.4	201.7 ± 4.0	212.6 ± 4.2	H	NS
Day 22	212.6 ± 4.2	218.7 ± 4.6	220.0 ± 4.9	210.9 ± 3.7	217.1 ± 3.3	205.9 ± 4.0	212.8 ± 5.4[a]	223.0 ± 4.2	H	NS
Day 29	220.9 ± 4.8	228.7 ± 4.7	228.1 ± 5.2	221.0 ± 4.3	225.1 ± 3.9	218.1 ± 4.9[a]	220.1 ± 6.0[a]	230.5 ± 4.8	H	NS
Body Weight Changes										
Day 8-Day 0	17.48 ± 2.10	19.46 ± 1.81	23.77 ± 1.32	15.65 ± 2.25	17.98 ± 2.09	16.74 ± 1.64	16.84 ± 1.89	25.13 ± 1.42*	H	NS
Day 15-Day 0	28.14 ± 2.55	31.27 ± 2.22	34.35 ± 1.50	25.15 ± 2.48	28.96 ± 3.28	27.19 ± 2.57	28.16 ± 2.36	39.88 ± 2.79**	H	NS
Day 22-Day 0	39.18 ± 3.70	45.35 ± 2.67	45.67 ± 3.33	36.94 ± 3.24	44.34 ± 4.00	[illegible] ± 3.58	[illegible].67 ± 3.56[a]	50.30 ± 3.33	H	NS
Day 29-Day 0	47.47 ± 4.20	55.33 ± 2.97	53.75 ± 3.28	47.03 ± 3.51	52.37 ± 4.66	45.79 ± 3.87[a]	46.96 ± 4.32[a]	57.78 ± 3.77	H	NS

Male Fischer 344 rats were administered nothing (naive), vehicle (35 cs PDMS fluid) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix E - Table 15

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 9

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	H/NH	Trend Analysis
				Body Weight				
Day 1	148.5 ± 1.6	148.2 ± 1.5	148.6 ± 1.5	147.2 ± 2.0	148.4 ± 1.4	147.2 ± 2.2	H	NS
Day 8	154.8 ± 1.7	154.5 ± 1.6	154.6 ± 1.9	152.5 ± 1.9	153.1 ± 1.9	150.6 ± 2.0	H	NS
Day 15	157.4 ± 1.9	157.6 ± 2.3	159.2 ± 2.3	157.2 ± 2.0	159.2 ± 2.2	153.2 ± 3.1	H	NS
Day 22	158.6 ± 1.6	158.6 ± 2.2	159.1 ± 2.7	159.7 ± 2.4	162.5 ± 2.1	154.2 ± 2.2	H	NS
Day 29	159.2 ± 1.6	159.3 ± 2.4	161.0 ± 2.6	159.2 ± 2.2[a]	164.4 ± 2.5	147.9 ± 2.5**	H	NS
				Body Weight Changes				
Day 8-Day 1	6.38 ± 0.95	6.27 ± 0.86	5.97 ± 0.96	5.29 ± 0.72	4.78 ± 1.11	3.45 ± 1.34	H	NS
Day 15-Day 1	8.89 ± 0.91	9.44 ± 1.24	10.56 ± 1.08	9.98 ± 0.72	10.88 ± 1.43	6.00 ± 1.62	H	NS
Day 22-Day 1	10.11 ± 1.23	10.39 ± 1.05	10.43 ± 1.43	12.56 ± 1.02	14.09 ± 1.54	7.01 ± 1.21	H	$p < 0.01$
Day 29-Day 1	10.73 ± 1.14	11.15 ± 1.02	12.37 ± 1.32	12.45 ± 0.61[a]	16.01 ± 1.96	0.78 ± 1.59**	NH	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix E - Table 16

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 9

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	CPS 25 mg/kg (6)
		Body Weight	
Day 1	149.7 ± 2.1	147.5 ± 2.1	146.4 ± 3.7
Day 8	155.0 ± 2.1	151.5 ± 2.6	152.1 ± 5.4
Day 15	158.5 ± 2.6	155.6 ± 2.6	155.0 ± 4.9
Day 22	158.6 ± 2.7	157.5 ± 2.7	157.2 ± 4.7
Day 29	158.8 ± 2.4	159.2 ± 2.8	158.1 ± 4.9
Day 36	164.9 ± 2.7	161.0 ± 2.8	162.3 ± 4.5
Day 43	167.2 ± 3.1	163.8 ± 2.5	158.6 ± 4.6
		Body Weight Changes	
Day 8-Day 1	5.29 ± 0.80	3.98 ± 0.95	5.71 ± 1.72
Day 15-Day 1	8.78 ± 0.85	8.06 ± 1.08	8.67 ± 1.44
Day 22-Day 1	8.86 ± 0.90	9.92 ± 1.16	10.85 ± 1.43
Day 29-Day 1	9.05 ± 0.92	11.61 ± 1.35	11.78 ± 1.71
Day 36-Day 1	15.19 ± 0.82	13.42 ± 1.47	15.90 ± 1.02
Day 43-Day 1	17.43 ± 1.33	16.26 ± 1.20	12.23 ± 1.27*

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, CPS, i.p. on days 39-42. Body weights were measured on days 1, 8, 15, 22, 29, 36 and 43. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix E - Table 17

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 10

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 25 mg/kg (6)	H/NH	Trend Analysis
				Body Weight				
Day 0	142.8 ± 1.4	142.2 ± 1.7	141.4 ± 1.5	143.1 ± 2.1	142.3 ± 1.6	141.6 ± 2.3	H	NS
Day 8	151.5 ± 2.2	149.4 ± 1.4	149.8 ± 2.3	150.3 ± 2.9	147.5 ± 3.2	149.4 ± 3.4	H	NS
Day 15	156.5 ± 2.6	155.9 ± 1.7	154.9 ± 2.5	158.2 ± 2.4	155.4 ± 2.7	155.7 ± 2.8	H	NS
Day 22	160.4 ± 3.0	158.4 ± 2.1	159.4 ± 2.3	160.9 ± 2.7	159.8 ± 3.5	158.4 ± 2.3	H	NS
Day 29	163.3 ± 2.9	162.2 ± 1.9	161.4 ± 2.5	163.3 ± 3.5	160.1 ± 3.2	153.0 ± 2.6*	H	NS
				Body Weight Changes				
Day 8-Day 0	9.14 ± 1.36	7.23 ± 0.62	8.35 ± 1.00	7.20 ± 1.52	5.29 ± 1.95	7.80 ± 1.36	H	NS
Day 15-Day 0	14.10 ± 1.99	13.71 ± 1.13	13.47 ± 1.39	15.09 ± 1.63	13.22 ± 1.75	14.06 ± 1.74	H	NS
Day 22-Day 0	17.56 ± 2.35	16.22 ± 1.52	17.97 ± 1.36	17.77 ± 1.33	16.64 ± 2.20	16.83 ± 2.17	H	NS
Day 29-Day 0	20.89 ± 2.34	19.96 ± 1.51	19.99 ± 1.53	20.14 ± 1.30	17.85 ± 2.13	11.34 ± 2.37*	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, MVE, was administered i.v. on day 28. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 18

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 10

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	MVE 25 mg/kg (6)
		Body Weight	
Day 0	142.8 ± 1.6	142.7 ± 2.4	143.2 ± 2.1
Day 8	150.1 ± 2.0	148.5 ± 2.7	150.2 ± 1.5
Day 15	155.7 ± 2.9	154.7 ± 3.5	157.7 ± 2.5
Day 22	159.4 ± 3.2	158.7 ± 4.6[a]	161.5 ± 2.3
Day 29	162.8 ± 2.9	160.4 ± 5.2[a]	162.9 ± 2.1
Day 36	164.3 ± 3.2	160.7 ± 5.1[a]	166.7 ± 2.8
Day 43	168.7 ± 3.5	164.1 ± 4.8[a]	161.2 ± 2.1
		Body Weight Changes	
Day 8-Day 0	7.32 ± 0.95	5.78 ± 1.14	6.95 ± 1.11
Day 15-Day 0	12.95 ± 1.59	11.93 ± 2.18	14.44 ± 2.03
Day 22-Day 0	16.66 ± 1.78	15.52 ± 3.01[a]	18.25 ± 2.82
Day 29-Day 0	20.04 ± 1.60	17.28 ± 3.47[a]	19.69 ± 2.24
Day 36-Day 0	21.52 ± 1.95	17.57 ± 3.43[a]	23.46 ± 3.02
Day 43-Day 0	25.92 ± 2.21	20.91 ± 3.70[a]	17.92 ± 2.64*

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 42. Body weights were measured on days 0, 8, 15, 22, 29, 36 and 43. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.
[a]N = 9 animals

Appendix E - Table 19

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 11

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Body Weight								
Day 1	187.3 ± 2.5	186.4 ± 2.2	185.4 ± 2.3	185.0 ± 1.8	184.9 ± 2.1	185.9 ± 2.3	H	NS
Day 8	211.9 ± 2.6	210.4 ± 2.5	210.5 ± 2.6	207.1 ± 2.1	200.1 ± 2.1**	215.7 ± 1.7	H	p < 0.01
Day 15	228.4 ± 3.4	226.2 ± 3.6	225.8 ± 3.2	221.5 ± 2.7	213.3 ± 2.4**	236.6 ± 2.0	H	p < 0.01
Day 22	237.0 ± 3.4	232.9 ± 4.2	235.6 ± 3.4	228.2 ± 2.8	218.9 ± 2.7**	247.7 ± 2.9*	H	p < 0.01
Day 29	245.6 ± 3.7	240.4 ± 4.5	243.0 ± 4.2	234.9 ± 3.1	224.6 ± 3.4**	240.6 ± 2.5	H	p < 0.01
Body Weight Changes								
Day 8-Day 1	24.60 ± 1.17	23.97 ± 1.31	25.13 ± 0.62	22.11 ± 0.87	15.25 ± 0.98**	29.75 ± 1.08*	H	p < 0.01
Day 15-Day 1	41.19 ± 2.13	39.80 ± 2.38	40.41 ± 2.28	36.55 ± 1.47	28.45 ± 1.34**	50.72 ± 1.05**	H	p < 0.01
Day 22-Day 1	49.74 ± 1.60	46.55 ± 3.06	50.23 ± 2.61	43.22 ± 1.57	34.05 ± 2.32**	61.75 ± 1.97**	H	p < 0.01
Day 29-Day 1	58.39 ± 1.94	54.01 ± 3.42	57.56 ± 3.33	49.87 ± 2.13	39.78 ± 3.32**	54.65 ± 1.17	H	p < 0.01

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, MVE, was administered i.v. on day 28. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 20

Body Weight (g) and Body Weight Changes (g) in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 11

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	MVE 50 mg/kg (6)
		Body Weight	
Day 1	185.5 ± 1.9	185.3 ± 1.6	186.1 ± 3.4
Day 8	208.1 ± 2.0	202.0 ± 2.3	211.6 ± 3.6
Day 15	223.7 ± 2.7	218.8 ± 2.7	230.2 ± 2.1
Day 22	232.4 ± 3.2	226.8 ± 3.5	242.2 ± 3.0
Day 29	239.1 ± 3.4	232.0 ± 3.7	251.4 ± 4.2*
Day 36	253.4 ± 3.5	243.0 ± 4.1	263.3 ± 4.5
Day 43	264.1 ± 4.2	256.9 ± 4.3	250.7 ± 3.9
		Body Weight Changes	
Day 8-Day 1	22.60 ± 1.54	16.69 ± 1.08**	25.47 ± 1.27
Day 15-Day 1	38.19 ± 2.21	33.52 ± 1.47	44.06 ± 1.32
Day 22-Day 1	46.89 ± 2.67	41.50 ± 2.34	56.04 ± 2.41*
Day 29-Day 1	53.64 ± 2.85	46.69 ± 2.41	65.29 ± 3.48*
Day 36-Day 1	67.88 ± 3.11	57.72 ± 2.65*	77.15 ± 3.76
Day 43-Day 1	78.55 ± 3.93	71.57 ± 2.83	64.54 ± 3.80*

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 42. Body weights were measured on days 1, 8, 15, 22, 29, 36 and 43. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix E - Table 21

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 12

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	H/NH	Trend Analysis
				Body Weight				
Day 0	141.7 ± 2.0	141.0 ± 2.1	139.8 ± 2.0	140.7 ± 2.7	140.0 ± 3.1	141.5 ± 3.9	H	NS
Day 8	149.9 ± 1.4	148.2 ± 2.6	148.3 ± 2.5	150.0 ± 3.0	145.3 ± 3.1	148.0 ± 5.3	H	NS
Day 15	152.9 ± 1.6	152.7 ± 2.7	153.3 ± 2.4	157.1 ± 3.6	152.0 ± 3.5	151.7 ± 4.7	H	NS
Day 22	157.3 ± 1.2	156.7 ± 2.8	156.9 ± 2.5	159.7 ± 3.4	154.5 ± 3.4	155.9 ± 5.1	H	NS
Day 29	158.6 ± 1.2	157.7 ± 3.0	157.4 ± 2.3	161.9 ± 4.0	156.5 ± 3.7	152.0 ± 4.6	NH	NS
				Body Weight Changes				
Day 8-Day 0	8.21 ± 1.01	7.29 ± 0.73	8.53 ± 0.87	9.31 ± 0.84	5.22 ± 0.63*	6.50 ± 1.72	H	NS
Day 15-Day 0	11.14 ± 1.12	11.77 ± 0.92	13.53 ± 0.94	16.37 ± 1.85*	12.02 ± 1.01	10.26 ± 1.18	H	$p < 0.05$
Day 22-Day 0	15.61 ± 1.30	15.70 ± 1.05	17.08 ± 0.98	19.00 ± 1.81	14.48 ± 1.56	14.45 ± 1.41	H	NS
Day 29-Day 0	16.82 ± 1.44	16.79 ± 1.18	17.59 ± 0.77	21.19 ± 2.05	16.44 ± 1.91	10.50 ± 1.11**	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. Body weights were measured on days 0, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 22

Body Weights (g) and Body Weight Changes (g) in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days in a 14 Day Recovery Study

Study 12

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane 300 mg/kg (10)	CPS 25 mg/kg (6)
		Body Weight	
Day 0	141.3 ± 2.1	140.9 ± 1.9	141.5 ± 3.9
Day 8	147.5 ± 2.2	147.0 ± 1.9	147.4 ± 3.8
Day 15	152.3 ± 2.5	153.6 ± 2.4	152.3 ± 4.0
Day 22	157.1 ± 2.3	157.9 ± 2.7	155.5 ± 3.9
Day 29	158.6 ± 2.9	159.4 ± 2.9	158.0 ± 4.5
Day 36	156.1 ± 2.6	153.5 ± 2.9	154.7 ± 4.9
Day 43	161.8 ± 2.8	157.3 ± 3.2	154.2 ± 4.4
		Body Weight Changes	
Day 8-Day 0	6.18 ± 0.68	6.57 ± 0.65	5.89 ± 0.53
Day 15-Day 0	10.95 ± 0.96	13.14 ± 1.28	10.73 ± 0.61
Day 22-Day 0	15.84 ± 0.91	17.42 ± 1.19	13.97 ± 0.74
Day 29-Day 0	17.27 ± 1.40	18.98 ± 1.24	16.46 ± 1.22
Day 36-Day 0	14.83 ± 1.79	13.05 ± 1.52	13.18 ± 1.73
Day 43-Day 0	20.52 ± 1.11	16.82 ± 1.52	12.71 ± 1.30**

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, CPS, i.p. on days 39-42. Body weights were measured on days 0, 8, 15, 22, 29, 36 and 43. Values represent the mean ± SE derived from the number of animals indicated in parentheses. The exposed group and positive control group were individually compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk.

Appendix E - Table 23

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 13

Parameter	Vehicle	Octamethylcyclotetrasiloxane (mg/kg)				H/NH	Trend Analysis
		10	30	100	300		
	(10)	(10)	(10)	(10)	(10)		
Body Weight							
Day 1	146.8 ± 1.8	146.6 ± 1.8	146.4 ± 1.9	146.2 ± 1.6	147.0 ± 1.8	H	NS
Day 8	150.0 ± 2.2	148.2 ± 2.4	148.4 ± 1.6	148.7 ± 1.4	149.9 ± 2.6	H	NS
Day 15	153.4 ± 2.3	151.9 ± 2.5	152.1 ± 1.8	154.7 ± 1.2	157.5 ± 2.9	H	NS
Day 22	156.0 ± 2.6	153.3 ± 2.6	155.1 ± 1.9	158.8 ± 1.6	160.4 ± 3.1	H	$p < 0.05$
Day 29	158.7 ± 2.6	157.1 ± 2.5	158.5 ± 2.1	162.0 ± 1.6	164.5 ± 3.4	H	$p < 0.05$
Body Weight Changes							
Day 8-Day 1	3.24 ± 0.65	1.64 ± 0.79	2.05 ± 0.96	2.48 ± 1.26	2.99 ± 1.02	H	NS
Day 15-Day 1	6.57 ± 0.83	5.33 ± 1.27	5.74 ± 0.94	8.54 ± 1.28	10.50 ± 1.29	H	$p < 0.01$
Day 22-Day 1	9.20 ± 1.12	6.74 ± 1.51	8.71 ± 1.25	12.55 ± 1.50	13.38 ± 1.47	H	$p < 0.01$
Day 29-Day 1	11.85 ± 1.11	10.55 ± 1.42	12.14 ± 1.14	15.81 ± 1.49	17.49 ± 1.74*	H	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix E - Table 24

Body Weight (g) and Body Weight Changes (g) in Female Fischer 344 Rats
Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 14

Parameter	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
			Body Weight				
Day 1	149.8 ± 2.4	150.0 ± 2.4	150.5 ± 2.3	149.8 ± 2.7	148.7 ± 2.2	H	NS
Day 8	150.0 ± 2.7	151.2 ± 2.5	152.5 ± 2.7	151.3 ± 2.9	148.9 ± 2.7	H	NS
Day 15	154.2 ± 3.2	154.9 ± 2.9	155.1 ± 2.9	154.4 ± 3.0	154.7 ± 2.5	H	NS
Day 22	158.3 ± 3.7	161.1 ± 3.2	156.8 ± 3.4	158.1 ± 3.4	158.1 ± 2.7	H	NS
Day 29	158.1 ± 3.6	161.3 ± 3.0	156.7 ± 3.4	158.9 ± 3.4	158.3 ± 2.7	H	NS
			Body Weight Changes				
Day 8-Day 1	0.19 ± 0.81	1.21 ± 0.60	2.01 ± 1.12	1.48 ± 0.70	0.18 ± 0.92	H	NS
Day 15-Day 1	4.42 ± 1.05	4.94 ± 1.05	4.58 ± 1.34	4.60 ± 0.54	5.97 ± 0.96	H	NS
Day 22-Day 1	8.56 ± 1.58	11.37 ± 1.32	6.23 ± 1.84	8.27 ± 1.26	9.34 ± 1.04	H	NS
Day 29-Day 1	8.32 ± 1.67	11.36 ± 1.34	8.21 ± 1.99	9.07 ± 1.17	9.56 ± 1.06	H	NS

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Body weights were measured on days 1, 8, 15, 22 and 29. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

APPENDIX F

FOOD CONSUMPTION DATA TABLES

Appendix F - Table 1

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 1

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	H/NH	Trend Analysis
Period 1-2	11.4 ± 0.3	11.8 ± 0.4	11.7 ± 0.2	11.8 ± 0.3	9.5 ± 0.3**	12.7 ± 0.7	H	p < 0.01
Period 2-3	12.0 ± 0.3	12.0 ± 0.4	12.2 ± 0.4	12.3 ± 0.3	11.1 ± 0.3	14.0 ± 0.8*	H	p < 0.05
Period 3-4	11.7 ± 0.3	11.7 ± 0.4	12.7 ± 0.3	13.2 ± 0.4*	12.8 ± 0.4	14.0 ± 0.5**	H	p < 0.01
Period 4-5	11.7 ± 0.3	11.4 ± 0.3	11.8 ± 0.4	12.6 ± 0.4	13.3 ± 0.3**	13.9 ± 0.5**	H	p < 0.01
Period 6-7	11.0 ± 0.3	11.3 ± 0.4	11.7 ± 0.3	12.0 ± 0.3	13.2 ± 0.4**	14.7 ± 0.4**	H	p < 0.01
Period 7-8	11.9 ± 0.3	11.7 ± 0.3	11.8 ± 0.3	12.8 ± 0.3	13.3 ± 0.4*	14.4 ± 0.4**	H	p < 0.01
Period 8-9	11.4 ± 0.3	11.4 ± 0.4	11.8 ± 0.3	12.2 ± 0.3	13.6 ± 0.3**	12.8 ± 0.7*	H	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix F - Table 2

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 2

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Period 1-2	19.2 ± 0.5	19.5 ± 0.3	20.5 ± 0.5	18.5 ± 0.5	18.7 ± 0.7	21.8 ± 0.5**	H	NS
Period 2-3	19.1 ± 0.5	20.1 ± 0.5[a]	20.3 ± 0.5	19.7 ± 0.5	20.4 ± 0.4	23.4 ± 1.5**	H	$p < 0.05$
Period 3-4	17.7 ± 0.5	18.2 ± 1.2	18.4 ± 0.5[b]	18.2 ± 0.5	19.4 ± 0.4**	21.0 ± 0.5**	NH	$p < 0.01$
Period 4-5	18.1 ± 0.3	18.4 ± 0.5	19.9 ± 0.5**	17.9 ± 0.3	19.1 ± 0.4	21.6 ± 1.0**	H	NS
Period 5-6	18.9 ± 0.5	18.4 ± 0.5	19.6 ± 1.5	18.5 ± 0.4	20.7 ± 0.4*	22.5 ± 0.8**	NH	$p < 0.05$
Period 6-7	19.2 ± 0.5	18.8 ± 0.4	20.1 ± 0.8	19.2 ± 0.4	23.6 ± 0.7**	23.4 ± 0.7**	H	$p < 0.01$
Period 7-8	17.3 ± 0.4	17.0 ± 0.9	18.0 ± 0.5	17.2 ± 1.6	24.3 ± 1.3**	22.2 ± 0.6**	NH	$p < 0.01$
Period 8-9	17.8 ± 0.4	18.6 ± 1.8	18.1 ± 1.0	18.2 ± 0.5	24.5 ± 1.1**	20.9 ± 0.8**	NH	$p < 0.01$

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

NS = Not Significant; [a]N = 8 animals; [b]N = 9 animals

Appendix F - Table 3

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 3

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	H/NH	Trend Analysis
Period 1-2	20.4 ± 0.3	20.3 ± 0.6	20.8 ± 0.4	20.2 ± 0.4	18.2 ± 0.4**	24.7 ± 2.0*	H	$p < 0.01$
Period 2-3	16.0 ± 0.2	15.8 ± 0.3	16.4 ± 0.4	16.2 ± 0.5	15.1 ± 0.4	17.4 ± 0.4**	H	NS
Period 3-4	20.7 ± 0.5	20.5 ± 0.5	21.4 ± 0.6	20.8 ± 0.6	20.5 ± 0.5	23.5 ± 0.7**	H	NS
Period 4-5	19.1 ± 0.4	19.0 ± 0.5	19.5 ± 0.9	19.4 ± 0.7	19.1 ± 0.4	22.1 ± 0.9**	NH	NS
Period 5-6	18.1 ± 0.5	18.5 ± 0.4	19.2 ± 0.7	19.4 ± 0.6	20.2 ± 0.5	21.9 ± 0.5**	H	$p < 0.01$
Period 6-7	17.6 ± 0.5	17.6 ± 0.4	18.2 ± 0.4	18.4 ± 0.5	19.0 ± 0.5	21.1 ± 0.8**	H	NS
Period 7-8	16.8 ± 0.6[a]	16.6 ± 0.5	17.2 ± 0.7	17.3 ± 0.4	19.3 ± 0.6*	20.3 ± 0.7**	H	$p < 0.01$
Period 8-9	17.3 ± 0.4[a]	17.0 ± 0.6	17.7 ± 0.6	17.4 ± 0.5	19.1 ± 0.5	19.4 ± 0.7*	H	$p < 0.05$

Male Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, cyclophosphamide, i.p. on days 25-28. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

NS = Not Significant; [a]N = 9 animals

Appendix F - Table 4

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 4

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	MVE 50 mg/kg (6)	H/NH	Trend Analysis
Period 1-2	12.0 ± 0.4	10.6 ± 0.2**	11.7 ± 0.2	11.2 ± 0.3	10.3 ± 0.3**	13.6 ± 0.4*	H	p < 0.01
Period 2-3	13.0 ± 0.6	11.8 ± 0.3	13.3 ± 0.2	13.5 ± 0.2	14.2 ± 0.3*	14.3 ± 0.5	NH	p < 0.01
Period 3-4	11.7 ± 0.4	10.9 ± 0.1	12.0 ± 0.2	12.4 ± 0.2	13.5 ± 0.2**	13.3 ± 0.4*	NH	p < 0.01
Period 4-5	11.9 ± 0.3	11.2 ± 0.2	12.0 ± 0.3	12.8 ± 0.2	14.5 ± 0.3**	13.1 ± 0.2*	H	p < 0.01
Period 5-6	11.6 ± 0.3	10.8 ± 0.3	11.8 ± 0.2	12.8 ± 0.3*	14.0 ± 0.3**	13.2 ± 0.4**	H	p < 0.01
Period 6-7	11.6 ± 0.4	10.9 ± 0.3	11.7 ± 0.3	12.7 ± 0.4	14.2 ± 0.4**	13.2 ± 0.4*	H	p < 0.01
Period 7-8	10.5 ± 0.4	10.5 ± 0.5	11.2 ± 0.4	12.1 ± 0.3*	13.9 ± 0.4**	11.9 ± 0.6	H	p < 0.01
Period 8-9	9.8 ± 0.3	9.9 ± 0.5	10.7 ± 0.3	11.3 ± 0.4*	13.7 ± 0.4**	9.6 ± 0.4	H	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil), octamethylcyclotetrasiloxane by gavage daily for 28 days or the positive control, MVE, i.v. on day 28. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Appendix F - Table 5

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 5

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	AAGMi 1:10 (6)	H/NH	Trend Analysis
Period 1-2	10.9 ± 0.3	10.5 ± 0.2	11.4 ± 0.3	11.6 ± 0.2	10.8 ± 0.4	12.7 ± 0.4**	13.3 ± 0.3**	H	NS
Period 2-3	11.2 ± 0.3	11.4 ± 0.3	12.1 ± 0.2	12.5 ± 0.3**	11.9 ± 0.4	13.2 ± 0.2**	13.9 ± 0.2**	H	p < 0.01
Period 3-4	11.1 ± 0.2	11.0 ± 0.2	11.6 ± 0.2	12.2 ± 0.3**	13.5 ± 0.2**	13.4 ± 0.3**	14.2 ± 0.3**	H	p < 0.01
Period 4-5	10.8 ± 0.2	11.2 ± 0.3	13.0 ± 1.3**	12.6 ± 0.3**	13.5 ± 0.4**	14.1 ± 0.3**	13.6 ± 0.3**	NH	p < 0.01
Period 5-6	11.0 ± 0.2	11.1 ± 0.2	11.7 ± 0.2	13.0 ± 0.3**	13.7 ± 0.4**	14.0 ± 0.2**	14.3 ± 0.2**	H	p < 0.01
Period 6-7	11.5 ± 0.3	11.0 ± 0.2	12.5 ± 0.5	13.1 ± 0.2**	13.9 ± 0.5**	14.2 ± 0.4**	14.6 ± 0.2**	NH	p < 0.01
Period 7-8	11.3 ± 0.4	11.1 ± 0.2	11.3 ± 0.3	12.2 ± 0.2	13.6 ± 0.4**	12.6 ± 0.2*	13.9 ± 0.2**	H	p < 0.01
Period 8-9	10.7 ± 0.4	11.7 ± 0.4	11.9 ± 0.2	12.7 ± 0.3**	13.2 ± 0.3**	11.9 ± 0.4	13.6 ± 0.3**	H	p < 0.01

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28 or the positive control, anti asialo GM1, was administered i.v. on day 28. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant

Appendix F - Table 6

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 6

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 10 (10)	30 (10)	100 (10)	300 (10)	CPS 25 mg/kg (6)	AAGM1 1:10 (6)	H/NH	Trend Analysis
Periods 1-2	17.6 ± 0.7[a]	18.2 ± 0.5	18.8 ± 0.6	18.9 ± 0.4	15.7 ± 1.0	22.1 ± 0.7**	21.0 ± 0.4**	H	NS
Periods 2-3	21.1 ± 0.6	21.3 ± 0.6	21.4 ± 0.5	21.9 ± 0.4	21.4 ± 0.7[b]	23.9 ± 0.2**	23.3 ± 0.4*	H	NS
Periods 3-4	19.5 ± 0.6[b]	19.6 ± 0.6	19.9 ± 0.4	20.8 ± 0.3	19.8 ± 0.6[a]	22.3 ± 0.6**	21.4 ± 0.5*	H	NS
Periods 4-5	18.7 ± 0.4	18.5 ± 0.6	19.3 ± 0.3	19.8 ± 0.4	19.9 ± 0.6[a]	22.0 ± 0.5**	21.5 ± 0.3**	H	$p < 0.05$
Periods 5-6	16.9 ± 0.7[b]	18.4 ± 0.6	18.6 ± 0.5	18.9 ± 0.4[b]	19.2 ± 0.7[a]	21.3 ± 0.6**	21.6 ± 0.3**	H	$p < 0.05$
Periods 6-7	16.4 ± 0.3	17.1 ± 0.7	17.3 ± 0.4	18.0 ± 0.4*	18.4 ± 0.4*[a]	20.3 ± 0.5**	20.0 ± 0.2**	H	$p < 0.01$
Periods 7-8	15.4 ± 0.5[b]	16.8 ± 0.7	17.6 ± 0.4*	17.5 ± 0.4	18.7 ± 0.9**[a]	20.4 ± 0.5**	20.2 ± 0.4**	H	$p < 0.01$
Periods 8-9	13.7 ± 0.4	12.1 ± 0.6	12.2 ± 0.4	11.7 ± 0.5*	9.7 ± 0.7**[a]	10.3 ± 0.4**	9.7 ± 0.4**	H	$p < 0.01$

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. The positive control, cyclophosphamide, was administered i.p. on days 25-28. The positive control, anti asialo GM1, was administered i.v. on day 28. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive controls were compared to the vehicle control using the Student's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 8 animals; [b]N = 9 animals

Appendix F - Table 7

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.1

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Period 1-2	10.7 ± 0.4	10.7 ± 0.4	11.0 ± 0.6	11.0 ± 0.5	10.8 ± 0.3	10.7 ± 0.5	10.4 ± 0.3	H	NS
Period 2-3	13.2 ± 0.3	12.8 ± 0.3	12.4 ± 0.4[a]	12.6 ± 0.3	12.9 ± 0.3	13.1 ± 0.5	12.5 ± 0.4	H	NS
Period 3-4	11.7 ± 0.4	11.4 ± 0.3	12.0 ± 0.5	12.0 ± 0.4	11.7 ± 0.3	12.1 ± 0.5	13.1 ± 0.4	H	$p < 0.05$
Period 4-5	11.1 ± 0.4	11.3 ± 0.3	11.6 ± 0.6	11.6 ± 0.4	11.5 ± 0.4[a]	11.9 ± 0.4	13.2 ± 0.4**	H	$p < 0.01$
Period 5-6	11.3 ± 0.3	11.5 ± 0.5	11.7 ± 0.4	11.9 ± 0.3	12.2 ± 0.4	12.2 ± 0.4	13.5 ± 0.5**[a]	H	$p < 0.01$
Period 6-7	11.5 ± 0.4	11.5 ± 0.4	11.7 ± 0.5[a]	12.1 ± 0.4	12.2 ± 0.4	12.7 ± 0.4	14.2 ± 0.5**	H	$p < 0.01$
Period 7-8	11.0 ± 0.3	11.5 ± 0.2[a]	11.2 ± 0.6	11.7 ± 0.3	12.3 ± 0.5	13.2 ± 0.5	14.3 ± 0.4**	H	$p < 0.01$
Period 8-9	11.3 ± 0.5	11.6 ± 0.4[a]	11.5 ± 0.5	11.7 ± 0.4	13.0 ± 0.5	11.9 ± 0.6	14.1 ± 0.4**	H	$p < 0.01$

Female Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix F - Table 8

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.2

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Period 1-2	12.7 ± 0.4	12.4 ± 0.4[a]	12.0 ± 0.3[a]	12.8 ± 0.3	12.9 ± 0.4[a]	12.2 ± 0.2	12.3 ± 0.6	H	NS
Period 2-3	14.5 ± 0.2	14.3 ± 0.2	14.5 ± 0.3	14.8 ± 0.2	14.7 ± 0.6	14.3 ± 0.4	14.4 ± 0.5	NH	NS
Period 3-4	13.8 ± 0.3	13.1 ± 0.3	13.3 ± 0.4	13.4 ± 0.3	13.4 ± 0.5	13.3 ± 0.2	13.8 ± 0.5	H	NS
Period 4-5	13.8 ± 0.5	13.1 ± 0.2	13.2 ± 0.6	13.5 ± 0.3	13.8 ± 0.5	13.1 ± 0.3	13.5 ± 0.4	H	NS
Period 5-6	14.4 ± 0.2	13.4 ± 0.2	13.1 ± 0.3*	13.8 ± 0.3	13.4 ± 0.4	13.6 ± 0.4	14.2 ± 0.2	H	NS
Period 6-7	13.9 ± 0.3	13.7 ± 0.3	13.8 ± 0.4	13.8 ± 0.2	13.9 ± 0.4	14.2 ± 0.4	14.0 ± 0.3	H	NS
Period 7-8	14.2 ± 0.3	13.7 ± 0.3	13.6 ± 0.4[a]	14.0 ± 0.3	14.2 ± 0.4	14.0 ± 0.4	14.2 ± 0.3	H	NS
Period 8-9	14.0 ± 0.3	14.0 ± 0.3	13.7 ± 0.3	13.7 ± 0.2	14.[illegible] ± 0.4	14.2 ± 0.4	14.7 ± 0.3	H	NS

Female Fischer 344 rats were administered vehicle (Maalox) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix F - Table 9

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.3

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Period 1-2	12.5 ± 0.4[a]	11.4 ± 0.5	12.5 ± 0.6	12.3 ± 0.4	12.4 ± 0.5	12.3 ± 0.6	12.3 ± 0.3	H	NS
Period 2-3	14.1 ± 0.4	13.0 ± 0.3	14.1 ± 0.5[b]	14.2 ± 0.4	13.9 ± 0.4	15.1 ± 0.6[a]	15.0 ± 0.3	H	p < 0.01
Period 3-4	13.2 ± 0.3	12.9 ± 0.4	13.1 ± 0.2	13.8 ± 0.4	13.4 ± 0.4	15.2 ± 0.5**	15.0 ± 0.4**	H	p < 0.01
Period 4-5	13.8 ± 0.2	12.6 ± 0.3	13.4 ± 0.5	13.3 ± 0.3	13.5 ± 0.4	14.2 ± 0.4[b]	15.0 ± 0.4[b]	H	p < 0.01
Period 5-6	13.8 ± 0.4	12.9 ± 0.3	13.7 ± 0.6	13.9 ± 0.4	14.0 ± 0.5	14.9 ± 0.5[b]	15.6 ± 0.3*[b]	H	p < 0.01
Period 6-7	14.1 ± 0.3	13.1 ± 0.4	13.5 ± 0.6	14.0 ± 0.4	14.1 ± 0.4	14.9 ± 0.4	16.6 ± 0.8	NH	p < 0.01
Period 7-8	14.0 ± 0.3	13.1 ± 0.3	13.8 ± 0.5	13.8 ± 0.4	14.0 ± 0.4	14.4 ± 0.5	16.2 ± 0.6**	H	p < 0.01
Period 8-9	14.8 ± 0.4	13.1 ± 0.4*	13.7 ± 0.6	13.9 ± 0.4	14.2 ± 0.5	15.0 ± 0.4	16.6 ± 0.3*	H	p < 0.01

Female Fischer 344 rats were administered vehicle (emulphor) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 8 animals; [b]N = 9 animals

Appendix F - Table 10

Food Consumption in Grams in Female Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 7.4

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	Naive (10)	H/NH	Trend Analysis
Period 1-2	12.5 ± 0.7[a]	13.0 ± 0.4	12.1 ± 0.6	13.2 ± 0.6	13.4 ± 0.5	12.5 ± 0.5	13.0 ± 0.7	12.5 ± 0.4	H	NS
Period 2-3	12.7 ± 0.7	14.3 ± 0.3	13.7 ± 0.4	14.5 ± 0.5*	14.6 ± 0.5*	14.4 ± 0.5	15.4 ± 0.4**	14.6 ± 0.4*	H	p < 0.01
Period 3-4	14.1 ± 0.6	13.8 ± 0.2	13.6 ± 0.5	13.7 ± 0.4	13.3 ± 0.4	14.3 ± 0.4	15.0 ± 0.4	14.1 ± 0.3	H	NS
Period 4-5	13.9 ± 0.5	14.1 ± [illegible]	13.3 ± 0.4	13[illegible].3	13.6 ± 0.5	13.7 ± 0.3	14.6 ± 0.4	13.6 ± 0.3	H	NS
Period 5-6	14.3 ± 0.5	14.2 ± 0.2[a]	13.7 ± 0.5	14.3 ± [illegible].3	13.7 ± 0.6	13.8 ± 0.3	14.8 ± 0.5	14.1 ± 0.3	NH	NS
Period 6-7	14.4 ± 0.5	13.9 ± 0.3	13.6 ± 0.4	14.4 ± 0.3	13.8 ± 0.4	13.5 ± 0.3	14.1 ± 0.3[a]	14.1 ± 0.5	H	NS
Period 7-8	14.4 ± 0.4	14.1 ± 0.3	13.9 ± 0.3	14.6 ± 0.3	13.3 ± 0.4	13.6 ± 0.3	14.8 ± 0.4	14.3 ± 0.4	H	NS
Period 8-9	14.5 ± 0.5	14.1 ± 0.3	14.2 ± 0.4	14.5 ± 0.4[b]	13.5 ± 0.5	14.0 ± 0.4	14.8 ± 0.5	14.0 ± 0.3	H	NS

Female Fischer 344 rats were administered nothing (naive), vehicle (35 cs PDMS fluid) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals; [b]N = 8 animals

Appendix F - Table 11

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.1

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Period 1-2	18.0 ± 0.5	16.7 ± 0.4	16.9 ± 0.4	17.5 ± 0.4	18.9 ± 1.3	17.0 ± 0.7	16.6 ± 0.8	NH	NS
Period 2-3	17.8 ± 0.4	16.9 ± 0.3	17.9 ± 0.4	17.7 ± 0.3	17.2 ± 0.5[a]	18.3 ± 0.8	17.3 ± 0.8	NH	NS
Period 3-4	17.5 ± 0.4	16.4 ± 0.2	17.4 ± 0.3	17.2 ± 0.3	16.9 ± 0.6	17.2 ± 0.5	17.8 ± 0.5	NH	NS
Period 4-5	16.8 ± 0.3	15.6 ± 0.3	16.8 ± 0.3	16.0 ± 0.4	16.5 ± 0.5	18.0 ± 0.6	17.9 ± 0.3	H	$p < 0.01$
Period 5-6	16.8 ± 0.3	15.9 ± 0.4	17.2 ± 0.4	16.5 ± 0.5	16.0 ± 0.5[a]	16.7 ± 0.6	18.1 ± 0.4	H	NS
Period 6-7	16.2 ± 0.4	16.1 ± 0.2	17.4 ± 0.4	16.3 ± 0.4	16.6 ± 0.5	17.1 ± 0.5	18.1 ± 0.4*	H	$p < 0.01$
Period 7-8	20.0 ± 0.5	19.7 ± 0.4	21.3 ± 0.4	20.1 ± 0.5	19.9 ± 1.0	20.9 ± 0.5	23.3 ± 0.6**	H	$p < 0.01$
Period 8-9	11.3 ± 0.3	10.7 ± 0.2	11.6 ± 0.3	11.3 ± 0.4	10.5 ± 0.4[a]	12.0 ± 0.3	13.9 ± 0.5**	H	$p < 0.01$

Male Fischer 344 rats were administered vehicle (corn oil) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix F - Table 12

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.2

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Period 1-2	19.3 ± 0.8	19.3 ± 0.6	19.3 ± 0.5	18.8 ± 0.9	18.7 ± 0.4	19.5 ± 0.6[a]	19.0 ± 0.6	H	NS
Period 2-3	19.2 ± 0.4	19.7 ± 0.5	19.4 ± 0.3	18.8 ± 0.5[a]	20.4 ± 0.3	19.5 ± 0.4[a]	21.0 ± 0.5*	H	p < 0.05
Period 3-4	18.8 ± 0.4	19.0 ± 0.5	18.9 ± 0.4	18.4 ± 0.4	18.6 ± 0.5	18.9 ± 0.6	19.5 ± 0.6	H	NS
Period 4-5	19.3 ± 0.4	19.3 ± 0.4	18.6 ± 0.3	18.5 ± 0.4	19.1 ± 0.5	19.5 ± 0.4	20.0 ± 0.5	H	NS
Period 5-6	19.0 ± 0.2	19.4 ± 0.4	19.1 ± 0.2	18.6 ± 0.5	19.7 ± 0.5	19.6 ± 0.2	20.1 ± 0.5	NH	p < 0.05
Period 6-7	18.9 ± 0.4	19.3 ± 0.6	19.0 ± 0.2	18.5 ± 0.6	18.8 ± 0.6	19.2 ± 0.4	19.6 ± 0.7	H	NS
Period 7-8	24.9 ± 0.5	24.6 ± 0.7	24.3 ± 0.5	23.5 ± 0.6	24.6 ± 0.4[a]	24.3 ± 0.4	24.6 ± 0.7	H	NS
Period 8-9	14.2 ± 0.3	13.7 ± 0.4	13.7 ± 0.4	12.9 ± 0.3	13.7 ± 0.4[a]	13.5 ± 0.3	14.0 ± 0.6	H	NS

Male Fischer 344 rats were administered vehicle (Masiox) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix F - Table 13

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.3

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	H/NH	Trend Analysis
Period 1-2	19.1 ± 0.6[a]	19.1 ± 0.6[a]	19.0 ± 0.5	19.1 ± 0.6	18.5 ± 0.5[a]	18.7 ± 0.5	18.3 ± 0.4	H	$p < 0.05$
Period 2-3	19.7 ± 0.5	19.4 ± 0.4	19.6 ± 0.4	20.3 ± 0.6	19.6 ± 0.5	20.3 ± 0.4	20.8 ± 0.6	F	NS
Period 3-4	18.1 ± 0.6	19.0 ± 0.3	19.2 ± 0.5	20.2 ± 0.6	19.7 ± 0.4	19.7 ± 0.4	19.3 ± 0.4	H	$p < 0.05$
Period 4-5	18.6 ± 0.5	19.0 ± 0.3	18.7 ± 0.5	19.9 ± 0.4	19.6 ± 0.4	20.1 ± 0.3	19.1 ± 0.3	H	$p < 0.05$
Period 5-6	18.8 ± 1.4	19.7 ± 0.5	19.2 ± 0.4	20.7 ± 0.5	20.3 ± 0.4	20.5 ± 0.4	19.6 ± 0.4	NH	NS
Period 6-7	18.9 ± 0.4[a]	18.5 ± 0.5	18.4 ± 0.5	19.3 ± 0.6	18.6 ± 0.4	19.6 ± 0.3	19.0 ± 0.4	H	NS
Period 7-8	22.7 ± 0.7	24.0 ± 0.7	24.0 ± 0.9	25.5 ± 0.8*	25.0 ± 0.4[a]	25.2 ± 0.4	25.5 ± 0.7*	H	$p < 0.01$
Period 8-9	12.9 ± 0.3	13.5 ± 0.5	13.7 ± 0.3	14.3 ± 0.4	13.8 ± 0.3	13.8 ± 0.3	13.6 ± 0.2	H	NS

Male Fischer 344 rats were administered vehicle (emulphor) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals

Appendix F - Table 14

Food Consumption in Grams in Male Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane Daily for 28 Days

Study 8.4

Parameter (Grams)	Vehicle (10)	Octamethylcyclotetrasiloxane (mg/kg) 1 (10)	3 (10)	10 (10)	30 (10)	100 (10)	300 (10)	Naive (10)	H/NH	Trend Analysis
Period 1-2	20.0 ± 0.6	20.0 ± 0.4[a]	20.8 ± 0.4	20.3 ± 0.4	19.7 ± 0.4[a]	19.7 ± 0.6	21.1 ± 0.5[a]	19.3 ± 0.3	H	NS
Period 2-3	19.8 ± 0.7[a]	20.1 ± 0.3[a]	21.6 ± 0.7	20.4 ± 0.6	19.7 ± 0.3	19.9 ± 0.5	20.1 ± 0.7	19.3 ± 0.3[a]	NH	NS
Period 3-4	19.8 ± 0.4	20.4 ± 0.7	21.1 ± 0.7	19.8 ± 0.4	19.3 ± 0.2	19.7 ± 0.4	20.7 ± 0.3[a]	19.3 ± 0.3	NH	NS
Period 4-5	19.6 ± 0.4	19.7 ± 0.6	21.2 ± 0.4**	19.8 ± 0.5	20.0 ± 0.2	19.2 ± 0.4	19.8 ± 0.5	19.4 ± 0.4[a]	H	NS
Period 5-6	20.0 ± 0.5	19.5 ± 0.8	21.6 ± 0.7[a]	20.6 ± 0.4	20.5 ± 0.4	20.2 ± 0.6	20.0 ± 0.7[a]	19.4 ± 0.4	H	NS
Period 6-7	20.0 ± 0.6	19.3 ± 0.5	20.0 ± 0.9	19.7 ± 0.4[b]	19.8 ± 0.4	19.1 ± 0.5	19.5 ± 0.5[a]	18.2 ± 0.2*	NH	NS
Period 7-8	25.3 ± 0.6[a]	26.6 ± 0.9	26.5 ± 0.8	25.9 ± 0.5	25.5 ± 0.6	25.2 ± 0.8[a]	25.8 ± 0.8[a]	23.5 ± 0.4	H	NS
Period 8-9	14.6 ± 0.3	15.0 ± 0.5	14.9 ± 0.5	14.8 ± 0.4	14.3 ± 0.3	14.1 ± 0.5[a]	14.4 ± 0.6[a]	13.2 ± 0.3**	NH	NS

Male Fischer 344 rats were administered nothing (naive), vehicle (35 cs PDMS fluid) or octamethylcyclotetrasiloxane by gavage daily for 28 days. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. Values significantly different from vehicle control at $p < 0.05$ are indicated by an asterisk while those significant at $p < 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.
NS = Not Significant; [a]N = 9 animals; [b]N = 8 animals

APPENDIX G

CHARACTERIZATION OF OCTAMETHYLCYCLOTETRASILOXANE

LOT #LL084732
LOT #LL121629
LOT #044530

EXACT COPY

QUALITY ASSURANCE MANAGEMENT SYSTEM
LOT ACCEPTANCE TEST RESULTS

PRODUCT NAME: 244 FLUID TR KEY: 000774268
LOT NUMBER: LL084732 CONTAINER RANGE:
STATUS: A STATUS DATE: 19AUG94
COMMENT: QAMS-LIMS AUTOMATIC FEED

MATL/ITEM ID:1706110 SAMPLE TYPE: FINAL LOT EFFECTIVE LAR DATE: 01JUL94

BLDG	LAB	CTM	SUB	NAME	TYP	CONTAINER	RESULT	USE	SPECIFICATION LOW LIMIT	SPECIFICATION HIGH LIM
***** SECTION: 00 LAST SECTION: 01 *****										
TESTS 0001A A AND 0004 A WILL BE DONE ON A QUARTERLY BASIS ONLY. THE SALES SPECIFICATION WILL INDICATE THE AUDIT NATURE OF THESE TESTS AND WILL REPORT THE SPECIFICATION RANGES ONLY.										
	0053	A	D4		S		99.74		96	100
							99.71	R		
				CHROMATOGRAM 21						
	0053	B	D3+D5+D6		S		0.260		0	4
							0.240	R		
	0053	C	D3		X		0.07		MAXIMUM	0.5
							0.06	R		
	0053	D	D5		X		0.19		MAXIMUM	4.0
							0.14	R		
	0053	E	D6		X		0.00		MAXIMUM	4.0
							0.04	R		
	0176	E	APPEAR		S		PASS		PASS	MINIMUM
							PASS	R		
				CRYSTAL CLEAR LIQUID, FREE FROM SUSPENDED MATTER.						
	0911	A	WATER		S		13	R	MAXIMUM	250
***** SECTION: 01 LAST SECTION: 01 *****										
SAMPLE PREPARATION SECTION: 01 THE FOLLOWING PROPERTIES ARE WARRANTED TO MEET THE INDICATED LIMITS BUT DOW CORNING DOES NOT PERFORM THESE TESTS IN LOT ACCEPTANCE.										
	0001A	A	SP GR		W		_____		0.945	0.959
				25C/15.6C						

EXACT COPY

QUALITY ASSURANCE MANAGEMENT SYSTEM
LOT ACCEPTANCE TEST RESULTS

PRODUCT NAME: 244 FLUID TR KEY: 000774268
LOT NUMBER: LL084732 CONTAINER RANGE:
STATUS: A STATUS DATE: 19AUG94
COMMENT: QAMS-LIMS AUTOMATIC FEED

MATL/ITEM ID:1706110 SAMPLE TYPE: FINAL LOT EFFECTIVE LAR DATE: 01JUL94

BLDG	LAB	CTM	SUB	NAME	TYP	CONTAINER	RESULT	USE	SPECIFICATION LOW LIMIT	SPECIFICATION HIGH LIMI
		0004	A	VISC 25C	W				2.0	2.8
		0005	A	COL APHA	W				MAXIMUM	35
		0051	A	ACID NO BCP	W				MAXIMUM	0.01
		1110	A	ODOR NONE DETECTABLE.	W				PASS	MINIMUM

LAST PAGE: 3

EXACT COPY

MATERIAL NUMBER: 01706110 PRODUCT NAME: 244 FLUID
SPECIFICATION DATE: 093001 FINAL LOT
STATUS CODES: 1= APPROVED 2= REJECT 3= WAIVED 4=NEW SAMPLE 5= IN PROCESS 6= OTHER
TEST CODES: 0= NIL OR PASS; 1= TRACE OR FAIL; 999= NO VALUE REPORTED; 000= NON DETECTABLE;
CONTAINER: 000 = NO CONTAINER WAS INPUT
SECTION NUMBER: 00

CTM NO	SUB	CTM NAME	UNITS	USE	FREQ	CALC NO. OF MODE	RESULTS	SPECIFICATION LOWER LIMIT	SPECIFICATION UPPER LIMIT	REASONABLE LOWER LIMIT	REASONABLE UPPER LIMIT
0001A	A	SPECIFIC GRAVITY 25C/15.6C		S	1	A	1	0.945	0.959		
0004	A	VISCOSITY 25C	CST	S	1	A	1	2.0	2.8		
0005	A	COLOR, APHA		S	1	A	1	MAXIMUM	35.		
0051	A	ACID NUMBER BCP		S	1	A	1	MAXIMUM	0.01		
0053	A	D4 (TETRAMER) CHROMATOGRAM 21	%	X	1	A	1	95.	100.		
0053	B	ALL OTHER CYCLICS (D3+D5+D6)	%	X	1	A	1	0.	5.		
0053	C	D3 (TRIMER)	%	T	1	A	1	RECORD			
0053	D	D5	%	T	1	A	1	RECORD			
0053	E	D6	%	T	1	A	1	RECORD			
0111	A	ODOR		S	1	A	1	PASS	MINIMUM		
				NONE DETECTABLE							
0176	B	APPEARANCE		S	1	F	1	PASS	MINIMUM		
				CRYSTAL CLEAR LIQUID, FREE FROM SUSPENDED MATTER							

SMPL	DATE	LOT	CONT	ST	SP GR	VISC	COL APHA	ACID NO	D4	D3+D5+D6	D3	D5	D6	ODOR	APP
121691	LL121629	000		1	0.953	2.3	5.	0.001	97.48	2.5	000	2.52	000	0	0

EXACT COPY

MATERIAL NUMBER: 01706110 PRODUCT NAME: 244 FLUID
SPECIFICATION DATE: 011193 FINAL LOT
STATUS CODES: 1= APPROVED 2= REJECT 3= WAIVED 4=NEW SAMPLE 5= IN PROCESS 6= OTHER
TEST CODES: 0= NIL OR PASS; 1= TRACE OR FAIL; 999= NO VALUE REPORTED; 000= NON DETECTABLE;
CONTAINER: 000 = NO CONTAINER WAS INPUT
SECTION NUMBER: 00

CTM NO	SUB	CTM NAME	UNITS	OSE	FREQ	CALC NO. OF MODE	RESULTS	SPECIFICATION LOWER LIMIT	SPECIFICATION UPPER LIMIT	REASONABLE LOWER LIMIT	REASONABLE UPPER LIMIT
0001A	A	SPECIFIC GRAVITY 25C/15.6C		S	1	A	1	0.945	0.959		
0004	A	VISCOSITY 25C	CST	S	1	A	1	2.0	2.8		
0005	A	COLOR, APHA		S	1	A	1	MAXIMUM	35		
0051	A	ACID NUMBER BCP		S	1	A	1	MAXIMUM	0.01		
0053	A	D4 (TETRAMER) CHROMATOGRAM 21	%	S	1	A	1	96	100		
0053	B	ALL OTHER CYCLICS (D3+D5+D6)	%	S	1	A	1	0	4		
0053	C	D3 (TRIMER)	%	S	1	A	1	MAXIMUM	0.5		
0053	D	D5	%	S	1	A	1	MAXIMUM	4.0		
0053	E	D6	%	S	1	A	1	MAXIMUM	6.0		
0121	A	ODOR		S	1	A	1	PASS	MINIMUM		
					NONE DETECTABLE.						
0176	B	APPEARANCE		S	1	F	1	PASS	MINIMUM		
					CRYSTAL CLEAR LIQUID, FREE FROM SUSPENDED MATTER.						
0931	A	WATER	PPM	S	1	A	1	MAXIMUM	250		

SMPL DATE LOT	CONT	ST	SP GR	VISC	COL APHA	ACID NO	D4	D3+D5+D6	D3	D5	D6	ODOR	APP
003494 LL444536 000		1	.954	2.27	5	.00	97.68	2.32	.04	2.28	.60	0	0

APPENDIX H

OCTAMETHYLCYCLOTETRASILOXANE ANALYSIS OF DOSING SOLUTIONS REPORT

LOT #LL121629

Samples of corn oil received from Paal Klykken on May 13, 1994 were weighed into hexane-rinsed 10-dram vials and diluted to 20 mL with toluene. An aliquot was removed and placed in an autosampler vial and analyzed by GC/FID, along with standards of known D4 concentration, using the following conditions:

injector temperature:	250°C
detector temperature:	250°C
initial temperature:	80°C
initial time:	1 minute
rate:	20°C/minute
final temperature:	320°C
final time:	7 minutes
air flow:	300 mL/minute
hydrogen flow:	30 mL/minute
helium makeup:	30 mL/minute
carrier flow:	1 mL/minute
purge vent:	3 mL/minute
split vent:	100 mL/minute
head pressure:	20 psi
data acquisition:	20 points/second

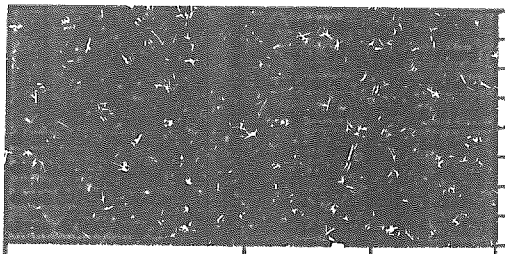
1 µL splitless injection (purge on at 0.20 minutes)

Samples were quantitated by obtaining a least-squares fit of the concentration versus average peak area plot for the standards, and applying the resulting slope and intercept values to the sample peak areas using the following equation

$$y = mx + b$$

where "y" is concentration in µg/mL, "m" is the slope, "x" is peak area and "b" is the intercept. All sample peak areas were corrected for the average peak area obtained for a sample of unspiked corn oil. Each dosing solution sample was prepared and analyzed in triplicate. A copy of the individual data is attached and a diskette copy of the spreadsheet is included. All sample weights and dilution volumes were entered directly into the spreadsheet. Copies of the chromatograms are also attached.

Mark W. Dykstra
5/18/94



						blank corn oil		
	calculated	sample	final	target		corrected	nonsample	corn oil
	D4 conc.	mass	volume	conc.	D4 peak	D4 peak	D4 conc.	D4 conc.
sample	(ug/g)	(grams)	(mL)	(ug/mL)	area	area	(ug/mL)	(mg/mL)
corn oil, rep 1	---	0.2115	20	---	937	-159	ND	---
corn oil, rep 2	---	0.2135	20	---	1127	31	ND	---
corn oil, rep 3	---	0.2085	20	---	1224	128	ND	---
dose 1/10 mg/kg, rep 1	2459	0.2015	20	24.77	51244	50148	23	2.3
dose 1/10 mg/kg, rep 2	2459	0.2074	20	25.50	50318	49222	21	2.1
dose 1/10 mg/kg, rep 3	2459	0.2234	20	27.47	54193	53097	23	2.1
dose 2/30 mg/kg, rep 1	7378	0.2137	20	76.83	161876	160780	77	7.2
dose 2/30 mg/kg, rep 2	7378	0.2167	20	79.94	161119	160023	72	6.6
dose 2/30 mg/kg, rep 3	7378	0.2052	20	75.33	151937	150841	67	6.6
dose 3/100 mg/kg, rep 1	22133	0.1973	20	218.3	520393	519297	250	25
dose 3/100 mg/kg, rep 2	22133	0.2101	20	232.5	520364	519268	234	22
dose 3/100 mg/kg, rep 3	22133	0.2054	20	227.3	508880	507784	229	22
dose 4/300 mg/kg, rep 1	66400	0.2113	20	701.5	1627335	1626239	785	74
dose 4/300 mg/kg, rep 2	66400	0.2039	20	676.9	1690819	1689723	763	75
dose 4/300 mg/kg, rep 3	66400	0.2063	20	684.9	1691552	1690456	759	74

	D4 conc.	D4 peak
1st GC run:	(ug/mL)	area (avg, n=3)
1 ug/mL standard	1.004	3274
10 ug/mL standard	10.04	22114
100 ug/mL standard	100.4	210457
1000 ug/mL standard	1004	2080430

	D4 conc.	D4 peak
2nd GC run:	(ug/mL)	area (avg, n=2)
1 ug/mL standard	1.004	3451
10 ug/mL standard	10.04	23896
100 ug/mL standard	100.4	224355
1000 ug/mL standard	1004	2223105

Note: rep 1 was quantitated using 1st GC run standards; reps 2 and 3 using 2nd GC run

5/18/94

1st GC Run Regression Statistics						
Multiple R	0.9999998					
R Square	0.9999996					
Adjusted R Square	0.9999994					
Standard Error	0.3687236					
Observations	4					
Analysis of Variance						
	df	Sum of Squares	Mean Square	F	Significance F	
Regression	1	707143.8684	707143.8684	5201237.4	1.92262E-07	
Residual	2	0.271914229	0.135957115			
Total	3	707144.1403				
	Coefficients	Standard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	-0.805619	0.221419898	-3.638420078	0.0357814	-1.758312193	0.147075
x1	0.000483	2.11767E-07	2280.619957	1.85E-10	0.000482048	0.0004839
2nd GC Run Regression Statistics						
Multiple R	1.000000					
R Square	1.000000					
Adjusted R Square	1.000000					
Standard Error	0.222205					
Observations	4					
Analysis of Variance						
	df	Sum of Squares	Mean Square	F	Significance F	
Regression	1	707144.0416	707144.0416	14321840	6.98234E-08	
Residual	2	0.098750449	0.049375224			
Total	3	707144.1403				
	Coefficients	Standard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	-0.76335	0.13342895	-5.721013029	0.010598	-1.337447595	-0.18925
x1	0.000452	1.19425E-07	3784.42064	4.07E-11	0.00045144	0.000452

Mark Van Dyke
5/18/94

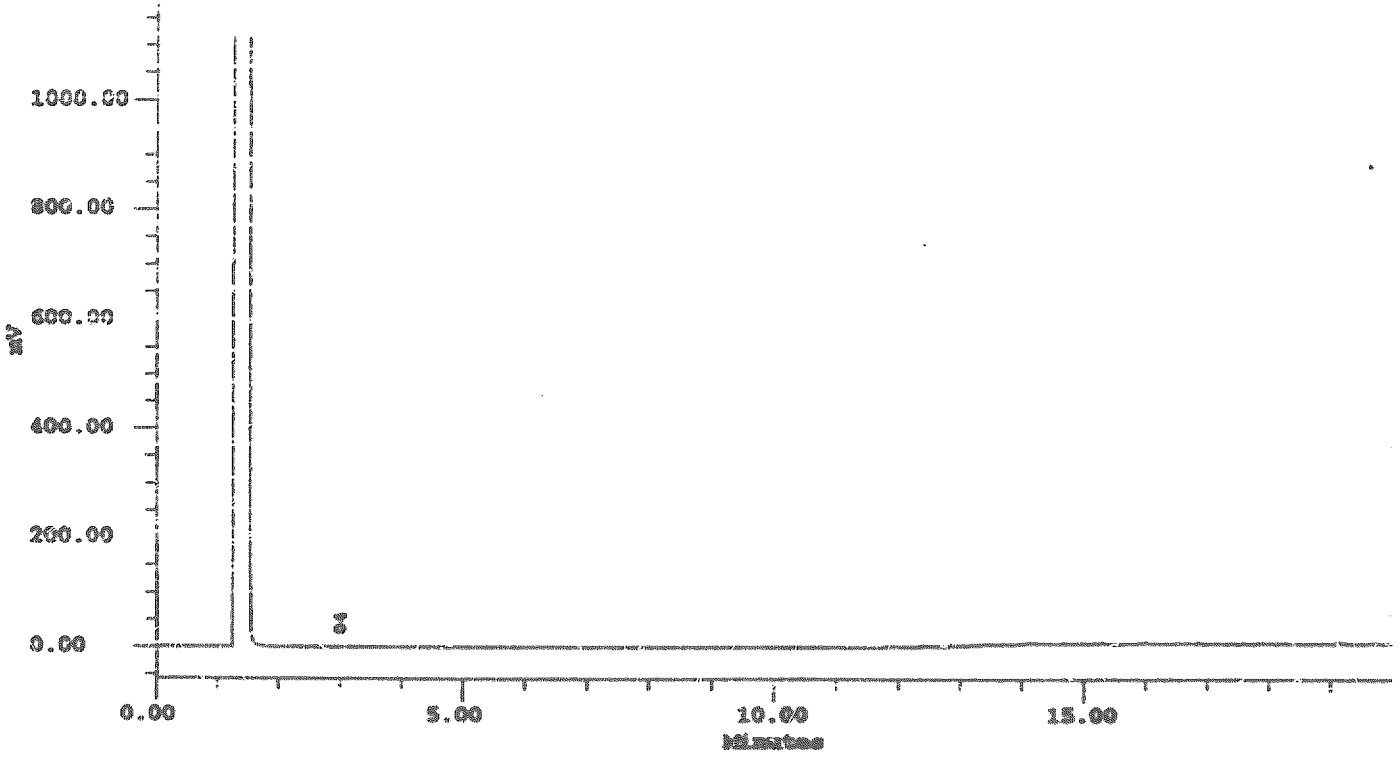
Millennium Results Report: HRS_pm Proc Chan: SATIN Page 1 o

For Sample: 1 ug/mL std Vial: 2 Inj: 1 Chan: SATIN Date Processed 05/12/94 02:47 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1 ug/mL std	File No.:	
Vial:	2	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 08:09 AM	Date Processed:	05/12/94 02:47 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Math Set:	GC_ms		
Processing Method:	HRS_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.011	3227	BB	100.00	

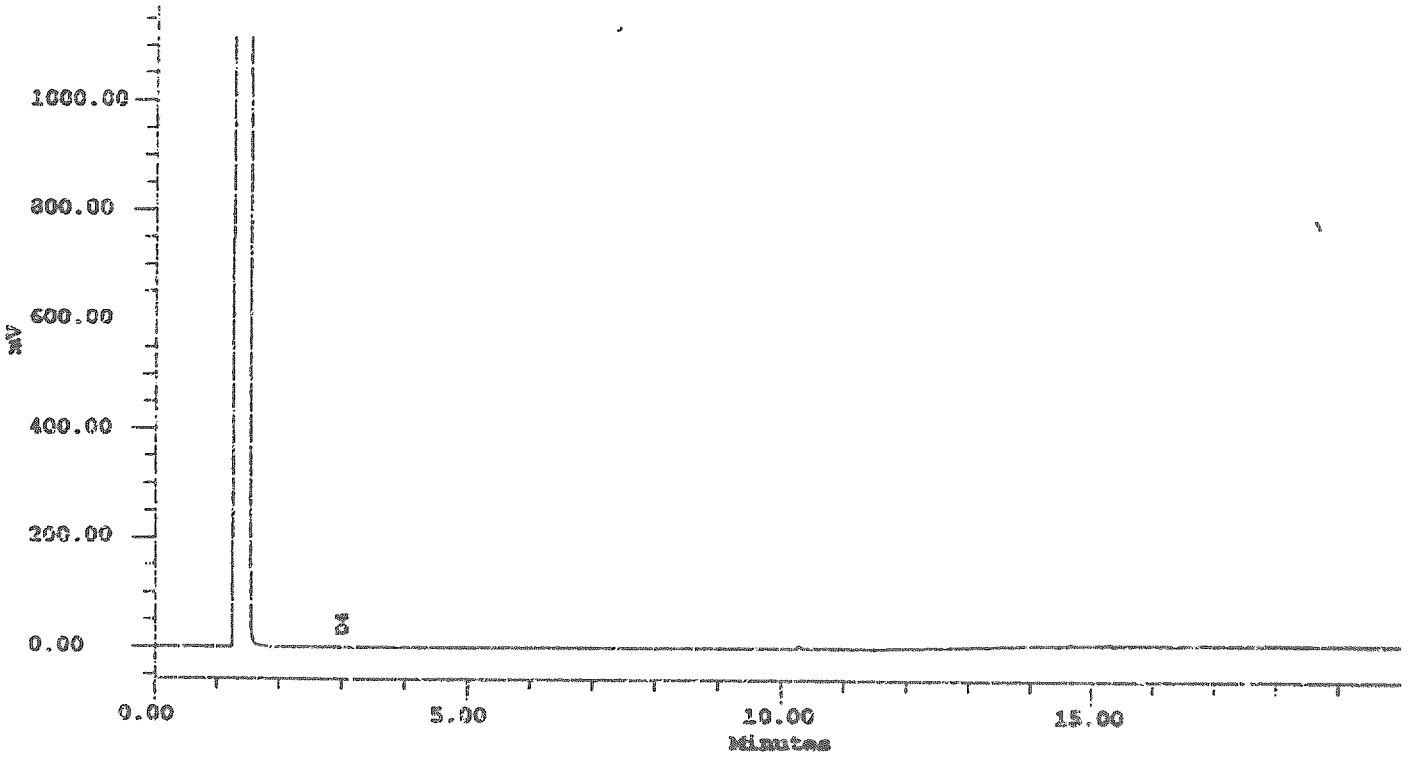
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: 1 ug/mL std Vial: 13 Inj: 1 Chan: SATIN Date Processed 05/15/94 08:31 AM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1 ug/mL std	File No.:	
Vial:	13	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 04:53 PM	Date Processed:	05/15/94 08:31 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.009	3320	BB	100.00	

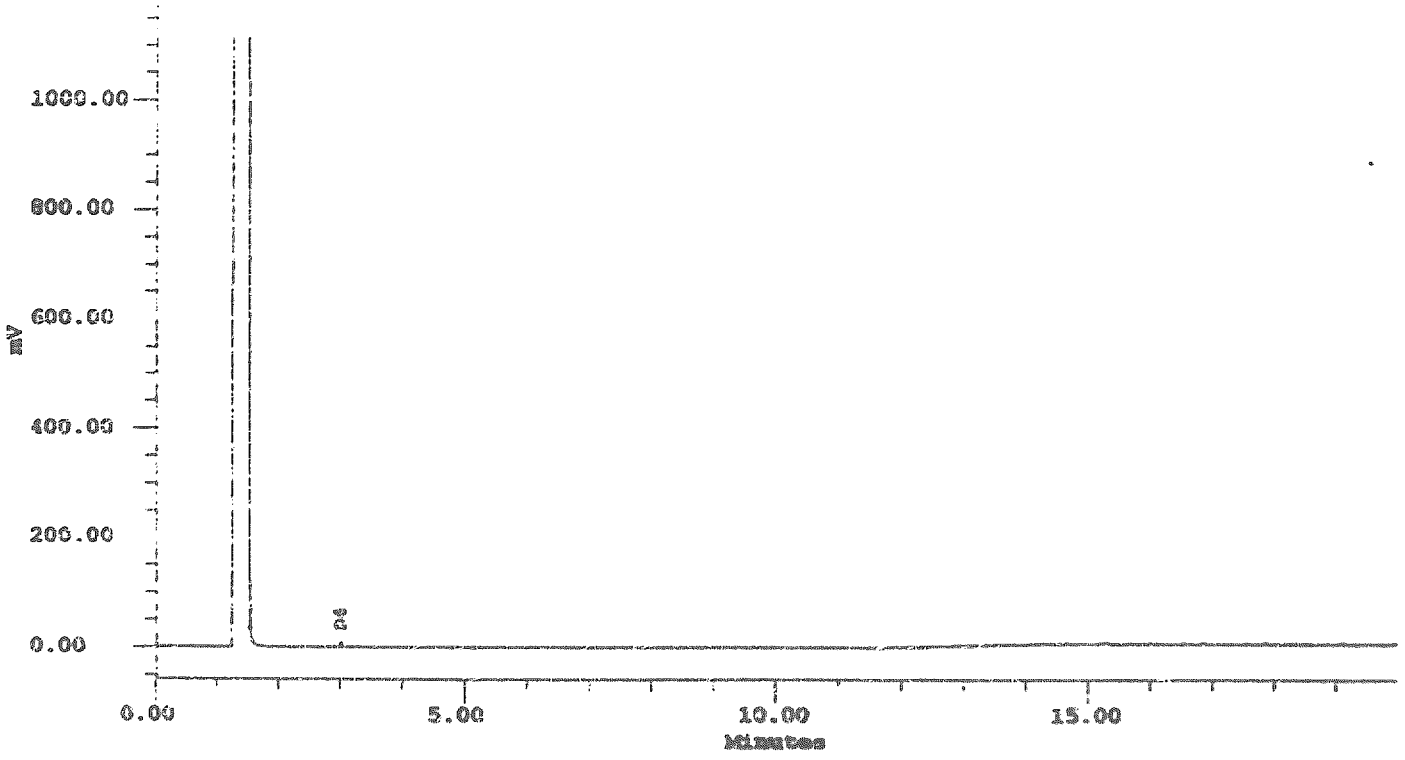
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1

For Sample: 10 ug/mL std Vial: 3 Inj: 1 Chan: SATIN Date Processed 05/12/94 02:48 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	10 ug/mL std	File No.:	
Vial:	3	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 08:34 AM	Date Processed:	05/12/94 02:48 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Math Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.011	21905	BB	100.00	

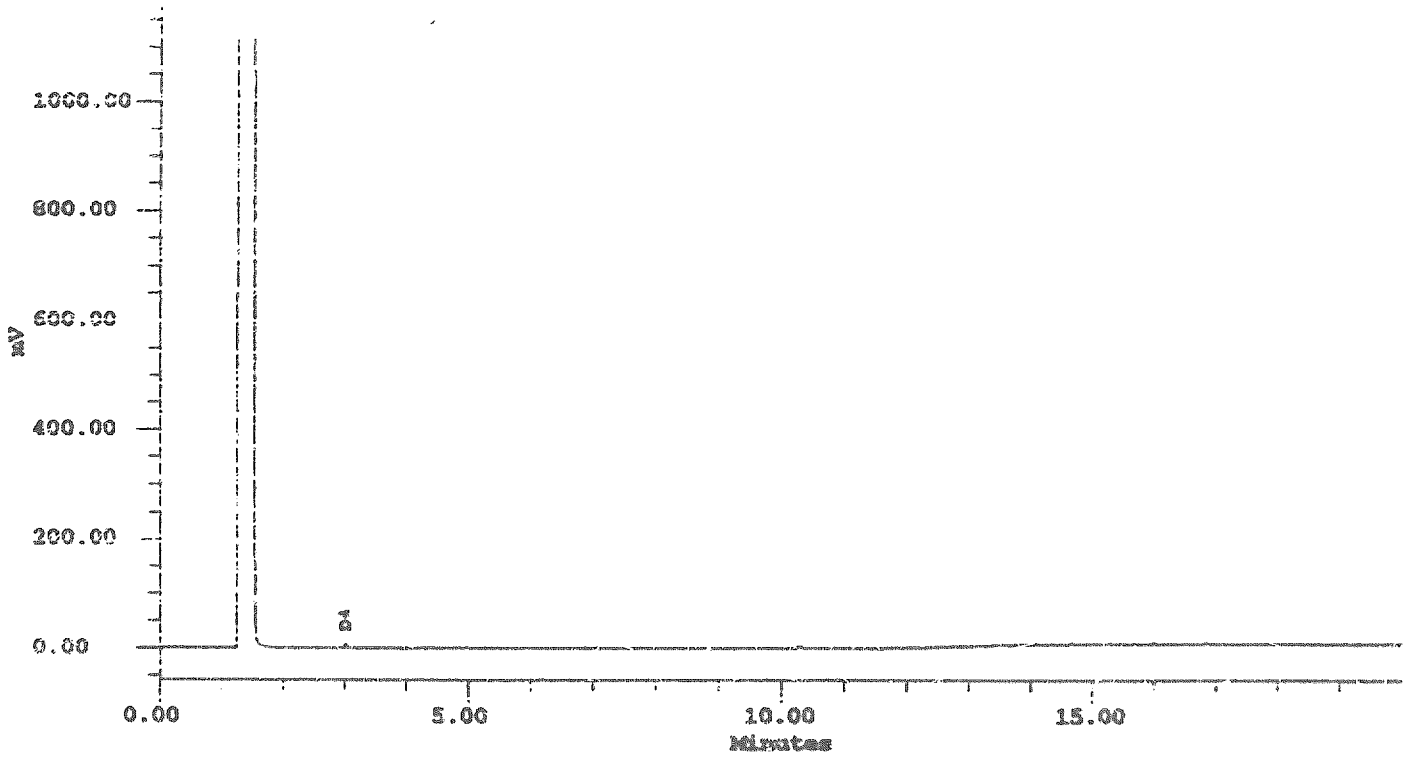
Millennium Results Report: HE3_rm Proc Chan: SATIN Page 1

For Sample: 10 ug/mL std Vial: 14 Inj: 1 Chan: SATIN Date Processed 05/15/94 08:31 AM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	10 ug/mL std	File No.:	
Vial:	14	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 05:18 PM	Date Processed:	05/15/94 08:31 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HE3_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	Usr Tailing
1	D4	3.008	23322	BB	100.00	

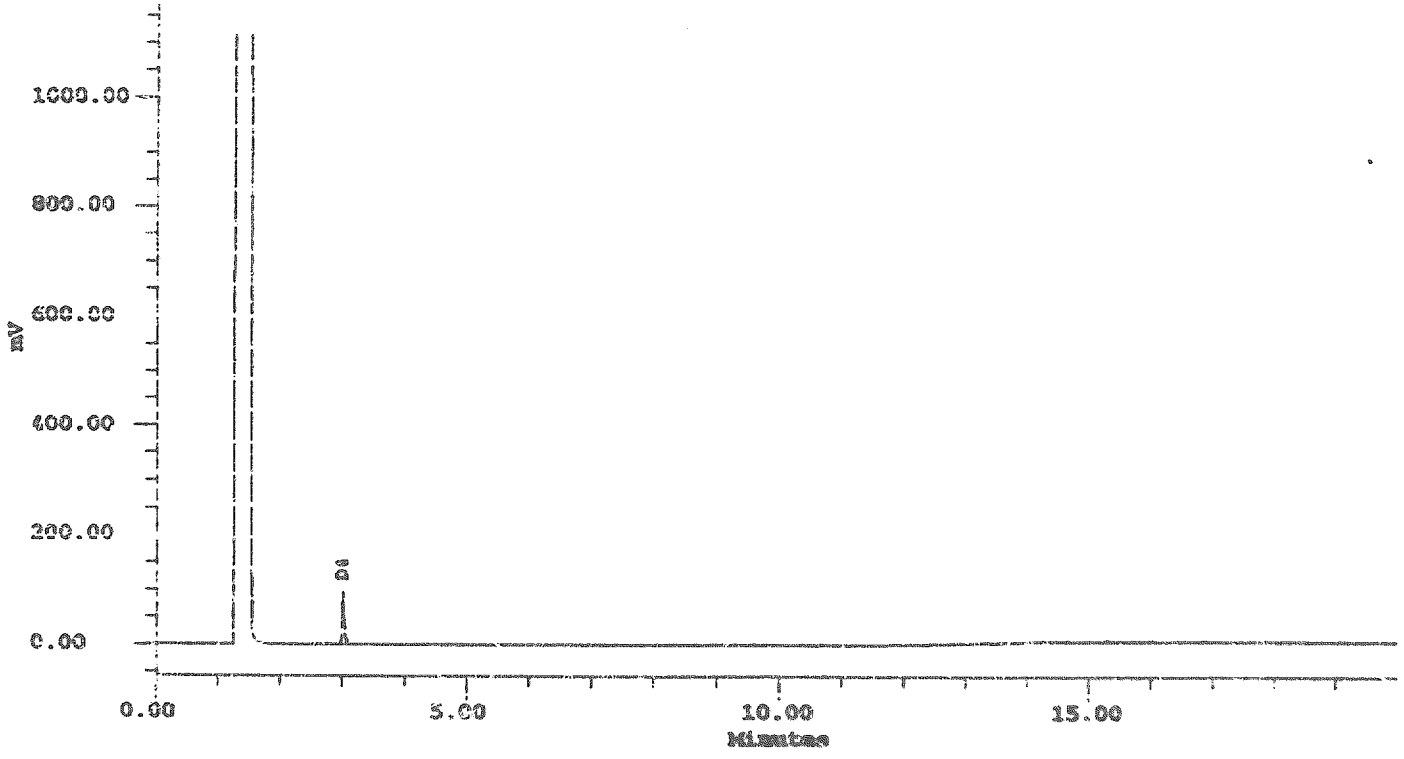
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: 100 ug/mL std Vial: 4 Inj: 1 Chan: SATIN Date Processed 05/12/94 02:49 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	100 ug/mL std	File No.:	
Vial:	4	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 08:58 AM	Date Processed:	05/12/94 02:49 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.012	208250	BB	100.00	

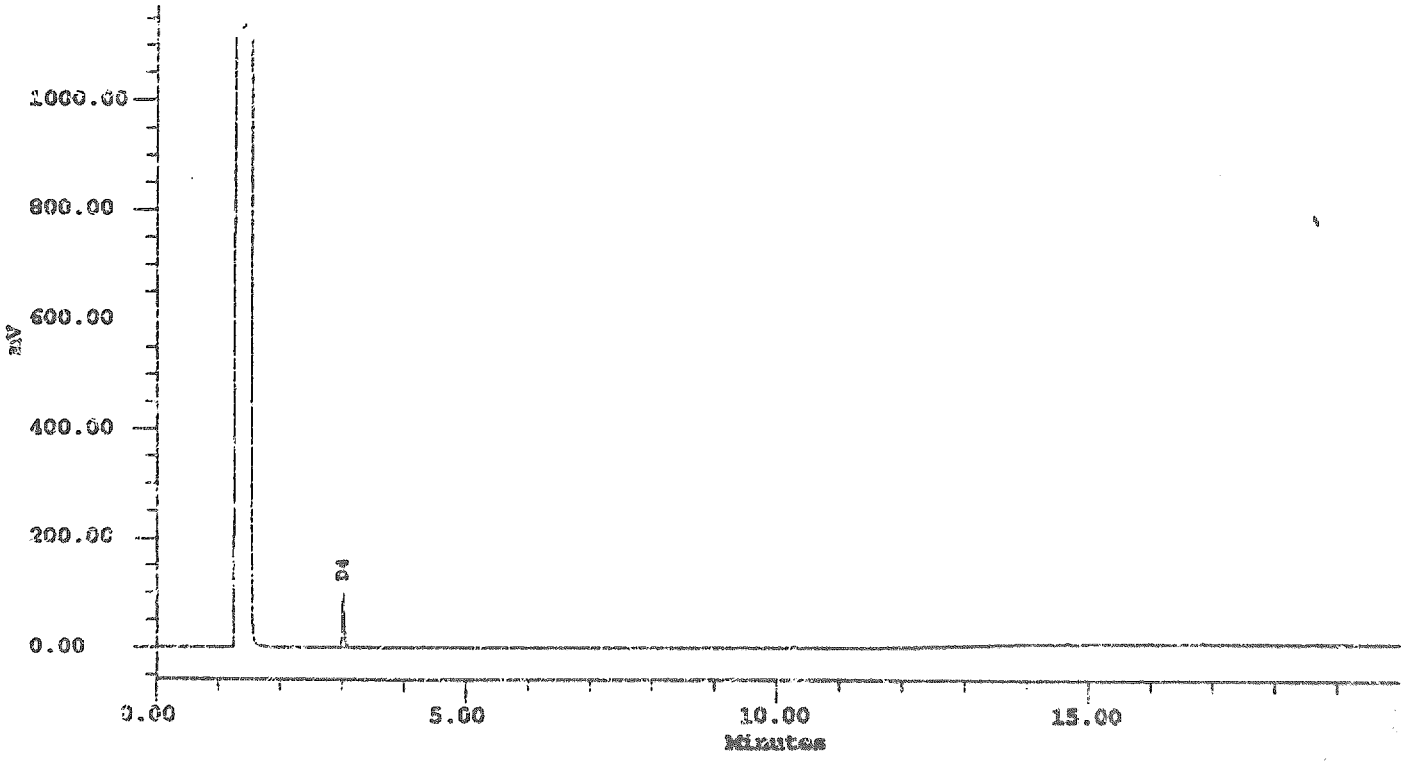
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: 100 ug/mL std Vial: 15 Inj: 1 Chan: SATIN Date Processed 05/15/94 08:32 AM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	100 ug/mL std	File No.:	
Vial:	15	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 05:42 PM	Date Processed:	05/15/94 08:32 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.010	212663	BB	100.00	

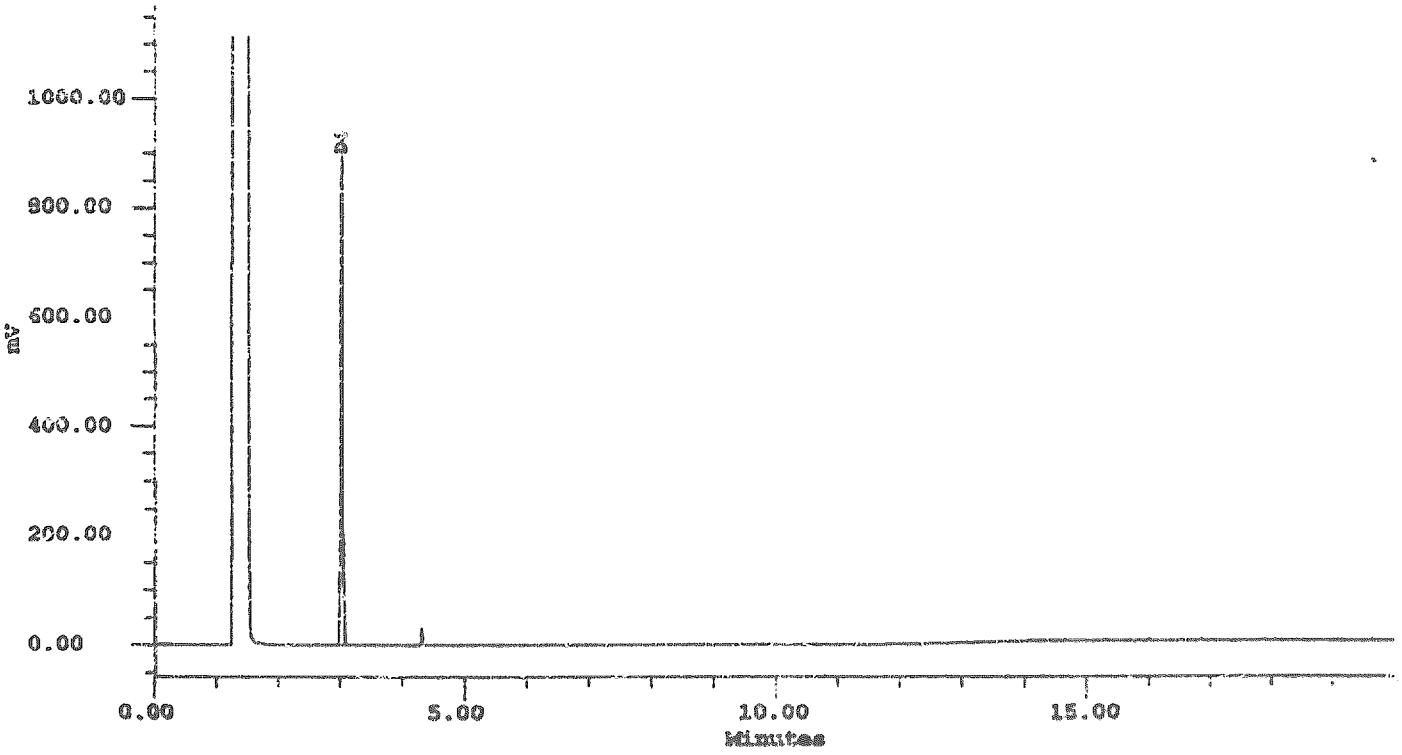
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: 1000 ug/mL std Vial: 5 Inj: 1 Chan: SATIN Date Processed 05/12/94 02:49 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1000 ug/mL std	File No.:	
Vial:	5	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 09:23 AM	Date Processed:	05/12/94 02:49 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



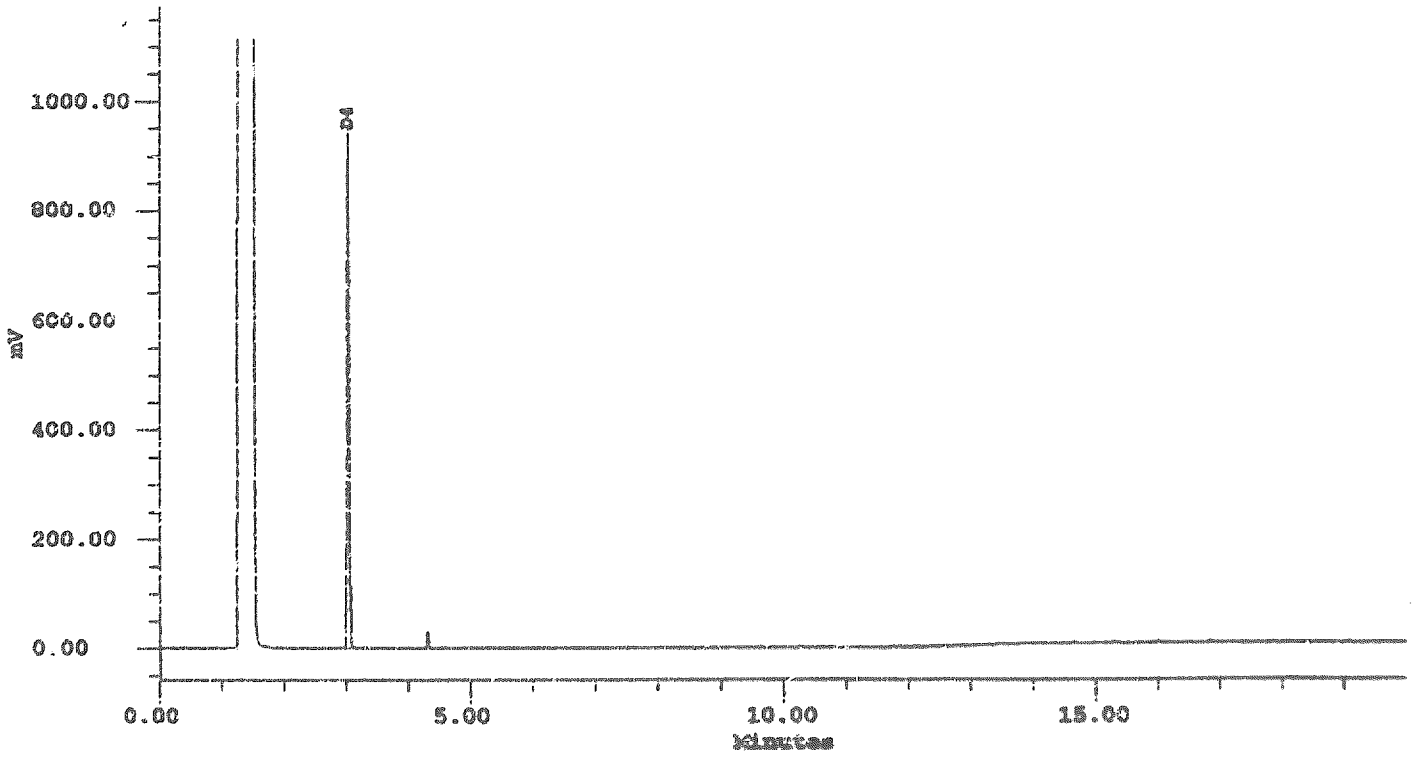
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.037	2043254	BB	100.00	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 1000 ug/mL std Vial: 16 Inj: 1 Chan: SATIN Date Processed 05/15/94 08:33 AM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1000 ug/mL std	File No.:	
Vial:	16	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 06:07 PM	Date Processed:	05/15/94 08:33 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.035	2117606	BB	100.00	

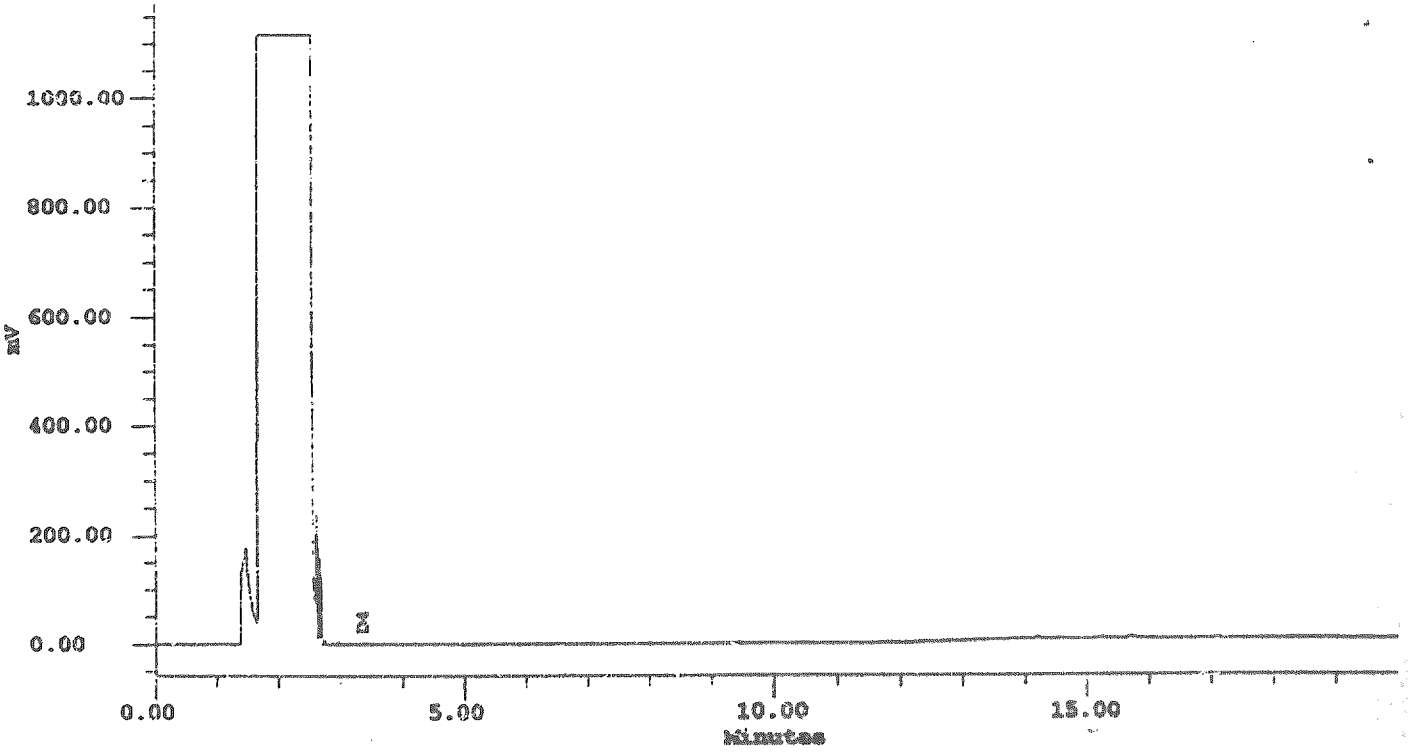
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 o

For Sample: corn oil blank Vial: 7 Inj: 1 Chan: SATIN Date Processed 05/12/94 05:26 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	corn oil blank	File No.:	
Vial:	7	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 02:24 PM	Date Processed:	05/12/94 05:26 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.362	937	BB	9.05	

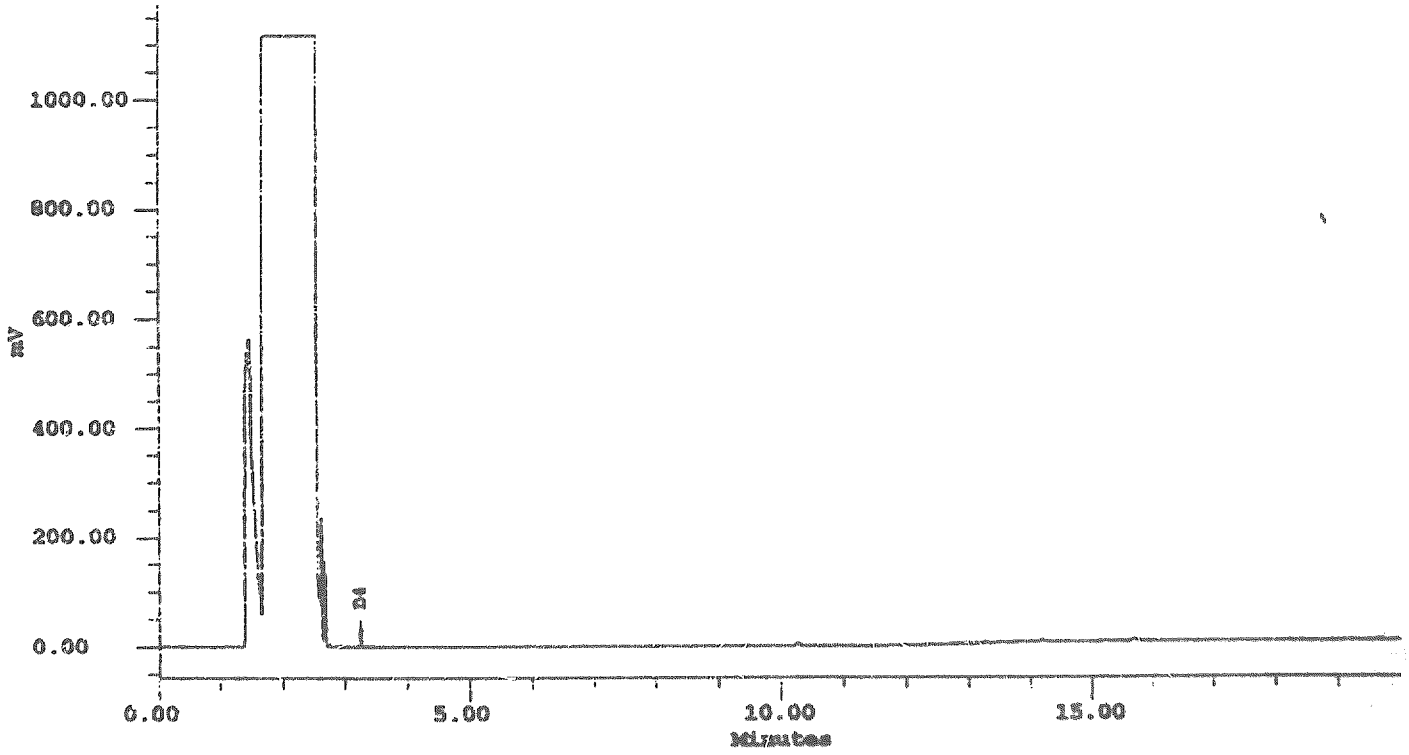
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 o

For Sample: 2 mg/mL smpl Vial: 8 Inj: 1 Chan: SATIN Date Processed 05/12/94 05:27 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	2 mg/mL smpl	File No.:	
Vial:	8	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 02:49 PM	Date Processed:	05/12/94 05:27 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



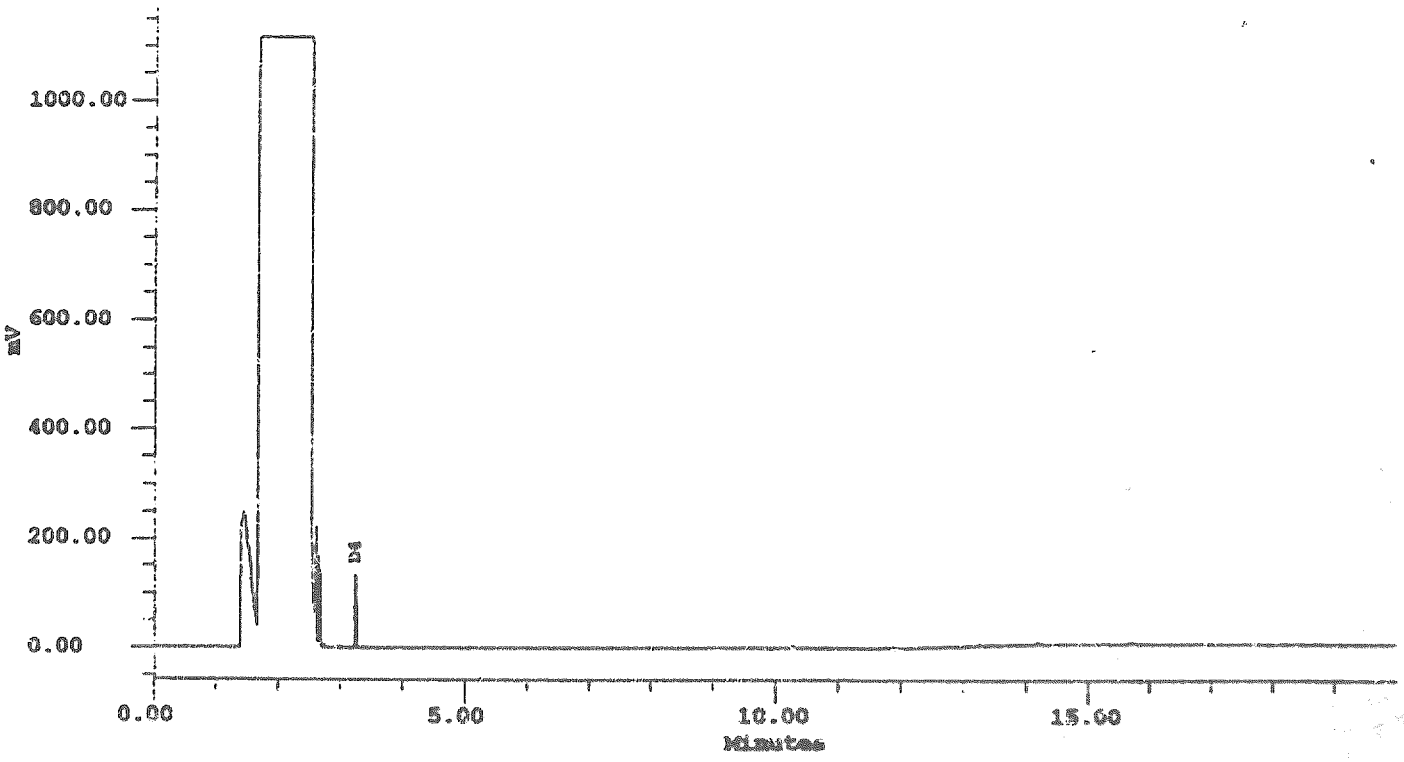
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.242	51244	BB	83.44	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 6 mg/mL smpl Vial: 9 Inj: 1 Chan: SATIN Date Processed 05/12/94 05:27 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	6 mg/mL smpl	File No.:	
Vial:	9	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 03:14 PM	Date Processed:	05/12/94 05:27 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



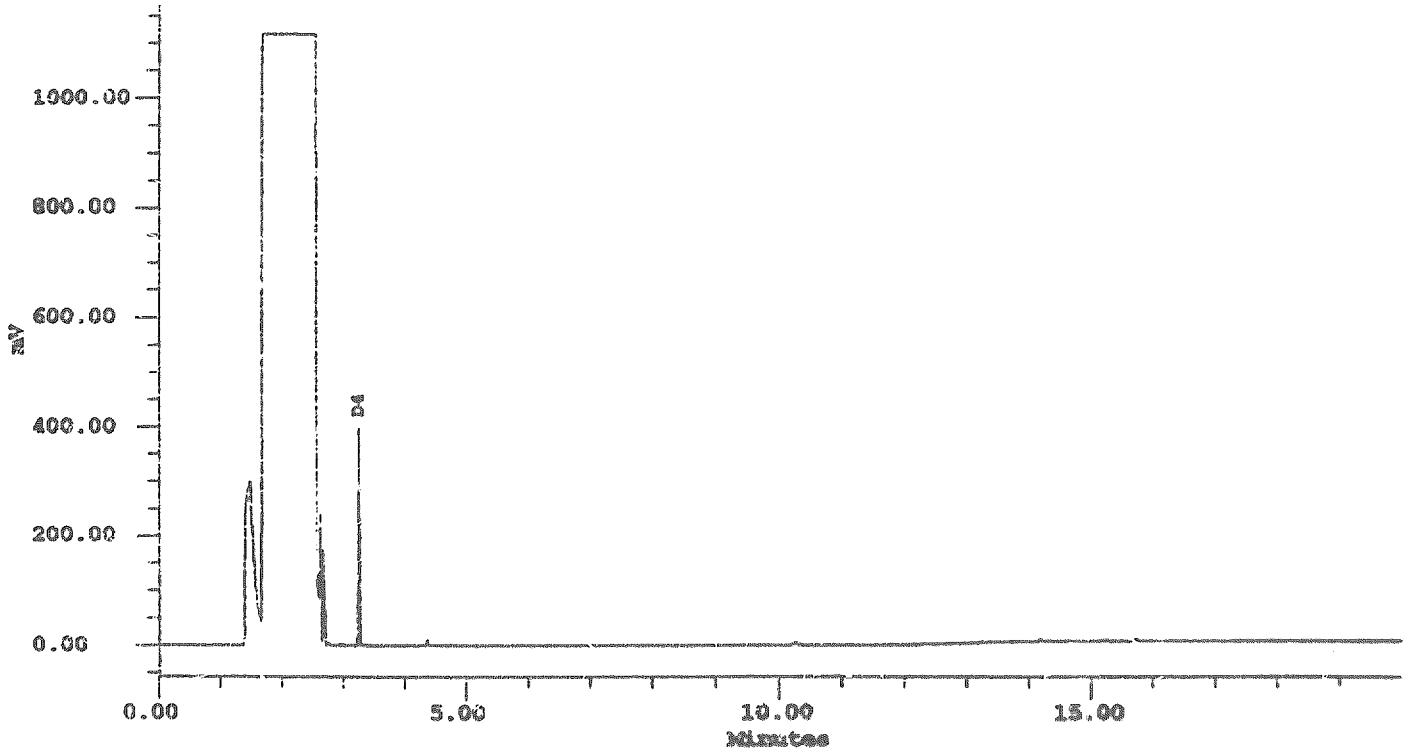
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.238	161879	BB	94.03	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1
For Sample: 20 mg/mL smpl Vial: 10 Inj: 1 Chan: SATIN Date Processed 05/12/94 05:28 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	20 mg/mL smpl	File No.:	
Vial:	10	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 03:39 PM	Date Processed:	05/12/94 05:28 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



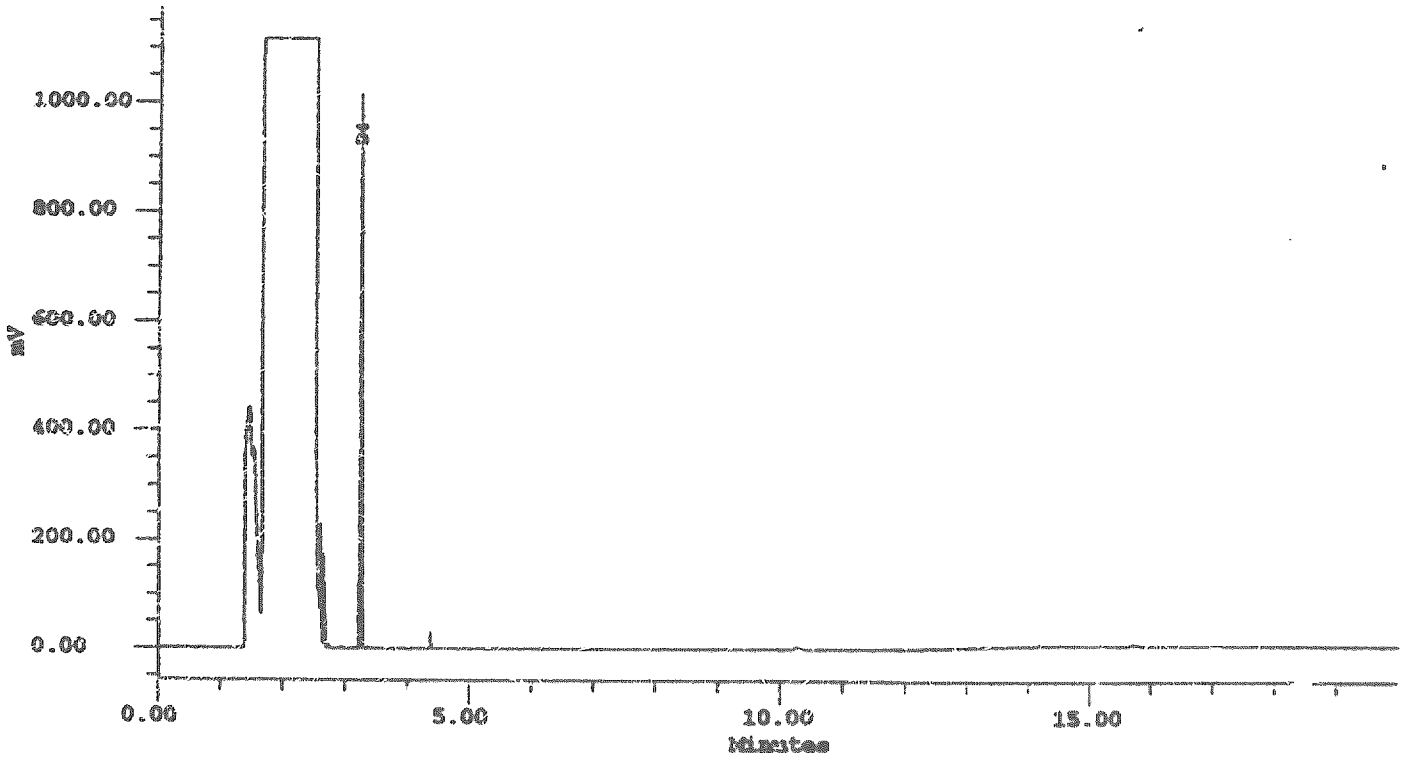
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.255	520393	BV	98.07	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 60 mg/mL smpl Vial: 11 Inj: 1 Chan: SATIN Date Processed 05/12/94 05:29 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	60 mg/mL smpl	File No.:	
Vial:	11	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/12/94 04:03 PM	Date Processed:	05/12/94 05:29 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.263	1627335	DB	99.38	

Millennium Results Report: HES_pm Proc Chan: SATIN Page 1 of

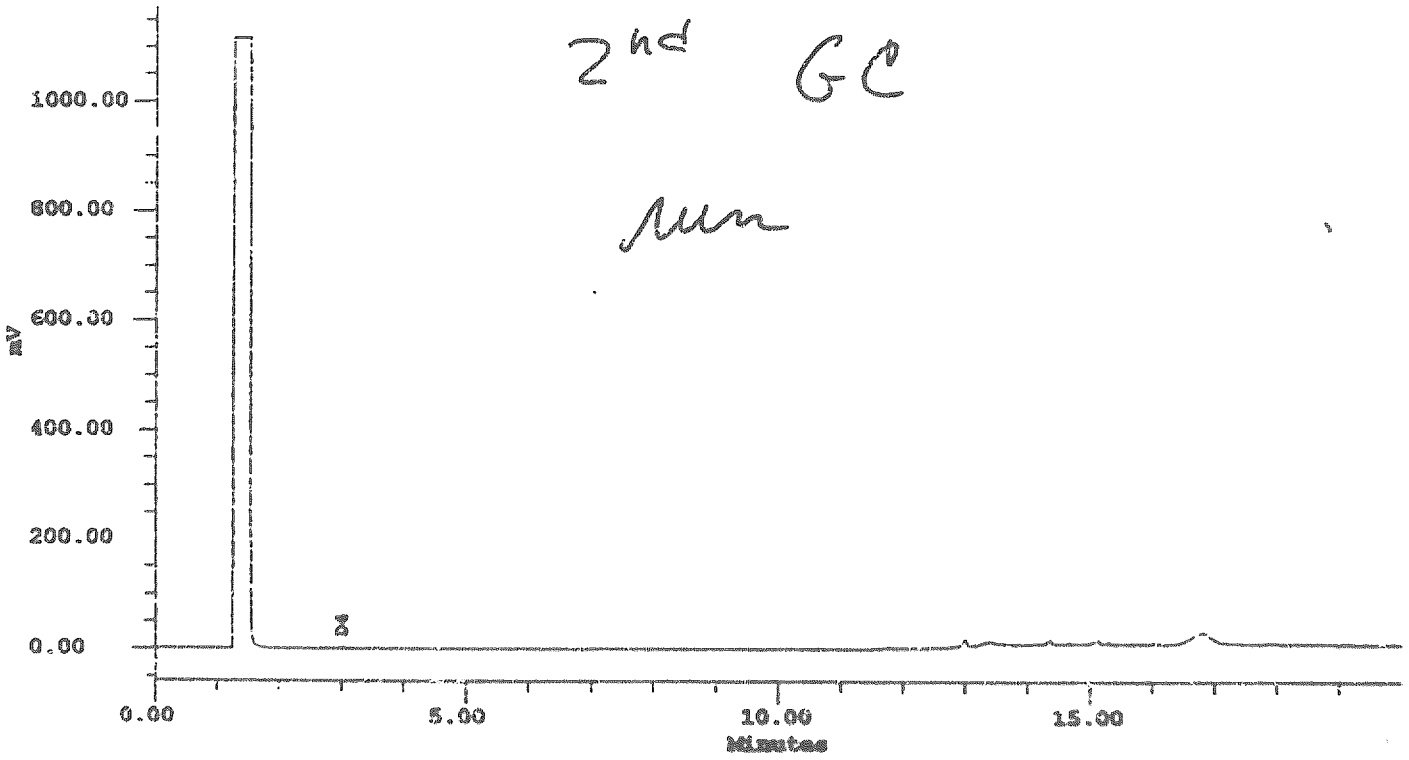
For Sample: 1 ug/mL std Vial: 2 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:05 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1 ug/mL std	File No.:	0
Vial:	2	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 02:00 AM	Date Processed:	05/16/94 09:05 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		

2nd GC



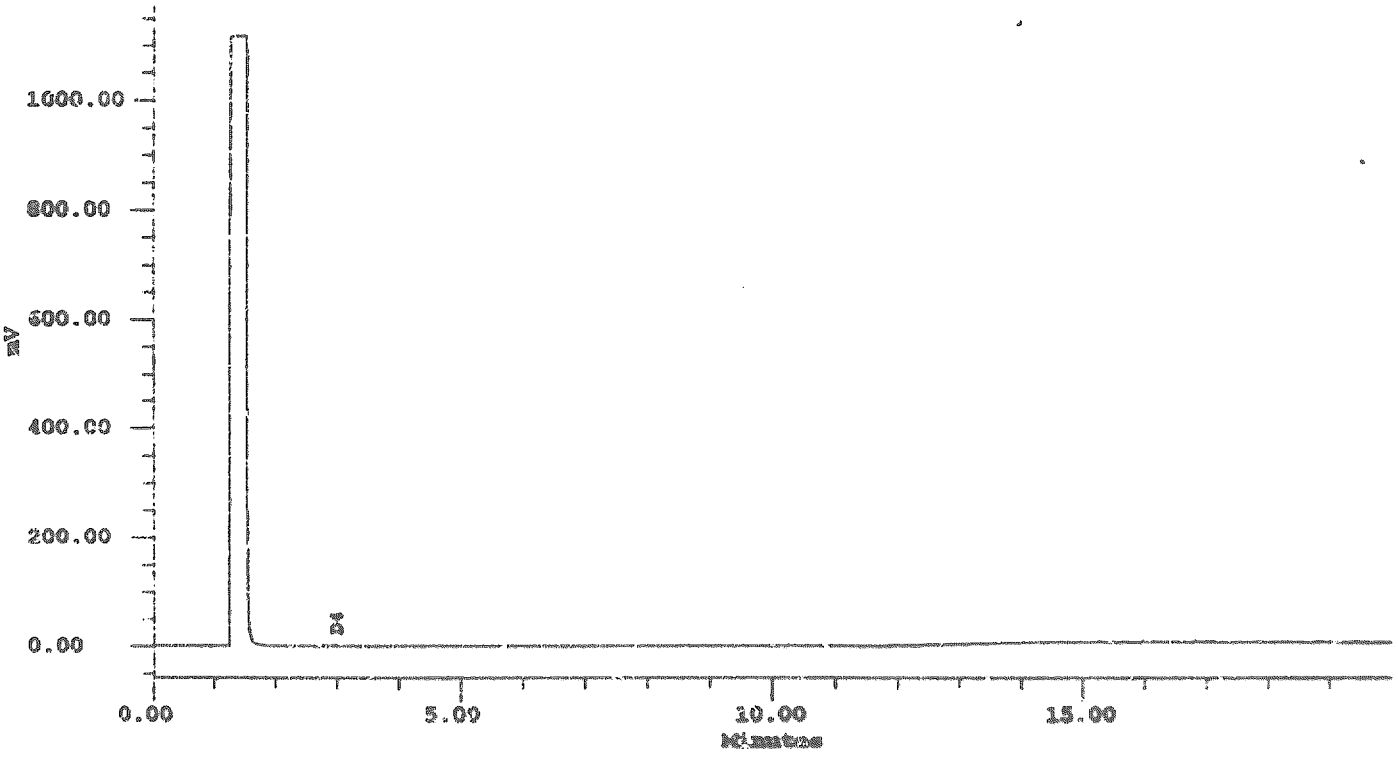
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.011	3278	BB	100.00	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1
For Sample: 1 ug/mL std Vial: 18 Inj: 1 Chan: SATIN Date Processed 05/17/94 03:25 AM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1 ug/mL std	File No.:	0
Vial:	18	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 11:34 PM	Date Processed:	05/17/94 03:25 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



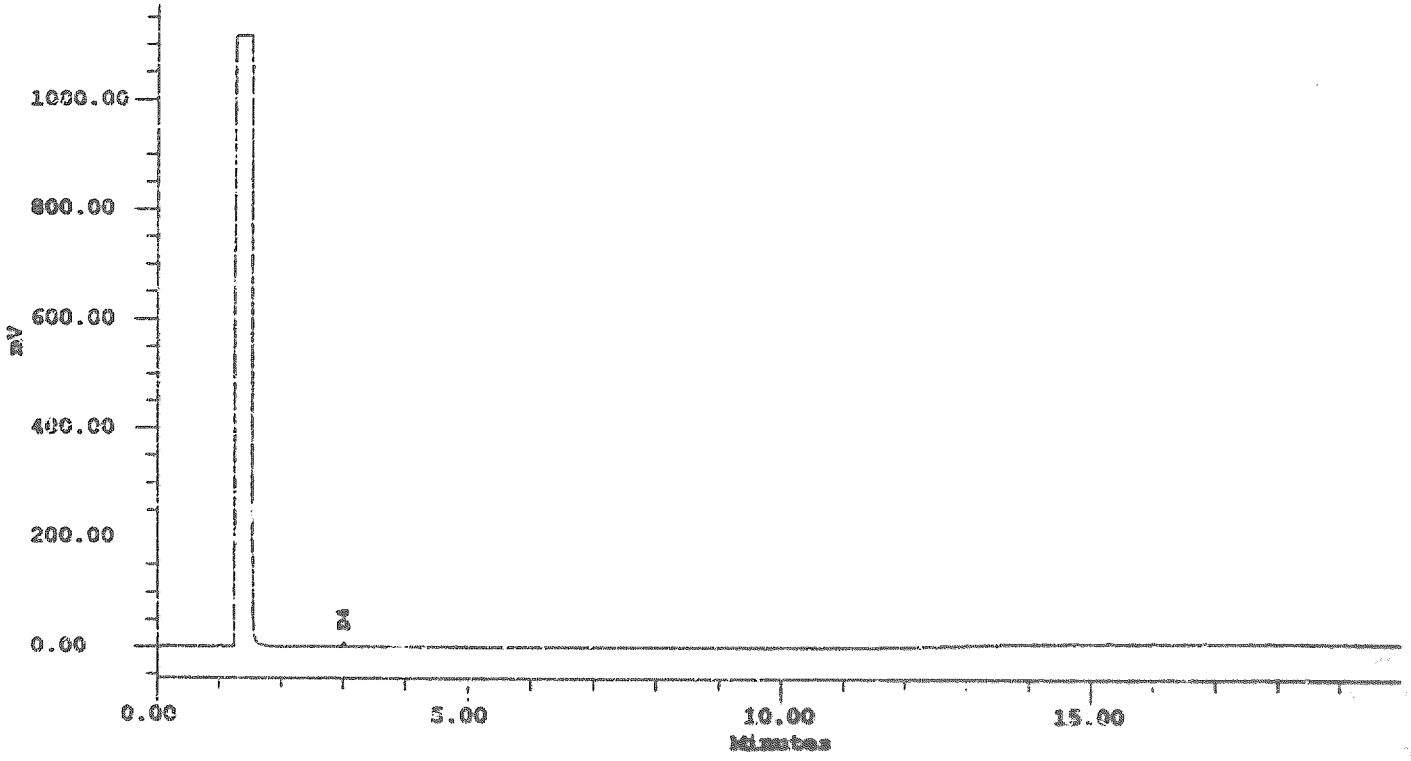
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.007	3624	BB	100.00	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 10 ug/mL std Vial: 3 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:06 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	10 ug/mL std	File No.:	0
Vial:	3	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 02:25 AM	Date Processed:	05/16/94 09:06 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



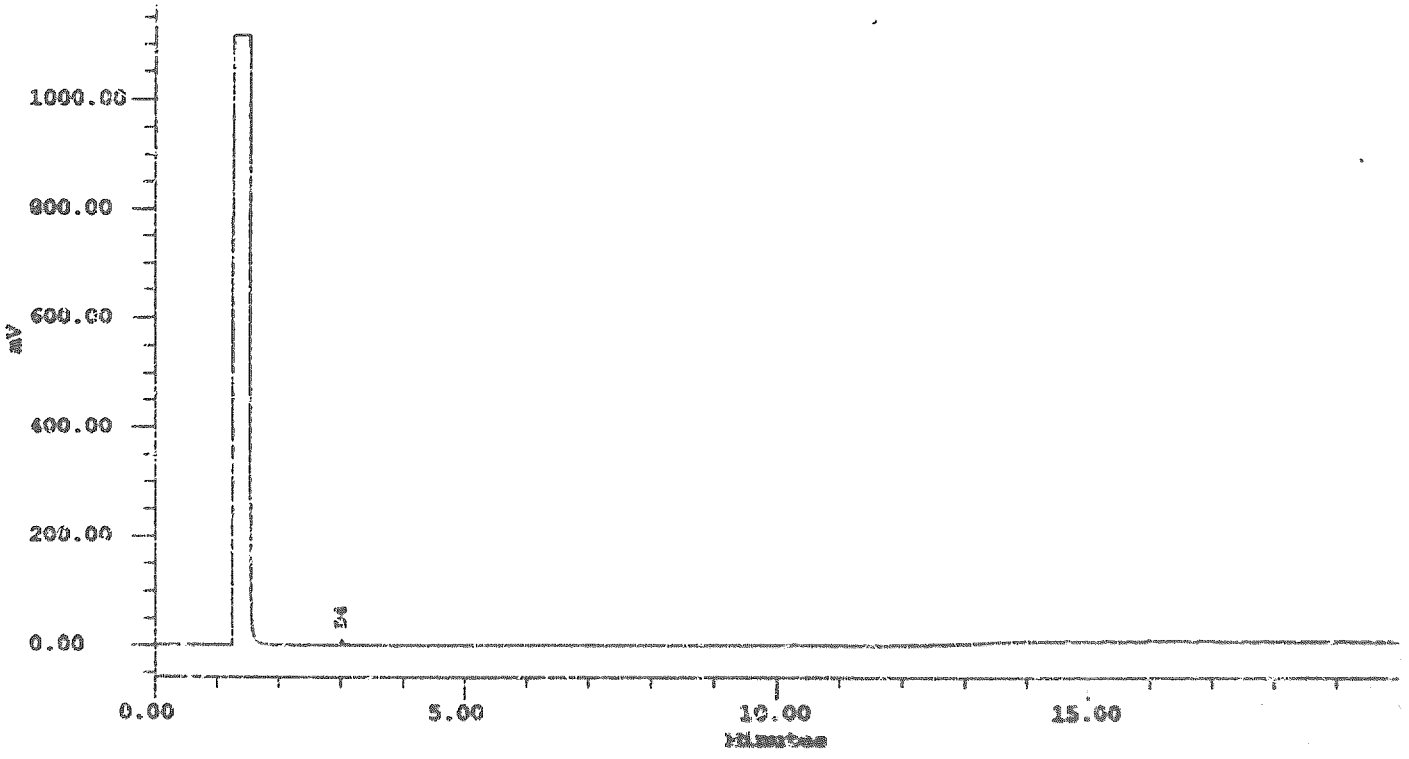
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.009	22728	BB	100.00	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 10 ug/mL std Vial: 19 Inj: 1 Chan: SATIN Date Processed 05/17/94 03:26 AM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	10 ug/mL std	File No.:	0
Vial:	19	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 11:59 PM	Date Processed:	05/17/94 03:26 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.007	25063	BB	100.00	

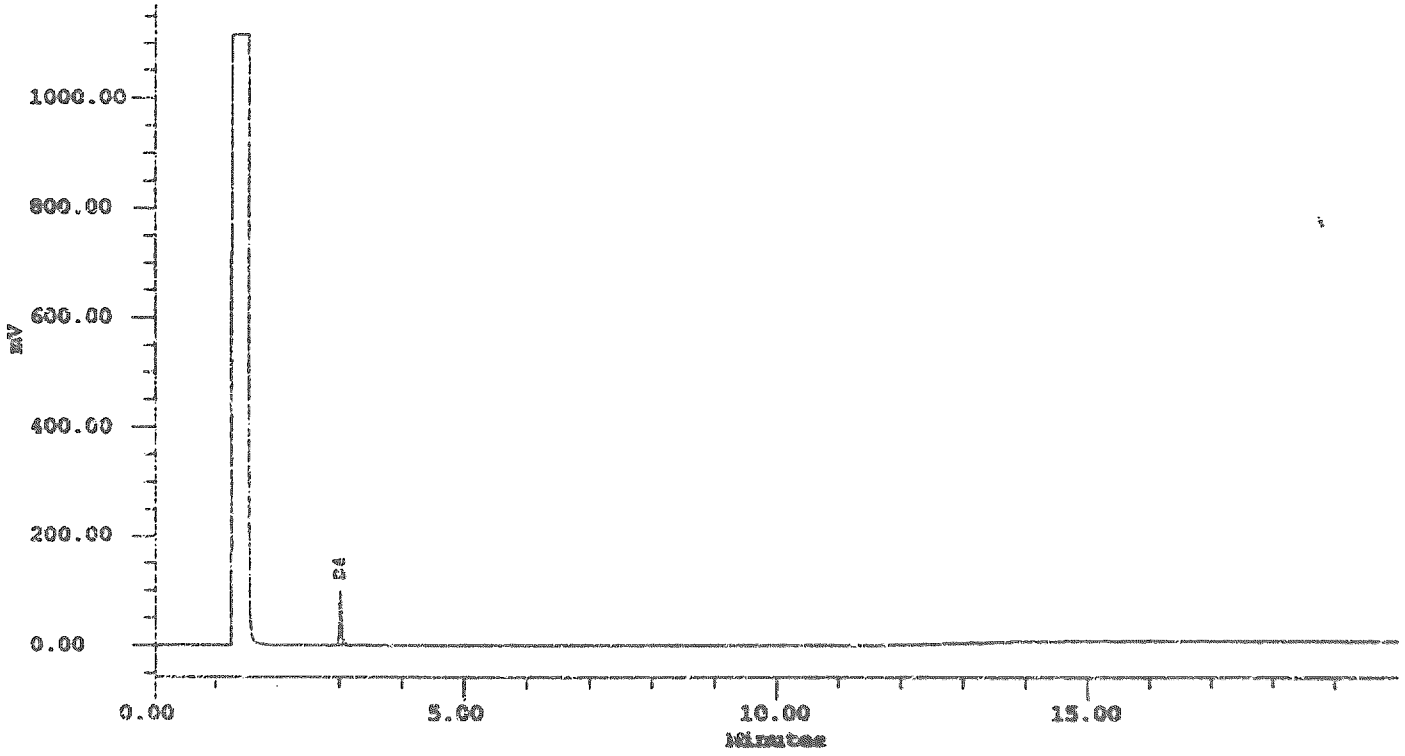
Millennium Results Report: HES_pm Proc Chan: SATIN Page 1

For Sample: 100 ug/mL std Vial: 4 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:06 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	100 ug/mL std	File No.:	0
Vial:	4	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 02:50 AM	Date Processed:	05/16/94 09:06 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_m9		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.010	213671	BB	100.00	

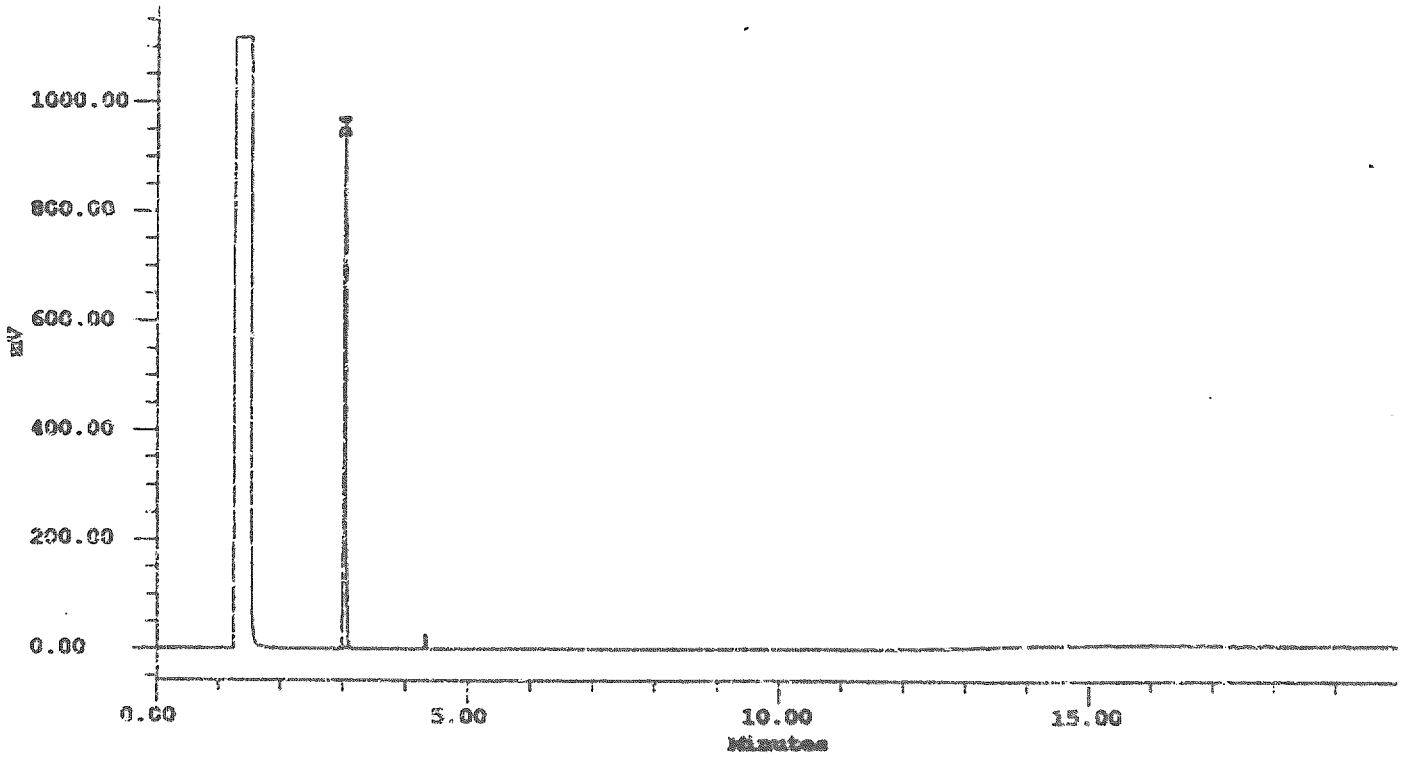
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: 1000 ug/mL std Vial: 5 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:07 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1000 ug/mL std	File No.:	0
Vial:	5	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 03:14 AM	Date Processed:	05/16/94 09:07 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.035	2091946	BB	100.00	

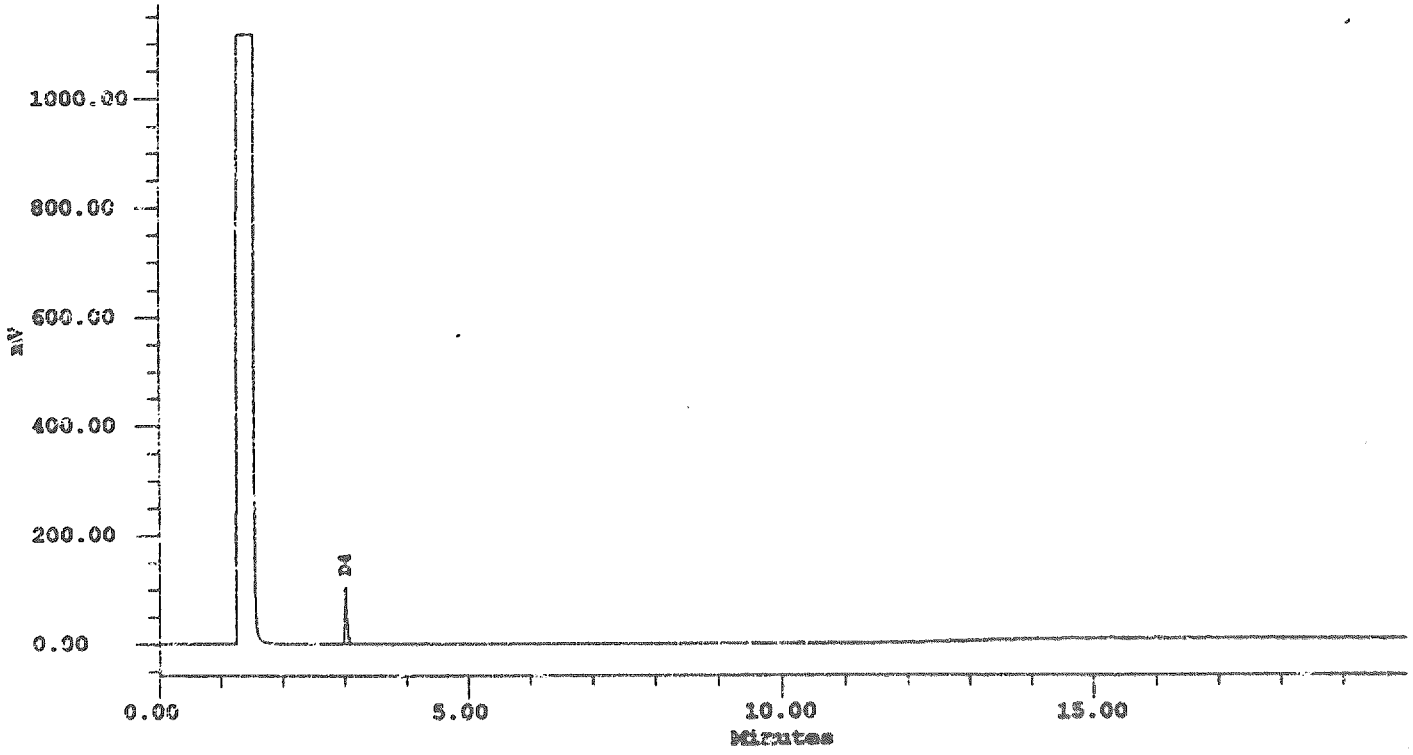
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: 100 ug/mL std Vial: 20 Inj: 1 Chan: SATIN Date Processed 05/17/94 03:26 AM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	100 ug/mL std	File No.:	0
Vial:	20	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/17/94 12:24 AM	Date Processed:	05/17/94 03:26 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



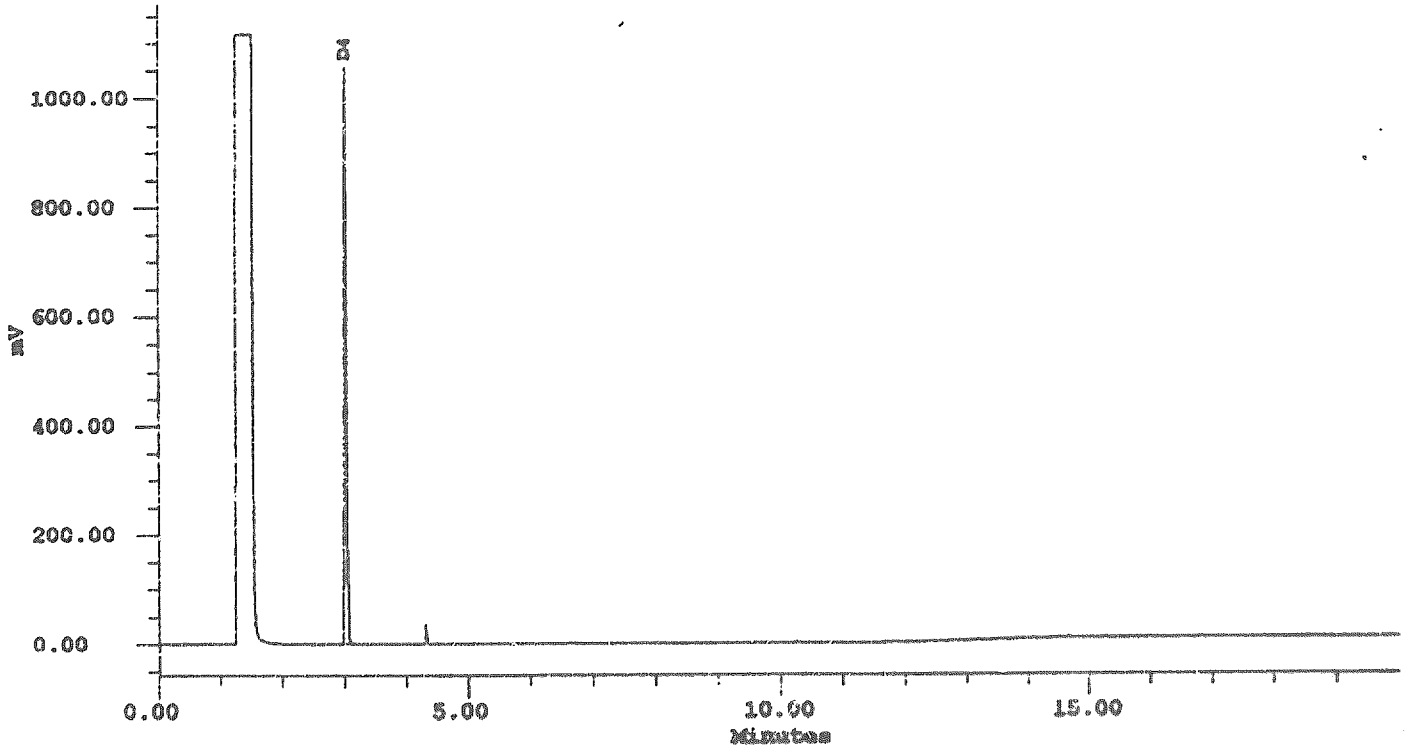
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.008	235039	BB	100.00	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 1000 ug/mL std Vial: 21 Inj: 1 Chan: SATIN Date Processed 05/17/94 03:27 AM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	1000 ug/mL std	File No.:	0
Vial:	21	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/17/94 12:48 AM	Date Processed:	05/17/94 03:27 AM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



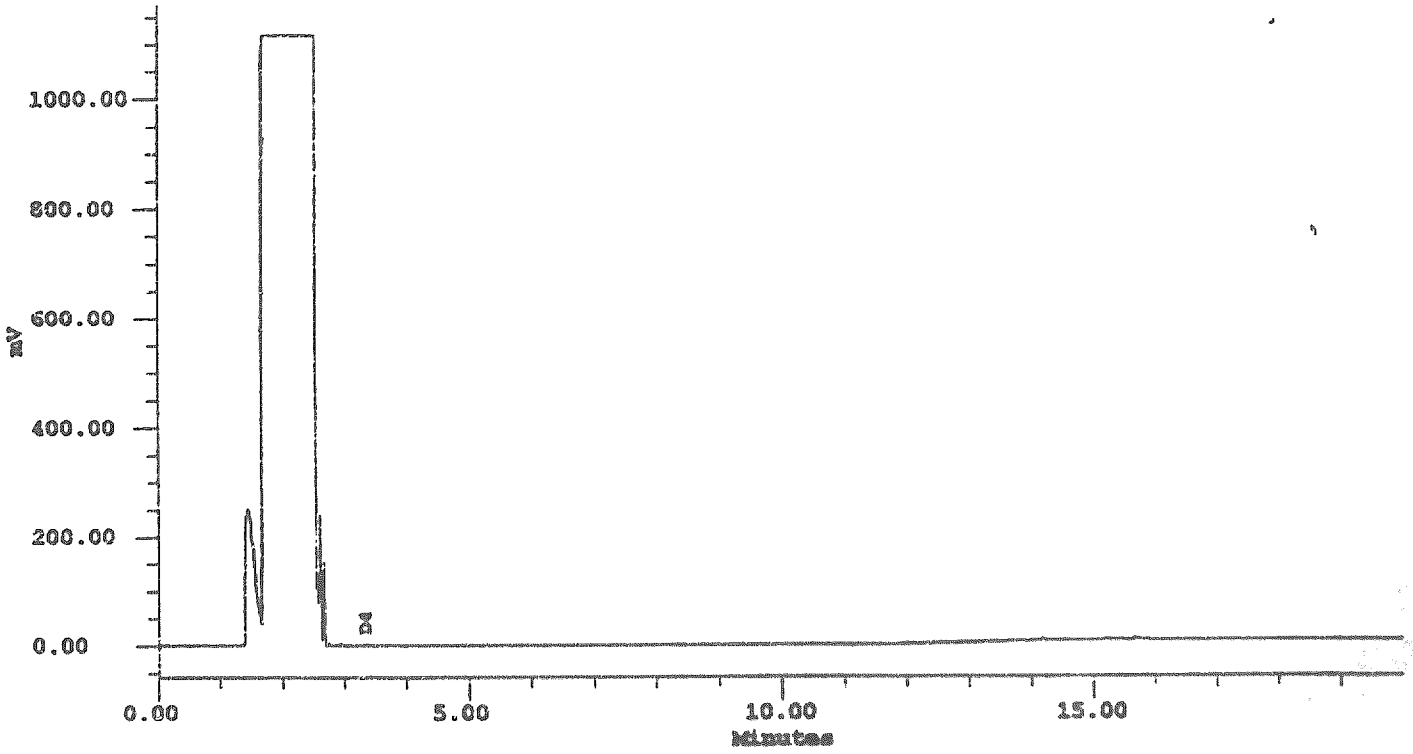
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.033	2354264	BB	100.00	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1
For Sample: corn oil, rep2 Vial: 7 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:19 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	corn oil, rep2	File No.:	0
Vial:	7	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 04:04 AM	Date Processed:	05/16/94 09:19 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.356	1127	BB	10.48	

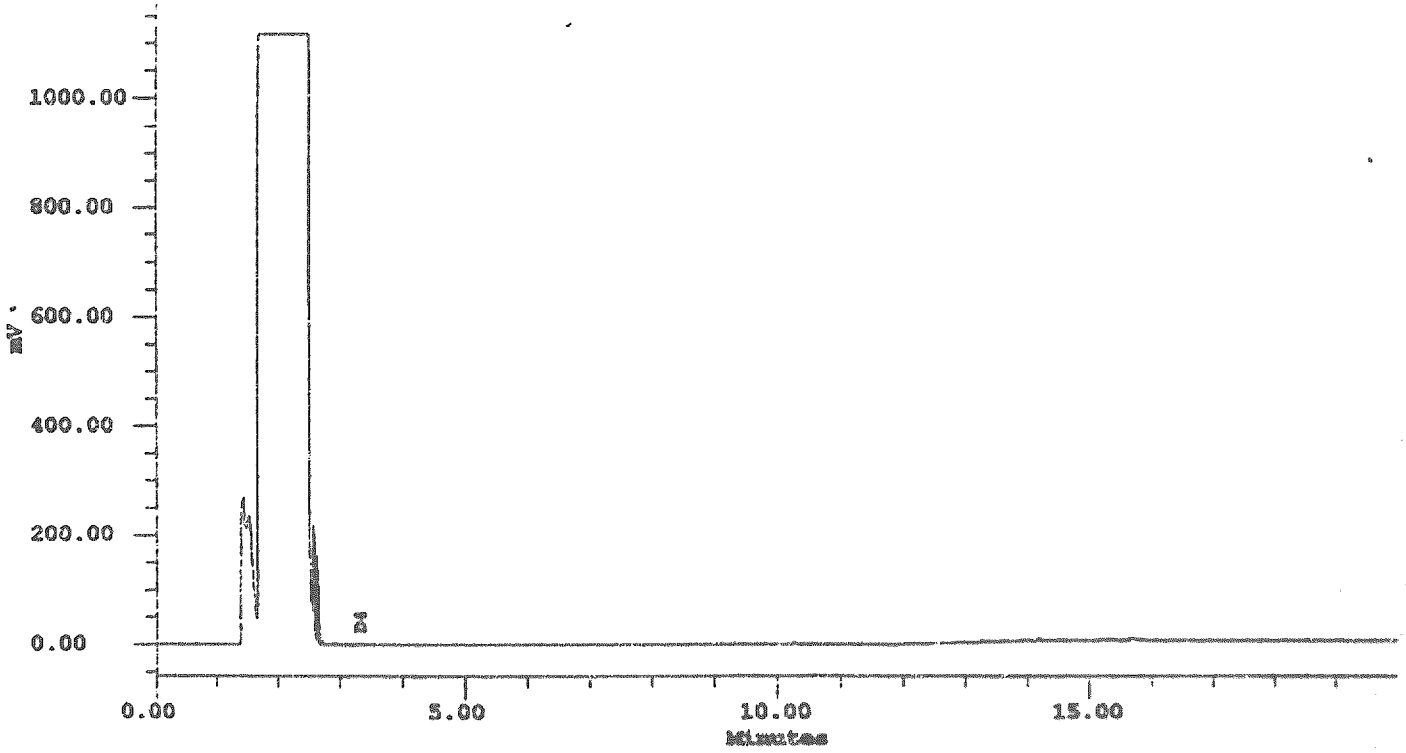
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of

For Sample: corn oil, rep3 Vial: 8 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:19 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	corn oil, rep3	File No.:	0
Vial:	8	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 04:29 AM	Date Processed:	05/16/94 09:19 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



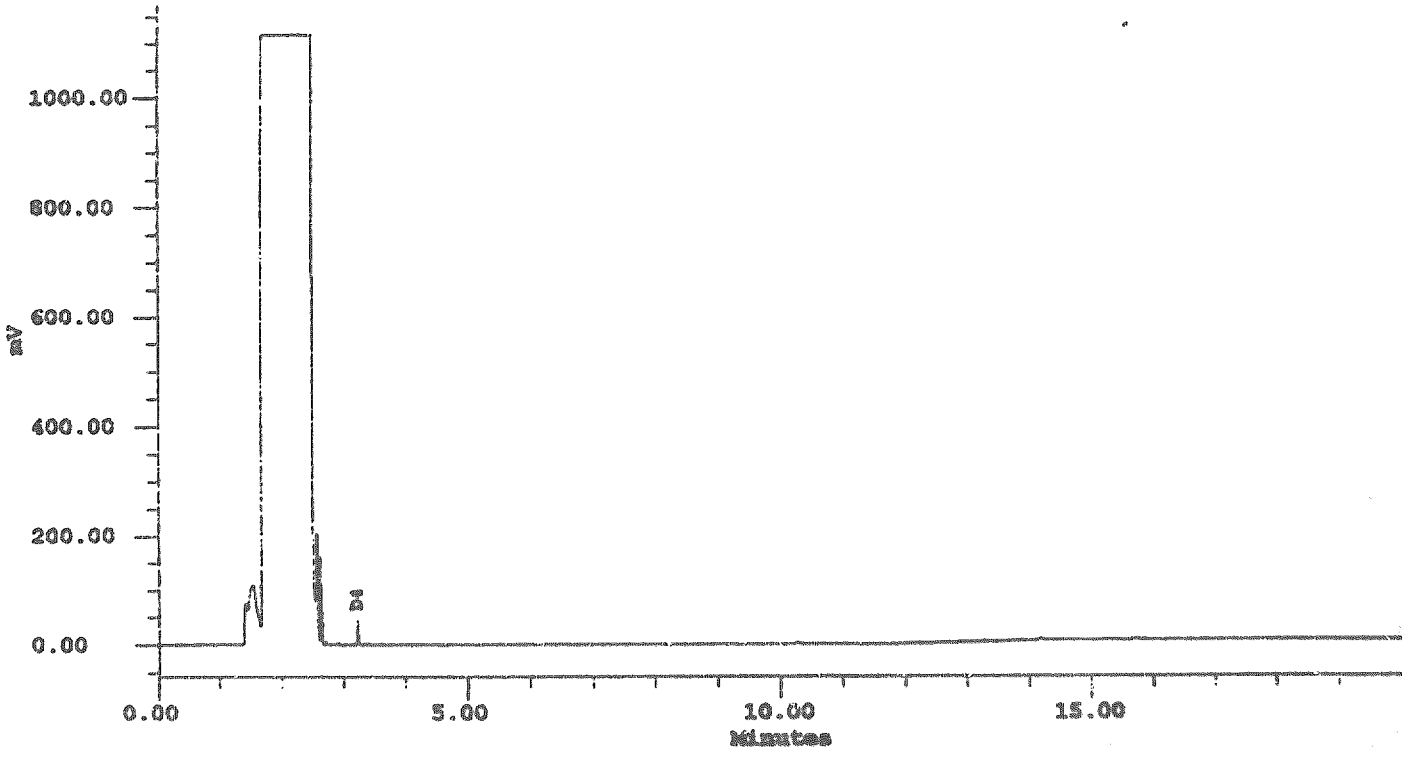
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.341	1224	BB	11.90	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 2 mg/mL, rep 2 Vial: 8 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:20 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	2 mg/mL, rep 2	File No.:	0
Vial:	~~8~~ 9 MEY wrong	Sample Type:	Unknown
Injection:	1 5/18/94 [illegible]	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 04:53 AM	Date Processed:	05/16/94 09:20 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



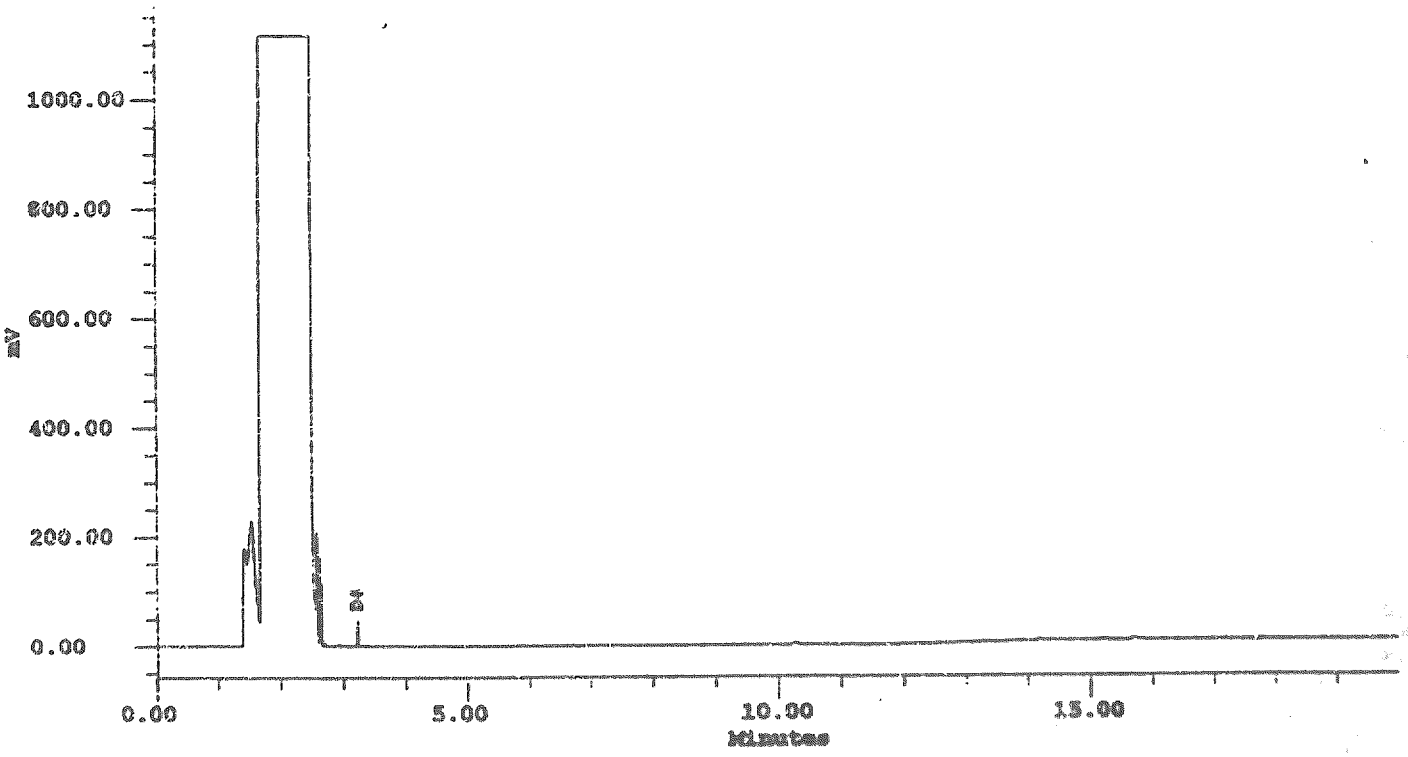
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.221	50318	BB	83.30	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 2 mg/mL, rep 3 Vial: 9 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:20 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	2 mg/mL, rep 3	File No.:	0
Vial:	~~9~~ 10 MEV 5/18/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 05:18 AM	Date Processed:	05/16/94 09:20 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



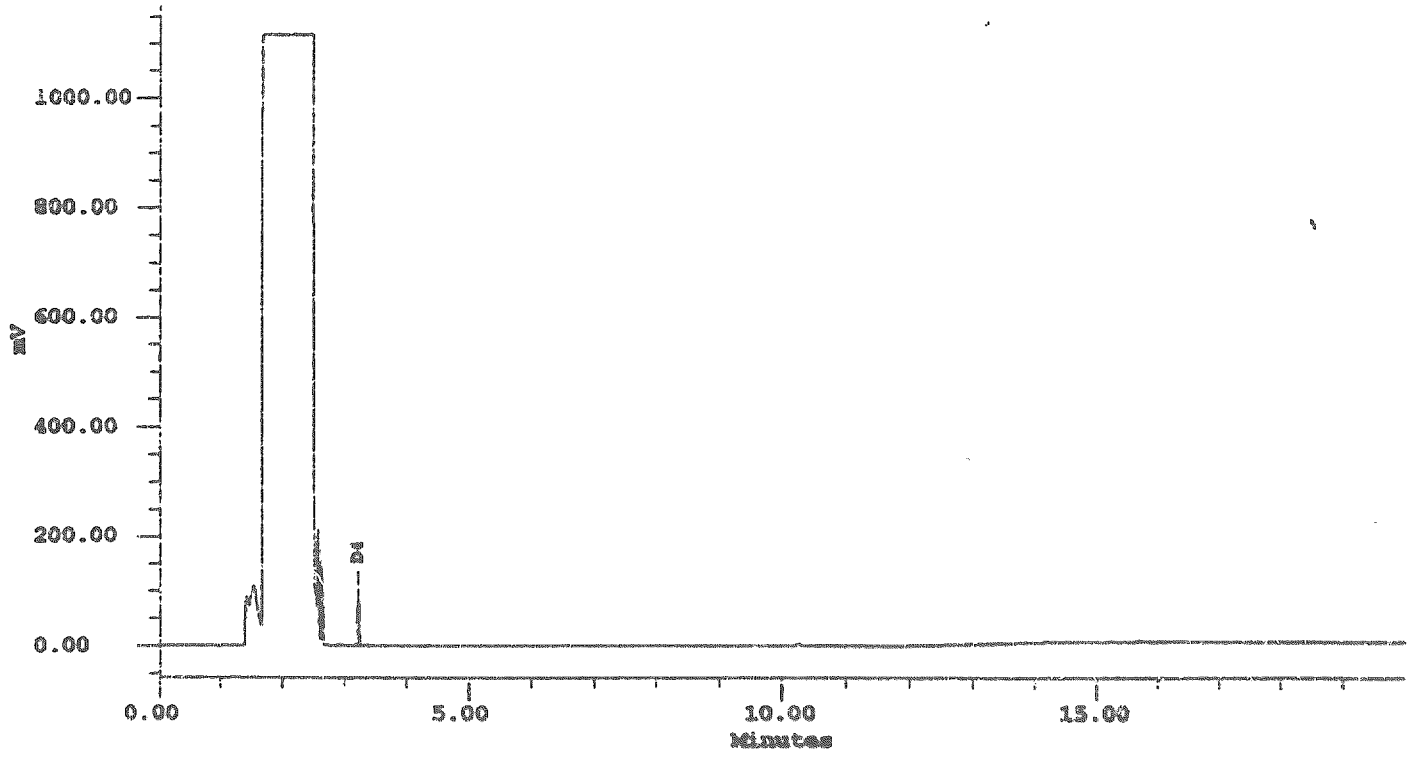
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.221	54193	BB	84.32	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 6 mg/mL, rep 2 Vial: 9 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:21 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	6 mg/mL, rep 2	File No.:	0
Vial:	~~9~~ 11 MJ 5/18/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 05:43 AM	Date Processed:	05/16/94 09:21 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.223	161119	BB	94.09	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 6 mg/mL, rep 3 Vial: 10 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:22 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	6 mg/mL, rep 3	File No.:	0
Vial:	~~10~~ 12 MEI 5/8/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 06:08 AM	Date Processed:	05/16/94 09:22 PM
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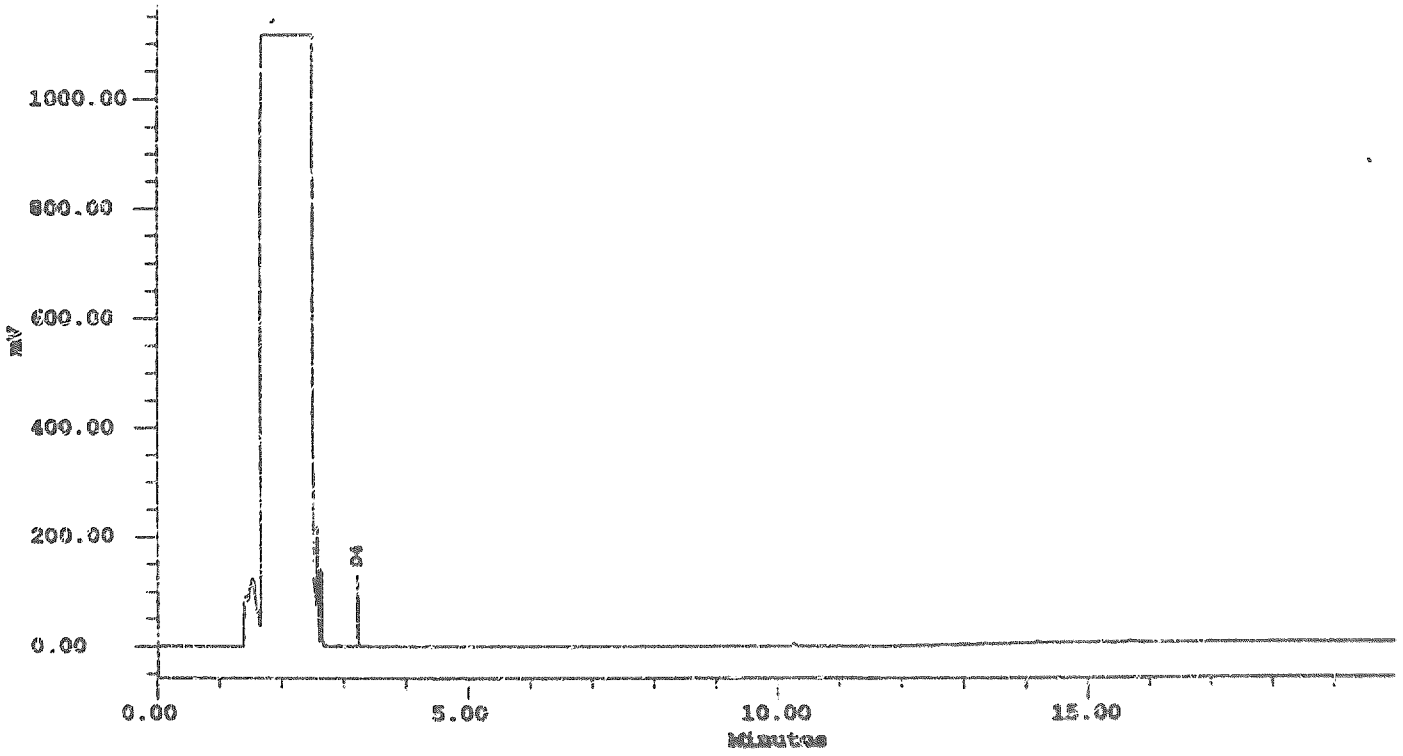
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



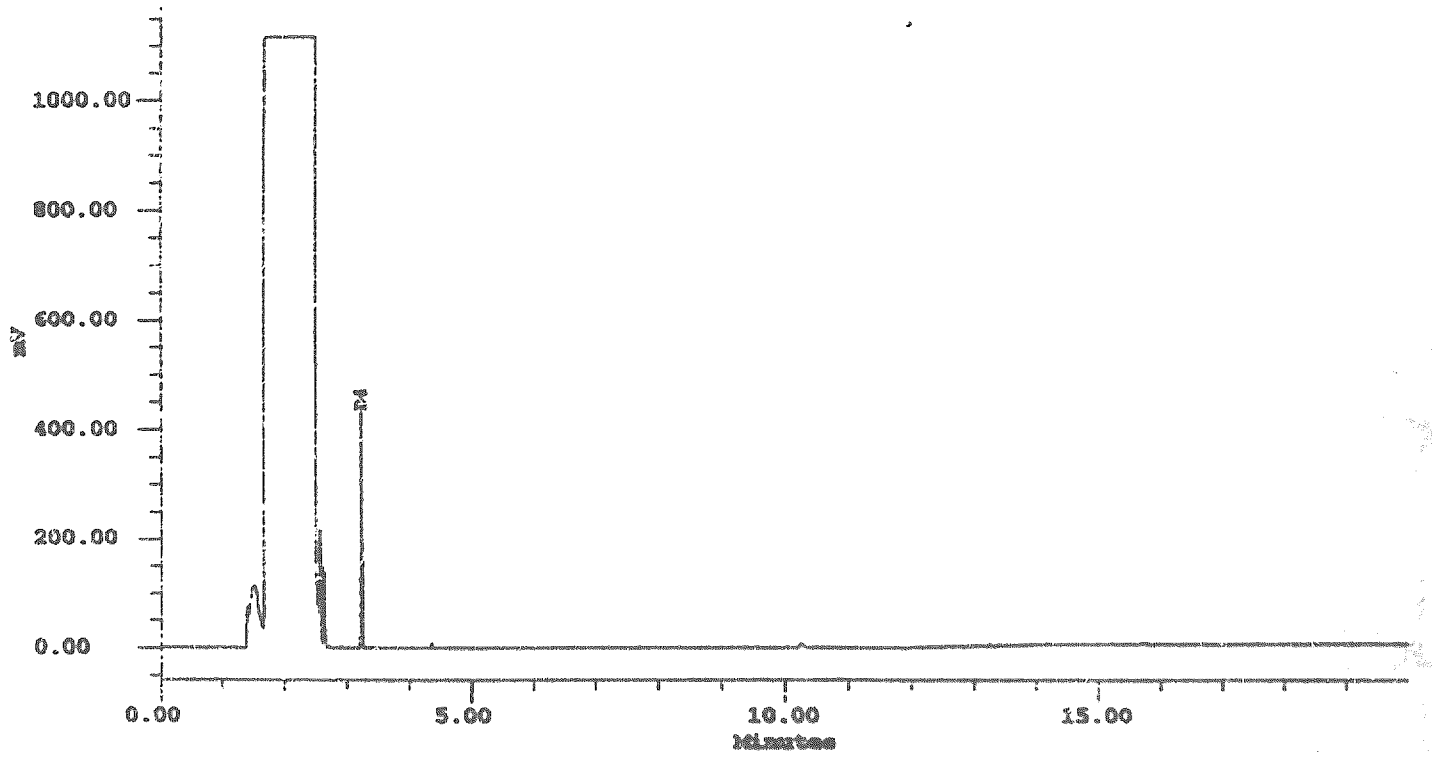
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.223	151937	BB	93.80	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 20 mg/mL, rep2 Vial: 10 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:23 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	20 mg/mL, rep2	File No.:	0
Vial:	~~10~~ 13 MEV 5/6/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 06:32 AM	Date Processed:	05/16/94 09:23 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.228	520364	BB	98.13	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 20 mg/mL, rep3 Vial: 11 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:23 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	20 mg/mL, rep3	File No.:	0
Vial:	~~11~~ 14 [illegible] 5/18/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 06:57 AM	Date Processed:	05/16/94 09:23 PM
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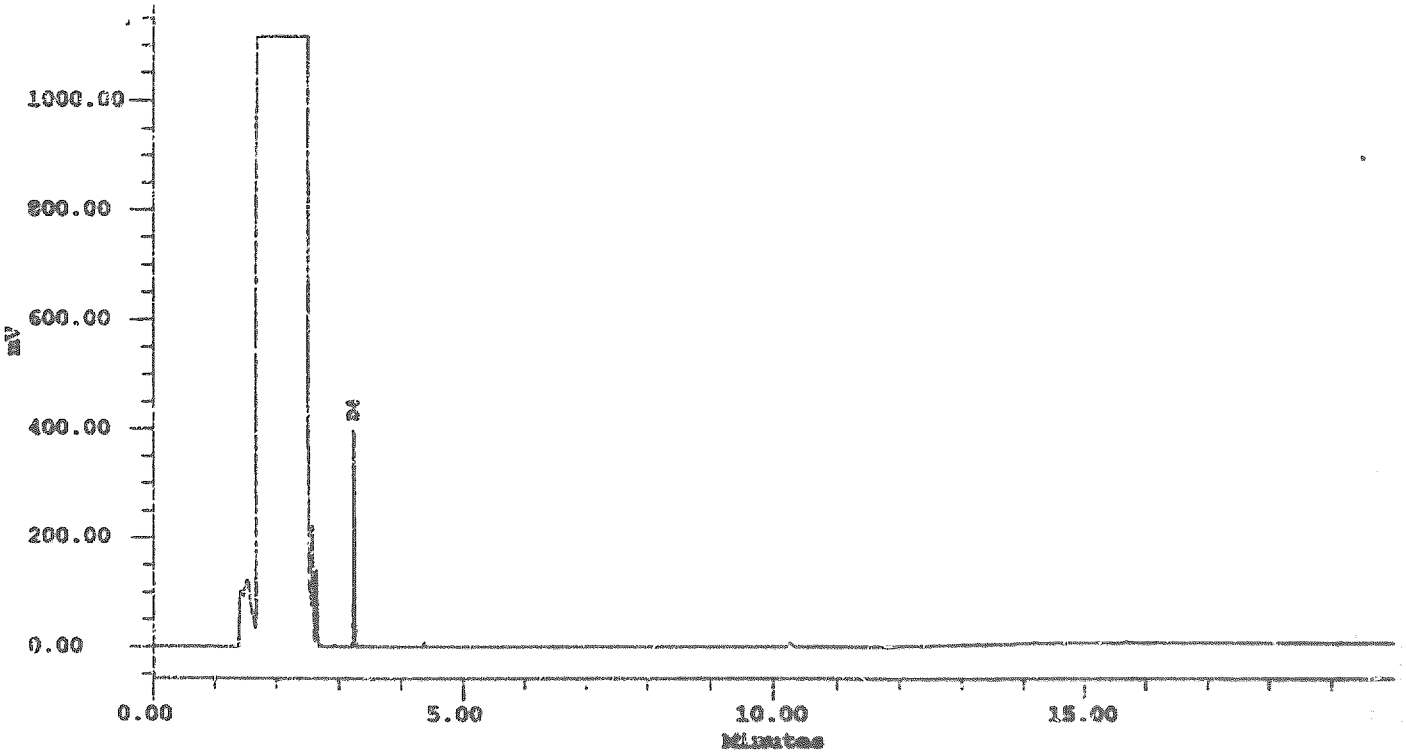
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.231	508880	BB	98.06	

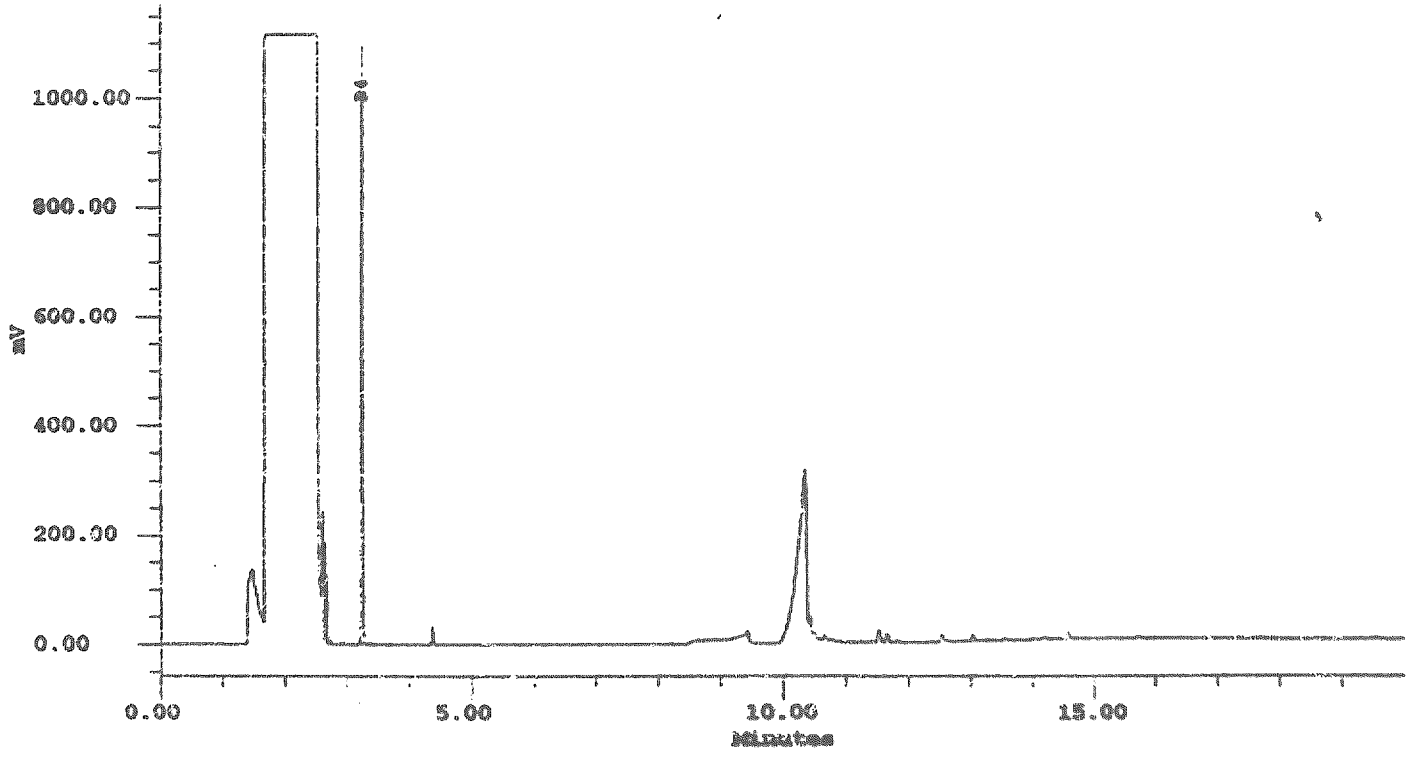
Millennium Results Report: HES_rm Proc Chan: SATIN Page 1

For Sample: 60 mg/mL, rep2 Vial: 11 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:24 PM

Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	60 mg/mL, rep2	File No.:	9
Vial:	~~11~~ 15 MVD 5/18/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 08:09 PM	Date Processed:	05/16/94 09:24 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



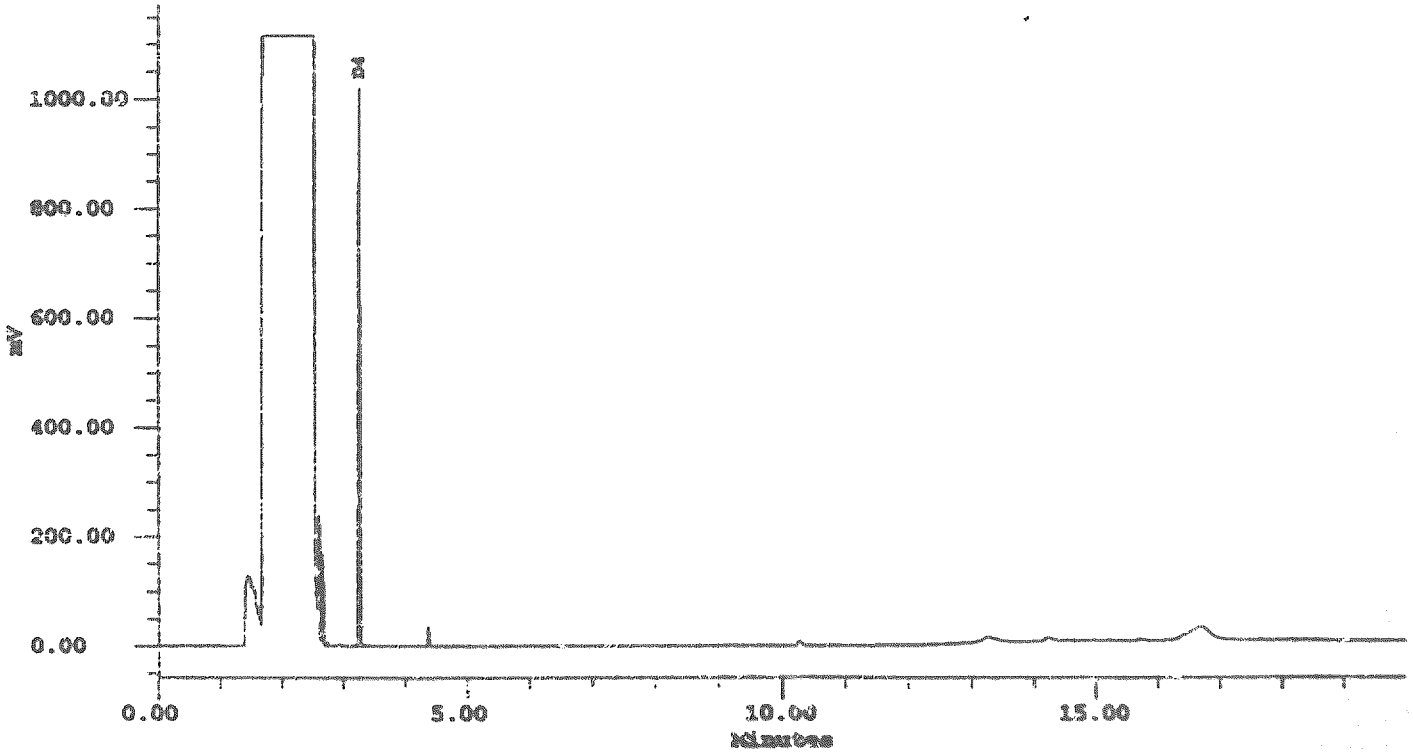
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.252	1690819	BV	99.34	

Millennium Results Report: HES_rm Proc Chan: SATIN Page 1 of
For Sample: 60 mg/mL, rep3 Vial: 12 Inj: 1 Chan: SATIN Date Processed 05/16/94 09:25 PM
Channel Descr:

Millennium Sample Information

Analyst:	Mark Van Dyke		
Project Name:	Dolly		
Sample Name:	60 mg/mL, rep3	File No.:	0
Vial:	~~12~~ 16 MEG 5/8/94 wrong entry	Sample Type:	Unknown
Injection:	1	Volume:	1.00
Channel:	SATIN	Run Time:	20.0 min
Date Acquired:	05/16/94 08:34 PM	Date Processed:	05/16/94 09:25 PM
Scale Factor:	1.00	Dilution:	1.00000
Acq Meth Set:	GC_ms		
Processing Method:	HES_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Int Type	% Area	USP Tailing
1	D4	3.257	1681552	BV	99.35	

APPENDIX I

STABILITY AND DOSING SOLUTIONS ANALYSIS

LOT #LL084732

Dow Corning Internal Memo
Page 1 of 34

To: Paal Klykken - C03101
From: Don Kleyer - C03101 Date 11/4/96
Thomas Galbraith - C03101
Subject: Analysis of dosing solutions D4-28-1R-PI#7 (22Aug95), D4-28-1R-PO#7 (29Aug95), D4-28-1R-PI#8 (3Oct95), D4-28-1R-PI#8 (10Oct95) from the Medical College of Virginia, Virginia Commonwealth University for study # 8389.

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Abstract

It was requested that dosing solutions from Virginia Commonwealth University, the Medical College of Virginia (MCV) Campus have their levels of Dow Corning® 244 Fluid (octamethylcyclotetrasiloxane, D_4) verified. There were four carriers used for the study; Corn Oil, 10% aqueous Emulphor, Dow Corning® X9-6370 (35 cSt PDMS) and Maalox TC. Dosing solutions of D_4 in each of the carriers was prepared daily. Dosing solutions samples that were analyzed were prepared on Day 21 and Day 28 of this 28 day study.

An infrared spectroscopy method for analysis of Dow Corning® 244 Fluid in corn oil was developed and applied to dosing solutions obtained from Day 21 (3Oct95) and 28 (10Oct95). The method used a linear regression of the peak height ratio of a D_4 absorbance at 812 cm^{-1} and a corn oil absorbance at 723 cm^{-1} versus concentration for calibration. The calculated results for the samples from Day 21 dosing were 56.23 $\pm$ 1.73 mg/mL (result $\pm$ standard deviation). The calculated results for samples from Day 28 dosing were 58.62 $\pm$ 0.13 mg/mL. The samples were true solutions, thus no homogeneity testing was required. Stability of the dosing solutions over 37 days was established by quantitative analysis. No spectral changes were observed during the analyses, a further indication of component stability.

Analysis of Dow Corning® 244 Fluid in Dow Corning® X9-6370 Fluid (35 cSt PDMS) was performed. The method consisted of extraction with acetone containing dodecane internal standard with analysis of the extract by gas chromatography. Dosing solutions from Day 21 (22Aug95) and Day 28 (29Aug95) were found to contain 59.67 $\pm$ 0.89 and 58.82 $\pm$ 0.39 mg/mL of Dow Corning® 244 Fluid (D_4) in Dow Corning® X9-6370 Fluid (35 cSt PDMS), respectively. The samples were true solutions, thus no homogeneity testing was required. Stability of the dosing solutions was not an issue since the test article and carrier or vehicle are chemically the same material, polydimethylsiloxane.

Analysis of Dow Corning® 244 Fluid in Maalox TC was performed. The analysis method consisted of extraction with methyl isobutyl ketone (MIBK) containing dodecane internal standard with analysis of the extract by gas chromatography. Dosing solutions from Day 21 (22Aug95) and Day 28 (29Aug95) were analyzed to contain 56.69 $\pm$ 1.84 and 54.99 $\pm$ 1.94 mg/mL Dow Corning® 244 Fluid (D_4) in Maalox TC, respectively. Homogeneity was established by repeated quantitative analysis of aliquots removed from the solution after vortex mixing. Stability was established by a repeated quantitative analysis of method development samples stored for 23 days.

Analysis of mL Dow Corning® 244 Fluid dispersed in 10% aqueous Emulphor was attempted. The analysis method consisted of extraction with MIBK containing dodecane internal standard with analysis of the extract by gas chromatography. Dosing solutions from Day 21 (3Oct95) and Day 28 (10Oct95) were analyzed to contain more than 51 and 52 mg/g D_4 in aqueous Emulphor, respectively.

It was discovered that there is no accurate way to verify the dosing level of D_4 in aqueous 10% Emulphor solutions which were prepared and allowed to age due to difficulty in redispersing D_4. Fortunately the dosing solutions were used shortly after preparation, when the dispersability was at its maximum. Also, the ability to accurately deliver the desired dosing level would increase with decreasing level of D_4 since ease of dispersion would go up. We were attempting to verify the maximum dose level, where maximum error would be expected. It could not be verified with certainty what the dosing solution level was on the day the samples were prepared. This work would suggest the concentrations were at least 51 ± 3 and 52 ± 2 mg/g for Day 21 and Day 28 respectively. Conversion to units of mg/mL would be expected to increase the values only very slightly. After review of the standard operating procedure for solution preparation and its application for the other carriers, the level was more probably 57 mg/mL. Our verification result, though expected to be low, was still within 11% of the expected result. It is recommended to review the sample preparation records and rely upon these for the actual dosing solution level.

A. Objective

It was requested that dosing solutions from Virginia Commonwealth University, MCV Campus (MCV) have their octamethylcyclotetrasiloxane (D_4) levels verified. There were four carriers used for the study; Corn Oil, 10% aqueous Emulphor, Dow Corning® X9-6370 Fluid (35 cSt PDMS) and Maalox TC. Cover letters were sent with each shipment on days 21 and 28 of the study. The cover letters are included with the raw data. Analysis of octamethylcyclotetrasiloxane in each of the four vehicles will be discussed in turn.

B. Test, Control, Reference Article Information

Information Supplied by MCV:
Name: Dow Corning® 244 Fluid
Lot Number: LL084732

Name: Dow Corning® X9-6370 (35 cSt PDMS fluid)
Lot Number: LL099608

Name: Maalox TC (therapeutic concentrate)
Lot Number: 98462 Exp. 6/97

Name: Mazola Corn Oil
CAS #: 8001-30-7

Materials used during analysis at Dow Corning:
Name: Dow Corning® 244 Fluid (D_4, octamethylcyclotetrasiloxane)
Lot Number: LL024S10

Name: Mazola 100% Pure Corn Oil
CAS #: 8001-30-7

Name: Maalox TC, (therapeutic concentrate) Rhone-Poulenc
Lot Number: 95213, exp. 4/96

Name: Dow Corning® X9-6370 Fluid (35 cSt PDMS)
Lot Number: LL099608

Name: Emulphor EL 620 - L
Lot Number: ref. #SP2H021180
CAS #: 9004-98-2

Name: Acetone, Certified ACS grade, Fisher Scientific
Lot Number: 952401
CAS #: 67-64-1

Name: Methyl Isobutyl Ketone, Certified ACS grade, Fisher Scientific
Lot Number: 952364
CAS #: 108-10-1

Name: Dodecane, Aldrich Chemical cat# D22,110-4
Lot Number: EE 092287
CAS #: 112-40-3

Throughout the work which follows, specific reference is made to the Dow Corning lab book (includes lab books numbered 12667, 12198 and 13046) and page number where all details are located. This was done of the sake of convenience and completeness.

A retrospective review of the MCV Standard Operating Procedure entitled " Procedure for Preparation and Handling of Octamethylcyclotetrasiloxane with Electronic Record using Four Different Vehicles" dated August 1995, was made. The density of octamethylcyclotetrasiloxane used for calculation was 990 mg/mL. For the 300 mg/kg dose, 0.06 mL of D_4 diluted up to a final volume of 1 mL would be needed to generate a working stock of 60 mg/mL, according to their calculations. The density of D_4 is actually 0.95 g/mL. Thus their preparation of a 60 mg/mL solution would have been in actuality a 57 mg/mL working stock solution. Since all subsequent solutions are made by dilution of the working stock solution, the error was carried through all dose solutions.

1. Octamethylcyclotetrasiloxane in Corn Oil

1C. Route of Exposure

A one mL glass syringe was used to make an injection of 0.1 mL of solution into a Spectra-Tech demountable cell kit (part # FT04-036) equipped with 3 X 32 mm NaCl plates and a 0.015 mm Teflon spacer. The contents of the cell were then analyzed by infrared spectroscopy.

1D. Test System

A Perkin Elmer Paragon 1000, SN 37949 with settings of weak apodization, ratio mode, 4400-450 cm^{-1} range, 16 scans and 4.0 cm^{-1} resolution was used for the analysis. A background spectrum, taken with the empty cell was ratioed with each sample spectrum. Peak heights were measured for D_4 and corn oil. For D_4, the baseline was drawn between 850 and 775 cm^{-1} and the data point at the maximum, 812 cm^{-1}, was used for peak height determination. For corn oil, the baseline was drawn between 775 and 650 cm^{-1} and the data point at the maximum, 723 cm^{-1}, was used for peak height determination.

The corn oil peak functions as an internal standard peak. The use of an internal standard corrects for any variation in cell path length which could be caused, for example, by a need to disassemble and reassemble a cell in the middle of a series of analyses. Fortunately no disassembles or reassembles were required during the analyses. Between samples, the cell was flushed with isopropanol (0.25 mL) and blown dry with a stream of nitrogen. A least squares linear regression analysis of the data, plotting the peak height ratio, 812 cm^{-1}/723 cm^{-1}, versus percent wt. (g)/volume (mL) D_4 in the standard provided a calibration curve. The calibration curve was then used to calculate the concentration of unknowns given their peak height ratio, 812 cm^{-1}/723 cm^{-1}.

1E. Experimental Design

An initial feasibility was completed (12667: 41-42) with standards at 0.95, 2.85, 4.75, 7.125, 9.025% wt (g) /volume (mL) (0.2, 0.6, 1.0, 1.5, 1.9 mL D_4 in 19.8, 19.4, 19, 18.5, 18.1 mL corn oil respectively) providing a calibration and six samples prepared at 5.7% wt (g) /volume (mL) (18.8 mL corn oil and 1.2 mL D_4) providing an opportunity to test the method. All samples and standards were prepared by volume using pipettes. All samples and standards were clear yellow solutions. For corn oil, a Costar 25 mL 2/10 non-pyrogenic TD-EX 20° C serological pipet was used with a drain time of 90 seconds. For D_4, a 2 mL in 1/100 TD-EX 20° C Pyrex™ disposable pipet was used. The density of D_4 used for calculating weight was 0.95 g/mL. A least squares linear regression analysis of the data, plotting the peak height ratio, 812 cm^{-1}/723 cm^{-1} (X-axis), versus percent wt. (g)/volume (mL) D_4 (Y-axis) in the standard, provided a calibration curve ($y = 1.97779x - 0.2890$, correlation coefficient $r^2 = 0.9988$). Analysis of a corn oil blank returned a value of -0.484 % g/mL. The calculated results for the 6 samples were: 5.685 % g/mL (99.7% of theory), 5.622 % (98.6%), 5.760 % (101.0 %), 5.771 % (101.3 %), 5.722 % (100.4%), 5.688% (99.8%). The first three samples had been prepared 6 hours prior to analysis and the latter three samples had been prepared 48 hours prior to analysis. The standards were prepared just prior to analysis. Please note that to convert from % wt. (g)/volume (mL) to mg/mL, multiply by a factor of 10.

Three of the samples were reanalyzed (12667: 74) at a later date to further establish stability. The calibration curve was not updated. A total of 37 days had elapsed between analyses. The calculated results for the 3 samples were: 5.951 % g/mL (104 % of theory), 5.919 % (104 %), 5.957 % (105 %).

Analysis of samples from MCV (12667: 97) was completed relative to standards prepared just prior to analysis at concentrations of 1.003, 2.990, 5.002, 7.625, 9.116 % wt/wt (0.201, 0.598, 1.001, 1.527, 1.625g D_4 in 19.833, 19.404, 19.009, 18.498, 16.200g corn oil respectively). A least squares linear regression analysis of the data, plotting the peak height ratio, 812 cm^{-1}/723 cm^{-1} (Y-axis), versus percent wt./wt. D_4 (X-axis) in the standard provided a calibration curve ($y = 0.520421x - 0.185154$, correlation coefficient $r^2 = 0.9995$). The calculated results for the 3 MCV samples from Day 21 dosing were: 5.902, 6.167, 6.262 % wt/wt D_4 in corn oil, for samples 1, 2 and 3 respectively. Analysis of the corn oil blank from MCV returned a calculated level of 0.108 % wt/wt D_4 in corn oil. The calculated results for the 3 MCV samples from Day 28 dosing were: 6.365, 6.385, 6.357 % wt/wt D_4 in corn oil, for samples 1, 2 and 3 respectively.

Corn oil density was determined (13046: 92). First the to contain volume of duplicate 25 mL volumetric flasks was verified using water. The flasks were then filled with corn oil and weighed. The measured corn oil density was 0.9179 and 0.9188 g/mL (average 0.9184 g/mL). This allows conversion of the MCV corn oil results. The calculated results for the 3 MCV samples from Day 21 (3Oct95) dosing were: 54.31, 56.75, 57.64 mg/mL D_4 in corn oil, for samples 1, 2 and 3 respectively. Analysis of the corn oil blank from MCV returned a calculated level of 0.99 mg/mL. The calculated results for the 3 MCV samples from Day 28 (10Oct95) dosing were: 58.58, 58.77, 58.51 mg/mL D_4 in corn oil, for samples 1, 2 and 3 respectively.

1F. Results and Discussion

One item regarding preparation of corn oil samples and standards deserves mention. Because of the differences in viscosity and surface tension of corn oil and D_4 relative to water, use of volumetric pipettes to deliver set volumes encounter difficulty in precision and accuracy. Remember that volumetric pipettes were designed to deliver aqueous solutions. Precision increases with consistency of the drain time of the pipet. Unfortunately accuracy cannot be corrected. Relative to an aqueous solution, less D4 is retained with complete draining. Ideally one should prepare solutions either by weight, positive displacement means or by filling a vessel to a graduated mark, e.g. volumetric flask, to counter these problems. This discussion only becomes important for purposes of dosing solution level verification. Because of the excellent precision which can be achieved with volumetric pipets, their use is sufficient for dosing solution preparation. A simple conversion between weight and volume is possible if one knows the density of the materials involved.

Low molecular weight polydimethylsiloxanes such as D_4 are soluble in all proportions with corn oil. Low molecular weight polydimethylsiloxanes such as D_4 are also indefinitely stable in corn oil. The combination of solubility and stability greatly eases the development of a method of analysis. Infrared spectroscopy was the method of choice because of the large spectral differences between corn oil and polydimethylsiloxane and the fact that no sample preparation was involved.

An initial feasibility was completed based on a calibration curve generated from 5 standards spread over the concentration range 1 - 9 % wt. (grams of D_4) / volume (mL total volume). A consistent drain time of 90 seconds ensured repeatability of the volume delivery although absolute accuracy was still questionable. Absolute accuracy at this point was not an issue. Six independently prepared samples at 5.7 % wt/volume were used to determine repeatability. The average calculated value was 5.708 $\pm$ 0.055 % weight/volume or 100.1 $\pm$ 1.0 % of the theoretical value of 5.7 % weight/volume. This suggested the method would readily determine the amount of D_4 in corn oil at the levels expected for the dosing solutions.

Reanalysis of three of the independently prepared samples 37 days later proved the stability of the D_4 in the corn oil matrix. The average calculated value was 5.94 $\pm$ 0.02 % weight/volume or 104 $\pm$ 0.4% of the theoretical value of 5.7% weight/volume. Greater accuracy could probably have been achieved if the standards had been reanalyzed and a new calibration curve prepared but this was felt to be unnecessary. No spectral change for any of the components was observed.

Analysis of the samples from MCV was completed relative to freshly prepared standards, range 1 - 9 % wt/wt. Figure 1 shows an overlay of infrared spectra for standards at three concentrations. Figure 2 shows an expansion of the region of interest of Figure 1. Weights were used instead of volumes for these standards to avoid any inaccuracies introduced by pipetting. The calculated results for the 3 MCV samples from Day 21 dosing were: 5.902, 6.167, 6.262 % wt./wt. The calculated results for the 3 MCV samples from Day 28 dosing were: 6.365, 6.385, 6.357 % wt./wt.. Corn oil density was determined to be 0.9184 g/mL. This allows conversion of the MCV corn oil results to wt./volume units. The calculated results for the 3 MCV samples from Day 21 dosing were: 54.31, 56.75, 57.64 mg/mL for samples 1, 2 and 3 respectively (average 56.23 $\pm$ 1.73 mg/mL). The calculated results for the 3 MCV samples from Day 28 dosing were: 58.58, 58.77, 58.51 mg/mL for samples 1, 2 and 3 respectively (average 58.62 $\pm$ 0.13 mg/mL). Figure 3 shows an overlay of infrared spectra for the 28 day samples. Figure 4 shows an expansion of the region of interest of Figure 3.

No spectral change for any of the components was observed. Analysis of the corn oil blank from MCV returned a calculated level of 0.108 % wt/wt. Analysis of the corn oil blank during the initial feasibility returned a calculated level of -0.484 % wt/volume. The calculated non zero blank values suggest the method should require standards bracketing the sample concentrations, as we did. Also, in the case of the blank measured relative to standards prepared by volume, the non-zero blank level has a significant contribution from a standard offset error associated with pipetting.

1G. Conclusions

An infrared spectroscopy method for analysis of Dow Corning® 244 Fluid (Octamethylcyclotetrasiloxane, D_4) in corn oil was developed and applied to dosing solutions obtained from Day 21 (3Oct95) and 28 (10Oct95) of a study conducted at the

Medical College of Virginia Campus. The method used a linear regression of the peak height ratio of a D_4 absorbance at 812 cm^{-1} and a corn oil absorbance at 723 cm^{-1} versus concentration for calibration. The calculated results for the 3 MCV samples from Day 21 dosing were: 54.31, 56.75, 57.64 mg/mL for samples 1, 2 and 3 respectively (average 56.23 ± 1.73 mg/mL). The calculated results for the 3 MCV samples from Day 28 dosing were: 58.58, 58.77, 58.51 mg/mL for samples 1, 2 and 3 respectively (average 58.62 ± 0.13 mg/mL). The samples were true solutions, thus no homogeneity testing was required. Stability of the dosing solutions over 37 days was established by quantitative analysis. No spectral changes were observed during the analyses, a further indication of component stability.

2. Octamethylcyclotetrasiloxane in Dow Corning® X9-6370 (35 cSt PDMS fluid)

2C. Route of Exposure

Dosing solutions were extracted with acetone and analyzed by injection into a gas chromatograph (GC).

2D. Test System

A VWR vortex mixer (model K550G, SN 3664, setting 10) was used to stir the vials prior to sampling with a 1 mL glass syringe from the middle of a 1/2 ounce vial. Samples were shaken during extraction with an Eberbach Corp. catalog # 6010 reciprocating shaker, speed setting high. Phase separation was assisted by centrifugation with a Hamilton Bell Co. centrifuge (model 1750, SN 6307). A Hamilton 25 µL syringe was used to measure all dodecane internal standard additions. For 35 cSt PDMS dispensed by volume, a Costar 25 mL 2/10 non-pyrogenic TD-EX 20° C serological pipet was used. For D_4 dispensed by volume, a 2 mL in 1/100 TD-EX 20° C Pyrex™ disposable pipet was used. For acetone dispensed by volume, a 5 mL in 1/10 TD-EX 20° C Pyrex™ disposable pipet was used. Samples were removed from vials using a Perfektum® glass 1 mL syringe manufactured by Popper & Sons, Inc. (Japan).

A Hewlett Packard 5890E Series II gas chromatograph, SN 3326A51216 with a Hewlett Packard capillary column HP-5, 5% phenylmethylsiloxane film, 0.25 µm thickness, 30 m length X 0.32 mm ID, part # 19091J-413 equipped with an autosampler, injection volume 1 µL, and flame ionization detector (FID) was used for analysis of the extracts. Conditions for the analysis include: split vent flow rate 126 mL/min., septum purge 3 mL/min., column constant flow mode set at 10 psi at 100° C, column flow 1.9 mL/min., detector helium makeup 6.9 mL/min., detector hydrogen 37 mL/min., detector air 430 mL/min., column temperature program 50° C for 2 min. then 15° C/min. ramp to 300° C for 5 min., injector temperature 250° C, detector temperature 300° C, threshold 1, peak width 0.04 min., area reject 500. Hewlett Packard Chemstation software G1201A SN US23000598 was used for the acquisition.

A Hewlett Packard 5890 series II gas chromatograph, SN2938A25833, equipped with a Hewlett Packard 5970 mass selective detector, SN 2923A12422 and a Hewlett Packard HP5 capillary column (crosslinked 5% phenylmethylsiloxane, 0.25 µm film thickness, 0.25 mm ID X 30 m length, part # 19091S-433) was used for acquisition of a mass spectrum. Conditions for the GC/MSD analysis were: manual injection, split flow 56 mL/min, septum purge 3 mL/min, 45-800 AMU scanned, 250° C injection port, 280° C detector (transfer line), constant He flow mode (2.2 psi at 50° C), vacuum compensation on, column oven program 50° C for 3 minutes then 15° C per minute to 280° C, held for 4 minutes. Hewlett Packard Chemstation software G1034C version C.03.00 was used for the acquisition.

2E. Experimental Design

Standards for gas chromatography (12667-29) were prepared in 25 mL volumetric flasks by adding a weighed amount of D_4 followed by dilution to volume with acetone. Internal standard (312 µL) was then added using a 25 µL syringe. Standards were prepared at D_4 concentrations of 1.536, 3.852, 8.02, 12.076 and 21.1316 mg/mL (38.4, 96.3, 200.5, 301.9, 528.29 mg into a 25 mL volumetric, respectively). The flasks were shaken to mix prior to use. Analysis was by GC using peak area, internal standard calibration and least squares linear regression. A set of standards and solvent blanks were run interspersed in each batch of samples. A new calibration was generated for each batch of samples by replacing the previous calibration table. All calibration curve correlation coefficients, r^2, were equal to, or in excess of, 0.999.

The first sample for method development was prepared (12198: 8C) by mixing 18.2 mL 35 cSt PDMS and 1.8 mL D_4 (8.55% wt (g)/volume (mL)). The density of D_4 used for calculating weight was 0.95 g/mL. The sample was a clear water white solution.

The initial procedure (12667: 29-34) followed for the duplicate analysis, 3 days after sample preparation, of D_4 in 35 cSt PDMS (sample 12198: 8C) consisted of: vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of acetone, reciprocating shaking for 60 minutes, centrifugation for 15 minutes, transfer of 2 mL of the acetone layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. It was noted during the sample preparation that a considerable portion of the 35 cSt PDMS dissolved in the acetone. Analysis of a 35 cSt PDMS blank returned a value of 84 ppm D_4 present. Analysis of the solutions returned values of 7.10% wt (g) /volume (mL) (83% of theory) and 7.24% (85% of theory). Please note that to convert from % wt (g)/volume (mL) to mg/mL, multiply by a factor of 10.

The second sample for method development was prepared (12198: 9B) by mixing 18.8 mL 35 cSt PDMS and 1.2 mL D_4 (5.70% g/mL). The density of D_4 used for calculating weight was 0.95 g/mL.

The procedure (12667: 29-34) for triplicate analysis, 2 days after sample preparation, of D_4 in 35 cSt PDMS (sample 12198: 9B) consisted of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of acetone, reciprocating shaking for 60 minutes, centrifugation for 15 minutes, transfer of 2 mL of the acetone layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. It was noted during the sample preparation that a considerable portion of the 35 cSt PDMS dissolved in the acetone. Analysis of the solutions returned values of 4.988% wt/volume (88% of theory), 5.004% (88% of theory) and 5.1805% (91% of theory). A second run of the procedure was made with the change that 62.5 µL of the internal standard was added to the 5 mL of acetone prior to shaking. . Analysis of the solutions returned values of 5.878% wt/volume (103% of theory), 5.260% (92% of theory) and 5.787% (102% of theory).

The third batch, consisting of three samples, for method development were prepared (12198: 11) by mixing 18.8 mL 35 cSt PDMS and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL.

The procedure (12667: 29-34, 36, 38-39) for analysis of D_4 in 35 cSt PDMS (sample 12198: 11 and sample Day 21 (22Aug95) MCV dosing solutions) consisted of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of acetone, reciprocating shaking for 45 minutes, centrifugation for 15 minutes, transfer of 2 mL of the acetone layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. It was noted during the sample preparation that a considerable portion of the 35 cSt PDMS dissolved in the acetone. Analysis of the three samples from 12198: 11, prepared 6 hours prior, returned values of 5.517% wt/volume (97% of theory), 5.517% (97% of theory) and 5.473% (96% of theory). Reanalysis of the three samples from 12198: 11, prepared 6 days prior, returned values of 5.365% wt/volume (94% of theory), 5.246% (92% of theory) and 5.383% (94% of theory). Analysis of the three Day 21 (22Aug95) MCV dosing solutions returned values of 5.105% wt/volume, 4.984% and 5.11%. A second run of the procedure was made with the change that 62.5 µL of the internal standard was added to the 5 mL of acetone prior to shaking. Analysis of the solutions returned values of 6.225% wt/volume (109% of theory), 6.146% (108% of theory) and 6.243% (110% of theory). Analysis of the three Day 21 (22Aug95) MCV dosing solutions returned values of 6.048% wt/volume, 6.038% and 6.025%.

The fourth batch of samples for method development were prepared (12667: 46-47) by mixing 18.8 mL 35 cSt PDMS and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. Every step of the triplicate sample preparation was also followed by weight.

The following procedure was used for all remaining analyses (12667: 46-47, 52-53, 63, 68-69) (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of acetone, addition of 62.5 µL of the internal standard, reciprocating shaking for 45 minutes, centrifugation for 15 minutes, transfer of an aliquot of the acetone layer (upper) to an autosampler vial for GC analysis. Samples from 12667:46 were reanalyzed after 20 and 23 days. Results are summarized in Tables 1, 2 and 3.

Dosing solution extracts (12667: 52-53) were analyzed by gas chromatography with a mass selective detector to verify peak identity. Extracts from both Day 21 (22Aug95) and Day 28 (29Aug95) were analyzed. The mass spectrum for octamethylcyclotetrasiloxane on Day 28 (29Aug95): m/z (% relative abundance) 283 (26%), 282 (36), 281 (base), 249 (11), 193 (9), 191 (10), 73 (10). The mass spectrum for octamethylcyclotetrasiloxane on Day 21 (22Aug95): m/z (% relative abundance) 283 (21%), 282 (31), 281 (base), 249 (7), 193 (6), 191 (5), 73 (3).

2F. Results and Discussion

One item regarding preparation of Dow Corning® X9-6370 (35 cSt PDMS) samples deserves mention. Because of the differences in viscosity and surface tension of 35 cSt PDMS and D_4 relative to water, use of volumetric pipettes to deliver set volumes encounter difficulty in precision and accuracy. Remember that volumetric pipettes were designed to deliver aqueous solutions. Precision increased with consistency of the drain time of the pipet. Unfortunately accuracy cannot be corrected. Relative to an aqueous solution, less D4 is retained with complete draining. Ideally one should prepare solutions either by weight, positive displacement means or by filling a vessel to a graduated mark, e.g. volumetric flask, to counter these problems. This discussion only becomes important for purposes of dosing solution level verification. Because of the excellent precision which can be achieved with volumetric pipets, their use is sufficient for dosing solution preparation. A simple conversion between weight and volume is possible if one knows the density of the materials involved.

Low molecular weight polydimethylsiloxanes such as D_4 are soluble in all proportions with higher molecular weight polydimethylsiloxanes such as Dow Corning® X9-6370, 35 cSt fluid. Low molecular weight polydimethylsiloxanes such as D_4 are also indefinitely stable in higher molecular weight polydimethylsiloxanes. The combination of solubility and stability greatly eases the development of a method of analysis. Extraction with acetone followed by gas chromatographic analysis was the method of choice. A non interfering internal standard, dodecane, was used to eliminate the possibility of volumetric errors in extraction and analysis.

All gas chromatography analyses were completed based on a calibration curve generated from 5 standards spread over the concentration range 7.7 - 105.6 mg/mL (based on dosing

solution). All calibration curve correlation coefficients, r^2, were equal to or in excess of 0.999. During initial method feasibility it was discovered that a significant portion of the 35 cSt PDMS carrier dissolved in the acetone used for extraction. Since the sample internal standard was added post extraction, to an measured aliquot, the result was low recoveries, e.g. 80-90% of theory. When the procedure was modified to addition of the internal standard prior to extraction the recoveries were higher, e.g. 90-110%, but not very precise. All samples were prepared assuming that a volumetric pipet would deliver stated volume and that the amount delivered did not change with drain time of the pipet. It turns out that both assumptions were incorrect. When pipet drain times were held constant at 90 seconds the amount delivered became consistent as determined by weight transferred. However, it was also determined that 1.2 mL of D_4 which should have weighed 1.14g based upon a density of 0.95 g/mL actually was 1.22g on average. Thus depending upon how the method development samples were prepared, by weight or by volume, the % recovery varied accordingly. Table 1 below summarizes the analyses. The average difference between a determination based on volume assumptions versus actual weight was 7.4%. This was quite similar to the 7% difference between assumed and delivered D_4 (1.14g assumed versus 1.22g delivered).

Table 1: Method development samples analyzed on a weight basis.

analyzed mg/mL[1]	actual mg/mL[2]	% volume recovery[3]	% weight recovery[2]	wt vehicle[4]	wt D_4[4]	wt 1 mL transfer
60.695	61.6	106.48	98.53	17.58	1.22	0.95
60.9	60.4	106.84	100.83	17.61	1.21	0.94
61.8	61.7	108.42	100.16	17.57	1.22	0.95
	average	107.25	99.84		1.22	

note 1: results are from 12667: 46-47
note 2: calculated on the basis of weight
note 3: calculated on the basis of volumes
note 4: weight data from sample preparation

A more rigorous examination of the volume assumption is summarized in Table 2. Each sample was extracted in duplicate with duplicate analysis of each extract. The standard deviations listed in Table 2 are based on 12 independent measurements. The results are again consistent with a problem in preparation of method development samples. Stability was verified by analysis of samples prepared 20 and 23 days prior to analysis.

Table 2: Comparison of D_4 recoveries calculated on a weight versus volume basis

Sample	% Recovery (wt)	% Recovery (vol.)
12667-46-4[1]	96.76	104.57
12667-46-4[2]	97.30	105.15
12667-46-5[1]	99.06	104.96
12667-46-5[2]	98.53	104.40
12667-46-6[1]	98.54	106.66
12667-46-6[2]	99.14	107.32
average	98.22 ± 0.93	105.51 ± 1.14

note 1: results are from 12667: 68-69
note 2: results are from 12667: 63

Fortunately, the dosing solution analyses are not affected by a volume assumption. The volume assumption only affected the method development work. The analysis of the 6 dosing solutions returned from MCV is summarized in Table 3. Figure 5 shows a representative chromatogram of an extract from Day 21 (22Aug95) #2. Analysis of extracts by gas chromatography with a mass selective detector confirmed the identity of the analyte.

Table 3: Average D_4 level in MCV dosing solutions

Sample[1]	Average Calculated Concentration (mg/mL)[2]	Average Concentration/Day (mg/mL)[3]
Day 21 (22Aug95) # 1	59.50 ± 0.16	59.67 ± 0.89
Day 21 (22Aug95) # 2	60.70 ± 0.62	
Day 21 (22Aug95) # 3	58.81 ± 0.23	
Day 28 (29Aug95) # 1	56.90 ± 0.36	56.82 ± 0.39
Day 28 (29Aug95) # 2	56.93 ± 0.41	
Day 28 (29Aug95) # 3	56.63 ± 0.39	

Note 1: results are from 12667: 52-3, 63, 68-69.
Note 2: standard deviations are calculated based on 5 measurements.
Note 3: standard deviations are calculated based on 15 measurements.

2G. Conclusions

Analysis of Dow Corning® 244 Fluid in Dow Corning® X9-6370 Fluid (35 cSt PDMS) was performed. The method consisted of extraction with acetone containing dodecane internal standard with analysis of the extract by gas chromatography. Dosing solutions from Day 21 (22Aug95) and Day 28 (29Aug95) were analyzed to contain 59.67 ± 0.89 and 56.82 ± 0.39 mg/mL D_4 in Dow Corning® X9-6370 Fluid (35 cSt PDMS), respectively. The standard deviations are the result of 15 independent measurements. The samples were true solutions, thus no homogeneity testing was required. Stability of the dosing solutions was not an issue since the test article and carrier or vehicle are chemically the same material, polydimethylsiloxane.

3. Octamethylcyclotetrasiloxane in Maalox TC

3C. Route of Exposure

Dosing solutions were extracted with methylisobutylketone and analyzed by injection into a gas chromatograph (GC).

3D. Test System

A VWR vortex mixer (model K550G, SN 3664, setting 10) was used to stir the vials prior to sampling with a 1 mL glass syringe from the middle of a 1/2 ounce vial. Samples were shaken during extraction with an Eberbach Corp. catalog # 6010 reciprocating shaker, speed setting high. Phase separation was assisted by centrifugation with a Hamilton Bell Co. centrifuge (model 1750, SN 6307). A Hamilton 25 µL syringe was used to measure all dodecane internal standard additions. For Maalox TC dispensed by volume, a Costar 25 mL 2/10 non-pyrogenic TD-EX 20° C serological pipet was used. For D_4 dispensed by volume, a 2 mL in 1/100 TD-EX 20° C Pyrex™ disposable pipet was used. For methyl isobutyl ketone (MIBK) dispensed by volume, a 5 mL in 1/10 TD-EX 20° C Pyrex™ disposable pipet was used. Samples were removed from vials using a Perfektum® glass 1 mL syringe manufactured by Popper & Sons, Inc. (Japan).

A Hewlett Packard 5890E Series II gas chromatograph, SN 3336A51216 with a Hewlett Packard HP-5, 5% phenylmethylsiloxane film, 0.25 µm thickness, 30 m length X 0.32 mm ID, part # 19091J-413 equipped with an autosampler, injection volume 1 µL, and FID was used for analysis of the extracts. Conditions for the analysis include: split vent flow rate 126 mL/min., septum purge 3 mL/min., column constant flow mode set at 10 psi at 100° C, column flow 1.9 mL/min., detector helium makeup 6.9 mL/min., detector hydrogen 37 mL/min., detector air 430 mL/min., column temperature program 50° C for 2 min. then 15° C/min. ramp to 300° C for 5 min., injector temperature 250° C, detector temperature 300° C, threshold 1, peak width 0.04 min., area reject 500. Hewlett Packard Chemstation software G1201A SN US23000598 was used for the acquisition.

A Hewlett Packard 5890 series II gas chromatograph, SN2938A25833, equipped with a Hewlett Packard 5970 mass selective detector, SN 2923A12422 and a Hewlett Packard HP5 column (crosslinked 5% phenylmethylsiloxane, 0.25 µm film thickness, 0.25 mm ID X 30 m length, part # 19091S-433) was used for acquisition of a mass spectrum. Conditions for the GC/MSD analysis were: manual injection, split flow 56 mL/min, septum purge 3 mL/min, 45-800 AMU scanned, 250° C injection port, 280° C detector (transfer line), constant He flow mode (2.2 psi at 50° C), vacuum compensation on, column oven program 50° C for 3 minutes then 15° C per minute to 280° C, held for 4 minutes. Hewlett Packard Chemstation software G1034C version C.03.00 was used for the acquisition.

3E. Experimental Design

Standards (12667-29) for gas chromatography were prepared in 25 mL volumetric flasks by adding a weighed amount of D_4 followed by dilution to volume with acetone. Internal standard (312 µL) was then added using a 25 µL syringe. Standards were prepared at D_4 concentrations of 1.536, 3.852, 8.02, 12.076 and 21.1316 mg/mL. The flasks were shaken to ensure uniformity prior to sampling. Analysis was by GC using peak area, internal standard calibration and least squares linear regression. A set of standards and solvent blanks were run interspersed in each batch of samples. A new calibration was generated for each batch of samples by replacing the previous calibration table. All calibration curve correlation coefficients, r^2, were equal to, or in excess of, 0.999.

The first sample for method development was prepared (12198: 8B) by mixing 18.2 mL Maalox TC and 1.8 mL D_4 (8.55% wt (g)/volume (mL)). The density of D_4 used for calculating weight was 0.95 g/mL. The sample was an opaque white suspension.

The initial procedure (12667: 29-34) followed for the duplicate analysis, 3 days after sample preparation, of D_4 in Maalox TC (sample 12198: 8B) consisted of: vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of MIBK, reciprocating shaking for 60 minutes, centrifugation for 15 minutes, transfer of 2 mL of the MIBK layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. Analysis of a Maalox TC blank returned a value of 4 ppm D_4 present. Analysis of the solutions returned values of 9.86% wt (g) /volume (mL) (115% of theory) and 9.35% (109% of theory). Please note that to convert from % wt/volume to mg/mL, multiply by a factor of 10.

The second sample for method development was prepared (12198: 9C) by mixing 18.8 mL Maalox TC and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL.

The procedure (12667: 29-34) for analysis, 2 days after sample preparation, of D_4 in Maalox TC (sample 12198: 9C) consisted of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of MIBK, reciprocating shaking for 60 minutes, centrifugation for 15 minutes, transfer of 2 mL of the MIBK layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. Analysis of the solutions returned values of 6.184% wt/volume (108% of theory), 6.410% (112% of theory) and 5.687% (99.8% of theory).

The third batch of three samples for method development were prepared (12198: 11) by mixing 18.8 mL Maalox TC and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL.

The procedure (12667: 29-34, 36, 38-39) for analysis of D_4 in Maalox TC (sample 12198: 11 and sample Day 21 (22Aug95) MCV dosing solutions) consisted of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of MIBK, reciprocating shaking for 45 minutes, centrifugation for 15 minutes, transfer of 2 mL of the MIBK layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. Analysis of three samples from 12198: 11, prepared 6 hours prior, returned values of 6.4235% wt/volume (113% of theory), 6.437% (113% of theory) and 6.4095% (112% of theory). Reanalysis of the three samples from 12198: 11, prepared 6 days prior, returned values of 6.338% wt/volume (111% of theory), 6.24% (110% of theory) and 6.34% (111% of theory). Analysis of the three Day 21 (22Aug95) MCV dosing solutions returned values of 5.453% wt/volume, 5.673% and 5.362%. A second run of the procedure was made with the change that 62.5 µL of the internal standard was added to the 5 mL of MIBK prior to shaking. Analysis of the 12198: 11 solutions returned values of 6.13% wt/volume (108% of theory), 6.019% (106% of theory) and 6.249% (110% of theory). Analysis of the three Day 21 (22Aug95) MCV dosing solutions returned values of 5.381% wt/volume, 5.442% and 5.440%.

The fourth batch of samples for method development were prepared (12667: 46-47) by mixing 18.8 mL Maalox TC and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. Every step of the triplicate sample preparation was also followed by weight.

The following procedure was used for all remaining analyses (12667: 46-47, 52-53, 63, 68-69) (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of MIBK, addition of 62.5 µL of the internal standard, reciprocating shaking for 45 minutes, centrifugation for 15 minutes, transfer of an aliquot of the MIBK layer (upper) to an autosampler vial for GC analysis. Samples from 12667: 46 were reanalyzed after 20 and 23 days. Results are summarized in Tables 5 and 6.

Dosing solution extracts (12667: 52-53) were analyzed by gas chromatography with a mass selective detector. Extracts from both Day 21 (22Aug95) and Day 28 (29Aug95) were analyzed. The mass spectrum for octamethylcyclotetrasiloxane on Day 28 (29Aug95): m/z (% relative abundance) 283 (24%), 282 (30), 281 (base), 249 (7), 193 (7), 191 (6), 73 (7). The mass spectrum for octamethylcyclotetrasiloxane on Day 21 (22Aug95): m/z (% relative abundance) 283 (20%), 282 (30), 281 (base), 249 (9), 193 (6), 191 (6), 73 (4).

3F. Results and Discussion

One item regarding preparation of Maalox TC samples deserves mention. Because of the differences in viscosity and surface tension of Maalox TC and D_4 relative to water, use of volumetric pipettes to deliver set volumes encounter difficulty in precision and accuracy. Remember that volumetric pipettes were designed to deliver aqueous solutions. Precision increased with consistency of the drain time of the pipet. Unfortunately accuracy cannot be corrected. Relative to an aqueous solution, less D_4 is retained with complete draining. Ideally one should prepare solutions either by weight, positive displacement means or by filling a vessel to a graduated mark, e.g. volumetric flask, to counter these problems. This discussion only becomes important for purposes of dosing solution level verification. Because of the excellent precision which can be achieved with volumetric pipets, their use is sufficient for dosing solution preparation. A simple conversion between weight and volume is possible if one knows the density of the materials involved.

Low molecular weight polydimethylsiloxanes such as D_4 are insoluble in aqueous based materials such as Maalox TC. However the high solids content of Maalox TC provides a large surface area for dispersed droplets of D_4 to cling. Thus low molecular weight polydimethylsiloxanes, such as D_4, are readily dispersible in Maalox TC while remaining easily extractable. Low molecular weight polydimethylsiloxanes such as D4 are chemically stable in Maalox TC. Extraction with MIBK followed by gas chromatographic analysis was the analysis method of choice. A non interfering internal standard, dodecane, was used to eliminate the possibility of volumetric errors in extraction and analysis.

All gas chromatography analyses were completed based on a calibration curve generated from 5 standards spread over the concentration range 7.7 - 105.6 mg/mL (based on dosing solution). All calibration curve correlation coefficients, r^2, were equal to or in excess of 0.999. Initial measurements returned higher than quantitative recoveries with higher than desired spread, e.g. 100-112% and 109-115% of theory. Since the sample internal standard was added post extraction, to an measured aliquot, the high recovery result was possibly due to MIBK solubility in Maalox TC. When the procedure was modified to addition of the internal standard prior to extraction the recoveries were very slightly, if at all, lower. All samples were prepared assuming that a volumetric pipet would deliver stated volume and that the amount delivered did not change with drain time of the pipet. It turns out that both assumptions were incorrect. When pipet drain times were held constant at 90 seconds the amount delivered became consistent as determined by weight transferred. However, it was also determined that 1.2 mL of D_4 which should have weighed 1.14g based upon a density of 0.95 g/mL actually was 1.20g on average. The quantitative delivery of Maalox TC via pipet was also questionable. Thus depending upon how the method development samples were prepared, by weight or by volume, the % recovery varied accordingly. Table 4 below summarizes the analyses. The average difference between a determination based on volume assumptions versus actual weight was 7.64%. This was similar to the 5% difference between assumed and delivered D_4 (1.14g assumed versus 1.20g delivered).

Table 4: Maalox TC method development samples analyzed on a weight versus volume basis.

analyzed mg/mL[1]	actual mg/mL[2]	% volume recovery[3]	% weight recovery[2]	wt vehicle[4]	wt D_4[4]	wt 1 mL transfer[4]
62.165	62.0	109.06	100.27	20.08	1.20	1.10
61.59	61.1	108.05	100.80	20.40	1.20	1.10
62.705	60.8	110.01	103.13	20.33	1.19	1.10
	average	109.04	101.40		1.20	

note 1: results are from 12667: 46-47
note 2: calculated on the basis of weight
note 3: calculated on the basis of volumes
note 4: weight data from sample preparation

A more rigorous examination of the volume assumption is summarized in Table 5. Each sample was extracted in duplicate with duplicate analysis of each extract. The standard deviation listed in Table 5 are based on 12 independent measurements. The results are again consistent with a problem in preparation of method development samples. Stability was established by analysis of samples prepared 20 and 23 days prior to analysis.

Table 5: Comparison of D_4 recoveries from Maalox TC calculated on a weight versus volume basis

Sample	% Recovery (wt)	% Recovery (vol.)
12667-46-1[1]	100.53	109.35
12667-46-1[2]	99.38	108.10
12667-46-2[1]	98.94	106.06
12667-46-2[2]	100.05	107.25
12667-46-3[1]	99.29	105.91
12667-46-3[2]	100.74	107.46
average	99.82 ± 0.73	107.35 ± 1.26

note 1: results are from 12667: 68-69
note 2: results are from 12667: 63

Fortunately, the dosing solution analyses are not affected by a volume assumption. The volume assumption only affected the method development work. The analysis of the 6 dosing solutions returned from MCV is summarized in Table 3. Figure 6 shows a representative chromatogram of an extract from Day 21 (22Aug95) #2. Analysis of extracts by gas chromatography with a mass selective detector confirmed the identity of the analyte.

Table 6: Average D_4 level in MCV Maalox TC dosing solutions

Sample[1]	Average Calculated Concentration (mg/mL)[2]	Average Concentration/Day (mg/mL)[3]
Day 21 (22Aug95) # 1	56.54 ± 1.98	56.69 ± 1.84
Day 21 (22Aug95) # 2	57.27 ± 1.90	
Day 21 (22Aug95) # 3	56.26 ± 1.69	
Day 28 (29Aug95) # 1	56.17 ± 1.29	54.99 ± 1.94
Day 28 (29Aug95) # 2	53.70 ± 1.85	
Day 28 (29Aug95) # 3	55.11 ± 1.92	

Note 1: results are from 12667: 52-3, 63, 68-69.
Note 2: standard deviations are calculated based on 9 measurements.
Note 3: standard deviations are calculated based on 27 measurements.

3G. Conclusions

Analysis of octamethylcyclotetrasiloxane in Maalox TC was performed. The method consisted of extraction with MIBK containing dodecane internal standard with analysis of the extract by gas chromatography. Dosing solutions from Day 21 (22Aug95) and Day 28 (29Aug95) were analyzed to contain 56.69 ± 1.84 and 54.99 ± 1.94 mg/mL D_4 in Maalox TC, respectively. The standard deviations are the result of 27 independent measurements. Homogeneity was established by repeated quantitative analysis of aliquots removed from the solution after vortex mixing. Stability was established by a repeated quantitative analysis of method development samples stored for 23 days.

4. Octamethylcyclotetrasiloxane in Aqueous 10% Emulphor

4C. Route of Exposure

Dosing solutions were extracted with methylisobutylketone and analyzed by injection into a gas chromatograph (GC).

4D. Test System

A VWR vortex mixer (model K550G, SN 3664, setting 10) was used to stir the vials prior to sampling with a 1 mL glass syringe from the middle of a 1/2 ounce vial. Samples were shaken during extraction with an Eberbach Corp. catalog # 6010 reciprocating shaker, speed setting high. Phase separation was assisted by centrifugation with a Hamilton Bell Co. centrifuge (model 1750, SN 6307). A Hamilton 25 µL syringe was used to measure all dodecane internal standard additions. For Maalox TC dispensed by volume, a Costar 25 mL 2/10 non-pyrogenic TD-EX 20° C serological pipet was used. For D_4 dispensed by volume, a 2 mL in 1/100 TD-EX 20° C Pyrex™ disposable pipet was used. For MIBK dispensed by volume, a 5 mL in 1/10 TD-EX 20° C Pyrex™ disposable pipet was used. Samples were removed from vials using a Perfektum® glass 1 mL syringe manufactured by Popper & Sons, Inc. (Japan).

A Hewlett Packard 5890E Series II gas chromatograph, SN 3336A51216 with a Hewlett Packard HP-5, 5% phenylmethylsiloxane film, 0.25 µm thickness, 30 m length X 0.32 mm ID, part # 19091J-413 equipped with an autosampler, injection volume 1 µL, and FID was used for analysis of most extracts. Conditions for the analysis include: split vent flow rate 126 mL/min., septum purge 3 mL/min., column constant flow mode set at 10 psi at 100° C, column flow 1.9 mL/min., detector helium makeup 6.9 mL/min., detector hydrogen 37 mL/min., detector air 430 mL/min., column temperature program 50° C for 2 min. then 15° C/min. ramp to 300° C for 5 min., injector temperature 250° C, detector temperature 300° C, threshold 1, peak width 0.04 min., area reject 500. Hewlett Packard Chemstation software G1201A SN US23000598 was used for the acquisition.

A Hewlett Packard 5890 series II gas chromatograph, SN2938A25833, equipped with a Hewlett Packard 5970 mass selective detector, SN 2923A12422 and a Hewlett Packard HP5 column (crosslinked 5% phenylmethylsiloxane, 0.25 µm film thickness, 0.25 mm ID X 30 m length, part # 19091S-433) was used for acquisition of a mass spectrum. Conditions for the GC/MSD analysis were: manual injection, split flow 56 mL/min, septum purge 3 mL/min, 45-800 AMU scanned, 250° C injection port, 280° C detector (transfer line), constant He flow mode (2.2 psi at 50° C), vacuum compensation on, column oven program 50° C for 3 minutes then 15° C per minute to 280° C, held for 4 minutes. Hewlett Packard Chemstation software G1034C version C.03.00 was used for the acquisition.

A Hewlett Packard 6890 gas chromatograph, SN 0001792 with a Hewlett Packard HP-5, 5% phenylmethylsiloxane film, 0.25 µm thickness, 30 m length X 0.25 mm ID, part # 19091J-433 column and equipped with an autosampler, injection volume 1 µL, and FID was used for analysis of 12667: 90-93 extracts. Conditions for the analysis include: split ratio 40:1, septum purge 3 mL/min., column constant flow mode set at 1.0 mL/min., detector helium makeup 25 mL/min., detector hydrogen 35 mL/min., detector air 400 mL/min., column temperature program 50° C for 3 min. then 15° C/min. ramp to 200° C for 3 min., injector temperature 250° C, detector temperature 300° C, threshold 0, peak width 0.04 min., 20 Hz data rate. Hewlett Packard Chemstation software G1034C version C.03.00 was used for the acquisition.

4E. Experimental Design

Standards (12667-29) for gas chromatography were prepared in 25 mL volumetric flasks by adding a weighed amount of D_4 followed by dilution to volume with acetone. Internal standard (312 µL) was then added using a 25 µL syringe. Standards were prepared at D_4 concentrations of 1.536, 3.852, 8.02, 12.076 and 21.1316 mg/mL. The flasks were shaken to ensure uniformity prior to sampling. Analysis was by GC using peak area, internal standard calibration and least squares linear regression. A set of standards and solvent blanks were run interspersed in each batch of samples. A new calibration was generated for each batch of samples by replacing the previous calibration table. All calibration curve correlation coefficients, r^2, were equal to, or in excess of, 0.999.

A batch of 10% Emulphor was prepared for preparation of known D_4 in Emulphor samples (12198: 7). The batch of 10% Emulphor was used to prepare samples designated 12198: 8D, 9D and 11. To an 8 oz. bottle was added 10 mL of Emulphor (EL620/L received 7/95 reference # SP2H021180, expiration date 7-98) and 90 mL of milli Q deionized water using serological pipettes. The contents were swirled until complete dissolution was observed.

The first sample for method development was prepared (12198: 8D) by mixing 18.2 mL Emulphor and 1.8 mL D_4 (8.55% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. The samples were an cloudy suspension upon mixing which with time would separate into two layers.

The initial procedure (12667: 29-34) followed for the analysis, 3 days after sample preparation, of D_4 in Emulphor (sample 12198: 8D) consisted of: vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 7 mL scintillation vial, addition of 5 mL of MIBK, reciprocating shaking for 60 minutes, and centrifugation for 15 minutes. The contents of the vial had gelled. The gel would not break upon centrifugation. The sample was discarded. Analysis of a Emulphor blank (did not gel) returned a value of 3 ppm D_4 present. Experiments extracting 1 mL of D_4 in Emulphor with 10 mL of MIBK or dilution of the 1 mL of D_4 in Emulphor with 9 mL of water prior to extraction with 5 mL MIBK did not gel. The procedure was followed in duplicate after changing to consist of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 1/2 ounce vial, addition of 10 mL of MIBK, reciprocating shaking for 60 minutes, centrifugation for 15 minutes, transfer of 2 mL of the MIBK layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. Analysis of the solutions returned values of 8.310% wt/volume (97.1% of theory), 8.409% (98.3% of theory). Please note that to convert from % wt/volume to mg/mL, multiply by a factor of 10.

The second sample for method development was prepared (12198: 9D) by mixing 18.8 mL Emulphor and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. Initial phase separation was noted within 1.75 hours of sample mixing.

The procedure (12667: 29-34) for the triplicate analysis, 2 days after sample preparation, of D_4 in Emulphor (sample 12198: 9D) consisted of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 1/2 ounce vial, addition of 10 mL of MIBK, reciprocating shaking for 60 minutes, centrifugation for 15 minutes, transfer of 2 mL of the MIBK layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC

analysis. Analysis of the solutions returned values of 5.602% wt/volume (98.3% of theory), 5.859% (102.8% of theory) and 5.764% (101.1% of theory).

The third batch of three samples for method development were prepared (12198: 11) by mixing 18.8 mL Emulphor and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL.

The procedure (12667: 32, 34) for analysis of D_4 in Emulphor, (samples 12198: 11) consisted of (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 1/2 ounce vial, addition of 10 mL of MIBK, reciprocating shaking for 45 minutes, centrifugation for 15 minutes, transfer of 2 mL of the MIBK layer (upper) to a 7 mL scintillation vial, 25 µL of internal standard was syringed in, contents were shaken and transferred to an autosampler vial for GC analysis. Analysis of three samples from 12198: 11, prepared 6 hours prior, returned values of 5.860% wt/volume (103% of theory), 5.607% (98.4% of theory) and 5.691% (99.8% of theory).

The basic procedure for analysis of D_4 in Emulphor (12667: 73, 76-78) was changed to (in order): vortex mixing of the sample vial for 15 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 1/2 ounce vial, addition of 10 mL of MIBK, addition of 125 µL of the internal standard, reciprocating shaking for 45 minutes, centrifugation for 15 minutes, transfer of an aliquot of the MIBK layer (upper) to an autosampler vial for GC analysis.

Reanalysis of the sample from 12198: 9D (12667: 73), prepared 51 days prior, returned average value of 2.101 $\pm$ 0.301% wt/volume (37% of theory). Analysis of the three Day 21 (3Oct95) MCV dosing solutions returned values of 3.600 $\pm$ 0.132% wt/volume, 3.855 $\pm$ 0.162% and 4.189 $\pm$ 0.246%. The standard deviations were calculated based upon triplicate extraction and duplicate analysis. This reanalysis run had one procedural change. The change was that after vortex mixing of the sample it was allowed to stand for 1-3 minutes, time enough for the foam dispersed within the sample to separate.

Reanalysis of the sample from 12198: 9D (12667: 76), prepared 61 days prior, returned an average value of 3.758 $\pm$ 4.44% wt/wt (66% of theory). The standard deviations was calculated based upon triplicate extraction and analysis. This reanalysis run had one procedural change. The change was that after vortex mixing of the vial it was immediately sampled. The amount of foam syringed up was accounted for by weighing the sample.

A new batch of 10% Emulphor was prepared for preparation of known D_4 in Emulphor samples (12667: 77). The new batch of 10% Emulphor was used to prepare samples designated 12667: 77 and 78. To an 8 oz. bottle was added 15.21g of Emulphor (EL620/L received 7/95 reference # SP2H021180, expiration date 7-98) with 136.90g of milli Q deionized water. The contents were shaken gently until complete dissolution was observed.

The fourth batch of samples for method development were prepared (12667: 77) by mixing 18.8 mL Emulphor and 1.8 mL D_4 (8.30% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. Every step of the triplicate sample preparation and triplicate extraction was also followed by weight. The average recovery from these samples was 77.2 $\pm$ 3.7 %. The standard deviation was determined based upon 9 measurements. This analysis was completed on a weight basis.

The fifth batch of samples for method development were prepared (12667: 78) by mixing 18.8 mL Emulphor and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. Every step of the triplicate sample preparation and triplicate extraction was also followed by weight. The average recovery from these samples was 96.79 $\pm$ 2.05 %. The standard deviation was determined based upon 9 measurements. This analysis was completed on a weight basis.

The fifth batch of samples for method development, 12667: 78, were reanalyzed (12667: 88-89) along with three MCV Day 21 (3Oct95) samples and three MCV Day 28 (10Oct95) samples. The results are listed in Table 7.

A new batch of 10% Emulphor was prepared for preparation of known D_4 in Emulphor samples (12667: 78). The new batch of 10% Emulphor was used to prepare samples designated 12667: 91. To an 8 oz. bottle was added 17.76g of Emulphor (EL620/L received 7/95 reference # sp2h021180, expiration date 7-98) with 159.85g of milli Q deionized water. The contents were shaken gently until complete dissolution was observed.

The sixth batch of samples for method development were prepared (12667: 91) by mixing 18.8 mL Emulphor and 1.2 mL D_4 (5.70% wt/volume). The density of D_4 used for calculating weight was 0.95 g/mL. Every step of the triplicate sample preparation was also followed by weight.

The following procedure was used for analysis (12667: 90-93) (in order): vortex mixing of the sample vial for 30 seconds, removal of 1 mL of sample from the middle of the glass 1/2 ounce vial using a glass syringe, transfer of the 1 mL to a 1/2 ounce vial, addition of 10 mL of MIBK, addition of 125 µL of the internal standard, reciprocating shaking for 60 minutes, the layers were allowed to separate by standing for 30 minutes, transfer of an aliquot of the MIBK layer (upper) to an autosampler vial for GC analysis. The sixth batch of samples for method development, 12667: 91, were reanalyzed (12667: 90-93) along with three MCV Day 21 (3Oct95) samples and three MCV Day 28 (10Oct95) samples. The results are listed in Table 8.

A method development solution extract (12667: 31) was analyzed by gas chromatography with a mass selective detector. The mass spectrum for octamethylcyclotetrasiloxane: m/z (% relative abundance) 283 (18%), 282 (29), 281 (base), 249 (6), 193 (4), 191 (4), 73 (2).

4F. Results and Discussion

One item regarding preparation of Emulphor samples deserves mention. Because of the differences in viscosity and surface tension of Emulphor and D_4 relative to water, use of volumetric pipettes to deliver set volumes encounter difficulty in precision and accuracy. Remember that volumetric pipettes were designed to deliver aqueous solutions. Precision increased with consistency of the drain time of the pipet. Unfortunately accuracy cannot be corrected. Relative to an aqueous solution, less D4 is retained with complete draining. Ideally one should prepare solutions either by weight, positive displacement means or by filling a vessel to a graduated mark, e.g. volumetric flask, to counter these problems. This discussion only becomes important for purposes of dosing solution level verification. Because of the excellent precision which can be achieved with volumetric pipets, their use is sufficient for dosing solution preparation. A simple conversion between weight and volume is possible if one knows the density of the materials involved.

Low molecular weight polydimethylsiloxanes such as D_4 are insoluble in aqueous based carriers such as 10 % Emulphor. However Emulphor provides a level of surfactancy allowing some stability of a dispersion of D_4. Thus low molecular weight polydimethylsiloxanes, such as D_4, are somewhat dispersible in Emulphor while remaining easily extractable. Low molecular weight polydimethylsiloxanes such as D4 are chemically stable in Emulphor. Extraction with MIBK followed by gas chromatographic analysis was the analysis method of choice. A non interfering internal standard, dodecane, was used to eliminate the possibility of volumetric errors in extraction and analysis.

All gas chromatography analyses were completed based on a calibration curve generated from 5 standards spread over the concentration range 15.4 - 211.2 mg/mL (based on dosing solution). All calibration curve correlation coefficients, r^2, were equal to or in excess of 0.999.

Initial attempts to extract 1 mL of a 10% Emulphor solution containing 8.5% wt./volume of D_4 with 5 mL of MIBK resulted in a gel which could not be broken. This contrasted with a 1:5 extraction of a 10% Emulphor solution containing no D_4 which did not gel. Experimentation showed that either a water or MIBK volume increase was sufficient to avoid the gellation problem. It was decided to use a MIBK volume increase for all subsequent extractions.

Initial measurements, based upon volume, returned near quantitative recoveries, e.g. 97-98%, 98-102% and 98-103% of theory for 8.5 and 5.7 % (twice) wt./volume D_4 in 10 % Emulphor dispersions. Since the sample internal standard was added post extraction, to a measured aliquot, the high recovery result was possibly due to MIBK solubility in Emulphor. When the procedure was modified to addition of the internal standard prior to extraction the recoveries were substantially lower, e.g. 37% of theory for 5.7 % wt./volume D_4 in 10 % Emulphor dispersions. Part of the problem with the analysis was traced to foam formation during mixing preventing withdrawal of a full 1 mL using a 1 mL syringe. A low recovery of 37% was obtained from samples that had been dispersed using

a vortex mixer but allowed to set 1-3 minutes for the foam to rise before sampling. When the same samples were reanalyzed, with immediate sampling but on a weight basis the recoveries increased to 66%. The weight basis was used to correct for the undervolume removed because of foam uptake via syringe.

All samples were prepared assuming that a volumetric pipet would deliver stated volume and that the amount delivered did not change with drain time of the pipet. It turns out that both assumptions were incorrect. When pipet drain times were held constant at 90 seconds the amount delivered became consistent as determined by weight transferred. However, it was also determined that 1.2 mL of D_4 which should have weighed 1.14g based upon a density of 0.95 g/mL actually weighed significantly more. This should have resulted in a greater than 100% recovery as seen during Maalox TC and 35 cSt PDMS extractions done by volume. When all sample preparations, extractions and calculations were completed on a 8.30 % wt./wt. dispersion of D_4 in 10% Emulphor the recovery averaged 77% of theory. Thinking that the low recovery was in part caused by increased difficulty in dispersing 8.3% instead of 5.7% of D_4 the latter concentration dispersion was prepared and immediately analyzed. The average recovery was 96.79 $\pm$ 2.05%, based upon 9 measurements.

Thinking that all was now resolved the latter method development samples prepared on a weight basis along with the three samples from each of Day 21 (3Oct95) and Day 28 (10Oct95) dosing were analyzed. The average recovery from the method development samples analyzed 4 weeks after preparation was 85.03 $\pm$ 4.60 %, based upon nine measurements. Tables 7 below summarizes the analyses of the samples from MCV. Figure 7 shows a representative chromatogram of an extract from Day 28 (10Oct95) #2.

Table 7: Average D_4 level in MCV dosing solutions.

Sample[1]	Average Calculated Concentration (mg/g)	Average Concentration/Day (mg/g)
Day 21 (3Oct95) # 1	49.93 $\pm$ 2.72[2]	51.07 $\pm$ 2.65[3]
Day 21 (3Oct95) # 2	53.09 $\pm$ 2.12[2]	
Day 21 (3Oct95) # 3	50.20 $\pm$ 2.16[2]	
Day 28 (10Oct95) # 1	53.10 $\pm$ 2.10[4]	52.14 $\pm$ 1.93[5]
Day 28 (10Oct95) # 2	52.74 $\pm$ 1.52[2]	
Day 28 (10Oct95) # 3	50.90 $\pm$ 1.78[2]	

Note 1: results are from 12667: 88.
Note 2: averages and standard deviations are calculated based on 6 measurements.
Note 3: averages and standard deviations are calculated based on 18 measurements.
Note 4: averages and standard deviations are calculated based on 4 measurements.
Note 5: averages and standard deviations are calculated based on 16 measurements.

The drop in recovery from 97% immediately after preparation, to 85%, four weeks later, as well as, the increase in standard deviation of the measurement with sample age are both consistent with increased difficulty in redispersing D_4 in aqueous Emulphor. Part of this increased dispersion difficulty tracks with the observation that it was increasingly difficult

to impart mechanical energy to the liquid fraction of the vial due to sample removal increasing foaming. This was verified by the amount of material transferred as 1 mL. The weight of the 1 mL transfer decreased with sample age. The 1 mL samples transferred for the 97% recovery averaged 0.90 ± 0.08 g while the 1 mL samples transferred for the 85% recovery averaged 0.77 ± 0.04 g in size. Averages and standard deviations were based upon 9 measurements.

To further verify this, the aged method development samples along with the three samples from each of Day 21 (3Oct95) and Day 28 (10Oct95) dosing were reanalyzed two days after the previous work. In addition, a set of freshly prepared method development samples were analyzed. The average recovery from the method development samples analyzed 4 weeks after preparation was 82.13 ± 3.63 % (1 mL = 0.83 ± 0.04g), based upon nine measurements. The average recovery from the method development samples analyzed immediately after preparation was 93.22 ± 1.83 % (1 mL = 0.89 ± 0.04g), based upon nine measurements. The analysis of the method development samples was again consistent with this hypothesis. Tables 8 below summarizes the reanalyzes of the samples from MCV.

Table 8: Average D_4 level in MCV dosing solutions upon reanalysis.

Sample[1]	Average Calculated Concentration (mg/g)[2]	Average Concentration/Day (mg/g)[3]
Day 21 (3Oct95) # 1	49.18 ± 1.78	48.56 ± 2.50
Day 21 (3Oct95) # 2	49.79 ± 3.41	
Day 21 (3Oct95) # 3	46.71 ± 1.50	
Day 28 (10Oct95) # 1	52.30 ± 1.61	52.28 ± 1.99
Day 28 (10Oct95) # 2	53.40 ± 0.32	
Day 28 (10Oct95) # 3	51.13 ± 3.04	

Note 1: results are from 12667: 90-93.
Note 2: averages and standard deviations are calculated based on 3 measurements.
Note 3: averages and standard deviations are calculated based on 9 measurements.

Thus there is no accurate way to verify the dosing level from D_4 in aqueous 10% Emulphor solutions which were prepared and allowed to age. Fortunately the dosing solutions were used shortly after preparation, when the dispensability was optimum. Also, the ability to accurately deliver the desired dosing level would increase with decreasing level of D_4 since ease of dispersion would go up. We were attempting to verify the maximum dose level, where maximum error would be expected. It is not known with certainty what the level was on the day the samples were prepared. This work would suggest the concentrations were at least 51 ± 3 and 52 ± 2 mg/g for Day 21 and Day 28 respectively.

4G. Conclusions

Analysis of mL Dow Corning® 244 Fluid dispersed in 10% aqueous Emulphor was attempted. The analysis method consisted of extraction with MIBK containing dodecane

internal standard with analysis of the extract by gas chromatography. Dosing solutions from Day 21 (3Oct95) and Day 28 (10Oct95) were analyzed to contain more than 51 and 52 mg/g D_4 in aqueous Emulphor, respectively.

It was discovered that there is no accurate way to verify the dosing level of D_4 in aqueous 10% Emulphor solutions which were prepared and allowed to age due to difficulty in redispersing D_4. Fortunately the dosing solutions were used shortly after preparation, when the dispersability was at its maximum. Also, the ability to accurately deliver the desired dosing level would increase with decreasing level of D_4 since ease of dispersion would go up. We were attempting to verify the maximum dose level, where maximum error would be expected. It could not be verified with certainty what the dosing solution level was on the day the samples were prepared. This work would suggest the concentrations were at least 51 ± 3 and 52 ± 2 mg/g for Day 21 and Day 28 respectively. Conversion to units of mg/mL would be expected to increase the values only very slightly. After review of the standard operating procedure for solution preparation and its application for the other carriers, the level was more probably 57 mg/mL. Our verification result, though expected to be low, was still within 11% of the expected result. It is recommended to review the sample preparation records and rely upon these for the actual dosing solution level.

H. Archive

(General) Cover letters (4) from MCV are included as well as an exact copy of Dow Corning lab book 12667 pages 37, 50-51, 71-72, 86-87 which cover receipt of the dosing solutions and a description of what was received.

(Corn Oil, 35 cSt PDMS, Emulphor and Maalox) An exact copy of Dow Corning lab book 12667 pages 28-34, 36, 38-39, 41-42, 52-53, 63, 68-69, 73-74, 76-78, 85, 88-93, and 97, lab boob 12198 pages 7-11 and lab book 13046 page 92 contain raw data and experimental descriptions. All GC data and calibration curve originals are included. A printout of all Microsoft Excel V5.0 spreadsheets, with/without formulas, used for manipulation of the raw data are included.

The lab books will be archived as per Dow Corning records retention guidelines. The data, described above, will be archived at: Dow Corning Corporation, Health and Environmental Sciences, 2200 W. Salzburg Road, Auburn, MI 48611.

I. References
none

This report, constituted of pages 1 - 34 signed this 4th day of November, 1996.

Authors: 11-4-96 / 11/4/96

Don L. Kleyer Date — Thomas W. Galbraith Date

Figure 1. Full Infrared Spectral Overlay of Dow Corning® 244 Fluid Standards in Corn Oil at 1.003, 2.990, 5.002 % wt/wt.

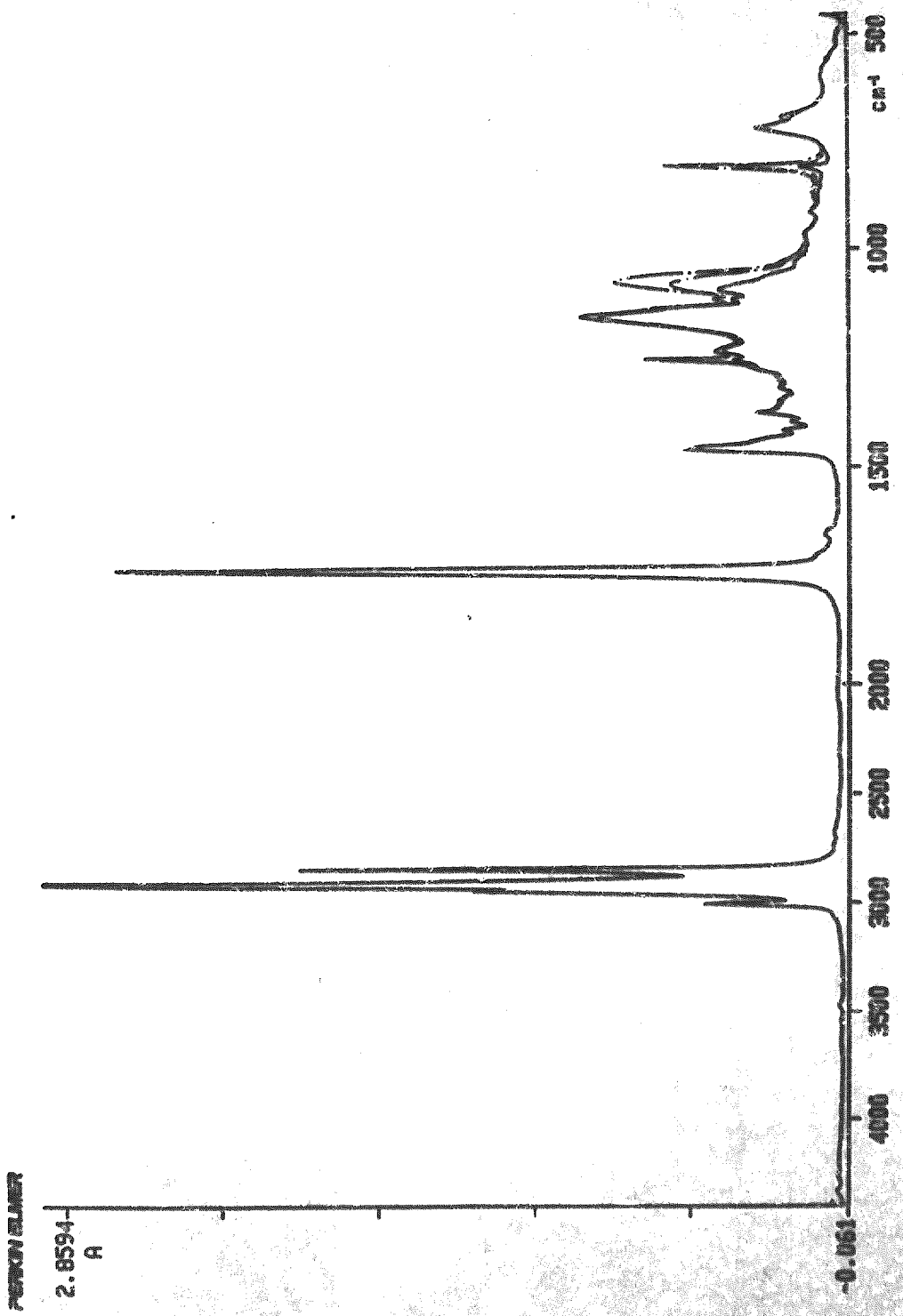


Figure 2. Expansion of Infrared Spectral Overlay of Dow Corning® 244 Fluid Standards in Corn Oil at 1.003, 2.990, 5.002 % wt/wt.

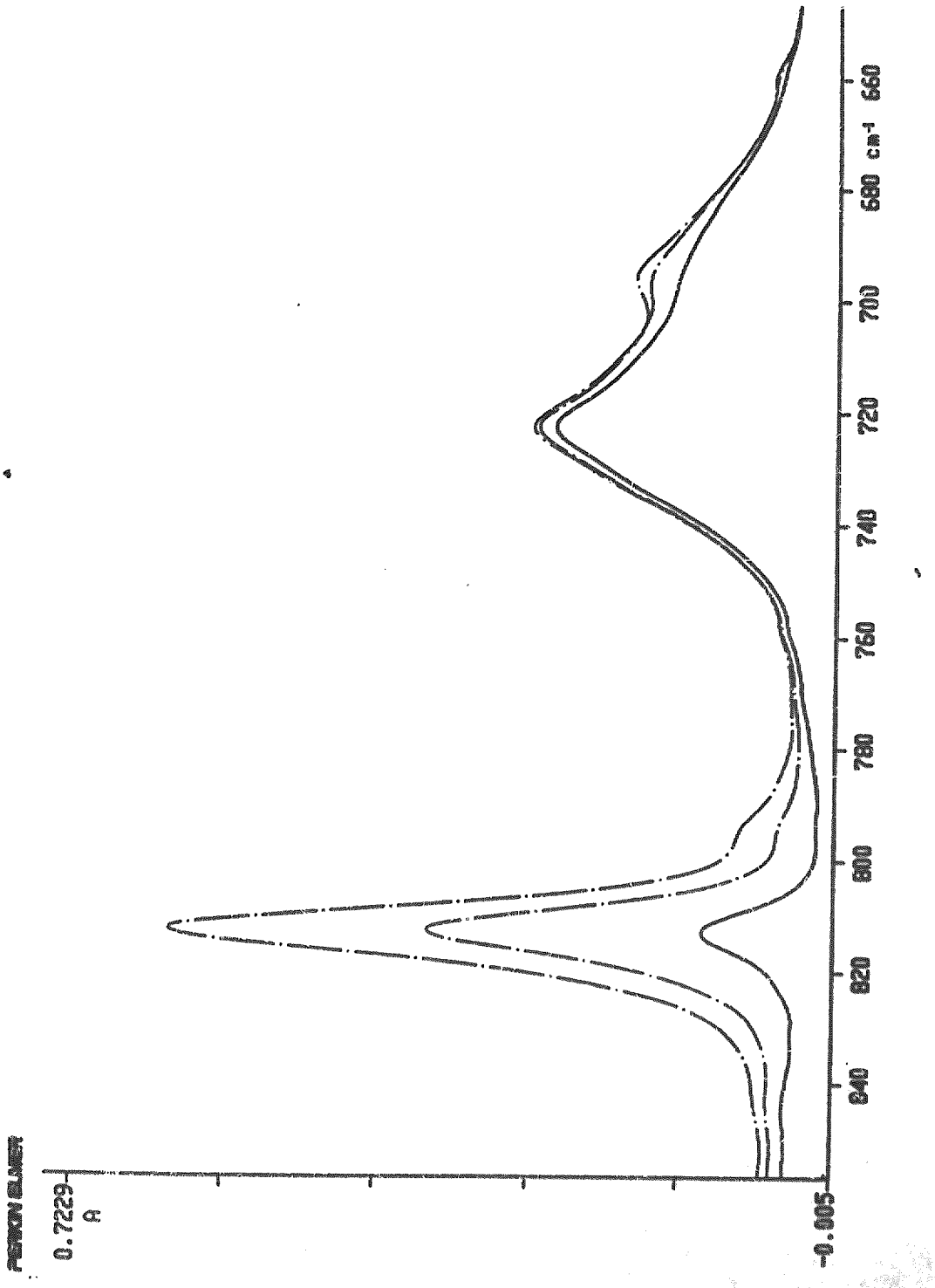


Figure 3. Full Infrared Spectral Overlay of Three Dow Corning® 244 Fluid in Corn Oil Dosing Solutions From MCV (Day 28, 10Oct95).

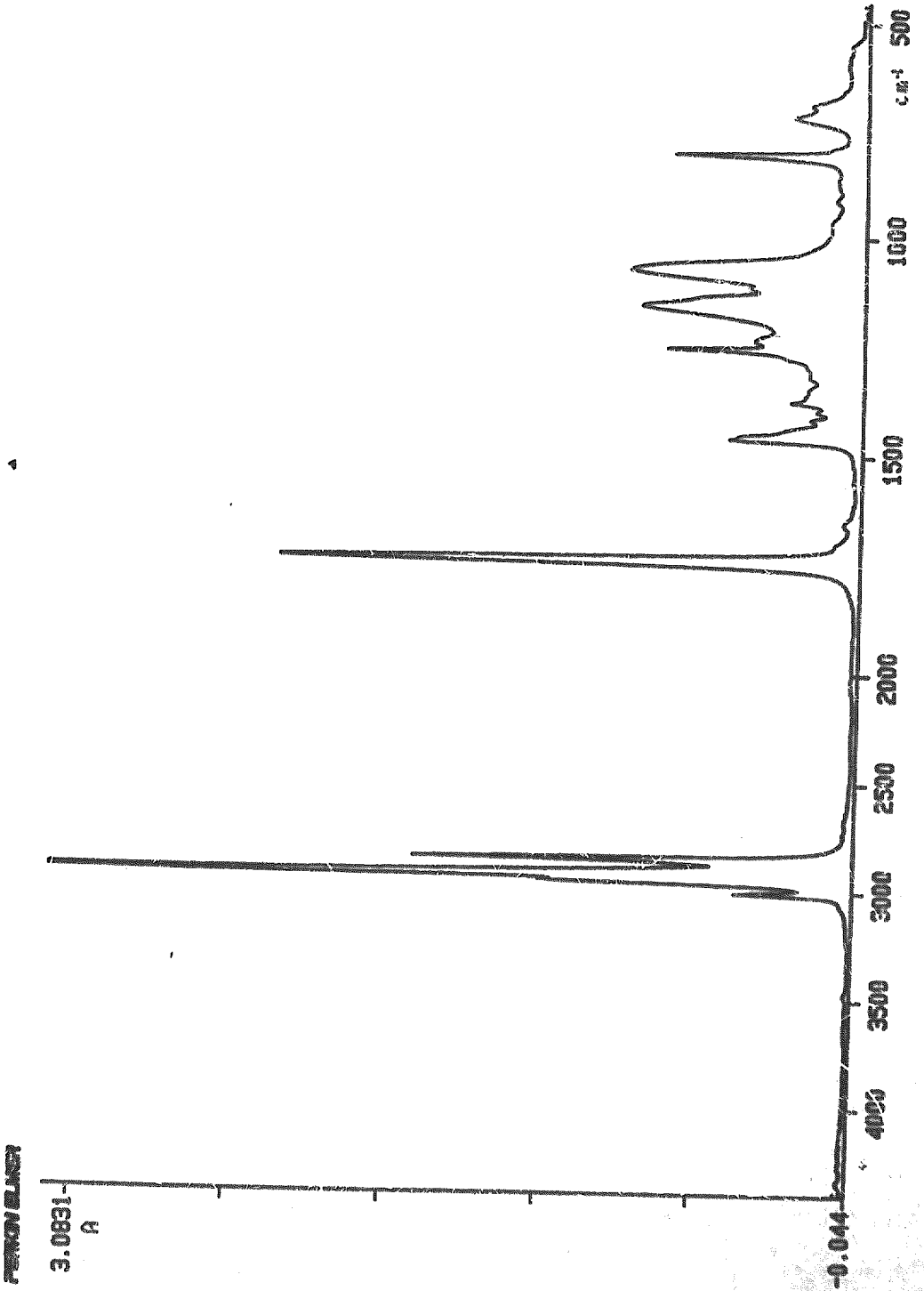


Figure 4. Expansion of Infrared Spectral Overlay of Three Dow Corning® 244 Fluid in Corn Oil Dosing Solutions From MCV (Day 28, 10Oct95).

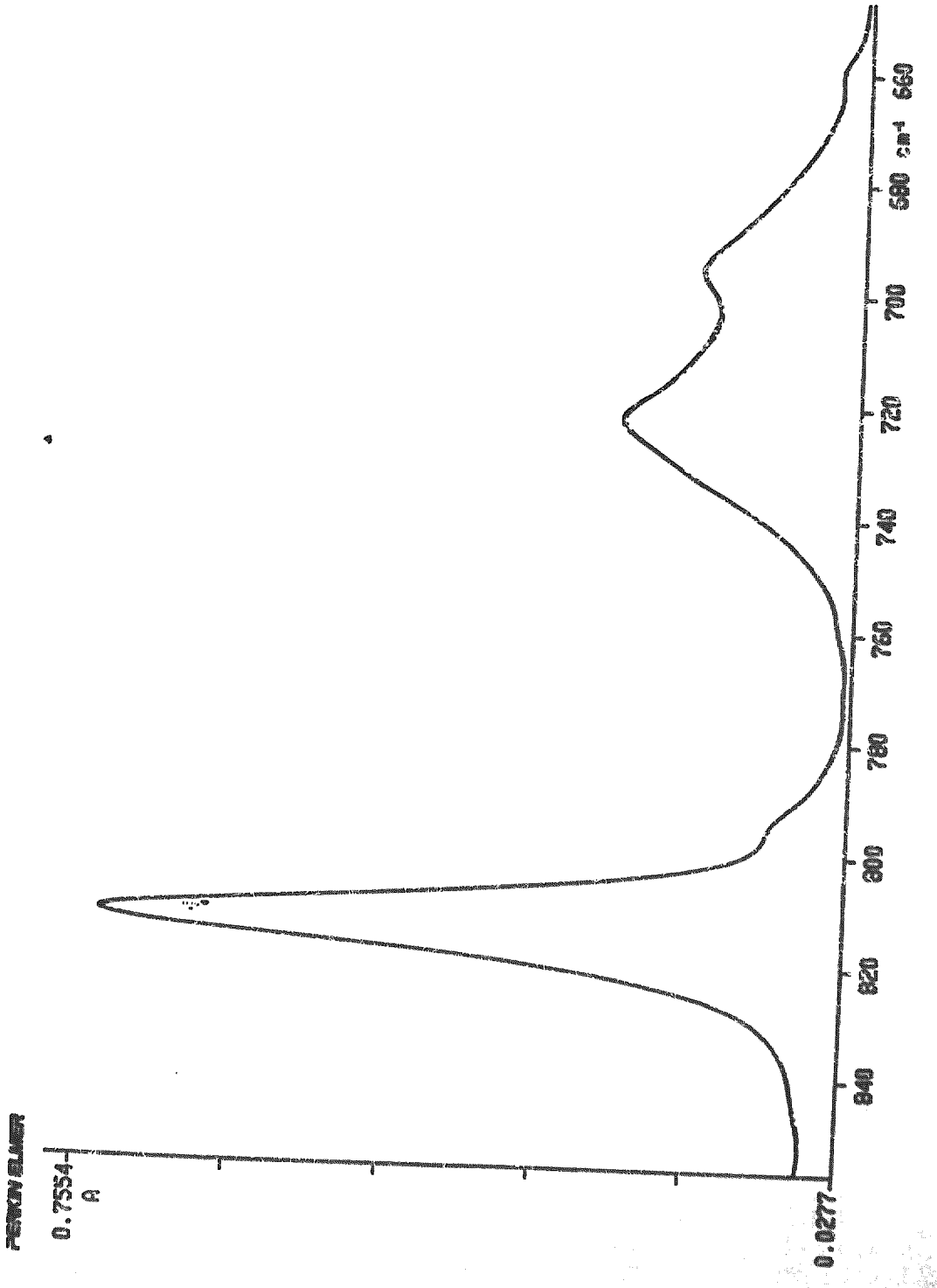
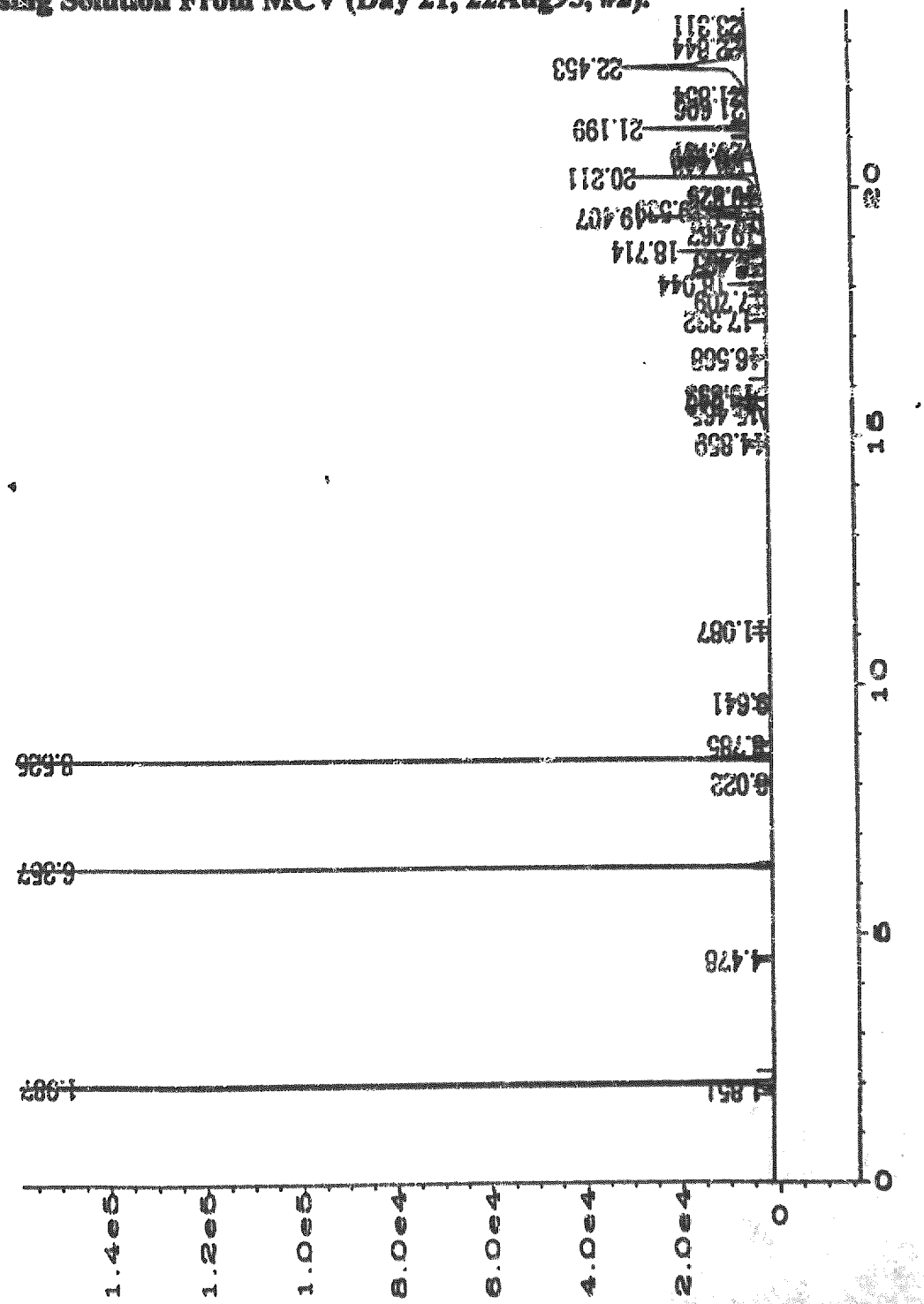
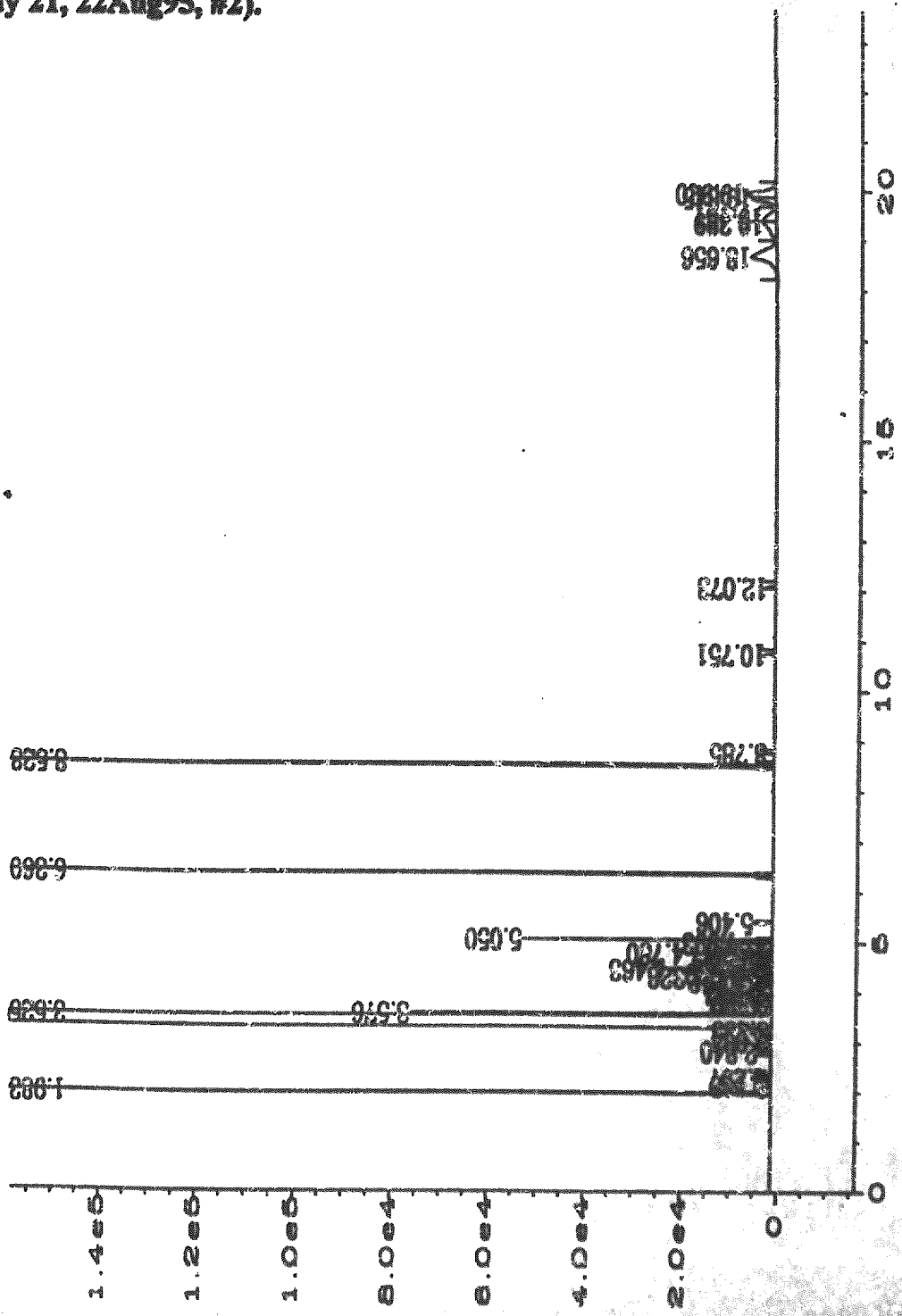


Figure 5. Chromatogram From GC/FID Analysis of an Acetone Extract of a Dow Corning® 244 Fluid in Dow Corning® X9-6370 (35 cSt PDMS) Dosing Solution From MCV (Day 21, 22Aug95, #2).



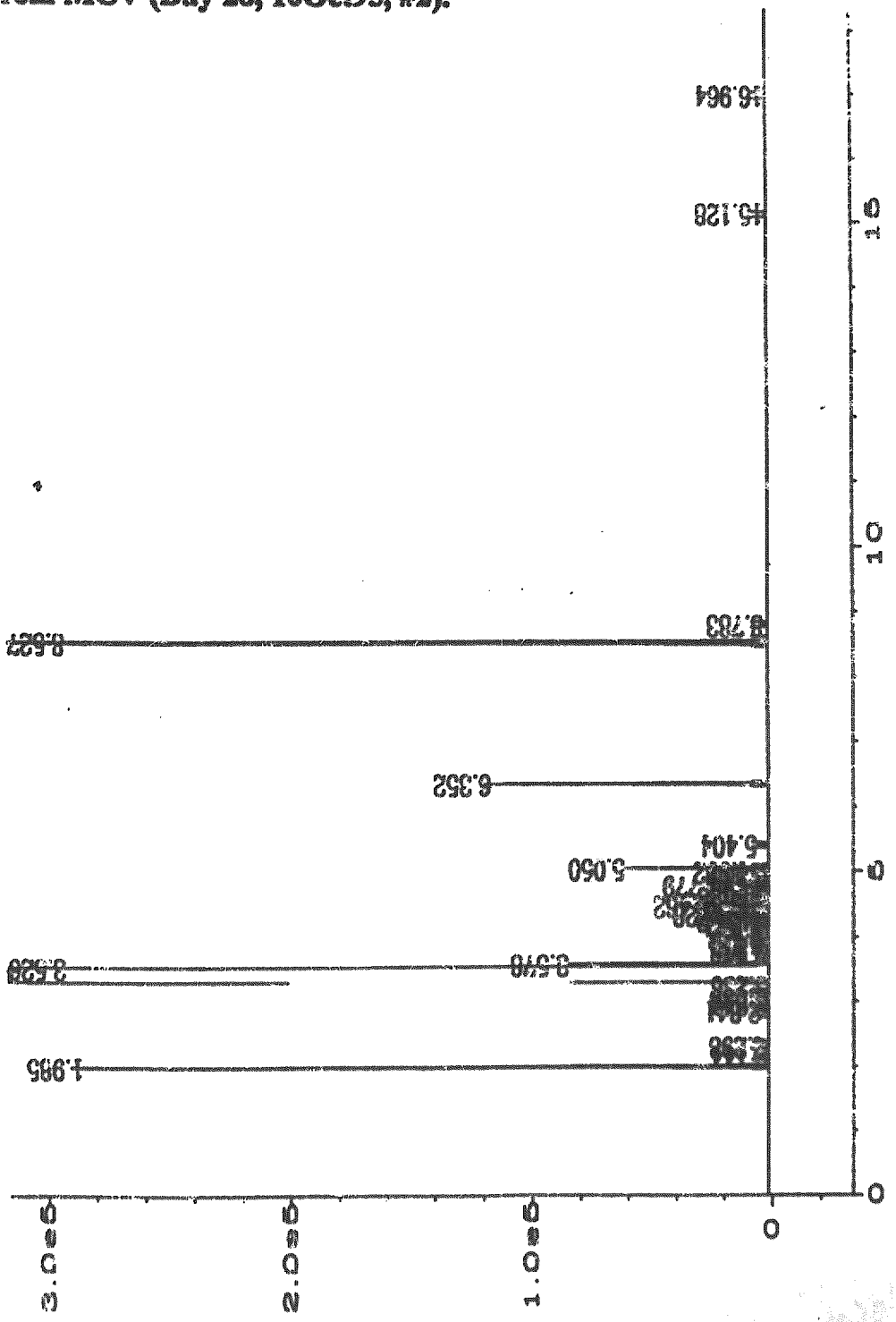
Note: Peak at 6.357 minutes is D_4, peak at 8.525 minutes is dodecane internal standard.

Figure 6. Chromatogram From GC/FID Analysis of a MIBK Extract of a Dow Corning® 244 Fluid in Maalox TC Dosing Solution From MCV (Day 21, 22Aug95, #2).



Note: Peak at 6.360 minutes is D_4, peak at 8.528 minutes is dodecane internal standard.

Figure 7. Chromatogram From GC/FID Analysis of a MIBK Extract of a Dow Corning® 244 Fluid in 10% Aqueous Emulphor Dosing Solution From MCV (Day 28, 10Oct95, #2).



Note: Peak at 6.352 minutes is D_4, peak at 8.527 minutes is dodecane internal standard.

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