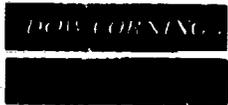


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TSCA Document Processing Center (7407)  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street S.W.  
Washington, D.C. 20460  
Attn: TSCA Sections 8(d) FYI Submission



FYI-99-001362

Re: For Your Information Submission:  
49 FR 46741 (November 28, 1984) [OPTS-84013; FRL-2725-1]  
TSCA Section 8(d) Health and Safety Data Reporting

Dear Sir:

The enclosed information is submitted on behalf of Dow Corning Corporation, Midland, Michigan, 48686-0994, on a For-Your -Information (FYI) basis as a follow-up to submissions made concerning octamethylcyclotetrasiloxane (OMCTS), which chemical substance was the subject of a health and safety data rule issued under Section 8(d) of the Toxic Substances Control Act (TSCA) and with an effective date of December 28, 1984 and a sunset date of December 28, 1994, as codified at 40 CFR 716 (Health and Safety Data Reporting). The information presented in this submission was generated as part of our Siloxane Research Program. This program was the subject of a memorandum of understanding, dated April 9, 1996, between Dow Corning and EPA.

**Chemical Substance:**

556-67-2                      Octamethylcyclotetrasiloxane

**Title of Recently Completed Study:**

ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF OCTAMETHYL-  
CYCLOTETRAILOXANE (D4) IN SPRAGUE-DAWLEY AND FISCHER 344  
IMMATURE FEMALE RATS USING A UTEROTROPHIC ASSAY

Dow Corning Corporation  
1998-10000-45425  
May 26, 1999

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CONFIDENTIAL

**Manufacturer:**

Dow Corning Corporation  
2200 West Salzburg Road  
Midland, Michigan 48686-0994

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

If you require further information regarding this submission, please contact Dr. Rays G. Daniels, Senior Regulatory Compliance Specialist, Regulatory Compliance Group, HERA Americas, at 517-496-4222 or at the address provided herein.

Sincerely,



Patrick W. Langvardt  
Director, Laboratory Health Sciences  
Health and Environmental Sciences  
(517) 496-4626

RGD99180

**A 05**

DC Study No. 9032  
MPI Study No. 416-148

DC Report No. 1998-I0000-45425  
Security - Internal

1

**ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF  
OCTAMETHYLCYCLOTETRASILOXANE (D4) IN SPRAGUE-  
DAWLEY AND FISCHER 344 IMMATURE FEMALE RATS USING  
A UTEROTROPHIC ASSAY**

**TEST ARTICLE:** Octamethylcyclotetrasiloxane (D4)

**PERFORMING LABORATORY:** MPI Research  
54943 North Main Street  
Mattawan, MI 49071-9399 U.S.A.  
(616) 668-3336

**MPI STUDY NUMBER:** 416-148

**SPONSOR STUDY NUMBER:** 9032

**STUDY DIRECTOR:** Patricia A. Turck, M.S.

**SPONSOR ADDRESS:** Dow Corning Corporation  
Health and Environmental Sciences  
2200 West Salzburg Road  
Midland, Michigan 48686-0944

**SPONSOR REPRESENTATIVE:** Kathleen Plotzke, Ph.D.

**DATE OF STUDY COMPLETION:** May 26, 1999

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Volume 1 of 2  
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DC Study No. 9032  
MPI Study No. 416-148

DC Report No. 1998-10000-45425  
Security - Internal

1

**ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF  
OCTAMETHYLCYCLOTETRASILOXANE (D4) IN SPRAGUE-  
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STATEMENT OF COMPLIANCE

This nonclinical laboratory study was conducted in accordance with the United States Environmental Protection Agency, TSCA Good Laboratory Practice Standards, 40 CFR Part 792, except that concentration, homogeneity and stability analyses of the dosing solutions for the intra-assay positive and negative (antiestrogen) controls were not conducted. Protocol deviations are presented in Appendix N.

Study Director Patricia A. Turck 5/26/99  
Patricia A. Turck, M.S. Date

Submitter Kathleen P. Plosino 5/27/99

A 08

DC Study No. 9032  
MPI Study No. 415-148

DC Report No. 1998-10000-46425  
Security - Internal

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5/26/99  
Date

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Kathleen F. Plotzke, Ph.D.  
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DC Study No. 9032  
MPI Study No. 416-148

DC Report No. 1998-10600-45425  
Security - Internal

**1. QUALITY ASSURANCE STATEMENT**

Below are the inspections conducted by the Quality Assurance Department and the dates the inspections were reported to the Study Director and Management:

<u>Date(s) of Inspection</u>	<u>Study Phase Inspected</u>	<u>Date(s) Reported to Study Director/Management</u>
6/17-18/98	Protocol Review	6/18/98, 7/8/98
6/25/98	Test Material Administration	6/25/98, 7/8/98
6/30/98	Body Weight Measurement	7/8/98
8/14-19/98	Report Review	8/19/98
8/14-19/98	Data Review	8/19/98
9/10-11/98	Report Review	9/11/98
3/17/99	Report Review	3/17/99
5/11/99	Report Review	5/11/99

  
Matthew A. Foster, B.S.  
Project Leader, Quality Assurance

5/12/99  
Date

## 2. SUMMARY

The objective of this study was to evaluate potential estrogenic and antiestrogenic effects of D4 by measuring uterine weights at euthanasia in 2 strains of immature female rats treated with D4 for 4 consecutive days. The study consisted of 6 groups of Sprague-Dawley rats (12 intact immature females/group) given 1 of 6 dose levels of D4, 12 positive control groups (4 dose levels for each of the 3 positive controls for estrogenic effects), 4 groups combining D4 and ethinyl estradiol (EE), 4 groups combining the antiestrogen ICI 182,780 (ICI) and ethinyl estradiol to evaluate antiestrogenic effects, and 1 vehicle control group of Sprague-Dawley rats (12 immature females/group). In addition, 6 D4 groups, 8 positive control groups (4 dose levels for each of 2 positive controls), 4 groups combining D4 and ethinyl estradiol, 4 groups combining the antiestrogen, ICI 182,780, and ethinyl estradiol, and 1 vehicle control group of Fischer 344 rats (12 immature rats/group) were also placed on study. D4 was administered by oral gavage at dose levels of 0 (sesame oil vehicle control), 10, 50, 100, 250, 500, and 1000 mg/kg/day. EE was administered at dose levels of 1, 3, 10, and 30 µg/kg/day. Diethylstilbestrol Dipropionate (DES-DP) was administered at dose levels of 0.5, 1.5, 5, and 15 µg/kg/day. Coumestrol (CE) was administered at dose levels of 10, 35, 75, and 150 mg/kg/day. D4 plus EE was administered at dose levels of 500 mg/kg/day D4 plus 1 µg, 3 µg, 10 µg, or 30 µg EE/kg/day. ICI 182,780 plus EE was administered at dose levels of 3 mg/kg/day ICI 182,780 plus 1 µg, 3 µg, 10 µg, or 30 µg EE/kg/day. Each dosage level was administered separately to the 2 strains of rats placed on study, with the exception of coumestrol, which was not administered to the Fischer 344 rats. The females were 18 (Sprague-Dawley) or 21 (Fischer 344) days of age at the start of dosing. The treatment, positive control, and vehicle control groups were dosed by oral gavage once per day for 4 consecutive days at a dose volume of 5 mL/kg body weight. Administration to the Fischer 344 rats began after administration to the Sprague-Dawley rats had been completed. Observations for clinical signs and body weights were measured during the test period. At termination of the in-life phase (22 days of age for Sprague-Dawley or 25 days of age for Fischer 344), the immature female rats were necropsied, and uterine weights were recorded.

No treatment-related effects were observed on mortality or clinical signs in Sprague-Dawley or Fischer 344 rats administered D4 by oral gavage at dose levels as high as 1000 mg/kg/day. Body weight gain was significantly decreased in a dose related manner in both strains of rats given 250, 500, or 1000 mg/kg/day D4. Absolute and relative liver weight were significantly increased at 100 mg/kg/day D4 and higher in the Fischer 344 rat. Relative liver weight was significantly higher at 250 mg/kg/day D4 and absolute and relative liver weight were significantly higher at 500 and 1000 mg/kg/day D4 in the Sprague-Dawley rat. Uterine weight (both absolute and relative) were significantly increased at 250 mg/kg/day D4 and higher in both strains of rats. The increased uterine weight was accompanied by a slight increase (1/10) in uterine distension at 1000 mg/kg/day D4 in both strains of rats and increased uterine and epithelial cell height at 250 mg/kg/day D4 and higher in both strains of rats. At the 50% of maximal response level for EE and DES-DP, D4 was approximately 1.2 to 25 million times less potent than EE or DES-DP, in Sprague-Dawley and Fischer 344 rats, respectively, as measured by its ability to produce increases in uterine weight. D4 administration also did not

produce the same magnitude (maximum effect) of uterine weight increases as either EE or DES-DP in either the Sprague-Dawley or Fischer 344 rat. When 500 mg/kg/day D4 was coadministered over a range of EE doses, D4 decreased the uterotrophic dose response of EE by approximately one-half (Sprague-Dawley) to one (Fischer 344) order of magnitude. This inhibitory effect indicates that D4 has weak antiestrogenic properties.

The positive controls, EE, DES-DP and CE, produced dose-related increases in uterine weight and demonstrated their strong (EE and DES-DP) or weak (CE) estrogenic potential. The negative control ICI virtually completely inhibited the uterotrophic response of EE when coadministered with EE.

In conclusion, under the conditions of this study, oral administration of D4 to intact immature Sprague-Dawley or Fischer 344 rats for 4 consecutive days at dose levels of 250, 500, or 1000 mg/kg/day was weakly estrogenic as measured by dose-related increases in uterine weight and increases in epithelial cell height. D4 was found to be weakly estrogenic in both species of rat. When compared to EE, D4 was approximately 77,000 (1/0.000013) to 1,500,000 (1/0.00000066) times less potent in Sprague-Dawley rats and 143,000 (1/0.000007) to 25,000,000 (1/0.00000004) times less potent in the Fischer 344 rat. When compared to DES-DP, D4 was approximately 48,000 (1/0.000021) to 1,200,000 (1/0.00000032) times less potent in Sprague-Dawley rats and 7,000,000 (1/0.00000014) times less potent in Fischer 344 rats. Due to the weak response of D4, potency ratios at 80% of the EE or DES-DP response could not be determined. At the 50% of maximal response level for EE and DES-DP, D4 was approximately 1.2 to 25 million times less potent than either EE or DES-DP in Sprague-Dawley and Fischer 344 rats, respectively, and 83 times less potent than CE in Sprague-Dawley rats. The estrogenic response of D4 occurred at dose levels that also produced decreases in body weight or body weight change and increases in liver weight. D4 also exhibited weak antiestrogenic properties by partially blocking EE induced uterine weight increases.

### 3. INTRODUCTION

#### 3.1. Objective

The objective of this study was to determine the estrogenic and antiestrogenic potential of D4 by measuring uterine weights at euthanasia of 2 strains (Sprague-Dawley and Fischer 344) of immature female rats treated orally with sesame oil for 4 consecutive days.

#### 3.2. Species Selection

The current state of scientific knowledge does not provide any acceptable alternatives, *in vitro* or otherwise, to the use of live animals to accomplish the purpose of this study. The rat is a universally used model for evaluating toxicity of various classes of chemicals and for which there is a large historical database.

#### 3.3. Study Schedule

Study initiation (Protocol signed by the Study Director)	June 11, 1998
Protocol Approved by the Sponsor	June 16, 1998
Arrival of Animals	June 18-19, 23, 1998 June 25-26, 30, 1998
Randomization	June 25 and 27, 28, 1998 July 2-6, 9-12, 1998
Commencement of Treatment	June 25-27, 1998 July 2-6, 9-12, 1998
Necropsy	June 29, 1998 July 1-2, 6-10, 13-16, 1998
Draft Report	September 15, 1998

## 4. MATERIALS AND METHODS

### 4.1. Experimental Design

#### 4.1.1. Animal Receipt and Distribution

On June 18, 19, 24, and 26, 1998, a total of 48 female Sprague-Dawley (CrI: CD® VAF/Plus®) foster dams with 9 to 11 day-old, fostered female pups (minimum of 10 pups/female) were received from Charles River Laboratories, Portage, Michigan. On June 23 and 30, 1998, a total of 42 female Fischer 344 [COBS® CDF® (F-344/CrlBR)] foster dams with 7 to 11 day-old, fostered female pups (minimum of 10 pups/female) were received from Charles River Laboratories, Raleigh, North Carolina. All pups were acclimated to the laboratory from the time they arrived at 7 to 11 days of age to the start of dosing at 18 (Sprague-Dawley) or 21 (Fischer 344) days of age. Pups were housed with the dam until selected for study at 18 (Sprague-Dawley) or 21 (Fischer 344) days of age. During the acclimation period, all animals were observed daily for any clinical signs of disease, and all animals were given a detailed clinical examination prior to selection for study.

Throughout the study, Sprague-Dawley and Fischer 344 rats were kept in separate, environmentally controlled rooms. Temperature and relative humidity were monitored and recorded daily and maintained between 66 to 71°F and 48 to 77%, respectively, in the room housing the Sprague-Dawley rats. Temperature and relative humidity were monitored and recorded daily and maintained between 68 to 72°F and 58 to 80%, respectively, in the room housing the Fischer 344 rats. Fluorescent lighting provided illumination 12 hours per day via an automatic timer. Airflow was maintained at a minimum of 10 exchanges per hour. Diet (Certified Rodent Chow #5002, PMI Feeds, Inc., St. Louis, Missouri) and tap water were available *ad libitum*. Upon arrival, the dams and litters were group housed in plastic shoe-box cages containing wood chip bedding. At the beginning of the work day on the day of dosing (18 days of age for Sprague-Dawley and 21 days of age for Fischer 344), the dams were removed from the cage. After randomization, pups were housed in plastic shoe-box cages (2 pups from the same group/cage) containing wood chip bedding. The wood chip bedding was periodically analyzed by the manufacturer. The results of this analysis were placed in the Study File. No contaminants were known to have been in the wood chip bedding which could have altered the outcome of the study. Each cage was equipped with water bottles.

Certification of analysis for each diet lot was performed by the manufacturer. Therefore, no additional analyses were conducted. Each lot of diet used was recorded in facility records. The drinking water used for the test animals was monitored for specified contaminants at periodic intervals according to the Standard Operating Procedures of MPI Research. Therefore, no further analysis was conducted. No contaminants were known to have been in the water or feed which could have altered the outcome of the study.

## 4.1.2. Assignment to Study

Rats considered suitable for study were weighed in the morning prior to the first exposure at 18 (Sprague-Dawley) or 21 (Fischer 344) days of age and randomized into treatment groups using a stratified, by weight, block randomization procedure. Only Sprague-Dawley pups weighing 35-50 grams and Fischer 344 pups weighing 25-40 grams were used. Separate randomizations were conducted for each strain of rat prior to each staggered start. For both strains, two animals began test article administration on six separate days. The Sprague-Dawley rats began test article administration on June 25, June 27, June 28, July 2, July 3, and July 4, 1998. The Fischer 344 rats began test article administration on July 5, July 6, July 9, July 10, July 11, and July 12, 1998. Animals were assigned to study as shown below and on the following page. Slightly older (21 days of age) Fischer 344 rats were used due to their small size and growth rate relative to the Sprague-Dawley rats.

Group	Strain	Dosage	Number on Study
1	Sprague-Dawley	0 (vehicle control)	12
2	Sprague-Dawley	10 mg/kg/day D4	12
3	Sprague-Dawley	50 mg/kg/day D4	12
4	Sprague-Dawley	100 mg/kg/day D4	12
5	Sprague-Dawley	250 mg/kg/day D4	12
6	Sprague-Dawley	500 mg/kg/day D4	12
7	Sprague-Dawley	1000 mg/kg/day D4	12
8	Sprague-Dawley	1 µg/kg/day EE	12
9	Sprague-Dawley	3 µg/kg/day EE	12
10	Sprague-Dawley	10 µg/kg/day EE	12
11	Sprague-Dawley	30 µg/kg/day EE	12
12	Sprague-Dawley	0.5 µg/kg/day DES-DP	12
13	Sprague-Dawley	1.5 µg/kg/day DES-DP	12
14	Sprague-Dawley	5.0 µg/kg/day DES-DP	12
15	Sprague-Dawley	15.0 µg/kg/day DES-DP	12
16	Sprague-Dawley	10 mg/kg/day CE	12
17	Sprague-Dawley	35 mg/kg/day CE	12
18	Sprague-Dawley	75 mg/kg/day CE	12
19	Sprague-Dawley	150 mg/kg/day CE	12
20	Sprague-Dawley	500 mg/kg/day D4 + 1 µg/kg/day EE	12
21	Sprague-Dawley	500 mg/kg/day D4 + 3 µg/kg/day EE	12

Group	Strain	Dosage	Number on Study
22	Sprague-Dawley	500 mg/kg/day D4 + 10 µg/kg/day EE	12
23	Sprague-Dawley	500 mg/kg/day D4 + 30 µg/kg/day EE	12
24	Sprague-Dawley	3 mg/kg/day ICI 182,780 1 µg/kg/day EE	12
25	Sprague-Dawley	3 mg/kg/day ICI 182,780 + 3 µg/kg/day EE	12
26	Sprague-Dawley	3 mg/kg/day ICI 182,780 + 10 µg/kg/day EE	12
27	Sprague-Dawley	3 mg/kg/day ICI 182,780 + 30 µg/kg/day EE	12
28	Fischer 344	0 (vehicle control)	12
29	Fischer 344	10 mg/kg/day D4	12
30	Fischer 344	50 mg/kg/day D4	12
31	Fischer 344	100 mg/kg/day D4	12
32	Fischer 344	250 mg/kg/day D4	12
33	Fischer 344	500 mg/kg/day D4	12
34	Fischer 344	1000 mg/kg/day D4	12
35	Fischer 344	1 µg/kg/day EE	12
36	Fischer 344	3 µg/kg/day EE	12
37	Fischer 344	10 µg/kg/day EE	12
38	Fischer 344	30 µg/kg/day EE	12
39	Fischer 344	0.5 µg/kg/day DES-DP	12
40	Fischer 344	1.5 µg/kg/day DES-DP	12
41	Fischer 344	5.0 µg/kg/day DES-DP	12
42	Fischer 344	15.0 µg/kg/day DES-DP	12
43	Fischer 344	500 mg/kg/day D4 + 1 µg/kg/day EE	12
44	Fischer 344	500 mg/kg/day D4 + 3 µg/kg/day EE	12
45	Fischer 344	500 mg/kg/day D4 + 10 µg/kg/day EE	12
46	Fischer 344	500 mg/kg/day D4 + 30 µg/kg/day EE	12
47	Fischer 344	3 mg/kg/day ICI 182,780 + 1 µg/kg/day EE	12

Group	Strain	Dosage	Number on Study
48	Fischer 344	3 mg/kg/day ICI 182,780 + 3 µg/kg/day EE	12
49	Fischer 344	3 mg/kg/day ICI 182,780 + 10 µg/kg/day EE	12
50	Fischer 344	3 mg/kg/day ICI 182,780 + 30 µg/kg/day EE	12

Each animal was individually identified by a metal ear tag bearing the animal number. The animal's cage was identified by the study number, strain, animal number, group, and sex. The individual animal number plus the MPI Research study number comprised a unique identification number for each animal. The adult female rats were 10 - 12 (Sprague-Dawley) or 10-16 (Fischer 344) weeks of age at arrival. The Sprague-Dawley pups were 9, 10, or 11 days of age at arrival. The Fischer 344 pups were 7, 8, 9, 10, or 11 days of age at arrival.

#### 4.2. Vehicle Control, Test Article, Positive Control and Antiestrogen Articles

##### 4.2.1. Test Article Identification

The test article, Octamethylcyclotetrasiloxane (D4) was received from Dow Corning Corporation, Midland, Michigan. Pertinent information is presented in Appendix A.

##### 4.2.2. Vehicle Control Article Identification

The vehicle control, sesame oil was received from Dow Corning Corporation, Midland, Michigan. Pertinent information is presented in Appendix A.

##### 4.2.3. Positive Control and Antiestrogen Article Identification

The positive control articles, diethylstilbesterol dipropionate (DES-DP), ethinyl estradiol (EE), and coumestrol (CE) were received from Dow Corning Corporation, Midland, Michigan. An additional lot of CE was received from Spectrum Quality Products, Inc., Gardena, California. The CE groups were prepared by combining both lots and then grinding the test article with an agate mortar and pestle. Two lots of the antiestrogen, ICI 182,780, were received, one from Dow Corning Corporation, Midland, Michigan, and one from Tocris Cookson Inc., Ballwin, Missouri. Pertinent information is presented in Appendix A.

EE was selected as the estradiol positive control because it has greater potency following oral administration than 17β-estradiol (Odum et al., 1997).

#### 4.2.4. Preparation of Dosing Material

##### 4.2.4.1. Preparation of Vehicle Control

The vehicle control dosing solution (0 mg/mL) was prepared by the Sponsor by placing approximately 100 mL of sesame oil into a 100 mL dosing jar. The vehicle control dosing preparation was prepared once and used throughout the study for both strains of rats. The sesame oil was stored in the dark at room temperature.

##### 4.2.4.2. Preparation of Test Article

Dosing solutions containing the test article (D4) were prepared by the Sponsor. Each dosing solution, containing D4 only, was prepared once and used throughout the study for both strains of rats. The dosing solutions with D4 + EE were prepared for each strain of rat on study (*i.e.*, once for the Sprague-Dawley rats and once for the Fischer 344 rats). Each dosing solution containing D4 was prepared in sesame oil by weighing the required amount of D4 into a volumetric flask and then adding the required volume of sesame oil. To facilitate complete solubilization, approximately one-half of the final volume of sesame oil was added to the flask containing D4 and mixed. The remaining amount of sesame oil was then added and the solution mixed by several inversions. The final solution was mixed for at least 1 hour with a magnetic stir bar. After mixing, the solutions were transferred into 100 mL dosing jars and wrapped in foil to protect them from light. The solutions were stored in the dark at room temperature.

##### 4.2.4.3. Preparation of Positive Control and Antiestrogen Articles

Dosing solutions containing only EE, DES-DP, or CE, or containing both ICI and EE were prepared by MPI Research. Each of the above dosing solutions was prepared once for each strain of rat (*i.e.*, once for Sprague-Dawley rats and once for Fischer 344 rats; only one set of dosing solutions was prepared for the CE groups as the Fischer 344 rats did not receive CE). Each dosing solution was prepared in sesame oil by weighing the required amount of stock solution, CE, or both EE and ICI 182,780, into a flask along with the required volume of sesame oil. To facilitate complete solubilization, approximately one half of the total required volume of sesame oil was added to each flask containing the appropriate positive control or antiestrogen articles and mixed. The remaining amount of sesame oil was then added and the solutions were mixed by several inversions. The final solution was mixed for at least 1 hour using a magnetic stir bar and stir plate. After mixing, the solutions were transferred into 100 mL dosing jars and stored in the dark at room temperature.

The EE, DES-DP, and EE plus ICI 182,780 dose solutions for the Sprague-Dawley and Fischer 344 rats were prepared separately. One set of dosing solutions was prepared for the Sprague-Dawley rats, and a second set was prepared for the Fischer 344 rats. Only one set of dosing solutions was prepared for the CE groups as the Fischer 344 rats did not receive CE.

The required amount of EE or both EE plus ICI was weighed and transferred into a volumetric flask. Approximately 250 mL of vehicle was added to the flask and dissolved with a magnetic stir bar and stir plate. The stir bar was removed, and additional vehicle was added to yield 500 mL of prepared stock solution. The flask was shaken thoroughly and the contents stirred with a magnetic stir bar and stir plate prior to dispensation. This stock solution was used for the preparation of EE Groups 8-11 and 35-38, and EE + ICI Groups 24-27 and 47-50. The EE groups were prepared by transferring the required volume of EE stock solution with a syringe to a graduated cylinder and adding vehicle to yield 18 mL of prepared dosing material. The cylinder was shaken thoroughly and stirred with a magnetic stir bar prior to dispensation. The EE plus ICI groups (24-27 and 47-50) were prepared by grinding ICI using an agate mortar and pestle. The appropriate amount of ICI was then weighed into a graduated cylinder and approximately 9 mL of vehicle was added. The required amount of EE stock solution was added to the cylinder with a syringe and stirred with a magnetic stir bar until dissolved. Additional vehicle was added to yield 18 mL of prepared dosing material. The cylinder was thoroughly shaken and stirred with a magnetic stir bar prior to dispensation. The suspension was transferred into amber glass containers and stored at room temperature.

The required amount of DES-DP was weighed and transferred to a volumetric flask. Approximately 500 mL of vehicle was added to the flask, and the contents were dissolved using a magnetic stir bar and stir plate. The stir bar was removed and additional vehicle was added to yield 1000 mL of prepared stock solution. The flask was thoroughly shaken and the contents stirred with a magnetic stir bar and stir plate prior to dispensation. This stock solution was used to prepare Groups 12-15 and 39-42. The required amount of the DES-DP stock solution was measured with a syringe and transferred to a volumetric flask. Additional vehicle was added to the flask to yield 50 mL of prepared dosing material. The flask was shaken thoroughly and the contents stirred with a magnetic stir bar prior to dispensation. The contents were transferred to an amber glass container using a syringe and stored at room temperature.

The CE groups (16-19) were prepared by grinding the CE using an agate mortar and pestle. The appropriate amount of CE was then weighed into a graduated cylinder, approximately 7 mL of vehicle was added, and the contents were suspended with a magnetic stir bar and stir plate. Additional vehicle was added to yield 15 mL of prepared stock suspension. The cylinder was shaken thoroughly and stirred with a magnetic stir bar and stir plate prior to dispensation. The contents were transferred into amber glass containers using a syringe and stored at room temperature.

#### 4.2.5. Analysis of Dosing Preparations

##### 4.2.5.1. Vehicle Control and Test Article Analysis

The vehicle control (0 mg/kg/day) dose preparation was analyzed for D4 concentration only according to the procedures outlined below.

Samples of D4 solutions and D4 solutions coformulated with EE were collected and analyzed by the Sponsor prior to distribution to MPI Research. The concentration of each dose solution

was verified by collecting a single aliquot from the 10, 20, 50, and 100 mg D4/mL solutions. Homogeneity and verification of D4 concentration were determined for the 2 and 200 mg D4/mL solutions by collecting 3 aliquots, one each from the top, middle, and bottom regions of the final dose preparations. A single aliquot was collected from the vehicle control and the concentration of D4 was measured. A single aliquot was collected from each dose solution containing D4 coformulated with EE to verify D4 concentration. Homogeneity was determined from the low and high concentration D4 dosing solutions and from the mixture D4 500 mg/kg/day and 1 µg/kg/day of EE (Ethinylestradiol). Retention samples of sesame oil, DES-DP, EE, CE, and ICI were taken and have been archived at MPI Research.

Following receipt of dose solutions by MPI Research, an additional set of samples was collected and analyzed. For this analysis, single aliquots were collected from each dose solution containing D4 on July 2, 1998 and sent to the Sponsor for analysis.

The analysis of D4 in sesame oil was done according to methods established at Dow Corning under study number 9039. Information on the concentration, homogeneity, and stability of D4 (up to 33 days) in sesame oil was provided to MPI Research as a contributing scientist report. The contributing scientist report is included as Appendix M.

#### **4.2.5.2. Positive Control and Antiestrogen Article Analysis**

Analysis of the positive control and antiestrogen dose preparations was not conducted.

#### **4.2.6. Test Article, Vehicle Control, Positive Control, and Antiestrogen Administration**

The dosing preparations were administered by oral gavage once per day for 4 consecutive days at 18, 19, 20, and 21 days of age for Sprague-Dawley rats and at 21, 22, 23, and 24 days of age for Fischer 344 rats using a 5.0 French polyurethane umbilical vessel catheter placed over the base of a 19-gauge, blunt hypodermic needle attached to a 1 cc syringe. The oral route represents one potential route of human exposure to D4. The test material was stirred continuously during administration. D4 was administered at dose levels of 0 (sesame oil vehicle control), 10, 50, 100, 250, 500, and 1000 mg/kg/day. EE was administered at dose levels of 1, 3, 10, and 30 µg/kg/day. DES-DP was administered at dose levels of 0.5, 1.5, 5, and 15 µg/kg/day. Coumestrol was administered at dose levels of 10, 35, 75, and 150 mg/kg/day. D4 plus EE was administered at dose levels of 500 mg/kg/day D4 plus 1, 3, 10, or 30 µg/kg/day EE. ICI 182,780 plus EE was administered at dose levels of 3 mg/kg/day ICI 182,780 plus 1, 3, 10, or 30 µg/kg/day EE. Each dosage level was administered separately to the 2 strains of rats placed on study, with the exception of Coumestrol, which was not administered to the Fischer 344 rats. The dosing preparations were administered in sesame oil at a constant volume of 5 mL/kg/day. The control animals received the vehicle, sesame oil, at the same dose volume. Individual dosages were based on the most recent body weights and were rounded to the nearest 0.01 mL.

The dose levels for EE, DES-DP, CE and ICI were selected based on previously reported dose-response data or effect levels (Odum et al., 1997), historical control data or previous unpublished data. The dose levels of D4 were selected by the Sponsor based on previous unpublished data.

#### 4.3. Observations

##### 4.3.1. Cageside and Detailed Observations

All rats were observed at least twice a day, 7 days a week, for morbidity, mortality, and signs of injury. Each pup was removed from the cage and given a detailed clinical examination on the following schedules: 1) prior to dosing and approximately 1 hour after dosing at 18, 19, 20, and 21 days of age and at 22 days of age prior to necropsy for Sprague-Dawley rats or 2) prior to dosing and approximately 1 hour after dosing at 21, 22, 23, and 24 days of age and at 25 days of age prior to necropsy for Fischer 344 rats. The examination included, but was not limited to, observations of the general condition, skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration. Palpation for masses was not conducted. Modifiers were included in the description of the clinical sign when necessary to describe the location, size, shape, color, or other characteristics.

##### 4.3.2. Body Weights

Individual body weights were recorded at 18, 19, 20, 21, and 22 days of age for Sprague-Dawley rats and at 21, 22, 23, 24, and 25 days of age for Fischer 344 rats. The body weights were collected prior to dosing during the first 4 days of the study and prior to necropsy on the last day of the study for each rat.

#### 4.4. Anatomic Pathology

A necropsy examination was performed by trained personnel under the supervision of a veterinary pathologist on all animals dying spontaneously, euthanized *in extremis*, or euthanized at the scheduled necropsy. At the termination of the study period at 22 days of age for Sprague-Dawley rats and 25 days of age for Fischer 344 rats, all surviving animals were euthanized and examined. Euthanasia was by carbon dioxide inhalation followed by exsanguination from the abdominal aorta. The animals were carefully examined for external abnormalities. The uterus and ovaries were removed together, caudal to the cervix. Each uterus from pups that survived to the scheduled necropsy at 22 (Sprague-Dawley) or 25 (Fischer 344) days of age was weighed after removal of the connecting adipose tissue and ovaries. Care was taken during the necropsy process to ensure that any fluid present in the uterus was not affected prior to weighing. Uterine weights were not taken on pups that were found dead or euthanized *in extremis*. After removal and weighing of the uterus, the liver was removed and weighed. The left ovary, left uterine horn with cervix and gross lesions from the first 6 pups/group necropsied were fixed in neutral, buffered 10% formalin and processed to paraffin blocks. The tissues were placed in the formalin for 24-48 (maximum) hours and then transferred to 70% ethyl alcohol for storage until further processing to paraffin blocks. The left

ovary, left uterine horn with cervix and gross lesions from the last 6 pups/group necropsied were fixed in Bouin's fixative and processed to paraffin blocks. The tissues were placed in the Bouin's fixative for 12-24 (maximum) hours and then rinsed for 15 minutes with tap water and placed in 50% ethyl alcohol for 24 hours. The 50% ethyl alcohol solution was changed once during the 24 hours. The tissues were then transferred to 70% ethyl alcohol for storage until further processing to paraffin blocks. The 70% ethyl alcohol solution was changed once prior to storage and processing. The right ovary, liver and right uterine horn without cervix from all rats were wrapped in aluminum foil and flash frozen in liquid nitrogen. The right ovary, liver and right uterine horn without cervix were frozen at  $-80 \pm 20^{\circ}\text{C}$  and sent to the Sponsor for possible future analysis.

The left uterine horn from the last 6 pups (Bouin's fixed tissues) in treatment groups 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, and 38 were processed to slides, stained with hematoxylin and eosin and microscopically evaluated for epithelial cell height of the endometrial surface lining (luminal surface). Using a 40x microscope objective and an eyepiece micrometer that had been calibrated with a stage micrometer (1 eyepiece unit =  $2 \mu\text{m}$ ) (Foreyt, 1989), the epithelial cell height was measured in 3 randomly selected microscope fields of the uterine endometrial surface lining. An average cell height of these 3 fields was then calculated for each animal. From these means, a group mean was calculated and analyzed. The uteri in the control groups (Groups 1 and 28) were evaluated first. Following evaluation of the control animals, the uteri in the remaining groups were evaluated blind to treatment.

**4.5. Statistical Analysis**

Statistical procedures were conducted using SAS<sup>®</sup> software, version 6.12 (SAS Institute Inc., Cary, NC).

**4.5.1. Potency Determinations - Uterine Weight**

The following is the statistical analysis plan used for determination of relative estrogenic potency between the materials evaluated. The analysis plan described below was conducted separately for each strain of rat. The table on the following page defines the sets of comparisons used in the statistical analyses. If more than one set of comparisons was required, all analyses were conducted separately on each set unless stated otherwise.

Statistical Comparisons	
Control Curve	Test Curves
Ethinyl Estradiol (EE)	Diethylstilbesterol dipropionate (DES-DP) Coumestrol (CE) D4 Only D4 + EE ICI 182,780 + EE
Diethylstilbesterol dipropionate (DES-DP)	Coumestrol (CE) D4 Only

The relationship between uterine weight vs. log dose was assessed by a linear regression (least square method) to estimate the slope and y intercept for each positive control and test response curve. Relative potency was calculated for each test group identified in the above table with respect to the indicated positive control group. The relative potency is defined as the ratio of the amounts of the two different test substances required to give the same response. A comparative assay method (Cornfield, 1964) was used for comparisons and estimates of potencies. If the two response curves were parallel, then the relative potency at 50% of the maximal uterine growth response for the positive controls was used. If the response curves were not parallel, 20%, 50%, and 80% responses of the positive controls were used. For the groups coadministered EE and D4 or EE and ICI, the dose used for the test agent in the potency calculation was the dose of EE coadministered with D4 or ICI that produced the appropriate response.

#### 4.5.2. Body Weights, Body Weight Change, Liver Weight, Uterine Weight and Uterine Epithelial Cell Height

The following table is the statistical analysis plan used for body weight, body weight change, liver weight, uterine weight (absolute and relative), uterine cell height. The table below defines the set(s) of comparisons that were used in the statistical analyses described below.

Statistical Comparisons	
Control Group	Treatment Groups
1	2,3,4,5,6,7
28	29,30,31,32,33,34

##### 4.5.2.1. Group Pair-Wise Comparisons

For body weight, body weight change, uterine weight (absolute and relative to body weight), uterine epithelial cell height, and liver weight (absolute and relative), Levene's test (Milliken and Johnson, 1992) was used to assess the homogeneity of group variances. If Levene's test was not significant ( $p \geq 0.01$ ), Dunnett's test (Dunnett, 1964) was used to compare each treatment group with the control group. If Levene's test was significant ( $p < 0.01$ ), comparisons with the control group were made using a Welch's t-test with a Bonferroni correction. Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels.

#### 4.6. Data and Specimen Retention

All raw data, documentation, records, protocol, reserve samples, dosing solutions, specimens (slides, blocks, and wet tissues), and the final report (copy) generated by MPI Research as a result of this study will be retained in MPI Research Archives for a period of 1 year following completion of the study (final report issue date). Retention of materials after the times stated above will be subject to future contractual agreements between the Sponsor and MPI Research. Raw data and records generated by Dow Corning will be retained at Dow Corning HES Archives. Copies of Dow Corning information was provided to MPI Research as required for reporting purposes.

## 5. RESULTS

### 5.1. Antemortem Observations

#### 5.1.1. Detailed Observations

Clinical observations for Sprague-Dawley rats are summarized in Table 1 and presented individually in Appendix B. Clinical observations for Fischer 344 rats are summarized in Table 2 and presented individually in Appendix C.

Two Sprague-Dawley rats, one at 3 µg EE/kg/day and one at 500 mg/kg/day D4 plus 3 µg EE/kg/day died on test. One Fischer 344 rat given 1000 mg/kg/day D4 was euthanized *in extremis*. The deaths or moribund conditions of these rats were not considered related to treatment. All other animals survived to the scheduled necropsy. No significant treatment-related effects were observed on clinical observations in any test group in either Sprague-Dawley or Fischer 344 rats. All observations, including the slight transitory increase in the incidence of material around the nose observed in the Fischer 344 rats administered 1000 mg/kg/day D4, were attributed to the combined stress of dosing and removal of the pups from the dam and their litter mates.

#### 5.1.2. Body Weights

Body weights for Sprague-Dawley rats are summarized in Table 3 and presented individually in Appendix D. Body weight changes for Sprague-Dawley rats are summarized in Table 4. Body weights for Fischer 344 rats are summarized in Table 5 and presented individually in Appendix E. Body weight changes for Fischer 344 rats are summarized in Table 6.

Statistical analyses were only performed on the animals that received D4 alone. Body weights in Sprague-Dawley rats given 1000 mg/kg/day D4 were decreased on Days 21 and 22, with the decrease on Day 21 being statistically significant. The body weight change in Sprague-Dawley rats was significantly decreased during Days 19-20 at 250 mg/kg/day D4, Days 19-20 and 18-22 at 500 mg/kg/day D4 and Days 19-20, 20-21, and 18-22 at 1000 mg/kg/day D4. The decreases in body weight change observed in the D4 dose groups occurred in a dose-related manner and were considered treatment related. Although not statistically compared, the body weight gain in Sprague-Dawley rats given 500 mg/kg/day D4 plus 1-30 µg EE/kg/day were also decreased compared to the concurrent control or EE only groups, further supporting an effect of D4 on body weight.

In Fischer 344 rats, significant decreases in body weight were observed on Days 23-25 in animals given 1000 mg/kg/day D4. The body weight change in Fischer 344 rats was also significantly decreased during Days 21-22 and 21-25 at 250 mg/kg/day D4, Days 22-23 and 21-25 at 500 mg/kg/day D4 and Days 21-22, 22-23, and 21-25 at 1000 mg/kg/day D4. Similar to that observed in Sprague-Dawley rats, the body weight change in Fischer 344 rats given 500 mg/kg/day D4 plus 1-30 µg EE/kg/day were decreased compared to the concurrent control or EE only groups.

## 5.2. Postmortem Observations

### 5.2.1. Necropsy Observations

Necropsy observations for Sprague-Dawley rats are summarized in Tables 7 and 9 and presented individually in Appendix F. Necropsy observations for Fischer 344 rats are summarized in Tables 8 and 10 and presented individually in Appendix G.

Uterine distention (fluid) was noted in only 1 animal of each strain in the 1000 mg/kg/day D4 dose group. Uterine distention also occurred in rats dosed at 3 µg/kg/day EE and higher; at 5 µg/kg/day DES-DP and higher; at 75 mg/kg/day CE and higher; and at 500 mg/kg/day D4 plus 30 µg/kg/day EE. Uterine fluid accumulation (imbibition) and uterine tissue growth are both well established responses to system estrogen exposure in rodents (Ree et al., 1996; Astwood, 1938). Therefore, the uterine distention observed in this study was attributed to the estrogenic effects of D4, EE, DES-DP or CE.

The only additional macroscopic observations were microphthalmia of the eye in one Fischer 344 rat and a small number of ovarian cysts noted in Fischer 344 rats. These changes were not dose related and were considered incidental findings.

### 5.2.2. Organ Weights

Organ weights for Sprague-Dawley rats are summarized in Table 11 and presented individually in Appendix H. Liver and uterine weights for Fischer 344 rats are summarized in Table 12 and presented individually in Appendix I.

Statistical analyses were only performed on the animals that received D4 alone. Uterine weights (both mean absolute and mean relative to body weight) were significantly increased compared to controls at 250 mg/kg/day D4 and higher in both strains of rat.

Dose-related increases in uterine weights occurred in all groups dosed with EE, DES-DP, CE (Sprague-Dawley only), and D4+EE. The uterine weight increases in the D4 plus EE groups in both the Sprague-Dawley and Fischer 344 rats were consistently lower than the weight increases observed at corresponding doses of EE alone. This relationship is supported by the graphical data (Figures 1 and 2) and relative potency calculations (Table 15) and indicates that D4 acting as a weak estrogen has antiestrogenic properties when coadministered with a potent estrogen. ICI administered at a dose level of 3 mg/kg/day virtually completely blocked the effects of EE at all doses of EE in both strains of rat, demonstrating the known antiestrogenic effects of ICI. The changes in uterine weight observed with EE, DES-DP, CE and ICI were consistent with previously published results (Odum et al., 1997).

Liver weights (both mean absolute and mean relative to body weight) were significantly increased at 100 mg/kg/day D4 and higher in the Fischer 344 rat. In the Sprague-Dawley rat,

mean relative liver weight was significantly increased at 250 mg/kg/day D4 and both mean absolute and mean relative liver weights were increased at 500 and 1000 mg/kg/day D4.

Liver weights were also increased in those rats receiving 500 mg/kg/day D4+EE. No other changes in liver weights were noted.

### 5.2.3. Microscopic Pathology

Epithelial cell field measurements for Sprague-Dawley and Fischer 344 rats are summarized in Tables 13 and 14, respectively, and presented individually in Appendix J.

In immature or ovariectomized adult female rats, very low estrogen levels are present. Under these conditions, the uterine epithelium is relatively inactive and consists of cuboidal cells. In the presence of estrogen, the uterine epithelium proliferates and changes from cuboidal to columnar-shaped cells (Branham et al., 1998; O'Connor et al., 1996).

Uterine epithelial cell height was increased in a dose-related manner in both strains of rats at 250 mg/kg/day D4 and higher, however, only the 500 and 1000 mg/kg/day D4 dose groups in Sprague-Dawley rats and 250 and 500 mg/kg/day D4 dose groups in the Fischer 344 rats were statistically significant. The lack of statistical significance at 250 mg/kg/day D4 in Sprague-Dawley rats and at 1000 mg/kg/day D4 in Fischer 344 rats was due to the high variability in cell height in these groups.

Uterine epithelial cell height was also increased (not statistically compared) at all dosage levels of EE in both strains of rats. The increased epithelial cell height at  $\geq 250$  mg/kg/day D4 and at  $\geq 1$   $\mu$ g/kg/day EE was consistent with the previously discussed increased uterine weights in these dose groups and was attributed to treatment.

### 5.2.4. Potency Determinations - Uterine Weight

The mean uterine weight values vs. log of dose (dose response curves) are plotted in Figure 2. The calculated regression lines for these data are presented in Figure 1. Potency calculations are presented in Table 15.

The uterine weight dose-response curves for Sprague-Dawley EE vs. CE, Sprague-Dawley EE vs. DES-DP and Fischer 344 EE vs. D4 plus EE were determined to be parallel lines (slopes not different). Therefore, for these potency comparisons, the relative potency at 50% of the maximal uterine growth response of EE was calculated. For all other potency calculations, the relative potency at 20, 50, and 80% of the maximal EE or DES-DP response was calculated, if possible. EE and DES-DP proved to be potent estrogens of roughly equal potency in both Sprague-Dawley and Fischer 344 rats. CE, a phytoestrogen known to be weakly estrogenic, was approximately 18,000 (1/0.000055) and 16,000 (1/0.000061) times less potent than EE or DES-DP, respectively, in Sprague-Dawley rats. CE was not tested in Fischer 344 rats due to inadequate supplies of this material. ICI, a strong antiestrogen, (Odum et al., 1997)

significantly blocked the response of EE at all doses of E. As a result of the inhibitory effect of ICI, the potency ratios at 50 and 80% of effect could not be estimated and the potency ratios at 20% of effect were highly variable.

D4 was found to be weakly estrogenic in both species of rat. When compared to EE, D4 was approximately 77,000 (1/0.000013) to 1,500,000 (1/0.00000066) times less potent in Sprague-Dawley rats and 143,000 (1/0.000007) to 25,000,000 (1/0.00000004) times less potent in the Fischer 344 rat. When compared to DES-DP, D4 was approximately 48,000 (1/0.000021) to 1,200,000 (1/0.00000082) times less potent in Sprague-Dawley rats and 7,000,000 (1/0.00000014) times less potent in Fischer 344 rats. When compared to CE, D4 was 83 times less potent in Sprague-Dawley rats. Due to the weak response of D4, potency ratios at 80% of the EE or DES-DP response could not be determined.

When coadministered with a range of doses of EE, 500 mg/kg/day D4 decreased the uterotrophic response of EE approximately one-half to one order of magnitude (50% potency ratios were 0.4656 in Sprague-Dawley and 0.1164 in Fischer 344 rats), demonstrating weak antiestrogenic properties. These results are consistent with the weak estrogenic properties of D4 and may result from competitive inhibition of estrogen receptor binding or be consistent with D4 acting as a partial estrogen agonist.

## 6. CONCLUSION

No treatment-related effects were observed on mortality or clinical signs in Sprague-Dawley or Fischer 344 rats administered D4 by oral gavage at dose levels as high as 1000 mg/kg/day. Body weight gain was significantly decreased in a dose related manner in both strains of rats given 250, 500, or 1000 mg/kg/day D4. Absolute and relative liver weight were significantly increased at 100 mg/kg/day D4 and higher in the Fischer 344 rat. Relative liver weight was significantly higher at 250 mg/kg/day D4 and absolute and relative liver weight were significantly higher at 500 and 1000 mg/kg/day D4 in the Sprague-Dawley rat. Uterine weight (both absolute and relative) were significantly increased at 250 mg/kg/day D4 and higher in both strains of rats. The increased uterine weight was accompanied by a slight increase (1/10) in uterine distension at 1000 mg/kg/day D4 in both strains of rats and increased uterine and epithelial cell height at 250 mg/kg/day D4 and higher in both strains of rats. At the 50% of maximal response level for EE and DES-DP, D4 was approximately 1.2 to 25 million times less potent than EE or DES-DP, as measured by its ability to produce increases in uterine weight. D4 administration also did not produce the same magnitude (maximum effect) of uterine weight increases as either EE or DES-DP. When 500 mg/kg/day D4 was coadministered with a range of doses of EE, D4 decreased the uterotrophic dose response of EE by approximately one-half (Sprague-Dawley) to one (Fischer 344) order of magnitude. This inhibitory effect indicates that D4 has weak antiestrogenic properties.

The positive controls, EE, DES-DP and CE produce dose-related increases in uterine weight and demonstrated their strong (EE and DES-DP) or weak (CE) estrogenic potential. The negative control ICI virtually completely inhibited the uterotrophic response of EE when coadministered with EE.

In conclusion, under the conditions of this study, oral administration of D4 to intact immature Sprague-Dawley or Fischer 344 rats for 4 consecutive days at dose levels of 250, 500, or 1000 mg/kg/day was weakly estrogenic as measured by dose-related increases in uterine weight and increases in epithelial cell height. D4 was found to be weakly estrogenic in both species of rat. When compared to EE, D4 was approximately 77,000 (1/0.000013) to 1,500,000 (1/0.0000066) times less potent in Sprague-Dawley rats and 143,000 (1/0.000007) to 25,000,000 (1/0.0000004) times less potent in the Fischer 344 rat. When compared to DES-DP, D4 was approximately 48,000 (1/0.000021) to 1,200,000 (1/0.0000082) times less potent in Sprague-Dawley rats and 7,000,000 (1/0.0000014) times less potent in Fischer 344 rats. Due to the weak response of D4, potency ratios at 80% of the EE or DES-DP response could not be determined. At the 50% of maximal response level for EE and DES-DP, D4 was approximately 1.2 to 25 million times less potent than EE or DES-DP in Sprague-Dawley and Fischer 344 rats, respectively, and 83 times less potent than CE in Sprague-Dawley rats. The estrogenic response of D4 occurred at dose levels that also produced decreases in body weight or body weight change and increases in liver weight. D4 also exhibited weak antiestrogenic properties by partially blocking EE induced uterine weight increases. These results are consistent with the weak estrogenic properties of D4 and may result from competitive inhibition of estrogen receptor binding or be consistent with D4 acting as a partial estrogen agonist.

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## GLOSSARY

**Cyst** - Any closed cavity or sac; usually contains a liquid or semi-solid material and is often lined by epithelium.

**Distended** - To become expanded or enlarged from internal pressure of a solid, liquid, or gaseous substance as in the lumen of an organ, usually making the walls stretched or thinned.

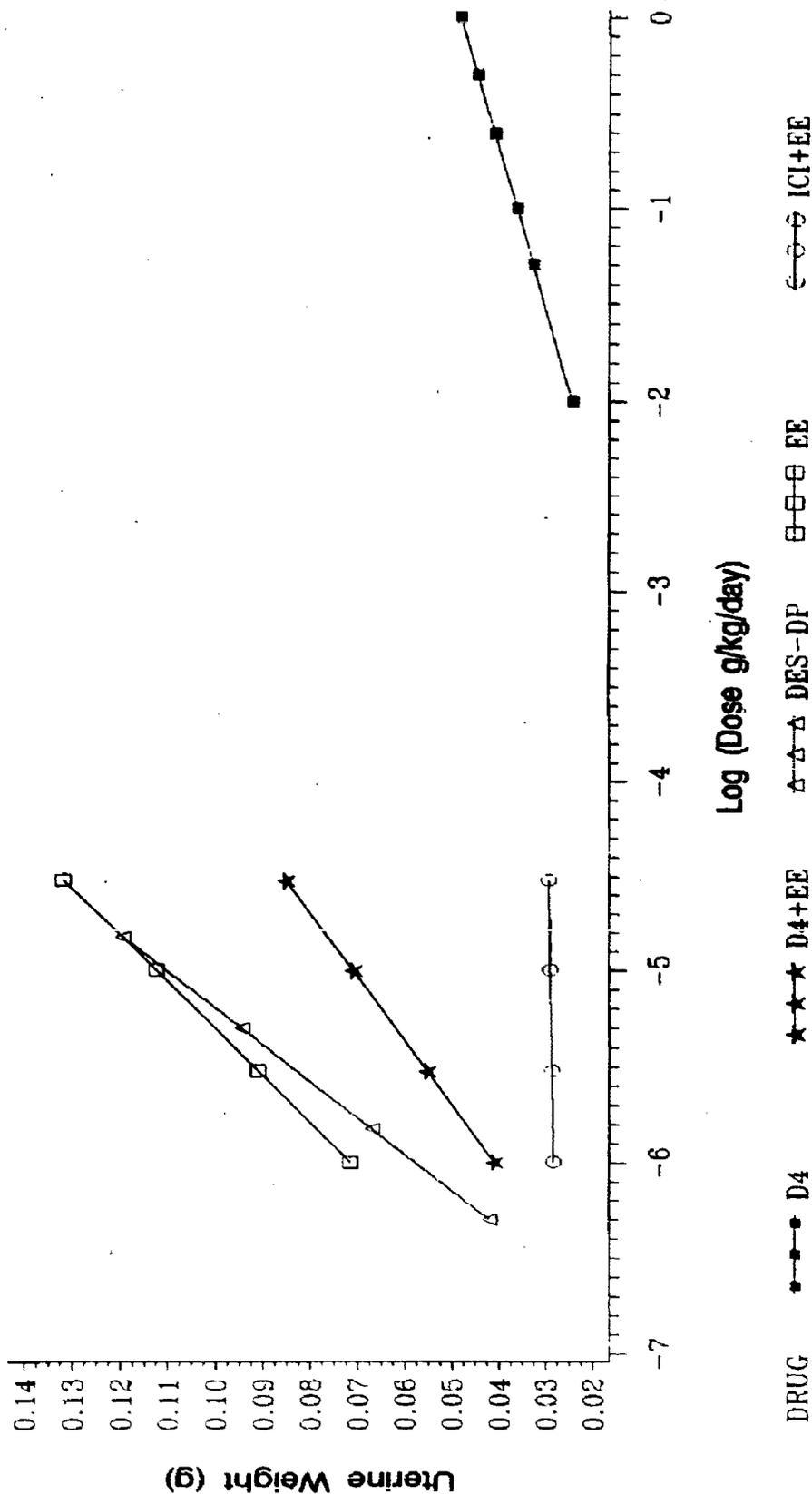
**Microphthalmia** - Congenital small eye.

**Within normal limits** - Tissue considered to be normal, under the conditions of the study and considering the age, sex and strain of the animal concerned.

Alterations may be present which, under other circumstances, would be considered deviations from normal.

Figure 1. Plot of Regression Lines

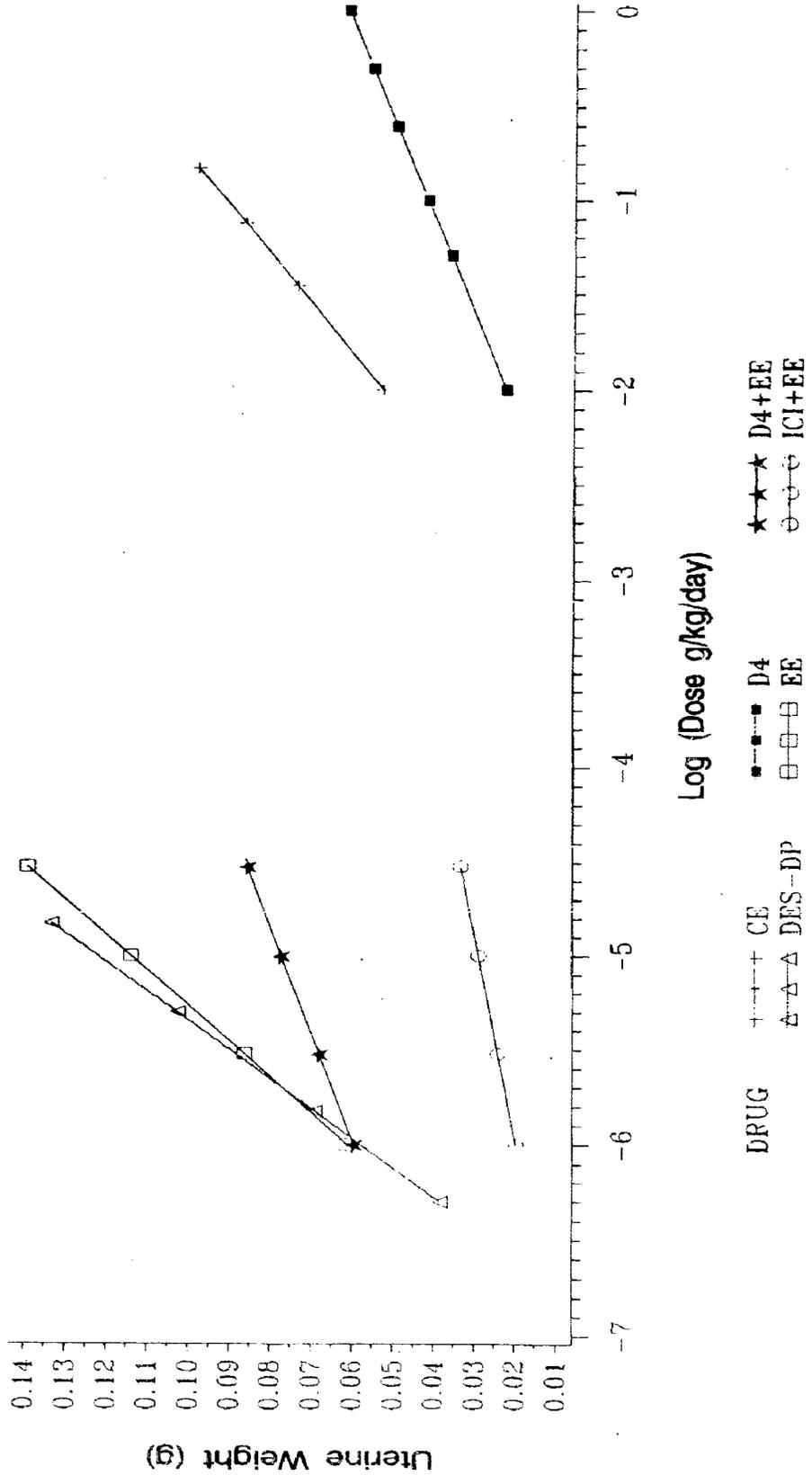
Study Number: 416-148  
STRAIN = FISCHER-344



\*

Figure 1 Cont. Plot of Regression Lines

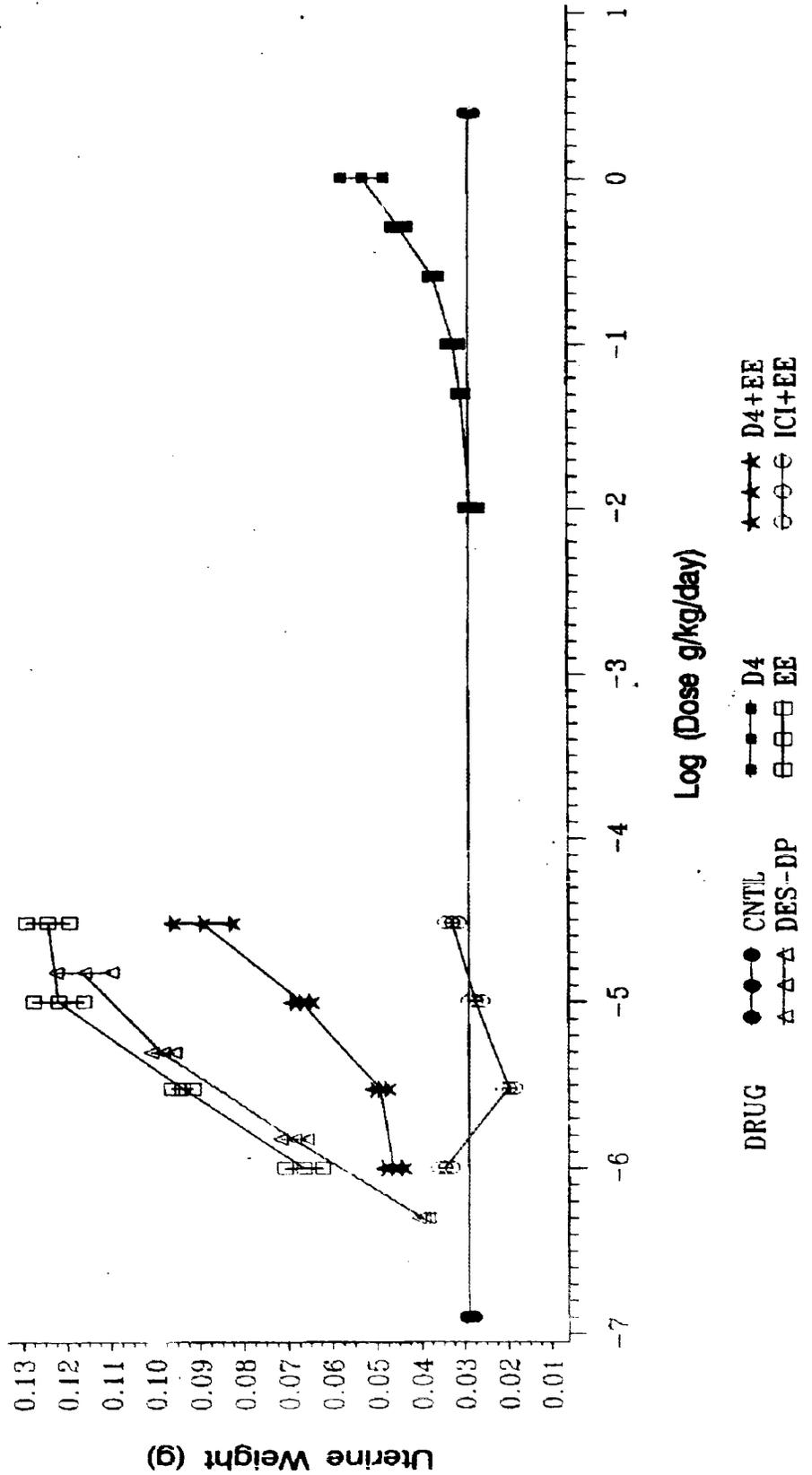
Study Number: 416-148  
STRAIN = SPRAGUE - DAWLEY



\*

Figure 2. Plot of Uterine Weight

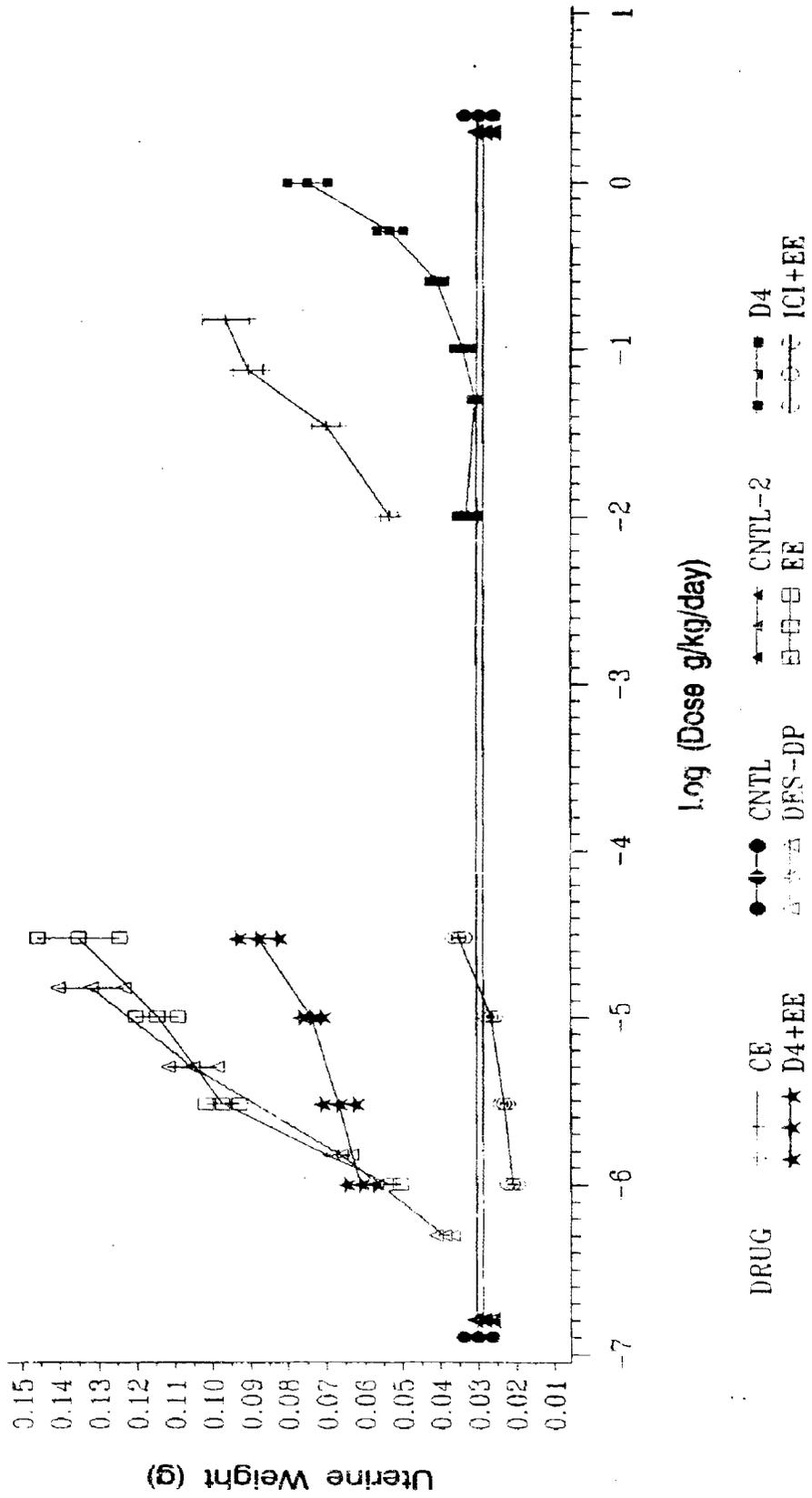
Study Number: 416-148  
STRAIN = FISCHER-344



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Figure 2 Cont. Plot of Uterine Weight

Study Number: 416-148  
STRAIN - SPRAGUE - DAWLEY



Summary of Detailed Clinical Examinations - Prior to Dosing  
Sprague-Dawley Rats

Table 1 Clinical Sign	Days	Groups			
		0 (Vehicle Control)	10 mg/kg/day D4	50 mg/kg/day D4	100 mg/kg/day D4
Total number of animals available		12	12	12	12
Normal/no visible abnormalities	18	12	11	12	12
	19	12	12	12	12
	20	12	12	12	10
	21	12	12	11	11
	22	12	12	11	11
Material around nose	18		1		
	19				
	20				
	21				
	22				
Anogenital staining	18				
	19				
	20				2
	21			1	1
	22			1	1



Summary of Detailed Clinical Examinations - Prior to Dosing  
 Sprague-Dawley Rats

Table 1 Cont.

Clinical Sign	Days			Groups		
	250 mg/kg day D4	500 mg/kg/day D4	1000 mg/kg/day D4	250 mg/kg day D4	500 mg/kg/day D4	1000 mg/kg/day D4
Total number of animals available	12	12	12	12	12	12
Normal/no visible abnormalities	18	12	12	12	12	12
	19	12	11	11	11	11
	20	12	12	12	9	9
	21	12	11	11	10	10
	22	12	9	9	11	11
Material around nose	18					
	19		1			1
	20					1
	21					
	22					
Anogenital staining	18					
	19					
	20					1
	21			1		1
	22			3		1

Summary of Detailed Clinical Examinations - Prior to Dosing  
 Sprague-Dawley Rats

Table 1 Cont. Clinical Sign	Days	Groups		
		250 mg/kg day D4	500 mg/kg/day D4	1000 mg/kg/day D4
Dehydrated appearance	18			
	19			
	20			1
	21			1
	22			
High carriage	18			
	19			
	20			
	21			1
	22			



Summary of Detailed Clinical Examinations - Prior to Dosing  
Sprague-Dawley Rats

Clinical Sign	Days	Groups			
		1 µg/kg/day EE	3 µg/kg/day EE	10 µg/kg/day EE	30 µg/kg/day EE
Total number of animals available		12	12	12	12
Normal/no visible abnormalities	18	12	12	12	12
	19	12	12	12	11
	20	11	12	12	12
	21	12	11	12	12
	22	11	11	12	12
Anogenital staining	18				
	19				1
	20	1			
	21		1		
	22	1			
Found dead	18				
	19				
	20				
	21				
	22		1		

Summary of Detailed Clinical Examinations - Prior to Dosing  
Sprague-Dawley Rats

Clinical Sign	Days	Groups			
		0.5 µg/kg/day DES-DP	1.5 µg/kg/day DES-DP	5.0 µg/kg/day DES-DP	15.0 µg/kg/day DES-DP
Total number of animals available		12	12	12	12
Normal/no visible abnormalities	18	12	12	12	11
	19	12	12	12	12
	20	11	11	12	11
	21	11	12	11	12
	22	11	12	12	12
Material around nose	18				
	19				
	20	1			
	21	1			
	22				
Anogenital staining	18				1
	19				
	20		1		
	21			1	
	22	1			



Summary of Detailed Clinical Examinations - Prior to Dosing  
 Sprague-Dawley Rats

Clinical Sign	Days					Groups
	10 mg/kg/day CE	35 mg/kg/day CE	75 mg/kg/day CE	150 mg/kg/day CE	150 mg/kg/day CE	
Total number of animals available	12	12	12	12	12	12
Normal/no visible abnormalities	18	12	12	12	12	12
	19	12	12	12	12	11
	20	12	12	12	12	11
	21	12	12	12	12	11
	22	12	12	12	12	11
Anogenital staining	18					1
	19					1
	20					1
	21					1
	22					1