Potential Estrogenic and Antiestrogenic Activity of the Cyclic Siloxane Octamethylcyclotetrasiloxane (D4) and the Linear Siloxane Hexamethyldisiloxane (HMDS) in Immature Rats Using the Uterotrophic Assay

James M. McKim, Jr.,* 1  Paul C. Wilga,*  William J. Breslin,† Kathy P. Plotzke,* Robert H. Gallavan,* and Robert G. Meeks*  

*Dow Corning Corporation, Health and Environmental Sciences, 2200 W. Salzburg Road, Midland, Michigan 48686–0994;  and †MPI Research, LLC, Mattawan, Michigan  

Received January 26, 2001; accepted May 29, 2001  

The cyclic siloxane octamethylcyclotetrasiloxane (D4) and the linear siloxane hexamethyldisiloxane (HMDS) have numerous industrial and consumer applications and thus have the potential for human exposure. The present study was undertaken to examine potential estrogenic and antiestrogenic activities of D4 and HMDS. To address potential differences in sensitivity between rat strains the study used both Sprague-Dawley (SD) and Fischer 344 (F-344) rats. Estrogenicity of the test compounds was determined by measuring absolute and relative uterine weights in immature rats and by monitoring uterine epithelial cell height. In order to place the data obtained for D4 into perspective relative to strong and weak estrogenic compounds, the response produced by D4 at 0, 10, 50, 100, 250, 500, and 1000 mg/kg/day was compared to responses produced by ethinyl estradiol (EE) (1, 3, 10, or 30 μg/kg/day), diethylstilbestrol dipropionate (DES-DP) (0.5, 1.5, 5, 15 μg/kg/day), and coumestrol (CE) (10, 35, 75, 150 mg/kg/day). Antiestrogenic effects were evaluated by co-administering D4 (500 mg/kg/day) with EE at 1, 3, 10, and 30 μg/kg/day. All compounds were administered in sesame oil at a volume of 5 mL/kg by oral gavage. Beginning on postnatal day 18 (SD) or 21 (F-344) each pup (12 per group) received a single dose of test compound once a day for 4 consecutive days. The pups were euthanized the morning after the last treatment and their uteri removed, weighed, and processed for histological examination. EE and DES-DP produced a significant dose-dependent increase in absolute and relative uterine weights and uterine epithelial cell height. The maximum increase produced by D4 in F-344 rats was 86%. D4 co-administered over a wide range of EE doses, resulted in a significant reduction in uterine weight compared to EE alone. HMDS was evaluated in SD rats only. The response produced by HMDS (600 and 1200 mg/kg/day) was compared to EE (3 μg/kg/day). Antiestrogenic effects were evaluated by co-administering HMDS (1200 mg/kg/day) with EE at 3 μg/kg/day. HMDS had no measurable effect on uterine weight under the experimental conditions described here. However, HMDS coadministered with EE did produce a small, but statistically significant reduction in uterine weight compared to EE alone. In conclusion, D4 showed weak estrogenic and antiestrogenic activity that was several orders of magnitude less potent than EE, and many times less potent than the weak phytoestrogen CE.  

Key Words: octamethylcyclotetrasiloxane (D4); hexamethyldisiloxane (HMDS); estrogenic activity; antiestrogenic activity; ethinyl estradiol; diethylstilbestrol dipropionate; coumestrol.

1  To whom correspondence should be addressed at Pharmacia Corporation, 333 Portage Road, Building 300–423, Kalamazoo, MI 49009. Fax: (616) 833-7722. E-mail: james.m.mckim@am.pnu.com.

The potential for xenobiotics to effect mammalian reproduction by mimicking or inhibiting endogenous estrogen activity has been the subject of several scientific studies in recent years. As a result of this heightened awareness, several chemical and pharmaceutical companies have started to evaluate potential estrogenic activity of compounds with a high probability of human environmental exposure. Octamethylcyclotetrasiloxane (D4) is a low molecular weight (296 Da) synthetically derived silicone fluid that is clear and odorless. D4 consists of alternating silicon-oxygen bonds connected in a ring (cyclic) ar-
arrangement with 2 methyl groups covalently bonded to each silicon atom (-(CH₃)₂SiO--; Fig. 1). Hexamethyldisiloxane (HMDS) is a low molecular weight (162 Da) linear siloxane consisting of a silicone–oxygen–silicone bond with 3 methyl groups covalently bonded to each silicone atom (CH₃)₃-Si-O-Si-(CH₃)₃ (Fig. 1). The primary use of D4 and HMDS is as intermediates in the manufacturing of high molecular weight silicone polymers. A secondary use of D4 and HMDS is as a vehicle or ingredient in consumer and precision cleaning products. Because of these uses and potential human exposure, studies have been initiated to examine the biological fate and effects of these siloxanes.

In rodents, inhalation of D4 produces a dose-related hepatomegaly, transient hepatic hyperplasia, hypertrophy, and induction of cytochrome P450 enzymes in a manner similar to phenobarbital (McKim et al., 1998, 2001). This pattern of induction was also observed following oral exposure to D4 (Zhang et al., 2000). Although several hepatic enzymes are affected by D4 exposure, CYP2B1/2 enzymes are induced to the greatest extent. In addition, it appears that these enzymes are also capable of recognizing D4 as a substrate and therefore may play an important role in its elimination (Salyers et al., 1996). In comparison, less has been reported on the effects of HMDS on biological systems.

Xenoestrogens are chemicals that have direct or indirect estrogenic activity in living systems. Recent evidence suggests that chemicals acting as estrogens can produce adverse effects in domestic and wildlife species (Kavlock et al., 1996; Klotz et al., 1996). These effects have been linked to high exposures to organochlorine compounds, or to naturally occurring phytoestrogens present in the primary food sources of grazing animals. In an early study designed to evaluate the estrogenic activity of several cyclic and linear siloxanes, D4 produced less than a 20% increase in uterine weight that was not statistically different from controls when administered to immature ovariectomized Wistar rats (Hayden and Barlow, 1972). The primary focus of the Hayden and Barlow study was to identify phenyl siloxanes with high estrogenic activity over a dose range of 0.01 mg/kg/day to 100 mg/kg/day. Although this dose range was broad, compounds with weak endocrine activity might not have been detected. Therefore, in the present study, the dose-range was expanded to better define the dose-response profile of D4 and to compare any estrogenic effects of D4 to reference compounds with either strong or weak estrogenic activity. This approach should allow positive results obtained at high doses in a uterotrophic assay to be placed into perspective relative to known estrogens. In addition, to understand structure activity relationships, the potential endocrine activity of the linear siloxane HMDS was evaluated.

There are considerable differences in the sensitivity of different rat strains to exogenous chemicals (Kacew et al., 1995). In addition, endocrine control of reproductive function is significantly different between Sprague-Dawley (SD) and F-344 rats (Chapin et al., 1996; Eldridge et al., 1999; Stevens et al., 1999). Thus, the primary objectives of the present study were (1) to evaluate the estrogenic and antiestrogenic potential of a cyclic (D4) and linear (HMDS) siloxane, (2) to investigate strain specific sensitivity, and (3) to determine the potency of D4 relative to both strong and weak estrogenic reference chemicals and the potency of HMDS relative to ethinyl estradiol (EE).

**MATERIALS AND METHODS**

**Chemicals.** D4 was obtained from Dow Corning and was > 98% pure. HMDS was purchased from Sigma Chemical Co. (St. Louis, MO) and was analyzed for purity by Dow Corning Corporation and found to be > 98% pure. Sesame oil, diethylstilbestrol dipropionate (DES-DP), and EE were also from Sigma Chemical Company (St. Louis, MO). Coumestrol (CE) was purchased from Spectrum Quality Products, Inc., (Gardena, CA). The antiestrogen ICI-182,780 was obtained from Tocris Cookson Inc. (Ballawin, MO).

**Animals (Experiment 1).** Previous studies with SD rats have suggested that matching pup weight at the start of dosing is more important than age because pups smaller than 35 g may not respond as effectively to estrogen and...
pups larger than 50 g may secrete endogenous estrogen (Reel et al., 1996). A similar comparison has not been conducted in the F-344 rat. Therefore, in this study, SD pup weights were approximately 40 g on the first day of dosing while F-344 pup weights were approximately 30 g on the first day of dosing. The starting pup weights for F-344 rats were estimated based on the growth and size differences between the SD and F-344. F-344 rats grow at a slower rate than SD rats, therefore, in order to achieve target weights the SD pups were started on day 18 while the F-344 pups were started on day 21.

Female SD (Cr:CD® VAF/Plus®) foster dams with 9- to 11-day-old, fostered pups (minimum of 10 pups/female) were obtained from Charles River Laboratories (Portage, MI). Female F-344 (COBS®CDF® (F-344/CrlBR)) foster dams with 7- to 11-day-old fostered female pups (minimum of 10 pups/female) were from Charles River Laboratories (Raleigh, NC). The pups were randomized by weight and assigned to the various treatment groups (12 pups/group). Pups were ordered over an age spread of 2 to 4 days in order to stagger the start of dosing for the 2 strains. All SD pups were started 18 days after birth and all F-344 pups were started 21 days after birth.

**Animals (Experiment 2).** Female SD (Cr:CD® VAF/Plus®) foster dams with 11-day-old, fostered pups (minimum of 10 pups/female) were obtained from Charles River Laboratories (Portage, MI). Juvenile rats were isolated from their dam on day 18 after birth and randomly assigned to the various treatment groups (12 pups/group).

**Animal housing.** All pups were acclimated to the laboratory from their time of arrival (7 to 11 days of age) to the start of dosing at 18 (SD) or 21 (F-344) days of age. For both experiments, SD and F-344 rats were group housed in plastic shoebox cages containing wood chip bedding in separate environmentally controlled rooms for the duration of the study. The animals were fed Certified Rodent Chow #5002, PMI Feeds, Inc. (St. Louis, MO) and tap water *ad libitum*. On the first day of dosing, the dams were removed from their cages. The pups were weighed and only pups weighing 35–50 gms (SD) or 25–40 gms (F-344) were included in the study.

**Rationale for selecting the SD and F-344 rats.** The F-344 rat has been the primary test animal for the biochemical evaluation of siloxane compounds in our laboratory for the past several years (McKim et al., 1998, 1999, 2001). Therefore, this strain was chosen in order to maintain consistency between this study and previous work. The SD rat is one of the most commonly used rat strains in reproductive toxicology studies. In addition, this strain has been used for reproductive toxicology studies in our laboratories (Stump et al., 2000). Therefore, this strain was chosen to allow comparisons to be made between studies done by other laboratories as well as reproductive toxicology studies done in our own laboratory.

**Administration of test compounds (Experiment 1).** Dosing solutions containing D4, EE, DES-EP, CE, D4 plus EE, or ICI-182,780 were prepared once for use throughout the study by dissolving a known amount (weight) of compound in sesame oil. D4 dosing solutions were verified by gas chromatography. The stability of the D4 dosing solutions was verified at the beginning, middle, and end of the dosing period. All compounds were administered once per day for 4 consecutive days (days 18, 19, 20, and 21 for SD and days 21, 22, 23, and 24 for F-344 pups) by oral gavage in a volume of 5 mL/kg. The gavage apparatus consisted of 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. D4 was administered at doses of 0 (sesame oil), 10, 50, 100, 250, 500, and 1000 mg/kg/day. EE was given at 1, 3, 10, and 30 mg/kg/day. DES-EP was administered at dose levels of 0.5, 1.5, 5, and 15 mg/kg/day. CE was dosed at 10, 35, 75, and 150 mg/kg/day. Animals were euthanized the morning after the last treatment.

**Administration of test compounds (Experiment 2).** Dosing solutions of HMDS and EE were prepared once for use throughout the study by mixing known weights of test compound with a known volume of sesame oil. All compounds were administered once per day for 4 consecutive days (days 18, 19, 20, and 21) to SD pups by oral gavage. HMDS was administered at doses of 0 (sesame oil), 600, or 1200 mg/kg/day. EE was given at 3 mg/kg/day. The gavage apparatus was the same as that described above under Experiment 1.

**Evaluation of D4 antiestrogenicity (Experiment 1).** At the start of this study, the estrogenic potential of D4 was not known. Consequently, it was not possible to design an experiment to evaluate antiestrogenicity that would have involved holding EE constant while increasing the exposure to D4. Therefore, in order to provide information on potential antagonistic effects of D4 on a normal EE response, a high dose of D4 (500 mg/kg/day) was chosen and co-administered with EE (1, 3, 10, or 30 mg/kg/day). Dosing was via oral gavage once a day for 4 consecutive days (*N* = 12 pups). The known antiestrogen (ICI-182,780) was co-administered (3 mg/kg/day) with each dose of EE (1, 3, 10, or 30 mg/kg/day) and was used as a positive control for detecting antiestrogenic activity. The primary objective of this experiment was to evaluate whether or not the presence of D4 at a high concentration would affect the normal activity of the endogenous ligand for ER over a broad concentration range.

**Evaluation of potential HMDS antiestrogenicity activity (Experiment 2).** HMDS (1200 mg/kg/day) was co-administered with EE (3 mg/kg/day) via oral gavage once a day for 4 consecutive days (*N* = 12 pups) according to the same dosing regimen described above. The rationale for the design of this experiment was the same as described above for D4.

**Effects on uterine weight (Experiments 1 and 2).** In both experiments all animals were euthanized the morning after the last dose by carbon dioxide inhalation followed by exsanguination via the abdominal aorta. Uteri and ovaries were removed as one piece caudal to the cervix. The uteri were weighed following the removal of adipose tissue and ovaries. Care was taken to ensure that any fluid in the uterus was not disturbed during the trimming and weighing procedures.

**Collection of tissues for histological examination (Experiment 1).** The left ovary and left uterine horn with cervix from 6 pups in each dose group were fixed in Bouin’s fixative and processed to paraffin blocks. The tissues were then sectioned, placed on glass slides, and stained with hematoxylin and eosin using standard techniques. The slides were then evaluated microscopically for changes in uterine epithelial cell height along the endometrial surface lining (luminal surface, Experiment 2). This was accomplished with a 40× objective and an eye piece micrometer that had been calibrated with a stage micrometer according to Foreyt (1989). An average cell height in 3 fields was calculated for each animal. The uteri of control animals were evaluated first. Following the evaluation of control animals, the uteri in the remaining groups were evaluated blind to treatment.

**Potency determinations in Experiment 1.** Potency calculations were based on the linear portion of the dose response curve for each agent. Linearity was determined using a regression analysis with log 10 dose and square of the log 10 dose included in the model. In cases where the dose response was quadratic, dose levels were removed one at a time, starting with the lowest dose in the case of the Fischer D4, D4 + EE, and ICI + EE and the SD D4 and ICI + EE curves and the highest dose in the Fischer EE dose response curve, until the quadratic term in the model was no longer significant at *p* < 0.05. Dose levels 0, 10, and 50 mg/kg/day were removed from the D4 treatment group for the F-344 strain, and 0, 10, 50, and 100 mg/kg/day were removed from the D4 treatment group for the SD strain. Dose 30 mg/kg/day was removed from the F-344 EE treatment group and dose 1 mg/kg/day was removed from the remaining treatment groups.

Considering only the linear portion of the uterine weight dose-response curves, EE vs. D4 (SD), EE vs. DES-EP (SD), EE vs. CE (SD), EE vs. D4 (F-344), DES-EP vs. D4 (F-344), and EE vs. D4 plus EE (F-344) were determined to have parallel lines (slopes not different). Therefore, for these potency comparisons, the relative potency at 50% of the maximal uterine growth response of EE or DES-EP was calculated. For all other potency calculations, the relative potency at 20, 50, or 80% of the maximal EE or DES-EP response was determined for values that fell within the range of the dose-response.

**Statistical analyses.** Statistical procedures were conducted with SAS software, version 8.0 (SAS Institute Inc. Cary, NC). Uterine weight (absolute and relative) and uterine epithelial cell height were evaluated with Levene’s test to
assess homogeneity of group variances. In those cases where Levene’s test was significant (\(p \leq 0.01\)), a suitable transformation to equalize variance was selected using a Box-Cox power transformation analysis. Treatment effects were assessed using a one-way ANOVA and Dunnett’s test was used to compare each treatment group to control when the global \(F\)-test indicated a significant treatment effect (\(p \leq 0.05\)).

RESULTS

In Experiment 1, there were no treatment-related clinical abnormalities observed in any of the treatment groups. SD pups treated with D4 had a statistically significant reduction in absolute body weight at 1000 mg D4/kg/day on day 21, but not on day 22 (Fig. 2A). In the F-344 pups, exposure to 1000 mg/kg/day D4 also caused a reduction in pup body weight that was statistically significant (\(p \leq 0.05\)) on days 23, 24, and 25 (B). Values represent the mean ± SEM of 12 animals. Statistical significance was not shown for clarity.

FIG. 2. Effects of D4 on body weight. Immature SD and F-344 pups were weighed on the first day of dosing and prior to necropsy. Body weight gain over the dosing period is shown for each dose administered. D4 administered at 1000 mg/kg/day reduced the body weights of SD pups on day 21, but not day 22 (A). These effects were statistically significant at \(p \leq 0.05\). In the F-344 pups, exposure to 1000 mg/kg/day D4 also caused a reduction in pup body weight that was statistically significant (\(p \leq 0.05\)) on days 23, 24, and 25 (B). Values represent the mean ± SEM of 12 animals. Statistical significance was not shown for clarity.

The strong (EE, DES-DP) and weak (CE) estrogenic compounds all increased uterine weight (absolute and relative-to-body weight) in a dose-dependent manner in both strains of rats (Figs. 4A, 4B, 5A, and 5B). Because of limited availability of CE, this compound was only administered to SD pups. In order to determine potencies, the data shown in Figures 4A and 4B were analyzed by linear regression analysis and the coefficients obtained were used to calculate relative potencies (Tables 1 and 2). By plotting the changes in uterine weight with increasing dose, it was possible to determine the potency of D4 relative to EE, DES-DP, and CE. When the slope of the reference compound was not different from the slope of the treatment compound, the regression lines were considered parallel. Under these conditions, relative potency would be the same at any response value. Therefore, when the regression lines of the dose-response data were parallel between reference and treatment compounds, potency was determined with the response produced by the reference compound that was 50% of the maximum response measured (Table 1). The curves for EE versus D4, CE, and DES-DP were parallel in SD pups (Table 1).

In F-344 pups the regression lines for EE and D4 were not parallel. Therefore, in order to understand how potency might change at different response points on the response curves, potency was determined with response values that were 20% and 50% of the maximum response of the reference. Regression analysis of the curves for EE and DES-DP in F-344 pups showed that these lines were parallel, and therefore the 50% response value of the reference compound (EE) was utilized (Table 1).

FIG. 3. Effects of octamethylcyclotetrasiloxane (D4) co-administered with ethinyl estradiol (EE) on body weight. Immature SD and F-344 pups were co-administered D4 and EE once a day for 4 consecutive days. There was a small decrease in body weight that was not statistically significant. This decrease was consistent with the small reduction in the body weights of pups exposed to 500 mg/kg/day D4 in the absence of EE. EE had no effect on body weight; therefore, the trend in body weight reduction was most likely due to the presence of D4. Values represent the mean ± SEM of 12 animals.
The 4 compounds evaluated could be ranked based on their uterotrophic responses as follows: EE $\geq$ DES-DP $> CE > D4$. When D4 was co-administered with EE, EE-dependent uterine growth was decreased by half in SD pups and by nearly an order of magnitude in F-344 pups (Figs. 4A and 4B). These data indicate that in addition to being weakly estrogenic, D4 also has weak antiestrogenic properties. The positive control for antiestrogenic activity (ICI-182,780) inhibited nearly all of EE-mediated uterine growth (Figs. 4A and 4B).

Uterine epithelium cell height, in both strains of rats, was increased in a dose-related manner following treatment with EE at 1, 3, 10, and 30 $\mu$g/kg/day and D4 at 250, 500, and 1000 mg/kg/day (Fig. 6). EE produced a maximal response of approximately 40 $\mu$m at 10 $\mu$g/kg/day, while the maximum response resulting from D4 was approximately 30 $\mu$m at a dose of 1,000,000 $\mu$g/kg/day. Based on a point estimate of these values, EE was about 100,000 times more potent than D4 in both strains of rats.

In Experiment 2, SD rats treated with the linear siloxane HMDS at 600 and 1200 mg/kg/day showed no treatment-related clinical abnormalities. There were also no dose-related changes in body weight. HMDS had no effect on uterine weight at either of the doses tested (Fig. 7). In this experiment, EE was evaluated at a single dose of 3 $\mu$g/kg/day, which

FIG. 4. Effects of octamethylcyclotetrasiloxane (D4), ethinyl estradiol (EE), diethylstilbestrol dipropionate (DES-DP), and coumestrol (CE) on absolute uterine weight. Immature SD (A) and F-344 (B) pups received dosages of each compound via oral gavage for 4 consecutive days. The dose range for each compound is listed in the Materials and Methods section. Co-administering EE and D4 assessed antiestrogenic activity. Absolute uterine weight increased in a dose-dependent manner following treatment with all of the test compounds. DES-DP and EE were the most potent, while D4 was the least potent. When D4 was co-administered with EE the increase in uterine weight was significantly less than that observed for EE alone. ICI 182,780 is a well-characterized antiestrogen that elicits its effect by direct interaction with the estrogen receptor. This compound was included as a positive control for antiestrogenic effects. ICI 182,780 produced a significant reduction in uterine weight in the presence of EE. The dashed line represents control uterine weights. Values represent the mean $\pm$ SEM of 12 animals. Statistical significance ($p \leq 0.05$) was as follows: All doses of EE, DES-DP, EE + D4, and CE were statistically significantly different from controls. D4 at doses of 250, 500, and 1000 mg/kg/day produced a statistically significant increase in uterine weight relative to controls. For clarity, statistical significance was not shown in the figure.

FIG. 5. Effects of octamethylcyclotetrasiloxane (D4), ethinyl estradiol (EE), diethylstilbestrol dipropionate (DES-DP), and coumestrol (CE) on uterine weight-to-body weight ratios. In order to show that the relative potency and shape of the dose-response curves was not influenced by small changes in body weight, the uterine weights shown in Fig. 4 were normalized to body weight. There was no detectable change in the dose-response profile for either SD (A) or F-344 (B) pups. Values represent the mean of 12 animals. Standard error bars not shown for clarity (see Figs. 4A and 4B). The dashed line represents control values. Statistical significance ($p \leq 0.05$) was as follows: All doses of EE, DES-DP, EE + D4, and CE were statistically significantly different from controls. D4 at doses of 250, 500, and 1000 mg/kg/day produced a statistically significant increase in uterine weight relative to controls. For clarity, statistical significance was not shown in the figure.
produced a statistically significant increase in uterine weight (Fig. 7). When HMDS (1200 mg/kg/day) was co-administered with EE (3 μg/kg/day) there was a small, but statistically significant reduction in uterine weight compared to EE alone (Fig. 7).

**DISCUSSION**

The present study has evaluated the estrogenic and antiestrogenic activity of D4, a cyclic siloxane, in immature SD and F-344 rats. The linear siloxane HMDS was evaluated in SD rats only. Uterine growth in immature rats following a 3- or 4-day exposure regimen has been used by several investigators as an indicator of estrogenic activity (Carthew et al., 1999; Christian et al., 1998; Fail et al., 1998; Reel et al., 1996). Other processes, such as high doses of progesterone, testosterone, and various synthetic progestagens, can also increase uterine weight.

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Reference</th>
<th>Relative potencya 20%</th>
<th>Relative potencya 50%</th>
<th>Y-intercept reference</th>
<th>Y-intercept treatment</th>
<th>Slopes reference</th>
<th>Slopes treatment</th>
<th>Response of the reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>D4</td>
<td>EE</td>
<td>0.00000171</td>
<td>0.37857</td>
<td>0.07407</td>
<td>0.052876</td>
<td>0.056888</td>
<td>0.0271</td>
<td>0.0678 0.1084</td>
</tr>
<tr>
<td>SD</td>
<td>D4</td>
<td>DES-DP</td>
<td>0.00000185</td>
<td>0.44409</td>
<td>0.07407</td>
<td>0.064373</td>
<td>0.056888</td>
<td>0.0264</td>
<td>0.0662 0.1059</td>
</tr>
<tr>
<td>SD</td>
<td>D4</td>
<td>CE</td>
<td>0.02167827</td>
<td>0.13065</td>
<td>0.07407</td>
<td>0.03887</td>
<td>0.056888</td>
<td>0.0194</td>
<td>0.0485 0.1059</td>
</tr>
<tr>
<td>SD</td>
<td>DES-DP</td>
<td>EE</td>
<td>0.92923917</td>
<td>0.37857</td>
<td>0.44409</td>
<td>0.052876</td>
<td>0.064373</td>
<td>0.0271</td>
<td>0.0678 0.1084</td>
</tr>
<tr>
<td>SD</td>
<td>CE</td>
<td>EE</td>
<td>0.00005490</td>
<td>0.37857</td>
<td>0.13065</td>
<td>0.052876</td>
<td>0.03887</td>
<td>0.0271</td>
<td>0.0678 0.1084</td>
</tr>
<tr>
<td>SD</td>
<td>CE</td>
<td>DES-DP</td>
<td>0.00010923</td>
<td>0.0006134</td>
<td>0.44409</td>
<td>0.13065</td>
<td>0.064373</td>
<td>0.03887*</td>
<td>0.0264 0.0662 0.1059</td>
</tr>
<tr>
<td>SD</td>
<td>D4 + EE</td>
<td>EE</td>
<td>4.40716824</td>
<td>0.4636836</td>
<td>0.16637</td>
<td>0.052876</td>
<td>0.017781*</td>
<td>0.0271</td>
<td>0.0678 0.1084</td>
</tr>
<tr>
<td>SD</td>
<td>ICI + EE</td>
<td>EE</td>
<td>0.00338815</td>
<td>NC</td>
<td>0.37857</td>
<td>0.08749</td>
<td>0.052876</td>
<td>0.011758*</td>
<td>0.0271 0.0678 0.1084</td>
</tr>
<tr>
<td>F-344</td>
<td>D4</td>
<td>EE</td>
<td>0.00000353</td>
<td>0.0000026</td>
<td>0.40099</td>
<td>0.05212</td>
<td>0.05638</td>
<td>0.020792*</td>
<td>0.0250 0.0625 0.0999</td>
</tr>
<tr>
<td>F-344</td>
<td>D4</td>
<td>DES-DP</td>
<td>0.00000528</td>
<td>0.0000051</td>
<td>0.37386</td>
<td>0.05212</td>
<td>0.052624</td>
<td>0.020792*</td>
<td>0.0233 0.0584 0.0999</td>
</tr>
<tr>
<td>F-344</td>
<td>DES-DP</td>
<td>EE</td>
<td>0.68287029</td>
<td>0.40099</td>
<td>0.37386</td>
<td>0.055638</td>
<td>0.052624</td>
<td>0.0250</td>
<td>0.0625 0.0999</td>
</tr>
<tr>
<td>F-344</td>
<td>D4 + EE</td>
<td>EE</td>
<td>0.12438578</td>
<td>0.40099</td>
<td>0.27207</td>
<td>0.055638</td>
<td>0.040480</td>
<td>0.0250</td>
<td>0.0625 0.0999</td>
</tr>
<tr>
<td>F-344</td>
<td>ICI + EE</td>
<td>EE</td>
<td>0.00090605</td>
<td>NC</td>
<td>0.40099</td>
<td>0.09274</td>
<td>0.055638</td>
<td>0.013109*</td>
<td>0.0250 0.0625 0.0999</td>
</tr>
</tbody>
</table>

Note. NC—Potency value was not calculated for those values outside the dose-response ranges used.

a = 10**((Y-intercept of treat/slope of treat) – (Y-intercept of ref/slope of ref)) + ((1/slope of ref) – (1/slope of treat))*response of the ref.

* Slope of treatment is significantly different from the slope of reference; p < 0.05.

**TABLE 2**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Reference</th>
<th>Inverse potency values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>D4</td>
<td>EE</td>
<td>584795.3</td>
</tr>
<tr>
<td>SD</td>
<td>D4</td>
<td>DES-DP</td>
<td>540540.5</td>
</tr>
<tr>
<td>SD</td>
<td>D4</td>
<td>CE</td>
<td>46.1</td>
</tr>
<tr>
<td>F-344</td>
<td>D4</td>
<td>EE</td>
<td>283286.1 3846153.8</td>
</tr>
<tr>
<td>F-344</td>
<td>D4</td>
<td>DES-DP</td>
<td>189393.9 1960784.3</td>
</tr>
</tbody>
</table>
weight. Although less sensitive than uterine weight, changes in vaginal or uterine epithelial cell height are considered highly specific for estrogen activity (Reel et al., 1996). When both endpoints are affected by treatment, there is a high degree of confidence that estrogenic processes have mediated the event. Thus, in the present study, uterine wet weight and uterine epithelial cell height following exposure to D4 were used to assess estrogenicity. In a similar study the potential endocrine activity of the linear siloxane, HMDS was evaluated. Because HMDS had essentially no effect on uterine weight at 1200 mg/kg, a detailed dose-response experiment was not performed.

Exogenous compounds can elicit estrogenic or antiestrogenic effects in a biological system by several mechanisms. The compound may mimic the effects of endogenous estrogen by direct association with the estrogen receptor (ER), resulting in an estrogen stimulated response, or through indirect mechanisms, such as perturbations in steroid synthesis pathways and up- or down-regulation of ER. The compound may compete with endogenous estrogen for ER binding without producing a direct estrogenic response; or may possess weak estrogenic activity along with some antiestrogenic activity (Ruh et al., 1995; Soto et al., 1998). Regardless of the mechanism involved, recent concerns regarding chemical estrogenicity and reproductive or developmental abnormalities have placed an emphasis on developing an understanding of the basic endocrine activity of chemicals to which both animals and humans may be exposed (Klotz et al., 1996).

In order to determine the potential reproductive effects of a compound with estrogenic activity, it is important to not only identify estrogenic activity, but also to put these data into context by comparing potency and efficacy relative to both strong and weak estrogens (Odum et al., 1997). Therefore, D4 was evaluated against the potent and well-characterized estrogens EE and DES-DP as well as the weak phytoestrogen CE. Each compound was evaluated at several doses and the log-dose response data were fitted to straight lines by regression analysis (Reel et al., 1996). The equations describing each line were used to determine the relative potencies of D4, DES-DP, and CE compared to EE (Finney, 1964). Because of its stability when administered orally, EE is considered a good reference standard for 17β-estradiol when the route of administration is by oral gavage (Edgren, 1994; Jones and Edgren, 1973; Reel et al., 1996).

Normal uterine growth in the rat is dependent on estrogen during postnatal days 16–26. In addition to uterine tissue growth, estrogen stimulates an increase in luminal fluid (imbibition). It has been reported that some estrogenic compounds may be more potent inducers of imbibition than cell proliferation and vice versa (Reel et al., 1996). However there is an equal number of investigators who have observed no differences between wet and dry uterine weight data (Odum et al., 1997). Therefore, by monitoring wet uterine weight, it is possible that a more conservative evaluation of an unknown compound can be achieved. Changes in uterine cell height are also stimulated by estrogen. Thus, uterine weight and uterine epithelial cell height serve as markers for estrogenic activity (Reel et al., 1996). Exposure to D4 resulted in a dose-dependent increase (NOAEL 100 mg/kg/day) in uterine weight and epithelial cell height indicating that D4 possesses weak estrogenic activity. It is not uncommon for compounds that are weakly estrogenic to also have antiestrogenic properties (Ruh et al., 1995). To evaluate whether D4 also possessed antiestrogenic activity, D4 was co-administered at a fixed dose level of 500 mg/kg/day with EE over a range of EE dose levels (1, 3, 10, and 30 µg/kg/day). The significant change in the EE + D4 uterine weight dose-response curve relative to EE alone, indicated that D4 also had weak antiestrogenic properties (Figs. 4A, 4B, 5A, and 5B). The dose-dependent increase in uterine weights, the estrogen mediated changes in uterine epithelial cell heights, the antiestrogenic data, and the similarities in the slopes between D4, EE, and CE in SD rats provide strong evidence for D4’s estrogen effects being mediated through the estrogen receptor system. However, additional studies de-

![FIG. 7. Effects of hexamethyldisiloxane (HMDS) and ethinyl estradiol (EE) on uterine weight. Immature SD pups received HMDS, EE, or HMDS + EE once daily for 4 consecutive days by oral gavage. HMDS was administered at dosages of 600 and 1200 mg/kg/day. The reference compound EE was dosed at 3 µg/kg/day and the co-administration of EE + HMDS was done at 3 µg/kg/day. EE + 1200 mg/kg/day HMDS. HMDS had no detectable effect on uterine weight, however there was a small, but statistically significant reduction in EE mediated uterine growth in the presence of HMDS. Values represent the mean ± SEM of 12 animals. A single asterisk indicates values were statistically significantly different from controls (p ≤ 0.05). The double asterisk indicates that the value was statistically significantly increased relative to controls and decreased relative to EE.](https://academic.oup.com/toxsci/article-abstract/63/1/37/1703135)
sioned to investigate the precise mechanism of D4s estrogen activity are currently in progress. In contrast to D4, which is a cyclic siloxane, the linear siloxane HMDS had no measurable effects on uterine weight. The highest dose of HMDS co-administered with EE produced a slight, albeit statistically significant reduction in absolute uterine weights (Fig. 7).

An understanding of a compound’s estrogenic potency relative to another, in combination with its physical and chemical properties and pharmacokinetic, pharmacodynamic, and exposure profiles can provide important information for understanding potential differences in reproductive parameters between 2 species or strains. If 1 species or strain of an animal is more sensitive than another it becomes difficult to combine information obtained in studies that used different models. Significant differences in endocrine controlled reproductive function in F-344 and SD rats have been reported (Chapin et al., 1996; Eldridge et al., 1999; Stevens et al., 1999). The magnitude of toxicity observed following exposure to chemicals could vary greatly between rat strains (Kacew et al., 1995). In addition, recent evaluations of various pharmacokinetic and biochemical effects of D4 have been done in both the F-344 and SD rats (McKim et al., 1998; Plotzke et al., 2000a,b). These studies indicated that the metabolism and hence availability of D4 at the target tissues may be different for SD and F-344 rats. Female F-344 rats showed an increased ability to metabolize D4 following inhalation exposure. Therefore, an important objective of the present study was to determine whether differences between rat strains could influence the relative potency calculations.

The data in Tables 1 and 2 and Figures 4A and 4B indicate that the SD rat is more sensitive in the uterotrophic assay than the F-344 rat. The potency of D4 relative to EE in F-344 rats was about 6-fold less than the potency observed in SD rats (Table 2). This trend was also apparent when the relative antiestrogenic potency data (D4 + EE) were reviewed. Therefore, although the same trend was observed in both strains of rat, comparisons of relative potency can vary by several fold. Whether or not this apparent difference in sensitivity was due to endocrine differences between strains, biochemical or pharmacokinetic differences, or variations in uptake from the gut could not be defined in the present study. However, it is clear that D4 and the positive reference compounds produced slightly different estrogenic responses in the 2 strains of rats. This implies that data obtained in 1 strain of rat may be difficult to compare to data collected from another, at least in terms of potency.

D4 was approximately 585,000 times less potent than EE in SD rats and 3.8 million times less potent than EE in F-344 rats (Table 2). D4 was about 46 times less potent than the phytoestrogen CE in SD rats. CE is an isoflavonoid found in many soy products, which has been shown to have weak estrogenic activity. Although some disagreement in the literature exists, several reports indicate that CE elicits its estrogenic effects by direct interaction with the ER (Kuiper et al., 1998; Martin et al., 1978; Whitten et al., 1994). Because CE is present in plant products, human exposure via diet is inevitable and the question of whether or not a weak estrogenic compound can have significant biological effects has been studied extensively. CE has been shown to be uterotrophic in immature SD rats at dietary concentrations similar to those found in the diets of animals and humans (Whitten et al., 1994). Six-week-old female B6D2F1 and ICR mice, exposed to 50, 100, 200, or 400 ppb CE in their diets for 14 consecutive days had increased uterine weights, reduced ovulation rates, and increased embryo degeneracy (Fredricks et al., 1981). In male SD pups treated with CE by sc injection for 5 days, beginning on postnatal day 1, there were no alterations in reproductive function or organ structure (Awoniyi et al., 1997).

It is clear that CE in the diets of female mice and rats can produce biological responses associated with estrogen activity. This was also observed in the present study (Figs. 4A, 4B, 5A, and 5B). Although there are many physiological and biochemical factors that make direct extrapolation from animal to human difficult, a key factor is the component in plasma that is free (Nagel et al., 1998). Endogenous estradiol circulates in blood bound to serum proteins. The primary high affinity binding protein for estradiol in rodents is alphafetoprotein while in humans the primary binding protein is sex hormone binding globulin (SHBG; Nagel et al., 1998; Sheehan and Young, 1979). Albumin provides a low affinity binding protein in both rodents and humans. Under steady state conditions of bound versus free estradiol, the concentration of free determines receptor occupancy and the level of biological response. Thus, protein binding provides a means of controlling cell uptake and hence the magnitude of biological response (Nagel et al., 1998). Any exogenous compound that does not bind to these carrier proteins would not be regulated by this system. Many xenoestrogens, such as CE, EE, and DES-DP, do not bind well to human SHBG and DES-DP, shows little binding to alphafetoprotein (Sheehan and Young, 1979). The ability of D4 to bind to either rodent alphafetoprotein or human SHBG has not yet been evaluated. In order to assess risk to human health, information on D4 human exposure scenarios, metabolism, receptor binding affinities, maximum absorbed dose levels, half-life, volume of distribution, and serum protein binding must be considered.

D4 has been shown to have reproductive effects in several reproductive studies including a 2-generation study (Stump et al., 2000). These effects include a reduction in the number of implantation sites, the number of live fetuses, and the mean live litter size at the highest inhalation exposure concentrations tested (i.e., 500 and 700 ppm). None of the estrogen sensitive endpoints assessed in this study appeared to be impacted by D4 exposure at the highest dose tested (700 ppm or 110 mg/kg). For example, in male rats treated with D4 there were no effects on testicular weight, seminal vesicle fluid, epididymal weight, or sperm counts. In females, there were no effects seen on anogenital distance, preputial separation, or vaginal patency in
the $F_1$ or $F_2$ generations. Although these data indicate that the effects seen in the reproductive studies are not related to D4s weak estrogenic activity, it is important to note that the NOAEL in the present study of 100 mg/kg/day was similar to the highest dose administered in the 2-generation study 110 mg/kg/day. Thus, differences in the route of administration, oral versus inhalation, may have resulted in different systemic exposure profiles and hence results. Studies are underway to provide additional mechanistic definition concerning these endpoints.

From the 2-generation study by Stump et al., 2000, several endpoints were selected for dose-response modeling using the benchmark dose (BMD) approach (Shipp et al., 2000). Margins of exposure (MOE), which are the ratio of the lowest lower bound on the BMD (BMDL) to the estimated intakes of D4, were calculated. The smallest BMDL that could be estimated from these data was 51 g/kg/day (323 ppm). Human exposure either in the workplace, through consumer products, or in the general environment that result in estimates of intake at least 100-fold lower than the BMDL (e.g., an MOE of 100 or greater) are not expected to cause any adverse reproductive effects in those populations. All MOEs calculated for the selected receptors were greater than 100 and with few exceptions were greater than 1000. MOEs may be further increased when the species- and strain-specific modes of action and absorption and kinetic data can be considered.

The biological effects of estrogens on target tissues are mediated by ER-$\alpha$ and the recently identified ER-$\beta$. Their distribution, tissue density, and expression varies among the different types of tissues in the female reproductive tract (Mowa and Iwanaga, 2000), the male reproductive tissues (Tena-Sempere et al., 2000), and in tissues outside of the reproductive system. These receptors have been shown to respond differently to various estrogen agonists and antagonists (Katzenellenbogen et al., 2000). In the rat uterus, ER-$\alpha$ is dominant and its expression can be induced by estradiol whereas ER-$\beta$ has very low expression and is nonresponsive to steroids. Thus, in the rat uterus ER-$\alpha$ is primarily responsible for the control of uterine physiology (Wang et al., 2000). These data indicate that a compound with a weak estrogenic effect on uterine weight, which is associated with ER-$\alpha$, might have a more pronounced effect in tissues where ER-$\beta$ predominates. Because of this, care must be taken in making direct extrapolations from rat uterotrophic data to effects in other female reproductive tissues, in male reproductive tissues, or in species other than rat. This would require knowledge of receptor type and density in each tissue tested and the relative binding of test compounds to these receptors.

In conclusion, the data presented here demonstrate that the cyclic siloxane D4 possesses weak estrogenic and antiestrogenic activity based on uterine weight and epithelial cell height in immature female SD and F-344 rats. In comparison, the linear siloxane HMDS had no effect on uterine weight when tested as an agonist. When HMDS was co-administered with EE there was a small but statistically significant reduction in uterine weight gain. The biological ramifications of this could not be assessed in the present study.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to Ms. Earnestine Stanton for her technical support throughout key phases of this study.

REFERENCES


