Toxicology Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Effects of chronic exposure to octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane in the aging female Fischer 344 rat

Paul A. Jean[a,*], Eddie D. Sloter[b], Kathleen P. Plotzke[a]

[a] Dow Corning Corporation, Midland, MI, 48686, United States
[b] Charles River Laboratories International, Inc, United States

ARTICLE INFO

Keywords:
Octamethylcyclotetrasiloxane
Decamethylcyclopentasiloxane
Pergolide mesylate
Dopamine agonism

ABSTRACT

Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) are used as intermediates or monomers in the synthesis of silicon-based polymers for industrial or consumer applications. D4 and D5 may remain as residual monomer in these polymers at less than 1000 ppm and may therefore be present as a minor impurity in consumer products. For D5, in addition to the manufacture of polymers, its uses include intentional addition to consumer products, personal care products and some dry- cleaning solvents. Two-year rodent chronic bioassays were conducted with both substances and borderline increases in the incidence of uterine tumors were observed, specifically, benign uterine adenoma with D4 and adenocarcinoma with D5. The effects profile and induction of uterine tumors share some similarity with that seen with chronic exposure to dopamine agonists. The current study investigated the potential for D4 and D5 to elicit dopamine agonist-like effects on estrous cyclicity. Separate groups of reproductively senescent female Fischer 344 rats (F344) were exposed via vapor inhalation to D4 (700 ppm, 9.3 mg/L) or D5 (160 ppm, 2.1 mg/L) or to a diet containing 0.0045, 0.045, or 4.5 ppm pergolide mesylate (PM), a potent dopamine agonist used here as a reference substance, from 11 through 24 months of age. The primary focus was to characterize the effects of D4 and D5 exposure on estrous cyclicity relative to that observed with PM. As a monitoring effort, circulating endogenous estradiol, progesterone, prolactin and corticosterone levels were evaluated monthly. A blood sample from each rat was obtained via tail vein in the afternoon after the daily inhalation exposure period once every 4 weeks. Histomorphologic examination of the major organs including the reproductive tract was conducted on all animals at study termination.

This study has shown that chronic exposure to D4 and D5 can affect cyclicity in the reproductively senescent F344 rat. For each substance the effect on cyclicity involved reduction in the incidence of pseudopregnancy with a shift toward cycles more typical of younger animals. D4 and D5 induced an increase in estrous cycle repetition whereas D4 also increased the incidence of extended estrus. These shifts resulted in animals entering proestrus/estrus significantly more times over the duration of the study than seen in the control group. Similar effects were observed with the reference substance, PM. However, distinct differences in the timing and magnitude of the effects on the estrous cycle and impact on prolactin, progesterone, estradiol, and corticosterone suggest that D4 and D5 are not classical dopamine agonists even though a similar increased incidence of proestrus/estrus was also observed with PM. These results may prove important with respect to understanding D4- and D5-induced uterine tumor response in the F344 rat, given the relationship between increased incidence of uterine endometrium stimulation by endogenous estrogen as a consequence of extended or more frequent proestrus/estrus, uterine tumor risk, and questions of relevance to humans. Recent publications have summarized the existing data on D4 and D5, with emphasis on exploring the biological relevance of the uterine tumors (Klaunig et al., 2016a,b; Franzen et al., 2017; Dekant and Klaunig, 2016; Dekant et al., 2017). The authors concluded that although the mode of action has not yet been fully established, the data, including the findings from this study, indicate that the D4- and D5-induced uterine tumors observed in the rodent chronic bioassays have no relevance for human risk characterization based not only on the distinct species differences in regulation of the reproductive systems, but also the high exposure levels and duration required for expression in rats.

* Corresponding author at: Dow Corning Corporation, 2200 West Salzburg Road, Midland, MI, 48686-0994, United States.
E-mail addresses: pajean4@gmail.com (P.A. Jean), eddie.sloter@crl.com (E.D. Sloter), kathy.plotzke@dowcorning.com (K.P. Plotzke).

http://dx.doi.org/10.1016/j.toxlet.2017.08.016
Received 27 May 2017; Received in revised form 9 August 2017; Accepted 16 August 2017

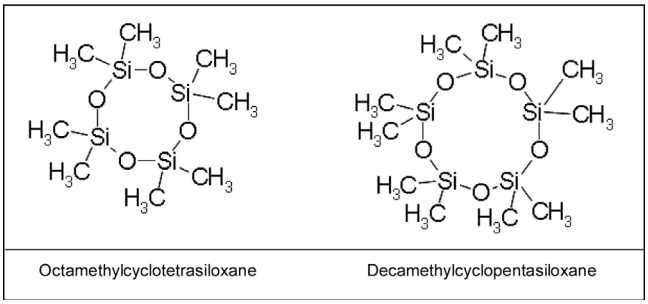


Fig. 1. Structure of Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5).

1. Introduction

Siloxane polymers are high-performance synthetic substances of commercial interest due to their unique chemical and physical properties. As a polymer of "siloxane units", two silicon atoms covalently bonded to one oxygen atom (–Si–O–Si–), siloxane polymers can vary greatly in degree of polymerization and configuration (linear, cyclic, and three dimensional structures). The simplest of the family of polymerized organo-silicon substances are polydimethylsiloxanes in which two methyl groups are covalently bound to each silicon atom ($–(CH_3)_2SiO–$).

Octamethylcyclotetrasiloxane (D4) and decamethylcyclopenta siloxane (D5) are cyclic siloxanes of 4 and 5 siloxane units (Fig. 1), respectively. They are volatile, colorless, and odorless liquids of low molecular weight and limited water solubility (Table 1) and both are primarily used in the synthesis of silicone polymers. D4 may remain as a residual monomer in these polymers at less than 1000 ppm and may therefore, be present as a minor impurity in consumer products. For D5, in addition to the manufacture of polymers, its uses include intentional addition to consumer products, personal care products and some dry-cleaning solvents. Persons who may be exposed to D4 or D5 include workers, consumers and the general public. Although dermal exposure is the most likely exposure route for consumers, testing by inhalation exposure (the second most prevalent route for consumers and predominate route for workers) is warranted due to the technical challenges associated with their spreading and evaporative characteristics and very low dermal absorption rate following dermal application. The similarity in kinetic behavior between these two exposure routes (Sarangapani et al., 2003) also supports the use of testing via the inhalation route to predict target organ exposure via the dermal route.

These cyclic siloxanes have been evaluated extensively with regard to understanding their safety profile, including examination of their potential toxicity hazards. The hazard profile of these substances has recently been reviewed (Dekant and Klaunig, 2016, Franzen et al., 2017).

Studies have identified that D4 and D5 are not extensively absorbed by the oral, dermal, and inhalation routes of exposure (Plotzke et al., 1994, 2000, 2002; Jovanovic et al., 2008; Tobin et al., 2008; McMahon et al., 2001; Reddy et al., 2007, 2008; Varaprath et al., 2003). The principal routes of elimination are exhalation of parent and urinary excretion of metabolites. Partitioning into fat occurs readily; however, bioaccumulation potential is low due to rapid elimination (Andersen et al., 2005, 2008). D4 and D5 have been shown to be phenobarbital-like with respect to adaptive effects on the liver such as liver enlargement, hepatocellular hypertrophy/hyperplasia, and hepatic cytochrome P450 induction (CYP2B1/2 primarily) in the rat (McKim et al., 1998, 1999, 2001a; Jean, 2005; Zhang et al., 2000). Effects of inhalation exposure to ≤500 ppm D4 in rat reproductive toxicity studies included reductions in the number of implantation sites and consequent reduction in litter size in the F_0 and F_1 generations, as well as prolonged estrous cycles and decreased mating and fertility indices in the F_1 generation (Siddiqui et al., 2007). Subsequent studies have shown that this is attributable to an effect of exposure on the female rats but not male rats. Meeks et al. (2007) reported that the time of ovulation was the critical period for exposure to elicit the reproductive effect. Follow-up studies support the hypothesis that inhibition of the pre-ovulatory surge of luteinizing hormone (LH) is a predominant factor (Quinn et al., 2007a). D4 has demonstrated very weak estrogenicity *in vivo* and *in vitro* (McKim et al., 2001b; Quinn et al., 2007b; Lee et al., 2015; He et al., 2003). No androgenic or progestogenic potential was observed for D4 when examined in *in vitro* and/or *in vivo* assays (Quinn et al., 2007b). D5 was similarly investigated for reproductive and endocrine toxicity and no effects were observed (Siddiqui et al., 2007; Quinn et al., 2007b). The toxicology profiles for D4 (Franzen et al., 2017) and D5 (Dekant and Klaunig, 2016) have been the subject of recent reviews.

The safety profile of these substances leading up to the 24-month chronic bioassays gave no indication that the uterus would have been a target organ. However, as recently reported, chronic vapor inhalation exposure to 700 ppm D4 was associated with a minimal increase in uterine benign epithelial adenoma at 24 months in the F344 rat (Jean et al., 2017). Following a similar study design 160 ppm D5 was found to give rise to a minimal increase in the incidence of uterine epithelial adenocarcinoma at 24 months (Jean et al., 2016).

Dopamine agonism was considered as a possible mode of action for both D4 and D5 given the noted selectivity of the target tissue, uterine endometrium. It has been reported that dopamine agonists, such as bromocriptine and pergolide mesylate (PM), elicit uterine endometrial tumors in rodent chronic bioassays (NDA 17-962; NDA 20-664; NDA 20-658; NDA 20-667; NDA 19-385; Richardson et al., 1984). The tumors occur by a Mode of Action (MOA) specific to rat strains like the F344 rat whose reproductive senescence involves loss of dopaminergic inhibition of pituitary prolactin secretion (Alison et al., 1994). In essence, aging F344 rats experience persistently elevated levels of prolactin in blood that has a luteotropic action on the ovary promoting prolonged occurrences of pseudopregnancy and elevated levels of progesterone. Administration of a dopamine agonist reverses this situation, lowers circulating prolactin levels promoting luteolysis and a return to cyclicity, which increases exposure to periods of estrogen dominance (proestrus/estrus). An increased exposure to periods of endogenous estrogen dominance associated with chronic administration of a dopamine agonist promotes an increased incidence of uterine tumors in the rat (Neumann, 1991; Gopinath, 1999). This manuscript details our investigation of the potential for chronic exposure to 700 ppm D4 and 160 ppm D5 to affect estrous cyclicity and other markers of reproductive senescence in aging female F344 rats. PM (a potent dopamine agonist) was included in the study as a concurrent reference group for comparison.

Table 1
Physical and Chemical Properties of Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5).

Property	D_4	D_5
Chemical Abstract Number	556 – 67-2	541 – 02-6
Molecular Weight (daltons)	297	371
Boiling Point	175 °C	211 °C
Melting Point	17.5 °C	–44 °C
Saturated Vapor Concentration at 23 °C	1161 ppm (14 mg/L)	195 ppm (2.9 mg/L)
Water Solubility[1] at 23 °C	56 ppb	17 ppb

[1]Varaprath et al. (1996).

2. Materials and methods

2.1. Test article

D4 and D5 were obtained from Dow Corning Corporation (Midland, MI). Purity was determined to be greater than 99% by gas chromatography utilizing both flame ionization and mass selective detection.

2.2. Reference material

Pergolide mesylate (Lot #: YE0365) was purchased from Spectrum Chemical Manufacturing Corporation (Gardena, CA). The substance was used as provided and the purity was reported to be greater than 99% pure (Supplier's Certificate of Analysis).

2.3. Animals and husbandry

Female F344 rats (Crl:CD(Fischer 344) BR VAF/Plus) were purchased from Charles River Laboratories (Wilmington, MA) and were 49–50 weeks of age at initiation of exposure. Animals were uniquely identified with Monel ® metal ear tags and implanted (subcutaneous, scapular region) with a programmable microchip (BMDS System) for identification. Animals were individually housed in elevated wire-mesh cages. Food (LLC Certified Rodent LabDiet #5002; PMI Nutrition International, St. Louis, MO) and reverse-osmosis purified water were provided *ad libitum* except during the daily inhalation exposure period. Animal room temperature and relative humidity ranges were 20.9–23.2 °C and 24.9–58.7% relative humidity. The light cycle consisted of 12 h of fluorescent light and 12 h of dark. Animals were observed twice daily for morbidity and mortality throughout the in-life phase of the study.

The study complied with all applicable sections of the final rules of the Animal Welfare Act regulations (9 CFR, Parts 1, 2, and 3) and all experimental procedures were conducted in accordance with the American Association for Laboratory Animal Science Policy on the Humane Care and Use of Laboratory Animals (AALAC, 1991) and approved by IACUC. Enrichment devices (Nylabones®) were provided to all animals throughout the study.

2.4. Inhalation exposure

Exposure of the F344 female rats from 11 through 24 months of age to D4 or D5 was conducted 5 days per week, excluding holidays, in 2.0 m^3 stainless steel and glass whole-body inhalation chambers. Daily exposures consisted of a six-hour period at the target exposure concentration between 20 min periods (T99) in which chamber concentrations were increasing to target concentrations or decreasing to non-detectable levels. Animals were individually housed and the chamber cage unit positions and the cage units were rotated among the rack positions weekly to limit the potential for positional effects. Adequate oxygen concentration (at least 19%) within the chambers was demonstrated pre-study and then approximately monthly. Inhalation chambers were set to maintain a temperature range of 20–27 °C and relative humidity range of 30–70% with at least 12 air changes per hour.

Maximal exposure concentrations of D4 (700 ppm; 9.3 mg/L) and D5 (160 ppm; 2.1 mg/L) were limited by the highest vapor concentrations that may be generated repeatedly with consistency and without formation of appreciable aerosol or condensation. Exposure concentrations were those with uterine tumor responses elicited in the 24-month chronic bioassay. The control group and the PM treatment group animals were placed into inhalation chambers and exposed to an atmosphere prepared identically to that for D4 and D5 treatment groups except D4 and D5 were absent.

Test atmospheres of D4 and D5 were produced by dilution of a D4 or D5 vaporization stream into the chamber inlet air stream. The vapor generator consisted of a glass column filled with glass beads. D4 and D5 were introduced onto the top of the glass bead column and evaporated into an air stream that flowed up through the column of glass beads and out of the vapor generator and directed to the chamber inlet. Chamber test atmosphere homogeneity was demonstrated prior to the initial exposure. Determination of test atmosphere concentration was performed by gas chromatography with flame ionization detection (GC/FID) (Hewlett Packard 5890 Series II).

Table 2
Treatment Group Details.

Group Number	Test Substance	Exposure Concentration	Route
1	Control	Not Applicable	Not Applicable
2	Pergolide Mesylate	0.0045%	Dietary
3	Pergolide Mesylate	0.045%	Dietary
4	Pergolide Mesylate	4.5%	Dietary
5	Octamethylcyclotetrasiloxane	700 ppm	Inhalation
6	Decamethylcyclopentasiloxane	160 ppm	Inhalation

2.5. Pergolide mesylate diet preparation and administration

A pre-mix formulation was prepared by mixing PM dissolved in ethanol with a quantity of basal diet using a Hobart Mixer. Quantities of this pre-mix were then blended with quantities of basal diet in a V-blender to prepare the high-dose diet (4.5 ppm PM). Medium (0.045 ppm) and low (0.0045 ppm) dose diets were then similarly prepared by dilution. The test diets were prepared weekly and stored at room temperature protected from light. The test diets were stable under these storage conditions for at least 11 days.

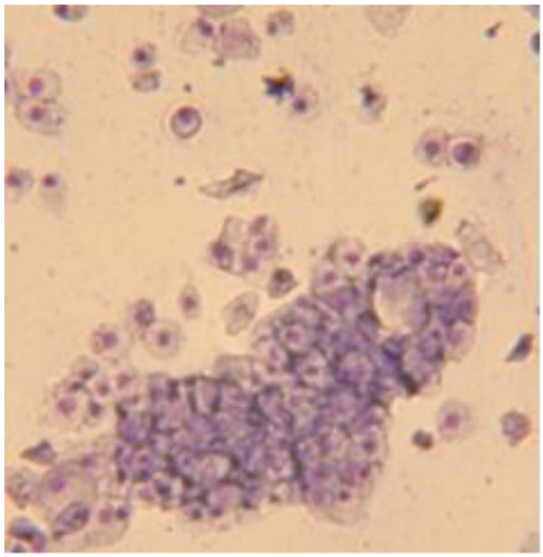
Administration of PM diets was performed such that animals received the PM diet 5 days a week in conjunction with the 5-day/week inhalation exposure for the D4/D5 treatment groups. The PM diets were replaced at the end of the 5-day period with basal diet for a 2-day non-treatment period. The D4/D5 treatment and untreated control group animals received basal diets for 7 days per week.

2.6. Study design and sample collection

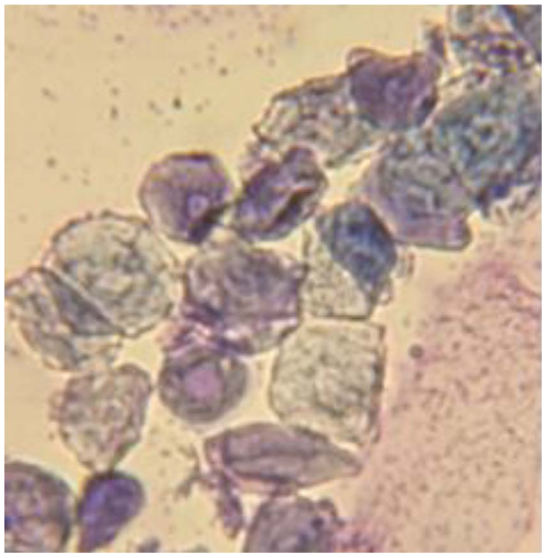
The aging female F344 rat has a high background incidence of pituitary tumors and elevated circulating prolactin levels associated with reproductive senescence. The allocation process used in this study was designed to promote an even distribution of animals entering reproductive senescence as well as to evenly distribute animals with pituitary tumors across the different treatment groups. Body weight and a pre-test determination of circulating prolactin levels were assessed at the end of the acclimation period for all animals. Assignment of animals to treatment groups was based on both body weight and circulating prolactin level. The first step in the process was to identify the pool of animals with normal circulating prolactin levels, defined as those animals with circulating prolactin values within 2 standard deviations of the mean for the total population. All animals in this pool were then allocated to treatment groups following a body weight-stratified randomization procedure. All remaining animals, considered to have "abnormal" prolactin values, were then allocated evenly (4–5 per group) to each treatment group. The allocation process resulted in treatment group sizes of 50 animals per group (Table 2).

Body weight data were collected at the time of randomization and twice each week during the treatment period (at the beginning of the 5-day exposure period and at the beginning of the 2-day non-exposure period). Morbidity/mortality observations were conducted twice daily, morning and afternoon. Clinical observations were recorded at least once each week. Food consumption was determined for each weekly 5-day treatment period and also, separately, for the 2-day non-treatment period.

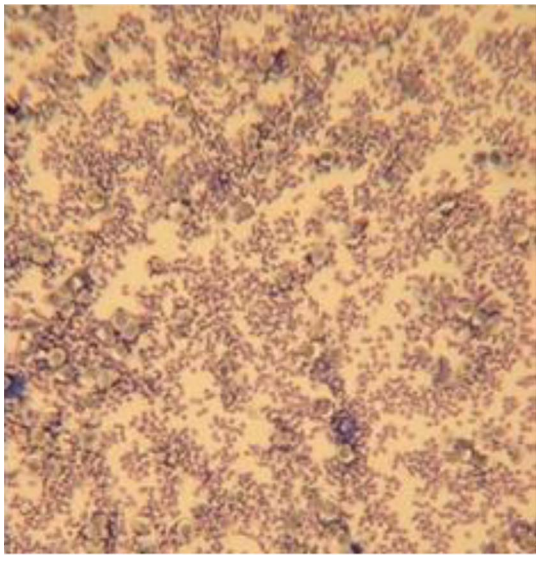
Estrous cycle monitoring was performed daily beginning approximately 1 week prior to initiation of treatments and continuing until the end of the treatment period (excluding major holidays). This was accomplished within 2 h of the start of the lights-on photoperiod via vaginal lavage performed using 0.1–0.2 mL deionized water aspirated 2–3 times. The vaginal slides were placed on a slide warmer for drying and then stained with Giemsa before being evaluated microscopically at 100X to determine the estrous stage (P = proestrus, E = estrus, M = metestrus, D = diestrus).



Proestrus (8–12 h duration)
This stage follows diestrus. Rat vaginal lavage samples collected during proestrus generally contain mostly nucleated epithelial cells. Cornified epithelial cells are also present during proestrus and become increasingly prevalent as estrus approaches, with up to 50% of the cells being cornified epithelial cells by the end of proestrus. Nucleated epithelial cells appear spherical in shape and are usually grouped in clusters. The epithelial cells tend to agglomerate and appear small and round with dark well-defined nuclei. This stage usually lasts from approximately 8–12 h.

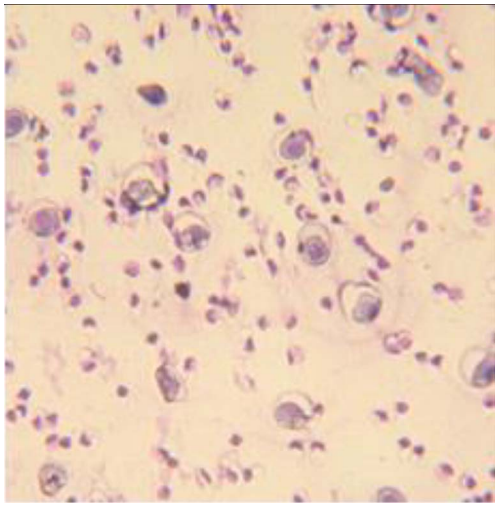


Estrus (18–24 h duration)
During estrus, the majority of cells are cornified epithelial cells that appear as irregular-shaped cells, which may or may not have remnants of a nucleus. Some nucleated epithelial cells may be present, and there are generally no leukocytes present. The cells can appear flat and separate or may be arranged in large masses of overlapping cells. At times, the cornified cells can appear “folded”. During late estrus or early metestrus, the majority of the cells are epithelial cells that appear nucleated and darker in color. This stage usually lasts from approximately 18–24 h.



Metestrus (~6 h duration)
During metestrus, the majority of the cells are leukocytes surrounding groups of nucleated epithelial cells. Some cornified epithelial cells may remain. Lavage samples from the metestrus stage usually contain dense populations of highly compacted cells. This stage is approximately 6 h in duration.

Diestrus (2–3 days duration)
Diestrus generally lasts 2–3 days and the majority of rats in a group will be in this stage at a given time. At the beginning of diestrus, the cells within a lavage sample may not appear as densely populated as the metestrus stage. Diestrus lavage samples are usually comprised of roughly equal numbers of leukocytes, cornified epithelial



and nucleated epithelial cells. Generally, as the stage progresses, the various cells become more sparsely populated. Towards late diestrus, the nucleated epithelial cells become more spherical in appearance. Mucus might also be found in this stage. At times, a field of view may appear ill-defined with increasing numbers of open spaces with only a few cells dispersed throughout.

These estrous data were evaluated to examine treatment-related alterations in aspects such as the percentage of days in each estrous stage and length of repetitive strings of estrogen-dominant days, proestrus and estrus (P or E).

Blood samples were taken pre-test and then approximately monthly for determination of serum concentrations of prolactin, progesterone, estradiol, and corticosterone. Blood was drawn from a lateral tail vein into tubes without anticoagulant. Serum was isolated and stored frozen (approximately −20 °C) until analyzed. Serum was also derived from blood collected from the vena cava at the scheduled euthanasia from anesthetized animals. Serum from the control group, D4, and D5 treatment groups were analyzed for FSH and for estradiol/estradiol metabolite profiles. Serum progesterone and estradiol were analyzed using a chemiluminescent immunoassay system (Immulite, Siemens Healthcare Diagnostics Inc.). Rat follicle stimulating hormone (rFSH), prolactin (rPRL), and corticosterone were measured in serum via an ^{125}I radioimmunoassay system (Izotop, Institute of Isotopes Ltd.) with magnetic separation for the quantitative determination of rFSH, rPRL, or corticosterone using a DPC Gamma C–12 gamma counter (Siemens Healthcare Diagnostics Products Corporation [formerly Diagnostics Products Corporation]).

A detailed necropsy was performed on all animals. Scheduled necropsy was performed staggered and counterbalanced among treatment groups on the 4th and 5th day of the last 5-day exposure period. The following organ weights were recorded for all animals surviving to the scheduled necropsy;

adrenals	brain	heart
kidneys	liver	lungs
pituitary gland	spleen	thymus
uterus	ovaries with oviducts	

Hematoxylin and eosin stained sections (4–8 µm thick) of fixed tissue (one ovary, one uterine horn, the uterine body with cervix, and the vagina) were evaluated by the study pathologist and subjected to a detailed peer review. In addition to descriptive findings, the review included ovarian corpora lutea sub-classification, enumeration of healthy and atretic antral follicles and primordial follicles, and an overall evaluation of the estrous cycle based solely upon the microscopic appearance of the reproductive tissues examined. In instances where histological features from multiple stages were present, the latest stage was assigned.

2.7. Statistical analysis

Numerical values are presented as the group mean with the standard deviation (S.D.), standard error (S.E.) and number of animals (n). Continuous data variables (mean body weights, body weight gains and food consumption for each interval, mean hormone values for each interval, organ weights [absolute and relative to body and brain weights]) for the control and PM-treat groups, Groups 1, 2, 3, and 4, were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup difference. If the results of the ANOVA were significant ($p < 0.05$), Dunnett's test (Dunnett, 1964) was applied to the data to compare the treated groups to the control group. Data from Group 5 (D4) and Group 6 (D5) were compared separately to the control Group 1 using two-sample *t*-tests. Statistics were conducted using SAS version 9.2 (SAS Institute, Inc., 2002–2008), or higher, software.

The Kolmogorov–Smirnov two-sample test was applied to the estrous cyclicity data for each of the 5 treated groups versus the control group using the cumulative P + E data. This was done over the entire time period and for each of the individual time intervals. Using the same data over the entire study period, a segmented regression model with 3 linear segments was used to estimate the inflection points and slopes for each of the segments. This was done individually for each of the treated and control groups. For the number of days that each animal contributes, the percentage of days in each stage of the estrous cycle (D, M, D + M, P, E, and P + E), the mean number of times in an estrogen-predominant state (E or P), and the mean length of strings of E or P, the nonparametric Dunn's test (Dunn, 1964) was used to compare each of the treated groups to the control group. The statistical test was performed for each time interval and for the entire study time period.

3. Results

3.1. Exposure

Inhalation exposures to D4 and D5 were at target concentrations achieving an overall mean ± SD analyzed concentration of 704 ± 22 ppm D4 and 161 ± 5 ppm D5. D4 and D5 were not detected in chambers containing the control and PM treatment groups.

The overall mean ± SD dietary concentrations of PM were 0.004 ± 0.0009, 0.04 ± 0.009 and 4.3 ± 0.3 ppm for the low-, middle-, and high-dose groups, respectively. Dose level mean, range, and calculated daily consumptions are summarized in Table 3.

Table 3
Pergolide Mesylate Dose Levels, Ranges, and Calculated Consumption Summary.

Dietary Concentration of Pergolide Mesylate[1]			Calculated Consumption (mg/kg/day)
Target	Mean (SD)	Range	
0.0045%	0.00395 (0.0009)	0.00252–0.00546%	0.0002
0.045%	0.040 (0.009)	0.021–0.051%	0.0023
4.5%	4.3% (0.3)	3.8–4.6%	0.2102

[1] Dietary concentrations expressed on a weight percentage.

3.2. Survival, clinical pathology, body and organ weight, food consumption

Most unscheduled deaths occurred in the months just prior to the terminal necropsy. The number of unscheduled deaths for the control, low, mid, high dose PM groups and D4 and D5 treatment groups were 13, 7, 9, 7, 15, and 10 animals, respectively.

Treatment related effects on body weight, food consumption, and clinical pathology markers were observed only in the high dose PM treatment group (Supplemental Tables S1–S10). For these animals, food consumption and food efficiency were reduced during the weekly 5-day exposure period and then elevated during the 2-day non-exposure period. These changes in food consumption gave rise to modest reductions in body weight and body weight gain, most notably after approximately 187 days of treatment. Group mean body weight was 13.5% lower than that of the control group at study termination. The most notable clinical observations for the high-dose PM treatment group involved an increased incidence of urogenital area staining and increased quantity of Nylabone® shavings under the cage.

Macroscopic observations for animals that survived to the scheduled necropsy demonstrated a dose-related increase in the incidence of uterine cysts among the PM treatment groups (Supplemental Table S11). The percent of animals with uterine cysts per group was 32, 35, 41, and 63% for the control, low-, middle-, and high-dose PM groups, respectively.

Treatment-related organ-to-body weight changes with correlating changes in absolute and/or organ-to-brain weight were seen in the D4, D5, and high-dose PM treatment groups (Supplemental Table S12). Liver (33%) and kidney (8%) weight increases were noted for the D4 exposed groups. Increases in liver (9%) and spleen (83%) weight were noted for the D5 treatment group.

3.3. Vaginal cytology profile

Vaginal lavage (performed within 2 h after the start of the daily photoperiod) afforded a daily assignment of estrous stage for each animal. The stage assignments included proestrus, estrus, metestrus, and diestrus. Proestrus and estrus were considered indicative of an “estrogen predominant” cycle phase and metestrus and diestrus as a “progesterone predominant” cycle phase. The number of days in an estrogen-predominant state (E or P), the duration of the proestrus-estrus cycle phase (consecutive days in P or E), cycle length (the time from the beginning of an estrogen-predominant state to the beginning of the next estrogen-predominant state), and cycling repetition were examined. Given the chronic nature of this investigation and to assess changes associated with aging, the data were organized into intervals of approximately 45 days.

3.4. Vaginal cytology profile of estrogen predominance

Estrous stages (vaginal cytology profiles) were evaluated from the perspective of identifying and examining the time spent under endogenous estrogen predominance (proestrus and estrus). The incidence of P and E were added together to generate a total number of days in an estrogen-predominant state per 45-day interval as well as for the entire study period (Table 4). The number of days in an endogenous estrogen-predominant state was also expressed as a percentage of days in an endogenous estrogen-predominant state (Table 5, Figs. 2 and 3).

The interval data show that the control group exhibited an age-related increase in the incidence of estrogen-predominant days with a peak at approximately 4.5–6 months from the start of the exposure period (16–17 months of age) followed by a steady decline through the remaining 7.5 months (intervals 5–9). The range of percent of days in an estrogen-predominant state for the control group was 13–35% depending on the interval. The temporal rise and fall was common to D4 and D5 treatment groups and the reference material, PM (low- and mid-dose PM groups,) with an exception of the high dose PM. However, there were notable differences relative to the control group with regard to the magnitude of the increases in incidence and temporal expression.

The interval incidence of estrogen-predominant days (expressed as number of days and by percentage of days) was slightly higher than control for the low dose PM group, giving rise to a statistically significant 16% increase in the total incidence of estrogen-predominant days for the entire study period (4827 days vs 4154 days) (Tables 4 and

Table 4
Cumulative Number of Estrogenic Days per Interval by Treatment.

Group						
Exposure Interval[a]	Control Group	Pergolide[b]			D4	D5
		0.0045 ppm	0.045 ppm	4.5 ppm	700 ppm[c]	160 ppm[c]
1	420	424	487	325	623	604
2	537	541	709	277	1048	960
3	767	867	857	749	1316	1016
4	732	842	890	808	1307	852
5	559	699	673	814	912	628
6	338	467	445	774	544	387
7	307	379	431	939	488	326
8	294	346	377	902	413	344
9	200	262	324	717	315	245
Total:	4154	4827**	5193**	6305**	6966**	5362**

** Statistically significant at $p < 0.01$ compared to the Control group (individual intervals were not examined for statistical significance).
[a] The exposure period was divided into 9 intervals of approximately 45 days duration each for the purposed of reporting.
[b] Dietary administration (concentrations reflect proportion of pergolide mesylate in the diet on a weight to weight basis).
[c] Vapor inhalation exposure concentration.

Table 5
Percentage (%) of Days in an Estrogenic State (Proestrus/Estrus).

Exposure Interval[a]	Control Group	Pergolide Mesylate[b]			D4	D5
		0.0045 ppm	0.045 ppm	4.5 ppm	700 ppm[c]	160 ppm[c]
1	19.1 ± 9.7	19.3 ± 12.0	22.1 ± 10.0	14.8 ± 7.4	28.3 ± 18.2	27.5 ± 12**
2	23.4 ± 9.8	23.6 ± 12.1	30.8 ± 9.7*	12.0 ± 9.8**	45.8 ± 19.8**	41.7 ± 15.1**
3	34.9 ± 12.2	39.5 ± 13.3	39.0 ± 8.4	34.1 ± 11.4	61.2 ± 14.7**	46.2 ± 14.3**
4	31.8 ± 14.3	36.6 ± 15.5	38.8 ± 11.9	35.9 ± 11.5	59.3 ± 20.8**	37.3 ± 15.2
5	24.9 ± 15.7	31.1 ± 18.1	30.8 ± 12.5	37.7 ± 12.7**	43.3 ± 21.6**	28.5 ± 16.7
6	15.4 ± 14.1	20.7 ± 16.2	20.7 ± 12.7	35.5 ± 11.2**	25.7 ± 17.5**	17.2 ± 14.4
7	14.3 ± 13.1	16.9 ± 16.1	20.4 ± 11.8*	44.9 ± 12.4**	23.4 ± 16.6**	14.5 ± 12.1
8	14.9 ± 12.6	16.9 ± 13.4	18.2 ± 9.8	44.7 ± 15.6**	22.5 ± 14.8**	15.9 ± 8.6
9	12.5 ± 7.1	14.9 ± 14.5	17.1 ± 12.7	38.8 ± 14.6**	20.4 ± 11.4**	13.6 ± 6.2

Values depict the group mean ± Standard Error.
* Statistically significant at $p < 0.05$ compared to the Control group.
** Statistically significant at $p < 0.01$ compared to the Control group.
[a] The exposure period was divided into 9 intervals of approximately 45 days duration each for the purposed of reporting.
[b] Dietary administration (concentrations reflect proportion of pergolide mesylate in the diet on a weight to weight basis).
[c] Vapor inhalation exposure concentration.

5, Fig. 2). The interval values for the percent of days in an estrogen-predominant state for the low dose PM group ranged from 15 to 40%.

Mid-dose PM induced essentially similar results as low-dose PM. However, the incidence of estrogen-predominant days for most intervals were slightly higher which contributed to a 25% increase in the total incidence of estrogen-predominant days (5193 days) relative to the control group (4154 days). The range of interval values for percentage of days in an estrogen-predominant state was 17–39%.

High-dose PM continued the dose trend with a 52% increase in the total incidence of estrogen-predominant days (6305 days) and the interval range for percentage of days in an estrogen-predominant state was 12–45%. The high-dose PM was unique in that it induced a marked reduction in the incidence of estrogen-predominant days for the first two 45-day intervals before exhibiting a rapid increase. A high incidence rate then ensued for the remainder of the study period. The overall result for PM treatment was a dose-responsive increase in the number of estrogen-predominant days across all three PM treatment groups relative to the control group.

Both D4 (6966 days) and D5 (5362 days) produced statistically significant increases in the total number of estrogen-predominant days for the overall treatment period relative to the control group (4152 days). For D4, the individual interval mean values for total number of estrogen-predominant days were markedly higher than the corresponding control values for each interval (Table 4). This was reflected also in the range of percent of estrogen-predominant days (range: 28–62%) with all being statistically significant except for the first interval (Table 5, Fig. 3). D4 had a higher interval incidence of total number of estrogen-predominant days compared to D5 and was higher than any of the PM groups. The age-related transient rise in incidence of estrogen-predominant days typical of the control group was also apparent in the D4 treatment group. D5 induced an increase in the total number of estrogen-predominant days, predominately in the

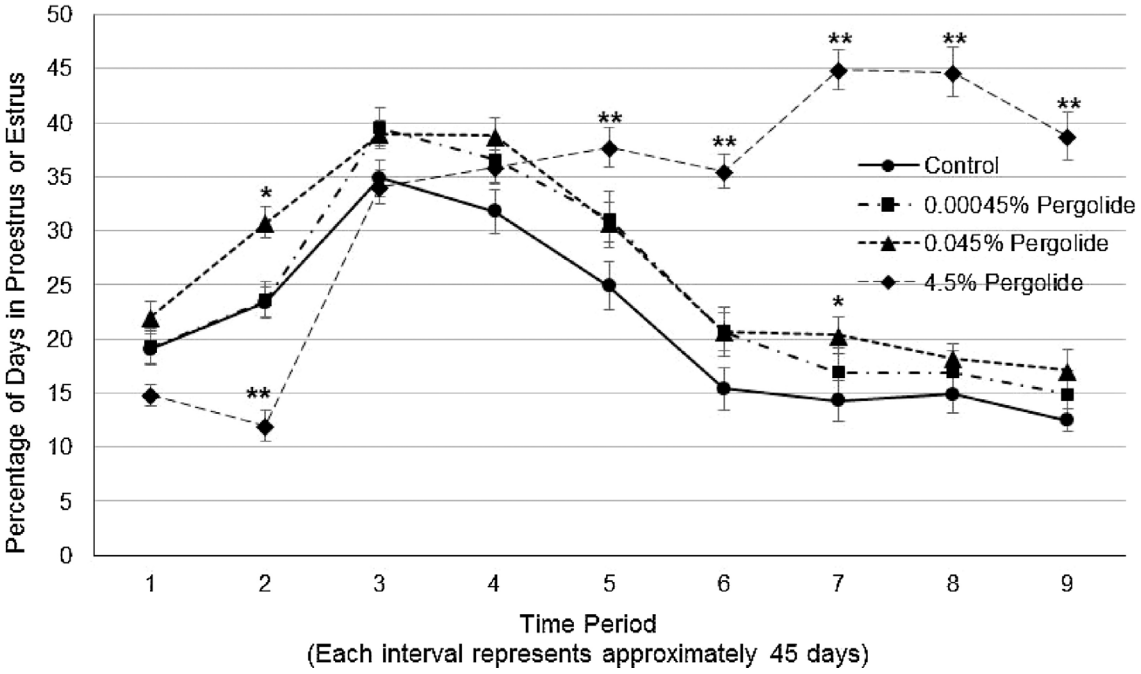


Fig. 2. Percentage of Days in an Estrogenic State (Proestrus-Estrus) in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
*Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error.

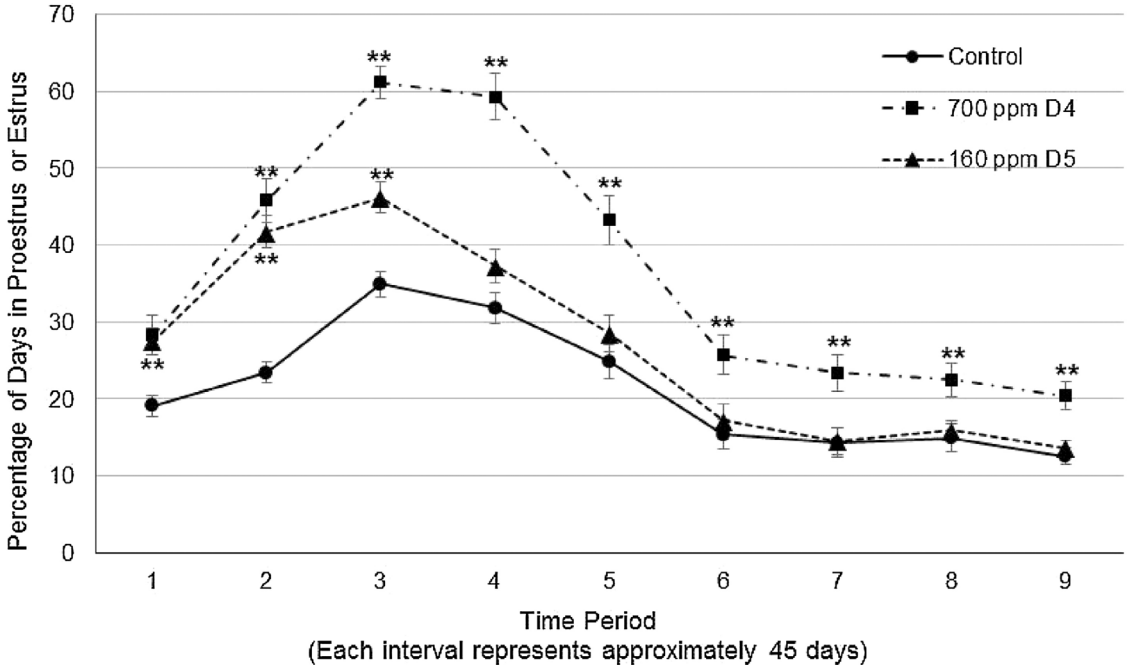


Fig. 3. Percentage of Days in an Estrogenic State (Proestrus-Estrus) in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

early intervals associated with the transient rise seen in the control group (Tables 4 and 5, Fig. 3). When expressed on the basis of percent of days in an estrogen-predominant state, the range of interval values for D5 was 14–46% as compared to 13–35% for the control group.

3.5. Cumulative incidence of proestrus and estrus stages

Figs. 4 and 5 present the cumulative incidence of proestrus/estrus as a function of time for the control and PM treatment groups and the control and D4, and D5 treatment groups, respectively. As shown, the control group's cumulative profile demonstrated an increasing incidence of days in proestrus/estrus that transitioned to a markedly higher incidence at exposure day 64. That increased incidence continued until exposure day 216 where the incidence slowed until the end of the exposure period. These shifts likely represent biological transitions inherent in the age-dependent process of reproductive senescence. The animals were approximately 13 and 18 months of age at exposure days 64 and 216, respectively.

As shown in Fig. 4, low- and mid-dose PM groups exhibited the same general trend as the control group with transition to an increased incidence of proestrus/estrus days at about nearly the same time (Day 62–70) as the control group. The second transition (to a lower incidence) occurred at essentially the same time as the control for the low-dose PM group. The second transition for the mid-dose PM group occurred almost 2 weeks later than that for the control group (Day 228 verses Day 216). Expressed in this way the graph depicts the marked dose-responsive increase in the number of estrogen-predominant days associated with PM exposure and potentially an additional influence on progression of reproductive senescence.

The incidence profile for the high-dose PM group was notably different from that of the other PM dose groups. High-dose PM induced initially a lower incidence of estrogen-predominant days followed by a marked increase in incidence far in excess of that seen in the control group. As with the control, low-, and mid-dose PM groups two transition points were identified in the high-dose PM cumulative data (Day 92 and 271). The first occurred 4 weeks later than that of the control group. The second was nearly 8 weeks later than that of the control group, and importantly the second transition point was to an even higher incidence and represents a marked difference in profile from that of the control and all other treatment groups.

The cumulative profile for estrogen-predominant days for D4 and D5 were generally similar to that of the control group with respect to having two transition points, the first to a higher incidence and the second to a lower incidence (Fig. 5). However, both D4 and D5 exhibited differences from the control group. One notable difference from the control, but also from the PM treatment groups, was that both D4 and D5 demonstrated a markedly higher incidence immediately after initiating exposures. Both continued to show higher incidences than control after the transition points. However this was markedly higher for D4 than for D5. The timing of the two transition points for D4 were not different from that of the control. In contrast both transition points for D5 were markedly earlier than the control group, a finding that was also distinctly different from PM. It is unknown if these shifts to an earlier time reflect a biologically significant change in the time course of reproductive senescence.

3.6. Estrous cyclicity

The effect of treatment on estrous cyclicity was examined with respect to the impact on cycle repetition (time between starting P/E stages of consecutive cycles) and duration of the within-cycle proestrus-estrus phase (Figs. 6–9).

Animals were approximately 11 months of age at initiation of exposure. Control group animals demonstrated an average cycle repetition of approximately 4.5 cycles/45- day interval (reflecting 10-day cycles). The repetition frequency nearly doubled by the 3rd exposure interval (to approximately 8 cycles/interval reflecting 6-day cycles) and then declined to approximately four cycles/interval by interval six. The frequency remained at this level until the end of the study.

Low- and mid-dose PM treatment exhibited a similar profile as the control group with respect to the transient increase in cycle repetition. However, the low-dose PM group's values for cycle repetition were slightly higher than controls for intervals that followed after the peak interval. Mid-dose PM demonstrated a consistent and marked elevation

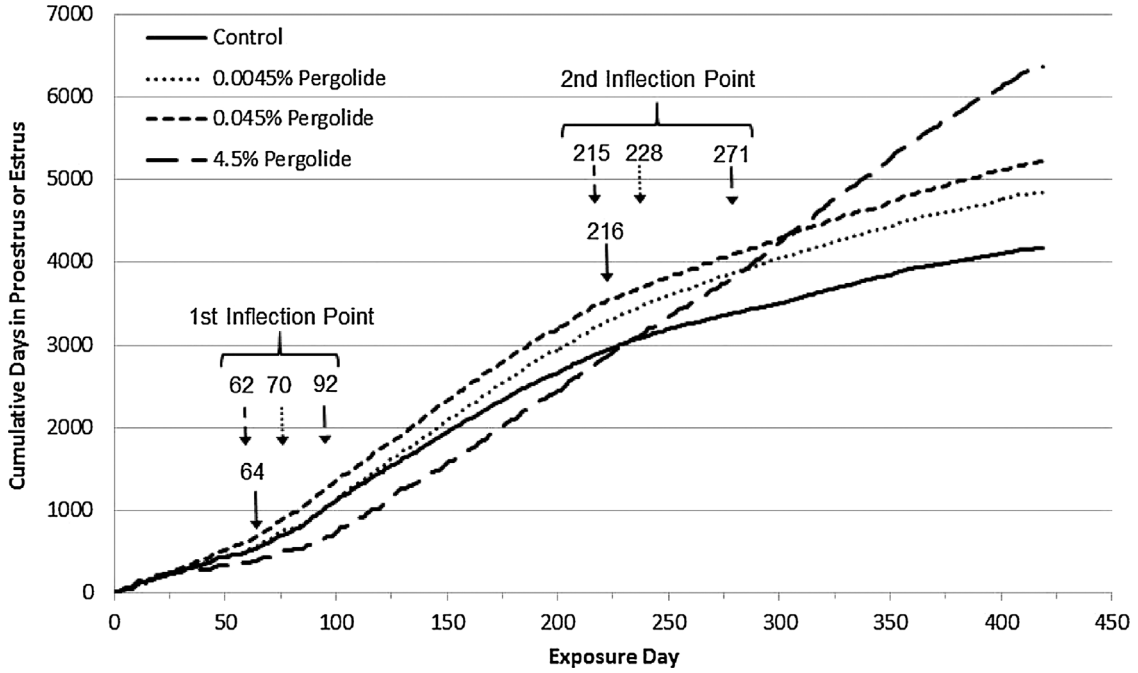


Fig. 4. Cumulative Incidence of Proestrus and Estrus in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)

in interval values across all of the intervals except interval 9.

The high-dose PM group demonstrated a unique profile in that cycle repetition was markedly reduced for the first two intervals followed by a dramatic increase in repetition to reach control group levels by the 5th interval. The frequency remained elevated for the duration of the exposure period.

D4 exposure gave rise to a similar temporal rise and fall in repetition frequency as was seen in the control group, however, there was a notable and consistent elevation in repetition frequency across all of the intervals.

D5-exposed animals also exhibited the transient increase in cycle repetition as seen in the controls. However, the repetition frequency for

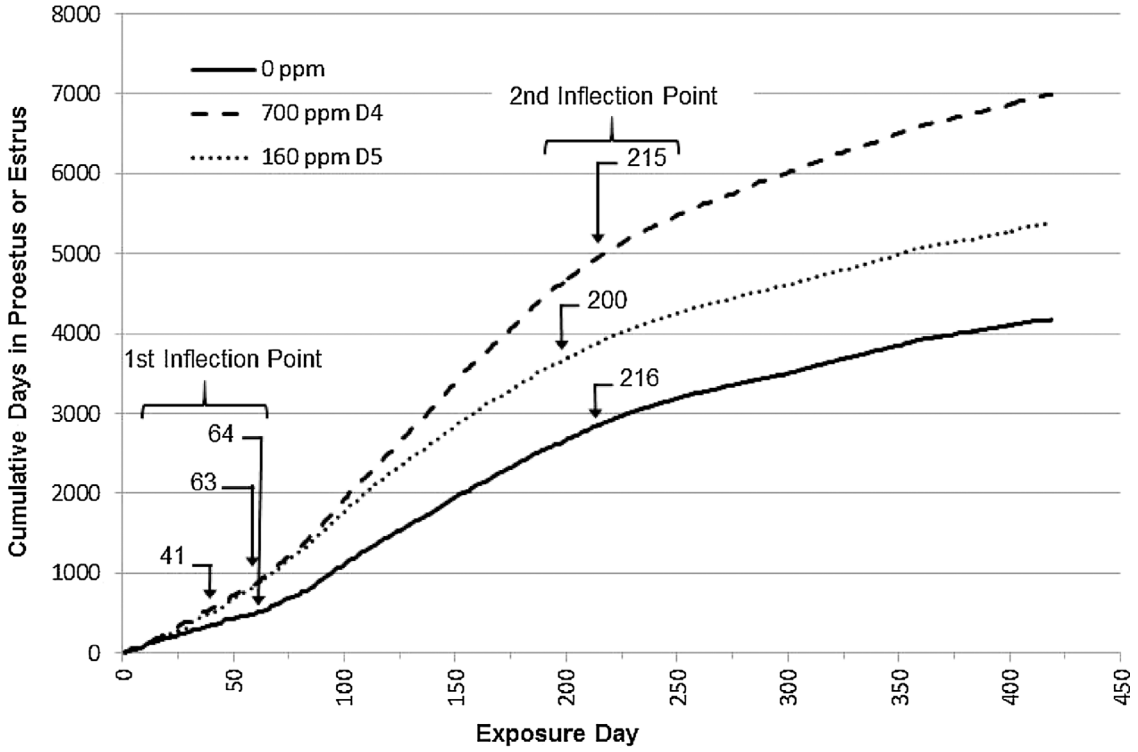


Fig. 5. Cumulative Incidence of Proestrus and Estrus in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)

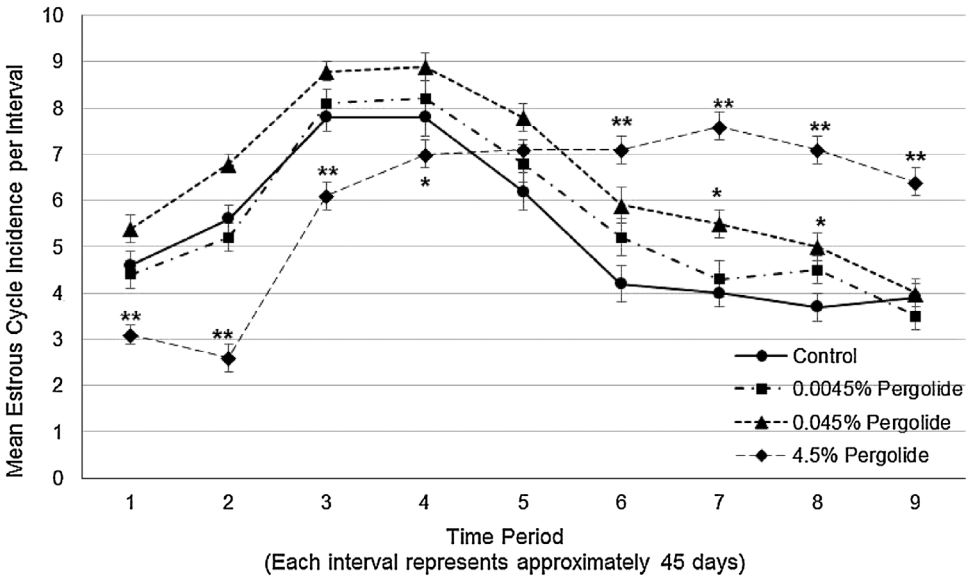


Fig. 6. Effect of Treatment on Estrous Cyclicity in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

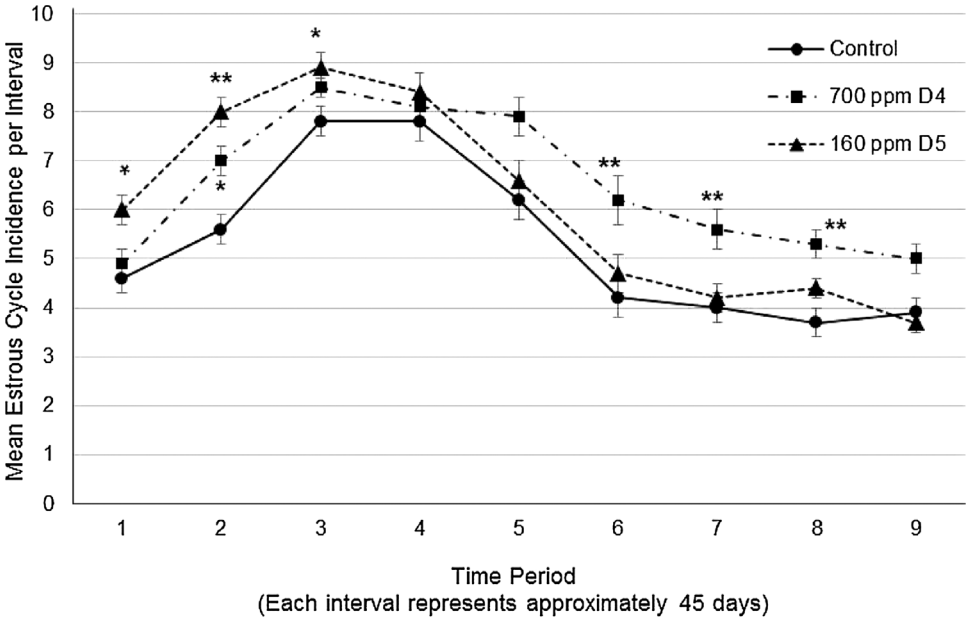


Fig. 7. Effect of Treatment on Estrous Cyclicity in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

D5 was markedly greater than that of the control group but only for the first three intervals. Interestingly, the cycle repetition over these three intervals was also notably greater than that seen for D4.

The duration of the within-cycle proestrus-estrus (P-E) phase is typically 1–2 days in young adult cycling rats. Control group females exhibited a consistent P-E phase duration of 1–2 days per cycle for the entire exposure period (Figs. 8 and 9).

Low- and mid-dose PM treatment groups were not notably different from the control group. The high-dose PM group demonstrated a P-E phase duration of 2–3 days per cycle over the entire study.

Increased P-E phase durations (2.4–3.5 days/cycle) were observed for the D4 exposure group in intervals 1–6. Slight elevations (2.2–2.3 days/cycle) were present only for intervals 2 and 3 for the D5 treatment group.

3.7. Hormone analysis

3.7.1. Prolactin

Circulating prolactin was assessed in all animals from all groups at about 4-week intervals. The data reflect a single point determination of late afternoon circulating prolactin concentration (Figs. 10 and 11).

Circulating prolactin concentrations determined prior to initiating exposures were higher than anticipated. A review of the sampling and analysis methods did not identify an obvious cause. The most likely contributor may have been stress related to the short acclimation to the laboratory setting, including the handling procedures and blood collection. Values observed at the 2-week sampling were more aligned with reported normal ranges.

Generally speaking, prolactin values were highly variable among animals of the same treatment group as well as between sampling dates. The driver for the high variability is unknown but may be related to sampling times not being optimized for estrous stage and/or time of day. The data for the control group demonstrated a mean prolactin level of more than 100 ng/mL at the end of the study period (rats were approximately 24 months of age) as compared to less than 50 ng/mL seen two weeks after initiating exposures (rats were approximately 11 months of age). The age-related elevation in prolactin is consistent with increases expected of reproductive senescence in aging female F344 rats (Estes et al., 1982).

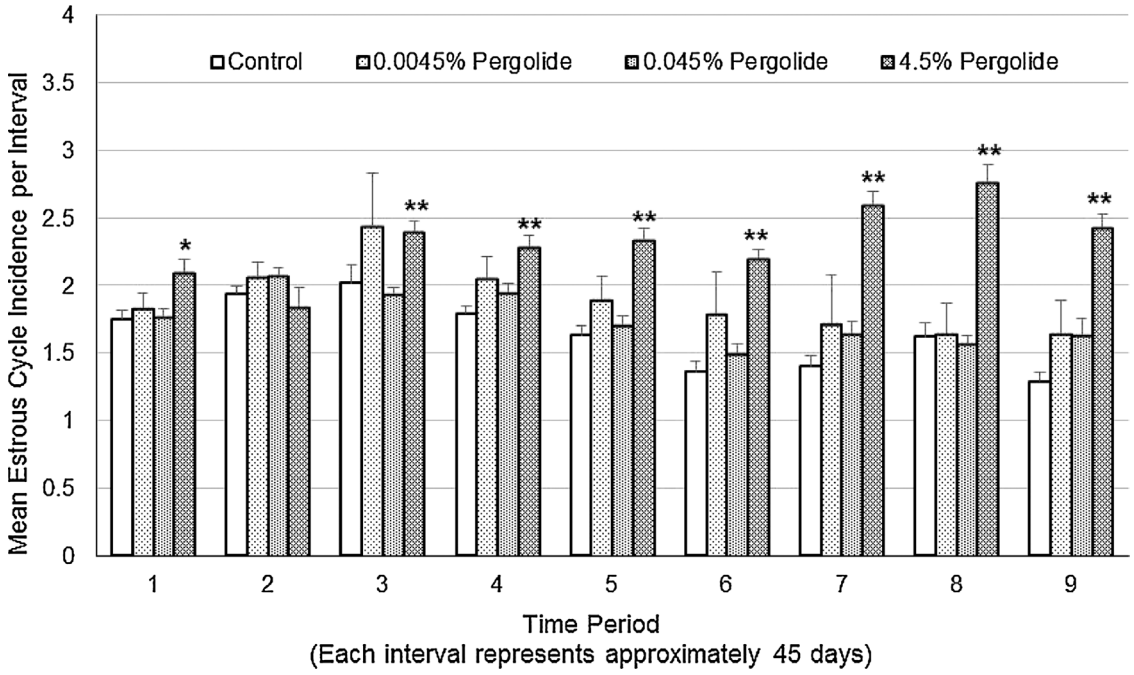


Fig. 8. Effect of Treatment on Length of Proestrus/Estrus Phase of the Estrous Cycle in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

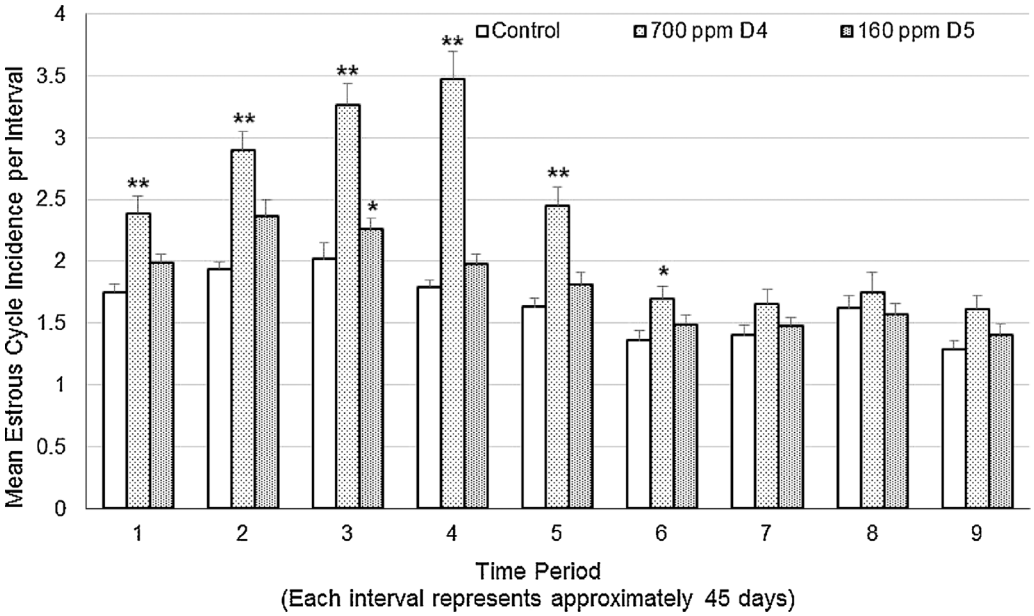


Fig. 9. Effect of Treatment on Length of Proestrus/Estrus Phase of the Estrous Cycle in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

Circulating prolactin levels in the low-dose PM treatment group were not different from that of the control group. In the mid-dose PM treatment group, there was an elevation in prolactin levels for animals through week 30, followed by a return to control group levels. In sharp contrast to this was the observed marked decrease in circulating prolactin concentration over the entire treatment period associated with high-dose PM administration. Reduced circulating prolactin concentration is an expected outcome associated with administration of a potent dopamine agonist.

D4 and D5 exposure were not associated with any notable effects on circulating afternoon prolactin concentration.

3.7.2. Progesterone

Circulating progesterone concentrations were assessed in all animals from all groups at about 4-week intervals. The data are summarized in Figs. 12 and 13.

Circulating progesterone concentration in these samples increased steadily with advancing age in the control group as shown in Fig. 12. With the exception of a slight elevation at Week 2, low-dose PM was essentially without an effect on the age-related rise in progesterone concentration. In contrast, exposure to both the mid- and high- dose PM treatment resulted in a marked influence on the age-related increase in circulating progesterone concentration. High-dose PM treatment resulted in maintaining low progesterone levels throughout the study. The mid-dose PM treatment demonstrated an intermediate level of activity giving rise to circulating

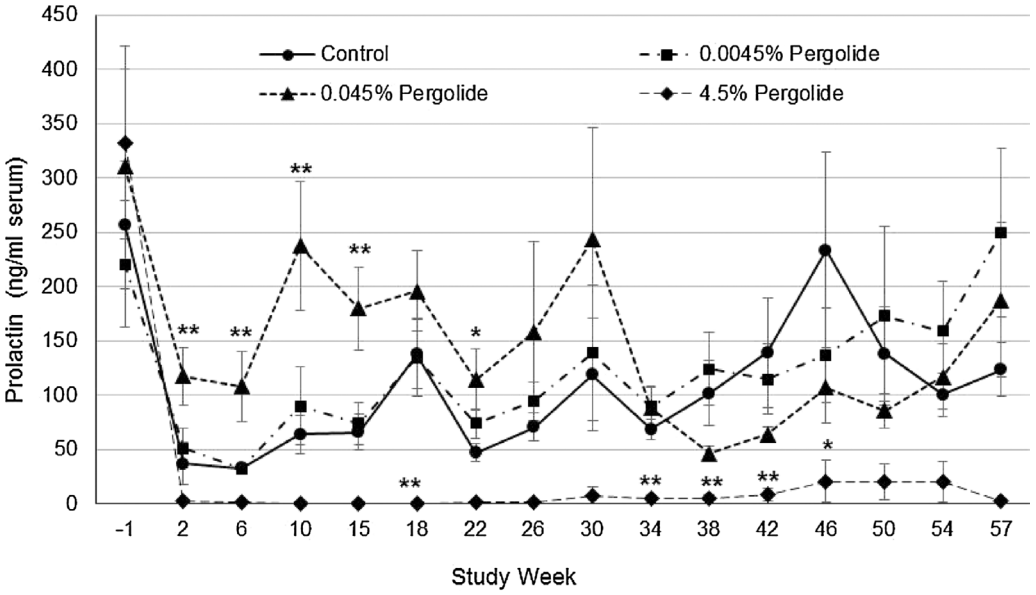


Fig. 10. Serum Prolactin Levels in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

progesterone concentrations that were approximately 50–70% of the increase seen in the control group. D4 exposure gave rise to slight statistically significant increases in circulating progesterone concentrations relative to controls at 2, 6, and 10 weeks only. D5 exposure had little to no effect on circulating progesterone level.

3.7.3. Estradiol

Circulating endogenous estradiol levels were determined in blood samples drawn from animals of all groups at about 4-week intervals. The data are summarized in Figs. 14 and 15.

Control group animals exhibited a relatively consistent circulating endogenous estradiol concentration (approximately 60 pg/mL) over the entire study period in these late afternoon samplings. PM administration increased circulating endogenous estradiol concentrations relative to the controls. This effect was most notable for the high-dose PM treatment group for which most all time point values were statistically different from the control. Group mean values for low- and mid-dose PM treatment groups were consistently higher than the control group but the increases were not statistically significant.

In contrast, exposure to D4 produced a moderate reduction in circulating endogenous estradiol concentration at each time point. Slight but statistically significant decreases in group mean endogenous estradiol concentrations were present only at weeks 2, 15, 18, and 26 in the D5-exposed animals. The concentrations were not notably different from control at all other time points.

3.7.4. Estradiol:progesterone ratio

The estradiol:progesterone (E:P) ratio was calculated for each time point for all animals from all groups. The data are summarized in Figs. 16 and 17.

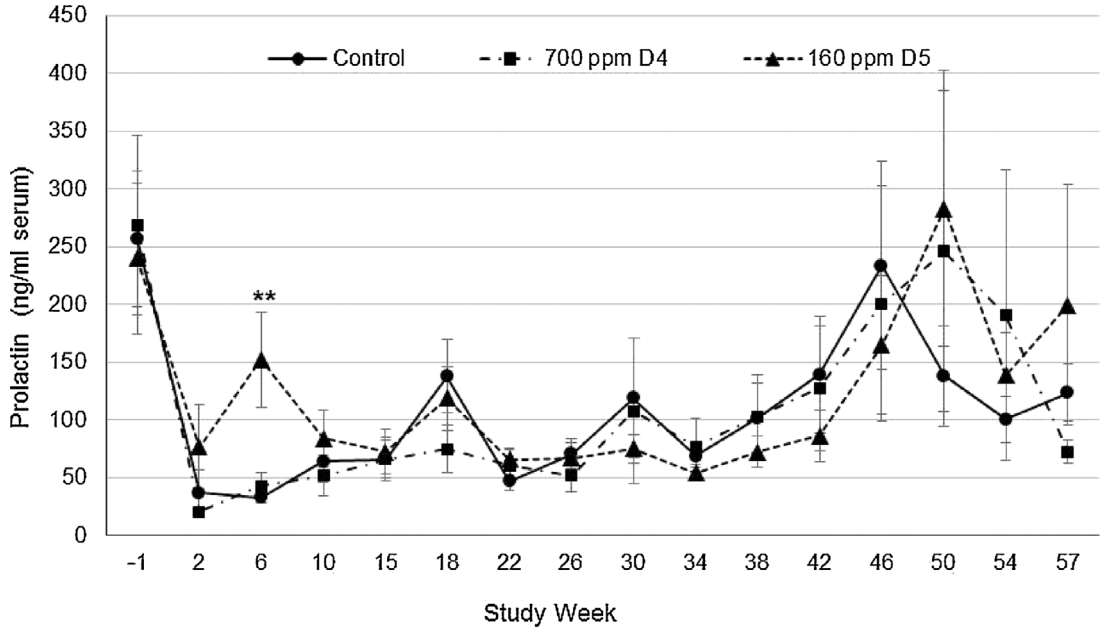


Fig. 11. Serum Prolactin Levels in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

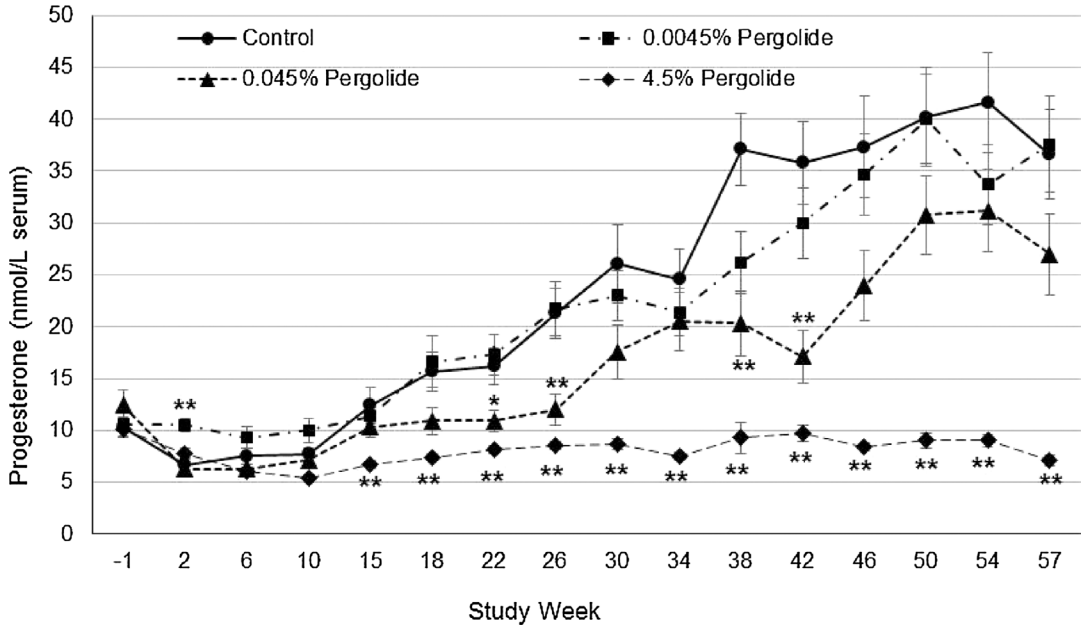


Fig. 12. Serum Progesterone Levels in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

The E:P ratio determined from the single point sampling data showed a steady decline with age in control group animals as a consequence of steady endogenous estradiol concentration and increasing progesterone concentrations. Low-dose PM was not different from controls with respect to the E:P ratio. However, both the mid- and high-dose PM treatment groups differed from the control group after the initial 6 weeks of exposure. Relative to the control group, the E:P ratios were slightly higher in the mid-dose group and markedly higher in the high-dose PM treatment group.

The D4 exposure group exhibited lower E:P ratios than the control group, as a consequence of lower endogenous estradiol levels. The age-related increase in progesterone levels in the control group was primarily responsible for minimizing the difference in E:P ratio between the control and D4 treatment groups with time. There was a slight decrease in the E:P ratio in the D5 exposure group for most of the time points during the initial 26 weeks on study. The ratio was essentially no different from control thereafter.

3.7.5. *Corticosterone levels*

Corticosterone concentrations were evaluated at each time point for all animals from all groups. The data are presented in Figs. 18 and 19.

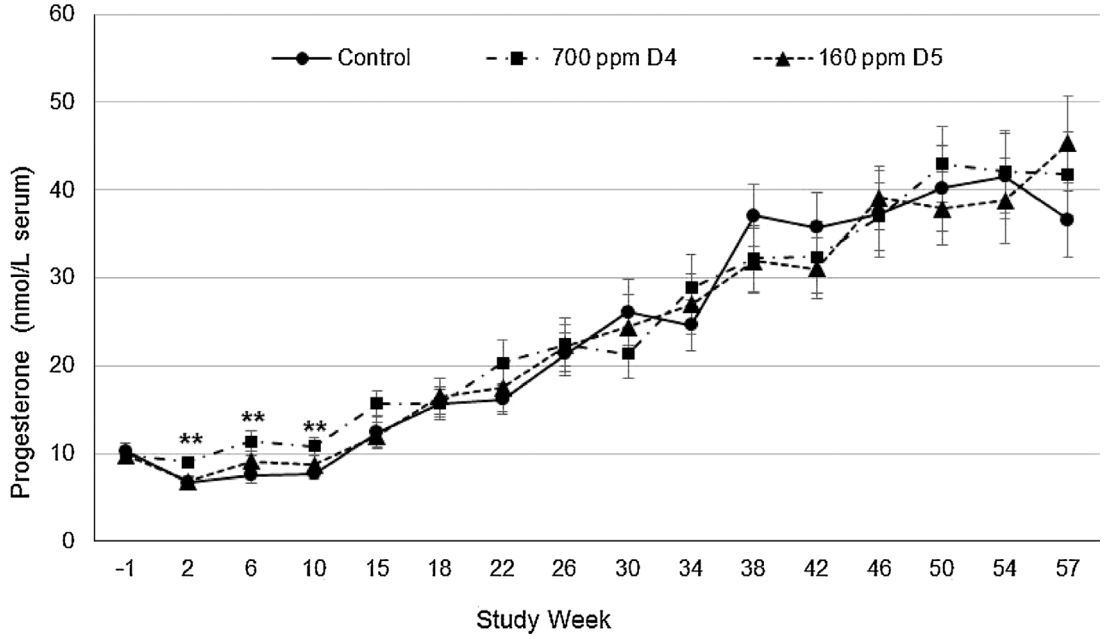


Fig. 13. Serum Progesterone Levels in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

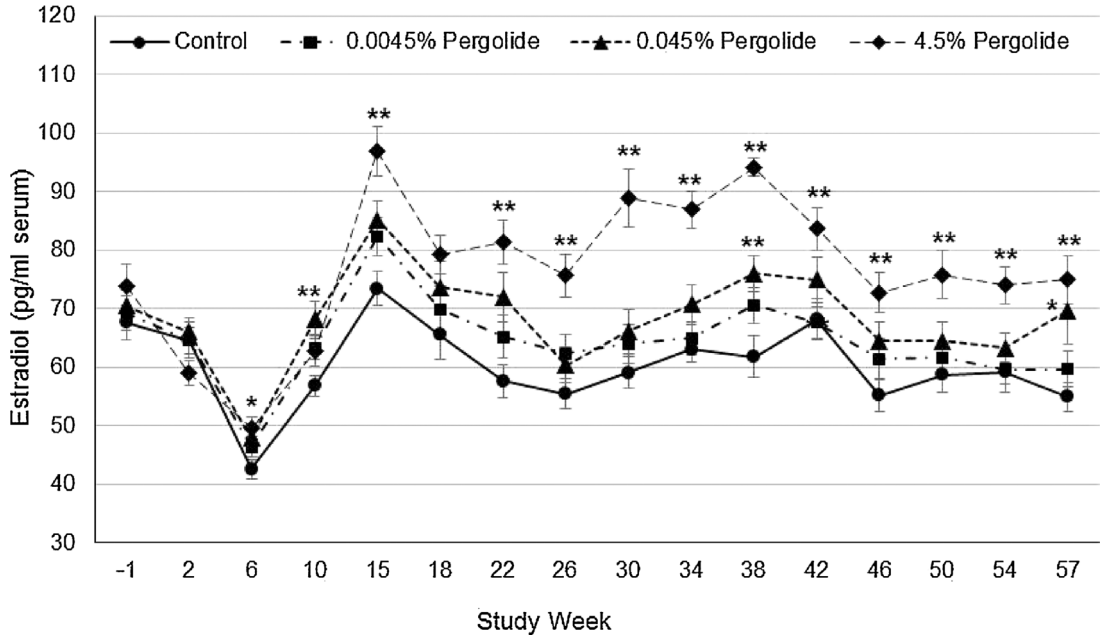


Fig. 14. Serum Estradiol Levels in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

Circulating corticosterone concentrations in all groups averaged around 95 ng/mL at the end of acclimation period, a high value perhaps reflective of a stress response associated with incomplete acclimation to the laboratory setting. The group mean circulating corticosterone concentrations in the control group remained relatively steady at about 60 ng/mL (+/−15 ng/mL) for the entire study period. PM administration gave rise to a notable and statistically significant increase in corticosterone concentration (10–20%), but only at the lowest dose. Mid- and high-dose PM groups were unaffected. Circulating corticosterone concentrations in D5-exposed animals were similar to that observed for the control group. In contrast, exposure to D4 resulted in a marked increase in corticosterone concentrations (10–30%) for almost the entire study period, weeks 2–51. Levels for the remaining 5 weeks were essentially no different than control.

4. Macroscopic examination and histomorphologic characterization of the ovary, uterus, and vagina

4.1. Control group

Microscopic examination of the ovarian, uterine, and vaginal tissue

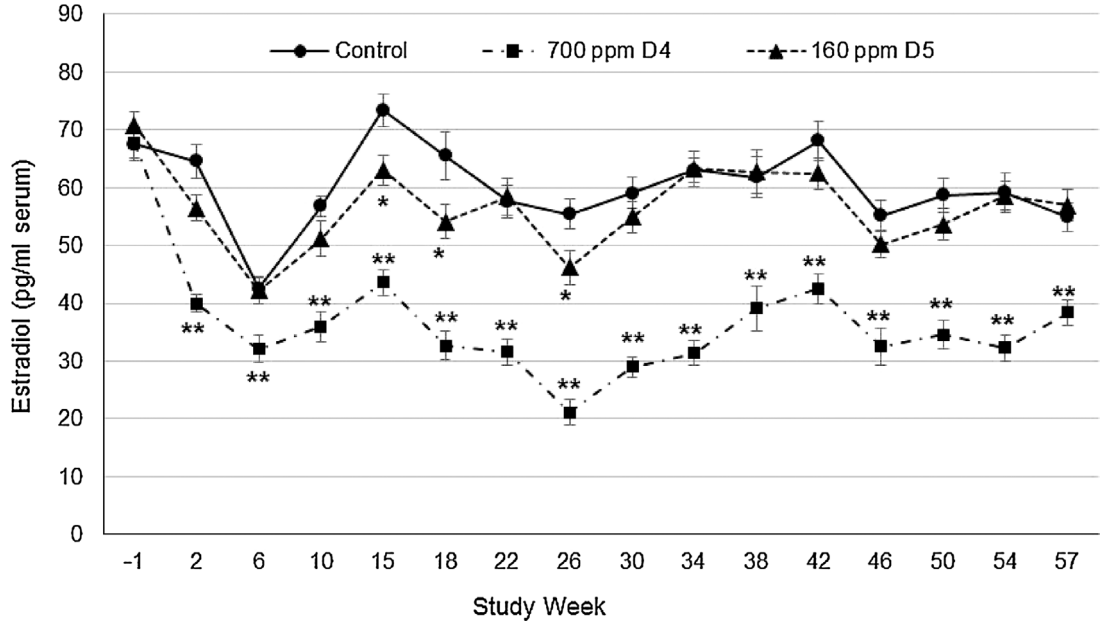


Fig. 15. Serum Estradiol in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

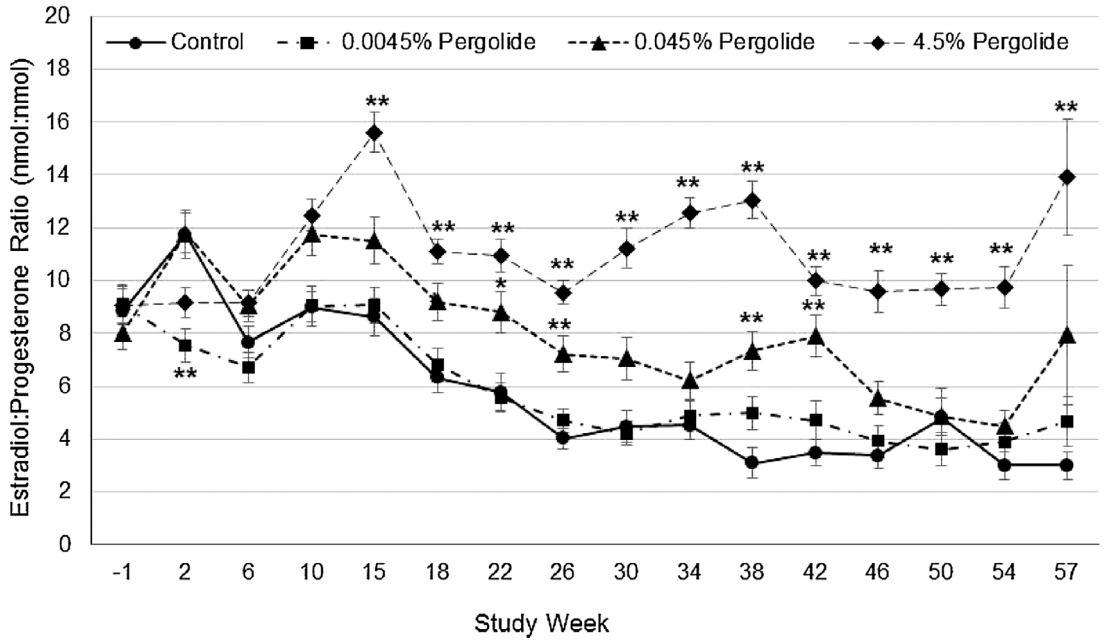


Fig. 16. Serum Estrogen:Progesterone Ratio in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

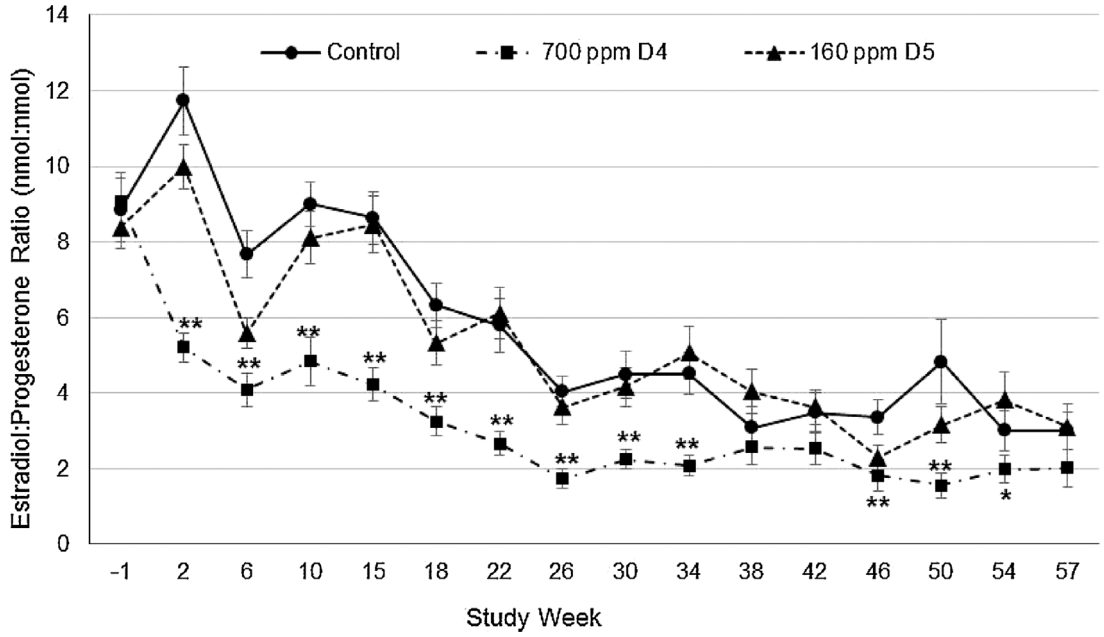


Fig. 17. Serum Estrogen:Progesterone Ratio in Control, D4, and D5 Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

from the control group animals demonstrated a profile consistent with reproductively senescent female F344 rats (Table 6). Histomorphological features of the tissues indicated that the animals were not cycling normally. The uterine profiles for all animals were indicative of diestrus I and II and there was a lack of ovarian indicators of recent ovulation. The vaginal profile was characterized by nearly all animals with mucification (minimal to severe), minimal to mild epithelial thickness, and only a few animals exhibiting cornification (minimal). These are responses expected of a progesterone-predominant influence on the tissues.

4.2. PM treatment groups

Histomorphology of the reproductive tissues at termination indicated a generally higher incidence of animals cycling normally in the PM groups as compared to the control group (Table 6). There was also a dose-related increase in the number of animals whose uterine histomorphology was indicative of proestrus/estrus in the PM treatment groups (5/43, 11/41, and 12/43 for the low-, mid-, and high-dose groups, respectively, as compared to 0/37 for the control). The incidence and severity of uterine cystic endometrial hyperplasia was

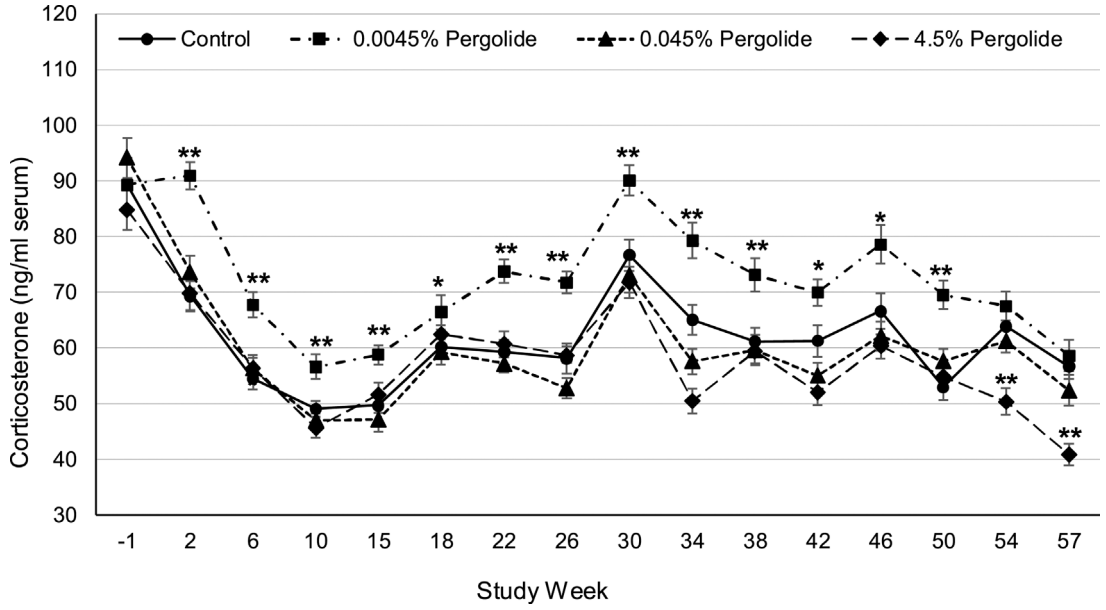


Fig. 18. Serum Corticosterone levels in Control and Pergolide Treatment Groups.
*Statistically significant at $p < 0.05$ compared to the Control Group (n = 35–50)
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

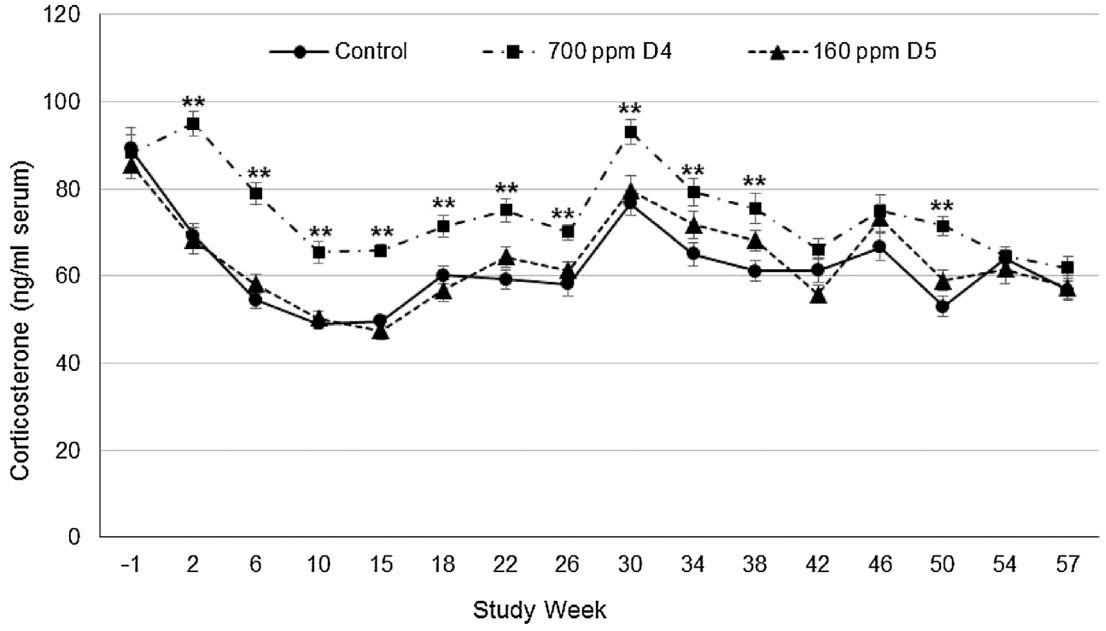


Fig. 19. Serum Corticosterone levels in Control, D4, and D5 Treatment Groups.
**Statistically significant at $p < 0.01$ compared to the Control Group (n = 35–50)
Values depict the group mean. Error bars represent the Standard Error

clearly increasing with increasing PM dose. Similarly, there was an increased incidence in the mid- and high-dose groups and severity (high dose) of dilated glands, a component of cystic endometrial hyperplasia. Vaginal findings suggestive of treatment-related effects include increases in the severity of vaginal epithelial thickness, degree of cornification, and extent of sloughed cornification, all of which are considered estrogen-predominant responses. There was also a clear dose-related decrease in the incidence of vaginal mucification among the PM treatment groups.

A number of ovarian characteristics were shown to be affected, principally at the high-dose PM. These include an increased incidence of old, eosinophillic, and basophilic incomplete corpora lutea, decreased number of antral-sized atretic follicles, increased number of primordial follicles, and increased incidence of clear cell change (vacuolation of the interstitial cells of the ovary).

4.3. D4-exposed groups

Exposure to D4 gave rise to only a few differences from control in the histomorphology of the ovary, uterus and vagina (Table 6). As with the control group, none of the animals were cycling normally at the scheduled termination and the uterine histomorphology for all animals was indicative of diestrus II. However, the incidence of cystic endometrial hyperplasia was slightly elevated compared to controls (8/37

Table 6
Histomorphologic Effects of Pergolide Mesylate, D4,and D5 Exposure.

Group:	Control	Pergolide Mesylate			D4	D5
		0.0045 ppm	0.045 ppm	4.5 ppm	700 ppm	160 ppm
Tissue/Diagnosis						
Ovary (no. examined)	**37**	**43**	**40**	**43**	**34**	**40**
Old Corpora Lutea (CL)	30	33	29	42	27	34
Eosinophilic CL	32	35	36	38	30	33
Basophilic Complete CL	4	4	4	9	5	5
Incomplete Luteinized CL	0	0	4	1	1	0
Activated CL	3	4	1	0	3	2
Increased CL (all types together)	0	0	0	21	0	0
Luteinized Follicle	0	1	1	2	3	0
Graafian Follicles[*,1]	1(1)	2(2)	2(3)	1(1)	1(1)	0(0)
mean score (includes all animals)	0.03	0.05	0.08	0.02	0.03	0
Antral Follicles[*,1]	11(14)	5(6)	10(11)	9(9)	7(12)	11(12)
mean score (includes all animals)	0.39	0.14	0.28	0.21	0.35	0.30
Basophilic Complete CL	4	4	4	9	5	5
Incomplete Luteinized CL	0	0	4	1	1	0
Activated CL	3	4	1	0	3	2
Increased CL (all types together)	0	0	0	21	0	0
Luteinized Follicle	0	1	1	2	3	0
Graafian Follicles[*,1]	1(1)	2(2)	2(3)	1(1)	1(1)	0(0)
mean score (includes all animals)	0.03	0.05	0.08	0.02	0.03	0
Antral Follicles[*,1]	11(14)	5 (6)	10 (11)	9 (9)	7 (12)	11 (12)
mean score (includes all animals)	0.39	0.14	0.28	00.21	0.35	0.30
Atretic Follicles, Antral Size[*,1]	27 (59)	29 (51)	24 (46)	22 (30)	16 (26)	25 (56)
mean score (includes all animals)	1.59	1.19	1.15	0.70	0.76	1.40
Primordial Follicles[*,1]	24 (57)	25 (49)	25 (52)	30 (71)	23 (42)	27 (53)
mean score (includes all animals)	1.54	1.14	1.30	1.65	1.24	1.33
Clear Cell Change[^]	2	3	2	8	1	1
minimal	2	2	1	8	1	1
Cyst, Follicular	0	0	0	0	0	1
Cyst, NOS	1	0	0	0	1	0
Cyst, Paraovarian	0	0	0	0	0	1
Hyperplasia, Sex Cord Stromal[^]	1	0	0	0	0	0
severe	1	na	na	na	na	na
Tumor, Granulosa Cell, Benign	0	1	0	0	0	0
Tumor, Sertoli Cell, Benign	0	1	0	0	0	0
Cystadenoma, Papillary	1	0	0	0	0	0
Uterus (w/Cervix) (no. examined)	**37**	**43**	**41**	**43**	**35**	**40**
Stage of Estrous Cycle						
Proestrus	0	2	7	6	2	2
Estrus	0	3	4	6	0	0
Diestrus I	1	0	3	4	0	1
Diestrus II	36	38	26	26	33	37
Not Determined	0	0	0	1	0	0
Adenomyosis[^]	0	0	1	0	0	0
minimal	na	na	1	na	na	na
Decidual Alteration[^]	0	2	0	0	0	0
minimal	na	1	na	na	na	na
mild	na	1	na	na	na	na
Dilatation, Lumen[^]	1	4	4	4	1	1
minimal	0	1	0	1	1	4
mild	1	1	1	0	0	0
moderate	0	1	1	3	0	1
severe	0	1	2	0	0	0
Dilated Glands[^]	1	0	3	5	2	1
minimal	1	na	3	2	2	1
mild	0	na	0	3	0	0
Epithelial Cytoplasmic Alteration[^]	0	0	0	0	1	0
minimal	na	na	na	na	Na	1
Hyperplasia, Cystic Endometrial[^]	8	10	17	18	14	20
minimal	8	8	11	8	13	17
mild	0	2	6	9	1	3
moderate	0	0	0	1	0	0
Hyperplasia, Glandular, Focal[^]	1	1	1	2	1	0
mild	0	1	1	2	0	na
severe	1	0	0	0	1	na
Hyperplasia, Squamous[^]	0	1	1	0	1	1
minimal	na	0	0	na	0	0
mild	na	1	1	na	1	1
Hyperplasia, Stromal[^]	0	1	2	1	1	2
minimal	na	1	0	0	0	1
mild	na	0	1	1	1	0

(continued on next page)

Table 6 *(continued)*

Group:	Control	Pergolide Mesylate			D4	D5
		0.0045 ppm	0.045 ppm	4.5 ppm	700 ppm	160 ppm
moderate	na	0	1	0	0	1
Metaplasia, Squamous	0	1	0	2	0	0
minimal	na	1	na	2	na	Na
Adenocarcinoma, Endometrial	0	1	0	0	0	0
Adenoma, Endometrial	2	0	0	1	0	1
Carcinoma, Squamous Cell	1	0	0	0	0	0
Leiomyoma	0	0	0	1	0	0
Leiomyosarcoma	0	0	0	0	0	1
Polyp, Endometrial Stromal	5	3	9	3	3	4
Polyp, Endometrial Stromal, multiple	2	1	0	1	0	0
Polyp, Glandular	1	0	0	2	0	1
Schwannoma, Malignant	0	0	0	1	0	0
Stromal Cell Sarcoma	1	0	0	0	0	0
Vagina (no. examined)	**37**	**43**	**41**	**42**	**35**	**40**
Epithelial Thickness^	37	43	41	42	35	40
minimal	26	28	18	18	19	27
mild	11	7	9	10	10	9
moderate	0	8	14	14	6	4
Degree of Cornification	6	6	12	15	6	4
minimal	6	3	7	7	6	3
mild		3	5	8	0	1
Sloughed Cornification^	0	1	6	6	1	1
minimal	na	1	4	6	1	1
mild	na	0	2	0	0	0
moderate	na	0	0	0	0	0
Mucification^	35	38	31	25	29	34
minimal	13	16	12	19	7	11
mild	15	11	9	6	3	8
moderate	6	9	4	0	12	13
severe	1	2	6	0	7	2
Aggregate, Granular Cell^	2	3	0	0	0	1
minimal	2	0	na	na	na	1
mild	0	3	na	na	na	0
Exudate, Luminal, Neutrophil^	20	26	19	10	15	24
minimal	16	21	15	10	12	20
mild	4	5	4	0	3	4
Hyperplasia, Basal Cell^	0	0	0	0	1	0
severe	na	na	na	na	1	na
Hyperplasia, Stromal^	0	0	1	0	0	0
mild	na	na	1	na	na	na
Polyp	0	0	0	1	0	0
Stromal Cell Sarcoma, Metastatic	1	0	0	0	0	0
Tumor, Granular	0	0	0	0	1	1
Estrous Cycle						
Normal	0	3	11	5	0	1
Abnormal	37	40	30	38	35	39

^ Graded (scale 1–4).
[1] (n) = total counted.

for control group, 14/35 for D4 group). With respect to the ovary, the only notable difference from control was a marked decrease (37%) in the incidence of antral-size atretic follicles. Vaginal tissue from the D4 treated animals presented with a shift in severity of epithelial thickness (6 animals from the D4-treatment group exhibited moderate severity whereas control group animals expressed only minimal/mild severity epithelial thickness). D4-exposed animals had a slightly lower incidence of mucification than controls, however for those D4-exposed animals expressing mucification there was a shift to moderate and severe severity.

4.4. D5-exposed groups

Only one animal exposed to D5 was cycling normally at the scheduled termination. Uterine histomorphology indicated that 37 of the females were in diestrus II, one in diestrus I, and two in proestrus (Table 6). There were no notable findings related to ovarian histomorphology to differentiate D5-exposed animals from control. There were, however, a couple of notable uterine and vaginal findings. In the uterus, there was a marked increased incidence of cystic endometrial hyperplasia (8/37 control group, 20/40 D5 group) and a slight increase in the incidence of dilated lumen (1/37 control group, 5/40 D5 group), both aspects considered to reflect recent endogenous estrogen-predominant influences. In contrast, and as seen with D4, a greater proportion of animals expressing mucification were of increased severity (13 minimal, 15 mild, 6 moderate and 1 severe in the control group as compared to 11 minimal, 8 mild, 13 moderate and 2 severe in the D5 group). Vaginal epithelial thickness appeared to shift slightly toward higher severity grades; 26 minimal, 11 mild, and 0 severe in the control verses 27 minimal, 9 mild, and 4 severe in the D5 exposed group.

5. Discussion

The effects of D4 and D5 on estrous cyclicity in the aging reproductively senescent female F344 rat were examined from 11 through 24 months of age. Chronic repeated vapor inhalation exposure to 700 ppm D4 and 160 ppm D5 induced an increase in estrous cyclicity in these aging reproductively senescent F344 rats resulting in a

significantly higher overall incidence of proestrus/estrus over the study duration. The potential implications for such changes with respect to the previously reported induction of uterine tumors in this particular strain of rat are discussed below. However it is important to note that reproductive senescence in this strain of rat differs from that of other rat strains and understanding the unique features of F344 rat reproductive senescence provides important context for examining the effects of D4 and D5 and potential relevance to humans.

Reproductive senescence develops in the aging female F344 rat in response to diminishing dopamine agonist inhibition of pituitary prolactin secretion from the hypothalamus (tuberoinfundibular dopaminergic (TIDA) neurons) (Demarest et al., 1982, 1985; Reymond, 1990). Circulating prolactin levels increase which promotes corpus luteum function and increased synthesis/release of progesterone (Demarest et al., 1982; Neumann, 1991). As a consequence the increased prolactin/progesterone levels induce a state of pseudopregnancy in which the corpora lutea persist and continue to secrete progesterone (Smith et al., 1975; Huang et al., 1976, 1978; Lu et al., 1980; Demarest et al., 1982, 1992). Levels of estrogen, LH, and FSH are typically low during pseudopregnancy (Smith et al., 1975; Huang et al., 1976, 1978; Lu et al., 1980; Demarest et al., 1982; Peluso and Gordon, 1992). As such progesterone is the predominant signal to the uterine endometrium during pseudopregnancy. The frequent episodes of pseudopregnancy and associated prolonged elevation in progesterone that characterizes reproductive senescence in the F344 rat is considered a principle factor driving the remarkably low background incidence in uterine endometrial tumors in this rat strain (Goodman et al., 1979; Goodman and Hildebrandt, 1987; Haseman et al., 1998; Solleveld et al., 1984; Maekawa et al., 1983). In F344 rats older than 24 months, the incidence of endometrial adenocarcinoma increases to about 8–12% after 30 months (Nyska et al., 1994) as they enter into a final persistent estrus stage. Additional reviews of data utilizing the F344 rat challenge the conclusion of a rare occurrence of endometrial adenocarcinomas in F344 rats younger than 24 months, particularly in certain sub-strains (Ando et al., 2008; CRL, 1990; Kuroiwa et al., 2013; Rao et al., 1990; Young and Morfeld, 2015).

In the current study the control group animals, as expected, were found to be in the reproductive senescent phase exhibiting repetitive pseudopregnancy at the start of the study. As they continued to age they demonstrated a transient shift toward cycles more typical of young animals (13–15 months of age) followed by a return to repeated pseudopregnancy for the remainder of the study. The control group animals also exhibited an age-related increase in circulating levels of prolactin and progesterone and a relatively consistent circulating level of estradiol and corticosterone in the afternoon blood sampling over the entire age range of the study. Altogether these findings in the control group females align well with expectations for the aging F344 female rat.

In addition, the dietary administration of the reference substance (PM) elicited a number of effects in these aging F344 rats consistent with that reported for dopamine agonists (Alison et al., 1994; Neumann 1991; NDA 19-385; Negishi and Koide, 1997; Estes et al., 1982; Thorner et al., 1980). These included inhibition of the age-related elevation in circulating levels of prolactin and progesterone and histologic changes indicative of increased endogenous estrogen predominance in the ovary, uterus, and vagina. There was also a marked effect on cyclicity as expected of this potent dopamine agonist. This was represented by a marked and dose-related increase in estrous cyclicity and an increased incidence of proestrus/estrus in all dose groups. Proestrus and estrus represent periods of rising levels of endogenous estrogen that can induce marked proliferation in the uterine endometrium. The increased incidence of periods of elevated endogenous estradiol, decreased endogenous progesterone, and uterine proliferation serve as the basis for an increased risk of uterine tumors from dopamine agonists in the F344 rat.

Repeated exposure of aging female F344 rats to 700 ppm D4 resulted in a marked increase in estrous cyclicity and, as a consequence, a marked increase (67%) in incidence of proestrus/estrus. The increase was the product of both an increase in the number of estrous cycles over the entire age range and an increase in the incidence of extended estrus in animals 11–18 months of age. In comparison with PM, the effect by D4 on increased estrous cyclicity was most similar to that seen with the mid-dose PM with respect to acting across the entire study period. However, unlike PM the increased incidence of extended estrus with D4 occurred earlier (11–18 months of age) than that seen with PM (≥15 months of age) and was generally coincident with the same period in which the control group exhibited the transient period of increased cyclicity. Interestingly, the induction of extended estrus by D4 (≥700 ppm D4, vapor inhalation exposure) was previously reported in young estrous cycling Sprague-Dawley rats (Siddiqui et al., 2007a; Quinn et al., 2007a). The authors concluded that D4 exposure induced a delay in the pre-ovulatory LH surge that resulted in a delay in ovulation and extended estrus. The effect of D4 on LH surge in the rat was not considered relevant to humans due to species differences in regulation of the reproductive systems (Plant, 2012; Quinn et al., 2007a; Dekant et al., 2017).

We also observed with exposure to 700 ppm D4 a modest and consistent decrease in endogenous estradiol levels in afternoon blood samples taken to monitor for generalized effects. The decrease seen with D4 was opposite to the increases seen with both mid- and high-dose PM. D4 also did not affect circulating levels of prolactin and progesterone in these same samples although both mid- and high-dose PM gave rise to significant changes in both hormones. Interestingly, circulating levels of corticosterone were elevated in the D4-exposed animals as well as in the low-dose PM exposed group, while mid- and high-dose PM groups were unaffected. It remains unclear if there are any toxicological implications associated with the lower endogenous estradiol and increased corticosterone levels following D4 exposure to 700 ppm. This is in large part due to the “point-in-time” sampling which fails to provide important context with respect to any role that either of these two hormones may have been serving at the time of blood collection and if the lower/higher levels had any biological implications. The sampling protocol was not designed to identify effects on specifically-timed surges that drive critical biological transitions, including those associated with the estrous cycle. As such, the hormone data from this study cannot address the potential impact, if any, of D4, D5, and PM on such purpose-driven modulations of these specific hormones.

Inhalation exposure to 160 ppm D5 gave rise to increased cyclicity and cumulative incidence of proestrus/estrus as a generalized outcome similar to D4. However, D5′s effects were essentially limited to the initial 6 months study period, occurring concurrent with the transient inherent increase in cyclicity as seen in the control group. Extended estrus contributed little to the overall 29% increase in cumulative incidence in proestrus/estrus and there were no notable changes in hormone levels within the hormone monitoring data except for slight decreases in circulating endogenous estradiol early in the study. Estrous cyclicity (and reproductive performance) was also not affected by inhalation exposure of young female Sprague-Dawley rats to D5 (0, 30, 70, and 160 ppm) in a two-generation reproductive toxicity study (Siddiqui et al., 2007b).

Analysis of the cumulative incidence of estrogen-predominant days over time identified two transition points. Though it is unclear what, if any, biological significance is represented by these transitions, the data show that D5-exposed animals experience these transitions at an earlier age. D4 had no impact on the transition points whereas PM demonstrated the potential to delay the timing of the transition.

The findings from the current study demonstrated that repeated exposure to 700 ppm D4 increased the incidence of estrous cycle in aged F344 rats that were otherwise exhibiting a low rate of estrous cyclicity among repeated episodes of pseudopregnancy typical of this age and strain of rat. The implications for this effect specific to the F344

rat include a higher risk of uterine adenoma, as a consequence of increased uterine stimulation (proestrus/estrus) associated with the additional estrous cyclicity as well as the reported increased incidence of extended estrus. Because of the nature of reproductive senescence in the F344 rat, this particular strain of rat has been shown to be susceptible to increased uterine tumor risk from dopamine agonists that increase endogenous estrogenic stimulation of the uterine endometrium as a consequence of reduced pituitary gland prolactin secretion. Uterine tumors arising from a dopamine agonism mode of action in the F344 rat are not considered relevant to humans due to species differences in regulation of the reproductive system generally, as well as differences in the role/activity of prolactin specifically.

A dopamine-related mode-of-action was considered as an explanation for the observed effects of D4 and D5 on the uterus in rats after chronic inhalation exposure to 700 ppm D4 (Dekant et al., 2017) or 160 ppm D5 (Klaunig et al., 2016a,b). However, after a review of the results from this study and a series of *in vitro* studies to evaluate the ability of D4 and D5 to stimulate prolactin release from specific cells and evaluate their affinity for dopamine receptors, the authors concluded that it is unlikely for D4 or D5 to interact directly with dopamine receptors. However, the authors indicated the results may suggest an indirect interaction with the dopamine pathway distal to the receptor.

The shift from pseudopregnancy to increased estrous cyclicity for D4 may be related to a more non-specific mechanism of cycle disruption derived from inhalation exposure to the high vapor concentrations of D4. The 700 ppm D4 exposure concentration was selected for investigation in this current study because it was the highest exposure level in the chronic bioassay and importantly the only exposure concentration to have indicated an increased risk of uterine tumor formation. However this concentration appeared to be above the kinetically-derived maximal dose indicating that the systemic dose derived from exposure to 700 ppm D4 exceeded the constitutive ADME processes and capacity (Jean and Plotzke, 2016). Toxicity occurring only under such conditions is of doubtful relevance to risk assessment when, as is true for D4, environmental, consumer, and workplace exposures are substantially lower than 700 ppm.

Chronic exposure to 160 ppm D5 in the two-year bioassay resulted in a low incidence of uterine adenocarcinoma. This was the highest concentration administered and the only exposure level to indicate a potential for uterine tumors. Background incidence of uterine adenocarcinoma in the F344 rat appears to be highly variable and sub-strain-dependent. The specific sub-strain of F344 rat used in the bioassay studies has been shown to have a higher spontaneous incidence than some other F344 sub-strains. As a spontaneous tumor, the incidence appears to be age related with incidence rates increasing markedly with advanced age (> 24 months). In the current study, 160 ppm D5 induced an increase in the estrous cyclicity in these aging F344 rats exhibiting repeated episodes of pseudopregnancy. However, unlike D4, the effect of D5 was specific to the initial 4.5 months of the study (11–15 months of age). The significance of the timing of D5′s effect, if any at all, is uncertain. However, the overall response to D5, like that with D4, suggests that D5 was not acting as a dopamine agonist.

If the shift in timing of the transitions by D5 represents a quickening of the progression of reproductive senescence then this observation may have some relevance with regard to the increased incidence of uterine adenocarcinoma in the chronic bioassay. This is a spontaneous tumor in aged F344 rats that is often not seen before 24 months of age in this strain of rat, although it is more common in the specific sub-strain of rat used in the bioassay studies. This is consistent with Klaunig et al. (2016a,b) who concluded that “the slight increase in uterine endometrial adenocarcinomas observed in the D5 chronic bioassay might not be the result of D5 exposure but may be related to variability of the spontaneous tumor incidence in this strain of rat”.

In conclusion, this study characterized the effect of 700 ppm D4 and 160 ppm D5 on estrous cyclicity in aging reproductively senescent F344 rats. Both substances, at very high exposure concentrations relative to consumer, workplace, and environmental exposures, induced an increase in estrous cycle repetition and, as a consequence, the overall incidence of proestrus/estrus. Though D4 and D5 appear not to be dopamine agonists, the induced increase in cyclicity in repetitive pseudopregnant F344 rats could represent a dopamine agonism-like mode of action from a non-specific mechanism. Though a dopamine agonism-mode of action or a dopamine agonism-like mode of action is relevant to the F344 rat it is not at all relevant to humans due to the significant species differences in regulation of the reproductive system and reproductive senescence (Alison et al., 1994; Gopinath, 1999; Neumann, 1991; Plant, 2012). Additionally, there is a significant difference between the exposure concentration required to induce the presumed uterine tumor response in the F344 rat as compared with the markedly lower environmental, consumer, and workplace exposures of D4 and D5.

Due to the marked species differences in the regulatory control of the reproductive system and senescence in F344 rats and the high dose nature of these effects, it is unlikely that effects observed here and in the D4 and D5 chronic bioassays have any relevance for assessing risks to humans.

Acknowledgements

This research was sponsored by the Silicones, Environmental, Health and Safety Center. The authors wish to thank the technical staff at WIL Research LLC (currently known as Charles River Ashland) for their efforts and expertise in performing this work. In addition the authors would like to recognize Dr. Karen Regan of Regan Path/Tox Services for her expert histomorphologic assessment of the reproductive tissues for hormonal influences. Such expertise is not common and we greatly appreciate Dr. Regan’s contributions to this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxlet.2017.08.016.

References

AALAC, 1991. American Association for Laboratory Animal Science Policy on the Humane Care and Use of Laboratory Animals. pp. 41–91.
Alison, R.H., Capen, C.C., Prentice, D.E., 1994. Neoplastic lesions of questionable significance to humans. Toxicol. Pathol. 22 (2), 179–186.
Andersen, M.E., Reddy, M.B., Plotzke, K.P., 2005. Lack of bioaccumulation with repeated, periodic exposures of cyclic siloxanes. Abstract #855. Toxicol. CD 84 (S-1) (March 2005).
Andersen, M.E., Reddy, M.B., Plotzke, K.P., 2008. Are highly lipophilic compounds expected to bioaccumulate with repeated exposures? Toxicol. Lett. 179, 85–92.
Ando, R., Nakamura, A., Nagatani, M., Yamakawa, S., Ohira, T., Takagi, M., Matsushima, K., Aoki, A., Fujita, Y., Tamura, K., 2008. Comparison of past and recent historical control data in relation to spontaneous tumors during carcinogenicity testing in F344 rats. J. Toxicol. Pathol. 21, 53–60.
CRL, 1990. Charles River Laboratories, Spontaneous Neoplastic Lesions in the CDF® (F-344)/CrlBR Rat.
Dekant, W., Klaunig, J.E., 2016. Toxicology of decamethylcyclopentasiloxane (D5). Regul. Toxicol. Pharmacol. 74, S67–76.
Dekant, W., Scialli, A.R., Plotzke, K., Klaunig, J.E., 2017. Biological Relevance of Effects Following Chronic Administration of Octamethylcyclotetrasiloxane (D4) in Fischer 344 Rats. (Submitted to Tox Letters as companion paper).
Demarest, K., Moore, K., Riegle, G., 1982. Dopaminergic neuronal function, anterior pituitary dopamine content, and serum concentrations of prolactin, luteinizing hormone and progesterone in the aged female rat. Brain Res. 247 (2), 347–354.
Demarest, K.T., Moore, K.E., Riegle, G.D., 1985. Adenohypophysial dopamine content and prolactin secretion in the aged male and female rat. Endocrinology 116, 1316–1323.
Dunn, O.J., 1964. Multiple comparisons using rank sums. Technometrics 6 (3), 241–252.
Dunnett, C.W., 1964. New tables for multiple comparisons with a control. Biometrics 20, 482–491.
Estes, K.S., Simpkins, J.W., Kalra, A.P., 1982. Normal LHRH neuronal function and hyperprolactinemia in old pseudopregnant Fischer 344 rats. Neurobiol. Aging 3, 247–252.
Franzen, A., Greene, T., Van Landingham, C., Kinslow, C., Gentry, R., 2017. Toxicology of Octamethylcyclotetrasiloxane (D4). (Submitted to Tox Letters as companion paper).
Gopinath, C., 1999. In: Harvey, P.W., Ruch, K.C., Cockburn, A. (Eds.), Comparative

Endocrine Carcinogenesis in Endocrine and Hormonal Toxicology. John Wiley & Sons Ltd., pp. 155–167.
Goodman, D.G., Hildebrandt, P.K., 1987. Adenocarcinoma, endometrium, rat. In: Jones, T.C., Mohr, U., Hunt, R.D. (Eds.), Genital System (Monograph on Pathology of Laboratory Animals). Springer, Berlin Heidelberg, pp. 80–82.
Goodman, D.G., Ward, J.M., Squire, R.A., Chu, K.C., Linhart, M.S., 1979. Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol. Appl. Pharmacol. 48 (2 (April)), 237–248.
Haseman, J.K., Hailey, J.R., Morris, R.W., 1998. Spontaneous Neoplasm Icidences in Fischer 344 Rats and B6C3F1 Mice in Two-Year Carcinogenicity Studies: A national Toxicology Program Update. Toxicol. Pathol. 26 (3), 428–441.
He, B., Rhodes-Brower, S., Miller, M.R., Munson, A.E., Germolec, D.R., Walker, V.R., Korach, K.S., Meade, B.J., 2003. Octamethylcyclotetrasiloxane exhibits estrogenic activity in mice via ERalpha. Toxicol. Appl. Pharmacol. 192 (3), 254–261.
Huang, H.-H., Marshall, S., Meites, J., 1976. Capacity of Old Versus Young Rats to Secrete LH, FSH and Prolactin. Biol. Reprod. 14, 538–543.
Huang, H., Steger, R., Bruni, J., et al., 1978. Patterns of sex steroid and gonadotropin secretion in aging female rats. Endocrinology 103, 1855.
Jean, P.A., Plotzke, K.P., 2016. Chronic toxicity and oncogenicity of octamethylcyclotetrasiloxane (D4) in the Fischer 344 rat. Toxicol. Lett.
Jean, P.A., Plotzke, K.P., Scialli, A.R., 2016. Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 rat. Regul. Toxicol. Pharm. 74, S57–S66.
Jean, P.A., 2005. Non-regulated Study: Effects of Decamethylcyclopentasiloxane (D5) on Cell Proliferation in the Liver of Female Fischer 344 Rats: a 28-day Inhalation Study Report on D5 Hypertrophy/hyperplasia, USEPA-OPPT. TSCA Document Processing Center, Washington, D.C (Document Control Number: FYI-0305-01491A).
Jovanovic, M.L., McMahon, J.M., McNett, D.A., Tobin, J.M., Plotzke, K.P., 2008. In vitro and in vivo percutaneous absorption of 14C-octamethylcyclotetrasiloxane (14C-D4) and 14C-decamethylcyclopentasiloxane (14C-D5). Regul. Toxicol. Pharmacol. 50 (2), 239–248.
Klaunig, J.E., Dekant, W., Plotzke, K., Scialli, A.R., 2016a. Biological relevance of decamethylcyclopentasiloxane (D5) induced rat uterine endometrial adenocarcinoma tumorigenesis: mode of action and relevance to humans. Regul. Toxicol. Pharmacol. S44–S56.
Klaunig, J.E., Dekant, W., Plotzke, K., Scialli, A.R., 2016b. Biological relevance of decamethylcyclopentasiloxane (D5) induced rat uterine endometrial adenocarcinoma tumorigenesis: mode of action and relevance to humans. Regul. Toxicol. Pharmacol. 74 (Suppl), S44–S56. http://dx.doi.org/10.1016/j.yrtph.2015.06.021. (Epub 2015 Jul 3).
Kuroiwa, Y., Ando, R., Kasahara, K., Nagatani, M., Yamakawa, S., Okazaki, S., 2013. Transition of historical control data for high incidence tumors in f344 rats. J. Toxicol. Pathol. 26, 227–230.
YLee, D., Ahn, C., An, B., Jeung, E., 2015. Induction of the estrogenic marker calbindn-D9k by octamethylcyclotetrasiloxane. Int. J. Environ. Res. Public Health 12 (11), 14610–14625.
Lu, J.K.H., Damassa, D.A., Gilman, D.P., et al., 1980. Differential patterns of gonadotropin responses to ovarian steroids and to LH-releasing hormone between constant-estrous and pseudopregnant states in aging rats. Biol. Reprod. 23, 345–351.
Maekawa, A., Kurokawa, Y., Takahashi, M., Kokubo, T., Ogiu, T., Onodera, H., Tanigawa, H., Ohno, Y., Furukawa, F., Hayashi, Y., 1983. Spontaneous tumors in F344/DuCrj rats. Gann 74, 365–372.
McKim Jr., J.M., Wilga, P.C., Kolesar, G.B., Choudhuri, S., Madan, A., Dochterman, L.W., Breen, J.G., Parkinson, A., Mast, R.W., Meeks, R.G., 1998. Evaluation of octamethylcyclotetrasiloxane (D_4) as an inducer of rat hepatic microsomal cytochrome P450, UDP-glucuronyl transferase, and epoxide hydrolase: a 28-day inhalation study. Toxicol. Sci. 41 (1), 29–41.
McKim Jr., J.M., Choudhuri, S., Wilga, P.C., Madan, A., Burns-Naas, L.A., Gallavan, R.H., Mast, R.W., Naas, D.J., Parkinson, A., Meeks, R.G., 1999. Induction of hepatic xenobiotic metabolizing enzymes in female fischer-344 rats following repeated inhalation exposure to decamethylcyclopentasiloxane (D_5). Toxicol. Sci. 50, 10–19.
McKim Jr., J.M., Kolesar, G.B., Jean, P.A., Meeker, L.S., Wilga, P.C., Schoonhoven, R., Swenberg, J.A., Goodman, J.I., Gallavan, R.H., Meeks, R.G., 2001a. Repeated inhalation exposure to octamethylcyclotetrasiloxane produces hepatomegaly, transient hepatic hyperplasia, and sustained hypertrophy in female Fischer 344 rats in a manner similar to phenobarbital. Toxicol. Appl. Pharmacol. 172, 83–92.
McKim Jr., J.M., Wilga, P.C., Breslin, W.J., Plotzke, K.P., Gallavan, R.H., Meeks, R.G., 2001b. Potential estrogenic and antiestrogenic activity of the cyclic siloxane octamethylcyclotetrasiloxane (D4) and the linear siloxane hexamethyldisiloxane (HMDS) in immature rats using the uterotrophic assay. Toxicol. Sci. 63, 37–46.
McMahon, J.M., Plotzke, K.P., Jovanovic, M.L., McNett, D.A., Gallvan, R.H., Meek, R.G., 2001. In vitro absorption of decamethylcyclopentasiloxane (D5) in human skin: a comparison octamethylcyclotetrasiloxane (D4). Toxicol. Sci. 54 (S-1) (Abstract 701).
Meeks, R.G., Stump, D.G., Siddiqui, W.H., Holson, J.F., Plotzke, K.P., Reynolds, V.L., 2007. An inhalation reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in female rats using multiple and single day exposure regimens. Reprod. Toxicol. 23, 192–201.
NDA 19-385, 2017a. New Drug Application Obtained from the Center for Drug Evaluation and Research. Office of Regulatory Policy, Division of Information Disclosure policy.
NDA 17-962, 2017b. New Drug Application Obtained from the Center for Drug Evaluation and Research. Office of Regulatory Policy, Division of Information Disclosure policy.
NDA 20-658, 2017c. New Drug Application Obtained from the Center for Drug Evaluation and Research. Office of Regulatory Policy, Division of Information Disclosure policy.
NDA 20-664, 2017d. New Drug Application Obtained from the Center for Drug Evaluation and Research. Office of Regulatory Policy, Division of Information Disclosure policy.
NDA 20-667, 2017e. New Drug Application Obtained from the Center for Drug Evaluation and Research. Office of Regulatory Policy, Division of Information Disclosure policy.
Negishi, H., Koide, S.S., 1997. Prevention and termination of pregnancy in rats by cabergoline, a dopamine agonist. J. Reprod. Fertil. 109, 103–107.
Neumann, F., 1991. Early indicators for carcinogenesis in sex-hormone-sensitive organs. Mutat. Res. 248, 342–356.
Nyska, A., Klein, T., Scolink, M., Waner, T., Klein, B., 1994. Unusually high incidence of spontaneous endometrial adenocarcinoma in aged virgin fischer rats. Exp. Toxic Pathol. 46, 7–9.
Plant, T.M., 2012. A comparison of the neuroendocrine mechanisms underlying the initiation of the preovulatory LH surge in the human, Old World monkey and rodent. Front. Neuroendocrinol. 33 (April (2)), 160–168. http://dx.doi.org/10.1016/j.yfrne.2012.02.002. (2012).
Plotzke, K.P., McMahon, J.M., Hubbell, B.G., Meeks, R.G., Mast, R.W., 1994. Dermal absorption of 14C-decamethylcyclopentasiloxane (D5) in rats. Toxicologist 14, 434 (Abstract 1720).
Plotzke, K.P., Utell, M.J., Looney, J.R., 2000. Absorption, Distribution, and Elimination of 13C-D5 in Humans After Dermal Administration EPA Document 840300000008.
Plotzke, K.P., Utell, M.J., Looney, J.R., 2002. Absorption, Distribution, and Elimination of 13C-D4 in Humans After Dermal Administration EPA Document 860100000007.
Peluso, J.J., Gordon, L.R., 1992. In: In: Mohr, U., Dungworth, D.L., Capen, C.C. (Eds.), Nonneoplastic and Neoplastic Changes in the Ovary. Pathobiology of the Aging Rat 1. Washington D.C., ILSI Press, pp. 351–364.
Quinn, A.L., Dalu, A., Meeker, L.S., Jean, P.A., Meeks, R.G., Crissman, J.W., Gallavan Jr., R.H., Plotzke, K.P., 2007a. Effects of octamethylcyclotetrasiloxane (D4) on the luteinizing hormone (LH) surge and levels of various reproductive hormones in female Sprague-Dawley rats. Reprod. Toxicol. 23 (4), 532–540.
Quinn, A.L., Regan, J.M., Tobin, J.M., Marinik, B.J., McMahon, J.M., McNett, D.A., Sushynski, C.M., Crofoot, S.D., Jean, P.A., Plotzke, K.P., 2007b. In vitro and in vivo evaluation of the estrogenic, androgenic, and progestagenic potential of two cyclic siloxanes. Toxicol. Sci. 96, 145–153.
Reddy, M.B., Looney, R.J., Utell, M.J., Plotzke, K.P., Andersen, M.E., 2007. Modeling of human dermal absorption of octamethylcyclotetrasiloxane (D(4)) and decamethylcyclopentasiloxane (D(5)). Toxicol. Sci. 99 (2), 422–431.
Reddy, M.B., Dobrev, I.D., McNett, D.A., Tobin, J.M., Utell, M.J., Morrow, P.E., Domoradzki, J.Y., Plotzke, K.P., Andersen, M.E., 2008. Inhalation dosimetry modeling with decamethylcyclopentasiloxane in rats and humans. Toxicol. Sci. 105 (2), 275–285.
Reymond, M., 1990. Age-related loss of the responsiveness of the tuberoinfundibular dopaminergic neurons to prolactin in the female rat. Neuroendocrinology 52 (5), 490–496.
Richardson, B.P., Turkalj, I., Fluckiger, E., 1984. Bromocriptine. In: Laurence, Mclean, A.E.M., Weatherall, M. (Eds.), In Safety Testing of New Drugs. Academic Press, London, pp. 19–63.
Rao, G., Boorman, G., 1990. History of the F344 rat. In: Pathology of the Fischer Rat. Reference and Atlas (G. Boorman, S. Eustis, M. Elwell, C. Montgomery Jr, W. MacKenzie (eds.), Academic Press, New York, pp. 5–8.
Sarangapani, R., Teeguarden, J., Andersen, M.E., Reitz, R.H., Plotzke, K.P., 2003. Route-specific differences in distribution characteristics of octamethylcyclotetra-siloxane in rats: analysis using PBPK models. Toxicol. Sci. 71 (2003), 41–52.
Siddiqui, W.H., Stump, D.G., Plotzke, K.P., Holson, J.F., Meeks, R.G., 2007a. A two-generation reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in rats exposed by whole-body vapor inhalation. Reprod. Toxicol. 23 (February (23)), 202–215 (2007).
Siddiqui, W.H., Stump, D.G., Reynolds, V.L., Plotzke, K.P., Holson, J.F., Meeks, R.G., 2007b. A two-generation reproductive toxicity study of decamethylcyclopenta-siloxane (D5) in rats exposed by whole-body vapor inhalation. Reprod. Toxicol. 23 (2), 216–225.
Smith, M., Freeman, M., Neill, J., 1975. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: Prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 96, 219–226.
Snedecor, G.W., Cochran, W.G., 1980. One-Way classifications; analysis of variance. In Statistical Methods, 7th edn. The Iowa State University Press, Ames, IA, pp. 215–237.
Solleveld, H.A., Haseman, J.K., McConnell, E.E., 1984. Natural history of body weight gain, survival, and neoplasia in the F344 rat. J. Natl. Cancer Inst. 72, 929–940.
Thorner, M.O., Fluckiger, E., Caine, D.B., 1980. Bromocriptine: A Clinical and Pharmacological Review. Raven Press, New York, NY, pp. 15–43.
Tobin, J.M., McNett, D.A., Durham, J.A., Plotzke, K.P., 2008. Disposition of decamethylcyclopentasiloxane in Fischer 344 rats following single or repeated inhalation exposure to 14C-decamethylcyclopentasiloxane (14C-D5). Inhal. Toxicol. 20 (5), 513–531.
Varaprath, S., McMahon, J.M., Plotzke, K.P., 2003. Metabolites of hexamethyldisiloxane and decamethylcyclopentasiloxane in Fischer 344 rat urine: a comparison of a linear and a cyclic siloxane. Drug Metab. Dispos. 31 (2), 206–214.
Young, L.J., Morfeld, P., 2015. Statistical considerations for a chronic toxicity study: exposure to decamethylcyclopentasiloxane (D5) and incidence of endometrial adenocarcinomas in a 2-Year inhalation study with fischer rats.
Zhang, J., Falany, J.L., Xie, X., Falany, C.N., 2000. Induction of rat hepatic drug metabolizing enzymes by dimethylcyclosiloxanes. Chem.-Biol. Interact. 124, 133–147.