Regulatory Toxicology and Pharmacology xxx (2015) 1–10

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat

Paul A. Jean [a, *], Kathleen P. Plotzke [a], Anthony R. Scialli [b]

[a] Dow Corning Corporation, 2200 West Salzburg Road, Midland, MI, 48686-0994, USA
[b] Scialli Consulting, 3033 Wilson Blvd, Arlington, VA, 22201, USA

ARTICLE INFO

Article history:
Received 8 May 2015
Accepted 22 June 2015
Available online xxx

Keywords:
Siloxane
Decamethylcyclopentasiloxane
Oncogenicity
Bioassay
Adenocarcinoma
Reproductive
Uterine

ABSTRACT

Decamethylcyclopentasiloxane (D5) is a cyclic polydimethylsiloxane used in the synthesis of silicon-based materials and as a component in consumer products. Male and female Fischer 344 rats were exposed to D5 vapor (0, 10, 40, 160 ppm; whole-body inhalation) for 6 h/d, 5 d/wk, for up to 104 weeks. Microscopic examination of tissues revealed test article effects at 160 ppm in the upper respiratory tract (hyaline inclusions in males and females at 6, 12, and 24 months) and an increased incidence of uterine endometrial adenocarcinoma at 24-months. The hyaline inclusions were considered a non-adverse tissue response for lack of any other respiratory tract non-neoplastic or neoplastic changes. Uterine endometrial adenocarcinoma was not anticipated. Toxicity testing (mutagenicity/genotoxicity, acute, sub-acute and sub-chronic descriptive toxicity) performed prior to the conduct of the chronic bioassay provided no indication that the uterus was a potential target organ. The target organ and tumor type specificity (adenocarcinoma is a common spontaneous tumor in the aged Fischer 344 rat) suggests the effect is associated with estrous cycle alteration. A robust assessment of potential mode(s) of action responsible for the uterine tumors and relevance to humans is addressed in a companion manuscript (Klaunig et al., 2015).

1. Introduction

Polysiloxanes are high-performance synthetic materials of commercial interest due to their unique chemical and physical properties. As a polymer of "siloxane units," two silicon atoms covalently bound to one oxygen atom (-Si-O-Si-), polysiloxanes can vary greatly in degree of polymerization and configuration (linear, cyclic, and three dimensional structures). The most basic and abundant polysiloxanes are polydimethylsiloxanes, in which two methyl groups are covalently bound to each silicon atom ($-(CH_3)_2SiO-$).

Decamethylcyclopentasiloxane (D5) is a cyclic siloxane of five siloxane units (Fig. 1). It is a volatile, colorless, and odorless liquid with low water solubility (Table 1). D5 is used primarily in the synthesis of larger siloxane polymers and also used in consumer and industrial products. This range of uses leads to potential human exposure, especially by the dermal and inhalation routes. A comprehensive testing program has been conducted utilizing primarily the inhalation route. This was warranted based on 1) relevance to human exposure, 2) the technical challenges associated with spreading/evaporative losses following dermal application, 3) D5's low dermal absorption potential (Jovanovic et al., 2008), and 4) the similarity in kinetic behavior between the dermal and inhalation routes (Sarangapani et al., 2003).

Studies, primarily in the rat, have included general descriptive toxicity studies as well as reproductive and immune system toxicology, disposition, and toxicokinetics of D5. These studies have revealed that D5 is not extensively absorbed by oral, dermal, or inhalation routes of exposure (Plotzke et al., 1994; Jovanovic et al., 2008; McMahon et al., 2001; Reddy et al., 2007, 2008; Tobin et al., 2008; Varaprath et al., 2003). Principal routes of elimination are by exhalation of parent and urinary excretion of metabolites. Partitioning into fat occurs readily; however, bioaccumulation has not been demonstrated due to rapid exhalation elimination and metabolism (Andersen et al., 2005, 2008). As is the case for phenobarbital, D5 has been shown to induce liver enlargement, hepatocellular hypertrophy/hyperplasia, and hepatic cytochrome P450 induction (CYP2B1/2 primarily) in the rat (McKim et al., 1999;

* Corresponding author. Dow Corning Corporation 2200 West Salzburg Road Midland, Michigan 48686-0994, USA.
E-mail address: paul.a.jean@dowcorning.com (P.A. Jean).

http://dx.doi.org/10.1016/j.yrtph.2015.06.014

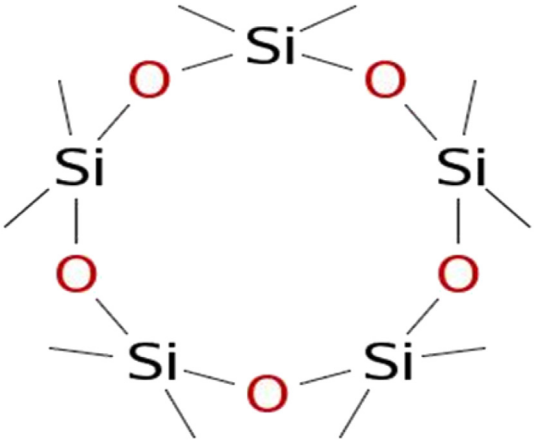

Fig. 1. Structure of decamethylcyclopentasiloxane.

Jean, 2005). D5 has been shown to function as a ligand for the pregnane × receptor (PXR) and constitutive androstane receptor (CAR) (Jean et al., 2006; Dow Corning Corporation, 2005a, 2005b). These effects have been considered non-adverse and adaptive due to the lack of apparent liver dysfunction and the reversibility seen upon cessation of exposure. D5 was without adverse reproductive and developmental effects in a two-generation reproductive study in rats (Siddiqui et al., 2007). D5 was also without estrogenic, androgenic or progestogenic activity in a series of *in vitro* and *in vivo* assays (Quinn et al., 2007). D5 has not demonstrated mutagenic/genotoxic potential in a battery of standardized testing (Bacterial Reverse Mutation Assay, In Vitro Mammalian Cell Gene Mutation Assay, In Vitro Mammalian Chromosomal Aberration Assay, In Vitro Sister Chromatid Exchange Assay, and In Vivo Micronucleus Assay).

This manuscript presents the results of the chronic toxicity/oncogenicity study of D5 in the Fischer 344 rat (F344). Potential modes of action that may be responsible for the unexpected uterine neoplastic response are suggested, however a detailed evaluation of specific modes of action are presented in a companion manuscript (Klaunig et al., 2015).

2. Materials and methods

2.1. Test material

D5 was obtained from Dow Corning Corporation (Midland, MI). Purity was determined prior to initiating exposures and at the start and end of sampling of each drum. Purity was greater than 99.4% by gas chromatography utilizing both thermal conductivity and mass selective detection.

2.2. Test species and animal husbandry

Male and female CDF F344 rats were purchased from Charles River Laboratories (Raleigh, NC) and were 6–8 weeks of age at initiation of exposure. Animals were housed in Makrolon® type IV cages at up to 5 animals/cage during the non-exposure periods and transferred daily to suspended wire-mesh exposure cages (2/cage) for the exposure. Food (Kliba 3433 rat maintenance diet, Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) and municipal water were provided *ad libitum* with the following exceptions. Food and water were withheld during the daily exposure period and food was withheld the night prior to blood collections, and during urine collection. Animals were housed in three rooms, one for controls, one for 10 and 40 ppm groups, and one for the 160 ppm D5 group. Recovery group animals were housed with controls during the second year. A 12 h/12 h light (fluorescent) and dark cycle was used. Animal room temperature (target range of 22 ± 3 °C and relative humidity (target range of 40–70%) were continuously monitored. Animals were observed at least twice daily for morbidity, moribundity and mortality throughout the study.

The study complied with all applicable 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).

Table 1
Physical and chemical properties of decamethylcyclopentasiloxane.

Property	D5
Chemical Abstract Number	541-02-6
Molecular Weight (daltons)	371
Boiling Point	211 °C
Melting Point	−44 °C
Saturated Vapor Concentration at 23 °C	195 ppm (2.9 mg/L)
Water Solubility at 23 °C	17 ppb

2.3. Inhalation exposure

Exposures were conducted five days/week for up to 24 months, excluding holidays, in whole-body inhalation chambers. Daily exposures consisted of a 6-h period at the target exposure concentration sandwiched between chamber equilibration times during which the chamber concentrations were increasing to 90% or decreasing to 10% of the target. The average daily mean chamber temperature (°C)/relative humidity (%) for the duration of the study were 24/48, 24/45, 25/45, and 25/44 for the control, 10, 40, 160 ppm exposure groups, respectively. Cage rack positions were rotated weekly to limit positional effects. Adequate chamber oxygen concentration and test atmosphere homogeneity were demonstrated periodically throughout the 24 month exposure period. Determination of test atmosphere concentration was performed by gas chromatography with flame ionization detection (GC/FID).

The maximum exposure concentration of 160 ppm D5 was limited by the highest vapor concentration that could be reliably generated without formation of appreciable aerosol or condensation. Intermediate exposure concentrations were selected based on responses elicited in previous studies.

2.4. Study design

Rats were weight stratified and randomized into test groups. Ninety-six rats of each sex were allocated to each exposure group; control (air exposed), 10, 40, and 160 ppm D5. Within each of the 4 exposure groups were 4 subgroups; A (6-month exposure), B (12-month exposure), C (12-month exposure/12-month recovery; Recovery), and D (24-month exposure).

Body weight data were collected each week for the first fourteen weeks and then at least once every four weeks. Clinical observations were recorded each week for the first fourteen weeks and then at least once every two weeks.

At terminal sampling, a complete necropsy was performed on all animals (except those in subgroup A (6-months exposure)) including gross and macroscopic evaluation and organ weight determinations. Necropsy of animals in the 6-month exposure subgroup included collection of liver, blood, and fat for tissue D5 determinations and a limited selection of organ weight determinations (liver and pituitary). For each necropsy, tissues were fixed in 10% neutral buffered formalin, except for eyes which were fixed in Davidson's solution, processed routinely, and stained with

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

hematoxylin and eosin. The list of tissues examined from the control and high-dose animals was typical of chronic toxicity and oncogenicity study protocols (EPA, 1998). Additionally the liver, lungs, kidneys, uterus, spleen, adrenals, nasal cavities, tissue masses and gross lesions from all animals (except those in subgroup A (6-months exposure)) were evaluated by the study pathologist. Additional pathology reviews were performed to more clearly detail the effects of exposure on the upper respiratory tract and on selected hormone sensitive tissues (ovary, uterus, vagina, mammary and pituitary glands). The nasal cavity was sectioned following a modification of the scheme reported by Young (1981) to yield five levels. The first section (level 1) was slightly more rostral than that specified by Young for level 1, the second section (level 2) represents tissue slightly rostral to the incisive papilla. A section cut just caudal to the papilla was taken as level 3 in this study. Levels 4 and 5 in this study represent sections taken at the first and second palatal ridges consistent with levels 3 and 4 as defined by Young.

Blood was collected from the orbital plexus from the Recovery subgroup animals (fasted overnight) for clinical pathology at 3, 6, and 12 months. Collections were performed on anesthetized animals (ether) prior to the daily exposure. Prothrombin time and activated partial thromboplastin time was determined only at 12 months and from blood collected by cardiac puncture from subgroup B (12-month exposure) animals. Urine was collected overnight in a chilled (ice) reservoir at 3, 6, and 12 months.

To determine D5 levels tissue samples (plasma, fat, and liver) from the 6-month exposure group were collected and extracted in tetrahydrofuran. The dried (magnesium sulfate) extracts were analyzed by GC/MS in accordance with the method of Varaprath et al. (1998).

Prior to the first exposure, ophthalmologic examinations were performed following mydriatic treatment on all rats and again on a portion of the animals of each exposure group after 12 and 24 months of exposure. Initial and periodic health screening found no evidence of confounding infectious disease.

2.5. Statistical analysis

Body and organ weight and clinical pathology data were analyzed by Dunnett's test if homogenous and by Steel test if non-homogenous ($p < 0.05$). Survival analysis was performed utilizing a Thomas, Breslow, and Gart program (Thomas et al., 1997), which includes the Kaplan–Meier product-limit procedure (Kaplan and Meier, 1958) and assessment of treatment-related effects by the Cox method (Cox, 1972). Statistical analysis of histomorphological non-neoplastic findings utilized the Poly3 Test (Bailer and Portier, 1988) to identify significant trend ($p < 0.05$) across the dose groups as well as for pair-wise comparisons of individual dose groups and control. Statistical analysis of neoplastic findings involved a tiered approach. In the first tier, Fisher's Exact Test (Fisher, 1934, 1935; Iwin, 1935) was applied to assess differences between control and individual dose groups ($p < 0.05$). Peto Mortality Prevalence Test (Peto et al., 1980) was used to determine if there was a significant trend across the dose groups ($p < 0.05$). In the second tier of testing the Poly3 test was applied to those neoplastic findings for which the Peto Mortality Prevence Test failed to detect a significant trend (See companion manuscript for a discussion of the statistical approach; Young and Morfeld, 2015).

3. Results

3.1. Exposure and tissue concentrations

The two year average mean daily exposure concentrations were within 2.4% of the target values of 0 (control), 10, 40, and 160 ppm D5. Exposure yielded a dose-responsive increase in tissue concentrations assessed at the end of 6 months of exposure. Tissue concentrations of D5 for the liver, plasma, and fat (brown, peri-renal, and abdominal) are provided in Table 2. Liver concentrations may have been higher than was detected at the time of analysis due to uncertainty with cold storage stability of the tissue. Thus, the values should only be used for relative comparisons. The toxicokinetic interpretation of these data have been reported (Andersen et al., 2008) and readers are referred to that publication for an in-depth review.

3.2. Survival and clinical pathology

Mortality data are presented in Table 3. Two animals (one male, one female) in the 12-month exposure group did not survive to the scheduled necropsy. The female was a 10 ppm group animal that died during blood collection procedures and the male was from the 160 ppm group and died as a consequence of malignant lymphoma. These deaths were not attributed to D5 exposure. Mortality in the 24-month exposure group among all exposure levels ranged between 40 and 53% for males and 15–23% for females. The lack of a dose–response suggests mortality was unaffected by the 24-months of treatment. A much broader mortality range was seen in the Recovery group (12 months exposure/12 months without exposure). A range of 15–75% for males and 5–30% for females was observed. The percent survival among the dose groups for males was 60, 35, 85, and 25% for the control, 10, 40, and 160 ppm exposure groups, respectively. The statistical analysis of these survival data for males was positive for trend ($p < 0.05$) and for the pair-wise comparison ($p < 0.05$) of the 160 ppm exposure group to control. However, the expressed mortality in these Recovery group males was not considered treatment related due to the lack of a similar effect in Recovery group females and in males and females of the 24-month exposure group.

Clinical observations for all groups/subgroups were similar across all exposure groups and not indicative of treatment-related effects (Supplemental Data Table 1). Examination of the eyes prior to initiation of the exposures identified corneal opacity in 81% of all animals. Corneal opacity, common in F344 rats, was present in all animals at the end of the first and second year of exposure. The incidence of clinically detectable masses was similar between the 24-month exposure group (16.9%) and the Recovery group (18.1%). Masses were found predominately in the genital, axillary, abdominal, and flank regions (Supplemental Data Table 2). Dose-response was lacking among exposure levels.

Clinical pathology assessments (serum chemistry, hematology, and urinalysis) resulted in a number of statistically identified differences in exposed verses control animals (Supplemental Data Table 3). However these differences were not considered to be of toxicological relevance because they were of low magnitude, were not dose responsive, and/or the effects were inconsistently present among the time points (3, 6, or 12 months).

3.3. Body and organ weight

Body weight was generally equal to or slightly higher in the D5 exposed groups for both males and females across time and exposure group/subgroups (Supplemental Data Table 4). The increase in body weight occasionally showed a dose response effect and at times the differences achieved statistical significance.

Organ weight was determined for the pituitary and liver for the 6-month exposure group and brain, pituitary, heart, lungs, liver, kidneys, adrenal glands, spleen, testes, epididymides, ovaries, and uterus for animals in all other exposure groups (Supplemental Data

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

Table 2
Tissue levels of Decamethylcyclopentasiloxane.

Concentration of D5 (μg D_5/g tissue)					
Dose (ppm)	Plasma	Liver	Fat		
			Abdominal	Perirenal	Brown
Male					
0	0.12 [0.022]	0.14 [0.02]	0.205 [0.167]	0.092 [0.054]	0.177 [0.041]
10	0.19 [0.037]	1.09 [0.21]	2.09 [0.922]	2.04 [0.473]	0.979 [0.247]
40	0.47 [0.074]	5.23 [0.45]	5.92 [3.01]	9.37 [2.70]	7.42 [1.74]
160	2.20 [0.39]	24.2 [0.95]	23.0 [8.77]	54.5 [12.8]	32.0 [4.94]
Female					
0	0.048 [0.039]	0.11 [0.02]	0.128 [0.046]	0.081 [0.03]	0.192 [0.136]
10	0.17 [0.024]	2.06 [0.19]	7.83 [2.82]	7.26 [0.64]	5.83 [0.562]
40	0.62 [0.032]	8.00 [0.91]	27.3 [9.06]	40.2 [10.5]	43 [18]
160	3.19 [0.758]	32.8 [2.97]	115 [42]	176 [58]	141 [23]
LOQ	0.027	0.09	0.013	0.023	0.023

Note: Values in brackets represent standard deviation of the mean D5 concentration. The limit of quantitation (LOQ) is also presented.

Table 3
Unscheduled mortality before the scheduled necropsy.

Sub-group	Sex	Group 1 0 ppm	Group 2 10 ppm	Group 3 40 ppm	Group 4160 ppm
B	Males	0/10	0/10	0/10	1/10
	Females	0/10	1[a]/10	0/10	0/10
C	Males	8/20	13/20	3/20	15/20
	Females	6/20	2/20	6/20	1/20
D	Males	26/60	29/60	24/60	32/60
	Females	14/60	14/60	9/60	9/60

Sub-group B animals = 1 year exposure.
Sub-group C animals = 1 year exposure/1 year recovery.
Sub-group D animals = 2 year exposure.
[a] Animal died during blood sampling.

Table 5). Statistically significant increases/decreases were identified for the liver, brain, uterus, adrenal gland, and epididymides. In all instances the organ weight changes appeared to occur without relationship to dose. In a number of animals, the increased organ weights were largely attributable to mononuclear cell leukemia infiltration (lung, liver, spleen), adenoma of the pars distalis in the pituitary, bursal dilation, follicular cysts or benign granulosa cell tumor in the ovary, lipomatous tumor in the adrenal gland, and stromal polyp or adenocarcinoma in the uterus.

3.4. Macroscopic examination and histomorphology

Macroscopic examination was performed at necropsy for all animals in all groups/subgroups. Macroscopic findings were recorded for 94% of the 6-month exposure group animals, 50% of the 12-month exposure group animals, and for all animals in the Recovery group and the 24-month exposure group (Supplemental Data Table 6). Differences between treated and control groups were generally not statistically significant, and none were considered toxicologically relevant or treatment-related. Treatment related histomorphological changes following exposure to D5 were limited to the upper respiratory tract and the uterus.

3.5. Upper respiratory tract effects

In both male and female rats the study pathologist's histopathology assessment of the upper respiratory tract identified a treatment-related increased incidence of hyaline inclusions in the olfactory (primarily) and respiratory (occasionally) epithelium of the nasal cavity. A second more focused assessment was performed to better characterize the presence of hyaline inclusions within the different tissue types and spatial location within the nasal cavity (Table 4). This review determined that the olfactory epithelium was principally affected and with hyaline inclusions present in, but without an apparent preference for, the dorsal and lateral meatus, septum, and turbinates. Photomicrographs are provided in Fig. 2a and b.

The hyaline inclusions were consistently present and at a highest incidence in the 160 ppm exposure group for each of the exposure periods (6, 12, and 24 months). Females were consistently more affected than males and nasal cavity levels 3 and 4 appear to be more sensitive (Table 4). There was a clear lack of inflammatory cell infiltration, tissue disorganization/degeneration, regeneration, pre-neoplastic or neoplastic lesions at any time point or exposure level.

3.6. Uterine effects

The incidence of uterine endometrial adenocarcinoma (adenocarcinoma) in the two year exposure groups was 0/60, 1/60, 0/60, and 5/60 in the control, 10, 40, and 160 ppm exposure groups, respectively; the increase in incidence was statistically significant in the 160 ppm exposure group (Table 5). Adenocarcinoma was not present in the 12-month exposure groups; however, adenocarcinoma was present in the Recovery group animals. The incidence among 20 animals per Recovery group included 1 in the control group, 1 in the 10 ppm group, 0 in the 40 ppm group, and 2 in the 160 ppm group. The adenocarcinomas were not associated with increases in precursor lesions such as uterine endometrial adenoma (none observed in this study) and focal glandular hyperplasia (a single incidence in the 10 ppm recovery group). There was no difference in incidence of cystic endometrial hyperplasia when the uteri from control and 160 ppm groups were evaluated specifically (see sensitive tissue review below) and no toxicologically relevant

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

Table 4
D5-Induced Incidence of Hyaline Inclusions in the upper Respiratory Tract.

	D5 exposure group	Proportion affected	Tissue type	Nasal level and Incidence(mean severity grade)				
				1	2	3	4	5
Males								
6 months	Control	1/6	olf	–	–	1(1)	–	–
			res	–	–	–	–	–
	10 ppm	0/6	olf	–	–	–	–	–
			res	–	–	–	–	–
	40 ppm	0/6	olf	–	–	–	–	–
			res	–	–	–	–	–
	160 ppm	6/6	olf	–	–	2(1)	5(1)	–
			res	–	–	–	–	–
12 months	Control	0/10	olf	–	–	–	–	–
			res	–	–	–	–	–
	10 ppm	0/10	olf	–	–	–	–	–
			res	–	–	–	–	–
	40 ppm	1/10	olf	–	–	–	1(1)	–
			res	–	–	–	–	–
	160 ppm	3/10	olf	–	1(1)	–	2(1)	–
			res	–	–	–	–	–
24 Months	Control	13/60	olf	–	5(1)	5(1.2)	3(1)	6(1)
			res	–	–	–	–	–
	10 ppm	6/60	olf	–	1(1)	2(1)	1(2)	3(1)
			res	–	–	–	–	–
	40 ppm	9/60	olf	–	5(1)	2(1)	3(1.3)	3(1.3)
			res	–	–	–	–	–
	160 ppm	43/60	olf	–	12(1.3)	21(1.2)	32(1.3)	17(1.1)
			res	–	–	–	–	3(1)
Recovery	Control	1/20	olf	–	–	–	–	1(1)
			res	–	–	–	–	–
	10 ppm	5/20	olf	–	1(1)	–	3(1)	2(1)
			res	–	–	–	–	–
	40 ppm	6/20	olf	–	2(1.5)	1(1)	1(1)	3(1)
			res	–	–	–	–	–
	160 ppm	9/20	olf	–	–	–	5(1)	6(1)
			res	–	–	–	–	–
Females								
6 months	Control	0/6	olf	–	–	–	–	–
			res	–	–	–	–	–
	10 ppm	0/6	olf	–	–	–	–	–
			res	–	–	–	–	–
	40 ppm	0/6	olf	–	–	–	–	–
			res	–	–	–	–	–
	160 ppm	4/6	olf	–	–	2(1)	1(1)	–
			res	–	4(1)	–	–	–
12 months	Control	1/10	olf	–	–	1(2)	–	–
			res	–	–	–	–	–
	10 ppm	1/10	olf	–	1(1)	1(1)	–	–
			res	–	–	–	–	–
	40 ppm	2/10	olf	–	1(1)	–	1(1)	1(1)
			res	–	–	–	–	–
	160 ppm	9/10	olf	–	3(1)	5/(1)	6(1.2)	3(1)
			res	–	–	–	–	–
24 Months	Control	48/60	olf	–	15(1.2)	32(1.6)	36(1.7)	13(1)
			res	–	–	–	2(1.5)	3(1.7)
	10 ppm	41/60	olf	–	16(1.3)	30(1.3)	33(1.4)	17(1.1)
			res	–	–	2(1.)	2(1)	2(1)
	40 ppm	48/60	olf	–	14(1.1)	32(1.2)	40(1.4)	17(1.1)
			res	–	–	–	2(2)	4(1.3)
	160 ppm	59/60	olf	–	15(1.5)	53(1.5)	55(2)	27(1.6)
			res	–	–	–	10(2.3)	23(1.7)
Recovery	Control	14/20	olf	1(1)	10(1.4)	10(1.6)	11(1.7)	7(1.1)
			res	1(1)	–	–	1(1)	–
	10 ppm	15/20	olf	–	4(1)	7(1.4)	11.(1.3)	6(1.2)
			res	–	–	–	1(1)	–
	40 ppm	12/20	olf	–	1(1)	7(1.3)	11(1.3)	7(1.3)
			res	–	–	–	–	1(1)
	160 ppm	20/20	olf	–	7(1)	19(1.7)	18(2.1)	12(1.5)
			res	–	–	–	3(2.3)	4(1.5)

Grading scale for Severity is as follows: 1. Minimal, 2. Slight, 3. Moderate, 4. Marked, 5. Severe.

differences in endometrial hyperplasia when evaluated more broadly.

Histomorphologically, the adenocarcinomas were characterized by malignant epithelial tissue consisting of glands, cords, or sheets of cells featuring cytological atypia, often characterized by nuclear pleiomorphism, vesiculization, and prominent nucleoli (Fig. 3).

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

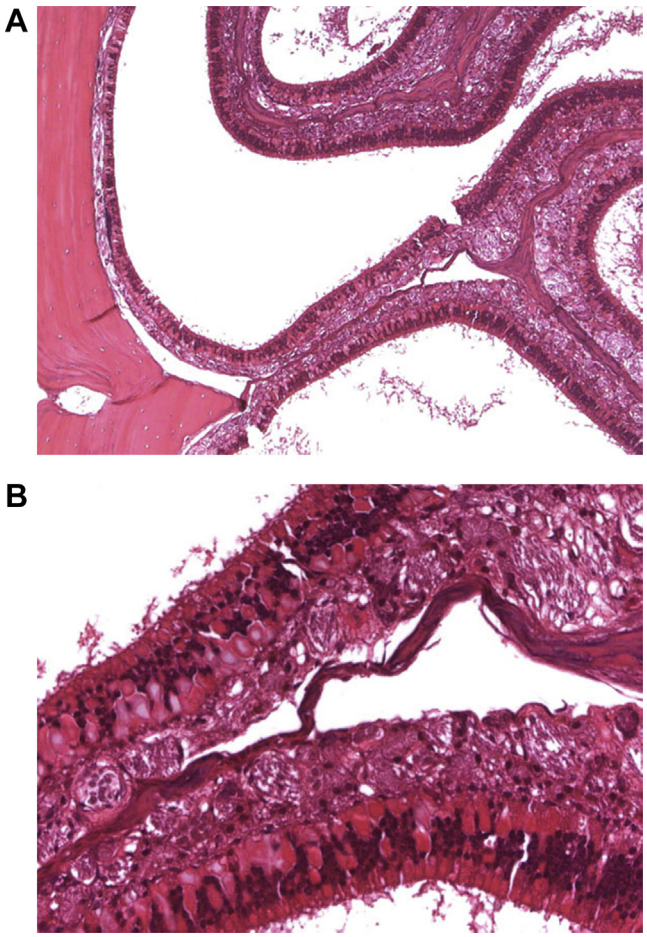

Fig. 2. A Nasal cavity level 4 (160 ppm female) according to Young (1985). Note diffuse distribution of eosinophilic inclusions at a moderate degree in the olfactory mucosa of the dorsal meatus and turbinates. Hematoxylin and eosin, lens ×10. B. Nasal cavity level 4 (160 ppm female) according to Young (1985). Higher magnification. The morphology and distribution of eosinophilic inclusions is not different from spontaneously occurring changes. Note: the olfactory mucosa is intact. No further degenerative, inflammatory or hyperplastic lesion is visible. The cilia on the surface are persevered. The submucosa is fully intact. Also there is no inflammatory or degenerative lesion notable. Bowmans glands and nerve fibers are normal. Hematoxylin and eosin, lens ×40.

Bizarre mitotic figures, evidence of necrosis/apoptosis, and cellular debris were also seen (Fig. 4). Stroma was often scanty, fibrotic, or contained a mononuclear infiltrate. Many of the tumors replaced the endometrial cavity or uterus or invaded myometrium, serosa, or neighboring fat (Fig. 5).

The adenocarcinomas present in the 160 ppm exposure group were indistinguishable from the one in the Recovery control group. A histomorphological comparison was also performed with 110 spontaneous adenocarcinomas from the untreated control groups

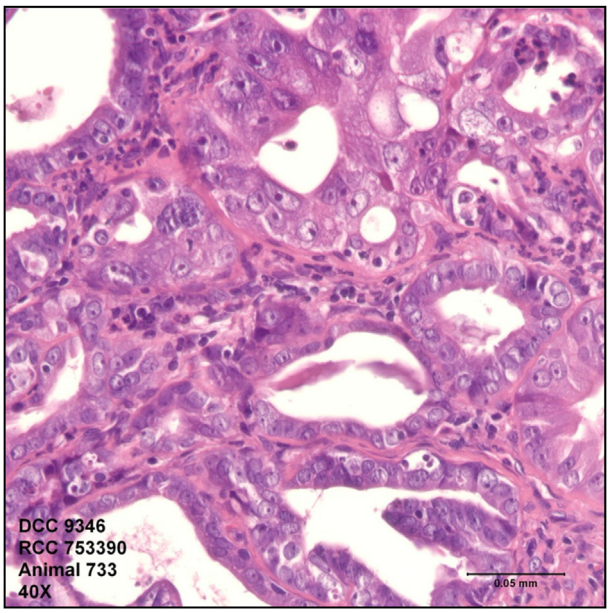

Fig. 3. Uterine endometrial adenocarcinoma showing pleiomorphic epithelial cells with vesicular nuclei and prominent nucleoli. This animal was exposed to 160 ppm D5 for 24 months. Scale bar is 50 μm.

of F344 rats from 107 NTP 2-year chronic bioassays (Klaunig et al., 2015). The review pathologist concluded that the histomorphology of the adenocarcinomas from the D5 chronic bioassay (treated and control groups) was not different from those present in the NTP bioassay control group animals.

3.7. Other neoplastic findings

Exposure to 160 ppm D5 vapor for 24-months was associated with a statistically significant decrease in incidence ($p < 0.05$) of mammary gland fibroadenoma in females and thyroid C-cell adenoma and combined C-cell adenocarcinoma in males (Table 6). These neoplasic lesions are common in the F344 rat (Kuroiwa et al., 2013; Chandra and Frith, 1992; Haseman et al., 1998).

3.8. Detailed histomorphological review of the ovarian, vaginal and uterine lesions

The increased incidence of uterine endometrial adenocarcinoma in the high dose group was unexpected, and in an effort to enhance our understanding of the circumstances surrounding its appearance a detailed histomorphological evaluation of the uterus, ovaries, mammary gland, pituitary and vagina was conducted. The objective was to assess specific non-neoplastic histomorphological features of these tissues to determine the potential hormonal influences relative to exposure to D5. Tissues from the control and 160 ppm exposure groups were evaluated for the 6-, 12-, and 24-

Table 5
Uterine effects associated with chronic D5 vapor inhalation Exposure in the Rat.

Exposure concentration (ppm D5)	2-Year Exposure group (subgroup D)		1-Year Exposure/Recovery group (subgroup C)	
	Cystic endometrial Hyperplasia[a]	Endometrial adenocarcinoma	Cystic endometrial Hyperplasia[a]	Endometrial adenocarcinoma
0	9/60 (1.8)	0/60	0/20	1/20
10	—	1/60	—	1/20
40	—	0/60	—	0/20
160	9/60 (2.4)	5/60	2/20 (1)	2/20

[a] Incidence (median severity grade) per sensitive tissue review pathologist (complete evalution of control and high dose only).

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

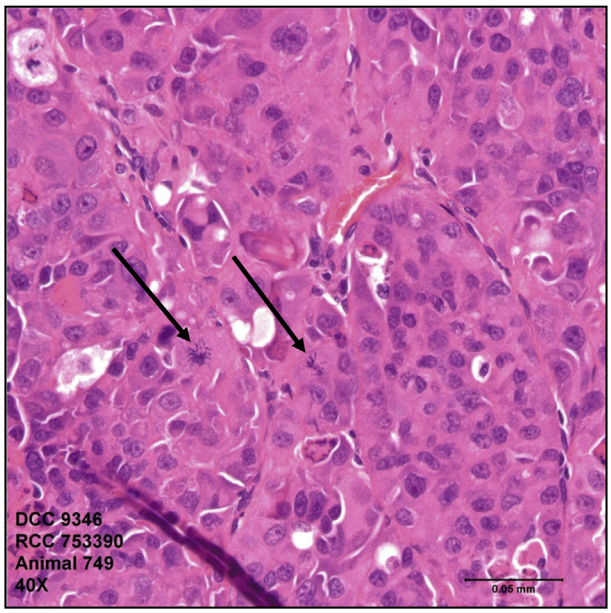

Fig. 4. Uterine endometrial adenocarcinoma show pleiomorphic endothelial cells, minimal stroma, and abnormal mitotic figures (arrows). This animal was exposed to 160 ppm D5 for 24 months. Scale bar is 50 μm.

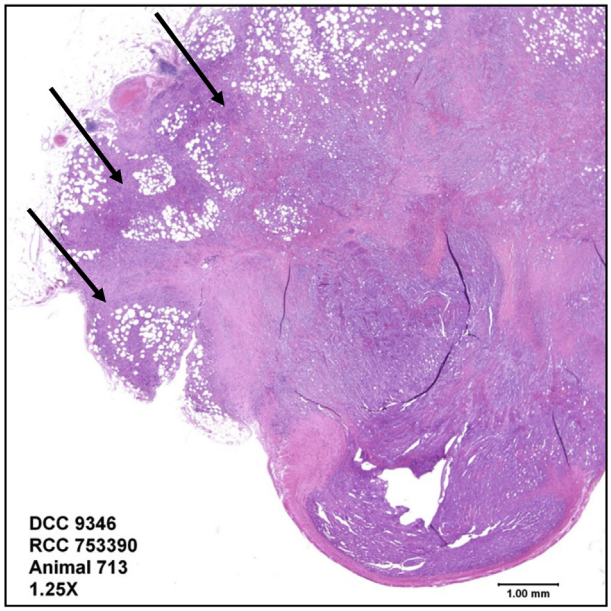

Fig. 5. Uterine endometrial adenocarcinoma has replaced much of the normal uterus and extends to neighboring adipose tissue (arrows). This animal was exposed to 160 ppm D5 for 24 months. Scale bar is 1 mm.

month exposure groups.

The non-neoplastic histomorphological features of the mammary and pituitary glands were typical for the age and strain of rat and lacked any obvious indication of a D5-related treatment effect. The assessment of the uterus, ovaries, and vaginal tissue suggested the potential for alteration of estrous cyclicity in the D5 exposed animals (Table 7). Following 6-months of exposure, the number of normally cycling rats and estrogenic verses progestogenic uterine estrous stages were similar in control vs treated groups. After 12-months of exposure, 6 of the 10 control and 6 of the ten 160 ppm group females were considered to be cycling abnormally. However there was a marked difference in uterine estrous phase between the two groups. The uteri of 8 of 10 control group animals appeared to be in an estrogenic estrous stage. In contrast, only 2 of ten 160 ppm group animals were in an estrogenic estrous stage, the remaining animals were in a progestogenic state (diestrus I or II). No differences in cyclicity or the uterine estrous phase was apparent in the 24-month exposure control vs treated groups. Only 8 of 60 animals in each group were showing signs suggesting normal cycling. The uteri in both groups were predominately demonstrating a progestogenic state (49 of 60 controls and 50 of sixty 160 ppm group animals). The recovery group animals demonstrated a difference in the proportion of "normally cycling" animals (3/20 controls vs. 7/20 in the 160 ppm group) and a difference in the proportion of animals in the uterine progestogenic phase (15/20 controls vs. 20/20 in the 160 ppm group).

4. Discussion

Vapor inhalation exposure of F344 rats to D5 for up to 2 years was well tolerated. There were no toxicologically relevant changes in body weight, appearance, or behavior suggestive of systemic toxicity. A reversible increase in liver weight has been reported following D5 vapor inhalation exposure of rats in sub-chronic toxicity studies (Burns-Naas et al., 1998a, 1998b; McKim et al., 1999). Hepatocellular hyperplasia and centrilobular hypertrophy were demonstrated after the first week of vapor inhalation exposure of female F344 rats to D5 but these effects proved transient in that they were not present after 2 or 4 weeks of exposure (Jean, 2005). D5 has also been shown to be a functional ligand for CAR and PXR in an *in vitro* reporter gene assay (Jean et al., 2006; Dow Corning Corporation, 2005a, 2005b) and, consistent with this property, to induce hepatic CYP 2B1/2 and CYP3A1 *in vivo* (McKim et al., 1999; Zhang et al., 2000). In total these effects likely represent an adaptive response purposed with increasing hepatic metabolic capacity in an effort to metabolize and remove D5.

The phenobarbital-like liver effect of D5 pointed to a potential for chronic exposure to yield liver tumors in the current rodent bioassay. However, as was observed in the current study, exposure to D5 vapor for 6, 12, and 24 months was without notable effects on liver weight or histomorphology. There were no preneoplastic or neoplastic changes in the liver of D5 exposed rats at any of the time

Table 6
Neoplastic lesions demonstrating a treatment-related Reduced Incidence.[a]

Tissue	Finding	Sex	Vapor concentration of D5			
			0 ppm	10 ppm	40 ppm	160 ppm
Mammary Gland	Fibroadenoma	Female	8 (60)[b]	7 (60)	7 (60)	2 (60)[c]
Thyroid Gland	C-Cell Adenoma	Male	9 (60)[b]	6 (32)	1 (25)	2 (60)[c]
Thyroid Gland	C-Cell Adenoma + Carcinoma	Male	10 (60)[b]	7 (32)	3 (25)	3 (60)[c]

[a] Neoplasia incidence for animals in the 24-month treatment groups. Values reflect the incidence observed (total number of animals evaluated).
[b] Significant negative Peto trend test ($p < 0.05$).
[c] Significant negative Fisher's Exact test ($p < 0.05$).

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

Table 7
Histomorphological features in Uterine, Vaginal, and Ovarian Tissue Following Inhalation Exposure to Decamethylcyclopentasiloxane.

Tissue/Diagnosis	6-Month Incidence		12-Month Incidence		24-Month Incidence		12/12 Recovery incidence	
	Control	D5	Control	D5	Control	D5	Control	D5
Ovary (No. Examined)	**6**	**6**	**10**	**10**	**60**	**60**	**20**	**20**
Old Corpora Lutea (CL)	6	6	10	10	52	50	17	20
Eosinophilic CL	6	5	7	7	34	36	15	20
Basophillic incomplete CL	1	3	3	3	5	2	3	5
Basophillic complete CL	4	3	2	6	22	18	13	5
Basophillic CL w/luteolysis	4	1	5	5	15	9	3	4
Antral Follicles [total]	5[18]	4[14]	5[7]	6[13]	14[18]	15[27]	6[6]	10[18]
Atretic Follicles, antral size[total]	6[36]	6[31]	10[56]	10[65]	55[252]	58[274]	19[86]	19[90]
Uterus (No. Examined)	**6**	**6**	**10**	**10**	**60**	**60**	**20**	**20**
Proestrus	4	2	5	0	7	6	3	0
Estrus	1	3	3	2	4	3	2	0
Diestrus I	0	1	1	3	7	0	4	5
Diestrus II	1	0	1	5	42	50	9	15
Vagina (No. Examined)	**6**	**6**	**10**	**10**	**60**	**60**	**17**	**20**
Epithelial Thickness								
mimimal	0	0	0	2	28	31	7	9
mild	1	1	4	7	21	21	7	9
moderate	5	5	6	1	11	7	3	2
Cornification								
minimal	1	1	4	0	8	5	1	0
mild	2	2	3	2	4	2	0	0
Sloughed Cornification								
minimal	1	3	2	0	10	4	5	1
mild	1	0	2	1	2	0	0	2
Mucification								
minimal	0	0	2	1	8	10	3	5
mild	3	0	3	3	19	21	4	5
moderate	0	1	0	0	12	13	1	4
severe	0	0	0	0	4	3	2	1

points. Such an outcome provides strong support for the assertion that the hepatic responses observed in the shorter term studies were representative of adaptive responses.

This study has also demonstrated a modest response to D5 vapor exposure within the upper respiratory tract. Hyaline inclusions, though present in the control group and demonstrating an increased incidence with age, occurred at a higher incidence in the 160 ppm exposure levels at each of the time points including the recovery group animals. Hyaline inclusions (also referred to as eosinophilic globules, eosinophilic droplets, and epithelial hyalinosis) in the nasal cavities are characterized as an "… accumulation of brightly eosinophilic cytoplasmic inclusions in sustentacular cells of olfactory epithelium, respiratory epithelial cells, and epithelial cells of the nasal seromucous glands …" (Renne et al., 2009), are believed to be proteinaceous in nature. The inclusions are commonly observed in laboratory animal studies involving inhalation exposure; however, they are not exclusive to this exposure route. The mechanism(s) involved in their emergence and persistence is not understood. Though they are often observed following inhalation exposure to noxious substances causing various degrees of nasal tissue injury, it is yet uncertain if they represent a non-specific adaptive response, a specific response to tissue injury, or an adverse effect in and of itself. The clear lack of nasal tissue injury (degeneration, necrosis, inflammatory infiltrate, dysplasia, and/or neoplasia) following repeated inhalation exposure to D5 vapor for 6–24 months suggests that their emergence in response to D5 represents an adaptive response.

In this chronic bioassay, D5 exposure was associated with a modest and borderline but statistically significant increased incidence of uterine endometrial adenocarcinoma at 160 ppm, the highest exposure level (See companion manuscript for a discussion of the statistical approach; Young and Morfeld, 2015). There was no effect on uterine weight or increased incidence of precursor lesions such as focal glandular hyperplasia. It has been reported that the incidence of spontaneous uterine adenocarcinoma in the F344 rat increases substantially with increasing age, especially ages beyond 24 months (Nyska et al., 1994). The incidence of spontaneous adenocarcinoma in the rat appears to vary markedly among strain and sub-strain and appears to reflect exposure to ovarian estrogens without sufficient anti-proliferative exposure to progesterone (Nagaoka et al., 1990).

The tumors in D5-exposed animals were advanced, raising the possibility that precursor lesions were obliterated by the spreading tumors. As discussed in more detail in a companion paper (Klaunig et al., 2015), the endometrial adenocarcinoma findings are most likely consistent with an increase in the time in estrus (due to cycle alteration) during the first year of treatment, with development of endometrial adenocarcinoma and progression over the second year of treatment (Group D) or recovery (Group C). Although there was no reported increase in focal glandular hyperplasia following D5 exposure for 24 months, at least one tumor in the 160 ppm D5 treated group was reported to have glandular hyperplasia associated with the tumor. Possible explanations for the lack of associated precursor lesions include focal precursor lesions that were lost as a consequence of sectioning or onset of low-grade adenocarcinoma months prior to the development of persistent diestrus/pseudopregnancy, a state during which precursor lesions may have regressed. The latter explanation is supported by the observation from studies in aging F344 rats (Dekant and Klaunig, 2015) that D5 treatment was associated with an increase in estrogen exposure during the first few months of treatment but not thereafter. Another possibility is that presence of focal glandular hyperplasia as a precursor lesion is lost as the tumors have progressed to such an advanced stage that the tumor is now the predominant lesion.

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014

The adenocarcinomas by this time may have achieved hormone-independence, preventing their regression in response to progesterone dominance.

D5 has been evaluated in a number of studies that cover a wide range of biological end points. This testing has suggested that D5 is without significant estrogenic/androgenic/progestogenic potential, and it has not demonstrated mutagenic or genotoxic potential. Therefore, direct effects on the endometrium by D5 or a genotoxic mechanism of endometrial carcinogenesis appear unlikely modes of action to explain the observed increase in endometrial adenocarcinoma.

5. Conclusion

Inducing an upper respiratory tract response from a chronic inhalation exposure is perhaps not a surprising outcome. In contrast an increased incidence of uterine endometrial adenocarcinoma was not an outcome anticipated for D5. General descriptive toxicity testing (acute, sub-acute and sub-chronic) performed prior to the conduct of the chronic bioassay provided no indication that the uterus was a potential target organ. This included a demonstrated lack of mutagenicity/genotoxicity. The target organ and tumor type specificity (adenocarcinoma is a common spontaneous tumor in the aged F344 rat) may suggest the effect is associated with a treatment related alteration in pituitary control of the estrous cycle. Potential modes of action responsible for the uterine tumors and their human relevance is presented in a companion manuscript (Klaunig et al., 2015).

Acknowledgments

The authors wish to thank the following key contributors; Dr. Paul A. Smith (Study Director, RCC ltd. currently Harlan), the late Dr. H. J. Chevalier (Study Pathologist, RCC ltd. currently Harlan), Dr. Karen Regan (Pathologist (Sensitive Tissue Review), Regan Path/Tox Services) and Dr. Klaus Weber (Pathologist (Upper Respiratory Tract Assessment), AnaPath Services GmbH). This research was sponsored by Dow Corning Corporation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2015.06.014.

References

AALAC., 1991. American Association for Laboratory Animal Science Policy on the Humane Care and Use of Laboratory Animals, pp. 41–91.
Andersen, M.E., Reddy, M.B., Plotzke, K.P., 2005. Lack of bioaccumulation with repeated, periodic exposures of cyclic siloxanes. Abstract #855. Toxicol. CD – An off. J. Soc. Toxicol. 84. Number S-1, March 2005.
Andersen, M.E., Reddy, M.B., Plotzke, K.P., 2008. Are highly lipophilic compouinds expected to bioaccumulate with repeated exposures? Toxicol. Lett. 179, 85–92.
Bailer, A.J., Portier, C.J., 1988. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics 44, 417–431.
Burns-Naas, L.A., Mast, R.W., Klykken, P.C., McCay, J.A., White, K.L., Mann, P.C., Naas, D.J., 1998a. Toxicology and humoral immunity assessment of decamethylcyclopenta-siloxane (D5) following a 1-Month whole body inhalation exposure in fischer 344 rats. Toxicol. Sci. 43, 28–38.
Burns-Naas, L.A., Mast, R.W., Meeks, R.G., Mann, P.C., Thevenaz, P., 1998b. Inhalation toxicology of decamethylcyclopentasiloxane (D5) following a 3-month nose-only exposure in fischer 344 rats. Toxicol. Sci. 43, 230–243.
Chandra, M., Frith, C.F., 1992. Spontaneous neoplasms in aged control Fischer 344 rats. Cancer Lett. 62 (1), 49–56.
Cox, D., 1972. Regression models and life-tables. J. R. Stat. Soc. B34, 187–220.
Dekant, W., Klaunig, J.E., Toxicology of decamenthylcyclopentasiloxane (D5), 2015.
Dow Corning Corporation, 2005a. Non-regulated Study: Assessment of Cyclic Siloxane Activation of the Constitutive Androstane Receptor Study. Report Number: 2005-I0000-55386.
Dow Corning Corporation, 2005b. Non-regulated Study: Assessment of Cyclic Siloxanes in an in Vitro Pregnane X Receptor (PXR) Reporter Gene Assay Study. Report Number: 2005-I0000-55384.
EPA, 1998. Health Effects Test Guidelines OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity. EPA 712-C-98–212. United States Environmental Protection Agency.
Fisher, R., 1934. Statistical Methods for Research Workers, fifth ed. Oliver and Boyd, Edinburgh.
Fisher, R., 1935. The logic of inductive inference. J. R. Stat. Soc. 98 (series A), 39–54.
Haseman, J.K., Hailey, J.R., Morris, R.W., 1998. Sponstaneous Neoplasm incidences in fischer 344 rats and B6C3F1 mice in two-year carcinoganicity studies: a national toxicology program update. Toxicol. Pathol. 26 (3), 428–441.
Irwin, J., 1935. Tests of significance for differences between percentages based on small numbers. Metron 12, 83–94.
Jean, P.A., Arthurton, J.A., You, L., Plotzke, K.P., 2006. Activation of pregnane x receptor (PXR) and constitutive androstane receptor (CAR) by octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5). Vitro Toxicol. 90 (1). ABS#1827.
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.
Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. J Am. Stat. Assoc. 53, 457–481.
Klaunig, J.E., Dekant, W., Plotzke, K., Scialli, A.R., Biological relevance of decamethylcyclopentasiloxane (D5): analysis of the potential mode of action of decamethylcyclopentasiloxane induced uterine tumorigenicity, 2015.
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 (2), 227–230.
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).
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 metabolizing enzymes in female fischer-344 rats following repeated inhalation exposure to decamethylcyclpentasiloxane (D5). Toxicol. Sci. 50, 10–19.
Nagaoka, T., Onodera, H., Matsushima, Y., Todate, A., Shibutani, M., Ogasawara, H., Maekawa, A., 1990. Spontaneous uterine adenocarcinomas in aged rats and their relation to endocrine imbalance. J. Cancer Res. Clin. Oncol. 116 (6), 623–628.
Nyska, A., Klein, T., Scolnik, M., Waner, T., Klein, B., 1994. Unusually high incidence of spontaneous endometrial adenocarcinoma in aged virgin Fischer rats. Exp. Toxicol. Pathol. 46, 7–9.
Peto, R., Pike, M.C., Day, N.E., 1980. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments in long-term and short-term screening assays for Carcinogens: a critical Appraisal. IARC Monogr. 311–426.
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.
Quinn, A.L., Regan, J.M., Tobin, J.M., Marinik, B.J., McMahon, J.M., McNett, D.A., Sushynski, C.J., Crofoot, S.D., Jean, P.A., Plotzke, K.P., 2007. *In vitro* and *In vivo* evaluation of the estrogenic, androgenic and progestagenic potential of two cyclic siloxanes. Toxicol. Sci. 96 (1), 145–153.
Renne, R., Brix, A., Harkema, J., Herbert, R., Kittel, B., Lewis, D., March, T., Nagano, K., Pino, M., Rittinghausen, S., Rosenbruch, M., Tellier, P., Wohrmann, T., 2009. Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol. Pathol. 37 (7), 5S–73S.
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.
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, 41–52.
Siddiqui, W.H., Stump, D.G., Reynolds, V.L., Plotzke, K.P., Holson, J.F., Meeks, R.G., 2007. A two-generation reproductive toxicity study of decamethylcyclopentasiloxane (D5) in rats exposed by whole-body vapor inhalation. Reprod. Toxicol. 23, 216–225.
Thomas, D.G., Breslow, N., Gart, J.J., 1997. Trend and homogeneity analysis of proportions and life table data. Version 2.1. Comput. Biomed. Res. 10, 373–381.
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.
Varaprath, S., Salyers, K.L., Plotzke, K.P., Nanavati, S., 1998. Extraction of octamethylcyclotetrasiloxane and its metabolites from biological matrices. Anal. Biochem. 256, 14–22.
Young, L.J., Morfeld, P., 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, 2015.
Young, J.T., 1981. Histopathologic examination of the rat nasal cavit. Toxicol. Sci. 1 (4), 309–312.
Zhang, J., Falany, J.L., Xie, X., Falany, C.N., 2000. Induction of rat hepatic drug metabolizing enzymes by dimethylcyclosiloxanes. Chemico-Biological Interact. 124, 133–147.

Please cite this article in press as: Jean, P.A., et al., Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat, Regulatory Toxicology and Pharmacology (2015), http://dx.doi.org/10.1016/j.yrtph.2015.06.014