Toxicology Letters 279 (2017) 75–97

Contents lists available at ScienceDirect

Toxicology Letters

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

Chronic toxicity and oncogenicity of octamethylcyclotetrasiloxane (D_4) in the Fischer 344 rat

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ARTICLE INFO

Keywords:
Siloxane
Oncogenicity
Octamethylcyclotetrasiloxane

ABSTRACT

Octamethylcyclotetrasiloxane (D_4) is a cyclic volatile methylsiloxane primarily used in the synthesis of silicon-based materials used in a variety of consumer products. This paper details the chronic toxicity and oncogenicity evaluation of D_4 in the Fischer 344 rat. Animals were exposed to 0, 10, 30, 150, or 700 ppm D_4 vapor for 6 h/day, 5 days/week for up to 104 weeks in whole-body inhalation chambers. Effects of two year chronic exposure included increased liver, kidney, testes, and uterine weight with correlating microscopic findings of hepatocellular hypertrophy (males only), chronic nephropathy (both sexes), interstitial cell hyperplasia, and cystic endometrial hyperplasia and endometrial adenoma, respectively. Upper respiratory tract irritation and lymphocytic leukocytosis were evident in both sexes. Increased neoplasia was demonstrated only in the uterus. Uterine endometrial adenomas were present in four of sixty animals exposed to 700 ppm D_4 for 24 months. None were present in the other treatment groups. In contrast, in 700 ppm D_4 group males the incidence of pituitary and pancreatic neoplasia was reduced as was thyroid c-cell adenoma/carcinoma in 700 ppm females. This study has identified that D_4 is a mild respiratory irritant and increases liver and kidney weight without inducing neoplasia in these tissues. The increased incidence of uterine adenoma was the only treatment-related neoplastic finding associated with chronic exposure to D_4.

1. Introduction

Silicones, more specifically 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 bonded 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 common polysiloxanes are polydimethylsiloxanes in which two methyl groups are covalently bound to each silicon atom ($-(CH_3)_2SiO-$) in repeating units.

Octamethylcyclotetrasiloxane (D_4) is a cyclic siloxane of 4 siloxane units (Fig. 1). It is a volatile, colorless, and odorless liquid of low molecular weight and water solubility (Table 1) and is primarily used in the synthesis of larger siloxane polymers. D_4 may be present as a low level impurity in Silicone polymers that are used in consumer products. The use in manufacturing and its presence as a low level impurity in a range of consumer product applications give rise to the potential for human exposure in both the workplace and consumer settings. Although dermal exposure is the most likely exposure route for consumers, testing by inhalation exposure (the second most prevalent route for consumers and most prevalent route in the manufacturing environment) was chosen due to the technical challenges associated with ensuring exposure to D_4 following dermal application (i.e. spreading beyond the dosing site and marked evaporation from the skin) and the similarity in kinetic behavior between dermal and inhalation exposure routes (Sarangapani et al., 2003). Oral exposure was not selected because it is the least relevant route of human exposure to D_4.

This study, a 24-month chronic toxicity and oncogenicity study of D_4, represents a critical effort in an ongoing comprehensive testing program to characterize potential health hazards, inform human risk assessment, and ensure its safe use in manufacturing and consumer applications. Testing has included sub-chronic general descriptive and reproductive toxicology, disposition, and toxicokinetics primarily in the rat. There also have been investigations in humans, other species, and *in vitro*. The outcome of this extensive effort has been recently summarized in an overall health hazard review (Dekant et al., 2017) and in a harmonized Global Risk Assessment (Franzen et al., 2017).

Testing has revealed that D_4 is absorbed by oral (up to 77%), but not extensively absorbed by the dermal (up to 0.5%), and inhalation (up to 6%) routes of exposure (Plotzke et al., 2000, 2002; Saranapani et al., 2003; Jovanovic et al., 2008; Domoradzki et al., 2017). Principal routes

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http://dx.doi.org/10.1016/j.toxlet.2017.06.003
Received 31 October 2016; Received in revised form 1 June 2017; Accepted 14 June 2017
Available online 01 July 2017
0378-4274/

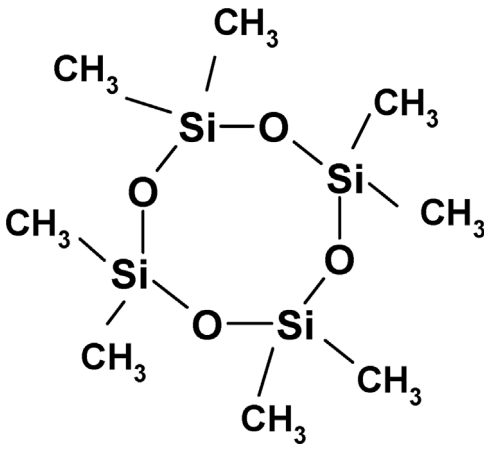


Fig. 1. Structure of Octamethylcyclotetrasiloxane (D_4).

Table 1
Physical and Chemical Properties of Octamethylcyclotetrasiloxane (D_4).

Property	D_4
Chemical Abstracts Number	556 – 67-2
Molecular Weight (daltons)	297
Boiling Point	175 °C
Melting Point	17.5 °C
Saturated Vapor Concentration at 23 °C	1161 ppm (14 mg/L)
Water Solubility[a] at 23 °C	56 ppb

[a] Varaprath et al., 1996.

of elimination are exhalation of D_4 and urinary excretion of metabolites. Partitioning into fat occurs readily, however bioaccumulation is not probable due to rapid elimination (Andersen et al., 2005). D_4 was shown to be phenobarbital-like with respect to non-adverse effects on the liver including liver enlargement, hepatocellular hypertrophy/hyperplasia, and hepatic cytochrome P450 induction (CYP2B1/2 primarily) in the rat (McKim et al.,1998, 1999, 2001a). Effects of inhalation exposure to D_4 in rat reproductive toxicity studies included reductions in litter size and number of implantation sites 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). Meeks et al. (2007) demonstrated that the short period around ovulation was the critical period of exposure for this reproductive effect. Follow-up studies suggest that inhibition of the pre-ovulatory surge of luteinizing hormone (LH) is a predominant factor (Quinn et al., 2007a). D_4 has demonstrated very weak estrogenicity *in vivo* and *in vitro* (McKim et al., 2001b; Quinn et al., 2007b).

This report presents the results of a chronic toxicity and oncogenicity study of D_4 in the Fischer 344 rat (F344). A comprehensive review of the biological relevance of the uterine tumors has been conducted that includes assessing various modes of action (Dekant et al., 2017).

2. Materials and methods

2.1. Test article

D_4 was obtained from Dow Corning Corporation (Midland, MI). D_4 was provided in 55-gallon steel drums and stored between 18 and 25 °C. Purity was determined prior to initiating exposures as well as twice for each drum (start and end of the drum's supply). Purity was determined to be greater than 99.5% by gas chromatography utilizing both thermal conductivity and mass selective detection.

2.2. Animals and husbandry

Male and female F344 rats were purchased from Charles River Laboratories (Raleigh, NC) and were 7–8 weeks of age at initiation of exposure. Animals were housed five/cage in suspended wire-mesh cages prior to randomization and identification, after which they were individually housed in suspended wire-mesh cages. Food (PMI Certified Rodent Chow #5002; PMI Nutrition International, St. Louis, MO) and municipal water was provided *ad libitum* with a few exceptions. Food was withheld during the daily exposure period, overnight prior to blood collections, and during urine collection.

Animals were housed individually in whole-body inhalation chambers (H-2000; Lab Products, Inc.; Seaford, DE) during the exposure phase of the study. Animals in the recovery groups were housed in the same wire cage racks used in the inhalation chambers but held in open racks in a separate animal room. Temperature and humidity (inhalation chambers and recovery animal room) ranges were 19–27 °C and 27–95% relative humidity, more than 99% of all measurements were within the protocol specified range for temperature (23 ± 3 °C) and relative humidity (50 ± 20%). The light cycle consisted of 12 h of light (fluorescent) and 12 h of dark. Animals were observed twice daily for morbidity, moribundity and mortality throughout the in-life phase of the study.

A pathogen screening program was used to monitor the animals' general health and disease status. Examinations were performed the first and third week after arrival. In each, five rats/sex were subject to gross necropsy and microscopic examination of selected tissues. Blood obtained from the third week examination and from the 6-, 12-, and 24-month scheduled necropsies was submitted for viral serology. There was no evidence of microbial infection or other pathogen-related disease (data not shown).

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).

2.3. Inhalation exposure

Exposures were conducted five days per week for up to 24 months, excluding holidays, in whole-body inhalation chambers (H-2000; Lab Products, Inc.; Seaford, DE). Daily exposures consisted of a six-hour period at the target exposure concentration sandwiched between 20 m periods in which chamber concentrations were increasing to, or decreasing from, the target concentration. Chamber cage units were rotated among the rack positions weekly to limit the potential for positional effects. Adequate oxygen concentration (21%) within the chambers was demonstrated with a Biosystems Oxy Plus Oxygen Detector (model 06-90; Rockfall, CT).

Exposure concentration targets were 0 (control group), 10, 30, 150, or 700 ppm D_4. This exposure concentration range represents the range of vapor concentrations investigated in the vast majority of previous repeated treatment studies. Though higher exposure concentrations (900 ppm) have been used in a few specific short-duration studies, 700 ppm D_4 was selected for this chronic bioassay because it was considered the highest concentration that could be reasonably achieved on a day-to-day basis without formation of appreciable aerosol or condensation on chamber surfaces.

Table 2
Group Allocations and Purpose.

Subgroup	Number of Animals (per sex)	Exposure Period	Purpose
A	6	6 months	Tissue D_4 Content
B	10	12 months	Chronic Toxicity Assessment
C	20	12 months[a]	Recovery Group
D	60	24 months	Oncogenicity Assessment

[a] 12 months exposure followed by 12 months without exposure.

Test atmosphere was produced by vaporization of D_4 into the chamber inlet air stream. The vapor generator consisted of a fiberglass wick in contact with a heated cylinder located within the chamber's inlet compartment. Chamber test atmosphere homogeneity was demonstrated prior to the initial exposure and periodically throughout the 24-month exposure period. Vapor generation did not result in an increase in aerosol within the chamber. Determination of test atmosphere concentration was performed by gas chromatography with flame ionization detection (GC/FID) (Agilent Model 6890, Palo Alto, CA).

2.4. Study design and sample collection

Rats were randomized into test groups using a weight-stratified procedure. There were 96 males and 96 females allocated to each of five primary exposure groups, control (air exposed), 10, 30, 150, or 700 ppm D_4 treatment groups. Within each treatment group were four subgroups (Table 2). This included a Toxicokinetic group (6 animals/sex) for which tissue levels of D4 would be determined at 6 months, a Chronic Toxicity Assessment group (10 animals/sex) with a scheduled euthanasia at 12 months, a Recovery group (20 animals/sex) exposed to D4 for 12 months followed by 12 months without exposure to D4, and the Oncogenicity group (60 animals/sex) receiving 24 months of exposure to D4.

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.

A complete necropsy was performed on all animals in subgroups B, C, and D. This included documenting macroscopic observations, collection of tissues/organs for microscopic evaluation (Table 3), and organ weight determinations. Necropsy of animals in subgroup A was limited to the collection of liver, blood, and fat for tissue determination of D_4 concentration in these tissues and collection of select tissues for possible microscopic examination (Table 3). Liver and pituitary gland weights were recorded.

After an overnight fasting, blood was collected pre-exposure from the orbital plexus from 10 rats/sex from subgroup C animals while under anesthesia (70% CO_2) for hematology and serum chemistry at 3, 6, 9 (serum chemistry only), and 12 months. Hematology determinations were performed utilizing a Roche'/ABX/Helios hematology instrument. Serum chemistry parameters were determined utilizing a Roche Cobas Fara centrifugal analyzer. Blood collected from subgroup B animals at 12 months was evaluated for prothrombin time and activated partial thromboplastin time (MLA 900 automated coagulation instrument). Urine was collected overnight in a chilled (ice) reservoir at 3-, 6- and 12 months from subgroup C animals. Food was withheld and water provided during the 16 h urine collection period.

Prior to the first exposure, ophthalmologic examinations were performed following mydriatic treatment on all rats in subgroup B and the first 10 rats/sex/group in subgroup D. A second ophthalmologic exam was performed approximately two weeks prior to the scheduled necropsy on the animals in the control and 700 ppm groups.

The tissues/organs collected at necropsy for potential microscopic examination were fixed in 10% neutral buffered formalin. Eyes were fixed in Davidson's solution. Tissues were processed routinely and stained with hematoxylin and eosin. A complete histologic examination was performed on all animals from the control and high dose group, as well as any animal found dead or euthanized due to moribund condition. The list of tissues examined is detailed in Table 3. Additionally the liver, lungs, kidneys, uteri, spleen, adrenal, nasal cavities, tissue masses and gross lesions from all animals in subgroups B, C, and D were evaluated. A generalized pathology peer review was performed and involved a review of all of the study pathologist's diagnoses by a second pathologist. Differences of opinion were documented and then resolved through collaborative discussion between the study and peer-review pathologists. The histopathology findings presented herein represent the resulting consensus opinion.

Tissue levels of D_4 were determined for the liver, plasma, and fat

Table 3
List of Tissues Collected at Necropsy.

Adrenal glands (2)	Larynx	Sciatic nerve
Aorta	Liver[a]	Seminal vesicles[a]
Bile duct (common)	Lungs[a] (including bronchi) [a]	Skeletal Muscle
Bone marrow smear[b]	Mammary gland[a] (female)	Skin
Brain	Nasal Cavities[a,d]	Spinal Cord (3 levels)
Cervix[a]	Ovaries[a]	Spleen
Esophagus	Pancreas	Stomach
Extra-orbital lachrymal gland	Parathyroid	Testes[a] (with epididymides)
Eyes with optic nerve[c] (2)	Penis[a]	Thymus (if present)
Gross lesions/Tissue masses	Pharynx[a]	Thyroid gland[a]
Heart	Pituitary gland[a]	Trachea[a]
Intestine, large (cecum, colon, rectum)	Prepuce[a]	Urinary bladder[e]
	Preputial gland[a]	Uterus[a,f]
Intestine, small (duodenum, jejunum, ileum)	Prostate gland[a]	Vagina[a]
	Salivary gland	Zymbals gland[a]
Kidneys (2) [a]		

[a] Denotes the partial tissue list for Subgroup A animals.
[b] Not collected from animals found dead.
[c] Perserved in Davidson's solution.
[d] Five cross-sections of the nasal cavities were prepared in general accordance with the method of Morgan (1991) including the nasal vestibule anterior to the incisor teeth.
[e] Inflated with fixative at the time of collection.
[f] Sections for microscopic examination included one cross-section of each uterine horn, longitudinal horizontal section of cervix with fused portion of uterus, and any observed gross lesions/masses.

(perirenal, abdominal, and brown) after 6 months of exposure (subgroup A). Blood was collected under anesthesia (100% CO_2) via cardiac puncture within minutes after being removed from the exposure chamber operating at the target concentration.

Plasma and fat samples were maintained on ice until processing. Plasma and fat D_4 content were determined by combining an aliquot of plasma or weighed portion of fat with magnesium sulfate and a solution of tetrahydrofuran containing tetrakis(trimethylsiloxy)silane as internal standard. Gas chromatographic analysis with mass spectrometric detection was performed after mixing and centrifugation of the samples and similarly prepared standards (Varaprath et al., 2000).

Liver was removed and flash frozen in liquid nitrogen immediately after blood collection. The frozen liver samples were shipped to Dow Corning for processing and analysis for D_4 content. Liver D_4 content determination required an initial homogenization of tissue in saline. An aliquot of homogenate was then extracted three times with tetrahyrofuran containing tetrakis(trimethylsiloxy)silane as internal standard. The extraction volumes were pooled for analysis by gas chromatographic analysis with mass spectrometric detection (Varaprath et al., 2000).

2.5. Statistical analysis

Statistical analyses were conducted using Xybion Path/Tox System (Xybion Medial Systems, Cedar Knolls, NJ) or Statistical Analysis System (SAS Institute Inc., Cary, NC) statistical programs.

Body and organ weight data were first analyzed for homogeneity using Barlett's test. Statistical significance ($p < 0.05$) was determined using Dunnett's test for homogeneous data and a Modified *t*-test for nonhomogeneous data.

Clinical pathology data were analyzed for homogeneity using Hartley's Fmax (equal n) or Cochran's C test (unequal n) followed by a Dunnett's two-tailed *t*-test (homogeneous data) or Behrens-Fisher *t*-test (nonhomogeneous data) for significance ($p < 0.05$).

Survival analysis was performed utilizing the Thomas, Breslow, and Gart program which includes the Kaplan-Meier product-limit procedure and assessment of treatment-related effects by the Cox method.

Statistical analysis of non-neoplastic findings utilized the Poly3 Test to identify significant trend ($p < 0.05$) across the treatment groups as well as for pair-wise comparisons of individual treatment groups and control.

Statistical analysis of neoplastic findings involved a tiered approach. In the first tier, Fisher's Exact Test was applied to assess differences between control and individual treatment groups ($p < 0.05$). Peto Mortality Prevalence Test was used to determine if there was a significant trend across the treatment groups ($p < 0.05$). In the second tier of testing the Poly3 test was applied to those neoplastic findings for which the Peto Mortality Prevalence Test failed to detect a significant trend.

3. Results

3.1. Exposure

At the end of each exposure day the average test atmosphere exposure concentration was derived from the individual sampling and analysis results for that day. The mean of these concentration values for each exposure group ranged between 100 and 101% of the target value (data not shown). The percent relative standard deviation ranged between 2 and 5% among the exposure groups. Greater than 99% of the individual test atmosphere concentration measurements were within 10% of the target concentration.

3.2. Tissue D4 concentration and dosimetry

The concentration of D_4 in plasma, liver, and fat were determined in sub-group A animals euthanized after six months of exposure (Table 4). Sample collections were performed within minutes of terminating the daily exposure and were expected to reflect peak blood levels for this material (Plotzke et al., 2000). The concentration of D_4 in each of the tissues increased with exposure concentration. However, the increases were not entirely exposure concentration-proportional (Fig. 2a–c) or linear over the exposure concentration range for both males and females in the various tissues. To be considered proportional the tissue level of D_4 in the 30, 150 and 700 ppm exposure groups would be expected to be approximately 3, 15, and 70 times greater than that observed at the 10 ppm lowest exposure group, respectively. Fig. 2 presents the tissue levels as observed and as would be expected based on

Table 4
Tissue Concentrations* of Octamethylcyclotetrasiloxane in Fischer 344 Rats after Inhalation Exposure for Six Months.

Tissue (unit measure)	Sex	Exposure Concentration (ppm)				
		0	10	30	150	700
Plasma (μg/ml)	Male	BLQ[e]	0.0722 [0.019]	0.237 [0.044]	1.19 [0.21]	8.21 [1.4]
Plasma(μg/ml)	Female	BLQ[e]	0.153[a] [0.014]	0.436[a] [0.13]	2.65[a] [0.63]	13.0[a] [2.6]
Liver[c](μg/g)	Male	0.30 [0.03]	0.58 [0.14]	0.92 [0.42]	3.34 [3.35]	71.19 [15.80]
Liver[d](μg/g)	Female	0.28 [0.01]	1.18[b] [0.13]	2.08[b] [0.36]	15.22[b] [1.28]	76.71 [6.21]
Fat: *peri*-renal(μg/g)	Male	BLQ[f]	3.35 [0.26]	12.9 [1.0]	91.3 [4.6]	755 [42]
Fat: Abdominal(μg/g)	Male	BLQ[f]	3.65 [0.67]	13.1 [0.54]	86.6 [5.7]	741 [30]
Fat: Brown(μg/g)	Male	BLQ[f]	4.53[g] [0.72]	16.0 [g] [2.0]	96.6 [h] [6.8]	866 [170]
Fat: Peri-renal(μg/g)	Female	BLQ[f]	11.1[a] [0.44]	32.5[a] [3.5]	188[a] [25]	1230[a] [110]
Fat: Abdominal(μg/g)	Female	BLQ[f]	11.2[a] [0.51]	32.8[a][2.7]	190[a] [30]	1240[a] [55]
Fat: Brown(μg/g)	Female	BLQ[f]	13.3[a,g] [1.1]	38.6[a,g][4.7]	196[a] [59]	1210 [330]

[a] Statistically greater than males at same exposure concentration (PROC TTest (SAS 6.12, SAS Institute, Inc.; Cary, NC), $p < 0.05$, $n = 6$).
[b] Statistically greater than males at same exposure concentration (Tukey's test for multiple comparisons ($p < 0.05$, $n = 6$).
[c] Each exposure level was statistically different from all others for this sex with exception of the 10 and 30 ppm exposure levels which were not statistically different from each other (Tukey's test for multiple comparisons ($p < 0.05$, $n = 6$).
[d] Each exposure level was statistically different from all others for this sex (Tukey's test for multiple comparisons ($p < 0.05$, $n = 6$).
[e] Below the limit of quantitation (9.6 ng/ml plasma).
[f] Below the limit of quantitation (47 ng/g fat).
[g] Statistically greater than peri-renal and abdominal fat at same exposure concentration (Tukey's studentized range multiple comparison test ($p < 0.05$, $n = 6$).
[h] Statistically greater than abdominal fat at same exposure concentration (Tukey's studentized range multiple comparison test ($p < 0.05$, $n = 6$).
* Values represent the mean D4 tissue concentration [standard deviation].

exposure concentration proportionality. Plasma levels for both males and females in the 700 ppm exposure level were markedly higher (63 and 21%, respectively) than predicted by exposure concentration proportionality. Liver levels of D_4 in females were essentially as predicted at all exposure concentrations. In contrast, males at the 700 ppm exposure concentration were 75% greater than would have been predicted by exposure concentration proportionality. In a similar fashion, D_4 concentrations in fat for females were exposure concentration proportional except at the 700 ppm exposure level where the tissue levels were notably greater than expected (30–58% depending on the type of fat sampled). Similarly for males, tissue levels of D_4 in fat were exposure concentration proportional at 30 ppm D_4 but higher than expected at 150 (42–82%) and 700 ppm (> 200%).

Tissue levels of D_4 in females were notably higher than those observed in males at each exposure concentration and tissue type, with the exception of the liver at 700 ppm where the levels were essentially the same. There were also notable differences in tissue D_4 concentration between types of fat at 10 and 30 ppm in both sexes and at 150 ppm for

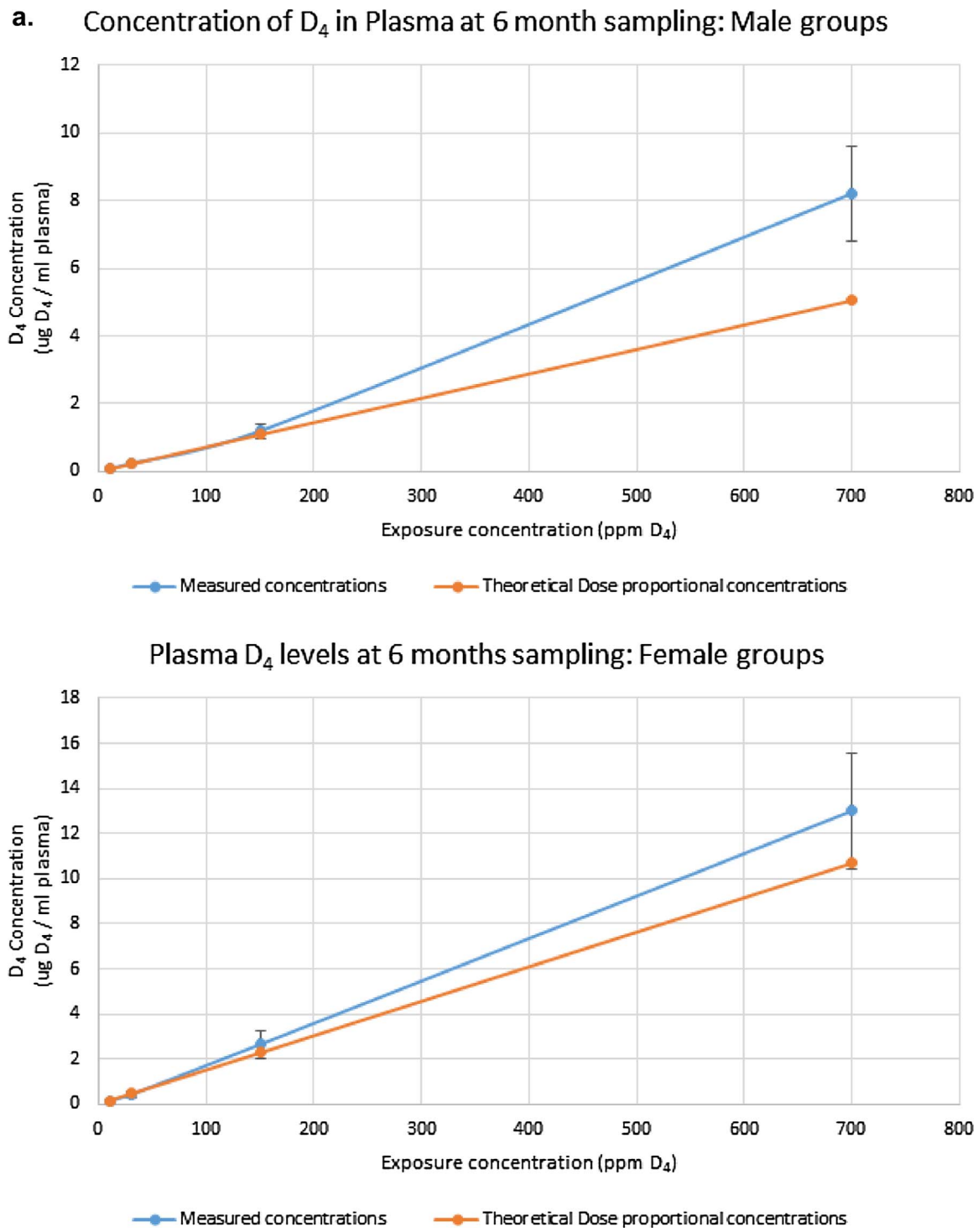
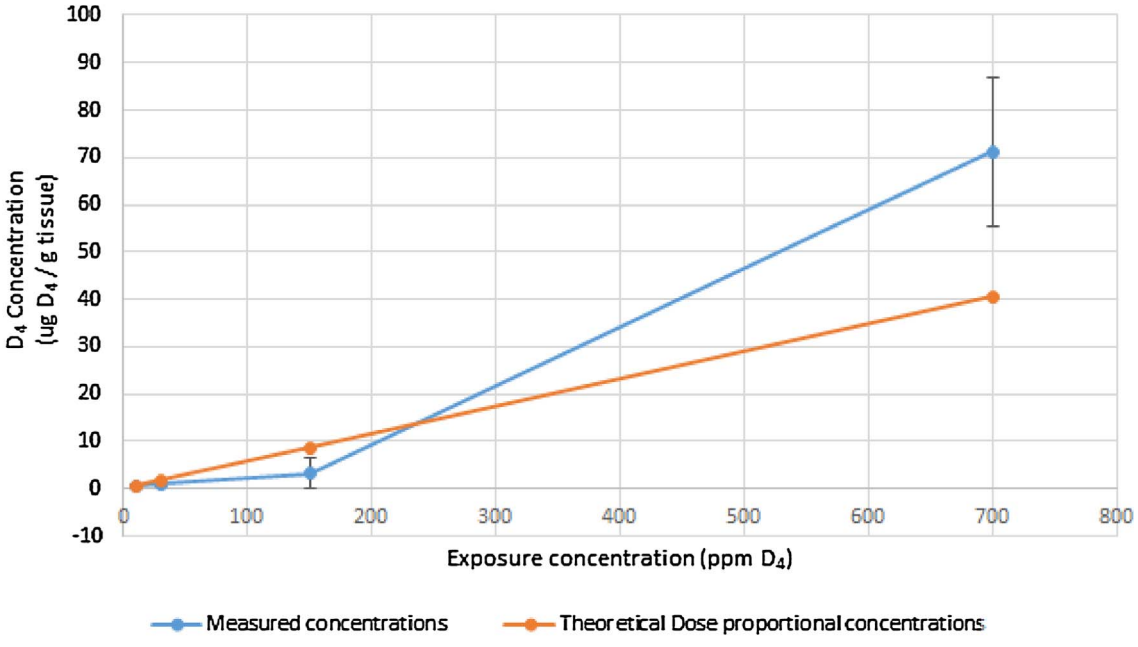


Fig. 2. a. Concentration of D_4 in Plasma of Males and Females. b. Concentration of D_4 in Liver of Males and Females. c. Concentration of D_4 in Brown Fat of Males and Females.

b. Concentration of D_4 in Liver at 6 month sampling: Male groups



Concentration of D_4 in Liver at 6 month sampling: Female groups

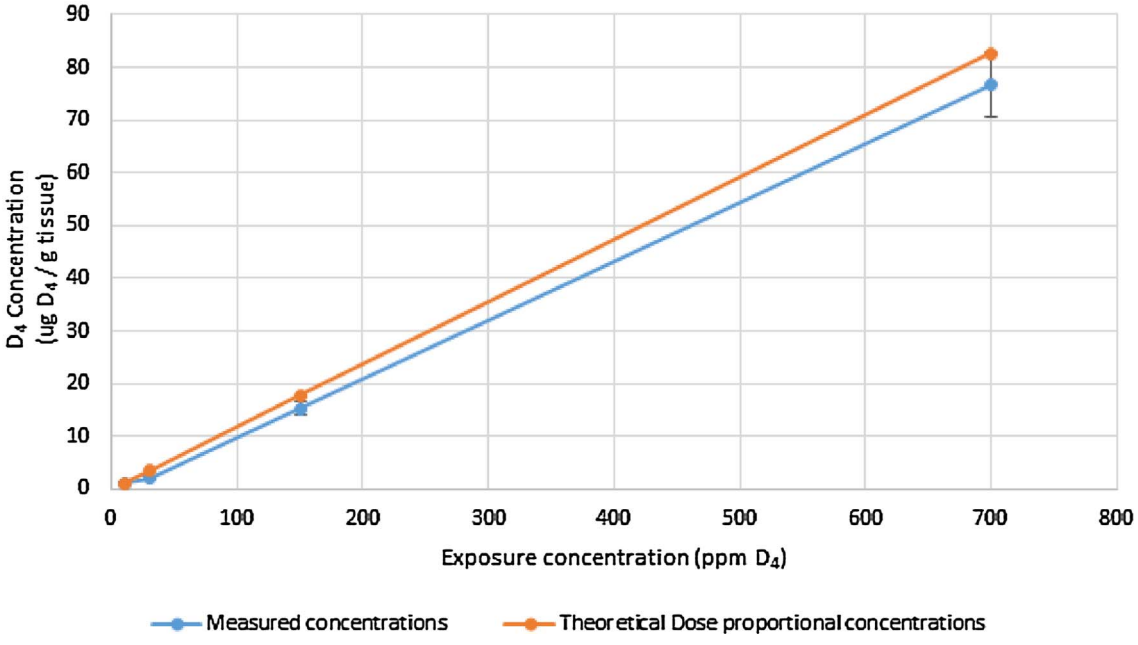


Fig. 2. (*continued*)

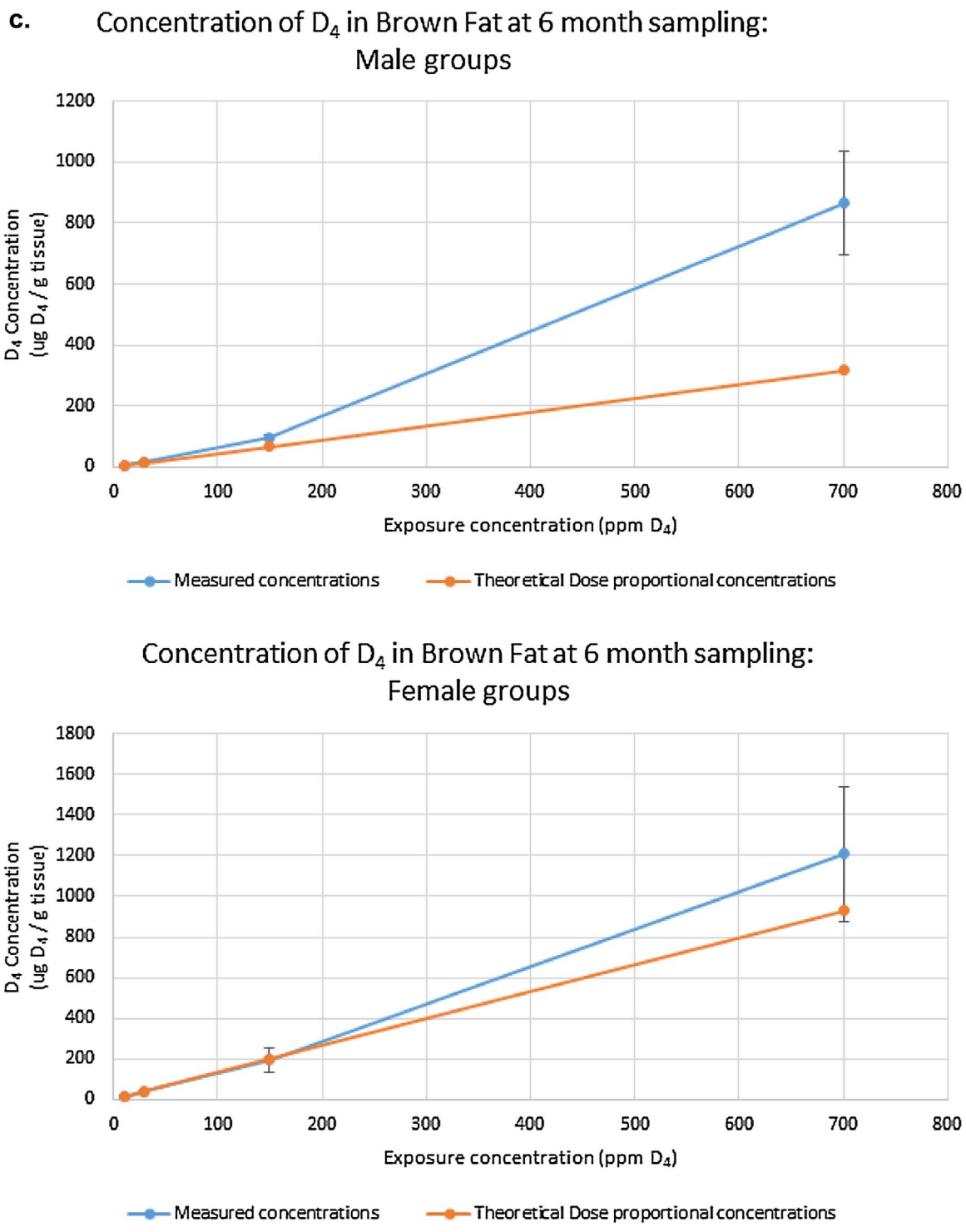


Fig. 2. (*continued*)

females. The concentration in brown fat was notably greater than abdominal or *peri*-renal fat, which were not different from each other.

3.3. Survival and clinical pathology

All animals in the 6- and 12-month exposure groups survived to their scheduled euthanasia. With the exception of the 700 ppm males in subgroup D, survival in all exposure subgroups C and D appeared unaffected by treatment (Table 5, Figs. 3–6). Survival rates were at or better than 50% after 2 years. The 700 ppm D_4 subgroup D males were the only group to exhibit a survival less than 50% at 24 months. The 38% survival rate for this group was statistically lower than the control

Table 5
Survival Statistics for Fischer 344 Rats Exposed to Octamethylcyclotetrasiloxane by Whole-Body Vapor Inhalation Exposure.

Exposure Concentration (ppm)	Subgroup C (12 months on exposure/ 12 months off exposure)		Subgroup D (24 months on exposure)	
	Male (% survival)	Female[a] (% survival)	Male (% survival)	Female[b] (% survival)
0	60	55	58	72
10	70	65	60	62
30	65	55	58	72
150	55	80	58	75
700	50	50	38*	58

[a] Three animals in the control group and 1 animal in the 10 ppm group died accidentally (anesthesia related).
[b] One female died accidentally (caging related).
* Statistically different from control (Chi-square test, $p < 0.05$).

group (58%) and may be related to an earlier onset of mononuclear cell leukemia (see Discussion section), a condition that commonly occurs in F344 rats.

Although there were no treatment-related effects with respect to the daily/weekly clinical observations and ophthalmologic examinations (data not shown), exposure to D_4 gave rise to treatment-related alterations of the following clinical pathology measures.

There was a consistent and marked increase (statistically significant) in white blood cell count attributable to an elevation in the number of leukocytes for males and females exposed to 700 ppm at 3, 6, and 12 months (Table 6). The high variability and lack of a clear dose-response confounds assigning the lower exposure concentrations as affected. There were no other effects on hematology that clearly distinguished treated groups from control groups.

Treatment-related alterations in serum chemistry parameters were observed in both sexes and included increased total protein, and decreases in creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine kinase, and lactate dehydrogenase activities (Tables 7 and 8). Total protein was slightly but consistently elevated in males (4–8%) among the sampling times (3, 6, 9, and 12-months) at 700 ppm D_4. In contrast slight treatment-related increased total protein (5%) was present only at the 12-month sampling time point in females at 700 ppm D_4. Serum creatinine levels were decreased from 6 to 18% across the 3, 6, 9, and 12-month sampling points in both males and females exposed to 700 ppm D_4, although it was not statistically significant at 3 and 9 months in males nor at 3 and 12 months in females. A similar trend was seen at the 150 ppm D_4 exposure, although the decrease was not statistically significant at 3 months in males and at 9 months in females. Perhaps related was a marked reduction in serum creatine kinase activity in 700 ppm D_4 males and females and across sampling times. Reductions in serum activities of AST, ALT, AP, and LDH were consistently present at 150 and 700 ppm D_4 exposure concentrations in males and females.

There were no toxicologically relevant treatment-related alterations in urinalysis parameters (data not shown).

3.4. Body and organ weight

Exposure had no effect on body weight or body weight gain with the exception of 700 ppm males in the 24-month exposure group (data not shown). Mean body weight for this group remained approximately 6% lower than controls for the last few months on study. The decrease was

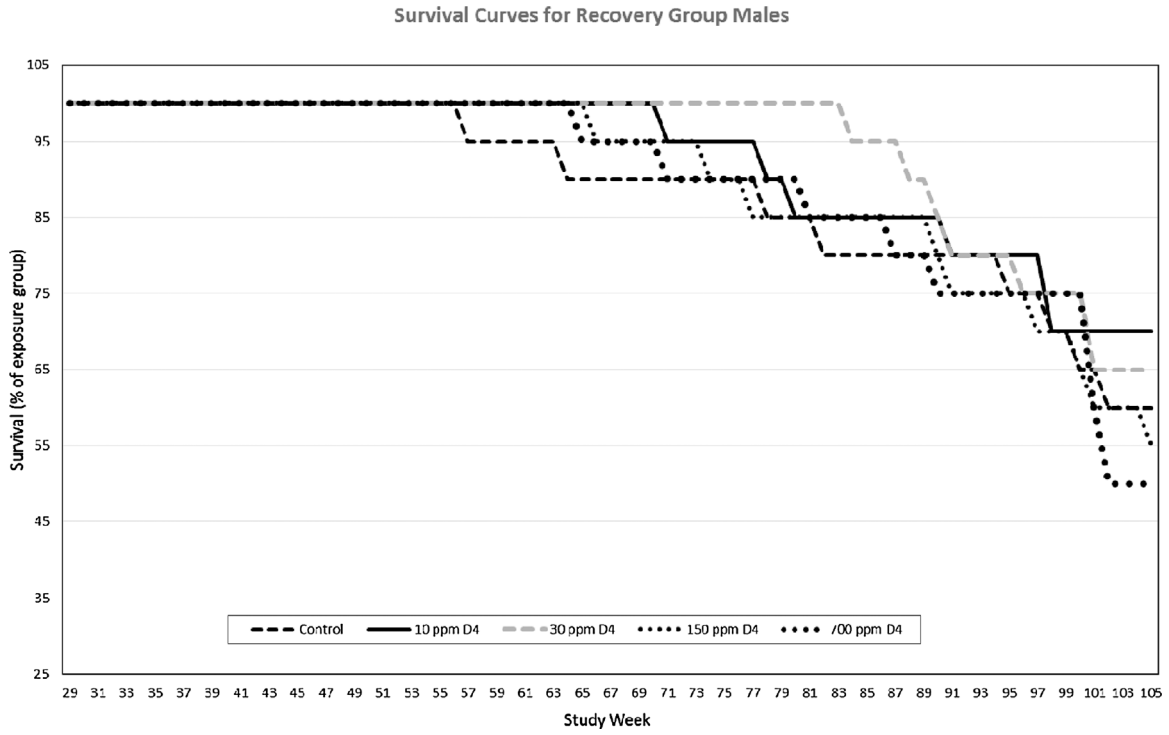


Fig. 3. Survival Curves for Recovery Group Males.

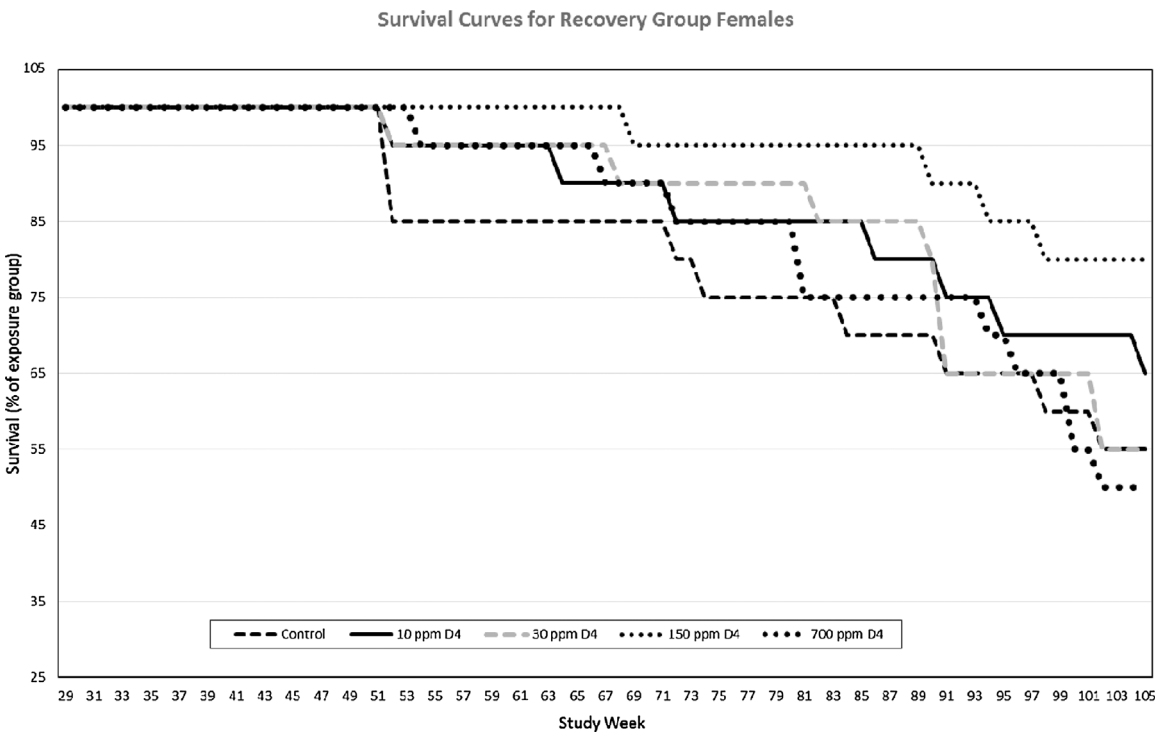


Fig. 4. Survival Curves for Recovery Group Females.

statistically significant only for week 97 and at necropsy.

Treatment-related increases in organ weight were evident for kidney, liver, testes, and uterus.

Kidney weight was evaluated in subgroups B (12-month), C (12-month recovery), and D (24-month) (Tables 9 and 10). There was a consistent and clear statistically significant increase in kidney weight for both males and females in subgroups B and D at the 700 ppm exposure concentration. The increased weight was apparent on the basis of both absolute and relative weight (kidney-to-body weight and kidney-to-brain weight). This was true also for the subgroup C females at 700 ppm. A similar effect on the subgroup C males was not observed. The noted increases in kidney weight may be related to a treatment-related increased severity in chronic nephropathy (see histology section).

Liver weight increases were evident at 6, 12, and 24-months exposure for both sexes (Tables 11 and 12). For males, the liver weight

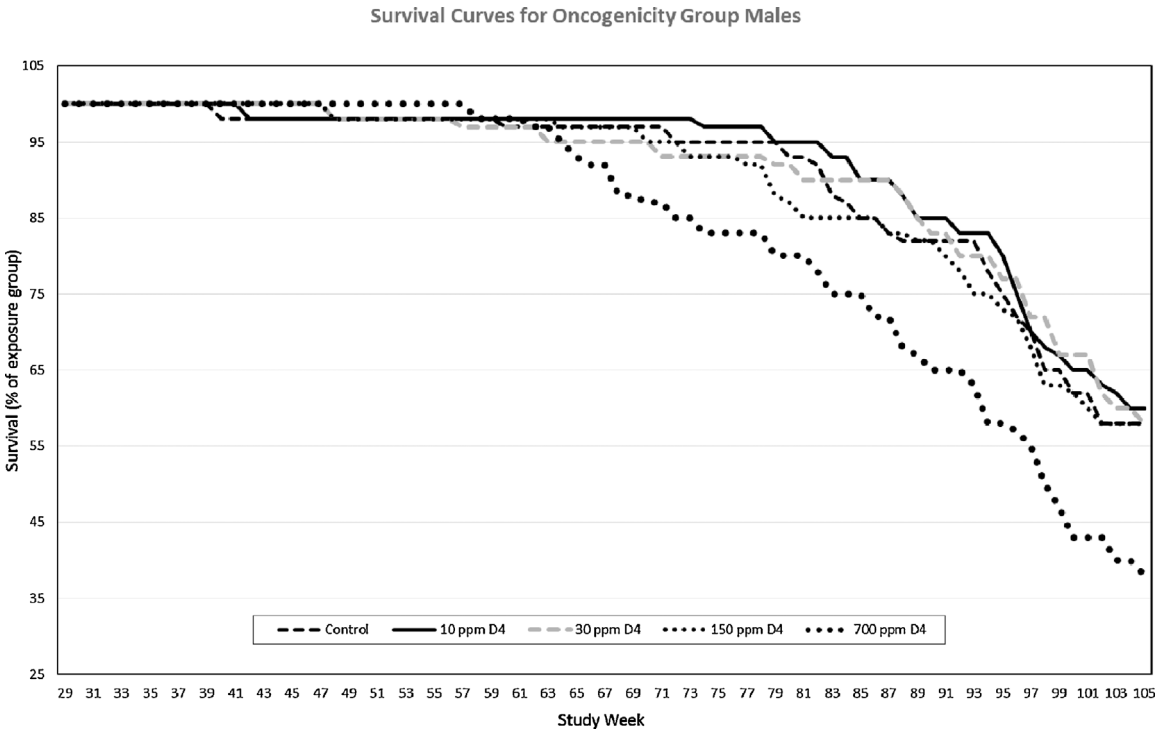


Fig. 5. Survival Curves for Oncogenicity Group Males.

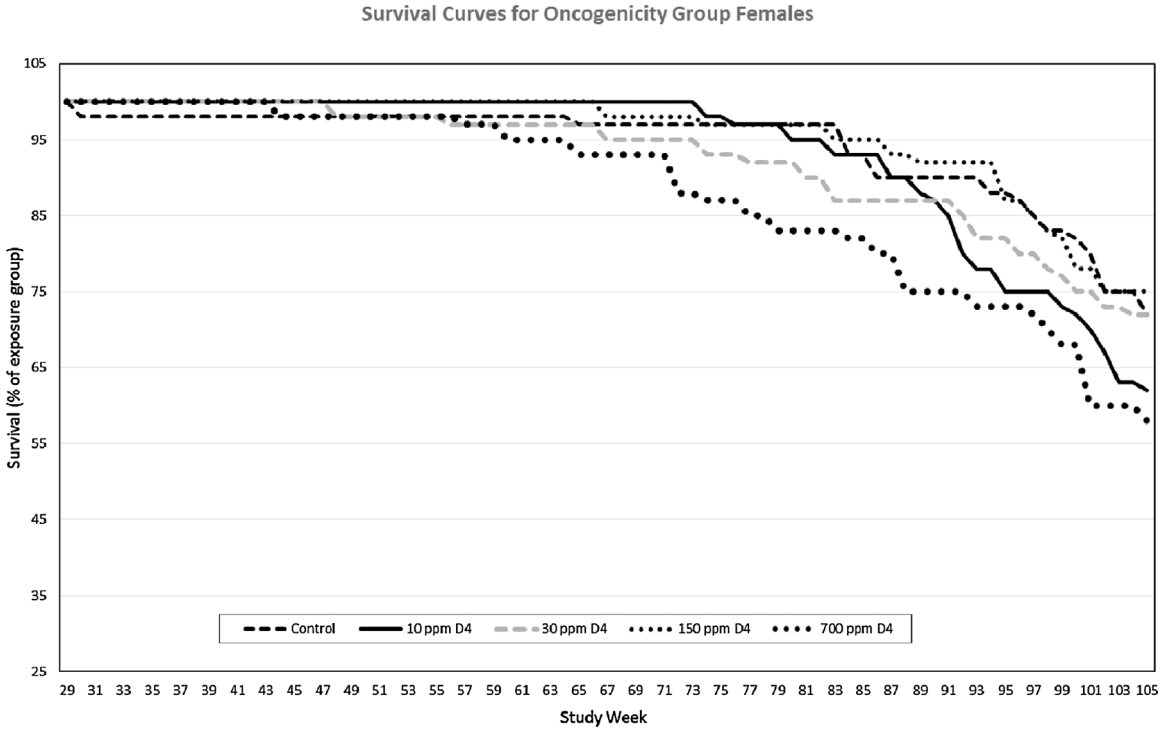


Fig. 6. Survival Curves for Oncogenicity Group Females.

increases were associated with exposure to ≥ 30 ppm D_4 at 6 months, ≥150 ppm at 12 months, and 700 ppm D_4 at 24-months. Group mean liver absolute weight, relative-to-body weight, and relative-to-brain weight ratios for recovery group males across the exposure levels were generally higher than the control values, although only the relative–to-body weight ratio at 700 ppm was statistically significant. Female liver weight increases were statistically significant at all three time points (6, 12, and 24 months) at 700 ppm and at 12 and 24 months with exposure to 150 ppm D_4. Increases were seen in absolute, relative–to-body weight and relative-to-brain weight ratios. There was no effect of treatment among the recovery group females.

Increased uterine weight (24%) was clearly discernable only in the 700 ppm treatment group at 24 months (Table 13). The increase was statistically significant on the basis of absolute and relative-to-body weight ratio. Group mean uterine weights among exposure groups at 12 months were generally increasing with exposure levels ≥30 ppm however, none were statistically different from control. There was no indication of a treatment-related effect on uterine weight among the recovery group females.

Testes weight increase was observed at 12- and 24-month exposure periods for males exposed to 700 ppm D_4 (Table 14). The increase was discernable on an absolute as well as on a relative-to-body weight basis, but only the testis-to-body weight ratio was statistically significant. Increased testes weight was present in the recovery group as an absolute value as well as relative-to-body weight and brain weight at 700 ppm D_4.

Table 6
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on White Blood Cell Count and Lymphocyte Numbers[a] in the Fischer 344 Rat.

Sex	Exposure Duration	Parameter	Exposure Level and group mean value [SD]				
			0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	3-Months	White Blood Cell ($\times 10^3/\mu l$)	9.6 [0.9]	9.8 [1.1]	11.1* [1.7]	10.8 [0.9]	11.5** [1.1]
Male	3-Months	Lymphocytes ($\times 10^3/\mu l$)	7.37 [0.85]	7.60 [1.11]	8.67* [1.39]	8.62* [0.98]	9.24** [0.91]
Male	6-Months	White Blood Cell ($\times 10^3/\mu l$)	7.8 [1.0]	8.5 [0.7]	8.7 [1.0]	8.8 [0.8]	9.2** [0.9]
Male	6-Months	Lymphocytes ($\times 10^3/\mu l$)	5.64 [0.91]	5.95 [0.38]	6.51 [1.07]	6.40 [0.89]	7.16** [1.05]
Male	12-Months	White Blood Cell ($\times 10^3/\mu l$)	7.6 [1.3]	9.0* [1.3]	8.3 [0.8]	8.9 [1.7]	9.0* [0.9]
Male	12-Months	Lymphocytes ($\times 10^3/\mu l$)	4.55 [1.3]	5.52 [1.1]	5.41 [0.56]	5.44 [0.73]	5.88* [0.64]
Female	3-Months	White Blood Cell ($\times 10^3/\mu l$)	6.4 [1.3]	8.4** [1.0]	7.5 [1.6]	8.4** [1.0]	9.7** [1.7]
Female	3-Months	Lymphocytes ($\times 10^3/\mu l$)	5.14 [1.08]	6.73* [0.80]	6.10 [1.20]	6.88** [0.75]	8.00** [1.67]
Female	6-Months	White Blood Cell ($\times 10^3/\mu l$)	6.4 [1.4]	7.4 [1.4]	6.6 [1.3]	8.6** [0.9]	9.5** [0.5]
Female	6-Months	Lymphocytes ($\times 10^3/\mu l$)	4.71 [0.71]	5.76* [0.1.04]	5.27 [1.06]	6.95** [1.07]	7.68** [0.48]
Female	12-Months	White Blood Cell ($\times 10^3/\mu l$)	4.3 [1.5]	5.3 [1.0]	5.0 [0.6]	5.3 [1.4]	6.2** [1.0]
Female	12-Months	Lymphocytes ($\times 10^3/\mu l$)	2.82 [0.93]	3.89** [0.50]	3.44 [0.58]	3.72* [0.35]	4.63** [1.0]

* Statistically different from control ($p < 0.05$, n = 10).
** Statistically different from control ($p < 0.01$, n = 10).
[a] Values represent the mean cell concentration [standard deviation].

Table 7
Clinical Chemistry Parameters[a] with Significant Alterations Related to Octamethylcyclotetrasiloxane Exposure in the Male Fischer 344 Rat.

Exposure Duration	Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
3-Months	Creatinine (mg/dL)	1.11 [0.11]	1.04 [0.07]	1.15 [0.12]	1.02 [0.12]	1.02 [0.08]
3-Months	Protein (g/dL)	7.3 [0.2]	7.3 [0.2]	7.5 [0.2]	7.4 [0.4]	7.6* [0.3]
3-Months	Aspartate Aminotransferase (U/L)	91.0 [8.7]	92.3 [12.6]	93.7 [36.7]	83.1 [10.8]	70.5* [4.2]
3-Months	Alanine Aminotransfease (U/L)	56.4 [7.7]	53.5 [10.9]	55.0 [11.8]	48.4 [7.0]	42.3* [3.2]
3-Months	Alkaline Phosphatase (U/L)	121 [8]	123 [9]	123 [9]	119 [9]	116 [10]
3-Months	Creatine Kinase (U/L)	489 [139]	466 [68]	369 [113]	434 [124]	346* [83]
3-Months	Lactate Dehydrogenase (U/L)	765 [232]	708 [80]	583 [185]	623 [215]	491** [116]
6-Months	Creatinine (mg/dL)	1.20 [0.12]	1.17 [0.05]	1.13 [0.08]	1.09** [0.06]	1.10* [0.05]
6-Months	Protein (g/dL)	7.0 [0.2]	7.3* [0.2]	7.2 [0.4]	7.0 [0.3]	7.6** [0.2]
6-Months	Aspartate Aminotransferase (U/L)	190 [89]	162 [71]	141 [31]	134* [38]	90** [36]
6-Months	Alanine Aminotransfease (U/L)	117 [38]	107 [45]	93 [15]	86 [21]	64** [28]
6-Months	Alkaline Phosphatase (U/L)	119 [10]	136 [19]	122 [11]	117 [21]	111 [12]
6-Months	Creatine Kinase (U/L)	383 [191]	301 [65]	271 [76]	205** [62]	144** [80]
6-Months	Lactate Dehydrogenase (U/L)	602 [164]	568 [141]	514 [138]	388** [112]	212** [149]
9-Months	Creatinine (mg/dL)	1.18 [0.06]	1.17 [0.07]	1.17 [0.08]	1.08** [0.06]	1.11 [0.07]
9-Months	Protein (g/dL)	7.4 [0.2]	7.5 [0.2]	7.4 [0.2]	7.5 [0.2]	7.8** [0.4]
9-Months	Aspartate Aminotransferase (U/L)	147 [42]	150 [83]	116 [28]	88** [9]	79** [24]
9-Months	Alanine Aminotransfease (U/L)	116 [34]	114 [44]	89* [15]	76** [8]	61** [21]
9-Months	Alkaline Phosphatase (U/L)	120 [10]	124 [18]	119 [12]	114 [13]	97** [20]
9-Months	Creatine Kinase (U/L)	202 [84]	160 [102]	222 [214]	150 [49]	110 [43]
9-Months	Lactate Dehydrogenase (U/L)	317 [116]	257 [150]	262 [121]	210* [56]	150** [82]
12-Months	Creatinine (mg/dL)	0.91 [0.07]	0.87 [0.07]	0.87 [0.07]	0.82* [0.04]	0.75** [0.07]
12-Months	Protein (g/dL)	7.1 [0.2]	7.2 [0.2]	7.3 [0.3]	7.2 [0.2]	7.6* [0.4]
12-Months	Aspartate Aminotransferase (U/L)	149 [29]	163 [79]	140 [53]	112 [21]	84* [25]
12-Months	Alanine Aminotransfease (U/L)	117 [18]	132 [63]	108 [37]	92 [20]	83 [35]
12-Months	Alkaline Phosphatase (U/L)	127 [16]	133 [21]	120 [15]	120 [15]	108 [19]
12-Months	Creatine Kinase (U/L)	490 [174]	434 [204]	415 [144]	367 [143]	266** [73]
12-Months	Lactate Dehydrogenase (U/L)	857 [261]	754 [346]	681 [212]	579* [149]	438** [129]

*Statistically different from control ($p < 0.05$, n = 10).
**Statistically different from control ($p < 0.01$, n = 10).
[a] Values represent the group mean [standard deviation].

3.5. Macroscopic examination and histopathology

3.5.1. Non-neoplastic observations

The incidences of non-neoplastic histopathological changes that gave a statistically significant trend and/or pair-wise comparisons were observed in the nasal cavity, lung, kidney, liver, spleen, eye, uterus, and testes. Lesions with a decreasing incidence with increasing exposure concentration were also identified in the liver, nasal cavity, cervix, thyroid, pancreas, and pituitary. In general, lesions were not considered treatment-related unless there was a clear dose-response in incidence/severity between treated and control groups.

Chronic inhalation exposure to D_4 resulted in nasal cavity responses typical of a mild irritant (Table 15). Photomicrographs of representative nasal lesions from control and affected animals are presented in Fig. 7. There was an increase in incidence and severity of intracytoplasmic eosinophilic globules for both males and females after 12 and 24 months of exposure to 700 ppm D_4. In addition, there was a dose-responsive increase in incidence and severity of intracytoplasmic eosinophillic globules among all treatment groups of females exposed for 24 months, although the increase was not significant in the lowest treatment group. The globules were most frequently present in respiratory and adjacent olfactory epithelium at the junction of these two tissue types of the dorsal meatus of the nasal section at the level of the incisive papilla. There was no indication of a treatment-related effect in recovery group males and females.

Goblet cell hyperplasia (primarily associated with respiratory epithelium) was increased in incidence, and in some instances, severity in both males and females exposed to 700 ppm D_4 for 12 and 24 months. The lesion was present in the section through the nasal vestibule (NS1), the maxilloturbinates in the nasal section immediately caudal to the upper incisor teeth (NS2) and/or the section taken at the level of the incisive papilla (NS3). The incidence was also increased at 24 months in males exposed to 150 ppm D_4. A return toward control levels was observed in the recovery groups for both males and females.

A marked increase in the incidence of nasal cavity squamous epithelium hyperplasia was present in both males and females exposed to 700 ppm D_4 for 12 months. The squamous epithelial hyperplasia was noted in the mucosa lining the atrioturbinates in the nasal vestibule. Though still elevated following 24-months exposure to 700 ppm D_4 the incidence at this time point was markedly lower than that observed at 12-months and was no longer statistically significant in the 700 ppm group females. The incidence in both male and female recovery groups had largely reversed after the 12- month recovery period.

Effects on the lungs in females involved a slight, but statistically significant increased incidence of hemorrhage after 24 months of exposure to 700 ppm D_4 (Table 16). In addition, there was a slight, but statistically significant increase in the incidence of alveolar sub-pleural chronic inflammation in females at 24 months in the 10, 30, and 700 ppm treatment groups. This finding was not observed in the recovery groups. Males showed a non-significant increase in hemorrhage incidence at 24 months, but not at 12 months or in the recovery groups.

Treatment-related, non-neoplastic findings in the liver included an increased incidence of centrilobular hypertrophy in males and a reduction in basophilic foci in females at 700 ppm at 12 and 24 months

Table 8
Clinical Chemistry Parameters[a] with Significant Alterations Related to Octamethylcyclotetrasiloxane Exposure in the Female Fischer 344 Rat.

Exposure Duration	Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
3-Months	Creatinine (mg/dL)	1.13 [0.08]	1.02** [0.08]	1.03* [0.08]	1.02* [0.08]	0.98** [0.06]
3-Months	Protein (g/dL)	7.1 [0.2]	6.9 [0.4]	6.9 [0.3]	7.1 [0.2]	7.2 [0.2]
3-Months	Aspartate Aminotransferase (U/L)	85.7 [15.0]	83.7 [12.0]	77.2 [7.5]	70.1** [5.3]	61.0** [7.8]
3-Months	Alanine Aminotransfease (U/L)	42.3 [7.4]	37.1 [4.6]	37.1 [8.5]	35.9** [2.7]	34.4* [3.3]
3-Months	Alkaline Phosphatase (U/L)	85 [11]	78 [9]	79 [6]	79 [11]	72** [6]
3-Months	Creatine Kinase (U/L)	234 [111]	339* [61]	351* [81]	269 [42]	197 [79]
3-Months	Lactate Dehydrogenase (U/L)	336 [205]	562** [113]	550** [122]	408 [55]	284 [118]
6-Months	Creatinine (mg/dL)	1.13 [0.08]	1.10 [0.09]	1.12 [0.13]	1.02* [0.08]	0.99** [0.06]
6-Months	Protein (g/dL)	7.4 [0.3]	7.3 [0.3]	7.3 [0.3]	7.4 [0.2]	7.5 [0.4]
6-Months	Aspartate Aminotransferase (U/L)	135 [66]	105 [26]	127 [40]	86* [25]	55** [4]
6-Months	Alanine Aminotransfease (U/L)	77 [37]	64 [21]	76 [26]	49* [10]	34** [3]
6-Months	Alkaline Phosphatase (U/L)	84 [9]	78 [9]	74 [9]	74 [10]	71* [10]
6-Months	Creatine Kinase (U/L)	364 [68]	380 [75]	343 [105]	293 [59]	200** [41]
6-Months	Lactate Dehydrogenase (U/L)	596 [149]	585 [85]	515 [154]	441* [95]	273** [70]
9-Months	Creatinine (mg/dL)	1.15 [0.05]	1.14 [0.08]	1.15 [0.08]	1.10 [0.05]	1.06* [0.05]
9-Months	Protein (g/dL)	7.9 [0.4]	7.8 [0.4]	8.2** [0.2]	8.2 [0.3]	8.0 [0.7]
9-Months	Aspartate Aminotransferase (U/L)	134 [65]	118 [51]	106 [54]	77** [16]	50** [3]
9-Months	Alanine Aminotransfease (U/L)	68 [27]	69 [32]	61 [14]	50 [12]	37** [4]
9-Months	Alkaline Phosphatase (U/L)	65 [8]	62 [6]	61 [14]	56 [5]	46** [6]
9-Months	Creatine Kinase (U/L)	313 [177]	274 [164]	248 [123]	197 [55]	152* [53]
9-Months	Lactate Dehydrogenase (U/L)	408 [172]	327 [118]	297 [169]	246* [68]	169** [49
12-Months	Creatinine (mg/dL)	0.92 [0.06]	0.93 [0.08]	0.86 [0.05]	0.81** [0.03]	0.78** [0.04]
12-Months	Protein (g/dL)	7.9 [0.4]	8.1 [0.3]	8.0 [0.3]	8.1 [0.1]	8.3* [0.3]
12-Months	Aspartate Aminotransferase (U/L)	142 [27]	126 [51]	107 [31]	105* [33]	51** [4]
12-Months	Alanine Aminotransferase (U/L)	77 [24]	58* [12]	57* [14]	48** [12]	37** [3]
12-Months	Alkaline Phosphatase (U/L)	65 [7]	61 [6]	58* [6]	53** [5]	47** [5]
12-Months	Creatine Kinase (U/L)	259 [58]	250 [65]	247 [56]	250 [57]	198 [40]
12-Months	Lactate Dehydrogenase (U/L)	522 [100]	497 [106]	518 [82]	462 [85]	311** [40]

* Statistically different from control ($p < 0.05$, n = 10).
** Statistically different from control ($p < 0.01$, n = 10).
[a] Values represent the group mean [standard deviation].

Table 9
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Kidney Weight[a] in Male Fischer 344 Rat.

Exposure Group	Organ: Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
12-Months	Kidney: absolute[b]	2.51 [0.15]	2.62 [0.15]	2.67 [0.17]	2.60 [0.15]	2.82** [0.19]
12-Months	Kidney: organ/body weight[c]	0.55 [0.02]	0.55 [0.02]	0.56 [0.02]	0.55 [0.03]	0.61** [0.02]
12-Months	Kidney: organ/brain weight[d]	126.5 [5.1]	132.7 [6.7]	134.4 [7.8]	131.2 [8.4]	141.2** [8.7]
	N	10	10	10	10	10
24-Months	Kidney: absolute	3.08 [0.32]	3.18 [0.53]	3.06 [0.29]	3.05 [0.35]	3.51** [0.35]
24-Months	Kidney: organ/body weight	0.64 [0.06]	0.67 [0.14]	0.64 [0.06]	0.65 [0.07]	0.77** [0.09]
24-Months	Kidney: organ/brain weight	148.4 [15.6]	154.6 [25.2]	149.4 [13.8]	148.7 [16.0]	170.3** [17.5]
	N	35	36	35	35	23
Recovery	Kidney: absolute	2.97 [0.21]	3.08 [0.37]	3.25 [0.45]	3.21 [0.42]	3.21 [0.22]
Recovery	Kidney: organ/body weight	0.68 [0.05]	0.70 [0.09]	0.75* [0.10]	0.74 [0.15]	0.74* [0.06]
Recovery	Kidney: organ/brain weight	145.3 [10.6]	150.5 [14.6]	158.7 [20.6]	154.3 [22.4]	156.3* [10.3]
	N	12	14	13	11	10

* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).
[a] Values represent the group mean [standard deviation].
[b] grams.
[c] (gram kidney/gram body weight)100.
[d] (gram kidney/gram brain weight)100.

Table 10
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Kidney Weight[a] in Female Fischer 344 Rat.

Exposure Group	Organ: Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
12-Months	Kidney: absolute[b]	1.59 [0.08]	1.59 [0.07]	1.58 [0.08]	1.63 [0.05]	1.67* [0.06]
12-Months	Kidney: organ/body weight[c]	0.64 [0.03]	0.60* [0.03]	0.64 [0.03]	0.64 [0.03]	0.65 [0.03]
12-Months	Kidney: organ/brain weight[d]	87.6 [4.6]	88.2 [3.0]	87.5 [4.4]	89.6 [3.4]	92.8* [3.8]
	N	10	10	10	10	10
24-Months	Kidney: absolute	2.01 [0.18]	2.04 [0.15]	2.02 [0.16]	2.13** [0.20]	2.25** [0.22]
24-Months	Kidney: organ/body weight	0.61 [0.05]	0.59 [0.03]	0.61 [0.04]	0.62 [0.05]	0.70** [0.08]
24-Months	Kidney: organ/brain weight	107.7 [10.4]	111.3 [8.0]	108.8 [9.5]	120.4** [29.5]	123.7** [12.2]
	N	43	37	43	45	35
Recovery	Kidney: absolute	2.04 [0.12]	2.00 [0.14]	2.00 [0.09]	2.07 [0.10]	2.18* [0.10]
Recovery	Kidney: organ/body weight	0.64 [0.05]	0.63 [0.04]	0.65 [0.03]	0.67 [0.05]	0.75* [0.12]
Recovery	Kidney: organ/brain weight	109.6 [6.4]	107.9 [8.3]	108.7 [6.7]	113.8 [6.6]	117.7* [7.7]
	N	11	13	11	16	10

* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).
[a] Values represent the group mean [standard deviation].
[b] grams.
[c] (gram kideny/gram body weight)100.
[d] (gram kidney/gram brain weight)100.

(Table 17).

Chronic nephropathy was a common finding, observed in males (80%-100%) and females (28%–92%) in all groups including controls at 12 and 24 months (Table 18). No treatment-related differences in incidence or severity were evident at 12 months or in recovery groups. The incidence of chronic nephropathy was elevated at 24 months for females at ≥ 30 ppm (statistically significant). Although not statistically significant, the severity scores were generally increasing with increasing exposure concentration for both males and females at 24 months.

There was an increased incidence of hematopoietic proliferation in the spleen of 700 ppm females and 30 ppm males at 24 months (Table 19). Severity scores were similar among exposure concentrations.

Effects of exposure on the female reproductive tract were limited to the 700 ppm treatment group (Table 20). The incidence of uterine cystic endometrial hyperplasia was increased approximately 3-fold in the 24-month exposure group. In the histologic sections of affected uteri, cystic endometrial hyperplasia was characterized by increased numbers of variably sized, cystically dilated endometrial glands (Fig. 8). These glands were mostly lined by low cuboidal epithelial cells and less frequently by columnar cells. Some of these dilated glands contained weakly stained secretory material. The endometrial stroma contained increased amounts of collagen. A mild infiltrate of neutrophils was variably present within some of the cystic glands.

There was a low incidence of cervical squamous epithelial hyperplasia in both the 24-month exposure and recovery groups and a slight reduction in cervical stromal hyperplasia in the 24-month exposure group. A marginal, but statistically significant, increase in incidence of ovarian atrophy was also present in the 24-month exposure group. These low incidence findings were not considered toxicologically significant.

A focused and detailed histomorphological review of the ovaries, uterus, vagina, pituitary and mammary gland from the control and high treatment group females was conducted with the purpose to assess the potential for treatment-related alteration of estrous cycle synchronicity among these tissues. Abnormal estrous cycle (lack of synchronicity among the tissues) was present in both control and 700 ppm D_4 group

Table 11
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Liver Weight[a] in Male Fischer 344 Rats.

Exposure Group	Organ: Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
6-Months	Liver: absolute	11.23 [1.40]	12.29 [0.66]	12.99** [0.79]	12.80* [0.86]	14.28** [0.44]
	N	6	6	6	6	6
12-Months	Liver: absolute[b]	12.96 [1.44]	14.02 [1.48]	14.55 [1.36]	15.08** [1.30]	16.81** [1.54]
12-Months	Liver: organ/body weight[c]	2.82 [0.14]	2.93 [0.14]	3.05** [0.12]	3.18** [0.20]	3.65** [0.14]
12-Months	Liver: organ/brain weight[d]	651.6 [58.7]	710.4 [70.2]	732.3* [64.2]	760.8** [61.7]	841.7** [74.3]
	N	10	10	10	10	10
24-Months	Liver: absolute	16.07 [3.45]	15.78 [2.53]	16.05 [2.64]	16.24 [2.57]	20.48** [2.84]
24-Months	Liver: organ/body weight	3.34 [0.85]	3.33 [0.53]	3.36 [0.53]	3.46 [0.47]	4.50** [0.63]
24-Months	Liver: organ/brain weight	776.1 [169.4]	767.4 [118.5]	784.3 [129.6]	792.8 [125.2]	993.9** [142.7]
	N	35	36	35	35	23
Recovery	Liver: absolute	14.02 [1.54]	14.42 [1.56]	15.49 [3.17]	15.82 [3.32]	17.12 [4.14]
Recovery	Liver: organ/body weight	3.21 [0.36]	3.28 [0.36]	3.61 [0.78]	3.66 [0.90]	3.95* [0.95]
Recovery	Liver: organ/brain weight	685.9 [76.3]	705.4 [62.5]	756.5 [150.0]	758.5 [157.9]	834.3 [198.6]
	N	12	14	13	11	10

* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).
[a] Values represent the group mean [standard deviation].
[b] gram.
[c] (gram liver/gram body weight)100.
[d] (gram liver/gram brain weight)100.

Table 12
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Liver Weight[a] in Female Fischer 344 Rats.

Exposure Group	Organ: Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
6-Months	Liver: absolute[b]	6.79 [0.66]	7.02 [0.48]	6.83 [0.87]	7.44 [0.41]	8.16** [0.68]
	N	6	6	6	6	6
12-Months	Liver: absolute	7.45 [0.68]	8.13 [1.13]	7.59 [0.41]	8.47** [0.48]	9.64** [0.66]
12-Months	Liver: organ/body weight[c]	2.98 [0.13]	3.06 [0.46]	3.08 [0.10]	3.29** [0.11]	3.74** [0.14]
12-Months	Liver: organ/brain weight[d]	409.2 [35.6]	450.6 [62.6]	419.3 [24.2]	464.8** [27.9]	535.0** [35.4]
	N	10	10	10	10	10
24-Months	Liver: absolute	9.93 [1.58]	10.47 [1.41]	10.40 [1.54]	11.36** [1.61]	12.85** [1.86]
24-Months	Liver: organ/body weight	3.00 [0.43]	3.02 [0.29]	3.13 [0.40]	3.30** [0.25]	3.98** [0.43]
24-Months	Liver: organ/brain weight	532.0 [86.3]	569.8* [75.4]	561.5 [86.1]	641.7** [173.3]	706.2** [106.3]
	N	43	37	43	45	35
Recovery	Liver: absolute	10.08 [1.02]	9.36 [1.10]	9.72 [1.01]	9.82 [0.93]	9.59 [1.34]
Recovery	Liver: organ/body weight	3.13 [0.27]	2.95 [0.22]	3.15 [0.30]	3.17 [0.25]	3.26 [0.33]
Recovery	Liver: organ/brain weight	539.6 [48.9]	506.6 [63.6]	529.7 [64.5]	540.5 [49.8]	519.3 [78.3]
	N	11	13	11	16	10

* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).
[a] Values represent the group mean [standard deviation].
[b] gram.
[c] (gram liver/gram body weight)100.
[d] (gram liver/gram brain weight)100.

animals at each time point. The proportion was the same for controls and treated at 6 months (3 of 6 females in each group). Those showing abnormal cycles in both groups exhibited features (basophilic corpora lutea (CL) with luteolysis) of extended inter-ovulatory interval (5–6 day cycle). However, the incidence of vaginal mucification was higher in the 700 ppm D_4 group (3 of 3) as compared to the control group (1 versus 3). At 12 months, the number of females with abnormal cycles (basophilic CL with luteolysis) was higher in the 700 ppm D_4 group females (4/10) as compared to the control group (2/10). This was accompanied by an increased incidence and severity of vaginal mucification (2/9 controls, 5/9 treated). However, at 24 months the only features in these aged animals to differentiate control from treated was the increased incidence of cystic endometrial hyperplasia and uterine adenoma (see Section 3.4.2).

A 60% incidence of testes interstitial cell hyperplasia was observed in control group males at 12 months and a slightly higher incidence was observed (80%) in the male rats exposed to 700 ppm D_4. Intermediate exposure group males for this time point were not evaluated. At 24 months, a modest increase in incidence and severity of testes interstitial cell hyperplasia was observed in the 150 and 700 ppm D_4 group males (Table 21). There was no indication of a treatment-related effect on testes interstitial cell hyperplasia among the recovery groups.

3.6. Neoplastic observations

Neoplastic findings in the controls were typical of those expected of F344 rats. Mononuclear cell leukemia was a common finding and a leading cause of mortality for males (Tables 22 and 23). A higher incidence of mortality with mononuclear cell leukemia as the probable cause of death was observed for 700 ppm males which accounted for the group's decreased survival. However, histological examination demonstrated no treatment-related increase in the overall incidence.

Endometrial adenomas were present in four of the sixty 700 ppm group females at 24 months (Table 24). None were present in any animal at 12 months and none were present in the control, 10, 30 and 150 ppm exposure groups at 24 months. However, endometrial

Table 13
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Uterine Weight[a] in Female Fischer 344 Rats.

Exposure Group	Organ: Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
12-Months	Uterus: absolute[b]	1.030 [0.202]	0.945 [0.148]	1.042 [0.169]	1.214 [0.303]	1.244 [0.401]
12-Months	Uterus: organ/body weight[c]	0.416 [0.101]	0.356 [0.062]	0.422 [0.060]	0.474 [0.131]	0.484 [0.156]
12-Months	Uterus: organ/brain weight[d]	56.56 [10.98]	52.28 [7.61]	57.48 [9.16]	66.45 [15.74]	69.33 [23.16]
	N	10	10	10	10	10
24-Months	Uterus: absolute	1.072 [0.551]	1.084 [0.637]	0.921 [0.246]	1.143 [0.858]	1.570* [1.279]
24-Months	Uterus: organ/body weight	0.325 [0.161]	0.316 [0.190]	0.279 [0.080]	0.331 [0.225]	0.499* [0.446]
24-Months	Uterus: organ/brain weight	57.34 [28.91]	58.89 [33.38]	49.66 [13.10]	63.90 [48.13]	86.47 [70.18]
	N	43	37	43	45	35
Recovery	Uterus: absolute	0.867 [0.142]	1.185 [0.364]	1.437 [1.028]	3.160 [7.996]	1.079 [0.301]
Recovery	Uterus: organ/body weight	0.270 [0.047]	0.376* [0.122]	0.473 [0.357]	1.129 [3.027]	0.370* [0.108]
Recovery	Uterus: organ/brain weight	46.39 [6.99]	64.17* [19.95]	78.17 [55.47]	178.87 [460.25]	58.51 [17.48]
	N	11	13	11	16	10

**Statistically different from control ($p < 0.01$).
* Statistically different from control ($p < 0.05$).
[a] Values represent the group mean [standard deviation].
[b] gram.
[c] (gram uterus/gram body weight)100.
[d] (gram uterus/gram brain weight)100.

Table 14
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Testes Weight[a] in Male Fischer 344 Rats.

Exposure Group	Organ: Parameter	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
12-Months	Testes: absolute[b]	3.2	3.198	3.161	3.342	3.475
		[0.212]	[0.280]	[0.359]	[0.275]	[0.243]
12-Months	Testes: organ/body weight[c]	0.698	0.672	0.667	0.706	0.757*
		[0.029]	[0.064]	[0.086]	[0.066]	[0.047]
12-Months	Testes: organ/brain weight[d]	161	162	159	169	174
		[7.9]	[13.3]	[17.9]	[13.1]	[12.2]
	N	10	10	10	10	10
24-Months	Testes: absolute	4.679	4.901	5.182	4.736	5.677
		[1.738]	[1.853]	[1.787]	[1.574]	[2.091]
24-Months	Testes: organ/body weight	0.97	1.032	1.085	1.015	1.243*
		[0.360]	[0.381]	[0.366]	[0.344]	[0.458]
24-Months	Testes: organ/brain weight	226	238	253	232	274
		[84.5]	[88.6]	[87.5]	[79.0]	[97.1]
	N	35	36	35	35	23
Recovery	Testes: absolute	4.081	4.898	3.981	5.168	6.832*
		[1.506]	[2.466]	[1.249]	[2.697]	[2.552]
Recovery	Testes: organ/body weight	0.939	1.105	0.912	1.174	1.573*
		[0.369]	[0.531]	[0.257]	[0.591]	[0.570]
Recovery	Testes: organ/brain weight	200	240	194	246	333*
		[73.2]	[119.5]	[60.5]	[122.3]	[123.9]
	N	12	14	13	11	10

* $p < 0.05$.
[a] Values represent the group mean [standard deviation].
[b] gram.
[c] (gram testes/gram body weight)100.
[d] (gram testes/gram brain weight)100.

adenoma was identified in one 30 ppm recovery group female and endometrial adenocarcinoma in one 150 ppm recovery group female. Photomicrographs of representative uterine lesions from control and affected animals are presented in Fig. 8. Histologically, the uterine adenomas were in general, well delineated, exophytic masses with papillary projections into the uterine lumen. The adenomas were composed of neoplastic epithelial cells which were mostly cuboidal to columnar, well differentiated, and variably arranged in glandular and/or papillary formations, generally in a single layer separated by variable amounts of stroma. Small amounts of pyknotic debris within vacuoles were noted within the neoplastic cells lining the glandular structures in some cases. Small numbers of scattered mitotic figures were noted in some of the adenomas. The neoplastic glandular structures were variably dilated and contained necrotic cell debris. Moderate numbers of neutrophils admixed with lesser numbers of mononuclear cells infiltrated the neoplastic glandular structures and the stroma in some cases. Cystic endometrial hyperplasia was noted in some sections of uterus unassociated with the adenoma.

In 700 ppm males, decreases in the incidence of pituitary pars distalis adenoma and pancreatic islet cell adenoma were evident as was the incidence of thyroid c-cell adenoma/carcinoma in 700 ppm females at 24 months (Tables 25, 26, and 27).

Interstitial cell adenoma of the testis was not present in controls or treated animals at 12 months [Table 21]. However, more than 80% of all animals in all exposure groups had developed testes interstitial cell adenoma by 24 months. The incidence of bilaterial interstitial cell adenoma was slightly higher (positive trend analysis among the exposure levels due to the higher value at 700 ppm D_4 group) and the incidence of unilateral interstitial cell adenoma was slightly lower in the 700 ppm D_4 group at 24 months. The incidence/severity of interstitial cell adenoma gave no indication of a treatment-related effect in recovery group males.

4. Discussion

Vapor inhalation exposure of male and female F344 rats at concentrations up to 150 ppm D_4 for up to 24 months was generally well tolerated. Vapor inhalation exposure at 700 ppm D_4 induced few effects of toxicological interest, some that were expressed only in males (increased testes weight and interstitial cell hyperplasia, and decreased survival), only in females (uterine endometrial cell hyperplasia and adenoma, chronic nephropathy) or in both males and females (liver and kidney weight increases, clinical chemistry alterations, lymphocytic leukocytosis, and respiratory tract irritation).

Decreased survival was apparent only in the 700 ppm males and was attributed to increased mononuclear cell leukemia-related mortality. However, the overall incidence of this common age-related tumor in Fischer 344 rats was not increased in either sex. Mononuclear cell leukemia (MCL), also known as large granular lymphocyte leukemia, is well characterized but not well understood with regard to the etiology involved (Thomas et al., 2007). The increased MCL-related mortality in males absent an increased overall incidence suggests exposure to 700 ppm D_4 may have supported progression of the inherent expression of this common tumor. Although there is insufficient information to define how this was achieved, there is the possibility that the lymphocytic leukocytosis observed at 3, 6, and 12 months in both males and females may have played a role. The lymphocytic leukocytosis was not characterized with respect to lymphocyte type and it remains unclear if the lymphocytosis is representative of a primary or secondary response to D_4 exposure. Lymphocytic leukocytosis was not apparent in prior inhalation studies with D_4 in rats or humans and tests of immune system function were negative for effects (Burns-Naas et al., 2002; Klykken et al., 1999; Looney et al., 1998).

Inhalation of irritant gases elicits a range of morphological alterations in the upper and lower respiratory tract (Dungworth et al., 1995;

Table 15
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on the Nasal Cavity[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	12-Months	Eosinophilic Globules	5/10[a] [1]	3/10 [1]	2/10 [1]	4/10 [1.3]	10/10** [1.3]
Male	24-Months	Eosinophilic Globules	16/58[a] [1.1]	21/58 [1.7]	21/59 [1.3]	22/58 [1.4]	45/56** [2.3]
Male	Recovery	Eosinophilic Globules	18/20 [1.1]	14/19 [1]	16/20 [1.2]	12/18 [1.1]	20/20 [1.7]
Male	12-Months	Respiratory Epithelium, Goblet Cell Hyperplasia	0/10[a] [–]	1/10 [1]	0/10 [–]	0/10 [–]	10/10** [1.4]
Male	24-Months	Respiratory Epithelium, Goblet Cell Hyperplasia	2/58[a] [2]	3/58 [2.3]	3/59 [1.3]	7/58* [2.3]	15/56** [1.3]
Male	Recovery	Respiratory Epithelium, Goblet Cell Hyperplasia	0/20[a] [–]	0/19 [–]	0/20 [–]	2/18 [1]	3/20* [1.3]
Male	12-Months	Squamous Epithelium, Hyperplasia	0/10[a] [–]	0/10 [–]	1/10 [1]	0/10 [–]	10/10** [1]
Male	24-Months	Squamous Epithelium, Hyperplasia	0/58[a] [–]	0/58 [–]	0/59 [–]	0/58 [1]	3/56* [1.7]
Male	Recovery	Squamous Epithelium, Hyperplasia	0/20 [–]	0/19 [–]	2/20 [2]	0/18 [–]	2/20 [1.5]
Male	12-Months	Suppurative Inflammation	1/10[a] [1]	3/10 [1]	1/10 [2]	1/10 [1]	6/10** [1.2]
Male	24-Months	Suppurative Inflammation	7/58 [1.4]	10/58 [1.6]	11/59 [1.3]	9/58 [2.3]	3/56 [2.3]
Male	Recovery	Suppurative Inflammation	4/20 [1.2]	0/19 [–]	4/20 [1.5]	3/18 [1]	3/20 [1.7]
Male	12-Months	Nasal Cavity: Lumen; Foreign Body	1/10	3/10	1/10	3/10	1/10
Male	24-Months	Nasal Cavity: Lumen; Foreign Body	20/58[a]	11/58	14/59	13/58	6/56*
Male	Recovery	Nasal Cavity: Lumen; Foreign Body	6/20	3/19	3/20	5/18	2/20
Female	12-Months	Eosinophilic Globules	7/10[a] [1]	9/10 [1]	7/10 [1]	9/10 [1.4]	10/10* [2]
Female	24-Months	Eosinophilic Globules	17/59[a] [1.4]	25/59 [1.6]	33/59** [1.9]	46/60* [1.9]	55/60** [2]
Female	Recovery	Eosinophilic Globules	16/20 [1.5]	15/20 [1.7]	14/20 [1.9]	15/20 [1.7]	19/20 [1.8]
Female	12-Months	Respiratory Epithelium, Goblet Cell Hyperplasia	1/10[a] [1]	0/10 [–]	0/10 [–]	2/10 [1]	8/10** [1.9]
Female	24-Months	Respiratory Epithelium, Goblet Cell Hyperplasia	1/59[a] [2]	0/59 [–]	0/59 [–]	2/60 [2]	36/60** [1.4]
Female	Recovery	Respiratory Epithelium, Goblet Cell Hyperplasia	0/20 [–]	0/20 [–]	1/20 [1]	0/20 [–]	2/20 [1]
Female	12-Months	Squamous Epithelium, Hyperplasia	0/10[a] [–]	1/10 [1]	1/10 [1]	1/10 [1]	9/10** [1]
Female	24-Months	Squamous Epithelium, Hyperplasia	1/59 [3]	0/59 [–]	0/59 [–]	1/60 [1]	4/60 [1]
Female	Recovery	Squamous Epithelium, Hyperplasia	0/20 [–]	0/20 [–]	0/20 [–]	1/20 [1]	2/20 [1]
Female	12-Months	Suppurative Inflammation	1/10 [1]	2/10 [1]	1/10 [1]	0/10 [–]	4/10 [1]
Female	24-Months	Suppurative Inflammation	0/59 [–]	2/59 [1]	5/59 [1.4]	4/60 [1.5]	2/60 [1]
Female	Recovery	Suppurative Inflammation	2/20 [1]	4/20 [1.2]	4/20 [1.8]	4/20 [1.5]	3/20 [2]
Female	12-Months	Nasal Cavity: Lumen; Foreign Body	1/10	1/10	1/10	1/10	1/10
Female	24-Months	Nasal Cavity: Lumen; Foreign Body	5/59	4/59	8/590	8/60	3/60
Female	Recovery	Nasal Cavity: Lumen; Foreign Body	1/20	5/20*	1/20	2/20	1/20

[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group mean incidence [average severity grade].
* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).

Harkema et al., 2006). The D_4-induced morphological changes in the nasal cavities were typical irritant effects (such as increased eosinophillic globules, squamous epithelium hyperplasia, and respiratory epithelium goblet cell hyperplasia) and did not progress to neoplasia in this repeated-exposure chronic bioassay.

The decreases in serum enzyme activities, modest decrease in creatinine, and slight increase in total protein are unexplained effects of treatment. However, they are not considered adverse for lack of any evidence of loss of tissue/organ function or associated impact on animal health and survival. The effect on total protein was primarily observed at the 700 ppm D_4 level and the decrease in creatinine and serum enzyme activities at treatment levels of ≥150 ppm D_4.

D_4 caused a reversible, treatment-related increase in liver weight in both sexes, which correlated with hepatocellular hypertrophy in 700 ppm males in the 12 and 24-month exposure groups. Hepatocellular hypertrophy was not apparent in females. Increased liver weight and hepatocellular hypertrophy and hyperplasia have been previously reported (McKim et al., 1998, 1999, 2001a) and are regarded as an adaptive and non-adverse response. The decrease in basophilic hepatocellular foci in females at 12 and 24 months was not considered adverse.

Chronic nephropathy is a common age-related spontaneous change in the Fischer 344 rat (Hard and Khan, 2004). Exposure to D_4 was associated with slight-modest increases in the severity of this condition (males and females) and an increased incidence in females. The modest increases in kidney weight observed at the high exposure concentration were considered associated with the advanced nephropathy. Though the mechanism(s) responsible for the D_4-induced exacerbation of this common disorder is unknown, the effect is considered to be of no toxicological significance.

Testicular interstitial cell adenoma is a common age-related tumor in the rat (Haseman et al., 1998). High incidences were demonstrated in

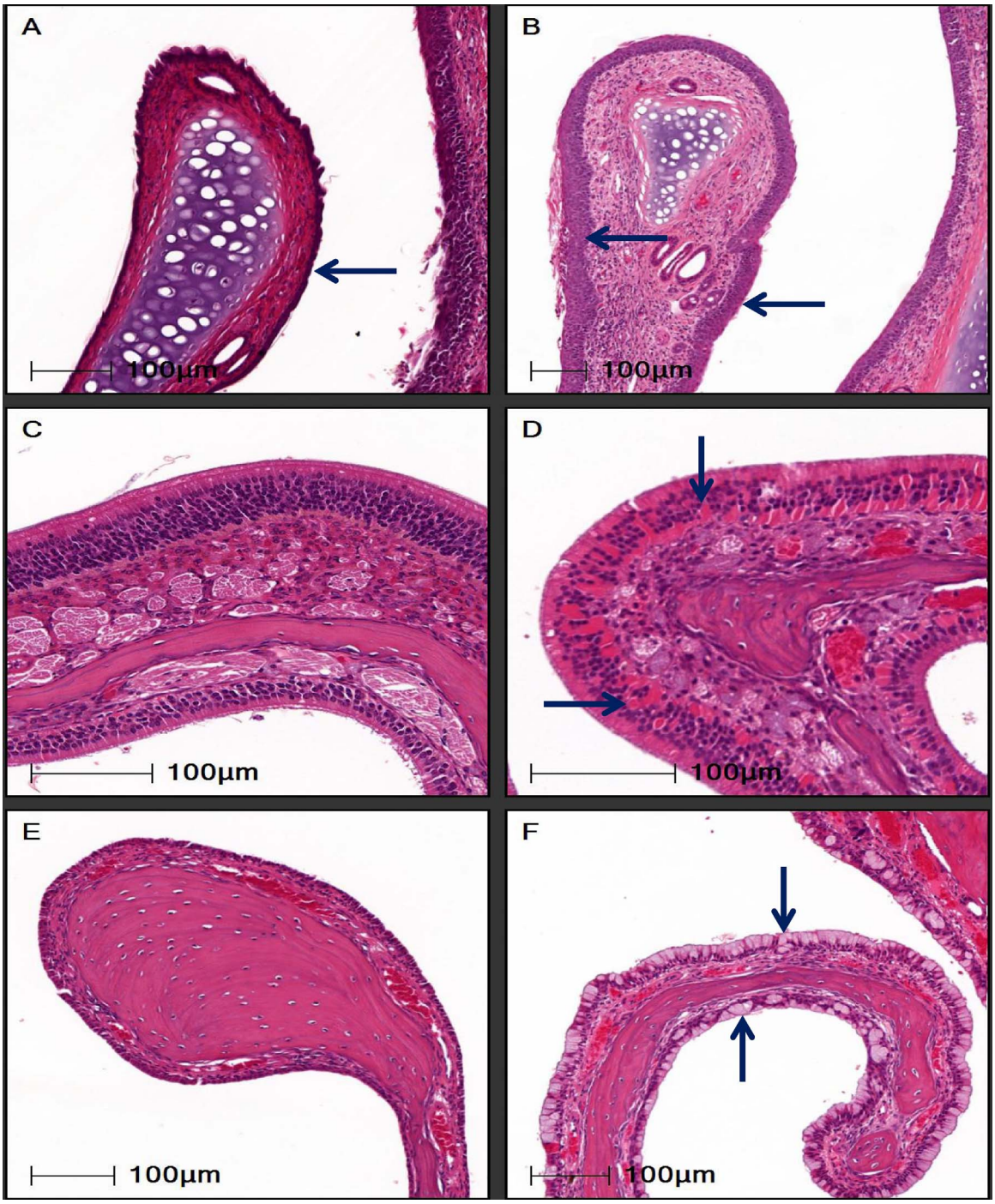


Fig. 7. Treatement-related Nasal Lesions; A: Cross section of the nares of a control rat showing an atrioturbinate lined by relatively thin stratified squamous epithelium (arrow). B: Section of the nares of D4 treated rat showing thickened (hyperplastic) keratinzed stratified squamous epithelium (arrow) lining the atrioturbinate. The lamina propria underlying the hyperplastic epithelium contains neutrophils admixed with mononuclear cells. C: Olfactory epithelium lining an ethmoturbinate of control rat. D. Note increased amounts of eosinophilic globular inclusions within the olfactory epithelial cells (arrows) lining an ethmoturbinate in D4 treated rat. E: Maxilloturbinate from a control rat lined by a transitional epithelium. F: Maxilloturbinate from a D4 treated rat with goblet cell hyperplasia. Note the increased numbers of goblet cells diffusely lining the maxilloturbinate (arrows).

both the 24-month exposure control group (88%) and the recovery control group (85%). As seen with chronic nephropathy there appears to be a slight exacerbation of this testicular neoplasia with exposure to 700 ppm D_4. Though the driver for the effect is unclear, the data suggest that chronic exposure to 700 ppm D_4 induces a slight shift from unilateral to bilateral tumor expression at 24 months. The slight-modest increase in testes weight and incidence/severity of interstitial cell hyperplasia at 12- and 24-months are believed associated with the increase in bilateral interstitial cell adenoma at 24 months. The noted increase in bilateral interstitial cell adenoma in the 700 ppm D_4

Table 16
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on the Lower Respiratory Tract[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	12-Months	Lung: Hemorrhage	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]
Male	24-Months	Lung: Hemorrhage	3/60[a] [3]	3/59 [1.7]	5/60 [2.6]	5/60 [2.4]	8/59 [2.1]
Male	Recovery	Lung: Hemorrhage	2/20 [1]	0/20 [–]	3/20 [2]	1/20 [2]	1/20 [2]
Male	12-Months	Subpleural Chronic Inflammation	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]
Male	24-Months	Subpleural Chronic Inflammation	2/60 [1.5]	1/59 [1]	2/60 [1.5]	3/60 [1.7]	3/59 [1.3]
Male	Recovery	Subpleural Chronic Inflammation	0/20 [–]	0/20 [–]	0/20 [–]	0/20 []	1/20 [2]
Female	12-Months	Lung: Hemorrhage	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]
Female	24-Months	Lung: Hemorrhage	0/59[a] [–]	1/60 [2]	0/59 [–]	2/60 [3]	4/60* [2.2]
Female	Recovery	Lung: Hemorrhage	0/20 [–]	1/20 [2]	0/20 [–]	0/20 [–]	1/20 [1]
Female	12-Months	Subpleural Chronic Inflammation	2/10 [1]	0/10 [–]	0/10 [–]	1/10 [1]	3/10 [1]
Female	24-Months	Subpleural Chronic Inflammation	0/59[a] [–]	2/60* [1]	3/59* [1]	2/60 [1]	8/60** [1.5]
Female	Recovery	Subpleural Chronic Inflammation	1/20 [1]	0/20 [–]	0/20 [–]	1/20 [2]	0/20 [–]

[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group mean incidence [average severity grade].
* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).

Table 17
Non-Neoplastic Changes in the Liver[b] of Fischer 344 Rats after Chronic Vapor Inhalation Exposure to Octamethylcyclotetrasiloxane.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	12-Months	Centrilobular Hypertrophy	0/10[a] [–]	0/10 [–]	0/10 [–]	0/10 [–]	6/10** [2]
Male	24-Months	Centrilobular Hypertrophy	0/60[a] [–]	0/60 [–]	0/60 [–]	0/60 [–]	5/60* [2.2]
Male	Recovery	Centrilobular Hypertrophy	0/20 [–]	0/20 [–]	0/20 [–]	0/20 [–]	0/20 [–]
Male	12-Months	Liver: Basophillic focus	0/10 [–]	0/10 [–]	0/10 [–]	2/10 [1]	0/10 [–]
Male	24-Months	Liver: Basophillic focus	25/60 [1.4]	27/60 [1]	15/60 [1]	15/60 [1.7]	20/60 [1.4]
Male	Recovery	Liver: Basophillic focus	6/20 [1.8]	7/20 [1]	6/20 [1]	8/19 [1.2]	9/20 [1.6]
Female	12-Months	Centrilobular Hypertrophy	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]	0/10 [–]
Female	24-Months	Centrilobular Hypertrophy	0/59 [–]	0/59 [–]	0/60 [–]	0/60 [–]	0/60 [–]
Female	Recovery	Centrilobular Hypertrophy	0/20 [–]	0/20 [–]	0/20 [–]	0/20 [–]	0/20 [–]
Female	12-Months	Liver: Basophillic focus	8/10[a] [1]	8/10 [1]	10/10 [1]	8/10 [1]	2/10* [1]
Female	24-Months	Liver: Basophillic focus	45/59[a] [1.2]	42/59* [1.4]	45/60 [1.1]	44/60 [1.4]	26/60* [1]
Female	Recovery	Liver: Basophillic focus	14/20[a] [1.1]	17/20 [1.2]	12/20 [1.5]	16/20 [1.2]	9/20* [1]

[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group mean incidence [average severity grade].
* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).

Table 18
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Chronic Nephropathy[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	12-Months	Chronic Nephropathy	9/10 [1]	10/10 [1]	9/10 [1]	8/10 [1.1]	10/10 [1.1]
Male	24-Months	Chronic Nephropathy	56/58 [2.4]	58/59 [2.6]	57/59 [2.6]	57/60 [2.6]	60/60 [3]
Male	Recovery	Chronic Nephropathy	20/20 [2.2]	20/20 [2.7]	20/20 [2.8]	19/19 [2.8]	20/20 [2.7]
Female	12-Months	Chronic Nephropathy	5/10 [1]	7/10 [1]	8/10 [1]	4/10 [1]	5/10 [1]
Female	24-Months	Chronic Nephropathy	17/59[a] [1.4]	25/59 [1.6]	33/59** [1.9]	46/60* [1.9]	55/60** [2]
Female	Recovery	Chronic Nephropathy	15/20 [1.7]	18/20 [1.4]	18/20 [1.7]	18/20 [2.1]	18/20 [1.9]

[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group mean incidence [average severity grade].
* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).

Table 19
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Hematopoiesis in the Spleen[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	24-Months	Spleen: Hematopoietic Proliferation	2/59 [4]	5/60 [3.4]	8/60* [3.5]	3/60 [3.7]	1/59 [3]
Male	Recovery	Spleen: Hematopoietic Proliferation	1/20 [4]	2/20 [3]	1/20 [2]	1/19 [2]	1/20 [4]
Female	24-Months	Spleen: Hematopoietic Proliferation	4/59[a] [3.5]	1/60 [2]	3/59 [3.3]	4/60 [3.2]	11/60* [3.4]
Female	Recovery	Spleen: Hematopoietic Proliferation	1/20 [4]	1/20 [3]	2/20 [2.5]	4/20 [3]	1/20 [4]

[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group mean incidence [average severity grade].
* Statistically different from control ($p < 0.05$).

Table 20
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Reproductive Organ Non-Neoplastic Changes[b] in Female Fischer 344 Rats.

Exposure Group	Diagnosis	Exposure Concentration (ppm D_4)				
		0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
24-Months	Endometrial Epithelial Hyperplasia	11/59[a] [1.7]	8/59 [1.8]	5/59 [1.8]	13/60 [1.8]	30/60* [2.5]
Recovery	Endometrial Epithelial Hyperplasia	1/20 [2]	6/20** [2]	4/20 [2.5]	3/20 [3]	3/20 [1.7]
24-Months	Ovarian Atrophy[b]	1/59[a] [3]	1/28 [4]	0/18 [–]	0/15 [–]	4/60* [3.5]
24-Months	Cervix: Squamous Epithelial Cell Hyperplasia[b]	0/59[a] [–]	0/22 [–]	1/18 [2]	2/17 [1.5]	3/60* [2]
Recovery	Cervix: Squamous Epithelial Cell Hyperplasia[b]	0/20[a] [–]	0/7 [–]	0/9 [–]	0/4 [–]	2/20* [4]
24-Months	Cervix: Stromal Hyperplasia[b]	3/59[a] [3.7]	1/22 [4]	0/18 [–]	0/17 [–]	0/60* [–]

[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group mean incidence [average severity grade].
* Statistically different from control ($p < 0.05$).
** Statistically different from control ($p < 0.01$).

exposure group is of doubtful toxicological significance given the low magnitude of change and lack of an overall increase in the total incidence of this common tumor (88% incidence of interstitial cell adenoma for both the control and 700 ppm D_4 exposure groups).

Chronic exposure to 700 ppm D_4 increased the incidence of cystic endometrial hyperplasia, endometrial adenoma, and elevated uterine weight at 24 months. Although no endometrial adenoma was observed in any 24-month treatment group other than the high dose group and the incidence in the 700 ppm D_4 group was low (6.7%) and not statistically different from the control (0% incidence) upon pairwise comparison, the incidence profile across the treatment groups was statistically significant for trend. Together the increase in uterine weight, increased incidence of endometrial hyperplasia and adenoma, and low historical incidence of uterine adenoma in F344 rats (0.3%, Haseman et al., 1998) identified the uterus as a target organ. There were no treatment-related increases in neoplasia in any other organs, including the mammary gland, ovary, vagina, and pituitary gland. The single incidence of uterine adenoma (30 ppm) and uterine adenocarcinoma (150 ppm) in the recovery groups were not considered treatment related for lack dose response and association with effects present in the 24-month exposure groups.

Cystic endometrial hyperplasia and endometrial adenoma in the rat are characteristic of prolonged unopposed estrogenic stimulation. It was proposed that if a systemic hormonal imbalance was present in the D_4-treated animals the estrogen-sensitive tissues may exhibit histomorphological features indicative of prolonged estrogenic stimulation. Thus, a focused microscopic review of the mammary gland, ovary, uterus, vagina, and pituitary was conducted (data not shown). No differences were observed for these hormone-sensitive tissues among the treated animals relative to the control group animals, with exception of the increased incidence of cystic endometrial hyperplasia and adenoma and a small increase at 12 months in the proportion of animals in the high treatment group histologically showing a prolonged cycle. Two of ten control and four of ten 700 ppm D_4 group females presented with scattered degenerating luteal cells within basophilic (new) corpora lutea, a finding typically associated with increased cycle length (≥6 day cycles). Such a finding is consistent with the observed delayed LH surge and ovulation demonstrated in the reproductive studies with Sprague-Dawley rats (Meeks et al., 2006). These observations are also consistent with the increased incidence of extended estrus demonstrated in a recent study of cyclicity in D_4 exposed aging F344 rats (Jean et al., 2017; Sloter, 2015). Vapor inhalation exposure of 11 to 18-month old female F344 rats to 700 ppm D_4 resulted in a larger proportion of the animals exhibiting prolonged estrogenic periods (consecutive days in proestrus/estrus as determined by vaginal cytology). This study also demonstrated that continued exposure to 700 ppm D_4 beyond 18 months was associated with a significantly increased incidence in cycling based on the vaginal cytology profile. These effects, prolongation of the estrogenic state and increased cyclicity, are suggestive of an overall increase in cumulative endogenous estrogenic stimulation of the uterine tissue over the lifetime of the rat.

The relevance of the uterine effects observed at 700 ppm D4 is perhaps uncertain as it relates to a high dose testing. Plasma and tissue levels of D4 after 6 months exposure were measured and the levels observed were generally dose-responsive and consistent with values observed in a shorter term (15-day) repeated dose toxicokinetic study

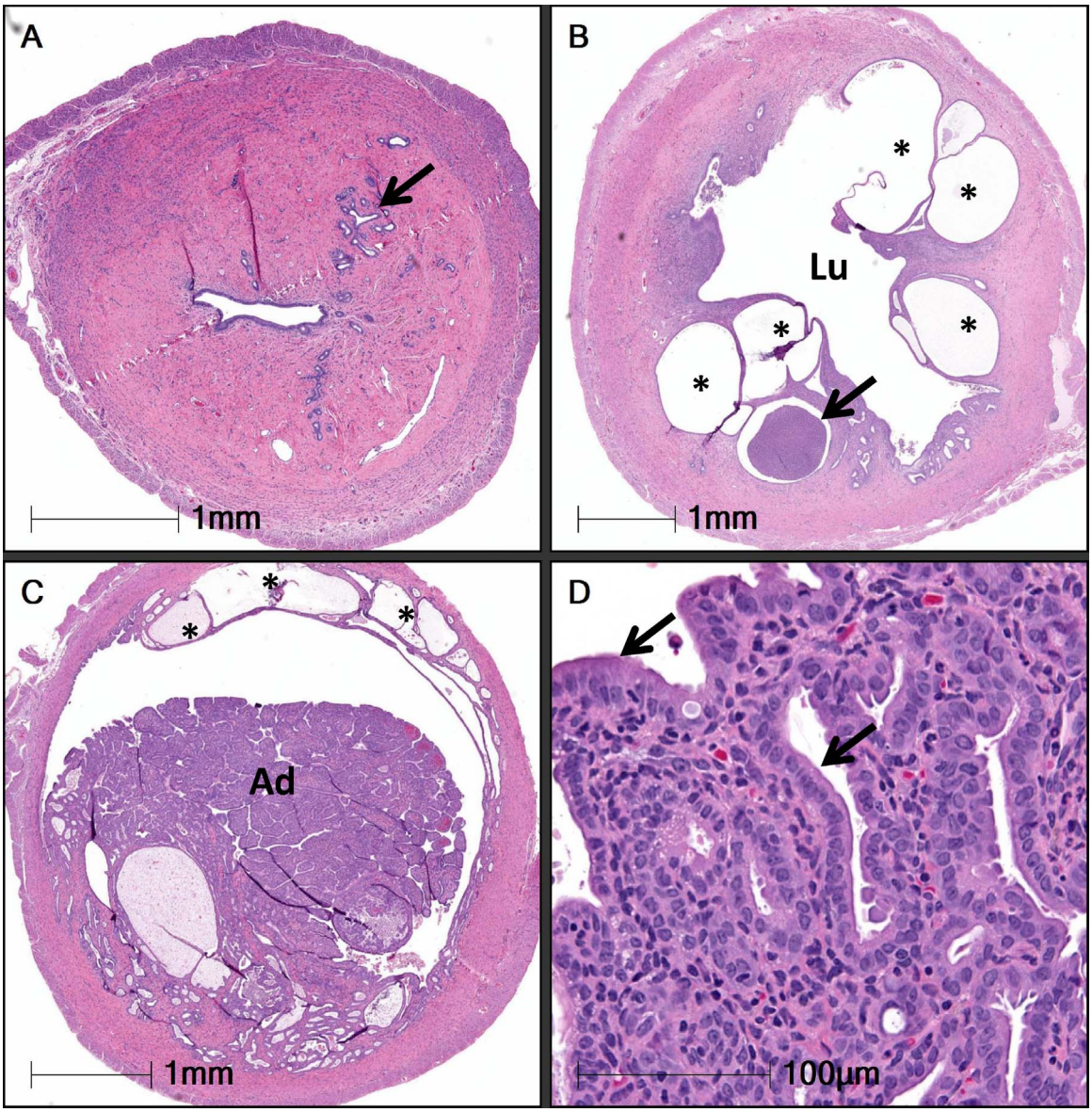


Fig. 8. Treatment-related changes in the uterus A: Cross section of uterus from a control rat. Note the relatively small endometrial glands (arrow). B: Cystic endometrial hyperplasia in the uterus of D4 treated rat. Note the cystically dilated endometrial glands (*) projecting into the uterine lumen (Lu). One of the cystic glands is filled with neutrophil infiltrate (arrow). C: Endometrial adenoma in the uterus of D4 rat. Note the solitary exophytic highly cellular adenoma (Ad) projecting into and occupying most of the dilated uterine lumen. Note the adjoining cystic endometrial glands (*) unassociated with the adenoma. D: Higher magnification of C: Note the well differentiated cuboidal to columnar neoplastic endometrial epithelial cells (arrows) supported by scant stroma which is infiltrated with small numbers of neutrophils admixed with mononuclear cells.

(Plotzke et al., 2000). In addition, plasma and tissue levels were comparable with values predicted by a PBPK prediction model and together, the observed and predicted values indicate a low bioaccumulation potential for D4 (Andersen et al., 2008). The kinetic data at 6 months also show a departure from dose proportionality for plasma D4 levels at 700 ppm. This apparent dose-related change in processes that govern absorption, distribution, metabolism, and/or elimination (ADME) kinetics may be toxicologically significant with respect to hazard identification. When toxicity is expressed in laboratory animal studies only at exposure levels that exceed the capacity of normal ADME processes and if human exposures to the substance are substantially lower than the adverse exposure levels in the animal study, the toxicity is unlikely to represent a hazard of relevance to humans (Saghir, 2015; Saghir et al., 2012). The uterine epithelial hyperplasia and adenoma observed in the current study are perhaps good examples as an increase in incidence of these effects occurred only in the 700 ppm exposure level. Gentry et al. (2016) has recently published a global human risk assessment on D4 and reported Margins of Safety (MOS) for occupational, consumer, and general public exposures to D4. The lowest MOS among these populations was 1500 based on an adverse effect level of 125 ppm D4. Thus the MOS of 1500 is conservative with respect to the 700 ppm D4 exposure level. Based on the observed departure from dose

Table 21
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Testes Interstitial Cell Hyperplasia and Adenoma in Male Fischer 344 Rats.

Exposure Group	Diagnosis		Exposure Concentration (ppm D_4)				
			0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
12-Months	Interstitial Cell Hyperplasia	Incidence	6/10				8/10
		mean severity	[1.2]				[1]
	Interstitial cell Adenoma	Incidence	0/10				0/10
	Interstitial cell Adenoma: bilateral	Incidence	0/10				0/10
24-Months	Interstitial Cell Hyperplasia	Incidence	7/60	12/60	9/60	13/60*	16/60**
		mean severity	[1.8]	[2.6]	[2.9]	[3.0]	[3.1]
	Interstitial cell Adenoma	Incidence	11/60	11/60	4/60	9/60	5/60
	Interstitial cell Adenoma: bilateral	Incidence	42/60[a]	40/60	44/60	41/60	48/60
Recovery	Interstitial Cell Hyperplasia	Incidence	4/20	6/20	2/20	5/20	5/20
		mean severity	[3.2]	[2.5]	[3.0]	[2.8]	[2.6]
	Interstitial cell Adenoma	Incidence	6/20	2/20	3/20	1/20	2/20
	Interstitial cell Adenoma: bilateral	Incidence	11/20	14/20	17/20	15/20	15/20

[a] Positive trend analysis (Peto Mortality Prevalence Test).
* $p < 0.05$.
** $p < 0.01$.

Table 22
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Mononuclear Cell Leukemia Incidence in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	12-Months	Mononuclear Cell Leukemia	0/10[a]	0/10	0/10	0/10	1/10
Male	24-Months	Mononuclear Cell Leukemia	43/60[a]	27/60	26/60	31/60	41/60
Male	Recovery	Mononuclear Cell Leukemia	8/20[a]	10/20	8/20	7/20	13/20
Female	12-Months	Mononuclear Cell Leukemia	0/10	0/10	0/10	0/10	0/10
Female	24-Months	Mononuclear Cell Leukemia	14/60	16/60	14/60	19/60	18/60
Female	Recovery	Mononuclear Cell Leukemia	6/20	4/20	4/20	4/20	7/20

[a] Statistically significant trend ($p < 0.05$).

Table 23
Comparison of Mononuclear Cell Leukemia-Related Mortality in the 24-Month Exposure Group Males.

Exposure Concentration (ppm)	Number of Early Deaths	Early Deaths Attributed to Mononuclear Cell Leukemia	
		Total number	Percent
0	25	14	56
10	24	10	42
30	25	11	44
150	25	15	60
700	37	28	76

proportionality at 700 ppm D4 and the low human exposure potential for D4 the increased incidence of uterine hyperplasia and adenoma is unlikely to represent a relevant hazard in assessing D4's potential human health risk.

In conclusion, vapor inhalation exposure of F344 rats to D_4 was generally well tolerated. Treatment-related effects include nasal epithelium irritation, lymphocytic leukocytosis, increased expression of chronic nephropathy, and increased liver, kidney, testes, and uterine weights with correlating microscopic findings of hepatocellular hypertrophy, chronic nephropathy, testes interstitial cell hyperplasia, and endometrial hyperplasia and adenoma, respectively. Examination of the potential modes of action for the uterine effects is beyond the scope of this manuscript. Companion manuscripts have been prepared that review the toxicology of D_4 (Franzen et al., 2017) and examine potential

Table 24
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Uterine Neoplasia[b] in Fischer 344 Rats.

Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
12-months	Stromal polyp	0/10	0/0	1/1	1/1	1/10
24-Months	Endometrial adenoma	0/59[a]	0/59	0/59	0/60	4/60
24-Months	Stromal polyp	11/59	17/59	14/59	17/60	15/60
24-Months	Stromal sarcoma	1/59	0/59	2/59	2/60	3/60
24-Months	Histiocytic sarcoma	0/59[a]	0/59	0/59	0/60	2/60
Recovery	Endometrial adenoma	0/20	0/20	1/20	0/20	0/20
Recovery	Endometrial adenocarcinoma	0/20	0/20	0/20	1/20	0/20
Recovery	Stromal polyp	1/20	6/20*	4/20*	6/20*	6/20*
Recovery	Stromal sarcoma	0/20	0/20	0/20	2/20	0/20
Recovery	Leiomyosarcoma	0/20	0/20	1/20	0/20	0/20

**Statistically different from control ($p < 0.01$).
[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group incidence.
* Statistically different from control ($p < 0.05$).

Table 25
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Pituitary Gland Par Distalis Neoplasia[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	12-Months	Pituitary Gland: Pars distalis adenoma	1/10	0/10	0/10	0/10	0/10
Male	24-Months	Pituitary Gland: Pars distalis adenoma	26/60[a]	19/38	20/34	16/34	15/60*
Male	24-Months	Pituitary Gland: Pars distalis carcinoma	1/60	1/38	0/34	0/34	1/60
Male	Recovery	Pituitary Gland: Pars distalis adenoma	4/20	7/15	5/12	6/14	8/20
Female	24-Months	Pituitary Gland: Pars distalis adenoma	27/58	21/34	20/33	17/30	24/60
Female	24-Months	Pituitary Gland: Pars distalis carcinoma	1/58	1/34	0/33	0/30	0/60
Female	Recovery	Pituitary Gland: Pars distalis adenoma	5/20	5/13	3/10	6/11	5/20
Female	Recovery	Pituitary Gland: Pars distalis adencarcinoma	0/20	0/13	1/10	0/11	0/20

**Statistically different from control ($p < 0.01$).
[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group incidence.
* Statistically different from control ($p < 0.05$).

Table 26
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Pancreatic Islet Cell Neoplasia[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	24-Months	Pancreas: Islet cell adenoma[b]	5/59[a]	1/23	3/26	2/25	0/59*
Male	24-Months	Pancreas: Islet cell carcinoma[b] (combined)	1/59	0/23	2/26	0/25	0/59
			6/60[a]	1/23	5/26	2/25	0/59*
Male	Recovery	Pancreas: Islet cell adenoma[b]	0/20	1/6	0/7	1/8	1/20
Female	24-Months	Pancreas: Islet cell adenoma[b]	0/59	0/22	0/16	0/16	0/60
Female	24-Months	Pancreas: Islet cell carcinoma[b]	0/59	0/22	0/16	0/16	1/60
Female	Recovery	Pancreas: Islet cell carcinoma[b]	1/20	0/7	0/9	0/4	0/20

**Statistically different from control ($p < 0.01$).
[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group incidence.
* Statistically different from control ($p < 0.05$).

Table 27
Effect of Vapor Inhalation Exposure of Octamethylcyclotetrasiloxane on Thyroid Gland C-cell Neoplasia[b] in Fischer 344 Rats.

Sex	Exposure Group	Diagnosis	0 ppm	10 ppm	30 ppm	150 ppm	700 ppm
Male	24-Months	Thyroid Gland: C-cell adenoma	9/60	0/28	3/25	1/26	4/60
Male	24-Months	Thyroid Gland: C-cell carcinoma combined	3/60	4/28	1/25	1/26	2/60
			12/60	4/28	4/25	2/26	6/60
Male	Recovery	Thyroid Gland: C-cell adenoma	2/20	0/6	1/9	0/9	2/20
Male	Recovery	Thyroid Gland: C-cell carcinoma combined	1/20	0/6	2/9	0/9	0/20
			3/20	0/6	3/9	0/9	2/20
Female	24-Months	Thyroid Gland: C-cell adenoma	4/59	1/25	0/18	2/16	1/60
Female	24-Months	Thyroid Gland: C-cell carcinoma combined	2/59	2/25	2/18	1/16	0/60
			6/59[a]	3/25	2/18	3/16	1/60
Female	Recovery	Thyroid Gland: C-cell adenoma	3/20	1/8	0/9	0/4	1/20
Female	Recovery	Thyroid Gland: C-cell carcinoma combined	1/20	1/8	0/9	0/4	0/20
			4/20[a]	2/8	0/9	0/4	1/20

*Statistically different from control ($p < 0.05$).
**Statistically different from control ($p < 0.01$).
[a] Statistically significant trend ($p < 0.05$).
[b] Values represent the group incidence.

modes of action that may be responsible for the observed uterine endometrial hyperplasia and adenoma (Dekant et al., 2017).

Acknowledgements

This study was conducted at Battelle, Toxicology Northwest, Richland, WA.

The authors would like to thank the Dr. K. M Lee (study director) and J. Blessing (study coordinator) for their expertise and dedication to the conduct and reporting of this study. Kind appreciation is also given to the Battelle TNW study pathologist Dr. Roger Renne, Dr. Karen Regan (Regan Path/Tox Services) and the late Frank McConnell for their histomorphologic review of the endocrine responsive tissues, and Dr. Johnson Thomas (The Dow Chemical Company) for preparing the photomicrographs of uterine and nasal cavity lesions. Similarly we thank Cynthia Van Landingham (Environ Health Sciences Institute) for her contributions regarding statistical analysis and R. Weigel, B. Hayden, and H. Ragan (Battelle TNW) for their excellent technical contributions to the conduct of the study.

Preparation of this review was supported in part by the Global Silicones Council.

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