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Toxicology of decamethylcyclopentasiloxane (D5)

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ABSTRACT

Decamethylcyclopentasiloxane (D5) is a cyclic siloxane used in the formulation of consumer products as well as an industrial intermediate. A summary of the previous studies on the toxicology of D5 is provided. Toxicokinetic studies with D5 after dermal administration demonstrate a very low uptake of due to rapid evaporation. Following inhalation exposure, exhalation of unchanged D5 and excretion of metabolites with urine are major pathways for clearance in mammals. Due to this rapid clearance by exhalation, the potential for bioaccumulation of D5 is considered unlikely. The available toxicity data on D5 adequately cover the relevant endpoints regarding potential human health hazards. D5 was not DNA reactive or mutagenic in standard *in vitro* and *in vivo* test systems. D5 also did not induce developmental and reproductive toxicity in appropriately performed studies. In repeated studies in rats with subacute, subchronic and chronic inhalation exposure, mild effects on the respiratory tract typically seen after inhalation of irritating materials, increases in liver weight (28- and 90-day inhalation studies), and a small increase in the incidence of uterine adenocarcinoma (uterine tumor) in female rats (two-year inhalation chronic bioassay) were observed. The liver effects induced by D5 were consistent with D5 as a weak "phenobarbital-like" inducer of xenobiotic metabolizing enzymes and these effects are considered to be an adaptive response. Mechanistic studies to elucidate the mode-of-action for uterine tumor induction suggest an interaction of D5 with dopamine signal transduction pathways altering the pituitary control of the estrus cycle. The resulting estrogen imbalance may cause the small increase in uterine tumor incidence at the highest D5-exposure concentration over that seen in control rats. A genotoxic mechanism or a direct endocrine activity of D5 is not supported as a mode-of-action to account for the induction of uterine tumors by the available data.

1. Introduction

Decamethylcyclopentasiloxane (D5), CAS 541-02-6, is a cyclic siloxane used as an intermediate in the production of polydimethylsiloxanes and has a number of secondary uses as a component in consumer products. D5 has a low water solubility (17 ppb) and a boiling point of 211 °C. Due to the high volatility and low surface energy, D5 readily volatizes. Therefore, inhalation and dermal contact are the major expected routes of human exposures to D5 for consumers, the general public and manufacturing workers.

Extensive toxicity testing and experimental studies on D5 have been performed addressing the acute, subchronic and chronic effects of D5 in rodents via dermal, oral and inhalation routes of exposure. The very limited dermal absorption (due to volatility) and a specific toxicokinetic behavior after oral administration has resulted in the selection of inhalation as the preferred route of D5 administration for toxicity studies. Several experimental studies *in vitro* have also been performed with D5 to provide further information on the possible underlying mechanisms for the toxic effects observed with D5 and the relevance of these effects in rodents for human risk assessment.

This manuscript reviews and evaluates the results of the toxicity and mechanistically based studies with specific interest in understanding the mechanisms by which D5 induces liver enlargement and increases the incidence of the uterine adenocarcinomas after chronic exposure in rats.

2. Absorption, distribution, metabolism, and excretion of D5

The toxicokinetics of D5 are well characterized. Studies covering both single and repeated inhalation exposures to D5, and following

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dermal application and oral administration have been performed in experimental animals. In addition, human data on extent of dermal absorption of D5 and the disposition of systemically available D5 have been generated (DCC, 1996; Burns-Naas et al., 1998a, 1998b; DCC, 2002; DCC, 2003c; DCC, 2003d; DCC, 2003a; DCC, 2003b; Varaprath et al., 2003; DCC, 2005a; DCC, 2007; Jovanovic et al., 2008; Reddy et al., 2008; Tobin et al., 2008).

2.1. Absorption and distribution of D5 after inhalation exposures

The disposition of D5 was evaluated in male and female rats after single and repeated inhalation exposures using nose only inhalation (Tobin et al., 2008).

Rats were either exposed once for six hours to 7 or 160 ppm ^{14}C-D5 or, after 14 consecutive six hour nose-only exposures to 160 ppm of unlabeled D5 over two weeks, to a single six hour exposure to 160 ppm ^{14}C-D5 on day 15. D5-derived radioactivity and parent D5 were quantified in blood, plasma, selected tissues, expired air, urine, and feces collected at different time points. After both single and repeated inhalation exposures, less then three % of the delivered doses of D5 (calculation based on respiratory minute volume, exposure duration, and D5 vapor concentration) were retained in the animals. Significant accumulation of D5 on the fur was observed. Retained D5 was widely distributed from blood to tissues with maximum concentrations observed in the majority of the tissues three hours after exposure. In the plasma, liver and lung, the majority of radioactivity immediately following exposure represented unchanged D5. However, the contribution of D5 to the total radioactivity in tissues decreased over time and D5 represented only a small fraction of total radioactivity at time points >24 h after the end of the exposure. Elimination of D5 and presumed metabolites from fat was slower as compared to plasma and other tissues (DCC, 2001). Repeated exposure gave rise to higher concentrations of parent D5 in the lung and fat of both sexes and in the liver in female rats as compared to a single exposure.

Exhalation of unchanged D5 was the major pathway of elimination after both single and multiple inhalation exposures and accounted for app. 50% of the retained D5. Elimination of D5-derived radioactivity with urine in the form of metabolites accounted for approximately 12% and fecal elimination for approximately 16% of retained radioactivity. Fecal elimination of D5 may in part be due to oral ingestion of D5 deposited on the fur absorbed during grooming. Elimination of D5-associated radioactivity was multiphasic, but most of the radioactivity was eliminated within 24 h after the end of inhalation exposure. As with other lipophilic chemicals, fat may serve as a reservoir of D5 since little decrease of D5-associated radioactivity in fat was observed over an observation period of 168 h. Most of the radioactivity present in fat was attributed to unchanged D5, a minor fraction was presumed to represent a hydroxylated metabolite (Tobin et al., 2008).

Analysis of the fecal extracts by high-pressure liquid chromatography (HPLC) indicated that the majority of the radioactivity in feces was unchanged D5, a hydroxylated derivative of D5 was a presumed minor metabolite. HPLC-analysis of urine samples revealed the presence of seven metabolites. The two major metabolites were dimethylsilanediol and methylsilane triol with $[MeSi(OH)_2{-}O{-}Si(OH)_3]$, $[MeSi(OH)_2{-}O{-}Si(OH)_2Me]$, $[MeSi(OH)_2{-}O{-}Si(OH)Me_2]$, $[Me_2Si(OH){-}O{-}Si(OH)Me_2]$, and $[Me_2Si(OH){-}OSiMe_2{-}OSi(OH)Me_2]$ representing minor metabolites (Varaprath et al., 2003). The metabolite structures suggest that D5 is initially oxidized to a hydroxylated derivative, presumable by cytochrome P450 (Fig. 1). This initial metabolite appears to rearrange and downstream products are degraded by hydrolysis to the short-chain siloxanes, which are excreted (Fig. 1).

The toxicokinetics of D5 after inhalation exposure were also studied in human subjects (three males and two females) after inhalation of D5 at a single concentration of 10 ppm for one hour using a mouthpiece exposure system under a mixed rest/exercise scheme (DCC, 2004c). During exposure, D5 concentrations in exhaled air rapidly reach a steady state between 7 and 10 ppm; after the end of the exposures, D5 levels in exhaled air rapidly declined and reached concentrations of less than 1 ppm within 20 min in most of the subjects. Concentrations of D5 in plasma increased from a baseline level of 0.15–3.3 μg/L to between 31 and 70 μg/L at the end of the inhalation exposure and rapidly declined after the end of the exposure to reach the basal levels within 24 h after the termination of the inhalation exposure (DCC, 2004c).

2.2. Bioavailability and deposition of D5 after dermal administration

Toxicokinetics of D5 after dermal exposure were assessed in humans and in rats. In addition, the dermal absorption of D5 was studied in a number of *in vitro* studies (Jovanovic et al., 2008).

Three male and three female human subjects applied 1.4 g (males) or 1.0 g (female) of ^{13}C-D5 to the axilla under unoccluded conditions. Blood samples were collected for up to six hours and exhaled air samples were collected for up to 24 h after application (DCC, 2002). Peak concentrations of ^{13}C-D5 in the plasma were achieved within two hours after application and were 1.22 ng/g at one hour, 0.61 ng/g at six hour sampling, and were below the limit of detection of 0.03 ng/g in all samples taken later than six hours after ^{13}C-D5 application. Concentrations of ^{13}C-D5 in exhaled air were below one ng/L at all time points.

^{14}C-D5 was applied under semi-occluded conditions to human skin from six donors using a flow-through diffusion cell technique with provisions to collect material volatilized from the skin by absorption to charcoal traps. Skin samples were dosed either with neat D5 or with a generic antiperspirant formulation containing D5. After 24 h, only 0.04% of the applied dose of neat D5 and 0.022% of the D5 present in the formulation was absorbed. The majority of the applied D5 was volatilized from the skin samples and was recovered on the charcoal traps (DCC, 1999). The cumulative penetration for neat D5 was 0.1 μg/cm^2 and 0.3 μ/cm^2 for formulated D5.

To assess the fate of D5 absorbed from the skin in intact animals, ^{14}C-D5 was applied to the dorsal surface of male and female rats. Hair at the application site was clipped prior to application and the application site was covered with a non-occlusive elastic wrap. The study was designed to permit differentiation between D5 exhaled after absorption and D5 evaporating from the application site. After application of D5, the animals were transferred to metabolic cages for the collection of urine and feces. The majority (about 85%) of the applied ^{14}C-D5 volatilized from the skin. After 96 h, 0.35% of the administered D5 remained at the application site and less than 1% of the applied ^{14}C-activity was recovered in urine and carcass with trace levels of ^{14}C-activity recovered in feces, CO_2 traps, and tissues. The total amount of D5 absorbed was <1% (DCC, 2003c).

2.3. Toxicokinetics of D5 after oral administration

Toxicokinetic studies were performed in rats after dosing by gavage with ^{14}C-D5 (single dose of 1000 mg/kg bw) dissolved in different vehicles (corn oil and simethicone fluid) and as a neat material (DCC, 2003a). The carrier had a significant influence on the extent of absorption of ^{14}C-D5. After administration of neat D_5, approximately 10% of the dose was absorbed from the gastrointestinal tract. Based on blood area under the curve (AUC), absorption increased after administration of D5 in corn oil and decreased after administration in simethicone fluid. Elimination half-lives for D5-associated radioactivity in blood ranged from 45 (simethicone)

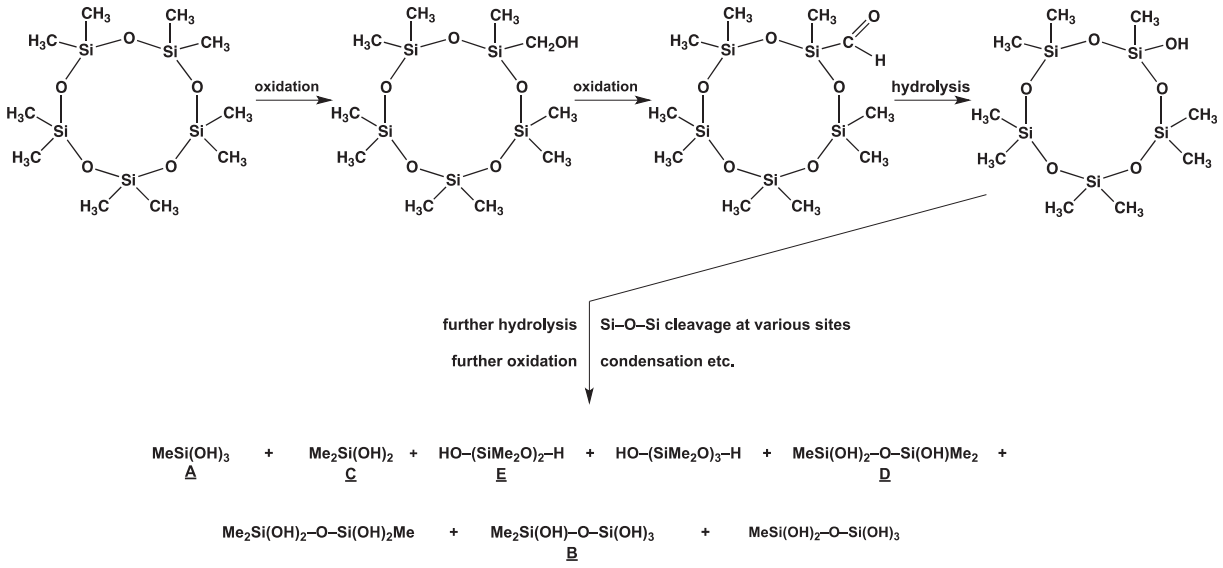

Fig. 1. Biotransformation of D5 in rodents.

to 240 h (corn oil), and were between 117 (neat) and 242 h (simethicone) for parent D5. The radioactivity eliminated in the urine consisted entirely of polar metabolites of D5. Mass balance analysis indicated that approximately 60–80 % of the administered D5 was excreted unchanged in the feces, and up to 20% of administered D5 as water soluble metabolites in urine. Half of the systemically available D5 was eliminated as unchanged D5 in exhaled air. However, the kinetics and tissue distribution observed after oral dosing were qualitatively different from the distributions after inhalation or dermal exposures. Higher relative concentrations of D5 were noted in liver and spleen as compared to exposure to D5 by inhalation and dermal application.

2.4. Physiologically based toxicokinetic modeling

The toxicokinetic data obtained in studies after D5 inhalation or after dermal application were used as a basis to develop and evaluate physiologically based toxicokinetic models (PBTK). The lipophilicity and volatility of D5 have a major influence on the toxicokinetics. The high lipophilicity results in high lipid partitioning and in retention of D5 in blood lipids. This is the cause of the differences in kinetic behavior after inhalation and dermal administration as compared to oral dosing. Due to the high volatility, D5 is rapidly cleared from blood by exhalation. Hepatic clearance by biotransformation of D5 to polar metabolites also occurs.

Inhalation and dermal administration result in a similar pharmacokinetic profile, presumably due to diffusion of D5 molecules through membranes. At the onset of inhalation, blood levels of D5 climb rapidly and equilibrium between inhalation and exhalation of unchanged D5 is rapidly reached. Only a relatively small amount of the inhaled D5 is retained. In blood, D5 may exist in a free pool available for exhalation and biotransformation and a sequestered pool associated with blood lipids. During dermal exposures, D5 is rapidly absorbed into the outer layers of skin, but readily evaporates before significant systemic absorption occurs. Physiologically based toxicokinetic modeling of the percutaneous absorption data from the human dermal absorption study predicts the dermal absorption of D5 as 0.05% (Reddy et al., 2007). Due to the efficient clearance of D5 by exhalation, approximately 90% of the D% systemically available after dermal application is exhaled.

The distribution and kinetics of D5 after oral dosing differed significantly from the predictions of the PBTK model that well described the inhalation and dermal exposure routes (Reddy et al., 2007, 2008). The differences in toxicokinetics after oral administration as compared to inhalation or skin contact suggest that D5 is transferred from the gastrointestinal tract to the blood by different mechanisms as compared to those operative after inhalation or dermal administration. The oral route may deliver microemulsions of D5 that do not readily dissolve in plasma and blood and are distributed as such. Uptake may be more associated with lipid transport, such as chylomicron formation and thus D5 may not be completely available for tissue interactions. The microemulsions may be removed from the circulation by the reticuloendothelial system in liver and spleen.

The oral dosing studies suggested a much higher persistence of D5 in blood compared to inhalation and dermal dosing. However, this apparent persistence after oral dosing is most likely due to the fact that a fraction of D5 is present in a pool that is unavailable to interact with tissues. These differences in toxicokinetics indicate that results from toxicity studies with D5 using oral dosing in oil vehicles likely have little relevance for assessing human health risks of D5 after inhalation and dermal exposures.

PBTK models also predict that D5 has no tendency to accumulate after repeated dosing. Absence of a potential for bioaccumulation is also indicated by an absence of an increase in D5-tissue concentrations after a 6-month inhalation exposure performed as a segment of the chronic bioassay (DCC, 2005b). While D5 is very lipophilic with fat:blood partition coefficients between 500 and 1,000, it is readily eliminated by exhalation or by biotransformation to polar metabolites.

3. Toxicity of D5

3.1. Acute and repeated dose toxicity

D5 has a low potential for toxicity after single oral administration with no overt signs of adverse effects after a single gavage dose of 5 g/kg bw. Therefore, the oral LD_{50} > 5000 mg/kg bw. After single aerosol inhalation exposures, the four hour LC_{50} is 560 ppm. D5 is not an eye or skin irritant in standard animal studies. D5 is not a skin sensitizer in animals or in humans.

The potential effects of D5-exposure in animals have been assessed after repeated dermal, oral, and inhalation exposure. Repeated dose dermal studies with D5 were performed for durations of up to 28 days (rats) or 21 days (rabbits) under occlusive conditions. These studies have performed detailed histopathological evaluation of a number of tissues at sacrifice and included analysis of clinical chemistry parameters, hematology, body weight gain and food consumption. Effects related to D5 treatment were not observed up to applied nominal doses of 1600 mg/kg bw (rats) or 1000 mg/kg bw (rabbits) to the skin. The lack of effects in toxicity studies using dermal exposure is consistent with a minimal dermal penetration of D5 (HRS, 1979; DCC, 1990b).

The oral toxicity of D5 was studied after repeated dosing for both 28 and 90 days (Table 1). Liver weight increases were reported in both studies. These were accompanied by an increased incidence and a dose-related increase in severity of periportal lipidosis in females in the 28-day study. In male rats, the liver weight changes were not accompanied by histopathological changes. In the 90-day study, increased liver weights without histopathological changes were reported for both male and female animals. In addition, no histopathological effect of D5-administration on the other organs examined nor effects on body weight gain, food consumption and clinical chemistry were reported (DCC, 1990a; REACH, 2011) (Table 1).

Due to the high volatility of D5, the very limited dermal absorption, and the specific processes of D5 uptake after oral administration, the pivotal toxicity studies relevant for hazard assessment of D5 used inhalation as the route of exposure. This exposure regimen results in higher systemic exposure as compared to dermal application. However, since the saturated vapor concentration of D5 is approximately 180 ppm, the maximum vapor concentrations of D5, which can be reproducibly produced without interference by aerosol formation, are around 160 ppm. These physicochemical properties of D5 limit the maximum vapor exposure concentrations achievable in inhalation exposures (Burns-Naas et al., 1998b) and results obtained in studies using higher concentrations of D5 in air have to be evaluated with caution due to the potential formation of aerosols and associated issues with doses delivered. The results of the available inhalation studies with D5 are compiled in Table 2.

In a 28-day study (RCC, 1995a) and a 90-day inhalation studies (RCC, 1995b; Burns-Naas et al., 1998b), nose-only exposure was used. The highest concentrations applied were a mix of D5 vapor and aerosol; in addition, a 28-day study and a 90-day study used whole-body exposures and exposure was to D5 vapor only (Burns-Naas et al., 1998a). The 28-day study with whole body exposure and both 90-day studies included recovery groups to assess the reversibility of potential effects resulting from D5 inhalation.

In all inhalation studies with D5, besides slight local effects on the respiratory tract, increased liver weights were observed and slight liver damage was also indicated by clinical chemistry parameters (Table 2). However, the liver weight changes were not accompanied by histopathological changes in the liver and were reversible. The respiratory tract was the other major target organ after D5 inhalation with exposure-related increases in absolute and relative lung weights, which remained elevated in females after the recovery phase. In addition, small increases in focal macrophage accumulation (both after 28-day and 90-day exposures) and interstitial inflammation (90-day exposure) were seen in the lungs of both male and female animals in the high concentration groups. In the 28-day study, D5 exposure also resulted in an increase in incidence and severity of goblet cell proliferation in the nasal cavity in both sexes at 160 ppm. Initially, histopathological effects were not reported in the other organs examined (Burns-Naas et al., 1998b). However, a detailed review of tissues potentially sensitive to D5 as indicated by the results of the 2-year inhalation chronic bioassay demonstrated slight changes in high dose female animals (160 ppm) from the 90-day study at terminal sacrifice. These included increased mean number of atretic, antral-sized follicles/animal, increased incidence and severity of vaginal mucification, and an increased incidence of male-type acinar development of the mammary gland. In the animals sacrificed after the recovery period, 10/10 animals in the control group and only 5/10 high dose animals had resumed normal estrous cycles. Most were in diestrus II but showed evidence of recent cycling. In addition, vaginal mucification and clear cell changes remained increased in the high-dose recovery animals and male-type acinar development persisted in two animals exposed to D5 (DCC, 2006).

D5 was also tested for carcinogenicity in a two year combined chronic toxicity/carcinogenicity study (chronic bioassay) by inhalation in male and female F344 rats (DCC, 2005b). The study was split in a main group (chronic, 24 month exposure) and three satellite groups exposed to D5 for different durations (6 months, 12 months and 12 months plus a 12 month recovery) (Table 2). An increased incidence of hyaline inclusions in the nasal cavity in both males and females exposed to 160 ppm D5 for six months were noted. Statistically significant increases in liver weights were seen at 6 and 12 months in females and at 24 months in males, but these increases were neither D5-concentration nor exposure duration related. Increased incidences of respiratory tract irritation were also observed in one or both sexes at the 6, 12, and 24 months sacrifices. In the animals of the main group (24 month exposure) and the recovery group (12 months exposure followed by 12 months recovery) sacrificed after 2 years, hyaline inclusions in the nasal cavity were seen in both males and females at 160 ppm and in males at the 40 ppm exposure levels. However, effects indicative of irritation (e.g., inflammatory cell infiltration or degenerative changes in the epithelium) were not observed.

The incidences of neoplastic changes were not statistically significantly different in males for all analyzed tissues and in females for all tissues except the uterus. In female F344 rats, inhalation exposure to D5 induced a small increase in the incidence of uterine endometrial adenocarcinoma at the highest exposure dose (160 ppm). This increase was statistically significant when all exposure levels (trend analysis) were included in the analysis (Table 2) but not significant when examined pair wise to the control rats. In addition, a significant decrease in the incidence of cystic changes in the clitoral gland was observed in the 160 ppm group

Table 1
Summary of oral toxicity studies with decamethylcyclopentasiloxane (D5) after oral administration.

Animal model	Study design and dosing	NOAEL/LOEL	Observations	Reference
Sprague–Dawley rats	28-day oral toxicity study using doses of 0, 25, 100, 400 and 1600 mg/kg bw, 5 days/week;	NOAEL 100 mg/kg bw/day in males, no NOAEL in females	Increases in absolute liver weight in males and females at doses > 100 mg/kg bw/day, slight, but not statistically significant increase in liver weight at 25 mg/kg bw in females	(DCC, 1990a)
Sprague–Dawley rats	90-day oral toxicity study using doses of 0, 100, 330 and 1000 mg/kg bw, 5 days/week;	NOAEL of >1000 mg/kg bw/day	Significant increases in liver weights at all dose levels in males and females without histopathological changes.	(REACH, 2011)

Table 2
Summary of results from key toxicity studies on Decamethylcyclopentasiloxane (D5) using inhalation as route of exposure.

Animal model	Study design and dosing	NOAEC/LOEC	Observations	Reference
Fischer F344 rats	28-day inhalation toxicity study with whole body exposure to 0, 10, 25, 75 and 160 ppm D5 for 6 h/day, 7 days/week; groups of 10 males and 10 females/group including recovery group of 5 male and 5 female rats observed for 14 days after termination of exposure	NOEC of 10 ppm for local effects in the respiratory tract; 75 ppm for liver weight increases	Increased liver weight in male and female animals at 160 ppm, increased mean lung weight and alveolar macrophage accumulation at 160 ppm, morphological alterations in nasal cavity at >10 ppm. Liver weight changes reversible	(Burns-Naas et al., 1998a)
Fischer F344 rats	28-day inhalation toxicity study with nose only exposure to 0, 32, 50, 105, and 148/239 ppm D5 for 6 h/day, 5 days/week; groups of 10 males and 10 females/group including recovery group of 5 male and 5 female rats observed for 14 days after termination of exposure	NOEC of 105 ppm for liver weight increases	Increased liver weight in females at 148/239 ppm, reversible after 14-day recovery; changes in hematology parameters highest concentration in females, reversible after 14-day recovery period, liver and lung weight increases at high concentration, increase in incidence and severity of goblet cell proliferation in nasal cavity in males and females at highest concentration, increased incidence of hepatocellular hypertrophy at highest concentration in males and females	(RCC, 1995a)
Sprague Dawley rats	90-day inhalation toxicity study with whole body exposure to 0, 20, 60 and 120 ppm D5 for 6 h/day, 7 days/week; groups of 10 males and 10 females/group including recovery group of 10 male and 10 female rats exposed to 0 and 120 ppm observed for 28 days after termination of exposure	NOEC of 60 ppm for liver weight increases (female)	Statistically significant increase in relative liver weight at 120 ppm in females, no effect in males; no difference in liver weights after 28 days recovery period	(DCC, 1990a)
Fischer F344 rats	90 day inhalation study with nose-only exposure to targeted concentrations of 0, 29, 46, 92, and 223 ppm D5 for 6 h/day, 5 days/week; groups of 20 male and 20 female rats including additional recovery group of 10 male and 10 female rats exposed to 0 and 223 ppm observed for 28 days after termination of exposure	NOEC of 46 ppm for liver weight increases (females)	Significant increases in liver weight in females at 46 and above and at 223 ppm in males; increased lung weights at 223 ppm; increased incidence of alveolitis in males and females at 92 and 223 ppm, increased incidence of ovarian interstitial gland hyperplasia and vaginal mucosal mucification and atrophy in females at 223 ppm; lung weights remained elevated in females after recovery phase. dose-related increases in γ-glutamyltransferase (>26 ppm) and serum lactate dehydrogenase (>46 ppm) not resolved during recovery;	(DCC, 1990a; RCC, 1995b; Burns-Naas et al., 1998b)
Fischer F344 rat	2-year inhalation study with whole body exposure to 0, 10, 40, and 160 ppm D5, 6 h/day, 5 days/week, main group with exposure for 2 years consisted of 60 animals/sex/D5 concentration Groups of 10 animals/sex/D5 concentration exposed for one year with immediate sacrifice group of 20 animals/sex/D5 concentration exposed for one year with sacrifice one year after termination of exposure group of 6 animals/sex/d5 sacrificed after 6 month, used mainly for calibration of Pk model	NOAEC of 160 ppm for systemic, non-neoplastic effects	Increased incidences of endometrial adenocarcinoma (0, 1, 0, 5) in the 0, 10, 40, and 160 ppm exposure groups after 2 year inhalation exposure No effects observed after one year inhalation and immediate sacrifice Endometrial adenomatous polyps and endometrial adenocarcinoma (combined incidences of 1, 1, 0, 3) in the 10, 40, and 160 ppm exposure groups after 1 year inhalation exposure to D5 and one year of recovery No histopathological effects after 6 month of exposure	(DCC, 2005b)
Sprague–Dawley rats	One-generation reproductive toxicity study by whole body inhalation to 0, 26, and 132 ppm D5 for 6 h/day for a min. of 28 days prior to mating and through the day of sacrifice (22 male and female animals/group). Exposures of females suspended from GD 21 to lactational day 4, offspring examined after sacrifice on PND 28	NOAEC of 132 ppm	No effects on food consumption, body weight gain, reproductive parameters (fertility, mating, days between pairing and coitus, gestation and parturition). Mean numbers of implantation sites and mean live litter size. No effects on pup viability, pup sex ratios and mean pup weights	(WIL, 1996)
Sprague–Dawley rats	2-generation reproductive toxicity study by whole body inhalation to 0, 30, 70, and 160 ppm D5 for 6 h/day for at least 70 consecutive days prior to mating through weaning of pups on postnatal day 21 performed in accordance with US EPA OPPTS Health Effects Test Guideline 870.3800	Overall NOAEC of 160 ppm	No effects on reproductive parameters, no effect on developmental parameters	(Siddiqui et al., 2007)

and a significant negative trend for the incidence of fibroadenoma of the mammary gland area (0 ppm, 8/60; 10 ppm, 7/60; 40 ppm, 7/60; 160 ppm, 2/60) was indicated.

3.2. Reproductive and developmental toxicity

The reproductive and developmental toxicity of D5 was assessed in a one-generation reproductive toxicity study and in a two-generation reproductive and developmental toxicity study in rats. The two-generation study included an assessment of developmental neurotoxicity (Table 2). In all reproductive and developmental toxicity studies, the animals were exposed to D5 by inhalation at concentrations up to 160 ppm.

In the two-generation study, the F_0 and F_1 female rats were exposed throughout mating and through gestational day 20 when exposures were stopped to allow parturition and to permit continuous maternal care for the neonates. Exposures were resumed on lactation day 5 and continued until the day prior to

sacrifice. F2 pups were not exposed to D5. All F_0 and F_1 females were allowed to deliver and rear their pups until weaning on lactation day 21. Offspring (30/sex/group) from the pairing of the F_0 animals were selected as the F_1 generation. Neonatal survival, growth, and development of the F_1 and F_2 generations and developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F_1 rats. Thirty pups/sex/group from the F_2 generation were selected for examination of development landmarks, neurobehavioral testing, neuropathology brain weights, and/or brain dimension measurements. Surplus F_1 and F_2 pups were necropsied on PND 21 or 28, and selected organs were weighed. Selected F_2 rats not allocated for neuropathology and brain dimension measurements were necropsied on PND 70. All surviving F_0 and F_1 parental animals were subjected to gross necropsy following the completion of weaning of the F_1 and F_2 pups. Evaluations of sperm parameters were performed in all F_0 and F_1 males, and ovarian primordial follicle and corpora lutea counts were recorded for F_0 and F_1 females in the control and the highest exposure concentration (160 ppm). Designated tissues from all F_0 and F_1 parental animals in the control and 160 ppm groups and from F_2 pups selected for neuropathological evaluation were examined microscopically (Siddiqui et al., 2007).

D5 inhalation did not affect any of the assessed reproductive parameters and had no effects on body weights, body weight gain, and organ weights in the F_0 and F_1 generations. In addition, the functional observational battery in F_1 females did not show D5-induced effects on gestation day 10 and lactation day 20. D5 inhalation did not induce effects on the parameters determined to assess reproductive performance or potential developmental effects. The only statistically significant effect was an increase in anogenital distance in male F_1 pups, but not male F_2 pups or in F_1 and F_2 female pups. Developmental landmarks in F_1 and F_2 and F_2 neurobehavioral responses were not affected by parental exposure to D5 and neither microscopic findings nor differences in mean brain weights and brain measurements were noted.

4. Genotoxicity of D5

Multiple assessments of genetic toxicity have been performed with D5 (Litton, 1978; Isquith et al., 1988; DCC, 2003d; DCC, 2004d). The genotoxicity of D5 was evaluated in bacteria, cultured mammalian cells, and in rats after D5 inhalation (Table 3). D5 did not show a genotoxic response when assessed for gene mutation in bacteria and in mammalian cells and did not induce structural chromosome aberrations (Table 3). The mutagenic potential of D5 was also assessed *in vivo* using unscheduled DNA Synthesis (UDS) in hepatocytes and induction of micronuclei in bone marrow (DCC, 2004b) after whole body inhalation exposure (160 ppm, 6 h/day for 7 consecutive days) in rats (Table 3). In these studies, positive controls (2-acetylaminofluorene for the UDS and cyclophosphamide in the micronucleus assays) were concurrently performed and produced the expected response. In the combined UDS/micronucleus assay, animals were sacrificed five and 16 h after the last inhalation exposure, and UDS was determined in primary hepatocytes. D5 did not induce UDS as compared to concurrent air controls. In bone marrow cells collected 24 h after the last inhalation exposure for micronucleus analysis, D5 did not cause bone marrow toxicity or increase the frequency of micronuclei.

5. Results from mechanistic studies with D5

5.1. Hepatomegaly and enzyme induction

The results of a number of repeated-dose toxicity studies with D5 resulted in a reversible hepatomegaly in rats. Several studies investigated the mechanistic basis for this effect (McKim et al., 1999; DCC, 2000a; Zhang et al., 2000; DCC, 2004e; DCC, 2004f). Most of these studies focused on enzyme induction as the potential mechanism for the liver weight increases. Enzyme activity and relative protein concentrations of cytochromes P4501A, 2B, 3A, and 4A), epoxide hydrolase, and UDP-glucuronyl transferase (UDPGT) were determined in liver microsomes obtained from female F344 rats exposed to D5 by inhalation for six hours/day and five days/week over 28 days. This exposure regimen increased liver size by 16% relative to controls. The increase in liver size in D5-exposed animals was partly reversed after a 14-day recovery period. Overall P450 activity in liver microsomes was not influenced by D5 inhalation; however, D5 inhalation resulted in small increases in the activity of NADPH-cytochrome *c* reductase and 7-ethoxyresorufin O-deethylase activity (EROD), but without an accompanying increase in the concentrations of the CYP1A1/2 protein. CYP1A1/2 is the major P450 enzyme that catalyzes EROD activity. A fourfold increase in 7-pentoxyresorufin 0-dependentylase activity (PROD) and a threefold increase in the relative concentrations of CYP2B1/2 protein were also observed. In addition, testosterone 6β-hydroxylase activity and CYP3A1/2 protein concentration were increased. The levels of CYP4A were unchanged, but a small increase in 11- and 12-hydroxylation of lauric acid was detected. D5-exposure also caused moderate increases in liver microsomal epoxide hydrolase protein concentration and activity. UDPGT activity was increased with chloramphenicol but not with 4-nitrophenol as substrate. These observations suggest that D5 acts as a "phenobarbital-like" inducer since the enzyme induction profile caused by D5 inhalation is similar to that reported for phenobarbital (McKim et al., 1999).

A direct comparison of the liver enzyme induction profile of D5 to that of phenobarbital used repeated oral administration of D5 at dose levels up to 100 mg/kg bw for four consecutive days and compared the liver enzyme profile to that induced by a single intraperitoneal dose of Phenobarbital. Relative liver weight was increased by D5 at the highest dose. In liver microsomes isolated from D5-treated rats, concentrations of CYP2B1/2 protein were significantly increased at D5 doses >5 mg/kg bw with a parallel increase in PROD activity in females at doses >5 mg/kg bw and in males at 20 and 100 mg/kg bw. In addition, EROD activity was increased in males and females at doses of above 5 mg/kg bw, but without changes in CYP1A1/2 concentrations. Increases in relative concentrations of the CYP3A1/2 protein also occurred in males at 100 mg/kg bw and females at D5 doses above 5 mg/kg bw. The P450 reductase protein was significantly induced at $\geq$5 mg/kg bw in males and $\geq$20 mg/kg bw in females. A similar induction pattern was observed with phenobarbital (Zhang et al., 2000) further confirming the ability of D5 to act as a "phenobarbital-like" inducer.

In incubations with human liver microsomes, the potential of D5 (0.040–3.5 μM) as a reversible or irreversible inhibitor of several P450 enzymes was evaluated. D5-mediated inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP4A9/11 activity was not observed. D5 was only a weak competitive inhibitor of human CYP3A4/5, but a potent metabolism-dependent inhibitor of human CYP3A4/5 (DCC, 2000b). For comparison, D5 was a potent reversible metabolism-dependent inhibitor of rat CYP1A1/2 activity but did not inhibit rat CYP2B1 activity.

5.2. Estrogenicity

The potential estrogenicity of D5 was assessed *in vivo* and in *in vitro* binding studies to the estrogen receptor (DCC, 2004e; DCC, 2004f; DCC, 2004g; DCC, 2004h; DCC, 2004a). In intact animals, estrogenic effects of D5 were assessed in an uterotrophic assay in both Sprague–Dawley and Fischer 344 rats after D5 inhalation

Table 3
Summary of results from genotoxicity testing with D5.

System	Experimental conditions	Endpoint assessed	Results	Reference
In vitro Salmonella typhimurium (TA-1535, TA-1537, TA-1538, TA-98, and TA-100) *Escherichia coli* (W3110/polA+, P3478/polA-)	Concentrations up to 5 mg/plate, with and without metabolic activation	Gene mutations	negative	(Litton, 1978)
In vitro Escherichia coli polA+	Concentrations up to 5 mg/plate	DNA-repair	negative	(DCC, 2003c)
In vitro Cultured mammalian cell (mouse lymphoma)	Concentrations up to 6.4 μl/ml with and 12.5 without metabolic activation	Gene mutation	negative	(Isquith et al., 1988)
In vitro Cultured mammalian cell (mouse lymphoma)	Concentrations up to 25 μl/ml with and without metabolic activation	Chromosome aberration	negative	(Isquith et al., 1988)
In vitro Cultured mammalian cell (Chinese hamster V79)	Concentrations up to with and without metabolic activation	Chromosome aberration	negative	(Isquith et al., 1988)
In vitro Cultured mammalian cell (mouse lymphoma)	Concentrations up to 25 μl/ml with and without metabolic activation	Sister chromatid exchange	negative	(Isquith et al., 1988)
In vitro Cultured mammalian cell (mouse lymphoma)	Concentrations up to 25 μl/ml with and without metabolic activation	Alkaline elution assay for general DNA-damage	negative	(Isquith et al., 1988)
In vivo Primary hepatocytes from rats	Isolated from rats exposed to D5 by inhalation (160 ppm, 6 h/day, 7 days)	Unscheduled DNA-synthesis	negative	(DCC, 2004a)
In vivo Bone marrow cells from rats	Isolated from rats exposed to D5 by inhalation (160 ppm, 6 h/day, 7 days) directly after the end of the inhalation exposure	Micronucleus formation	negative	(DCC, 2004a)

(160 ppm, whole body, for 16 h/day for three days). Animals were sacrificed immediately following the last exposure. No effects of D5-exposure on wet and blotted uterine weight were observed. D5 was also negative in a Hershberger assay (a short-term *in vivo* assay to detect androgenic or antiandrogenic chemicals or chemicals that inhibit 5α-reductase).

In an additional study to assess a role of membrane and nuclear estrogen receptors in a potential estrogenic response of D5 in animals, rats were exposed by inhalation to D5 (whole body, 160 ppm for 6 or 16 h) and possible D5-mediated effects on estrogen-dependent endpoints were compared to the responses induced by ethinylestradiol (activates both the membrane and nuclear ER) and 4-hydroxytamoxifen (activates membrane ER but is a partial agonist/antagonist for nuclear ER). The study determined uterine weight (wet and blotted), estrous state, epithelial cell height (luminal and glandular), histopathology (uterus and mammary gland) and cell proliferation in luminal, glandular, stromal and total cells of the uterus and mammary gland epithelium. Both positive controls resulted in the expected response, but D5 did not influence any of the estrogen responsive parameters measured (DCC, 2012).

In vitro, D5 (0.28 μM) did not show a capacity to compete with estradiol for binding to the estrogen receptor. D5 (10 μM) also did not give a response in a reporter gene assay using MCF-7 cells transiently transfected with a plasmid for estrogen receptor alpha and the luciferase gene. D5 did not interact with progesterone receptors alpha and beta (Quinn et al., 2007).

5.3. Dopamine agonism

A series of experiments investigated the ability of D5 to act as dopamine agonist and effect prolactin secretion. Such a mode-of-action may explain the formation of uterine tumors in rats observed after inhalation to 160 ppm D5 for two years (Klaunig et al., 2015). Dopamine agonists inhibit prolactin secretion from the pituitary in rats causing estrogen dominance, and results in persistent endometrial stimulation leading to endometrial tumors.

Studies investigating a possible role of dopamine agonism were performed in two animal model systems and in a series of *in vitro* systems (DCC, 2005d; DCC, 2005e; DCC, 2010a; DCC, 2010b; DCC, 2011). The reserpine pretreated rat and the aging Fisher F344 rat were used as animal model systems. Reserpine administration to rats depletes brain dopamine (Metzger et al., 2002), which blocks the dopamine inhibition of prolactin secretion into blood and results in a pronounced increase in circulating prolactin. Administration of dopamine D2 receptor agonists following reserpine administration will decrease prolactin. Therefore, this model is often used to investigate the potential for a chemical to interact with the dopamine D2-receptor *in vivo* (Graf et al., 1976; Horowski and Graf, 1976). In the aging F344 rat, altered hypothalamic control of dopamine causes an elevated prolactin secretion and results in increased prolactin levels in blood. Administration of dopamine receptor agonists also reduces prolactin in this system.

Support for D5 as a dopamine agonist was examined in rats treated with D5 by inhalation and reserpine (DCC, 2005c). In reserpine-treated rats, D5-exposure (nose-only inhalation, 160 ppm for six hours) reduced the reserpine-induced increase in prolactin levels by 50% (DCC, 2005c). The dopamine receptor antagonist sulpiride blocked the prolactin-lowering effect of D5.

In another study, a time course exposure of D5 on prolactin concentrations was investigated in reserpine-pretreated female rats, since effects on dopamine may only occur during and shortly after the end of D5 exposures due to the rapid clearance of D5 by exhalation. The study design used Pergolide in a positive control group. In this study, serum prolactin levels were not decreased in reserpine-treated rats at the end of the six hour exposure to 160 ppm D5 and at four hours after the end of the inhalation exposure. At eight hours after exposure, levels of serum prolactin were decreased in the D5 group by 36% as compared to reserpine controls (not statistically significant). Decreases in serum prolactin levels were most prominent after the 18-h post-exposure time point in the D5 exposed group. At this time, circulating serum prolactin levels were returning to normal due to a loss of the reserpine effect. Prolactin levels in the D5 exposed group were similar to the reserpine-treated group. The weak effect of D5 at 18 h after the end of inhalation exposure may suggests some potential of D5 for a direct or indirect modulation of prolactin secretion (DCC, 2010b).

The aged Fischer 344 rat (female rats > 20 month old) model was utilized (DCC, 2010a) to evaluate the effect of repeated inhalation exposure to D5 (160 ppm, 6 h/day, for five days) on circulating prolactin levels and the estrus cycle. Repeated D5 inhalation did not significantly affect circulating prolactin levels at the end of the inhalation exposure on days one and five. However, the average prolactin levels animals exposed to D5 strongly trended higher at four and eight hours after the end of the inhalation exposure on day five. The dopamine receptor agonist Pergolide consistently reduced

prolactin levels. A strong trend was observed in the D5 group (only one animal cycled throughout the six day period) suggesting that D5 exposure may inhibit cyclicity and D5 inhalation may have stabilized the pseudopregnant/anovulatory state as evidenced by decreased number of estrogenic days and maintaining estrogen, progesterone, and the estrogen:progesterone ratio. D5 inhalation did not modulate blood levels of corticosterone or uterine weight (DCC, 2010a).

Aged Fischer 344 rats were also utilized (DCC, 2013) to evaluate the effect of exposure to D5 on cyclicity, prolactin, and estrogen:progesterone ratio. Female F344 rat aged 22 months were exposed to D5 by inhalation (160 ppm, 6 h/day, 5 days/week for 13 weeks?). Dietary Pergolide was used as positive control. The rats were already in a state of persistent diestrus/pseudopregnancy when the study began. At the end of the study, pergolide administration resulted in decreased prolactin and decreased progesterone in blood and also inhibited increased estrogen concentration and an increased estrogen/progesterone ratio. In addition, Pergolide markedly altered cyclicity increasing the incidence of proestrus/estrus. D5 exposure produced few changes in the markers assessed except a marked increase in the incidence of proestrus/estrus.

An aged F344 rat model (age 49–50 weeks at study initiation) assessed the effects of repeated inhalation exposure to D5 (inhalation exposure to 160 ppm, 6 h/day, five days/week for at least 58 weeks). The study included assessment of vaginal cytology and reproductive senescence and monitored estrous cycle stage by daily vaginal lavage (WIL, 2013). Serum prolactin, estradiol, progesterone and follicle stimulating hormone were also determined at predetermined time-points. At the beginning of the exposure and for the first 90 days in the study, the rats were in an estrogenic state 20–25 % of the time. Afterwards, in control rats, age-specific changes in the reproductive status occurred as expected during the aging process. In the D5-exposed rats, the percent of days spent in an estrogenic state was increased by an average of 44% during the first 45-day interval and 78% during the second 45-day interval after initiation of D5 exposure. The percentage of estrogenic days remained increased during the third 45-day interval but the control percentage had increased markedly during this time. Later on, frequency of estrogenic state was not different between controls and D5-exposed animals, as both control and D5-exposed animals converted to a repeated pseudopregnant state characteristic of the reproductively senescent F344 rat. Evaluation of the reproductive tract of the animals by histopathology was consistent with animals in pseudopregnancy and only very few differences between controls and D5-exposed animals were seen. A slightly more marked vaginal mucification and epithelial thickening in the D5-exposed rats suggest a more advanced aging in these animals.

In vitro studies were performed to determine the ability of D5 to stimulate prolactin release from specific cells and its affinity to dopamine receptors (DCC, 2005e; DCC, 2005d; DCC, 2009). An initial study was performed in a cell line derived from rat pituitary tumor (MMQ cells) to assess the potential of D5 to act as a dopamine D2-receptor agonist (DCC, 2005e). MMQ cells synthesize and secrete prolactin and contain functional dopamine D2-receptors. Prolactin secretion in MMQ cells is inhibited by dopamine and the extent of inhibition of prolactin secretion is thus a function of dopamine receptor agonist concentration and potency. This model system requires maitotoxin to elevate prolactin secretion. The maitotoxin-induced prolactin secretion is dopamine D2-receptor agonist-sensitive (Judd et al., 1988; Judd and MacLeod, 1991; Forget et al., 1993). The MMQ cells used produced and secreted prolactin, with and without induction by maitotoxin. The maitotoxin-induced increase in prolactin secretion was sensitive to dopamine. In support of the hypothesis that D5 may act as a dopamine receptor agonist, D5 inhibited maitotoxin-induced prolactin secretion by 55%.

However, a direct interaction of D5 with dopamine receptors was not seen in a series of studies using competition experiments. D5 did not displace dopamine receptor ligands from recombinant human D_1, D_2, D_3, $D_{4.4}$ and D_5 dopamine receptor. Competitive binding was observed only for the dopamine D_2 receptor. However, competition was minimal and only occurred at high nominal D5 concentrations. As a consequence, the response with the D_2 receptor was considered equivocal (DCC, 2009).

Another dopamine receptor binding study was initiated utilizing rat striatal membranes to assess the potential for the interaction of D5 with the D_2 receptor and the D_2 receptor second messenger protein (GTPyS) (Baker, 2010). Binding of D5 to the D_2 receptor was not observed, but a suppression of the basal activity (guanosine 5′(γ-thio) triphosphate (GTPγS)) occurred. An additional study utilizing the MMQ pituitary cell line further assessed D_2 receptor activation as indicated by suppression of cellular cAMP production following exposure to D5 (DCC, 2011). Utilizing alteration of forskolin-induced cAMP production as the marker of dopamine D_2 receptor activation demonstrated that the dopamine agonistic effect of D5 was not mediated through activation of the dopamine receptor. The activity was not inhibited by inclusion of a dopamine receptor antagonist and the effect was not lost following pertussis toxin uncoupling of the G-protein and receptor. The effects of D5 appeared to be associated with competitive inhibition of forskolin activation of adenylate cyclase.

Female rat brain striatal membranes have a high density of dopamine receptors. Therefore, a potential interaction of D5 with dopamine D2-receptors was studied in this isolated membrane system. In isolated membranes from the brain striatum, exposure to D5 did not result in activation of the dopamine D2 receptor. In addition, the effect of D5 on iodosulpiride binding (used to determine an interaction with the receptor and the stimulation of GTPγS binding) showed that D5 did not have any effect on receptor activation of coupled G-protein or stimulated GTPγS binding (Baker, 2010).

The potential of D5 and its metabolites (nonamethylcyclopentasiloxanol (nona-D5) and dimethylsilanediol) to interfere with signaling cascades downstream to binding to the dopamine receptor was assessed. After the binding of an agonist to the dopamine D2-receptor, an activated receptor activates a G-protein, which then inhibits adenylate cyclase, resulting in decreased production of 3′-5′-cyclic adenosine monophosphate (cAMP). The capacity of D5 to act as dopamine D2-receptor and/or adenylate cyclase agonist was assessed in MMQ pituitary cells by analyzing a possible modulation of forskolin-induced increases in cAMP production. Transdecalin, which does not interact with the dopamine D2 receptor or influences adenylate cyclase activity, was included to assess a possible modulation of cAMP production by highly lipid soluble chemicals. In these studies, while D5 and nona-D5 attenuated forskolin-induced cAMP increases in MMQ cells, effects of D5 was not modulated by the dopamine receptor antagonist Raclopride (DCC, 2011).

6. Discussion and conclusions

D5 is a highly lipophilic and volatile compound with a particular kinetic behavior after oral administration (predicted uptake and distribution of D5 in the form of microemulsions). D5 concentrations in rodent feed are rapidly reduced due to evaporation and feeding studies are therefore extremely difficult to conduct. In contrast, absorption of D5 after inhalation and dermal contact, two exposure routes considered most relevant to humans, occurs by D5 molecules diffusing through cell membranes. Therefore, only

inhalation and dermal toxicity studies will provide useful information on hazard properties of D5 potentially relevant to humans. Due to the very low permeability of D5 through human skin and the rapid evaporation of D5 when applied to skin, dermal uptake is expected to be of low significance with regard to the generation of sufficient plasma concentrations of D5 to produce potential systemic toxicity. Moreover, due to the volatility and the surface-spreading characteristics of D5, repeated dose dermal studies cannot be conducted with reasonable confidence. In contrast, after inhalation, D5 is absorbed though the lungs and inhalation therefore is the only reasonable route of exposure resulting with a potentially important contribution to D5 systemic availability.

As other volatile lipophilic molecules, absorbed D5 is preferentially distributed to lipid-rich tissues. However, due to the high volatility, exhalation of unchanged D5 is the major route of elimination of absorbed D5. Due to this rapid elimination by inhalation, a bioaccumulation of D5 in lipid-rich tissues is not expected and increased tissue levels of D5 were not observed after repeated inhalation for six month.

Data from repeated dose inhalation studies showed that exposure of experimental animal to D5 results only in a limited number of potentially adverse effects and did not produce reproductive or developmental toxicity. In repeated chronic exposure inhalation studies, effects observed were limited to local effects in nasal cavity and the lungs, reversible liver weight increases without histopathological changes, and to a small increase in the incidence of uterine adenocarcinoma in female rats after two year inhalation exposure. D5 was determined to be not genotoxic.

In summary:

i) The respiratory tract effects (increased lung weights, submucosal inflammation in the lung, goblet cell proliferation) seen in the chronic bioassay inhalation study with D5 can be considered as a non-specific response of the respiratory tract to repeated inhalation exposures to a mildly irritating agent.
ii) The liver weight increases observed in several toxicity studies with D5 of shorter duration, but not in the two-year chronic bioassay study at termination, were not accompanied by histological changes in the liver. These observations suggest that the liver weight increases represent adaptive responses and are not adverse. Mechanistic studies confirm that D5 is a weak “phenobarbital-like” inducer of cytochromes P450 in the rat. The “phenobarbital-like” enzyme induction pattern explains the small changes in liver weights seen in the inhalation studies.
iii) In the two-year chronic bioassay, D5 inhalation caused a small, but (borderline) statistically significant increase in the incidence of uterine adenocarcinoma at the highest exposure concentration of 160 ppm. A number of mechanistic studies have been performed to elucidate the biological processes which may result in uterine adenocarcinoma induction. Due to the absence of both genotoxicity and estrogenicity of D5 in a variety of test systems, it must be concluded that neither genotoxicity nor a direct estrogen effect produced the slight increase in uterine tumors. Discussed in the detail in the accompanying paper (Klaunig et al., 2015), the likely mechanism for the induction of uterine adenocarcinoma is an interference with prolactin secretion. While D5 does not appear to be a direct dopamine agonist, the experimental data are suggestive of an indirect interaction of D5 on the dopamine system to alter the pituitary control of the estrus cycle. Like other dopamine receptor agonists, D5 decreases pituitary lactotroph release of prolactin *in vitro* and decreases circulating prolactin levels *in vivo*. Further studies *in vitro* suggest that D5 may interfere with one or more downstream components of the dopamine signal transduction pathway. The observed effects of D5 on estrus cyclicity are consistent with a dopamine-like effect, and further suggest that D5 may be accelerating the aging of the reproductive endocrine axis in this strain of rat.

Conflicts of interest

Dr. Dekant and Dr. Klaunig both report personal fees from the American Chemistry Council.

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