A complete histopathology examination was performed on all animals that were either sacrificed or died *in extremis.* The percent survival of animal exposed to 700 ppm was decreased (38% in treated males compared to 58% for controls and 58% in treated females compared to 72% for controls). The survival in the other groups was similar to that found in the control groups. It is stated that this effect on mortality was likely due to early onset and increased incidence of mononuclear cell leukaemia (MNCL) that occurred at 700 ppm. The terminal mean body weights and weight gains of the 700-ppm male rats were significantly lower than those of the controls (~6 and 8%, respectively). This difference from the controls was apparent only during the last few months of study. No other exposure-related effect on body weight was noted in the male groups. In females, there were a few occasions during the study when exposed groups had significantly different body weight from the control. This occurred sporadically and the difference from the controls was minor.

Statistically significant increases in absolute and relative kidney weights in both sexes were observed in the high dose group. It is stated that these increases in kidney weights may reflect the observed increases in severity of chronic nephropathy. Statistically significant increases in absolute and relative liver weights were observed in males in the high dose group and in females at the two highest dose groups. There were no accompanying histopathological effects.

A 51% increase in absolute and relative uterine weight was seen in the high dose female rats. Histopathologically the total incidence of cystic endometrial hyperplasia was 78% compared to 19% in the control group. Four of the 35 (11%; $p<0.04$) female animals in the high dose group that survived two years were diagnosed with endometrial adenomas. No uterine adenomas were diagnosed in the intercurrent mortality animals or in any of the other groups.
The neoplastic effect observed in the high dose (700 ppm) female rats has been attributed to a hormonal dysregulation resulting from interaction of D4 with the dopamine D2-receptor.
Pre-treatment of F344 rats with sulpiride, a dopamine receptor antagonist, blocked the effect of D4 on the serum prolactin levels suggesting that D4 acts on the pituitary as dopamine D2-receptor agonist *in vivo* (Ref. AR 5). These results and the known species differences in reproductive physiology provide support for a potential mode of action that is not relevant for humans. Additional investigations on the mode of action are described in Section (3.3.12. Special studies).

The frequency of mononuclear cell leukaemia (MNCL) in male rats was: 73% in the controls (43/59; historical controls 474/1059 [45%]; $p < 0.0001$), 10-ppm group 45% (27/60), 30-ppm group 43% (26/60), 150-ppm group 48% (29/60), and 700-ppm group 69% (41/59). The frequency of MNCL in the high dose group is similar to the control. The frequency in the control group was significantly higher than in the historical controls. This finding is not discussed by the study authors. However, it is stated that the incidence of MNCL was increased in early death and moribund sacrificed males exposed to 700 ppm compared to male controls. This increase was statistically significant ($p < 0.05$) using the Peto analysis. It is apparent that the frequencies of MNCL in the 10 ppm and 30 ppm groups are similar to the historical controls. If the MNCL in the 700 ppm group is compared to the 10 ppm group, the increase in the 700 ppm group is significant ($p<0.0094$). No increase in MNCL was found among the exposed female rats. It is likely that a threshold exists in the induction of MNCL. If it is considered that the very high frequency in the control group may be erroneous, the NOAEL for MNCL induction is 150 ppm (320 mg/kg bw/d).

Ref.: 79, AR 4

Comment
In the lifetime study with D4, uterine (endometrial) adenomas and hyperplasia were observed at the highest dose level of 700 ppm. The lack of a genotoxic potential supports the view that tumour formation by D4 is due to threshold effects. The applicant has proposed that the endometrial adenomas and hyperplasia are due to dopamine-agonist like activity of D4 and thus not relevant to humans. But, this position is not supported to date

by international reviews due to a lack of a published data and a thorough mode of action analysis.
An increased incidence of mononuclear cell leukaemia was observed in male rats. Since this tumour type is unique to F344 rats, its relevance to humans is questionable. Thus, SCCS as well as Health Canada (Ref.: AR 8) dismissed this tumour type in their evaluations of D4.

3.3.8. Reproductive toxicity

3.3.8.1. Teratogenicity (Embryo-foetal developmental studies)

The effects of D4 on embryo-foetal development have been investigated in rats and rabbits. The study designs and the results are summarised in the following table:

Table 4: Embryo-foetal development studies

Species/strain	Route	Treatment	No./group	Dose levels of D4	Results	Ref.*
DOSE RANGE-FINDING STUDIES						
Rabbit/New Zealand White	Oral gavage	Daily oral doses on GD7 through GD19	6F	0, 50, 100, 500, 1000 mg/kg/day	Maternal toxicity at ≤50 mg/kg. No teratogenicity	24
Rat/Sprague Dawley	Whole body inhalation	6h/day on GD6 through GD15	6F	0, 10, 100, 300, 700 ppm	Maternal toxicity at 700 ppm. No teratogenicity	22
Rabbit/New Zealand White	Whole body inhalation	6h/day on GD6 through GD18	6F	0, 10, 100, 300, 700 ppm	Maternal toxicity at 300 ppm. No teratogenicity	23
EMBRYOFOETAL TOXICITY STUDIES						
Rat/Sprague Dawley	Whole body inhalation	6h/day on GD6 through GD15	30F	0, 100, 300, 700 ppm	Maternal toxicity at 700 ppm. No teratogenicity	25
Rabbit/New Zealand White	Whole body inhalation	6h/day on GD6 through GD18	20F	0, 100, 300, 500 ppm	Maternal toxicity at 500 ppm. No teratogenicity	26

GD = gestation day, F = female
* The studies summarized in this table refer to GLP studies

Dose range-finding studies were conducted in pregnant Sprague Dawley rats (Ref. 22) and New Zealand White rabbits (Ref. 23) with whole body exposure to D4 vapour concentrations of 10, 100, 300 or 700 ppm. Also, an oral gavage study was conducted in pregnant New Zealand White rabbits at D4 dose levels of 50, 100, 500 or 1000 mg/kg/day (Ref. 24). Rats were exposed on gestation days (GD) 6 through 15 whereas rabbits were exposed on GD7 through 19 (oral study) or GD6 through 18 (inhalation study). On gestation day 20 (rats) or 29 (rabbits), the dams were killed for Caesarean section and uterine examination.

In the **oral study in rabbits**, the death of one rabbit in the 500 mg/kg/day group on GD 26 was considered unlikely to be treatment-related in the absence of death at 1000 mg/kg/day (Ref. 24). Clinical signs included mucoid stool at 500 and 1000 mg/kg/day, anogenital staining and hair loss at 1000 mg/kg/day, and tissue and/or red fluid on cage tray (often associated with abortion) at 500 and 1000 mg/kg/day. Body weight and food consumption reductions were recorded at all D4 dose levels. Treatment-related abortions were observed at 500 and 1000 mg/kg/day with markedly increased post implantation

losses at 1000 mg/kg/day. This correlated with reductions in the number of live foetuses and gravid uterine weights at 1000 mg/kg/day.
By gestation day 13 most rabbits at 500 or 1000 mg/kg/day were consuming less than 20 g/day or not eating at all. Therefore, it is considered likely that the increase in abortions and post implantation losses are the consequence of reduced food consumption and not a direct effect of D4.
This conclusion is substantiated by an oral gavage study in non-pregnant rabbits at dose levels of 500 or 1000 mg/kg/day for 14 days (Ref. 6). These doses caused marked reductions in food intakes and body weights, similar to those seen in the reproductive study (Ref. 24) indicating the foetal losses in the earlier study were probably due to weight loss and stress and not to the direct action of D4.
In the **inhalation dose-range finding studies in rats and rabbits**, there was no treatment-related maternal mortality, although both rats and rabbits showed reduced food consumption and reduced body weight gains at 700 ppm. Rabbits exhibited decreased defecation, soft stool and/or anogenital staining at 300 and 700 ppm. In neither species was there any evidence of developmental toxicity.
Embryofoetal toxicity studies were conducted by whole body inhalation exposure of 30 dams/group at dose levels of 100, 300 or 700 ppm (rats) (Ref. 25), and of 20 dams/group to 100, 300 or 500 ppm (rabbits) (Ref. 26). Rats and rabbits were exposed for 6 hours/day on gestation days 6 through 15 (rats) or gestation days 6 through 18 (rabbits).
All animals survived the treatment period with no overt signs of toxicity. Food consumption was reduced in rabbits at 500 ppm and in rats at 700 ppm although a reduction in body weight gain was only noted for rats. Reproduction and Caesarean parameters were not affected by treatment. Morphological evaluation of the foetuses did not demonstrate any test article-related malformations or developmental variations.
The NOAEL for maternal toxicity was 300 ppm for rats and rabbits. D4 was not teratogenic at the highest dose levels tested, i.e. 700 ppm for rats and 500 ppm for rabbits.

Conclusion
Embryofoetal inhalation studies in Sprague Dawley rats and New Zealand White rabbits revealed no evidence of developmental toxicity (teratogenicity) up to the highest dose levels tested, i.e. 700 ppm for rats and 500 ppm for rabbits.

Ref.: 22, 23, 24, 25, 26

3.3.8.2. One-generation studies (general reproduction and fertility)

A series of one-generation studies with inhaled D4 has been conducted: They include two range finding (Refs. 27, 28), two male (Refs. 29, 30) and one female (Ref. 31) crossover, and two "phased female" (Refs. 32, 33) studies in rats. (Note: A two-generation study has also been completed and is described in section 3.3.8.3.)

In all these studies male and/or female Sprague Dawley rats were exposed by whole body vapour inhalation to D4 at concentrations ranging from 70 ppm to 700 ppm for 6 hours/day, 7 days/week. The general protocol for each study was similar and included continuous exposure for at least 28 or 70 days prior to mating, with exposure to females continuing in some studies throughout gestation and lactation.
The study designs and results of these studies are summarized below in two tables: The first table describes range-finding studies; the second one describes the phased-female studies.

Table 5: One-generation range-finding inhalation studies

Species/ strain	Treatment	Nr/group	Dose levels of D4	Termination day	Results	Ref. *
Rat/Sprague Dawley	Males and females exposed 6h/day for 28 days prior to mating, through-out mating to GD 21, then LD 4 to termination	F0 – 20M, 20F	0, 70, 700 ppm	F0 females on LD 21 F1 pups on PND 28	Parental toxicity at 700 ppm; reduced number of implantation sites at 700 ppm. No postnatal toxicity.	27
Rat/Sprague Dawley	Males and females exposed 6h/day for 28 days prior to mating, throughout mating to GD 20	F0 – 22M, 22F	0, 700 ppm	F0 females on LD 4, F1 pups on PND 4	Parental toxicity at 700 ppm. No postnatal toxicity.	28
Rat/Sprague Dawley	Males exposed 6h/day for 70 days prior to mating, throughout mating to GD 13	F0 – 40M, 40F (females exposed to filtered air only)	0, 500, 700 ppm	F0 females and F1 pups on PND 21. -- F0 males 5 weeks later following a 5 week recovery period	Toxicity to F0 males at 700 ppm. No toxicity at 500 ppm or to F1 pups.	29
Rat/Sprague Dawley	Males exposed 6h/day for 70 days prior to mating, throughout mating	F0 – 22M, 22F (females exposed to filtered air only)	0, 70, 300, 500, 700 ppm	F0 males after mating, F1 pups on PND 4, F0 females following F1 pups	No parental or neonatal toxicity at 70 or 300 ppm.	30
Rat/Sprague Dawley	Females exposed 6h/day for 70 days prior to mating, throughout mating to GD 21 and from LD 3 to 21	F0 – 22M, 22F (males exposed to filtered air only)	0, 70, 300, 500, 700 ppm	F0 males after mating, F0 females on LD 21, F1 pups on PND 28,	No maternal toxicity at 70 ppm. No postnatal toxicity at 70, 300 or 500 ppm	31

GD = gestation day, LD = lactation day, PND = postnatal day, M = males, F = females F0 = Parent generation, F1 = First generation
* The studies summarized in this table are GLP compliant

Results
In one *range-finding study* (Ref. 28), the gestation length was reported to be statistically significantly increased compared to concurrent controls (21.8 days in control and 22.3 days in the treatment group); however, the gestation length in the treated group was within the historical control range for the laboratory (21.5-22.8 days). Exposure to D4 did not have any treatment-related effects on pup viability as measured by the number of pups born dead or the pup viability indices on postnatal days (PND) 1 and 4 (Refs. 27, 28).
The major findings noted in females exposed to D4 at 700 ppm in the two ***range-finding studies*** (Refs. 27, 28) and in the two studies (described below) in which treated females were mated to control males (Refs. 31, 33), and at 500 ppm or 700 ppm in the *"phased female" study* (Ref. 32) were statistically significant treatment-related decreases in: number of corpora lutea, number of uterine implantation sites, total number of pups born, and mean live litter size. These parameters are all inter-related in that the number of eggs ovulated (represented by the number of corpora lutea) should be equivalent to the number of implantation sites, the number of foetuses, and therefore the potential litter size. The mean live litter size in the 700-ppm exposure groups was consistently 60% to 70% of the control

values. Yet, while the mean live litter size was decreased in the higher exposure groups, the percentage live births of the total number of pups born was comparable to control values.
A clinical observation noted in some of these studies (Refs. 27, 28, 29, 30) was an apparent increase in ejaculatory plugs in male rats exposed to D4. However, when the number of ejaculatory plugs was expressed as number of plugs/rat/day, the values were all within the historical control range for this parameter, indicating that there was no effect of D4 on ejaculatory plug production in male Sprague Dawley rats.
No effects on the number of uterine implantation sites, the litter size, or the mean live litter size were found in the ***male crossover studies*** (Refs. 29, 30) in which males were exposed to D4 concentrations up to 700 ppm and were mated to control (unexposed) females. In these studies, exposure to D4 did not affect sperm production, motility, or morphology, nor did it result in either weight changes or histopathological changes of male reproductive or accessory sex organs. Therefore, it can be concluded that the effects on litter size are not male-mediated.

Phased female studies
Two studies were conducted (i.e., "phased-female" studies) in which female rats were exposed to D4 during selected phases of the reproductive cycle (Refs. 32, 33). In these studies females were mated with unexposed males. The design and results of these studies, recently also published in a peer reviewed article (Ref. AR 14) are summarised in table 6.

Table 6: One-generation "phased-female" inhalation studies (males unexposed)

Species/ Strain	Treatment	No./group	Dose levels of D4	Termination day	Results	Ref.*
Rat/Sprague Dawley	**Overall phase**: females exposed 6h/day for 28 days prior to mating and until GD 19	24 F	0, 70, 300, 500, 700 ppm	GD 20	Maternal toxicity at 300 ppm and above due to food and body weight decreases. Reduced number of corpora lutea at >300 ppm and foetal survival at ≥500 ppm.	32
	Ovarian phase: females exposed 6h/day for 31 days prior to mating until 3 days before to mating (total 28 days exposure)	60F (30F controls)	0, 700 ppm	GD 20	Food and body weight decreased. Number of corpora lutea and foetal survival unaffected	32
	Fertilisation phase: females exposed 6h/day for 3 days prior to mating, throughout mating period until GD 3	60F (30F controls)	0, 700 ppm	GD 20	Food and body weight decreased. Number of corpora lutea reduced and lower intrauterine survival	32
	Implantation phase: females exposed 6h/day from GD 2 through GD 5	24F	0, 700 ppm	GD 20	Lower food intakes and body weights during GD 2 – 6. Number of corpora lutea and foetal survival unaffected	32
Rat/Sprague Dawley	**Premating phase:** Gps 2-5: single 6h exposure on 1,2,3 or 4 days prior to mating. Gp 6 - 6h/day exposure from 3 days prior to mating until one day before mating. Gp 7 6h/day exposure from 3 days prior to mating through to GD3.	25F in Gp 1, 125F total in Gps 2-5, 125F / Gp 6, 70F in Gp 7	0, 700 ppm	GD 8	Maternal and reproductive toxicity expressed by effects on body weight gains, reduced food intakes and reduced corpora lutea and implantation sites	33

Species/ Strain	Treatment	No./group	Dose levels of D4	Termination day	Results	Ref.*
Rat/Sprague Dawley	**Post mating phase:** Single 6h exposure on GD0 (Gps 2-5), on GD1 (Gp 3), or GD2 (Gp4), or daily from GD0 through GD2 (Gp 5).	25F	0, 700 ppm	GD 8	Maternal toxicity expressed by reduced body weights gains and food consumption in GD0 through GD2 group.	33

GD = gestation day, LD = lactation day, PND = postnatal day
M = males, F = females Gps 1-6 = group number in phase study
* The studies summarized in this table are GLP compliant

In the first "phased-female" study, four groups of female rats were exposed to D4 by whole body inhalation for 6 hrs/day according to the following schedule:

- **Overall Phase**: Groups of 24 female Sprague Dawley rats were exposed to D4 at concentrations of 70, 300, 500, or 700 ppm beginning at least 28 days prior to mating and continuing through gestation day (GD) 19.
- **Ovarian Phase**: Sixty female rats were exposed to 700 ppm beginning 31 days prior to mating and stopping three days prior to mating.
- **Fertilisation Phase**: Sixty female rats were exposed to 700 ppm for three days prior to mating and continuing through to GD3.
- **Implantation Phase**: Sixty females were exposed to 700 ppm from GD2 through to GD5.

In the Overall phase study, the following observations were made: a reduction in the number of corpora lutea (300, 500 and 700 ppm), reduction in the number of uterine implantation sites and foetuses (500 and 700 ppm), an increase in pre-implantation loss (500 and 700 ppm), and increased post-implantation loss (700 ppm).
No significant effects were noted on the number of corpora lutea or indices of intrauterine survival in females exposed at 700 ppm in the Ovarian and Implantation phase studies. In the Ovarian phase study, no effects were seen on uterine implantation sites, viable foetuses, or on any other reproductive parameters measured.
In the Fertilisation phase study, the numbers of corpora lutea, uterine implantation sites, and viable foetuses were reduced at 700 ppm (the only dose tested) while the mean pre-implantation and post-implantation losses were increased. The effects on corpora lutea and intrauterine survival were similar for both the fertilization phase in which exposure began 3 days pre-mating and continued through gestation day 3 and the overall phase in which exposure began 28 days pre-mating and continued through gestation day 19.

A second study was performed to investigate the relative temporal responsiveness of female rats to D4 (Ref. 33). Female Sprague Dawley rats were treated by whole body exposure to 700 ppm D4 vapour for 6 hrs / day with the following regimens:

– **Pre-mating phase**:
- a single 6-hour exposure, on either the first (D-1), second (D-2), third (D-3) or fourth (D-4) day prior to mating
- daily 6-hour exposures from three days prior to mating until one day prior to mating (3 exposures)
- daily 6-hour exposures three days prior to mating through a two-day mating phase and until gestation day 3 (8 exposures)

– **Post-mating phase**
- a single 6-hour exposure on either gestation days 0, 1 or 2
- daily 6-hour exposures from gestation day 0 until gestation day 2 (3 exposures)

Maternal and reproductive toxicity were expressed in the pre-mating phase by a reduced pregnancy rate in the group exposed one day before mating (Day –1 group), but not in

groups exposed on Days –2, -3 or –4, and by effects on mean body weight gains in the group exposed from 3 days before mating until one day prior to mating, and by effects on mean body weight gains, reduced food consumption, reduced numbers of corpora lutea and implantation sites, increased numbers of small implantation sites, and reduced mean uterine weights in the group exposed from 3 days before mating until gestation day 3. Apart from the reduced pregnancy rate in the group exposed one day before mating (Day –1 group), no impairment in pregnancy rates was observed in any other group. The temporal nature of this effect in the Day –1 group was demonstrated by the lack of effect on pregnancy rates of animals exposed two, three or four days before mating.
Maternal toxicity was expressed in the post-mating phase by reduced mean body weight gain and food consumption in the gestation day 0 through GD 2 group. There was no evidence of reproductive toxicity in parameters monitored through the uterine examination on gestation day 8.

Conclusion
The weight-of-the-evidence from the above studies indicates that octamethylcyclotetrasiloxane's effect on fertility occurs some time around the time of ovulation, i.e. within the 24 hours before mating.

Ref.: 27 to 33; AR 14

3.3.8.3. Two-generation study (reproduction toxicity and developmental neurotoxicity)

Guideline:	2-generation study protocol (EPA OPPTS test guidelines)
Species/strain:	Rats, Sprague Dawley (Crl:CD IGS BR rats)
Group size:	groups of 30 male and 30 female (F0 and F1)
Treatment:	Inhalation exposure for 6 hours daily for at least 70 consecutive days prior to mating, and during mating, and gestation until day 20 and during lactation day 5 to termination.
Dose level:	0, 70, 300, 500 or 700 ppm
Test substance:	D4
Batch no:	Lot # LL0847; purity at least 99.7 %
GLP statement:	yes

In a 2-generation study with D4 following the US EPA OPPTS (Office of Prevention, Pesticides and Toxic Substances) protocol, groups of 30 male and 30 female Sprague Dawley rats (F0) were exposed to 70, 300, 500, or 700 ppm D4 for 6 hours daily for at least 70 consecutive days prior to mating, and during mating, and gestation until day 20 and during lactation day 5 to termination. Exposure of F1 females at the same concentrations was from weaning (i.e. day 22) through the second F1 mating and to gestation day 20 (i.e., about 274 days of age). A control group of males and females were exposed to filtered air on a comparable regimen.
Animals were checked twice daily for clinical signs. Their food consumption and body weights were recorded at intervals. All females were allowed to rear their pups to weaning (lactation day 21). F0 males and females were necropsied after weaning F1 pups. Following weaning the F1 pups were selected to produce F2a and F2b litters. F2a pups were selected for neurobehavioral and neuropathological examination. All decendents were necropsied. Selected tissues were examined microscopically from F0 and F1 adults in control and 700 ppm groups, and from all F0 and F1 parental animals dying during the study.

The results of this study are summarized as follows:

Toxicity parameters:
- Deaths: Two F0 females at 700 ppm died of dystocia and two had liver necrosis and kidney failure, which may have been contributory factors to their deaths. One F1 female at 500 ppm died of dystocia and one female at 700 ppm died with liver necrosis as a possible contributory factor. The relationship of treatment to these events is uncertain.

- Reductions in mean body weight gain for F0 at 500 and 700 ppm and for F1 at 700 ppm.
- Organ weight changes: in F0 animals: statistically significantly increased kidney weights in males at 300, 500 and 700 ppm, statistically significantly increased liver weights in females at 300, 500 and 700 ppm and in males at 700 ppm. In F1 animals: statistically significantly increased kidney weights in males at 500 and 700 ppm and females at 700 ppm, statistically significantly increased liver weights in males and females at 300, 500 and 700 ppm and statistically significantly increased pituitary gland weights in 700 ppm females.
- Renal tubular mineralization: increased incidence at 500 and 700 ppm (F0 and F1), statistically significant at 700 ppm only.
- Hepatocyte hypertrophy: increased incidence at 500 and 700 ppm (F0), at 70, 300, 500 and 700 ppm (significant only at 500 and 700 ppm)(F1) with hepatic pigment at 300, 500 and 700 ppm (F1) and bile duct hyperplasia at 500 and 700 ppm (F1)
- Thyroid follicular cell hypertrophy at 700 ppm (F1)
- Lung interstitial inflammation and alveolar histiocytosis: The F0 incidence: (control, 70, 300, 500 and 700 ppm, respectively) was in males:1/20 (control), 0/30, 4/30, 1/29, 5/28 and in females: 0/30 (control), 7/30, 5/30, 7/30, 8/26. The F1 incidence of alveolar histiocytosis was in males: 10/30 (control) vs 22/29 (700 ppm); in females: 3/30 (control) vs 8/30, 9/30, 7/29, 13/29 and interstitial inflammation was increased at 700 ppm only, males: 3/30 in controls vs 10/29, females: 4/30 in controls vs 9/29.

Reproduction parameters:

- Reduced mating and fertility indices occurred in F1 animals at 700 ppm. In the second F1 mating, the indices were reduced in all treated groups, but, the difference from controls only attained statistical significance at exposure concentrations ≥ 500 ppm.
- Reductions in mean live litter size and mean number of pups born were recorded at 500 and 700 ppm (F0, F1). Similar changes (not statistically significant) were noted sporadically at 70 and 300 ppm in both F0 and F1 animals without a clear dose-response relationship
- Extended parturition and/or dystocia: in F0 females: two (of 30) at 500 ppm and three at 700 ppm; in F1 females: one each at 300, 500 and 700 ppm. The relationship of treatment to these events is uncertain.
- Increased oestrous cycle length noted in F1 females at 700 ppm
- Histopathological change: There were no reported changes in ovary, uterus, vagina, mammary gland and pituitary gland in F0 animals. In F1 animals, oestrus cycle irregularities, reductions in corpora lutea and reduced numbers of pregnancies were reported. However, there was no clear dose-response and the differences from controls were only obvious at 700 ppm. The subtle change reported in the ovaries (anovulatory), and mammary glands (ductal/acinar proliferation and evidence of secretion) were considered to be part of the oestrous cycle perturbation. Effects seen in the F_1 generation were possibly a combination of D4's effect on the LH surge as well as a slight acceleration of the spontaneous process of reproductive senescence in the F1 females.

The differences in *general toxicity responses* between the F0 and F1 generations after inhalation of D4 were minimal. Overall the responses were slightly more severe in F1 than in F0 animals except for respiratory tract reactions. In reproduction toxicity, it is interesting to note a general lack of response to D4 treatment in the F0 generation compared to the F1 generation. This may be associated with the small difference in age at start of treatment (F0 – 44 days old, F1 – 22 days old).

Ref.: 34; AR 15

Conclusions on Reproductive Toxicity

There is no evidence that D4 causes developmental toxicity in rats or rabbits or an adverse effect on male rat fertility. However, the following effects on female rat fertility were identified:

- An effect on fertility which occurs at ovulation apparently with reduced numbers of eggs ovulated as demonstrated by the 'phased' studies in female rats.
- Decreases in number of corpora lutea, number of uterine implantation sites, total number of pups born, and mean live litter size were noted in the one-generation general reproduction and fertility studies at high exposures. Two multidose studies (0, 70, 300, 500 or 700 ppm) allow estimates of NOAELs. In one study (Ref. 31**),** reductions in reproductive parameters were recorded only at 700 ppm, while in the other study (Ref. 32), reduced implantation sites and viable foetuses and increased pre-implantation losses were noted at 500 and 700 ppm. In addition, reduced numbers of corpora lutea were found at ≥ 300 ppm. However, as the reduction in corpora lutea was marginal at 300 ppm (14.6/dam vs. 16.2/dam in controls) without a clear exposure-related response and within the range of values in the historical control database, (14.2/dam-20.5/dam), the NOAEL is considered to be 300 ppm.
- Similar reproductive changes were recorded in the two-generation study at 500 and 700 ppm, but, in addition increased oestrous cycle length in F1 females at 700 ppm as well as increased pituitary gland weights were noted. Also in F1 females there were histopathological changes in ovaries and mammary glands at *all* exposure levels. These histopathological changes were:

  1) minor, and not clearly treatment-related except at 700 ppm,
  2) reported only in the F1 and not in the F0 generation,
  3) similar in nature to those found in concurrent controls and,
  4) considered to be probably a combination of D4's effect on the LH surge, as well as a manifestation of the spontaneous, age-related waning of the female reproductive system in the rat (i.e. F1 female Sprague Dawley rats were about 274 days of age at sacrifice),

  Considering these points, it appears justified to set 300 ppm as the NOAEL.

General comment on reproductive toxicity
From the reproductive toxicology studies and taking the weight of evidence approach for reproduction parameters, the NOAEL is considered to be 300 ppm.
There is evidence (see Special studies, section 3.3.12.) suggesting that the effect of D4 on reproduction in females is due to a delayed ovulation caused by a treatment-related delay in or blockage of the luteinising hormone surge on the day of pro-oestrus. The reproduction findings in the two-generation study are consistent with a long-term suppression of LH release.

3.3.9. Toxicokinetics

Octamethylcyclotetrasiloxane (D4), randomly labelled with carbon-14, was used in a number of studies to examine its absorption, distribution, metabolic fate and elimination following oral, inhalation and intravenous administration to rodents and/or humans.

Oral Study

Guideline: /
Species/strain: female Fisher 344 rats
Group size: 49 (preliminary groups I (15), II (26) and III (8))

	183 (definitive groups I (122) and II (61))
Test substance:	D4 ^{14}C-D4, specific activity of 2.0 mCi/mmol
Batch:	D4, lot n° LL084732 ^{14}C-D4, lot n° 921217
Purity:	D4, approximately 99% (GC/MS) ^{14}C-D4, 99.2 (radiochemical purity by HPLC)
Test formulation:	^{14}C-D4 diluted with unlabeled D4 dosed orally in corn oil, Emulphor, Simethicone fluid, or neat
Dose level:	Dosing solutions prepared to achieve a radioactivity concentration of approximately 25µCi and a nominal dose of 300 mg/kg D4. Rats received a single oral dose.
GLP statement:	in compliance

Absorption was studied in female Fischer rats following a single oral dose of 300 mg/kg ^{14}C-D4 in corn oil, Simethicone fluid or undiluted. Absorption of radioactivity, expressed as percentage of total recovered radioactivity from urine, carcass, expired volatiles and expired CO_2 was 51.95±4.97%, 12±1.21% and 28.14±5.78% with ^{14}C-D4 in corn oil, Simethicone or neat, respectively. The area under the curve (AUC) generated from blood data also indicated D4 was most readily absorbed when delivered in corn oil (AUC in µg ^{14}C-equivalents D4•hr/g of blood was 933±26 and AUC in µg D4• hr/g of blood was 159±17) and least available in Simethicone fluid (AUC in µg ^{14}C-equivalents D4•hr/g of blood was 77±13 and AUC in µg D4• hr/g of blood was 19±5). Blood radioactivity concentrations were highest 24 h after dosing.

Conclusion
An oral dose of D4 is rapidly absorbed when administered in corn oil, with radiolabelled D4 in tissues generally following plasma levels. Examination of the blood radioactivity and parent D4 concentration and the mass balance of radioactivity indicated that D4 was most readily absorbed when delivered in corn oil and least available for absorption when administered in Simethicone fluid. Qualitative assessment by Whole-Body autoradiography showed comparatively similar patterns of absorption and disposition of radioactivity, but differences in transit time of radioactivity through the gastrointestinal tract following administration of ^{14}C-D4 in the various carriers or neat. This study indicated that the oral absorption of D4 can be significantly influenced by the carrier used to deliver D4.

Ref.: 35

Whole Body Inhalation study

Guideline:	/
Species/strain:	Rat, Fisher 344 CDF(F-344)/CrlBR
Group sizes	
Absorption study:	81 males and 13 females. Additional 8 males and 8 females were used to measure respiratory minute volume (RMV)
Elimination study:	56 males and 8 females
Test substance:	D4 ^{14}C-D4, specific activity of 30.1 mCi/mmol
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot number 940512, radiochemical purity 99.58% (HPLC),
Test formulation:	^{14}C-D4 diluted with unlabeled D4 vapour
Dose level:	700 ppm for 6 hours
GLP statement:	Yes

The rats were divided into subgroups to determine 1) retention and total body burden of radioactivity 2) tissue distribution of radioactivity up to 7 days post exposure, and 3) elimination of radioactivity – up to 7 days post exposure.

Results
Based on the mean total body burden and the achieved dose, the percent ^{14}C-D4 retained by the animals during the 6 hour exposure was 5.63% of the delivered radioactivity. Radioactivity was readily taken up in tissues. Maximum concentrations in most tissues were observed at the end of exposure. In blood and plasma, Cmax was achieved at 1 and 3 h, respectively. In fat, the tissue exhibiting the highest concentration of radioactivity, Cmax was achieved at 12 h post exposure. Except for fat, the elimination of radioactivity from the tissues was at approximately the same rate as from plasma. The apparent terminal elimination $t_{1/2}$ was 13 h in blood, 59 h in plasma and ranged from 34 h to 158 h in tissues. Most of the radioactivity was recovered as expired volatiles (30.68±2.26%) and by renal excretion (urine, 47.01±2.49%). The faecal recovery was 12.33±0.95%. Approximately 10.56±0.53% of the body burden (mainly muscle, fat and bones) was recovered in the carcasses at 168 h. The overall recovery as expired $^{14}CO_2$ was low (1.83±0.21% body burden) indicating that its formation was not a major route of elimination.

No ^{14}C-D4 was detected in the urine samples pooled over the collection intervals up to 48 h indicating that the retained ^{14}C-D4 was rapidly metabolized. Radioactivity in urine and faeces was mainly due to polar metabolites.

Ref.: 36

Nose only Inhalation Studies

Guideline:	/
Species/strain:	Rat, Fisher 344 CDF(F-344)/CrlBR
Group size	
Absorption study:	191 male and 189 female. Groups of 50 males and 50 females
Elimination study:	50 males and 50 females
Test substance:	D4 ^{14}C-D4, specific activity of 30.1, 33.2 and 39.7 mCi/mmole
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot number 940512, 940519 and 940809 radiochemical purity 99.58%- 99.8%
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Dose level:	7, 70 or 700 ppm for 3 hours (4 animals/sex/group) or 6 hours (46 animals/sex/group)
GLP statement:	Yes

Results
Actual mean achieved chamber D4 concentrations were 7.52, 70.4 and 716 ppm (exposure 29.0 - 40.4 µCi; body burden corresponding to 1.595 – 2.203 µCi). The overall recoveries of the radioactivity (% of body burden) in the excreta were: urine (39.9 – 42.3% male and 32.3 – 43.1% female; faeces (12.3 – 14.6% male and 9.53 – 14.0% female); expired volatiles (25.7 – 27.3% male and 23.2 – 34.8% female); expired CO_2 (0.59 – 5.24% male and 0.38 – 5.42% female); cage wash (1.34 – 2.84%).
Plasma values of ^{14}C-D4 at the three concentrations (7, 70 and 700 ppm) showed an increase that was approximately proportional to increasing dose (see Table below)

Table 7: Concentration of radioactivity (µg eq/ml) in blood and plasma after a 6-hour nose only exposure

	7 ppm	70 ppm	700 ppm
Males			
Blood	0.270±0.013	2.21±0.12	14.16±1.00
Plasma	0.349±0.018	2.875±0.044	16.15±1.53
Females			
Blood	0.248±0.019	1.54±0.16	11.13±1.17

	7 ppm	70 ppm	700 ppm
Plasma	0.310±0.031	1.728±0.288	11.14±1.73

Of the delivered radioactivity 4.99 - 5.47 % was retained in males and 5.19 - 5.52 % in females with no apparent gender or dose effect 6 hrs post nose-only exposure. Radioactivity was readily taken up by the tissues, especially by fat, and was eliminated at rates similar to or somewhat slower than from plasma. Maximum concentrations were observed at end of exposure to 3 h post exposure (except fat). Radioactivity in fat was sustained up to 48 h post exposure. Except for fat and adrenals (and to a lesser extent, trachea and pancreas) there was no apparent gender effect in the blood, plasma or tissue radioactivity AUC values. Except for the testes the combined (male and female) mean radioactivity $t_{1/2}$ ranged from 68 h in plasma to 154 h in skin. There were no apparent gender or dose effects in the terminal elimination half-lives. The tissues having the longest half-lives were testes (combined mean $t_{1/2}$, 273 h), skin, lung, nasal mucosa, fat, eye, uterus and vagina. Increases in the tissue radioactivity AUC values were generally proportional or less than proportional to the increase in exposure level except for fat, uterus and vagina which showed higher than proportional increases. Small amounts of radioactivity were recovered in all tissues analyzed at 168 h: the total (sum of the mean values) ranged between 0.442 and 0.793% of the body burden. The excretion of radioactivity was mainly via the pulmonary (expired volatiles: 23.2 – 34.8%; expired CO_2: 0.38 – 5.42%) and renal routes (urine: 32.3 – 43.1%), and to a lesser extent via faeces (9.53 – 14.6%). Elimination of radioactivity was most rapid during the first 0 - 12 h interval and more prolonged up to 7 days (168 h).

Ref.: 37

Guideline: /
Species/strain: Rat, Fisher 344 CDF(F-344)/CrlBR
Group size: 50 males and 50 females
Subsets: Absorption: 5 males / 5 females
Elimination: 4 males / 4 females
Test substance: D4
^{14}C-D4, specific activity of 36.4 mCi/mmole
Batch: D4, lot number LL024S10, purity 99.8% by GC-MS
^{14}C-D4, lot numbers 950309 and 951031 radiochemical purity 99% and 98.2%,
Test formulation: ^{14}C-D4 mixed with unlabeled D4
Dose level: 7 and 700 ppm unlabelled D4, Day 1-14, 6 hour nose-only followed on Day 15, single 6 hours exposure to ^{14}C-D4
GLP statement: in compliance

Following exposure to ^{14}C-D4, each group was divided into subsets. 5 animals per sex were killed immediately following exposure and a further subset kept in metabolic cages to collect urine, exhaled volatiles, faeces and CO_2 at intervals up to 168 h post exposure. The remainder were in subsets of 4 animals per sex, killed at 0, 1, 3, 12, 24, 48, 72, 96, 120, 168 h to check ^{14}C-D4 in blood and tissues.

Results
The mean body burden of radioactivity in the animals at the end of the exposure was 1.486 – 2.170 µCi, corresponding to retained radioactivity ranging from 4.38 - 6.14 % with no apparent gender or dose effects. In blood, plasma and all tissues, maximum concentrations of radioactivity were observed between 0 h (end of exposure) to 3 h post exposure. Radioactivity was readily taken up by the tissues, especially by fat (sustained up to 24-48 h post exposure), and was eliminated at rates similar to or somewhat lower than from plasma ($t_{1/2}$ 56 ± 10 h). High levels of radioactivity were found in the respiratory tract functionally involved in the intake and elimination of the administered ^{14}C-D4. AUC and Cmax values were comparatively low in the reproductive tissues, somewhat higher in the liver, thymus,

lungs, nasal mucosa and highest in the fat. The increase in radioactivity (AUC and Cmax) in whole blood, plasma, and all analyzed tissues was proportional or less than proportional to the exposure level. With the exception of fat, the elimination profile in blood, plasma and tissues appeared to be multiphasic and was characterized by an initial, relatively rapid decline up to 24 h post exposure followed by a long apparent terminal elimination phase (mean radioactivity $t_{1/2}$ ranged from 56 h to 253 h); (mean radioactivity $t_{1/2}$ ranged from 56 h to 155 h). The blood-to-plasma ratios were approximately 1 or somewhat lower over the time course of the study indicating that the radioactivity was readily taken up by the red blood cells and eliminated at approximately the same rate as from plasma. In each dose group, the tissue-to-plasma ratios remained approximately the same or increased up to 168 h indicating that the rate of radioactivity elimination from tissues was approximately the same or slower than that from plasma.
The tissues containing the highest amount of radioactivity were the liver and fat. There was no apparent effect in the blood, plasma and tissue levels. Recovery of radioactivity in both sexes in excreta was for both dosages (7 and 700 ppm): urine, 37.4 - 40.0 %; faeces, 12.6 - 19.1 %; expired volatiles, 25.9 - 35.4 %; expired $^{14}CO_2$, 2.06 - 4.54 %; cage wash, 1.31 - 1.86 %. Significantly higher proportions of radioactivity were eliminated via the lung both as volatiles and CO_2 while a significantly lower proportion was eliminated via the gastro-intestinal tract in the faeces at the high dose level when compared with the low dose level. However, there was no significant dose level effect in the urinary recoveries. Based on normalized values, the portion of radioactivity remaining in the carcasses at 168 h post exposure ranged from 6.53 - 8.50 %. Small amounts of radioactivity were recorded in all analyzed tissues at 168 h; the total (sum of the mean values) ranged between 0.193 and 0.468% of the body burden.

Ref.: 38; AR 16

Comment
In three inhalation studies, male F344 rats were exposed to ^{14}C-D4 at 700 ppm for 6 hours [Ref. 36], male and female F344 rats were exposed to ^{14}C-D4 at 7, 70 or 700 ppm for 3 or 6 hours [Ref. 37], and male and female F344 rats were exposed to unlabelled D4 at 7 or 700 ppm for 14 consecutive days followed by a single 6-hour exposure of ^{14}C-D4 at 7 or 700 ppm [Ref. 38; AR 16]. After 6 hours exposure, the percent of ^{14}C-D4 retained by males ranged from 5.23% to 5.96% and in females from 5.75% to 6.14% of the delivered radioactivity, at 7 or 700 ppm, respectively. Similar retention levels were achieved in males and females exposed to 7 or 700 ppm for 14 consecutive days. Plasma values of ^{14}C-D4 at the three concentrations (7, 70 and 700 ppm) showed an increase that was approximately proportional to increasing dose (see Table 7, above). Radioactivity was taken up by the tissues, especially fat, and was eliminated at rates similar to or somewhat slower than from plasma. In blood, plasma and all tissues (except fat), maximum concentrations of radioactivity occurred at 0 h (end of exposure) to 3 h post exposure.

Fat appeared to be a depot for radioactivity as maximum concentrations were sustained up to 48 h post exposure. The combined (male and female) mean radioactivity $t_{1/2}$ ranged from 68 h in plasma to 154 h in skin. Tissues having the longest half-life were testes, skin, lung, nasal mucosa, fat, eye, uterus and vagina.

The data show that approximately 5-6% of an inhaled D4 dose is absorbed. Higher D4 levels were found in lung tissue and fat compared to other tissues although this could be expected, as D4 is lipid soluble and would preferentially deposit in fat and highly lipophilic tissues.

Intravenous Study

Guideline:	/
Species/strain:	Rat, Sprague-Dawley (CD)
Group size:	10 males and 10 females
Group size:	3 Groups of 10 rats
Test substance:	D4
Batch:	^{14}C-D4, lot number 921210, radiochemical purity > 97 %, specific activity of 1.48 mCi/mmole Unlabelled D4, lot number AJ844, purity not provided
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Dose level:	intravenous 1.1 ml.: single 7 mg/kg, single 70 mg/kg repeat (x14) 7 mg/kg
Vehicle:	Ethanol, Emulphor EL 620 and saline (0.9%) 1:1:7 by volume
GLP statement:	in compliance

This was a toxicokinetics study in rats following intravenous administration of D4. 6 groups of 10 animals / sex were assigned to determine different endpoints, (3 doses - for plasma radioactivity kinetics/tissue distribution: 1 group - single low dose for excretion balance/tissue distribution; 2 groups - low and repeat dose for whole body autoradiography) were performed by liquid scintillation counting.
The whole-body autoradiography demonstrated that radioactivity was well distributed throughout the animal shortly after administration. Male animals appeared to metabolize D4 more extensively than females. A greater proportion of the mean administered radioactivity (AR) was excreted in male urine (48.1 %) and faeces (10.4 %) than in female urine (28.5 %) and faeces (7.9 %). The expired air of female animals contained more radioactivity than that of male animals, 35.2 and 22.4 % AR respectively, but the expired air from male animals contained more $^{14}CO_2$ (6.5 % for males, 3.2 % for females). Retention of radioactivity in the total tissues of animals 120 hours after dosing was 19.0 % in female tissues and 11.3 % in male tissues. The tissue with the highest concentration of radioactivity at 120 hours was fat with a higher concentration in female fat. There were no marked differences in plasma radioactivity pharmacokinetics observed between males and females following either 7 mg/kg or 70 mg/kg. A ten-fold increase in dose from 7 mg/kg bw (66.1 µg.h/ml male and 48.3 µg.h/ml female) to 70 mg/kg bw (546 µg.h/ml male and 485.4 µg.h/ml female) resulted in a proportional increase in the AUC in males and females. A comparison of the results after single and repeated dosing suggested no accumulation of plasma radioactivity in males and females. The concentration of radioactivity in tissues suggested an approximate proportional increase with dose. The concentration of radioactivity in fat was higher in females than in males but was similar in the liver and kidneys. Fat radioactivity concentration appeared to decline at a similar rate compared to the other tissues sampled (liver, kidneys) after initial peaks but the rate appeared to be slow. The concentration of radioactivity in the liver and kidneys was substantially lower than that measured in fat 30 - 48 hours after dosing. In male animals this difference ranged from 5 - 15 times lower and in females 12 - 25 times lower at 7 mg/kg. The concentration of radioactivity measured in the tissues after 14 consecutive doses of 7 mg/kg suggested that radioactivity accumulated in the tissues: the concentrations measured were 4 - 5 times higher in all tissues than after the single dose at 7 mg/kg. The whole-body autoradiography demonstrated that radioactivity was well distributed throughout the animal within short time after administration.

Ref.: 39

Conclusion
A single IV dose of ^{14}C-D4 as a microemulsion at 7 mg/kg appeared to be more extensively metabolized by male rats than by female rats. Peak concentrations of radioactivity in liver, kidneys and lungs were seen 0.5 hours after dosing in both sexes. These concentrations declined slowly up to the final sampling point 120 hours after dose administration. The

tissue with the highest concentration of radioactivity at 120 hours was fat, with a higher concentration in females.

Additional kinetic studies in rats, with different routes of application (inhalation, i.v. *per os*), focussed on **Elimination and Metabolism**

Guideline:	/
Species/strain:	Rat, Fisher rats
Group size:	3 animals (body burden), 5 sets 3 animals (tissue distribution); 3 animals (excretion)
Test substance:	D4 ^{14}C-D4, specific activity of 39.7 mCi/mmole
Batch:	D4, lot number LL084732, >99,7% by GC-MS ^{14}C-D4, lot number 941128, radiochemical purity 97.3% (HPLC with radiochemical detector)
Dose level:	700 ppm for 6 hours
GLP statement:	in compliance

This was a pilot study to determine the ^{14}C-D4 vapour pharmacokinetics following a single 6h nose only inhalation exposure to D4.

Results
Maximum concentration in blood, plasma and tissues was at the end of exposure period. The total body burden of radioactivity, which was retained by the animals during the 6 hour exposure was 6.53 %. Elimination of radioactivity from tissues was approximately at the same rate as from plasma (except for perirenal fat and lung). The overall mass balance of radioactivity in the excreta was the following: urine, 35.75 ± 1.09%; faeces 29.68 ± 2.84%; expired volatiles, 33.72 ± 14.72; expired CO_2, 1.72 ± 0.10%; and cage washes, 0.24 ± 1.97%. After the inhalation exposure to ^{14}C-D4, radioactivity was rapidly excreted by the animals; in the first hour, 12.43 ± 3.36% of the body burden was exhaled. Approximately 85% of the expired volatiles were recovered during the 0 – 24 hour interval after removal from the inhalation chamber. In the urine most of the radioactivity (86%) was recovered during the 0 – 48 hour interval after removal from the inhalation chamber.

Ref.: 42

Guideline:	/
Species/strain:	Rat, Fisher 344 and Sprague-Dawley IGS, female
Group size:	groups of 4 or 5 rats
Test substance:	D4 ^{14}C-D4, specific activity of 17.6 mCi/mmole
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot number 9074-1, radiochemical purity > 98 %
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Dose level:	700 ppm ^{14}C-D4 mixed with unlabeled D4 as a single 6 h nose only exposure
GLP statement:	Yes

The animals were divided into subsets:

- body burden immediately post exposure;
- cannulated animals for blood levels at 1, 2, 6, 12, 24, 48, 72, 96, 120 h and tissue distribution from the same animals at 2, 12, 72 and 120 h
- excretion group: in metabolic cages for 168h

Results
Fischer 344 rats retained a significantly higher amount ($p<0.05$) of radioactivity (8.3 ± 0.22%) than Sprague-Dawley rats (5.9 ± 0.13%) at the end of the 6-hour exposure.

Excretion of retained radioactivity was similar in both strains, with similar amounts being excreted in urine (25.5–32.2%), faeces (19.4–19.2%), expired volatiles (23.5-25.4%) and expired $^{14}CO_2$ (3.94-3.57) for female Fisher and Sprague Dawley IGS rats, respectively. The concentration of radioactivity over time in blood and lung was also similar over the 168 hour post exposure period while differences were seen in fat, liver, faeces and urine (AUC in µg equivalent D4/g*hr was 21685 and 14036 for fat, 1778 and 1510 for liver, 1137 and 600 for faeces and 5175 and 6679 for urine) from Fischer 344 and Sprague-Dawley rats, respectively. Analysis of fat for parent D4 revealed the concentration of D4 in these samples was essentially the same as the concentration of radioactivity found in both strains. Analysis of blood, liver, lung, faeces and expired volatiles samples for parent D4 demonstrated differences in the percent of radioactivity that could be attributed to metabolites in blood (61 vs. 81%), liver (18 vs. 49%), lung (82 vs. 90%), faeces (98 vs. 98%) and expired volatiles (48 vs. 33%) for Sprague-Dawley vs. Fischer 344 rats, respectively. Fischer 344 rats generally showed a lower percentage of the total radioactivity present as parent D4, suggesting that the Fischer 344 rats may more readily metabolize D4 as compared to Sprague-Dawley rats. Faeces demonstrated the least amount of parent D4 present at 2% and 2.3% for the female Fisher 344 and Sprague Dawley rats, respectively, suggesting that D4 is largely metabolized prior to faecal excretion.
No parent D4 was found in the urine samples from either strain suggesting all radioactivity present in the urine was as metabolites. The radioactivity present in the urine consisted entirely of polar metabolites of D4. Two major metabolites comprising 70-100% of the urinary radioactivity for both strains were identified as dimethylsilanediol [$Me_2Si(OH)_2$] and methylsilanetriol [$MeSi(OH)_3$]. No significant differences in urinary metabolism are found between the Fisher 344 and Sprague Dawley rats. Following sacrifice at the 168 hour post exposure time point the total percent of body burden dose remaining in the tissues (combined) was 0.4% for female Fisher 344 and Sprague Dawley IGS rats. Radioactivity remaining in the carcasses, mainly in muscle, bone and fat was 9.17% and 15.95% of the body burden dose for female Fischer 344 and Sprague Dawley IGD rats, respectively. These kinetic differences between female Sprague-Dawley and Fischer 344 rats suggest that there may be important biochemical differences leading to a decreased metabolism of D4 in the female Sprague-Dawley rat.

Ref.: 43

Guideline:	/
Species/strain:	Rat, Fisher 344, female
Group size:	Groups of 3 – 5
Test substance:	D4 ^{14}C-D4, specific activity was 2.0 mCi/mmole
Batch:	D4, lot LL084732, purity 99.8% (GC-MS) ^{14}C-D4, lot 921217, radiochemical purity 99.03%.
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Pre-treatment:	Days 1-4, phenobarbital (80 mg/kg i.p.), 3-methylcholanthrene (30 mg/lg i.p.) or vehicle Control groups either 0.9% saline, corn oil or no pretreatment.
Dose level:	Day 5, a single i.v. dose of ^{14}C-D4 (70 mg/kg). Additional group oral dose of ^{14}C-D4 (70 mg/kg) no pretreatment
GLP statement:	/

This was a pilot study to see if classical inducing agents such as Phenobarbital (PB) or 3-methyl-cholanthrene (3-MC) altered the metabolism of D4.

Results
PB-pretreated rats excreted 55 % of the administered dose in the urine, while control and 3-MC-pretreated rats excreted 24 - 27 % over the same 72 hour period. Rats pretreated with PB excreted 14 % of the dose as CO_2, while 3-MC-pretreated and control rats excreted less than 3 % as CO_2. However, only 9 % of the dose was excreted as expired volatiles in

PB-treated rats, while 3-MC-pretreated rats excreted 29 % and control rats excreted 38 %. The majority of the expired radiolabelled material was collected in the volatile trap, which suggests it was likely parent compound due to its higher volatility compared to its metabolites. At 72 hours following administration of D4, 29 % of the dose remained in the carcass of control rats and 35 % in 3-MC-pretreated rats compared with 7 % of PB-pretreated rats.
Following a single oral dose of ^{14}C-D4, 22 % of the dose was excreted as expired volatile, while rats administered a single i.v. dose excreted 38 % over 72 hours. Urinary excretion was similar between the different routes of D4 administration. At 72 hours i.v.-treated rats excreted 24 % in the urine, while orally treated rats excreted 31 %. Elimination of $^{14}CO_2$ appeared to be independent of the route of administration (3 % over 72 hours). Elimination in the faeces was a minor route of excretion after i.v. administration (< 8 %), but accounted for 29 % after oral dosing. This suggests that the majority (about 20%) of the radioactivity excreted in the faeces following oral administration is most likely non-absorbed dose. At 72 hours, 18 % (oral) and 29 % (i.v.) of the radioactivity remained in the carcass. The parent compound was not excreted in the urines of the control or of either group of pretreated animals over the 72 hr collection period. There were at least six metabolites in urine collected from control and pretreated rats. The profile did not change over the 72 hr collection period. The urinary profile in animals administered ^{14}C-D4 via oral gavage was quantitatively very similar to that seen in control rats administered ^{14}C-D4 intravenously.

Conclusion
This study indicates that there were differences in the major route of excretion following different routes of administration, that phenobarbital but not 3-MC pre-treatment increased the amount and rate of urinary excretion of radioactivity following a single i.v. dose of ^{14}C-D4; however PB pretreatment did not change the metabolic profile of D4. This provides compelling evidence for the involvement of PB inducible enzymes in the metabolism of D4 in rats.

Ref.: 44

Guideline:	/
Species/strain:	Rat, Fisher 344, male and female
Group size:	see study #8464 (Ref. 44) and Study #8496
Test substance:	D4 ^{14}C-D4, specific activity was 2.0 mCi/mmole
Batch:	D4, lot LL084732, purity 99.8% (GC-MS) ^{14}C-D4, lot 921217, radiochemical purity 99.03%.
Test formulation:	unlabeled D4 mixed with 14C-D4
Dose level:	single i.v. dose of 70 mg/kg
GLP statement:	/

This was a pilot study to determine the urinary metabolites of D4

Results
Analysis was performed using an HPLC system equipped with a radioisotope detector. The metabolites identified have clearly established that some demethylation occurs at the silicon-methyl bond. The 2 major metabolites, constituting 75 - 85 % of the total components, were identified as dimethylsilanediol [$Me_2Si(OH)_2$] and methylsilanetriol [$MeSi(OH)_3$]. Formation of $MeSi(OH)_3$ clearly established demethylation at the silicone-methyl bonds of D4. No parent D4 was present in urine. The minor metabolites identified were [$MeSi(OH)_2$-O-$Si(OH)_3$], [$MeSi(OH)_2$-O-$Si(OH)_2Me$], [$MeSi(OH)_2$-O-$Si(OH)Me_2$], [$Me_2Si(OH)$-O-$Si(OH)Me_2$], [$Me_2Si(OH)$-O$SiMe_2$-O$Si(OH)Me_2$].

Ref.: 45; AR 17

In Vitro Data on Metabolism

Guideline:	/
Test system:	Human liver microsomes (from a pool of 15 donors); Rat liver microsomes (from untreated and PB induced Sprague Dawley rats)
Test substance:	^{14}C-D4, specific activity 20.6 Ci/mol
Batch:	^{14}C-D4, lot 990316, radiochemical purity 99.67%.
Dose levels:	3 M and 5 M D4, 49.5 nCi / incubation Incubation conditions: 0-60 min (and several amounts of microsomal protein)
Controls:	No substrate and no protein samples
GLP statement:	/

Incubations with ^{14}C-octamethyltetracyclosiloxane were carried out to assess species differences and investigate the role of human liver microsomal enzymes in its *in vitro* metabolism.

Results
^{14}C-D4 was converted by liver microsomes from the phenobarbital-treated rats to at least eight metabolites, designated M1 through M8, based on their HPLC retention times, but not further characterised. M8 was the major metabolite formed in incubations with human liver microsomes and also in liver microsomes from saline-treated rats, suggesting a similarity in the metabolism of D4 for rats and humans. The conversion of D4 to M8 did not exceed 10%, yet, the formation of M8 was not proportional to protein concentration or incubation time. Results of an experiment to assess ^{14}C-D4 binding to human liver microsomes indicated that this was not due to the binding of ^{14}C-D4 and its metabolite(s) to the microsomal protein. The observation that incubations with microsomes from phenobarbital-treated rats caused extensive metabolism of D4 suggested also that microsomal metabolism of D4 in the uninduced system is a complex blend of enzyme action and inhibition.

Ref. 46

Guideline:	/
Test system:	Human liver microsomes (from a pool of 7 individuals); Rat liver microsomes (from MC and PB induced rats)
Test substance:	^{14}C-D4, specific activity 47 Ci/mol, lot #970310-4, and D4 LL024S10, purity 96%
Dose levels:	0.032 µM to 2.9 µM
GLP statement:	/

The study was conducted to evaluate the ability of D4 to inhibit the major cytochrome P450 (CYP) enzymes in human and rat liver microsomes.

Results: The study showed D4 to be a non-competitive inhibitor of human CYP2B6, CYP2D6 and CYP3A4/5, a competitive inhibitor of human CYP1A2, and either a competitive or non-competitive inhibitor of CYP2C19. D4 appeared to have no capacity to inhibit rat CYP1A2 or human CYP2A6, CYP2C9 and CYP4A9/11 activity. Because D4 is an activator, not an inhibitor of human CYP2E1, D4 has little or no capacity to function as a metabolism-dependent inhibitor of any of the CYP enzymes examined with the possible exception of rat CYP1A1/2 and human CYP3A4/5.

Ref. 48

Conclusion
Based on results of two *in vitro* studies with human liver microsomes (Refs. 46, 48), it was concluded that ^{14}C-D4 is primarily metabolized to metabolite M8, and that CYP2B6 and CYP3A4 are largely responsible for its formation.

Human data

Guideline:	/
Species/strain:	Human volunteers
Group size:	8 males and 4 females aged 25 to 49 years
Test substance:	D4 ^{14}C-D4, specific activity was 2.0 mCi/mmole
Batch:	D4, lot LL084732, purity 99.8% (GC-MS) ^{14}C-D4, lot 921217, radiochemical purity 99.03%
Test formulation:	Air containing 10 ppm D4 (122 µg/l)
Dose level:	10 ppm D4 for 1 hour, two exposures separated by one week Three months later the exposure was repeated. Blood samples after D4 exposure and at 1, 6 and 24 hours postdose
Control:	double-blind cross over study
GLP statement:	/

The respiratory intake and uptake of D4 were measured in 12 healthy volunteers (25-49 years; 8 males and 4 females) on two occasions. Subjects inhaled 10 ppm D4 (122 micrograms/liter) or air (control) during a 1h exposure via a mouthpiece in a double-blind, randomized fashion. Inspiratory and expiratory D4 concentrations were continuously measured. Exhaled air and plasma D4 levels were measured before, during, and after exposures. Individual D4 uptakes were measured under steady-state conditions during three rest periods (10, 20, and 10 min, respectively) alternating with two 10-min exercise periods.
At the end of the 1h exposure to D4, the mean D4 concentration in the blood plasma was 56 ng/g of plasma. Symptoms and finding in pulmonary function tests were minimal and not treatment related. No significant change in forced vital capacity (FVC), and FVC in 1 sec (FVC1) was observed immediately after exposure or 24 h post-exposure for either the air or D4 compared with the baseline measurements immediately prior to exposures.
Mean D4 intake was 137 ± 25 mg (SD) and the mean deposition efficiency was equivalent to 0.74/(1 + 0.45 VE), where VE is the minute ventilation. No changes in lung function were induced by the D4 vapour. Plasma measurements of D4 gave a mean peak value of 79 ± 5 ng/g (SEM) and indicated a rapid nonlinear blood clearance. A model was developed, using lung volume and respiratory surface area estimates based on functional residual capacity measurements, to determine the effective mass transfer coefficient for D4 ($5.7 \times 10^{(-5)}$ cm/s from lung air to blood). In additional eight subjects, a comparison of mouthpiece and nasal breathing on D4 deposition, at resting ventilations, was made. Mean deposition was similar for the two exposure protocols, averaging 12% after correction for exposure system losses.
Ref.: 40; AR 18

Assays were chosen to screen for immunotoxicity or a systemic inflammatory response. Assessment of immunotoxicity included enumeration of peripheral lymphocyte subsets and functional assays using peripheral blood mononuclear cells. Because in humans there is no direct test for adjuvant effect of respiratory exposure, proinflammatory cytokines and acute-phase reactants in peripheral blood, markers for a systemic inflammatory response, as surrogate markers for adjuvancy were analyzed. These tests were repeated when the volunteers were reexposed to D4 approximately 3 months after this initial exposure. Blood was obtained prior to exposure, immediately after exposure, and 6 and 24 h postexposure. In vivo cytokine production using a supersensitive ELISA for IL-6 in serum and serum levels of acute phase reactants, serum amyloid A (SSA) and C-reactive protein (CRP) were measured. The erythrocyte sedimentation rate and lipopolysaccharide-induced production of TNFα was assessed.
The baseline values were 1.0 pg/ml for IL-6, 24 mg/ml for SAA, 0.28 mg/dl for CRP, and 5.4 mm/h for ESR. At no time point was there a significant "treatment effect". At every time point after air or D4 exposure, there was no difference between groups in the total white blood cell count or in the percentage of lymphocytes (determined by complete blood count

and differential, data). Lymphocyte subsets were measured by flow cytofluorometry. The percentages of CD41, CD81, CD191, and CD561/CD161 lymphocytes were unaffected by exposure to D4. Functional Studies of peripheral blood mononuclear cells (PBMCs) have shown that there was no "treatment effect" seen in phytohemagglutinin (PHA)-induced proliferation. There was also no significant difference in allogeneic stimulation immediately post-exposure. The data have shown that there was no significant difference in NK cell cytotoxicity either immediately or 24 h following exposure. The production of IL-2, interferon γ, and TNFα measured by ELISA in the supernatant from PBMCs stimulated for 48 h with PHA was measured. The only statistically significant effect of exposure to D4 ($p < 0.05$) was for IL-2 production 6 h post-exposure. The PHA-induced IL-2 production was 28 U/ml for air and 18 U/ml for D4 exposure (p for treatment effect = 0.045). This effect was not seen immediately after exposure or 24 h post-exposure TNFα production by diluted whole blood was not different in the two exposure groups.
Rechallenge several months later (double blind, crossover protocol as with the initial study), no "treatment" effect of D4 occurred, i.e., no difference between the second D4 exposure and the second air exposure, in terms of pulmonary function tests, white blood cell count, percentages lymphocytes/neutrophils/monocytes in the differential, percentage of lymphocytes staining for CD4, CD8, CD19, or CD56/CD16, ESR, serum levels of IL-6, SAA, or CRP, proliferative response to PHA or alloantigens, NK cell-mediated cytotoxicity, or production of IL-2 or interferon g after PHA stimulation. In particular, a decrease in IL-2 production at the 6-h point after D4 exposure was not seen. The only statistically significant effect ($p < 0.05$) was seen for PHA-induced TNFa production at the 24-h time point. With PHA stimulation of PBMCs, TNFα production for the 24-h post-exposure time point was 5958 pg/ml for the air-exposed group and 10,081 pg/ml for the D4-exposed group (treatment effect $p = 0.018$). Immediately and 6 h post-exposure there was no difference. In addition, with the whole blood assay there was no significant difference in LPS-induced TNFα production at that same time point. LPS induced TNFα production was 948 pg/ml for air versus 1117 pg/ml for D4 (treatment effect $p = 0.51$). There was no significant effect of D4 on total protein at 6 h and aspartate transaminase post-exposure at other times in the first exposure or at any time in the second set of exposures.

Conclusion
Most individuals had minimal symptoms. However, the total symptom score was similar for D4 and air exposures. These are the first human data describing the intake and absorption of D4.

Ref.: 40, 41; AR 18

Guideline:	/
Species/strain:	Human, 6 male volunteers, 24 to 52 years old.
Group size:	see study #8464 (Ref 44) and Study 8496 (Ref)
Test substance:	D4
Batch:	D4, Lot number LL024S10, purity 99.75% by GC-MS. ^{14}C-D4, lot number 971210 radiochemical purity 98.91%, specific activity 49.39 mCi/mmol.
Test formulation:	D4 vapour and ^{14}C-D4 diluted with unlabeled D4 vapour.
Dose level:	10 ppm ^{14}C-D4 with intermittent exercise, for one hour.
GLP statement:	/

The purpose of this study in humans was to increase the analytical sensitivity of D4 measurements and to quantify D4 hydrolysis products in blood and urine. Blood samples were obtained 5 minutes after an exercise period. Following exposure to D4, volunteers were switched to room air for a 20-minute wash-out period. A 24-hour urine sample was then collected. Blood and exhaled air were also collected 3 and 6 hours post exposure.

Results
The mean respiratory intake increased to 154±39 mg and the uptake to 19±6 mg. A rapid respiratory elimination of 28% of the absorbed dose was observed. Plasma measurements

immediately post exposure revealed a mean peak value of 115±50 for D4 in ng/g and 161±53 in ^{14}C activity equivalents, respectively, and indicated a rapid non-linear clearance from plasma. Similar relationships were found in blood. Metabolites were far more persistent in blood and plasma than parent D4 and were still present at 24 hours post-exposure. Approximately 25-30 % of the D4 uptake was found in urine when the ^{14}C activity of the metabolites was expressed in D4 equivalents. Human urine chromatograms were qualitatively very similar to those of the rat. One metabolite in man, tentatively identified as trimethyldisiloxane-1,3,3-triol, is not detected in rat.

Ref.: 47

Pharmacokinetic modelling

A physiologically based pharmacokinetic (PBPK) model to describe the tissue dosimetry, plasma concentration and clearance in the rat following inhalation, dermal, oral and i.v. exposure indicated that the pharmacokinetics of D4 delivered by the inhalation or dermal routes were similar, and that it is different from the i.v. or oral delivery routes.

Recent toxicokinetic and pharmacokinetic modelling studies [**65, 66**] investigated the question how the exposure route (inhalation, dermal and oral) affects bioavailability of D4 and hence the biologically relevant internal dose: when absorbed through the lungs, D4 enters the arterial systemic circulation where it is distributed throughout the body to potentially all organ systems. When absorbed by the dermal route, D4 enters the venous circulation, which moves directly to the heart and lungs where the majority of the D4 is then eliminated via exhaled air and therefore unavailable systemically. A series of studies (described above) were conducted and a PBPK model constructed to evaluate the magnitude of the difference. D4 has been shown to have a very low blood:air partition coefficient. Consistent with this low blood:air partition coefficient, exhalation is the major route of elimination following dermal absorption of D4 with 80% or more of D4 that reached the systemic circulation being eliminated by exhalation within 24 hours **[67** or AR 13**]**. This model also indicates that the percent of a dermally applied dose of D4 that penetrates into the systemic circulation is about 0.3% or less**.**

In a recent publication **[78]**, it was emphasized that the oral route of exposure is an inappropriate route of exposure for the purposes of risk characterization and, therefore, risk assessment. The reason for this is that D4 appears to enter the blood in a different form following oral administration from that for the inhalation or dermal routes of exposure. For the oral route, D4 appears to be delivered via the lymphatics with the lipid core of chylomicrons and other lipoproteins. Given the route-specific nature of D4 pharmacokinetics, oral pharmacokinetic data collected is not as useful in understanding the bioavailability or tissue kinetics of D4. The oral pharmacokinetic data therefore, may not be practical for safety assessments and can lead to misleading or erroneous conclusions.

Ref.: 65, 66, 67 (AR 13), 78

3.3.10. Photo-induced toxicity

No data was submitted.

Siloxanes (such as D4) contain only methyl groups, which have no double bonds and do not absorb ultra violet radiation. Consequently, no phototoxicity studies have been performed.

3.3.11. Immunotoxicity

Studies in rats and humans have been conducted with different routes of application in order to examine the potential effects of D4 on the immune system.

Male and female Fischer 344 rats were used in a series of studies with gavage administration of D4: Immunotoxicity was assessed by splenocyte phenotyping, peripheral blood phenotyping, spleen IgM antibody response to the T-dependent antigen sRBC, serum IgM antibody titres, mixed leukocyte response to Long Evans and Brown Norway rat spleen cells, mixed leukocyte response, clearance of sRBC by the reticulo-endothelial system and natural killer cell activity. These biological parameters were measured one day after 28 days of oral gavage doses of 10, 30, 100 or 300 mg/kg D4 in corn oil. The studies [Ref. 54] showed that D4 does not cause immune suppression in male or female Fischer 344 rats.

An *in vitro* study with cultured human peripheral blood mononuclear cells showed that in the absence of serum, D4 was toxic to these cells, inhibiting proliferation induced by phytohaemaglutinin at concentrations greater than 10 µM/ml. This inhibitory effect was completely reversed by the addition of small amounts of serum or plasma to the serum-free medium. The serum factors responsible for this protection are lipoproteins. However, this inhibitory effect is irrelevant systemically since high levels of phospholipids in plasma would neutralise such effects. Nevertheless, culturing human peripheral blood mononuclear cells and D4 with or without serum was not associated with the production of TNFα [Ref 55].

Human volunteers were exposed to an oral dose of 12 mg D4/day in corn oil for 14 days in a double blind, placebo-controlled crossover study design. Assays for immunotoxicity included enumeration of peripheral lymphocyte subsets and functional assays using peripheral blood mononuclear cells. Pro-inflammatory cytokines and acute phase reactants in peripheral blood were used as surrogate markers for adjuvancy. No immunotoxic or pro-inflammatory adjuvant effect of D4 ingestion was found [Ref. 56].

Human volunteers were also exposed by inhalation to 10 ppm D4 for one hour on two occasions each separated by one week. The exposure was repeated 3 months later. Assessment of immunotoxicity was as described above [Ref. 56]. No immunological effect of respiratory exposure to D4 was found. Furthermore, no evidence of sensitisation was detected in another study [Ref. 41], described in more detail in section 3.3.9.

3.3.12. Special studies

Rat studies to elucidate the hepatomegaly

Guideline:	/
Species/strain:	Rat, Fischer 344, male and female
Group size:	24/sex/group except for positive controls (7/sex).
Test substance:	D4
Batch:	D4, lot LL024S10, purity 99.8% (GC-MS)
Test formulation:	D4 vapour/air mixture
Dose level:	Whole body; 70 or 700 ppm; 6h/day, 5 days/week; 4 weeks Interim sacrifices exposure day 3, 7, 14, 21 28 and post exposure days 7, 14
Vehicle/Control:	Positive controls Phenobarbital (40mg/ml in 0.9% saline), 80 mg/kg i.p. for 2 to 4 consecutive days prior to sacrifice. Negative and positive controls exposed to air, 6h/day, 5 days/week, 4 weeks
GLP statement:	/

Two identically designed studies to demonstrate the effects of D4 on liver size and enzyme induction in the rat.
Clinical signs were monitored daily, body weight recorded on day 1, then every 7 days throughout the study. Following sacrifice, liver and brain tissue were removed (brain was not used in this study). Liver tissue was placed in homogenisation buffer for preparation of hepatic microsomes. CYP and NADPH-cytochrome c reductase activities were determined.

Results
No effects on clinical signs, mortality or body weights were noted. There was a significant increase in liver weight (17%) at 700 ppm compared with controls (trend also at 70 ppm). Liver size decreased to within control values during the 14-day post-exposure (recovery) period.
There was a small increase in total hepatic CYP enzymes and a modest increase in NADPH-cytochrome *c* reductase activity. A slight induction in ethoxyresorufin *O*-deethylase (EROD) activity and in CYP3A1 immunoreactive protein was detected. A large increase in pentoxy-resorufin *O*-dependentylase (PROD) activity correlated with an increase in CYP2B1/2 protein levels. A modest induction of microsomal epoxide hydrolase (mEH) mRNA, immunoreactive protein, and activity was observed. (Dose-dependent and partly strong induction of hepatic CYP enzymes *i.e.* ethoxyresorufin O-deethylase, pentoxyresorufin O-dependentylase, 6ß-testosterone-hydroxylase, p-nitrophenol hydroxylase and P450 proteins (total P450, CYP 2B1/2, CYP 3A1/2, CYP 2E1) at 70 and 700 ppm; induction of hepatic phase-II conjugation enzymes (UDP-glucu-ronosyltransferase towards chloramphenicol at 70 ppm, mEH in 70 and 700 ppm males and females) and increase of mEH mRNA protein in 70 ppm females and 700 ppm males and females; dramatic and dose-dependent decrease in lung PROD activity in 70 and 700 ppm males and females.)
The magnitude of this induction was nearly identical to that observed in the phenobarbital-treated positive control animals.

Ref.: 57, 58

In this peer reviewed publication, a similar response was obtained on liver weight and CYP enzyme activity in Sprague Dawley rats treated with D4 in corn oil by oral gavage at dose levels of 1, 5, 20 or 100 mg/kg body weight for 4 consecutive days. Negative controls received corn oil, positive controls received 50 mg/kg phenobarbital in phosphate buffer by intraperitoneal injection for 4 days.

Ref.: 59

Another study examined CYP induction in female young, mature and pregnant Sprague Dawley rats treated with D4 by oral gavage at dose levels of 0, 5, 20 or 100 mg/kg body weight for 8 consecutive days. The peer reviewed publication reports dose and age-dependent increases in CYP2B and CYP3A isoforms: the increases were significant at doses of 20 mg/kg bw and higher, and there was a 20% increase in the liver to body weight ratio in mature rats treated with 100 mg/kg D4. No histological examination was conducted. At 100 mg/kg-bw/day, additional effects included decreased foetal body weights and liver weight / body weight ratio in foetuses.

Ref.: AR 19

Guideline:	/
Species/strain:	Rat, Fischer 344, female
Group size:	10 per group
Test substance:	D4
Batch:	D4, lot LL024S10, purity >99% (GC-MS)
Test formulation:	D4 vapour
Dose level:	0, 1, 7, 30, 70, 150, 300, 500, 700 or 900 ppm; whole body; 6h/day for 5 days
Vehicle/Control:	Positive controls Phenobarbital in the drinking water at 500 ppm Negative controls received filtered room air
GLP statement:	in compliance

This was a metabolism study to investigate the effects of D4 on hepatic microsomal enzyme induction in rats. Within each group, three subgroups were used for liver biochemical analyses and two subgroups used for determining D4 in blood, fat and liver.
Results

There were no effects on body weight. A dose-related increase in liver weight occurred. The liver-to-plasma D4 ratio remained constant over the dose range.
The lowest dose level to induce significant hepatomegaly was 150 ppm. D4 content in fat, liver and plasma increased proportionally with increasing exposure concentrations. A dose-dependent increase occurred in PROD activity and in CYP2B1/2 proteins with a maximum response at 500 ppm D4. These findings are consistent with those reported for phenobarbital and confirm that D4 is a qualitative "phenobarbital-like" inducer of rat hepatic cytochrome P450 enzymes.
A NOEL of 70 ppm D4 is based on enzyme induction in female rats.

Ref.: 60

Guideline:	/
Species/strain:	Rat, Sprague-Dawley, male
Group size:	10 per group
Test substance:	D4
Batch:	Lot number LL108831, purity approx. 98%
Test formulation:	D4 in 0.5% aqueous methylcellulose
Dose level:	Two groups received 1600 mg/kg/day D4 oral gavage for 14 consecutive days
Vehicle/Control:	Negative controls (two groups of 10 males) vehicle only at 4 ml/kg
GLP statement:	in compliance

This was a morphometric study and DNA analysis designed to define the mechanism that caused the hepatomegaly in rats exposed to D4. The animals were examined daily for clinical signs and weighed weekly. At termination, rats were euthanised by perfusion fixation accomplished by flushing the vascular system with glutaraldehyde/formaldehyde solution. Livers were collected for light and electron microscopy and for determination of DNA concentration.

Results
No deaths occurred and no clinical signs of toxicity were observed. No differences in body weights were noted.
Morphometric analysis revealed no significant difference in the number of cells per given volume of liver between control and treated rats. However, there was a significant increase in the total number of hepatocytes in the treated rat liver demonstrating that D4 causes hepatocellular hyperplasia. The mean hepatocyte profile diameter in the three lobular zones did not differ significantly between treated and control rats.
No significant difference in DNA values between treated and control rats was detected. Further, as the DNA content of the liver was similar for treated and control rats, this suggests that the same number of nuclei was present in each sample of liver (control vs. treated). The results of these studies indicate that hepatomegaly is due in part to hepatocellular hyperplasia.

Ref.: 61, 62, 63

Additional studies by McKim et al. compared D4 and phenobarbital treatment in F344 female rats. Both compounds produced hepatomegaly, transient hepatic hyperplasia and sustained hepatic hypertrophy. At 700 ppm D4 (6 hours/day, 5 days/week), the hyperplastic effect was greatest at day 6 and was still present at day 13 to a small extent, but had declined to normal by day 27. This pattern of transient hyperplasia followed by sustained hypertrophy leading to hepatomegaly is consistent with the pattern observed for phenobarbital. Data from this study, namely hepatic CYP2B1/2 activity, plasma and tissue D4 concentrations, were also used to develop a pharmacokinetic model that describes the dose-response curves for enzyme induction (Ref.: AR23).

Ref.: 64, AR23

Comment
Administration of D4 by oral or inhalation routes to rats causes hepatomegaly as a result of hepatocellular hyperplasia and hypertrophy. The enzyme expression profile observed in rats following exposure to D4 is similar to that observed following exposure to phenobarbital. Therefore, D4 may be considered a "phenobarbital-like" inducer in rat liver. Interestingly, and inspite of being 'phenobarbital-like', chronic administration of D4 to F344 rats in the carcinogenicity study did not induce hepatomegaly or any hepatic lesions of relevance, including tumours, even at the high dose of 700 ppm (see Section 3.3.7.). It is clear that not all chemicals that induce hepatomegaly are tumorigenic in lifetime bioassays.

Studies to elucidate estrogenic and antiestrogenic effects of D4 in rats and mice

Guideline:	Prior to, but in line with OECD 440
Species/strain:	Rat, immature female Sprague-Dawley and Fischer 344
Group size:	12 per group
Test substance:	D4
Test formulation:	D4 in sesame oil
Dose level:	0, 50, 100, 250, 500 and 1000 mg/kg/day D4 by daily oral gavage for 3 consecutive days in uterotrophic assays
Vehicle/Control:	received sesame oil by gavage
Positive Controls:	1, 3, 10 or 30 µg ethinylestradiol (EE), and antiestrogen ICI182,780 plus ethinyl estradiol administered at 3 mg/kg and 1-30 µg/kg EE; DES-dipropionate at 0.5, 1.5, 5 and 15 g/kg per day
GLP statement:	in compliance

Results
Decreased body weight gain was seen at 250 mg/kg D4 and above in both strains of rats. Increased liver weights at 100 mg/kg D4 (Fischer 344) or 250 mg/kg D4 (Sprague-Dawley) and above. Increased uterus weight at 250 mg/kg D4 and above (both strains). D4 inhibited uterotrophic response of EE in both strains at 500 mg/kg, showing a weak antiestrogenic activity. At the 50 % of maximal response D4 was approx. 1.2 to 25 million times less potent than EE or DES-DP in Sprague-Dawley and Fischer 344 rats, respectively. Decreased body weight gain was observed at 250 mg/kg D4 and above. Increased liver weights were seen at 100 mg/kg D4 (Fischer 344) or 250 mg/kg D4 (Sprague-Dawley) and above. Increased uterus weight occurred at 250 mg/kg D4 and above (both strains).

Conclusion
D4 inhibited uterotrophic response of EE in both strains at 500 mg/kg indicative of a weak antiestrogenic activity. At the 50 % of maximal response D4 was approx. 1.2 to 25 million times less potent than ethinyl estradiol or diethylstilbestrol diproprionate in Sprague-Dawley and Fischer 344 rats, respectively.

Ref.: 69; AR 20

Guideline:	/
Species/strain:	female estrogen receptor knockout (ERKO) mice and wild type mice (129/J/C57BL/6J) and female B6C3F1 mice-
Group size:	5 per group (uterotrophic); 8 per group (serum hormones)
Test substance:	D4
Test formulation:	D4 in corn oil
Dose level:	a) uterotrophic assay: 0, 50, 100, 250, 500 and 1000 mg/kg/day D4 by daily oral gavage for 3 consecutive days; sacrificed 24 h later b) serum hormone: level: 1 to 1000 mg/kg/day D4 by daily oral gavage for 7 consecutive days; sacrificed 24 h later
Vehicle/Control:	Negative controls received corn oil

Positive Controls: estradiol (E2) 10 µg/kg by s.c., and antiestrogen ICI 182,780 at 20 mg/kg 30 min given s.c. prior before to dosing with D4 or estradiol
GLP statement: /

Results
Uterine weight was significantly increased by 250 to 1000 mg/kg D4 administered orally. Uterine peroxidase levels (a marker for estrogenic activity) were also significantly increased in D4 exposed mice. Pretreating mice with ICI 182,780 completely blocked D4-induced increase in uterine weight. Further, ovariectomized estrogen receptor-knockout mice showed no increase in uterine weights when exposed to D4 or estradiol. These uterotrophic effects of D4 were ablated by pre-treatment with ICI 182,780, an estrogenic receptor (ER) antagonist. Ovariectomised αER KO mice showed no increases in uterine weights when treated with D4. Studies with adrenalectomized mice showed that decreased serum estradiol levels, which were decreased upon oral administration of D4, were not due to elevated serum corticosterone levels.

Conclusion
The data indicate that D4 has weak estrogenic activity, and these effects are mediated through estrogen receptor (ER), probably by direct receptor binding via ERα. The data indicate that the stimulatory effect of D4 on the mouse uterus is not related to estradiol activity.

Ref.: 70

Studies to investigate effects of D4 on LH surge and reproductive hormone levels in rats

Guideline: /
Species/strain: Rat, ovariectomized female Sprague-Dawley; three days prior to inhalation exposure, each female received a surgical implant of silastic tubing containing 17ß-estradiol
Group size: 10 per group
Test substance: D4, Lot LL084732, 99.78% pure
Test formulation: D4 vapour (in air)
Dose level: 700 and 900 ppm D4 as a single whole body exposure for 6 hours
Vehicle/Control: negative controls received filtered air
GLP statement: in compliance

The purpose of this study was to evaluate the potential of D4 to affect preovulatory luteinizing hormone (LH) surge in ovariectomized rats. Blood samples were collected for LH, prolactin and estradiol and/or estrone analysis after 6 hours exposure to D4 (at end of exposure), or 2, 4, 6 or 8 hours post exposure.

Results and Conclusion
Group mean LH levels in ovariectomized females treated with estradiol via a subcutaneous implant were similar to the control group mean following a single six-hour D4 exposure at 700 or 900 ppm. However, several animals in the 700 and 900 ppm exposure groups had reduced LH levels (< 7 ng/ml) at the peak time of 6:00 p.m. relative to the control group. Because the LH surge is required for ovulation to occur, these results suggest that the reduced fertility rate observed in a previous study (Dow Corning Report No. 1999-I0000-47049) in which rats were exposed to D4 at 700 ppm on the day of proestrus may have been the result of a reduction in peak serum LH levels.

Ref.: 74

Guideline: /
Species/strain: Rat, female Sprague-Dawley (13 weeks old)

Group size:	30 per group (Phase I); 20 per group (Phase II)
Test substance:	D4, Lot LL0024S10, 99.6% pure
Test formulation:	D4 vapour (in air)
Dose level:	700 and 900 ppm D4
Exposure:	Phase I: Cannulated rats received 6 hours/day exposure to D4 for 3 days on diestrus –1, diestrus –2 and proestrus. Phase II: non-cannulated rats exposed 6h/day to D4 for 2 consecutive days of diestrus and 2.5 h on proestrus
Vehicle/Control:	negative controls received filtered air
GLP statement:	no

This study assessed the ability of D4 to attenuate the preovulatory LH surge, and to assess the ability of D4 to block or delay ovulation, and to evaluate the levels of other reproductive hormones in D4 exposed rats. Prior to treatment, estrous cycle was staged for 10-12 days. Blood collected from Phase I rats on the day of proestrus at 2, 4, 6, 8 and 10 p.m. for plasma LH and prolactin measurements. Following necrospsy on morning of next estrus, blood was collected for FSH, estradiol, estrone and progesterone measurements. Brain, uterus and ovary weights recorded and ovaries evaluated histopathologically. Phase II rats blood samples were taken for FSH, estradiol, estrone and progesterone measurements.
Results and Conclusion: D4 exposure resulted in significant reductions of proestrus LH levels in 900 ppm group at 4, 6 and 8 p.m., A smaller effect was seen at 700 ppm. As a consequence, only 42% and 31% of rats at 700 or 900 ppm ovulated compared to 79% in the controls. On the morning of estrus, higher levels of estradiol were found in rats at 700 or 900 ppm D4 relative to controls, indicating failure of mature follicles to ovulate. Therefore, high exposure to D4 attenuates the preovulatory LH surge and significantly decreases the proportion of females that ovulate.

Ref.: 75; AR 21

The mechanism for delayed ovulation status resulting in reduced fertility has been studied. The observed changes were shown to be reversible (Ref. 32, 33). Other studies have shown that D4 has very weak estrogenic and anti-estrogenic activity in vitro and in a Rat Uterotrophic Assay in both immature Sprague Dawley and F344 rats (Ref. 69; AR 21). Rather than a direct estrogen receptor (ER)-mediated mechanism (of very low potency), an indirect mode of action appears to be more relevant for explaining the reproductive toxicity and carcinogenicity of D4 observed at high doses: There are data in rats indicating that D4 can cause a delay or blockage of the luteinizing hormone (LH) surge necessary for optimal timing of ovulation (Ref. 74). Support for a role of LH was obtained from a study of estrous cycle staged female Sprague Dawley rats exposed to 700 or 900 ppm D4 for 6h/day for 3 days, i.e. on diestrus 1, diestrus 2, and pro-oestrus. Measurement of LH on the day of pro-estrus at 2, 4, 6, 8 and 10 p.m. showed a significant reduction of LH levels at 4, 6 and 8 p.m. which correlated with blocked ovulation (Ref. 75). The majority of reproduction findings in the two-generation study are also consistent with a long-term suppression of LH release.
While it is possible that D4 can affect the secretion of LH in female F344 rats in a manner similar to that observed for SD rats (Ref. 75, AR 21), it is also possible that the mode of action for the cystic endometrial hyperplasia and endometrial adenomas observed in the combined chronic/carcinogenicity study is by effects on prolactin through interaction with the dopamine receptor in the pituitary. This view is supported by as yet unpublished studies in vitro and in vivo indicating that D4 can inhibit prolactin release from the pituitary by acting as a dopamine agonist (Ref. AR5). However, at present there is insufficient data for this suggested neuroendocrine mode of action in rats to preclude a relevance for humans. The absence of genotoxic potential supports the view that tumour formation is due to threshold effects.

3.3.13. References for D4 (section 3.3)

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3.4. Toxicological Evaluation of D5 (Decamethylcyclopentasiloxane; Cyclopentasiloxane)

The description is largely based on SEHC, CES, SIAJ Decamethylcylopentasiloxane (D5): A White paper on Health Research Findings (Ref.: 66). Since this document did only cover studies and publications on D5 up to 2005, SCCS did take into consideration the available open literature and more recent evolutions on health effects by other panels (Refs. AR12**,** AR13, AR14).

3.4.1. Acute toxicity

3.4.1.1. Acute oral toxicity

Five male and five female Wistar rats survived a single oral gavage dose of 4800 mg D5/kg bw with no overt signs of toxicity.

Ref.: 1

3.4.1.2. Acute dermal toxicity

No study submitted

3.4.1.3. Acute inhalation toxicity

Four groups of 5 male and 5 female Fischer 344 rats were exposed by whole body inhalation to D5 during a ***single, continuous 4-hour period,*** followed by an observation period of 15 days. The achieved test atmosphere concentrations (sum of the aerosol and vapour phase) were 4.64, 6.73, 9.82, and 15.37 mg D5/l of air (300, 434, 634, 1000 ppm, respectively). Animals were observed for clinical signs, abnormal behaviour, mortality, body weight, body weight gain, and food consumption during the 15-day observation period. All animals were necropsied and all macroscopic abnormalities recorded at the end of the study. The food consumption and mean body weights were decreased in animals exposed to concentrations of 6.73 mg D5/l (434 ppm) and above but these effects were reversible during the observation period. The clinical signs that were observed following exposure were of low incidence. In animals dying spontaneously post exposure, the lungs were affected (red lungs partly collapsed) while no macroscopic observations were recorded in any animals necropsied at the scheduled sacrifice date. No animals died at the lowest exposure concentration. At the intermediate exposure concentrations, four animals of each sex died. All animals at the highest exposure concentration died during the exposure phase. The LC_{50} for both sexes was calculated to be 8.67 mg D5/1 (560 ppm).

Ref.: 2

Five male and 5 female Wistar rats survived a ***single four hour whole body vapour exposure*** of >545 ppm of D5 with no overt signs of toxicity and no mortality.

Ref.: 3

3.4.2 Irritation and corrosivity

3.4.2.1. Skin irritation

No special study submitted, but a subacute study with dermal application of D5 is relevant:

Six male and female New Zealand white rabbits were treated 6 hrs/day, 7 days/wk for ***21 consecutive days*** with 1000 mg D5/kg bw under occlusive conditions. The skin was abraded in one-half of the animals. Skin reactions were scored daily for signs of oedema and

erythema. At necropsy, the heart, lungs, liver, kidneys, spleen, testes, epididymides, ovaries, and urinary bladder were weighed and preserved. No clinical signs of toxicity were observed. There was no effect on body weight, no mortality, no effect on organ weights, and no treatment related gross pathology findings. No signs of skin irritation were observed.

Ref.: 16

3.4.2.2. Mucous membrane irritation

Eye Irritation

A standard Draize test was performed using New Zealand White rabbits. A single treatment with 100 µl of neat D5 liquid instilled into the conjunctival sac of the eye of 3 male and 3 female rabbits elicited no response. In this study, D5 was not an eye irritant.

Ref.: 13

Five rabbits (strain and sex not specified) received a single treatment of 500 µl of undiluted D5 in the conjunctival sac. The eyes were stained with fluorescein at 24 hours and read for irritation. There was no corneal injury following treatment with D5. Minor capillary injection of the eyelids was noted. D5 was judged to be non-irritating.

Ref.: 14

Five hundred microliters of undiluted D5 were instilled into the conjunctival sac of six rabbits (strain and sex not specified), 2 times/day for four consecutive days. No corneal injury or eye irritation was observed among treated rabbits and controls receiving comparable amounts of a saline solution.

Ref.: 15

3.4.3. Skin sensitisation

Magnusson-Kligman Maximization test

Guinea pigs were pretreated intracutaneously with a 1% solution of D5 in paraffin oil and epicutaneously with undiluted D5 using the "Maximization test" of Magnusson and Kligman. Challenge with undiluted D5 and a 10% solution in paraffin oil did not elicit a hypersensitivity skin response. The results of this study indicate that D5 and paraffin oil are not skin sensitizers in the guinea pigs.

Ref.:18

HRIPT

In a Human Repeated Insult Patch Test (HRIPT) designed to assess skin irritation and sensitization of D5 in humans, 28 males and 22 females were treated with a dermal application of 0.05 ml test material three times/week for a total of 9 applications. The D5 was applied using an occlusive patch that remained in place for 24 hours. Skin was graded for erythema, eschar, and oedema after the patch was removed. Twelve days after the ninth application, the site was graded and 0.05 ml of D5 was applied to a new site and covered for 24 hours with an occlusive patch. The site was then re-graded for erythema, eschar, and oedema immediately and at 24 and 48 hours after removal of the occlusive patch. No dermal irritation or sensitization was reported following D5 exposure.

Ref.: 19

Comment
The SCCS considers HRIPT-studies as unethical.

3.4.4. Dermal / percutaneous absorption

In Vitro

Excised split-thickness skin, ranging from 381-629 µm, from young adult Sprague-Dawley rats was mounted on static Franz diffusion cells with 6% polyoxyethylene-20oleyl ether and 1% penicillin/streptomycin in saline solution as a receptor fluid. An initial screening to check barrier integrity of the skin was accomplished by applying 970 µl of $^{3}H_2O$ (0.77 µCi) to the surface of the skin for 20 minutes. Following administration of the $^{3}H_2O$, the unabsorbed material was removed from the skin; the receptor fluid was sampled and analysed for ^{3}H at 60 minutes. Once the $^{3}H_2O$ had been removed, ^{14}C-D5 (6.4 mg/cm²) was applied to each skin sample. Measurements were made of the ^{14}C-labelled material that could be washed from the skin, were associated with skin, or penetrated through the skin into the receptor fluid over a 24-hour period. The washing procedure was performed using a 1% soap solution-moistened gauze 3 times, followed by 3 washes with gauze moistened with 70% ethanol. The skin was solubilised in 40% tetraethylammonium hydroxide. The cumulative penetration was calculated based on the amount of radioactivity in the receptor fluid over the 24-hour sampling period. The percentage of radioactivity found in the skin was 0.67% and 1.19% in males and females respectively. The total absorbed (% radioactivity in the skin and receptor fluid) was 1.08% and 1.54% in males and females respectively. According to the applicant, these data need to be evaluated cautiously due to unrecognized technical problems associated with working with this material at the time this study was conducted. More reliable studies are reported below.

Ref.: 20

^{14}C-D5 was applied to semi-occluded human skin using a flow-through diffusion cell technique. Human epidermis was prepared from intact abdominal skin. The epidermis was separated from the dermis using a Padgett® dermatome. Skin disks from 6 donors were mounted in replicate in the flow-through chambers. A physiological receptor fluid was pumped underneath the skin samples and collected in a fraction collector. The barrier integrity of each piece of skin was evaluated prior to dosing using $^{3}H_2O$. Skin samples were evaluated on two separate days. In experiment 1, skin samples from each of 3 donors were dosed with neat D5 and the remaining 3 were dosed with a generic antiperspirant formulation containing D5. In experiment 2, a second set of skin samples from the same 6 donors were dosed with the other test article (i.e. antiperspirant or neat D5), and immediately after dosing, charcoal baskets were placed above the skin and secured into a custom designed cap to capture any volatilized material. At the end of 24 hours, the charcoal baskets were removed and extracted, skin was washed and solubilised, and the receptor fluid was collected. The radioactivity content in each sample was measured by liquid scintillation counting. The percent dose absorbed was determined as the amount of radioactivity in the receptor fluid and the amount left in the skin after washing and tape stripping. At the end of the assay, only 0.04% ± 0.007% of the applied dose of neat D5 was absorbed which was not significantly different from that seen with formulated D5 (0.022% ± 0.005% of the applied dose). The percent of applied dose recovered from all analysed samples for neat D5 was 91.45% ± 1.60% and for D5 formulated in generic antiperspirant formulation was 98.05% ± 1.17%. The majority of the dose was evaporated from the dosing site and was collected from the charcoal baskets.

Ref.: 21; AR1

In Vivo

^{14}C-D5 was applied to the dorsal surface of male and female Sprague-Dawley rats from which the hair was clipped. The skin depot chamber (a Teflon® gasket attached to the dosing site with cyanoacrylate glue, an activated charcoal trap, and a plastic cap with a hole to allow for air circulation) was covered with a non-occlusive elastic wrap. At the termination of a 24-hour exposure period, animals were removed from the metabolism cages and the exposure site

was washed. Animals were rewrapped with a fresh nonocclusive bandage and returned to metabolism cages for continued collection of samples. Animals were removed from the metabolism cages 96 hours post-initial exposure, sacrificed, and the exposure site carefully excised. The majority (about 85%) of the ^{14}C-D5 volatilised from the skin surface. The dose site, which was washed prior to excision at 96 hours, contained 0.35% of the administered dose. Less than 1% of the dosed ^{14}C was recovered in urine and carcass. There were trace levels of ^{14}C in faeces, CO_2 traps, and tissues. Total radioactivity in excreta, carcass, and dose site, which was considered to be the amount absorbed, was 0.80 ± 0.62% (n=11) with a recovery of about 89%. According to the applicant, these data need to be evaluated cautiously due to unrecognized technical problems associated with working with this material at the time this study was conducted. The studies reported below were considered more reliable.

Ref.: 22

In another study, the percutaneous absorption of neat ^{14}C-D5 was evaluated in Fischer 344 rats when applied topically at 10.9 mg/cm² of skin. Four animals per group were exposed for 6 or 24 hours. Two control animals were euthanized at the 24-hour time point. In order to differentiate expired air from ^{14}C-D5 that escaped from the skin depot, an additional group of four euthanized rats (i.e., no expired air) was included in the study design. An additional 24-hour exposure group was added to evaluate disposition of the residual D5 following a soap and water wash (i.e., wash group). During exposure, rats were housed in Roth-style metabolism cages to enable collection of urine, faeces, and expired or escaped volatiles associated with D5. Dose sites were washed, charcoal baskets were replaced and the animals were returned to the metabolism cages for continued collection of excreta and expired volatiles for a total 168 hours. All rats were exposed in a semi-occluded manner using an aluminium skin depot with a charcoal basket for collection of volatilized D5. At the termination of exposure at 24 hours or at 168 hours post exposure, rats were euthanized by CO_2 asphyxiation, the charcoal baskets were removed and extracted, skin was washed, tape stripped, excised, and solubilised in 35% tetraethylammonium hydroxide (TEAH). Remaining carcasses were also solubilised in 35% TEAH. Radioactivity content in each sample was measured by liquid scintillation counting. Total radioactivity in charcoal tubes was compared to unchanged D5 determined by GC-MS analysis. The percent dose absorbed was determined as the amount of radioactivity in carcasses, faeces, urine, skin dosing sites, and cage rinses. Absorption of ^{14}C-D5 after 168 hours was determined to be 0.089 ± 0.0302%.

Ref.: 23; AR1

Normal, healthy human volunteers (3 male, 3 female) were exposed to either 1.4 g (males) or 1.0 g (female) of ^{13}C-D5 by applying the D5 to the axilla. The dose was split between axilla and applied once while the subject was breathing from a clean air source. Blood samples were obtained prior to exposure and at 0.5, 1, 2, 4, and 6 hours. Exhaled air samples were obtained prior to exposure and at 15, 30, 45, 60, 75, 90, 105, 120, 240, and 360 minutes and at 24 hours after application. D5 levels were significantly elevated above baseline in blood, plasma, and in exhaled air at all time points after application. The plasma and blood levels of D5 after dermal application were less than 2.0 ng/g blood or plasma. With dermal application of 1.0 g (female) or 1.4 g (males) of D5, peak plasma D5 levels were 1.22 ng/g at 1 hour and 0.61 ng/g at 6 hr post exposure. There was a relatively poor correlation between blood levels and exhaled air levels of D5 especially at one hour after application, i.e., considerably higher levels were found in exhaled air than would have been expected based on blood levels. D5 levels did not differ significantly between male and female volunteers.

Ref.: 24

General comment on dermal absorption

There is some variation in results of in vitro and in vivo studies with D5. In vitro an average of 0.04% of decamethylpentacyclosiloxane (D5, applied neat or in antiperspirant formulation) was absorbed in human cadaver skin and the receptor fluid after 24 h of exposure (Ref. 20). Higher values were found in an in vitro study with rat skin, where

dermal absorption of ^{14}C-D5 was maximum 1.5% (Ref. 20). Similar to in vitro studies with human or rat skin, also the *in vivo* rat study demonstrated that the majority (~ 85%) of D5 applied volatilized from the skin surface before being absorbed. Less than 1.0% of applied D5 appeared to be absorbed *in vivo in one rat study of lower quality (Ref. 22), whilst a newer rat study showed 0.09% dermal absorption of D5 (ref. 23).* Consistent with *in vitro* results for human cadaver skin, pharmacokinetic modelling of dermal absorption in human volunteers indicated for men and women that 0.05% of applied D5 was absorbed into systemic circulation (Ref. AR2).

A value of 0.17% for dermal absorption of D5 was taken by the Canadian authorities (Ref. AR13), based on the publication by Jovanovic et al. (2008; Ref. AR1).

3.4.5. Repeated dose toxicity

3.4.5.1. Repeated Dose (14 days and 28 days) oral / dermal / inhalation toxicity

Oral

Five groups of eight male and female Sprague-Dawley rats were administered by oral gavage 0, 25, 100, 400, or 1600 mg D5/kg bw, 5-days/ week for two weeks. All animals were observed for signs of local and systemic toxicity, general appearance, behavioural abnormalities, and mortality throughout the study. No treatment related deaths were observed. No effects were seen on body weight or body weight gain, on behaviour or on gross pathology. A treatment related increase in absolute and relative liver weight was seen in females at 100 mg D5/ kg bw and in males at 25 mg D5/kg bw.

Ref.: 4

A similar study on short-term oral toxicity of D5 by Crofoot et al. (1990) was cited in a recent report of the US Cosmetic Ingredient Review Expert Panel, 2009 (Ref. AR14). Five groups of 8 male and 8 female Sprague-Dawley rats received oral doses (via gavage) of 0, 25, 100, 400, and 1600 mg/kg bw, five days/week for two weeks. Neither treatment-related deaths, overt signs of toxicity, nor changes in behaviour were observed in any of the groups. Treatment-related increases in liver weights were observed at doses as low as 100 mg/kg in female rats (LOEL). A no-observed-effect-level (NOEL) for liver weight of 100 mg/kg was reported for male rats. No significant changes were observed at gross pathological examination.

Ref.: AR3

Comment
Similar observations were made in a 13-week oral gavage study (see section 3.4.5.2) that derived a LOEL of 100 mg/kg bw/day.

A four week study was conducted in which 6 male and 6 female rats were given 1500 mg/kg bw/day of the test substance via oral gavage 5 days a week for the duration of the study. Animals were observed for signs of local or systemic toxicity, general appearance, behavioural abnormalities and mortality. Body weight and food consumption were determined weekly. A control group received distilled water. No treatment-related deaths, overt signs of toxicity or changes in the behaviour were noted in any of the groups. Statistical comparison of mean body weight and food consumption data indicated no treatment-related effects between the control and the test groups. A statistically significant increase in absolute liver weight was observed in female rats treated with 1500 mg D5/kg bw. No gross pathological changes were observed in any of the organs or tissues of male and female rats in the control and test groups.

Ref.: 5

Inhalation

Five groups of ten male and female Fischer 344 rats were exposed by nose-only inhalation for 6 hrs/day, five days/week for four weeks to 0, 0.44, 0.65, 1.50, or 2.29/3.71 mg D5/l (0, 28, 42, 96, 151/197 ppm, respectively). Animals were exposed to the highest exposure concentration of 3.71 mg/l (197 ppm) for days 1 to 6 while the exposure to 2.29 mg/l (151 ppm) was for the remaining duration of the study (week 2 to 4). (According to AR#4**,** 160 ppm is the highest D5 exposure concentration that can be reliably generated and maintained as a vapour without interference by aerosol formation). No behavioural abnormalities or mortalities were seen during the study. No effects were seen on mortality, body weight or body weight gain, food intake, or clinical signs. Urinalysis and biochemistry data indicated no changes of toxicological significance at termination of the treatment. However, a few minor changes with statistical significance were recorded in rats exposed to 1.5 and 2.29 mg/L (42 and 96 ppm). These were characterised by a slightly increased mean corpuscular volume, slightly decreased mean corpuscular haemoglobin concentration and slightly increased leukocyte and lymphocyte counts in males. There was no apparent relationship between these effects and the treatment. An increase in absolute and relative liver weight with slight hepatocellular hypertrophy (females only), increased incidence of goblet cell proliferation in the nasal cavity (males and females), and minimal to slight interstitial inflammation in the lungs (males and females) were observed at the highest exposure concentration of 2.29/3.71 mg D5/L. i.e. 151 and 197 ppm.

Ref.: 6

Comment
The changes observed at the highest exposure concentrations (increased incidence of goblet cell proliferation in the rat nasal cavity, and minimal to slight interstitial inflammation in the lungs) are consistent with changes due to inhalation of a mild irritant. The local effects are considered to be of little/no relevance for consumer exposure to much lower concentrations of D5.
In female rats a LOEL of 160 ppm was set for systemic effects on liver (an increase in absolute and relative weight with slight hepatocellular hypertrophy).

In a 28-day study in rats, D5 was administered via whole body inhalation to four groups of male and female Fischer 344 rats for a period of six hours/day, seven days/week for four consecutive weeks. The target exposure concentrations were 10, 25, 75 and 160 ppm. A concurrent negative control group of identical design received only filtered air. After completion of 28 days of exposure, 10 rats/sex/group were necropsied and 5 rats/sex/group entered a two-week recovery period. Animals were observed for clinical signs, effects on body weight, food consumption and ophthalmologic effects. Complete necropsies were performed, selected organs weighed and selected tissues were examined grossly and microscopically. No test article-related effects on survival, clinical condition, body weights, body weight gains, food consumption or ophthalmoscopy at any exposure level were observed in this study. No test article-related gross findings were observed. An increased mean lung weight and alveolar macrophage accumulation was observed in the 160-ppm group. Treatment-related morphological alterations (Goblet cell proliferation) were also noted in the nasal cavity of both sexes at concentration of 10 ppm of D5 or greater. These changes were reversible following a two-week recovery period. The mean (absolute and relative) liver weights in the 160 ppm group, especially the females, were increased at the week 4 primary necropsy. No histopathological changes were noted. At the week 6-recovery necropsy, no effects on liver weights were observed.

Ref.: 7

Comment
The treatment-related changes observed in lung and nasal cavity of rats at 160 ppm are local effects of little/no relevance for consumer exposure to low concentrations of D5. A LOEL of 160

ppm was found for systemic effects on liver (an increase in absolute and relative weights, with no histopathological changes). The effect was reversible upon cessation of exposure.

Ten male and 10 female Wistar rats were exposed via whole body inhalation to 0.081, 0.432 or 2.00 mg D5/l (5, 28, or 129 ppm, respectively), 6 hrs/day, 5 days/week for four weeks. Five animals of each sex were allowed a 14-day recovery period following the four weeks of exposure. A concurrent control of identical design was exposed to only filtered air. Animals were observed for clinical signs, effects on body weight, food consumption, and organ weights. Haematology, clinical biochemistry, urinalysis and gross and microscopic pathology were performed. In this study, no effects were seen on body weight, body weight gain, food consumption, or clinical condition. There were no gross findings. At 0.432 mg D5/l (28 ppm) and above, there was an increase in white blood cell and neutrophil counts (males only) a decreased number of red blood cells and mean corpuscular haemoglobin concentration. An increase in relative liver weight (percentage not stated) was also observed in male and female rats at 0.432 mg D5/l (28 ppm) and above. All effects were reversible during the 14-day recovery period. The NOAEL reported in this study was 0.081 mg D5/L air (5 ppm).

Ref.: 8

Comment
A LOEL of 28 ppm D5 was reported based on changes of some haematological parameters and reversible effects on liver (an increase in relative weight, percentage not stated) in this study of 1984. However, neither two more recent rat studies with 4-week inhalation nor studies with longer exposure did observe such changes at similar concentrations. Thus, SCCS as well as the Canadian authorities (Ref. AR13) have not further considered this study of 1984 in their assessment of D5.

Dermal

A subacute dermal toxicity study of D5 was conducted in rats. In this study, 10 male and 10 female Sprague-Dawley rats were treated with D5 dermally under occlusive conditions at dose levels of 0, 200, 800, and 1600 mg/kg bw/day. Treatments were for 6 hours per day, 7 days per week, for 28 days. A control and a test group, each consisting of five male and five female rats, were treated respectively with 0 and 1600 mg/kg bw and observed for 14 days after the treatment period for reversibility, persistence and delayed effects. Animals were observed for signs of local or systemic toxicity, general appearance, behavioural abnormalities and mortality. Food consumption and body weights were determined weekly. After 28 days, blood and urine samples were collected and the animals were sacrificed and examined for histopathological changes. No mortality, overt signs of toxicity or behavioural changes were noted in any of the groups. A comparison of mean body weight, food consumption or haematological data between control and test groups showed no treatment-related effects. The few statistically significant differences in clinical chemistry parameters between the control and test groups were within normal biological variation. No treatment-related effects were identified by histopathology at either the terminal or recovery sacrifices. Based on urinalysis, there was some evidence of dermal absorption and metabolism. Under the test conditions, dermal applications of D5 at a dose level of up to 1600 mg/kg bw did not produce significant toxicological effects.

Ref.: 9

Six male and female New Zealand white rabbits were treated 6 hrs/day, 7 days/wk for 21 consecutive days with 1000 mg D5/kg bw under occlusive conditions. The skin was abraded in one-half of the animals. Skin reactions were scored daily for signs of oedema and erythema. At necropsy, the heart, lungs, liver, kidneys, spleen, testes, epididymides, ovaries, and urinary bladder were weighed and preserved. No clinical signs of toxicity were observed. There was no effect on body weight, no mortality, no effect on organ weights, and no treatment related gross pathology findings. No signs of skin irritation were observed.

Ref.: 16

An unspecified number of male and female New Zealand white rabbits were exposed dermally to 0, 96, 288, or 960 mg D5/kg bw for 5 day/wk, for three weeks. The treatment period was followed by a two-week recovery period. Animals were observed for clinical signs of toxicity, mortality, and body weight changes. Haematology, clinical biochemistry, gross examination, and histopathology were performed. No effects were seen on clinical condition, survival, or body weight. No substance related findings were seen on gross examination or histopathology.

Ref.: 17

Conclusion
LOEL/NOEL for D5
Studies on repeated dose toxicity with two week oral application of D5 (Ref. 4) revealed a LOEL of about 100 mg/kg bw. The treatment related increases in absolute and relative liver weight in rats at 100 mg D5/kg bw can be considered as an adaptive response.
In studies on repeated dose toxicity with inhalation exposure a LOEL of 160 ppm was found for systemic effects of D5 on rat liver (increase in absolute and relative weights). The effect was reversible upon cessation of exposure.
A NOAEL of 1600 mg/kg bw was found in studies conducted in rats with dermal application of D5 up to 4 weeks.

3.4.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral

Male and female Wistar rats were administered 100, 330, or 1000 mg neat D5/kg bw daily for 13 weeks by gavage. Animals were observed for clinical signs, effects on body weight, food consumption and ophthalmologic effects. Complete necropsies were performed, selected organs weighed and selected tissues grossly and microscopically examined. No effects were seen on body weight or body weight gain or on food consumption. No effects were seen on blood or haematopoetic organs. Increases in liver weight in both males and females were seen at all dose levels. No significant effects related to D5 exposure were seen on histopathology.

Ref.: 10

Inhalation

Male and female Fischer 344 rats of both sexes were exposed by nose only inhalation to D5 6 hours/day, 5 days/week for 13 weeks. Each exposure concentration group had 20 male and female rats except for the control and highest exposure concentration groups, which contained 30 males and females each. Ten of the control and high exposure concentration male and female rats were used for a treatment-free recovery period of 4 weeks. The achieved test atmosphere concentrations, based upon analytical determination of the vapour phase, were 0, 28.6, 49.2, 87.7 or 233 ppm (0.44, 0.758, 1.35, or 3.59 mg D5/l, respectively). No mortality was observed in any of the treated or control groups and no clinical signs of toxicity were noted which were considered treatment-related.
Following the recovery phase, initial differences in body weight gains between 233 ppm and the control group diminished. Analysis of organ weight data indicated statistically significant increases in liver weight (relative and absolute) for the 49.2 and 87.7 ppm (female) and 233 ppm (female/male) groups after treatment. The most apparent clinical biochemistry findings included a dose-related increase in gamma-glutamyltransferase activity in female rats. Lung weights remained elevated in the 233-ppm group (female) after the recovery phase. Reductions in thymus, testis and ovary organ weights were observed after the recovery phase in the high exposure (233 ppm) group only. Possible treatment-related histopathological findings included an increased incidence of ovarian interstitial gland hyperplasia and vaginal mucosal mucification and atrophy in the female rats exposed to 233 ppm.
Slight, not statistically significant, ovaries and testes weight decrease was also observed.

Ref.: 11

Comment
The achieved levels of D5 were slightly higher than the target exposure levels (0, 26, 46 and 224 ppm) which have been quoted in previous assessments of D5 (Refs. AR12, AR13).

Two groups of ten male and ten female Sprague-Dawley rats each were exposed to D5 vapours at 1 and 120 ppm for six hours/day, seven days/week for 28 days. Four other groups were exposed to 0, 20, 59 and 119 ppm D5 in a similar regime for a period of 13 weeks. A control and test group consisting of 10 male and female rats each were exposed, respectively, to 0 and 120 ppm for 13 weeks and were observed for 28 days for reversibility, persistence or delayed toxic effects. The 120-ppm female 90-day terminal sacrifice group had a statistically significant increase in relative liver weight when compared to controls. The liver weight in females returned to normal values at the end of the recovery period. Under the same test conditions, D5 caused no biological or toxicological effect in male rats.

Ref.: 12

Conclusion
A subchronic (13 week) toxicity study with oral application of D5 revealed a LOEL of 100 mg/kg bw, based on liver weight increases in both male and female rats (Ref. 10).

In studies with 3 months inhalation exposure of D5 a LOEC of 49/46 ppm was found, based on effects on rat liver, i.e. an increase in liver weight and gamma-glutamyltransferase activity, and a decrease in LDH activity in female rats (Ref. 11).

3.4.5.3. Chronic (> 12 months) toxicity

See section 3.4.7. Carcinogenicity

3.4.6. Mutagenicity / Genotoxicity

3.4.6.1 Mutagenicity / Genotoxicity *in vitro*

D5 was evaluated for genetic activity in a battery of microbial assays and *in vitro* mammalian cell culture assays employing *Salmonella typhimurium* (TA-1535, TA-1537, TA-1538, TA-98, and TA-100) and *Escherichia coli* (W3110/polA+, P3478/polA-) indicator organisms and L5178Y mouse lymphoma cells, respectively. The test substance showed no mutagenic activity in the Ames bacterial test with and without S9 microsomal activation and was inactive in the mammalian cell culture system at concentrations up to 25 µl/ml.

Ref.: 31, 32

Bacterial Reverse Mutation Assay

The potential for D5 to induce gene mutations was further investigated using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100 and the *Escherichia coli* strain WP2 uvrA. The assay was performed in two independent experiments both with and without liver microsomal (S9-mix) activation. Each concentration, including controls, was tested in triplicate. D5 was tested at the following concentrations: 33, 100, 333, 1000, 2500, and 5000 µg/plate. The plates incubated wit D5 showed normal background growth up to 5000 µg/plate with and without metabolic activation in all strains used. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with or without metabolic activation. No substantial increase in revertant colony numbers in any of the five tester strains was observed following treatment with D5 at any dose level with or without metabolic activation. Under the experimental conditions of these studies, D5 did not induce gene mutations by base pair changes or frameshift mutations in the genome of the strains tested. Therefore, it is concluded that D5 is non-mutagenic.

Ref.: 33

In vitro Chromosomal Aberration Test

In another *in vitro* experiment, D5 was dissolved in ethanol and was assessed for its potential to induce structural chromosome aberrations using Chinese hamster V79 cells in the absence and presence of S9 metabolic activation. In each experimental group, two parallel cultures were set up. One hundred metaphase plates were scored for structural chromosome aberrations per culture. In the absence of the S9-mix in both experiments, toxic effects indicated by reduced cell numbers and/or mitotic indices of below 50% of control were observed. When the S9 fraction was present, there were no toxic effects seen on the cells. In both independent experiments, neither a statistically significant nor a biologically relevant increase in the number of cells carrying structural chromosomal aberration was observed after treatment with the test material. No increase in the frequencies of polyploid metaphases was found after treatment with the test material as compared to the frequencies of the controls. In conclusion, under the experimental conditions reported, the test material did not induce an increase in cells with structural chromosome aberrations as determined by the chromosome aberration test in Chinese hamster V79 cells *in vitro.* Therefore, D5 is considered to be non-clastogenic in this chromosome aberration test with and without metabolic activation when tested up to cytotoxic concentrations and up to the highest recommended concentrations.

Ref.: 34

3.4.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo UDS and Micronucleus Test

The mutagenic potential of D5 was assessed using *in vivo* Unscheduled DNA Synthesis (UDS) and micronucleus assays. The D5 was administered to Fischer male and female rats by whole body vapour inhalation. The animals were treated daily for 6 hours with 160 ppm of D5 for 7 consecutive days. Filtered air was used for the negative control group. The animals of the positive control groups were treated in the same way as the air control groups. However, following the last exposure they were treated orally (by gavage) with the corresponding positive control. 2-Acetylamino-fluorene (2-AAF) and cyclophosphamide were used as the positive controls for the UDS and micronucleus assays, respectively.

For analysis of DNA repair (UDS) in treated rat hepatocytes, the animals were killed five hours and 16 hours after the last treatment. Primary hepatocytes were obtained by liver perfusion and hepatocyte cultures were established and exposed for four hours to methyl-^{3}H-thymidime (^{3}HTdR), which was incorporated if UDS occurred. For each experimental group including the controls, hepatocytes from 6 treated animals per sex were assessed for the occurrence of UDS. The viability of the hepatocytes was not affected by the *in vivo* treatment with D5 and D5 did not cause UDS induction at any dose level in the hepatocytes as compared to concurrent air controls. Treatment with 2-AAF (100 mg/kg) revealed distinct increases in the number of nuclear and net grain counts.
Twenty-four hours after the last treatment, bone marrow cells of the respective animals were collected for micronucleus analysis. For each experimental group including controls, bone marrow cells from six treated animals per sex were assessed for the occurrence of micronuclei. The test material did not exert any cytotoxic effect. There was no biologically relevant or statistically significant enhancement in the frequency of detected micronuclei compared to air controls following treatment with D5. Cyclophosphamide (40 mg/kg) showed a substantial increase of induced micronucleus frequency, indicating the test system was sensitive and valid.

Exposure to D5 neither induced DNA damage leading to increased repair synthesis in the hepatocytes of treated rats nor induced micronuclei. Therefore, D5 is considered to be non-genotoxic in these assays.

Ref.: 35

Conclusion on mutagenicity
The negative results obtained in the bacterial reverse mutation assay, *in vitro* chromosomal aberrations in Chinese Hamster V79 cells, unscheduled DNA synthesis, and *in vivo* micronucleus assays indicate that D5 does not possess mutagenic or genotoxic potential.

3.4.7. Carcinogenicity / Chronic Toxicity

In a 24-month combined chronic/oncogenicity inhalation study, male and female Fischer 344 rats were exposed to vapour concentrations of 0, 10, 40, or 160 ppm D5 for 6 hr/day, 5 days/week, for up to 24 months. The study animals were divided into four groups (Table 8). Group A animals consisting of six animals per sex were exposed for six months and then sacrificed for the determination of the D5 concentration in liver, fat, and plasma. Group B, consisting of 10 animals per sex, were exposed to D5 for 12 months and then sacrificed. Group C animals, consisting of 20 animals per sex, were exposed to D5 for 12 months only and then observed for an additional 12 months to determine the possible reversibility of any effects. Group D animals, consisting of 60 animals per sex, were exposed to D5 for 24 months. Both group C and D animals were sacrificed at 24 months. All animals were monitored for mortality, clinical signs, food consumption and body weights. Clinical laboratory investigations included haematology, clinical biochemistry, and urinalysis at 3, 6, and 12 months. The lungs, liver, kidney, nasal cavity, gross lesions and tissue masses from all group B, C, and D animals were submitted for histological examination. A complete histopathology examination was performed on all tissues from the control and high dose group animals from groups B, C, and D as well as suspected target organ tissue from intermediate exposure level groups.

Ref.: 36

Table 8: D5 Combined Chronic/Oncogenicity Study Design

Exposure Concentration	**Subgroups**			
	A 6-month tissue level determination	B 12-month chronic toxicity group	C chronic toxicity recovery group	D oncogenicity group
0 ppm	6M/6F	10M/10F	20M/20F	60M/60F
10 ppm	6M/6F	10M/10F	20M/20F	60M/60F
30 ppm	6M/6F	10M/10F	20M/20F	60M/60F
160 ppm	6M/6F	10M/10F	20M/20F	60M/60F

Group A Results

Group A animals were used for the analysis of D5 levels in blood, fat, and liver in order to validate the PBPK model developed for D5. An increased incidence of hyaline inclusions in the nasal respiratory/olfactory epithelium was noted in male and female rats at 160 ppm. This finding was considered a non-specific treatment-related effect (changes consistent with chronic inhalation of a mild irritant).

Group B Results

No effects were seen at one year of exposure that could be related to D5.

Group C Results

Endometrial adenomatous polyps and adenocarcinomas were observed in animals exposed to D5 for one year followed by one year of recovery (Table 9). The incidence of endometrial

adenocarcinoma was 1, 1, 0 and 2 for female rats in the 0, 10, 40 and 160-ppm exposure groups. Endometrial adenomatous polyp was diagnosed in one female rat in the 160-ppm exposure group. Combining adenomatous polyps with the adenocarcinoma data, the combined incidence becomes 1, 1, 0 and 3 for female rats in the 0, 10, 40 and 160-ppm exposure groups, respectively. Uterine endometrial adenoma was not present in Group C female rats.

Peto's test showed there was no significant trend among the groups (p=0.4159) when all tumours were combined. Likewise, when the adenocarcinomas were analysed separately, there was no significant trend (p=0.8227). Fisher's Exact test showed there was no significant difference in the proportion of tumour occurrences among the groups (p=0.3867) when all tumours were combined or when the tumours were analysed separately (p=O.8988). The poly-3 test showed there was no significant trend among the groups when all tumours were combined (p=0.0580). When the adenocarcinoma tumours were analysed separately using the poly-3, there was no significant trend (p=0.1754).

Group D Results

An increased incidence of hyaline inclusions in the nasal respiratory/olfactory epithelium was noted in male and female rats at 160 ppm. This finding was considered a non-specific, treatment-related effect.
The incidence of endometrial adenocarcinoma in Group D was 0, 1, 0 and 5 for female rats in the 0, 10, 40 and 160-ppm exposure groups, respectively (Table 10). One female rat in the 0 and one female in the 40-ppm exposure groups, respectively, were diagnosed with endometrial adenomatous polyps. The combined tumour incidence for female rats in Group D was, therefore, 1, 2, 1 and 5 in the 0, 10, 40 and 160-ppm exposure groups, respectively.
For animals exposed for 2 years (Group D), Peto's test showed there was no significant trend among the groups when all tumours were combined (p=0.1314). When the adenocarcinomas were analysed separately, a significant trend was found ($p < 0.05$). Fisher's Exact test showed there was no significant difference in the proportion of tumour occurrences among the groups when all tumours were combined (p=0.3233). There was a significant difference when the adenocarcinoma tumours were analysed separately ($p< 0.05$). Analysis of the tumour incidence in females exposed for two years using the poly-3 test showed a significant trend among the groups when all tumours were combined ($p<0.05$) and when the adenocarcinomas were analysed separately ($p<0.001$).

Table 9: Group C Uterine Tumour Incidence after 12 months exposure to D5 plus 12 months recovery in air

Exposure Concentration	Endometrial Adenocarcinomas	Adenomatous Polyps *	Total
0 ppm	1/20	0/20	1/20
10 ppm	1/20	0/20	1/20
40 ppm	0/20	0/20	0/20
160 ppm	2/20	1/20	3/20

* significant positive trend test ($p<0.05$) by the Poly3 test

Table 10: Group D Uterine Tumour Incidence after 24 months exposure to D5

Exposure Concentration	Endometrial Adenocarcinomas [a,*]	Adenomatous Polyps	Endometrial Adenomas	Total
0 ppm	0/60	1/60	0/60	1/60
10 ppm	1/60	0/60	1/60	2/60
40 ppm	0/60	1/60	0/60	1/60
160 ppm	5/60	0/60	0/60	5/60

a significant positive trend test ($p<0.05$) by the Peto test
* significantly higher ($p<0.05$)than control by the Fisher's Exact Test

The study authors note that there was a complete lack of an increase in incidence or severity of uterine endometrial hyperplasia in Group B, C and D females. Endometrial hyperplasia is considered an essential precursor lesion commonly associated with uterine adenoma/carcinoma. Some hyperplasia was found in a later analysis of the pathology slides [Ref. AR5].

For uterine endometrial adenocarcinomas alone, the data show a statistically significant increase at a D5 exposure concentration of 160 ppm for two years. Work has been performed to address the potential mode of action for the formation of these tumours in this study. The results of this work are described in section 3.3.12 (special investigations).

Ref.: 36

Comment
Carcinogenicity of D5 was significant only at the highest concentration of 160 ppm. There is uncertainty whether the uterine tumours are relevant or are not relevant to humans. It is recognized that D5 may possibly act as a dopamine agonist, thus contributing to the observed tumourigenic effects in female rats. The applicant states that this mode of action is not relevant to humans (Ref.: 66). However, this position has not been adopted so far by the California OEHHA (2007) due to insufficient data for a thorough mode-of action analysis. The SCCS considered that there is insufficient data for this suggested neuroendocrine mode of action in rats to preclude a relevance for humans. The absence of a genotoxic potential supports the view that tumour formation is due to threshold effects.

3.4.8. Reproductive toxicity

3.4.8.1. One generation reproduction toxicity

A screening study was designed to determine the exposure levels appropriate for studying the potential adverse effects of D5 vapours on male and female reproduction in rats. The test article was administered via whole body inhalation to three groups each of 22 male and 22 female Sprague Dawley rats. Exposure levels were 26 or 132 ppm. A control group was exposed to clean, filtered air. The exposure period was 6 hours per day, 7 days/wk for a minimum of 28 days prior to mating and lasted until the day of necropsy for each animal, with the following exception: exposure of the females was suspended from gestation day 21 through lactation day 4. All animals were observed twice daily for appearance and behaviour. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were also recorded on gestation days 0, 7, 10, 14 and 20 as well as on lactation days 1, 4, 7, 14 and 21. Food consumption was measured for corresponding intervals prior to mating, during gestation and during lactation. All of the females were allowed to deliver and rear their pups to weaning on postnatal day 21 (PND 21). The offspring were euthanized on PND 28. The surviving dams were necropsied on lactation day 21. The males were necropsied after the breeding period. In these animals, reproductive parameters (fertility, mating, days between pairing and coitus, gestation and parturition), mean body weights, body weight gains and food consumption, mean numbers of implantation sites and mean live litter size were not adversely affected by test material exposure at exposure levels of 26 or 132 ppm. No exposure-related effects on pup viability throughout lactation and no exposure-related clinical signs were noted in the pups in either the 26 or 132-ppm groups. Pup sex ratios and mean pup weights were unaffected by exposure to the test material at any exposure level. No internal findings related to the test material were noted at either exposure level in females necropsied on post-mating day 25 (10). The NOAEL for this study was > 132 ppm.

Ref.: 25

3.4.8.2. Two generation inhalation reproduction and developmental neurotoxicity study

A 2-generation reproductive study was conducted with D5 to evaluate the potential adverse reproductive effects of whole-body vapour inhalation exposure of F0 and F1 animals to D5. Neonatal survival, growth, and development of the F1 and F2 generations were evaluated. The potential for D5 to cause functional and morphological changes to the nervous system of the developing F2 rats following exposure of the F0 and F1 generations was also evaluated. Groups of male and female Crl:CD®(SD)BR rats (30/sex/group) were exposed to D5 for six hours daily for at least 70 consecutive days prior to mating. Target test article concentrations were 30, 70 or 160 ppm. A control group of identical design was exposed to clean, filtered air on a comparable regimen. Exposure of the F0 and F1 males continued throughout mating and through the day prior to euthanasia. The F0 and F1 females were exposed throughout mating and through gestation day 20 at which time exposures were stopped. Exposures were re-initiated on lactation day 5 and continued through the day prior to euthanasia. All animals were observed twice daily for appearance and behaviour. All F0 and F1 females were allowed to deliver and rear their pups until weaning on lactation day 21. Offspring (30/sex/group) from the pairing of the F0 animals were selected to constitute the F1 generation. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F1 rats. Thirty pups/sex/group from the F2 generation were selected for development landmarks, neurobehavioral testing, neuropathology brain weights and/or brain dimension measurements. Surplus F1 and F2 pups were necropsied on PND 21 or 28, and selected organs were weighed. Selected F2 rats not allocated for neuropathology and brain dimension measurements were necropsied on PND 70; selected organs were weighed. All surviving F0 and F1 parental animals received a complete detailed gross necropsy following the completion of weaning of the F1 and F2 pups, respectively; selected organs were weighed. Spermatogenic evaluations were performed on all F0 and F1 males and ovarian primordial follicle and corpora lutea counts were recorded for F0 and F1 females in the control and high-exposure groups. Designated tissues from all F0 and F1 parental animals in the control and 160 ppm groups, from all parental animals that were found dead or euthanized *in extremis,* and from F2 pups selected for neuropathological evaluation were examined microscopically.

No clear exposure-response relationship for the mortalities was evident for the six F0 animals that died or were euthanized *in extremis* and no consistent target organ could be identified at the gross and microscopic examinations of these animals. The mortalities and moribundity of these F0 males and females were not attributed to test article exposure. All other F0 and all F1 parental animals survived to the scheduled necropsy. No exposure-related clinical signs were noted at any test article concentration.

Reproductive parameters (days between pairing and coitus, mating indices, fertility indices, duration of gestation, and parturition) in the F0 and F1 generations were not adversely affected by exposure to the test article. Mean weekly, gestation, and lactation body weights, body weight gains, and food consumption were not adversely affected by test article exposure at any concentration in the F0 and F1 generations. Functional observational battery (FOB) data (home cage, handling, and open field observations) for the F1 females revealed no exposure-related effects at the gestation day 10 and lactation day 20 evaluations.

No exposure-related gross internal findings were noted at any concentration in the F0 and F1 animals at the scheduled necropsy. No exposure-related differences in mean organ weight data (absolute and relative to final body weights and brain weights) were noted at any concentration in the F0 and F1 generations. F0 and F1 mean ovarian primordial follicle counts were unaffected by exposure in the 160 ppm group. Spermatogenic endpoints (testicular and epididymal sperm numbers, sperm production rate, sperm motility, and sperm morphology) were not affected by test article exposure at concentrations of 30, 70 or 160 ppm.

F1 and F2 mean live litter sizes, numbers of pups born, percentages of males per litter at birth, postnatal survival, and anogenital distances were not affected by parental exposure at any concentration. One F0 female in the 160-ppm group had total litter loss on lactation day 0. Because no exposure-related decreases in postnatal survival of the F1 and F2 litters were noted at any concentration, the single occurrence of total litter loss in the 160-ppm group was not attributed to D5 exposure. Mean pup body weights and the general physical condition of the F1 and F2 pups were similar in control, 30, 70 and 160 ppm groups both before and after weaning. Necropsy findings for the F1 and F2 pups that were found dead or euthanized *in extremis* were not suggestive of any correlation with parental exposure. At the scheduled necropsies of F1 and F2 surplus pups on PND 21 or 28, no gross internal findings or differences in mean organ weight data, which could be attributed to parental exposure, were noted at any concentration. F1 and F2 developmental landmarks (balanopreputial separation and vaginal patency) and F2 neurobehavioural responses (motor activity, startle response, Biel maze and FOB data) were not affected by parental exposure. At the PND 11 and PND70 neuropathological evaluations, no microscopic findings or differences in mean brain weights and brain measurements related to parental exposure were noted for any of the selected F2 rats. No gross internal findings or differences in mean brain weights, which could be attributed to parental exposure, were noted at the PND 70 necropsy of F2 rats not selected for neuropathological evaluation.

In conclusion, no parental toxicity in the F0 and F1 generations was seen at exposure concentrations of 30, 70 or 160 ppm. F0 and F1 reproductive performance was not affected at any concentration. No test-article-related total litter losses occurred. No neonatal toxicity was evident in the F1 and F2 generations at concentrations of 30, 70 or 160 ppm. No F2 developmental neurotoxicity was evident at any concentration. Based on the results of this study, the NOAEL (no-observed-adverse-effect level) for parental toxicity, reproductive toxicity, neonatal toxicity, and developmental neurotoxicity is considered to be 160 ppm.

Ref.: 26

Comment
Results of the 2-generation reproductive toxicity study were published recently in a peer reviewed journal (Ref.: AR4).
In line with the above description, the authors found no treatment-related gross findings or organ weight effects at the F0 and F1 necropsies, except for a 10% increase in liver weight at 160 ppm in F0 females, and minimal alveolar histiocytosis in all exposed groups. No significant changes between D5-treated and control groups were noted in reproductive parameters in the F0 and F1 parental animals. Mean live litter sizes, number of pups born, sex ratios, pup body weights, postnatal pup survival, and the general physical condition of offspring in each generation were not affected. There was a slight, but statistically significant, increase in the mean F1 male pup anogenital distance (AGD; was not measured in F1 male pups exposed to 30 and 70 ppm D5). An increase in male pup anogenital distance may indicate an anti-estrogenic or androgenic effect. Yet, other studies (see section 3.3.12) failed to show such hormonal activity for D5. Vaginal patency and balanopreputial separation were unchanged compared to controls. The authors suggested a NOAEL of 160 ppm D5 for parental and reproductive toxicity.
Conclusion
For developmental and reproductive toxicity a NOAEL of 160 ppm D5 can be derived from the two-generation study.

3.4.8.3. Teratogenicity

Prenatal developmental study

See above section 3.4.8.2

3.4.9. Toxicokinetics

Absorption and Distribution

In an exploratory study to determine the time points and dosing for a definitive study, 48 female and 3 male F344 rats were exposed to 160 ppm ^{14}C-D5 via nose only inhalation for a single six-hour time period. An additional 3 female rats were used as controls to establish background matrix effects on measurement of radioactivity. Body burden animals were counted *in toto*. Pelts and carcasses were counted separately. Blood, plasma, and/or selected tissues were collected at 1.5, 3, and 4.5 hours during exposure and at 8 time points post-exposure. Expired air, urine and faeces were collected at specified intervals during a 168-hour period post-exposure. The mean achieved dose was 88 ± 2 µCi and the mean body burden dose was 2 ± 0.6 µCi (approximately 3% of the achieved dose). A mean of 97 ± 26% of the body burden dose was recovered from the mass balance group. Plasma toxic kinetic values were calculated: $t_{½}$ = 58.9 hours; AUC = 77 µg x hr/gm; t_{max} = 0 hr post-exposure and C_{max} = 3.39 µg/ml.

Ref.: 27

The disposition of D5 in male and female Fischer 344 rats following single or multiple inhalation exposures was evaluated. Animals were administered a single 6 hour nose-only exposure to 7 or 160 ppm ^{14}C-D5 or fourteen 6-hour nose-only exposures to unlabeled D5 followed on the 15th day by a 6-hour exposure to ^{14}C-D5. Subgroups of exposed animals were established to evaluate body burden, distribution, and elimination. Samples of plasma, fat, liver, lung, faeces and expired air were also processed for parent D5 analysis. Retention of D5 following single exposures was relatively low (~4-5% of inhaled D5), with ~8-10% retained following multiple exposures. Approximately 50-80% of this retained dose was attributed to deposition on the fur for males and ~60-70% for the females. Parent compound and radioactivity were widely distributed to tissues of both males and females, with maximum concentrations observed in the majority of the tissues by 3 hours post-exposure. D5 was distributed to fat, with elimination of parent and radioactivity occurring slower compared to plasma and other tissues.

Ref.: 28, AR6

Elimination

The elimination of D5 from male and female Fischer 344 rats following single and multiple inhalation exposures was evaluated. Elimination of retained radioactivity in urine (~12%) and faeces (~16%) was similar for both sexes following all exposures. The radioactivity in exhaled air was similar for both sexes following multiple exposures and females following single exposure (~45%) with significantly higher amounts for the males following a single exposure (~72%). In the plasma, liver and lung, the majority of radioactivity immediately following exposure could be attributed to parent, with this decreasing over time to a small fraction attributable to parent from 24 to 168 hours post exposure. In the urine samples, several peaks were present, but none corresponded to the retention time of parent D5. In contrast, the major peak found in the faeces corresponded to the retention time for parent D5.

Ref.: 28, AR6

Metabolism

The metabolic profile of D5 in rats was obtained using a high-pressure liquid chromatography (HPLC) system equipped with a radioisotope detector. The HPLC chromatogram revealed two major metabolites and five minor metabolites in the urine of female rats administered ^{14}C-D5 orally. The two major metabolites were dimethylsilanediol [$Me_2Si(OH)_2$] and methylsilanetriol [$MeSi(OH)_3$]. No parent D5 was found in the urine. The minor metabolites were identified as: [$MeSi(OH)_2$-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 [$Me_2Si(OH)$-O-$SiMe_2$-$OSi(OH)Me_2$].

In addition, the presence of D4D'OH and D4D'CH_2OH also were detected in the urine using GC-MS. The formation of D4D'OH and $MeSi(OH)_3$ clearly shows demethylation at the silicon-methyl bonds.

Ref.: 29, 30

Comment
Results on the metabolism of D5 in Fisher 344 rats have been published also in a peer reviewed journal (AR7). The authors identified at least ten metabolites by GC-MS analysis (see above), and report that D5 metabolism in rats is extensive, with no parent compound detected in urine.

Bioaccumulation

A concern with lipophilic compounds is the possibility of bioaccumulation. Usually, this occurs with compounds that are both lipophilic and very slowly cleared from the body. While D5 is very lipophilic with fat:blood partition coefficients between 500 and 1000, it also has high clearance by both metabolism and exhalation following all routes of exposure. The possibility of bioaccumulation of this compound has been tested by evaluating blood and tissue concentrations of D5 after long-term inhalation exposures. In toxicity studies, rats were exposed 5 days per week for 6 months and killed immediately after the last day of exposure (group A animals of the studies described in section 3.4.7). D5 levels in plasma and fat increased with exposure levels (10-160 ppm). D5 reached 2.2 and 3.19 µg/ml in plasma of male and female rats at the highest dose, and about ten-fold higher levels in the fat of male rats. In the fat of females, the levels were three to six times higher than those in male rats.

Ref.: 36

The PBPK model developed based on the 1 and 15 day exposure studies (Ref. 28; AR#6) successfully predicted tissue levels in the 6-month group. No appreciable increase in any tissue was predicted between the 15-day exposures and the 6-month exposure period, and none was observed. The 6-month fat concentrations were actually somewhat lower than the 15-day exposures because of the different exposure regimens, i.e., every day versus 5 days per week. Because D5 is rapidly eliminated by pulmonary and metabolic clearance, tissue concentrations, even in fat, do not increase with repeated exposures.

For more information on PBPK models developed to predict D5 kinetics for dermal, inhalation and oral exposure see section 3.4.12. (special investigations).

3.4.10. Photo-induced toxicity

Siloxanes (such as D5) contain only methyl groups, which have no double bonds and do not absorb ultra violet (UV) light. Consequently, no phototoxicity studies have been performed.

3.4.11. Human data

Studies with D5 which involved humans are described above in section 3.4.3. (HRIPT, Ref. 19) and 3.4.4. (*in vivo* percutaneous absorption, Ref. 24).

These data obtained from humans subjects at the University of Rochester were used in Pharmacokinetic modelling and are described below in section 3.4.12 (Ref. 61).

3.4.12. Special investigations

Immunotoxicology

In order to assess the potential immunomodulatory consequences of inhalation exposure to D5, male and female Fischer 344 (F344) rats (25/group) were exposed by whole body vapour inhalation to 0, 10, 25, 75, or 160 ppm of D5, 6 hours/day, for 28 days. Clinical signs, body

weights, and food consumption were recorded. On the day following the final exposure, 10 rats/group/sex were euthanized and a complete necropsy performed. Following a 14-day non-exposure recovery period, the remaining 5 rats/group/sex were necropsied. Body and organ weights were obtained and a complete set of tissues taken for histopathology. Samples were also collected for serum chemistry, haematology, and urinalysis. Immunotoxicology-designated rats (10/sex/group) were immunized with sheep erythrocytes (sRBC) 4 days prior to euthanasia and cyclophosphamide was administered i.p. to positive controls on days 24 through 28. The anti-sRBC antibody-forming cell (AFC) response was evaluated in a standard plaque assay. Blood was also collected for examination in the anti-sRBC enzyme-linked immunosorbant assay (ELISA). D5 inhalation exposure did not alter humoral immunity and caused only minor, transient changes in haematological, serum chemistry, and organ weight values. Histopathological changes were confined to the respiratory tract and appeared to be reversible.

Ref.: 37

Hepatomegaly and Enzyme Induction

A number of studies conducted have shown that D5 causes a reversible hepatomegaly in male and female rats following oral administration by gavage or inhalation exposure. Studies have been conducted to examine this effect.

Female Fischer 344 rats were exposed by whole-body vapour inhalation to either 0 or 160 ppm D5, six hours/day, five days/week for 28 days. Changes in the activity and relative abundance of hepatic microsomal CYPs (CYP1A, CYP2B, CYP3A and CYP4A), EH, and UDP-glucuronosyltransferase (UDPGT) were measured. Repeated inhalation exposure of rats to D5 increased liver size by 16% relative to controls by day 28. During a 14-day post-exposure period, liver size in D5-exposed animals showed significant recovery. Exposure to D5 did not change total hepatic CYP, but increased the activity of NADPH-cytochrome *c* reductase by 1.4 fold. An evaluation of CYPs in hepatic microsomes prepared from D5-exposed rats revealed a slight (1.8-fold) increase in 7-ethoxyresorufin O-deethylase (EROD) activity, but no change in immunoreactive CYP1A1/2 protein. A moderate increase (4.2-fold) in both 7-pentoxyresorufin 0-depentylase (PROD) activity and immunoreactive CYP2B1/2 protein (3.3-fold) was observed. Testosterone 6ß-hydroxylase activity was also increased (2.4-fold), as was CYP3A1/2 immunoreactive protein. Although a small increase in 11- and 12-hydroxylation of lauric acid was detected, no change in immunoreactive CYP4A levels was measured. Liver mEH activity and immunoreactive protein was increased 1.7- and 1.4-fold, respectively, in the D5-exposed group. UDPGT activity toward chloramphenicol was elevated 1.8-fold, while no change in UDPGT activity toward 4-nitrophenol was seen. These results suggest that the profile for enzyme induction following inhalation exposure of female Fischer 344 rats to D5 vapours is qualitatively similar to that reported for phenobarbital, and therefore, D5 may be considered as a weak "phenobarbital-like" inducer.

Ref.: 38

Groups of 3-4 rats per sex were administered D5 in corn oil by gavage at dose levels of 0 (control), 1, 5, 20 or 100 mg/kg for 4 consecutive days. Positive control animals received 50 mg/kg phenobarbital by intraperitoneal injection for 4 days. At the end of each experiment the liver was removed, weighed, homogenized and microsomes prepared. The activities of 7-pentoxyresorufin O-depentylase (PROD) and 7-ethoxyresorufin O-deethylase (EROD) were determined. Immuno-chemical analysis was performed using different anti-CYP polyclonal antibodies to determine CYP1A1/2, CYP2B1/2, CYP3A1/2, NADPH cytochrome P-450 reductase. Relative liver weight was increased in females at 20 and 100 mg/kg and in males at 100 mg/kg. CYP2B1/2 immunoreactive protein was significantly increased at 5 mg/kg and above as was PROD activity in females (males at 20 and 100 mg/kg). EROD activity was increased in males and females at 5 mg/kg and above (however, no changes were detected in CYP1A1/2 immunoreactive protein in rats of either sex). CYP3A1/2 immunoreactive protein was significantly increased in males at 100 mg/kg and females at 5 mg/kg and above. NADPH cytochrome P450 reductase immunoreactive protein was significantly induced at ≥ 5

mg/kg in males and ≥ 20 mg/kg in females, induction pattern was similar to that observed with phenobarbital.

Ref.: 39

Human liver microsomes from a pool of seven individuals were incubated with marker substrates in the presence or absence of D5 at concentrations ranging from 0.040 to 3.5 µM. In addition, D5 was evaluated for its ability to function as a metabolism-dependent reversible or irreversible inhibitor. For comparison, D5 was also evaluated for its ability to inhibit CYP1A1/2 and CYP2B1/2 in liver microsomes from rats treated with 3-methylcholanthrene and phenobarbital, respectively. D5 does not appear to be a 2B1 inhibitor. D5 appears to be a strong reversible metabolism-dependent inhibitor of rat CYP1A1/2. D5 appears to be a weak competitive inhibitor of human CYP3A4/5 and a strong metabolism-dependent inhibitor of human CYP3A4/5. D5 has little or no capacity to inhibit rat CYP1A1/2 and human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP4A9/11 activity in a reversible metabolism-independent manner. D5 has little or no capacity to function as a metabolism-dependent inhibitor of rat CYP2B1/2, human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP4A9/11 activity.

Ref.: 40

Estrogenicity

In order to assess whether the adenocarcinomas seen in the two-year combined chronic / carcino-genicity study could be due to direct estrogenic effects, D5 was evaluated in a rat uterotrophic assay using both ovariectomized Sprague-Dawley and Fischer 344 rats that were exposed to the highest achievable vapour concentration of D5 (160 ppm) *via* whole body inhalation for 16 hours/day for 3 days. Immediately following exposure animals were euthanized and the effect of test article on uterine weight (wet and blotted) was evaluated. Exposure to D5 did not result in an increase in any of the estrogenic endpoints measured in either strain of rat. Subsequently, D5 was evaluated for its ability to bind to the oestrogen receptor alpha. In a typical competition experiment, D5 (160 ppm) did not displace any of the estradiol indicating that this material does not compete for the receptor-binding site. In an *in vitro* luciferase reporter gene assay using MCF-7 cells transiently transfected with a plasmid for estrogen receptor alpha and the luciferase gene, D5 did not activate the reporter gene at 10 µM, while 17β-estradiol resulted in the expected increase (35-fold increase at 10 nM dose range). Using *in vitro* and *in vivo* assays, D5 did not elicit any estrogenic responses.

Ref.: 41, 42, published in Ref. AR8

Studies in mice dosed orally with either D5 or D4 (100 up to 1000 mg/kg bw) revealed uterotrophic/estrogenic activity for D4; in contrast D5 was not estrogenic in this assay.

Ref.: AR9

Dopamine Agonism

To assess whether D5 could affect prolactin secretion through dopamine agonism, both *in vitro* and *in vivo* studies were performed. Utilizing an *in vitro* cell line, derived from a rat pituitary tumour (MMQ), 10 µM of D5 was shown to decrease maitotoxin-induced prolactin release by 55% without affecting viability of the cell line. In a short-term *in vivo* study, F344 rats were pre-treated with reserpine, which causes a reduction in dopamine, which, in turn, causes prolactin levels to increase. Animals were exposed via nose-only for six hours to 160-ppm of D5 or air (controls). Blood samples were collected immediately at the end of the six-hour exposure and analysed for prolactin. In this study, serum prolactin levels in the reserpine treated controls were six fold greater than in the untreated controls. Exposure to 360-ppm of D5 caused a 50% decrease in serum prolactin levels relative to the reserpine treated control group, in phase 2 of this study, F344 rats were pre-treated with reserpine and then administered 6 mg sulpiride/kg body weight prior to a six-hour exposure to 160-ppm of D5. Sulpiride is a highly specific dopamine receptor antagonist. At the end of the six-hour exposure, blood samples were collected immediately for prolactin measurements. In this

study, sulpiride pre-treatment blocked the effect of D5 on prolactin levels. This indicates that D5 acts on the pituitary as a dopamine receptor agonist *in vivo.*

Ref.: 43 (Abstract)

Comment
A related study (Ref. AR10, Dow Corning 2005b) was not submitted to SCCP/SCCS. The OEHHA (2007) pointed out that some desirable controls were missing and that no attempt to determine a dose-response relationship was reported (Ref.: AR12).

Physiologically Based Pharmacokinetic Modelling

A comprehensive pharmacokinetic data set was developed on D5 using both single and repeated inhalation exposures to D5 at concentrations up to 160 ppm. A single one-hour inhalation exposure to 10 ppm D5 in humans also was conducted, as were both *in vitro* and *in vivo* percutaneous absorption studies. These data were used to develop PBPK models.

From the point of view of absorption and retention of D5 in the body, there are three important attributes that control the disposition of this compound. First, it is a volatile compound with a blood:air partition coefficient (Pb) between 0.3 and 1.0. When Pb is 1.0, half of the free D5 in venous blood entering the lung would be eliminated by exhalation. Secondly, it is very soluble in fat. In PBPK models the parameter that determines the relative fat:blood distribution is the fat: blood partition coefficient (Pf). For this compound, Pf is somewhere between 600 and 1000, i.e., highly partitioned into lipids in the body. Lastly, it is rapidly cleared by metabolism, possessing high hepatic clearance. In a single pass through the liver, 60 to 90% of the free D5 in the blood is removed by metabolism and eliminated via polar metabolites in the urine. Thus, the pharmacokinetics of this compound is heavily influenced by an unusual set of properties, high lipid partitioning coupled with very high blood clearance due to exhalation and metabolism.

In addition, the pharmacokinetics is influenced by the ability of D5 to be retained in blood lipids and by unusual differences in kinetic behaviour for different dosing routes. Inhalation and dermal absorption lead to similar pharmacokinetics, presumably since they provide uptake of molecular forms of the D5. Oral dosing leads to more complex pharmacokinetics that appears to be associated with uptake of D5 as a micro-emulsion. This behaviour is likely a reflection of the low water solubility of D5. The overview here focuses on three aspects of D5 pharmacokinetics. The first section describes inhalation and dermal dosing routes. The second outlines the differences noted with oral dosing with relatively high doses of D5 in gavage dosing studies. Lastly, it is noted that the combination of properties - high metabolic clearance and low blood:air partitioning - serve to prevent significant bioaccumulation of this compounds despite its tendency to be stored in lipids within the body following all routes of exposure.

<u>Inhalation Exposure</u>

In 6-hr inhalation studies, blood levels of D5 climb rapidly and reach a plateau within a relatively short time. Although the blood concentrations do not vary very much, fat loading continues throughout the 6 hour exposure. Only a relatively small amount of the total D5 inhaled is retained, about 10% for a 6 hr exposure and correspondingly smaller percentages for longer exposure durations. After the major tissues reach steady state, the continued uptake of inhaled compound would be related to metabolism or continued distribution into fat.

Following inhalation exposure, the measured D5 in blood appears to exist in two pools, a free pool available for exhalation and metabolic clearance and a sequestered pool, believed to be associated with blood lipids, that is unavailable for these processes. During inhalation, almost all D5 in the blood would be in the free pool. Many hours after cessation of exposure when blood concentrations have fallen by several orders of magnitude from their peaks, the proportion of total in a bound or sequestered pool becomes much greater.

PBPK models have also been developed using data obtained from human subjects at the University of Rochester (Ref. 61). While these data sets are more limited than those available for rats, the kinetics in humans is controlled by similar processes and the same conclusions about uptake, tissue loading and blood sequestration of a lipid bound pool appear equally valid. Although not directly validated by any direct measurements, the time required to approach steady levels for fat is expected to be 3 or 4 times longer than in the rats. However, the same concentrations would be reached in each species for a given exposure regimen in relation to daily exposure patterns (See section 3.4.9 on bioaccumulation).

Ref. 61 and AR11

Dermal Exposure

During dermal exposures to D5, this chemical is rapidly absorbed into the outer layers of skin, but it evaporates back out of the skin before significant systemic absorption can occur. *In vivo* rat dermal absorption studies showed that ~90% of D5 volatilizes from the skin surface and that the remaining D5 in the skin at the end of the 24 hr dosing period actually migrates to the skin surface and continues to evaporate, significantly decreasing the amount remaining in the skin to 0.03% D5 (Refs. 63, 64). The results of rat or human *in vivo* dermal absorption studies are also consistent with human *in vitro* dermal absorption studies (Ref. 65). Using human cadaver abdominal skin, only a small amount of the D5 applied to the skin is actually absorbed (~0.04%). PBPK modelling of the percutaneous absorption data from an *in vivo* human dermal absorption study also predicts dermal absorption of D5 to be about 0.05% respectively (Ref. 62). Of the amount systemically absorbed, ~90% of D5 is exhaled unchanged. This result appeared contradictory to those obtained for inhalation in that most of the D5 eliminated from the body after a 6 hour inhalation exposure was as metabolite(s). The PBPK model for skin absorption (Ref. 62) clarified this discrepancy, showing that all the processes were in fact consistent between inhalation and dermal exposures. Dermal exposure traces the uptake of discrete amounts of absorbed D5, showing that most of any amount taken up into the body is removed by exhalation with a significant first-pass loss from lung due to venous blood return directly to the pulmonary circulation for oxygenation. The inhalation exposures integrate processes occurring over the 6 hour period providing for retention of metabolite during the 6 hour period. As with inhalation exposures, the same conclusions hold for D5 and the PBPK model for humans requires the same structure to simulate absorption, distribution and elimination of these compounds in human subjects as were required in laboratory studies with rats.

Ref. 62 – 65 and AR2

Oral Dosing Routes

Pharmacokinetic studies have also been completed for rats dosed by gavage with D5 dissolved in corn oil. Mass balance of systemic radioactivity indicated ~80% of the administered dose was excreted unchanged in the faeces. In addition, of the ~20% that was absorbed 50-60% was eliminated as unchanged D5 in exhaled air and ~20% as water soluble metabolites in urine. However, the kinetics and tissue distribution observed after this dose route were qualitatively different from the distributions after inhalation or dermal exposures. Higher concentrations were noted in liver and spleen than seen previously via the inhalation and dermal route. The distribution and kinetics differed significantly from predictions of the PBPK model that successfully described the inhalation and dermal exposure routes. The dose route differences are most consistent with complications that arise from different forms of D5 that reach the blood. It appears that the oral route delivers micro-emulsions that do not readily dissolve into plasma and blood. These micro-emulsions would be removed from the circulation initially by actions of cells of the reticuloendothelium system, in liver (where it will be readily metabolized) and spleen. After the oral dosing, it is likely that uptake may be more associated with lipid transport, such as chylomicron formation and thus may not be completely available for tissue interactions as free material. In contrast, the kinetics

and tissue distribution with preferential uptake into fat and body lipids that follows inhalation and dermal uptake indicate that the D5 is absorbed as a free molecule rather than some aggregate of D5 and dosing vehicle.

The differences seen after the oral dosing studies compared to inhalation and dermal dose studies suggested a much higher persistence in blood for oral dosing. However, this apparent persistence is most likely due to that fraction of D5 in a pool that is unavailable to interact with tissues and represents the deep blood compartment required to describe all routes of administration. These dose route differences argue that the oral dose route may have little relevance for assessing risks of D5 and results from oral studies using oral gavage in corn oil have to be used with caution for drawing broad conclusions about safety or risks arising from more common routes of exposure or from oral dosing studies with much lower doses associated or mixed with feed.

Comment
PBPK models for the cyclomethicone components D5 and D4 have been under development for several years (Refs. 61 – 65), and have been published now in peer-reviewed journals [Ref.: AR2, AR11]

PBPK Summary

The pharmacokinetic properties of D5 have been well documented over the past 10 years and can be described with comprehensive multi-dose-route, multi-species PBPK models. The pharmacokinetics of this compound is heavily influenced by an unusual set of properties, high lipid partitioning coupled with very high blood clearance due to exhalation and metabolism. The more unusual characteristic with this compound is the qualitative differences for inhalation/dermal routes versus oral route. The former appear to involve absorption of molecular forms of D5 and the latter appears to involve absorption of micro-emulsions or chylomicrons.

3.4.13. References for D5 (section 3.4)

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3.5. Discussion

3.5.1. Discussion on D4 (Octamethylcyclotetrasiloxane; Cyclotetrasiloxane)

The reference numbers given in this chapter refer to the D4 references listed in chapter 3.3.13.

Physico-chemical properties
Octamethylcyclopentasiloxane (D4) is a clear, colourless synthetically derived silicon-based fluid with a molecular weight of 296 Daltons. It is used in cosmetics with a wide range of concentrations as well as in a variety of other applications.

Skin/eye irritation and sensitisation
According to rather old non-GLP studies, cyclomethicone appears not to be irritant to skin or mucous membranes. A GLP study performed according to OECD guideline demonstrated that cyclomethicone is not a skin sensitiser.

Dermal absorption
Several *in vivo* and percutaneous absorption studies with D4 have been performed with some variation in results. In vitro an average of 0.5% of octamethyltetracyclosiloxane (D4, applied neat or in antiperspirant formulation containing 62% (w/w) D4 was absorbed in human cadaver skin and the receptor fluid after 24 h of exposure, with dermal absorption of 0.94% of the applied dose as an upper value (Ref. 19). Lower values were found in an in vitro study with pig skin, where dermal absorption of ^{14}C-D4 was maximum 0.05% from the three formulations and different concentrations tested (Ref. 80). Similar to in vitro studies with human or pig skin, also the *in vivo* rat study demonstrated that the majority (~90%) of D4 applied volatilized from the skin surface before being absorbed. On average less than 1.0% of applied D4 appeared to be absorbed *in vivo,* with the majority remaining in the skin. Consistent with *in vitro* results for human cadaver skin, pharmacokinetic modelling of dermal absorption in human volunteers (AR 13) indicated that 0.12% and 0.30% of applied D4 was absorbed into systemic circulation for men and women, respectively.

A value of 0.5% for dermal absorption of D4 is taken for the safety assessment of D4.

Pharmacokinetics
Radiolabelled octamethyltetracyclosiloxane (D4) is rapidly absorbed orally when administered in corn oil, with tissue levels generally following plasma levels over time. The same pattern of disposition of radioactivity was seen with neat D4 or Simethicone, but the oral absorption and transit times in gastrointestinal tract were altered. This study indicated that the oral absorption of D4 can be significantly influenced by the vehicle/carrier used to deliver D4.
When inhaled, approximately 5% D4 in rat and 12% D4 in humans is absorbed. High D4 levels were found in lung tissue and fat as compared to other tissues. This could be expected, as D4 is lipid soluble and would preferentially deposit in fat and highly lipophilic tissues. There is evidence that D4 accumulates in adipose tissue; the toxicological relevance of this is unknown.
Pharmacokinetics as well as PBPK modelling has revealed that 80% of the systemically available dose is exhaled. Possibly a certain percentage of the dermally absorbed dose is eliminated in exhaled air as the venous blood passes through the lung. This can lead to a reduction in the amount of D4 in the arterial blood delivered to a target organ and may result in a somewhat lower actual systemic dose than calculated on the basis of dermal absorption alone.

Mutagenicity
The negative results obtained in bacteria (reverse mutation assay) or in mammalian cells, i.e. *in vitro* chromosomal aberration and SCE test, along with an *in vivo* micronucleus assay and dominant lethal test, indicate that D5 does not possess mutagenic or genotoxic potential.

Toxicity
D4 has been extensively evaluated for its safety in a full range of toxicity studies by a number of routes of exposure. The results of these studies show that D4 has a very low acute oral, inhalation and dermal toxicity. Repeated dose studies at relatively high oral and inhalation doses revealed few systemic effects. In repeated-dose studies, clinical signs of toxicity were minimal and the histological changes observed were reversible. One consistent finding was a reversible liver enlargement (hypertrophy) and phenobarbital-like increases in xenobiotic metabolising enzyme activities.
D4 was a mild irritant to the respiratory tract when inhaled. A major adverse effect seen with D4 has been evidence of reproductive toxicity in rats. These effects consist of reductions in corpora lutea, implantation sites and number of pups born to dams exposed to high concentrations of D4, and are all inter-related. Mechanistic research in female rats indicates that suppression of the preovulatory luteinizing hormone surge could cause the delayed ovulation stage and reduced fertility (see below).

For D4, appropriate pivotal studies to define a meaningful NOAEL are considered to be the three 90-day repeat-dose studies in rats (Ref. 13, 14, 15) together with the reproduction toxicity studies in rats (Ref. 34; AR 14) and a combined chronic/carcinogenicity study (Ref.: 37; AR 4). These inhalation studies were conducted with the 90-day study in Sprague Dawley rats (Ref. 14) and the two-generation study in SD rats (Ref. 34) using 7 days per week exposure to D4, and 5 days per week in the chronic 2 year study (Ref.: 37; AR 4). Data from other animal species (mice, guinea pig, rabbit, hamster) and other routes of administration (dermal, oral) are insufficient and/or inappropriate for D4 risk assessment.

In the pivotal studies, the NOAELs were largely determined by the three organ toxicities, which have morphological counterparts i.e. liver, respiratory tract and ovary. The NOAELs for general and reproduction toxicity in the 90-day repeat-dose studies in rats and the two-generation reproduction toxicity study in rats can reasonably be set at 300 ppm. The results of the other repeated-dose toxicity studies (up to 13 weeks duration) and the one-generation reproduction toxicity studies do not conflict with this conclusion regarding reprotoxicity. Yet, the number of corpora lutea was decreased at 300 ppm, and increases in

liver and kidney weights were also noted at 300 ppm in the latter study. Thus, 300 ppm could be considered a LOEC rather than a NOAEL.

The main toxicities are viewed differently in terms of their importance in safety assessment and in setting NOAELs as discussed below.

The ***liver weight*** increase with centrilobular hepatocyte hypertrophy has been attributed to a "phenobarbital-like" induction of rat hepatic CYP enzymes (Ref. 57, 58). This change was reversible (Ref. 13, 14, 15) and was not associated with overt hepatotoxicity, i.e. morphological evidence of necrosis/degeneration or increases in hepatic serum enzymes. Mild enzyme induction is considered to be an adaptive response to xenobiotics and has no significant impact on determining NOAELs or on human risk assessment. Indeed, D4 in the 24-month combined chronic/oncogenicity inhalation rat study (doses 0, 10, 30, 150 or 700 ppm) did not induce hepatic tumours but hepatic hypertrophy (see Section 3.3.7).

The ***respiratory tract*** changes in the nose and lungs are considered to be adaptive responses to a mild, non-specific irritant. Again, this change was reversible (Ref. 15). Considering that rats were exposed to D4 for 6 hours/day for at least 5 days/week for the duration of the various studies, the magnitude of the response was minute. When one further considers that the application route for cosmetics is primarily dermal, then rat respiratory tract changes can be largely discounted in determining NOAELs for safe human use of D4. In the 24-month combined chronic/oncogenicity inhalation rat study, D4 appears to be devoid of respiratory tract effects.

Exposure to D4 produced some effects on the ***thymus***. Few studies in the provided dossier examined the thymus. The results were equivocal; seeming not to be species or sex related and occurred with oral and inhalation exposure in rat and rabbit. In two short term (28 day) studies, reduced thymus weights were marked (Ref. 10, 15). In the 2-generation rat study, no significant changes were noted in F1 and F2a animals (Ref. 34). In nose only inhalation pharmacokinetic studies in rats (Ref. 36, 38), the AUC and C_{max} values were highest in fat, mid range in liver, thymus, lungs and nasal mucosa, and lowest in the reproductive organs. Elimination was relatively slow in these tissues ($t_{1/2}$ >168 h). No immunological effects in humans were seen in the study provided (Ref. 56). It would be interesting to know if there is more data as this could be a possible indicator of altered immune response.

The mechanism for delayed ovulation status resulting in reduced ***fertility*** has been studied. The observed changes were shown to be reversible (Ref. 32, 33). Other studies have shown that D4 has very weak estrogenic and anti-estrogenic activity in a Rat Uterotrophic Assay in both immature Sprague Dawley and F344 rats. D4 was 77,000 to 25 million times less potent than ethinyloestradiol or diethylstilbestrol in Sprague Dawley or Fischer 344 rats (Ref. 69). Many observations in the reproductive studies (Ref. 27 – 32) are inconsistent with a significant estrogenic and anti-estrogenic activity, thus indicating the very weak hormonal potency of D4. In a series of studies in mice, in which D4 was administered orally, D4 significantly reduced serum estradiol levels (Ref. 70). On the other hand, uterine peroxidase activity, a marker for estrogenic activity, and uterine weights were significantly increased. The uterotrophic effects of D4 were ablated by pre-treatment with ICI 182,780, an estrogenic receptor (ER) antagonist, and ovariectomised αER knock-out mice showed no increases in uterine weights when treated with D4. It can be concluded that D4 exhibits estrogenic activity in mice via ERα, probably by direct receptor binding. The data also indicate that the stimulatory effect of D4 on the uterus is not related to (endogenous) estradiol activity.

Rather than a direct (ER) receptor mediated mechanism, an indirect mode of action appears to be more relevant for explaining the reproductive toxicity and carcinogenicity of D4 observed at high doses: There are data in rats indicating that D4 can cause a delay or blockage of the luteinizing hormone (LH) surge necessary for optimal timing of ovulation. Barbiturates given during a critical time period, which is about seven to eight hours before the LH release on the day of proestrus, can block or delay the LH surge and delay ovulation

for 24 hours (Ref. 71). The extent of the decrease in ovulation is time- (Ref. 71, 72) and dose-dependent (Ref. 72, 73). Repeated administration of barbiturates during this critical period on subsequent days continues to suppress the LH surge and consequently ovulation. A similar mechanism could provide an explanation for the reduced fertility rate encountered in the female groups exposed to D4 for one to three days prior to mating (Ref. 33). Initial support for this mechanism was obtained by exposing ovariectomized Sprague Dawley rats with a subcutaneous implant of estradiol to inhaled D4. Several animals had reduced LH levels at the peak time (Ref. 74). In a well designed follow-up study, definitive support of the role of LH was obtained from a study of estrous cycle staged female Sprague Dawley rats exposed to 700 or 900 ppm D4 for 6h/day for 3 days, i.e. on diestrus 1, diestrus 2, and pro-oestrus. Measurement of LH on the day of pro-estrus at 2, 4, 6, 8 and 10 p.m. showed a significant reduction of LH levels at 4, 6 and 8 p.m. which correlated with blocked ovulation (Ref. 75). The majority of reproduction findings in the two-generation study are also consistent with a long-term suppression of LH release.

The NOAEL of 300 ppm for reproductive toxicity of D4 is higher than the NOAEL of 150 ppm derived from the chronic/carcinogenicity studies (below).

Carcinogenicity
A 2-year combined chronic/carcinogenicity study was conducted by whole body vapor inhalation of D4 in Fischer 344 rats. Changes were identified at the highest exposure concentration (700 ppm) only, and included increases in kidney weights associated with chronic nephropathy, increases in mean uterine weight and uterus-to-body weight ratios, an increase in cystic endometrial hyperplasia, and an increased incidence of uterine endometrial adenomas (Ref. 37, submission II). An earlier onset and increased incidence of mononuclear cell leukaemia (MNCL) was observed in male rats, not in exposed female rats. Since this tumour type is unique to F344 rats, its relevance to humans is questionable.
A NOAEL of 150 ppm was identified in this study based on endometrial adenomas. Since D4 is not genotoxic, an epigenetic mode-of-action was considered to be responsible for its neoplastic effect. Special studies (section 3.3.12) conducted to understand the mode-of-action in the chronic study support a secondary effect rather than a direct effect of D4 on the uterus: It is unlikely that D4's very weak estrogenic activity can account for the effects seen in this study. Rather, the data support the conclusion that D4 can act as a dopamine agonist causing a reduction in prolactin. A reduction of prolactin in the rat then causes luteolysis and new ovarian follicle stimulation resulting in estrogen dominance, which leads to persistent endometrial stimulation and finally to uterine tumours. However, it is important to point out that prolactin is not luteotropic in primates and humans (Ref. 27, 28).
On the other hand, from the reproductive studies (section 3.3.8) it is possible that D4 could affect LH secretion from the pituitary, which would also result in elevated endogenous estrogen as a consequence of prolonged stimulation of tissues of ovarian origin.

While it is possible that D4 can affect the secretion of LH in female F344 rats in a manner similar to that observed for SD rats (AR 21), it is also possible that the mode of action for the cystic endometrial hyperplasia and endometrial adenomas observed in the combined chronic/carcinogenicity study is by effects on prolactin through interaction with the dopamine receptor in the pituitary. This view is supported by as yet unpublished studies in vitro and in vivo indicating that D4 can inhibit prolactin release from the pituitary by acting as a dopamine agonist (AR 5).

In conclusion, the uterine (endometrial) adenomas and hyperplasia observed at the highest dose level of 700 ppm in a lifetime study in rats are due to threshold effects on the rat endocrine system. This view is supported by the lack of genotoxic potential. On the other hand, there is at present insufficient (published) data to dismiss altogether the proposed neuroendocrine mode of action in rats as not relevant for humans.

In the final safety assessment of cyclomethicone (3.6), effect levels were selected/chosen that cover the most relevant toxicities observed for both D4 and D5.

3.5.2. Discussion on D5 (Decamethylcyclopentasiloxane; Cyclopentasiloxane)

The reference numbers given in this chapter refer to the D5 references listed in chapter 3.4.13

Physico-chemical properties
Decamethylcyclopentasiloxane (D5) is a clear, odourless, synthetically derived silicon-based fluid with a molecular weight of 371 Daltons. It is used in cosmetics with a wide range of concentrations as well as in a variety of other applications.

Skin/eye irritation and sensitisation
Tests with D5 provided no evidence that it is irritant to skin or mucous membranes or that it is a skin sensitiser.

Dermal absorption
In vitro studies using rat and human skin and *in vivo* studies in rats and humans have shown very low dermal absorption rates of ≤ 0.1% to 0.17% for D5 (AR1) Furthermore, PBPK modelling of the percutaneous absorption data predict dermal absorption of D5 to be about 0.05%. The PBPK models also show that about 90% of the small amount of systemically absorbed D5 is eliminated by exhalation within 24 hours (AR2).

Mutagenicity/genotoxicity
The negative results obtained in the bacterial reverse mutation test, *in vitro* chromosomal aberrations in Chinese Hamster V79 cells, and *in vivo* unscheduled DNA synthesis and micronucleus assays indicate that D5 does not possess mutagenic or genotoxic potential.

Toxicity
D5 has a low acute oral and inhalation toxicity in rats. No overt toxicity occurred following a single oral dose of 4800 mg D5/kg bw (Ref. 1). The inhalation LC50 was 8.67 mg/l air (560 ppm) with the lungs as the target organ of toxicity (Ref. 2).
Dermal application to rats of up to 1600 mg D5/kg bw for 28 days did not produce any test material related effects (Ref. 9).
Oral studies for 14 and 28 days at dose levels up to 1600 mg D5/kg/day revealed liver weight increases in Sprague Dawley rats, and a LOEL of 100 mg/kg bw/day (Ref. 4). Test material related effects on liver weight were also found in Wistar rats administered between 100 up to 1000 mg D5/kg bw by oral gavage for 13 weeks (Ref. 5). No significant histopathological effects related to D5 exposure were seen.
Three whole-body inhalation studies of four weeks in Fischer 344 rats (two studies; Refs. 6, 7) or Wistar rats (one study, Ref 8), at doses up to 197 ppm were conducted. In the Fischer 344 rats, there was an increased incidence of goblet cell proliferation in the nasal cavity and minimal interstitial inflammation in the lungs, which is consistent with exposure to a mild respiratory irritant. Increases in absolute and relative liver weight also were seen which were accompanied by slight hepatocellular hypertrophy. These effects were reversible upon cessation of treatment. In Wistar rats, there was an increase in relative liver weight in male and female rats. All other effects were considered unrelated to D5 exposure.
One nose-only vapour inhalation study of 90 days duration in Fischer 344 rats at exposure concentrations up to 233 ppm (Ref. 11) and one 90-day whole body inhalation study in Sprague-Dawley rats at exposure concentrations up to 120 ppm (Ref. 12) were conducted. Male and female Fischer 344 rats had a significant increase in absolute and relative liver weight, and a dose-related increase in gamma glutamyltransferase could be observed in females only. Possible treatment related histopathological findings included an increased incidence of ovarian interstitial gland hyperplasia and vaginal mucification and atrophy in female rats at 233 ppm. In Sprague-Dawley rats at the end of 90 days of exposure to 120 ppm, there was an increase in relative liver weight, which had returned to normal in females at the end of 28 days of recovery.

Reproductive toxicity
In both, one- and two-generation studies (Refs. 25, 26) with male and female Sprague-Dawley rats there were no significant effects on any of the parameters examined upon exposure by whole-body vapour inhalation to D5 up to 160 ppm. Moreover, *in vivo* rat studies with short-term inhalation exposure to D5 showed no increase in uterine wet or blotted weights and no increase in male reproductive organ weights. In vitro, D5 did not bind to human estrogen receptors α and β or progesterone receptors and was negative in ERα and ERβ reporter gene assays. On the other hand, in a nose-only inhalation study of 90 days duration in Fischer 344 rats, treatment-related histopathological findings included an increased incidence of ovarian interstitial gland hyperplasia and vaginal mucosal mucification and atrophy in the female rats exposed to 233 ppm, and a slight, not statistically significant, decrease in ovaries and testes weight of the animals.
In conclusion, a NOAEL of 160 ppm for reproductive toxicity of D5 is appropriate.
In the two-generation study, a statistically significant increase in the incidence of pulmonary vascular mineralization was observed in all F0 and F1 animals at 30 ppm and above. Also, increased incidences of minimal alveolar histiocytosis were observed at the high concentration (160 ppm) in F0 and F1 females, consistent with exposure to a mild irritant. When one further considers that the application for cosmetics is primarily dermal, then rat respiratory tract changes can be largely discounted in determining NOAELs for safe human use of D5.

Carcinogenicity
In the two-year combined chronic/carcinogenicity study (Ref. 36), exposure to D5 caused uterine endometrial adenocarcinomatous polyps and adenocarcinomas. From additional evaluations of the mode of action for this effect in the uterus, there are indications that D5 might act, as also suggested for D4, as a dopamine agonist and thereby affects prolactin secretion in the rat. The relevance of this mode of action in humans is unclear at present. The lack of genotoxic effects for D5 (based on limited genotoxicity data) suggests that the uterine tumours observed in the chronic toxicity/carcinogenicity study could be due to threshold effects.

In the final safety assessment of cyclomethicone (3.6), effect levels were selected/chosen that cover the most relevant toxicities observed for both D4 and D5.

3.6. Safety assessment of D4 and D5

Toxicities

D4 and D5 exert a rather similar profile of toxicities. Since the two compounds are apparently used together in cosmetic products in varying proportions, the SCCS has decided to perform a combined risk assessment for the two compounds. For the safety evaluation, SCCS has considered the available database, and derived the following critical effect levels: a NOAEL of 150 ppm from chronic studies with inhalation exposure and a LOEL of 100 mg/kg bw/d from subchronic toxicity studies with oral exposure. These values should also cover some possible differences in potency between D4 and D5.

The NOAEL for *reproductive toxicity* of D4 in rats in the 2-generation study was defined as 300 ppm (Siddiqui et al. 2007a). The NOAEL for D5 in the 2-generation rat study was 160 ppm, i.e. the highest concentration that can be applied without aerosol formation (Siddiqui et al 2007b).

In *chronic toxicity* studies, D4 caused endometrial adenomas only at 700 ppm; the NOAEL was 150 ppm (Dow Corning 2004). D5 induced endometrial adenocarcinomas only at 160 ppm (Dow Corning 2005). Other non-neoplastic effects (e.g. liver weight increases) were observed at 150 ppm D4 and 160 ppm D5 in these studies.

Effects on liver were also observed in subchronic toxicity studies with D4 and D5, with either inhalation exposure or oral administration: The oral LOEL for liver weight increase with D5 in rats was 100 mg/kg bw/d (Jäger & Hartmann 1991), which for D4 in rats and rabbits was 500 mg/kg bw/d (Dow Corning 1986). Liver weight increases in mature rats after oral D4 dosing, and decreased fetal body weights and liver to body weight ratios after one week of administration to pregnant rats indicate a LO(A)EL of 100 mg/kg bw/day for D4 (Falany & Li 2005). Liver weight increases after subchronic inhalation of D4 or D5 (≥ 35 or 46 ppm) were reversible upon cessation of exposure (Burns-Naas et al. 2002, Burns-Naas et al. 1998).

Other systemic effects, such as increases in hepatic CYP enzymes have been reported at relatively low concentrations of D4 or D5 (≥ 30 ppm), but are considered as adaptive responses.

Local effects, such as goblet cell proliferation in the nasal cavity and minimal alveolar histiocytosis (pulmonary vascular mineralization) can be related to a mild irritant effect of D4/D5 and were not considered for safety calculations.

In conclusion, a NOAEL of 150 ppm is chosen with regard to the most critical effects of cyclomethicone in rats, namely reproductive toxicity and potential carcinogenicity, and a LOAEL of 100 mg/kg body weight to cover organ weight changes in liver, kidney and thymus.

Exposure

Use levels of Cyclomethicone in cosmetic products

Cyclomethicone is used in various cosmetic products as an antistatic / emollient / humectant / solvent / viscosity controlling / hair conditioning ingredient and for the good spreadability of the products. The applicant had initially described that cyclomethicone (octamethyltetracyclo-siloxane, D4) is used in all cosmetic products at an average concentration of 1%. However, published data in the scientific literature indicated that cyclomethicone may be present in some cosmetic products, for instance in antiperspirants, at concentrations >40% concentration. The SCCP (2004) requested clarification from COLIPA on typical use patterns and concentrations. The response (provided in a letter to SCCP) was not sufficiently detailed. Therefore, the SCCS considered information provided by the Norwegian authorities (Talberg 2006) and the Danish EPA (2005) on use concentrations and relevant subgroups of cosmetic products. This and recent surveys on organosilicone compounds in personal-care and household products (Horii & Kannan 2008; Wang et al. 2009; CIR 2009) indicate the presence of both D4 and D5 in several cosmetic products. Thus, it is indicated to assess the safety of cyclomethicone by considering exposure to both compounds.

Table 11: Concentration of D4 + D5 in different types of cosmetic products according to Norwegian report [Talberg 2006]
The information on D4 and D5 concentrations was taken from the American branch periodical Cosmetic & Toiletries Magazine (C&TM) that regularly provides formulations for many different types of cosmetics

Type of product	Number of product within group	Average concentration (%)	Range (%)
Sun protection products (SPP)	25	7.2	0.5 - 24
Skin care products (SCP)	17	5.7	1 – 16.5
SPP and SCP combined	42	6.6	
Hair styling products	4	2.0	1 - 5
Hair care (not colours)	4	14.2	3 -28

Hair care combined	8	8.1	
Rouge, powder	8	9.9	2 – 33.6
Deodorants and antiperspirants	4	23	8 - 45
All product	*62*	*8.3*	

The SCCS used the reported average concentration (8.3 %) of D4 and D5 in cosmetic products (Ref. Talberg) to calculate the systemic exposure resulting from dermal absorption (see below) for different types of products.

It is worth noting that cyclomethicone (D4 and/or D5) is not present in all cosmetic products: The Danish EPA has a database in which 766 cosmetic products are registered with respect to their chemical content: 61 products (8% of these) contain D4 or cyclomethicone (D4 and/or D5) (http://www.mst.dk/NR/rdonlyres/13C1D483-54CE-48BC-B281-5F51EBCF7460/0/engudgavelayout.pdf). These are hair styling products, shampoos, conditioners and stick deodorants (Lassen et al. 2005). Another database (Skin Deep), established by the American environmental organisation the Environmental Working group, lists 14900 cosmetic products and their ingredients. According to this database 719 products contain cyclomethicone, and 964 products contain D5), that is about 11% of all cosmetic products (cited from Talberg). Data published in the recent final report of the Cosmetic Ingredient Review Expert Panel (CIR 2009) support similar conclusions on use patterns in various product categories. For example, D5 is reportedly used in 60 out of 499 mascara products, suggesting that about 12% of mascara products on the market contain cyclomethicone (Tab. 3 in CIR 2009).

Dermal absorption

Experimental studies showed a low absorption of D4 and D5. Despite some variation in the results of *in vitro* and *in vivo* studies with the compounds, it is apparent that dermal absorption of D5 is lower than that of D4. The majority (~90%) of cyclomethicone applied to human skin *in vitro* and rat skin *in vivo*, volatilized from the skin surface before being absorbed. *In vitro* an average of 0.5% of D4, applied neat or in antiperspirant formulation containing 62% (w/w) D4 was absorbed in human cadaver skin and the receptor fluid after 24 h of exposure. For D5 comparable *in vitro* studies showed an average of 0.04% dermal absorption. In rat studies on average less than 1.0% of applied D4 and between 0.1 to <1% of applied D5 were absorbed *in vivo,* with the majority remaining in the skin. Pharmacokinetic modelling of dermal absorption in human volunteers indicated that 0.12% and 0.30% of applied D4 was absorbed into systemic circulation for men and women, respectively; for D5 it was 0.05% for both sexes.

In the following calculations **dermal absorption** of cyclomethicone (D4 plus D5) in the products has been considered as **0.5%** of the applied dose, and only single application per day has been considered.

The value of 0.5%, taken for both components, is considered a conservative approach: Since the ratios of D4 and D5 in cosmetic products are unknown, dermal absorption of cyclomethicone is calculated with the average experimental D4 value (0.5%) that is in accordance with PBPK modelling for D4 indicating dermal absorption of 0.3% in females. In comparison, a value of 0.17% (the highest experimental value reported) for dermal absorption of D5 was taken by the Canadian authorities in 2008, based on the publication by Jovanovic et al. (2008).

Calculation of systemic exposure dose (SED) from all cosmetic products excluding sun protection products and oral products

Applied amount (17.8 g – oral 3.5 g)	=	14.3 g/day
Average concentration	=	8.3 %
Dose cyclomethicone	=	1.2 g/day
Dermal absorption	=	0.5 %
Cyclomethicone absorbed	=	6 mg/day
Typical human body weight:	=	60 kg
SED ($14.3*10^3$ mg/day * 8.3/100 * 0.5/100) / 60 kg	=	0.1 mg/kg bw/day

Calculation of systemic exposure dose (SED) from sun protection products

Applied amount	=	18.0 g/day
Average concentration	=	7.2 %
Dose cyclomethicone	=	1.3 g/day
Dermal absorption	=	0.5 %
Cyclomethicone absorbed	=	6.5 mg/day
Typical human body weight:	=	60 kg
SED ($18*10^3$ mg/day * 7.2/100 * 0.5/100) / 60 kg	=	0.1 mg/kg bw/day

Calculation of SED (rat) at the NOAEL

Converting ppm concentrations to a systemic dose following inhalation exposure requires the respiratory minute volume and the % of the inhaled dose absorbed.

NOAEL:	150 ppm (exposure 6 hours, 5 days per week)
Conversion factor:	1 ppm = 0.012 mg/l (D4) and 0.015 mg/l (D5)
Combined conversion factor:	1 ppm = 0.0135 mg/l
Converted NOAEL:	150 ppm = 0.0135 mg/l x 150 = 2.0 mg/l (exposure 6 hours, 5 days per week)
Inhalation volume[1], male rat	20.5 l/h;
Weight male rat:	0.5 kg;
Exposure by inhalation, male rat:	
[(2.0 x 20.5 x 6) x 5/7]/0.5	356 mg/kg bw/day
Absorption by inhalation, rat	5%
NOAEL male rats (356 x 0.05)	**17.8 mg/kg bw/d**
Inhalation volume female rat	15.7 l/h
Weight female rat:	0.35 kg
Exposure by inhalation, female rat	
[(2.0 x 15.7 x 6) x 5/7]/0.35	389 mg/kg bw/day
Absorption by inhalation, rat	5%
NOAEL female rats (389 x 0.05)	**19.5 mg/kg bw/d**

[1] Default inhalation values for rat from REACH (chapter R.8 – Dose p. 70)

Calculation of the Margin of Safety (MoS) from cosmetic products excluding sun protection products and oral products, based on inhalation studies

Systemic exposure dose (SED)	=	0.1 mg/kg bw/day
NOAEL (inhalation, male rats):=	=	17.8 mg/kg bw/day

Margin of Safety	**NOAEL / SED =**	**178**

Calculation of the Margin of Safety (MoS) from cosmetic products (excluding oral products) and sun protection products, based on inhalation studies

Systemic exposure dose (SED) human	=	0.2 mg/kg bw/day
NOAEL (inhalation, male rats):	=	17.8 mg/kg bw/day

Margin of Safety	**NOAEL / SED =**	**89**

Comment
If MoS calculations were based on the LOAEL of 100 mg/kg bw/day from oral studies, adjusted for 52% oral absorption and a factor of 3 for converting the LOAEL to a NOAEL, the MoS for all cosmetic products (excluding sun protection and oral care products) would be 173; the MoS for cosmetic products including sun protection products (excluding oral care products) would be 87. These values are very similar to the figures calculated on the basis of a NOAEL of 150 ppm from inhalation studies.
In this particular case SCCS considers calculated MoS values <100 for an exposure scenario that includes also sun protection products with cyclomethicone acceptable for the following reasons: (i) sun protection products are not regularly used. (ii) Recent surveys on product uses and cyclomethicone concentrations show that D5 is now used in a higher proportion of products than D4 (Talberg 2006; CIR 2009; Hori & Kannan 2008). (iii) The SED calculation is based on a value for dermal absorption which overestimates D5 absorption.

Uncertainties

There are considerable uncertainties in exposure estimates due to wide range of D4/D5 levels present in various cosmetic products (and frequency of use per day). Thus, external exposure may differ from that based on average concentration values. Current data indicate the use of cyclomethicone at concentrations between 0.06 to 89% in a total of 1499 products (CIR 2009). An estimated 10% of products on the market contain cyclomethicone, and some product categories are more important for the overall exposure than others. A conservative estimate of exposure is made by assuming that all products in the relevant product categories contain cyclomethicone, rather than a fraction of these.

The actual systemic dose might be somewhat lower than calculated since a certain percentage of the dermally absorbed dose (SED) is eliminated in exhaled air as the venous blood passes through the lungs. Moreover, the SED for combined human exposures to D4 and D5 is based on the average value of 0.5% dermal absorption for D4 as conservative approach, since information on the proportion of D4/D5 in various product categories was not available.

The margin of safety calculation for cyclomethicone further depends upon the critical effect level derived from animal studies with D4 and/or D5. As explained in section 3.6, SCCS has chosen a NOAEL of 150 ppm based on chronic inhalation studies with D4 and D5, and a LOEL of 100 mg/kg bw/day from subchronic oral studies with D5, taking into account similar uses and function of D4 and D5, and their similar profile of toxicities in the animal studies (section 3.5.1 and 3.5.2). Critical effects of the compounds covered by the NOAEL of 150 ppm include carcinogenicity as well as reproductive toxicities. It is recognized that both are

due to a mode of action that is thresholded. On the other hand, there is at present insufficient (published) data to dismiss altogether the proposed mode of action in rodents as not relevant for humans.

Non-neoplastic changes in chronic inhalation and reprotoxicity studies with D4 included increases in liver and kidney weights >150 ppm, and a decreased number of corpora lutea at 300 ppm. Thus, 300 ppm could be considered a LOEC rather than a NOAEL.

Other systemic effects observed at lower exposure levels of D4 and D5, namely increases in hepatic CYP enzymes, are considered as adaptive responses. Liver weight increases after subchronic inhalation of D4 and D5 (≥ 35 or 46 ppm) were reversible upon cessation of exposure. Local effects, such as goblet cell proliferation in the nasal cavity and minimal alveolar histiocytosis (pulmonary vascular mineralization) can be related to a mild irritant effect of D4/D5 and were not considered for safety calculations. The applicant noted that this is justified, considering that consumer exposure to cyclomethicone containing cosmetic products is mainly dermal, and that particle size in hair spray products (≈ 38 µm in aerosol hair sprays and >80 µm in pump hair sprays) is large, compared to respirable particulate sizes (<10 µm) [CIR 2009]. The SCCS notes that these particle sizes refer to mean particle sizes, but both the aerosol and pump sprays generate particles with a certain distribution in particle size. For the pump sprays it is accepted that the inhalable fraction is negligible. Aerosol hair sprays however, will result in a small fraction of respirable particles. Given the (only) mild irritant effect of D4 and D5 in combination with a very short exposure duration there is no need for a separate risk assessment for D4/D5 in spray applications.

Although the majority of animal toxicity studies involved inhalation, SCCS also considered data from studies with oral exposure to cyclomethicone: For D5 a LOEL of 100 mg/kg bw/day was derived, based on liver weight increases reported in rat studies with gavage administration for two and for 13 weeks. For D4 a LO(A)EL of 100 mg/kg bw/day was derived, based on liver weight increases in mature rats after one week of dosing, and decreased foetal body weights and liver to body weight ratios after administration of D4 to pregnant rats.

Environmental aspects
The SCCS, in accordance with its mandate, has not considered possible environmental effects resulting from the use of cyclomethicone containing cosmetic products or other uses of D4 and D5.

Health Canada (2008), in recent reports covering this issue, concluded:
"Based on the available information on its potential to cause ecological harm, it is concluded that D4 is entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity."

„Based on the available information on its potential to cause ecological harm, it is concluded that D5 is entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity."

The Commission Services should consider whether an environmental risk assessment associated with the use of cyclomethicone (D4/D5) in cosmetic products is required.

4. CONCLUSION

The SCCS is of the opinion that cyclomethicone (D4, D5) does not pose a risk for human health when used in cosmetic products. Other uses were not considered in this risk assessment.

This conclusion is based on the currently available in-use concentrations as cited in this opinion.

It should be noted that D4 is classified as a reprotoxic substance, category 3 [ECB 2006]. The NOAEL for systemic toxicity (150 ppm) used for this risk assessment also covers reprotoxic effects (NOAEL = 300 ppm).

The Commission Services should consider whether an environmental risk assessment associated with the use of cyclomethicone (D4/D5) in cosmetic products is required.

5. MINORITY OPINION

Not applicable

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