Octamethylcyclotetrasiloxane (D4), CAS no. 556-67-2

Synonyms: Cyclic dimethylsiloxane tetramer, OMCTS.

Octamethylcyclotetrasiloxane (C8H24O4Si4) is a cyclic siloxane/ silicone with low molecular weight (Figure 1). D4 is used in the synthesis of larger silicone polymers and organosilicon as well as in consumer products such as cosmetics and personal care products, washing and cleaning products, polishes and wax blends. D4 is used in 100000-1000000 tonnes per annum. D4 is classified in ECHA as a substance suspected of damaging fertility or the unborn child (Repr. 2) and may cause long lasting harmful effects in aquatic life (Aquatic Chronic 4)).

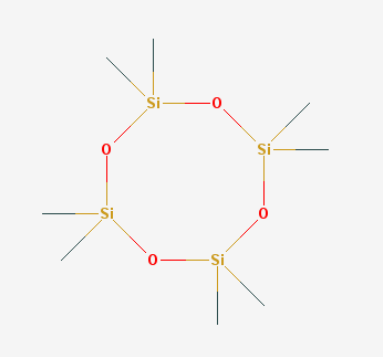

Figure 1. 2D structure from PubChem

4. Human health hazard assessment

4.10.3 Endocrine disruption

4.10.3.1 General approach – human health

4.10.3.2 In vitro information indicative of endocrine activity

Lee et al. (2015)

Summary: The oestrogenic activity of D4 was investigated *in vivo* and *in vitro* in the present study. The GH3 cell line (from rat pituitary) was exposed to D4 ($1x10^{-5}$ M) or to 17β-estradiol (E2) ($1x10^{-9}$ M) for 1 day. Gene and protein expression levels of CaBP-9K, ERα, PR and CYP2B1 were measured in the cells. Simultaneous exposure to ICI 182-780 (ICI) was used to investigate if the effects on gene expression level were regulated by the ER mediated pathway.

CaBP-9K gene expression was increased in GH3 cells exposed to E2 or D4. When cells were exposed to ICI 182-780 in combination with D4 or E2, the gene expression level of CaBP-9K was not affected. Protein expression of CaBP-9K was slightly increased by E2 and D4 but not when ICI was administered simultaneously. Similarly, PR gene- and protein expression levels were increased by D4 and E2, an effect that was blocked by ICI. Conversely, gene-and protein expressions of ERα were downregulated by E2 and D4 and ICI blocked the effect.

Study quality and assessment: The study is well described and thorough and it is assessed to be of high quality although no CAS-number or purity of the chemical is given. The study provides strong evidence of an estrogenic mode of action of D4.

Quinn et al. (2007a)
Summary: The purpose of the study was to determine the estrogenic, progestagenic and androgenic activity of two cyclic siloxanes in vitro and in vivo. In the in vitro part of the study receptor binding experiments and luciferase reporter gene assays were performed for oestrogen receptor (ER)α, ERβ, progesterone receptor (PR)α or PRβ. Androgenic activity was investigated in an in vivo assay (see below). Weak binding of D4 to ERα (compared to diethylstilbestrol) was observed. D4 did not appear to bind to ERβ, PRα or PRβ. The ERα reporter gene assay showed activity at 10 µM of D4, whereas no activation of the PRβ reporter gene assay was observed for any of the tested doses of D4 (0.1-10 µM).

This paper is included in the REACH registration dossier for D4 on toxicity to reproduction.

Study quality and assessment: The in vitro studies are well described, the tests are performed in triplicates and although it is not stated whether cytotoxicity is evaluated, the findings do not reflect results from cytotoxicity. The study is assessed to be of high quality. The study provides strong evidence of an estrogenic mode of action of D4 through binding and activation of ERα.

He et al. (2003)
Summary: The objective of this study was to characterise the estrogenic mode of action of D4 *in vivo* and to investigate the estrogenic mechanism of D4 *in vitro*. An oestrogen receptor (ER) binding assay using human ERα and ERβ receptors was performed. The siloxane D4 bound competitively to ERα but not to ERβ.

Study quality and assessment: The study is well-described and appears to be well-performed. The study is assessed to be of high quality. The study provides strong evidence of an estrogenic mode of action and the *in vivo* data support this conclusion.

4.10.3.3 In vivo effects with regard to an endocrine mode of action

Jean and Plotzke (2017)
Summary: This study presents data of a chronic toxicity and oncogenicity study of D4. In the oncogenicity part of the study, male and female rats (60/sex/group) were exposed to D4 (0, 10, 30, 150 or 700 ppm) for 6 hours per day for 24 months in whole-body inhalation chambers. Organ weights were recorded and histologic examination of organs was performed.

Uterine (absolute and relative to body weight) and testis weights (relative to body weight) were increased in animals in the 700 ppm group. An increased incidence of uterine cystic endometrial hyperplasia (from 19 % in controls to 50 % in treated females) was found in females exposed to 700 ppm D4. Although few high-dose females had cervical squamous epithelial hyperplasia and/ or ovarian atrophy, the incidence of these changes (5 % and 6.7 %, respectively) was statistically significantly increased compared to controls (0 % and 1.7 %, respectively). The severity of the changes in uterus and ovaries were increased by the exposure, but no information on the statistical significance for these is given. Similarly, a modest but significant increase in the incidence of testicular interstitial cell hyperplasia was observed after 24 months of exposure in the two highest exposure groups (150 and 700 ppm).

Study quality and assessment: The study is well-described and assessed to be of high quality although it would have been preferred if the CAS-number of the substance had been given. The study provides moderate evidence of reproductive adverse effects.

Jean et al. (2017)

Summary: This study describes the effects of chronic exposure to 700 ppm of D4 on markers of reproductive senescence in female rats (49-50 weeks of age). The animals (50 per group) were exposed through whole-body inhalation from 11 to 24 months of age. Estrous cycle was monitored throughout the study and blood samples were collected monthly (for measurement of prolactin, progesterone, estradiol and corticosterone). At necropsy, blood samples were collected (FSH, estradiol and estradiol metabolites), organ weights were recorded (adrenal glands, pituitary glands, uterus, ovaries with oviducts and other non-endocrine related organs) and histopathology (of ovaries, uterus and vagina) was performed. Histological assessment of reproductive organs was also used to evaluate the estrous cycle and the types of corpora lutea and to record the number of atretic and healthy antral follicles and primordial follicles.

Increased number or percent of estrogen-predominant days (proestrous and estrous) in the estrous cycle was found in D4 treated animals compared to controls. Progesterone levels were elevated in exposed rats 2-10 weeks after start of treatment and estradiol was reduced over the total study period after exposure start. As a consequence, lower estradiol:progesterone ratios compared to controls were found in D4 treated females. Corticosterone concentrations was increased in exposed animals during the almost the entire study period. Statistical analysis of histological changes was not performed, but a marked decrease was observed in the incidence of antral-size atretic follicles and an increase in severity was observed for vaginal mucification.

Study quality and assessment: The study is well described and thorough. Although information on CAS-number and feed had been preferred, the study is assessed to be of high quality. The study provides strong evidence of an endocrine mode of action of D4 and moderate evidence for reproductive adverse effects.

Lee et al. (2015)

Summary: The oestrogenic activity of D4 was investigated *in vivo* and *in vitro* in the present study. A Uterotrophic assay in immature female rats (18 days old) dosed s.c. for 4 days (5/group) with ethinyl estradiol (EE) (3 µg/kg) or D4 (500 or 1000 mg/kg) was performed. 4 more groups of rats were co-treated with the ERα inhibitor ICI 30 min before EE or D4 administration. Animals were killed 1 day after the last dosage. Livers were weighed and gene- and expression of CYP2B1/2 was measured. Gene and protein expression was measured in uteri from the Uterotrophic assay for evaluation of the expression levels of the estrogenic biomarkers CaBP-9K, ERα and PR.

Relative liver weights were increased by D4 but not by EE. Gene expression of CYP2B1/2 was increased markedly compared to controls in the D4 dose-group in a dose-dependent manner and ICI did not change the effect significantly compared to D4. In contrast, CYP2B1/2 gene expression was decreased by EE and co-administration of ICI and EE decreased the gene expression additionally compared to EE alone. In the Uterotrophic assay, the positive control EE increased the relative uterus weight markedly. No effects on uterus weight were seen with administration of D4. Gene-and protein

expression of CaBP-9K, ERα and PR was measured in rat immature uterus. The gene expression levels of CaBP-9K were increased by EE and D4 and the effect was blocked by ICI. Protein expression of CaBP-9K was similarly induced by EE and D4 and the effect was inhibited by ICI. Both protein and gene expression levels were more elevated after EE exposure than with D4. Expression of PR mRNA was suppressed by EE and by the high dose of D4. Co-treatment of ICI further decreased PR gene expression levels in controls, in EE treated animals and in the low-dose D4 group. In the high-dose D4 exposure group ICI treatment increased PR levels compared to D4 alone. For ERα, gene expression was reduced by EE and D4, and simultaneous exposure to ICI reduced the expression level further for EE exposure and increased expression in the high D4-group compared to D4 alone.

Study quality and assessment: The study is generally well described and the Uterotrophic assay followed the OECD guideline for this test. However in the *in vivo* part of the result section there seem to be a mistake in referring to E2 instead of EE and there is conflicting description of data compared to the depicted data in a figure (PR gene expression levels in uterus). Moreover, no CAS-number or purity of the chemical is given. The study is assessed to be of medium quality. Data on the estrogenic mode of action of D4 are somewhat conflicting as some effects of D4 are similar to EE and the ERα inhibitor ICI block some of the effects of D4, but some effects are not comparable between EE and D4. Also, D4 induced no effect on uterus weight. The study provides weak evidence of an estrogenic mode of action of D4.

Meeks et al. (2007)

Summary: The aim of this study was to investigate which phases of the female reproductive cycle are affected by D4. A study design allowing investigation of the complete female reproductive cycle from pre-mating and throughout gestation was performed. Several different cohorts were performed in female rats exposed by whole-body vapour inhalation for 6 hours per day. In the first experiment, female rats were exposed to D4 (0, 70, 300, 500 or 700 ppm) from 28 days prior mating to GD19 (n=24/ group) or for a shorter period covering the ovarian phase, the fertilisation phase or the implantation phase. In these experiments, females were exposed to 700 ppm of D4 from 31 days prior to mating to 3 days before mating (n=26 and 50 pregnant dams in controls and exposed groups, respectively), from 3 prior to mating until GD3 (n=28 and 19 pregnant dams in controls an exposed groups, respectively) or from GD2-GD5 (n=23 pregnant females/ group), respectively. In the second experiment with focus on the fertilisation and implantation phases, females were exposed during 1 day only on days 1, 2, 3 or 4 prior to mating or daily either for 3 days prior to mating or from 3 days prior to mating until GD3 (n=11-40 pregnant dams). Necropsies were performed on GD20 in the first experiment and on GD8 in the second experiment. The body weight gain, number of corpora lutea (CLs) in ovaries, number of foetuses, early resorptions and implantation sites and weight of uterus, ovaries, thyroid gland, adrenal glands and brain were assessed in all the studies.

Exposure concentrations were found to be very close to the targeted exposure levels, e.g. in the 700 ppm group, mean exposure concentrations were found to range between 689 and 702 ppm in the different studies. Overall, the paper shows effects of D4 on female fertility. In the first experiment, effects on the investigated parameters were seen in the study with exposure to D4 during the fertilisation phase and in the study with exposure during the whole mating to gestation period. In the second study, decreased fertility was seen in females exposed at a time-point close to mating.

In the females exposed from before mating and throughout gestation reduced body weight gain during gestation and increased weight of adrenal glands was seen in the highest exposure group. The number of CLs was reduced by D4 in the three highest exposure-groups, but only statistically significantly in

the 300 and 500 ppm groups and increased pre-implantation loss was seen in the two highest dose-groups of 500 and 700 ppm. The number of viable foetuses was lower compared to controls as a result of lower numbers of CLs and the higher level of pre-implantation losses. The gravid uterine weight was reduced in the highest exposure group due to a lower number of viable foetuses. In the fertilisation phase, pregnant females exposed to 700 ppm of D4 had a lower body weight gain, a lower number of CLs and an increased percentage of pre-implantation loss. The number of implantation sites, the number of viable foetuses and the uterine weight were reduced. No effects were found in females exposed during the ovarian or the implantation phases. In the second study, a reduced number of pregnant dams were found among the confirmed mated females exposed to D4 1 day prior to mating. A decreased number of CLs, decreased uterine weight and an increased number of small implantation sites were seen in females exposed from 3 days before mating until GD 3.

This paper is included in the REACH registration dossier for D4 on toxicity to reproduction.

Study quality and assessment: The study is well-described and although the paper includes many sub-studies with different exposure periods it is clear how it was done, what the results are and the link between the different results is well explained. The study is assessed to be of high quality even though no CAS-number of D4 is given. The study provides strong evidence of adverse effects on female fertility around ovulation and fertilisation.

Quinn et al. (2007a)

Summary: The purpose of the study was to determine the estrogenic, progestagenic and the androgenic activity of two cyclic siloxanes *in vitro* and *in vivo*. In the *in vivo* part of the study the rat Uterotrophic and Hershberger assays for investigation of the estrogenic and androgenic activity, respectively, were performed. In both assays exposure to D4 (700 ppm) was through whole-body inhalation, 16 hours/day. In the Uterotrophic assay, exposure in Wistar and Fisher 344 rats (n=6 controls and 10 treated for each strain) lasted 3 days and in the Hershberger assay exposure of Fisher 344 rats (n=10/group) lasted 10 days.

In the Uterotrophic assay uterine (wet and blotted) weight was increased, uterus was fluid filled and had increased luminal and epithelial cell height in both strains tested. The anti-estrogenic effect was investigated, however the results are unclear. The D4 but not the ER antagonist ICI 182,789 suppressed the ethinyl estradiol induced increase in uterine weight, indicating a weak anti-estrogenic activity of D4. The Hershberger assay showed no androgenic or anti-androgenic activity of D4.

Study quality and assessment: The study is well described but the CAS-number of the substance and housing conditions are not described. The study is assessed to be of high quality. The study provides strong evidence of an estrogenic mode of action of D4 and weak evidence of an anti-estrogenic mode of action.

Quinn et al. (2007b)

Summary: The objective of the study was to investigate the effects of D4 on ovulation and on reproductive hormones, including luteinizing hormone. Two studies in female rats (13 weeks old) were performed with whole-body vapour inhalation exposure (generally 6 hours per day) to D4 at 700 or 900 ppm for 3 days from the day of diestrous until proestrous. In the first study, called phase I (n=24, 22 and 27 for controls, 700 and 900 ppm, respectively), rats were euthanized on the day of proestrous. Blood was collected for hormone analysis (Follicle stimulating hormone (FSH), 17β-

estradiol, estrone and progesterone). In the second study, called phase II (n=138 in total at study start), blood was collected at 2, 4, 6, 8 and 10 p.m. on the day of proestrous and the animals were euthanized on the day of oestrous. The serial blood samples from phase II were analysed for luteinizing hormone (LH) and prolactin levels. On the day of necropsy body weight and weight of uterus, ovaries and brain were assessed and the number of ova in the oviducts was counted in both studies/ phases. Step sections of ovaries from phase II were evaluated histologically for assessment of large follicles, new corpora lutea and atretic antral follicles for classification of ovulators and non-ovulators. Terminal blood samples were used for hormone analysis (FSH, estradiol, estrone and progesterone).

Body weight was reduced in both treatment groups in phase I and in the 900 ppm group in phase II. The absolute ovary and relative ovary and uterus weights were reduced in the highest exposure group in phase II as well as the ovary relative to brain weight. More non-ovulating females were seen in the treatment groups (n=26, 12 and 10 ovulating females in controls, 700 ppm and 900 ppm groups, respectively compared to n=9, 19, 23 non-ovulating females in the controls, 700 ppm, 900 ppm groups, respectively). In general, plasma LH levels and the peak levels of LH were lower compared to controls, but the changes in LH peaks were related to the ovulatory status, i.e. lower mean levels of LH were related to a higher number of non-ovulators in the treatment groups. However, in ovulating females, prolactin levels were reduced at 2 p.m. in the 900 ppm group. In phase I and II, estrone hormone levels were increased in both treatment groups and in phase II, 17β-estradiol levels were elevated in both exposure groups, both in ovulating and in non-ovulating rats. The ratio between estrone and 17β- estradiol was reduced in the 900 ppm group in non-ovulating females. FSH was decreased in both treatment groups. In phase I, progesterone was increased in the highest exposure group on the day of proestrous. Histological assessment of phase II ovaries showed a tendency to increased number of large antral follicles, which correlated well with the increased estradiol levels. The number of ova found in the oviducts was reduced in the treated groups compared to controls, but not in the ovulating females. Overall, there were no signs of follicular toxicity, but D4 appeared to disrupt the LH surge and decreased the portion of ovulating females.

This paper is included in the REACH registration dossier for D4 on toxicity to reproduction.

Study quality and assessment: The study is well described and the interpretation of data and relations between the different observed effects are elucidated. The number of animals used per group in the phase II study is not stated, but the number of ovulators and non-ovulators in the different groups are listed in a figure. The study is assessed to be of high quality although no CAS-number of D4 is given. The study provides strong evidence of an endocrine mode of action of D4 and strong evidence of adverse effects on female reproduction. However, disruption of the LH surge that probably lead to the observed effects may be a rodent species specific mode of action and thus the effects on ovulation may not be relevant to humans.

Siddiqui et al. (2007)

Summary: The aim of the study was to evaluate the effects of D4 on reproductive function in F0 and F1 animals and on survival, growth and development of the offspring. A two-generation toxicity study with whole-body vapour inhalation of 0, 70, 300, 500 or 700 ppm of D4 for 6 hours per day was performed in rats. Male and female rats (F_0, 165 per sex, 44-45 days old) were exposed from 70 days before mating and until weaning of the pups (F_1, n=23-27 litters per group). The F_1 offspring were exposed in utero and through lactation and directly exposed from weaning throughout adulthood with at least 70 days of exposure prior to first mating. F_1 offspring were mated twice delivering two litters (F_{2a} and F_{2b}, n= 17-29 and n=12-26 litters per group, respectively) and male offspring were mated a

third time with untreated females while males were still exposed. Exposure was interrupted for 5 days at the time of delivery from GD20 until PND4 for all pregnant female rats.

General toxicity (food consumption, body weight gain and weight and histology of liver, kidney and lung) was assessed. Reproductive (e.g. mating and fertility index, number of implantation sites, pups born per litter etc.) and developmental parameters (e.g. sperm parameters, AGD and sexual maturation) were assessed.

Food consumption in F_0 males was reduced compared to controls in the 700 ppm group during weeks 1-2 which was consistent with a reduced weight gain observed in these males at that time-point. Increased organ weights were seen in livers (absolute and relative weights in F_0 and F_1 males and females), kidneys (absolute and relative weights in F_0 males and F_1 females and relative weights in F_1 males) and pituitary glands (absolute weights in F_1 females and relative weights in F_0 males and F_1 females). Histologically, changes were seen in livers, kidneys and lungs. Hepatocytic hypertrophy was increased in F_1 females in 500 and 700 ppm groups and in F_1 males in the 700 ppm group. In the F_1 males in the 700 ppm group, increased bile duct hyperplasia and bile pigment were observed. Tubular mineralisation was seen in the kidneys of F_0 and F_1 males in the 700 ppm group.

Reproductive parameters were also affected. Female body weight gain was reduced during GD14-20 in the 700 ppm group (F_0 and F_1), a change considered to be related to a decreased number of foetuses in the uterus, as a reduced number of pups were born in this group. The percentage of mated rats with successful delivery of litters was reduced and the gestation length was increased in F_1 offspring in the 700 ppm treatment group. The oestrous cycle length was increased in F_1 females in the highest exposure-group. Most (8 out of 9) females with extended diestrous did not show evidence of mating and this led to decreased mating indices (number of successful matings out of number of animals used for mating) during the second mating in F_1 animals. Histologically, the number of corpora lutea was decreased in F_1 females and half of the females (15 out of 30) in the highest exposure group were anovulatory or had disturbed oestrous cycles. In males, no effects were seen on sperm parameters. The number of litters was reduced in the 700 ppm group for the first mating in the F_1 generation and in the two highest dose-groups for the second mating. The number of live pups per litter was reduced in the 500 and 700 ppm groups in the F_0 and F_1 generations and the number of implantation sites was reduced in the 700 ppm group in F_0 females. Pup survival on PND 0 was reduced in F_{2b} pups from the 700 ppm group.

This paper is included in the REACH registration dossier for D4 on toxicity to reproduction. Reduction of fertility indices of F_{1b} males and females (second mating) in the 500 and 700 ppm groups is reported as statistically significant compared to control levels in the REACH dossier but not in the published paper.

Study quality and assessment: The study was conducted according to EPA OPPTS Health Effects Test guideline (which is equivalent or similar to the OECD guideline 416). It looks like there is a mistake in table 1 showing data on reproductive and developmental parameters from F_0, F_{1a}, F_{1b} and F_{1c} animals. The table covers more than one page and it is probably data from F_{1b} and F_{1c} that are shown on page 2 and not data from F_0 and F_{1a} as stated in the first line of the table on page 2. Apart from this detail, the study is well-described and thorough and the study is assessed to be of high quality. The study provides strong evidence of adverse effects on female reproduction of rats exposed as adults and especially *in utero* and throughout lactation. However, the F_1 females showed more signs of toxicity (increased organ weights and histological changes in livers indicating metabolising activity) compared to F_0 females and this may explain why more marked effects on reproductive parameters such as

gestation length and oestrous cycle length were seen in the F1 females. Thus, the study provides moderate evidence of adverse effects on female reproduction that could be explained by an endocrine disrupting mode of action.

He et al. (2003)

Summary: The objective of this study was to characterise the estrogenic mode of action of D4 *in vivo* and *in vitro*. Several *in vivo* studies were performed and other siloxanes were also tested in the Uterotrophic assay. Female mice (6-7 weeks old, n=6/group) were dosed orally with 1000 mg/kg D4 for 7 days and serum oestradiol was measured. Subsequently intact, sham operated and adrenalectomised (ADX) mice were dosed similarly as the previous study and serum oestradiol and corticosterone were measured. The siloxane D4 was tested in an Uterotrophic assay in ovariectomised female mice dosed orally to 1000 mg/kg/day D4 for 3 days (n=5/group). A uterine peroxidase assay was performed on uteri from the ovariectomised mice. A dose-response study was additionally performed for the Uterotrophic assay using 0, 1, 10, 50, 100, 250, 500 and 1000 mg/kg D4 for a 3-days exposure period (n=5/group) in ovariectomised mice. To investigate if the effects on uterine weight were mediated through ER, two more studies were performed. The Uterotrophic assay was repeated in ovariectomised mice with pre-treatment with the ER antagonist ICI 182,780. Finally, ERα knockout mice (αERKO) and wild-type controls were ovariectomised (n=5) and used in an Uterotrophic assay with oral dosing of 1000mg/kg D4 for 3 days.

Serum oestradiol was reduced by approximately 50 % by D4 exposure. In the ADX study serum oestradiol was reduced in intact, sham operated and ADX mice. Serum corticosterone was increased in intact and sham operated mice, whereas corticosterone was reduced in ADX mice. This suggested that the D4 induced decreased in oestradiol was independent of corticosterone levels and an association between stress-like elevated corticosterone levels and suppression of the hypothalamic-pituitary-gonadal axis could be excluded. D4 induced increased uterine weight in the Uterotrophic assay and increased uterine peroxidase activity in ovariectomised rats. The dose-response study in the Uterotrophic assay showed effects at doses of 250mg/kg D4 and above. Pre-treatment with ICI 182,780 blocked the D4-induced increase in uterine weight, indicating that the effects on uterus weight are ER-mediated. Additionally, the D4 induced increase in uterus weight was absent in exposed αERKO mice, suggesting that the effects on uterus weight were more specifically mediated through ERα.

Study quality and assessment: Information of the CAS-number and purity of the compound would have been preferred, but otherwise the study is well-described although it is a complex study describing several smaller studies. The study is assessed to be of high quality. The study provides strong evidence of an estrogenic mode of action of D4.

Burns-Nass et al. (2002)

Summary: The objective of the study was to investigate the subchronic toxicity of D4 in a 3 months inhalation study. Male and female rats (approximately 10 weeks old) were exposed to 0, 35, 122, 488 or 898 ppm for 6h/day, 5 days a week for 3 months (20/sex/group) to D4 by nose-only inhalation. A recovery study was performed with 10 rats per group exposed to 0 or 898 ppm as described previously for 3 months and with a following recovery period of 1 month. Blood was collected for hematologic and clinical chemistry, urine analysis was performed and organs were weighed, including liver,

spleen, heart, lung, thymus, ovaries, testes, kidneys, adrenals and brain. Histopathological assessment of a full set of organs was conducted.

Changes in endocrine related endpoints were found. Testes weight was increased in the 488 ppm group but not at the highest dose, and the change was considered not to be exposure related. Ovary weight was decreased (by 38% compared to controls) in the highest exposure group. Histopathological evaluation of the ovaries showed an increased incidence of hypoactivity seen as a lack of active corpora lutea in the 898 ppm dose-group after a 3 months exposure period. Follicular development appeared normal. The morphological changes indicated a decreased ovulatory activity of the ovaries and the reduced ovary weight was most likely due to the lower number of corpora lutea. After the 1 month recovery period, the ovaries showed normal follicular development. Increased incidence and thickness of mucification of the vaginal mucosa was seen in the uterus in females exposed to 898ppm for 3 months.

Study quality and assessment: The study is well-described and is assessed to be of high quality. The changes observed in female reproductive organs may be related to an endocrine mode of action of D4. The study provides strong evidence of adverse effects on ovaries which can be explained by an endocrine mode of action of D4.

McKim et al. (2001)

Summary: The objective was to investigate the estrogenic and anti-estrogenic activity of the cyclic D4 and the linear siloxane hexamethyldisiloxane in an Uterotrophic assay. Immature female rats (12 pups per group) from two different strains were used in the study. Sprague-Dawley rats (18 days old) and Fisher-344 rats (21 days old) were dosed by oral gavage with 0, 10, 50, 100, 250 or 1000 mg/kg/day of D4 for 4 days. For evaluation of anti-estrogenic properties of D4, EE was co-administered with either D4 (500 mg/kg/day) or ICI 182-780 (positive control). The uterine weight was measured and the epithelial cell height in uterus was measured histologically (n=6 per group). Relative potency of the different chemicals tested (EE, diethylstilbestrol dipropionate and Coumestrol) was evaluated based on the effects on uterine weight.

Female body weight was decreased in the highest D4 dose-group on day 21 (SD rats) or on days 23, 24 and 25 (Fisher rats). Compared to the other compounds tested, D4 was the least potent in increasing uterine weight (absolute and relative). Higher doses of D4 were necessary to get the same increase in uterine weight compared with the weak estrogenic compound Coumestrol. Uterus weight was significantly increased by 250, 500 and 1000 mg/kg/day of D4. Co-administration of D4 with EE attenuated the effect of EE on uterine weight suggesting an anti-estrogenic effect of D4. The compound D4 was less effective in inhibiting the effect of EE on uterine weight compared to ICI. Uterine epithelial cell height was increased by EE and D4 in a dose-dependent manner. In conclusion, D4 appeared to have weak estrogenic and anti-estrogenic properties.

Study quality and assessment: The study has some limitations as the number of litters is not mentioned, which means it is unclear if litter mates were used. Moreover, more details on housing conditions would have been preferred as well as the CAS-number of D4. The study is assessed to be of medium quality. The study provides strong evidence of a weak estrogenic mode of action of D4.

Hayden and Barlow (1972)
Summary: The purpose of the study was to investigate the effects of a series of organosiloxanes in immature females rats and to establish structure-activity relationships. However D4 does not appear to have been investigated in this study

REACH registration dossier

Toxicity to reproduction:

Key Experimental results and Supporting Experimental results
Summary: The applicant reported data from 7 additional study reports. Data from two of these studies are published in Meeks et al. (2007) and Siddiqui et al. (2007) and these papers have already been included here. The other 5 studies are one-generation reproductive toxicity studies in male and/or female rats with inhalation exposure. The studies do not add significant value or new information on the endocrine mode of action or endocrine-related adverse effects of D4, except for a reversible increase in the weight of the thyroid gland in adult males. In general, the studies show decreased weight gain and food consumption in adult rats and adverse effects on reproduction (e.g. reduced mean litter size, reduced pup viability and reduced number of implantation sites).

Study quality and assessment: The quality of the unpublished studies cannot be assessed based in the summaries available. The studies are performed under GLP with 20-22 animals per dose-group, but not all of them follow test guidelines. The studies provide strong evidence of adverse effects on reproduction based on reduced litter size, pup viability and number of implantation sites.

Developmental toxicity/ teratogenicity:

001 Key Experimental results
Summary: The first key study in the REACH registration dossier with data on developmental toxicity is published as an abstract in The Toxicologist in 1994 (York and Schardein 1994). This publication was not possible to retrieve from PubMed or by a Google search (24/11-2017). In this study pregnant rats (n=30 per group) were exposed through whole-body inhalation to 100, 300 or 700 ppm D4 for 6 hours per day for 4 weeks (GD6-15). A caesarean section was performed GD20 for assessment of developmental parameters. No effects were found on embryolethality, litter size, sex distribution and foetal body weight. Mean maternal food consumption was reduced as well as the body weight gain in the highest exposure group.

Study quality and assessment:
The study is performed under GLP with 30 females per dose-group and the study design was equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) with some (unspecified) missing details. Overall the study seems to be of good quality, but as only a summary is available and details on data and methods are not given, the quality of the study cannot be assessed. The study provides no evidence of relevance for evaluating adverse effects related to endocrine mode of action.

002 Key Experimental results:

Summary: The second key study in the REACH registration dossier for D4 with data on developmental toxicity was published in the same abstract in The Toxicologist in 1994 (York and Schardein 1994) as the data from the first key study on rats. The publication was not possible to retrieve from PubMed or by a Google search (24/11-2017). In this study 20 rabbits per group were exposed to D4 through whole-body inhalation of 0, 100, 300 or 500 ppm on GD6-18 or during different intervals in this gestational period. Decreased maternal food consumption was observed. No effects were found on mortality, malformations, developmental variations or body weight gain. Slight increase in postimplantation loss was seen but the levels were within historical control levels. No treatment-related effects were found on the number of viable foetuses per dam, mean foetal body weight, foetal malformations or developmental variations.

Study quality and assessment: The study is performed under GLP with 20 females per dose-group and the study design was equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) with some (unspecified) missing details. Overall the study seems to be of good quality, but as only a summary is available and details on data and methods are not given, the quality of the study cannot be assessed. The study provides no evidence of relevance for evaluating adverse effects related to endocrine mode of action.

Supporting Experimental results

Summary: Several supporting experimental results are included in the REACH registration dossier of D4 on developmental toxicity and teratogenicity. Overall, the (3 GLP) studies showed decreased food consumption and reduced body weight gain in dams and 1 study showed increased abortions (from 500 mg/kg) and increased post implantation loss (1000 mg/kg), but the reduced food consumption was considered responsible for these findings on reproduction. One study in rabbits showed decreased weight of gravid uterus and a reduced number of live foetuses (1000 mg/kg).

Study quality and assessment: Three out of 5 studies were performed under GLP compliance with 6 female rats or rabbits/ group and the study design was equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study). Overall the studies seem to be of good quality, but as only summaries are available and details on data and methods are not given, the quality of the studies cannot be assessed. Two study summaries were retrieved from IUCLID and included in this part of the REACH registration dossier of D4 but very few information on those studies are available. They are not GLP compliant and no details on the doses or the number of animals used are given. In conclusion, the quality of the unpublished studies cannot be assessed based on the summaries available. The studies appears to provide strong evidence of maternal toxicity based on food consumption and body weight gain and weak evidence of reproductive adverse effects but no conclusion can be made on the relation to endocrine disruption.

Toxicity to reproduction: other studies

Summary: Fifteen additional studies on reproductive toxicity and *in vitro* studies on endocrine related activity are included in the REACH registration dossier for D4. Some of these refer to published papers already assessed here (Meeks et al. 2007, He et al. 2003, Quinn et al. 2007 and McKim et al 2001). No significant additional data on fertility and reproduction are given on these studies in the REACH registration dossier compared to the published papers. The only supplemental information given in the REACH registration dossier for the published studies is reduced food consumption in

females with reduced weight gain. The remaining studies included supporting data published in the papers assessed above. In these studies, D4 had an estrogenic effect in MCF-7 cells and in the Uterotrophic assay, D4 exposure led to fewer ovulating female rats, attenuated the LH surges, decreased serum prolactin levels and *in vitro* studies showed a lack of receptor binding and receptor activation of PR. Some data appear to support the hypothesis that D4 may be a dopamine D2-receptor agonist.

Study quality and assessment: Most of the studies are non-GLP studies and do not follow specific guidelines. The quality of the studies cannot be assessed based on the restricted information given in the summaries. The studies appear to provide strong evidence of an endocrine mode of action of D4 on female reproductive system, including strong evidence of an estrogenic mode of action and strong evidence for adverse effects, although the mechanism behind reduced ovulations may be species specific.

Repeated dose studies

Summary: Overall, repeated dose toxicity studies with exposure to D4 through inhalation showed effects on the female reproductive tract. Several of the studies found decreased ovary weight, ovary atrophy/ hypoactivity and vaginal mucification. Additionally, increased uterus weight and endometrial epithelial hyperplasia was seen in a study after 24 months of D4 exposure. Some studies found increased weight of adrenal glands in females and vacuolation of zona fasciculate of the adrenal glands was shown. Effects were also observed in the male reproductive tract, more specifically the testes. One study showed increased testes weight without histopathological changes after 24 months of exposure while another study found testes tubular atrophy after exposure to D4 for 13 weeks.

Study quality and assessment: The studies referred to above were all GLP compliant and followed a test guideline. In these studies 10 rats or more were used per group. Overall the studies seem to be of good quality, but as only summaries are available and details on data and methods are not given, the quality of the studies cannot be assessed. The studies appear to provide strong evidence of reproductive adverse effects.

Specific investigations: Other studies

Summary: In vivo studies investigating effects of D4 on thyroid glands and on serum prolactin levels are included. One of the studies showed increased thyroid gland weights, hyperplasia and increased proliferation in thyroid glands after 6 or 13 days of exposure. No such effects were seen after 5 days of exposure. This suggests a proliferative effect of D4 on cells in the thyroid glands after continuous exposure. Other studies showed time-dependent effects on serum prolactin levels. No changes in prolactin levels were seen in any of the studies immediately after exposure. However, 18 hours after D4 exposure decreased prolactin levels were observed in one study whereas another study showed increased prolactin levels 4 and 8 hours after D4 exposure.

Study quality and assessment: The studies are not performed under GLP compliance and do not follow any guidelines. The quality of the studies cannot be assessed based on the restricted information given in the summaries. The studies provide weak evidence of an endocrine mode of action of D4 based on effects on serum prolactin levels *in vivo* and hyperplasia in thyroid glands.

4.10.3.4 Summary of the plausible link between adverse effects and endocrine mode of action

A large number of studies have found adverse effects on female reproductive organs and reproductive function in rodents (Table 2). Most studies were performed in adult rodents and only one study in the published literature investigated developmental effects (Siddiqui et al. 2007). The reproductive effects reported include reduced fertility, disturbed oestrous cycles, reduced ovulations, increased uterus weights with endometrial hyperplasia, vaginal mucification, reduced ovary weight and atrophy of ovaries. These effects could be related to an endocrine disrupting mode of action involving female reproductive hormones such as oestrogens, LH or FSH or a combination of these (OECD GD 106). No additional effects were found in offspring in the developmental study, but the effects appeared to be more marked than in adult females (Siddiqui et al. 2007). This could be due to more general toxicity in the offspring as evidenced by increased organ weights of liver, kidney and pituitary glands and histologically observed hypertrophy of hepatocytes, indicative of increased metabolising activity of the liver. The evidence of effects on reproductive function related to endocrine disruption observed in the developmental study is thus weak. Reproductive effects observed in adult animals in the other studies are more convincingly related to an endocrine disrupting mode of action and the studies provide strong evidence of adverse effects on female reproduction.

One published paper and a study registered in the REACH registration dossier reported work that has been done to identify whether D4 could lead to changes in plasma LH levels and if the reduced ovulations and fertility observed *in vivo* could be related to such changes (Quinn et al. 2007b). Lower plasma LH peaks in non-ovulating females compared to controls was reported and it is very likely that the link between low LH peak levels and reduced ovulations can explain some of the effects observed on lower fertility in females (e.g. reduced litter size) and disturbed oestrous cycles (Siddiqui et al. 2007; Meeks et al. 2007; Jean et al. 2017). However, disruption of the LH surge may be a species specific mode of action and thus the effects on ovulation may not be relevant to humans.

In concordance with the observed adverse effects in females (e.g. uterus and vaginal changes), several *in vivo* and *in vitro* studies showed an estrogenic mode of action of D4, although some studies show conflicting data on the estrogenic effects *in vivo* (Table 1). Overall, *in vitro* studies showed binding to and activation of ERα and estrogenic effects at gene- and protein expression level in GH3 cells. Compared to the positive controls ethinyl estradiol and 17β-estradiol, D4 showed similar effects *in vitro* (i.e. gene- and protein expression of the estrogenic biomarker CaBP-9K and of ERα in pituitary GH3 cells) and *in vivo* (i.e. gene- and protein expression of the estrogenic biomarker CaBP-9K and of ERα in the uterus and increase in uterine epithelial cell height) and the effects were blocked by the anti-estrogenic (ERα inhibitor) substance ICI 182-780 both for the effects observed after exposure to D4 and the positive control (Lee et al. 20015; McKim et al. 2003). Other *in vitro* studies showed binding of D4 to ERα (but not ERβ) and activation of the ERα reporter gene assay (Quinn et al. 2007a; He et al. 2003) and this was supported by estrogenic effects of D4 in MCF-7 cells reported in the REACH registration dossiers.

Some conflicting data on *in vivo* estrogenic effects in the Uterotrophic assay have been reported. One study did not find effects of D4 on uterus weight in the assay (Lee et al. 2015), whereas other studies did (Quinn et al. 2007a; He et al. 2003; McKim et al. 2001). This could be explained by the administration route as D4 was dosed s.c. in the study by Lee et al. (2015) whereas first-pass metabolism was possible in two of the other studies, where exposure was conducted through oral gavage (HE et al. 2003; MicKim et al. 2001). Moreover, the studies that found increased uterus

weight in the Uterotrophic assay had a larger number of animals per group compared to the study performed by Lee et al. (2015). Supporting studies submitted by the applicant in the REACH registration dossier also showed an estrogenic effect of D4 in the Uterotrophic assay. The anti-estrogenic compound, ICI 182-780, was shown to have an inhibiting effect on the increase in uterine weight after D4 exposure in the Uterotrophic assay, indicating an ER-mediated effects of D4 on the uterus (He et al. 2003). Conflicting data on estrogenic effects of D4 were also seen at the hormonal level in rat studies. Some studies showed reduced serum estradiol levels (Jean et al. 2017; He et al. 2003), whereas another study showed increased estrone and 17β-estradiol levels in plasma (Quinn et al. 2007b). The lack of effect in the study by Jean et al. (2015) could be explained by the differences in dosing period. In the study by Jean et al (20017), exposure was conducted in aging females. In contrast, females in the Quinn et al. (2007b) study were in the reproductive stage (13 weeks old), and the data on hormone levels are evaluated to be more reliable in the study performed by Quinn and co-workers (2007b). In summary, the weight of evidence of the available *in vivo* and *in vitro* studies point to an estrogenic mode of action of D4. Altogether, the published data provide strong evidence of estrogenic mode of action of D4 and strong evidence for adverse effects linked to anti-estrogenic mode of action of D4.

In addition to the effects on female reproductive function and female reproductive organs, some studies reported adverse effects on male reproductive organs. Increased testis weight and histological changes of testis (i.e. testicular interstitial cell hyperplasia and testis tubular atrophy) were described in the REACH registration dossiers and in the published literature (Burns-Nass et al. 2002; Jean and Plotzke, 2017). However, studies on the androgenic or anti-androgenic mode of action of D4 are sparse. A Hershberger assay was performed in a single study but no signs of androgenic or anti-androgenic activity of D4 were found (Quinn et al. 2007b). There is moderate evidence of adverse effects on male reproductive organs, but the mode of action behind the testicular effects is unclear. However, it could be speculated, that the estrogenic potential of D4 could play a role in the testis. Thus, there is weak evidence for a plausible link between the estrogenic mode of action of D4 and adverse effects on male reproductive organs.

In addition to the above mentioned effects on reproductive organs, one mechanistic *in vivo* study reported in the REACH registration dossiers showed increased thyroid gland weights, thyroid hyperplasia and increased proliferation in thyroid glands. Based on this study there appear to be moderate evidence of a thyroid disrupting potential of D4, and more studies on these endpoints are necessary to conclude on this kind of endocrine disrupting capacity of D4. Thyroid hormones play a role in the regulation of prolactin and the effects observed on plasma prolactin levels (Quinn et al. 2007b; REACH registration dossier) may be related to the thyroid gland effects reported in the REACH registration dossier.

Summary and conclusions

All in all there is strong evidence that D4 has adverse effects on the reproductive system as well as weak evidence of adverse effects on the thyroid gland. There is strong evidence for an estrogenic mode of action of D4, and strong evidence for adverse effects on female reproductive system that can be related to this estrogenic mode of actin of D4 together with an endocrine mode of action through LH. However, changes in LH levels may be species specific. Changes in LH levels are probably responsible for some of the adverse effects observed, but D4 also had a strong estrogenic activity and it is unclear which adverse effects can be linked to this mode of action alone. The male reproductive effects are likely related to an endocrine mode of action as well, but the few data available on androgen-related mode of action did not confirm an anti-androgenic mode of action of D4. It is possible that the estrogenic mode of action of D4 could be responsible for the testicular effects

observed. The mode of action behind the effects observed on thyroid glands cannot be determined based on the available data.

In conclusion, D4 does meet the WHO definition of an endocrine disruptor with an estrogenic mode of action leading to adverse effects on the female reproductive system.

Additional literature not included in the evaluation

Reviews on D4 have been used to check for additional literature not found in our literature search (Dekant *et al.* 2017; Franzen *et al.* 2017). Both reviews referred to an abstract with *in vitro* data on ligand binding assays and reporter gene assay for progesterone receptors, but this abstract was not accessible (Jean *et al.* 2005).

Table 1. Overview of *in vitro* and *in vivo* endocrine disrupting (ED) mode(s) of action (MoA(s)) of octamethylcyclotetrasiloxane (D4).

Reference	**MoA**		**Quality of study**	**Evidence for ED MoA**
	In vitro	*In vivo*		
Jean et al. 2017		Progesterone levels were elevated in exposed rats 2-10 weeks after start of treatment and estradiol was reduced over the total study period. As a consequence, lower estradiol:progesterone ratios compared to controls were found in D4 treated females. Corticosterone concentrations were increased in exposed animals during almost the entire study period.	High	Strong
Lee et al. 2015	CaBP-9K gene expression was increased in GH3 cells exposed to E2 or D4. When cells were exposed to ICI 182-780 in combination with D4 or E2, the gene expression level of CaBP-9K was not affected. Protein expression of CaBP-9K was slightly increased by E2 and D4 but not when ICI was administered simultaneously. Similarly, PR gene- and protein expression levels were increased by D4 and E2, an effect that was blocked by ICI. Conversely, gene-and protein expressions of ERα were downregulated by E2 and D4 and ICI blocked the effect.	In the Uterotrophic assay, no effects on uterus weight were seen with s.c. administration of D4. Gene- and protein expression levels of CaBP-9K, an estrogenic biomarker, were increased in the uterus by EE and D4 and co-administration of ICI inhibited the effect. PR gene expression in the uterus was decreased by EE and the high D4 dose. ERα gene expression was reduced in the uterus by EE and D4. Gene expression of CYP2B1/2 in the livers was increased markedly compared to controls in the D4 dose-group in a dose-dependent manner.	High	Strong (in vitro)-Weak (in vivo)
Quinn et al. 2007b		A decrease in plasma LH peak levels in female rats were related to the ovulatory status, i.e. lower mean levels of LH were related to a higher number of non-ovulators in the treatment groups. In ovulating females, prolactin levels were reduced. In the 900 ppm group. Plasma estrone and 17β-estradiol hormone levels were increased in both	High	Strong

Reference	MoA		Quality of study	Evidence for ED MoA
	In vitro	*In vivo*		
		treatment groups (700 and 900 ppm). The ratio between estrone and 17β- estradiol was reduced in the 900 ppm group in non-ovulating females. FSH was decreased in both treatment groups. Progesterone was increased in the highest exposure group.		
Quinn et al. 2007a	Weak binding of D4 to ERα was observed. D4 did not bind to ERβ, PRα or PRβ. Activation of the ERα reporter gene assay was seen, whereas no activation of the PRβ reporter gene assay was observed for D4.	In the Uterotrophic assay with inhalation exposure to D4, uterine weight was increased, uterus was fluid filled and had increased luminal and epithelial cell height in both strains tested. D4 but not ICI 182,789 showed weak anti-estrogenic activity. The Hershberger assay showed no androgenic or anti-androgenic activity of D4.	High	Strong
He et al. 2003	D4 bound competitively to ERα but not to ERβ in an ER binding assay.	Serum oestradiol was reduced by approximately 50% by D4 exposure. In adrenalectomised (ADX) mice serum oestradiol was reduced with D4 exposure. D4 exposure induced increased serum corticosterone in intact and sham operated mice, whereas corticosterone was reduced in ADX mice. D4 induced increased uterine weight in the Uterotrophic assay and increased uterine peroxidase activity. Pre-treatment with ICI 182,780 blocked the D4-induced increase in uterine weight, indicating that the effects on uterus weight are ER-mediated. Additionally, the D4 induced increase in uterus weight was absent in exposed αERKO mice.	High	Strong
McKim et al. 2001		Body weight was decreased in the highest D4 dose-group. Uterus weight was significantly increased by 250, 500 and 1000 mg/kg/day of D4. Co-administration of D4 with EE	Medium	Strong

Reference	MoA		Quality of study	Evidence for ED MoA
	In vitro	*In vivo*		
		attenuated the effect of EE on uterine weight suggesting an anti-estrogenic effect of D4. D4 inhibited the effect of EE on uterine weight. Uterine epithelial cell height was increased by EE and D4 in a dose-dependent manner.		
REACH registration dosier, Toxicity to reproduction: other studies	An estrogenic effect of D4 was found in MCF-7 cells. A lack of receptor binding and receptor activation of PR was found for D4.	An estrogenic effect of D4 was found in the uterotrophic assay. Moreover, D4 attenuated the LH surges and decreased serum prolactin levels.	Not assessable	Appears to be strong
REACH registration dossier, Specific investigations		Studies showed time-dependent effects on serum prolactin levels. No changes in prolactin levels were seen in any of the studies immediately after exposure. However, 18 hours after D4 exposure decreased prolactin levels were observed in one study whereas another study showed increased prolactin levels 4 and 8 hours after D4 exposure.	Not assessable	Weak

Ethinyl estradiol (EE); 17β-estradiol(E2), estrogen receptor (ER); progesterone receptor (PR), luteinizing hormone (LH).

Table 2. Overview of potential endocrine-related adverse effects of octamethylcyclotetrasiloxane (D4).

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
Jean and Plotzke, 2017	Rats, n=60/sex/group	Uterine and testis weights were increased in animals in the 700 ppm group exposed through inhalation for 24 months. An increased incidence of uterine cystic endometrial hyperplasia was found in high-dose females. Small but statistically significantly increased incidence of cervical squamous epithelial hyperplasia and/ or ovarian atrophy was observed. Similarly, a modest but significant increase in the incidence of testicular interstitial cell hyperplasia was observed after 24 months of exposure to 150 and 700 ppm D4.	High	Moderate
Jean et al. 2017	Rats, n=50/group	Increased number or percent of estrogen-predominant days (proestrous and estrous) in the estrous cycle was found in D4 treated animals compared to controls. A marked decrease was observed in the incidence of antral-size atretic follicles and an increase in severity was observed for vaginal mucification.	High	Moderate
Lee et al. 2015	Rats, n=5/group	D4 but not ethinyl estradiol increased the relative liver weights in immature female rats after s.c. exposure for 4 days.	Medium	Weak
Meeks et al. 2007	Rats, n=24/group or 60/group	Decreased fertility was observed in female rats exposed at a time-point close to mating and fertilisation or when the exposure period included this time-point.	High	Strong
Siddiqui et al. 2007	Rats, n=23-27 litters/ group in the first generation	Reproductive parameters were adversely affected, e.g. reduced number of litters, reduced number of pups per litter and extended oestrous cycles.	High	Moderate
Quinn et al. 2007b	Rats, n=22-27 in phase I, n=31-35 in phase II	Body weight was reduced in both treatment groups (700 and 900 ppm) in phase I and in the highest exposure group in phase II. The absolute ovary and relative ovary and uterus weights were reduced in the highest exposure group in phase II as well as the ovary relative to brain weight. More non-ovulating females were seen in the treatment groups. Histological assessment of ovaries showed a tendency to increased number of large antral follicles, which correlated well with the increased estradiol levels. The number of ova found in the oviducts was reduced in	High	Strong

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
		the treated groups compared to controls, but not in the ovulating females.		
Burns-Nass et al. 2002	Rats, n=20/group	Ovary weight was decreased in the 898 ppm group and histopathological evaluation of the ovaries showed an increased incidence of hypoactivity in the 898 ppm dose-group after a 3 months exposure period. Increased incidence and thickness of mucification of the vaginal mucosa was seen in the uterus in females exposed to 898 ppm. Testes weight was increased in the 488 ppm group but not at the highest dose, and the change was considered not to be exposure related.	High	Strong
REACH registration dossier, toxicity to reproduction	Rats, n=20-22/ group	The studies show decreased weight gain and food consumption in adult rats and adverse effects on reproduction (e.g. reduced mean litter size, reduced pup viability and reduced number of implantation sites).	Not assessable	Strong
REACH registration dossier, developmental toxicity	Rats, n=30 females/group	Maternal toxicity was observed as reduced food consumption and reduced body weight gain in the 700 ppm group.	Not assessable	None
REACH registration dossier, developmental toxicity	Rabbits, n=20/ group	Decreased maternal food consumption was observed. Slight increase in postimplantation loss was seen but the levels were within historical control levels.	Not assessable	None
REACH registration dossier, Repeated dose: inhalation	Rats, n=10 or more/group	Several of the studies found decreased ovary weight, ovary atrophy/ hypoactivity and vaginal mucification. Additionally, increased uterus weight and endometrial epithelial hyperplasia was seen in a study after 24 months of D4 exposure. Some studies found increased weight of adrenal glands in females and vacuolation of zona fasciculate of the adrenal glands was shown. One study showed increased testes weight without histopathological changes after 24 months of exposure while another study found testes tubular atrophy after exposure to D4 for 13 weeks	Not assessable	Strong

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
REACH registration dossier, Specific investigations	NA	Increased thyroid gland weights, hyperplasia and increased proliferation in thyroid glands after 6 or 13 days of exposure. No such effects were seen after 5 days of exposure, suggesting a proliferative effect of D4 on cells in the thyroid glands after continuous exposure.	Not assessable	Weak

NA: not applicable (e.g. if represents several studies)

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