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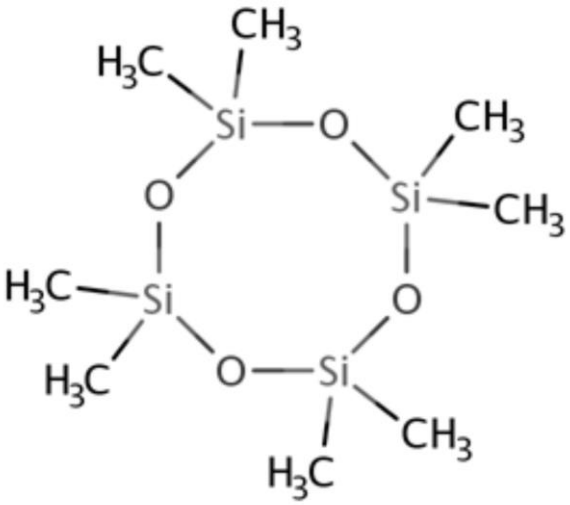
Draft Human Health Hazard Assessment for Octamethylcyclotetrasiloxane (Cyclotetrasiloxane, 2,2,4,4,6,6,8,8-octamethyl-) (D4)

Technical Support Document for the Draft Risk Evaluation

CASRN 556-67-2



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ABBREVIATIONS AND ACRONYMS

α ERKO	Estrogen receptor-α knockout (mouse strain)
AST	Aspartate aminotransferase
AUC	Area under the curve
BMD(L)	Benchmark dose (lower 95th percentile)
BMR	Benchmark response
BrdU	5-bromo-2'-deoxyuridine (used in cell proliferation assays)
CaBP-9K	Calcium-binding protein 9K
CHO	Chinese hamster ovary (cells)
CO_2	Carbon dioxide
COU	Condition of use
CYP	Cytochrome P450
D2	Dopamine receptor 2
D4	Octamethylcyclotetrasiloxane
DAF	Dosimetric adjustment factor
DMSD	Dimethylsilanediol
DNA	Deoxyribonucleic acid
E1	Estrone
E2	Estradiol
EC	European Commission
EC/HC	Environment Canda/Health Canada
ECOD	7-ethoxycoumarin O-deethylase
ER	Extra risk
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
EROD	7-ethoxyresorufin-O-deethylase
F344	Fischer 344 (rat)
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
F0,F1,F2	Successive generations in multigeneration reproductive toxicity study
GABA	Gamma aminobutyric acid
GC/MS	Gas chromatography/mass spectroscopy
GC-FID	Gas chromatography - flame ionization detection
GD	Gestational day
GnRH	Gonadotropin releasing hormone
GSD	Geometric standard deviation
HEC	Human Equivalent Concentration
HED	Human Equivalent Dose
ICI	ICI 182,780
i.v.	Intravenous
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
LOAEL	Lowest-observed-adverse-effect level
Log K_{OW}	Logarithm of the octanol-water partition coefficient
MLP	Mobile lipoprotein pool
MMAD	Mass median aerodynamic diameter
MNCL	Mononuclear cell leukemia
MOA	Mode of action
MOE	Margin of exposure

mRNA	Messenger ribonucleic acid
NOAEL	No-observed-adverse-effect level
NTP	National Toxicology Program
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Cooperation and Development
OPP	Office of Pesticide Programs
OPPT	Office of Pollution Prevention and Toxics
OQD	Overall Quality Determination
ORD	Office of Research and Development
PBPK	Physiologically based pharmacokinetic
PCNA	Proliferating cell nuclear antigen
PECO	Population, Exposure, Comparator, Outcome
PESS	Potentially exposed or susceptible subpopulations
PM	Pergolide mesylate
PND	Postnatal day
POD	Point of departure
PRL	Prolactin
PROD	7-Pentoxyresorufin-O-depentylase
RD	Relative deviation
RfC	Reference concentration
SCCS	Scientific Committee on Consumer Safety
SCE	Sister chromatid exchange
SD	Sprague Dawley (rat) (Main body of TSD) /Standard deviation (Appendix B)
SEHSC	Silicones Environmental, Health, and Safety Center
TSCA	Toxic Substances Control Act
UF	Uncertainty factor

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Docket
Supporting information can be found in the public docket, Docket ID EPA-HQ-OPPT-2018-0443

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SUMMARY

This technical support document (TSD) for octamethylcyclotetrasiloxane (D4) summarizes the non-cancer and cancer hazards associated with exposure to D4 and identifies the points of departure (PODs) to be used to estimate non-cancer risks from D4 exposures in the risk evaluation of D4. This TSD supports the Toxic Substances Control Act (TSCA) *Draft Risk Evaluation for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025i).

D4 is a volatile lipophilic liquid at room temperature. EPA quantitatively evaluated hazards through systematic review of reasonably available human and animal toxicology data. EPA rated the data quality of each individual study as high, medium, low, or uninformative for dose-response. EPA considered studies that received medium or high overall quality determinations for hazard identification, integration of evidence streams (animal, human, mechanistic), and dose-response analysis. Information from studies of low or uninformative quality were discussed on a case-by-case basis for hazard identification and evidence integration and were considered but not used quantitatively for dose-response analysis.

Studies show D4 is readily absorbed through the lungs and gastrointestinal tract but exhibited low absorption via the dermal route because the majority volatilized from skin before it could be absorbed. D4 that is retained in the body was distributed widely with higher partitioning to adipose tissue. The primary metabolites are dimethylsilanediol (DMSD) and methylsilanetriol as identified in oral and inhalation toxicokinetic laboratory animal studies. D4 induces multiple enzymes, and the largest increases are for cytochrome P450 (CYP) 2B1 and 2B2 based on both enzyme activity and protein levels. In oral and inhalation animal studies, 68 to 91 percent D4 is eliminated through exhalation and via urine and feces within a week. The toxic moiety is not well-established but concentrations in target tissues (*e.g.*, female reproductive organs) is primarily parent D4, not metabolites. See Sections 3.1 through 3.4 for more information on toxicokinetics and metabolism.

Multiple physiologically based pharmacokinetic (PBPK) models have been developed for D4, many of which were revisions to earlier models. EPA used a recent PBPK model to predict human equivalent concentrations and doses (HECs/HEDs) by extrapolating inhalation toxicity data in rats to humans and extrapolating across exposure routes and durations associated with exposure scenarios used in the risk evaluation. EPA concluded that using the model was preferable to relying on default approaches to estimate HECs and HEDs because D4 has a complex toxicokinetics and metabolism profile. EPA determined the PBPK model provided a reasonable fit of the experimental data on toxicokinetics and metabolism in rats and humans (see Section 4.2.1).

Previous assessments by European Commission (EC)'s Scientific Committee on Consumer Safety (SCCS) (SCCS, 2010), Environment Canada and Health Canada (EC/HC, 2008), the United Kingdom (Brooke et al., 2009), and American Chemistry Council's Silicones Environmental, Health, and Safety Center (SEHSC, 2020) focused on reproductive, liver, lung, and developmental toxicity with some consideration for additional isolated outcomes. These previous assessments differed in their choice of PODs and endpoints as the basis of their PODs. The assessments also differed as to whether the liver endpoints represented adverse or adaptive changes and whether lung effects represented mild irritating effects or could be used as the basis of a POD.

Previous assessments note D4's association with uterine tumors (SEHSC, 2020; SCCS, 2010; Brooke et al., 2009). Brooke et al. (2009) and SEHSC (2020) suggested that the mechanism was not relevant to humans but EC/HC (2008) and SCCS (2010) concluded that there was not a thorough mode of action (MOA) analysis to support this position. All previous assessments noted the lack of evidence of

genotoxicity, with Brooke et al. (2009) stating that a chromosomal aberrations assay resulted in ambiguous results when conducted with metabolic activation.

EPA identified several studies with hazard endpoints that could be considered for POD selection. These studies included two short-term human studies (one via oral and one via inhalation), multiple short-term inhalation toxicity studies in rats and one short-term inhalation study in multiple species, two 90-day studies in rats, a combined chronic bioassay and oncogenicity inhalation study in rats, and a chronic study in older rats via inhalation. EPA also identified multiple 1- and 2-generation inhalation reproductive toxicity studies in rats that examined a variety of exposure durations within various stages of reproduction. EPA found three pre-natal developmental toxicity studies – an inhalation study in rats and an inhalation and oral study in rabbits. Given the different approaches for POD selection used in the previous assessments, EPA opted to evaluate all identified D4 hazard studies for data quality and considered the full set of studies when integrating evidence associated with D4 human health hazards. EPA evaluated non-cancer hazards associated with liver, pulmonary, reproductive, and developmental toxicity. EPA also evaluated D4 for cancer.

After data evaluation of individual studies and hazard effects and evidence integration across animal, human, and mechanistic evidence streams, EPA identified toxicity related to female fertility and associated reproductive outcomes as the most sensitive and well supported of the non-cancer hazard outcomes. There were no human data that evaluated reproductive outcomes. EPA characterized the strength of the evidence for each endpoint according to considerations described in Chapter 7 of the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021). Based on moderate animal evidence and moderate mechanistic evidence, EPA concluded that D4 likely causes effects on the female reproductive system when integrating the evidence for reproductive toxicity endpoints. Evidence is inadequate to assess whether D4 exposure may cause developmental effects. In addition, EPA concluded that evidence suggests but is not sufficient to conclude that D4 may cause liver or lung effects. See Section 4.1 for more information on these non-cancer outcomes.

EPA identified a single D4 cancer bioassay in rats via the inhalation route and mechanistic data related to mutagenicity and non-mutagenic biochemical changes that generally lead to increases in estrogen hormone levels (unopposed estrogen) that could contribute to proliferation lesions in the uterus. EPA did not identify human data that examined cancer from exposure to D4. EPA concluded that D4 has suggestive evidence of carcinogenic potential based on slight evidence of D4's carcinogenic potential in animals for uterine tumors combined with indeterminate human and mechanistic evidence. D4 is not expected to act via a mutagenic MOA. Although other mechanistic data evaluated whether D4 may result in high uterine levels of unopposed estrogen, no MOAs have been established. Based on this conclusion, and in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA did not conduct cancer dose-response modeling for D4 (see Section 4.2.1). This approach is consistent with previous assessments of D4 cancer hazard (SEHSC, 2020; SCCS, 2010; Brooke et al., 2009; EC/HC, 2008). While specific characterization of the available cancer hazard data, mode of action evidence and potential relevance for humans varies across assessments, none identified sufficient evidence to support quantitative cancer risk assessment.

Across studies and endpoints with multiple concentrations, most reproductive effects resulted in NOAECs of 3639 mg/m^3 (300 ppm) and LOAECs of 6066 mg/m^3 (500 ppm) (as nominal[1] values). Supporting studies that evaluated single D4 air concentrations often identified reproductive effects at the nominal concentration of 8492 mg/m^3 (700 ppm). EPA selected the 2-generation inhalation reproductive

[1] Nominal concentrations are the intended air concentration (as opposed to the analytically verified concentration).

toxicity study by WIL Research (2001a) as the basis for dose-response because it had a high overall quality determination. Also, the authors evaluated a wide range of doses and a comprehensive set of sensitive reproductive endpoints across parental (F0 and F1) and offspring (F1 and F2) generations. Effects were identified in F1 parents (decreased fertility and mating indices), F1 offspring (decreased mean live litter size and mean number of pups), and F2 offspring (decreased mean live litter size). The study also included exposures for both males and females and identified sensitive endpoints following long-term exposure across life stages. Other than the decreased mating index (which was observed only at the highest concentration), EPA was able to conduct dose-response modeling for the outcomes observed in this study. EPA considered the selected study and endpoints to be relevant for acute, intermediate, and chronic exposure durations because the study included dosing of parents during premating, mating, and gestation with indirect exposure through lactation by offspring spanning subchronic to chronic durations. Also, related reproductive effects (fertility) were observed in supporting studies after a single day of exposure, supporting use of the endpoints for acute exposure (see Section 4.2.1).

EPA performed dose-response analysis by first using the PBPK model and relevant physiologic and toxicokinetic parameters with the D4 concentrations in air from the above-mentioned reproductive study to obtain internal doses representing blood concentrations. EPA then performed benchmark dose (BMD) modeling for each of the selected reproductive endpoints using the PBPK-converted internal doses and responses from the reproductive toxicity study to obtain the BMDLs (the lower confidence limits on the exposure at which the model predicts a given percent benchmark response). From these BMDLs, EPA chose decreased live litter size in the second generation of offspring because the endpoint demonstrated a dose-response relationship with good model fit and included parental exposure as well as indirect exposure of offspring, providing a robust and sensitive endpoint with which to calculate risks. The BMDLs based on blood concentrations for the other modeled hazard outcomes were within a factor of two of the BMDL for decreased live litter size. For the chosen endpoint (decreased live litter size), EPA modeled a $BMDL_5$ using a 5 percent benchmark response based on severity (viability of offspring) that is consistent with previous TSCA risk evaluations (Section 4.2.4).

EPA then ran the PBPK model again, this time using the resulting $BMDL_5$ using the D4 blood AUC to estimate HECs and HEDs using the physiologic and toxicokinetic parameters appropriate for humans. The PBPK model estimated PODs for acute, intermediate, and chronic durations used in the risk evaluation and included HECs for the inhalation route and HEDs for the oral and dermal routes. Because the HECs and HEDs for intermediate and chronic durations were similar, EPA used the values for chronic duration, which were slightly more sensitive than the intermediate HEC and HEDs (*i.e.*, 1 to 2 percent lower).

For the chosen hazard endpoint, the PBPK-predicted toxicity values used to calculate risks were as follows (Section 4.2.5):

- *Inhalation:* HECs were 8.82 ppm (107 mg/m^3) and 4.60 ppm (55.8 mg/m^3) for acute and intermediate/chronic exposures, respectively.
- *Oral:* HEDs were 8.93 and 3.60 mg/kg-bw/day for acute and intermediate/chronic exposures, respectively.
- *Dermal:* HEDs for unoccluded conditions were 394 and 326 mg/kg-bw/day for acute and intermediate/chronic exposures, respectively. These values were used for exposure scenarios in which D4 can freely evaporate from skin. HEDs for occluded conditions were 216 and 179 mg/kg-bw/day for acute and intermediate/ chronic exposures, respectively. These occluded

values were used for a limited set of situations where D4 is trapped at the skin surface (*e.g.*, consumer clothing uses) or is undepleted (*e.g.* swimming scenario) with limited potential for evaporation.

These internal dose inhalation HECs were lower than the internal dose NOAEC and LOAEC values in the 2-generation reproductive toxicity study because: 1) they use a specific response rate for the hazard endpoint (5 percent) to allow consistency across endpoints; 2) they account for differences in toxicokinetics between rats and humans (allowing for a lower uncertainty factor to be used); and 3) they account for specific exposure durations considered in the risk evaluation, relying on continuous exposure instead of a 6 hour exposure.

EPA used a total uncertainty factor (UF) of 30 for the benchmark margins of exposure (MOEs) for acute, intermediate, and chronic exposure durations. This is based on a UF_A of 3 to account for toxicodynamic differences across species and a UF_H of 10 to account for toxicokinetic and toxicodynamic variation in sensitivity within the human population. (Section 4.2.4).

EPA has moderate overall confidence in the chosen hazard values for female reproductive toxicity, which will be used for risk estimation. These confidence ratings were based on the weight of scientific evidence considering data quality of the individual studies, integration of evidence across studies, selection of the critical study and endpoint, relevance to exposure scenarios, dose-response considerations, and consideration of potentially exposed or susceptible subpopulations (PESS). This overall confidence rating differs from individual study ratings and considers the overall quality determination given to the chosen toxicity study and hazard endpoint (Section 6.1.1).

1 INTRODUCTION

On March 19, 2020, EPA received a request, pursuant to 40 CFR 702.37, from Dow Silicones Corporation, Elkem Silicones USA Corporation, Evonik Corporation, Momentive Performance Materials, Shin-Etsu Silicones of America, Inc., and Wacker Chemical Corporation through the American Chemistry Council's Silicones Environmental, Health, and Safety Center (SEHSC), to conduct a risk evaluation for octamethylcyclotetrasiloxane (D4) (CASRN 556-67-2) (Docket ID: EPA-HQOPPT-2018-0443). This chemical substance is listed in the 2014 update to the TSCA Work Plan as "octamethylcyclotetra- siloxane" and is assigned CA Index Name "Cyclotetrasiloxane, 2,2,4,4,6,6,8,8-octamethyl-." It is most referred to as octamethylcyclotetrasiloxane and is abbreviated in this document as "D4."

On June 17, 2020, EPA opened a 45-day public comment period to gather information relevant to the requested risk evaluation. EPA reviewed the request (along with additional information received during the public comment period) and assessed whether the circumstances identified in the request constitute conditions of use under 40 CFR 702.33 and whether those conditions of use warrant inclusion within the scope of a risk evaluation for D4. EPA determined that the request meets the applicable regulatory criteria and requirements, as prescribed under 40 CFR 702.37. The Agency granted the request on October 6, 2020 and published the draft scope document for D4 in 2021, with the final scope document published in 2022 (U.S. EPA, 2022).

Following publication of the final scope document, one of the next steps in the TSCA risk evaluation process is to identify and characterize the human health hazards of D4 and conduct a dose-response assessment to determine the points of departure (PODs) to be used to estimate risks from D4 exposures. This technical support document for D4 describes the non-cancer and cancer hazards associated with exposure to D4 and identifies the PODs to be used to estimate risks from D4 exposures.

This technical document for D4 presents the draft human health hazard assessment in support of the TSCA *Draft Risk Evaluation for Octamethylcyclotetra- siloxane* (U.S. EPA, 2025i), which includes a short summary of the human health hazards described in this TSD.

2 APPROACH AND METHODOLOGY

EPA used the approach described below and in the identified documents to evaluate, extract, and integrate evidence for D4 human health hazard and conduct dose-response modeling. Figure 2-1 provides an overview of the approach, which is based on the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021), updates to the systematic review processes presented in the *Draft Systematic Review Protocol for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025j) and the *Framework for Human Health Risk Assessment to Inform Decision Making* (U.S. EPA, 2014).

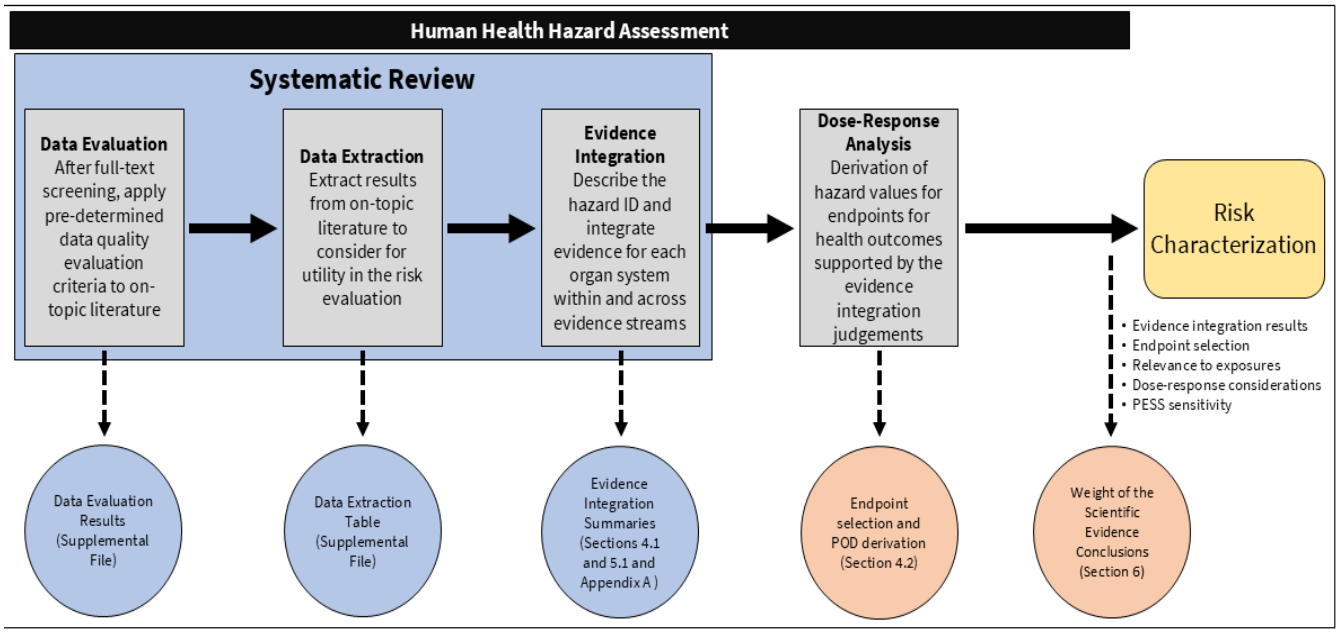

Figure 2-1. EPA Approach to Hazard Identification, Evidence Integration, and Dose-Response Analysis for D4

For the human health hazard assessment, EPA systematically reviewed data sources identified in the literature searches conducted in 2020 and 2021. EPA first screened titles and abstracts and then full texts for relevancy using population, exposure, comparator, and outcome (PECO) screening criteria. Inhalation, oral, and dermal studies that met the PECO criteria were then evaluated for data quality using pre-established quality criteria and metrics. The data quality rating for each individual study is reported as an overall quality determination (OQD). EPA did not evaluate studies by routes of exposure not evaluated in the risk evaluation (*e.g.*, intramuscular, intraperitoneal), even if they met the PECO criteria.

While the complete systematic review process was not formally updated since 2021, EPA has identified and relied on additional relevant toxicokinetic studies published after 2021 for the human health hazard assessment. EPA used a recent PBPK model described in (Campbell et al., 2023) that also relied on toxicokinetics studies published after the literature searches were conducted. EPA intends to bring the formal systematic review process up to date prior to finalization of the risk evaluation.

Although EPA used data quality criteria for many studies, EPA has not developed such criteria for toxicokinetics data other than dermal absorption studies. Also, EPA relied primarily on the studies with apical endpoints that were possible candidates for dose-response analysis and did not formally evaluate the supporting mechanistic studies for data quality. EPA did assess whether selected genotoxicity studies followed existing guidelines.

Following data quality evaluation, EPA extracted the toxicological information from each evaluated study into a supplemental file. The results of data quality evaluation and extraction of key study information for dermal absorption studies as well as human and animal phenotypic toxicity studies are presented in the following supplemental files:

- *Draft Data Quality Evaluation and Data Extraction Information for Dermal Absorption for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025b)
- *Draft Data Quality Evaluation Information for Human Health Hazard Epidemiology for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025d)

- *Draft Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025c)
- *Draft Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025a)

EPA gave greater weight to studies that received medium or high overall quality determinations for hazard identification, evidence integration, and dose-response analysis. Information from studies of low or uninformative quality were only discussed on a case-by-case basis for hazard identification and evidence integration to support the weight of scientific evidence as appropriate and were not used quantitatively for dose-response analysis.

After evaluating individual studies for data quality, EPA summarized hazard information by hazard outcome and considered the strengths and limitations of individual evidence streams (*i.e.*, human studies of apical (phenotypic) endpoints if available, animal toxicity studies with phenotypic endpoints, and supplemental mechanistic information). The Agency integrated data from these evidence streams to arrive at an overall evidence integration conclusion for each health outcome category (*e.g.*, reproductive toxicity). When weighing and integrating evidence to estimate the potential that D4 may cause a given human health hazard outcome, EPA uses several factors adapted from Hill (1965). These elements include consistency, dose-response relationship, strength of the association, temporal relationship, biological plausibility, and coherence, among other considerations. Section 4.1 presents hazard identification and evidence integration conclusions for key non-cancer outcomes. For the cancer assessment, Section 5.1 presents hazard identification and Section 5.2 discusses mode of action (MOA) considerations.

EPA conducted dose-response analysis for the health outcome categories that received a judgment of *likely* ("evidence indicates that D4 exposure likely causes [health effect]") during evidence integration. EPA only considered the health outcomes and associated specific health effects from the *likely* evidence integration judgments to use as toxicity values when estimating risks from exposure to D4.

If supported by statistically and/or biologically significant results and if the dose-response data could be reasonably modeled, EPA conducted benchmark dose (BMD) modeling. The dose-response assessment of relevant non-cancer endpoints, including selection of studies and chosen PODs, is discussed in Section 4.2. EPA did not conduct a dose-response assessment for cancer.

Finally, EPA assigned confidence ratings for each human health hazard outcome chosen for acute, intermediate, and chronic exposure scenarios. These ratings considered the evidence integration conclusions as well as additional factors such as relevance of the health outcome (and associated health effect[s]) to the exposure scenario (acute, intermediate, or chronic) and biological factors that increase susceptibility to D4 human health hazards (*e.g.*, identification of susceptible subpopulations). This overall weight of scientific evidence analysis is presented in Section 6.

2.1 Consideration of Previous Assessments

Throughout each of these human health hazard analysis steps, EPA considered results of previous analyses (summarized in Table 2-1). In the following sections, EPA compared the currently available information on human health hazards with the results of these previous assessments when relevant.

(SCCS, 2010)(EC/HC, 2008)(SEHSC, 2020)(Brooke et al., 2009)EPA reviewed key studies and critical endpoints identified in previous assessments. The studies and the endpoints selected as the basis for the POD of the previous assessments were not consistent. Given the relatively small set of studies identified

in systematic review and the different approaches in the previous assessments, EPA opted to consider the full set of studies with relevant routes of exposure identified through systematic review rather than narrowing the focus based on the previous assessments.

Table 2-1. Summary of Previous Assessments of D4 Hazard

Previous Assessments of D4 Human Hazard	PODs	Basis for Non-Cancer PODs	Uncertainty Factors	Cancer Hazard Conclusions
Environment Canada and Health Canada's *Screening Assessment for the Challenge: Octamethylcyclotetrasiloxane (D4): CASRN 556-67-2* (EC/HC, 2008)	Inhalation: 420 mg/m^3 (35 ppm)	LOEC for increased liver weights, increased adrenal weights, decreased thymus weights and alveolar macrophage foci and chronic interstitial inflammation of the lung in a 3-month rat inhalation study (Burns-Naas et al., 2002; RCC, 1995a)	N/A	No quantitative cancer assessment. Assessment provides a brief description of uterine tumors observed at high doses and a statement that the position that uterine tumors are not relevant to humans "has not been supported to date by international reviews due to lack of a thorough mode-of-action analysis."
	Oral and dermal: 100 mg/kg/day	LOEC for decreased serum estradiol in 7-day mouse studies (He et al., 2003) and decreased body weights and relative liver weights in fetuses in 8-day rat studies (Falany and Li, 2005),	N/A	
The UK's Environment Agency *Environmental Risk Assessment Report: Octamethylcyclotetrasiloxane (D4; CASRN: 556-67-2)* (Brooke et al., 2009)	Inhalation 363 mg/m^3 (30 ppm)	NOAEL for local effects on the respiratory tract in a two year inhalation study in rats (Battelle PNL, 2004a).	N/A	No quantitative cancer assessment. Assessment states D4 causes uterine tumours in F344 rats, but the underlying mechanism is not relevant to humans, noting that species differences in reproductive aging "render this mechanism irrelevant to humans"
	Oral: 25 mg/kg/day	NOAEL 17% increase in liver weight at 100 mg/kg/day in a 14 day gavage study in rats (Dow Corning, 1990)	AF = 300	
The European Commission (EC)'s Scientific Committee on Consumer Safety (SCCS) *Opinion on Cyclomethicone: Octamethylcyclotetrasiloxane (Cyclotetrasiloxane, D4) and Decamethylcyclopentasiloxane (Cyclopentasiloxane, D5)* (SCCS, 2010)	Inhalation: 1820 mg/m^3 (150 ppm)	NOAEL for non-neoplastic changes (increased liver weights and centrilobular hypertrophy of hepatocytes in male rats receiving D4 for 12 months, 6 hours/ day, 5 days/ week (Plotzke et al., 2005; Battelle PNL, 2004b)	N/A	No quantitative cancer assessment. Assessment provides a description of uterine tumors observed at high doses and a statement that the position that uterine tumors are not relevant to humans "is not supported to date by international reviews due to lack of published data and a thorough mode-of-action analysis."
	Dermal: 17.8 mg/kg/day	Extrapolated from inhalation POD 150 ppm	N/A	
SEHSC's *Request for Risk Evaluation Under the Toxic Substances Control Act; Octamethylcyclotetrasiloxane (D4; CASRN: 556-67-2)* (SEHSC, 2020)	Internal dose: 30 mg-hr/L	BMDL for internal dose 24 hr AUC associated with reduced live litter weights in a two-generation reproductive toxicity study (WIL Research, 2001a)	10x for human variability 10x database uncertainty	No quantitative cancer assessment. Authors suggest that the effects were not relevant at lower concentrations that would be encountered by humans

3 TOXICOKINETICS

Toxicokinetic and metabolism studies were available in humans and rodents for D4 via the oral, inhalation, dermal, and intravenous routes. Information on absorption, distribution, metabolism, and elimination are described in Sections 3.1 through 3.4 below. The paragraphs in this section (directly below) outline the available human, animal, and *in vitro* studies for each route (oral, inhalation, dermal), and Appendix E presents details on methods and results from these studies by route of exposure.

Via the oral route, EPA identified one human study that measured D4 in plasma after oral administration (Dow Corning, 1998c). Three studies in Fischer 344 (F344) rats investigated D4 absorption, distribution, and elimination using single doses from 10 to 300 mg/kg-bw (Domoradzki et al., 2017; Dobrev et al., 2008; Dow Corning, 1998d). Two of the rat studies analyzed for specific metabolites in urine (Dow Corning, 1998d) and in urine and feces (Domoradzki et al., 2017). Additional studies focused primarily on enzyme induction (Falany and Li, 2005; Zhang et al., 2000).

Via inhalation, EPA identified a study in which humans were exposed to D4 vapor for one hour (Utell et al., 1998). EPA also identified multiple inhalation studies in F344 and Sprague-Dawley (SD) rats that evaluated toxicokinetics and/or enzyme induction after single or repeated exposures (Schmitt et al., 2023; Meeks et al., 2022; Dow Corning, 2001b; Plotzke et al., 2000; McKim et al., 1998) and enzyme induction in guinea pigs (Dow Corning, 2001b).

Studies conducted to primarily investigate absorption from dermally applied D4 included two *in vivo* studies in humans (Biesterbos et al., 2015; University of Rochester Medical Center, 2001) and three *in vivo* studies in rats and mice (one in which nude mice were grafted with human skin) (Dow Corning, 2001a; University of Rochester Medical Center, 2000; GE, 1994a). EPA identified *in vitro* studies using human skin (Dow Corning, 1998a, 1991), miniature swine skin (Dow Corning, 2006), and rat skin (GE, 1994b). EPA also conducted dermal absorption modeling with IH SkinPerm ™ (AIHA, 2024) using physical and chemical properties of D4 as a comparison to the empirical data.

EPA used a PBPK model described in Campbell et al. (2023) that used input parameters based on multiple D4 toxicokinetics studies to extrapolate across exposure routes, species, and exposure durations associated with conditions of use (COUs) and exposure scenarios used in the risk evaluation. Section 4.2.3 describes the model, provides a high-level review, and identifies the toxicokinetics studies used to estimate parameters for the model.

3.1 Absorption

D4's highly lipophilic and volatile nature affects absorption. After oral and inhalation exposure, absorption in rat studies was estimated to be up to 100 percent (Schmitt et al., 2023; Domoradzki et al., 2017; Dobrev et al., 2008; Plotzke et al., 2000; Dow Corning, 1998d).

Dermal absorption estimates were much lower, with most studies showing less than 1 percent (Dow Corning, 2006, 2001a; University of Rochester Medical Center, 2000; Dow Corning, 1998a) except one study that identified absorption of approximately 20 percent (GE, 1994a). These dermal absorption estimates are from *in vivo* and *in vitro* studies using unoccluded conditions (no covering over the skin).

3.1.1 Humans

In human studies, D4 was absorbed after oral and inhalation exposure, and less through dermal exposure. Dow Corning (1998c) found that D4 parent compound (measured using gas chromatography and mass spectroscopy (GC/MS)) was absorbed after volunteers were exposed to 12 mg/day

(approximately 0.15 mg/kg-bw/day) oral D4 doses for 2 weeks, with amounts in plasma of 17.4 and 23.8 ng/mL (ng/g) after 1 and 2 weeks, respectively compared with baseline (pre-exposure) and placebo levels that ranged from 3.6 to 6.3 ng/mL. Quantitative percent absorption estimates were not possible because Dow Corning (1998c) did not provide information on elimination.

Utell et al. (1998) exposed humans to 122 mg/m^3 D4 vapor via inhalation through the mouth for one hour with intermittent exercise in two separate experiments and combined the results. The authors also conducted a second exercise in which humans were exposed through the mouth for part of the exposure period and through the nose for part of the exposure. This alternate mouth and nose exposure was conducted at rest No information on excretion (in urine, feces) was provided, and body burden information was not available because it is a human study. *OECD Guideline for the Testing of Chemicals (Number 417): Toxicokinetics* (OECD, 2010) recommends estimating absorption from animal studies using the amount excreted and in the carcass. Human studies do not include all of these measurements, and Utell et al. (1998) did not provide information on excretion, which limited EPA's ability to obtain an accurate measurement of absorption from this study.

Given D4's high volatility, some may be exhaled relatively quickly without becoming systemically available. The authors estimated D4 "uptake" as the amount of parent compound vapor deposition in lungs (using GC/MS) after excluding the amount exhaled. In the mouthpiece experiments, the intake was 137 mg and the uptake was 11 mg. The deposition across the experiments in which exposure was by mouth was 12 percent and rest, 6 percent when exercising, and 9 percent when averaged across rest and exercise periods. The peak plasma D4 level was 79.4 ng/g during exposure (via mouth). In the intermittent mouth and nasal inhalation experiments, the deposition was 12 percent for each (Utell et al., 1998).

University of Rochester Medical Center (2001) applied 1.0 and 1.4 g D4 dermally to axilla (underarm skin) of female and male volunteers, respectively. D4 concentration in plasma peaked at 0.85 to 7.02 ng/g one hour after application; information on amount of D4 volatilized from the application site was not provided, and the authors did not estimate percent absorption. Female volunteers had mean D4 levels in plasma that were approximately three times higher than males ($p < 0.05$). The mean peak D4 levels in expired air were also approximately three times higher in females than males but this difference was not statistically significant.

3.1.2 Laboratory Animals

In rats and mice, EPA identified information for the oral, inhalation, and dermal routes of exposure. The highest absorption rates in rats were via the oral and inhalation routes. For the oral studies, measured values were vehicle and dose dependent. In studies with good recovery, dermal absorption was mostly 1.02 percent or lower for *in vivo* and *in vitro* studies, with some higher values (*e.g.*, 19.96 percent without occlusion in a rat *in vivo* study).

Oral

Dobrev et al. (2008) administered D4 via oral gavage (in corn oil) as a single administration to F344 rats. Absorption of parent D4 (measured by gas chromatography - flame ionization detection (GC-FID)) was estimated as 100 percent at the lowest dose (10 mg/kg-bw) and decreased with increasing dose, with 50 percent absorbed at the highest dose (300 mg/kg-bw). In another study (Domoradzki et al., 2017), F344 rats were administered 30 mg ^{14}C-D4/kg-bw by oral gavage in a liquid diet dosing solution one time, and the mean percent of total radioactivity absorbed (inclusive of D4 parents and any metabolites) was 77.2 and 72.5 percent in female and male rats, respectively (inclusive of the amounts in urine, tissues, carcass, expired volatiles, and expired carbon dioxide (CO_2)). Dow Corning (1998d)

administered 300 mg/kg-bw of ^{14}C-D4 a single time to female F344 rats in two different vehicles or without a vehicle and estimated absorption as a percent of total radioactivity to be 12.11 percent (in Simethicone, a polydimethylsiloxane fluid), 28.14 percent (no vehicle), and 51.95 percent (corn oil).

Inhalation

EPA estimated absorption from a study by Schmitt et al. (2023), who exposed female SD and F344 rats in a nose-only inhalation study to 0 or 8,492 mg/m^3 (0 or 700 ppm) of ^{14}C-D4 for a single 6-hour exposure or to non-radiolabeled D4 for 14 days followed by a single exposure to ^{14}C-D4 on day 15. EPA added the percent of total radioactivity identified in urine, feces, carcass, expired volatiles, and expired CO_2 presented in Table 1 from Schmitt et al. (2023) to estimate the amount of D4 and any metabolites absorbed. After a single exposure, 82 and 95.8 percent was absorbed by F344 and SD rats, respectively. After repeated exposure, the amount of total radioactivity absorbed by F344 and SD rats was estimated at 78.8 and 85.4 percent, respectively. The percent exhaled as volatiles ranged from 18.2 to 25.4 percent across experiments.

Plotzke et al. (2000) exposed F344 rats (male and female), via a nose-only inhalation exposure, to 84.9, 849, or 8,492 mg/m^3 (7, 70, or 700 ppm) ^{14}C-D4 vapor for a single 6-hour exposure. The authors also exposed F344 rats (male and female) to unlabeled D4 for 14 days to 84.9 or 8,492 mg/m^3 followed by ^{14}C-D4 for a single day (day 15). The authors also used control groups. EPA added the percent of total radioactivity identified in feces, urine, expired volatiles, as CO_2, and carcass (estimated from Figures 4 and 6 in Plotzke et al. (2000)), and estimated absorption as between approximately 94 and 96 percent for the lowest concentration across experiments and sexes. The amount of D4 exhaled as volatiles was approximately 27 to 29 percent of total radioactivity.

Dermal

D4 is highly volatile and across dermal studies, a large amount volatilized when applied to skin and was not available for absorption. In two *in vivo* studies in mice with human skin grafts or F344 rats, absorption of neat D4 by the dermal route was estimated to range from approximately 0.35 to 1.09 percent (Dow Corning, 2001a; University of Rochester Medical Center, 2000). Dow Corning (2001a) applied nominal neat ^{14}C-D4 doses of up to 10.0 mg/cm^2 skin to rats for 1, 6, and 24 hours and evaluated absorption when exposure ended or at 168 hours after exposure. University of Rochester Medical Center (2000) applied neat ^{14}C-D4 for 24 hours to female nude mice with human skin grafts.

A third *in vivo* study (GE, 1994a) in which neat D4 was applied to SD rats for 6 hours resulted in higher percentages absorbed: 18.61 and 19.96 in females and males for unoccluded experiments and 33.91 in females and 41.77 in males for occluded scenarios. *In vitro* estimates for studies with recovery greater than 80 percent were 1.02 percent for human skin when using neat D4 (Dow Corning, 1998a) and 0.004 to 0.24 percent for dilutions of D4 in personal care products using miniature swine skin (Dow Corning, 2006). Two additional studies with recoveries of less than 80 percent are presented in Appendix E.[2]

It is not clear why dermal absorption estimates were higher in one study with adequate recovery (GE, 1994a). A different study (University of Rochester Medical Center, 2000) identified significant leaking from the skin depot used to administer D4, and the authors were able to revise the methods to minimize leaking. GE (1994a) did not suggest that there was any leaking from the skin depot or elsewhere but it did use more sampling times compared with other studies, which may have increased opportunities for leaking. As discussed in Campbell et al. (2023), differences in dermal absorption across studies may also

[2] Dow Corning (1991) reported absorption of 0.29 to 4.5 percent for individual human donors using neat D4 in an *in vitro* study, but recovery ranged from 38.6 to 63.8 percent. GE (1994b) reported absorption as 11.64 percent for neat D4 in an *in vitro* study using rat skin but recovery was 76.9 percent.

be due to genetic variation and hepatic differences between SD rats used in GE (1994a) and F344 rats used in Dow Corning (1998a).

Modeling dermal absorption of D4 (50 or 10 percent solution or neat) via the IH SkinPerm ™ model (AIHA, 2024) using D4's physical and chemical properties as input values resulted in absorption estimates ranging from 0.143 to 1.1 percent. D4's log K_{OW} of 7.13 was higher than the upper end of the range (log K_{OW} of 5.49) used to train the model (AIHA, 2017), and therefore, EPA's confidence in these modeled results is lower than if D4's log K_{OW} was within the range of training values.

3.2 Distribution

After single oral doses, two studies measured percentages of total radioactivity that were retained in the carcass when measured at 168 hours after exposure. After a dose of 30 mg/kg-bw, 8.68 and 10.73 percent were retained in male and female F344 rats (Domoradzki et al., 2017). After a single 300 mg/kg-bw dose, Dow Corning (1998d) identified differences based on the vehicle with only 0.76 percent retained in the carcass using Simethicone but up to 7.64 percent retained when D4 was administered in corn oil.

Via inhalation, the percentages of total radioactivity in carcasses were also measured 168 hours after exposure to D4 (Schmitt et al., 2023; Plotzke et al., 2000). After a single 6-hr exposure, 9.2 and 16.0 percent were observed in carcasses of female F344 and SD rats, respectively; in F344 rats, 7.8 to 12.3 percent (inclusive of muscle, fat, and bone) across sexes and air concentrations remained in the carcasses after a single exposure (Plotzke et al., 2000). After a 14-day exposure to unlabeled D4 with a final 1-day exposure to ^{14}C-D4, 4.5 and 6.9 percent of total radioactivity were observed in the carcasses in F344 and SD females, respectively (Schmitt et al., 2023) and from 6.53 to 8.50 percent in F344 rats (Plotzke et al., 2000).

After dermal exposure, less than one percent of the total administered dose was found in the carcass when measured at 72 and 96 hours post exposure and values as low as 0.01 percent remained in the carcasses when measured at 168 hours post-exposure (Dow Corning, 2001a; University of Rochester Medical Center, 2000; GE, 1994a).

D4 (and metabolites) were distributed to blood and a wide variety of tissues (*e.g.*, fat, liver, lungs, adrenals, uterus, ovaries, testes, spleen) after oral and inhalation exposure by rats (Schmitt et al., 2023; Meeks et al., 2022; Domoradzki et al., 2017; Jean and Plotzke, 2017; Plotzke et al., 2000; Dow Corning, 1998d). The highest concentrations were consistently identified in fat (*e.g.*, perirenal, brown, abdominal) whether via oral or inhalation exposure. Liver, lungs and sometimes adrenals had lower values than fat but usually higher concentrations than other tissues. Maximum (peak) concentrations (*i.e.*, the C_{max}) were observed within 1 to 2 hours after the start of exposure in blood and most tissues but occurred later (*e.g.*, 12 to 24 hours) in fat. In one study, the terminal half-life was longest in the uterus; however, the area under the curve (AUC), which is an estimate of the amount that takes into consideration total concentration over the full exposure duration, was lower in the uterus than in most other tissues (Domoradzki et al., 2017).

Female rats generally had higher tissue levels than males, and SD females often had the highest concentrations when both F344 and SD rats were examined (*e.g.*, after repeated exposure).

3.2.1 Oral Studies in Laboratory Animals

Domoradzki et al. (2017) measured distribution at multiple times after a single oral gavage dose of ^{14}C-D4 at 30 mg/kg-bw to F344 rats. D4 was distributed in all tissues that were measured: blood, perirenal

fat, liver, lungs, adrenals, the digestive tract, spleen, uterus, ovaries, and testes. The C_{max} was observed 2 hours post-dosing in most tissues but at 12 or 24 hours in fat. C_{max} was observed at 6 hours in a couple tissues – in testes when measuring both D4 parent and total radioactivity and in adrenals only when measuring total radioactivity in females. The highest amounts in tissues measured as AUCs were in perirenal fat and adrenals (both total radioactivity and parent D4) with highest levels in females. The longest terminal half-lives were in the uterus (400-700 hours), a primary target organ in the risk evaluation. Perirenal fat, lungs (total radioactivity only), and ovaries also had longer half-lives than other tissues, generally greater than 200 hours.

Total radioactivity and parent D4 AUCs in the uterus were 700.66 µg equivalents*h/g and 547.2 µg*h/g respectively. Several tissues had higher AUCs than the uterus. Values for total radioactivity in females for other tissues with higher AUCs than the uterus ranged from 793.74 (liver) to 7774.09 (perirenal fat) with values for lung, digestive tract, and adrenals within this range. Ovaries had AUCs at 1,465.07 for total radioactivity and 1,316.02 for parent D4. In females, two tissues had AUCs lower than the uterus: blood and spleen total radioactivity values were 174.93 and 329.41, respectively. Testes also had a lower AUC, measured at 220.56 for total radioactivity (Domoradzki et al., 2017).

At 168 hours after dosing, 8.68 and 10.73 percent remained in carcasses in males and females, respectively, and 0.98 and 1.43 percent of the administered dose remained in unspecified tissues in males and females, respectively (Domoradzki et al., 2017).

Dow Corning (1998d) administered 300 mg/kg-bw of ^{14}C-D4 once to female F344 rats via oral gavage in two different vehicles or as neat D4. Total cumulative percentages of administered doses in the carcass at 168 hours after exposure were 7.64 (corn oil), 0.76 (Simethicone), and 3.59 (neat). Of the tissues measured, the highest percentages measured at 168 hours were as follows: fat > liver > blood > lung - each had less than 1 percent for each vehicle/neat condition. Radioactivity was not detected in adrenals, ovaries, or spleen as a percent of administered dose for any condition.

3.2.2 Inhalation Studies in Laboratory Animals

EPA identified multiple inhalation studies in rats, with results discussed in this section. Summarizing the findings across studies, the highest C_{max} values were most often observed in fat after both single and repeated exposures. Distribution to blood, liver, lung, testes, ovaries and other tissues was also observed in multiple studies after single and repeated inhalation exposures in rats.

Plotzke et al. (2000) exposed F344 rats (male and female) via a nose-only exposure to 84.9, 849, or 8,492 mg/m^3 (7, 70, or 700 ppm) ^{14}C-D4 vapor for a single 6-hour exposure. The authors also exposed F344 rats (male and female) to unlabeled D4 for 14 days to 84.9 or 8,492 mg/m^3 followed by ^{14}C-D4 for a single day (day 15).

Samples were taken from exposed animals up to 168 hours after exposure – to assess radioactivity of blood and plasma, liver, lungs, perirenal fat, ovaries, vagina, and testes. Tissues were also collected from control animals to measure background radioactivity, but the authors do not mention whether controls were placed in air chambers (Plotzke et al., 2000). Directly after exposure ceased, rats retained 5.0 to 6.1 percent of the total radioactivity (carcass as well as urine and feces in exposure tube). The highest amounts of radioactivity (D4 µg equivalents) were in the lungs, liver, fat and ovaries immediately after exposure. Results did not differ significantly between single and repeated exposures, by sex, or across concentrations (Plotzke et al., 2000).

In plasma, radioactivity increased through the single exposure period and showed a multiphasic time course with a rapid decline within 24 hours and then a slower terminal phase. Slower elimination occurred from tissues than blood/plasma (Plotzke et al., 2000).

Female rats had somewhat lower values in plasma but higher values in fat than males after a single exposure; similar but smaller differences occurred after repeated exposure (Plotzke et al., 2000).

The C_{max} for most tissues and plasma occurred within 1 hour but the C_{max} for fat occurred from 3 to 24 hours post exposure. The AUC values in fat were 12- to 61-fold higher than those for plasma in the single exposure experiment, with similar ratios after repeated exposure. Other tissue AUCs were between fat and plasma levels, except that the testes AUCs were similar to plasma values (Plotzke et al., 2000).

After 168 hours in the single exposure study, 7.8 to 12.3 percent of total radioactivity remained in the carcass (which was mostly muscle, fat, and bone). Only 0.495 to 0.794 percent was found in tissues after 168 hours. Lower percentages of total radioactivity were observed in tissues (0.193 to 0.468 percent) and carcass (6.53 to 8.50 percent) after repeated exposure (Plotzke et al., 2000). Almost all radioactivity in fat was parent D4, even up to 168 hours after exposure.

Schmitt et al. (2023) exposed female SD and F344 rats in a nose-only inhalation study to 0 or 8,492 mg/m^3 (0 or 700 ppm) of ^{14}C-D4 for a single 6-hour exposure or to non-radiolabeled D4 for 14 days followed by a single exposure to ^{14}C-D4 on day 15. The authors identified 9.2 and 16.0 percent of total radioactivity in the carcass of F344 and SD females, respectively after the single 6-hour D4 exposure (measured at 168 hours after exposure). Lower levels – 4.5 and 6.9 percent in F344 and SD females, respectively – were observed in the carcass after repeated D4 exposure (also measured 168 hours after the final dose) (Schmitt et al., 2023).

The authors sampled blood, plasma, liver, lung, and perirenal fat through 168 hours post-exposure for total radioactivity and parent D4. During the single 6-hour exposure, parent D4 blood AUCs for F344 and SD females were similar (45.45 and 46.95 µg ^{14}C-D4 x h/g, respectively) but diverged soon after exposure, resulting in AUCs of 39.06 and 69.52 µg ^{14}C-D4 x h/g, respectively through the 168-hour post-exposure period. During the 15-day exposure period, parent D4 AUC was already lower in F344 rats (26.82 µg ^{14}C-D4 x h/g) compared with SD rats (34.19 µg ^{14}C-D4 x h/g) and the AUC values were 31.32 and 57.31 µg ^{14}C-D4 x h/g in F344 and SD rats, respectively through the 168-hour post-exposure period (Schmitt et al., 2023).

Of the tissues sampled, the C_{max} for total radioactivity was reached just after exposure ended for all exposed groups for all tissues except fat, which reached C_{max} at 12 hours after exposure in F344 rats and 2 hours after exposure in SD rats. Fat had the highest levels followed by lung and then liver when measured as AUC. Comparable amounts of total radioactivity were seen in lungs of both rat strains. In perirenal fat, the AUC in F344 rats was about 50 percent higher than in SD rats (both total radioactivity and D4) after single exposures. This changed after repeated exposure, in that total radioactivity AUC in fat of SD rats was more than 20 percent higher than F344 rats (Schmitt et al., 2023).

Meeks et al. (2022) exposed F344 and SD rats to 0 or 8,492 mg/m^3 (0 or 700 ppm) D4 via whole-body inhalation for up to 28 days with a recovery period up to 14 days. The authors measured parent D4 in plasma and fat. Plasma D4 levels were maintained at higher levels through the exposure period with F344 females showing some decrease starting at day 15 ($p \leq 0.05$). day 28. During exposure, plasma levels ranged from 4.67 to 8.33 µg D4/g plasma in male SD rats and from 4.37 to 6.23 µg D4/g plasma

in male F344 rats. In female SD rats, values were 10.45 to 10.98 µg D4/g plasma during exposure, whereas female F344 rats showed 5.44 to 9.15 µg D4/g plasma during exposure. On recovery day 1, plasma D4 decreased substantially for all groups – 0.20 to 0.25 µg D4/g plasma among SD and F344 males and F344 females, with a somewhat higher value for SD females (0.87 µg/g). By recovery day 14, levels were 0.03 to 0.4 across sexes and strains.

D4 in fat increased to at least day 15 but usually through day 28 with the highest levels (849 to 1,656 µg D4/g fat) observed at recovery day 1. Levels then decreased by recovery day 14 (ranging from 151 to 384 µg/g). SD females always had the highest levels at each timepoint except on recovery day 14. The authors hypothesized that the decrease of D4 from fat after exposure may indicate that D4 was transported to lungs and subsequently exhaled, although this was not measured in the study (Meeks et al., 2022).

Jean and Plotzke (2017) measured parent D4 in plasma, liver, and fat of male and female F344 rats after 6 months of exposure via inhalation (6 hours/day, 5 days/week). The samples were measured just after the daily exposure ended and were expected to represent peak values. Across the full range of D4 air concentrations used in the study (121 to 8,492 mg/m^3), the D4 concentration in fat ranged from 3.35 to 866 µg/g in males and 11.1 to 1,240 µg/g in females compared with levels below detection in controls. D4 concentrations in liver ranged from 0.58 to 71.19 µg/g in males and 1.18 to 76.71 µg/g in females, with levels of 0.30 and 0.28 µg/g in livers of control males and females, respectively. In plasma, D4 levels ranged from 0.0722 to 8.21 µg/mL in males and from 0.153 to 13.0 µg/mL in females and was not detected in plasma of controls. Females had consistently higher tissue levels of D4 than males for most tissues and exposure concentrations (Jean and Plotzke, 2017).

3.2.3 Dermal Studies in Laboratory Animals

Dow Corning (2001a) applied nominal neat ^{14}C-D4 doses of 2.0, 4.8, and 10.0 mg/cm^2 skin to rats for 1, 6, and 24 hours with samples taken directly after exposure ended. Additional groups were exposed for 24 hours, with subsequent skin washing, and samples were taken through 168 hours. For all doses tested, less than 1% of the applied dose was absorbed at each time point. Absorption appears to be time-dependent between 1 and 24 hours. After 24 hours of exposure, the average percentage of dose absorbed was significantly less than that seen after 1 h of exposure (however, no significant difference for time-dependence absorption was seen at 6 hours post exposure).

A blood kinetics group was assessed at 0.5, 2,4, and 10 hours for both total radioactivity and D4 content. The authors evaluated total radioactivity, with blood and expired volatiles also evaluated for presence of parent D4 using GC/MS. The carcass had 0.01 to 0.05 percent of total radioactivity across doses and when measured from 24 to 168 hours after exposure. From 0.5 to 168 hours in the mass balance and blood kinetics experimental groups, total radioactivity in blood (measured only after the high dose) was below detection. Parent D4 (high dose) was not higher than background. Total radioactivity content in individual tissues other than skin was not assessed.

University of Rochester Medical Center (2000) applied neat ^{14}C-D4 for 24 hours to female nude mice with human skin grafts. Only 0.02 percent of total administered radioactivity was found in the carcass 72 hours after exposure began, and only 0.02 percent remained in skin at the application site after 72 hours. Other tissues were not examined.

GE (1994a) applied radiolabeled D4 to skin of SD rats for 6 hours. In unoccluded experiments, 6 percent of total radioactivity administered was found at the skin application site and less than one percent was

found in the carcass at 96 hours after exposure. In the occluded scenario, 5 to 10 percent was found in skin and one percent or less found in the carcass at 96 hours. Other tissues were not evaluated.

3.3 Metabolism

3.3.1 Metabolites

Dimethylsilanediol and methylsilanetriol were identified in urine after oral, inhalation, and intravenous (i.v.) routes as major metabolites of D4 along with several minor metabolites (Schmitt et al., 2023; Domoradzki et al., 2017; Plotzke et al., 2000; Varaprath et al., 1999; Dow Corning, 1998d). Methylsilanetriol and dimethyldisiloxane-1, 3, 3, 3-tetrol were found in feces after oral exposure (Domoradzki et al., 2017).

3.3.1.1 Oral Studies in Laboratory Animals

Dow Corning (1998d) administered single oral gavage doses of 300 mg/kg-bw of ^{14}C-D4 in two vehicles and as a neat preparation to female F344 rats and measured D4 in various matrices up to 168 hours after exposure. Selected urine samples showed similar metabolic profiles for both vehicles and the neat experiment (Dow Corning, 1998d). Five metabolites were identified:

- Methylsilanetriol [$MeSi(OH)_3$]
- Dimethyldisiloxane-1,3,3,3-tetrol [Me_2-Si(OH)-O-Si$(OH)_3$]
- Monomer diol [Me_2-Si$(OH)_2$], identified as dimethylsilanediol in other studies
- Trimethyldisiloxane-1,1,3-triol [$MeSi(OH)_2$-O-Si(OH)Me_2]
- Dimer triol [Me_2-Si(OH)-O-Si(OH)Me_2]

Domoradzki et al. (2017) administered a single 30 mg ^{14}C-D4/kg-bw dose by oral gavage in a liquid diet dosing solution to male and female F344 rats. The authors analyzed for specific metabolites in urine and feces.

Urine was sampled at the 12-, 24-, and 48-hour collection timepoints. In urine of female rats, dimethylsilanediol represented the greatest percentage of total radioactivity - from 51 to 59 percent across the three collection intervals. Methylsilanetriol ranged from 20 to 25 percent, and dimethyldisiloxane-1, 3, 3, 3-tetrol ranged from 9.5 to 13.1 percent. Similar ranges were identified for males. Hexamethyltrisiloxane-1, 5-diol was only present in female animals and only after the first two collection times.

In feces, methylsilanetriol was present at 13 and 26 percent (at 24 hours) and increased to 55 and 53 percent (at 48 hours) in females and males, respectively. Dimethyldisiloxane-1, 3, 3, 3-tetrol was observed at 17 percent in feces of males at 24 hours and 12.2 and 13.9 percent in males and females at 48 hours.

Domoradzki et al. (2017) found that the percent of total radioactivity in female rats that was be attributed to metabolites was 100 percent in urine, 48.26 percent in feces, and 30.93 percent in expired volatiles, with similar results in males.

3.3.1.2 Inhalation Studies in Laboratory Animals

Schmitt et al. (2023) exposed female SD and F344 rats in a nose-only inhalation study to 0 or 8,492 mg/m^3 of ^{14}C-D4 for a single 6-hour exposure or to non-radiolabeled D4 for 14 days followed by a single exposure to ^{14}C-D4 on day 15. Major metabolites in urine were dimethylsilanediol and methylsilanetriol, and the metabolite profile was similar for both strains. F344 rats, after repeated

exposure, showed slight shifts to higher molecular weight metabolites such as di- and trisiloxanols; the difference was statistically significant in the first six hours post-exposure. Fifteen to twenty-two percent of the D4 methyl groups excreted in urine were replaced with hydroxyl groups, with slightly greater replacement in F344 rats.

Only half the AUC in liver was the D4 parent in F344 rats, whereas about 80 percent of the liver AUC was parent D4 for SD rats. In lungs of both rat strains, only 10.6 to less than 20 percent was present as parent D4. In perirenal fat after single exposures, all radioactivity was identified as parent D4(Schmitt et al., 2023).

Plotzke et al. (2000) exposed F344 rats (male and female), via a nose-only exposure, to 0, 84.9, 849, or 8,492 mg/m^3 (0, 7, 70, or 700 ppm) ^{14}C-D4 vapor for a single 6-hour exposure. The authors also exposed F344 rats (male and female) to unlabeled D4 for 14 days to 84.9 or 8,492 mg/m^3 followed by ^{14}C-D4 for a single day (day 15). Control rats were also used.

Two major metabolites, dimethylsilanediol and methylsilanetriol, comprised 75 to 85 percent of the radioactivity in urine, and at least five minor metabolites were identified (with no D4 parent identified). Formation of methylsilanetriol indicated oxidative demethylation at the silicon-methyl bonds. The five minor metabolites indicated hydrolysis and/or oxidation after original oxidated metabolism of D4 by P450 enzymes (Plotzke et al., 2000). Almost all radioactivity in expired volatiles (up to 9 hours post-exposure) and all radioactivity in fat was parent D4, even up to 168 hours after exposure. The authors found no unmetabolized D4 in urine collected from 0 to 48 hours after single or multiple exposures.

3.3.1.3 Intravenous Studies in Laboratory Animals

Similar to the oral and inhalation routes, single i.v. injections of 70 mg/kg-bw (^{14}C-D4 emulsion with unlabeled D4) to F344 rats (Varaprath et al., 1999) yielded two major metabolites in urine - dimethylsilanediol and methylsilanetriol, which comprised 75 to 85 percent of the total radioactivity. Minor metabolites included tetramethyldisiloxane-1,3-diol [Me2Si(OH)-O-Si(OH)Me2], hexamethyltrisiloxane-1,5-diol [Me2Si(OH)-OSiMe2-OSi(OH)Me2], trimethyldisiloxane-1,3,3-triol [MeSi(OH)2-O-Si(OH)Me2], dimethyldisiloxane-1,1,3,3-tetrol [MeSi(OH)2-O-Si(OH)2Me], and dimethyldisiloxane-1,1,1,3,3-pentol [Si(OH)3-O-Si(OH)2Me]. Parent D4 was not detected in urine.

3.3.2 Enzyme Induction

D4 has been shown to induce enzyme activity and protein levels when measured in liver from oral and inhalation studies from four days to four weeks in SD and F344 rats (Meeks et al., 2022; Falany and Li, 2005; Dow Corning, 2002a, 2001b; Zhang et al., 2000; McKim et al., 1998) but not in guinea pigs after oral dosing or inhalation (Dow Corning, 2002a, 2001b).

Of all enzymes measured, D4 induced cytochrome P450 (CYP) 2B1 and 2B2 more than any of the other enzymes. CYP 2B1 and 2B2 enzyme activity and/or protein levels increased up to 100-fold. Meeks et al. (2022) evaluated both SD and F344 rats and suggested that the lower enzyme activity induced by SD rats may have been due to some inhibition of CYP2B1/2 activity that did not limit its expression (based on increased protein levels in SD rats as well as F344 rats).

CYP 3A1 and 3A2 induction was more modest; studies found increases in protein levels that were highest in SD females; one study also found increased enzyme activity (Meeks et al., 2022). Except for young rats, Falany and Li (2005) found consistent and modest increases in enzyme activity of CYP 2A1/2 (*e.g.*, 2-3-fold) but protein levels were not induced or were suppressed. NADPH cytochrome

P450 reductase (also called NADHP cytochrome c reductase) was modestly induced after oral (Zhang et al., 2000) and inhalation exposure (Meeks et al., 2022; McKim et al., 1998).

The inhalation studies (Meeks et al., 2022; Dow Corning, 2001b; McKim et al., 1998) evaluated several other enzymes. All three studies showed increases in epoxide hydrolase from less than 2- to 3-fold based on protein levels or enzyme activity or both. Meeks et al. (2022) found little change in protein or activity levels for CYP 2A1 and CYP 2C11 and McKim et al. (1998) found a slight increase in uridine diphosphate (UDP)-glucuronosyltransferase activity (but no dose-response) but no differences in CYP 4A activity from controls. Dow Corning (2001b) found that SD rats exposed via inhalation had increased enzyme activity of glutathione S-transferase, epoxide hydrolase, and 7-ethoxycoumarin O-deethylase (ECOD) by less than 2 times the control, but guinea pigs exhibited no increased activity of these enzymes.

3.3.2.1 Oral Studies in Laboratory Animals

Zhang et al. (2000) administered 0, 1, 5, 20, or 100 mg/kg-bw/day D4 in corn oil via gavage to male and female SD rats for four days. D4 induced CYP 2B1 and 2B2 isozymes the most based on increased enzyme activity when using 7-pentoxyresorufin-O-depentylase (PROD) as the substrate, with increases up to 13-fold at 20 mg/kg-bw/day in female rats. Increased protein levels of CYP 2B1 and 2B2 occurred at $\geq$ 5 and $\geq$ 1 mg/kg-bw/day D4 in males and females, respectively. CYP 3A1 and 3A2 also showed a dose-related increase in protein levels in females and minor increases in males. NADPH cytochrome P450 reductase increased by a small statistically significant amount. CYP 1A1/2 protein levels did not increase but enzyme activity using 7-ethoxyresorufin-O-deethylase (EROD), exhibited a modest statistically significant increase.

Falany and Li (2005) administered 0, 5, 20, and 100 mg/kg-bw/day D4 via oral gavage to groups of young, pregnant, and retired breeder female SD rats for 8 days and assayed liver microsomes for enzyme activity and protein concentrations of CYP 2B1 and 2B2, CYP 3A1 and 3A2, and CYP 1A. D4 induced CYP 2B, with statistically significant increases in enzyme activity (based on PROD) in young, pregnant, and mature rats; young rats exhibited the highest increase (16-fold increase at 100 mg/kg-bw/day compared with controls). Increases in CYP 2B protein were also observed in all groups, with a maximum of 90-fold increase in mature rats at 100 mg/kg-bw/day (Falany and Li, 2005). In pregnant dams, fetal CYP 2B1 and CYP2B2 levels and enzyme (PROD) activity were higher than controls (Falany and Li, 2005).

CYP 3A1/2 protein levels increased for young, mature, and pregnant rats with the highest increase observed at 100 mg/kg-bw/day in pregnant rats (55 times higher than controls). Mature rats had the lowest increase (8.5-fold at 100 mg/kg-bw/day) but all doses were statistically significantly higher than controls whereas only the 20 and 100 mg/kg-bw/day dose groups were statistically significantly higher in young and pregnant rats. No changes in enzyme activity were observed for CYP 3A1 and3A2 after D4 exposure (Falany and Li, 2005). CYP 1A activity (based on EROD) exhibited a modest increase (> 2-fold at 20 and 100 mg/kg-bw/day compared with controls) in mature and pregnant rats but not in young rats. None of the groups of rats showed significant changes in CYP 1A protein levels (Falany and Li, 2005).

In a short-term oral toxicity study, F344 rats and female Hartley guinea pigs were exposed via oral gavage to D4 at doses of 0 or 301 mg/kg-bw/day for 14 continuous days. Microsomal protein, total microsomal cytochrome P450, microsomal NADPH cytochrome C reductase, microsomal enzymatic activity (PROD, BROD, EROD, and MROD), and CYP 1A1 and 1A2, CYP 2B1 and 2B2, CYP 3A1 and 3A2, CYP 4A1 and 4A3, and epoxide hydrolase protein content were measured in the liver

microsomes (Dow Corning, 2002a). Total microsomal cytochrome P450 was unchanged in treated rats as compared to controls. Microsomal NADPH cytochrome C reductase activity was increased 142 percent in treated rats relative to controls. Treated rats exhibited a statistically significant increase of 61 percent in microsomal EROD activity (indicator of CYP 1A enzyme activity) and a statistically significant decrease of 3 percent in microsomal MROD activity (another indicator of CYP 1A activity). There was no statistically significant change in CYP 1A protein among treated rats. Treated rats exhibited a statistically significant increase in microsomal PROD and BROD activities (indicators for CYP 2B enzyme activity), as well as an increase in CYP 2B protein as compared to the undetectable level in controls. Treated rats exhibited decreased CYP 4A and increased microsomal epoxide hydrolase and CYP 3A relative to controls. Based on the data presented in the study, a LOAEL of 301 mg/kg-bw/day was identified for rats based on a significant increase in liver weight (absolute and relative) and changes in microsomal enzymatic activity (Dow Corning, 2002a). Under similar experimental conditions, study authors did not observe statistically significant treatment-related effects liver weight (absolute or relative) in treated guinea pigs. No significant changes in microsomal enzymatic activity were reported for guinea pigs (Dow Corning, 2002a).

3.3.2.2 Inhalation Studies in Laboratory Animals

Meeks et al. (2022) exposed male and female SD and F344 rats 6 hours per day to 0 or 8,492 mg/m^3 (0 or 700 ppm) D4 via whole-body inhalation for 1, 15, or 28 days. The authors also used two groups of recovery animals — 1 day or 14 days after the 28-day exposure period. Liver microsomes from the 1-day recovery group were assayed for enzyme activity using substrates expected to be specific for the enzyme and/or were measured for amount of enzyme present. The following enzymes (and substrates) were measured: total protein, total P450, CYP 1A1 and 1A2 (EROD), CYP 2A1 (testosterone 7α hydroxylase), CYP 2B1 and 2B2 (PROD and testosterone 16β-hydroxylase), CYP 3A1 and 3A2 (testosterone 6β-hydroxylase), and CYP 2C11 (testosterone 2α hydroxylase), epoxide hydrolase, and NADPH-cytochrome c reductase activity. The liver microsomes from the 14-day recovery groups were assayed for total protein, total P450, and CYP 2A1 and 2B2. Phenobarbital was used as a positive control during the male study.

On the first recovery day after exposure, enzyme induction was highest for CYP 2B1 and 2B2 (with protein levels 28 to 100 times higher than controls and activity levels of 7.3 to 40 times higher); SD rats had higher protein levels but F344 rats had similar or higher activity levels. The CYP 2B1 and 2B2 activity and protein levels decreased significantly by recovery day 14. Meeks et al. (2022) suggested that either the parent D4, a metabolite, or other mediator present or generated specifically in SD females was likely to have resulted in inhibited CYP2B1/2 activity but did not limit its expression.

For CYP 3A1 and 3A2, female rats given D4 had protein and activity levels of 4.1- to 17-fold higher than controls with higher levels in SD rats compared with F344 rats. There were less than 2 to 3-fold increases (protein and/or activity) for NADPH-cytochrome c reductase and epoxide hydrolase (both sexes) and CYP 3A1 and 3A2 (males) after D4 exposure. For CYP 1A1 and 1A2, both rat strains and sexes had increases of 2 to 3-fold in enzyme activity but decreases in protein levels (down to 22 to 40 percent of controls). CYP 2C11 and CYP 2A1 showed little change in protein levels or activity. Meeks et al. (2022) stated that the results suggest D4 has a similar enzyme induction profile as phenobarbital.

McKim et al. (1998) exposed both male and female F344 rats to 0, 849, and 8,492 mg/m^3 (0, 70, and 700 ppm) D4 vapor via whole-body exposure for 6 hours per day, 5 days per week for 4 weeks. Phenobarbital was used as a positive control. Tissues were taken on days 3, 7, 14, 21, and 28. Microsomal fractions from the liver were examined for enzyme activity using substrates considered to be specific for each enzyme examined. Enzyme levels were also analyzed using immunoreactive

protein. CYP 2B1/2 activity using the substrate PROD increased by more than 10 and 20 times at 849 and 8,492 mg/m^3, respectively by day 10, and showed increases using immunoreactive protein. CYP 3A1 and CYP 3A2 showed modest increases in activity based on 6B-hydroxylation of testosterone and immunoreactive protein levels. Although CYP 1A1 and 1A2 exhibited a 2-to-3-fold increase in activity with EROD, D4 resulted in no induction of CYP 1A1 and a suppression of CYP 1A2 when measured by immunoreactive protein. NADPH cytochrome P450 reductase showed increased induction at both D4 exposure concentrations. Epoxide hydrolase activity and immunoreactive protein levels were increased two to three times in a dose-dependent manner, whereas UDP-glucuronosyltransferase exhibited only slight increases in activity that were not dose related and CYP4A enzyme activity did not differ from negative controls. There were some similarities to the enzyme induction profile of phenobarbital.

Dow Corning (2001b) exposed SD rats and Hartley guinea pigs via inhalation to 0 and 700 ppm for 6 hours per day for 5 days and evaluated whether D4 exposure induced the following enzymes: glutathione S-transferase using cytosolic liver fractions, and both epoxide hydrolase and ECOD using microsomal liver fractions. The authors noted that ECOD is a substrate for multiple CYP enzymes. Male rats showed significantly increased induction of all three enzymes compared with controls whereas female rats had increases in epoxide hydrolase and ECOD compared with controls. In contrast, none of the enzymes were increased in the guinea pigs.

3.4 Elimination

Rat data show that elimination via feces ranged from 22.56 to 80.6 percent of oral administered doses (higher with a dose of 300 mg/kg-bw ^{14}C-D4) and from 10 to 19.4 percent after inhalation with decreasing amounts after repeated exposure. Dermal exposure yielded less than one percent in feces. Excretion in urine after oral exposure ranged from 4.3 to 40.0 percent and depended on the method of administration (*e.g.*, neat, corn oil or Simethicone vehicle). After inhalation, the percent of D4 metabolites in urine ranged from 26.0 to 48 percent. Similar to fecal excretion, less than one percent was eliminated in urine after dermal exposure.

Expired volatiles ranged from 6.49 to 35 percent among oral and inhalation studies, and the lowest value was after oral administration in Simethicone (Dow Corning, 1998d). In two dermal studies, only 0.47 percent or less was eliminated as expired volatiles, but another study identified 12 to 25 percent as expired volatiles. CO_2 exhalation was less than 5 percent after inhalation and less than 1 percent after dermal exposure.

Dow Corning (1998d) administered ^{14}C-D4 at 300 mg/kg-bw orally to F344 rats as single doses both neat and in two separate vehicles and reported the cumulative percentages eliminated in feces through 168 hours as 41.0 (corn oil), 54.6 (neat), and 80.6 (Simethicone). The cumulative percentages eliminated in urine were 25.8 (corn oil), 12.8 (neat), and 4.30 (Simethicone). The cumulative percentages in expired volatiles through 168 hours were 14.5 (corn oil), 9.65 (neat), and 6.49 (Simethicone).

Domoradzki et al. (2017) administered a single 30 mg ^{14}C-D4/kg-bw dose by oral gavage in a liquid diet dosing solution to male and female F344 rats. Urine was sampled at the 12-, 24-, and 48-hour collection timepoints. The authors measured total radioactivity (inclusive of parent D4 plus metabolites) in multiple tissues and analyzed for parent D4 in feces and expired air. Parent D4 was not measured in urine because it was expected that only metabolites would be observed. The percent of total radioactivity recovered in expired volatiles was 29.90 and 18.41 in females and males, respectively. Amounts in urine were 32.08 and 40.04 percent in females and males, respectively. Finally, the amounts in feces were 22.56 and 27.21 percent in females and males, respectively. Domoradzki et al. (2017) found that the

percent of total radioactivity in female rats that was be attributed to metabolites was 100 percent in urine, 48.26 percent in feces, and 30.93 percent in expired volatiles, with similar results in males.

Schmitt et al. (2023) exposed female SD and F344 rats in a nose-only inhalation study to 0 or 8,492 mg/m^3 (0 or 700 ppm) of ^{14}C-D4 for a single 6-hour exposure or to non-radiolabeled D4 for 14 days followed by a single exposure to ^{14}C-D4 on day 15. At 168 hours after exposure, the authors collected urine, feces, expired volatiles, and CO_2. After single exposures, the percent in urine (26.0 and 32.6 percent) was similar to the percent in expired volatiles (23.5 and 25.4 percent). However, after 14 days, the percent in urine was roughly double (39.8 and 40.2 percent in F344 and SD rats, respectively) the percent in expired volatiles (18.2 and 19.5 percent in F344 and SD rats, respectively). The percent of total radioactivity excreted in feces decreased with longer exposure (18.2 to 19.4 percent after one day; 12.8 to 15.4 percent after repeated exposure). Amounts exhaled as CO_2 were 3.4 to 3.9 percent of total radioactivity (Schmitt et al., 2023).

Plotzke et al. (2000) exposed F344 rats (male and female) via a nose-only exposure to 84.9, 849, or 8,492 mg/m^3 (7, 70, or 700 ppm) ^{14}C-D4 vapor for a single 6-hour exposure. The authors also exposed F344 rats (male and female) to unlabeled D4 for 14 days to 84.9 or 8,492 mg/m^3 followed by ^{14}C-D4 for a single day (day 15). After single exposures, most recovered radioactivity after 168 hours was found in urine (approximately 33 to 48 percent) and expired volatiles (approximately 28 to 35 percent). In females, at the highest exposure (8,492 mg/m^3), more of the radioactivity that was eliminated was found in expired volatiles compared with urine. A similar difference based on air concentration was not found in males. Feces contained 10 to 15 percent and expired CO_2 had approximately 5 percent or less of total radioactivity. Similar results regarding elimination were seen after repeated exposure Almost all radioactivity in expired volatiles (up to 9 hours post-exposure) and all radioactivity in fat was parent D4, even up to 168 hours after exposure. The authors found no unmetabolized D4 in urine collected from 0 to 48 hours after single or multiple exposures (Plotzke et al., 2000).

Dow Corning (2001a) applied neat D4 doses to skin of F344 rats up to 24 hours and measured elimination directly after exposure or up to 168 hours. The authors applied 2.0, 4.8, or 10.0 mg/cm^2 to skin and measured total radioactivity (inclusive of D4 and any metabolites) in expired volatiles, urine, feces, and CO_2. Parent D4 was also analyzed in expired volatiles using GC/MS methodology. Percent of total radioactivity in expired volatiles 0.47 percent or less. Feces had 0.02 percent of total radioactivity and urine had 0.08 percent or lower. Exhaled CO_2 was only 0.03 percent or lower.

University of Rochester Medical Center (2000) applied neat ^{14}C-D4 for 24 hours dermally to female nude mice with human skin grafts. Samples were taken at one or more timepoints up to 72 hours. The amount of radioactivity exhaled after absorption through skin was 0.46 percent, amount in excreta (feces and urine) was 0.54 percent, and the amount exhaled as CO_2 was 0.05 percent (University of Rochester Medical Center, 2000).

In another dermal study, GE (1994a) applied radiolabeled D4 to skin of SD rats for 6 hours. For the unoccluded experiments, approximately twelve percent was exhaled, and urine, feces, carcass, and exhaled CO_2 each accounted for less than 1 percent each (GE, 1994a). In the occluded scenario, approximately 25 percent was found in expired volatiles, approximately 1 percent or less was identified in urine and in feces and less than one percent was exhaled as CO_2. Total recovery was lower than the unoccluded scenario: 77.14 percent for females and 84.05 percent for males, respectively (GE, 1994a).

4 NON-CANCER HAZARD ASSESSMENT

The sections below summarize available information on adverse outcomes and mechanistic data and present evidence integration conclusions for relevant human health hazard outcomes for D4. The data quality rating for each individual study is reported as an overall quality determination (OQD). For complete details on evidence integration judgments within and across evidence streams, see the evidence profile tables for each organ system in Appendix A.

Evidence integration judgments were determined based on considerations described in Chapter 7 of the 2021 Draft Systematic Review Protocol (U.S. EPA, 2021). In short, strength of the evidence judgments (robust, moderate, slight, indeterminate, or compelling evidence of no effect) for individual evidence streams (*i.e.*, human, animal, mechanistic) were determined by expert judgment based on the considerations described in the protocol. These considerations include quality of the database, consistency, magnitude and precision, dose-response, and biological significance. These were then integrated into an overall summary classification (see Appendix A for overall judgment classifications).

As described in Section 2, EPA used results from systematic review and previous assessments to independently evaluate the weight of scientific evidence for each hazard outcome. Hazard outcomes with sufficient confidence and quantitative study data then underwent dose-response analysis (Section 4.2).

4.1 Key Human Health Hazard Outcomes

The sections below summarize the hazard identification and evidence integration of liver toxicity, pulmonary toxicity, reproductive toxicity, and developmental toxicity, which are the endpoints for which the most data are available. Details on the evidence database and evidence integration judgments are presented in Appendix A. See full data extraction for all relevant studies in Draft Data Extraction for Environmental and Human Health (U.S. EPA, 2025a). Details on the data quality evaluations are provided in Draft Data Quality Evaluation for Animal Toxicology (U.S. EPA, 2025c) and Draft Data Quality Evaluation for Epidemiology (U.S. EPA, 2025d).

4.1.1 Hepatic Toxicity

4.1.1.1 Human Evidence

EPA identified two studies in humans that evaluated the effect of controlled D4 exposure on liver function. A high-quality cross-over controlled exposure study of 12 male and female volunteers (20-62 years old) exposed to D4 (12 mg in corn oil, or corn oil alone) orally (via syringe). Blood was collected at days 0, 7, and 14 as well as 1, 2, and 3 weeks after exposure ended and was analyzed for clinical chemistry. Oral exposure to 12 mg D4 per day did not result in statistically significant changes in total protein, albumin, bilirubin, aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), or cholesterol at any time point (Dow Corning, 1998c).

In a medium-quality cross-over controlled exposure study, 12 male and female volunteers (20-50 years old, nonsmoking) were exposed by inhalation to D4 vapor (10 ppm (12 µ/l, or 122 mg/m^3) or clean air via mouthpiece or via nasal delivery for 1 hour. Blood was collected immediately after exposure and 6 and 24 hours after exposure and was analyzed for clinical chemistry. Inhalation of D4 vapor in male and female volunteers did not result in statistically significant changes in total protein, albumin, bilirubin, alanine aminotransferase (ALT), AST, or LDH at any time point. Immune endpoints were also considered but no effects were observed in the controlled exposure studies in humans (University of Rochester, 1997).

4.1.1.2 Laboratory Animal Evidence

EPA identified multiple medium and high-quality animal inhalation studies that evaluated the effect of D4 exposure on the liver. Hepatic effects, including liver weight and increased incidence of hepatocyte hypertrophy were reported in multiple inhalation rodent studies of D4. Studies that evaluated other liver endpoints like clinical chemistry or other liver lesions generally did not report significant effects. Table 4-1 summarizes the available animal studies that provide evidence relevant to reproductive effects.

In a 1-month repeated inhalation toxicity study, F344 were exposed to D4 at concentrations of 0, 226, 416, 700, or 1154 ppm (0, 2,742, 5,047, 8,492, or 14,000 mg/m^3), 6 hours per day for 5 days per week (Burns-Naas et al., 2002; RCC, 1995a). Each test group consisted of 10 males and 10 females. Assessment of the organ weight data revealed a slight to moderate, dose-dependent and statistically significant increase in absolute and relative liver weight in animals of all groups exposed to D4. Compared to controls, liver weights increased by 16, 21, and 26 percent in males and by 19, 33, and 43 percent in females for concentrations of 417, 700, and 1154 ppm, respectively (Burns-Naas et al., 2002; RCC, 1995a).

In another 28-day study, F344 rats via whole body inhalation were exposed to 0 (room air), 7, 20, 60, 180 and 540 ppm (for 6 hours per day, 6 days per week)(Klykken et al., 1999). Statistically significant increases in liver weight and liver to body weight were observed in both sexes, with effects seen as low as 20 ppm in female rats. However, the difference in liver weight increase observed in both male and female rats had resolved back to baseline after a 14-day recovery period. Similar to other findings, the sex difference and dose-response of the liver weight increase in female rats was reproducible (Burns-Naas et al., 2002; Klykken et al., 1999). McKim et al. (2001a) exposed female F344 rats via whole body inhalation to 0 or 700 ppm D4 vapor for 6 hours per day for 5 days per week for 1 month and found liver-to-body weight ratio increases over controls at the highest concentration (700 ppm) produced an increase in hepatomegaly, transient hepatic hyperplasia (seen at 6 but not 27 days), and sustained hypertrophy (McKim et al., 2001a).

In a 90-day inhalation study, SD rats were exposed to 0, 60, 121, or 3588 mg/m^3 (0, 5, 10, 300 ppm) for 6 hours per day, 5 days per week. The study authors reported notable liver weight changes at the highest dose, with statistically significant increase in absolute (28 percent) and relative (21 percent) in females only when compared to control group. Liver weight changes occurred in the absence of any associated pathology and were comparable to controls after the 4-week recovery period (IRDC, 1991).

In another 90-day inhalation vapor study, SD rats were exposed to 0, 50, 300, and 700 ppm (0, 607, 3,639, 8,492 mg/m^3) D4 for 6 hours per day, 7 days per week for 13 weeks (Dow Corning, 1989b). Findings showed male and female rats had significant dose responsive increases in absolute and relative liver weights. However, for male rats, the increases had returned to normal at the end of the recovery period. Female rat liver weights had not decreased to normal at the end of the recovery period of 28 days and remained significantly increased (Dow Corning, 1989b).

In another 90-day inhalation vapor study, F344 rats were exposed to (0, 0.42, 1.48, 5.91, and 10.87 mg/L) 0, 420, 1,480, 5,910, or 10,870 mg/m^3 D4 for 6 hours per day, 5 days per week for 13 weeks (Burns-Naas et al., 2002; RCC, 1995b). The authors reported a marginal to slight, dose-dependent increase in group mean liver absolute weight in both male and female rats. Statistically significant mean liver absolute weight effect ($p < 0.01$) in male rats occurred at the highest dose (10,870 mg/m^3), whereas statistical significances in mean liver absolute weight effect ($p < 0.01$; $p < 0.05$) in females were recorded in doses 1,480 mg/m^3 and, predominantly, 5,910 mg/m^3 and 10,870 mg/m^3 when compared to controls. Following a recovery period of 4 weeks, females in the higher group only had marginally

higher, but not statistically significant mean absolute and relative liver weight than the control group. This observation was attributed to the treatment (Burns-Naas et al., 2002; RCC, 1995b).

Other liver endpoints like liver enzyme changes and histopathology were also evaluated in some studies (Jean and Plotzke, 2017; Dow Corning, 1997; WIL Research, 1997b; RCC, 1995a, b; IRDC, 1991; Dow Corning, 1989b; Civo Institute TNO, 1984; FDRL, 1966) but no significant changes were observed. Studies that examined clinical chemistry did not observe adverse effects on these parameters. Histopathology evaluations in rats exposed by inhalation showed inconsistent findings, with some studies reporting increased incidences of hyperplasia and hypertrophy, and others reporting no treatment-related lesions.

Table 4-1. Studies Evaluating Hepatic Effects of D4 Exposure

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Hepatic Effects at LOAEL	Remarks [b]
28-day inhalation toxicity study in F344 rats (n= 10/sex/group) (Burns-Naas et al., 2002; RCC, 1995a)	Rats exposed 6 hours/day, 5 days/week via nose-only inhalation to 0, 226, 416, 700, or 1154 ppm (0, 2,742, 5,047, 8,492, or 14,000 mg/m^3)	NOAEL = N/A LOAEL = 226 ppm (2,742 mg/m^3)	Significant increase in absolute and relative liver weight in males and females	Changes in clinical chemistry were generally described as not exposure-related; The highest concentration tested resulted in mortality and was above the level of saturation (resulting in aerosolization) OQD=High
28-day inhalation study in F344 rats (n= 15/sex/group) (Klykken et al., 1999; Dow Corning, 1997):	Rats exposed 6 hours/day, 6 days/week via whole body inhalation to 0, 7, 20, 60, 180 or 540 ppm (0, 85, 243, 728, 2,184, and 6,551 mg/m^3)	NOAEL = 7 ppm (85 mg/m^3) LOAEL = 20 ppm (243 mg/m^3)	Significant increase in absolute and relative liver weights in females; effect resolved to baseline after 14 day recovery period; liver effects also reported in males at higher doses	Changes in hematological parameters seen in terminal and 14-day recovery animals were marginal and within normal range OQD=High
28-day inhalation study in female F344 rats (n= 10/group) (Dow Corning, 2002b; McKim et al., 2001a)	Female rats exposed 6 hours per day for 5 days per week via whole body inhalation to 0 or 700 ppm (0 or 8,492 mg/m^3) D4 vapor	NOAEL = N/A LOAEL = 700 ppm (8,492 mg/m^3)	Significant increase in liver-to-body weight ratio; increase in hepatomegaly, transient hepatic hyperplasia (seen at 6 but not 27 days), and sustained hypertrophy	OQD=High

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Hepatic Effects at LOAEL	Remarks [b]
28-day study in male and female Wistar rats (n=10/sex/group) (Civo Institute TNO, 1984)	Rats exposed 6 hrs/day, 7 days/week, for 4 weeks to 0, 17, 83, 418 ppm (0, 201, 1,004, and 5,074 mg/m^3) D4 aerosol; an additional 5 rats/sex in the control and high-dose groups were held for a 14-day recovery period	NOAEL = 83 ppm (1004 mg/m^3) LOAEL = 418 ppm (5074 mg/m^3)	Significant increase in relative liver weights in males and relative and absolute liver weights in females; liver weights returned to normal after a 14-day recovery period	Significant clinical chemistry changes were reported to be unrelated to exposure concentration or were within normal ranges and of no toxicological significance. OQD=Medium.
13-week inhalation toxicity study in male and female SD rats (n= 60/sex/group in control and high dose; 20/sex/group at other doses) (IRDC, 1991)	Rats exposed 6 hours/ day, 5 days/week for 13 weeks via whole body inhalation to 5, 10 or 300 ppm (59.8, 121, 3588 mg/m^3) D4 vapor	NOAEL = 10 ppm (121 mg/m^3) LOAEL = 300 ppm (3588 mg/m^3)	Significant increase in liver weights in females; After a 28-day recovery period liver weights returned to control levels	Clinical chemistry not assessed OQD=Low
13-week inhalation toxicity study in male and female SD rats (n = 10/sex/group) (Dow Corning, 1989b)	Rats exposed 6 hours/ day, 7 days/week via whole body inhalation to 0, 51, 301, or 700 ppm (0, 619, 3,652, or 8,492 mg/ m^3) D4 vapor; Additional groups were exposed to control or high dose concentrations and allowed a 28-day recovery	NOAEL = N/A LOAEL = 51 ppm (619 mg/ m^3)	Significant increase in absolute and relative liver weights in males at 51 ppm and females starting at 301 ppm; After a 28-day recovery period, the effect in male rats resolved, but significant effects in female rats remained	No change in clinical chemistry or histopathology OQD=High
90-day inhalation study in F344 rats (n= 20/sex/group) (Burns-Naas et al., 2002; RCC, 1995b)	Rats exposed via nose-only inhalation to 0, 0.42, 1.48, 5.91, and 10.87 mg/L (0, 420, 1,480, 5,910, or 10,870 mg/m^3)	NOAEL = 35 ppm (420 mg/m^3 LOAEL = 122 ppm 1480 mg/m^3	Significant increase in absolute liver weights in female rats; effect no longer significant after 4-week recovery period	Significant changes in some clinical chemistry parameters (increased GGT activity, increased ALT, increased total protein) reported at higher doses

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Hepatic Effects at LOAEL	Remarks [b]
				OQD=High
Range-finding one-generation reproductive toxicity study in in male SD rats (n= 22/group) (WIL Research, 1997b)	Male rats exposed 6 hours/day for at least 70 consecutive days prior to mating, and throughout the mating interval via whole-body inhalation to 0, 71, 300, 492, and 694 ppm (0, 861, 3639, 5968, 8419 mg/m^3)	NOAEL = 300 ppm (3639 mg/ m^3) LOAEL = 492 ppm (5968 mg/ m^3)	Significant increases in absolute and relative liver weights in F0 males	OQD=High
12-month study F344 rats with a 12-month recovery period (n=20/sex/group) (Jean and Plotzke, 2017)	Rats exposed 6 hours/day, 5 days/week for 12 months to 0, 10, 30, 150, or 700 ppm (0, 121, 364, 1,820, 8,492 mg/m^3) followed by a 12-month untreated recovery period	NOAEL = 30 ppm (364 mg/m^3) LOAEL = 150 ppm (1,820 mg/m^3)	Significant increase in absolute and relative liver weights in males and females; no effect remained after 12-month recovery period	Clinical chemistry changes include increased total protein, decreased creatinine, CK and LDH in both sexes in high exposure groups OQD=High.
52-week dietary study in albino rats (n= 5/sex/group; 10/sex/group for controls) (FDRL, 1966)	Rats exposed to 770-870 mg/kg-bw/day by diet for 52 weeks	NOAEL = 770-870 mg/kg/day LOAEL =N/A	No treatment-related changes in liver weight, histopathology or clinical chemistry	Study limitations include substantial ambiguity about how the test substance was formulated and prepared, making the true administered dose difficult to determine and flaws with sample size and ambiguity with statistical analysis. OQD= Low
OQD = Overall quality determination, as discussed in Section 2 and at the beginning of Section 4				

4.1.1.3 Mechanistic and Supporting Evidence

EPA identified mechanistic studies in liver and liver cells from both *in vivo* and *in vitro* studies. Several studies have shown that repeated inhalation exposure to D4 causes dose-dependent liver weight

increases, reversible hepatomegaly and induction of hepatic microsomal enzymes. Dow Corning (1999) tested a wide range of exposure concentrations to define the dose-response curve for liver enlargement and hepatic CYP2B1 and 2B2 induction. Liver weight increased with increasing exposure concentrations. The data showed an increased in hepatic CYP2B1 and 2B2 proteins with a dose-dependent increase in PROD activity at the highest exposure concentration tested. The increased expression of CYP 2B in the centrilobular regions occurred at the lowest dose and expanded across the hepatic lobule in a dose-dependent manner (Dow Corning, 1999).

SD rats were exposed to D4 at dose levels of 0, 1, 5, 20, or 100 mg/kg-bw/day D4 in corn oil by gavage for 4 days to evaluate induction of drug metabolizing enzymes. Significant increases were observed in liver to body weight ratios in female rats at doses greater than 20 mg/kg-bw/day. D4 produced a 10 and 19 percent increase in liver to body weight ratio in the 20 and 100 mg/kg-bw/day groups, respectively. Treatment also increased the induction of CYP1A1 and1A2(Zhang et al., 2000).

In a short-term oral toxicity study, F344 rats and female Hartley guinea pigs were exposed via oral gavage to D4 at doses of 0 or 301 mg/kg-bw/day for 14 continuous days. Microsomal protein, total microsomal cytochrome P450, microsomal NADPH cytochrome C reductase, microsomal enzymatic activity (PROD, BROD, EROD, and MROD), and CYP 1A1 and 1A2, CYP 2B1 and 2B2, CYP 3A1 and 3A2, CYP 4A1 and 4A3, and epoxide hydrolase protein content were measured in the liver microsomes (Dow Corning, 2002a). Total microsomal cytochrome P450 was unchanged in treated rats as compared to controls. Microsomal NADPH cytochrome C reductase activity was increased 142 percent in treated rats relative to controls. Treated rats exhibited a statistically significant increase 61 percent in microsomal EROD activity and a statistically significant decrease of 3 percent in microsomal MROD activity. There was no statistically significant change in CYP 1A protein among treated rats. Treated rats exhibited a statistically significant increase in microsomal PROD and BROD activities, as well as an increase in CYP 2B protein as compared to the undetectable level in controls. Treated rats exhibited decreased CYP 4A and increased microsomal epoxide hydrolase and CYP 3A relative to controls. Based on the data presented in the study, a LOAEL of 301 mg/kg-bw/day was identified for rats based on a significant increase in liver weight (absolute and relative) and changes in microsomal enzymatic activity (Dow Corning, 2002a). Under similar experimental conditions, study authors did not observe statistically significant treatment-related effects liver weight (absolute or relative) in treated guinea pigs. No significant changes in microsomal enzymatic activity were reported for guinea pigs (Dow Corning, 2002a).

Cell proliferation assays were measured by 5-bromo-2’-deoxyuridine (BrdU) and proliferating cell nuclear antigen (PCNA) labeling index to determine hepatocellular proliferation Dow Corning (2001b). A range of experiments were conducted in multiple species to assess differences in liver responses to inhalation exposure to D4 vapor. In experiment 1, rats and guinea pigs were exposed to 0 or 700 ppm for 5 days. In Experiment 2, rats, mice, guinea pigs, rabbits, and hamsters were exposed to 0, 10, or 700 ppm for 28 days Dow Corning (2001b). No statistically significant differences were noted between D4 treated groups and controls.

4.1.1.4 Evidence Integration Summary

The overall judgment for hepatic/liver effects based on human evidence is *indeterminate*. High (Dow Corning, 1998c) and medium (University of Rochester, 1997) quality cross-over controlled exposure studies in humans assessed liver function based on clinical chemistry from blood samples collected immediately after exposure. No statistically significant changes in total protein, albumin, bilirubin, AST, alkaline phosphate, LDH, or cholesterol were observed at any time point. Available data are limited to a 2-week controlled oral exposure study and a single 1-hour controlled inhalation exposure study in which

small numbers of clinical chemistry endpoints were measured and no effects seen. These data are insufficient to draw conclusions regarding hepatic effects in humans.

The evidence in laboratory animals is *slight* based on increased liver weights consistently seen in rats after short-term, subchronic, or chronic exposure to D4 by inhalation and after short-term oral administration. Studies that examined clinical chemistry did not observe adverse effects on these parameters. Histopathology evaluations in rats exposed by inhalation showed inconsistent findings, with some studies reporting increased incidences of hyperplasia and hypertrophy, and others reporting no treatment-related lesions. Also, the absence of PCNA or BrdU staining in the Dow Corning (2001b) study that indicates lack of a proliferative response is inconsistent compared with the hyperplasia finding. According to U.S. EPA (2002a), increased liver weights and hepatocellular hypertrophy in the absence of clinical chemistry changes, other liver lesions, or a clear mechanism of toxicity are not considered adverse. There were isolated reports of other hepatic lesions in rats (bile duct hyperplasia in F1 rats in a 2-generation inhalation study and inflammation in a 2-week gavage study) but these were not supported by other studies. Limited studies did not report histopathology changes in the livers of mice, hamsters, rabbits, or guinea pigs exposed to D4 by inhalation or in guinea pigs exposed orally (Dow Corning, 2002a, 2001b; Zhang et al., 2000).

Overall, evidence *suggests*, but it is not sufficient to conclude that D4 may cause hepatic/liver effects in humans under relevant exposure circumstances. Evidence integration tables in Appendix A provide a detailed summary of lines of evidence that support these overall conclusions.

4.1.2 Pulmonary Toxicity

4.1.2.1 Human Evidence

EPA identified one controlled exposure study of 12 male and female volunteers (20-50 years old, nonsmoking) that evaluated effects of D4 on pulmonary function. Volunteers were exposed by inhalation to 121 mg/m^3 (10 ppm) D4 vapor or clean air via mouthpiece or via nasal delivery for 1 hour. Volunteers recorded symptoms and researchers measured respiratory function and spirometry using two measures – forced vital capacity (FVC) and forced expiratory volume within 1 second (FEV1) – before and after exposure and 24 hours after exposure. No statistically significant changes in symptom scores, FVC, or FEV1 were measured after 1 hour of exposure (University of Rochester, 1997). Available data were limited to the single volunteer study that showed no changes in respiratory function after 1 hour of inhalation exposure. No major limitations were reported for this study.

4.1.2.2 Laboratory Animal Evidence

EPA identified several animal studies that evaluate the effects of D4 inhalation on the respiratory tract. Table 4-2 summarizes the available animal studies that provide evidence relevant to reproductive effects.

In a 2-year inhalation (via whole body exposure) toxicity study, male and female F344 rats were exposed to vapor concentrations of 0, 121, 364, 1,820, 8,492 mg/m^3 (0, 10, 30, 150, or 700 ppm) D4 for 6 hours per day, 5 days per week, for up to 24-months (Jean and Plotzke, 2017).[3] Treatment-related effects within the nasal cavity were observed following 12 months of exposure to D4. The effects included increased incidence and severity of goblet cell hyperplasia, squamous epithelial cell hyperplasia, suppurative inflammation, and eosinophilic globules. Among animals exposed for 12

[3] EPA evaluated Jean and Plotzke (2017) as the primary published journal article of the 24-month combined chronic toxicity and oncogenicity study because it is the most comprehensive presentation of the study. EPA also consulted other references with individual data from the study: Dow Corning (2004), Battelle PNL (2004a), and Battelle PNL (2004b).

months and then sacrificed, both males and females in the 8,492 mg/m^3 dose groups had statistically significant ($p<0.01$) increases in incidence and severity grade of eosinophilic globules in the respiratory epithelium. At 12 months after exposure, female rats in the 8,492 mg/m^3 dose group had statistically significant increased incidence and grade severity of eosinophilic globules but the incidence was no longer statistically significantly increased in the recovery period (Jean and Plotzke, 2017). After 24 months exposure to D4, the effects reported included increased incidence and severity of goblet cell hyperplasia, squamous epithelial cell hyperplasia, and eosinophilic globules of 1,820 mg/m^3 and 8,492 mg/m^3 for males at exposure concentrations of 364 mg/m^3 and higher in females. In the recovery group exposed to D4 for 12 months followed by 12 months exposure to D4, animals showed an increase in severity of eosinophilic globules (8,492 mg/m^3 males and females) and an increase in incidence of goblet cell hyperplasia (8,492 mg/m^3 males) . There were also statistically significant increased incidences of lung hemorrhage at 8,492 mg/m^3 and subpleural chronic inflammation at 121, 364, and 8,492 mg/m^3 (but not 1,820 mg/m^3) in female F-344 rats after 24 months of exposure. The incidence at 1,820 mg/m^3 for subpleural chronic inflammation was the same as the lowest concentration, but it was not statistically significant (Jean and Plotzke, 2017). The study authors conclude that these findings and observations are consistent with chronic inhalation exposure to materials with slight irritant properties such as D4. A NOAEC of 10 ppm and a LOAEC of 30 ppm were determined for nasal lesions in female rats (Jean and Plotzke, 2017). Incidence of some nasal lesions (*e.g.*, goblet cell hyperplasia in females) that had been observed at 12 months was lower and no longer statistically significant after the recovery period. No major limitations were reported. When evaluated using data quality criteria for animal toxicity, EPA assigned this study a high overall quality determination during systematic review.

In a 90-day inhalation (via nose only exposure) toxicity study, male and female F344 rats were exposed to vapor concentrations of 0, 35, 122, 488, or 898 ppm (0, 425, 1,480, 5,920, 10,894 mg/m^3) D4 for 6 hours per day, 5 days per week (Burns-Naas et al., 2002; RCC, 1995b). Increased incidence and severity of alveolar macrophage accumulation in one or both sections of the lung were found in male and female rats exposed to 898 ppm. Similar effects of elevated alveolar macrophage accumulation were observed in females exposed to lower doses in the 122 and 488 ppm groups. Interstitial inflammation was increased in males and females. Males had the highest incidence and severity in the highest exposure group (898 ppm), and females exposed to 122 to 898 ppm had also high incidence and severity. Other adverse effects in the lungs included increased eosinophilic influx in both males and females. The authors of this study reported the presence of aerosol droplets in the mixed aerosol and vapor test atmospheres of the high dose group. This mixed aerosol and vapor atmosphere appeared to be correlated with the adverse effects observed. After a 1-month recovery, male and female rats had lower incidence of alveolar macrophage accumulation, interstitial inflammation, and reversible histopathological changes (Burns-Naas et al., 2002; RCC, 1995b). The authors recognized that even though these adverse effects were observed in the lungs of male and female rats exposed to D4, similar study designs at similar concentrations have failed to show similar effects.

Another 90-day inhalation study (whole body exposure) (Dow Corning, 1989b) evaluated organ weights and histopathological changes in SD rats exposed to actual mean concentrations of 0, 619, 3,652, or 8,492 mg/m^3 (0, 51, 301, or 700 ppm) of D4 6 hours per day, 7 days per week. The study found no pulmonary tract effects. No major limitations were reported. However, histopathological data did not include a measure of severity for any of the tissues and organs including lungs, nasal passages, larynx, and trachea. A LOAEC of 51 ppm was identified for increased absolute and relative weights in this study (Dow Corning, 1989b). EPA gave this study and associated hazard endpoints high overall quality determination.

In a 28-day whole body vapor inhalation study, F344 rats were exposed to concentrations at 0, 85, 243, 728, 2,184, and 6,551 mg/m^3 (0, 7, 20, 60, 180, and 540 ppm) for 6 hours per day, 5 days per week (Klykken et al., 1999). The outcomes evaluated included body weight, organ weight, gross pathology, histopathology, serum chemistry, and urinalysis. There were no treatment related histopathological findings in male or female rats at the end of the exposure period or at the end of the 14 day recovery period (Klykken et al., 1999).

Groups of Wistar rats were exposed, whole body, to aerosolized D4 at analytical concentrations of 0, 201, 1,004, and 5,074 mg/m^3 for 6 hours per day, 7 days per week, for 4 weeks. No treatment-related lung histopathology changes were observed in Wistar rats exposed to aerosol ≤ 5,074 mg/m^3 (Civo Institute TNO, 1984). It was noted that no particles were detected in the atmospheres and no mass median aerodynamic diameter (MMAD) or geometric standard deviation (GSD) values were reported. No further information was provided, but it is assumed that animals were instead exposed to test substance vapors. EPA assigned this study a medium overall quality determination.

In a short-term, repeated dose oral toxicity study, female Wistar rats were exposed via gavage to D4 at concentrations of 0 or 1,600 mg/kg-bw. Histopathological changes showed increased incidence of interstitial inflammation with alveolar macrophages in the lungs of Wistar rats after 2 weeks of gavage exposure at 1,600 mg/kg-bw/day (Mobay Chemical, 1991). However, a major limitation of the study was that it did not include incidence data for quantitative and qualitive findings. The missing statistical significance made analysis and interpretation of the findings difficult. EPA assigned this endpoint and study an uninformative overall quality determination.

In the D4 literature, vapor exposures have been historically used to conduct inhalation toxicity animal studies. Some, but not all, studies of rats exposed by vapor inhalation of D4 reported increased incidences of inflammation and/or histiocytosis in the lungs after short-term, sub chronic, and chronic exposure. However, in earlier D4 range-finding studies, investigators noticed aerosol formation appears to occur at certain high temperatures. There are no hazard values derived from aerosols that are acceptable for aerosol risk assessment. EPA inhalation guidance states that aerosol risk assessment must have inhalation toxicity studies that include particle characterization that provides an MMAD with accompanying GSD (U.S. EPA, 1994, 6488). Some of the D4 studies noted that no particles were detected in the atmospheres. Particle size is a critical factor to predict the regional deposition and no MMAD or GSD were reported. Most COUs that include inhalation exposure are associated with vapor exposures. However, one COU that has aerosol and/or mist exposure is the application and/or use of paints and coatings. Thus, the focus of most D4 inhalation toxicity studies on use of vapor exposure at lower concentrations might not capture any aerosol-specific adverse effects, if such unique effects exist from D4 exposure.

Table 4-2. Studies Evaluating Respiratory Effects of D4 Exposure

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Respiratory Effects at LOAEL	Remarks [b]
2-year inhalation toxicity study in male and female F344 rats (n= 10/sex/group) (Jean and Plotzke, 2017)	0, 10, 30, 150, or 700 ppm (0, 121, 364, 1,820, 8,492 mg/m^3), 6 hours per day for 5 days per week	NOAEL = 10 ppm (121 mg/m^3) LOAEL = 30 ppm (364 mg/m^3)	Increased incidence of nasal lesions including eosinophilic globules in female rats exposed to ≥360 mg/m^3 for	OQD=High

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Respiratory Effects at LOAEL	Remarks [b]
			24 months and goblet cell hyperplasia in the respiratory epithelium of males exposed to ≥1,820 mg/m^3 for 24 months. Additional nasal lesions, including squamous epithelial hyperplasia and suppurative inflammation were seen at 8490 mg/m^3 in one or both sexes after 12 or 24 months	
90-day inhalation toxicity study in male and female F344 rats (n= 20/sex/group) (Burns-Naas et al., 2002; RCC, 1995b)	0, 35, 122, 488, or 898 ppm (0, 425, 1,480, 5,920, 10,894 mg/m^3), 6 hours per day for 5 days per week	NOAEL = 35 ppm (425 mg/m^3) LOAEL = 122 ppm (1480 mg/m^3)	Increased incidences of chronic interstitial lung inflammation and alveolar macrophage foci at ≥ 122 ppm (1,480 mg/m^3)	OQD=High
90-day inhalation toxicity study in SD rats (n= 10/sex/group) (Dow Corning, 1989b)	0, 51, 301, or 700 ppm (0, 619, 3,652, or 8,492 mg/m^3), 6 hours per days for 7 days per week	NOAEL = N/A LOAEL = 51 ppm (619 mg/m^3)	no pulmonary tract effects were reported in this study	OQD=High
28-day inhalation toxicity study in male and female F344 rats (n= 10/sex/group) (Klykken et al., 1999; Dow Corning, 1997)	0, 7, 20, 60, 180, and 540 ppm (0, 85, 243, 728, 2,184, and 6,551 mg/m^3), 6 hours per day for 5 days per week	NOAEL = N/A LOAEL = N/A	No treatment related histopathological findings in male or female rats at the end of the exposure period or at the end of the 14 day recovery period	OQD=High
28-day inhalation toxicity study in Wistar rats to aerosolized D4 (n= 10/sex/group) (Civo	0, 201, 1,004, and 5,074 mg/m^3 6 hours per day for 7 days per week	NOAEL = N/A LOAEL = N/A	No treatment-related lung histopathology changes were observed	OQD=Medium

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Respiratory Effects at LOAEL	Remarks [b]
Institute TNO, 1984)				
2-week repeated dose oral toxicity study (n= 10/female/group) (Mobay Chemical, 1991)	0 or 1,600 mg/kg-bw/day	NOAEL = N/A LOAEL = N/A	Histopathological changes showed increased incidence of interstitial inflammation with alveolar macrophages in the lungs	Study limitations include substantial ambiguity, missing important information on test animal characteristics, purity of test substance, animal husbandry conditions, methods were sparely reported, and flaws with sample size, and ambiguity with statistical analysis. Study limitations include substantial ambiguity missing important information on test animal characteristics, purity of test substance, and animal husbandry conditions, methods were sparsely reported, and flaws with sample size and ambiguity with statistical analysis. OQD= uninformative
OQD = Overall quality determination, as discussed in Section 2 and at the beginning of Section 4				

4.1.2.3 Mechanistic and Supporting Evidence

No mechanistic studies for D4 exposure were identified for potential pulmonary effects.

4.1.2.4 Evidence Integration Summary

The overall judgment for respiratory tract effects based on human evidence is *indeterminate*. Available human data for respiratory effects is based on a single 1-hour controlled inhalation exposure study in which small numbers of clinical chemistry endpoints and symptoms of respiratory function and

spirometry were measured and no effects were seen. These data are insufficient to draw conclusions regarding pulmonary effects in humans (University of Rochester, 1997).

No statistically significant changes in symptoms score were observed. The overall judgment for pulmonary/lungs effects based on human evidence is *indeterminate*.

The evidence in laboratory animals is *moderate* based on increased incidences of nasal lesions after chronic exposure, and *slight* based on increased incidences of inflammation and histiocytosis in lungs after short-term, intermediate, and chronic exposure.

No mechanistic studies for D4 exposure were identified for potential pulmonary effects.

Overall, evidence *suggests*, but it is not sufficient to conclude that D4 may cause pulmonary/lung effects in humans under relevant exposure circumstances. Evidence integration tables in Appendix A provide a detailed summary of lines of evidence that support these overall conclusions.

4.1.3 Reproductive Toxicity

EPA guidance defines reproductive toxicity as a range of possible hazard outcomes that may occur after treatment periods of adequate duration to detect such effects on reproductive systems (U.S. EPA, 1996). This section describes male and female reproductive system toxicity (*e.g.,* effects on estrous cycle, follicle reduction, ovarian atrophy, uterine weight in female rats and absolute and relative testes weights effects and testicular lesions in male rats) as well as effects on reproductive outcomes, including mating, mean live litter size, mean number of pups born, and fertility index.

4.1.3.1 Human Evidence

EPA did not identify any human dosing studies that evaluated potential reproductive effects from D4 exposure in the literature search.

4.1.3.2 Laboratory Animal Evidence

Animal toxicity studies that evaluated reproductive effects after D4 exposure include 1- and 2-generation reproductive assessments by continuous breeding in rats (WIL Research, 2005, 2001a, 1997a, 1996a, b) and several repeated-dose studies that evaluated reproductive organs in adult rats (Jean and Plotzke, 2017; Jean et al., 2017; Burns-Naas et al., 2002; RCC, 1995b; Dow Corning, 1989a). Table 4-3 summarizes the available animal studies that provide evidence relevant to reproductive effects.

Reproductive Outcomes: D4 inhalation exposure has been associated with effects on a range of related reproductive outcomes, including live litter size, number of pups born, numbers of implantation sites, fertility index, pre-implantation loss, post-implantation loss and/or reduced pup viability (WIL Research, 2005, 2001a, 1997a, 1996a, b).

In a 2-generation reproductive toxicity study, Crl:CD (SD)IGS BR rats were exposed to 0, 71, 298, 502 or 700 ppm (0, 861, 3,615, 6,090, 8,492 mg/m^3) vapor concentrations of D4 via (whole-body) inhalation for 6 hours a day for at least 70 days prior to mating through weaning of F1 pups on postnatal day (PND 21) (WIL Research, 2001a). Exposure in females was suspended from gestational day (GD21) through postnatal day 4 (PND4) to allow for parturition. On PND 22, F1 offspring were exposed to the same target concentrations of D4 as their F0 parents (analytical concentrations of 0, 71, 301, 502 and 702 ppm) for 70 days. Reproductive effects including reductions in fertility indices, litter size, and the number of pups were observed following exposure as low as 502 ppm (WIL Research, 2001a).

Significant reductions in mean live litter size and/or number of pups born were reported at 502 ppm in F0 and the first mating of F1 generation. Implantation sites were also reduced at 700 ppm in the F0 generation. In the F1 generation, fertility indices for both sexes were significantly decreased at 702 ppm. No differences in reproductive performance were observed when exposed F1 males were paired with unexposed females, suggesting that observed effects on reproductive outcomes are likely the result of female exposures. This finding is consistent with that lack of effects on reproductive outcomes reported following male-only exposures in a range-finding one-generation reproductive toxicity study (WIL Research, 1997b, c).

A subsequent 2-generation study designed to evaluate the effect of specific exposure periods in females replicated the significant reductions in litter size and mean number of pups born following exposure to 700 ppm, the only dose tested in the study (WIL Research, 2005). The study included multiple groups of exposed animals. One group was exposed throughout the F0 and F1 generations, generally replicating the conditions of the 2001 study (WIL Research, 2001a). In another group, direct exposure for the F1 generation was not started until PND44. In another group, exposure only occurred during the F0 generations (meaning F1 was exposed only in utero), and in yet another group, exposure only occurred during F1. Similar to WIL Research (2001a), WIL Research (2005) direct exposures to 700 ppm in females resulted in significant effects on reproductive outcomes, including reduced mean live litter size and mean number of pups born. The study did not identify any adverse reproductive effects in the F1 generations when exposure was limited to F0, suggesting that in utero exposures alone do not produce the adverse reproductive outcomes observed in 2-generation studies.

Several 1-generation reproductive studies also describe significant effects on litter size, number of pups born, implantation sites and/or pre-implantation loss at similar effect levels 700 ppm (WIL Research, 1997a, 1996a, b). A set of phased 1-generation studies evaluated effects of exposure in adult females during specific periods of time or on specific days prior to or after mating (Meeks et al., 2007; WIL Research, 1999; Dow Corning, 1998b). These studies replicate effects on reproductive outcomes (including reduced viable fetuses and implantation sites and increased pre-implantation loss and post-implantation loss) following a shorter period of exposure to 700 ppm during the fertilization phase (prior to mating through gestation day 3). Significant effects on fertility index were observed following a single 6-hour period of exposure to 700 ppm one day prior to mating.

Female reproductive toxicity: In females, D4 inhalation exposure has been associated with a range of reproductive effects, including altered estrous cycle, follicle reduction, ovarian atrophy, increased uterine weight and increased incidences of endometrial epithelial hyperplasia. Decreases in reproductive outcomes like fertility indices, implantation sites, and litter sizes may be related to estrous cycle disruptions, failure to ovulate, and/or decreases in corpora lutea.

One of the two available 2-generation studies reported significant reductions in corpora lutea counts, increased estrous cycle lengths in the F1 generation at 700 ppm relative to controls (WIL Research, 2001a). In the same study, dystocia was also observed in two and three females in the 700 ppm group, which the authors considered to be test-article related; dystocia was the cause of death in two of three high-exposure females.

Several 1-generation studies also report significant decreases in corpora lutea (Meeks et al., 2007; Dow Corning, 1998b; WIL Research, 1996a, b), decreases in gravid uterine weights, and/or ovarian weights (Meeks et al., 2007; WIL Research, 1999; Dow Corning, 1998b). One study reported significant reductions in the number of corpora lutea at exposure concentrations as low as 301 ppm (Meeks et al., 2007; Dow Corning, 1998b). A related study demonstrated that significant decreases in corpora lutea

and gravid uterine weight can occur following a relatively short period of exposure to 700 ppm D4 3 days prior to mating through gestation day 3 (during mating and fertilization) (Meeks et al., 2007; WIL Research, 1999).

Another inhalation toxicity study demonstrated that 3 days of exposure timed to occur during diestrus and proestrus is sufficient to result in significant effects on hormonal activity and ovulation. Female SD rats were exposed to concentrations of 0, 702, or 905 ppm (0, 8,516, or 10,979 mg/m^3). Exposure was associated with significant changes in luteinizing hormone (LH), prolactin (PRL) and estrone (E1) and estradiol (E2) level, a decreased percentage of ovulatory animals and dose-dependent reduction of eggs in the oviduct (Quinn et al., 2007a). The study is limited by issues with unmeasured respiratory irritant effects of D4 at concentrations >700 ppm, which may be due to the formation of particles at concentrations higher than 700 ppm, which is consistent across the D4 literature. There is also inconsistent reporting of sample sizes for some endpoints, which may imply that not every endpoint was measured in every animal.

A chronic inhalation study in reproductively senescent female F344 rats, Jean et al. (2017) suggests that D4 inhalation exposure can alter estrous cyclicity in aging females. Rats were treated with 704 ppm (8,540 mg/m^3) D4 vapor for 14 months (11 to 24 months of age). The authors reported a 37 percent decrease in antral-size atretic follicles, increased cumulative number of estrogenic days and percentage days in an estrogenic state, increased severity of vaginal epithelial thickness and decreased vaginal mucification at the only dose tested (Jean et al., 2017).

Other inhalation toxicity studies report effects on reproductive organ weight, histopathology and/or estrus cycles. In a 2-year inhalation toxicity study and cancer bioassay, F-344 rats were exposed to concentrations of 0, 10, 30, 150, or 700 ppm D4 (Jean and Plotzke, 2017). Significant effects on reproductive endpoints were reported in the highest exposure group, including increased incidence of ovarian atrophy, increased uterine weights, and higher incidences of endometrial epithelial hyperplasia and cervical squamous epithelial cell hyperplasia (Jean and Plotzke, 2017).

In a 90-day inhalation toxicity study, F344 rats were exposed to D4 concentrations of 0, 35, 122, 488, or 898 ppm (0, 425, 1,480, 5,920, 10,894 mg/m^3) (Burns-Naas et al., 2002; RCC, 1995b). At the highest dose tested, there was a significant decrease in ovarian weights as well as histopathological changes in the ovary and vaginal mucification. In addition, more females appeared to be in the diestrous stage of the rat estrous cycle (Burns-Naas et al., 2002; RCC, 1995b). Other 90-day studies report both increases (IRDC, 1991) and decreases (Dow Corning, 1989b) in ovary weights, while 4-week studies report no significant effects on organ weights (Dow Corning, 1997; RCC, 1995a).

Additional animal evidence provides mechanistic information relevant to many of the observed female reproductive effects. As further discussed in Section 4.1.3.3, estrogenic effects of D4 exposure have been investigated in uterotrophic assays via oral gavage, subcutaneous administration, and whole-body inhalation exposure with female F344 and SD rats, female B6C3F1 mice, and female estrogen receptor-α knockout (αERKO) mice with increased in uterine weight (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003; MPI Research, 1999).

Male reproductive toxicity: There is some evidence of changes in male reproductive organs following D4 inhalation exposure, but many of the available studies report no effect on these endpoints as reported in Appendix A.

In the 2-year inhalation toxicity study and cancer bioassay, increased incidence and severity of testicular interstitial (Leydig) cell hyperplasia were observed after 24 months of exposure to 150 and 700 ppm (Jean and Plotzke, 2017). In a 13-week toxicity study, Male F-344 rats exposed to vapor (nose-only) to the test substance at concentrations of 0, 420, 1,480, 5,910, 10,870 mg/m^3 for 6 hours per day, 5 days per week, absolute and relative testes weights were significantly increased after 13 weeks of exposure to 5,910 mg/m^3 but not 10,870 mg/m^3 (Burns-Naas et al., 2002; RCC, 1995b).

In one 28-day repeated dose inhalation toxicity study, male and female LVG Golden Syrian hamsters, mice, guinea pigs, and rabbits were exposed to vapor (whole body) to the test substance at concentrations of 0, or 697 ppm (8,460 mg/m^3) for 6 hours a day, and 7 days a week. Treated male hamsters displayed a significant 22 percent increase in relative testes weights (Dow Corning, 1989a). The study authors did not consider the histopathological changes to be treatment related and attribute them to be spontaneous changes typical of hamsters of this age and strain.

Other 90-day and 28-day inhalation toxicity studies generally reported no treatment-related effects on testes weights or histopathological changes (Dow Corning, 1997; RCC, 1995a; IRDC, 1991; Dow Corning, 1989b).

The 2-generation study that included male exposures reported no effects on male reproductive tissues or spermatogenic endpoints (WIL Research, 2001a). Similarly, the one-generation study that evaluated effects of male exposure reported no significant effects on terminal endpoints for males following a 5-week recovery period (WIL Research, 1997b, c).

1708 **Table 4-3. Studies Evaluating Reproductive Effects of D4 Exposure**

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
Reproductive Toxicity Inhalation Studies				
2-generation inhalation toxicity in male and female SD rats (n = 30/sex/group) (WIL Research, 2001a)	F0 exposure (male and female): 6 hours/day, 70 days prior to mating, through weaning of F1 pups on postnatal day (PND 21). Exposure in females was suspended for parturition. 0, 71, 298, 502 or 700 ppm (0, 861, 3,615, 6,090, 8,492 mg/m^3)	NOAEL=298 ppm (3620 mg/m^3) LOAEL = 502 ppm (6,090 mg/m^3)	Significant decreases in mean live litter size and number of pups born	Implantation sites were also significantly reduced in the F0 generation at 700ppm. In the F1 generation, the mean number of pups born and fertility indices for both sexes significantly decreased and estrous cycle length increased in the first mating at 702 ppm. Microscopic evaluation of reproductive tissues in non-pregnant rats in the F1 generation suggest estrous cycle perturbation and accelerated reproductive senescence at 71, 301, 502 and 702 ppm Other effects reported in this study include increased liver and kidney weights. *Limitations:* The study authors noted that the advanced age of some of the females used for breeding may confound some of the results. OQD=High
	F1 exposure (male and female): 6 hours/ day, 70 days prior to mating and during mating/gestation/lactation; rats mated twice to produce F2a and F2b litters 0, 71, 301, 502, or 702 ppm (0, 861, 3,652, 6,090, 8,516 mg/m^3)	NOAEL= 301 ppm (3650mg/m^3) LOAEL = 502 ppm (6,090 mg/m^3)	Significant decrease in mean live litter size during first mating Significant decrease in fertility index after second mating	
	F1 exposure (male only): 6 hours/day, 70 days prior to mating and during mating; mated with unexposed females to produce F2c litters; 0, 71, 301, 502, or 702 ppm (0, 861, 3,652, 6,090, or 8,516 mg/m^3)	NOAEL = 702 ppm (8,516 mg/m^3) LOAEL= N/A	No significant effects on implantation sites, gestation length, pup body weights, number of pups born, % males, post-natal survival, or deaths in offspring; No significant effects on male reproductive tissue or spermatogenic endpoints	
2-generation reproductive toxicity study in female	Female rats exposed 6 hours/ day to 0 or 700	NOAEL= N/A	Significantly decreased litter size, mean number of pups born	The authors note that exposures limited to the F0 generation resulted in no

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
Sprague Dawley rats exposed over varying durations and timing and mated with unexposed males (n = 30/group) (WIL Research, 2005)	ppm (0 or 8,492 mg/m^3) D4 To evaluate the impact of in utero or early life exposures vs. adult exposures on F1 fertility, exposure periods either continue across both generations, (with F1 exposure starting at PND22 or PND44) or are limited to only F0 or only F1.	LOAEL= 700 ppm (8,492 mg/m^3)	in exposed F0 females and in some groups of F1 females	effect on F1 females, suggesting that in utero exposure is not required to produce adverse reproductive outcomes. The authors also note that initiating direct F1 exposure at PND44 instead of PND22 appears to reduce effect on F1 fertility rates (though the effect is not statistically significant in either group). Reductions in fertility indices and estrous cycle length in F1 females are reported but not statistically significant. *Limitations:* The authors could have taken more steps to minimize observational bias for some endpoints as well as confounding bias. OQD=High.
14-month inhalation toxicity study in reproductively senescent female F344 rats (Jean et al., 2017)	0 or 704 ppm (0 or 8,540 mg/m^3) [a]	NOAEL= N/A LOAEL= 704 ppm (8,540 mg/m^3)	Significant increase in cumulative number of estrogenic days and percentage days in an estrogenic state in aged rats, significantly decreased serum estadiol; Histomorphologic changes include 37 % decrease in antral-size atretic follicles in the ovary, increased total incidences of cystic endometrial hyperplasia, decreased incidence of vaginal mucification, and increased severity of vaginal epithelial thickness.	Other effects reported in the study include increased absolute and relative liver and kidney weights. No increases in incidences of tumors in reproductive tissues were observed. *Limitations:* The concentration tested was high and not representative of expected exposure levels. The concentration tested was near the point of vapor saturation leading to uncertainty that the entirety is a vapor. OQD=Medium
Phased one-generation reproductive toxicity study in female SD rats exposed via whole body inhalation in multiple experiments at varying timepoints and durations; exposed females	"Overall phase" exposure: females (n = 24/group) exposed 6 hours/day via whole body inhalation of 0, 72, 301, 503, or 698 ppm (0, 873, 3,652, 6,102, or 8,468 mg/m^3) D4 vapor	NOAEL = 72 ppm (873 mg/m^3) LOAEL= 301 ppm (3,652 mg/m^3)	Significant decrease in number of corpora lutea	In the overall phase, significant decreases in viable fetuses, implantation sites were observed at 503 and 698 ppm. A significant increase in pre-implantation loss and decrease in gravid uterine weight and GD 0-20

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
were mated with unexposed males (Meeks et al., 2007; Dow Corning, 1998b)	28 days prior, through mating and gestation days 0-19			body weight gain were reported at 698 ppm. *Limitations:* No major limitations were identified. OQD=High.
	"Ovarian phase" exposure: females (n = 60) exposed 6 hours/day via whole body inhalation of 0 or 702 ppm D4 vapor 31 days prior to mating to 3 days prior to mating	NOAEL= 702 ppm LOAEL = N/A	No significant reproductive effects reported	
	"Fertilization phase" exposure: females (n = 60) exposed 6 hours/day via whole body inhalation of 0 or 696 ppm D4 vapor 3 days prior to the start of mating through gestation day 3	NOAEL= N/A LOAEL = 696 ppm	Significant decreases in viable fetuses, implantation sites, corpora lutea, gravid uterine weights and absolute ovarian weights; Increased early resorption, pre-implantation loss and post-implantation loss	
	"Implantation phase" exposure: females (n = 24) exposed 6 hours/day via whole body inhalation of 0 or 702 ppm D4 vapor from gestation days 2-5	NOAEL= 702 ppm LOAEL = N/A	No significant reproductive effects reported	
Phased one-generation reproductive toxicity study in female Sprague Dawley rats exposed for 1-4 days prior to mating and mated with unexposed males	Single 6 hr exposure during the pre-mating phase (either 1, 2, 3, or 4 days prior to mating): females exposed via whole body inhalation of 0, 700 ppm D4 vapor	NOAEL= N/A LOAEL = 700 ppm	Significant decrease in fertility index in females only for females exposed 1 day prior to mating (not for the other single exposures)	*Limitations:* The study reports differences in timing of control group's exposure to air (GD 0) compared to two of the test groups (GD 1 and GD2). OQD=Medium.

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
(Meeks et al., 2007; WIL Research, 1999).	Single 6 hr exposure during the post-mating phase (either 0, 1, or 2 days post mating): females exposed via whole body inhalation of 0, 700 ppm D4 vapor	NOAEL= 700 ppm LOAEL = N/A	No significant reproductive effects reported	
	Day 3 to 1 prior to mating (inclusive) 0, 700 ppm	NOAEL= 700 ppm LOAEL = N/A	No significant reproductive effects reported	
	Premating (3 days), mating (up to 10 days), GD 0-3 0, 700 ppm (0, 8,492 mg/m^3)	NOAEL= N/A LOAEL = 700 ppm	Significant decreases in number of corpora lutea and gravid uterine weight; increase in number of *small* implantation sites	
	GD 0-2 (inclusive) 0, 700 ppm	NOAEL= 700 ppm LOAEL = N/A	No significant reproductive effects reported	
3-day inhalation exposure in 13-week-old female SD rats (Quinn et al., 2007a)	Females exposed 6 hours/ day for 3 days (timed to occur on diestrus days 1 and 2 and proestrus) via whole body inhalation to 0, 700, or 900 ppm (0, 8,492, or 10,918 mg/m^3) D4 vapor (nominal concentrations)	NOAEL= N/A LOAEL= 700 ppm (8,492 mg/m^3)	Significantly decreased LH, PRL and E1/E2 ratio, increased E1 and E2, decreased percentage of ovulatory animals, and decreased ova in the oviduct/rat	*Limitations:* There is inconsistent reporting of sample sizes for some endpoints, which may imply that not every endpoint was measured in every animal. OQD=Medium
Range-finding one-generation reproductive toxicity study in female SD rats mated with unexposed males (n = 22/group) (WIL Research, 1997a)	Females exposed 6 hours/ day for 70 days prior to mating through gestation and lactation via whole body inhalation to 0, 72, 302, 498, or 700 ppm (0, 873, 3634, 6,041, or 8,492 mg/m^3) D4 vapor	NOAEL= 498 ppm LOAEL= 700 ppm (8,492 mg/m^3)	Significantly decreased implantation sites, litter size, and number of pups born; significant increase in the difference between the number of implantation sites and the number of offspring.	No effects observed on estrous cyclicity, oocyte/ovarian follicle counts, mating indices, fertility indices, duration of gestation and parturition, histological changes, or pup body weights, survival or sex ratio. Other effects reported include significant increases in absolute and relative liver weights and reductions in maternal body weight gain

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
				Limitations: No major limitations were identified. OQD=High
Range-finding one-generation reproductive toxicity study in male and female SD rats (n = 22/sex/group) (WIL Research, 1996a, b)	Males and females exposed 6 hours/ day via whole body inhalation to 0 or 700 ppm (8,492 mg/m^3) D4 vapor for 28 days prior to mating and throughout mating. Female exposures continued through GD 21, and then PNDs 4-21	NOAEL= N/A LOAEL= 700 ppm (8,492 mg/m^3)	Significantly reduced litter size, number of pups born, implantation sites and corpora lutea, pup viability at PND 1 and 4; increased pre-implantation loss and pup weight.	No significant effects observed on fertility, mating, pup weights or sex ratios, sperm numbers or sperm production rate *Limitations:* Lack of additional test substance concentrations and lack of independent analytical verification of the test substance purity OQD=High
Range-finding one-generation reproductive toxicity study in in male SD rats (n=40/group) mated with unexposed females (WIL Research, 1997b, c)	Male rats exposed 6 hours/day via whole body inhalation to 0, 500, or 693 ppm (0, 6066, or 8407 mg/ m^3) D4 vapor 70 days prior to mating and throughout mating followed by 5-week recovery	NOAEL= >693 ppm (>8407 mg/m^3) LOAEL = N/A	No significant findings for organ weights nor histopathology were reported in F0 males. No exposure related differences in reproductive performance were observed. No significant differences in litter size, number of pups, sex ratio of offspring, pup survival, malformations, pup weight or pup gross necropsy at PND21 were observed.	*Limitations:* While the study is generally well constructed, the 5- week recovery period may have contributed to the negative results for terminal endpoints in adult males. OQD=High
Other Inhalation Studies				
13-week inhalation toxicity study in male and female SD rats (n= 60/sex/group in control and high dose; 20/sex/group at other doses) (IRDC, 1991)	Rats exposed 6 hours/ day, 5 days/week for 13 weeks via whole body inhalation to 5, 10 or 300 ppm (59.8, 121, 3588 mg/m^3) D4 vapor	NOAEL= 10 ppm (120 mg/m^3) LOAEL= 300 ppm (3588 mg/m^3)	Statistically significant increase in ovary weights and ovary/brain weights in females	Authors conclude that increased ovary weights are "considered normal biological variability". Other effects reported include increased absolute and relative liver weights in females at 13 weeks, decreased lung weights in females, and increased lung weights in males. *Limitations:* No major limitations were identified

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
				OQD=High
13-week inhalation toxicity study in male and female SD rats (n = 10/sex/group) (Dow Corning, 1989b)	Rats exposed 6 hours/ day, 7 days/week via whole body inhalation to 0, 51, 301, or 700 ppm (0, 619, 3,652, or 8,492 mg/ m^3) D4 vapor; Additional groups were exposed to control or high dose concentrations and allowed a 28-day recovery	NOAEL= 301 ppm (3,652 mg/m^3) LOAEL= 700 ppm (8,492 mg/m^3)	Significantly decreased ovary weights in females after a 28-day recovery period	Other effects reported in this study include increased absolute and relative liver weights in males and females *Limitations:* No major limitations were identified OQD=High
4-week inhalation toxicity study in F344 rats (n = 10/sex/group) (RCC, 1995a)	Rats exposed 6 hours/ day, 5 days/week via nose-only inhalation to 0, 2.78, 5.13, 8.62, or 14.21/13.25 mg/L air	LOAEL= 13.25 mg/L air (1093 ppm; 13,250 mg/m^3)	Significant increase in vaginal mucification and a decrease in mean corpora lutea score	Other effects reported in this study include increased absolute and relative liver and adrenal weights, decreased absolute and relative thymus weights, changes in clinical chemistry, and changes in leukocyte and reticulocyte counts, *Limitations:* The high exposure concentration was too high resulting in mortality. This concentration was also above the level of saturation resulting in aerosolization and was evaluated separately. OQD=High
28-days inhalation toxicity study in F344 rats (Dow Corning, 1997)	Rats exposed 6 hours/day, 5 days/week via whole body inhalation to 0, 7, 20, 60, 180, or 540 ppm (0, 85, 243, 728, 2,184, or 6,551 mg/ m^3)	NOAEL = 540 ppm (6,551 mg/m^3) LOAEL= N/A	No effects reported on ovaries or testes weights or gross pathological changes.	Other effects reported in this study include increased absolute and relative liver weight in male and female rats, but no effect following a 14-day recovery period; marginal but statistically significant changes in hematological and serum chemistry parameters *Limitations:* No major limitations were identified. OQD=High

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
90-day inhalation toxicity study in F344 rats (20/sex/group) (Burns-Naas et al., 2002; RCC, 1995b).	Rats exposed 6 hours/day, 5 days/week for 3 months via nose-only inhalation 0, 35, 122, 488, or 898 ppm (0, 420, 1,480, 5,910, or 10,870 mg/m^3) D4 vapor. Additional groups exposed to control or high dose concentrations and allowed 4-week recovery.	NOAEL= 488 ppm (5,910 mg/m^3) LOAEL= 898 ppm (10,894 mg/m^3)	Significantly decreased ovarian weight (but not after recovery period); histopathological changes in the ovary, vaginal mucification; more females appeared to be in the diestrous stage of the rat estrous cycle	Other effects reported in this study include increased liver and adrenal weights and decreased thymus weights in females, and changes in hematology and serum chemistry, increased incidence and severity of macrophage accumulation, inflammation and leukocyte infiltration in the lungs *Limitations:* No major study limitations identified. OQD=High
24-month inhalation toxicity study and cancer bioassay in male and female F344 rats (Jean and Plotzke, 2017)	Rats exposed 6 hours/day, 5 days/week via whole body inhalation to 0, 10, 30, 150 or 700 ppm (0, 121, 364, 1,820, or 8,492 mg/m3mg/m^3) D4 vapor for up to 104 weeks	NOAEL= 150 ppm (1,820 mg/m^3) LOAEL= 700 ppm (8,492 mg/m^3)	Increased incidence of ovarian atrophy, increased uterine weights, and higher incidences of endometrial epithelial hyperplasia and cervical squamous epithelial cell hyperplasia; Significantly increased incidence of Leydig cell hyperplasia.	Other effects reported in this study include increased liver and kidney weights, upper respiratory tract irritation, lymphocytic leukocytes, and uterine adenomas (discussed in Section 5.1.1.1) *Limitations:* No major study limitations identified. OQD=High
Oral and Dermal Studies				
Range-finding oral developmental toxicity study in pregnant New Zealand White SPF rabbits (n=6/group) (IRDC, 1993c)	Pregnant rabbits exposed by oral gavage to 0, 50, 100, 500, or 1,000 mg/kg/day on GD 7-19	NOAEL= 50 mg/kg/day LOAEL = 100 mg/kg/day	Significantly reduced maternal body weights, body weight gain, and food consumption.	Significant increased numbers of abortions were reported at 500 and 1000 mg/kg/day and significant increased post-implantation loss, decreased number of fetuses and gravid uterine weight were observed at 1,000 mg/kg/day. *Limitations:* Due to attrition and spontaneous abortions in the 500 and 1000 mg/kg/day groups, uterine examination endpoints did not have sufficient data for statistical analysis and were omitted from the analysis OQD=Medium

Brief Study Description	Exposure	NOAEL/LOAEL[a] (ppm or mg/kg/day)	Reproductive Effects at LOAEL	Remarks [b]
Chronic oral toxicity study in albino FDLR rats (5/sex/group, 10/sex/group for controls) (FDRL, 1966)	Rats exposed via diet to 1 % D4 (770-870 mg/kg-bw/day) for 52 weeks	NOAEL= 770-870 mg/kg-bw/day LOAEL = N/A	No significant effects on reproductive organ weights or microscopic changes (in gonads, uterus or seminal vesicles) reported	*Limitations:* The test substance purity is not reported and there is substantial ambiguity about how the test substance was formulated and prepared, making the true administered dose difficult to determine. There are also serious flaws with sample size and ambiguity with statistical analysis. OQD=Low
Sub-acute dermal toxicity study in NZW rabbits (n=6/sex/group) (FDRL, 1979)	Rabbits dermally exposed to 0 or 1 g/kg D4 6 hours/day for 3 weeks	NOAEL = N/A LOAEL = 1 g/kg	Significant decrease in absolute ovary weights (25 % reduction compared to controls)	Although not statistically significant, dosed groups showed lower ovary and uterus weights (relative ovary weights were reduced by 15 %, and absolute and relative uterus weights were decreased by 21-33 %), which provide some support to the more significant reproductive effects. *Limitations:* Lack of blinding and a lack of scoring for skin irritation. The authors also had issues with animal attrition (possibly due to stress) OQD=Medium

[a] LOAEL for reproductive effects. In some studies, other effects may occur at lower doses
[b] OQD = Overall quality determination, as discussed in Section 2 and at the beginning of Section 4

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4.1.3.3 Mechanistic and Supporting Evidence

EPA considered available mechanistic evidence that may contribute to the weight of evidence for reproductive effects. The effect of D4 on several mechanisms related to hormonal regulation have been explored in the available literature, including estrogen, progesterone and androgen activity.

4.1.3.3.1 Estrogen and Progesterone Activity

Estrogenic effects of D4 have been investigated in uterotrophic assays via oral gavage, subcutaneous administration, and whole-body inhalation exposure with female F344 and SD rats, female B6C3F1 mice, and female estrogen receptor-α knockout (αERKO) mice (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003; MPI Research, 1999).[4] Receptor-binding and transcription assays have also investigated the capability of D4 to bind to estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β) as well as progesterone receptors (Quinn et al., 2007b; He et al., 2003).

Uterotrophic and related assays

Several uterotrophic assays suggest weak estrogenic and/or anti-estrogenic activity of D4.

In one high-quality uterotrophic assay, MPI Research (1999) administered D4 to immature female F344 and SD rats via oral gavage at 0, 10, 50, 100, 250, 500, or 1000 mg/kg-bw/day for 4 days. Several positive controls with known estrogenic compounds were also assessed: ethinyl estradiol (EE, a potent estrogen), coumestrol (a weak phytoestrogen), and diethylstilbestrol dipropionate (nonsteroidal estrogen). Both rat strains exhibited increased uterine weight and uterine epithelial cell height when dosed with D4 at 250 to 1000 mg/kg-bw/day. To evaluate anti-estrogenic effects, 500 mg/kg-bw/day D4 was also co-administered with EE in both rat strains. D4 significantly inhibited the uterotrophic response of EE, indicating weak anti-estrogenic activity of D4. Overall, D4 was many orders of magnitude less potent than the known estrogenic compounds (MPI Research, 1999).

Quinn et al. (2007b) conducted a uterotrophic assay using ovariectomized female F344 and SD rats exposed via whole body inhalation of 0 or 8,492 mg/m^3 (0 or 700 ppm)[5] D4 for 16 hours per day for 3 days. EPA gave this study an overall quality determination of medium. Both strains exhibited increases in uterine weight and epithelial cell height indicating a weak estrogenic response. When given in combination with EE, D4 exhibited a mild suppression of EE-induced increase of uterine weight in the F344 rat; D4 did not exhibit a similar anti-estrogenic effect in SD rats.

He et al. (2003) administered D4 via oral gavage to ovariectomized female B6C3F1 mice and investigated the effects on uterine weight, uterine peroxidase activity, and serum levels of estradiol. EPA gave this study an overall quality determination of medium. D4 administered in the dose range of 250 to 1000 mg/kg-bw/day (but not from 1 to 100 mg/kg-bw/day) resulted in increases in uterine weight. A D4 dose of 1000 mg/kg-bw/day for three days (only dose level tested) resulted in increased uterine peroxidase activity, a marker for estrogenic activity. When pre-treated with the estrogen receptor antagonist ICI 182,780 (ICI), ovariectomized mice did not exhibit the increased uterine weight that had been induced by D4 alone. Furthermore, ovariectomized ER-α knockout (α ERKO) mice did not exhibit increases in uterine weights when exposed to D4 or estradiol via oral gavage. These combined effects suggest weak estrogenic activity.

[4] Information in MPI Research (1999) is also described in McKim et al. (2001b).

[5] Values were converted from ppm to mg/m^3 using the following equation: X mg/mg^3 = (Y ppm*296.61)/24.45, where 296.61 is the molecular weight for D4 and 24.45 is the number of liters occupied by 1 mole of gas at standard temperature and pressure (STP; 25°C and 760 mm Hg).

When female B6C3F1 mice in this study were given oral D4 doses of 100 to 1000 mg/kg-bw/day for seven days, they exhibited decreased serum estradiol in a dose-dependent manner. Additional studies with adrenalectomized mice demonstrated that the decreased serum estradiol levels were not due to elevated serum corticosterone levels and thus, not associated with stress induced activation of the hypothalamic-pituitary-adrenal axis (He et al., 2003). The decreases in estradiol contrasts with results of the uterotrophic assays, and He et al. (2003) hypothesizes that decreased estradiol may be due to enhanced metabolism associated with D4 exposure; such changes in metabolism from D4 exposure have been identified by McKim et al. (1998). Given the findings on decreased serum estradiol in mice exposed to D4, He et al. (2003) concludes that the contradictory results of 1) stimulatory effect of D4 on uterine weight and peroxidase activity and 2) decreased estradiol production show that D4 has multiple and complex effects on estradiol-regulated processes.

Lee et al. (2015) gave D4 (at 500 or 1000 mg/kg-bw) subcutaneously for 4 days to immature female SD rats in a uterotrophic assay. The authors also administered EE, and both compounds were given with and without ICI, an estrogen receptor antagonist. Although D4 did not significantly increase uterine weight, it induced gene expression of both calcium-binding protein 9K (CaBP-9K), a biomarker for estrogenicity, and the progesterone receptor. The authors concluded that D4 has estrogenic potential.

In Vitro Receptor Binding Assays

D4's ability to activate ER-α, ER-β, or progesterone receptors was also investigated using *in vitro* receptor binding and luciferase reporter gene assays.

Quinn et al. (2007b) showed that D4 had a low binding affinity for ER-α but did not bind with ER-β when evaluated using a receptor binding assay. In the reporter gene assay, D4 affected ER-α activity at the highest concentration but was not a ligand for the progesterone β receptor. For the ER binding assays, the authors used airtight septa and ensured that the air concentrations were maintained at target levels. Quinn et al. (2007b) indicate that for the reporter gene activation experiments, the positive results suggest that D4 was retained.

He et al. (2003) also conducted an *in vitro* estrogen receptor binding assay in which D4 competed with ^{3}H-estradiol in binding to ER-α, but not with ER-β. The results suggest that D4 is a weak competitor, supporting the results that D4 has weak estrogenic activity via acting on ER-α. The authors did not state whether they controlled for evaporation and did not measure the D4 concentrations. Therefore, it is not known whether results were influenced by any volatilization.

Lee et al. (2015) exposed GH3 rat pituitary cells *in vitro* to D4 or 17β-estradiol with or without ICI. Both D4 and 17β-estradiol alone upregulated the estrogenic biomarker CaBP-9K and the progesterone receptor, and the effects were completely blocked by ICI. D4 and 17β-estradiol decreased the transcription of ER-α, whereas ICI increased transcription. The authors did not describe whether volatility controls were used.

4.1.3.3.2 Luteinizing Hormone

Suppression of the preovulatory luteinizing hormone (LH) surge by D4 on the day of proestrus was associated with decreased ovulation, a small increase in persistent of mature follicles, and increased serum estradiol (Quinn et al., 2007a).

Quinn et al. (2007a) exposed SD rats via whole-body inhalation to nominal D4 concentrations of 0, 8,492, or 10,918 mg/m^3 (0, 700, or 900 ppm) to assess the potential to suppress the pre-ovulatory LH surge and delay ovulation. Hormone levels were also assessed. The study authors used two groups of

rats. Phase I rats were not cannulated and were exposed to D4 on diestrus days 1 and 2 for 6 hours per day and on a proestrus day for 2.5-hours (with blood taken at 10 a.m.). Measured D4 concentrations were 0, 8,516, or 10,979 mg/m^3 (0, 702, or 905 ppm). Trunk blood was evaluated for follicle stimulating hormone, estrone, estradiol, and progesterone.

Phase II rats were cannulated and exposed to D4 levels of 0, 8625, or 10,858 mg/m^3 (0, 711, or 895 ppm) for six hours per day over three days and multiple blood samples were collected for LH and prolactin from 2 pm starting in proestrus through 10 a.m. during the first day of estrus. Trunk blood was again evaluated for follicle stimulating hormone, estrone, estradiol, and progesterone.

During the morning of proestrus (Phase I), rats at both 8,516 and 10,979 mg/m^3 exhibited significantly higher estrone levels than controls. The high concentration rats had significantly higher progesterone levels than controls. In the afternoon of proestrus (Phase II), rats had lower prolactin levels and higher progesterone.

On the morning of estrus (Phase II), both D4 doses had significantly higher levels of estrone, estradiol, and lower ratios between estrone and estradiol; follicle stimulating hormone concentrations were lower in both groups than controls. During Phase II, fewer rats ovulated when exposed to D4 (42 and 31 percent at 8625 and 10,858 mg/m^3, respectively) vs. 79 percent among controls. Rats had fewer mean numbers of ova at each concentration. The females that failed to ovulate had no LH surge, whether they were in the control or exposed groups. Quinn et al. (2007a) states that the observed hormone changes indicate that exposures during proestrus delayed the mid-cycle LH surge, delayed ovulation, and extended estrus.

4.1.3.3.1 Androgen Activity

To assay for androgenic activity, Quinn et al. (2007b) conducted a Hershberger assay by exposing castrated male F344 rats via whole body inhalation 0 or 8,492 mg/m^3 D4 (0 or 700 ppm) 16 hours per day for 10 days and measuring increases in relevant organ weights. Testosterone propionate was used as a positive control. EPA gave this study an overall quality determination of low. D4 was negative in the Hershberger assay, indicating no androgenic activity. When co-administered with the positive control to determine whether D4 affected study outcomes, no changes to the results were observed, and thus D4 showed no anti-androgenic activity.

This lack of activity correlates with lack of related effects in the 2-generation reproductive toxicity study (WIL Research, 2001a), which found no adverse effects related to male functional reproductive parameters, spermatogenic endpoints, or microscopic evaluation of male reproductive tissue when both sexes received D4. This difference was further strengthened when D4-exposed F1 males were mated with unexposed females and resulted in no adverse effects (WIL Research, 2001a).

4.1.3.4 Evidence Integration Summary

There were no human epidemiological studies on reproductive effects available for D4. The human evidence is indeterminate for reproductive effects.

Animal evidence for female reproductive effects is moderate. High- and medium-quality studies show concordant and dose-responsive effects that are relevant for human health risk assessment. In female rats, D4 exposure was associated with increased uterine weights, increased incidences of endometrial hyperplasia (Jean and Plotzke, 2017), and increased severity of vaginal epithelial thickness (Jean et al., 2017), reduction of ovarian corpora lutea score and presence of numerous follicles without ovulation in

rats (Quinn et al., 2007a; WIL Research, 2005; Burns-Naas et al., 2002; WIL Research, 2001a; RCC, 1995a), estrous cycle irregularities (Burns-Naas et al., 2002; WIL Research, 2001a), and increased incidence of ovarian atrophy (Jean and Plotzke, 2017; Quinn et al., 2007a; RCC, 1995b). Effects on reproductive function include decreases in fertility, implantation sites, and litter sizes (WIL Research, 2005, 2001a, 1997a, 1996a, b), and increased gestation lengths (WIL Research, 2005). The biological plausibility of these effects is supported by moderate mechanistic evidence. Mechanistic data suggest that reduced ovulation resulting from D4 exposure may be related to a suppression of the preovulatory LH surge, but the mechanism by which this occurs is not known (Quinn et al., 2007a). A role for estrogenic activity is suggested by positive findings in multiple uterotrophic assays and the blockade of the positive response during cotreatment with an ER antagonist and in ERα knockout mice. *In vitro* studies demonstrate binding to ER-α receptor and increased expression of biomarkers of estrogenicity (Quinn et al., 2007b); He et al. (2003).

Animal evidence for male reproductive effects is slight. In males, testicular lesions and increases in absolute and relative testes weights were reported after 12 months exposure, but the results were not statistically significant (Jean and Plotzke, 2017). Effects on reproduction function include decreases in fertility, implantation sites, and litter sizes (WIL Research, 2005, 2001a, 1997a, 1996a, b), increased gestation lengths (WIL Research, 2005). Mechanistic data on male reproductive effects is indeterminate. D4 did not induce androgenic effects in a Hershberger assay, and the results of a single study of the D4 metabolite dimethylsilanediol did not provide evidence for a role for this metabolite in male reproductive effects of D4. The overall judgment for male reproductive effects based on mechanistic evidence is indeterminate.

Based on integration of information across evidence streams, evidence indicates that D4 *likely* causes effects on female reproductive system in humans under relevant exposure circumstances. Evidence *suggests* but it is not sufficient to conclude that D4 may cause effects on male reproductive function in humans under relevant exposure circumstances. Evidence integration tables in Appendix A provide a detailed summary of lines of evidence that support these overall conclusions.

4.1.4 Developmental Toxicity

U.S. EPA (1991) identifies death, structural abnormalities, altered growth, and functional deficits as the four major manifestations of developmental toxicity. This section describes developmental assessments.

4.1.4.1 Human Evidence

EPA did not identify any human studies that evaluated potential developmental effects from D4 exposure in the literature search conducted in 2019.

4.1.4.2 Laboratory Animal Evidence

Section 4.1.3, describes the 1- and 2-generation reproductive toxicity studies that assessed reproductive and developmental outcomes. All significant effects on these outcomes are described above. This section focuses on effects of gestational exposure to D4.

EPA identified two inhalation studies that evaluated developmental effects including maternal toxicity, uterine contents and weights, litter size, fetal viability, sex ratio, fetal body weights, and malformations and variations in rats and rabbits. Pregnant SD female rats were exposed to 0, 100, 300, or 700 ppm D4 via whole-body inhalation daily for 6 hours on gestation days 6 through 15. No overt clinical sings of toxicity related to the test substance were observed. Sporadic decreases in maternal body weight were observed in rats at 700 ppm. There were body weight gain and food consumption decrease observed

throughout. No maternal clinical signs or uterine weight changes in female rats and no fetal effects (litter size, fetal viability, sex ratio, or fetal body weight), or changes to uterine contents (number of implantations, early and late resorptions, or corpora lutea, or pre- or post-implantation loss) in female rats. No fetal malformations/variations were observed in rats exposed to concentrations ≤700 ppm (IRDC, 1993b).

In another inhalation study, female New Zealand White (NZW) rabbits were exposed to mean measured concentrations of 0, 100, 300, or 501 ppm of D4 daily for 6 hours on gestation days 6 through 18. On GD 29, sacrificed females were evaluated for any gross morphological changes in the abdominal or thoracic cavities (including organs). Uteri were excised and pregnancy status was determined. Gravid uteri were weighed, fetuses were removed, and uteri were assessed for location of viable and nonviable fetuses, early and late resorptions, and number of total implantations and corpora lutea. Individual fetuses were weighed, sexed, and examined for internal (visceral and skeletal) and external malformations. No clinical signs of toxicity were observed. No significant changes in maternal body weight or body weight gains were seen, compared with controls, despite a significant decrease in maternal food consumption at 501 ppm (22 percent from GD 6-9; 17 percent from GD 9-12, compared with controls). No significantly increased incidences of gross abnormalities were seen and mean post-implantation loss (resorption) was slightly increased in the 501-ppm group compared to controls; however, the authors indicated that the loss was within historical control ranges (not provided). No differences in the number of viable fetuses per dose or mean fetal body weights were seen relative to controls. There was no compound-related developmental toxicity or increases in internal or external malformations or variations (IRDC, 1993a).

EPA also identified an oral study that evaluated the effects of oral exposure to D4 during gestation in rabbits (IRDC, 1993c). In a range-finding developmental toxicity study, pregnant NZW rabbits were exposed to 0, 50, 100, 500, or 1,000 mg/kg-bw/day of D4, via gavage in methocel vehicle, each day between GD 7-19. Exposures were paused after day 19, but animals were kept alive until GD 29. Significant increases in post-implantation loss, decreased number of fetuses and gravid uterine weight were observed at 1,000 mg/kg/day. Uterine examinations in the 50 and 100 mg/kg-bw/day groups were comparable with controls. The authors suggest that these effects may have been due to decreased food consumption (IRDC, 1993c). A significant limitation was the increased numbers of abortions that occurred at 500 and 1000 mg/kg-bw/day, respectively, that may have limited the data to sufficiently analyze uterine examination endpoints.

4.1.4.3 Mechanistic and Supporting Evidence

EPA did not identify any relevant mechanistic information that may inform the mechanism of action of observed developmental effects.

4.1.4.4 Evidence Integration Summary

There were no human epidemiological studies available for D4, and the human evidence is indeterminate for developmental effects.

Animal evidence evaluating the potential for developmental effects is moderate. Available evidence in high- and medium-quality studies indicates no effect on fetal viability, fetal body weight or sex ratio in female rats or rabbits exposed to concentrations ≤8,492 mg/m^3 (IRDC, 1993a, b, d, e). Sporadic decreases in maternal body weight were observed in rats at 700 ppm (IRDC, 1993b, e). Similarly, female rabbits were reported to have decreased body weight gain and decreased food consumption at 700 ppm (IRDC, 1993d). Decreased maternal body weight and weight gain were reported in rabbits

exposed via gavage at ≥100 mg/kg-bw/day, with decreased food consumption reported at higher doses (≥500 mg/kg-bw/day) (IRDC, 1993c). No maternal clinical signs or uterine weight changes in female rats or rabbits exposed to concentrations ≤ 700 ppm (IRDC, 1993a, b, d, e), or female rabbits administered doses ≤1000 mg/kg-bw/day via gavage (IRDC, 1993c). Inhalation exposure to D4 did not induce any developmental effects in rats or rabbits following inhalation exposures. Some developmental effects were reported following oral exposure. However, these studies were not comprehensive. For instance, two oral developmental studies were available but did not perform complete maternal and fetal evaluations (Falany and Li, 2005; IRDC, 1993c). IRDC (1993c) also reported significant increased numbers of abortions, at the two highest doses (500 and 1000 mg/kg/day), respectively, which impacted the number of animals available for analysis.

Overall, evidence is inadequate to assess whether D4 exposure may cause developmental effects in humans under relevant exposure circumstances. Evidence integration tables in Appendix A provide a detailed summary of lines of evidence that support these overall conclusions.

4.2 Dose-Response Assessment

4.2.1 Dose-Response Derivation for Non-Cancer Hazard Values

As described in Section 4.2.1, EPA identified reproductive endpoints reported in the 2-generation inhalation study (WIL Research, 2001a) as the primary basis for quantitative dose-response analysis. The endpoints considered in the dose-response analysis included decreases in mean live litter size, mean number of pups born, and fertility.

The sections below described the steps used to derive the hazard values that are used to calculate acute and chronic risks for inhalation, oral, and dermal exposures to D4. Specifically, EPA used a PBPK model for D4 (Campbell et al., 2023) to convert administered dose-response data reported in WIL Research (2001a) into internal dose-response data, with blood AUC of parent D4 serving as the internal dose metric. Following this conversion, EPA then performed benchmark dose (BMD) modeling on the internal dose-response data and identified the best model (based on adequate fit and lowest AIC) and BMDL for each selected endpoint. After completing the BMD modeling, EPA identified the most robust and sensitive endpoint with a good model fit to determine the $BMDL_5$ (the exposure at which the model predicts a 5 percent benchmark response). This POD was then converted back to external human equivalent concentrations (HECs) and human equivalent doses (HEDs) for all relevant exposure routes and durations.

EPA selected the $BMDL_5$ for decreased live litters in the F2 offspring as the basis for HEC and HED derivation because it was among the most sensitive endpoints with a good model fit (as illustrated in Appendix B.1) supported by the weight of evidence across multiple studies. The HECs and HEDs derived from the selected POD were used to calculate risks for both adults and children and for all exposure durations and routes applicable to the D4 COUs: acute (one-day), intermediate (approximately 30 days), and chronic exposure (which varies based on age and group (workers or the general population)).

The steps in running the PBPK model as well as assumptions, parameter choices, and conversions used in the dose-derivation and PBPK modeling are described below. Details are presented in the Draft PBPK Model Description and Review (U.S. EPA, 2025f). BMD modeling results for PBPK-converted exposure levels are presented for each endpoint in Appendix B. PBPK model results, including internal

doses and predicted HECs and HEDs, are presented in the Draft PBPK Model Results (U.S. EPA, 2025g). Appendix C presents a review of the model by an EPA PBPK model expert.

4.2.2 Selection of Studies and Endpoints for Non-cancer Toxicity Dose-Response Analysis

EPA considered studies and endpoints from the suite of available animal toxicological studies for which the weight of evidence supported adverse health outcomes following D4 exposure, as described in Section 4.1. Reproductive effects were identified as the only critical hazard domain supported by sufficient evidence to support a dose-response analysis. EPA determined that evidence for other hazard domains, including liver toxicity, pulmonary toxicity, and developmental toxicity is suggestive or indeterminate. Evidence for these endpoints is not sufficient to conclude that D4 causes adverse effects in humans and is therefore not sufficient to support a dose-response analysis. Therefore, when considering non-cancer PODs for estimating risks for acute, intermediate, and chronic exposure scenarios, EPA reviewed the available evidence and studies on reproductive effects. EPA selected studies and specific hazard endpoints relevant to adverse reproductive effects based on the following considerations:

- Overall quality determinations
- Exposure durations
- Dose range
- Relevance (*e.g.*, what species was the effect in, was the study directly assessing the effect, is the endpoint the best marker for the tox outcome?)
- Uncertainties not captured by overall quality determination
- Endpoint sensitivity
- Total uncertainty factors (UF)
- Uncertainty and sensitivity of BMR selection from BMD modeling
- Relevance and uncertainty of dose metric for PBPK model (if applicable)

4.2.2.1 Non-cancer Endpoints for Acute, Intermediate, and Chronic Exposures

EPA considered available studies and endpoints evaluating reproductive effects to identify the most appropriate ones for dose-response.

Although there were no human studies evaluating reproductive effects of D4, available animal and mechanistic evidence led EPA to conclude that D4 *likely* causes effects on the female reproductive system. Rats consistently exhibited signs of reproductive effects across studies and exposure durations. In the one- and 2-generation reproductive toxicity studies in which females were exposed, the authors reported significant effects for a range of inter-related reproductive endpoints, including decreases in the numbers of implantation sites, estrous cycle disruptions, decreased litter size, failure to ovulate, decreases in corpora lutea and decreases in maternal body weight changes during gestation and lactation at 700 ppm (WIL Research, 2005, 2001a, 1997a, 1996a, b). Decreases in fertility, implantation sites, and litter sizes may be related to estrous cycle disruptions, failure to ovulate, and/or decreases in corpora lutea, as described above in Section 4.1.3.

For most of the available studies evaluating reproductive endpoints, the dose range tested is limited to one high concentration exposure group (Jean et al., 2017; WIL Research, 2005, 1999) or limited to nominal concentrations of 8,492 mg/m^3 (700 ppm) or higher (Quinn et al., 2007a). Therefore, although these studies may contribute to the broader weight of evidence for reproductive effects, they are not informative for dose-response and are not further considered here.

EPA used four studies that evaluated reproductive effects over a broader dose range. These included high-quality 1-generation (Dow Corning, 1998b) and 2-generation (WIL Research, 2001a) reproductive studies that assess a comprehensive set of reproductive endpoints. Other studies that include endpoints relevant to reproductive toxicity included a high-quality 90-day inhalation study (Burns-Naas et al., 2002) and a high-quality 24-month inhalation toxicity study in rats (Jean and Plotzke, 2017).

In the 90-day inhalation toxicity study, F344 rats were exposed to concentrations of 0, 425, 1,480, 5,920, 10,894 mg/m^3 (0, 35, 122, 488, or 898 ppm). Treated females at the highest exposure (10,894 mg/m^3) exhibited significant histopathological changes in their reproductive tracts, more females appeared to be in the diestrous stage of the rat estrous cycle. Other effects included vaginal secretion, and increased incidence of ovarian atrophy (Burns-Naas et al., 2002; RCC, 1995b). In the 24-month inhalation toxicity study in F344 rats exposed to concentrations 0, 10, 30, 150, or 700 ppm (0, 121, 364, 1,820, 8,492 mg/m^3), 6 hours per day, 5 days per week, Jean and Plotzke (2017) observed increased incidence of ovarian atrophy, increased uterine weights, and higher incidences of endometrial epithelial hyperplasia were observed in females at 700 ppm (8,492 mg/m^3). Male rats showed increased incidence of interstitial cell hyperplasia severity in testes at the 150 ppm (1,820 mg/m^3) (Jean and Plotzke, 2017).

In a 1-generation study (Dow Corning, 1998b), rats were exposed to 0, 849, 3,639, 6066, or 8,492 mg/m^3 (0, 70, 300, 500, or 700 ppm) D4 vapor for six-hours daily for 28 days prior to mating through gestation day 19. Rats exposed to 500 or 700 ppm (6066, or 8,492 mg/m^3) during this period showed significant decreases in viable fetuses, implantation sites, and the number of corpora lutea. Significant changes in the number of corpora lutea were also reported at 300 ppm. This study also evaluated the effects of exposure on the same reproductive endpoints during shorter period of exposure (*i.e.*, prior to mating, during fertilization, or during implantation) but the longer duration exposure resulted in the most sensitive effects. A NOAEL of 301 ppm (3,652 mg/m^3) and a LOAEL of 501 ppm (6078 mg/m^3) were determined based on decreased viable fetuses, implantation sites, and corpora lutea.

In a 2-generation study, Crl:CD (SD)IGS BR rats were exposed to 0, 71, 298, 502 or 700 ppm (0, 861, 3,615, 6,090, 8,492 mg/m^3) vapor concentrations of D4 via (whole-body) inhalation for 6 hours a day for at least 70 days prior to mating through weaning of F1 pups on postnatal day (PND 21). Statistically significant and dose-related decreases in F1 litter size and number of F1 pups born were observed at 500 ppm (6066 mg/m^3). Apparent decreases in these endpoints also occurred at 300 ppm (3,639 mg/m^3) but did not reach statistical significance. In F1 adult females, there were no differences observed for mean ovarian primordial follicle counts, but reduced corpora lutea counts were observed at 702 ppm (8,516 mg/m^3). For the first mating, F1 male and female mating indices were decreased at 700 ppm (8,492 mg/m^3). The reduction was significant for females. Fertility indices for both sexes were significantly decreased at the same concentration. Estrous cycle length was significantly increased at 702 ppm (8,516 mg/m^3), compared with controls (WIL Research, 2001a).

On PND 22, F1 offspring were exposed to the identical target concentrations to their corresponding F0 parents (with analytical concentrations being 0, 71, 301, 502 and 702 ppm (0, 861, 3,652, 6,090, 8,516 mg/m^3)) for 70 days. During the second mating, significant decreases in the mating indices occurred in high-exposure males and females, and fertility indices were decreased in both sexes at 502 ppm. Dystocia was observed in 2 and 3 females in the 502 and 702 ppm (6,090 and 8,516 mg/m^3) groups, which the authors considered to be test-article related; dystocia was the cause of death in two of three high-exposure females. For F1 males paired with unexposed females, no differences in reproductive performance were observed. Sperm parameters were not significantly affected by D4 exposure in F1 males. F2 litter effects included significant reductions in litter size and number of pups born at 502 and 702 ppm (6,090 and 8,516 mg/m^3), respectively. Overall, reproductive effects including significant

reductions in fertility indices, litter size, and the number of pups were observed at 502 ppm (6,090 mg/m^3). This was identified as the overall LOAEL for the study (WIL Research, 2001a).

The first parent generation (F0) were dosed starting in adulthood. F1 were assessed as offspring and became parents of the F2s. The F1 generation was exposed during gestation, indirectly via lactation after birth, and directly as adults. The F1 generation therefore covered more lifestages (*e.g.*, with additional indirect exposure from lactation) to D4 than the F0 generation. The NOAECs for the F2 offspring are the same as the F1 offspring (all at 300 ppm) with LOAECs of 500 ppm. The effects on fertility/number of pups born/decreased live litter size in each generation are somewhat similar to fertility of the parents.

In all 1- and 2-generation toxicity studies in which females were exposed, the authors reported a decrease in the numbers of implantation sites and decreases in maternal body weight changes during gestation and lactation at 700 ppm (WIL Research, 2005, 2001a, 1997a, 1996a, b). Decreases in fertility, implantation sites, and litter sizes may be related to estrous cycle disruptions, failure to ovulate, and/or decreases in corpora lutea as described above under female reproductive toxicity.

After considering hazard identification and evidence integration, EPA chose several reproductive endpoints to include in the dose-response analysis for derivation of PODs for acute, intermediate, and chronic exposure scenarios in the D4 Risk Evaluation. The endpoints selected for dose-response include decreased mean live litter size, mean number of pups born, fertility index, and females without confirmed pregnancy in the 2-generation reproductive toxicity study (WIL Research, 2001a). EPA selected this study as the basis for dose-response because it was a high-quality study that evaluated a wide range of doses and a comprehensive set of sensitive reproductive endpoints. The study also included exposures for both males and females and identified the most sensitive endpoints following long-term exposure across lifestages. WIL Research (2001a) was one of a limited set of studies that evaluate effects across a range of exposure levels. The significant effects on reproductive endpoints reported in WIL Research (2001a) were consistent with those reported in other studies that tested effects at a more limited range of higher exposure levels. The effects reported in WIL Research (2001a) were also consistent with the effects reported at similar exposure levels in shorter duration exposures in WIL Research (1999).

EPA considered several of the inter-related reproductive endpoints in this study to be relevant to acute, intermediate and chronic exposure durations. In one supporting study, a single six-hour exposure in females at 8,492 mg/m^3 (700 ppm) during premating resulted in decreased in pregnancy rates and decreased fertility (WIL Research, 1999). Although this acute study only evaluated effects at a single dose level and cannot be used for dose-response, it demonstrated that a single exposure was sufficient to produce reproductive effects being considered for dose-response. Therefore, the dose-response information derived for related reproductive effects in the 2-generation study (WIL Research, 2001a) is considered applicable for acute, intermediate, and chronic POD derivation.

Limited hazard data were available for reproductive toxicity following dermal exposure. A prenatal dermal toxicity study in NZW rabbits reported significant decrease in absolute ovary weights following dermal exposure to D4, but the study did not evaluate other endpoints relevant to reproductive toxicity. In addition, the study is limited to a single exposure level and did not provide dose-response information (FDRL, 1979).

For the oral route, the available evidence was not a suitable basis for dose-response. A range-finding oral toxicity study between gestational day (GD) 7-19 in pregnant New Zealand White SPF rabbits exposed to 0, 50, 100, 500, or 1000 mg/kg/day resulted in significant increased numbers of abortions

that occurred at 500 and 1000 mg/kg/day. At the highest concentration, 1000 mg/kg-bw/day, there were significant increases in post-implantation loss, decreased number of fetuses and gravid uterine weight. However, study limitations made the study uninformative for dose-response. Due to animal attrition and abortions in the 500 mg/kg-bw/day group, uterine examination endpoints did not have sufficient data for statistical analysis and were omitted from the analysis, making the oral route unsuitable for deriving route-specific POD (IRDC, 1993c). This study received a high overall quality determination. In the absence of adequate dose-response information for oral and dermal exposure routes, EPA used the PBPK model to derive oral and dermal PODs for D4 based on reproductive effects reported in the 2-generation inhalation study (WIL Research, 2001a) used as the basis for the inhalation POD.

4.2.3 PBPK Modeling Approach

4.2.3.1 D4 Model Description

The toxicokinetics of D4 is complex and differs depending on the route of exposure and the species being studied. PBPK models can incorporate route-specific and species-specific difference to estimate internal doses (*e.g.*, concentrations in blood, liver, fat) associated with adverse health outcomes. By using a common dose metric, a PBPK model can facilitate various extrapolations (*e.g.*, across species) based on the biologically effective dose, rather than the administered doses.

The most recent D4 PBPK model described in Campbell et al. (2023) allowed for extrapolation across different exposure routes, species, and exposure durations used in the risk evaluation. The model was developed using toxicokinetic data for male and female SD and F344 rats and humans. Generally, Campbell et al. (2023) addressed deficiencies in previous models that inaccurately predicted blood and fat D4 levels after longer exposures by including post-exposure toxicokinetics, such as from an inhalation study by Meeks et al. (2022). Figure 4-1 provides the overall model structure.

PBPK modeling is particularly informative for D4 for several reasons. D4 is highly lipophilic and volatile. It has complex toxicokinetic properties such as the bidirectional movement in and out of lipoprotein pools, transfer to and release from deep tissues, and different toxicokinetic behaviors among exposure routes.

D4 modeling started in the early 2000s and has undergone several revisions to incorporate additional species and routes of administration. These revisions leveraged findings from toxicokinetic studies published over the years. The initial model by Andersen et al. (2001) was built using data from a rat inhalation study and it identified D4's unique properties, including low blood:air and high fat:blood partitioning, high hepatic and exhalation clearance, and plasma lipid storage (McMullin et al., 2016; Andersen et al., 2001). Sarangapani et al. (2003) expanded the model to include oral and dermal routes of exposure, and Reddy et al. (2007) further developed the model to include inhalation and dermal exposure in humans.

McMullin et al. (2016) integrated the available models into a single unified model structure applicable for both rats and humans, as well as for multiple exposure routes. Campbell et al. (2017) further refined the model, using oral absorption information to account for sequestration of D4 in blood, and it included hepatic induction of metabolism.

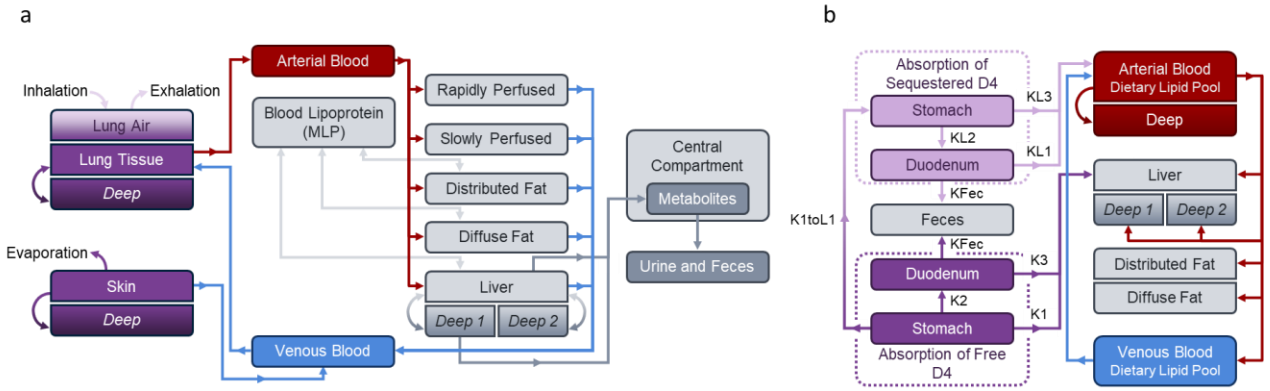

Figure 4-1. PBPK Model Diagram for Rats and Humans Adapted from Campbell et al. (2023), Figure 1

(a) Inhalation and dermal exposure. (b) Oral submodel. K1, K2, K3, KL2, KL3, and KFec are first order rate constants for transfer between different compartments (1/h). K1toL1 is the rate constant for conversion of free D4 to sequestered D4 (1/h).

The updated model (Campbell et al., 2023) addressed specific deficiencies related to predicting D4 levels in blood and fat after longer term exposures and used additional inhalation toxicokinetic data for SD and F344 rats after 14 to 28 days of D4 exposure and a 14-day postexposure period (Schmitt et al., 2023; Meeks et al., 2022) that were not incorporated into previous models. Major updates described by Campbell et al. (2023) included:

- Accounting for differences in toxicokinetic data by rat strain (SD and F344 rats) such as differences in enzyme induction among the strains;
- Converting a mobile lipoprotein pool (MLP) from a single directional distribution (liver to fat) to a bidirectional recirculating distribution between liver and fat;
- Using a parallelogram approach for MLP parameters between rats and humans to account for the lack of long-term clearance data in humans using ratios between relevant human and rat parameters.

In addition to D4-specific toxicokinetic data, the model relied on general rat and human physiological parameters (body weight, tissue volume blood flows, cardiac output, and alveolar ventilation rates) from Brown et al. (1997), which is typically a source of such values for PBPK models. The model code was updated from acsIX used in previous models to R in the updated model (Campbell et al., 2023).

Campbell et al. (2023) could predict both C_{max} and AUC for blood (also described as plasma in some places) [6], liver, fat, and exhaled D4. Following EPA guidance (U.S. EPA, 2006), EPA chose the AUC of blood concentrations of parent D4 as the dose metric. Due to limited information available on the biologically active form in a relevant target tissue for D4, blood concentration serves as a reasonable surrogate for the target tissue dose (U.S. EPA, 2006). Additionally, the PBPK model does not predict D4 concentrations in reproductive tissues.

For assessing health risks related to repeated exposure to systemically acting chemicals, EPA U.S. EPA (2006) recommends that the integrated concentration of the toxic form of a chemical, such as the AUC, is considered appropriate. Although EPA uses the D4 hazard endpoints for acute, intermediate, and

[6] Campbell et al. (2023) uses some parameter inputs that are specific to blood (*e.g.*, blood:air coefficients, blood volume, blood flow). The authors also use parameters based on toxicokinetic studies with information on D4 in plasma. The model outputs report blood values, but the figures based on these outputs in Campbell et al. (2023) are shown as plasma values.

chronic exposure scenarios, exposure durations in the 2-generation reproductive toxicity study (WIL Research, 2001a) from which hazard endpoints were modeled were subchronic to chronic (approximately 70 to 241 days).

The model detail is described in *Draft PBPK Model Description and Review for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025f). Model results, including internal doses and predicted human equivalent concentrations (HECs) and human equivalent doses (HEDs) are presented in *Draft PBPK Model Results for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025g). Section 4.2 presents the steps used to derive HECs and HEDs using the PBPK model and the resulting HEC and HED values.

4.2.3.2 Toxicokinetics Studies Used to Parameterize the PBPK Model

The PBPK model for D4 used parameter input values for rats and humans. The rat parameters were mostly developed from F344 rat data, but two of these parameters have values that are derived from SD rats, Human parameter values were derived from human data if available. In cases for which human data were not available, rat values were used as a substitute for humans. The studies used for model parameterization, as chosen by Campbell et al. (2023), are summarized below.

Data from a human inhalation study (Utell et al., 1998) were used to estimate the blood:air partition coefficient, a diffusion coefficient for slowly perfused tissue, and a metabolic parameter (maximum velocity of the enzymatic reaction, or V_{maxc}). Data from a human dermal study (University of Rochester Medical Center, 2001) were used to estimate human dermal parameters such as evaporation from skin, absorption into blood, and movement into and out of deep skin compartments.

Data from several rat inhalation toxicokinetic studies were used to estimate rat-specific values in the PBPK model. Data for F344 rats exposed to D4 for 1 or 15 days from Plotzke et al. (2000) formed the basis of most rat partition coefficients (*e.g.*, blood:air, diffuse fat:air). Many of these partition coefficient values were also used for humans when human data were lacking. Plotzke et al. (2000) data for F344 rats were also used to estimate the metabolic parameters V_{maxc} and the affinity constant K_m (strength of enzyme binding to D4). McKim et al. (1998), a 4-week inhalation study using F344 rats, provided inputs for rat enzyme induction parameters. The 28-day inhalation data for F344 rats from Meeks et al. (2022) were used for parameters associated with MLP production and clearance from liver and fat. MLP parameter values for humans were adjusted by scaling the rat values to tissue volumes (Campbell et al., 2023).

SD rat data were used when the parameter values differed from the F344 rat parameters. Two parameters use SD-rat specific values in addition to F344 rat values. These are V_{maxc} with SD rat data provided in Schmitt et al. (2023) and the maximum CYP enzyme production rate (K_{max}).

Domoradzki et al. (2017) was the only oral rat study used in the PBPK model. The study used a liquid diet and a lower single dose (30 mg/kg-bw/day) for F344 rats than an earlier oral study. The data were used for diffuse fat and distributed fat diffusion coefficients, a deep liver tissue metabolic induction parameter, mass transfer coefficients for the deep liver tissue, oral absorption rate constants, and parameters associated with uptake into tissue from lipoprotein-associated absorption. Many of the parameter values for rats from Domoradzki et al. (2017) were also used for humans.

4.2.3.3 D4 Model Review

EPA solicited a thorough review of the updated PBPK model. The review was performed for EPA by ICF and included checking the model code for errors, running model simulations to reproduce model

runs published in Campbell et al. (2023), comparing model simulations with available D4 toxicokinetic data, and conducting sensitivity analyses to determine which parameters had the largest influence on model outputs. Model simulations included inhalation and oral routes in rats; and inhalation, oral, and dermal routes in humans. The model review is described in the Draft PBPK Model Description and Review (U.S. EPA, 2025f).

Two EPA PBPK modeling experts also reviewed the model. One expert also provided responses to charge questions related to the model in a high-level review, using the Draft PBPK Model Description and Review U.S. EPA (2025f) and the original model publication Campbell et al. (2023) as background information (see Appendix C for the expert review).

As shown in Figure 4-2, the PBPK model provided good fit of the experimental data after inhalation exposure for F344 rats to 8,492 mg/m^3 D4. Compared with an earlier version of the model Campbell et al. (2017), the updated model reported a 15-fold reduction in sums of squared errors when predicting the D4 concentration in the blood of rats.

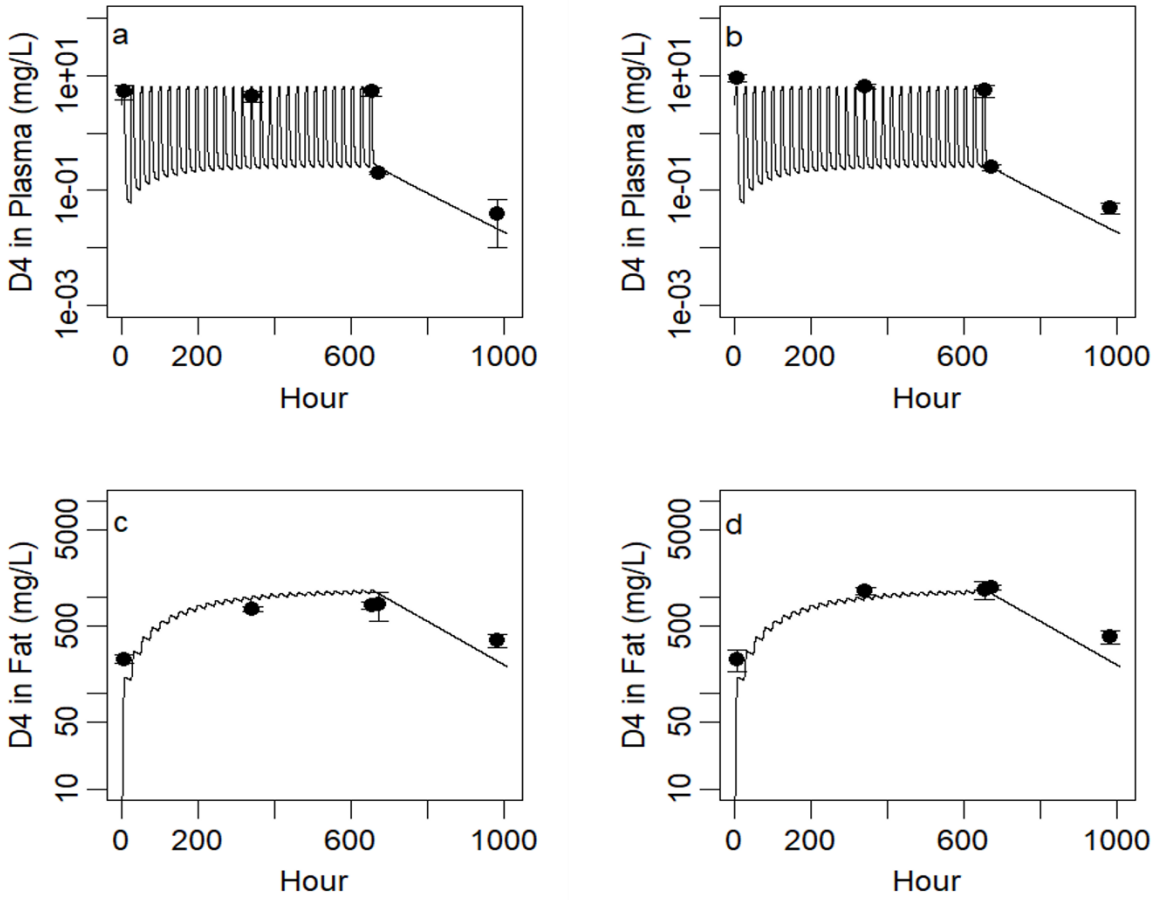

Figure 4-2. Model Simulations Compared with F344 Rat Inhalation Experimental Data

Experimental data (Meeks et al., 2022) for F344 rats (closed circles) and time series from the Campbell et al. (2023) model simulation (lines) for the concentration of D4 in blood/plasma of males (a) and females (b) and fat for males (c) and females (d). Rats were exposed via inhalation to 8,492 mg/m^3 (700 ppm) of D4 (Meeks et al., 2022). The plots were regenerated using the model codes from Campbell et al. (2023).

Simulations of D4 concentrations in plasma and fat using the refined model (Campbell et al., 2023) were compared with observed oral data from male F344 rats in Domoradzki et al. (2017). These simulations were comparable to those obtained from an earlier version of the model Campbell et al. (2017), and the updated model provided a closer prediction of the combined concentrations of D4 and its metabolites in plasma (see Figure 4-3).

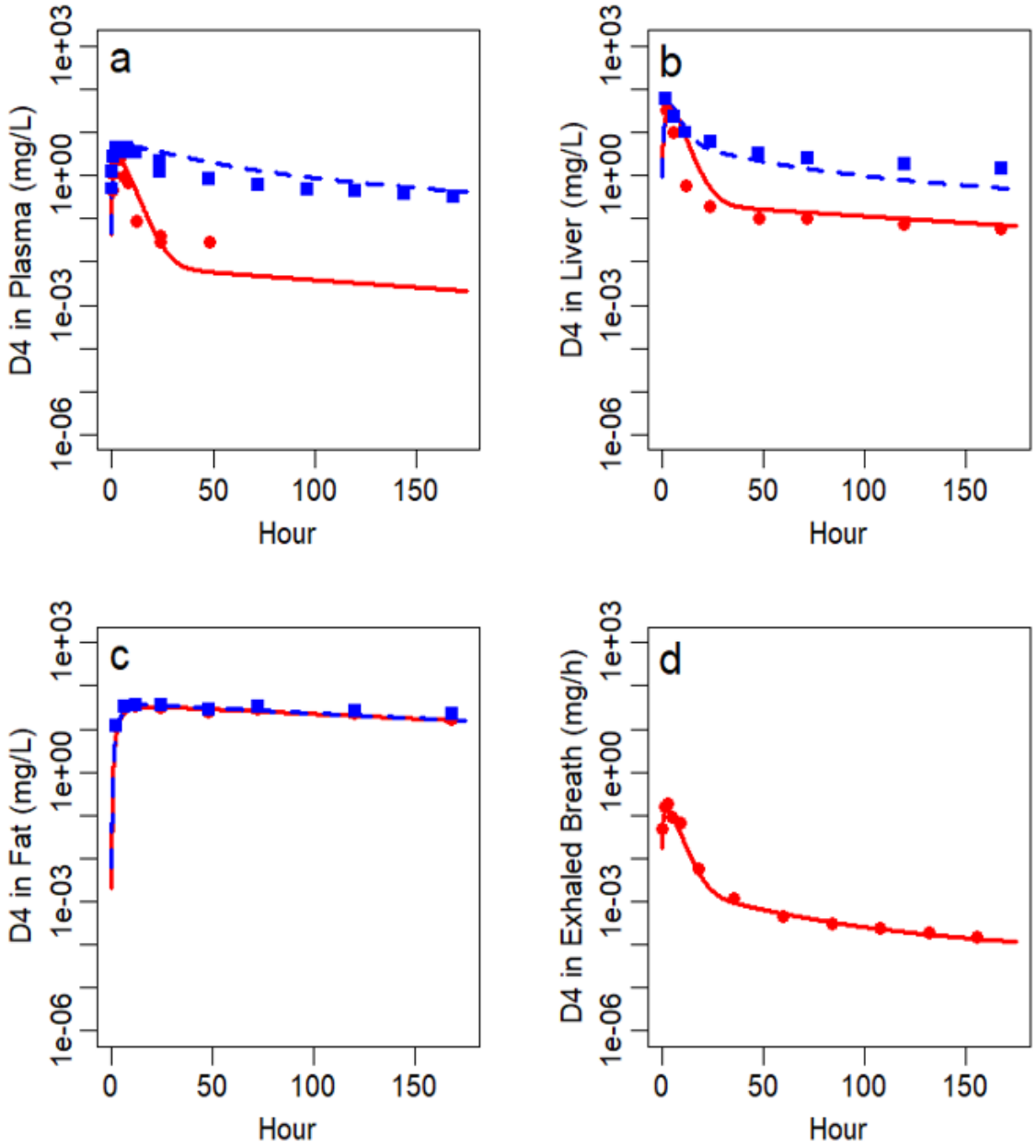

Figure 4-3. Model Simulations Compared with Male F344 Rat Oral Experimental Data

Experimental data (Domoradzki et al., 2017) shown as closed circles or squares and time series from the Campbell et al. (2023) model simulation (lines) for the concentration of D4 in (a) plasma, (b) liver, and (c) fat in male F344 rats after oral administration of 30 mg/kg. The rate of D4 generated in exhaled breath is shown in (d). Red solid lines and circles (red) represent the parent D4 and blue dashed lines and blue squares represent the total concentration of D4 and its metabolites. The plots were generated using the model codes from Campbell et al. (2023) but the plots were not published in Campbell et al. (2023).

The model also reasonably predicted D4 concentrations in exhaled breath and plasma/blood for both inhalation Utell et al. (1998) and dermal (University of Rochester Medical Center, 2001) exposure routes in humans (see Figure 4-4).

The model was also applied to simulate a second human dermal study (Biesterbos et al., 2015), but it underpredicted D4 concentrations in exhaled breath U.S. EPA (2025f).

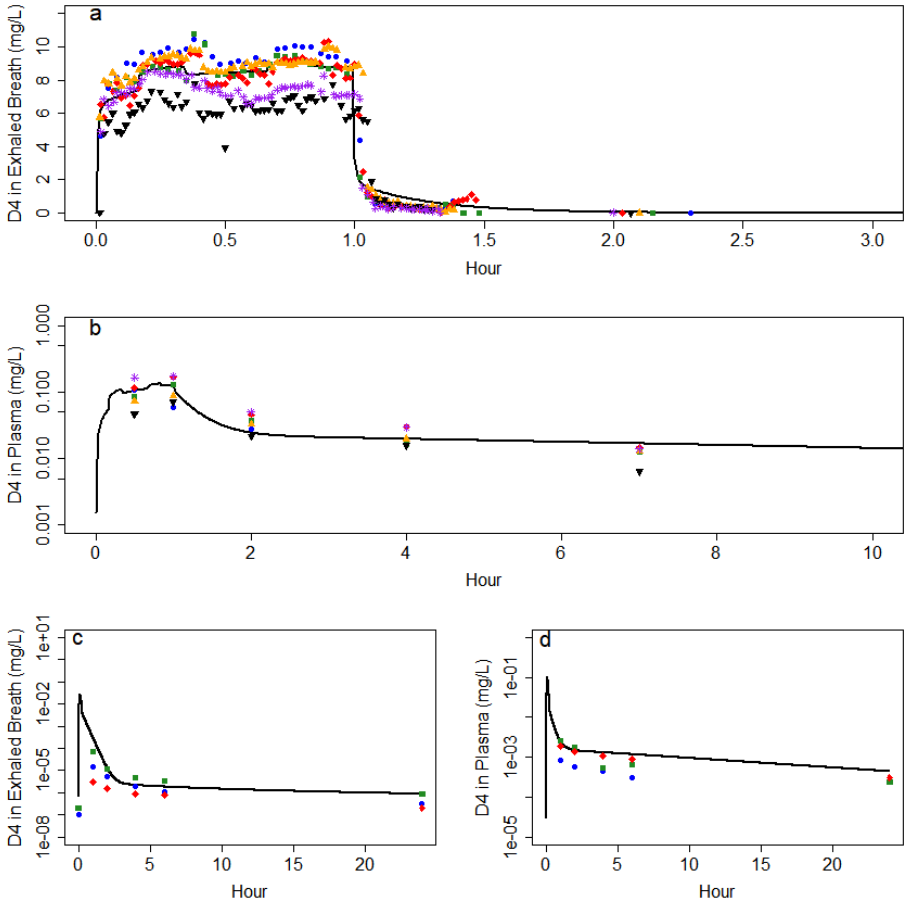

Figure 4-4. Model Simulations Compared with Human Inhalation and Dermal Data

Experimental data (closed shapes) and the Campbell et al. (2023) model simulations of D4 in (a) exhaled breath and (b) plasma of human subjects during and after 10 ppm D4 vapor (Utell et al., 1998) or (c) exhaled breath and (d) plasma during and after a single application of 1.0 or 1.4 g ^{13}C-D4 to skin axilla of women and men, respectively (University of Rochester Medical Center, 2001). The plots are generated from the model codes to reproduce Figure 7 in Campbell et al. (2023).

The only oral exposure data in humans was from a 2-week study (Dow Corning, 1998c). The predicted D4 concentrations in exhaled breath and plasma were similar to the experimental data, as shown in Figure 4-5.

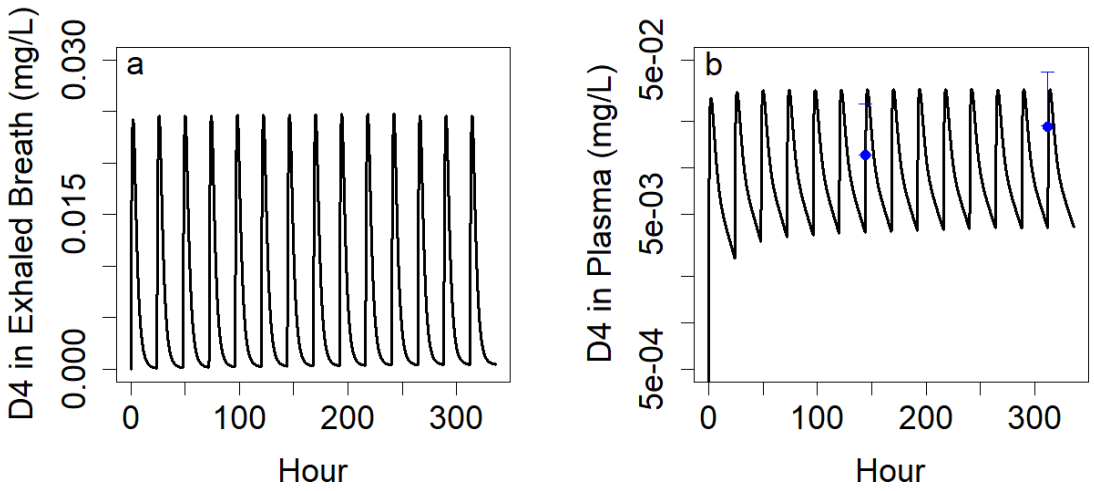

Figure 4-5. Model Simulations Compared with Repeated-Dose Oral Exposure in Humans

The Campbell et al. (2023) model was run with an exposure scenario from Dow Corning (1998c) to simulate the repeated-dose oral exposure in humans (lines) in (a) exhaled breath and (b) plasma of the subjects. Closed circles show weekly average concentrations offset by placebo concentrations from the experimental data presented in Dow Corning (1998c).

EPA conducted sensitivity analyses by varying individual parameters by one percent to determine the effect on model results. EPA calculated sensitivity coefficients as (percent change in output)/(percent change in input). Parameters with sensitivity coefficients greater than 0.5 were considered to have a high influence on outcomes, and parameters with sensitivity coefficients of 0.2 to 0.5 had a moderate influence.

The 2-generation reproductive toxicity study (WIL Research, 2001a), conducted in SD rats, forms the basis of the human health hazard values used to calculate risks from D4. Varying the blood:air coefficient resulted in several high and moderate sensitivity coefficients across tissues, especially blood and fat concentrations. Several other coefficients and blood flows w highly influential for predicted D4 concentrations in fat during the 14-day post exposure period.

In humans, parameters were evaluated for their influence on D4 concentrations in blood and exhaled breath both during and immediately after exposure. For dermal exposure, parameters with moderate and high sensitivity coefficients included absorption into blood from skin, release out of deep skin compartments, dermal surface area, as well as body weight and the blood:air partition coefficient. For inhalation exposure by humans, exercise as a binary variable was highly influential for all D4 concentrations. Varying the blood:air partition coefficient also had high sensitivity coefficients for many model outputs. For oral exposure, alveolar ventilation, cardiac output, liver blood flow, and enzyme induction highly influenced D4 in exhaled breath. The length of oral dose administration had a high influence on all D4 concentrations during and after exposure. Uptake to liver from lipid and blood volume highly influenced D4 concentrations in blood after oral exposure by humans.

See the Draft PBPK Model Description and Review (U.S. EPA, 2025f) for details regarding the sensitivity analyses for SD rats and humans, as well as a sensitivity analysis for F344 rats.

4.2.4 Steps to Estimate HECs and HEDs

Step 1: Estimate Internal Doses Associated with Toxicity Study Air Concentrations

EPA entered the measured air chamber concentrations from the 2-generation reproductive toxicity study (WIL Research, 2001a) as ppm into the PPBK model. These air concentrations reflect exposures by F1 rats for 6 hours per day for 7 days per week (see Table 4-4).

Parameters for SD Rats: The model used parameters specific to SD rats, which were the test species and strain used in WIL Research (2001a), to estimate internal doses. The values are presented, along with the sources of the values, in Table 3 of the Draft PBPK Model Description and Review (U.S. EPA, 2025f).

Rat Body Weight from 2-Generation Toxicity Study: EPA used female body weights for rats because hazard was mediated primarily from exposure of the female parents to D4 as identified in multiple studies. When males were administered D4 in WIL Research (2001a) and mated with unexposed females, no adverse effects were identified. Also, mechanistic evidence was associated with changes in estrous cycles, consistently weak estrogenic effects (ERα), and other effects studied in female rodents. Furthermore, a Hershberger assay that evaluated androgenic effects did not identify agonistic or antagonistic effects of D4 exposure (Quinn et al., 2007b).

Toxicity Study Exposure Duration: One of the PBPK model input parameters was the exposure duration from the toxicity study (WIL Research, 2001a), which allowed the internal D4 concentration estimates in rats to appropriately reflect the amount of time they were exposed (for a given hazard endpoint). The first-generation modeled outcomes were associated with an exposure of 122 days. The hazard endpoints for the second generation (fertility and live litter size) were determined to be more closely aligned with the lower end of the exposure duration range (70 vs. 241 days).

Calculating Rat Blood AUCs: For each of the air concentrations from WIL Research (2001a), EPA ran PBPK model simulations to estimate parent D4 blood AUCs. Although the PBPK model can predict the AUC and C_{max} for fat and liver as well as exhaled D4, EPA considered the AUC in blood to be the most appropriate internal dose metric (see Section 4.2.3 for additional discussion). Table 4-4 presents the AUCs associated with each exposure concentration from the toxicity study. See Draft PBPK Model Results (U.S. EPA, 2025g) for other internal doses.

Table 4-4. Internal Doses (AUC) in Blood Associated with Toxicity Study Exposures

Air Concentration from Toxicity Study[a]		Internal Dose Metric: AUC_{blood} Last 24 Hours (mg/L-h)
ppm	mg/m^3	
F0 Generation		
0	0	0
70	850	4.45
298	3,620	19.95
502	6,090	34.86
700	8,490	49.70
F1 Generation		

Air Concentration from Toxicity Study[a]		Internal Dose Metric: AUC $_{blood}$ Last 24 Hours (mg/L-h)
ppm	mg/m^3	
0	0	0
71	860	4.44
301	3,650	20.21
502	6,090	34.97
702	8,520	50.01

[a] Values were rounded to three significant figures before modeling.

EPA considered the blood AUC associated with the last 24 hours of the simulated time course to be more appropriate than total AUC in blood because the HECs/HEDs are daily concentrations/doses and using the last 24-hour AUC metric allowed for better comparison among different exposure durations (*e.g.*, between durations used in the toxicity study and the assumptions used for humans in the exposure scenarios used in the risk evaluation).

Step 2: Conduct BMD Modeling to Predict Blood AUC BMDLs

EPA used the PBPK-predicted blood AUC values and hazard endpoints from DuPont (1997) to conduct BMD modeling consistent with U.S. EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). See Appendix B for more information on the BMD modeling for these endpoints.

Table 4-5. BMDLs of AUC in Blood for 2-Generation Study Hazard Endpoints

Exposed Generation	Endpoint	PBPK-Predicted AUC$_{blood}$ Last 24 h[a] (mg/L-h)
F0	Live litter size (F1 offspring) 5 % BMR	6.51 [Model: Linear CV] [b]
	No. pups (F1 offspring) 5 % BMR	6.73 [Model: Linear CV]
F1	Live litter size (F2 offspring) 5 % BMR	6.31 [Model: Linear CV]
	Fertility in F1	10.34 [Model: Logistic]

Exposed Generation	Endpoint	PBPK-Predicted AUC_{blood} Last 24 h[a] (mg/L-h)
	adult females 10 % BMR	
	Fertility in F1 adult males 10 % BMR [c]	10.36 [Model: Logistic]

[a] The AUC_{blood}s for NOAECs are 19.95 (F0 exposure) and 20.21 (F1 exposure) mg/L-h.
[b] CV = Constant variance
[c] Male reproductive outcomes were less supported by the weight of the scientific evidence, but EPA presented this endpoint as a comparison with other endpoints.

EPA chose a BMR of 5 percent for measures of live litter size and number of pups due to the severity of the hazard endpoint (association with viability). Use of this BMR also matched the choice of BMR for two previous TSCA risk evaluations (*e.g.*, for live litter size): *Risk Evaluation for 1-Bromopropane* (U.S. EPA, 2020) and *Risk Evaluation for Tris(2-chloroethyl) phosphate* (U.S. EPA, 2024). For the fertility indices, EPA used a BMR of 10 percent extra risk (ER).

EPA selected the internal 24-hour blood AUC $BMDL_5$ of 6.31 mg/L-hour for live litter size in the F2 offspring as the basis for HED and HEC derivation. EPA chose this hazard endpoint based on sensitivity but also because it was based on exposure of the F1 rats and included indirect exposure of young rats to D4 (through lactation). The $BMDL_5$ of 6.31 is based on the model with the lowest AIC and adequate model fit for liver litter size. Of the endpoints modeled using F1 exposures, the model fit based on visual inspection was better for decreased litter size than for the fertility endpoint. See Appendix B for details associated with the benchmark dose modeling.

Step 3: Use PBPK Model to Calculate External HECs and HEDs from $BMDL_5$ Blood AUC

EPA used the human PBPK model to convert the blood AUC $BMDL_5$ estimated in step 2 to relevant HECs and HEDs for acute, intermediate, and chronic exposure scenarios associated with conditions of use (COUs).

Exposure Durations: EPA calculated HECs and HEDs associated with the exposure scenarios used for estimating risks to humans. These exposure durations were acute (= 1 day), intermediate (= 30 days), and chronic (= 40 years as a representative value). HECs and HEDs were lower for longer exposure durations. However, calculating dermal values was computationally burdensome when modeling the 40-year simulation. Therefore, EPA tested the model by running simulations using several different durations to identify when AUCs reached steady state (or pseudo steady state). Only negligible differences were observed among one, two, and five-year dermal simulations and EPA used five years as a reasonable value for model simulations of chronic dermal exposure.

Human Body Weight and Age: To generate HEDs for oral and dermal routes, EPA chose a body weight for reproductive age female humans of 72.4 kilograms, which is a simple average for multiple age

ranges covering 16 to < 50 years from Table 8-5 in the *Exposure Factors Handbook* (U.S. EPA, 2011a). Female human body weights were used for the same reason that female body weights were used for rats given the likelihood that effects were mediated through exposures by females.

Age was entered as a parameter because it influences cardiac output independently of body weight. Simulations were run using the low and high end of the age range associated with 72.4 kg body weight. EPA used the HEDs for the simulations using 49 years, which were slightly more sensitive than using an age of 16 years. See *Draft PBPK Model Results for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025g).

Unit Conversion for Hazard Values: It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in toxicity studies and the default units for different OPPT exposure models. Therefore, EPA presented all inhalation hazard values in both units.[7] The following equation converts air concentrations from ppm to mg/m^3.

Equation 4-1. Converting ppm to mg/m^3 (*e.g.*, for HECs) [8]

$$\text{HEC (mg/m}^3\text{)} = \text{HEC (ppm)} \times (\text{Molecular Weight (g/mol)}/\ 24.45\ \text{(L/mol)})$$

$$\text{HEC (mg/m}^3\text{)} = \text{HEC (ppm)} \times (296.61\ \text{(g/mol)}/\ 24.45\ \text{(L/mol)})$$

Step 4: Adjusting PBPK-Predicted Dermal HED for Occluded Scenarios

The PBPK model used dermal absorption parameters appropriate for unoccluded scenarios. However, for the general population and consumers, EPA also estimated risks scenarios in which D4 contacted skin throughout the duration of the event (*i.e.*, an infinite dose/occluded scenario in which D4 remained in contact with skin and was not depleted). Accounting for the difference between unoccluded and occluded scenarios is important given how rapidly D4 volatilizes.

EPA used the relationship between absorption for an occluded and an unoccluded scenario from an *in vivo* dermal absorption study (GE, 1994a) in female SD rats to adjust the dermal HED for the occluded, infinite scenarios. The differences were 18.61 and 33.91 percent, a ratio of 0.549. Adjusting the unoccluded dermal HED from the PBPK model by the same fraction resulted in HEDs of 216 and 179 mg/kg-bw/day for acute and chronic occluded conditions, respectively.

Using this simple ratio to account for occluded scenarios replaced the dermal absorption parameters of permeability coefficient (Kp) and flux that EPA typically uses for such scenarios to represent infinite doses. EPA considered the simple ratio of percent absorption between occluded and unoccluded scenarios to be preferable because none of the dermal absorption studies had non-depletable doses given D4's high volatility, even though doses applicable for infinite dosing scenarios were used in some dermal absorption studies (*e.g.*, 15.7 mg/cm^2 D4 on skin in the *in vivo* study by University of Rochester Medical Center (2000)).

[7] WIL Research (2001a) presented all air concentrations in ppm. Using Equation 4-1 (and reporting results as significant figures), the second-generation chamber concentrations of 0, 71, 301, 502, and 702 ppm used for the hazard endpoint (decreased live litter size in F2 offspring) were converted to 0, 861, 3650, 6,090, and 8520 mg/m^3.

[8] The Ideal Gas Law can be used to convert between ppm and mg/m^3. At standard temperature and pressure (STP; 25 °C and 760 mm Hg), 1 mole of gas occupies 24.45 L. However, when conditions differ from STP, a different gas conversion factor can be calculated using the reported experimental temperature or pressure. Note that g/L is equivalent to mg/m^3.

Applicability of Model to Lifestages

Pregnant Women: The PBPK model for D4 was not designed to be used for pregnant animals or humans. WIL Research (2001a) administered D4 to both sexes of rats (both F0 and F1) prior to mating, during mating, during gestation, and lactation. Although the full exposure period includes pregnancy, there is solid evidence that the most sensitive lifestage is likely to occur prior to pregnancy (see Section 4.2.1. for more discussion). Therefore, EPA considered the PBPK model appropriate for the chosen hazard endpoint.

Infants and Children: The D4 PBPK model used parameters that are specific for adults. Of the available information and methods to calculate HECs and HEDs, EPA considered the PBPK model to be the preferred method for all age groups because information was lacking on toxicokinetics for children (as well as a toxicity study that doses young rats directly) and the alternative would be to use a default method that relies on route-to-route extrapolation. Therefore, there is no alternative method to the PBPK model for estimating HECs and HEDs *specifically* for infants and children.

There is uncertainty in applying the model to younger age groups. In several ways, infants have a lower metabolic capacity than adults. For example, metabolism through cytochrome P450 and UDP glucronosyltransferase enzyme pathways in infants are lower than adult levels (Anderson, 2010). In addition, none of the D4 toxicokinetics studies were performed in very young animals. Given the complexity of D4's toxicokinetic profile, EPA cannot estimate how parameters such as liver metabolism rates, enzyme induction rate, mobile lipid pool parameters, and mass transfer parameters for deep compartments would differ for infants and children.

Given this uncertainty in using the PBPK model values for children, EPA compared the PBPK model predictions for HECs and HEDs with toxicity values estimated using default methods that involved BMD modeling of air concentrations directly from the 2-generation inhalation toxicity study and route-to-route extrapolation to dermal and oral routes. Most of the PBPK model-predicted HECs and HEDs were more sensitive than default HECs and HEDs. The only exception was the acute oral HED, which was about two times higher using the PBPK model compared with the default approach. Appendix D presents the default approach and comparison with the PBPK model outputs.

Benchmark MOEs/Uncertainty Factors

In typical risk assessments, PODs are derived directly from laboratory animal studies and inter and intra-species extrapolation is accomplished by use of 10X factors. The Agency's 2014 Data-Derived Extrapolation Factors (DDEF) guidance[9] allows for the separation of standard inter- and intra-species extrapolation factors into PK and PD components. In the case of D4, PBPK modeling is being used as a data-derived approach to estimate PODs based on differences in PK between laboratory animals and humans. Thus, PK differences between rats and humans are accounted for with human equivalent PODs which alleviates the need for the PK portion of the interspecies factor. Since the D4 PBPK model does not address the pharmacodynamic component of intraspecies extrapolation, a factor of 3X was retained.

Similarly, the PBPK model does not account for within human variability; thus the 10X intra-species is being used. Therefore, as shown in Table 4-6, the total UF is 30X (3X for interspecies and 10X for intraspecies variability of 30 for the benchmark MOEs for acute, intermediate, and chronic exposure durations.

[9] https://www.epa.gov/risk/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies

4.2.5 Model-Predicted HECs and HEDs for Acute, Intermediate, and Chronic Durations

Table 4-6 presents the external HECs and HEDs for each exposure duration considered for the COUs and exposure scenarios evaluated in the D4 risk evaluation. The values for intermediate and chronic durations were very similar. Therefore, for simplicity, EPA used the chronic values for both the intermediate and chronic exposure scenarios, and therefore, the intermediate column in Table 4-6 is grayed out.

Table 4-6. D4 HECs/HEDs from the PBPK Model and HEDs Adjusted for Dermal Occlusion

Exposure Route	Units	Acute (1 day)	Intermediate (30 days)	Chronic (steady state)	Benchmark MOE
Inhalation	mg/m^3	107	56.7	55.8	30 [$UF_A = 3$ $UF_H = 10$]
	ppm	8.82	4.67	4.60	
Oral	mg/kg-bw/day	8.93	3.67	3.60	
Dermal (unoccluded)		394	329	326	
Dermal (occluded)		216	181	179	

5 CANCER HAZARD ASSESSMENT

The sections below outline animal evidence (Section 5.1.1) and introduce mechanistic evidence (Section 5.2) for carcinogenicity. Section 5.2.2 presents the cancer classification and summary of evidence integration conclusions. The genotoxicity data are provided in Section 5.2, and possible modes of action (MOA) are described in Section 5.2.1.1.3.

For complete details on the evidence for cancer, see the evidence profile table organized by cancer type (Table_Apx A-5) in Appendix A. Full details on all evaluated health outcomes from the key cancer bioassay and selected studies used for mechanistic evidence are in Draft Data Quality Evaluation for Human Health Hazard Animal Toxicology (U.S. EPA, 2025c) and Draft Data Extraction for Environmental and Human Health (U.S. EPA, 2025a).

Previous assessments have summarized human health hazards of D4. None of the previous assessments have identified sufficient evidence to support quantitative cancer risk assessment. Specific characterization of the available evidence and the potential relevance of the mode of action for humans varies across assessments as follows:

- Environment Canada and Health Canada's *Screening Assessment for the Challenge: Octamethylcyclotetrasiloxane (D4): CASRN 556-67-2* (EC/HC, 2008) acknowledged evidence of increased incidence of uterine adenomas in rats exposed to D4 at the highest dose in a 2-year cancer bioassay. The assessment stated that the lack of human relevance of uterine tumors suggested by SEHSC had not been thoroughly analyzed in a mode-of-action analysis and was not supported by international reviews. (EC/HC, 2008) also stated that the relevance to humans of the mononuclear cell leukemia (MNCL) was unknown because it is unique to rats and common only in F344 rats. Tumor data were not used quantitively in the Canadian screening assessment.

- The European Commission's SCCS, in their Opinion on Cyclomethicone [10](SCCS, 2010) essentially provided the same conclusion as EC/HC (2008) that support was lacking with which to dismiss the uterine tumors as relevant to human health but there was questionable relevance of MNCL to humans.
- The UK's Environment Agency *Environmental Risk Assessment Report: Octamethylcyclotetrasiloxane (D4; CASRN: 556-67-2)* (Brooke et al., 2009) cited an industry assessment from 2005 indicating that the uterine endometrial tumors were associated with D4's action as a dopamine agonist and subsequent actions: inhibition of prolactin secretion; delayed female reproductive senescence; and prolonged stimulation of the endometrium that eventually leads to tumors. Brooke et al. (2009) also stated that the mechanism was not relevant to humans due to differences in the reproductive aging process between rodents and humans.
- SEHSC's request for risk evaluation of D4 under TSCA (SEHSC, 2020) discussed various possible mechanisms for the uterine tumors and indicated that a direct interaction with the dopamine receptor was not likely. The submission described multiple investigations into other mechanisms, including non-specific mechanisms and secondary effects associated with toxicity resulting from the high concentration (possibly stress responses related to aerosol exposure) at which tumors were observed. Thus, although no conclusion was reached within the SEHSC submission regarding a mode of action, the authors suggested that the effects were not relevant at lower concentrations that would be encountered by humans (SEHSC, 2020).

5.1 Hazard Identification

EPA did not locate any human epidemiological studies that examined tumor incidence and located one 24-month combined chronic toxicity and oncogenicity bioassay in male and female F344 rats (Jean and Plotzke, 2017).[11] EPA identified mechanistic evidence, which is introduced below but is described in more detail in Section 5.2: Mode of Action Considerations. Much of the mechanistic evidence was also described in 4.1.3.3 as supporting evidence for the observed reproductive effects.

5.1.1 Laboratory Animal Studies

EPA located one combined chronic toxicity and cancer bioassay in which male and female F344 rats were exposed to D4 vapor (whole body) at 0, 121, 364, 1,820, and 8,492 mg/m^3 (0, 10, 30, 150, or 700 ppm)[12] for 6 hours per day, 5 days per week (Jean and Plotzke, 2017). Animals were assessed for toxicity in three main groups: 12-month exposure; 12-month exposure with 12-month recovery; or 24-month exposure. Another group was exposed for six months and levels of D4 were evaluated in various tissues.

Despite a few limitations (*e.g.*, lack of information on whether experimenters were 'blinded' to the study groups dose regimen and a lack of data on food consumption or respiratory rates), EPA assigned high overall quality determinations for all three parts of the study based on high-quality methods that followed established guidelines (*e.g.*, chemicals analytically measured, animals randomized to dose groups, and appropriate exposure durations), full reporting of results that included individual animal data, and other factors.

[10] Cyclomethicone is a generic name that can refer to multiple cyclic dimethyl polysiloxane compounds. SCCS (2010) gives opinions for both D4 and decamethylcyclopentasiloxane (D5).
[11] EPA evaluated Jean and Plotzke (2017) as the primary published journal article of the 24-month combined chronic toxicity and oncogenicity study because it is the most comprehensive presentation of the study. EPA also consulted other unpublished references with individual data from the study: Dow Corning (2004), Battelle PNL (2004a), and Battelle PNL (2004b).
[12] Values were converted from ppm to mg/m^3 using the following equation: X mg/mg^3 = (Y ppm*296.61)/24.45, where 296.61 is the molecular weight for D4 and 24.45 is the number of liters occupied by 1 mole of gas at standard temperature and pressure (STP; 25°C and 760 mm Hg).

The authors chose 8,492 mg/m^3 (700 ppm) as the highest D4 concentration that could be achieved without generating appreciable aerosol or condensing on chamber surfaces. Jean and Plotzke (2017) indicated that aerosol generation did not occur in the chambers.

All rats survived to their 6- and 12-month scheduled sacrifices (Jean and Plotzke, 2017). Control group survival in the 24-month exposure group was 58 and 72 percent in males and females, respectively. Male and female controls in the 12-month exposure/12-month recovery group had 60 and 55 percent survival rates, respectively. D4 exposed groups had greater than 50 percent survival, except for males exposed for 24 months at 8,492 mg/m^3, which had a 38 percent survival, and this was the only statistically significant decrease among all groups (Jean and Plotzke, 2017). Table 5 in Jean and Plotzke (2017) showed that survival for females was 72, 62, 72, 75, and 58 percent at 0, 121, 364, 1,820, and 8,492 mg/m^3, respectively; although the highest concentration showed somewhat lower survival, the dose-response was not clear, and the difference was not statistically significant.

Jean and Plotzke (2017) stated that D4 tissue levels were higher in females than males at each exposure concentration and most tissue types. Oral and inhalation toxicokinetics studies in F344 and SD rats showed females had higher concentrations of D4 and metabolites in fat and several other tissues (Meeks et al., 2022; Domoradzki et al., 2017; Plotzke et al., 2000). Also, in F344 rats exposed via inhalation, higher levels were most often observed in female reproductive tissues (ovaries, uterus, vagina) than in testes, although levels in the uterus were only slightly higher than those in the testes (Plotzke et al., 2000).

Jean and Plotzke (2017) identified clinical signs that included statistically significant increases in white blood cells, specifically leukocytes ($p < 0.05$ or 0.01). The increases were consistently observed at the highest concentration but also occurred at lower concentrations. Both sexes exhibited increased total protein, and decreased creatinine, alkaline phosphatase and creatine kinase (Jean and Plotzke, 2017).

Increased kidney weights at the highest concentration correlated with increased severity of chronic nephropathy. There were no increases in incidence in most groups, except for females exposed for 24 months, in which a statistically significant dose-response trend with pairwise changes starting at 364 mg/m^3 and higher ($p < 0.01$ or 0.05) was observed. Liver weights were increased starting at 6 months, with increased absolute liver weight in males at $\geq$ 364 mg/m^3 ($p < 0.01$ or 0.05, depending on concentration) and females at 8,492 mg/m^3 ($p < 0.01$). Relative liver weight values were not reported at the 6-month time point. At 24 months, males had increased absolute liver weights and relative weights (when compared with both body and brain weight) at the highest concentration ($p < 0.01$)., and females had increases in all liver weight measures at both 1,820 and 8,492 mg/m^3 ($p < 0.01$). Uterine and testes weight changes identified by Jean and Plotzke (2017) are described below in Sections 5.1.1.1 and 5.1.1.2 respectively.

Non-neoplastic histopathological changes with statistically significant trends and/or pair-wise comparisons occurred in the nasal cavity, lung, kidney, liver, spleen, eye, uterus, and testes (Jean and Plotzke, 2017).In the nasal cavity, males and females exhibited statistically significant increasing dose-response trends ($p < 0.05$) in eosinophilic globules and globlet cell hyperplasia at 12 and 24 months. For both measures, Jean and Plotzke (2017) also identified pairwise differences ($p < 0.05$ or $p < 0.01$) between 8,492 mg/m^3 and controls at both time points and in both sexes. Males showed additional pairwise increases in goblet cell hyperplasia after 24 months at 1,820 mg/m^3 ($p < 0.05$) and in the 12-month exposure/12-month recovery group at the highest concentration ($p < 0.05$). Additional pairwise

differences in females were seen for increased eosinophilic globules after 24 months at lower concentrations – 364 mg/m^3 ($p < 0.01$) and 1,820 mg/m^3 ($p < 0.05$).

Also in the nasal cavity, in the 12-month evaluation, males and females exhibited increased dose-response trends in squamous epithelium hyperplasia ($p < 0.05$) and pairwise differences from controls for females ($p < 0.05$) and males ($p < 0.01$) at 8,492 mg/m^3. Males showed similar responses after 24 months (with statistical significance reaching only < 0.05 at the highest concentration) but the change was not statistically significantly for females at 24 months although there was an increased incidence at the highest concentration (4 of 60) compared with controls (1 of 59). Males had suppurative inflammation (dose trend at $p < 0.05$ and pairwise difference at 8,492 mg/m^3 at $p < 0.01$) after 12 months but not at 24 months or in the recovery group. Females did not exhibit statistically significant differences in suppurative inflammation. Males exhibited a statistically significant increase in foreign bodies in the nasal lumen at 24 months (increasing dose trend and pairwise difference at 8,492 mg/m^3; $p < 0.05$) but this pattern was not seen in females (Jean and Plotzke, 2017).

After 24 months exposure, males and females exhibited a positive dose-response trend in lung hemorrhage ($p < 0.05$) with a pairwise difference ($p < 0.05$) at 8,492 mg/m^3 compared with controls for females. After 24 months, females also exhibited subpleural chronic inflammation with dose-response trend ($p < 0.05$) and all concentrations different from controls ($p < 0.05$ or 0.01) except at 1,820 mg/m^3.

Females exhibited nephropathy in the kidney, with an increasing dose-response trend ($p < 0.05$) and pairwise differences at all concentrations except the lowest ($p < 0.01$ or 0.05).

Males exhibited increased centrilobular hypertrophy in the liver that included a trend ($p < 0.05$) and pairwise increases at 8,492 mg/m^3 ($p < 0.01$ or 0.05) after both 12 and 24 months.

Females experienced an increase in the incidence of hematopoietic proliferation in the spleen showing a dose-response trend and a pairwise difference from controls at the highest concentration (both $p < 0.05$).

Non-neoplastic histopathological changes with statistically significant trends and/or pair-wise comparisons occurred in the eye that was not further described (Jean and Plotzke, 2017).

5.1.1.1 Female Reproductive Tumors

At 6 or 12 months, no uterine changes other than non-statistically significant increases in uterine weight were observed. At 24 months, the highest D4 concentration (8,492 mg/m^3) was associated with endometrial adenomas, histiocytic sarcomas, and an increase in cystic endometrial epithelial hyperplasia. Table 5-1 reports the percent incidence of tumors and hyperplasia, calculated from values reported by Jean and Plotzke (2017). The incidence was based on all examined tissues (whether the animal survived or died) for all lesions. Table 5-1 also provides incidence among surviving females for endometrial adenomas because these were observed only in animals that survived.

Among all exposed female rats that started the study (n = 60), incidence was 7 and 3 percent for endometrial epithelial adenomas and histiocytic sarcomas, respectively. Both tumors exhibited statistically significant dose-response trends ($p < 0.05$) as presented by Jean and Plotzke (2017). For both tumors, however, tumors were observed only at the highest concentration and thus, limited information was available for assessing trends. Among animals that survived, the increased incidence of endometrial adenomas was 11 percent. No uterine tumors were observed in controls.

Endometrial epithelial hyperplasia was also increased at the highest concentration — 31 percent higher than controls among all animals. The hyperplasia was identified by variable sizes of cystically dilated endometrial glands. The dilated glands were lined by low cuboidal epithelial cells and some columnar cells. Also, some of the dilated glands contained "weakly stained secretory material." Endometrial stroma contained increased collagen, and some glands contained infiltrated neutrophils (Jean and Plotzke, 2017). The endometrial hyperplasia and adenomas were described as a hyperplastic/neoplastic response in a final unpublished report of the study (Dow Corning, 2004), which showed that the severity of hyperplasia increased from 1.7 in controls to 2.5 at the highest concentration. A study of aging female F344 rats exposed to 8,540 mg/m^3 (704 ppm) D4 for 6 hours per day from 11 to 24 months of age (Jean et al., 2017) also identified an elevated incidence of cystic endometrial hyperplasia (14/35 for the D4 group vs. 8/37 for controls).

Jean and Plotzke (2017) reported a statistically significant dose-response trend of increased uterine histiocytic sarcoma incidence. There was a 3.33 percent incidence at the highest concentration, and no sarcomas were observed at lower concentrations. Histiocytic sarcoma is a rare neoplasm in which the malignant cells have a lineage consistent with histiocytes (immune cells) (Elsevier, 2025).

In addition to the neoplastic and pre-neoplastic changes shown in Table 5-1, other uterine neoplastic and pre-neoplastic changes were observed.

In the recovery group (12 months exposure and 12 months recovery), rats at all D4 concentrations had higher incidence of stromal polyps in uterine connective tissue (by 15 or 25 percent) compared with controls ($p < 0.05$). However, no stromal sarcomas were observed at the highest concentration (Jean and Plotzke, 2017) after recovery. In comparison, no statistically significant increases in incidence of stromal polyps or sarcomas occurred in the 24-month exposure group — only slightly (non-significant) higher incidences at higher concentrations without any dose-response trends. In an associated original report of the 2-year cancer bioassay, historical control incidence of stromal polyps was identified as 17 percent, with 0.3 percent incidence for stromal sarcomas (Battelle PNL, 2004a).

Jean and Plotzke (2017) reported increased squamous epithelial cell hyperplasia of the cervix at the highest concentration compared with controls after 24 months exposure and in the recovery group ($p < 0.05$) but there were no increased neoplastic changes in the cervix.

Table 5-1. Percent Incidence of Female Reproductive Lesions in F344 Rats After 24 Months Exposure

Concentration (mg/m^3)	Number of Females		Uterine Endometrium			Uterine Histiocytes
	Dosed	Surviving[a]	Endometrial Epithelial Hyperplasia (All Dosed Females) [b c d]	Endometrial Adenomas		Histiocytic Sarcomas (All Dosed Females)[c d]
				All Dosed Females [c d]	Surviving Females[f g]	
0	59	43	18.6	0	0	0
121	59	37	13.6	0	0	0
364	59	43	8.47	0	0	0
1,820	60	45	21.7	0	0	0
8,492	60	35	50.0 [d]	6.67	11.4	3.33

[a] Survival from Table III-2 in Battelle PNL (2004a); percent incidence is mostly provided only for all dosed animals because it is not clear whether the study counted tumors among animals that died. The exception is that the study stated that endometrial adenomas were found only in the surviving animals.

[b] Also identified as uterine cystic endometrial hyperplasia[c] Significant dose-response trend ($p < 0.05$)

[d] Calculated from incidence values in Jean and Plotzke (2017): Table 20 for hyperplasia and Table 24 for adenomas and sarcomas [e] Statistically different from controls ($p < 0.05$)

[f] Calculated from survival values (Table III-2 in Battelle PNL (2004a)) and adenoma incidence values in Table 24 in Jean and Plotzke (2017)

[g] Statistics not calculated

Mean uterine weight was statistically higher than controls at 8,492 mg/m^3 after 24 months exposure — by 46.5 and 53.5 percent for absolute and relative to body weight, respectively ($p < 0.05$). At the earlier timepoint of 12 months, uterine weight increased across the D4 exposures of 364 to 8,492 mg/m^3 but was not statistically different for trend or by pairwise comparison with controls at $p = 0.05$.

F344 rats have been shown to have low historical control incidence of uterine adenomas. Therefore, Jean and Plotzke (2017) concluded that the increases in endometrial hyperplasia and adenomas and increased uterine weight are associated with D4 exposure. The authors did not discuss the significance of the observed histiocytic sarcomas.

No increases in neoplasms were observed in the mammary gland, ovary, vagina, or pituitary gland (Jean and Plotzke, 2017). Other non-uterine pre-neoplastic effects of the female reproductive system observed by Jean and Plotzke (2017) included a statistically significant increase in ovarian atrophy after 24 months exposure ($p < 0.05$).

5.1.1.2 Testicular Tumors

Table 5-2 presents incidence of interstitial (Leydig) cell lesions, including hyperplasia and unilateral and bilateral adenomas. Male rats showed a positive dose-related trend for increased incidence of bilateral adenomas after 24 months exposure but no increased pairwise comparisons with controls. At 24 months and in the recovery group, the incidence of unilateral tumors did not increase and was somewhat lower than controls in the exposed male rats (Jean and Plotzke, 2017). Jean and Plotzke (2017) indicated that the increase in bilateral adenomas was not clear but may have indicated a shift from unilateral to bilateral adenomas. Pairwise comparisons showed increased incidence of Leydig cell hyperplasia at 24 months at 1,820 ($p < 0.05$) and 8,492 mg/m^3 ($p < 0.01$) compared with controls. Severity of hyperplasia also increased with D4 concentration.

Table 5-2. Percent Incidence of Testicular Interstitial (Leydig) Cell Lesions in F344 Rats After 24 Months Exposure

Concentration (mg/m^3)	Number of Males		Hyperplasia[b]	Adenomas, Unilateral [b, e]	Adenomas, Bilateral [b, f]
	Dosed	Surviving[a]			
0	60	35	11.7	18.3	70.0
121	60	36	20.0	18.3	66.7
364	60	35	15.0	6.67	73.3
1,820	60	35	21.7 c[bc]	15.0	68.3
8,492	60	23	26.7 [cd]	8.33	80.0

[a] Survival from Table III-1 in Battelle PNL (2004a); provided as comparison with number of all dosed males; incidence was calculated for ma

[b] Calculated from incidence values in Table 21 of Jean and Plotzke (2017) and provided for all dosed males because it is not clear whether incidence was inclusive of effects observed in males that died early.

[c] Statistically different from controls ($p < 0.05$)

Concentration (mg/m^3)	Number of Males		Hyperplasia[b]	Adenomas, Unilateral [b, e]	Adenomas, Bilateral [b, f]
	Dosed	Surviving[a]			

[d] Statistically different from controls ($p < 0.01$) [e] Described in Jean and Plotzke (2017) as interstitial cell adenomas, but presumed to be only unilateral adenomas (not the total of unilateral plus bilateral adenomas) based on the incidence numbers. [f] Significant dose-response trend ($p < 0.05$)

At 8,492 mg/m^3, males exhibited increased testes weights after 12 and 24 months of exposure. The relative organ-to-body weight basis was statistically significant ($p < 0.05$) but increases were also seen in absolute weight. In the recovery group, testes weight increases were statistically significantly higher at 8,492 mg/m^3 than controls based on all measures (absolute, relative to body weight, relative to brain weight) ($p < 0.05$) (Jean and Plotzke, 2017).

F344 rats have a high spontaneous background rate of Leydig cell tumors that range from 86 to 87 percent (Cook et al., 1999). This can make it difficult to detect treatment-related increases in this tumor type in this strain of rat. The incidences at all D4 exposure levels were within the range of spontaneous tumors for this strain (Jean and Plotzke, 2017).

5.1.1.3 Hematopoietic Tumors

High rates of MNCL were reported in both control and treated male and female rats after 24 months. There were no significant dose-related trends for increasing MNCL with D4 concentrations or differences between D4 exposed rats and concurrent controls using pairwise comparisons in rats exposed to D4 after 24 months of exposure. Although males exhibited higher mortality at 8,492 mg/m^3 due to MNCL than controls between 12 and 24 months, there was no treatment-related increase in overall incidence of MNCL among males or females (Jean and Plotzke, 2017). Exposure at 8,492 mg/m^3 in males may have been associated with progression of MNCL.

In a related effect, females exhibited an increased incidence of hematopoietic proliferation in the spleen at 24 months at 8,492 mg/m^3 ($p < 0.05$ for trend and pairwise difference). Males exhibited an increased incidence at 364 mg/m^3 but not at higher concentrations.

As noted above, significant increases in white blood cells, specifically leukocytes ($p < 0.05$ or 0.01), were seen consistently at the highest concentration but also occurred at lower concentrations; increases occurred at 3, 6, and 12 months. The authors speculated that these changes might have contributed to an increased progression to MNCL in males that led to increased mortality at the highest concentration (Jean and Plotzke, 2017).

MNCL is a spontaneously occurring neoplasm of the hematopoietic system that reduces the lifespan and is one of the most common tumor types that occurs at a high background rate in the F344 strain of rat. It is also referred to as Fisher rat leukemia because it is so common. Historical control data from NTP demonstrated an increase in the spontaneous background incidence of MNCL in untreated male and female F344 rats from 7.9 and 2.1 percent in males and females, respectively, in 1971 to 52.5 and 24.2 percent in males and females, respectively, from 1995 through 1998 (Thomas et al., 2007).

5.2 Mode of Action Considerations

EPA considered available mechanistic information for cancer. This section focuses on mechanistic evidence for uterine tumors, for which animal evidence is slight. This section does not further consider mechanistic data for other tumor types (testicular and hematopoietic tumors), for which animal evidence is indeterminate.

Several review papers (Dekant et al., 2017; Franzen et al., 2017; Gentry et al., 2017) considered possible MOAs but concluded that an MOA was not well defined or clearly confirmed for uterine tumors associated with D4.

Environment Canada and Health Canada's Screening Assessment of D4 (EC/HC, 2008) and EC's SCCS's Opinion on Cyclomethicone (SCCS, 2010) both suggested that the uterine tumors had not been thoroughly analyzed in an MOA analysis and support was lacking to dismiss them as relevant for humans. Alternately, the UK Environment Agency's Environmental Risk Assessment of D4 (Brooke et al., 2009) had suggested that the uterine endometrial tumors were associated with D4's action as a dopamine agonist and not relevant to humans due to differences in the reproductive aging process between rodents and humans.

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) defines MOA as "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes and resulting in cancer formation" and provides a framework for conducting MOA analysis. For D4, EPA did not perform a complete MOA analysis as described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) because there are no completed proposed MOAs with sufficient detail or supporting evidence and EPA is not conducting a quantitative cancer assessment. Instead, EPA considered available mechanistic evidence that may support interpretation of the observed increases in endometrial hyperplasia and adenomas associated with D4 inhalation in rats identified by Jean and Plotzke (2017).

In this section, EPA considered available evidence on genotoxicity and mutagenicity endpoints (Section 5.2.1) and evidence for a set of mechanisms related to hormonal regulation (Section 5.2.1.2). Overall, although there are a variety of associated mechanistic effects of D4 that may contribute to uterine tumors, a single MOA is not well understood or supported.

5.2.1 Genotoxicity and Mutagenicity

This section summarizes the database of studies on chromosomal aberrations, gene mutations, and other genotoxicity endpoints for D4. Although EPA did not evaluate these studies using formal data quality criteria, studies were reviewed by comparing against current OECD or EPA test guidelines and important deviations are noted below. When interpreting the results of these studies, EPA also consulted OECD's *Overview of the Set of OECD Genetic Toxicology Test Guidelines and Updates Performed in 2014-2015* (OECD, 2017).

EPA did not identify *in vivo* studies that evaluated any of the following relevant effects specifically in uterine tissue, the target of tumors that may be caused by D4: (1) oncogene or tumor suppressor gene mutations, (2) other gene mutations and chromosomal aberrations, (3) DNA adducts, or (4) DNA damage. EPA did identify two *in vivo* studies in other tissues – a study that was negative for chromosomal aberrations in bone marrow of rats after inhaling D4 for five days (Vergnes et al., 2000) and a study negative for dominant lethal effects after parental male rats were exposed to D4 via oral gavage for eight weeks and mated with unexposed females (Dow Corning, 1982). The Agency did not

identify any additional *in vivo* studies that evaluated DNA damage, DNA adducts, or other measures of DNA damage or repair in surrogate tissues.

EPA also did not locate any *in vitro* studies of genotoxicity in uterine endometrial tissues. *In vitro* assays using other tissues exposed to D4 provided no evidence for chromosomal aberrations in mammalian cells or gene mutations in mammalian cells or bacteria. Some *in vitro* assays resulted in sister chromatid exchanges (SCEs) and DNA damage, and one test showed D4 resulted in decreases in DNA repair proteins. None of the *in vitro* assays described whether measures were taken to control the volatility of D4, resulting in uncertainty in the conclusions from the *in vitro* assays.

Genetic toxicology studies can be divided into two overall types: 1) mutagenicity studies, which measure direct, irreversible damage to DNA that is transmissible to the next cell generation; and 2) genotoxicity studies, which measure early, potentially reversible effects to DNA, or the effect of mechanisms involved in the preservation of the integrity of the genome (OECD, 2017).

OECD (2017) stated that mutagenicity studies can measure: "1) changes in a single base pair; partial, single or multiple genes; or chromosomes; 2) breaks in chromosomes that result in the stable (transmissible) deletion, duplication or rearrangement of chromosome segments; 3) a change (gain or loss) in chromosome number (*i.e.* aneuploidy) resulting in cells that have not an exact multiple of the haploid number; and, 4) DNA changes resulting from mitotic recombination" (p. 12). The mutagenicity studies for D4 are divided into two sections below: Studies of chromosomal aberrations (*e.g.*, breaks, changes in number) in Section 5.2.1.1; and gene mutation studies (*e.g.*, single base pair changes) in Section 5.2.1.1.1.

Tests of mutagenicity are within the broader term "genotoxicity," which also includes "DNA damage which may be mutagenic but may also be reversed by DNA repair or other cellular processes, and, thus, which may or may not result in permanent alterations in the structure or information content in a surviving cell or its progeny" (OECD, 2017)\, p. 12). Section 5.2.1.1.2 describes tests that measured sister chromatid exchanges (SCEs), DNA damage, and dominant lethal effects from D4 exposure.

5.2.1.1 Chromosomal Aberrations

EPA identified an *in vivo* and an *in vitro* study of chromosomal aberrations. Vergnes et al. (2000) found no increase in chromosomal aberrations in bone marrow of rats exposed via inhalation to 8,492 mg/m^3 (700 ppm)[13] D4 for six hours per day for five days. The authors referred to multiple test guidelines as guidance for conducting this test. EPA found that the methods generally adhered to the current OECD Test Guideline (TG) 475 (OECD, 2016a), except that a total of 100 metaphases per animal were scored (compared with 200 recommended by OECD TG 475) and only 500 cells vs. the recommended 1000 cells were evaluated for mitotic indices. These limitations are not expected to significantly impact the conclusions of the test.

Vergnes et al. (2000) also evaluated chromosomal aberrations *in vitro* using Chinese hamster ovary (CHO) cells. The authors cited OECD and EPA guidelines on cytogenetic testing *in vitro* as well as other methods. Cytotoxicity was observed at $\geq$ 0.01 mg/mL without S9 and $\geq$ 0.10 mg/mL with S9, and the authors selected 0.01 mg/mL without S9 and 0.03 mg/mL with S9 as the maximum concentrations for testing. Vergnes et al. (2000) found no statistically significant increases in mean percent aberrant cells either with or without metabolic activation.

[13] Conversion from ppm to mg/m3: (700 ppm * 296.61 g/mol)/(24.45 L/mol)

OECD TG 473 (OECD, 2016b) indicates that the solvent (ethanol) used by Vergnes et al. (2000) is not a well-established solvent and data should be included to indicate compatibility with the test chemical. The amount of ethanol should be one percent or less; however, Vergnes et al. (2000) did not state the percent used. The positive control without activation was triethylenemelamine (TEM; CASRN No. 51-18-3), which is not one of the compounds listed by OECD TG 473. However, the guideline does indicate that other positive controls are acceptable.

In the main assay of chromosomal aberrations, only 200 metaphases were scored (100 for each duplicate culture) (Vergnes et al., 2000) compared with 300 recommended by OECD TG 473. The authors did not include a longer exposure duration than 4 hours for the experiment without metabolic activation but the OECD TG 473 states that a longer duration of exposure to D4 is also needed without metabolic activation. Vergnes et al. (2000) also noted that they used a harvest time without activation to keep the cells within one cell cycle from the start of treatment, but OECD TG 473 recommends that the sampling time should include 1.5 cell cycles after the beginning of treatment. Finally, even though OECD TG 473 states that methods should be used to accommodate volatile chemicals (such as the use of sealed culture vessels), the authors did not describe any measures to control for volatilization. The sum of these deviations suggest that the conclusions of this test were not thorough enough to indicate that the results were negative (as noted specifically in OECD TG 473), and the assay could have been significantly compromised.

In an *in vitro* study using mouse lymphoma cells (Isquith et al., 1988; Litton Bionetics, 1978), there was an increase in chromosomal aberrations (12.5 percent) over controls (0 percent). The aberrations occurred at the highest exposure and were based on analysis of a small number of cells (8 vs. 50) due to cytotoxicity. The authors did not reference an OECD or EPA test guideline. EPA concluded that the study methods generally followed OECD TG 490 (OECD, 2016d) but the assay did not describe any measures to control for volatilization. If such measures were not used, the assay could have been significantly compromised.

Many of the *in vitro* tests used ethanol as the vehicle in which D4 was dissolved. Ethanol is a polar solvent, although it is less polar than water, meaning D4 is likely to have a greater affinity to remain in ethanol compared to water. However, even with this likely greater affinity and D4's higher solubility in ethanol, D4 has an extremely large Henry's Law constant and a high vapor pressure. Therefore, D4 is expected to volatilize readily from open solutions in ethanol, although it would volatilize more slowly from ethanol than from water.

5.2.1.1.1 Gene Mutations

EPA identified two studies of gene mutations in mammalian cells, two reverse mutation assays in bacteria, and one study of gene mutations in yeast for D4 (Vergnes et al., 2000; Felix et al., 1998; Isquith et al., 1988; Litton Bionetics, 1978).

No increases in point mutations were observed in mouse lymphoma cells (Isquith et al., 1988; Litton Bionetics, 1978) both with and without metabolic activation. The study methods generally followed OECD TG 490 (OECD, 2016d) but used bromodeoxyuridine as the selection medium instead of triflurothymidine, which is the only selection medium recommended by the guideline. Also, relative growth (a measure of cytotoxicity) for the highest concentration should be 10-20 percent. However, the study resulted in 6.6 percent relative growth (somewhat low) in the non-activation experiment and 61.2 percent in the activation experiment. The assay also did not describe any measures to control for volatilization; if such measures were not used, the assay could have been significantly compromised.

Felix et al. (1998) exposed Rat2λlacI fibroblasts to D4 to measure mutant frequencies *in vitro*. To investigate whether a depletion of glutathione (GSH) might lead to increased mutations based on an increased pro-oxidative state, some of the experiments were conducted with the addition of L-buthionine (S,R) sulfoximine (BSO) to decrease intracellular GSH. The fibroblasts were derived from Rat2 embryo cells by co-transfecting the λlacI shuttle vector with plasmid pSV2Neo. The authors did not identify any test guidelines in their study. D4 exposure either without or with BSO did not increase in gene mutations in rat fibroblast cells. To address poor bioavailability due to hydrophobicity of D4, the authors administered D4 in an 'inclusion complex' of a cyclic α (1–4) linked D-glucopyranose octamer. Upon contact with the lipid phase of the plasma membrane, D4 partitioned from the complex into the lipid bilayer (Felix et al., 1998). It is possible that the use of the identified complexes for administration of D4 may have resulted in limited volatility. However, the authors do not describe methods to minimize volatility and if D4 volatilized from the cell cultures, the results may have been compromised.

Vergnes et al. (2000) exposed *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 to D4 and tested for reverse mutations using the pre-incubation method at five concentrations each both with and without metabolic activation. Many of the methods followed OECD TG 471 (OECD, 2020). However, OECD TG 471 also recommends including *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102, which have AT base pairs at the primary reversion site, but the authors did not include any of these strains; the impact of this exclusion is not known. The authors used only 2-aminoanthracene as the positive control for the experiments using metabolic activation, even though OECD TG 471 states that 2-aminoanthracene should not be used as the only positive control with the S9-mix. However, the impact of this limitation was likely to be minimal because all experiments showed clear responses using this positive control. The assay did not describe any measures to control for volatilization; OECD TG 471 strongly recommends considering methods other than the standard plate incorporation or pre-incubation method for volatile chemicals. If measures were not used to control for volatilization, the assay could have been significantly compromised.

Like Vergnes et al. (2000), Isquith et al. (1988) also evaluated *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, but used the plate incorporation method. Detailed methods are described in Litton Bionetics (1978). In addition to bacterial strains, Isquith et al. (1988) also evaluated the yeast *Saccharomyces cerevisiae* (strain D4) to investigate a eukaryotic species. The first TA1535 experiment without metabolic activation resulted in an increased number of revertants; the second test did not show an increase.[14] The authors stated that slight toxicity occurred with this and other strains but did not describe the level of cytotoxicity at each concentration. Therefore, it is not known whether cytotoxicity interfered with the results. The authors concluded that the experiment was negative for reverse mutations but without further information on cytotoxicity, EPA concluded that the results are equivocal. Limitations of Isquith et al. (1988) were the same as those identified for Vergnes et al. (2000), namely that recommended strains were not included, and the authors did not describe measures to minimize volatilization.

5.2.1.1.2 Other Genotoxicity Assays

EPA identified multiple SCE assays (Vergnes et al., 2000; Isquith et al., 1988; Litton Bionetics, 1978), assays evaluating DNA damage in mammalian cells and bacteria (Farasani and Darbre, 2017; Vergnes et

[14] Number of revertants with increasing dose: First experiment - 14, 48, 28, 33, 20 at 0.001, 0.01, 0.1, 1.0, and 5.0 µL/plate (vs. 23 in solvent control); Second experiment – 19, 27, 25, 29, 12 (vs. 28 in solvent control).

al., 2000; Isquith et al., 1988; Litton Bionetics, 1978) and one dominant lethal assay in rats (Dow Corning, 1982).

Vergnes et al. (2000) found D4 resulted in increased SCEs with S9 in Chinese hamster ovary cells *in vitro*. Isquith et al. (1988) and Litton Bionetics (1978) identified an increase in SCEs in mouse lymphoma cells that was less than 2-fold greater than the solvent control. The OECD test guideline related to evaluation of SCEs (OECD TG 479) was rescinded in 2014 because the mechanism for this effect is not known (OECD, 2017).

Farasani and Darbre (2017) conducted tests of DNA damage *in vitro* (comet assays), effects on DNA repair proteins, and a measure that correlates with cell transformation. No information was provided for these tests to indicate controls for volatility. Also, no test guidelines were cited, and there are no accompanying OECD or EPA guidelines for these tests.

Farasani and Darbre (2017) identified increased DNA damage in an *in vitro* comet assay using human breast epithelial cells exposed to 10^{-5} M D4 for 1 hour. In addition to these results, decreases in levels of DNA repair proteins (one of which was BRCA1) were observed after long-term exposure to D4. Although one week in the presence of 10^{-5} M D4 resulted in *increased* mRNA for BRCA1 in one cell line, 30 weeks exposure to D4 (also at 10^{-5} M) resulted in reduced mRNA and protein levels of BRCA1 in two breast epithelial cell lines. The mRNA levels of 3 or 4 other repair proteins (of 5 proteins tested) were reduced when exposed to 10^{-5} M D4 after one week with one cell line and after 30 weeks for both cell lines (Farasani and Darbre, 2017).

Farasani and Darbre (2017) also measured the ability of D4 to induce the growth of epithelial cells (MCF-10A and MCF-10F) *in vitro* under anchorage-independent conditions (without attachment to solid surface). Usually, these cells don't grow under such conditions without addition of 17β-estradiol, and the authors conducted this test because it is expected to be correlated with cell transformation *in vivo*. For these cell lines, colony numbers increased in a dose-dependent manner above the control for D4 concentrations from 10^{-9} M to 10^{-5} M. The maximum colony size was greatest at 10^{-5} M.

Isquith et al. (1988) (with unpublished report by Litton Bionetics (1978)) found no DNA damage in mouse lymphoma cells using an alkaline elution method, which can primarily measure single strand breaks in DNA. The authors referenced a published method, but there are no comparable OECD or EPA methods. No information on controls for volatilization were described.

Isquith et al. (1988) and Litton Bionetics (1978) evaluated DNA damage by measuring growth inhibition of a bacterial strain deficient in a DNA repair enzyme — *Escherichia coli* strain P3078 (*pol* A-) as compared with the wild type *E. coli* strain W3110 (Vergnes et al., 2000; Isquith et al., 1988; Litton Bionetics, 1978). The authors referenced only a published method. EPA did publish a test guideline in 1998 — the Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.5500: Bacterial DNA Damage or Repair Tests. The methods of Isquith et al. (1988) followed the guideline with some deviations. The enzyme-deficient strain used by Isquith et al. (1988) is not listed in OPPTS Guideline 870.5500, although the guideline acknowledges the possibility of using other strains. No negative chemical control was used, and it is not apparent that duplicate cultures were assessed. No information on control for volatilization was included.

Dow Corning (1982) did not find any dominant lethal effects as assessed by embryonic death after male parental rats were exposed at 100, 500, or 1000 mg/kg-bw/day for 8 weeks via gavage and mated with unexposed females that were sacrificed on day 14 of pregnancy. The authors referenced U.S. Food and Drug Administration procedures from 1977, and the methods generally complied with OECD TG 478 (OECD, 2016c). The volatility of the D4 should not have been of concern during the conduct of this test because D4 was not diluted in a vehicle and doses were administered orally.

5.2.1.1.3 Summary of Evidence on Genotoxicity and Mutagenicity

Based on mutagenicity and genotoxicity data described above5.2, EPA concluded that there is a lack of evidence that D4 acts via a mutagenic MOA. All studies were negative for genotoxicity or mutagenicity except one equivocal result in a bacterial assay for one bacterial strain (*S. typhimurium* TA1535 was positive in one of two experiments) (Isquith et al., 1988; Litton Bionetics, 1978). There is some ambiguity about results of one chromosomal aberrations assay that identified increased aberrations but these occurred at a concentration that resulted in significant cytotoxicity and identified in a small number of cells (Isquith et al., 1988; Litton Bionetics, 1978). The *in vitro* studies were limited by uncertainty about whether the assays controlled for loss of D4 due to volatility.

All previous assessments of D4 (SEHSC, 2020; SCCS, 2010; Brooke et al., 2009) noted the lack of evidence of genotoxicity for D4, with Brooke et al. (2009) stating that the chromosomal aberrations assay (Isquith et al., 1988; Litton Bionetics, 1978) resulted in ambiguous results when conducted with metabolic activation.

5.2.1.2 Other Mechanistic Information

Increases in endometrial hyperplasia and adenomas associated with D4 exposure in rats (Jean and Plotzke, 2017) could be associated with chronic unopposed estrogen (estrogen dominance) resulting in endometrial stimulation and proliferative endometrial lesions (Dekant et al., 2017; Jean and Plotzke, 2017).

EPA considered available evidence for several inter-related mechanisms associated with hormonal regulation that may have contributed to the potential carcinogenicity of D4, including estrogen and progesterone activity, dopamine pathway changes, effects on the estrous cycle, and repression of the luteinizing hormone surge.

Given the complex interactions of endocrine systems, D4 may operate via multiple related pathways. Furthermore, dominant mechanisms may vary depending on the timing and magnitude of exposure.

5.2.1.2.1 Estrogen And Progesterone Activity

Xenoestrogens may exhibit estrogenic or anti-estrogenic activity by interacting with estrogen receptors directly (He et al., 2003), and both the α and β subtypes of the estrogen receptor (ER-α and ER-β) are expressed in uterine tissue (He et al., 2003). As described above in Section 4.1.3.3, D4's estrogenic effects have been investigated in uterotrophic assays via oral gavage, subcutaneous administration, and whole-body inhalation exposure with female F344 and SD rats, female B6C3F1 mice, and female estrogen receptor-α knockout (αERKO) mice (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003; MPI Research, 1999).[15] Receptor binding and transcription assays also investigated the capability of D4 to bind to ER-α and ER-β as well as progesterone receptors or show increased gene transcription (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003).

[15] Information in MPI Research (1999) is also described in McKim et al. (2001b).

Although results of the uterotrophic assays are not consistent across all studies and strains, most of them indicated weak estrogenic (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003; MPI Research, 1999) and some evidence of anti-estrogenic activity (MPI Research, 1999). Similarly, available receptor binding assays suggested weak binding to ER-α, but no binding to ER-β or to progesterone receptors even though D4 exposure was associated with a statistically significant increase in gene expression of the progesterone receptor (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003). For most *in vitro* assays, there was no information on use of controls for volatilization.

The greater potency of estrogen suggests that the effect of D4 directly on estrogen receptors would be limited due to expected competition with endogenous estrogen. However, it is possible that high D4 levels evaluated in Jean and Plotzke (2017) might compete with endogenous estrogen.

5.2.1.2.2 Dopamine Pathway

Dopamine agonists inhibit prolactin secretion from the pituitary in rats, leading to estrogen dominance that results in endometrial stimulation and proliferative endometrial lesions (Dekant et al., 2017).

EPA examined the evidence regarding whether a dopamine-related MOA could explain D4's induction of adenomas in the uterus of F344 rats. Dopamine can influence pituitary regulation of the estrous cycle in rats (Dekant et al., 2017). Two types of *in vivo* experimental models can be used to examine whether a compound may act as a dopamine agonist. The first model relies on the fact that in aging female F344 rats, a change in the hypothalamic control of dopamine leads to increased prolactin secretion into blood. Researchers can identify dopamine agonists by the subsequent decreases in prolactin in the aging females (Dekant et al., 2017). Jean et al. (2017) evaluated the effect of 8,540 mg/m^3 D4 on aged, reproductively senescent female F344 rats dosed for six hours per day from 11 to 24 months of age when compared with pergolide mesylate (PM), a known dopamine agonist. D4 had no effect on serum prolactin, whereas PM decreased serum prolactin to nearly undetectable levels throughout the study. Serum estradiol levels decreased after D4 exposure and increased with PM exposure at all time points. Finally, serum progesterone was increased only during dosing weeks 2-10 with D4 exposure but decreased during weeks 15-57 with PM exposure. These results did not support a dopamine agonist mechanism for D4.

The second *in vivo* model that can be used to investigate dopamine agonism is pre-treatment of female F344 rats with reserpine. Reserpine depletes dopamine levels in the brain leading to increases in circulating prolactin and allowing chemicals to interact with the dopamine D2-receptor (Dekant et al., 2017). Dow Corning (2005b) administered reserpine to female F344 rats and exposed them nose-only to 8,492 mg/m^3 (700 ppm) D4 for 6 hours. Controls included 1) ovariectomized rats; 2) reserpine treatment; and 3) bromocriptine (a known dopamine agonist) treatment. Prolactin blood levels were 80 percent lower during the D4 treatment compared with the reserpine-treated rats and were also lower than the ovariectomized controls. Pre-treatment with a dopamine antagonist (sulpiride) resulted in a decrease in D4's ability to decrease prolactin. These results suggested D4 was operating as a dopamine D2 receptor agonist in this study. The authors suggested that other mechanisms (*e.g.*, indirect effects on dopamine) were unlikely but noted that they could not be ruled out Dow Corning (2005b).

In another study, Dow Corning (2010) administered reserpine to female F344 rats and exposed them nose-only to 8,492 mg/m^3 (700 ppm) D4 for 6 hours. The authors used the following controls: 1) ovariectomized rats; 2) reserpine-treated rats; and 3) reserpine plus pergolide treatment. In this experiment, D4 exposure did not result in decreased serum prolactin levels at the end of exposure compared with the reserpine group (in fact, prolactin was 12 percent higher in the D4 group) or in the 4-

or 8-hour post-exposure evaluations. Only at 18 hours post-exposure were prolactin levels in the D4 group 87 percent lower than the reserpine group ($p < 0.02$). The authors noted the lack of prolactin decrease at the end of exposure and suggested that D4 was not a dopamine agonist, but later effects suggested some direct or indirect modulation of prolactin. Overall, the authors concluded that the contradictory influences were not clear and required further investigation (Dow Corning, 2010).

Several *in vitro* studies also investigated D4's ability to bind to dopamine receptors. Dopamine receptor assays using MMQ pituitary cells *in vitro* (Domoradzki, 2011) demonstrated that D4 did not act as a dopamine D2 receptor agonist but did act upon adenylate cyclase directly or indirectly; the authors used sealed reaction vessels to control for volatilization.

Using multiple assays including dopamine D2 receptors from isolated F344 rat brain striatum membranes, Baker (2010) did not identify direct interaction of D4 with the receptor, although suppression of basal [^{35}S] GTPγS by D4 was consistent with inverse receptor agonism. D4 suppressed the pergolide stimulation of [^{35}S] GTPγS binding suggesting D4 may have acted as a D2 antagonist, although the authors suggested this was not likely and that the observed effects may have been unrelated to interactions with the receptor (Baker, 2010). D4 concentrations decreased throughout these experiments, likely due to evaporation, and in some cases the levels decreased to less than the detection limit Baker (2010). Thus, there is uncertainty associated with these results.

In a study by Thackery (2009), D4 appeared to compete with radioligand [N-methyl-^{3}H]-methylspiperone for binding to the dopamine D2 receptor (16-20 percent reduction) at lower D4 concentrations, suggesting dopamine D2 receptor agonism. No effect of D4 exposure was observed on other dopamine receptors (1; 3; 4,4; or 5). Although the authors controlled for some evaporation by covering the reaction vessels, the D4 nominal concentrations decreased due to volatilization and were sometimes lower than detection limits. Levels were still higher than blood, brain, or uterine tissue concentrations observed in *in vivo* studies.

The two *in vivo* studies in F344 rats and *in vitro* assays did not show consistent results. It is possible that D4 does not act as a dopamine receptor agonist or antagonist but still indirectly influences some portions of the dopamine pathway. *In vitro* studies were limited by uncertainty in exposure concentrations due to D4's volatility. Dekant et al. (2017), Franzen et al. (2017), and (Matthews, 2021) also concluded that D4 was not likely to act directly on dopamine receptors, and Dekant et al. (2017) suggested that D4 may act indirectly on the dopamine pathway.

5.2.1.2.3 Estrous Cycle Changes

As noted by Jean and Plotzke (2017), increased cyclicity suggests an overall increase in endogenous estrogen stimulation of uterine tissue over the life of the rat. Multiple studies identified changes in estrous cyclicity among female rats (Jean and Plotzke, 2017; Jean et al., 2017; WIL Research, 2001a).

Jean and Plotzke (2017) showed that female F344 rats exhibited changes in estrous cycles and prolonged estrogenic states in the 104-week cancer bioassay. At 12 months, an increased percentage of rats at the highest concentration of 8,492 mg/m^3 (700 ppm) had longer cycles. An increased incidence (4 of 10 F344 rats at 8,492 mg/m^3 vs. 2 of 10 controls) of degenerating luteal cells within new corpora lutea also suggested longer cycle length (≥ 6 days) (Jean and Plotzke, 2017).

In reproductively senescent female F344 rats, Jean et al. (2017) also found that 8,540 mg/m^3 D4 led to changes in estrous cyclicity that included an increased number and percentage of days in proestrus and

estrus (estrogen-dominant states) and an increased mean estrous cycle incidence within a 45-day interval. D4-exposed rats had 6,966 estrogen-predominant days compared with 4,152 days for the controls ($p < 0.01$) (Jean et al., 2017). Across the individual 45-day intervals that formed the basis of evaluation, the percent of estrogen-predominant days for D4-exposed rats ranged from 28 to 62 percent and were all statistically significantly greater than controls ($p < 0.01$) except for the first interval.

Despite this increased estrogen dominant state, the D4 exposure group exhibited lower estrogen to progesterone ratios than the control group ($p < 0.01$ for most intervals), which was a consequence of lower endogenous estradiol levels in the D4 group compared with controls ($p < 0.01$). The decease may have been due to the "point-in-time" sampling of estradiol and was not designed to identify specifically timed surges such as those that may be associated with the estrous cycle. Therefore, the significance of the decreased estradiol concentrations associated with D4 exposure is difficult to interpret (Jean et al., 2017).

As noted in Section 5.1.1.1, the incidence of cystic endometrial hyperplasia was slightly elevated in the D4-exposed rats compared to controls (14/35 for D4 vs. 8/37 for controls). In the ovary, the D4-exposed rats exhibited a marked decrease (37 percent) in the incidence of antral size atretic follicles vs. controls. Although incidence was not greater for increased vaginal thickness, D4 exposed rats had greater severity (6 animals had moderate severity vs. only minimal/mild severity epithelial thickness in controls) (Jean et al., 2017).

The higher risk of uterine adenoma may have been a consequence of increased uterine stimulation based on more time in proestrus and estrus associated with the additional estrous cyclicity and extended estrus (Jean et al., 2017).

WIL Research (2001a), the 2-generation reproductive toxicity study in SD rats, also showed that F1 rats had prolonged estrous cycles. No change in estrous cycle length was observed in the F0 generation after exposure to D4 vapor at concentrations up to 8,492 mg/m^3 (700 ppm) for 6 hours per day for 70 consecutive days prior to mating, through mating and weaning of F1 pups on PND 21. However, the first mating of the F1 generation that was exposed in a similar fashion resulted in a mean estrous cycle length of 5.3 days at the highest concentration, which was statistically significantly greater than the control mean length of 4.2 days ($p < 0.01$); at 3640 and 6070 mg/m^3 (300 and 500 ppm), the number of days was 4.4 and 4.6 but these were not statistically significantly different from the controls. No differences in estrous cycle length from controls were observed for offspring of F1 males mated with unexposed females, suggesting that the changes were mediated by females. Other effects in the 2-generation study included decreases in mean number of pups born and mean live litter size at 6070 and 8,492 mg/m^3 in both the F0 and F1 generations. Implantation sites were also reduced at 8,492 mg/m^3 for both generations. No adverse effects were observed at any exposure level on anogenital distance, vaginal patency, or preputial separation.

In a high-quality study, WIL Research (1999)[16] conducted multiple experiments in which female SD rats were exposed to D4 for varying exposure durations across reproductive lifestages and identified a narrow time frame around fertilization and ovulation during which D4 primarily exerts effects. D4 was associated with decreases in implantation sites and litter size in the overall and fertilization phases, but not in the ovarian or implantation phases. To further define the sensitive period for D4 exposure, additional groups of rats were exposed on single days. A single 6-hour exposure to D4 on the day prior to mating resulted in a significant reduction in fertility. Based on these effects that were observed after

[16] Information in WIL Research (1999) is also described in Meeks et al. (2007).

dosing during different stages, Andersen (2022) noted that the effects of D4 on rat reproduction were dependent on the timing of exposure during the estrous cycle.

5.2.1.2.4 Luteinizing Hormone

Andersen (2022) suggested that D4 may result in prolonged endogenous estrogen exposure via reduced gonadotropin releasing hormone (GnRH) during the middle of the estrous cycle in rats that results in decreased LH and subsequent delayed ovulation.

As described in Section 4.1.3.3, D4 exposure by suppression of the preovulatory LH surge on the day of proestrus was associated with decreased ovulation, persistence of mature follicles, and increased serum estradiol (Quinn et al., 2007a).

Andersen (2022) hypothesized several possible mechanistic changes that may have led to D4's effect on the LH surge, decreased ovulation, and increased estradiol. These include non-specific changes in membrane fluidity from that high lipid phase concentrations due to D4's lipophilicity that affect neurotransmission such as activity of GnRH neurons; possible changes in the activation of gamma aminobutyric acid (GABA), with subsequent decrease in GnRH release; or
uptake of D4 into the nasal epithelium or vomeronasal organ and diffusion into the olfactory bulb might have led effects on neuronal pathways that communicate with the hypothalamus and subsequently affect GnRH release. Limited information exists to understand whether D4 affects one or more of these changes that precede the changes identified by Quinn et al. (2007a).

There may be overlap among purported MOAs. For instance, longer estrous cycles described in Section 5.2.1.2.3 are consistent with delays in both the luteinizing hormone surge and in ovulation, as identified by Quinn et al. (2007a).

5.2.1.3 Summary of Mechanistic and Supporting Evidence

EPA considered available mechanistic evidence for uterine tumors (Section 5.2). Mechanistic studies evaluated mutagenic and carcinogenic properties of D4. As described in Section 5.2.1, genotoxicity and mutagenicity studies generally indicated no evidence for direct mutagenic activity of D4 in *in vivo* or *in vitro* studies. One study reported an equivocal result in a reverse mutation bacterial assay for one bacterial strain. Although there are a variety of associated mechanistic effects of D4 that may contribute to uterine tumors, a single MOA is not well understood or supported.

5.2.2 Cancer Classification and Evidence Integration Conclusions

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), D4 is considered to have "suggestive evidence of carcinogenic potential" based on the slight evidence of D4's carcinogenic potential in animals for uterine tumors.

EPA made evidence integration judgments based on considerations described in Chapter 7 of the *2021 Draft Systematic Review Protocol* (U.S. EPA, 2021). Specifically, EPA developed strength of the evidence judgments (slight, indeterminate) for individual evidence streams (*i.e.*, human, animal, mechanistic) by expert judgment based on quality of the database, consistency, magnitude and precision, dose-response, and biological significance. For cancer, the overall cancer classification incorporated considerations across evidence streams for all cancers, consistent with U.S. EPA (2005).

Table 5-3 summarizes the evidence integration judgments for each evidence stream across all cancer types. See Appendix A (Table_Apx A-5) for complete details on evidence integration judgments within and across evidence streams in evidence integration. The table is organized by cancer type.

No human data were available for any tumor type and thus, EPA identified the human evidence as indeterminate.

The conclusion of "suggestive evidence" is based on evidence of rare uterine tumors in a bioassay using one species (F344 rats), with accompanying non-neoplastic and presumed pre-neoplastic effects (increased uterine weight, hyperplasia). There was an increased incidence of malignant histiocytic sarcomas and benign endometrial adenomas at the highest concentration in F344 rats with statistically significant positive dose-response trends. Although no malignant endometrial adenocarcinomas were observed in the 24-month exposure group, one was observed at 1,820 mg/m^3 (150 ppm) during recovery within the 12-month exposure/12-month recovery group. The use of only one species and the observation of two tumor types (histiocytic sarcoma [malignant] and endometrial epithelial adenoma [benign]) at only the highest concentration resulted in characterizing the animal evidence as *slight.*

EPA determined the animal evidence for testicular tumors to be *indeterminate.* Although a dose-related trend for bilateral Leydig cell adenomas was observed in male rats exposed for 24 months, unilateral tumor incidence was lower than controls at the highest three concentrations. There were no statistically significant differences in unilateral or bilateral adenomas when evaluating pairwise comparisons with the controls. Leydig cell tumors occur spontaneously at high incidence in F344 rats, and incidences of bilateral adenomas in Jean and Plotzke (2017) were within the range seen in control F344 rats.

Animal evidence for MNCL is also *indeterminate.* An increase in mortality occurred at the highest concentration in males, but overall incidence was not greater than controls.

Although no clear MOA was identified, mechanistic data suggest that D4 has weak estrogenic activity, may affect other hormones related to reproduction, and has been shown to affect timing of the estrous cycle and ovulation. Thus, it is possible that more than one MOA may have been operating and contributing to uterine tumors observed at the highest concentration in the chronic animal bioassay. EPA concluded that none of the mechanistic studies are definitive for a single MOA, and mechanistic evidence is indeterminate for all three types of tumors.

According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), when there is suggestive evidence "the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one." Consistent with those guidelines, EPA did not conduct a dose-response assessment for D4 and did not quantitatively evaluate it for carcinogenic risk to human health.

Importantly, EPA expects the non-cancer HECs and HEDs to be protective of cancer effects if cancer occurs via a threshold mechanism. None of the benign endometrial epithelial tumors or hyperplasia observed at the end of the study could be modeled using BMD software, and an incidence of hyperplasia and tumors occurred only at the highest concentration. Thus, the NOAEC is 1,820 mg/m^3. When converted to a continuous value of 325 mg/m^3 (1,820 x 5/7 x 6/24), the value is 5.8 times higher than the non-cancer continuous HEC of 55.8 mg/m^3. Assuming that endometrial epithelial hyperplasia is a precursor to tumors, EPA would use the same benchmark MOE for a threshold cancer assessment that

was used for the non-cancer HECs. Although evidence for the testes tumors is indeterminate, BMD modeling of testes interstitial cell hyperplasia resulted in a $BMDL_{10}$ of 460 mg/m^3, which is 8.2 times higher than the non-cancer HEC.

Table 5-3. Evidence Integration Judgments for Each Cancer Type

Cancer Type	Human	Animal	Mechanistic
Uterine	Indeterminate	Slight	Indeterminate
Testicular	Indeterminate	Indeterminate	Indeterminate
Hematopoietic	Indeterminate	Indeterminate	Indeterminate

6 WEIGHT OF THE SCIENTIFIC EVIDENCE CONCLUSIONS FOR HUMAN HEALTH HAZARD

EPA considered evidence integration conclusions and dose-response considerations from Section 4 to describe overall hazard confidence levels in the PODs selected for risk assessment based on the following characteristics:

- Evidence integration conclusion
  - *Demonstrates*
  - *Likely*
  - *Suggests*
- Selection of most critical endpoint and study
- Relevance to exposure scenario
- Dose-response considerations
- PESS sensitivity

Section 6.1 summarizes the strengths and limitations from summary table of confidence for each hazard endpoint and exposure duration, drawing upon information from previous sections and additional considerations as needed. Section 6.1.1 presents the overall confidence for the non-cancer hazard based on the above characteristics. Section 6.1.2 briefly discusses reasons the uterine tumors were not used to calculate risks.

6.1 Strengths, Limitations, Assumptions, and Key Sources of Uncertainty for the Hazard Identification and Selection of PODs for Human Health Hazard Assessment

6.1.1 Acute, Intermediate, and Chronic Non-cancer

Evidence Integration Conclusions

The main critical health effect domain was supported by the weight of scientific evidence and considered appropriate for dose-response analysis. EPA concluded that D4 likely causes effects on female reproductive system and function in humans under relevant exposure circumstances based on moderate evidence in animals and moderate mechanistic evidence.

Selection of Most Critical Endpoint and Study

EPA identified reproductive effects as the most sensitive endpoints supported by the strongest evidence. Available studies consistently show effects on a set of related reproductive endpoints, including reduced fertility indices, implantation sites, litter size and number of pups born, altered estrous cycle, reduced

corpora lutea, and ovarian atrophy (Quinn et al., 2007a; WIL Research, 2001a, 1996a). EPA selected WIL Research (2001a) as the basis for dose-response because it is a high-quality study that evaluates a wide range of doses and a comprehensive set of sensitive reproductive endpoints. The study also includes exposures for both males and females and identifies the most sensitive endpoints following long-term exposure across lifestages. WIL Research (2001a) is one of a limited set of studies that evaluate effects across a range of exposure levels. The significant effects on reproductive endpoints reported in WIL Research (2001a) are consistent with those reported in other studies that tested effects at a more limited range of higher exposure levels. In dose-response analysis, reduced mean live litter size (WIL Research, 2001a) was selected as the basis for the PODs used for characterizing risk from exposure to D4 because it was the most robust and sensitive endpoint with a good model fit.

Relevance to Exposure Scenarios

The POD is based on effects in a 2-generation reproductive toxicity study WIL Research (2001a) that EPA considers to be relevant for acute, intermediate, and chronic exposures in humans. Rats in the 2-generation study were exposed prior to mating, during mating, throughout gestation and lactation. While this provides an exposure period that includes sensitive phases of development, this study does not provide information about which exposure periods or durations contribute most to the observed effects including significant increases absolute and relative liver weights. Evidence from other studies demonstrates that short-term and acute exposures in adult females prior to mating are sufficient to produce similar reproductive effects.

For instance, after a 3-day exposure at 700 ppm (8,492 mg/m^3) (nominal concentration) in rats, (Quinn et al., 2007a) reported a reduction of eggs in the ovary. In another study, a single six-hour exposure one day prior to mating at 700 ppm (8,492 mg/m^3), F0 rats had decreased pregnancy rate and fertility index outcomes (Dow Corning, 1998b). While the PODs are primarily derived based on adult female exposures and endpoints, they are based on the most sensitive endpoints in a 2-generation study. They are therefore expected to be protective of a range of sensitive endpoints across lifestages and are considered appropriate for assessing risks for exposures to several lifestages and populations.

Dose-Response Considerations

EPA has confidence in the dose-response analysis for female reproductive effects. The analysis is based on decreased live F2 litter size, one of the most sensitive and robust endpoints in a 2-generation inhalation reproductive toxicity study (WIL Research, 2001a). A PBPK model for D4 converted dose-response data to internal dose metric representing blood concentrations. BMD modeling was performed for this endpoint in a manner consistent with consistent with EPA's *BMD Technical Guidance* (U.S. EPA, 2012). The data of four treatment levels adequately fit several BMD models based on statistics and visual inspection and resulted in similar BMDLs among the fit models. For decreased live litter size, a BMR of 5 percent relative deviation was used to address the severity of the endpoint. The POD for the decreased live litter size was a BMDL of 279 mg/m^3.

PESS Sensitivity

The POD is expected to be protective of sex-specific effects across most lifestages. It is based on the most sensitive effect observed in a 2-generation study and is therefore expected to be protective of effects resulting from exposure in males and females during sensitive phases of development across most lifestages but some lifestages were exposed only indirectly. While the POD is based on a female reproductive endpoint most relevant to adult females, it is intended to be protective of other endpoints that may be affected by D4, including respiratory and liver effects relevant to other groups and lifestages. As discussed in Section 7.2, other factors that may contribute to biological susceptibility were considered qualitatively but not quantitatively incorporated into the POD. As described in Section 4.2.4,

a 10 × UF_H factor was applied to account for human toxicokinetic and toxicodynamic variability, which is expected to account for differences in dose-response and toxicokinetics due to factors such as limited coverage of infants and children's exposure as well as interindividual variability. For example, genetic polymorphisms in metabolizing enzymes or transporters can vary chemical clearance and distribution within subpopulations. Similarly, disease states (such as inflammatory bowel diseases) that result in variations in GI tract compositions may affect absorption and overall distribution (especially for first-pass metabolism in the gut wall), thereby adding to unaccounted for interindividual differences. Hepatic diseases can change the liver volume and metabolic processes, changing internal kinetics as well.

PBPK Modeling

Exposure duration and related issues. Because D4 has a complex toxicokinetic and metabolic profile, predicting HECs and HEDs using the D4 PBPK model is preferable to using default approaches. Yet, some uncertainty exists when applying the D4 PBPK model.

The model relies on experimental data from acute and short-term studies. Therefore, there is some uncertainty when modeling longer exposure durations associated with the exposure scenarios used to calculate risk. Furthermore, extrapolating from the inhalation route to other exposure routes may also be affected by limited long-term toxicokinetics information.

Parameters related to induction of D4 metabolism via CYP pathways (*e.g.*, CYP2B1/2) were used from a study that measured induction for 3 to 28 days in rats. Therefore, uncertainty exists when applying the parameters to acute or chronic durations.

Dermal parameters: The differences in HEC and HED values by exposure route that were predicted by the PBPK model suggested dermal absorption/bioavailability to be 3.5 percent relative to the inhalation route (after conversion of the inhalation HEC to mg/kg-bw/day)[17] and 1.1 percent compared with the oral route. However, one of the *in vivo* rat dermal absorption studies (GE, 1994a) identified higher dermal absorption (up to approximately 20 percent for the unoccluded scenario). Therefore, there is some uncertainty about the amount of D4 that may be available internally through the dermal route.

Although there is some uncertainty, EPA expects that dermal absorption of D4 is low based on its volatility and because most *in vivo* and *in vitro* dermal absorption studies with acceptable recovery (more than 80 percent) estimate absorption percentages of 1.09 or less. Also, absorption through rat skin is often higher than human skin as noted in *Guidance Notes on Dermal Absorption Studies* (OECD, 2022) and the PBPK model relied on human dermal data to predict absorption through human skin. Also, it is possible that the higher value in GE (1994a) was due to leaking from the skin depot (possibly due to frequent sampling), resulting in mischaracterization of volatilized D4 as exhaled volatiles within the experimental metabolism chamber.

Overall Confidence

Based on the above factors, EPA has moderate to robust overall confidence in the evidence integration, study/endpoint selection, exposure scenario applicability, dose-response analysis, PESS sensitivity, and PBPK modeling supporting derivation of PODs for D4 based on reproductive endpoints.

6.1.2 Cancer: Uterine Tumors

As described in Section 5.2.2, EPA did not perform quantitative dose-response analysis for uterine tumors. Based on the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the "suggestive

[17] 55.8 mg/m^3 * ((0.6125 m3/h *24 h)/72.4 kg) = 11.3 mg/kg-bw/day

evidence of carcinogenic potential" for D4 based on slight evidence in animals and indeterminate mechanistic evidence is not sufficient to support a quantitative dose-response analysis or risk assessment. EPA concluded that the non-cancer PODs selected for characterizing non-cancer risk from exposure to D4 are expected to be health-protective, including for PESS.

7 CONSIDERATION OF PESS AND AGGREGATE EXPOSURE

7.1 Hazard Considerations for Aggregate Exposure

Section 2605(b)(4)(F)(ii) of TSCA requires EPA, as a part of the risk evaluation, to describe whether aggregate or sentinel exposures under the conditions of use were considered and the basis for their consideration. EPA has defined aggregate exposure as "the combined exposures to an individual from a single chemical substance across multiple routes and across multiple pathways" (40 CFR § 702.33).

Most of the available D4 toxicity studies were inhalation studies in rats. EPA concluded that the systemic effects observed in these inhalation studies were relevant to all routes of exposure. EPA therefore used a PBPK model to estimate adult HECs and HEDs based on a decrease in live litter size in F2 offspring in a 2-generation reproductive inhalation study (WIL Research, 2001a). The PBPK model included route-specific (inhalation, oral, and dermal) and species-specific (two rat species and humans) toxicokinetic information for D4. Because the PODs are based on systemic effects relevant to all routes, EPA concludes that it is biologically appropriate to aggregate risks across exposure routes.

7.2 PESS Based on Greater Susceptibility

In this section, EPA addresses subpopulations and lifestages expected to be more susceptible to D4 exposure than other populations. Table 7-1 identifies susceptibility factors that may contribute to biological susceptibility and describes the extent to which these factors were addressed quantitatively or qualitatively throughout the hazard identification and dose-response analysis.

EPA examined sources of biological susceptibility for each of the factors in Table 7-1. The Agency quantitatively incorporated these considerations into hazard values and subsequent risk estimates when possible; however, for many factors EPA did not identify any reasonably available information to support quantitative adjustment of hazard/risk values. For these other factors, the Agency acknowledges either direct or indirect information suggesting additional susceptibility of certain subpopulations.

EPA is directly incorporating lifestage susceptibility into hazard values for the non-cancer endpoint used in the risk evaluation. The modeled hazard endpoint was a decrease in live litter size in the F2 generation from WIL Research (2001a). For the single robust hazard domain (reproductive toxicity), this endpoint was among the most sensitive endpoints that resulted from inhalation exposure to D4 across lifestages, including pre-mating, gestation, lactation, and post-weaning in both males and females. Inhalation reproductive toxicity studies that tested multiple exposures consistently identified effects at approximately 6070 mg/m^3 (500 ppm), with NOAECs of approximately 3640 mg/m^3 (300 ppm). Several other inhalation reproductive toxicity studies tested only at approximately 8400 mg/m^3 (700 ppm) and identified a variety of effects at this concentration. Thus, the chosen HECs (and HEDs) are expected to protect for multiple reproductive effects in both males and females across the lifecycle.

The D4 PBPK model does not account for within human variability; thus the 10X intra-species is being used. This UF accounts for interindividual variability that is not represented in the model as described in detail in Table 7-1.

3454 **Table 7-1. PESS Evidence Crosswalk for Biological Susceptibility Considerations**

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to D4		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to D4		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Pre-mating/ mating	This lifestage is one of the most sensitive reproductive lifestages, resulting in decreased fertility and pregnancy rates when rats were dosed only during premating phases.	Dow Corning (1998b) WIL Research (1999)			The chosen POD protects for changes in pregnancy and fertility that are observed after exposure during *only* these lifestages.
	Embryo/ fetuses	The outcome modeled in the risk evaluation (decreased live litter size from the 2-generation study) may be associated with these lifestages, although exposure of the parents included premating, mating, gestation, and lactation. One-generation study findings include pre- and post-implantation losses along with decreased viable fetuses and other effects.	WIL Research (2001a) Dow Corning (1998b)			The chosen POD is based on decreased live litter size in the F2 offspring, which is the most sensitive endpoint in a study that includes exposure during gestation.
	Pregnancy/ lactating status	Most studies that investigated effects of D4 exposure only during gestation in rats for one or more days from GD 0 through 15 show no effects; an inhalation prenatal developmental toxicity study in rabbits from GD 6-18 showed no effects. Longer exposure durations - from premating through gestation and lactation - result in more effects than just during premating/mating	Dow Corning (1998b) WIL Research (1999) IRDC (1993a) WIL Research (2001a)			The chosen POD includes these lifestages and protects for effects that may occur in dams during pregnancy.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to D4		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to D4		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Males of reproductive age	Lack of effects after exposed males were mated with unexposed females, and no evidence of androgenic or anti-androgenic activity indicates little effects on males of reproductive age.	WIL Research (2001a) WIL Research (1997c) Quinn et al. (2007b) Anderson et al. (1998); Anderson et al. (1996); BIBRA (1996); Hackett et al. (1988)			
	Infants/ children	No direct exposure. Indirect exposure during lactation. Observed effects occurred prior to lactation.	WIL Research (2001a)			Although the F1 generation was exposed indirectly during lactation, there is no direct evidence available on effects of D4 on this lifestage (*i.e.*, no direct D4 exposure during early life – infants or children). Any susceptibility related to exposure in early life is expected to be covered by the 10 × UF_H.
	Elderly	Aging female rats (possibly in reproductive senescence) may be more susceptible to the effects of D4, based on tumors, hyperplasia, changes in estrous cycles, and other effects late in life.	Jean et al. (2017) Jean and Plotzke (2017)	Physiological changes associated with aging (including decreased muscle mass, increased body lipids, reduced liver function and reduced hepatic blood flow) may lead to slower metabolism and clearance and longer retention of lipophilic chemicals.	Ginsberg et al. (2005)	Any susceptibility related to effects in aging females is expected to be covered by the 10 × UF_H.
Pre-existing disease or disorder	Health outcome/ target organs			Any preexisting condition affecting a target organ could possibly increase or decrease susceptibility to D4 in that organ.		Any susceptibility related to chronic disease is covered by the 10× UF_H.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to D4		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to D4		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Toxicokinetics	Toxicokinetic parameters in the PBPK model use data from rats and humans. Parent D4 concentrations are higher in female reproductive and related target organs than metabolites (F344 rats). D4 induces enzymes in rats as shown in multiple studies. D4 did not induce enzymes in guinea pigs in one study. Thus, if D4 parent is the toxic moiety, species differences in metabolism/enzyme induction will likely result in different tissue concentrations and possibly different levels of adverse effects.	Campbell et al. (2023) Domoradzki et al. (2017) Zhang et al. (2000) Falany and Li (2005) Meeks et al. (2022) McKim et al. (1998) Dow Corning (2001b)	In humans, there is no information on enzyme inductions or D4 concentrations vs. metabolites in target organs.		Any susceptibility associated with toxicokinetic differences among humans not reflected in the pbpk model is expected to be covered by the 10× UF_H.
Lifestyle Activities	Smoking	No direct evidence identified		Heavy smoking and other tobacco usage may increase susceptibility for reproductive outcomes and cancer throughout the body (including uterine cervical cancer).	CDC (2023a) CDC (2023b)	Qualitative discussion in this section and table only
	Alcohol consumption	No direct evidence identified		Alcohol consumption increases risk for infertility in women.	CDC (2023b)	Qualitative discussion in this section and table only
	Physical activity	Limited information via the inhalation route suggests less D4 may be absorbed during exercise than when at rest.	Utell et al. (1998)	Insufficient activity may increase susceptibility to multiple health outcomes, including cancer and obesity. Overly strenuous activity may also increase susceptibility. Having overweight or low body weight is a risk factor for infertility in women.	CDC (2023a) CDC (2023b) CDC (2022)	Although Utell et al. (1998) was used to estimate parameters for the PBPK model, data are limited. Thus, this factor is addressed mostly by the qualitative discussion in this table.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to D4		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to D4		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Sex	Female rats are expected to be the most sensitive based on lack of reproductive effects when males are mated with unexposed females and based on lack of androgenic effects in mechanistic studies. Toxicokinetic studies also show higher D4 and metabolite concentrations in fat and several other tissues in females than in males. In F344 rats exposed via inhalation, higher levels were most often observed in female reproductive tissues (ovaries, uterus, vagina) than in testes, although levels in the uterus were only slightly higher than those in the testes.	WIL Research (2001a) Domoradzki et al. (2017) Meeks et al. (2022) Plotzke et al. (2000)			The most sensitive sex from rodent assays was used for non-cancer dose-response modeling.
Nutrition	Malnutrition	No direct evidence identified		Micronutrient (vitamins/minerals) deficiencies can have negative health consequences.	CDC (2021)	Qualitative discussion in this table only
Genetics/ epigenetics	Health outcome/ target organs	No direct evidence identified				Any susceptibility associated with toxicodynamic differences among humans is expected to be covered by the 10× UF_H.
	Toxicokinetics	No direct evidence identified				Any susceptibility associated with toxicokinetic differences among humans not accounted for in the PBPK model is expected to be covered by the 10× UF_H.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to D4		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to D4		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Other chemicals and non-chemical stressors	Built environment	No direct evidence identified		Poor-quality housing (resulting in exposure to mold, lead, cold, pests, etc) is associated with a variety of negative health outcomes, including effects on respiratory health.	ODPHP (2023a)	Qualitative discussion in this table only These categories are primarily relevant to increased exposure.
	Social environment	No direct evidence identified		Social isolation and other social determinants (*e.g.*, decreased social capital, stress) can lead to negative health outcomes. Physical or emotional stress can be a risk factor for infertility in women.	ODPHP (2023b) CDC (2023b)	
	Chemical co-exposures	D4 may inhibit effects associated with estrogen (ethinyl estradiol) in F344 but not SD rats when administered together in the same study. When pre-treated with an estrogen-receptor antagonist (ICI-182,780), D4 exposure did not result in the same weak estrogenic effects in mice.	(MPI Research, 1999) Quinn et al. (2007b) He et al. (2003)			Qualitative discussion in this table only

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8 HUMAN HEALTH HAZARD ASSESSMENT CONCLUSION

In this human health hazard assessment of D4, EPA determined that decreased mean live litter size in a 2-generation reproductive toxicity inhalation study was among the most sensitive and robust endpoints for risk characterization of acute, intermediate, and chronic exposure scenarios for D4. Overall, EPA concluded that D4 *likely* causes female fertility and associated reproductive outcomes in humans when there is sufficient exposure. Among other hazard outcomes, EPA concluded that evidence *suggests* but is not sufficient to conclude that D4 may cause liver or lung effects. EPA determined that evidence is inadequate to assess whether D4 exposure may cause developmental effects. EPA also concluded that D4 has *suggestive evidence of carcinogenic potential* and did not conduct cancer dose-response modeling for D4. However, EPA notes that based on available data and information, the hazard value being used for non-cancer effects in this assessment, would be protective of any possible cancer effects if cancer occurs via a threshold mode of action through the same key events that contribute to non-cancer effects (as suggested by some evidence presented in Section 5.2).

Across studies and endpoints with multiple concentrations, most reproductive effects resulted in NOAECs of 3639 mg/m^3 (300 ppm) and LOAECs of 6066 mg/m^3 (500 ppm) (as nominal values). EPA chose decreased mean live litter size, mean number of pups born, and fertility indices for dose-response analysis from the 2-generation inhalation reproductive toxicity study (WIL Research, 2001a), which had a high overall quality determination. This study evaluated a wide range of doses and a comprehensive set of endpoints and identified effects across both generations.

EPA considered the selected study and endpoints to be relevant for acute, intermediate, and chronic exposure durations because the study included dosing of both parents during premating, mating, and gestation with indirect exposure by offspring spanning subchronic to chronic durations. Also, related reproductive effects (fertility) were observed in supporting studies after a single day of exposure, supporting use of the endpoints for acute exposure.

EPA performed dose-response analysis by first using the PBPK model with physiological and toxicokinetic parameters for SD rats to obtain internal doses in blood that were associated with each air concentration in the 2-generation reproductive toxicity study in rats. EPA then obtained BMDLs through BMD modeling of these internal doses. EPA used the $BMDL_5$ for decreased live litter size in the second generation as a sensitive dose. EPA then ran the PBPK model again using physiologic and toxicokinetic parameters appropriate for humans, this time using the $BMDL_5$ for decreased live litter size in the second generation to obtain external PODs.

Table 8-1 lists the HECs and HEDs and corresponding UFs that EPA derived for evaluating risks from acute and intermediate/chronic inhalation, oral, and dermal exposures. The HEDs are expressed as daily doses and the HECs are based on daily, continuous concentrations (24 hours/day) assuming a breathing rate for individuals at rest. Any adjustments needed to exposure durations and frequencies and breathing rates are made in the exposure estimates used to calculate risks for individual exposure scenarios.

Because HECs and HEDs for intermediate and chronic durations were very similar, EPA used the values for chronic duration when estimating risks for both intermediate and chronic exposure scenarios. The chronic values were slightly more sensitive (1 to 2 percent lower). EPA adjusted the dermal POD from the PBPK model using empirical data on the difference in absorption for unoccluded and occluded experiments to obtain the occluded HED, which was used for exposure scenarios in which D4 remained undepleted when in contact with skin.

Table 8-1. Non-cancer HECs and HEDs and Critical Endpoint Used for Risk Estimates for Each Exposure Pathway

Exposure Route	Units	Acute (1 day) [a]	Intermediate (30 days)/ Chronic (steady state) [a,b]	Benchmark MOE	Critical Endpoint
Inhalation	mg/m^3	107	55.8	30 *[UF_A = 3 UF_H = 10]*	Decreased mean live litter size in a 2-generation reproductive inhalation study (WIL Research, 2001a)
	ppm	8.82	4.60		
Oral	mg/kg-bw/day	8.93	3.60		
Dermal (unoccluded)		394	326		
Dermal (occluded)		216	179		

[a] HECs and HEDs for each route and duration were extrapolated from BMDLs calculated in terms of internal doses using the PBPK model (BMD= 8.4 mg/L-hour AUC; $BMDL_5$= 6.31 mg/L-hour AUC) as described in Section 4.2.2 and Appendix B.1.2 of the *Draft Human Health Hazard Assessment for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025e)

[b] Because the PBPK model results for chronic HECs and HEDs were very similar to intermediate values (but slightly lower), EPA used the chronic HEC and HEDs for both intermediate and chronic exposure scenarios when calculating risks.

EPA has moderate overall confidence in the hazard values based on female reproductive toxicity, which are used for risk estimation in the *Draft Risk Evaluation for Octamethylcyclotetra- siloxane* (U.S. EPA, 2025i). This moderate confidence rating is based on the weight of scientific evidence considering evidence integration, selection of the critical endpoint and study, relevance to exposure scenarios, dose-response considerations, and incorporation of PESS.

EPA concluded that D4 likely causes effects on female reproductive system and function in humans under relevant exposure circumstances based on moderate evidence in animals and moderate mechanistic evidence. The high-quality two-generation study of reproduction used as the basis for dose-response analysis (WIL Research, 2001a) includes exposures for both males and females and indicates that reproductive endpoints are the most sensitive endpoints following long-term exposure across lifestages. WIL Research (2001a) is one of a limited set of studies that evaluate effects across a range of exposure levels. The significant effects on reproductive endpoints reported in WIL Research (2001a) are consistent with those reported in other studies that tested effects at a more limited range of higher exposure levels. In dose-response analysis, reduced mean live litter size (WIL Research, 2001a) was selected as the basis for the PODs used for characterizing risk from exposure to D4 because it was the most robust and sensitive endpoint with a good model fit.

Although the PODs are primarily derived based on adult female exposures, they are based on the most sensitive endpoints in a two-generation study. They are therefore expected to be protective of a range of sensitive endpoints across lifestages and are considered appropriate for assessing risks for exposures to all lifestages and populations.

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WIL Research. (1996b). Support: An inhalation range-finding reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in rats, with cover letter dated 5/10/96 [TSCA Submission]. (1995-I0000-40919. 8305. WIL-51035. OTS0558459. 89960000133. 8EHQ-0596-13585. TSCATS/444267). Dow Corning Corporation.

WIL Research. (1997a). An inhalation range-finding reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in female rats, with cover letter dated 8/15/1997 [TSCA Submission]. (1997-I0000-42936. Study No.: 8463. OTS0559010. 86970000847. TSCATS/445058). Dow Corning Corporation.

WIL Research. (1997b). An inhalation range-finding reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in male rats [TSCA Submission]. (1997-I0000-43725; Study No.: 8462. WIL-51042. OTS0559387. 86-980000049). Dow Corning Corporation.

WIL Research. (1997c). An inhalation range-finding reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in male rats, with cover letter dated 12/3/1997 [TSCA Submission]. (1997-I0000-43726. 8601. WIL-51051. OTS0559399. 86-980000061. TSCATS/445540). Dow Corning Corporation.

WIL Research. (1999). An inhalation reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in female rats using multiple and single day exposure regimens, with cover letter dated 07/07/1999 [TSCA Submission]. (Report No. 1999-I0000-47049. Study No. 8864. OTS0573894. 86990000058. TSCATS/453901). Dow Corning Corp.

WIL Research. (2001a). 2-Generation inhalation reproductive toxicity and developmental neurotoxicity study of octamethylcyclotetrasiloxane (D4) in rats, w/cvr ltr dtd 010302 [TSCA Submission]. (2001-I0000-50855. 8713. OTS0574394. 86020000003. TSCATS/454735). Dow Corning Corporation.

WIL Research. (2001b). An inhalation study of the effects of octamethylcyclotetrasiloxane (D4) exposure on the preovulatory LH surge in ovariectomized female rats, with cover letter dated 121301 [TSCA Submission]. (2001-I0000-50592. 9377. WIL-51057. OTS0001419. FYI-OTS-0102-01419. TSCATS/454741). Dow Corning Corporation.

WIL Research. (2005). An inhalation F1 generation reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in female Sprague-Dawley rats [TSCA Submission]. (2004-I0000-54361. Study No.: 9621. FYI-0705-01498. 84050000019). Dow Corning Corporation.

Zhang, J; Falany, JL; Xie, X; Falany, CN. (2000). Induction of rat hepatic drug metabolizing enzymes by dimethylcyclosiloxanes. Chem Biol Interact 124: 133-147. http://dx.doi.org/10.1016/s0009-2797(99)00153-2

Appendix A EVIDENCE INTEGRATION TABLES

This appendix presents evidence integration tables for the human health hazard outcomes associated with D4. The data quality ratings for each individual study included in these evidence integration table is summarized as an overall quality determination (OQD).

Table_Apx A-1. Evidence Integration for Hepatic/Liver Effects[1]

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Evidence in Studies of Exposed Humans Considered for Deriving Toxicity Values				
• Cross-over controlled exposure study of 12 male and female volunteers (20-62 years old) exposed to D4 in corn oil orally (via syringe) or placebo once daily for 2 weeks to assess immune effects; blood collected at days 0, 7, and 14 as well as 1, 2, and 3 weeks after exposure ended was analyzed for clinical chemistry (Dow Corning, 1998c). OQD=High. • Cross-over controlled exposure study of 12 male and female volunteers (20-50 years old, nonsmoking) exposed by inhalation of D4 vapor or clean air via mouthpiece or via nasal delivery for 1 hour; blood collected immediately after exposure and 6 and 24 hours after exposure was		• Oral exposure to 12 mg D4 per day did not result in statistically significant changes in total protein, albumin, bilirubin, AST, alkaline phosphatase, LDH, or cholesterol at any time point. • Inhalation of D4 vapor did not result in statistically significant changes in total protein, albumin, bilirubin, ALT, AST, or LDH at any time point.	*Key findings:* Available data are limited to a 2-week controlled oral exposure study and a single 1-hour controlled inhalation exposure study in which small numbers of clinical chemistry endpoints were measured and no effects seen. These data are insufficient to draw conclusions regarding hepatic effects in humans. *Overall judgment for hepatic/liver effects based on human evidence*: • Indeterminate	*Overall judgment for hepatic/liver effects based on integration of information across evidence streams:* **Evidence suggests, but is not sufficient to conclude, that D4 exposure may cause hepatic/liver effects in humans under relevant exposure circumstances.**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
analyzed for clinical chemistry (University of Rochester, 1997). OQD=Medium.				
Evidence from *in vivo* Mammalian Animal Studies[2] Considered for Deriving Toxicity Values				
Studies that evaluated histopathology, liver weight, and clinical chemistry • Wistar rats exposed to aerosol; ≤5,074 mg/m^3 for 4 weeks (Civo Institute TNO, 1984). OQD=Medium. • F344 rats exposed to vapor (nose only); ≤8620 mg/m^3 for 1 month or ≤5,910 mg/m^3 13 weeks (RCC, 1995a, b). OQD=High.	Biological gradient/dose-response: • Increased incidences of hepatocellular hypertrophy and/or hyperplasia were seen in F344 rats after 1 week of exposure to ≥850 mg/m^3 (Dow Corning, 2002b), 1 month of exposure to 8620 mg/m^3 (RCC, 1995a), or 12 or 24 months of exposure to 8490 mg/m^3 (Jean and Plotzke, 2017).	Consistency • No treatment-related liver histopathology changes were observed in SD or F344 rats exposed to ≤8490 mg/m^3 for 4 or 13 weeks (RCC, 1995b; IRDC, 1991; Dow Corning, 1989b) or in male SD rats exposed to ≤8520 mg/m^3 for 1 generation (WIL Research, 1997b). • No treatment-related liver histopathology changes were	*Key findings:* Increased liver weights were consistently seen in rats after short-term, subchronic, or chronic exposure to D4 by inhalation and after short-term oral administration. Studies that examined clinical chemistry did not observe adverse effects on these parameters. Histopathology evaluations in rats	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• SD rats exposed to vapor; ≤8490 mg/m^3 for 13 weeks (IRDC, 1991; Dow Corning, 1989b). OQD=High. • F344 rats exposed to vapor; ≤8490 mg/m^3 for 12 or 24 months (Jean and Plotzke, 2017). OQD=High. • Albino rats exposed by diet; ~770-870 mg/kg-bw/day for 52 weeks (FDRL, 1966). OQD=Low. Studies that evaluated histopathology and liver weight • Female F344 rats exposed to vapor; ≤8500 mg/m^3 for 5 days or 8490 mg/m^3 for 1, 2, or 4 weeks (Dow Corning, 2002b). OQD=High. • SD rats exposed to vapor; ≤3588 mg/m^3 for 4 weeks (IRDC, 1991) or ≤8520 mg/m^3 for 1 or 2 generations (WIL Research, 2001a, 1997b). OQD=High. • CD-1 mice, LVG Golden Syrian hamsters, Hartley guinea pigs, and NZW	• Increased incidence of hepatocellular hypertrophy was seen at ≥6,090 mg/m^3 in F1 SD rats in a 2-generation study (WIL Research, 2001a). • Increased incidence of bile duct hyperplasia was seen at 8520 mg/m^3 in F1 male rats, with nonsignificant increase in F1 females. At 8520 mg/m^3, F1 males also had increased liver pigment that was "morphologically compatible with bile pigment" (WIL Research, 2001a). • Exposure-related increases in liver weights were observed in rats after short-term, subchronic, and chronic inhalation exposures at concentrations as low as 121 mg/m^3 (Jean and Plotzke, 2017; Jean et al., 2017; Dow Corning, 2002b, 2001b; WIL Research, 2001a; Dow Corning, 1999, 1997; WIL Research, 1997a, b; Dow Corning, 1996a, b; RCC, 1995a, b; IRDC, 1991; Dow Corning, 1989b; Civo Institute TNO, 1984).	observed in F344 female rats exposed to 301 mg/kg-bw/day by gavage for 14 days (Dow Corning, 2002a) or in albino rats exposed to ~770-870 mg/kg-bw/day by diet for 52 weeks (FDRL, 1966). • No treatment-related liver histopathology changes were observed in mice, rabbits, guinea pigs, or hamsters exposed to 8,460 mg/m^3 for 28 days (Dow Corning, 1989a), or in guinea pigs gavaged with 301 mg/kg-bw/day for 14 days (Dow Corning, 2002a). • Liver weights were not increased in rabbits or guinea pigs exposed by inhalation to ≤8550 mg/m^3 for 4-5 weeks (Dow Corning, 2001b, 1989a) or by oral administration at doses of 301 mg/kg-bw/day for 2 weeks (guinea pigs) or ≤1000 mg/kg-bw/day (rabbits) on GDs 7-19 (Dow Corning, 2002a; IRDC, 1993c). Biological plausibility and human relevance • No adverse effects on clinical chemistry parameters were	exposed by inhalation showed inconsistent findings, with some studies reporting increased incidences of hyperplasia and hypertrophy, and others reporting no treatment-related lesions. According to EPA (2002) guidance, increased liver weights and hepatocellular hypertrophy in the absence of clinical chemistry changes, other liver lesions, or a clear mechanism of toxicity are not considered adverse. There were isolated reports of other hepatic lesions in rats (bile duct hyperplasia in F1 rats in a 2-generation inhalation study and inflammation in a 2-week gavage study) but these were not supported by other studies. Limited studies did not report histopathology changes in the livers of mice, hamsters, rabbits, or guinea pigs exposed to D4 by inhalation or in guinea pigs exposed orally.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
rabbits exposed to vapor; 8,460 mg/m^3 for 28 days (Dow Corning, 1989a). OQD=Medium (hamsters) or High (all others). • Female Wistar rats and male and female SD rats exposed by gavage; 1,600 mg/kg-bw/day for 2 weeks (Mobay Chemical, 1991). OQD=Low. (Dow Corning, 1992c). OQD=High. • Female F344 rats and Hartley guinea pigs exposed by gavage; 301 mg/kg-bw/day for 14 days (Dow Corning, 2002a). OQD=High. Studies that evaluated liver weight and clinical chemistry • F344 rats exposed to vapor; ≤ 6,424 mg/m^3 for 28 days (Dow Corning, 1997) OQD=High. Studies that evaluated liver weight and gross pathology • Male SD rats exposed to vapor; ≤8,410 mg/m^3 for 1 generation (WIL Research, 1997c). OQD=High.	• Increased liver weights were observed in mice and hamsters exposed to ≥8,460 mg/m^3 for 4-5 weeks (Dow Corning, 2001b, 1989a). • Short-term gavage exposure to 1,600 mg/kg-bw/day resulted in increased incidences of inflammation and hepatocellular hyperplasia in male SD rats (Dow Corning, 1992c). • In a low-quality study in which incidences were not reported, hepatocellular hypertrophy and loss of glycogen were observed in female Wistar rats and both sexes of SD rats exposed to 1,600 mg/kg-bw/day for 2 weeks (Mobay Chemical, 1991). • Increased liver weights were seen in rats after 28 days of exposure to ≥10 mg/kg-bw/day by gavage (Virginia Commonwealth University, 1997).	seen in rats at inhaled concentrations ≤8490 mg/m^3 (Jean and Plotzke, 2017; Dow Corning, 1997; RCC, 1995a, b; IRDC, 1991; Dow Corning, 1989b; Civo Institute TNO, 1984) or in rats exposed to ~770-870 mg/kg-bw/day via diet (FDRL, 1966). Quality of the database • Clinical chemistry was not examined in studies of species other than rats.	*Overall judgment for hepatic/liver effects based on animal evidence*: • Slight	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Studies that evaluated only liver weight • Female SD rats exposed to vapor; ≤8490 mg/m^3 on GDs 6-15 (IRDC, 1993b). OQD=High. • SD rats, CD-1 mice, NZW rabbits, Dunkin Hartley guinea pigs, and Syrian Golden hamsters exposed to vapor; ≤8550 mg/m^3 for 5 weeks (Dow Corning, 2001b). OQD=Low (rats). High (all others). • F344 rats exposed to vapor; ≤8490 mg/m^3 for up to 28 days (Dow Corning, 1999, 1996a, b). OQD= Medium or High. • Female F344 rats exposed to vapor; 8,540 mg/m^3 for 14 months (Jean et al., 2017). OQD=Medium. • Female SD rats exposed to vapor; ≤8490 mg/m^3 for 1 generation (WIL Research, 1997a). OQD=High. • Pregnant and non-pregnant female SD rats exposed by gavage; ≤100 mg/kg-bw/day for 8 days (Falany and Li, 2005). OQD=Medium.				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• Female NZW rabbits exposed by gavage; ≤1000 mg/kg-bw/day on GDs 7-19 (IRDC, 1993c). OQD=High. • SD rats exposed by gavage; ≤100 mg/kg-bw/day for 4 days (Zhang et al., 2000) or ≤1,600 mg/kg-bw/day for 2 weeks (Dow Corning, 1990). OQD=Medium. • Female SD and F344 rats exposed by gavage; ≤1000 mg/kg-bw/day for 4 days (MPI Research, 1999). OQD=High. • F344 rats exposed by gavage; ≤300 mg/kg-bw/day for 28 days (Virginia Commonwealth University, 1997). OQD=High. • NZW rabbits exposed dermally; 1 mg/kg for 3 weeks (FDRL, 1979). OQD=Medium. <u>Studies that evaluated only gross pathology</u> • SD rats exposed to vapor; ≤8,540 mg/m^3 for 1 or 2 generations (WIL Research, 2005, 1996b). OQD= Medium or High.				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• Male Wistar rats exposed once by gavage to ≤10 mg/kg or by dermal application to ≤5000 mg/kg for 4 hours (Union Carbide, 1993). OQD=Medium. Studies Rated Uninformative • Rats, mice, and guinea pigs (unspecified strains), exposed to aerosol; 39,700 mg/m^3 for 2 weeks; (Hazleton Labs, 1971). • F344 rats exposed to aerosol (nose only); 13,250 mg/m^3 for 1 month; (RCC, 1995a). • F344 rats exposed to aerosol (nose only); 10,870 mg/m^3 for 13 weeks (RCC, 1995b). • Female Hilltop Wistar rats exposed by diet to unspecified doses for 7 days (Carnegie Mellon University, 1978). • Female NZW rabbits exposed by gavage; ≤1000 mg/kg-bw/day for 14 days (Dow Corning, 1992a). • Rabbits (unspecified strain), exposed via diet (1				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
% in food, dose could not be calculated) for 36 weeks (FDRL, 1965).				
Evidence in Mechanistic Studies and Supplemental Information				
Metabolite studies • Combined repeat-dose and reproductive/developmental screening test in rats exposed orally to D4 metabolite DMSD (Dow Corning, 2009a). • SD rats exposed by gavage to ≤1200 mg/kg-bw/day DMSD for 14 days (Dow Corning, 2007). CYP induction • CYP enzyme activity following inhalation or oral gavage exposure to D4 (Dow Corning, 2002a, 2001b; Zhang et al., 2000; Dow Corning, 1999; McKim et al., 1998; Dow Corning, 1996b). • CYP protein levels (Zhang et al., 2000; Dow Corning, 1999; McKim et al., 1998; Dow Corning, 1996b). • CAR and PXR reporter gene assays in HepG2 cells (CIIT, 2005; Dow Corning, 2005a).	• In rats exposed to 250 mg/kg-bw/day DMSD, increased liver weights and hepatocellular hypertrophy were seen, with more severe effects (increased serum ALT, liver inflammation and/or vacuolation, protoporphyrinosis, and bile duct hyperplasia) occurring at 500 mg/kg-bw/day (Dow Corning, 2009a). Liver weights were increased in SD rats at ≥600 mg/kg-bw/day after 28 days of oral exposure to DMSD (Dow Corning, 2007).	• Hepatomegaly and hepatocellular hypertrophy were associated with CYP induction (measured by increased enzyme activities, CYP2B1/2 and CYP3A1/2 protein levels, and CAR and PXR expression) and cell proliferation (BrdU and PCNA labeling).	*Key findings*: The D4 metabolite, DMSD, may play a role in the liver effects in animals exposed to D4. Hepatomegaly and hepatocellular hypertrophy after D4 exposure were associated with CYP induction and cell proliferation. No data were available to evaluate other liver effects (pigmentation and bile duct hyperplasia). *Overall judgment for hepatic/liver effects based on mechanistic evidence*: • Slight	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Cell proliferation • Hepatic cell proliferation *in vivo* study measured by BrdU and PCNA labeling index (Dow Corning, 2002b, 2001b; McKim et al., 2001a).				

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BrdU: bromodeoxyuridine; CAR: constitutive androstane receptor; CYP: cytochrome P450; DMSD: dimethylsilanediol; GD: gestation day; LDH: lactate dehydrogenase; PCNA: proliferating cell nuclear antigen; PXR: pregnane X receptor; SD: Sprague-Dawley or Sprague-Dawley-derived; NZW: New Zealand White

[1] Reported effects are statistically significant unless otherwise noted.
[2] Except where specified, all studies included both male and female animals. Inhalation exposures were whole-body except where noted.

Related HERO IDs (linked in Distiller)
Linked to (Jean and Plotzke, 2017): 7310083, 6991447
Linked to (Dow Corning, 1997): 6834019
Linked to (Dow Corning, 1999): 5884183
Linked to (Dow Corning, 1996a): 5887837
Linked to (Dow Corning, 2002b): 1415970
Linked to (MPI Research, 1999): 1310507.
Linked to (RCC, 1995b): 6833996
Linked to (WIL Research, 2001a): 7002248, 4924646, 7002247
Linked to (WIL Research, 1996b): 5887777

4146 **Table_Apx A-2. Evidence Integration for Respiratory Tract Effects[1]**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Evidence in Studies of Exposed Humans Considered for Deriving Toxicity Values				*Overall judgment for respiratory tract effects based on integration of information across evidence streams:* **Evidence suggests, but is not sufficient to conclude, that D4 exposure may cause respiratory tract effects in humans under relevant exposure circumstances.**
• Controlled exposure study of 12 male and female volunteers (20-50 years old, nonsmoking) exposed by inhalation of D4 vapor or clean air via mouthpiece or via nasal delivery for 1 hour; volunteers recorded symptoms of respiratory function and spirometry (FVC, FEV1) was measured before and after exposure and 24 hours after exposure (University of Rochester, 1997). ODQ=TBD.		No statistically significant changes in symptom score, FVC, or FEV1 were observed after D4 exposure.	*Key findings:* Available data are limited to a single volunteer study that showed no changes in respiratory function after 1 hour of inhalation exposure. *Overall judgment for respiratory tract effects based on human evidence*: • Indeterminate	
Evidence from *in vivo* Mammalian Animal Studies[2] Considered for Deriving Toxicity Values				
Studies that evaluated upper and lower respiratory tract histopathology (including nasal cavity) and lung weights • Wistar rats exposed to aerosol; ≤5,074 mg/m^3 for 4 weeks (Civo Institute TNO, 1984). OQD=Medium. • SD rats exposed to vapor; ≤8490 mg/m^3 for up to 13 weeks (IRDC, 1991; Dow Corning, 1989b). OQD=High. • F344 rats exposed to vapor; ≤8490 mg/m^3 for 3 months (Dow Corning, 1997), 12 months, or 24 months (Jean and Plotzke, 2017). OQD = High.	Nasal cavity: biological gradient/dose-response • Chronic exposure resulted in exposure-related increased incidences of nasal lesions including eosinophilic globules in female rats exposed to ≥360 mg/m^3 for 24 months and goblet cell hyperplasia in the respiratory epithelium of males exposed to ≥1,820 mg/m^3 for 24 months. Additional nasal lesions, including squamous epithelial hyperplasia and	Nasal cavity: biological gradient/dose-response • The absence of treatment-related nasal histopathology changes in rats after short-term and subchronic inhalation exposures to concentrations up to 8490 mg/m^3 (Dow Corning, 1997; IRDC, 1991; Dow Corning, 1989b; Civo Institute TNO, 1984) suggests that the lesions develop only with long exposure durations.	*Key findings:* Rats exposed to D4 by inhalation exhibited increased incidences of nasal lesions after chronic (at least 12 months), but not subchronic exposure. Some, but not all, studies of rats exposed by inhalation reported increased incidences of inflammation and/or histiocytosis in the lungs after short-term, subchronic, and chronic exposure.	

Studies that evaluated lower respiratory tract histopathology (and lung weights) • CD-1 mice, NZW rabbits, Hartley guinea pigs, and LVG Golden Syrian hamsters exposed to vapor; 8,460 mg/m^3 for 28 days (Dow Corning, 1989a). OQD=Medium-High. • F344 rats exposed to vapor (nose only); ≤8620 mg/m^3 for 1 month (RCC, 1995a) or ≤5,910 mg/m^3 for 13 weeks (RCC, 1995b). OQD = High. • SD rats exposed to vapor; ≤8520 mg/m^3 for 1 or 2 generations (WIL Research, 2001a, 1997c). OQD=High. • Female Wistar rats exposed by gavage; 1,600 mg/kg-bw/day for 2 weeks (Mobay Chemical, 1991). OQD=Low. • Albino rats exposed via diet; ~770-870 mg/kg-bw/day for 52 weeks (FDRL, 1966). OQD=Low. Studies that evaluated only lung weight • Female F344 rats exposed to vapor; 8,540 mg/m^3 for 14 months (Jean et al., 2017). OQD=Medium. • SD rats exposed to vapor; ≤8490 mg/m^3 for 1 generation (WIL Research, 1997a, b). OQD=High.	suppurative inflammation were seen at 8490 mg/m^3 in one or both sexes after 12 or 24 months (Jean and Plotzke, 2017). Lung: biological gradient/dose-response • Increased incidences of lung hemorrhage at 8490 mg/m^3 and subpleural chronic inflammation at 120, 360, and 8490 mg/m^3 (but not 1,820 mg/m^3) in female F344 rats after 24 months of exposure (Jean and Plotzke, 2017). • Increased incidences of alveolar inflammation in F344 rats after 1 month at ≥2780 mg/m^3 (RCC, 1995a). • Increased incidences of chronic interstitial lung inflammation and alveolar macrophage foci in F344 rats after 13 weeks at ≥1,480 mg/m^3 (RCC, 1995b). • Increased incidence of alveolar histiocytosis at 8490 mg/m^3 in F0 female SD rats and at 8520 mg/m^3 in both sexes of F1 rats. Nonsignificant increased incidences of interstitial inflammation in both sexes of both F0 and F1	Nasal cavity: quality of the database • Nasal cavity histopathology was not examined in studies of species other than rats. Lung: consistency • No treatment-related lung histopathology changes were observed in Wistar rats exposed to aerosol ≤5,074 mg/m^3 for 4 weeks (Civo Institute TNO, 1984), in SD or F344 rats exposed to vapor ≤8520 mg/m^3 for 13 weeks (Dow Corning, 1997; IRDC, 1991; Dow Corning, 1989b) or in SD rats exposed to vapor ≤8520 mg/m^3 for 1 generation (WIL Research, 1997c). • No treatment-related lung histopathology changes were observed in mice, rabbits, guinea pigs, or hamsters exposed to 8,460 mg/m^3 for 28 days (Dow Corning, 1989a) • Lung weights were not increased with exposure to D4 at concentrations up to ≤8520 mg/m^3 in any study.	*Overall judgment for respiratory tract effects based on animal evidence*: • Moderate (Nasal Effects) • Slight (Lung Effects)	

• Male Wistar rats, exposed once by gavage; ≤10 mg/kg (Union Carbide, 1993). OQD=Medium. • NZW rabbits exposed dermally; 1000 mg/kg for 3 weeks (FDRL, 1979). OQD=Medium <u>Studies that evaluated only gross pathology</u> • SD rats exposed to vapor; 8560 mg/m^3 for 1 or 2 generations (WIL Research, 2005, 1996b). OQD=High. <u>Studies rated uninformative</u> • Acute-duration vapor exposures in male Wistar rats (Union Carbide, 1993; Carnegie Mellon University, 1972). • Rats, mice, and guinea pigs (unspecified strains), exposed to aerosol; 39,700 mg/m^3 for 2 weeks (Hazleton Labs, 1971). • F344 rats exposed to aerosol (nose only); 13,250 mg/m^3 for 1 month (RCC, 1995a). • F344 rats exposed to aerosol (nose only); 10,870 mg/m^3 for 13 weeks (RCC, 1995b). • SD rats exposed by gavage; 1,600 mg/kg-bw/day for 2 weeks (Mobay Chemical, 1991). • Female NZW rabbits exposed by gavage; ≤1000 mg/kg-bw/day for 14 days (Dow Corning, 1992a). • Rabbits (unspecified strain), exposed via diet (1 % in food,	generations (WIL Research, 2001a). • Increased incidence of interstitial inflammation with alveolar macrophages in the lungs of Wistar rats after 2 weeks of gavage exposure at 1,600 mg/kg-bw/day (Mobay Chemical, 1991).			

dose could not be calculated) for 36 weeks (FDRL, 1965).				
Evidence in Mechanistic Studies and Supplemental Information				
• None			Indeterminate	
FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity				

[1] Reported effects are statistically significant unless otherwise noted.
[2] Except where specified, all studies included both male and female animals. Inhalation exposures were whole-body except where noted.

Related HERO IDs (linked in Distiller)

Linked to (Jean and Plotzke, 2017): 7310083, 6991447
Linked to (Dow Corning, 1997): 6834019
Linked to (RCC, 1995b): 6833996
Linked to (WIL Research, 2001a): 7002248, 4924646, 7002247
Linked to (WIL Research, 1996b): 5887777

4156 **Table_Apx A-3. Evidence Integration for Reproductive Effects[1]**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Evidence in Studies of Exposed Humans Considered for Deriving Toxicity Values				*Overall judgment for reproductive effects based on integration of information across evidence streams:* **Evidence indicates that D4 likely causes effects on female reproductive system structure and function in humans under relevant exposure circumstances.** **Evidence suggests, but is not sufficient to conclude, that D4 may cause effects on male reproductive structure without deficits to male reproductive function in humans under relevant exposure circumstances.**
• None		• Indeterminate		
Evidence from *in vivo* Mammalian Animal Studies[2] Considered for Deriving Toxicity Values				
Effects on Male Reproductive Organs				
Studies that evaluated reproductive organ histopathology and weights and sperm parameters • A 2-generation study performed with male SD rats exposed to vapor; ≤8490 mg/m^3 (F0) or ≤8520 mg/m^3 (F1) for at least 70 days prior to mating (WIL Research, 2001a). ODQ=High. • Range-finding one-generation studies in male SD rats exposed to vapor; 8490 mg/m^3 for 28 days prior to mating or ≤8420 mg/m^3 for 70 days prior to mating (WIL Research, 1997b, 1996a). ODQ=High. Studies that evaluated reproductive organ histopathology and weights • Male F344 rats exposed to vapor; ≤8490 mg/m^3 for 12 or 24 months (Jean and Plotzke, 2017). ODQ=High. • Male SD rats exposed to vapor; ≤3588 mg/m^3 for 13 weeks (IRDC, 1991). ODQ=High. • Male F344 rats exposed to vapor (nose-only); ≤5,910 mg/m^3 for 13 weeks (RCC, 1995b). ODQ=High.	Biological gradient/dose-response: • Increased incidence and severity of testicular interstitial (Leydig) cell hyperplasia were observed in F344 rats after 24 months of exposure to ≥1,820 mg/m^3 (Jean and Plotzke, 2017). • Relative (but not absolute) testes weights were increased in F344 rats exposed to 8490 mg/m^3 for 12 or 24 months (Jean and Plotzke, 2017). • Absolute and relative testes weights were increased in F344 rats after 13 weeks of exposure to 5,910 mg/m^3 (RCC, 1995b). • Relative (but not absolute) testes weights were increased in LVG Golden Syrian hamsters exposed to 8,460 mg/m^3 for 28 days (Dow Corning, 1989a).	Consistency: • No histopathological changes or male reproductive organ weight changes were observed in F344 rats exposed to ≤6,424 mg/m^3 for 28 days (Dow Corning, 1997; RCC, 1995a). No histopathological changes or male reproductive organ weight changes were observed in SD rats exposed to ≤8520 mg/m^3 in one- and 2-generation studies (WIL Research, 2001a, 1997b, c, 1996a, b) or ≤8490 mg/m^3 for 13 weeks (IRDC, 1991; Dow Corning, 1989b), or in Wistar rats exposed to ≤5,074 mg/m^3 for 28 days (Civo Institute TNO, 1984). • No histopathological changes or male reproductive organ weight changes were observed in Hartley guinea pigs, NZW rabbits, or CD-1 mice exposed to 8,460 mg/m^3 for 28 days (Dow Corning, 1989a). • No histopathology changes or reproductive organ weight changes were observed in rats exposed by diet for 1 year (FDRL, 1966).	*Key findings:* D4 exposure resulted in increased incidence and severity of testicular interstitial (Leydig) cell hyperplasia and increased testes weights in F344 rats. Leydig cell hyperplasia is common in F344 rats, and rats may be more sensitive than humans to this effect. No histopathology or reproductive organ weight changes were observed in reproductive toxicity studies of SD rats or in short-term studies of Wistar rats, mice, guinea pigs, or rabbits. *Overall judgment for male reproductive tract effects based on animal evidence*: • Slight	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• Male SD rats exposed to vapor; ≤8490 mg/m^3 for 13 weeks (Dow Corning, 1989b). ODQ=High. • Male F344 rats exposed to vapor; ≤6,424 mg/m^3 for 28 days (Dow Corning, 1997). OQD=High. • Male F344 rats exposed to vapor (nose-only); ≤8620 mg/m^3 for 28 days (RCC, 1995a). OQD=High. • Male LVG Golden Syrian hamsters, Hartley guinea pigs, NZW rabbits, and CD-1 mice exposed to vapor; 8,460 mg/m^3 for 28 days (Dow Corning, 1989a). OQD=Medium (hamsters) or High (all others). • Male Wistar rats exposed to aerosol; ≤5,074 mg/m^3 for 28 days (Civo Institute TNO, 1984). OQD=Medium. • Male Albino rats exposed by diet; ~770-870 mg/kg-bw/day for 52 weeks (FDRL, 1966). OQD=Low. Studies that evaluated reproductive organ weights and/or gross pathology • Range-finding one-generation studies in male SD rats exposed to vapor; ≤8,410 mg/m^3 for 70 days prior to mating or ≤8490 mg/m^3 for 28 days prior to mating (WIL Research, 1997c). OQD=High (WIL Research, 1996b). OQD =Medium. • NZW rabbits exposed dermally; 1 mg/kg for 3 weeks (FDRL, 1979). OQD=Medium.		Biological plausibility/human relevance: • Interstitial (Leydig) cell hyperplasia is a common lesion in aging F344 rats (Maronpot et al., 2016; Creasy et al., 2012). • Rats may be more sensitive than humans to LH-mediated Leydig cell hyperplasia, in part because humans have fewer LH receptors per Leydig cell than rats (Thayer and Foster, 2007).		

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Studies rated uninformative • F344 rats exposed to aerosol (nose only); 13,250 mg/m^3 for 1 month (RCC, 1995a). • F344 rats exposed to aerosol (nose only); 10,870 mg/m^3 for 13 weeks (RCC, 1995b). • A palatability study in male SD rats exposed via diet at 2.1 % for 28 days (Dow Corning, 1988). • Male rabbits (strain not specified) exposed via diet (1 % in food, dose could not be calculated) for 36 weeks (FDRL, 1965). • 7-day gavage study in male rats evaluated seminal fluid, seminal vesicle, and prostate weights (Dow Corning, 1972). OQD =TBD.				
Effects on Female Reproductive Organs				
Studies that evaluated reproductive organ histopathology and weights and estrous cyclicity • A 2-generation study in female SD rats exposed to vapor; 8,540 mg/m^3 beginning 7 days prior to mating (WIL Research, 2005). OQD=High. • A 2-generation study in female SD rats exposed to vapor ≤8490 mg/m^3 (F0) or ≤8520 mg/m^3 (F1) for at least 70 days prior to mating	Biological plausibility/human relevance: • The proportion of non-ovulating female SD rats increased after a 3-day exposure to ≥8,492 mg/m^3 (nominal), and the mean number of eggs in the oviducts of treated rats decreased in a dose-dependent manner (Quinn et al., 2007a). • Reduced ovarian corpora lutea score was observed in F344 rats	Consistency: • No changes to estrous cyclicity were identified in a range-finding one-generation study in rats exposed to ≤8490 mg/m^3 (WIL Research, 1997a). • No changes to gross and/or histopathological findings in female reproductive organs were observed in rats in several after subchronic exposures to ≤8,492 mg/m^3 (Dow Corning, 1997; WIL Research, 1997a, 1996b; IRDC,	*Key findings:* D4 exposure resulted in decreased ovulation, decreased corpora lutea, estrous cycle irregularities, increased incidences of vaginal mucification and ovarian atrophy, and decreased ovary weight. These effects were observed in multiple repeat-dose and multigeneration studies. Increased uterine weight and increased incidence	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
(WIL Research, 2001a). OQD=High. • A range-finding one-generation study in female SD rats exposed to vapor; ≤8490 mg/m^3 for 70 days prior to mating, throughout mating to GD 21, and then PNDs 5-21 (WIL Research, 1997a). OQD=High. • Female F344 rats exposed to vapor (nose-only); ≤10,870 mg/m^3 for 13 weeks (Burns-Naas et al., 2002; RCC, 1995b). OQD=High. • Female F344 rats exposed to vapor; 8,540 mg/m^3 for 14 months (Jean et al., 2017). OQD=Medium. <u>Studies that evaluated reproductive organ histopathology and weights</u> • Female SD rats exposed to vapor; ≤10,918 mg/m^3 nominal concentration for 3 days (Quinn et al., 2007a). OQD=Medium. • Female F344 rats exposed to vapor; ≤8490 mg/m^3 for 12 or 24 months (Jean and Plotzke, 2017). OQD=High. • Female SD rats exposed to vapor; ≤3588 mg/m^3 for 13 weeks (IRDC, 1991). OQD=High. • Female SD rats exposed to vapor; ≤8490 mg/m^3 for 13 weeks (Dow Corning, 1989b). OQD=High.	exposed to ≥2780 mg/m^3 for 1 month (RCC, 1995a). • Absence of corpora lutea in the ovary and presence of numerous developing follicles with no evidence of recent ovulation were observed in F344 rats exposed to 10,870 mg/m^3 for 13 weeks (Burns-Naas et al., 2002). • Decreased number of corpora lutea was observed at 8490 mg/m^3 in a one-generation study (WIL Research, 1996a) and in F1 females exposed postnatally to 8,540 mg/m^3 in a 2-generation study (WIL Research, 2005). • Decreased corpora lutea count with pregnancy was observed in F1 rats at >6,090 mg/m^3 (WIL Research, 2001a). • In phased one-generation studies, decreased numbers of corpora lutea were observed at ≥3650mg/m^3 with exposure 28 days prior to mating through gestation (Dow Corning, 1998b) and ≥8440 mg/m^3 with exposure 3 days prior to mating to GD 3 (WIL Research, 1999; Dow Corning, 1998b). • Decreased incidence of antral-size atretic follicles was observed in F344 rats exposed to 8,540 mg/m^3 for 14 months (Jean et al., 2017).	1991; Dow Corning, 1989b; Civo Institute TNO, 1984) or after a 1-year oral exposure to ~770-870 mg/kg-bw/day (FDRL, 1966). • No changes to female reproductive organ weights were identified in one and 2- generation studies in SD rats exposed to ≤8,540 mg/m^3 (WIL Research, 2005, 2001a, 1997a, 1996a) or in repeated-dose studies with up to 14-month exposures ≤8620 mg/m^3 (Jean et al., 2017; Dow Corning, 1997; RCC, 1995a), or a 1-year oral exposure at ~770-870 mg/kg-bw/day (FDRL, 1966). <u>Quality of the Database:</u> • Available studies of female reproductive effects endpoints were conducted in rats; no other species have been evaluated for these effects.	of endometrial epithelial hyperplasia and cervical squamous epithelial cell hyperplasia were also observed in rats exposed chronically to D4. Not all studies identified effects to the female reproductive system following D4 exposure. Inconsistencies across studies may be due to differences in strains, exposure durations, or timing of evaluations. For example, ovarian atrophy was only identified in F344 rats following at least subchronic exposures. *Overall judgment for female reproductive tract effects based on animal evidence*: • Moderate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• Female F344 rats exposed to vapor (nose-only); ≤8620 mg/m^3 for 28 days (RCC, 1995a). OQD=High. • Female F344 rats exposed to vapor; ≤6,424 mg/m^3 for 28 days (Dow Corning, 1997). OQD=High. • Female Albino rats exposed by diet; ~770-870 mg/kg-bw/day for 52 weeks (FDRL, 1966). OQD=Low. Studies that evaluated reproductive organ gross pathology and weights • A phased one-generation study in female SD rats exposed to vapor; ≤8520 mg/m^3 from 28 days prior to mating until GD 19 (overall phase), 31 days to 3 days prior to mating (ovarian phase), 3 days prior to mating to GD 3 (fertilization phase), or on GDs 2-5 (implantation phase) (Dow Corning, 1998b). OQD=High. • A phased one-generation study performed with in female SD rats exposed to vapor; ≤8490 mg/m^3 for 4, 3, 2, or 1 days prior to mating; 3 days to 1 day prior to mating; 3 days prior to mating to GD 3; GD 0, 1, or 2; or GD 0-2 (WIL Research, 1999). OQD=Medium. • A range-finding one-generation study in female SD rats exposed to vapor; 8490 mg/m^3 for 28 days prior	• Estrous cycle irregularities were noted in nonpregnant F1 females exposed to 8520 mg/m^3 (WIL Research, 2001a). • Estrous cycle disruptions included increased estrous cycle length in F1 females exposed to 8520 mg/m^3 (WIL Research, 2001a), increased cumulative number of estrogenic (proestrus, estrus) days and percentage of days in an estrogenic state in aged F344 rats exposed to 8,540 mg/m^3 for 14 months (Jean et al., 2017), and an increased incidence of F344 rats in diestrus following a 13-week exposure to 10,870 mg/m^3 (Burns-Naas et al., 2002). • Increased incidence of vaginal mucification was seen in F344 rats at 10,870 mg/m^3 for 3 months (RCC, 1995b) or ≥2780 mg/m^3 for 1 month (RCC, 1995a). • Increased incidence of ovarian atrophy and/or congestion was observed in F344 rats at 8490 mg/m^3 after 24 months (Jean and Plotzke, 2017) and at 10,870 mg/m^3 after 13 weeks (RCC, 1995b). • Decreases in ovary weights were observed in SD rats exposed to 10,918 mg/m^3 (nominal) for 3 days through proestrus (data presented qualitatively) (Quinn et al., 2007a), to 8,440 mg/m^3 in the			

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
to mating, throughout mating to GD 21, and then PNDs 4-21 (WIL Research, 1996a). OQD=High. • NZW rabbits exposed dermally; 1 mg/kg for 3 weeks (FDRL, 1979). OQD=Medium. Studies that evaluated only reproductive organ histopathology • Female Wistar rats exposed to aerosol; ≤5,074 mg/m^3 for 28 days (Civo Institute TNO, 1984). OQD=Medium. Studies that evaluated only reproductive organ gross pathology • A range-finding one-generation study in female SD rats exposed to vapor; ≤8490 mg/m^3 for 28 days prior to mating, throughout mating to GD 21 and then PNDs 4-21 (WIL Research, 1996b). OQD=Medium. Studies rated uninformative • F344 rats exposed to aerosol (nose only); 13,250 mg/m^3 for 1 month (RCC, 1995a). • F344 rats exposed to aerosol (nose only); 10,870 mg/m^3 for 13 weeks (RCC, 1995b). • Female rabbits (strain not specified) exposed via diet (1% in food, dose could not be	fertilization phase of a phased one-generation study (Dow Corning, 1998b), and to 10,870 mg/m^3 for 3 months (RCC, 1995b). Biological gradient/dose-response: • Increased uterine weights in F344 rats exposed to 8490 mg/m^3 for 24 months (Jean and Plotzke, 2017). • Increased incidences of endometrial epithelial hyperplasia and cervical squamous epithelial cell hyperplasia in F344 rats at 8490 mg/m^3 after a 24-month exposure (Jean and Plotzke, 2017). • Increased incidences of mammary gland duct ectasia in F1 females SD rats exposed to 8560 mg/m^3 (WIL Research, 2005). • Increased severity of vaginal epithelial thickness in F344 rats exposed to 8,540 mg/m^3 for 14 months (Jean et al., 2017).			

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
calculated) for 36 weeks (FDRL, 1965). • Female NZW rabbits exposed by gavage; ≤1000 mg/kg-bw/day for 14 days (Dow Corning, 1992a).				
Effects on Reproductive Function				
• A 2-generation study in male and female SD rats exposed to vapor; ≤8520 mg/m^3 for 70 days prior to mating and during mating and gestation until GD 20, and during PNDs 5-21 (WIL Research, 2001a). OQD=High. • A 2-generation study in female SD rats exposed to vapor; ≤8560 mg/m^3 beginning 7 days prior to mating, with F1 exposed beginning PND 22 or 44 (WIL Research, 2005). OQD=High. • A phased one-generation study in female SD rats exposed to vapor; ≤8520 mg/m^3 from 28 days prior to mating until GD 19 (overall phase), 31 days to 3 days prior to mating (ovarian phase), 3 days prior to mating to GD 3 (fertilization phase), or on GDs 2-5 (implantation phase) (Dow Corning, 1998b). OQD=High. • A phased one-generation study performed with female SD rats exposed to vapor; 8490 mg/m^3 for 4, 3, 2, or 1 days prior to mating; 3 days to 1 day prior to mating; 3 days prior to mating to GD 3; GDs 0, 1,	Biological gradient/dose-response: • Decreased female mating and fertility[3] indices were observed in F1 matings at 8520 mg/m^3, with decreased fertility index at ≥6,090 mg/m^3 in the second mating, in a 2-generation study. These effects are reflected in decreased numbers of litters and/or litter sizes in these groups (WIL Research, 2001a). Decreased fertility index was also observed when females were exposed to 8490 mg/m^3 1 day prior to mating in a phased one-generation study (WIL Research, 1999). • Decreased numbers of implantation sites were observed at 8490 mg/m^3 in all one- and 2-generation studies in which females were exposed (WIL Research, 2005, 2001a, 1997a, 1996a, b). • In a phased one-generation study, decreased numbers of implantation sites were observed at ≥6100 mg/m^3 with exposure 28 days prior to mating through gestation and at 8440 mg/m^3 with	Consistency: • No effects on female mating or fertility indices in one-generation studies in which females were exposed ≤8490 mg/m^3 (WIL Research, 1997a, 1996a, b). • No effects on gestation length were observed in one-generation reproduction studies; where increases relative to the concurrent control were noted, the treatment group values were within the historical control ranges (WIL Research, 2001a, 1997a, b, c, 1996a, b). • No clear effects on neonatal pup survival were observed in one-generation studies, in F1 litters or in the first F2 litters in 2-generation studies. Where decreases relative to the concurrent control were noted, the treatment group values were within the historical control ranges (WIL Research, 1997a, b, c, 1996a, b). Quality of the Database:	*Key findings:* Exposure of female SD rats to D4 concentrations ≥6,090 mg/m^3 resulted in decreases in fertility, implantations, numbers of litters, and/or numbers of pups per litter in multiple one- and 2-generation reproduction studies. Decreases in fertility, implantation sites, and litter sizes may be related to effects on female reproduction including estrous cycle disruptions, failure to ovulate, and/or decreases in numbers of corpora lutea (see *Effects on Female Reproductive Organs.)* D4 did not adversely affect fertility or reproductive function in exposed male SD rats mated with untreated females.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
or 2; or GDs 0-2 (WIL Research, 1999). OQD=Medium. • Range-finding one-generation studies in male SD rats exposed to vapor; ≤8420 mg/m^3 for 70 days prior to mating (WIL Research, 1997b, c). OQD=High. • A range-finding one-generation study in female SD rats exposed to vapor; ≤8490 mg/m^3 for 70 days prior to mating, throughout mating to GD 21, and then PNDs 5-21 (WIL Research, 1997a). OQD=High. • Range-finding one-generation studies in male and female SD rats exposed to vapor; 8490 mg/m^3 for 28 days prior to mating, throughout mating to GD 21, and then PNDs 4-21 (WIL Research, 1996a, b). OQD= High or Medium.	3 days prior to mating to GD 3. Both exposure regimens resulted in increased pre-implantation losses, decreased numbers of pups per litter, and decreased gravid uterine weights at ~8440 mg/m^3. In the group exposed 3 days prior to mating to GD 3, increased early resorptions and post-implantation losses were observed at 8440 mg/m^3 (Dow Corning, 1998b). • Exposure of female rats from 3 days prior to mating through GD 3 resulted in increased number of small implantation sites and decreased gravid uterine weight at 8490 mg/m^3 (WIL Research, 1999). • Decreases in maternal body weights and/or body weight changes during gestation and/or lactation were observed at ~8490 mg/m^3 in multiple studies (WIL Research, 2005, 2001a, 1997a, 1996a, b). • Increased gestation lengths were observed at 8,540 mg/m^3 in F0 dams in a 2-generation study (WIL Research, 2005). • Decreased neonatal survival was observed in F2 pups (in some, but not all matings) at ≥8520 mg/m^3 in 2-generation studies (WIL Research, 2005, 2001a).	• Available studies of reproductive function endpoints were conducted in SD rats; no other strains or species have been evaluated for these effects.	*Overall judgment for effects on reproductive function based on animal evidence*: • Moderate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
	Biological plausibility and human relevance: • Evidence for fertility effects mediated by female exposure is provided in one- and 2-generation studies of male-only exposure in which neither mating nor fertility was affected (WIL Research, 2001a, 1997b, c, 1996a, b). • Decreases in fertility, implantation sites, and litter sizes may be related to estrous cycle disruptions, failure to ovulate, and/or decreases in corpora lutea as described above under *Effects on Female Reproductive Organs.*			
Evidence in Mechanistic Studies and Supplemental Information				
Mechanistic Evidence for Male Reproductive Effects				
• A Hershberger assay in castrated male F344 rats exposed to vapor; 8490 mg/m^3, 16 hours/day for 10 consecutive days (Quinn et al., 2007b). • A gavage study in F344 rats exposed to ≤1200 mg/kg-bw/day DMSD (D4 metabolite) for 14 days (Dow Corning, 2007).	• Oral exposure to DMSD resulted in decreased absolute, but not relative, testes weight in F344 rats (Dow Corning, 2007).	• Reproductive tract organ weights were not altered in castrated rats (Quinn et al., 2007b).	*Key findings*: D4 did not induce androgenic effects in a Hershberger assay, and the results of a single study of DMSD did not provide evidence for a role for this metabolite in male reproductive effects of D4. *Overall judgment for male reproductive effects based on mechanistic evidence*: • Indeterminate	
Mechanistic Evidence for Female Reproductive Effects				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Suppression of the LH surge • Ovariectomized rats with a with a subcutaneous estradiol implant, exposed by inhalation to ≤11,100 mg/m^3 for 6 hours (WIL Research, 2001b). • Female rats exposed by inhalation to ≤10,918 mg/m^3 (nominal conc.), for 3 days (Quinn et al., 2007a). ER agonism • Immature female rats orally exposed to ≤1000 mg/kg-bw/day for 4 days (gavage), uterine weight and epithelial cell height (McKim et al., 2001b). • Immature female rats orally exposed to ≤1000 mg/kg-bw/day for 4 days (gavage), uterine weight and epithelial cell height (MPI Research, 1999). • Immature female rats exposed s.c. to ≤1000 mg/kg-bw/day for 4 days, uterine weight, uterine levels of calcium binding protein 9K (CaBP-9K) and progesterone receptor (PR) (Lee et al., 2015). • Ovariectomized rats exposed by inhalation to 8490 mg/m^3 16 hours/day for 3 days, uterine weight (Quinn et al., 2007b). • Ovariectomized wild type and ERα knockout mice orally exposed to ≤1000 mg/kg-bw/day for 3 days (gavage), uterine weight (He et al., 2003).	Suppression of the LH surge • Suppression of the preovulatory LH surge (Quinn et al., 2007a; WIL Research, 2001b). • Suppression of the preovulatory LH surge on the day of proestrus was associated with decreased ovulation, persistence of mature follicles, and increased serum estradiol (Quinn et al., 2007a). ER agonism • Increased uterine weight in rats and mice exposed orally (He et al., 2003; McKim et al., 2001b; MPI Research, 1999) and in rats exposed by inhalation (Quinn et al., 2007b); increased epithelial cell height was correlated with increased weight (Quinn et al., 2007b; McKim et al., 2001b; MPI Research, 1999). • No increase in uterine weight in ERα knockout mice, effect in wild type mice was blocked by an ER antagonist (He et al., 2003). • Increased mRNA and protein levels of CaBP-9K and PR in rat uterus *in vivo* and in GH3 cells *in vitro* as markers of estrogenicity, blocked by an estrogen receptor antagonist (Lee et al., 2015). • Demonstrated binding to the ERα receptor, no effect on the ERβ receptor (Quinn et al., 2007b; He et al., 2003); increased activity in	ER agonism • No increase in uterine weight in rats exposed by s.c. injection (Lee et al., 2015). Dopamine agonism • Inconsistent results in reserpine pretreated rats; one study reduced serum prolactin immediately following exposure (Dow Corning, 2005b); no reduction in serum prolactin in a study using the same rat strain and study protocol (Dow Corning, 2010). • *In vitro* assays showed that D4 does not act as dopamine receptor agonist or antagonist but may indirectly influence some portions of the dopamine pathway (GTPγS activity; cAMP production) (Domoradzki, 2011; Baker, 2010; Thackery, 2009). • Studies that measured D4 concentrations in culture medium showed that concentrations declined with incubation time and were often much less than nominal concentrations (Baker, 2010; Thackery, 2009). Not all *in vitro* studies measured concentrations. Metabolite studies • DMSD exposure did not affect reproductive parameters at doses up to 500 mg/kg-bw/day (Dow Corning, 2009a) or ovary weight	*Key findings*: Mechanistic data suggest that reduced ovulation by D4 is related to a suppression of the preovulatory LH surge, but the mechanism by which this occurs is not known. A role for ER agonism is suggested by positive findings in multiple uterotrophic assays and the blockade of the positive response during cotreatment with an ER antagonist and in ERα knockout mice. *In vitro* studies demonstrate binding to ERα receptor and increased expression of biomarkers of estrogenicity. Inconsistent findings were reported pertaining to the role of dopamine agonism in the female reproductive effects of D4. *Overall judgment for female reproductive effects based on mechanistic evidence*: • Moderate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• GH3 rat pituitary cells, levels of CaBP-9K and PR (Lee et al., 2015). • ER binding assays using human recombinant ERα and ERβ (Quinn et al., 2007b; He et al., 2003) • MCF-7 human epithelial cells, ERα reporter gene assay (Quinn et al., 2007b). <u>Dopamine agonism</u> • Female rats exposed to ≤10,918 mg/m^3 (nominal), 6 hours/day for 3 days, measurement of plasma prolactin levels (Quinn et al., 2007a). • Female rats pretreated with reserpine (depletes brain dopamine and increases prolactin) or sulpiride (dopamine receptor antagonist), exposed by inhalation to 8490 mg/m^3 for 6 hours, measurement of serum prolactin levels (Dow Corning, 2005b). • Female rats pretreated with reserpine exposed by inhalation to 8490 mg/m^3 for 6 hours, measurement of serum prolactin levels (Dow Corning, 2010). • Dopamine receptor binding/agonism/antagonism assays in MMQ pituitary cells *in vitro* (Domoradzki, 2011), isolated rat brain striatum membranes (Baker, 2010), and commercial membrane preparations (Thackery, 2009). <u>Metabolite studies</u>	ERα reporter gene assay (Quinn et al., 2007b). <u>Dopamine agonism</u> • Dopamine D2 receptor agonism suggested by reduced plasma prolactin levels, preceding the suppression of LH (Quinn et al., 2007a). • Dopamine D2 receptor agonism suggested by reduced serum prolactin levels in reserpine pretreated rats and blockade of this effect by sulpiride pretreatment (Dow Corning, 2005b). • In MMQ pituitary cells, D4 inhibited Forskolin-induced cAMP production via a mechanism that was not modified by dopamine D2 receptor antagonist (Domoradzki, 2011). <u>Metabolite studies</u> • DMSD induced increased uterine weights at 1000 mg/kg-bw/day but showed no antiestrogenic effects (Dow Corning, 2009b).	at doses up to 1200 mg/kg-bw/day (Dow Corning, 2007). <u>Biological plausibility/Human Relevance:</u> • While the pre-ovulatory LH surge in both rodents and primates is controlled by a sustained increase in estradiol levels, the upstream regulation of the surge differs between species. The LH surge in rats governed by the preoptic area of the hypothalamus, is controlled by circadian rhythm, and is blocked by barbiturates. In contrast, the LH surge in primates is controlled by the mediobasal hypothalamus-pituitary unit and is not affected by circadian rhythm or barbiturates (Goodman et al., 2022; Plant, 2012). However, the mechanism by which D4 attenuates the LH surge has not yet been determined, and there are no data on effects of D4 on ovulation in primates.		

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• Uterotrophic assay in rats orally exposed to DMSD (Dow Corning, 2009b). • Combined repeat-dose and reproductive/developmental screening test in rats exposed orally to DMSD (Dow Corning, 2009a). • Gavage study in F344 rats exposed to ≤1200 mg/kg-bw/day DMSD for 14 days (Dow Corning, 2007).				

DMSD: dimethylsilanediol; ER: estrogen receptor; GD: gestation day; LH: luteinizing hormone; PND: postnatal day; s.c.: subcutaneous; SD: Sprague-Dawley or Sprague-Dawley-derived; NZW: New Zealand White

[1] Reported effects are statistically significant unless otherwise noted.
[2] Except where specified, all studies included both male and female animals. Inhalation exposures were whole-body except where noted.
[3] Using EPA method (number of females with confirmed pregnancy/number of females used for mating).

Related HERO IDs (linked in Distiller)
Linked to (Jean and Plotzke, 2017): 7310083, 6991447
Linked to (Dow Corning, 1989b): 5903742
Linked to (Dow Corning, 1998b): 4922978
Linked to (Dow Corning, 1997): 6834019
Linked to (MPI Research, 1999): 1310507, 11504289
Linked to (RCC, 1995b): 6833996
Linked to (WIL Research, 2001a): 7002248, 4924646, 7002247
Linked to (WIL Research, 1996b): 5887777

4171 **Table_Apx A-4. Evidence Integration for Developmental Effects[1]**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Evidence in Studies of Exposed Humans Considered for Deriving Toxicity Values				*Overall judgment for developmental effects based on integration of information across evidence streams:* **Evidence is inadequate to assess whether D4 exposure may cause developmental effects in humans under relevant exposure circumstances.**
• None			• Indeterminate	
Evidence from *in vivo* Mammalian Animal Studies[2] Considered for Deriving Toxicity Values[3]				
Studies that included evaluation of malformations/variations • Female SD rats exposed to vapor; ≤8490 mg/m^3 on GDs 6-15; evaluated maternal toxicity, uterine contents and weights, litter size, fetal viability, sex ratio, fetal body weights, and malformations and variations (IRDC, 1993b). ODQ= High. • Female NZW rabbits exposed to vapor; ≤6080 mg/m^3 on GDs 6-18; evaluated maternal toxicity, uterine contents and weights, fetal viability, fetal body weight, and fetal malformations and variations (IRDC, 1993a). ODQ=Medium. Studies that evaluated pup viability, weight, liver weight, and/or sex ratio • Female Crl:CD rats exposed to vapor; ≤8490 mg/m^3 on GDs 6-15; evaluated maternal body weight, uterine contents and weights, number of live fetuses, sex ratio, and fetal weight (IRDC, 1993e). ODQ=Medium.	Biological gradient/dose-response • Sporadic decreases in maternal body weight were observed in rats at 8490 mg/m^3; decreased body weight gain and decreased food consumption were observed throughout exposure (IRDC, 1993b). • Decreased maternal body weight gain was reported in rats at 8490 mg/m^3; no corresponding body weight or food consumption decreases were observed (IRDC, 1993e). • Decreased body weight gain and decreased food consumption were reported in female rabbits at 8490 mg/m^3; no corresponding body weight changes were reported (IRDC, 1993d). • Decreased maternal body weight and weight gain were reported in rabbits exposed via gavage at ≥100 mg/kg-bw/day, with decreased food	Consistency • No maternal clinical signs or uterine weight changes in female rats or rabbits exposed to concentrations ≤8490 mg/m^3 (IRDC, 1993a, b, d, e) or female rabbits administered doses ≤1000 mg/kg-bw/day via gavage (IRDC, 1993c). • No fetal effects (litter size, fetal viability, sex ratio, or fetal body weight), or changes to uterine contents (number of implantations, early and late resorptions, or corpora lutea, or pre- or post-implantation loss) in female rats or rabbits exposed to concentrations ≤8490 mg/m^3 (IRDC, 1993a, b, d, e) or in in female rabbits exposed to oral doses ≤100 mg/kg-bw/day; these parameters could not be evaluated at oral doses ≥500 mg/kg-bw/day due to the high incidence of abortion (IRDC, 1993c).	*Key findings:* Inhalation exposure to D4 did not induce any developmental effects in rats or rabbits following inhalation exposures; however, malformations/ variations were not evaluated in all studies. Some developmental effects were reported following oral exposure; however, these studies were not comprehensive. Decreased maternal body weight gain was frequently reported; however, this was typically observed along with decreased food consumption. Body weights themselves were generally not affected by treatment. *Overall judgment for developmental effects based on animal evidence*: • Moderate evidence of no effect.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• Female NZW rabbits exposed to vapor; ≤8490mg/m^3 on GDs 6-18 (range finding); evaluated maternal weight, uterine contents and weights, and fetal weights (IRDC, 1993d). ODQ=Medium (developmental) or High (maternal). • Female NZW rabbits exposed by gavage; ≤1000 mg/kg-bw/day on GDs 7-19; evaluated maternal toxicity, uterine contents and weights, and fetal viability (IRDC, 1993c). ODQ=High. <u>Other studies</u> • Female SD rats exposed by gavage; ≤100 mg/kg-bw/day GDs 12-20; evaluated fetal body weight and relative liver weight (Falany and Li, 2005). ODQ=Medium. <u>Studies rated uninformative</u> • Female NZW rabbits exposed by gavage; ≤100 mg/kg-bw/day on GDs 5-18; evaluated clinical signs, maternal toxicity (Dow Corning, 1983).	consumption reported at higher doses (≥500 mg/kg-bw/day) (IRDC, 1993c). • 83 and 67 % increases in the incidence of rabbits with abortions at 500 and 1000 mg/kg-bw/day via gavage, respectively (IRDC, 1993c). • Decreased fetal body weight and decreased relative liver weights at 100 mg/kg-bw/day via gavage (Falany and Li, 2005).	• No fetal malformations/ variations were observed in rats (≤8490 mg/m^3) or rabbits (≤6080 mg/m^3) (IRDC, 1993a, b). <u>Quality of the database:</u> • Four prenatal developmental studies were available in two species for the inhalation route, but only two of these studies evaluated malformations/ variations (IRDC, 1993a, b). • Two oral developmental studies were available (one in rats and one in rabbits), but neither performed complete evaluations of the maternal and fetal animals (Falany and Li, 2005; IRDC, 1993c). <u>Biological plausibility and human relevance:</u> • Decreased body weight gains were reported following both inhalation and oral exposures; however, these decreases were often observed with decreased food consumption, making the toxicological significance of the decreased body weight gains unclear (IRDC,		

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
		1993b, c, d). In addition, decreased maternal body weight was not consistently reported in tandem with decreased body weight gains.		
Evidence in Mechanistic Studies and Supplemental Information				
Metabolite studies • Combined repeat-dose and reproductive/developmental screening test in CRl:CD (SD) IGS BR VAF/Plus rats exposed orally to DMSD (D4 metabolite) (Dow Corning, 2009a).	• None	• DMSD exposure did not affect developmental parameters at doses up to 500 mg/kg-bw/day (Dow Corning, 2009a).	*Key findings*: Oral exposure to the D4 metabolite, DMSD, did not result in developmental effects in a screening study in rats. *Overall judgment for developmental effects based on mechanistic evidence*: • Indeterminate	
GD: gestation day				

[1] Reported effects are statistically significant unless otherwise noted.
[2] Only studies with gestational exposure after implantation (*i.e.*, after GD 6) were considered for this endpoint. Studies with gestational exposure prior to GD 6 are included in the reproductive evidence integration table.
[3] All inhalation exposures were whole-body.

4175 **Table_Apx A-5. Evidence Integration for Carcinogenicity[1]**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
Uterine Tumors				*Overall judgment for carcinogenicity based on integration of information across evidence streams:* Based on EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), EPA concludes that there is suggestive evidence that D4 may be carcinogenic to humans.
Evidence for Uterine Tumors in Studies of Exposed Humans				
• None			• Indeterminate	
Evidence for Uterine Tumors from *in vivo* Mammalian Animal Studies				
• Male and female F344 rats exposed to vapor (whole body); ≤8,492 mg/m^3 for 6 hours/day, 5 days/week for up to 104 weeks (Jean and Plotzke, 2017). OQD=High.	• Female rats exhibited significant dose-related trends for endometrial adenomas and histiocytic sarcomas after 2 years (Jean and Plotzke, 2017). • The historical incidence of uterine adenoma in F344 rats is low (0.3 % in chamber studies) (Haseman et al., 1998). • Increased incidence and severity of endometrial epithelial hyperplasia were also seen with D4 exposure (Jean and Plotzke, 2017; Jean et al., 2017).	• The tumors incidence was 7 % for adenomas and 3 % for histiocytic sarcomas compared with 0 % in controls when considering all dosed females, and the tumors were observed only at the highest exposure level (8,492 mg/m^3). There were no significant pairwise comparisons with concurrent controls when considering the incidence among all dosed rats (Jean and Plotzke, 2017).	*Key findings:* Dose-related trends for endometrial adenomas and histiocytic sarcomas were observed in female rats after 2 years. The tumors occurred only at the highest exposure level, but uterine adenomas are rare in F344 rats and the tumors were accompanied by hyperplasia in the endometrium. *Overall judgment for uterine tumors based on animal evidence*: • Slight	
Testicular Tumors				
Evidence for Testicular Tumors in Studies of Exposed Humans				
• None			• Indeterminate	
Evidence for Testicular Tumors from *in vivo* Mammalian Animal Studies				
• Male and female F344 rats exposed to vapor (whole body); ≤8,492 mg/m^3 for 6 hours/day, 5 days/week for up to 104 weeks	• Male rats exhibited a dose-related trend for increased incidence of bilateral testicular interstitial	• No significant pairwise comparison with concurrent control for Leydig cell adenomas or bilateral	*Key findings:* A dose-related trend for bilateral Leydig cell adenomas was observed in male F344 rats exposed for	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
(Jean and Plotzke, 2017). OQD=High.	(Leydig) cell adenomas (Jean and Plotzke, 2017). • Increased incidence and severity of Leydig cell hyperplasia were also seen with D4 exposure (Jean and Plotzke, 2017).	adenomas (Jean and Plotzke, 2017). • Leydig cell tumors occur spontaneously at high incidence in aging F344 rats (Maronpot et al., 2016; Creasy et al., 2012; Thayer and Foster, 2007); historical control incidence of 70-90 % (Maronpot et al., 2016; Thayer and Foster, 2007). • The incidences at all D4 exposure levels were within the range of spontaneous tumors for this strain (Jean and Plotzke, 2017).	2 years; however, this tumor occurs spontaneously at high incidence, there were no significant pairwise comparisons, and the incidences were within the range seen in control F344 rats. *Overall judgment for testicular tumors based on animal evidence*: • Indeterminate	
Hematopoietic Cancer				
Evidence for Hematopoietic Cancers in Studies of Exposed Humans				
• None			Indeterminate	
Evidence for Hematopoietic Cancer from *in vivo* Mammalian Animal Studies				
• Male and female F344 rats exposed to vapor (whole body); ≤8,492 mg/m^3 for 6 hours/day, 5 days/week for up to 104 weeks (Jean and Plotzke, 2017). OQD=High.	• MNCL was the likely cause of early death in males at 700 ppm (Jean and Plotzke, 2017).	• There were no significant dose-related trends or pairwise comparisons with concurrent controls for MNCL in male or female rats exposed to D4 (Jean and Plotzke, 2017). • MNCL occurs spontaneously at high but variable incidence (13-50 %) in aging F344 rats (Maronpot et al., 2016)	*Key findings:* Although there was no trend or increase in the incidence of mononuclear cell leukemia in rats exposed for 2 years, early deaths in males of the highest exposure group were attributed to MNCL. *Overall judgment for hematopoietic cancers based on animal evidence*:	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
			• Indeterminate	
Evidence in Mechanistic Studies and Supplemental Information				
Genotoxicity				
• CAs in bone marrow of rats exposed by inhalation (Vergnes et al., 2000). • Dominant lethal assay in rats exposed via gavage (Dow Corning, 1982). • Mutagenicity, CAs, and/or SCEs in mouse lymphoma cells (Isquith et al., 1988; Litton Bionetics, 1978), rat fibroblast cells (Felix et al., 1998), and CHO cells (Vergnes et al., 2000). • Mutagenicity in bacteria and yeast (Vergnes et al., 2000; Isquith et al., 1988; Litton Bionetics, 1978). • DNA damage in bacteria (Isquith et al., 1988; Litton Bionetics, 1978), human breast epithelial cells (Farasani and Darbre, 2017), and mouse lymphoma cells (Isquith et al., 1988; Litton Bionetics, 1978).	• Increased SCEs with S9 and nonsignificant increase in CAs with S9 in mouse lymphoma cells *in vitro* (Isquith et al., 1988; Litton Bionetics, 1978). • Increased SCEs with S9 in CHO cells *in vitro* (Vergnes et al., 2000; SEHSC, 1994). • Increased DNA damage in human breast epithelial cells exposed to 10^{-5} M (Farasani and Darbre, 2017).	• No increase in CAs in bone marrow of rats exposed to 700 ppm (Vergnes et al., 2000). • No dominant lethal effects in rats exposed up to 1000 mg/kg/day for 8 weeks (Dow Corning, 1982). • No increase in mutations in bacteria or yeast or DNA damage in bacteria (Vergnes et al., 2000; Isquith et al., 1988; Litton Bionetics, 1978). • No increase in mutations or DNA damage in mouse lymphoma cells (Isquith et al., 1988; Litton Bionetics, 1978). • No increase in mutations in rat fibroblast cells (Felix et al., 1998). • The increase SCEs in mouse lymphoma cells was <2-fold compared to solvent control (Isquith et al., 1988; Litton Bionetics, 1978). • The increase CAs in mouse lymphoma cells occurred only at the highest exposure and was based on analysis of	*Key findings*: *In vivo* studies in rats have not shown CAs in bone marrow after inhalation exposure or germ cell mutations after oral exposure. *In vitro* assays provided equivocal evidence for SCEs and/or DNA damage and no evidence for mutagenicity. No studies of genotoxicity in endometrial or Leydig cells/tissues were located. Reports of *in vitro* studies lacked information on whether the assays controlled for volatility. *Overall judgment for carcinogenicity based on mechanistic evidence (genotoxicity)*: • Indeterminate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
		a small number of cells due to cytotoxicity (Isquith et al., 1988; Litton Bionetics, 1978).		
Estrogen and Progesterone Activity				
Assays have been conducted to measure D4's direct binding potential to estrogen and progestrone receptors and related effects. Uterotrophic assays (oral/inhalation): • D4 and positive controls given orally to immature female F344 and SD rats. D4 was also co-administered with estrogens to determine anti-estrogenic effects (MPI Research, 1999). • D4 given to ovariectomized female F344 and SD rats via inhalation. D4 was also co-administered with estrogen to check anti-estrogenic effects (Quinn et al., 2007b). OQD = medium • D4 given orally to ovariectomized female B6C3F1 mice and ER-α KO mice. D4 was also administered after B6C3F1 mice were dosed with an estrogen antagonist (ICI). (He et al., 2003) OQD = medium *In vitro* receptor binding assays: • D4 binding and luciferase reporter gene assays with ER-α, ER-β, and progesterone receptors (Quinn et al., 2007b) • D4 competition with estradiol for ER-α and ER-β binding (He et al., 2003)	Uterotrophic assays: • Rats exhibited increased uterine weight and epithelial cell height when dosed with D4 alone. In most cases, responses were inhibited when administered with estrogen (Quinn et al., 2007b; MPI Research, 1999) • Mice exhibited increased uterine weight and uterine peroxidase activity when dosed with D4 alone. When ICI was given first or when D4 was given to ER-α KO mice, effects were not observed (He et al., 2003) *In vitro* assays: • Receptor binding assays suggest weak binding to ER-α, but no binding to ER-β or to progesterone receptors although D4 exposure was associated with a statistically significant increase in gene expression of the progesterone receptor. (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003)	• D4's ability to bind to ER-α is much weaker than endogenous estrogen, and it is not known whether or how D4 can compete with the endogenous estrogen for binding to ER-α in uterine tissue. • Except for ER binding assays conducted by Quinn et al. (2007b), it is not clear whether the other *in vitro* assays controlled for volatilization (Lee et al., 2015; Quinn et al., 2007b; He et al., 2003).	*Key findings*: Uterotrophic assays in rats and mice and *in vitro* binding and transcription assays showed D4 has weak estrogenic activity. However, it is not known whether D4's potency is adequate to compete with endogenous estrogen and to result in increased uterine tumors. *Overall judgment for carcinogenicity based on mechanistic evidence (estrogen/progesterone activity)*: Indeterminate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
• D4 binding assay with ER and progesterone receptors. Estradiol also given. An estrogen-receptor antagonist (ICI-182,780) also used with and without D4 (Lee et al., 2015)				
Androgen Activity				
Hershberger assay: D4 administered to male F344 rats via inhalation and organ weights were measured. Testosterone propionate was given as a positive control, separately and with D4 (Quinn et al., 2007b). OQD = low	None	Only a single low-quality study was conducted to measure androgen activity.	*Key findings*: One mechanistic study did not identify D4 as having androgenic or anti-androgenic activity. *Overall judgment for carcinogenicity based on mechanistic evidence (androgenic activity)*: Indeterminate	
Dopamine Agonism				
Summary of putative MOA: dopaminergic inhibition of prolactin secretion leads to delay of female reproductive senescence, prolonging endometrial stimulation/proliferation, and increasing adenomas: • *In vivo* studies comparing estrous cyclicity and hormone levels in aged female F344 rats exposed to 8,540 mg/m^3 D4 or dopamine agonist PM (Jean et al., 2017). • Dopamine receptor binding/agonism/antagonism assays in MMQ pituitary cells *in vitro* (Domoradzki, 2011), isolated rat brain striatum membranes	• In aged female rats, some *in vivo* effects of D4 were similar to effects of PM (Jean et al., 2017): o Increased number and percentage of days in proestrus and estrus. o Increased mean estrous cycle incidence per 45-day interval. • In MMQ pituitary cells, D4 inhibited Forskolin-induced cAMP production via a mechanism that was not modified by a dopamine D2	• In aged female rats, some *in vivo* effects of D4 differed from effects of PM (Jean et al., 2017): o D4 had no effect on serum prolactin at any time, while PM decreased serum prolactin to nearly undetectable levels throughout the study. o Serum E2 levels were decreased with D4 exposure and increased with PM exposure at all time points.	*Key findings*: *In vivo* and *in vitro* assays suggest that D4 does not act as a dopamine receptor agonist or antagonist but may indirectly influence some portions of the dopamine pathway. *In vitro* studies are limited by uncertainty in exposure concentrations. *Overall judgment for carcinogenicity based on mechanistic evidence (dopamine agonism)*:	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
(Baker, 2010), and commercial membrane preparations (Thackery, 2009).	receptor antagonist (Domoradzki, 2011).	o D4 induced slight increase in serum progesterone during weeks 2-10 while PM decreased serum progesterone during weeks 15-57. • *In vitro* assays showed that D4 does not act as a dopamine receptor agonist or antagonist but may indirectly influence some portions of the dopamine pathway (GTPγS activity; cAMP production) (Domoradzki, 2011; Baker, 2010; Thackery, 2009). • Studies that measured D4 concentrations in culture medium showed that concentrations declined with incubation time and were often much less than nominal concentrations (Baker, 2010; Thackery, 2009). • Domoradzki (2011) does not identify any controls for volatilization and did not measure D4 concentrations. indicate whether Some D4 may have been lost if evaporation was not controlled.	• Indeterminate	
Luteinizing Hormone (LH) Suppression				
D4 was given to SD rats via inhalation at 0, 8,516, or 10,979	• D4 exposure was associated with LH surge suppression,	• Although putative MOAs (or steps within such MOAs)	*Key findings*:	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
mg/m^3. LH surge and delays in ovulation as well as hormone levels were assessed (Quinn et al., 2007a). *Putative MOAs* Andersen (2022) suggests D4 may result in LH suppression through non-specific changes in cell membrane fluidity or changes in GABA activation that could result in decreased GnRH release that could result in decreased LH surge and subsequent decreased or delayed ovulation.	decreased ovulation, persistence of mature follicles, and increased serum estradiol (Quinn et al., 2007a).	have been suggested, they have not been fully elaborated and thus, it is not clear how it explains increased uterine tumors from D4 exposure. • If the putative MOA is plausible, there is still very limited evidence for D4's effect for multiple steps in a possible mechanism (*i.e.*, from a possible MIE to the final adverse outcome).	An *in vivo* assay shows that D4 suppresses the LH surge and decreases ovulation with increases in estradiol in serum. An MOA for this change that leads to increases in uterine tumors is not well defined; evidence for D4 changes associated with putative MOA is lacking. *Overall judgment for carcinogenicity based on mechanistic evidence (luteinizing hormone suppression)*: Indeterminate	
Estrous Cycle Changes				
Putative MOA (or steps in an MOA): Changes in estrous cycles that lead to increases in endogenous estrogen could result in uterine tumors by increased stimulation by estrogen. • Two studies evaluated changes in estrous cycles in F344 female rats after inhalation of D4 (Jean and Plotzke, 2017; Jean et al., 2017). • One study evaluated changes in estrous cycles in female SD rats after inhalation of D4 (WIL Research, 2001a).	• D4 administered to F344 rats resulted in changes in estrous cycles in females that included prolonged estrogenic states (Jean and Plotzke, 2017; Jean et al., 2017; WIL Research, 2001a). D4 administered to SD rats resulted in prolonged estrous cycles in the F0 generation but not the F1 generation (WIL Research, 2001a).	• Despite increases in estrogenic states, D4 exposure resulted in lower estrogen to progesterone ratios than controls (Jean et al., 2017). • It is not known whether the increase in estrous cycle length in SD rats is associated with prolonged estrogenic states (WIL Research, 2001a).	*Key findings*: D4 has been shown to result in changes in estrous cycles and greater time in estrogenic states. It is possible that this could lead to increased estrogen stimulation of uterine tissues and tumors. However, D4 exposure was associated with decreased estrogen/ progesterone ratio in one study, which would suggest that there is less likelihood for estrogen	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgment	Inferences across Evidence Streams and Overall Evidence Integration Judgment
			stimulation of uterine tumors. *Overall judgment for carcinogenicity based on mechanistic evidence (estrous cycle changes)*: Indeterminate	

CA: chromosomal aberrations; CaBP-D9k: Calbindin-D9k, a calcium binding protein; cAMP: cyclic adenosine monophosphate; CHO: Chinese hamster ovary; DNA: deoxyribonucleic acid; E2: estradiol; ER-α: alpha estrogen receptor; ER- β: beta estrogen receptor; GABA: gamma aminobutyric acid; GnRH: gonadotropin releasing hormone; GTPγS: guanosine gamma thio-phosphate; ICI: the estrogen receptor antagonist ICI 182, 780; KO: knock out; LH: luteinizing hormone; MIE: molecular initiating event; MMQ: type of pituitary cell; MNCL: mononuclear cell leukemia; MOA: mode of action; OQD: overall quality determination; PM: pergolide mesylate; SCE: sister chromatid exchange; .

[1] Reported effects are statistically significant unless otherwise noted.

Related HERO IDs (linked in Distiller)

Linked to (Jean and Plotzke, 2017): 7310083, 6991447

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Appendix B BENCHMARK DOSE MODELING RESULTS FOR KEY ENDPOINTS

EPA performed benchmark dose (BMD) modeling using EPA's BMD modeling software (BMDS Version 3.3.2) for continuous and dichotomous data for any hazard domain that received a judgment of "likely" during evidence integration. Evidence indicated that D4 likely causes effects on female reproductive system (and associated changes in reproductive function) in humans under relevant exposure circumstances. Other domains received "suggestive" or "inadequate" evidence integration conclusions. EPA conducted BMD modeling in a manner consistent with EPA's *Benchmark Dose (BMD) Technical Guidance* (U.S. EPA, 2012).

EPA considered endpoints that had statistically significant pairwise changes between individual doses and controls for multiple doses or any endpoints with significant dose-response trends from the 2-generation reproductive toxicity study (WIL Research, 2001a), the most robust reproductive toxicity study. Hazard endpoints that could be modeled were decreases in live litter size, number of pups born, and fertility. Although evidence suggests but is not sufficient to conclude that D4 may cause effects on male reproductive structure, EPA presented the results of BMD modeling for the fertility endpoint for males as a comparison. For both males and females, fertility was measured in two ways – by an EPA method and an OECD method. Significant differences were observed in the EPA method, but not the OECD method, and those are presented here. Decreased mating indices were statistically significantly lower than controls only at the highest concentration and were therefore not amenable to BMD modeling.

EPA relied on the BMD guidance and other information to choose benchmark responses (BMRs) appropriate for each endpoint. Although the *BMD Technical Guidance* (U.S. EPA, 2012) does not recommend default BMRs, the guidance does recommend calculating one standard deviation for continuous data and 10 percent extra risk (ER) for quantal data to compare modeling results across modeled hazard endpoints, even when other BMRs are preferred. For the continuous endpoints (live litter size, number of pups born), EPA also modeled five percent relative deviations (RD) in addition to one standard deviation. EPA's choice of BMRs is described in more detail in the following sections that present BMD modeling results for each hazard endpoint. EPA modeled decreased fertility as a dichotomous endpoint.

When modeling dichotomous dose-response relationships, the data can be modeled as either ER or additional risk. EPA modeled the data as ER. EPA's BMD Technical Guidance defines ER as "a measure of the proportional increase in risk of an adverse effect adjusted for the background incidence of the same effect." Mathematically, ER is equal to $[P(d) - P(0)]/[1 - P(0)]$. P(d) is the probability of the effect at dose d, and P(0) is the probability of risk with no exposure to a hazard (U.S. EPA, 2012).

For dichotomous data, the Dichotomous Hill, Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, Weibull, and Quantal Linear dichotomous models available within the software were fit using the selected BMR(s). Adequacy of model fit was judged based on the $\chi 2$ goodness-of-fit p-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied > 3-fold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) was selected.

For continuous measurement data, the Exponential, Hill, Linear, Polynomial, and Power continuous models available within the software were fit employing the selected BMR(s). An adequate fit was judged based on the chi-square goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (*i.e.*, Test 2; p-value > 0.05), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected (p-value < 0.05), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model also did not adequately fit the data (*i.e.*, Test 3; p-value < 0.05), the dataset was considered unsuitable for BMD modeling. Among all models providing adequate fit, EPA selected the lowest BMDL if the BMDLs estimated from different models varied > 3-fold; otherwise, EPA selected the BMDL from the model with the lowest AIC.

B.1 Mean Live Litter Size

B.1.1 F1 Litters

Table_Apx B-1 presents BMD model inputs that include the PBPK-predicted last 24-hour blood AUCs and associated mean litter sizes and standard deviations (SDs) from the first generation in WIL Research (2001a). EPA chose a BMR of five percent RD to calculate the human equivalent concentrations and doses (HECs/HEDs) based on the severity of the endpoint. *Risk Evaluation for 1-Bromopropane* (U.S. EPA, 2020) and *Risk evaluation for Tris(2-chloroethyl) phosphate* (U.S. EPA, 2024) also used a BMR of five percent RD for this hazard endpoint.

Table_Apx B-1. Model Inputs: Decreased Mean Live Litter Size[a] in F1 Rats

Last 24-h Blood AUC (mg/L-h)	Number of F0 Dams that Delivered[b]	Mean Number of Viable Pups Among Litters with Viable Pups	Standard Deviation
0	27	13.3	3.27
4.45	24	13.4	3.75
19.95	27	11.9	3.14
34.86	23	10.4	3.83
49.7	23	9.7	3.84

[a] [Total viable pups on day 0] / [number of litters with viable pups on day 0]
[b] From Table 2, p. 167 in WIL Research (2001a)

Table_Apx B-2 summarizes modeling results for decreased mean live F1 litter size. All constant variance models provided adequate fit and BMDLs were within 3-fold of each other. The $BMDL_5$ from the linear model is preferred because it is the most parsimonious model with the lowest AIC. The table also presents the BMD and BMDL for one standard deviation.

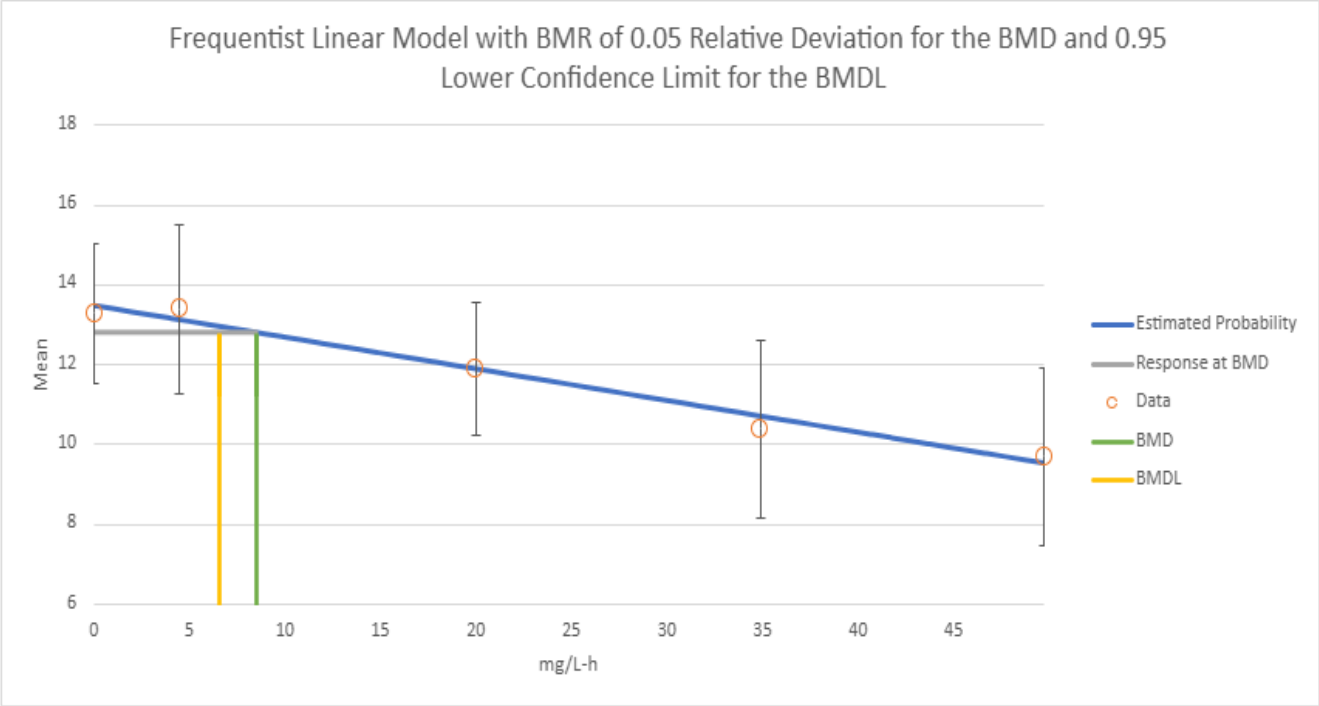
Table_Apx B-2. BMD Modeling Summary Results for Decreased Mean Live Litter Size in F1 Rats (Constant Variance Model)[a] [b]

Model	Goodness of Fit (Means)		BMD 1 SD mg/L-h	BMDL 1 SD mg/L-h	BMD 5 % RD mg/L-h	BMDL 5 % RD mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC					
Exponential 3	0.830	670	43.9	29.8	8.94	5.44	The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, all models provided an adequate fit to the means (test 4 p-value > 0.1). BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. The Power model converged on the Linear model and these had the lowest AIC; the Linear model was selected as the more parsimonious choice.
Exponential 5	0.850	671	43.4	20.6	12.9	3.22	
Hill	0.885	671	44.5	21.3	13.7	2.82	
Polynomial Degree 3	0.929	668	44.2	32.1	8.52	6.51	
Polynomial Degree 2	0.929	668	44.1	32.1	8.50	6.51	
Power	0.929	668	44.0	32.1	8.49	6.51	
Linear	**0.929**	**668**	**44.0**	**32.1**	**8.49**	**6.51**	

[a] Selected model in bold

[b] Values rounded to three significant figures – The lowest AIC is 667.8817 for both the Power and Linear models; the next higher AIC is 667.8818 for the Polynomial Degree 2.

Figure_Apx B-1 presents the plot of decreased live mean F1 litter size using the linear model along with the resulting $BMDL_5$ considered for the risk evaluation. Visual inspection showed an adequate model fit.



Figure_Apx B-1. Response by Dose with Fitted Curve for the Selected Model (Linear, Constant Variance): Mean Live Litter Size in F1 Litters

Figure_Apx B-2 provides additional modeling results, including estimates and confidence limits for each model parameter, goodness of fit at each dose, log likelihood, and tests of interest.

Benchmark Dose	
BMD	8.488434163
BMDL	6.512312467
BMDU	12.61731812
AIC	667.8817053
Test 4 P-value	0.928798212
D.O.F.	3

4264

Model Parameters				
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	13.4658177	0.47455439	12.53571	14.3959272
beta	-0.07931862	1.71E-02	-0.11275	-0.04588674
alpha	12.1810154	1.88E+01	-24.75269	49.1147217

4265

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	27	13.46581771	13.3	13.3	3.49013114	3.27	3.27	-0.246871553
4.45	24	13.11284985	13.4	13.4	3.49013114	3.75	3.75	0.40306299
19.95	27	11.88341124	11.9	11.9	3.49013114	3.14	3.14	0.024697562
34.86	23	10.70077061	10.4	10.4	3.49013114	3.83	3.83	-0.413292548
49.7	23	9.523682292	9.7	9.7	3.49013114	3.84	3.84	0.242280301

4266

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-330.71367	6	673.4273481
A2	-329.83352	10	679.6670472
A3	-330.71367	6	673.4273481
fitted	-330.94085	3	667.8817053
R	-340.9083	2	685.816604

4267 * Includes additive constant of -113.94838. This constant
4268 was not included in the LL derivation prior to BMDS 3.0.
4269

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	22.14955674	8	0.004646219
2	1.760300918	4	0.779736955
3	1.760300918	4	0.779736955
4	0.454357187	3	0.928798212

4270
4271 **Figure_Apx B-2. Details for Selected Model (Linear, Constance Variance Model) for Decreased**
4272 **Live Litter Size in F1 Rat Offspring**
4273
4274

B.1.2 F2 Litters

Table_Apx B-3 presents BMD model inputs for the second generation of WIL Research (2001a). EPA chose a BMR of five percent based on the severity of the endpoint. *Risk Evaluation for 1-Bromopropane* (U.S. EPA, 2020) and *Risk Evaluation for Tris(2-chloroethyl) phosphate* (U.S. EPA, 2024) also used a BMR of five percent RD for this hazard endpoint.

Table_Apx B-3. Model Inputs: Decreased Mean Live Litter Size [a] in F2 Sprague-Dawley Rat Offspring and D4 Blood AUCs

Last 24-h Blood AUC (mg/L-h)	Number of F1 Gravid Dams that Delivered[b]	Mean Number of Pups per Litter	SD
0	29	13.1	3.43
4.44	26	12.0	3.94
20.21	25	12.0	3.66
34.97	26	10.5	3.36
50.01	17	8.6	3.74

[a] [Total viable pups on day 0] / [number of litters with viable pups on day 0]
[b] From Table 55, p. 345 in WIL Research (2001a)

Figure_Apx B-4 summarizes modeling results for decreased mean live F1 litter size. All constant variance models provided adequate fit and BMDLs were within 3-fold of each other. The $BMDL_5$ from the linear model is preferred because it is the model with the lowest AIC. The BMD and BMDL for one SD are also presented.

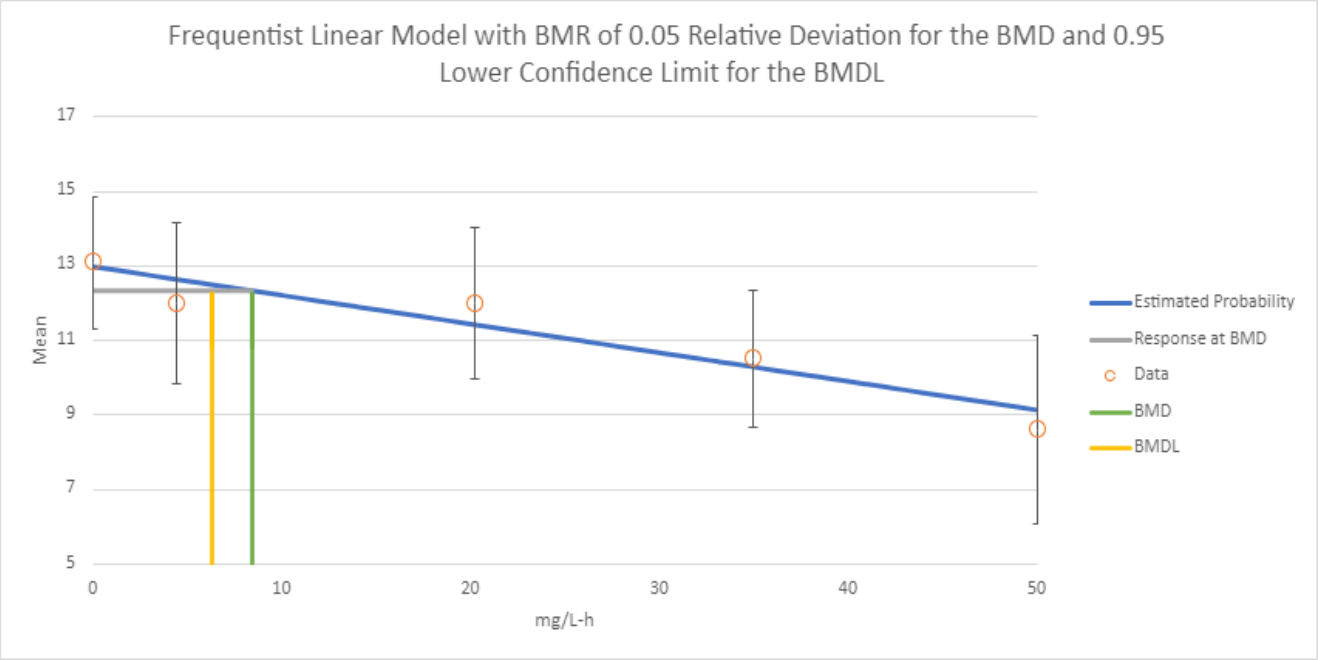
Table_Apx B-4. BMD Modeling Summary Results for Decreased Mean Live Litter Size in F2 Rats (Constant Variance Model)[a] [b]

Model	Goodness of Fit (Means)		BMD 1 SD mg/L-h	BMDL 1 SD mg/L-h	BMD 5 % RD mg/L-h	BMDL 5 % RD mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC					
Exponential 3	0.532	670	46.6	34.7	18.9	5.78	The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, all models provided an adequate fit to the means (test 4 p-value > 0.1). BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Exponential 5	0.262	672	46.6	34.7	18.9	5.78	
Hill	0.264	672	46.6	34.6	18.1	4.61	
Polynomial Degree 3	0.279	671	47.1	34.8	18.1	6.58	
Polynomial Degree 2	0.559	669	46.2	34.8	15.6	6.58	
Power	0.537	670	46.6	43.9	17.8	6.55	
Linear	**0.574**	**668**	**46.4**	**33.1**	**8.42**	**6.31**	

[a] Selected model in bold

Model	Goodness of Fit (Means)		BMD 1 SD mg/L-h	BMDL 1 SD mg/L-h	BMD 5 % RD mg/L-h	BMDL 5 % RD mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC					
[b] Values rounded to three significant figures							

Figure_Apx B-3 presents the plot of decreased live mean F2 litter size using the linear model along with resulting $BMDL_5$ considered for the risk evaluation. Visual inspection shows an adequate model fit.



Figure_Apx B-3. Response by Dose with Fitted Curve for the Selected Model (Linear): Decreased Live Litter Size in the F2 Litters

Figure_Apx B-4 provides additional modeling results, including estimates and confidence limits for each model parameter, goodness of fit at each dose, log likelihood, and tests of interest.

Benchmark Dose	
BMD	8.424440746
BMDL	6.312656327
BMDU	13.19555015
AIC	668.3156889
Test 4 P-value	0.574441984
D.O.F.	3

Model Parameters				
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	12.96086	0.475747	12.028411	13.8933068
beta	-0.07692	1.81E-02	-0.1123821	-0.0414662
alpha	12.76623	2.08E+01	-27.9649	53.4973584

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	29	12.96086	13.1	13.1	3.572986	3.43	3.43	0.209711916
4.44	26	12.61932	12	12	3.572986	3.94	3.94	-0.883827274
20.21	25	11.40622	12	12	3.572986	3.66	3.66	0.830927369
34.97	26	10.27082	10.5	10.5	3.572986	3.36	3.36	0.327062034
50.01	17	9.113882	8.6	8.6	3.572986	3.74	3.74	-0.593002138

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-330.16274	6	672.325483
A2	-329.74174	10	679.483477
A3	-330.16274	6	672.325483
fitted	-331.15784	3	668.315689
R	-339.58699	2	683.173977

* Includes additive constant of -113.02944. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	19.6904997	8	0.01157261
2	0.842005978	4	0.93272951
3	0.842005978	4	0.93272951
4	1.990205944	3	0.57444198

Figure_Apx B-4. Details for Selected Model (Linear, Constance Variance Model) for Decreased Live Litter Size in F2 Rat Offspring

B.2 Pups Born

Table_Apx B-5 presents BMD model inputs for decreases in mean number of pups born in F1 rat litters WIL Research (2001a). EPA chose a BMR of five percent RD based on the severity of the endpoint. Also, the endpoint is similar to the hazard endpoint of decreases in live litter size, which also used a BMR of five percent RD.

Table_Apx B-5. Model Inputs: Decreased Number of F1 Rat Pups Born and D4 Blood AUCs

Last 24-h Blood AUC (mg/L-h)	Number of F0 Gravid Dams that Delivered[a]	Mean Number of Rat Pups Born per Litter	SD
0	27	13.7	3.11
4.45	24	13.5	3.81
19.95	27	12.2	3.26
34.86	23	10.8	3.65
49.7	23	10.0	3.88

[a] From Table 2, p. 167 in WIL Research (2001a)

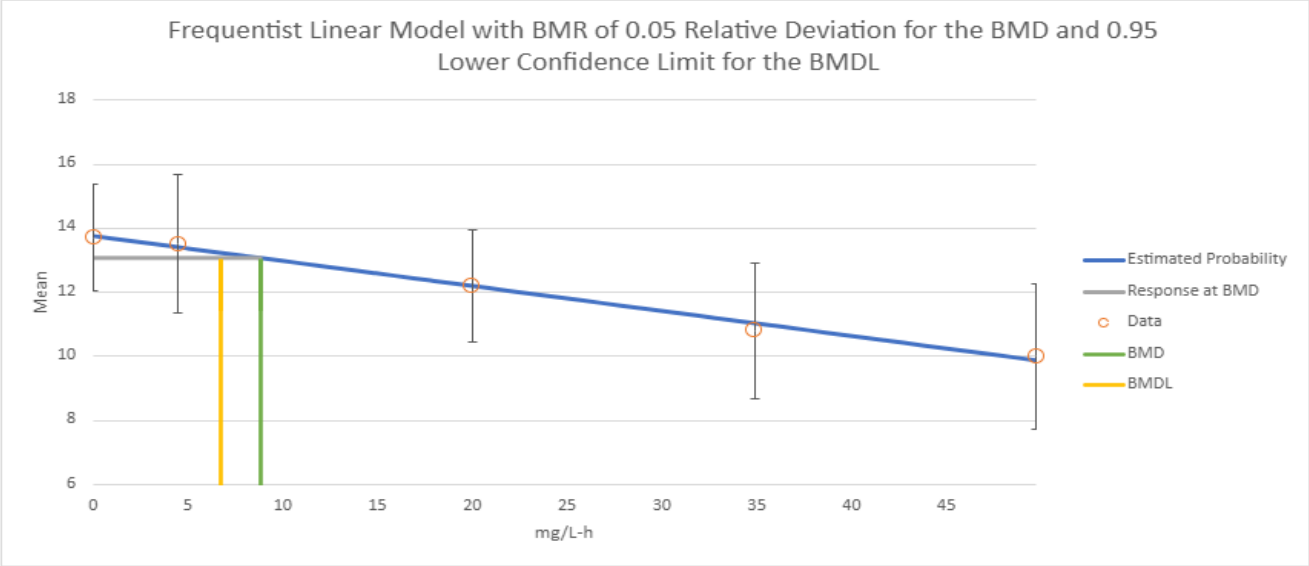
Table_Apx B-6 summarizes modeling results for decreased mean number of F1 pups born. All constant variance models provided adequate fit and BMDLs were within 3-fold of each other. The $BMDL_5$ from the linear model is preferred because it is the most parsimonious model with the lowest AIC. The table also presents the BMD and BMDL for one SD.

Table_Apx B-6. BMD Modeling Summary Results for Decreased Mean Number of Pups Born in F1 Rat Litters (Constant Variance Model)[a] [b]

Model	Goodness of Fit (Means)		BMD 1 SD mg/L-h	BMDL 1 SD mg/L-h	BMD 5 % RD mg/L-h	BMDL 5 % RD mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC					
Exponential 3	0.947	668	44.1	30.1	8.67	5.66	The constant variance model provided an adequate fit to the variance data and all models provided an adequate fit to the means (test 4 p-value > 0.1). BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. The Power model converged on the Linear model and these had the lowest AIC; the Linear model was selected as the more parsimonious choice.
Exponential 5	0.741	670	44.1	30.1	8.67	5.66	
Hill	0.912	670	45.1	21.6	12.1	2.49	
Polynomial Degree 3	0.984	666	44.5	32.3	8.82	6.73	
Polynomial Degree 2	0.984	666	44.7	32.3	8.86	6.73	
Power	0.984	666	44.4	32.3	8.81	6.73	
Linear	**0.984**	**666**	**44.4**	**32.3**	**8.81**	**6.73**	

Model	Goodness of Fit (Means)		BMD 1 SD mg/L-h	BMDL 1 SD mg/L-h	BMD 5 % RD mg/L-h	BMDL 5 % RD mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC					
[a] Selected model in bold; [b] Values rounded to three significant figures – The lowest AIC is 666.01114 for the Linear and Power models and the next lowest value is 666.01117 for the Polynomial Degree 3 model.							

Figure_Apx B-5 presents the plot of decreased mean F1 pups born using the linear model along with resulting $BMDL_5$ considered for the risk evaluation. Visual inspection showed an adequate model fit.



Figure_Apx B-5. Response by Dose with Fitted Curve for the Selected Model (Linear, Constant Variance): Mean Number of Pups Born in F1 Litters

Figure_Apx B-6 provides additional modeling results, including estimates and confidence limits for each model parameter, goodness of fit at each dose, log likelihood, and tests of interest.

Benchmark Dose	
BMD	8.806254417
BMDL	6.733339711
BMDU	13.17316251
AIC	666.0111366
Test 4 P-value	0.984278811
D.O.F.	3

Model Parameters				
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	13.74017108	0.470933	12.817159	14.6631834
beta	-0.078013696	1.69E-02	-0.1111877	-0.0448397
alpha	11.99864107	1.83E+01	-23.836793	47.8340755

Goodness of Fit								
ɔse	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	27	13.74017108	13.7	13.7	3.463905465	3.11	3.11	-0.06026
4.45	24	13.39301013	13.5	13.5	3.463905465	3.81	3.81	0.1513151
.95	27	12.18379784	12.2	12.2	3.463905465	3.26	3.26	0.0243046
34.86	23	11.02061364	10.8	10.8	3.463905465	3.65	3.65	-0.305443
49.7	23	9.86289039	10	10	3.463905465	3.88	3.88	0.1898304

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-329.9272943	6	671.8545885
A2	-329.0037652	10	678.0075303
A3	-329.9272943	6	671.8545885
fitted	-330.0055683	3	666.0111366
R	-339.8077143	2	683.6154285

* Includes additive constant of -113.94838. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	21.60789818	8	0.00569644
2	1.847058197	4	0.763862464
3	1.847058197	4	0.763862464
4	0.15654806	3	0.984278811

Figure_Apx B-6. Details for Selected Model (Linear, Constance Variance Model) for Decreased Numbers of Pups Born in F1 Rat Litters

B.3 Fertility

B.3.1 Females

Table_Apx B-7 presents BMD model inputs for decreased fertility in F1 females from WIL Research (2001a). EPA used a BMR of ten percent ER to calculate the human equivalent concentrations and doses (HECs/HEDs). *Risk Evaluation for 1-Bromopropane* (U.S. EPA, 2020) and *Risk Evaluation for n-Methylpyrrolidone* (U.S. EPA, 2020) at EPA-HQ-OPPT-2019-0236 also used a BMR of ten percent ER for this hazard endpoint.

Table_Apx B-7. Model Inputs: Decreased Fertility Index[a] in Second Mating of F1 Females and D4 Blood AUCs

Last 24-h Blood AUC (mg/L-h)	Number of Females Used for Mating	Incidence of Females with Confirmed Pregnancy	Incidence of Females without Confirmed Pregnancy [b]
0	30	26	4
4.44	30	20	10
20.21	30	21	9
34.97	30	18	12
50.01	30	12	18

[a] EPA method to calculate female fertility: [No. females with confirmed pregnancy]/[Total no. females used for mating] (see p. 73 in WIL Research (2001a)

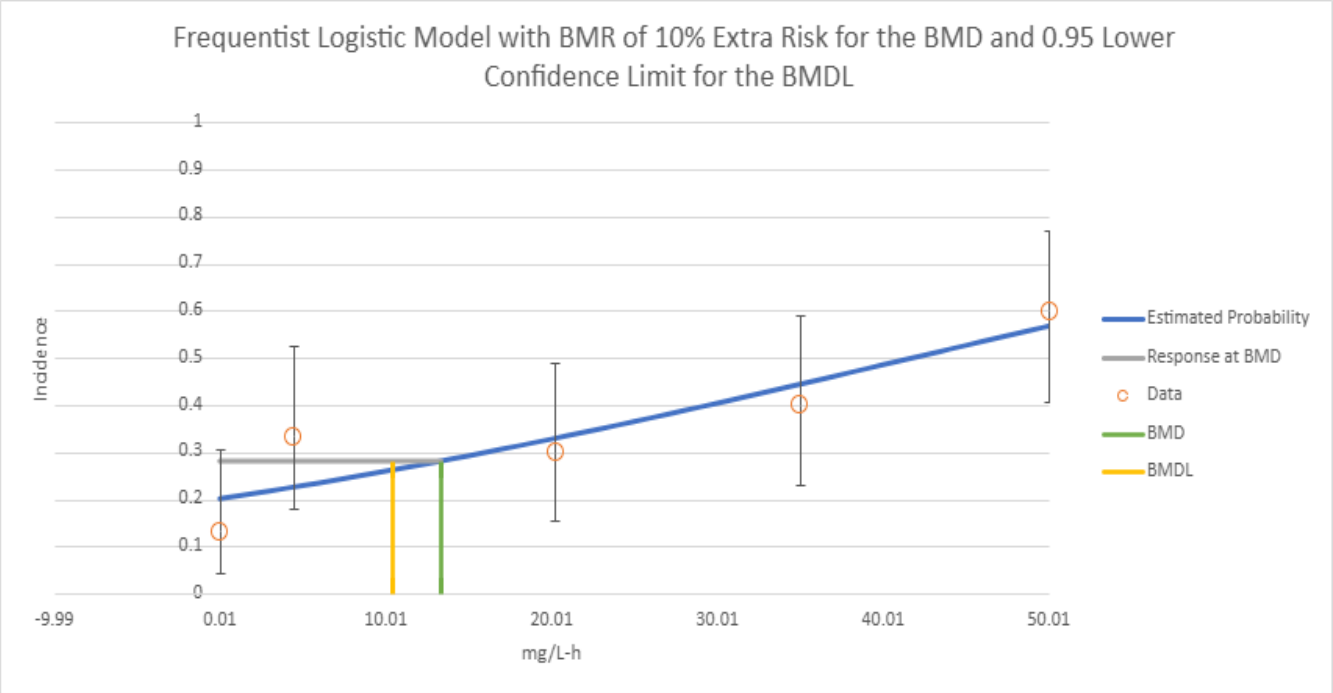
[b]Modeled data for females confirmed to be pregnant (as the inverse of the reported number of fertile females); BMDS dichotomous models can only model positive dose-response trends, where incidence increases with dose.

Table_Apx B-8. BMD Modeling Summary Results for Decreased Fertility Index in Second Mating of F1 Female Rats[a] [b]

Model	Goodness of Fit (Means)		BMD 10 % ER mg/L-h	BMDL 10 % ER mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC			
Dichotomous Hill	0.182	189	24.0	4.48	All models provided adequate fit to the data (chi-square p-value > 0.1). The BMDL computation failed for the Log-Probit Model because the lower limit included zero; thus, the model was unusable. BMDLs of the remaining fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Gamma	0.181	189	23.7	6.05	
Log-Logistic	0.182	189	24.0	4.48	
Multistage Degree 3	0.221	188	15.9	6.29	
Multistage Degree 2	0.187	188	14.7	6.15	
Multistage Degree 1	0.301	187	9.21	6.03	
Weibull	0.186	189	22.5	6.08	
Logistic	**0.344**	**186**	**13.3**	**10.3**	
Log-Probit	0.177	189	24.7	0	
Probit	0.341	186	12.9	9.94	
Quantal Linear	0.301	187	9.21	6.03	

[a] Selected model in bold
[b] Values rounded to three significant figures – The lowest AIC is 186.41 for the Logistic model and the second lowest is 186.42 for the Probit model

Figure_Apx B-7 presents the plot of decreased fertility using the linear model along with resulting $BMDL_{10}$ considered for the risk evaluation. Although EPA considered the model fit to be adequate upon visual inspection, other modeled hazard endpoints exhibited better fits.



Figure_Apx B-7. Response by Dose with Fitted Curve for the Selected Model (Logistic): Decreased Fertility of F1 Female Rats

Figure_Apx B-8 provides additional modeling results, including estimates and confidence limits for each model parameter, goodness of fit at each dose, and analysis of deviance.

Benchmark Dose	
BMD	13.33258381
BMDL	10.34474481
BMDU	20.3734351
AIC	186.4075832
P-value	0.343871721
D.O.F.	3
Chi^2	3.327222464

4379

Model Parameters				
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	-1.379746363	0.299655864	-1.96706107	-0.7924317
b	0.032999325	9.66E-03	0.01407154	0.05192711

4380

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.201049738	6.031492141	4	30	-0.925428546
4.44	0.225616712	6.768501362	10	30	1.411496258
20.21	0.328973815	9.869214438	9	30	-0.33776598
34.97	0.44379851	13.31395531	12	30	-0.482848596
50.01	0.567227895	17.01683686	18	30	0.362290162

4381

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-89.58228815	5	-	-	NA
Fitted Model	-91.2037916	2	3.2430069	3	0.355654119
Reduced Model	-97.42285778	1	15.6811393	4	0.003478277

4382

4383 **Figure_Apx B-8. Details for Selected Model (Logistic) for Decreased Fertility Index in Second**
4384 **Mating of F1 Female Rats**

B.3.2 Males

Table_Apx B-9 presents BMD model inputs for decreased fertility in F1 males from WIL Research (2001a). EPA used a BMR of ten percent ER to calculate the human equivalent concentrations and doses (HECs/HEDs). *Risk Evaluation for 1-Bromopropane* (U.S. EPA, 2020) and *Risk Evaluation for n-Methylpyrrolidone* (U.S. EPA, 2020) at EPA-HQ-OPPT-2019-0236 also used a BMR of ten percent ER for this hazard endpoint.

Table_Apx B-9. Model Inputs: Decreased Fertility Index[a] in Second Mating of F1 Males and D4 Blood AUCs

Last 24-h Blood AUC (mg/L-h)	Number of Males Used for Mating	Incidence of Males Siring a Litter	Incidence of Males Not Siring a Litter [b]
0	30	26	4
4.44	30	20	10
20.21	30	21	9
34.97	29	17	12
50.01	29	12	17

[a] EPA method to calculate fertility: [No. males siring a litter]/Total no. males used for mating] (see p. 73 in WIL Research (2001a)
[b] Modeled data for males not siring a litter (as the inverse of the reported number of fertile males); BMDS dichotomous models can only model positive dose-response trends, where incidence increases with dose.

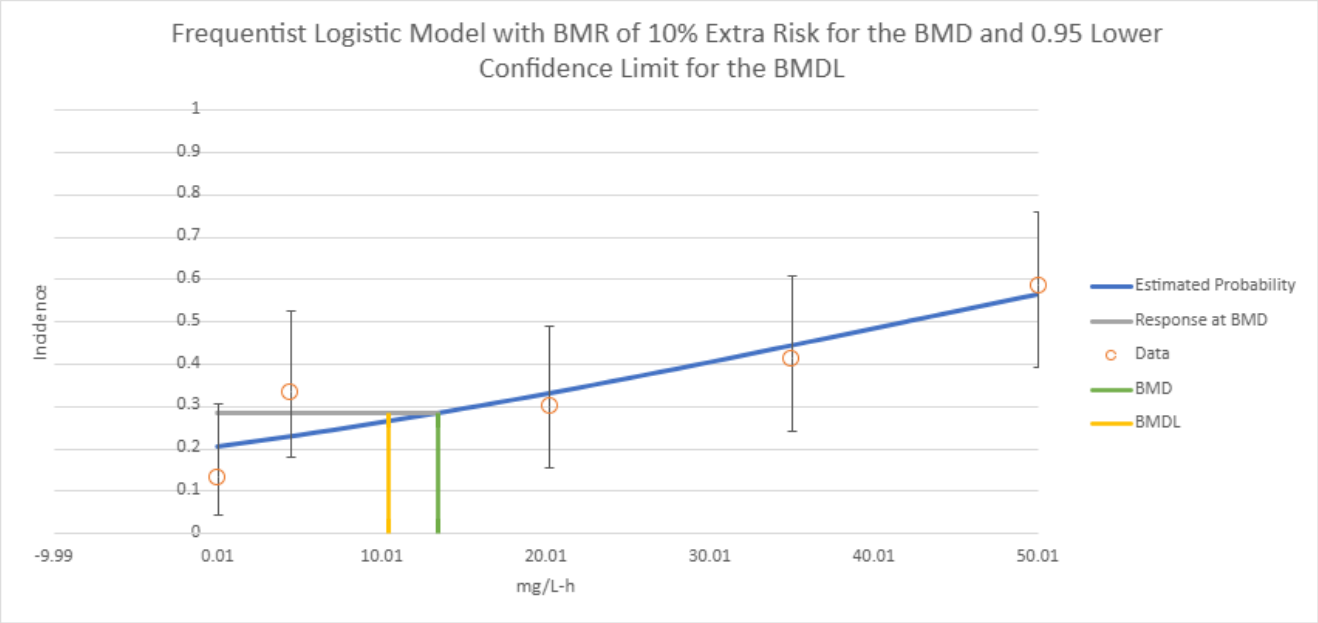
Table_Apx B-10. BMD Modeling Summary Results for Decreased Fertility Index in Second Mating of F1 Male Rats[a b]

Model	Goodness of Fit (Means)		BMD 10 % ER mg/L-h	BMDL 10 % ER mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC			
Dichotomous Hill	0.187	186	21.5	4.34	All models provided adequate fit to the data (chi-square p-value > 0.1). The BMDL computation failed for the Log-Probit Model because the lower limit included zero; thus, the model was unusable. BMDLs of the remaining fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Gamma	0.347	184	9.30	6.04	
Log-Logistic	0.187	186	21.5	4.34	
Multistage Degree 3	0.235	186	14.2	6.20	
Multistage Degree 2	0.209	186	13.1	6.11	
Multistage Degree 1	0.347	184	9.30	6.04	
Weibull	0.347	184	9.30	6.04	
Logistic	**0.379**	**184**	**13.4**	**10.4**	
Log-Probit	0.184	187	23.0	0	
Probit	0.377	184	13.0	9.96	

Model	Goodness of Fit (Means)		BMD 10 % ER mg/L-h	BMDL 10 % ER mg/L-h	Basis for Model Selection
	Test 4 p-value	AIC			
Quantal Linear	0.347	184	9.30	6.04	

[a] Selected model in bold
[b] Rounded to three significant figures – The lowest AIC is 184.096 for the Logistic model and the second lowest is 184.100 for the Probit model.

Figure_Apx B-9 presents the plot of decreased male fertility in the second generation using the logistic model along with resulting $BMDL_{10}$ considered for the risk evaluation. Although EPA considered the model fit to be adequate after visual inspection, other modeled hazard endpoints exhibited better fits.



Figure_Apx B-9. Response by Dose with Fitted Curve for the Selected Model (Logistic): Decreased Fertility of F1 Males

Figure_Apx B-10 provides additional modeling results, including estimates and confidence limits for each model parameter, goodness of fit at each dose, and analysis of deviance.

Benchmark Dose	
BMD	13.4484113
BMDL	10.35855572
BMDU	21.04681884
AIC	184.0964678
P-value	0.37859779
D.O.F.	3
Chi^2	3.08571298

4410
4411

Model Parameters				
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	-1.36447997	0.298947986	-1.950407261	-0.778552678
b	0.032394048	9.72E-03	0.013350625	0.05143747

4412

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.203513155	6.105394636	4	30	-0.95474488
4.44	0.227822013	6.834660384	10	30	1.37785379
20.21	0.329643859	9.88931577	9	30	-0.345398204
34.97	0.442342594	12.82793521	12	29	-0.309552445
50.01	0.563541172	16.342694	17	29	0.246112762

4413

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-88.53773297	5	-	-	NA
Fitted Model	-90.04823392	2	3.021001893	3	0.388398418
Reduced Model	-95.94531677	1	14.81516759	4	0.005100332

4414
4415 **Figure_Apx B-10. Details for Selected Model (Logistic) for Decreased Fertility Index in Second**
4416 **Mating of F1 Male Rats**

Appendix C D4 PBPK MODEL REVIEW – RESPONSE TO CHARGE QUESTIONS

This review was conducted by Elaina Kenyon, Ph.D., DABT, ATS, a research toxicologist in U.S. EPA's Office of Research and Development (ORD), Center for Computational Toxicology and Exposure. The review is based on the PBPK description and review document provided on 10/29/24 (final document available as U.S. EPA (2025f)) and review of the published PBPK models (Campbell et al., 2023; Campbell et al., 2017). Review and testing of the model code itself was not requested as part of this review. The charge questions below were provided to Dr. Kenyon to guide her review.

Charge Question 1: Is it reasonable to use the model to predict doses and exposure concentrations for all routes of exposure for humans (inhalation, oral, dermal)?

- Overall, the Campbell et al. (2023) D4 model can reasonably be used for route extrapolation for healthy adult humans with acknowledgement of some uncertainties.
- A strength of the model specific to route-to-route extrapolation is the existence of studies that allow evaluation of kinetic behavior for oral, dermal and inhalation exposure in rats and dermal and inhalation exposure in humans.
- The biological basis for the behavior of D4 at barrier tissues is adequately documented and appears reasonable.
- Shorter duration route-to-route extrapolation can be conducted with relatively greater confidence compared to longer duration route-to-route extrapolation (see below).

Charge Question 2: Is the model appropriate for exposure durations ranging from acute to chronic? For example, how does use of short-term human studies in the model affect the confidence in our predictions? Would ORD expect the parameters to change with subchronic or chronic durations?

- The PBPK model is applicable for duration extrapolation from rats to healthy adult humans with some caveats and uncertainties.
- Ideally, given that PK data for chronic exposure in humans is rarely available for environmental chemicals, it is highly desirable to have available PK data in animal models that is equivalent to the desired exposure duration extrapolation (*i.e.*, acute to subchronic or chronic, or subchronic to chronic). Based on my reading of the report, such data are available for acute and subacute rat inhalation exposure, but possibly not longer duration exposures.
- Given that the desired duration extrapolation is in turn driven by the duration of exposure required to produce the toxic effect of concern, there would be more uncertainty associated with longer duration PK extrapolation for the inhalation route.
- If pharmacokinetic factors that impact PK for acute vs chronic or subchronic exposures are known, this increases the uncertainty and decreases the confidence in such extrapolations. Induction of metabolism of D4 via a cytochrome P450 pathway (CYP2B1/2) following subacute inhalation exposure occurs in rats. Effectively this means that there will be differences in measures of internal dose (*e.g.*, AUC for parent chemical) for acute compared to longer term exposure. These factors can be accounted for using an appropriately parameterized PBPK model, but this does increase the uncertainty associated with extrapolation to exposure durations beyond those explicitly measured in experimental studies.
- In the case of dermal exposure, there would be relatively greater confidence in extrapolation from acute to longer term exposures (compared to inhalation) because skin penetration is relatively low compared to inhalation uptake and the volatility of D4 enables rapid evaporation

from the skin under practical conditions and thus more likely to be low and remain in the linear range.

Additional Comments

- Based on reading the papers describing the PBPK models, two overarching issues that arise are that it is unclear in some cases: (1) what data were used to estimate which parameters and (2) whether different data not used to estimate parameters were used to evaluate the performance of enhanced or modified versions of the model. This is a common issue with published PBPK models (McLanahan et al., 2012).
- While a complete review of the model is not feasible in the context of information available and scope of charge questions, the biological rationale for differences in kinetics and the increasing complexity in model structure through various model versions is biologically sound and supported by experimental studies in many instances.
- It would be desirable to consider the potential pharmacokinetic and pharmacodynamic impacts of simultaneous multi-route exposure.

Appendix D DEFAULT METHOD TO ESTIMATE HECs AND HEDs

D.1 Unit Conversion for Hazard Values

It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in toxicity studies and the default units for different OPPT exposure models. Therefore, EPA presented all inhalation hazard values in both units.[18] The following equation converts air concentrations from ppm to mg/m^3, using HEC as an example.

Equation_Apx D-1. Converting ppm to mg/m^3 (*e.g.*, for HECs) [19]

$$HEC\ (mg/m^3) = HEC\ (ppm) \times (Molecular\ Weight / 24.45)$$
$$HEC\ (mg/m^3) = HEC\ (ppm) \times (296.61 / 24.45)$$

D.2 Duration Adjustments

The study selected for dose-response assessment (WIL Research, 2001a) administered D4 to rats for six hours per day for seven days per week. To better compare results across exposure scenarios, EPA adjusted the administered concentrations linearly to continuous exposure (twenty-four hours per day, seven days per week) [20] prior to POD derivation based on Haber's Law (Haber, 1924) using the following equation:

[18] WIL Research (2001a) presented all air concentrations in ppm. Using Equation_Apx D-1 (and reporting results as significant figures), the second-generation chamber concentrations of 0, 71, 301, 502, and 702 ppm used for the hazard endpoint (decreased live litter size in F2 offspring) were converted to 0, 861, 3650, 6,090, and 8520 mg/m^3.

[19] The Ideal Gas Law can be used to convert between ppm and mg/m^3. At standard temperature and pressure (STP; 25 °C and 760 mm Hg), 1 mole of gas occupies 24.45 L. However, when conditions differ from STP, a different gas conversion factor can be calculated using the reported experimental temperature or pressure.

[20] The second-generation air concentrations (for decreased live litter size in F2 offspring) expressed as 6 hours/day, 7 days/week (0, 861, 3650, 6,090, and 8520 mg/m^3) were converted to 0, 215, 913, 1520, and 2130 mg/m^3, expressed as 24 hours per day, 7 days per week.

Equation_Apx D-2. Adjusting Exposure Concentrations for Differences in Days and Hours of Exposure

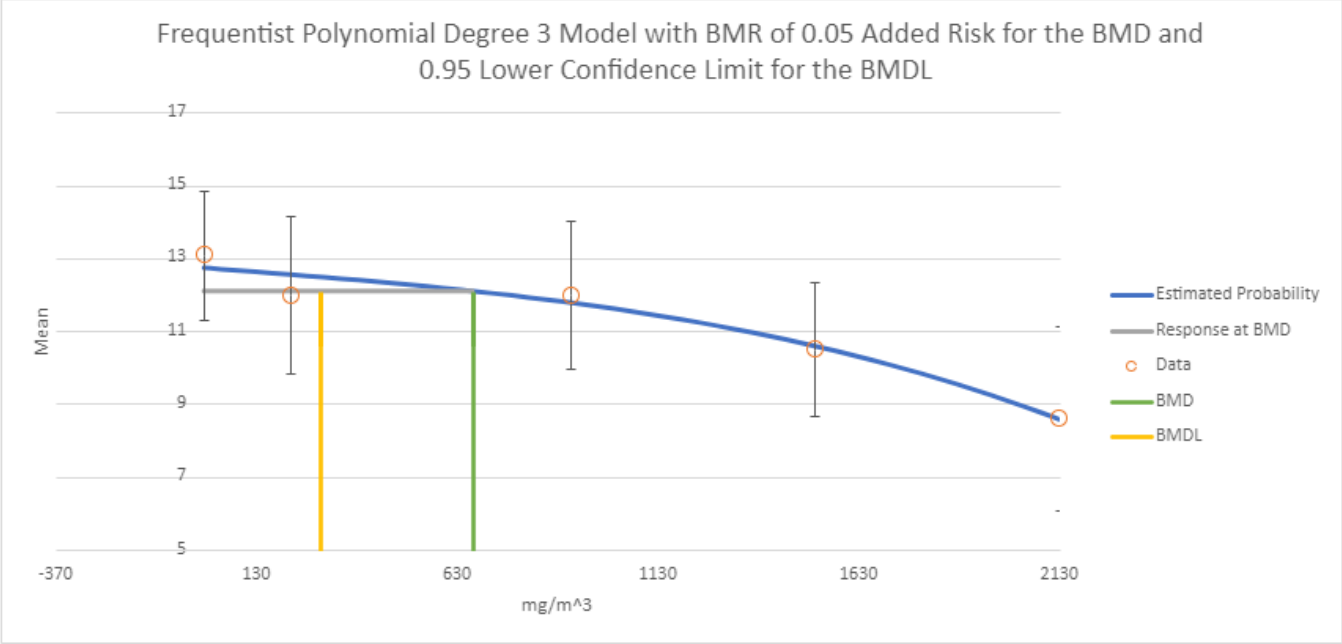
Where:

$Concentration_{continuous}$ = Adjusted air concentration/inhalation POD
$Concentration_{study}$ = Air concentrations and inhalation PODs from study dataset
D_s = Days per week exposure in study dataset (7 days)
H_s = Hours per day exposure in study dataset (6 hours)

EPA used the continuous concentrations to conduct BMD modeling for the same hazard endpoint used in the PBPK model. Thus, all HECs and HEDs are expressed on this continuous basis (or daily basis for oral and dermal routes).

D.3 Benchmark Dose Analysis

Using the adjusted exposure concentrations using Equation_Apx D-2, EPA conducted BMD modeling in accordance with BMD guidance (U.S. EPA, 2012) for decreased live litter size in the F2 offspring to demonstrate results using default methods. This is the endpoint that was also chosen for PBPK modeling. The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, all models provided an adequate fit to the means (test 4 p-value > 0.1). BMDLs for each of the fit models were sufficiently close (differed by < 3-fold); therefore, EPA selected the model (Polynomial 3-Degree) with the lowest Akaike information criterion (AIC) that showed adequate fit based on visual inspection (see Figure_Apx D-1). The resulting $BMDL_5$ was 288 mg/m^3.



Figure_Apx D-1. Response by Dose with Fitted Curve for the Selected Model (Polynomial Degree 3): Decreased Live Litter Size in the F2 Offspring

D.4 Inhalation HEC

Although D4 is a volatile liquid, EPA considers D4 to be a category 3 gas for dosimetry of all systemic endpoints, in accordance with EPA's reference concentration (RfC) guidance (U.S. EPA, 1994). Therefore, the relative blood:air partition coefficient between the test organism and humans has a large

influence on relative dosimetry. Although the estimated blood:air coefficient used in the PBPK model was slightly higher for humans than rodents (1.3 vs 0.85) (U.S. EPA, 2025f), EPA defaulted to a relative ratio of one and considered internal dose to be equivalent for rodents and humans for purposes of using this default approach.

D.5 Oral HED

D.5.1 Extrapolation from Inhalation HEC

EPA extrapolated the daily continuous inhalation HEC to an oral POD using human body weight and breathing rate relevant to daily doses for an individual at rest, as follows:

Equation_Apx D-3. Extrapolating from Inhalation HEC to Oral (or Dermal) POD

Where:

POD_{Daily}	=	Oral (or dermal) POD based on daily exposure (58.5 mg/kg-bw/day)
$HEC_{Daily,cont.}$	=	Inhalation HEC based on continuous daily exposure (288 mg/m^3)
IR_R	=	Inhalation rate for an individual at rest (m^3/h) = 0.6125
ED_C	=	Exposure duration for a continuous exposure (h/day) = 24
BW_H	=	Body weight of females of reproductive age (kg) = 72.4

Based on information from U.S. EPA (2011a), EPA assumed an at rest breathing rate of 0.6125 m^3/h.

D.5.2 Adjustment for Differences in Absorption

EPA adjusted the oral POD based on estimated differences in absorption and bioavailability between the oral and inhalation routes, reflected by the differences in HECs/HEDs predicted by the PBPK model. EPA first converted the PBPK-predicted chronic HEC of 55.8 mg/m^3 to 11.3 mg/kg-bw/day using Equation_Apx D-3. Then the ratio of the chronic oral value of 3.60 mg/kg-bw/day to the converted inhalation POD of 11.3 mg/kg-bw/day was used to adjust the oral POD (using default methods) as follows:

Equation_Apx D-4. Daily Oral (or Dermal) POD Accounting for Differences in Absorption

Where:

$POD_{Daily,\ absorp}$	=	POD adjusted by relative absorption (oral = 18.6 mg/kg-bw/day; dermal = 1682 mg/kg-bw/day)
POD_{Daily}	=	POD converted from inhalation HEC (58.5 mg/kg-bw/day)
Rel Absorp	=	Relative absorption predicted from PBPK model (unitless, oral:inhale = 0.318, dermal:inhale = 28.8)

EPA considered the use of these PBPK-model predicted values to more accurately reflect differences in absorption than using values directly from toxicokinetics studies.

D.5.3 Application of the Dosimetric Adjustment Factor (DAF)

EPA then adjusted the POD using allometric BW$^{3/4}$ scaling based on guidance from U.S. EPA (2011b). Allometric scaling accounts for differences in physiological and biochemical processes, mostly related

to kinetics. For application of allometric scaling, EPA used dosimetric adjustment factors (DAFs), which can be calculated using Equation_Apx D-5.

Equation_Apx D-5. Dosimetric Adjustment Factor (DAF)

Where:
DAF = Dosimetric adjustment factor (unitless, 0.234 for rat to human adjustment)
BW_A = Body weight of species used in toxicity study (0.25 kg for rats)
BW_H = Body weight of adult human (80 kg)

Using the DAF from Equation_Apx D-5. EPA adjusted the POD to obtain the daily HED as follows:

Equation_Apx D-6. Daily Oral POD Adjusted by DAF

Where:

$HED_{Daily,\ DAF}$	=	HED adjusted by relative absorption (4.39 mg/kg-bw/day)
POD_{Daily}	=	Oral POD assuming daily doses (18.6 mg/kg-bw/day)
DAF	=	DAF (unitless, 0.234)

D.6 Dermal HED

Like the oral route, EPA used Equation_Apx D-3 to extrapolate the daily continuous inhalation HEC to a dermal POD using human body weight and breathing rate relevant to daily doses for an individual at rest.

EPA then adjusted the dermal POD using the ratio of the dermal HED to the converted inhalation HEC predicted by the PBPK model using Equation_Apx D-4. The ratio is 326/11.3 (=28.8) and reflected estimated differences in absorption and bioavailability. The resulting dermal HED is 1682 mg/kg-bw/day.

D.7 Uncertainty Factors in the Benchmark Margins of Exposure

D.7.1 Interspecies Uncertainty Factor (UF_A) of 3 or 10

Inhalation

Interspecies toxicokinetic dosimetry for systemic endpoints assumed the blood:air coefficients for rats and humans are essentially the same, and this consideration is expected to account for interspecies toxicokinetic differences. Therefore, EPA used a 3x intraspecies uncertainty factor (UF_A) in the benchmark margin of exposure (MOE) to account for the remaining uncertainty associated with toxicodynamic differences across species.

Oral

EPA used allometric scaling by applying the DAF for the oral route of exposure to account for toxicokinetic differences among species. Therefore, EPA used a 3x UF_A in the benchmark MOE to account for the remaining uncertainty associated with toxicodynamic differences across species.

Dermal

EPA used a 10x UF_A in the benchmark MOE to account for uncertainty associated with toxicokinetic and toxicodynamic differences across species due to limited information regarding the degree to which such differences may impact the disposition of or response to D4 in humans.

D.7.2 Intraspecies Uncertainty Factor (UF_H) of 10

The D4 PBPK model does not account for within human variability; thus the 10X intra-species is being used. This UF accounts for interindividual variability that is not represented in the model.

D.8 Toxicity Values and Comparison to PBPK Model Predictions

Table_Apx D-1 presents the HEC and HED values using the default method and accounting for occlusion along with the benchmark MOEs based on the composite uncertainty factors.

Table_Apx D-1. HECs and HEDs Using Default Method

Exposure Route	Units	HEC or HED	Benchmark MOE
Inhalation	mg/m^3	288	30
	ppm	23.7	
Oral	mg/kg-bw/day	4.39	30
Dermal (unoccluded)	mg/kg-bw/day	1682	100
Dermal (occluded)	mg/kg-bw/day	923	100

Table_Apx D-2 presents the ratios of default HECs and HEDs to the PBPK model-predicted values after normalizing by the benchmark MOEs, which differed for the dermal route (30 for the PBPK-model HED and 100 for the default HED) but were the same for the inhalation and oral routes (30 for each method). Except for the acute oral HED, all normalized toxicity values were less sensitive using the default method.

Table_Apx D-2. Ratios of Default to Predicted HECs/HEDs

Exposure Route	Acute	Intermediate/Chronic
Inhalation	2.69	5.16
Oral	0.492	1.22
Dermal (Unoccluded)	1.28	1.55

Appendix E TOXICOKINETICS STUDIES BY ROUTE OF EXPOSURE

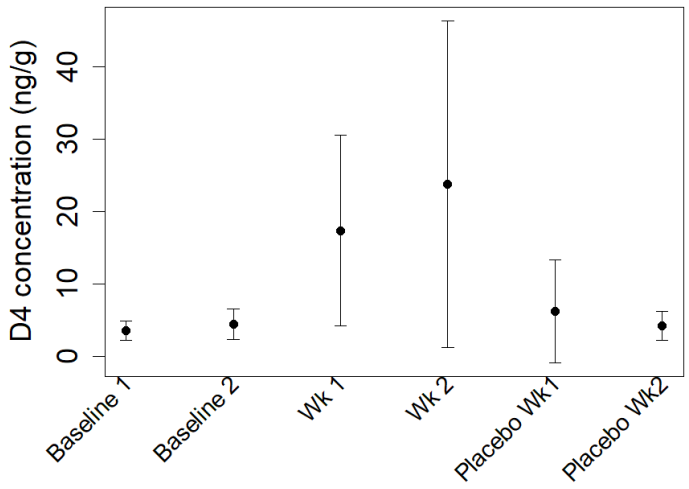
E.1 Oral Route

EPA identified one human study that measured D4 in plasma after oral administration (Dow Corning, 1998c). Three studies in Fischer 344 (F344) rats investigated D4 absorption, distribution, and elimination using single doses from 10 to 300 mg/kg-bw (Domoradzki et al., 2017; Dobrev et al., 2008;

Dow Corning, 1998d). Two of the rat studies analyzed for specific metabolites (Domoradzki et al., 2017; Dow Corning, 1998d).

E.1.1 Humans

Dow Corning (1998c) administered 12 mg D4 per day in corn oil or placebo (corn oil alone) to 12 human subjects (7 females; 5 males) daily for two weeks. The exposures were then stopped for two weeks and subjects were again dosed for two weeks with the substance (either D4 or placebo) they had not received in the first 2-week exposure period. The study was conducted using a double-blind design (neither researchers nor volunteers knew whether they received placebo or D4). Body weights were not provided; to approximate dose per kilogram body weight, 12 mg was divided by the average body weight for male and female adults (80 kg), which yields a D4 dose of 0.15 mg/kg-bw/day. The authors measured D4 concentrations in plasma at baseline and after the first and second weeks of D4 or placebo administration (blood was taken 2 hours after the daily dose was administered) using gas chromatography and mass spectroscopy (GC/MS). Average D4 plasma concentrations and standard deviations are presented in Figure_Apx E-1. After one and two weeks of D4 exposure, plasma D4 values were 17.4 and 23.8 ng/mL, respectively (standard deviations: 13.1 and 22.5 ng/mL). Mean plasma D4 at baseline and after placebo ranged from 3.6 to 6.3 ng/mL. Information on elimination was not obtained and therefore, percent absorption cannot be estimated.



Figure_Apx E-1. Plasma D4 Concentrations in Humans After Oral Dosing
Dow Corning (1998c) measured D4 in plasma before (baseline 1 and 2) and during administration of 12 mg D4 in corn oil (wk 1 and 2) or corn oil only (Placebo wk1 and 2).

E.1.2 Rats

EPA obtained three rat studies that all administered D4 to F344 rats in a single (one-time) gavage dose. Dow Corning (1998d) administered 300 mg/kg-bw D4 to female rats in three separate vehicles. Domoradzki et al. (2017) administered 30 mg/kg-bw D4 to both sexes of F344 rats via gavage in a liquid rat diet. Finally, Dobrev et al. (2008) gave groups of male F344 rats a range of doses from 10 to 300 mg/kg-bw/day in corn oil as a single dose. The studies taken together suggest that absorption differs by vehicle and magnitude of dose.

Dow Corning (1998d) administered single oral gavage doses of 300 mg/kg-bw of ^{14}C-D4 in two vehicles and as a neat preparation to female F344 rats and measured D4 in various matrices up to 168 hours after exposure. Corn oil, Simethicone (a polydimethylsiloxane fluid), and neat D4 resulted in percent absorption values of 51.95, 12.11, and 28.14 percent, respectively. Absorption was expressed as

the percent of total radioactivity recovered in expired volatiles, expired carbon dioxide (CO_2), urine, and carcass relative to the amount of administered radioactivity. Biliary excretion was not measured. The relative AUCs (µg ^{14}C-D4-equivalents per gram blood) also suggested that D4 in corn oil was most highly absorbed with the amount in Simethicone least absorbed. An analysis of the AUC of parent compound (D4) compared with total radioactivity AUC in blood indicated the percent that was the parent compound calculated from information Figure 4 of Dow Corning (1998d) was 17.0, 21.5, and 24.7 when D4 was administered in corn oil, neat, and Simethicone, respectively.

Selected urine samples showed similar metabolite profiles for all vehicles (Dow Corning, 1998d). Five metabolites were identified:

- Methylsilanetriol [$MeSi(OH)_3$]
- Dimethyldisiloxane-1,3,3,3-tetrol [Me_2-Si(OH)-O-Si(OH)$_3$]
- Monomer diol [Me_2-Si(OH)$_2$]
- Trimethyldisiloxane-1,1,3-triol [$MeSi(OH)_2$-O-Si(OH)Me_2]
- Dimer diol [Me_2-Si(OH)-O-Si(OH)Me_2]

Evaluation of radioactivity from whole body autoradiography showed that at one hour, radioactivity appeared in the stomach, upper gastrointestinal (GI) tract, with a moderate amount in fat for all three vehicles. At 6 and 12 hours, highest values were in the GI tract for all three vehicles, with some differences at 12 hours – the neat and corn oil groups had radioactivity in the stomach whereas the Simethicone group showed most radioactivity in the small intestine. At 12 hours, all groups had some radioactivity in fat, brown fat, and clitoral glands.

At 24 hours, rats with neat or corn oil administration had highest amounts in small intestines and cecum; these animals had residual amounts in the stomach. Among rats in these two groups, brown fat and urine contained moderate amounts, and liver, Harderian gland, and bone marrow had levels slightly higher than background. At 24 hours, the rats that received D4 in corn oil had a moderate amount of radioactivity in the clitoral gland. For rats in the Simethicone vehicle group, radioactivity was concentrated in cecum and lower portion of the colon and was detectable in the brown fat and clitoral gland.

At 48 hours, the neat and corn oil administrations had similar profiles with moderate amounts in fat, colon, and clitoral gland. The liver, cecum, small intestine contained smaller amounts, Harderian gland, bone marrow, skin, urine, and fat had lower amounts; residual amounts were observed in the stomach. The Simethicone rat had very low levels in brown fat, colon, and clitoral gland.

At 96 hours, rats that had neat ^{14}C-D4 or ^{14}C-D4 in corn oil had moderately high amounts in the fat and clitoral gland; lower amounts were observed in liver, lower GI tract, skin, bone marrow, and Harderian gland. The rat receiving D4 in Simethicone had only low levels in brown fat and slightly detectable levels in fat (Dow Corning, 1998d).

The cumulative percentages eliminated in feces through 168 hours were 41.0 (corn oil), 54.6 (neat), and 80.6 (Simethicone fluid). The cumulative amounts eliminated in urine were 25.8 (corn oil), 12.8 (neat), and 4.30 (Simethicone fluid). The cumulative percentages exhaled through 168 hours were 14.5 (corn oil), 9.65 (neat), and 6.49 (Simethicone).

Total cumulative percentages of administered doses found in the carcass at 168 hours were 7.64 (corn oil), and 3.59 (neat), and 0.76 (Simethicone (Dow Corning, 1998d).

Domoradzki et al. (2017) administered a single 30 mg ^{14}C-D4/kg-bw dose by oral gavage in a liquid diet dosing solution to male and female F344 rats. Blood was collected at 15, 30, and 60 minutes as well as 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours. Urine, CO_2, and feces were collected from 24 through 168 hours (with an additional urine sample at 12 hours). Expired volatiles were collected at 1, 2, 4, 6, 12, and 24 through 168 hours. Selected tissues and the carcass were collected at 168 hours. Tissues were collected in additional rats at 2, 6, 12, 24, 48, 72, 120, and 168 hours. These tissues included adrenals, digestive tract (without contents), perirenal fat, liver, lung, ovaries, spleen, testes, and uterus. Both the parent compound and total radioactivity were measured in blood, excretions, and tissues (Domoradzki et al., 2017).

Domoradzki et al. (2017) reported percent of radioactivity recovered in the mass balance portion of the study (see Table_Apx E-1). The tissues and carcass were measured at 168 hours and EPA assumes that other measures were cumulative (adding all measurements through 168 hours) even though Domoradzki et al. (2017) does not clearly state the basis of the measurements.

Table_Apx E-1. Radioactivity Eliminated, in Tissues, or in Carcass as Percent of Recovered Radioactivity

Measurement	Females	Males
Percent radioactivity recovered	*87.01*	*85.86*
Expired volatiles	29.90	18.41
CO_2	3.10	4.42
Urine	32.08	40.02
Feces	22.56	27.21
Tissues	1.43	0.98
Carcass	10.73	8.68

Source: Table 1, (Domoradzki et al., 2017)

Mean percent absorption of the recovered radioactivity in female and male rats was 77.2 and 72.5 percent, respectively and was measured as the sum of expired volatiles, exhaled CO_2, urine, tissues, and carcass. Feces represented an unabsorbed dose (assuming no enterohepatic circulation). Domoradzki et al. (2017) suggests this higher absorption is due to the lower doses than the dose used by Dow Corning (1998d). Another difference, however, is the use of a different vehicle (liquid diet dosing solution).

Both parent and total radioactivity were measured in blood. C_{max} for total radioactivity occurred at two and four hours in females and males, respectively. Terminal half-lives of total radioactivity in blood were 104.5 and 80.6 hours in females and males, respectively whereas the half-lives of D4 parent in blood were 20 and 18.7 hours in females and males. C_{max} of parent D4 in blood was lower than in other tissues, and terminal half-lives of parent D4 were also shorter in blood than in other tissues. The percent (based on AUC) of the total radioactivity that was parent compound in blood was 16.7 and 12.6 percent in females and males, respectively (Domoradzki et al., 2017).

Of the small amount found in tissues (0.98 to 1.43 percent), D4 was distributed widely throughout the body, including in blood, perirenal fat, liver, lungs, adrenals, the digestive tract, spleen, uterus, ovaries,

and testes (Domoradzki et al., 2017). The tissue with the highest C_{max} for females when considering total radioactivity occurred in adrenals (247.22 µg eq D4/g tissue) with a terminal half-life of 125.5 hours. The highest C_{max} in males for total radioactivity was the in the digestive tract (151.27 µg eq D4/g), with a terminal half-life of 61.0 hours. The C_{max} of total radioactivity in tissues occurred most often at 2 hours except for perirenal fat in both sexes (12 hours) and adrenals in females and testes in males (both occurred at 6 hours).

The C_{max} of parent D4 in tissues occurred at 2 hours except for fat and testes. The C_{max} for fat occurred at 24 hours in females and 12 hours in males, and the C_{max} for testes occurred at 6 hours. Parent D4 was measurable in tissues through 168 hours. The highest C_{max} for D4 parent was in adrenals (127.51 and 75.08 µg D4/g) with terminal half-lives of 62 and 65.8 hours in females and males, respectively. Of all tissues, the uterus had the longest terminal half-lives: 700.1 hours for total radioactivity, but C_{max} was lower than all tissues except blood. Fat (both sexes) and ovaries (females) showed long half-lives for both total radioactivity and parent D4 (greater than 200 hours, except the D4 parent half-live in fat in males was 166.8 hours). Half-lives for total radioactivity in lungs were 311.9 and 212.0 hours in females and males but parent D4 half-lives in lungs were much shorter.

The largest areas under the curve (AUCs) in tissues were for perirenal fat. Females had the highest AUCs: 7774.09 µg eq* h/g fat (total radioactivity) and 7034.63 µg*h/g fat (D4 parent). In males, AUCs in fat were 4918.3 and 4278.85 for total radioactivity and parent D4, respectively. Adrenals had the next highest AUCs for both sexes.

Although the uterus had long terminal half-lives, based on low concentrations in the uterus, the AUCs in were lower than most other tissues: 700.66 µg eq* h/g (total radioactivity) and 547.2 µg*h/g (D4 parent).

Most of the radioactivity in fat was parent D4 whereas other tissues had lower percentages of D4 parent as a percentage of the total radioactivity, although many tissues (more for females) still showed most of the total radioactivity was parent D4. The percent of radioactivity estimated to be parent D4 is 78.1 in uterus, 89.8 in ovaries, and 61.7 in adrenals (major/potential target tissues), whereas 40.6 percent retained in lungs was associated with D4 parent (Domoradzki et al., 2017).

Domoradzki et al. (2017) analyzed for metabolites in urine and feces. Urine was sampled at the 12-, 24-, and 48-hour collection timepoints. In urine of female rats, dimethylsilanediol represented the greatest percentage of total radioactivity - from 51 to 59 percent across the three collection intervals. Methylsilanetriol ranged from 20 to 25 percent, and dimethyldisiloxane-1, 3, 3, 3-tetrol ranged from 9.5 to 13.1 percent. Similar ranges were identified for males. No sex differences in metabolites were observed other than hexamethyltrisiloxane-1, 5-diol, which was only present in female animals and only after the first two collection times.

In feces collected from 0 to 24 hours, 13 and 26 percent of total radioactivity was methylsilanetriol in females and males, respectively. The amount increased to 55 and 53 percent in females and males at the 24-to-48-hour collection. Parent D4 decreased from 71 to 41 in females and 51 to 24 in males between the 24- and 48-hour timepoints. The only sex difference in identified metabolites was that dimethyldisiloxane-1, 3, 3, 3-tetrol was not present at the 0–24 h collection interval in females.

Dobrev et al. (2008) administered D4 at 0, 10, 50, 100, 200, or 300 mg/kg-bw in corn oil by oral gavage to male F344 rats in open and closed chamber experiments as a single exposure. In the closed chamber, D4 was distributed into the blood and then throughout the body, and free D4 became available to be exhaled through the lungs and back into the chamber; the exhaled D4 was then ‘rebreathed’ by the rats.

The open chamber introduced fresh air and removed a portion of exhaled D4 from the chamber atmosphere, and the authors used information from the open chamber to estimate oral kinetics parameters (Dobrev et al., 2008).

The authors recorded exhaled D4 concentrations for up to 10 hours after dosing using gas chromatography with flame ionization detection (GC-FID). Peak concentrations of exhaled D4 were reached in the open chamber within 4 to 6 hours and declined until 10 hours. The increase in dose from 200 to 300 mg/kg-bw did not result in a proportional increase in exhaled D4 in the open chamber and showed that absorption was higher at lower doses. In the closed chamber, the exhaled concentrations had not reached a peak by 10 hours but simulations suggested that the peaks would be at about 13 to 15 hours after exposure. The authors calculated absorption factors of 1.0 for 10 and 50 mg/kg-bw, 0.85 for 100 mg/kg-bw, 0.65 for 200 mg/kg-bw, and 0.5 at 300 mg/kg-bw. EPA assumes these are fractions (not percentages) (Dobrev et al., 2008).

Dobrev et al. (2008) suggested that various processes such as saturation of uptake/transport mechanisms and nonlinear metabolism and storage might contribute to the toxicokinetic profile. For example, the authors suggest that D4 from oral administration goes directly to a deep lipid pool in the blood compartment and can re-enter the blood or enter deep blood lipid pools in the fat and liver. Thus, D4 appears to act in a unique manner depending on exposure route that differs from other chemicals (Dobrev et al., 2008).

Zhang et al. (2000) administered 0, 1, 5, 20, or 100 mg/kg-bw/day D4 in corn oil via gavage to male and female SD rats for four days. The authors obtained liver microsomes and assayed for enzyme induction CYP 1A1/2, CYP 2B1/2, CYP 3A1/2 and NADPH cytochrome P-450 reductase based on changes in enzyme activity and/or immunoreactive proteins. The CYP 2B1/2 enzyme was increased more than the other enzymes based on both activity (up to 13-fold increase at 20 mg/kg-bw/day in females) and protein levels ($\geq$ 5 and $\geq$ 1 mg/kg-bw/day in males and females, respectively). For CYP 3A1/2, females showed a dose-related increase in protein levels at 20 and 100 mg/kg-bw/day with minor increases in males at 100 mg/kg-bw/day. NADPH cytochrome P450 reductase levels measured using immunochemical analysis were increased by small amounts at 100 mg/kg-bw/day in females, which was statistically significant. For CYP 1A1/2, no changes in protein levels were observed but a modest statistically significant increase in activity was seen at 20 and 100 mg/kg-bw/day.

Falany and Li (2005) administered 0, 5, 20, and 100 mg/kg-bw/day D4 to groups of young, pregnant, and retired breeder female SD rats for 8 days and assayed liver microsomes to assess enzyme induction. PROD and EROD enzyme activities were used to detect increases in CYP 2B1/2 and CYP 1A, respectively. Immunohistochemical analysis was used to detect CYP 2B1/2, CYP 3A1/2, and CYP 1A. Finally, reverse transcription polymerase chain reaction (RT-PCR) was used to detect increases in CYP 2B1/2 in fetal liver and in pregnant dams.

D4 induced CYP 2B isoforms, with statistically significant increases in enzyme activity (based on PROD) at $\geq$ 20 mg/kg-bw/day for young and pregnant rats, and at $\geq$ 5 mg/kg-bw/day in mature rats; young rats had the highest increase (16-fold increase at 100 mg/kg-bw/day compared with controls). Increases in CYP 2B protein were observed at $\geq$5 mg/kg-bw/day in young and mature rats and at $\geq$20 mg/kg-bw/day in pregnant rats, with a maximum of approximately 90-fold increase in mature rats at 100 mg/kg-bw/day. In fetuses, PROD activity was increased by about 4-fold at 100 mg/kg-bw compared with control fetuses. RT-PCR identified increased RNA in dams at 20 and 100 mg/kg-day and the fetuses of the dams from these two dose groups also had low levels of RNA (Falany and Li, 2005).

In pregnant and mature rats, CYP1 A1/2 was increased modestly (but statistically significantly) at ≥ 20 mg/kg (based on increased EROD activity) but not in young rats. No increases were observed using immunohistochemical analysis. Fetal liver

CYP 3A1/2 protein levels increased for young, mature, and pregnant rats with the highest increase observed at 100 mg/kg-bw/day in pregnant rats (55 times higher than controls) but separate isoforms could not be distinguished. Mature rats had the lowest increase (8.5-fold at 100 mg/kg-bw/day) but all doses were statistically significantly higher than controls whereas only the 20 and 100 mg/kg-bw/day dose groups were statistically significantly higher in young and pregnant rats (Falany and Li, 2005).

CYP 1A activity (based on EROD) exhibited a modest increase (> 2-fold at 20 and 100 mg/kg-bw/day compared with controls) in mature and pregnant rats but not in young rats. None of the groups had significant changes in CYP 1A protein levels. Overall, induction differs based on female rat age and reproductive status (Falany and Li, 2005).

E.2 Inhalation Route

EPA identified a study in which humans were exposed to D4 vapor for one hour (Utell et al., 1998). EPA also identified multiple inhalation studies in F344 and SD rats that evaluated toxicokinetics after single or repeated exposures (Schmitt et al., 2023; Meeks et al., 2022; Plotzke et al., 2000; McKim et al., 1998), as well as a study in rats, mice, rabbits, hamsters, and guinea pigs (Dow Corning, 2001b).

E.2.1 Humans

Utell et al. (1998) exposed twelve healthy non-pregnant humans (8 males; 4 females) to 122 mg/m^3 (10 ppm) of D4 vapor using a mouthpiece (plus a nose-clip to avoid nose breathing) for one hour while they exercised intermittently (10 min. rest; 10 min. exercise; 20 min. rest; 10 min. exercise; 10 min. rest). The experiment was conducted in a double-blind fashion so that the researchers and volunteers did not know whether the subjects were exposed to D4 or air.

Subjects acted as their own controls; control (using air only) and D4 exposures were separated by at least one week. Expired air was collected before and immediately after exposure, during a washout phase of 65-75 minutes after exposure, and at 6 hours post exposure. Blood samples were collected before, during the experiment (for plasma and only for 3 subjects), immediately after, and at 1, 6, and 24 hours post exposure. A sample of end expiratory air was also taken later. Urine samples were taken immediately before and after exposure and at 6 and 24 hours post exposure, but results were not reported. To investigate the effects of re-exposure, these subjects were exposed again three months later to D4 or air (Utell et al., 1998).

Blood D4 measurements were initially measured based on whole blood but Utell et al. (1998) discovered that peaks were better defined using plasma. The D4 amounts were similar in plasma and whole blood, which indicated that there was little D4 in blood cells. The recovery of plasma was greater than 90 percent. Thus, D4 was subsequently extracted from plasma and analyzed using GC/MS.

Utell et al. (1998) pooled the results from the mouthpiece experiments that occurred three months apart because there were no significant differences in the results. The mean intake was calculated as the product of the minute ventilation and initial concentration. A mean uptake was calculated as the product of the mean uptake and the deposition fraction, which was calculated using the following equation: (concentration of inspired D4 – concentration of expired D4)/concentration of inspired D4. The authors then summed the products for each exposure period within the hour to obtain total intake and total uptake. The authors calculated an overall mean intake of 137 mg and overall mean uptake of 11 mg

across both experiments. The mean deposition fractions across experiments were 12 percent at rest and 6 percent during exercise with an overall deposition of 9 percent (when considering both exposure during rest and exercise).

The authors assumed that uptake was equivalent to the absorbed dose. However, no information on excretion (in urine, feces) was available, and because this is a human study, no body burden information was available. Thus, this study cannot be used to estimate absorption according to official guidance (*e.g.*, as described by *OECD Guideline for the Testing of Chemicals (Number 417): Toxicokinetics* (OECD, 2010)) in a way that can be compared directly with animal toxicokinetics studies.

In plasma, the mean peak concentration was 79.4 nanogram D4 per gram plasma and occurred during the one-hour exposure period. The amount in plasma decreased to 56.0 ng/g immediately post-exposure, then 42.8 ng/g at one hour, 25.0 ng/g at 6 hours, and below quantitation at 24 hours, showing rapid non-linear decrease.

Utell et al. (1998) conducted a second experiment in eight volunteers (6 males; 2 females) to compare deposition from nasal and oral exposure. The volunteers were exposed for 16 minutes to 122 mg/m^3 (10 ppm) D4 via a nasal device (for nasal exposure), then no exposure for 10 minutes, and then 16 minutes via a mouthpiece (for oral exposure). The experiment was also conducted using the mouthpiece first and then the nasal exposure in the second exposure period. In this nasal/oral comparison, the volunteers were at rest and blood samples were not taken.

In this second experiment, average total intake was 11.5 mg compared with 14.8 mg for mouthpiece vs. nasal breathing, respectively (with estimated uptake of 1.1 and 2.0 mg, respectively). Utell et al. (1998) determined that the mean deposition percent for the nose-only and mouthpiece experiments were both 12 percent after correcting for exposure system losses and adjusting for differences in minute ventilation.

Information from Utell et al. (1998) was used for some parameters in the PBPK model (Campbell et al., 2023).

E.2.2 Rats

Meeks et al. (2022) exposed male and female SD and F344 rats 6 hours per day to 0 or 8490 mg/m^3 (0 or 700 ppm) D4 via whole-body inhalation for 1, 15, or 28 days. The authors also used two groups of recovery animals – 12 hours or 14 days after the 28-day exposure period. The authors measured D4 in plasma and fat by gas chromatography/mass selective detector (GC/MSD) after extraction. Liver microsomes from the 12-hour recovery group after 28 days exposure were assayed for enzyme activity using substrates expected to be specific for the enzyme and/or were measured for amount of enzyme present. The following enzymes (and substrates) were measured for the 12-hour recovery: total protein, total P450, CYP 1A1/2 (based on 7-ethoxyresorufin-O-deethylase (EROD) activity and CYP 1A1/2 protein levels), CYP 2A1 (based on testosterone 7α hydroxylase activity), CYP 2B1/2 (based on 7-pentoxyresorufin-O-depentylase (PROD) and testosterone 16β-hydroxylase activity and increased protein levels), CYP 3A1/2 (testosterone 6β-hydroxylase and protein levels), and CYP 2C11 (based on testosterone 2α hydroxylase activity), epoxide hydrolase protein levels, and NADPH-cytochrome c reductase activity. The liver microsomes from the 14-day recovery groups for both males and females were assayed for total protein, total P450, and CYP **2**B**1**/2. Phenobarbital was used as a positive control during the male study.

Absolute and relative (to body weight) liver weights were increased in both sexes and strains at 1 day after exposure ceased and at 14-days post-exposure remained elevated only for F344 females. Plasma D4 levels were maintained at higher levels through the exposure period with F344 females showing some decrease starting at day 15 ($p \leq 0.05$). day 28.

During exposure, D4 plasma levels ranged from 4.67 to 8.33 µg D4/g plasma in male SD rats and from 4.37 to 6.23 µg D4/g plasma in male F344 rats. In female SD rats, values were 10.45 to 10.98 µg D4/g plasma during exposure, whereas female F344 rats showed 5.44 to 9.15 µg D4/g plasma during exposure. On recovery day 1, plasma D4 decreased substantially for all groups – 0.20 to 0.25 µg D4/g plasma for F344 and SD males and F344 females, with a somewhat higher value for SD females (0.87 µg/g). By recovery day 14, levels were 0.03 to 0.4 across sexes and strains.

Due to its lipophilicity, D4 partitioned primarily to fat and increased to at least day 15 but usually through day 28 with the highest levels per sex and strain (849 to 1,656 µg D4/g fat) observed at recovery day 1. Levels then decreased by recovery day 14 (ranging from 151 to 384 µg/g). SD females always had the highest levels at each timepoint except on recovery day 14. The authors note that the decrease of D4 from fat after exposure indicates slow release of this lipophilic yet volatile compound (Meeks et al., 2022).

On the first recovery day after exposure, enzyme induction was highest for CYP 2B1/2 (with protein levels 28 to 100 times higher than controls and activity from 7.3 to 40 times higher); SD rats had higher protein levels but F344 rats had similar or higher activity levels. The CYP 2B1/2 activity and protein levels were lower by recovery day 14 but some levels were still statistically significantly greater than controls. Meeks et al. (2022) suggested that either the parent D4, a metabolite, or other mediator present or generated specifically in SD females results in inhibited CYP2B1/2 activity but does not limit its expression.

For CYP 3A1/2, female rats given D4 had protein and activity levels of 4.1- to 17-fold higher than controls with the greatest increases in SD rats but SD rats had overall lower protein and activity levels in controls and in exposed rats. There were < 2 to 3-fold increases (protein and/or activity) for NADPH-cytochrome c reductase and epoxide hydrolase (both sexes) and CYP 3A1/2 (males) in among D4-exposed rats. For CYP 1A1/2, both rat strains and sexes had increases of 2 to 3-fold in enzyme activity but decreases in protein levels (down to 22 to 44 percent of controls). CYP 2C11 and CYP 2A1 showed little change in enzyme activity.

Meeks et al. (2022) state that the results suggest D4 has a similar enzyme induction profile as phenobarbital.

Schmitt et al. (2023) exposed female SD and F344 rats in a nose-only inhalation study to 0 or 8490 mg/m^3 (0 or 700 ppm) of ^{14}C-D4 for a single 6-hour exposure or to non-radiolabeled D4 for 14 days followed by a single exposure to ^{14}C-D4 on day 15. The authors sampled blood, plasma, liver, lung, and perirenal fat at multiple times through 168 hours post-exposure. At the end of exposure, the authors collected urine, feces, expired volatiles, and CO_2. Tissues (liver, lung, perirenal fat) and excreta were analyzed for total radioactivity and parent D4, with the exception that the carcass and urine were not analyzed for parent D4. A fraction of blood was processed to obtain plasma. Mass balance and body burden were also determined.

Among single and repeated-dose experiments, recovery of total radioactivity ranged from 78.5 to 98.0 percent, with greater recovery in SD rats. After single exposures, the percent in urine and expired

volatiles was similar (23.5 to 32.6 percent across strains). However, after 14 days, the percent in urine was roughly double (39.8 and 40.2 percent in F344 and SD rats, respectively) the percent in expired volatiles (18.2 and 19.5 percent in F344 and SD rats, respectively). The percent of total radioactivity excreted in feces decreased with longer exposure (19.4 and 18.2 percent after one day in F344 and SD rats, respectively; 12.8 and 15.4 percent in F344 and SD rats after repeated exposure). CO_2 was 3.4 to 3.9 percent of total radioactivity across strains and exposure durations. The amount in carcass was 9.2 and 16.0 percent in F344 and SD rats after single exposures and 4.5 and 6.9 in F344 and SD rats after repeated exposure (Schmitt et al., 2023).

EPA estimated percent absorption from the information presented in Schmitt et al. (2023) by adding the percentages in urine, feces, expired volatiles, expired CO_2, and carcass as a percent of administered radioactivity. Absorption was 82 and 78.8 percent for F344 rats after single and repeated-dose exposures, respectively. In SD rats, absorption was 95.8 and 85.4 percent after single and repeated-dose exposures, respectively.

In blood, total radioactivity and parent D4 AUCs were similar among F344 and SD rats during the single exposure experiment but differed post exposure for the single dose, with parent D4 being higher in SD rats. In the repeated exposure experiment, total radioactivity was similar between strains during exposure but differed post-exposure and parent D4 was higher for SD rats both during and after repeated exposure.
Plasma concentrations of total radioactivity and parent D4 did not differ significantly from blood concentrations (Schmitt et al., 2023).

For the liver, the C_{max} for total radioactivity and parent D4 was reached just after exposure ended across strains and exposure durations. For total radioactivity, the decline was similar For F344 and SD rats for both the single and repeated exposures. The decline of parent D4 in liver after exposure was greater in F344 than SD rats in the first 24 hours and is reflected in differences in AUC for both single and repeated exposures. ;For example, after a single exposure, only half the AUC in liver was the D4 parent in F344 rats, whereas more than 80 percent of the liver AUC was parent D4 for SD rats. Comparable AUCs based on total radioactivity were seen in lungs of both rat strains with 10.6 and 17.9 percent as parent D4 for F344 and SD rats, respectively.

In perirenal fat after single exposures, all radioactivity was identified as parent D4. In F344 rats, the AUC was about 50 percent higher than in SD rats for both total radioactivity and parent D4 after single exposures. This changed after repeated exposure, in that total radioactivity AUC in fat of SD rats was more than 20 percent higher than F344 rats. Time course data suggests delayed distribution into fat.

Urine AUC of total radioactivity in SD rats was two times higher than F344 rats after the single exposure. In contract, the AUC for total radioactivity was 70 percent higher inf F344 than SD rats after repeated exposure. Major metabolites in urine were dimethylsilanediol and methylsilanetriol, and the metabolite profile was similar for both strains. F344 rats, after repeated exposure, showed slight shifts to higher molecular weight metabolites such as di- and trisiloxanols; there was a statistically significant difference from SD rats in the first six hours post-exposure. Fifteen to twenty-two percent of the D4 methyl groups subject to excretion in urine were replaced with hydroxyl groups, with slightly greater replacement in F344 rats (Schmitt et al., 2023).

Jean and Plotzke (2017) measured parent D4 in plasma, liver, and fat of male and female F344 rats after 6 months of exposure via inhalation (6 hours/day, 5 days/week) using GC/MSD. The samples were obtained just after the daily exposure ended and are expected to represent peak values (Jean and Plotzke,

2017). The D4 concentration ranges in various tissues associated with the D4 air exposures of 121 to 8490 mg/m^3 (10 to 700 ppm) were as follows:

- Plasma:
  - 0.0722 to 8.21 µg/mL (males)
  - 0.153 to 13.0 µg/mL (females)
- Liver:
  - 0.58 to 71.19 µg/g (males)
  - 1.18 to 76.71 µg/g (females)
- Fat (brown, peri-renal, and abdominal):
  - 3.35 to 866 µg/g (males)
  - 11.1 to 1240 µg/g (females)

Most control animals had levels below the limit of quantitation except in the liver for which levels were 0.30 and 0.28 µg/g in males and females respectively.

Females had consistently higher tissue levels of D4 for most tissues and exposure concentrations. Jean and Plotzke (2017) compared the measured D4 tissue concentrations with "expected" concentrations for higher exposures (using the 121 mg/m^3 value as the comparison). To be proportional to air concentrations, the tissue concentrations would be 3, 15, and 70 times higher at the 364, 1,820, and 8,492 mg/m^3 air concentrations compared with 121 mg/ m^3. Most tissue values were higher than expected except D4 levels in the liver were slightly lower than expected at all exposures in females and lower at 364 and 1,820 mg/m^3 (30 and 150 ppm) in males.

Plotzke et al. (2000) exposed male and female F344 rats via a nose-only exposure, to 84.9, 849, or 8490 mg/m^3 (7, 70, or 700 ppm) ^{14}C-D4 vapor for a single 6-hour exposure. The authors also exposed both sexes of F344 rats to unlabeled D4 for 14 days to 84.9 or 8490 mg/m^3 followed by ^{14}C-D4 for a single day (day 15). Control rats were also used.

Animals were split into groups and analyzed for body burden, distribution, or elimination information. For both single and repeated-dose body burden experiments, the carcass, urine, and feces were solubilized together directly after exposure. In the distribution analyses, samples were taken up to 168 hours after exposure - radioactivity in blood and plasma, liver, lungs, perirenal fat, ovaries, vagina, and testes were measured. In the elimination analysis, authors measured total radioactivity in urine, feces, expired volatiles, and exhaled CO_2 up to 168 hours after exposure. Cages were rinsed at 72, 120, and 168 hours in the elimination group. Elimination results were compared with a control group(Plotzke et al., 2000).

In the body burden experiment, rats retained 5.0 to 6.1 percent of the total radioactivity at the end of the single 6-hour exposure (as measured in the carcass plus urine and feces in exposure tube). As determined from the distribution experiments, the highest amounts of D4 µg equivalents were in the lungs, liver, fat and ovaries immediately after exposure (Plotzke et al., 2000).

In plasma for the single exposure experiments, radioactivity increased through exposure and showed a multiphasic time course with a rapid decline within 24 hours and then a slower terminal phase. In general, the tissue to plasma ratios were 1 or higher for most tissues (except testes) and indicated that radioactivity was readily taken up by tissues. In general, the tissue-to-plasma ratios increased through time indicating slower elimination from tissues than from plasma (Plotzke et al., 2000).

In fat, retained amounts increased at a higher rate than the overall increase in exposure concentrations. At the highest concentration after a single exposure, the retained amount was 137 (female) to 188 (male) times greater than at the lowest concentration even though the difference in exposure concentrations was only 95 times higher at the highest vs. lowest concentrations. Concentrations in plasma increased at a much lower rate (36 to 46 times at the highest vs. lowest concentrations). Female rats had somewhat lower radioactivity concentrations in plasma but higher values in fat than their male counterparts just after single exposure; similar but less pronounced differences occurred after repeated exposure (Plotzke et al., 2000).

After a single exposure to D4, the peak concentrations (C_{max}) for most tissues and plasma occurred within 1 hour but the C_{max} for fat occurred from three to 24 hours post exposure. The AUC values in fat were 12- to 61-fold higher than those for plasma after a single exposure, with similar ratios after repeated exposure. Other tissue AUCs were somewhere between those for plasma and fat for both exposure durations except that those for testes were very similar to plasma AUCs. Uterus, vagina, and ovary AUCs were higher than the testes AUCs (Plotzke et al., 2000).

The authors reported results for the single exposure elimination experiments. Most recovered radioactivity was found in urine (approximately 33 to 48 percent) and expired volatiles (approximately 28 to 35 percent). In females, at the highest exposure (8490 mg/m^3), more of the radioactivity that was eliminated was found in expired volatiles compared with urine, but in males, a higher percent was found in urine compared with expired volatiles. For urine and expired volatiles, most was recovered by 48 hours after exposure. Feces contained 10 to 15 percent and expired CO_2 had approximately five percent or less of total radioactivity. After 168 hours in the single exposure study, 7.8 to 12.3 percent of total radioactivity remained in the carcass (which was mostly muscle, fat, and bone). Only 0.495 to 0.794 percent was found in tissues after 168 hours. Similar results regarding elimination were seen after repeated exposure although it is interesting that lower percentages were observed in tissues (0.193 to 0.468 percent of total radioactivity and carcass (6.53 to 8.50 percent) when measured at 168 hours after exposure (Plotzke et al., 2000).

EPA added the percent of total radioactivity identified in feces, urine, expired volatiles, as CO_2, and carcass (estimated from Figures 4 and 6 in Plotzke et al. (2000)), and estimated absorption as between approximately 94 and 96 percent for the lowest concentration across experiments and sexes. The amount of D4 exhaled as volatiles was approximately 27 to 29 percent of total radioactivity.

Almost all radioactivity in expired volatiles (up to 9 hours post-exposure) and all radioactivity in fat was parent D4 up through 168 hours after exposure. The authors found no unmetabolized D4 in urine collected from 0 to 48 hours after single or multiple exposures. Two major metabolites, dimethylsilanediol and methylsilanetriol, comprised 75 to 85 percent of the radioactivity in urine, and at least five minor metabolites were identified. Formation of methylsilanetriol indicated oxidative demethylation at the silicon-methyl bonds. The five minor metabolites were suggested by the authors as indicating hydrolysis and/or oxidation after original oxidated metabolism of D4 by cytochrome P450 enzymes (Plotzke et al., 2000).

McKim et al. (1998) exposed both male and female F344 rats to 0, 849, and 8490 mg/m^3 (0, 70, and 700 ppm) D4 vapor via whole-body exposure for six hours per day, five days per week for four weeks. Phenobarbital was used as a positive control. Rats were killed on days 3, 7, 14, 21, and 28. Microsomal fractions from the liver were examined for enzyme activity using substrates considered to be specific for each enzyme examined. Enzyme levels were also analyzed using immunoreactive protein for most enzymes.

Cytochrome P450 2B1 and 2B2 activity using the substrate PROD was statistically significantly increased from day 3 to 28 during exposure at both air concentrations and through day 14 post exposure at the highest air concentration. Males exhibited a maximum of 27-fold higher activity at the highest concentration on day 28. Females exhibited maximum activity levels of 46-fold on day 7 at the highest concentration. CYP 2B1/2 immunoreactive protein was statistically significantly increased compared with controls by day 28 at 849 and 8490 mg/m^3 (female data shown). CYP 3A1 and CYP 3A2 showed modest increases in activity using 6β-hydroxylation of testosterone and by immunoreactive protein. Although CYP 1A1 and 1A2 exhibited a < 2-to-3-fold increase in activity with EROD, D4 resulted in no induction of CYP 1A1 and a suppression of CYP 1A2 when measured by immunoreactive protein. NADPH cytochrome P450 reductase showed slightly increased induction at both D4 exposure concentrations. Epoxide hydrolase activity and immunoreactive protein levels were increased two to approximately three times higher than controls in a dose-dependent manner, whereas UDP-glucuronosyltransferase activity exhibited only slight increases in activity that were not dose related and CYP 4A enzyme activity and protein levels did not differ from negative controls. There were some similarities to the enzyme induction profile of phenobarbital.

Dow Corning (2001b) exposed male and female SD rats and Hartley guinea pigs via inhalation to 0 and 700 ppm for six hours per day for five days. The authors then evaluated whether D4 exposure induced the following enzymes based on enzyme activities: glutathione S-transferase activity was measured using cytosolic liver fractions, and epoxide hydrolase and 7-ethoxycoumarin O-deethylase (ECOD) activities were measured using microsomal liver fractions. ECOD is a substrate for multiple cytochrome P450 enzymes. Male rats showed significantly increased induction of all three enzymes compared with controls whereas female rats had increases in epoxide hydrolase and ECOD compared with controls; all increases were less than two times the control. In contrast, guinea pigs did not show increased enzyme induction that was statistically significantly greater than controls.

E.3 Dermal Route

When investigating kinetics from the dermal route (focusing on absorption in particular), EPA identified two *in vivo* studies in humans (Biesterbos et al., 2015; University of Rochester Medical Center, 2001) and three *in vivo* studies in rats and mice (one in which nude mice were grafted with human skin) (Dow Corning, 2001a; University of Rochester Medical Center, 2000; GE, 1994a). EPA identified *in vitro* studies using human skin (Dow Corning, 1998a, 1991), miniature swine skin (Dow Corning, 2006), and rat skin (GE, 1994b).

D4 volatilizes from skin; over the time periods studied, the volatilized amounts were upwards of 90 to near 100 percent for several studies. Most studies show very low absorption (1 percent or less) but some have higher values (up to 19.96 percent in an *in vivo* rat study after 6 hours of exposure for unoccluded scenarios, which are the relevant scenarios used in the risk evaluation).

EPA has formal data quality criteria for some dermal absorption studies – 1) animal *in vivo* studies, and 2) *in vitro* studies. Data quality criteria for dermal absorption studies are critical because dermal absorption data represent a very important aspect of almost all EPA risk evaluations conducted under TSCA. Formal criteria for human *in vivo* studies are in development. Details related to each of the studies with OQDs of high or medium are listed in Table_Apx E-2. Studies that have low or uninformative OQDs are discussed in the narrative below only if they provide important aspects for comparison with the acceptable studies. Data quality evaluations (with comments related to all metrics) and basic data extractions for all acceptable and uninformative studies are provided in the Draft Data Quality Evaluation and Extraction of Dermal Absorption Information for D4 (U.S. EPA, 2025b).

Some amounts of D4 loaded to skin in the available dermal absorption studies could be considered 'infinite' doses as noted by the Organisation of Economic Cooperation and Development's (OECD's) *Guidance Document for the Conduct of Skin Absorption Studies* (OECD, 2004) and could possibly be used to estimate Kp and flux. However, fractional absorption is best used to characterize non-steady state absorption of liquids or solids where evaporation is expected to be uninhibited (*i.e.*, for any unoccluded scenarios).

E.3.1 Humans

EPA identified two *in vivo* studies in which D4 was applied to skin using human volunteers (Biesterbos et al., 2015; University of Rochester Medical Center, 2001).[21]

University of Rochester Medical Center (2001) applied target doses of 1.4 grams to the axilla (armpits) to males (3 volunteers) and 1 gram to the axilla of females (3 volunteers). Axilla were not washed, and the authors collected information on exhaled breath, blood, and plasma concentrations at 0, 1, 6, and 24 hours after application. The volunteers collected their own urine over 24 hours, but results were not presented. Peak levels in exhaled air and in blood/plasma occurred one hour after D4 was applied to skin. Mean peak values in air were 30 and 111 ng/L in males and females, respectively. Peak levels in plasma occurred at 1 hour and ranged from 0.85 to 7.02 ng/g among individual volunteers. Mean peak blood values were 1.30 and 4.45 ng D4/g blood in males and females, respectively. Although urine was not analyzed and both feces and skin amounts were not measured, *in vivo* rodent studies indicate these levels are generally very low (see Section E.3.2). University of Rochester Medical Center (2001) is used to estimate some of the dermal parameters in the D4 PBPK model (Campbell et al., 2023).

Biesterbos et al. (2015) applied neat D4 every 10 minutes for a total of one hour to the forearms of 15 volunteers[22]; the forearms were placed in a hood so that the volunteers would not inhale D4 from the exposed arms. The mean sum of D4 doses for each 10-minute exposure throughout the hour exposure was 10.1 mg/cm^2. Biesterbos et al. (2015) used a control group exposed only via inhalation to artificial forearms dosed with D4 placed *next* to the individuals acting as controls. Although Biesterbos et al. (2015) stated that dermal absorption was barely higher than background levels, the range of exhaled D4 after dermal exposure (range: 7.5 to 280 ng/L) was higher than in the controls (range: 0.8 to 3.5 ng/L).

E.3.2 Rats and Mice

Three *in vivo* studies in rodents (Dow Corning, 2001a; University of Rochester Medical Center, 2000; GE, 1994a) with medium or high OQDs estimated dermal absorption ranging from 0.35 to 19.96 percent for unoccluded scenarios.[23] All three studies cite the same methods publication (Susten et al., 1986) that described application of an aluminum skin depot on the skin using cyanoacrylate glue and vapor traps. Susten et al. (1986) checked for leaking/migration from the skin application site using the described method and found the results to be acceptable. A fourth study (Dow Corning, 1992b), in rats, received a low OQD.

Dow Corning (2001a) applied neat D4 doses to skin of F344 rats for 1, 6, and 24 hours with evaluation of absorption at the end of exposure. Additional groups were exposed for 24 hours, and then skin was

[21] Powell et al. (1996), an abstract, was not available as a full study; the median amount of D4 in the epidermis in humans for under occluded conditions for 48 hours using Finn chambers was 2.1 percent of the administered dose and 0.7 percent in the dermis.

[22] D4 concentrations in personal care products were too low for quantification.

[23] The authors identify the scenarios as semi-occluded, but they were only semi-occluded due to use of the vapor traps; therefore, it appears to be more appropriate to describe these as unoccluded.

washed, a new charcoal basket used to capture volatilized D4, and absorption was measured through 168 hours. For each condition, the authors applied low, medium, and high nominal amounts of 2.0, 4.8, and 10.0 mg/cm^2 skin. The authors measured total radioactivity (inclusive of D4 and any metabolites) in blood (high dose only), carcass, excised skin, the skin depot, the stratum corneum, skin washes, expired volatiles, urine, feces, and CO_2. Parent D4 was also analyzed in blood and expired volatiles using GC/MS methodology.

The amount of total radioactivity and parent D4 in blood of the high-dose group was similar to background levels. Percent of total radioactivity in expired volatiles was 0.47 percent or less.

Most of the test substance evaporated from skin within 6 hours. Less than 1 percent of the applied dose was recovered from the skin surface at all time points. The highest amount found in feces was 0.02 percent of total radioactivity and urine had 0.08 percent and lower. The carcass had no higher than 0.05 percent and exhaled CO_2 was only 0.03 percent or lower.

The percent absorbed ranged from 0.35 to 0.95 percent across exposure durations and doses (Dow Corning, 2001a); these values do not include the tape strips because some of the amount left in skin, even after tape stripping, likely volatilized over time based on slight decreases in the amount absorbed from 1 to 24 hours. The authors note that the differences between these two time points were significant and were in good correlation with decreases in amount in the skin; however, no statistical analysis was presented for the difference between 1 and 24 hours. Dow Corning (2001a) did state, however, that there were no differences in absorption between 1 and 6 hours or between 6 and 24 hours, noting that the p value was greater than 0.05. Low recovery for the one-hour exposure at the high dose may be due to volatilization of the amount in skin (9.37 percent) after the charcoal baskets were removed; the high-dose experiments at 6 and 24 hours had much lower amounts in skin, which lends support to the possibility that amounts in skin continue to volatilize over time.

University of Rochester Medical Center (2000) applied neat ^{14}C-D4 for 24 hours to female nude mice with human skin grafts. To end exposure, skin was tape stripped at 24 hours, and volatilized D4 was measured at this timepoint. Other measures were taken at one or more timepoints up to 72 hours. The authors reported a value of 1.09 percent absorption under the following conditions: 1) activated charcoal to capture volatile D4; 2) Vaseline® to control D4 from leaking out of the skin depot; and 3) Soluene-350 and isopropanol instead of tetraethylammonium hydroxide (TEAH) to solubilize feces to avoid chemiluminescence that can interfere with liquid scintillation counting. The 1.09 percent absorption value does not include the amount found in tape strips, but the authors did not wash skin and the exclusion of the small percent in the tape strips (0.015) had minimal impact on the overall value.

The vast amount of the applied dose (94.59 percent) evaporated from the application site. The amount of radioactivity exhaled after absorption through skin was 0.46 percent, amount in excreta (feces and urine) was 0.54 percent, the amount in the carcass was 0.02 percent, and the amount exhaled as CO_2 was 0.05 percent. Only 0.1 percent remained at the application site (University of Rochester Medical Center, 2000).

University of Rochester Medical Center (2000) investigated other methods to measure absorption that included no Vaseline®, use of TEAH for feces solubilization, and use of ethoxyethanol bubblers to catch volatilized D4. The authors reported that the ethoxyethanol bubblers created back pressure and only a small amount of D4 was captured (University of Rochester Medical Center, 2000). This back pressure may have resulted in volatilized D4 from the skin surface being captured inadvertently by the

charcoal tubes that were supposed to capture only exhaled D4. It is not clear why the skin depots leaked but it may have been due to detachment of the skin depot.

GE (1994a) measured absorption of radiolabeled D4 applied to skin of Sprague-Dawley rats for 6 hours. The authors did not state whether they used Vaseline®. The study did use TEAH when preparing the feces, which may have led to possible interference of chemiluminescence; however, excreted values were routinely low across studies (about one percent or less).

For the experiments using vapor traps without added occlusion, the authors reported 18.61 and 19.96 absorption for female and male rats, respectively. Approximately 70 percent of the applied dose volatilized from skin, twelve percent was exhaled, and 6 percent was found at the skin application site. Urine, feces, carcass, and exhaled CO_2 each accounted for less than one percent each. Total recovery was 102.78 and 97.73 percent in males and females, respectively (GE, 1994a).

In the occluded scenario (Saran wrap secured with adhesive tape with an outer elastic wrap), 41.77 and 33.91 percent was absorbed in males and females, respectively. Approximately 40 percent of the administered dose was found in the bandages, 5-10 percent found in the skin, 25 percent in expired volatiles, approximately one percent or less each in urine, feces, and carcass; less than 1 percent was exhaled as CO_2. Total recovery was lower than the unoccluded scenario: 77.14 percent for females and 84.05 percent for males, respectively (GE, 1994a).

Dow Corning (1992b) applied neat D4 as 100 mg/kg-bw to male rats for 24 hours under occluded conditions and measured expired volatiles, urine, feces, tissues, and organs up to 96 hours after exposure. EPA assigned a low OQD to this study, and based on the low quality of the study, EPA considered the overall absorption values from this study to be less reliable (and they are not summarized in Table_Apx E-2). Reasons for the low OQD include the following: lack of information on body weights to calculate the dose applied to skin; low recovery of D4 (35.1 percent); lack of information on storage conditions and whether washing was conducted; high coefficients of variation; and other issues.

The fraction of D4 absorbed among three *in vivo* studies in rodents (Dow Corning, 2001a; University of Rochester Medical Center, 2000; GE, 1994a) that used vapor traps without full occlusion ranged from 0.35 to 19.95 percent. It is not clear why there are such differences in absorption values. The three studies all referenced the same methods publication (Susten et al., 1986) that described application of an aluminum skin depot on the skin using cyanoacrylate glue and vapor traps; the publication also described experiments that checked for leaking/migration of test substance from the skin application site and found the results to be acceptable.

Two studies (Dow Corning, 2001a; University of Rochester Medical Center, 2000) directly referenced use of cyanoacrylate glue. GE (1994a), which measured the highest absorption values, used Skin Bond ®. Although EPA could not verify whether this adhesive is a cyanoacrylate compound, the authors cite Susten et al. (1986), and EPA assumes they did use a cyanoacrylate glue. However, if Skin Bond ® did not strongly bond to skin, this could have inadvertently led to leaking with subsequent capture by the charcoal tube that measures expired volatiles. This would result in an erroneously high estimate of absorption and could possibly explain the higher expired volatiles content in the GE (1994a) study, which was 12 percent 0.12 to 0.32 percent for Dow Corning (2001a) and 0.46 percent for University of Rochester Medical Center (2000).

There were other differences among the rodent *in vivo* studies: 1) skin was exposed to D4 for different time periods (1, 6, or 24 hours); 2) the species/strains were Sprague-Dawley CD rat, F344 rat, or nude

mice (with human skin grafts); 3) the amount of D4 applied to the skin surface ranged from 1.88 to 9.85 mg/cm^2; and 4) the number of measurements used to calculate absorption during and after exposure differed – once vs. multiple time points. Leaking may have also occurred depending on the sampling (*e.g.,* if multiple sampling times required multiple instances of opening the skin depot, it is possible that D4 evaporated to the charcoal tubes and was inadvertently counted as expired D4). GE (1994a), which had the highest absorption percentages, also had the largest number of sampling times.

E.3.3 *In Vitro* Studies

EPA identified four acceptable dermal exposure studies using excised skin – from humans (Dow Corning, 1998a, 1991), miniature swine (Dow Corning, 2006), and rats (GE, 1994b). Absorption estimates range from 0.004 to 11.64 percent.

Dow Corning (1998a) evaluated dermal absorption using neat D4 applied to excised human skin (split thickness) for 24 hours in a flow-through system. Charcoal baskets captured D4 that volatilized from the skin. Although the study authors did not include tape strips in their estimate of percent absorption, OECD's *Guidance Notes on Dermal Absorption Studies* (OECD, 2022) recommends that tape strips (or tape strips minus the first two) be included if less than 75 percent of cumulative doses was absorbed at half the full exposure duration. Dow Corning (1998a) showed that 50.0 and 64.4 percent of the total amount absorbed ultimately absorbed was absorbed at 12 hours (the mid-point of the full 24-hour exposure). Therefore, EPA included the tape strips for a total absorption value of 1.02 percent; the tape strips could not be separated because the study only reported the radioactivity for all tape strips combined. Continued volatilization of D4 from skin may occur over time; if this does occur, adding tape strips results in a slightly conservative absorption estimate.

Dow Corning (1991) ran three dermal absorption experiments each using excised human skin from separate donors. D4 was applied neat and semi-occluded for 24 hours as 8.33 mg/cm^2 in two experiments and 0.44 mg/cm^2 in the third. The authors could not easily explain the poor recoveries obtained from at least seven of 12 cells across the three donors; the authors suggested the need for a different apparatus for volatilized D4 such as using bubblers instead of Amberlite adsorbent used in this study. The authors also note that a higher amount of radioactivity may result in a better estimation of penetration through skin. The higher absorption by the first donor may have been explained by her advanced age and possible thinner stratum corneum (Dow Corning, 1991).

Dow Corning (2006) applied D4 in personal care products (skin moisturizer, antiperspirant, and cuticle coat, with percentages of D4 ranging from 5 to 95.8) unoccluded to the skin of Yucatan miniature swine for 24 hours. Estimates of dermal absorption ranged from 0.004 to 0.24 percent. Recovery of D4 from all matrices ranged from 90.52 to more than 100 percent. EPA assigned this study an OQD of medium.

GE (1994b) evaluated skin absorption using neat D4 applied to rat skin for a 6-hour exposure duration. A greater amount of D4 was found in the skin than in several other studies. EPA calculated a total absorption of 11.64 percent, including the amount in the receptor fluid, amount in skin after tape stripping and washing, and the amount in the tape strips. EPA included the tape strips in this experiment because, according to OECD (2022), the amount absorbed did not reach 75 percent at 3 hours (the mid-point of 6 hours). EPA assigned a medium OQD to the female experiment. For the experiment in males, EPA assigned a low overall quality determination and therefore, it is not discussed here (total absorption in males was 6.73 percent).

Krenczkowska et al. (2019), Krenczkowska et al. (2020) and Mojsiewicz-Pieńkowska et al. (2022) evaluated the distribution of different quantities of neat D4 applied under occlusion to excised human

skin for 24 hours. In addition to other limitations, the studies were not conducted using methods that allowed quantitation of dermal absorption. Therefore, EPA assigned uninformative OQDs to these studies.

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5387 **Table_Apx E-2. Summary of D4 Dermal Absorption Values**

Species/ Dose type	Sex/ Model	Exposure Duration (h)/Timing of Absorption Measurements (h)	Occlusion/ Radiolabeling/ Tape Stripping/ Washing/ Vehicle (if diluted)	Mass/skin SA - mg/cm^2 (volume/ skin SA - µl/cm^2) *[Percent D4 in applied dose]*	Percent Absorption *[Total recovery]*	Basis for absorption *[Amount volatilized]*	Reference/ Rating
In Vivo							
Rat – F344 Finite	Female	Exposure: 1 h Absorption: 1 h	Unoccluded (vapor traps) [a]/ Radiolabeled / Tape strips (5)/ Skin washed 3x with a cotton swab, soaked with a 1 % soap solution, followed by a dry swab, then washed 3x with a cotton swab soaked with 70 % ethanol, and one dry swab /	1.88, 4.85, 9.85 *[100]*	0.79, 0.80, 0.95 *[101.88, 94.77, 76.32]*	Blood (high dose only), charcoal tube (exhaled volatiles), excised skin, urine, feces, CO_2 trap, carcass, cage rinse [b] *[100.56, 93.05, 66.00]*	Dow Corning (2001a) OQD = High
		Exposure: 6 h Absorption: 6 h		2.15, 4.8, 9.78 *[100]*	0.78, 0.69, 0.79 *[92.80, 98.61, 96.03]*	Blood (high dose only), charcoal tube (exhaled volatiles), excised skin, urine, feces, CO_2 trap, carcass, cage rinse *[91.75, 97.66, 94.98]*	
		Exposure: 24 h Absorption: 24 h		1.63, 4.8, 9.35 *[100]*	0.76, 0.57, 0.61 *[96.42, 94.57, 93.01]*	Blood (high dose only), charcoal tube (exhaled volatiles), excised skin, urine, feces, CO_2 trap, carcass, cage rinse *[95.43, 93.86, 92.24]*	

Species/ Dose type	**Sex/ Model**	**Exposure Duration (h)/Timing of Absorption Measurements (h)**	**Occlusion/ Radiolabeling/ Tape Stripping/ Washing/ Vehicle (if diluted)**	**Mass/skin SA - mg/cm^2 (volume/ skin SA - µl/cm^2) *[Percent D4 in applied dose]***	**Percent Absorption *[Total recovery]***	**Basis for absorption *[Amount volatilized]***	**Reference/ Rating**
		Exposure: 24 h Absorption: 168 h		2.1, 4.88, 9.78 *[100]*	0.51, 0.47, 0.35 *[92.99, 96.00, 91.69]*	Blood (high dose only), charcoal tube (exhaled volatiles), excised skin, urine, feces, CO_2 trap, carcass, cage rinse *[92.32, 95.44, 91.24]*	
Mouse (nude) with human skin graft Finite	Female	Exposure: 24 h Absorption: 24 h for tape strips, charcoal baskets (unabsorbed, volatilized D4) 24, 48, and 72 h for urine, feces, exhaled CO_2 72 h for exhaled volatiles, skin (human, mouse), carcass, cage wash	[a] Radiolabeled/ No washing	15.7 (10 µl/cm^2) *[100]*	1.09 *[96.35]*	Human skin application site, animal skin, carcass, feces, urine, volatile trap (from exhaled air), CO_2 trap[c] *[94.59]*	University of Rochester Medical Center (2000) OQD = Medium
Sprague Dawley CD rats Finite [hair was clipped]	Female, Male	Exposure: 6 h Absorption: 1, 2, 3, 6, 9, 12, 24, 48, 72, 96 h for expired volatiles 6, 12, 24, 48, 72, 96 h for urine, feces	Unoccluded (vapor traps) [a]/ Radiolabeled/	5.73 (5.9 µl/cm^2) *[100]*	18.61 (F), 19.96 (M) *[102.78 (F), 97.7 (M)]*	Dose sites (assumed to be skin after tape stripping), feces, urine, CO_2, charcoal tubes, carcass [d] *[66.26 (F), 68.51 (M)]*	GE (1994a) OQD = Medium

Species/ Dose type	Sex/ Model	Exposure Duration (h)/Timing of Absorption Measurements (h)	Occlusion/ Radiolabeling/ Tape Stripping/ Washing/ Vehicle (if diluted)	Mass/skin SA - mg/cm^2 (volume/ skin SA - µl/cm^2) *[Percent D4 in applied dose]*	Percent Absorption *[Total recovery]*	Basis for absorption *[Amount volatilized]*	Reference/ Rating
		6, 96 h for the exposure site 96 h for whole body	Occluded	5.73 (5.9 µl/cm^2) *[100]*	33.91 (F), 41.77 (M) *[77.14 (F), 84.05 (M)]*	Dose sites (assumed to be skin after tape stripping), feces, urine, CO_2, charcoal tubes, carcass [d]	
In Vitro							
Human Finite (high doses, but D4 is very volatile)	Split thickness (~ 407 µm)/ Flow-through	Exposure: 24 h Absorption: Every hour until six hours, then every three hours through 24 h (receptor fluid)	Un-occluded (vapor traps) [a]/ radiolabeled/ washing dry swab, 3 soap swabs, dry swab, and then 3 ethanol swabs	10.7 *[100]*	1.02 *[91.63]*	Receptor fluid, skin (after tape stripping and washing), Saran Wrap (for excised skin), tape strips	Dow Corning (1998a) Medium
Human Finite	Split thickness (~500 µm)/ Flow-through	Exposure: 24 h Absorption: Every hour for 24 h (receptor fluid)	Un-occluded (vapor traps) [a]/ Radiolabeled/ No tape stripping/ Skin washed at 24 h, once with 20 % Ivory liquid soap, then two rinses with water	0.44 (5 µl/cm^2) [78-yr-old female] 8.33 (8 µl/cm^2) [52-year-old male] 8.33 (8 µl/cm^2) [40-year-old male] *[100]*	4.5, 0.29, 0.32 *[38.6, 63.8, 51.1]*	Skin, receptor fluid [e] *[33.00, 62.75, 41.12]*	Dow Corning (1991) OQD = Medium

Species/ Dose type	Sex/ Model	Exposure Duration (h)/Timing of Absorption Measurements (h)	Occlusion/ Radiolabeling/ Tape Stripping/ Washing/ Vehicle (if diluted)	Mass/skin SA - mg/cm^2 (volume/ skin SA - µl/cm^2) *[Percent D4 in applied dose]*	Percent Absorption *[Total recovery]*	Basis for absorption *[Amount volatilized]*	Reference/ Rating
Yucatan miniature swine Finite (high doses, but D4 is very volatile)	Split thickness/ flow-through	Exposure: 24 h Absorption: Every hour to 6 h, then at 9, 12, 15, 18, 21, and 24 h (receptor fluid); 24 h for all other measures	Personal care matrices/ Un-occluded (vapor traps)[a] / Radiolabeled/ Tape stripping/ Washing with dry cotton-tipped applicators and then blotting with 3-4 cotton-tipped applicators moistened with 1 % aqueous soap solution and then dry swabs/personal care matrices	11.2 and 10.6 mg/cm^2 *[5 and 41.7, in skin moisturizer]*	0.07, 0.24 *[91.16, 90.52]*	skin (after tape stripping), receptor fluid, and tape strips [f] *[90.90, 90.27]*	Dow Corning (2006) OQD = Medium
		Exposure: 24 h Absorption: Every hour to 6 h, then at 8, 10, 12, 14, 16, 18, 20, 22, and 24 h (for receptor fluid) 24 h for all other measures		10.3 and 10.6 mg/cm^2 *[10.6 and 62.2 in antiperspirant]*	0.004, 0.02 *[99.00, 95.18]*	skin (after tape stripping), receptor fluid [f] *[98.57, 94.85]*	
				12.1 and 10.5 mg/cm^2 *[51.6 and 95.8 in cuticle coat]*	0.142, 0.230 *[98.46, 105.44]*	skin (after tape stripping), receptor fluid, and tape strips [f] *[98.18, 105.13]*	
Rat Finite	Full thickness/ Static	Exposure: 6 h Absorption: 30 min and every hour up to 6 h (receptor fluid)	Un-occluded (vapor traps) [a] / Radiolabeled/ Tape stripping/ Skin wiped with dry filter, then with gauze moistened by alcohol, then cotton swab moistened with alcohol	4.69 (4.84 µl/cm^2) *[100]*	11.64 [g] *[76.9]*	Receptor fluid, amount in skin after tape stripping and "washing", tape strips (all) *[60.75]*	GE (1994b) OQD = Medium [h]

[a] These studies referred to the methods as semi-occluded. However, EPA prefers calling the results un-occluded because the ability of D4 to volatilize appears to be uninhibited, with the volatilized amounts captured by the charcoal basket vapor traps.

[b] The amount of D4 in blood was below detection and therefore, was not obtained for the low and medium doses.

[c] Because the authors did not wash skin and did not separate tape strips, EPA did not include tape strips in the estimate of percent absorption. The exclusion of tape strips (0.015 percent) had a minimal effect on the estimate of absorption (1.09 vs. 1.11 percent). The authors also did not collect blood. EPA expects the amount in blood to be minimal because Dow Corning (2001a) found that D4 in blood was not above background using the highest dose in the study of close to 10 mg/cm^2]

[d] Absorption includes: dose sites (assumed to be the amount remaining in skin after tape stripping), feces, urine, CO^2 traps, charcoal tubes (expired volatiles), carcass; Absorption excludes: charcoal basket, dosing bandage, back-up bandages, filter wipes, swab wipes, tape strips. Tape strips were excluded because it appears that more than 75 percent of the total test substance (if considering the expired volatiles amount) that was ultimately absorbed had been absorbed prior to the mid-point of the exposure duration.

[e] Some calculations were errors in the reference. For the 78-year old, cells 1 and 3 added receptor fluid and skin wash (not the amount remaining in the skin). Therefore, the percent absorbed was recalculated and changed to 4.5 vs. 4.4 %. Also, the authors presented the average absorption (across 4 cells) for the 40-year old as 0.47 % but the value was actually 0.32 %. The authors did not clearly identify whether the receptor percent absorption also included D4 from the receptor rinses; if rinses were not included, the absorption may be underrepresented. On the other hand, tape stripping was not conducted, and therefore, the values may be somewhat higher than if tape stripping was conducted, and the top two tape strips were excluded.

[f] Absorbed amount for skin moisturizer and cuticle coat included the cumulative percent in the receptor fluid at 24 h, the amount in skin after washing and tape stripping, tape strips. Tape strips were added because the amount absorbed into receptor fluid at 12 hours vs. the cumulative amount in receptor fluid at 24 h was less than 75 percent. The antiperspirant absorption value does not include the tape strips because more than 75 percent of the total in the receptor fluid penetrated by 12 hours See OECD (2022).

[g] The precent absorption included the amount in the receptor fluid (0.03 %), in skin after washing and tape stripping (10.37 %), and in tape strips (1.24 %). Tape strips were added because the cumulative amount that was in receptor fluid was not 75 %.

[h] The experiment in males is not presented because it resulted in a low OQD. The overall absorption for males was 6.73 %.

E.3.4 Modeled Values

Because the absorption values varied across studies, EPA conducted modeling using the IH SkinPerm ™ model to compare predictions based on D4's properties with the measured values from the *in vivo* and *in vitro* studies. IH SkinPerm ™ is available from the American Industrial Hygiene Association (AIHA) and is a product of the AIHA's Dermal Project Team (DPT) and Exposure Assessment Strategies Committee (EASC). It was developed in collaboration with the original author of the SkinPerm model, Wil ten Berg (AIHA, 2024).

This model uses physical and chemical properties and other values as inputs to estimate dermal absorption. EPA used the D4-specific values presented in Table_Apx E-3. When assuming deposition of neat D4 onto uncovered skin over an 8-hour period at a rate of 0.05 mg/cm^2-h, the model predicted that 1.01 percent of the D4 deposited would be absorbed. If 5 mg/cm^2 neat D4 is deposited instantaneously on uncovered skin (like the amount used in several of the D4 dermal absorption studies), the model predicted that 0.0716 percent would be absorbed. The percent absorbed increased slightly with greater dilution. For a 50 percent solution at 5 mg/cm^2, absorption was predicted to be 0.143 percent, and for a 10 percent solution, the prediction was 0.716 percent.

It should be noted that the log K_{OW} is beyond the domain of values in the model (-3.70 to 5.49) to predict permeation and the partition coefficient (AIHA, 2017). It is not known how D4's log K_{OW} affects the model predictions.

Table_Apx E-3. Input Parameters for IH SkinPerm™ Model to Estimate Dermal Absorption

Input Parameter	Value [a]	Units
CASRN	556-67-2	N/A
Molecular Weight	296.61	g/mole
Temperature	32	° C
Vapor Pressure	124.5 @ 25 °C [b]	Pa
Water Solubility	5.6E-02 @ 23 °C	mg/L
Log K_{OW}, pH 5.5	7.13 @ 34.8°C	N/A
Density	956.03 @ 20 °C	mg/cm^3
Melting Point	17.5	° C

[a] **Source:** *Draft Physical Chemistry and Fate Assessment for Octamethylcyclotetrasiloxane (D4)* (U.S. EPA, 2025h), Table 2-1

[b] Converted from 0.9338 mm Hg

E.4 Other Routes

Varaprath et al. (1999) injected F344 rats intravenously (by i.v.) once with 70 mg/kg-bw D4 (as a ^{14}C-D4 emulsion with unlabeled D4). Additional rats were induced with phenobarbital for four days and similarly given D4 at 70 mg/kg-bw i.v. via a jugular vein cannula; this procedure was done to obtain greater amounts of metabolites in urine.

Two major metabolites were identified. Dimethylsilanediol [Me2Si(OH)2] and methylsilanetriol [MeSi(OH)3] constituted 75 to 85 percent of the total radioactivity. Minor metabolites included

tetramethyldisiloxane-1,3-diol [Me2Si(OH)-O-Si(OH)Me2], hexamethyltrisiloxane-1,5-diol [Me2Si(OH)-OSiMe2-OSi(OH)Me2], trimethyldisiloxane-1,3,3-triol [MeSi(OH)2-O-Si(OH)Me2], dimethyldisiloxane-1,1,3,3-tetrol [MeSi(OH)2-O-Si(OH)2Me], and dimethyldisiloxane-1,1,1,3,3-pentol [Si(OH)3-O-Si(OH)2Me].

Appendix F OTHER UNCERTAINTY FACTORS NOT APPLIED IN THIS ASSESSMENT

LOAEL-to-NOAEL Uncertainty Factor (UF_L)

A UF_L is applied when adverse effects are identified at the lowest dose/concentration tested and the POD cannot be refined through BMD modeling. A value of 3 or 10 can be applied based on the magnitude of the observed effect and the dose-response curve. The POD chosen to calculate acute, intermediate, and chronic risks is a BMDL and therefore, EPA did not apply this UF.

Subchronic-to-Chronic Uncertainty Factor (UF_S)

A UF_S may be justified when a POD from a shorter study is used to characterize a longer duration. For D4, the acute, intermediate, and chronic PODs were all based on intermediate reproductive and/or pre-mating exposures. The 2-generation reproduction exposure durations spanned a subchronic duration (*i.e.*,70 days) to a chronic duration (241 days), assuming a 2-year lifetime for rats. Also, no effects were seen at lower doses than the POD at this longer time point (241 days) in the reproduction study. Based on these reasons, no extrapolation across durations was required.

Database Uncertainty Factor (UF_D)

EPA may consider application of a UF_D on a case-by-case basis when the available quantitative data may insufficiently account for expected adverse effects from chemical exposure. EPA identified sufficient and reasonably available hazard information in repeated-dose, cancer, neurotoxicity, reproduction and developmental, and respiratory studies to conduct the D4 risk evaluation and support the used of the chosen hazard endpoint. For D4 EPA is utilizing the most sensitive and well-supported POD from the more sensitive species for risk estimates. Therefore, a UF_D is not applied for this assessment.