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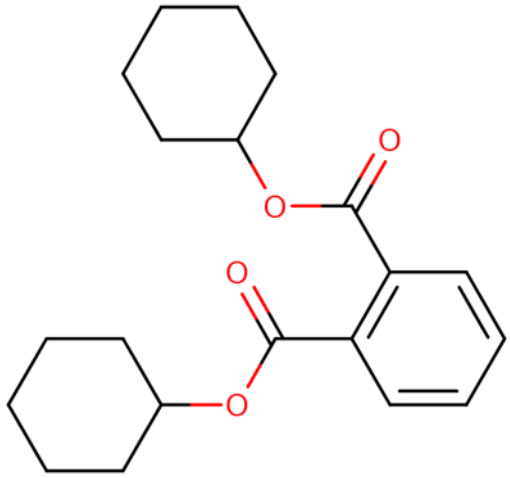
Non-Cancer Human Health Hazard Assessment for Dicyclohexyl Phthalate (DCHP)

Technical Support Document for the Risk Evaluation

CASRN 84-61-7



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ABBREVIATIONS AND ACRONYMS

ADME	Absorption, distribution, metabolism, and excretion
AGD	Anogenital distance
BBP	Butyl benzyl Phthalate
BMD	Benchmark dose
BMDL	Benchmark dose lower bound
BMR	Benchmark response
CASRN	Chemical abstracts service registry number
CPSC	Consumer Product Safety Commission (U.S.)
DBP	Dibutyl phthalate
DCHP	Dicyclohexyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthalate
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency (U.S.)
GD	Gestational day
HEC	Human equivalent concentration
HED	Human equivalent dose
LOAEL	Lowest-observable-adverse-effect level
LOEL	Lowest-observable-effect level
MCHP	Monocyclohexyl phthalate
MOA	Mode of action
NHANES	National Health and Nutrition Examination Survey
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPT	Office of Pollution Prevention and Toxics
PBPK	Physiologically based pharmacokinetic
PECO	Population, exposure, comparator, and outcome
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal day
POD	Point of departure
SACC	Science Advisory Committee on Chemicals
SD	Sprague-Dawley
TSCA	Toxic Substances Control Act
UF	Uncertainty factor
U.S.	United States

SUMMARY

This technical support document is in support of the Toxic Substances Control Act (TSCA) *Risk Evaluation for Dicyclohexyl phthalate (DCHP)* (U.S. EPA, 2025n). This document describes the use of reasonably available information to identify the non-cancer hazards associated with exposure to DCHP and the points of departure (PODs) to be used to estimate risks from DCHP exposures in the risk evaluation of DCHP. The U.S. Environmental Protection Agency (EPA, or the Agency) summarizes the cancer and genotoxicity hazards associated with exposure to DCHP in the *Cancer Human Health Hazard Assessment for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP), Butyl Benzyl Phthalate (BBP), and Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025a). See the risk evaluation for a complete list of all the technical support documents for DCHP.

EPA identified effects on the developing male reproductive system as the most sensitive and robust non-cancer hazard associated with oral exposure to DCHP in experimental animal models (Section 3.1). Existing assessments of DCHP—including those by the U.S. Consumer Product Safety Commission (CPSC, 2014, 2011), Health Canada (Health Canada, 2020; EC/HC, 2015), European Chemicals Agency (ECHA, 2014), and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2016, 2008)—also consistently identified effects on the developing male reproductive system as a sensitive and robust non-cancer effect following oral exposure to DCHP. EPA also considered epidemiologic evidence qualitatively as part of hazard identification and characterization. However, epidemiologic evidence for the one DCHP study was not considered further for dose-response analysis due to limitations and uncertainties in exposure characterization (discussed further in Section 1.1). Use of epidemiologic evidence qualitatively is consistent with phthalates assessments by Health Canada and U.S. CPSC.

EPA selected a POD of 10 mg/kg-day (human equivalent dose [HED] of 2.4 mg/kg-day) based on phthalate syndrome-related effects on the developing male reproductive system (decreased fetal testicular testosterone; decreased anogenital distance (AGD); Leydig cell effects; decreased mRNA and/or protein expression of steroidogenic genes) to estimate non-cancer risks from oral exposure to DCHP for acute, intermediate, and chronic durations of exposure in the risk evaluation of DCHP. The selected POD is the most sensitive no-observed-adverse-effect level (NOAEL) and is further supported by one study reporting a NOAEL of 17 mg/kg-day (Hoshino et al., 2005) and four other studies reporting effects on the developing male reproductive system consistent with a disruption of androgen action and phthalate syndrome in rats at lowest-observed-adverse-effect levels (LOAELs) ranging from 20 to 33 mg/kg-day (Ahbab et al., 2017; Ahbab and Barlas, 2015; Furr et al., 2014; Ahbab and Barlas, 2013). EPA performed ¾ body weight scaling to yield the HED and applied the animal to human uncertainty factor (*i.e.*, interspecies uncertainty factor; UF_A) of 3× and the within human variability uncertainty factor an (*i.e.*, intraspecies uncertainty factor; UF_H) of 10×. Thus, a total UF of 30× was applied for use as the benchmark MOE. Overall, based on the strengths, limitations, and uncertainties discussed in Section 4.3, EPA has robust overall confidence in the selected POD based on effects on the developing male reproductive system. This POD was used to characterize risk from exposure to DCHP for acute, intermediate, and chronic exposure scenarios.

The applicability and relevance of this POD for all exposure durations (acute, intermediate, and chronic) is described in the introduction to Section 4 and additionally in Section 4.2 and Appendix C. For purposes of assessing non-cancer risks, the selected POD is considered most applicable to women of reproductive age, pregnant women, and infants based on the observation that exposures during these specific life stages encompass the masculinization programming window and produce the identified most-sensitive hazard (phthalate syndrome-related effects on the developing male reproductive system)

in rodents. Use of this POD to assess risk for other age groups (*e.g.*, older children, adult males, and the elderly) is considered to be conservative and appropriate for a screening level assessment for these other age groups.

No data are available for the dermal or inhalation routes that are suitable for deriving route-specific PODs. Therefore, EPA is using the acute/intermediate/chronic oral POD to evaluate risks from dermal exposure to DCHP. Differences between oral and dermal absorption are accounted for in dermal exposure estimates in the risk evaluation for DCHP. For the inhalation route, EPA is extrapolating the oral HED to an inhalation human equivalent concentration (HEC) per EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994) using the updated human body weight and breathing rate relevant to continuous exposure of an individual at rest provided in EPA's *Exposure Factors Handbook* (2011 version) (U.S. EPA, 2011b). The oral HED and inhalation HEC values selected by EPA to estimate non-cancer risk from acute/intermediate/chronic exposure to DCHP in the risk evaluation of DCHP are summarized in Table ES-1 below and Section 6.

This non-cancer human health hazard assessment for DCHP was released for public comment and was peer-reviewed by the Science Advisory Committee on Chemicals (SACC) during the August 4–8, 2025 SACC Meeting (U.S. EPA, 2025o). Following SACC peer-review and public comment, this technical support document was revised to incorporate recommendations from the SACC and public.

Table ES-1. Non-cancer HEDs and HECs Used to Estimate Risks

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect at LOAEL	HED[a] (mg/ kg-day)	HEC[a] (mg/m^3) [ppm]	Benchmark MOE[b]	Reference (TSCA Study Quality Rating)
Acute, intermediate, chronic	Developmental toxicity	Rat	10 days during gestation	NOAEL (LOAEL)[c] = 10	Phthalate syndrome-related effects (*e.g.*, ↓ fetal testicular testosterone; ↓ AGD; Leydig cell effects; ↓ mRNA and/or protein expression of steroidogenic genes; ↓INSL3)	2.4	13 [0.95]	UF_A= 3 UF_H=10 *Total UF=30*	(Li et al., 2016) (Medium)

HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor

[a] HED and HEC values were calculated based on the most sensitive NOAEL of 10 mg/kg-day.

[b] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011c), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics. EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations.

[c] Statistically significant effects at 10 mg/kg-day are limited to fetal Leydig cell effects, decreased expression of genes and proteins involved in steroidogenesis, and decreased protein expression of INSL3 (all of which are not considered adverse in isolation). The remaining effects listed reached statistical significance at higher doses.

1 INTRODUCTION

In December 2019, EPA designated dicyclohexyl phthalate (DCHP; CASRN 84-61-7) as a high-priority substance for risk evaluation following the prioritization process as required by section 6(b) of the Toxic Substances Control Act (TSCA) and implementing regulations (40 CFR part 702). The Agency published the draft and final scope documents for DCHP in 2020 (U.S. EPA, 2020a, b). 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 DCHP and conduct a dose-response assessment to determine the toxicity values to be used to estimate risks from DCHP exposures. This technical support document for DCHP summarizes the non-cancer human health hazards associated with exposure to DCHP and summarizes the selected non-cancer toxicity values to be used to estimate risks from DCHP exposures. Cancer human health hazards associated with exposure to DCHP are summarized in EPA's *Cancer Human Health Hazard Assessment for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP), Butyl Benzyl Phthalate (BBP), and Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025a).

Over the past several decades, the human health effects of DCHP have been reviewed by several regulatory and authoritative agencies, including the U.S. Consumer Product Safety Commission (U.S. CPSC), Health Canada, European Chemicals Agency (ECHA), and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS). EPA relied on information published in these assessments as a starting point for its human health hazard assessment of DCHP. Additionally, EPA considered literature published since the most recent existing assessments of DCHP to determine if additional data might support the identification of new human health hazards or lower PODs for use in estimating human health risk. EPA's process for considering and incorporating DCHP literature is described in the *Systematic Review Protocol for Dicyclohexyl Phthalate (DCHP)* (also referred to as the DCHP Systematic Review Protocol) (U.S. EPA, 2025q). EPA's approach and methodology for identifying and using human epidemiologic data and experimental laboratory animal data is described in Sections 1.1 and 1.2.

1.1 Human Epidemiologic Data: Approach and Conclusions

To identify and integrate human epidemiologic data into the DCHP Risk Evaluation, EPA first reviewed existing epidemiologic assessments of DCHP conducted by regulatory and authoritative agencies. Existing assessments reviewed by EPA are listed below. As described further in Appendix A, most of these assessments have been subjected to peer-review and/or public comment periods and have employed formal systematic review protocols.

- *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters* (Health Canada, 2018b); and
- *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for effects on behavior and neurodevelopment, allergies, cardiovascular function, oxidative stress, breast cancer, obesity, and metabolic disorders* (Health Canada, 2018a).

In developing the epidemiology human health hazard assessment for DCHP, EPA conducted literature searches and updates at two different timepoints, including 2018–2019 and 2025. These literature updates are described further below.

Next, EPA sought to identify population, exposure, comparator, and outcome (PECO)-relevant literature published since the most recent existing assessment(s) of DCHP by applying a literature inclusion cutoff date. For DCHP, the applied cutoff date was based on existing assessments of epidemiologic studies of phthalates by Health Canada (2018a, b), which included literature up to January 2018. The Health Canada (2018a, b) epidemiologic evaluations were considered the most appropriate existing assessments for setting a literature inclusion cutoff date because those assessments provided the most robust and recent evaluation of human epidemiologic data for DCHP. Health Canada evaluated epidemiologic study quality using the Downs and Black method (Downs and Black, 1998) and reviewed the database of epidemiologic studies for consistency, temporality, exposure-response, strength of association, and database quality to determine the level of evidence for association between urinary DCHP metabolites and health outcomes. EPA identified additional PECO-relevant epidemiological literature through a literature search of papers published in 2018 to 2019 and through public comment submissions received through the DCHP Docket (https://www.regulations.gov/docket/EPA-HQ-OPPT-2018-0504). All additional PECO-relevant literature was evaluated for data quality and extracted consistent with EPA's *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021). Data quality evaluations for studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025d).

As described further in the DCHP Systematic Review Protocol (U.S. EPA, 2025q), EPA considers phthalate metabolite concentrations in urine to be an appropriate proxy of exposure from all sources—including exposure through ingestion, dermal absorption, and inhalation. As described in the *Application of US EPA IRIS Systematic Review Methods to the Health Effects of Phthalates: Lessons Learned and Path Forward* (Radke et al., 2020), from EPA's Integrated Risk Information System (IRIS) Program, the "problem with measuring phthalate metabolites in blood and other tissues is the potential for contamination from outside sources [(Calafat et al., 2015)]. Phthalate diesters present from exogenous contamination can be metabolized to the monoester metabolites by enzymes present in blood and other tissues, but not urine." Therefore, EPA has focused its epidemiologic evaluation on urinary biomonitoring data; epidemiologic studies that examined DCHP metabolites in matrices other than urine were considered supplemental and not evaluated for data quality.

EPA used epidemiologic studies of DCHP qualitatively. This is consistent with Health Canada and U.S. CPSC assessments of DCHP. EPA did not use epidemiology studies quantitatively for dose-response assessment due to uncertainty associated with the source(s) of exposure, timing of exposure assessment that may not be reflective of exposure during outcome measurements, and use of spot-urine samples, which may not be representative of average urinary concentrations that are collected over a longer term due to rapid elimination kinetics and are calculated using pooled samples. The majority of epidemiological studies introduced additional uncertainty by considering DCHP in isolation and failing to account for confounding effects from co-exposure to mixtures of multiple phthalates (Shin et al., 2019; Aylward et al., 2016). Conclusions from Health Canada (2018a, b) regarding the level of evidence for association between urinary DCHP metabolites and each health outcome were reviewed by EPA and used as a starting point for its human health hazard assessment. EPA also evaluated and summarized epidemiologic studies captured after Health Canada's 2018 assessment and up to 2019 that were identified by EPA's systematic review process to use qualitatively during evidence integration to inform hazard identification and the weight of scientific evidence.

Following release of the draft non-cancer human health hazard assessment of DCHP in December 2024, EPA updated the literature considered as part of the DCHP human health hazard assessment. As described further in the DCHP Systematic Review Protocol (U.S. EPA, 2025q), studies submitted to the

docket by the SACC and by public commenters were screened for PECO-relevance and, if relevant, were included in this non-cancer human health hazard assessment. Overall, EPA did not identify any epidemiological studies suitable for quantitative dose-response analysis.

1.2 Laboratory Animal Findings: Summary of Existing Assessments, Approach, and Methodology

1.2.1 Existing Assessments of DCHP

The human health hazards of DCHP have been evaluated in existing assessments by U.S. CPSC (2014, 2011), Health Canada (Health Canada, 2020; EC/HC, 2015), ECHA (2014), and Australia NICNAS (2016, 2008). Across risk assessments of DCHP conducted by U.S. CPSC and Health Canada, effects on the developing male reproductive system consistent with a disruption of androgen action were identified as the most sensitive effect for use in extrapolating human risk from exposure to DCHP. Accordingly, U.S. CPSC and Health Canada selected PODs for use in their DCHP risk assessments that are based on these effects (Table 1-1). Although ECHA did not conduct a human health risk assessment of DCHP or derive a POD, DCHP was classified (harmonized) for developmental toxicity (*i.e.*, Repr. 1B (H360D)) in the European Union based on effects on the developing male reproductive system consistent with an antiandrogenic mode of action (ECHA, 2014). Similarly, Australia NICNAS summarized data on developmental and reproductive effects of DCHP exposure (NICNAS, 2008) and later recommended DCHP "for classification and labelling for reproductive effects under the current approved criteria and adopted Globally Harmonized System of Classification and Labeling of Chemicals (GHS)" in a human health tier II assessment (NICNAS, 2016). EPA further considers developmental and reproductive toxicity in Section 3.1.

In addition to effects on the developing male reproductive system, effects on the liver and skin sensitization have also been identified as potential human health hazards of concern in existing assessments of DCHP. Specifically, U.S. CPSC (2011) concluded "the weight of evidence from the above studies supported the conclusion that there was "sufficient animal evidence" for the designation of DCHP as a "hepatotoxicant." Similarly, the most sensitive effect (other than developmental toxicity) reported following repeated oral exposure to DCHP by Health Canada (EC/HC, 2015) were effects on the liver, which Health Canada selected to support a POD for use in the DCHP risk assessment (see Table 1-1). EPA further considers liver toxicity in Section 3.3.

There is inconsistency across existing assessments regarding the skin sensitizing potential of DCHP. In the European Union, DCHP has been classified (harmonized) as a category 1 skin sensitizer (ECHA, 2014), while U.S. CPSC (2011) concluded there was "inadequate evidence for the designation of DCHP as a strong dermal sensitizer", and Australia NICNAS (2016) did not consider DCHP classifiable as a skin sensitizer. EPA further discusses evidence for skin sensitization, in Section 3.2.

Table 1-1. Summary of DCHP Non-cancer PODs Selected for Use by Other Regulatory Organizations

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	U.S. CPSC (2014)	ECCC/HC (2020)
Male and female SD rats fed diets containing 0, 240, 1,200, or 6,000 ppm DCHP (mean achieved intake of 0/0, 17/21, 85/106, and 430/523 mg/kg-day in males/females across both generations) starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two generations (Hoshino et al., 2005) (Adhered to OECD TG 416)	16/80[a]	↓ AGD and ↑ nipple retention in F2 males	✓	
	18/90[a]	↓ spermatid head counts, testicular atrophy, ↓ body weight gain, ↓ food consumption in F1 males		✓[b]
Pregnant Wistar rats gavaged with 0, 20, 100, 500 mg/kg-day DCHP on GDs 6–19. Dams sacrificed on GD 20 and male fetuses examined (Ahbab and Barlas, 2015)	10–20[c] (LOAEL)	↓ AGD, testicular pathology, increase resorptions (GD 6–19)		✓[c]
Pregnant SD rats gavaged with 0, 10, 100, 500 mg/kg-day DCHP on GD 12–21. Dams allowed to deliver litters naturally and then pups were sacrificed on PND 1 (Li et al., 2016)				
Male and female rats fed diets containing 0, 0.05, 0.15, 0.4, 1.0% DCHP for 90 days (equivalent received doses: 25, 75, 200, 500 mg/kg-day) (de Ryke and Willems, 1977)[e]	25/75	↑ relative liver weight (females)		✓[d]

[a] Achieved intake for 240, 1200, and 600 ppm diets was 0, 16, 80, and 402 mg/kg-day for the F0 males and 18, 90, and 457 mg/kg-day for the F1 males. See Table_Apx B-1.

[b] Health Canada selected a NOAEL of 18 mg/kg-day from Hoshino et al. to calculate hazard quotients for children (prepubertal) as part of its phthalate cumulative risk assessment (see Tables F-5 and F-9 of (Health Canada, 2020)).

[c] Health Canada selected a LOAEL of 10-20 mg/kg-day based on results from 3 co-critical studies (Li et al., 2016; Ahbab and Barlas, 2015; Hoshino et al., 2005). The LOAEL was used to calculate MOEs for adolescents 12-19 years of age exposed to DCHP in indoor air and dust via inhalation and dermal routes, and hazard quotients for pregnant women and women of childbearing age and infants as part of Health Canada's phthalate cumulative risk assessment (see Table 9-52 and Tables F-5, F-7, and F-8 of (Health Canada, 2020)).

[d] Health Canada selected a NOAEL of 25 mg/kg-day, which was used to calculate an MOE for children 6 months to 4 years of age exposed to DCHP in indoor air and dust via inhalation and dermal routes (see Table 9-52 of (Health Canada, 2020)).

[e] Study by de Ryke and Willems (1977) was not reasonably available to U.S. EPA and was not evaluated for data quality.

1.2.2 Approach to Identifying and Integrating Laboratory Animal Data

Figure 1-1 provides an overview of EPA's approach to identifying and integrating laboratory animal data into the DCHP Risk Evaluation. EPA reviewed the existing assessments of DCHP conducted by various regulatory and authoritative agencies listed below. The purpose of this review was to identify sensitive and human relevant hazard outcomes associated with exposure to DCHP and to identify the key studies used by these agencies to establish PODs for estimating human risk. As described further in

Appendix A, most of these assessments have been subjected to external peer-review and/or public comment periods but have not employed formal systematic review protocols:

- *Toxicity Review of Dicyclohexyl Phthalate (DCHP)* (CPSC, 2011);
- *Chronic Hazard Advisory Panel on phthalates and phthalate alternatives* (CPSC, 2014);
- *State of the Science Report: Phthalate Substance Grouping: Medium-Chain Phthalate Esters: Chemical Abstracts Service Registry Numbers: 84-61-7; 84-64-0; 84-69-5; 523-31-9; 5334-09-8;16883-83-3; 27215-22-1; 27987-25-3; 68515-40-2; 71888-89-6* (EC/HC, 2015);
- *Screening Assessment – Phthalate Substance Grouping* (Health Canada, 2020);
- *Committee for Risk Assessment RAC Opinion Proposing Harmonised Classification and Labelling at EU Level of Dicyclohexyl Phthalate, EC number: 201-545-9, CAS number: 84-61-7* (ECHA, 2014);
- *Phthalates Hazard Compendium: A Summary of Physicochemical and Human Health Hazard Data for 24 Ortho-Phthalate Chemicals* (NICNAS, 2008); and
- *C4-6 Side Chain Transitional Phthalates: Human Health Tier II Assessment* (NICNAS, 2016).

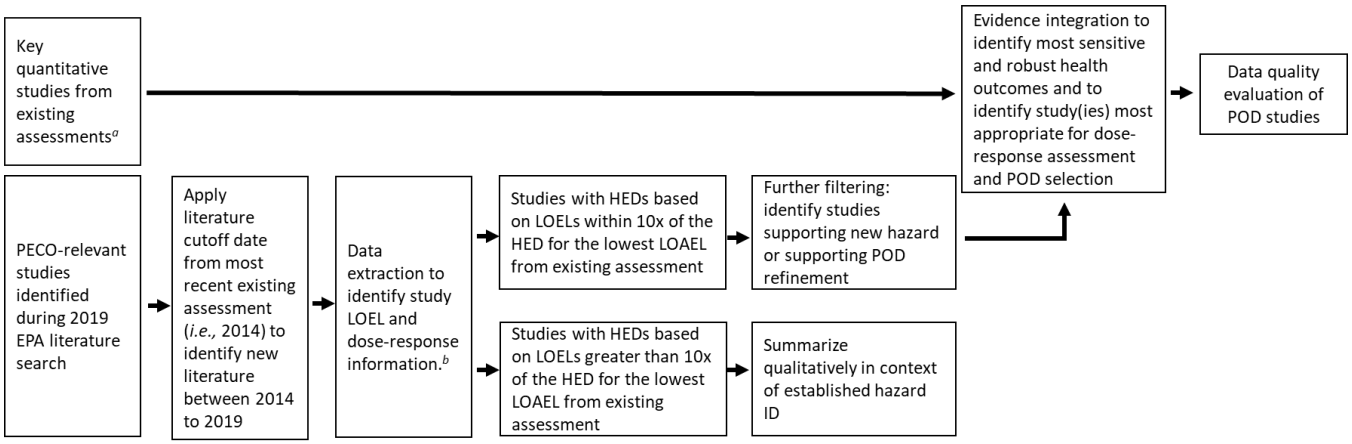

Figure 1-1. Overview of DCHP Human Health Hazard Assessment Approach

[a] Any study that was considered for dose-response assessment, not necessarily limited to the study used for POD selection.

[b] Extracted information includes PECO relevance, species, exposure route and type, study duration, number of dose groups, target organ/systems evaluated, study-wide LOEL, and potentially exposed or susceptible subpopulations (PESS) categories.

In developing the human health hazard assessment for DCHP, EPA conducted literature searches and updates at three different timepoints, including 2014–2019, 2022, and 2025. These literature updates are described further below.

EPA sought to identify PECO-relevant literature published since the most recent existing assessment(s) of DCHP by applying a literature inclusion cutoff date. EPA used the 2015 Health Canada assessment (EC/HC, 2015) as the key starting point for this document. The Health Canada assessment included scientific literature up to August 2014 and considered a range of human health hazards (*e.g.*, developmental and reproductive toxicity, systemic toxicity to major organ systems, genotoxicity, carcinogenicity) across all durations (*i.e.*, acute, short-term, subchronic, chronic) and routes of exposure (*i.e.*, oral, dermal, inhalation). Therefore, EPA considered literature published between 2014 to 2019 further as shown in Figure 1-1. For the DCHP human health hazard assessment, EPA also considered

literature related to effects on the developing male reproductive system identified through development of EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b), which included a literature search in 2022. EPA first screened titles and abstracts and then full texts for relevancy using PECO screening criteria described in the DCHP Systematic Review Protocol (U.S. EPA, 2025q). Next, for PECO relevant studies, EPA then extracted key study information as described in that protocol (U.S. EPA, 2025q)—including PECO relevance; species tested; exposure route, method, and duration of exposure; number of dose groups; target organ/systems evaluated; information related to potentially exposed or susceptible subpopulations (PESS); and the study-wide lowest-observable-effect level (LOEL) (Figure 1-1).

Information for DCHP, which was identified during the 2014 to 2019 and 2022 literature searches described above and which reflects reasonably available information since the most recent existing assessment (EC/HC, 2015) was limited to oral exposure studies. No studies were reasonably available for other exposure routes (*i.e.,* dermal or inhalation). Study LOELs were converted to HEDs by scaling allometrically across species using the ¾ power of body weight ($BW^{3/4}$) for oral data, which is the approach recommended by the Agency when physiologically based pharmacokinetic models (PBPK) or other information to support a chemical-specific quantitative extrapolation is absent (U.S. EPA, 2011c). EPA's use of allometric body weight scaling is described further in Appendix D. Studies with HEDs within an order of magnitude of the lowest LOAEL-based HED identified across existing assessments were considered sensitive and potentially relevant for POD selection. These studies were further reviewed by EPA to determine if they support a different human health hazard or potentially lower POD than those identified in existing assessments of DCHP. Studies with HEDs more than an order of magnitude above the HEDs associated with the lowest LOAELs from previous assessments were integrated into the hazard identification process but did not undergo formal TSCA study quality evaluations. Instead, as discussed further in the Systematic Review protocol for DIBP (U.S. EPA, 2025p), these studies were evaluated in a manner consistent with the Office of Pesticide Programs *Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment* (U.S. EPA, 2012b).

In 2025, EPA updated the literature considered as part of the DCHP human health hazard assessment. As described further in the DCHP Systematic Review Protocol (U.S. EPA, 2025q), studies submitted to the docket by the SACC and by public commenters were screened for PECO-relevance and, if relevant, included in this human health hazard assessment. Overall, EPA did not identify any studies that support selection of a lower POD for DCHP.

Data quality evaluations for DCHP animal toxicity studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025c).

1.2.3 Literature Identified and Hazards of Focus for DCHP

As described further in the Systematic Review Protocol for DCHP (U.S. EPA, 2025q), EPA identified three PECO-relevant studies published between 2014 to 2019, one additional PECO-relevant study during its 2022 search in support of the *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b), and one additional PECO-relevant study from its 2025 literature update. These studies provided information pertaining to reproduction, development, and the liver. EPA identified three oral exposure studies of rats that evaluated effects of DCHP on the developing male reproductive

system consistent with phthalate syndrome (Gray et al., 2021; Lv et al., 2019; Ahbab and Barlas, 2015). Additionally, one publication (Ahbab et al., 2017) examined effects of DCHP on skeletal development/ossification, hematology, and placental histopathology in offspring of both sexes; it also reported measurement of AGD in female offspring. One publication of gestationally exposed rats evaluated effects of DCHP on the liver (Aydemir et al., 2023). These studies of DCHP are discussed further in Section 3 and Appendix B.

Based on information provided in existing assessments of DCHP for developmental and reproductive toxicity, liver effects, and skin sensitization, in combination with information identified by EPA, the Agency focused its non-cancer human health hazard assessment on effects on the developing male reproductive system (Section 3.1), skin sensitization (Section 3.2), and liver toxicity (Section 3.3).

Genotoxicity and carcinogenicity data for DCHP are summarized in EPA's *Cancer Human Health Hazard Assessment for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP), Butyl Benzyl Phthalate (BBP) and Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025a).

2 TOXICOKINETICS

2.1 Oral Route

No controlled human exposure studies or *in vivo* laboratory animal studies are available that evaluate the absorption, distribution, metabolism, and excretion (ADME) properties of DCHP for the oral route.

Regarding metabolism of DCHP, two *in vitro* studies using non-human primate, rat, and ferret hepatic and intestinal preparations and rat gastrointestinal contents are available (Lake et al., 1977; Rowland et al., 1977). These preparations have been shown to hydrolyze DCHP *in vitro* to its corresponding monoester, monocyclohexyl phthalate (MCHP). Lake et al. (1977) further concluded that orally ingested phthalate diesters, including DCHP, would most probably be absorbed from the gut of the rat, baboon, ferret, and human primarily as the corresponding monoester derivatives and that any toxic effects due to oral exposure would be governed by the properties of the constituent phthalate monoester and/or alcohols. However, rates of DCHP hydrolysis vary by species; baboon hepatic and intestinal preparations hydrolyze DCHP at a faster rate relative to similar preparations from rats and ferrets (Lake et al., 1977). DCHP hydrolysis rates are also faster in hepatic preparations relative to intestinal preparations across the rat, baboon, and ferret. An additional study found that the rate of hydrolysis of DCHP are greatest in the presence of rat small intestine contents relative to caecal or stomach contents, suggesting that that enzymes of mammalian rather than bacterial origin are responsible for DCHP metabolism. Consistently, Saito et al. (2010) found that bovine and porcine pancreatic cholesterol esterases completely metabolize DCHP to MCHP *in vitro* within 24 hours and that cholesterol esterases from *Pseudomonas aeruginosa* are not able to hydrolyze DCHP.

Consistent with *in vitro* studies, human biomonitoring studies have detected MCHP, the monoester metabolite of DCHP, in urine. Specifically, low levels of MCHP were detected in the urine of the general U.S. population as part of the U.S. Centers for Disease Control and Prevention (CDC) NHANES Biomonitoring Program. This provides potential qualitative evidence of metabolism of DCHP to MCHP in humans and excretion of MCHP in urine; however, NHANES does not provide associated exposure data, and it is uncertain whether the MCHP detected specifically originated from DCHP. Furthermore, because human biomonitoring data reflects recent aggregate exposure, it cannot quantitatively be attributed to a specific route although it is likely predominately from oral exposure. MCHP was excluded from the NHANES survey following the 2009 to 2010 survey (CDC, 2013) due to low detection levels and a low frequency of detection in human urine. Additionally, DCHP was detected in 17 percent of human milk samples at a maximum level of 9.1 ng/g in a study conducted in Germany (Fromme et al., 2011). MCHP was not measured in this study. Given that DCHP is rapidly metabolized to MCHP, it is possible that the DCHP detected in the human milk samples was due to contamination from sampling equipment.

In the absence of chemical-specific information for DCHP, *EPA assumes 100 percent absorption for the oral route for the DCHP risk evaluation*. This assumption is consistent with the assumptions of other regulatory agencies (Health Canada, 2020; EC/HC, 2015; CPSC, 2014) and with EPA's consideration for other phthalates currently undergoing risk evaluation, including DIDP (U.S. EPA, 2024a) and DINP (U.S. EPA, 2025m).

2.2 Inhalation Route

No controlled human exposure studies or *in vivo* animal studies are available that evaluate the ADME properties of DCHP for the inhalation route. In the absence of chemical-specific information for DCHP,

EPA assumes 100 percent absorption via inhalation for the DCHP risk evaluation. Other regulatory agencies have also consistently assumed 100 percent absorption via the inhalation route (Health Canada, 2020; EC/HC, 2015; CPSC, 2014). EPA has also assumed 100 percent absorption via the inhalation route for other phthalates undergoing risk evaluation, including DIDP (U.S. EPA, 2024a) and DINP (U.S. EPA, 2025m).

2.3 Dermal Route

No controlled human exposure studies or *in vivo* animal studies are available that evaluate the ADME properties of DCHP for the dermal route. As discussed further in Section 2.4.4 of EPA's *Environmental Release and Occupational Exposure Assessment for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025f), for the draft DCHP risk evaluation, EPA is using physical chemistry parameters for DCHP to estimate average dermal absorptive flux, which is used to calculate occupational and consumer dermal exposure estimates. Briefly, because DCHP exists in solid form at room temperature, EPA assumes that DCHP will first migrate from the DCHP-containing matrix to a thin layer of moisture on the skin surface and that absorption of DCHP is limited by its aqueous solubility. Therefore, EPA used the Consumer Exposure Model (CEM) (U.S. EPA, 2023a) to estimate the steady-state aqueous permeability coefficient, K_p (cm/hr), and then Equation 3.2 from the *Risk Assessment Guidance for Superfund (RAGS), Volume I: Human Health Evaluation Manual (Part E: Supplemental Guidance for Dermal Risk Assessment)* (U.S. EPA, 2004) to calculate the dermally absorbed dose (mg/cm^2) over a given time period to determine the dermal absorptive flux (mg/cm^2/hour).

3 NON-CANCER HAZARD IDENTIFICATION

As was stated in Section 1.2.3, EPA is focusing its hazard identification on effects on the developing male reproductive system, skin sensitization, and liver toxicity. These include all endpoints that were assessed in available studies and considered by previous existing risk assessments. Effects on fetal testicular testosterone and other male developmental reproductive effects related to disruption of androgen action are presented in Section 3.1.2.1. Other hazards considered by EPA, including skin sensitization and liver toxicity, are presented in Sections 3.2 and 3.3, respectively.

3.1 Effects on the Developing Male Reproductive System

EPA has developed detailed hazard characterization and mode of action (MOA) analysis for the effects on fetal testicular testosterone and other male developmental reproductive effects consistent with a disruption of androgen action. EPA's MOA analysis for effects on fetal testicular testosterone and other male developmental reproductive effects consistent with a disruption of androgen action was previously presented in EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b). The scientific MOA analysis includes a description of the state of the science with regards to key outcomes, pathways of toxicity, and weight of scientific evidence following the modified Bradford Hill criteria consistent with the IPCS Mode of Action Framework (IPCS, 2007) and EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).

3.1.1 Summary of Available Epidemiological Studies

Epidemiologic studies investigating associations between urinary metabolites of DCHP and developmental and reproductive outcomes were identified by EPA and other organizations. Health Canada (2018b) concluded that there was *inadequate evidence of association* for DCHP and several developmental and reproductive outcomes, including: changes in sex and thyroid hormones (*e.g.*, follicle stimulating hormone, luteinizing hormone, testosterone, triiodothyronine, thyroxine, etc.); changes in birth measures (*e.g.*, birth weight, birth length, head circumference, femur length, etc.); changes in preterm birth (*i.e.*, occurring before 37 weeks of gestation) and gestational age; altered fertility (*e.g.*, ovary response to stimulation during IVF, male infertility, other self-reported fertility problems); sexual dysfunction in males (*i.e.*, erectile dysfunction); and infant sex ratio at birth. Health Canada defined "inadequate evidence" as "the available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of an association." Health Canada concluded that there was *no evidence of association* for DCHP and time to pregnancy, occurrence of endometriosis and/or adenomyosis, and occurrence of uterine leiomyoma. Health Canada defines the descriptor of 'no evidence of association' as "the available studies are mutually consistent in not showing an association between the phthalate of interest and the health outcome measured."

EPA identified four epidemiologic studies (two medium quality, one low quality and one uninformative) published between 2018 and 2019 that measured the association between DCHP and its metabolites and health outcomes (Moreira Fernandez et al., 2019; Albert et al., 2018; Arbuckle et al., 2018; Strassle et al., 2018). The authors indicated that, MCHP, the primary urinary metabolite measured in these studies, was below the limit of detection in urine in all but one study, and thus three of the studies were not analyzed for associations with any health outcome. The one study (Moreira Fernandez et al., 2019), a low-quality study, that adequately detected MCHP in urine was a case-control study that examined urinary MCHP and endometriosis among Brazilian women aged 18 to 45 years. Urine samples from all women detected MCHP in 33.3 percent of cases and 13.6 percent of controls. The study calculated odds

ratios (OR) for the association between endometriosis and MCHP, dichotomized at the median value and reported a positive but non-significant association between exposure to MCHP and endometriosis (OR: 5.25; 95% confidence interval: 0.58, 47.22). These findings support the conclusion made by Health Canada in their 2018 report.

Overall, conclusions of the study identified by the EPA were consistent with that of Health Canada (2018b). EPA concluded that the existing epidemiological studies do not support quantitative dose-response assessment, but rather provide qualitative support as part of weight of scientific evidence. Further information on the studies identified by the EPA can be found in the *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025d) and *Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025b).

3.1.2 Summary of Laboratory Animal Studies

Using the approach described in Sections 1.2.2 and 1.2.3, which involved using data from existing assessments on DCHP alongside literature searches that capture the data available since these assessments, EPA identified 10 oral exposure studies of rats that investigated effects of DCHP on the developing male reproductive system consistent with phthalate syndrome (Gray et al., 2021; Lv et al., 2019; Li et al., 2016; Ahbab and Barlas, 2015; Furr et al., 2014; Ahbab and Barlas, 2013; Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005). Additionally, one publication (Ahbab et al., 2017) examined effects of DCHP on skeletal development/ossification, hematology, and placental histopathology in offspring of both sexes, and reported measurement of AGD in female offspring. These studies include all animal developmental and reproductive toxicology studies on DCHP that were identified by other agencies in previous assessments and additional studies identified by EPA that were published since these assessments. No studies evaluating the developmental and/or reproductive toxicity of DCHP were identified for mice or non-rodent species (*e.g.*, rabbits, dogs, etc.) or routes of exposure other than oral (*e.g.*, inhalation or dermal exposure routes).

All available oral exposure studies of DCHP evaluating developmental and reproductive outcomes are summarized in Table 3-1. More detailed descriptions of the studies summarized in Table 3-1 are provided in Appendix B. Most of the available studies evaluate effects on the developing male reproductive system consistent with a disruption of androgen action following gestational, perinatal, or pre-pubertal oral exposures to DCHP. Several studies also evaluate other developmental outcomes (*e.g.*, resorptions, fetal body weight, skeletal variations, etc.). Effects on the developing male reproductive system and other developmental outcomes are discussed in Sections 3.1.2.1 and 3.1.2.2, respectively.

Table 3-1. Summary of Studies of DCHP Evaluating Effects on Development and Reproduction

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
Developmental and reproductive studies on DCHP available for consideration in existing assessments			
Pregnant SD rats (3–4 per dose) gavaged with 0, 33, 100, 300 mg/kg-day DCHP on GDs 14–18. Dams sacrificed on GD 18 (Block 33 rats) (Furr et al., 2014)[c] (High)	None/33	↓*ex vivo* fetal testicular testosterone (25% decrease [not statistically significant])	No effect on fetal viability or dam weight gain
Pregnant SD rats (2-3 dams per dose) gavaged with 0, 100, 300, 600, and 900 mg/kg-day DCHP on GD 14-18. Dams sacrificed on GD 18 (Block 23 rats) (Furr et al., 2014) [c] (High)	None/100	↓ *ex vivo* fetal testicular testosterone (69% decrease at LOAEL)	No effect on fetal viability or dam weight gain
Pregnant SD rats (3–5 dams per dose) gavaged with 0 or 750 mg/kg-day DCHP on GD 14–18. Dams sacrificed on GD 18 (Block 7 rats) (Furr et al., 2014) [c] (High)	None/750	↓ *ex vivo* fetal testicular testosterone (79% decrease at LOAEL)	No effect on fetal viability or dam weight gain
Pregnant SD rats (22-25 per dose) gavaged with 0, 250, 500, 750 mg/kg-day DCHP on GDs 6–20. Dams sacrificed on GD 21 (Saillenfait et al., 2009) (Medium)	None/250	↓ AGD in male fetuses (absolute and normalized to cube root of body weight)	Maternal Effects - ↓ body weight gain & food consumption (750) - ↑ ALT & AST (750) - ↑absolute & relative liver weights (500 & 750) - ↑hepatic palmitoyl CoA oxidase activity (250, 500, & 750) Developmental Effects - ↓ fetal body weight in both sexes (10–11% decrease) (750) Unaffected outcomes - Post-implantation loss; resorptions; # live and dead fetuses per litter; sex ratio; AGD in female fetuses; incidence of external, soft tissue and skeletal variations; malformations; cryptorchidism; trans-abdominal testicular migration

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
Pregnant SD rats (10 per dose) gavaged with 0, 20, 100, and 500 mg/kg-day DCHP on GD 6–PND 20. Dams allowed to deliver litters naturally and then male and female offspring were carried out to at least post-natal week 10 (Yamasaki et al., 2009) (Medium)	100/500	↓ viability index on PND 4 (slight); ↓ offspring body weights (both sexes); ↓ male AGD; ↑ male nipple retention; hypospadias; delayed PPS; ↓ relative prostate and LABC weights	Maternal Effects - ↑ absolute and relative liver weight (≥100) Developmental Effects - ↓ viability index on PND 4 (500) - ↓ F1 body weights on PND 14 and/or 21 (both sexes) (500) - ↓ F1 male relative ventral prostate & LABC weight (500) - ↓ Male AGD (absolute and normalized to cube root of body weight) on PND 4 (500) - ↑ nipple/areolae retention on PND 13 (500) - ↑ hypospadias in 2 F1 males (500) - Delayed PPS (500) Unaffected outcomes - Dam body weight gain; gestation index; gestation length; # live pups; delivery index; live birth index; sex ratio; # of live pups on PND 4 and 21; #weaning index on PND 21; F1 female relative ovary and uterus weight on PNW 10; F1 male relative testis, epididymis, seminal vesicle weight on PNW 10; F1 male and female relative brain, pituitary, thyroid, adrenal, kidney, liver weight on PNW 10; vaginal opening; estrous cyclicity
Pregnant Wistar albino rats (10 per dose) gavaged with 0, 20, 100, 500 mg/kg-day DCHP on GD 6–19. Dams allowed to deliver litters naturally and then male offspring evaluated on PND 20, PND 32, and PND 90 (Ahbab and Barlas, 2013) (Medium)	None/20	↑ Abnormal sperm; ↑ histopathology findings in testes, epididymis, and prostate	- ↓ absolute & relative testes weights on PND32 (≥100) - ↑ relative and absolute prostate weight on PND 32 (500) - ↑ absolute epididymis, prostate weights on PND90 (500) - ↓ serum testosterone on PND 32 (500) - ↓ normal sperm and ↑ abnormal sperm (≥20) - ↑ incidence of testicular pathology on PND 20, 32, 90 (*e.g.*, tubular atrophy, Sertoli cell vacuolation, oedema) (≥20) - ↑ incidence of epididymal pathology on PND 20, 32, 90 (*e.g.*, atrophic tubules, spermatogenic cells in lumen, decrease sperm number in lumen) (≥20)

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
			- ↑ incidence of prostate pathology on PND 20, 32, 90 (*e.g.,* Prostatic intraepithelial neoplasia, atrophic tubules) (≥20) - ↓ bodyweight (not dose-dependent) in prepubertal males at 20 mg/kg-day only Unaffected outcomes - Bodyweight in pubertal and adult males; absolute/relative testes, epididymis, prostate, and seminal vesicle weight on PND 20; absolute/relative epididymis and seminal vesicle weight on PND 32; absolute/relative testes seminal vesicle, caput weight on PND 90; serum hormone levels of MIS, inhibin B, FSH, LH, estradiol on PND 20, PND 32, PND 90.
Two-generation study of reproduction (Adhered to OECD TG 416) Male and female SD rats fed diets containing 0, 240, 1200, or 6,000 ppm DCHP (0/0, 17/21, 85/106, and 430/523 mg/kg-day in males/females) starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two-generations (Hoshino et al., 2005) (Medium)	Developmental: 17/ 85	Seminiferous tubule atrophy and ↓ sperm count in F1 adult males; ↓ F2 male AGD on PND 4 (absolute and bodyweight corrected); ↑ nipple retention in F2 males	Parental Effects - ↓ food consumption in F0 females and F1 males (≥1,200 ppm) - ↓ body weight gain in F0 and F1 males and females (≥1,200 ppm) - ↓ spermatid head counts in the testis of adult F1 males (≥1,200 ppm) - Organ weight changes [*e.g.,* ↑ Absolute/relative thyroid and liver weight in F0 males and females (6,000 ppm); ↑ relative liver weight in F1 males and females (6,000 ppm); ↓ absolute prostate weight in F1 males (≥240 ppm)] - ↑ Histopathologic findings [*i.e.,* ↑ hepatocellular hypertrophy in F0 males and female (≥1,200 ppm) and F1 males and females (6,000 ppm); ↑ thyroid follicular cell hypertrophy in F0 males (≥1,200 ppm) and F0/F1 females and F1 males (6,000 ppm); seminiferous tubule atrophy in F1 males (≥1,200 ppm)] Offspring Effects - ↓ male and female F1 offspring bodyweight on PND 0, 4, 6, 14, 21 and male and female F2 offspring bodyweight on PND 21 (≥1,200 ppm) - ↓ AGD (absolute & BW adjusted) on PND 4 in F1 (6,000 ppm) and F2 males (≥1,200 ppm)

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
			- ↑ nipple retention in F1 (6,000 ppm) F2 males (≥1,200 ppm) Unaffected outcomes - Clinical signs (F0, F1 parental animals); survival of F0, F1 parental animals; F0 male and F1 female food consumption; F0 and F1 estrous cyclicity; gestation length, gestation index, birth index, # offspring at birth, # offspring born alive, and sex ratio (both generations); serum levels of testosterone, FSH, LH in F0 and F1 male and female parents; sperm effects (F0 parents); F1 and F2 pup viability index on PND 0, 4, 21; F1/F2 male and female pinna unfolding, incisor eruption, eye opening, vaginal opening, preputial separation
Studies of DCHP Since Health Canada (EC/HC, 2015)			
Pregnant SD rats (6 dams/dose) gavaged with 0, 10, 100, and 500 mg/kg-day DCHP on GD 12–21. Dams allowed to deliver litters naturally and then pups were sacrificed on PND 1 (Li et al., 2016) (Medium)	10/100[a]	↓ testicular testosterone; ↓ absolute male AGD; Leydig cell effects (aggregation, ↓size, cytoplasm size, and nuclear size); ↓ mRNA and/or protein expression of steroidogenic genes (*Star*, *Hsd3β1*, *Hsd17β3*); ↓ INSL3	Maternal Effects - None evaluated Developmental Effects - ↓ (16–17%) male pup body weight on PND 1 (≥10) [b] - ↓ male pup AGD (absolute) on PND 1 (≥100) (9% decrease at 10 mg/kg-day was not statistically significant) - ↑ MNGs per tubules (≥100) - ↓ Testicular testosterone (≥100) (10% decrease at 10 mg/kg-day was not statistically significant) - Testis dysgenesis (not statistically significant, incidence across dose groups: 0/6, 0/6, 1/6, 3/6) - Fetal Leydig cell aggregation (↑ #cells/cluster), ↓ fetal Leydig cell size, cytoplasm size, nuclear size (≥10) -↓ gene and protein expression related to steroidogenesis (*Star*, *Hsd3β1* & *Hsd17β3*); ↓ protein expression of INSL3 Unaffected outcomes - Birth rate, litter size, sex ratio
Pregnant Wistar rats (10 dams per dose) gavaged with 0, 20, 100, 500 mg/kg-day DCHP on GDs 6–19. Dams sacrificed on	None/20	↓ male AGD; serum hormone changes (↓ testosterone [not statistically significant] and	Developmental Effects - ↑ resorptions (≥20)

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
GD 20 and male fetuses examined (Ahbab and Barlas, 2015) (High)		MIS, ↑ inhibin); ↑ resorptions; ↑ testicular pathology (*e.g.*, seminiferous tubule atrophy); Leydig cell aggregation	- ↓ male AGD (absolute and normalize to cube root of body weight (≥20) - ↓ serum testosterone (≥100) (12% decrease at 20 mg/kg-day not statistically significant) - ↓ serum Mullerian inhibiting substance (MIS) and ↑ serum inhibin (≥20) - ↑ Testicular pathology (atrophic and small seminiferous tubules, ↓ germ cells in tubules, detached cells from tubular wall at ≥20) (Sertoli cell only tubules, MNGs at ≥100) -↓ Expression of 3βHSD & androgen receptor (≥20) - Bodyweight of male fetuses (not dose-dependent) (↑ at 20 and 100 mg/kg-day only) Unaffected Outcomes - Maternal weight gain; maternal food or water intake during gestation; gestation length; # of implantation sites, # of live fetuses; sex ratio
Pregnant Wistar rats (10 dams per dose) gavaged with 0, 20, 100, 500 mg/kg-day DCHP on GDs 6–19. Dams sacrificed on GD 20 and female fetuses examined (Ahbab et al., 2017) (Medium)	None/20	↓ AGD & AGD (corrected for BW) in females; skeletal retardation & delayed ossification; Changes in hematological parameters; ↓ MCH in males and females, ↓ MCHC in males; placental histopathologic findings	Maternal Effects - ↑ relative (but not absolute) liver and kidney weight (500) - Altered placental parameters [↑ placental weight (≥100), ↓ placental thickness (500), ↑ placental index (≥20), ↓ diameter of placenta (≥20)] - ↓ Expression of proliferating cell nuclear antigen (PCNA), peroxisome proliferator-activated receptor (PPAR)γ, estrogen receptor (ER)α, ERβ, and androgen receptor (AR) - Placental histopathology [Polymorphism in the nucleus and degeneration in the cytoplasm of trophoblastic giant cells (500 mg/kg-day); degeneration of spongiotrophoblast (≥100), hemorrhage of spongiotrophoblast (≥20), decreased and irregular vessel formation in spongiotrophoblast (≥20), hemorrhage in the basal zone (≥100), and edema in the basal zone (≥20)] Developmental Effects - ↑ resorptions and # of live fetuses (≥20)

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
			- ↓ fetal female AGD (absolute & BW corrected) (≥20) - ↓ ossification and alizarin red staining (both sexes) (≥20) - Hematologic findings in male and female fetuses (*e.g.*, ↓ mean cell hemoglobin, ↓ hemoglobin concentrations, ↑ lymphocytes and monocytes, ↓ neutrophil granulocyte) (≥20) - Bodyweight of female fetuses (not dose-dependent) (↑ at 20 and 100 mg/kg-day and ↓ at 500) Unaffected outcomes - Maternal weight gain and food consumption
Male SD rats (6 per dose) received an intraperitoneal injection of ethane dimethane sulfone (EDS) to eliminate all Leydig cells in the testis and were then gavaged with 0, 10, 100, 1,000 mg/kg-day DCHP from post-EDS day 7 to 21 or 28. Rats sacrificed on post-EDS day 21 or 28 (Lv et al., 2019) (Medium)[d]	None/10 (LOEL)	↓ mRNA levels of *Lhcgr*, *Scarb1*, *Star*, *Cyp11a1*, *Hsd3b1*, *Cyp17a1*, *Hsd17b3*, *Hsd11b1*, and *Insl3*	Effects on post-EDS day 21 - Serum testosterone (not dose-dependent) (↑ at 100 mg/kg-day and ↓ at 1,000 mg/kg-day) - ↓ Serum FSH (1,000) - ↑ Leydig cell number and labeling index (10 and 100 mg/kg-day) - ↓ mRNA levels of *Lhcgr*, *Scarb1*, *Cyp11a1*, *Hsd3b1* (at 1,000 mg/kg-day) and ↓ *Hsd17b3* (≥100 mg/kg-day) Effects on post-EDS day 28 - ↓ Serum testosterone and FSH (1,000) - ↓ Leydig cell size and cytoplasm size (≥100) - ↓ Leydig cell number and labeling index (1,000) - ↓ mRNA levels of *Lhcgr*, *Scarb1*, *Star*, *Cyp11a1*, *Hsd3b1*, *Cyp17a1*, *Hsd17b3*, *Hsd11b1*, *Insl3* (≥10) Unaffected Outcomes -Body weight on post-EDS day 0, 7, 21, 28; testes and epididymis weight (post-EDS day 21, 28); serum luteinizing hormone (post-EDS day 21, 28)
Pregnant SD rats gavaged with 0, 100, 300, 600, 900 mg/kg-day on GD 14-18. Dams sacrificed on GD 18 (block 148 rats) (Gray et al., 2021)[c] (High)	None/100	↓ in *ex vivo* fetal testicular testosterone; ↓ mRNA expression of steroidogenic genes	- 41–88% decrease in *ex vivo* fetal testicular testosterone (≥100)

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg/day)	Effect at LOAEL	Remark
[a] Statistically significant effects at 10 mg/kg-day are limited to fetal Leydig cell effects, decreased expression of genes and proteins involved in steroidogenesis, and decreased protein expression of INSL3 (all of which are not considered adverse in isolation). The remaining effects listed reached statistical significance at higher doses. [b] Effects on bodyweight were not dose-related and were not replicated until higher doses in other studies. See Section 4.2 and Appendix B. [c] These studies were conducted by EPA's Office of Research and Development (ORD). [d] As discussed in the Systematic Review protocol for DCHP (U.S. EPA, 2025q) and consistent with Office of Pesticide Programs *Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment* (U.S. EPA, 2012b), the study was of sufficient quality to be considered qualitatively as part of the weight of scientific evidence and was assigned a quality score of medium.			

3.1.2.1 Developing Male Reproductive System

EPA previously developed a weight of scientific evidence analysis and concluded that oral exposure to DCHP can induce effects on the developing male reproductive system consistent with a disruption of androgen action (see EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b)). Notably, EPA's conclusion was supported by the Science Advisory Committee on Chemicals (SACC) (U.S. EPA, 2023c). A summary of the MOA for phthalate syndrome and data available for DCHP supporting this MOA is provided below. Readers are directed to see EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b) for a more thorough discussion of DCHP's effects on the developing male reproductive system and EPA's MOA analysis. Effects on the developing male reproductive system are considered further for dose-response assessment in Section 4.

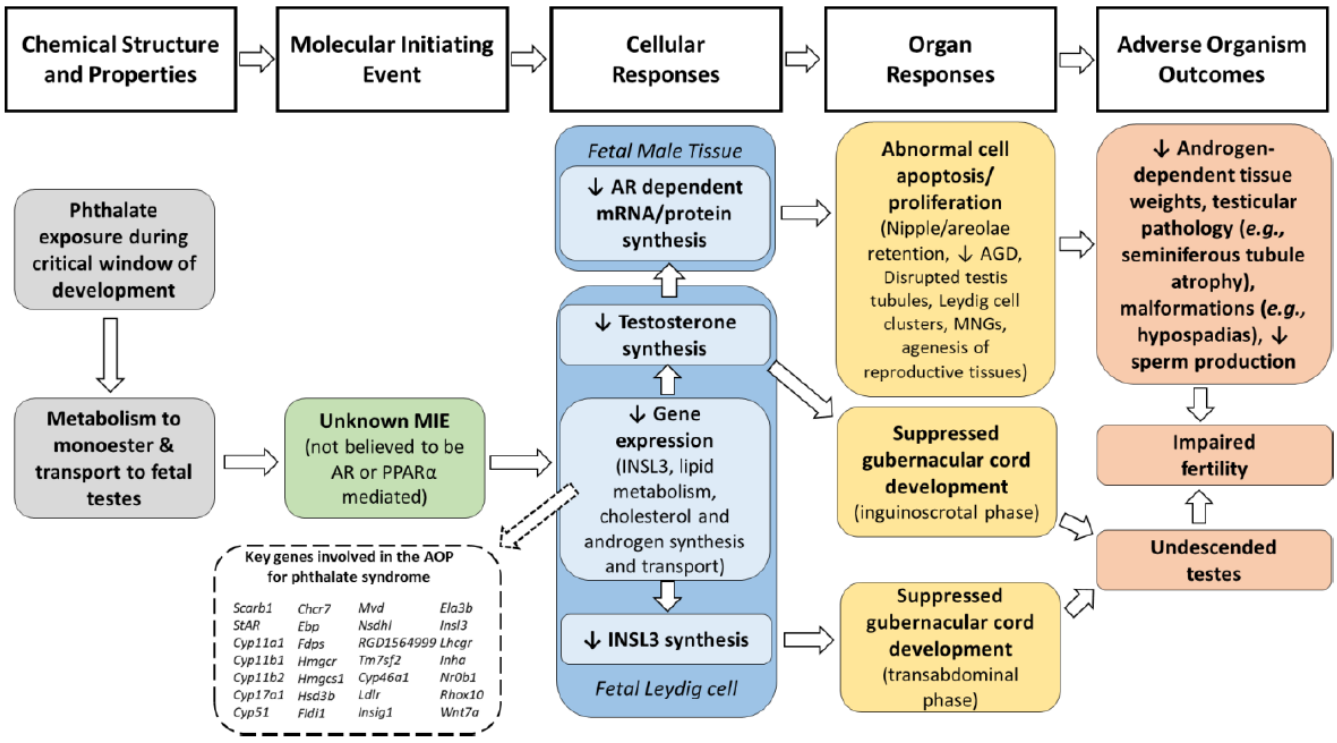

Figure 3-1. Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure
Figure taken directly from (U.S. EPA, 2023b) and adapted from (Conley et al., 2021; Gray et al., 2021; Schwartz et al., 2021; Howdeshell et al., 2016).
Abbreviations: AR = androgen receptor; INSL3 = insulin-like growth factor 3; MNG = multinucleated gonocyte; PPARα = peroxisome proliferator-activated receptor alpha.

As shown in Figure 3-1, a MOA for phthalate syndrome has been proposed to explain the link between gestational or perinatal exposure to DCHP and effects on the male reproductive system in rats. The molecular events preceding cellular changes remain unknown. Although androgen receptor antagonism and peroxisome proliferator-activated receptor alpha activation have been hypothesized to play a role, studies have generally ruled out the involvement of these receptors (Foster, 2005; Foster et al., 2001; Parks et al., 2000).

Exposure to DCHP during the masculinization programming window (*i.e.*, GDs 15.5–18.5 for rats; GDs 14–16 for mice; gestational weeks 8–14 for humans) in which androgen action drives development of the male reproductive system can lead to antiandrogenic effects on the male reproductive system (MacLeod et al., 2010; Welsh et al., 2008; Carruthers and Foster, 2005). Consistent with the MOA outlined in Figure 3-1, three rat studies of DCHP have demonstrated that oral exposure to DCHP during the masculinization programming window can reduce expression (mRNA and/or protein) of insulin-like growth factor 3 (INSL3), as well as genes involved in steroidogenesis in the fetal testes of rats (Gray et al., 2021; Li et al., 2016; Ahbab and Barlas, 2015). Consistently, four rat studies have also demonstrated that oral exposure to DCHP during the masculinization programming window can reduce fetal testicular testosterone concentration and/or testosterone production (Gray et al., 2021; Li et al., 2016; Ahbab and Barlas, 2015; Furr et al., 2014). Oral exposure of rats to DCHP during the masculinization programming window has also been shown to reduce male pup anogenital distance (AGD) in five studies (Li et al., 2016; Ahbab and Barlas, 2015; Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005) and cause male pup nipple retention (NR) in two studies (Yamasaki et al., 2009; Hoshino et al., 2005), which are two hallmark effects of antiandrogenic chemicals (see Sections 3.1.3.3 and 3.1.3.4 of (U.S. EPA, 2023b) for additional discussion). Additional effects consistent with phthalate syndrome observed in rats following oral exposure to DCHP during the critical window of development include: reproductive tract malformations (*i.e.*, hypospadias) in one study (Yamasaki et al., 2009); delayed preputial separation in on one study (Yamasaki et al., 2009); testicular pathology (*e.g.*, tubular atrophy, Leydig cell aggregation, Sertoli cell vacuolation, multinucleated gonocytes) in four studies (Li et al., 2016; Ahbab and Barlas, 2015, 2013; Hoshino et al., 2005); decreased sperm count in one study (Hoshino et al., 2005); and abnormal sperm morphology in one study (Ahbab and Barlas, 2013).

Collectively, reasonably available studies consistently demonstrate that oral exposure to DCHP during the masculinization programming window in rats can disrupt androgen action, leading to a spectrum of effects on the developing male reproductive system consistent with development of phthalate syndrome. As noted above, this conclusion was supported by the SACC (U.S. EPA, 2023c), and readers are directed to EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b) for a more thorough discussion of DCHP's effects on the developing male reproductive system and EPA's MOA analysis. Epidemiological studies on DCHP are limited, and as discussed further in EPA's *Environmental Media and General Population and Environmental Exposure Assessment for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025e), MCHP was removed from the National Health and Nutrition Examination Survey (NHANES) following the 2009 to 2010 survey (CDC, 2013). However, there is evidence of an association between exposure to DEHP, DBP, DINP, DIBP, and BBP and male reproductive effects in humans (U.S. EPA, 2023b). Therefore, EPA further considers effects on the developing male reproductive system for dose-response assessment in Section 4.

3.1.2.2 Other Developmental and Reproductive Outcomes

In addition to effects on the developing male reproductive system, oral exposure to DCHP has been associated with other developmental and reproductive effects in rats (*e.g.*, decreased offspring viability, decreased offspring bodyweight, delayed skeletal ossification, resorptions, and placental histopathology); however, these effects generally occurred at higher doses (>400 mg/kg-day) when determined to be treatment-related. As can be seen in Table 3-1, one study reported decreased offspring viability in rats exposed to 500 mg/kg-day DCHP on GD 6 through PND 20 (Yamasaki et al., 2009) and four studies reported decreased fetal or offspring bodyweight in rats gestationally and/or perinatally to DCHP (Li et al., 2016; Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005). Saillenfait et al. (2009) reported a 10 to 11 percent decrease in fetal body weight in SD rats gavaged with 750 mg/kg-

day DCHP on GD 6 through 20, while Hoshino et al. (2005) reported decreased F1 and F2 male and female offspring bodyweight gain at doses ranging from 430 to 523 mg/kg-day. Similarly, Yamasaki et al. (2009) reported a decrease (magnitude of effect was not specified) in male and female F1 body weights on PND 14 and PND 21 in rats exposed perinatally to 500 mg/kg-day DCHP on GD 6 through PND 20; however, offspring body weight was no longer affected by postnatal week 10. Finally, Li et al. (2016) reported a statistically significant 15 to 16 percent decrease in male pup body weight on PND 1 at doses ranging from 10 to 500 mg/kg-day, however, the effect on body weight did not occur in a dose-dependent manner. Although these four studies report effects on offspring bodyweight following gestational and/or perinatal exposure to DCHP, three additional studies that evaluated offspring bodyweights found no treatment-related changes following gestational exposure to doses ranging from 20 to 500 mg/kg-day on GD 6 through 19 (Ahbab et al., 2017; Ahbab and Barlas, 2015, 2013).

One study reported increased resorptions in pregnant Wistar rats exposed to 20 to 500 mg/kg-day DCHP on GDs 6 through 19 (Ahbab and Barlas, 2015); however, the percentage of resorptions in this study did not occur in a dose-dependent manner (percentage of resorptions was 33, 31, and 26 percent across low-, mid- and high-dose groups). In a second study, no increase in resorptions was reported when pregnant SD rats were exposed to up to 750 mg/kg-day DCHP on GD 6 through 20 (Saillenfait et al., 2009). Finally, Ahbab et al. (2017) report delayed skeletal ossification in male and female fetuses and placental abnormalities in dams (increased diameter, decreased thickness, microscopic lesions in the spongiotrophoblast and basal zone, and polymorphisms in the nucleus and degeneration in the cytoplasm in trophoblastic giant cells) exposed to 20, 100, and 500 mg/kg-day DCHP on GD 6 through 19. However, in another developmental study, Saillenfait et al. (2009) found no effect on incidence of skeletal malformations or skeletal variations in fetuses exposed to up to 750 mg/kg-day DCHP on GD 6 through 20.

Collectively, available studies provide some evidence that gestational and/or perinatal oral exposure to DCHP can cause developmental and reproductive effects other than those associated with phthalate syndrome in rats, including effects on fetal and/or offspring body weight, reduced offspring viability, increased resorptions, and delayed skeletal ossification. Although, effects are not observed consistently across studies and/or do not occur dose-dependently, EPA further considers these developmental effects for dose-response assessment in Section 4.

3.2 Skin Sensitization

As discussed in Section 1.2, dermal sensitization has been identified as a potential human health hazard associated with exposure to DCHP in some existing assessments, however, there is little consensus across assessments. U.S. CPSC (2011) concluded that there is "inadequate evidence for the designation of DCHP as a dermal 'strong sensitizer' and Australia NICNAS (2016) concluded that DCHP is "not considered classifiable as a skin sensitizer", while ECHA (2014) classified (Harmonized) DCHP as a "category 1 skin sensitizer". This section summarizes the available human and laboratory animal evidence for dermal sensitization.

Humans

EPA did not identify any human studies (including studies conducting patch testing) that evaluated exposure to DCHP and/or its metabolites and skin sensitization.

Laboratory Animals
EPA identified three references that have evaluated DCHP for dermal sensitization. The first study by Eastman Kodak (1965) reported that DCHP is not a sensitizer when applied to the skin of guinea pigs; however, no further details were provided in the report.

DCHP has also been evaluated in one local lymph node assay (LLNA) that adhered to OECD TG 442B, which has been previously reported by ECHA (2014). However, the original study report (Company Withheld, 2012) was not reasonably available to EPA, although ECHA's robust summary was available (ECHA, 2014). Briefly, ECHA report that female CBA/JN mice were topically treated with solutions of 10 (minimal irritant concentration), 5, and 2.5 percent DCHP (in acetone:olive oil, 4:1 [v/v]). Stimulation indices (SI) were 1.80, 1.91, and 1.24 for solutions of 2.5, 5, and 10 percent DCHP, respectively. Under OECD TG 442B, as reported in the ECHA robust study summary, the initial result was considered to provide a borderline positive result (*i.e.,* when SI values between 1.6–1.9 were obtained). Because the result of the initial study was inconclusive, the study was repeated. In the second study, SI values were 2.22, 2.82, and 1.94 for solutions of 2.5, 5, and 10 percent DCHP, respectively. The effective concentration needed to produce a SI of 3 (EC3) could not be determined. Because the SI values for all three solutions of DCHP were greater than 1.9, the study was considered to provide a positive result for skin sensitization as reported in the ECHA robust summary.

On the basis of the LLNA test result, ECHA (2014) classified DCHP as a category 1 skin sensitizer. Alternatively, noting the limitations of the LLNA test result, Australia NICNAS (2016) did not consider DCHP to be classifiable as a skin sensitizer.

Conclusions on Dermal Sensitization
Overall, there is a limited database of studies to support the conclusion that DCHP is a dermal sensitizer. The study by Eastman Kodak (1965) is poorly reported. Although DCHP has been evaluated in one LLNA that adheres to OECD TG 442B (Company Withheld, 2012), the original study was not available to EPA for independent review. Therefore, EPA considers there to be inadequate information to draw a conclusion on the sensitizing potential of DCHP and will therefore not consider this endpoint further in the dose-response assessment.

3.3 Liver Toxicity

As discussed in Section 1.2, U.S. CPSC and Health Canada have identified the liver as a target organ following oral exposure to DCHP. This section summarizes the available human epidemiologic and laboratory animal evidence supporting the liver as a target organ.

Humans
EPA did not identify any epidemiologic studies on liver injury for DCHP and/or its metabolites.

Laboratory Animals
Liver effects of DCHP have been reported in orally exposed rats. Available studies include two intermediate (>1–30 days) oral exposure studies (Lake et al., 1982; Grasso, 1978); two subchronic (>30–90 days) oral exposure studies (de Ryke and Bosland, 1978; de Ryke and Willems, 1977); four developmental studies (Aydemir et al., 2023; Ahbab et al., 2017; Saillenfait et al., 2009; Yamasaki et al., 2009); and one two-generation reproductive toxicity study (Hoshino et al., 2005). These studies are discussed further below. No studies were identified for the inhalation or dermal routes of exposure.

Of the available studies investigating hepatic effects, several were unpublished technical reports that were not reasonably available to EPA (de Ryke and Bosland, 1978; Grasso, 1978; de Ryke and Willems, 1977). For these three references, only study summaries provided in existing assessments by other regulatory agencies were available. Because original study reports for these three references were not available to EPA to independently review, they are only considered qualitatively in the context of liver hazard identification and characterization.

Evidence of Liver Effects from Studies Not Reasonably Available to EPA

Health Canada (EC/HC, 2015) and U.S. CPSC (2011) reported liver enlargement following 21 days of exposure of rats to 4,170 mg/kg-day DCHP. However, the study is poorly reported (*e.g.*, no information on strain, sex, or sample size is provided, and minimal information of study design are reported), and some conflicting information is presented by Health Canada and U.S. CPSC. For example, Health Canada report the study by Grasso (1978) to be a dietary exposure study, while U.S. CPSC reports the method of DCHP administration to be oral gavage.

Health Canada (Health Canada, 2020; EC/HC, 2015) and U.S. CPSC (2011) also reported the results of a study in which albino rats (10/sex/group) were administered 0, 0.05, 0.15, 0.4 or 1 percent DCHP in the diet (equivalent to 0, 25, 75, 200 and 500 mg/kg-day) for 90 days (de Ryke and Willems, 1977). Liver effects included increased (magnitude of effect not reported) serum alkaline phosphatase in males (at 25 mg/kg-day and above) and females (500 mg/kg-day), increased (magnitude of effect not reported) relative liver weight in males (at 200 mg/kg-day and above) and females (at 75 mg/kg-day and above), and unspecified histopathological changes to the liver of male and female rats in the two highest dose groups.

Health Canada (2015) and CPSC (2011) also reported a follow-up study wherein de Ryke and Bosland (1978) exposed albino rats (10/sex/dose) to 0, 0.075, 0.1, 0.15, or 1 percent DCHP in the diet (equivalent to 0, 37.5, 50, 75, 500 mg/kg-day) for 90 days. Liver effects included increased serum alkaline phosphatase, increased relative liver weight, and unspecified histopathological changes in the liver in both sexes. It is not clear from the reporting in previous assessments whether these effects were seen in both the 75 and 500 mg/kg-day groups or only the 500 mg/kg-day group.

Overall, these three studies provide some limited evidence to indicate that the liver is a target of DCHP toxicity. However, due to poor/partial reporting, it is unclear whether the observed liver effects in these studies were statistically or biologically significant.

Evidence of Liver Toxicity from Studies Reasonably Available to EPA

Lake et al. (1982) gavaged young (30 days old) male SD rats (5 per dose) with 0, 500, 1,000, 1,500, 2,000, and 2,500 mg/kg-day DCHP for 7 days. Hepatic 7-ethoxycoumarin 0-deethylase, microsomal cytochrome P-450 content and relative liver weight were increased at 500 mg/kg-day and above. Histologic examination of tissue sections from the 1,500 and 2,500 mg/kg-day groups revealed slight hypertrophy of centrilobular cells (reported qualitatively only). Ultrastructural examination revealed marked proliferation of the smooth endoplasmic reticulum of centrilobular cells; however, no evidence of peroxisome proliferation was apparent. In a second study, male Sprague-Dawley (SD) rats were gavaged with either 1,500 mg/kg-day DCHP or 1,130 mg/kg-day MCHP for 7 days. Relative liver weight increased 39 to 42 percent compared to controls in both treatment groups. Treatment with both DCHP and MCHP induced activity of numerous hepatic enzymes involved in xenobiotic metabolism.

Increases in liver weight have been consistently reported in three developmental studies of rats (Ahbab et al., 2017; Saillenfait et al., 2009; Yamasaki et al., 2009) and in one two-generation reproductive study

in rats (Hoshino et al., 2005) (see Table 3-1 and Appendix B for further study details). Ahbab (2017) reported a 12.5 percent increase in relative liver weight (treatment-related effects on absolute liver weight not observed) in pregnant Wistar rats administered 500 mg/kg-day DCHP (highest dose tested) on GD 6 through GD 19 and sacrificed on GD 20. Relative liver weight in dams was unaffected at lower doses (*i.e.*, 20 and 100 mg/kg-day). Similarly, Yamasaki et al. (2009) observed a 7 and 24 percent increase in relative liver weight in female SD rats administered 100 and 500 mg/kg-day DCHP, respectively, on GD 6 through PND 20 and that were sacrificed the day after weaning. No effect on relative liver weight was observed in low-dose dams administered 20 mg/kg-day DCHP, and no effect on relative liver weight was observed in 10-week-old F1 male or female offspring exposed to up to 500 mg/kg-day DCHP during gestation and lactation. Neither study evaluated serum chemistry markers of liver toxicity nor conducted microscopic examinations of the liver.

In a third developmental study, Saillenfait et al. (2009) observed a 17 and 28 percent increase in relative, but not absolute, liver weight of pregnant SD rats exposed to 500 and 750 mg/kg-day DCHP, respectively, on GDs 6 through GD 20 and sacrificed on GD 21. Changes in liver weight were accompanied by 49 and 120 percent increases in serum AST and ALT, respectively, at the highest dose tested (750 mg/kg-day); however, no histopathologic lesions or changes in serum cholesterol or triglycerides were observed. Consistent with a slight induction of PPARα activation, hepatic palmitoyl CoA oxidase activity increased dose-dependently from 75 to 108 percent at the lowest dose tested (250 mg/kg-day) and above.

In a fourth developmental study, Aydemir et al. (2023) reported increased incidence of certain histopathological changes (*i.e.*, congestion, inflammatory cell infiltration, cells with pyknotic nuclei, lysis of hepatocytes, and degeneration of hepatic parenchyma) in the livers of F1 adult male and female Wistar rats that were gestationally exposed to DCHP. Dams (10/group) were gavaged with 0, 20, 100, or 500 mg/kg-day DCHP during GD 6 to GD 19 and allowed to deliver naturally. In adult F1 males (PND 90), incidence of congestion (incidence: 0/10, 4/8*, 2/10, 5/10*), inflammatory cell infiltration (incidence: 0/10, 6/8*, 6/10*, 7/10*), and lysis of hepatocytes (incidence: 0/10, 4/8*, 6/10*, 6/10*) were significantly increased relative to control starting at 20 mg/kg-day, and the number of cells with pyknotic nuclei (incidence: 0/10, 2/8, 8/10*, 7/10*) increased relative to control starting at 100 mg/kg-day. However, the incidence of several of these lesions (*i.e.*, congestion, inflammatory cell infiltration, lysis of hepatocytes) displayed a flat dose-response. In adult F1 females, incidence of inflammatory cell infiltration (incidence: 0/10, 6/10*, 6/10*, 7/9*) increased starting at 20 mg/kg-day, and the number of cells with pyknotic nuclei (incidence: 0/10, 2/10, 8/10*, 6/9*) and lysis of hepatocytes (incidence: 0/10, 4/10, 6/10*, 6/9*) increased starting at 100 mg/kg-day. However, the incidence of all of these lesions displayed a relatively flat dose-response. Liver weights and serum chemistry markers of liver toxicity (AST, ALT, protein, and/or albumin, cholesterol) were either not changed significantly relative to control animals or lacked dose-response. Since most liver lesions displayed a flat dose-response, and since there were no treatment related effects on liver weight or serum chemistry markers of liver toxicity, is it uncertain as to whether these effects are adverse or even due to DCHP treatment. Increases in liver weight and hepatocellular hypertrophy have also been observed F0 and F1 adult male and female SD rats in a two-generation study of reproduction by Hoshino et al. (2005). In F0 adults, relative and absolute liver weight increased 21 to 24 percent in high-dose males (430 mg/kg-day) and 6 to 19 percent in mid- (106 mg/kg-day) and high-dose (523 mg/kg-day) females. Similarly, for F1 adults, relative (but not absolute) liver weight was increased 14 to 16 percent in high-dose males and females. Liver weight changes were accompanied by histopathologic findings in the liver, including increased incidence of diffuse hepatocellular hypertrophy in high-dose F0 and F1 adult male and female rats (see Table_Apx B-2).

Conclusions on Liver Toxicity

Overall, there is consistent evidence to indicate that the liver is a target organ following oral exposure to DCHP. However, the observed liver effects across reasonably available studies are generally not indicative of an adverse response or may be considered to be of questionable adversity. Consistent with previous guidance (Hall et al., 2012; U.S. EPA, 2002a), EPA considered hepatocellular hypertrophy and corresponding increases in liver size and weight to be adaptive non-adverse responses, unless accompanied by treatment-related, biologically significant changes (*i.e.*, 2- to 3-fold) in clinical markers of liver toxicity; that is, decreased albumin; or increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), bilirubin, cholesterol), and/or histopathology indicative of an adverse response (*e.g.*, hyperplasia, degeneration, necrosis, inflammation).

Across the five studies reasonably available to EPA that evaluated liver effects in directly exposed animals, consistent dose-related increases in liver weight were observed in all studies (Ahbab et al., 2017; Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005; Lake et al., 1982). Three of the five studies conducted histopathologic examinations of the liver. Lake et al. (1982) and Hoshino et al (2005) report dose-related increases in hepatocellular hypertrophy, while Saillenfait et al. (2009) report no microscopic findings in the liver. Notably, none of the three reasonably available studies that included histopathologic examinations reported any pathology indicative of an adverse response (*e.g.*, necrosis, inflammation, hyperplasia, etc.). Only one of the five studies reported serum chemistry. Saillenfait et al. (2009) reported 49 and 120 percent increases in serum AST and ALT (coinciding with a 17 to 28 percent increases in relative liver weight) at high-doses of DCHP (750 mg/kg-day). These changes in serum chemistry provide equivocal evidence of an adverse liver response because the magnitudes of the changes were below (in the case of AST) or just above (in the case of ALT) a 2-fold increase and were not accompanied by any microscopic findings indicative of liver toxicity.

Across the three studies reasonably available to EPA that evaluated liver effects in offspring after developmental exposures, findings did not occur until high doses (> 500 mg/kg-day) and were of uncertain adversity. In the study by Yamasaki et al.(2009), liver weights were unaffected in F1 males and females at gestational doses up to 500 mg/kg-day at post-natal week 10, and serum chemistry and liver histopathology were not measured. Although Hoshino et al. (2005) reported liver weight increases at a higher gestational dose of 523 mg/kg-day, concurrent histopathology findings were limited to increased incidence of diffuse hepatocellular hypertrophy; no additional histopathological changes were reported that would be indicative of an adverse response (*e.g.*, hyperplasia, degeneration, necrosis, inflammation). Consistently, Aydemir et al. (2023) observed no significant, treatment-related changes in liver weights or clinical chemistry markers at gestational doses up to 500 mg/kg-day in F1 males and females during adulthood. Although this study reported increased incidence of certain liver histopathology lesions (*i.e.*, congestion, inflammatory cell infiltration, cells with pyknotic nuclei, lysis of hepatocytes, and degeneration of hepatic parenchyma) starting at a gestational dose of 20 mg/kg-day, the adversity of some of the reported lesions are uncertain, and no other effects on liver weight or clinical chemistry markers of liver toxicity were observed.

Given that the liver effects observed in the oral exposure studies that were available to the Agency are generally not indicative of an adverse response according to guidance from EPA (2002a) and Hall et al. (2012), the Agency is not further considering liver effects for dose-response assessment.

4 DOSE-REPONSE ASSESSMENT

EPA is focusing its dose-response analysis on developmental and reproductive toxicity—particularly effects relevant to phthalate syndrome in male rats. These effects are consistently observed across different strains of rat, and durations of exposure and occur in a dose-related manner. Other non-cancer hazard endpoints considered by EPA (*i.e.,* skin sensitization and liver toxicity) were not considered for dose-response analysis due to limitations in the number of studies and uncertainties that reduce EPA's confidence in using these endpoints for estimating risk to human health.

For the DCHP dose-response assessment, EPA first identified NOAEL and LOAEL values from the nine developmental toxicity studies considered for dose-response assessment (Table 4-1). Eight of the nine studies provided dose-response information and tested doses at or below 100 mg/kg-day, and data for decreased testicular testosterone and other endpoints associated with phthalate syndrome from these eight studies were further considered for benchmark dose (BMD) analysis. One study of the initial nine (Saillenfait et al., 2009) was not considered for BMD analysis because it was not very sensitive (*i.e.,* the lowest dose evaluated was 250 mg/kg-day). For one hazard endpoint (*i.e.,* reduced fetal testicular testosterone in rats), EPA conducted meta-analysis and benchmark dose modeling using the approach previously published by the National Academies of Science, Engineering, and Medicine (NASEM)(2017), which is further described in EPA's *Meta-analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), Dicyclohexyl Phthalate (DCHP), and Diisononyl Phthalate* (U.S. EPA, 2025g). Fetal testicular testosterone data from three studies, reported in two publications, was included in EPA's meta-analysis (Gray et al., 2021; Furr et al., 2014). Data from all three of the individual studies was also subjected to BMD analysis using EPA's BMD Software, so that results between the two analyses could be compared. In subsequent sections below the extent to which BMD modeling was or was not conducted for each study is discussed further.

No dermal or inhalation studies were reasonably available that could be used for dose-response assessment. Acute, intermediate, and chronic non-cancer NOAEL/LOAEL values identified by EPA are discussed further in Section 4.2. As discussed in Section 4.2, the Agency considers effects on the developing male reproductive system consistent with a disruption of androgen action relevant for setting a POD for acute exposure durations. However, because these acute effects are the most sensitive effects following exposure to DCHP, they are also considered protective of intermediate and chronic duration exposures. As described in Appendix D, EPA converted oral PODs derived from animal studies to HEDs using allometric body weight scaling to the three-quarters power (U.S. EPA, 2011c). Differences in dermal and oral absorption are corrected for in the dermal exposure assessment, allowing the same HEDs to be used for both oral and dermal routes. In the absence of inhalation studies, EPA performed route-to-route extrapolation to convert oral HEDs to inhalation HECs (Appendix D).

4.1 Selection of Studies and Endpoints for Non-cancer Health Effects

EPA considered the suite of oral animal toxicity studies primarily indicating effects on the developing male reproductive system consistent with phthalate syndrome when considering non-cancer PODs for estimating risks for acute, intermediate, and chronic exposure scenarios (see Section 4.2). The Agency considered the following factors during study and endpoint selection for POD determination from relevant non-cancer health effects:

- Exposure duration;
- 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 toxicological outcome?);
- Uncertainties not captured by the overall quality determination;
- Endpoint/POD sensitivity; and
- Total uncertainty factors (UFs). EPA considers the overall uncertainty with a preference for selecting studies that provide lower uncertainty (*e.g.*, lower benchmark MOE) because they provide higher confidence (*e.g.*, use of a NOAEL vs. a LOAEL with additional UF_L applied).

The sections that follow provide comparisons of the above attributes for studies and hazard outcomes relevant to each of these exposure durations and details related to the studies considered for each exposure duration scenario.

4.2 Non-cancer Oral Points of Departure for Acute, Intermediate, and Chronic Exposures

4.2.1 Studies Considered for Dose-Response Assessment

EPA considered nine developmental and reproductive toxicity studies of rats with endpoints relevant to acute exposure duration (U.S. EPA, 1996, 1991), in addition to being relevant for intermediate and chronic durations. These studies were previously discussed in Section 3.1, and are summarized below in Table 4-1. Notably, the study by Lv et al. (2019) was not considered quantitatively for dose-response because the animals were altered by ethane dimethane sulphonate (EDS) administration to destroy Leydig cells. Primary endpoints considered relevant to acute exposure durations include effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of male reproductive development in rats and other developmental effects, such as resorptions. Although single dose studies evaluating the effects DCHP on the developing male reproductive system are not available, studies of the toxicologically similar phthalate dibutyl phthalate (DBP) have demonstrated that a single exposure during the critical window of development can disrupt expression of steroidogenic genes and decrease fetal testes testosterone. Therefore, EPA considers effects on the developing male reproductive system consistent with a disruption of androgen action to be relevant for setting a POD for acute duration exposures (see Appendix C for further discussion). Notably, SACC agreed with EPA's decision to consider effects on the developing male reproductive system consistent with a disruption of androgen action to be relevant for setting a POD for acute durations during the July 2024 peer reviewed meeting of the DINP human health hazard assessment (U.S. EPA, 2024b). Studies considered for dose-response assessment are summarized in Table 4-1.

Of the nine studies considered by EPA, three support LOAELs (no NOAEL identified) ranging from 100 to 250 mg/kg-day (Table 4-1) (Gray et al., 2021; Furr et al., 2014; Saillenfait et al., 2009). In studies conducted by Furr et al. and Gray et al., pregnant SD rats were gavaged with 100 to 900 mg/kg-day DCHP on GDs 14 through 18. In both studies, *ex vivo* fetal testicular testosterone production was reduced 31 to 69 percent in the low-dose group compared to concurrent controls, supporting a LOAEL of 100 mg/kg-day for both studies (Table 4-2). As discussed further below in Section 4.2.2, testosterone data from each individual study was BMD modeled using EPA's BMD Software (BMDS Online Version 25.1) and as part of a meta-analysis. Similarly, Saillenfait et al. (2009) gavaged pregnant SD rats with 250, 500, and 750 mg/kg-day DCHP on GD 6 through 20 and observed a treatment-related decrease in male fetus AGD at 250 mg/kg-day DCHP and above, supporting a LOAEL of 250 mg/kg-day. EPA did not attempt BMD modeling of data from Saillenfait et al. because the study was not very sensitive and was limited by dose selection, and other studies of DCHP tested lower doses (*i.e.*, 10–20 mg/kg-day). These studies were not selected for the final POD because they are limited by dose

selection and do not support the identification of a NOAEL. Other developmental studies of DCHP, which test lower doses, are available and support identification of more sensitive candidate PODs.

In another study, Yamasaki et al. (2009) gavaged pregnant SD rats with 20, 100, and 500 mg/kg-day DCHP on GD 6 through PND 20 and then evaluated F1 male offspring. Effects were limited to the high-dose group and included decreased F1 male AGD on PND 4, F1 male pup nipple retention on PND 13, delayed preputial separation, increased hypospadias, and decreased relative ventral prostate and LABC muscle weight in F1 males at 10 weeks of age, supporting a NOAEL of 100 mg/kg-day. EPA considered BMD modeling of data from Yamasaki et al., however, there were limitations in data reporting that prevented BMD modeling. For example, study authors state that two high-dose males had hypospadias, but do not provide incidence data for the number of pups examined. Additionally, study authors report the mean and variation for AGD, nipple retention, and preputial separation data, but do not state the type of variation reported (*i.e.,* standard deviation or standard error), and do not report the number of F1 male pups examined for each endpoint. This study was not selected for the final POD because other developmental studies of DCHP support identification of more sensitive candidate PODs.

Six medium-to-high quality developmental studies in rats, including a two-generation study, support candidate PODs ranging from 10 to 33 mg/kg-day DCHP (Ahbab et al., 2017; Li et al., 2016; Ahbab and Barlas, 2015; Furr et al., 2014; Ahbab and Barlas, 2013; Hoshino et al., 2005). In an additional experiment in their 2014 study, Furr et al. (2014) dosed pregnant SD rats (block 33 rats) with 33, 100, and 300 mg/kg-day DCHP on GD 14 through 18. As can be seen from Table 4-2, *ex vivo* fetal testicular testosterone was reduced 25 to 69 percent across doses. The effect was statistically significant at 100 mg/kg-day DCHP and above. However, the study is limited by a small sample size (3 to 4 dams per dose group), which may contribute to the lack of statistical significance in the lowest dose group where a 25 percent reduction in testosterone was observed. Notably, the magnitude of the reduction in testicular testosterone observed across dose groups in this study is remarkably consistent with changes in testicular testosterone observed in other developmental studies of DCHP (Table 4-2). Given the magnitude of the effect (25 percent decrease) and consistency in the testosterone response across studies, EPA considers this study to support a LOAEL of 33 mg/kg-day. As discussed further below in Section 4.2.2, testosterone data from Furr et al. was BMD modeled using EPA's BMD Software (BMDS Online Version 25.1) and as part of a meta-analysis.

Of the six previously mentioned studies, three studies conducted by Ahbab et al. (2017; 2015, 2013) used a consistent study design to evaluate the effect of *in utero* exposure to DCHP on a range of reproductive and developmental endpoints at different life stages. Collectively, these three studies support a LOAEL of 20 mg/kg/day for effects on fetal development and male reproductive development. Briefly, Ahbab et al. (2017; 2015, 2013) gavaged pregnant Wistar rats with 20, 100, and 500 mg/kg-day DCHP on GD 6 through 19 and then evaluated fetal effects on GD 20 or postnatal effects in F1 male offspring on PND 20, 32, and 90. Ahbab et al. (2013) reported a non-dose-dependent increase in sperm abnormalities in adult male offspring on PND 90 and dose-dependent increases in testes, epididymal, and prostate histopathological lesions in pre-pubertal (PND 20), pubertal (PND 32), and adult (PND 90) F1 male offspring—supporting a LOAEL of 20 mg/kg-day. EPA considered BMD modeling of increased histopathological lesions in the testes, epididymis, and prostate of pre-pubertal, pubertal, and adult F1 males. However, as can be seen from Table_Apx B-5, the majority of lesions displaying a dose-response trend were observed in at least 30 to 70 percent of examined males in the low-dose group (compared to 0% of control males for all lesions). EPA did not attempt to BMD model this data because this type of response is not amenable to BMD modeling because of the lack of data in the low-end range of the curve near the BMR of 5 to 10%. Notably, many of the statistically significant effects observed at 20 mg/kg-day did not show dose-response (*i.e.,* sperm abnormalities, AGD, and a significant portion of

histopathological findings). Furthermore, no instances of histopathological abnormalities were reported in control animals and the authors do not state whether the investigators were blind to the treatment status of the animals, which raises additional uncertainty.

Ahbab et al. (2015) reported significant increases in resorptions, fetal Leydig cell effects (*e.g.*, cell aggregation), fetal testicular histopathologic lesions (*i.e.*, MNGs, atrophic and small seminiferous tubules, decreased germ cells in tubules, Sertoli cell only tubules, detached cells from tubular wall), and decreased fetal male AGD (absolute and body weight normalized), supporting a LOAEL of 20 mg/kg-day. EPA considered BMD modeling of data from this study, but the majority of observed effects where not amenable to modeling. For example, the increase in resorptions did not show dose-response, while AGD and Leydig cell effect data were reported graphically only, and the sample size was not reported, nor did study authors report if the litter or pup was the statistical unit for comparison. Finally, statistically significant increases in incidence of histopathologic lesions were observed in 50 to 80 percent of males in the low-dose group (verses 0% of controls). EPA did not attempt to BMD model this histopathology data because this type of response is not amenable to modeling because of the lack of data in the low-end range of the curve near the BMR of 5 to 10%. As discussed further below Section 4.2.2, fetal plasma testosterone data from Ahbab et al. (2015) was BMD modeled using EPA's BMD Software (BMDS Online Version 25.1); however, no models adequately fit the dataset. Finally, Ahbab et al. (2017) reported additional developmental effects supporting a LOAEL of 20 mg/kg-day, including skeletal retardation and delayed ossification in male and female fetuses on GD 20 and dose-dependent increases in placental histopathologic lesions in dams.

The two-generation study of reproduction by Hoshino et al. (2005), which adhered to OECD TG 416, supports a NOAEL of 17 mg/kg-day (Table 4-1). In this study, male and female SD rats were dosed continuously in the diet with 17, 85, and 430 mg/kg-day (males) or 21, 106, and 523 mg/kg-day (females) DCHP for two generations. Effects consistent with a disruption of androgen action and phthalate syndrome were observed in mid- and high-dose groups, including decreased sperm counts and seminiferous tubule atrophy in F1 adult males, decreased F2 male AGD on PND4, and increased nipple retention in F2 males on PND14. EPA considered BMD modeling of decreased F2 male AGD, however, previous NASEM (2017) meta-analyses of decreased AGD for DEHP, DBP, and BBP have demonstrated that reduced testosterone is a more sensitive outcome than AGD (*i.e.*, BMD_5 estimates for reduced testosterone and AGD were 15 and 270 mg/kg-day for DEHP; 12 and 150 mg/kg-day for DBP; 23 and 250 mg/kg-day for BBP). Reduced AGD being less sensitive than reduced fetal testicular testosterone is consistent with the phthalate syndrome MOA, since reduced AGD is mechanistically linked to and occurs subsequent to reduced fetal testicular testosterone. Therefore, EPA did not model this outcome for DCHP. Compared to the studies conducted by Ahbab et al. (2017; 2015, 2013), which reported reduced male AGD at 20 mg/kg-day and above, Hoshino et al. reported a dose-dependent reduction in AGD in F1 male offspring at the highest dose of 430 mg/kg-day DCHP and in F2 male offspring at 85 mg/kg-day DCHP and above. Similar differences in testicular, epididymal, and prostate histopathologic findings are apparent between the two studies. Ahbab et al. reported increased lesions in all three tissues at 20 mg/kg-day and above, while Hoshino et al. reported increased testicular atrophy in F1 adult males at 85 mg/kg-day and above, and no histopathologic findings in the prostate or epididymis of F0 or F1 adult males. Overall, studies by Hoshino et al. and Ahbab et al. found a consistent pattern of effects, although doses at which effects are significant vary across these studies (85 mg/kg-day and 20 mg/kg-day, respectively).

In the sixth study, Li et al. (2016) gavaged pregnant SD rats with 10, 100, and 500 mg/kg-day DCHP on GD 12 through 21 and reported developmental effects at all doses. Body weight of male pups was statistically significantly reduced 16 to 17 percent across treatment groups; however, the effect lacked a

clear dose-response relationship and was considered to be of uncertain toxicologic significance. At 10 mg/kg-day DCHP and above, treatment-related effects on Leydig cells were observed, including statistically significant: decreases in Leydig cell size and Leydig cell cytoplasmic and nuclear size, and significantly increased Leydig cell aggregation. Additionally, at 10 mg/kg-day DCHP and above, decreased expression of INSL3 protein (which promotes testes descent) and decreased expression of genes (*Star*, *Hsd3b1*, and *Hsd17b3*) and proteins (3β-HSD) involved in testosterone biosynthesis were observed in the testes. Additional dose-dependent effects including decreased testosterone (10% decrease in low-dose group), decreased absolute male AGD (9% decrease in low-dose group), and increased MNGs per seminiferous tubule were also observed at 10 mg/kg-day but did not reach statistical significance until 100 mg/kg-day. Although this study provides clear evidence of effects on the developing male reproductive system consistent with phthalate syndrome, the effects observed at 10 mg/kg-day are not clearly adverse (*i.e.*, observed Leydig cell effects, decreased steroidogenic gene and protein expression, and INSL3 protein expression, though involved in the MOA of rat phthalate syndrome, are of uncertain toxicological significance by themselves), did not occur dose-dependently (*i.e.*, decrease in fetal body weight), or were not statistically significant (*i.e.*, effects on AGD and testosterone were significant at 100 mg/kg-day DCHP and above). EPA considered BMD modeling of AGD and testis testosterone data from Li et al. (2016). However, AGD data were not modeled because absolute AGD and pup body weight were both reduced, and study authors did not report AGD normalized to body weight as recommended by OECD guidance (2013), and it is unclear if the effect on AGD was related to the decrease in animal size. As discussed further below in Section 4.2.2, testicular testosterone data from Li et al. was BMD modeled using EPA's BMD Software (BMDS Online Version 25.1). Regarding the decreased fetal bodyweight in Li et al (2016), other studies provide additional evidence that this effect lacks dose-response or does not occur until higher doses. Specifically, Ahbab et al. also reported changes in fetal body weight that were inconsistent across doses (Ahbab et al., 2017; Ahbab and Barlas, 2015, 2013), and three other studies reported decreased bodyweight in male and female offspring that did not occur until 85, 500, and 750 mg/kg-day, respectively (Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005). Therefore, EPA considers this study to support a NOAEL of 10 mg/kg-day.

4.2.2 Benchmark Dose Modeling of Testosterone Data

In 2017, NASEM (2017) assessed experimental animal evidence for effects on fetal testicular testosterone following *in utero* exposure to several phthalates (*e.g.*, DEHP, DBP, DIBP, DINP, BBP) using the systematic review methodology developed by the National Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT). NASEM further analyzed the fetal rat testosterone data via meta-analysis and BMD, but did not include DCHP in their 2017 analysis. Using the publicly available R code (https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose), EPA applied the same meta-analysis and BMD modeling approach used by NASEM to DCHP. *Ex vivo* fetal rat testicular testosterone production data from three studies reported in two publications by Furr et al. (2014) and Gray et al. (2021) was included in EPA's analysis. Consistent with the NASEM approach, testosterone data from Li et al. (2016) were excluded from the analysis because testosterone was measured in male offspring on PND 1, not the fetal lifestage. Overall, the meta-analysis found a statistically significant overall effect and linear trends in $\log_{10}$(dose) and dose, with an overall effect that is large in magnitude (>50% change) (Table 4-3). There was substantial, statistically significant heterogeneity in all cases (I^2>80%). The linear-quadratic model provided the best fit (based on lowest AIC) (Table 4-3). For the BMD analysis, EPA modeled a range of BMRs based on biological and statistical considerations, including BMRs of 5, 10, and 40 percent. However, as discussed further in Appendix E, EPA considers a BMR of 5 percent the most supportable for deriving a health protective candidate POD, when supported by available data. BMD estimates from the linear-quadratic model were

8.4 mg/kg-day (95% confidence interval: 6.0, 14 for a 5 percent change [BMR = 5%], 17 mg/kg-day [12, 29] for a 10 percent change [BMR = 10%], and 90 mg/kg-day [63, 151] for a 40 percent change [BMR = 40%] (Table 4-4). Further methodological details and results (*e.g.*, forest plots, figures of BMD model fits) for the meta-analysis and BMD modeling of fetal testicular testosterone data are provided in the *Meta-analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), Dicyclohexyl Phthalate (DCHP), and Diisononyl Phthalate* (U.S. EPA, 2025g).

Although meta-analysis and BMD modeling of fetal testicular testosterone data support a $BMDL_5$ of 6.0 mg/kg-day, there is some uncertainty associated with the BMDL estimate. As can be seen in Table 4-2 only one data point below a dose of 100 mg/kg-day was available for inclusion in the meta-analysis (*i.e.*, at 33 mg/kg-day, where a 25 percent decreased in testosterone was observed) and the estimated BMD_5 (8.4 mg/kg-day) and $BMDL_5$ (6.0 mg/kg-day) values for DCHP are both well below the lowest dose tested. Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the lack of data to inform the low-end of the dose-response curve reduces EPA's confidence in using the $BMDL_5$ of 6.0 mg/kg-day for use in risk characterization.

EPA also conducted BMD modeling of the individual *ex vivo* fetal rat testicular testosterone data from the three studies included in the meta-analysis. BMD modeling was conducted using all standard continuous models included in EPA's BMD Software (BMDS) online (https://bmdsonline.epa.gov/). EPA evaluated BMRs of 5, 10, and 40 percent relative deviation, which were included for consistency with EPA's meta-analysis and benchmark dose analysis of fetal testicular testosterone. However, as described in Appendix E, EPA considers a BMR of 5 percent to be the most appropriate and health protective response level for evaluating decreased fetal testicular testosterone for POD determination. No viable model fits were obtained for the *ex vivo* fetal testicular testosterone production data from Block 23 rats reported by Furr et al. (2014) (Appendix F.3). For the *ex vivo* fetal testicular testosterone production data from Block 33 rats reported by Furr et al. (2014), the Exponential 3 model provided the best fit, and supports BMD_5 and $BMDL_5$ estimates of 9.0 and 5.2 mg/kg-day (Appendix F.4). For the *ex vivo* fetal testicular testosterone production data reported by Gray et al. (2021), the Exponential 3 model provided the best fit, and supports BMD_5 and $BMDL_5$ estimates of 13.7 and 10.0 mg/kg-day (Appendix F.5). Although this BMD analysis of fetal testis testosterone data from individual studies provides BMD_5 and $BMDL_5$ estimates similar to the BMD_5 and $BMDL_5$ estimates from the meta-analysis (*i.e.*, BMD_5/$BMDL_5$ values of 8.4/6.0 mg/kg-day), there is some uncertainty associated with these BMD_5 and $BMDL_5$ estimates. This is because the $BMDL_5$ estimate of 5.2 mg/kg-day from Furr et al. is factor of approximately 6.3× below the lowest dose included in the study (33 mg/kg-day), while the $BMDL_5$ estimate of 10.0 mg/kg-day from Gray et al. is an order of magnitude below the lowest factor dose included in the study (100 mg/kg-day). Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the lack of data to inform the low-end of the dose-response curve reduces EPA's confidence in using the $BMDL_5$ estimates of 5.2 and 10 for use in risk characterization.

EPA also conducted BMD modeling on testosterone data from two additional studies not included in the meta-analysis, including fetal serum testosterone data reported by Ahbab et al. (2015) (not included in the meta-analysis because only serum, and not testis testosterone was evaluated). Testis testosterone content data in postnatal day one rats reported by Li et al. (2016) was also modeled (not included in the meta-analysis because testosterone was not measured during the fetal lifestage). BMD modeling was conducted using all standard continuous models included in EPA's BMD Software (BMDS) online (https://bmdsonline.epa.gov/). EPA evaluated BMRs of 5, 10, and 40 percent relative deviation, which were included for consistency with EPA's meta-analysis and benchmark dose analysis of fetal testicular testosterone. However, as described in Appendix E, EPA considers a BMR of 5 percent to be the most

appropriate and health protective response level for evaluating decreased fetal testicular testosterone for POD determination. Results of this BMD analysis are summarized in Appendix F. No viable model fits were obtained for the fetal serum testosterone data from Ahbab et al. (2015) (Appendix F.2), and as discussed above this study is considered to support a LOAEL of 20 mg/kg-day (no NOAEL identified). For the postnatal testicular testosterone data from Li et al. (2016), the Hill model provided the best fit and supports BMD_5 and $BMDL_5$ estimates of 6.9 and 1.2 mg/kg-day (Appendix F.1). However, there is uncertainty associated with these BMD_5 and $BMDL_5$ estimates, as the BMD/BMDL ratio is large (approximately 6), while the $BMDL_5$ of 1.2 is nearly an order of magnitude below the lowest dose included in the study (10 mg/kg-day). Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the lack of data to inform the low-end of the dose-response curve reduces EPA's confidence in using the $BMDL_5$ of 1.2 mg/kg-day for use in risk characterization.

4.2.3 Selection of the Non-cancer Oral Point of Departure

Across available studies, effects on the developing male reproductive system are observed at doses ranging from 10 to 33 mg/kg-day. EPA has selected the NOAEL of 10 mg/kg-day from Li et al. (2016) as the POD for assessing acute, intermediate, and chronic risk in the DCHP risk evaluation. Although some changes associated with the MOA of rat phthalate syndrome were observed at 10 mg/kg/day, these are of uncertain toxicological significance, did not occur in a dose-dependent manner, and/or were inconsistent with other studies. As such, the observations at 10 mg/kg-day in the study by Li and colleagues were not considered adverse.

Using allometric body weight scaling to the three-quarters power, EPA extrapolated an HED of 2.4 mg/kg-day from the NOAEL of 10 mg/kg-day. A total uncertainty factor of 30 was selected for use as the benchmark MOE based on an interspecies uncertainty factor (UF_A) of 3 and an intraspecies uncertainty factor (UF_H) of 10. Consistent with EPA guidance (2022, 2002b, 1993), EPA reduced the UF_A from a value of 10 to 3 because allometric body weight scaling to the three-quarter power was used to adjust the POD to obtain a HED (see Appendix D). EPA considered reducing the UF_A further to a value of 1 based on apparent differences in toxicodynamics between rats and humans. As discussed in Section 3.1.4 of EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b), several explant (Lambrot et al., 2009; Hallmark et al., 2007) and xenograft studies (van Den Driesche et al., 2015; Spade et al., 2014; Heger et al., 2012; Mitchell et al., 2012) using human donor fetal testis tissue have been conducted to investigate the antiandrogenicity of mono-2-ethylhexyl phthalate (MEHP; a monoester metabolite of DEHP), DBP, and monobutyl phthalate (MBP; a monoester metabolite of DBP) in a human model. Generally, results from human explant and xenograft studies suggest that human fetal testes are generally less sensitive to the antiandrogenic effects of phthalates. However, as discussed in EPA's draft approach document (U.S. EPA, 2023b), the available human explant and xenograft studies have limitations, which preclude definitive conclusions related to species differences in sensitivity. Therefore, EPA did not reduce the UF_A further.

Table 4-1. Dose-Response Analysis of Selected Studies Considered for Acute, Intermediate, and Chronic Exposure Scenarios

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
Meta-analysis of data from 3 studies reported in Furr et al. (2014) and Gray et al. (2021) (High for both references)	$BMDL_5$ = 6.0	↓ *ex vivo* fetal testicular testosterone production	1.4	UF_A= 3 UF_H=10 *Total UF=30*	- See (U.S. EPA, 2025g) for BMD results
Pregnant SD rats (6 dams/dose) gavaged with 0, 10, 100, and 500 mg/kg-day DCHP on GD 12–21 (Li et al., 2016) (Medium)	NOAEL [c] = 10	↓ testicular testosterone; ↓ absolute male AGD; Leydig cell effects (aggregation, ↓size, cytoplasmic size, and nuclear size); ↓ mRNA and/or protein expression of steroidogenic genes; ↓ INSL3	2.4	UF_A= 3 UF_H=10 *Total UF=30*	- See Appendix F.1 for BMD results F.1
	$BMDL_5$ = 1.2	↓ testicular testosterone	0.28	UF_A= 3 UF_H=10 *Total UF=30*	
2- gen reproduction study with SD rats administered DCHP in diet at 0, 240, 1,200, or 6,000 ppm (0/0, 17/21, 85/106, and 430/523 mg/kg-day in males/females) (Adhered to OECD TG 416) (Hoshino et al., 2005) (Medium)	NOAEL = 17	Seminiferous tubule atrophy and ↓ sperm count in F1 adult males; ↓ F2 male AGD on PND 4 (absolute and bodyweight corrected); ↑ nipple retention in F2 males	4.0	UF_A= 3 UF_H=10 *Total UF=30*	- Study considered for BMD modeling, however, data not considered sensitive enough to warrant BMD modeling (Section 4.2.1)
Pregnant Wistar albino rats (10 per dose) gavaged with 0, 20, 100, 500 mg/kg-day DCHP on GD 6–19. Dams allowed to deliver litters naturally and then male offspring evaluated on PND 20, PND 32, and PND 90 (Ahbab and Barlas, 2013) (Medium)	LOAEL = 20	↑ Abnormal sperm; ↑ incidence of histopathology lesions in testes, epididymis, and prostate	4.7	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- Study considered for BMD modeling, however, majority of data was not considered amenable to modeling due data reporting issues and/or nature of response (*i.e.*, all or nothing response) (Section 4.2.1)
Pregnant Wistar rats (10 dams per dose) gavaged with 0, 20, 100, 500 mg/kg-day DCHP on GDs 6–19. Dams sacrificed on GD 20 and male fetuses examined (Ahbab and Barlas, 2015) (High)	LOAEL = 20	↓ male AGD; serum hormone changes (↓ testosterone and MIS, ↑ inhibin); ↑ resorptions; ↑ testicular pathology (*e.g.*, seminiferous tubule atrophy); Leydig cell aggregation	4.7	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- BMD modeling of fetal serum testosterone data attempted - No models adequately fit the data set (Appendix F.2)
Developmental toxicity with Wistar albino rats administered DCHP at 0, 20, 100 and	LOAEL = 20	↓ AGD & AGD (corrected for BW) in females; skeletal retardation & delayed	4.7	UF_A= 3 UF_H=10	- Study considered for BMD modeling, however, no BMD

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
500 mg/kg-day by gavage on GD 6–19. Dams terminated on GD 20 & fetuses removed by cesarean section. (Ahbab et al., 2017) (Medium)		ossification; Changes in hematological parameters; ↓ MCH in males and females, ↓ MCHC in males; placental histopathologic findings		UF_L=10 *Total UF=300*	modeling was conducted, as outcomes were measured in female, not male, fetuses.
Pregnant SD rats (3–4 per dose) gavaged with 0, 33, 100, 300 mg/kg-day DCHP on GDs 14–18. Dams sacrificed on GD 18 (Block 33 rats) (Furr et al., 2014) (High)	LOAEL = 33	↓ in *ex vivo* fetal testicular testosterone (25% decrease, not significant at 33 mg/kg-day)	7.8	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- See Appendix F.4 for BMD results
	$BMDL_5$ = 1.2	↓ in *ex vivo* fetal testicular testosterone production	1.2	UF_A= 3 UF_H=10 *Total UF=30*	
Pregnant SD rats (2–3 dams per dose) gavaged with 0, 100, 300, 600, and 900 mg/kg-day DCHP on GD 14-18. Dams sacrificed on GD 18 (Block 23 rats) (Furr et al., 2014) (High)	LOAEL = 100	↓ in *ex vivo* fetal testicular testosterone; ↓ mRNA expression of steroidogenic genes	23.6	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- BMD modeling of fetal testosterone data attempted - No models adequately fit the data set (Appendix F.3)
Pregnant SD rats gavaged with 0, 100, 300, 600, 900 mg/kg-day on GD 14–18. Dams sacrificed on GD 18 (block 148 rats) (Gray et al., 2021) (High)	LOAEL = 100	↓ in *ex vivo* fetal testicular testosterone production; ↓gene expression (*e.g.*, steroidogenesis)	23.6	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- See Appendix F.5 for BMD results
	$BMDL_5$ = 10	↓ in *ex vivo* fetal testicular testosterone production	2.4	UF_A= 3 UF_H=10 *Total UF=30*	
Pregnant SD rats (22–25 per dose) gavaged with 0, 250, 500, 750 mg/kg-day DCHP on GDs 6–20. Dams sacrificed on GD 21 (Saillenfait et al., 2009) (Medium)	LOAEL = 250	↓ AGD in male fetuses	59.1	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- Study not considered for BMD analysis, as other studies evaluated lower doses and provided more sensitive outcomes for modeling (Section 4.2.1)
Pregnant SD rats (10 per dose) gavaged with 0, 20, 100, and 500 mg/kg-day DCHP	NOAEL = 100	↓ viability index on PND 4 (slight); ↓ offspring body weights (both sexes); ↓ male AGD; ↑ male nipple retention;	23.6	UF_A= 3 UF_H=10 *Total UF=30*	- Study considered for BMD modeling, however, no modeling was conducted due to uncertainties

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
on GD 6–PND 20. Dams allowed to give birth naturally (Yamasaki et al., 2009) (Medium)		hypospadias; delayed PPS; ↓ relative prostate & LABC weights			associated with data reporting (Section 4.2.1)

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011c), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics.

[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to DCHP. EPA used a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 to account for the uncertainty inherent in extrapolating from the LOAEL to the NOAEL.

[c] Statistically significant effects at 10 mg/kg-day are limited to fetal Leydig cell effects, decreased expression of genes and proteins involved in steroidogenesis, and decreased protein expression of INSL3 (all of which are not considered adverse in isolation). The remaining effects listed reached statistical significance at higher doses.

Table 4-2. Summary of Effects of Gestational Exposure to DCHP on Testicular Testosterone Across Studies

Study Details (Species, Duration, Exposure Route/ Method, Endpoint, Measurement timing) (TSCA Study Quality Rating) (Reference)	% of Control Testosterone Response by Dose (mg/kg-day) [a]								
	0	10	33	100	300	500	600	750	900
SD Rats (Block 33); GD 14–18; Oral/gavage; *ex vivo* fetal testicular testosterone production; GD 18 (High) (Furr et al., 2014) [c]	100% (n = 4)	–	75% (n = 4)	45%* (n = 4)	31%* (n = 3)	-	–	–	–
SD Rats (Block 23); GD 14–18; Oral/gavage; *ex vivo* fetal testicular testosterone production; GD 18 (High) (Furr et al., 2014) [c]	100% (n = 3)	–	–	31%* (n = 3)	22%* (n = 2)	–	20%* (n = 3)	–	55%* (n = 3)
SD Rats; GD 12–21; Oral/gavage; testicular testosterone; PND 1 (Medium) (Li et al., 2016) [d]	100% (n = 6)	90% (n = 6)	–	62%* (n = 6)	–	33%* (n = 6)	–	–	–
SD Rats (Block 148); GD 14–18; Oral/ gavage; *ex vivo* fetal testicular testosterone production; GD 18 (High) (Gray et al., 2021) [b] [c]	100% (n = 3)	–	–	59% (n = 3)	28% (n = 3)	–	17% (n = 3)	–	12% (n = 3)

* Denotes statistically significant compared to control ($p < 0.05$)
[a] Effect on fetal testicular testosterone production reported as percent of control. Asterisks indicate statistically significant pairwise comparison to control, as reported by study authors.
[b] Data from Block 148 rats reported in supplemental information file associated with Gray et al. (2021). *Ex vivo* testosterone production data from Block 148 rats was subjected to statistical analysis.
[c] Data used in meta-analysis and BMD modeling analysis of fetal testosterone.
[d] Data from Li et al. (2016) not used in meta-analysis or BMD analysis because testosterone was measured on PND 1, not during the fetal lifestage.

Table 4-3. Overall Analyses of Rat Studies of DCHP and Fetal Testosterone

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P value	Tau	I^2	P value for Heterogeneity	AICs
Overall	intercept	−113.99	–146.03	–81.95	3.1E–12	50.13	88.36	3.6E–12	114.46
Trend in log10(dose)	log10(dose)	–77.00	–135.97	–18.04	1.0E–02	39.19	81.97	5.5E–08	104.45
Linear in dose100	dose100	–22.14	–28.75	–15.54	5.0E–11	49.12	88.03	8.1E–13	121.53
Linear Quadratic in dose 100	dose100	–61.83	–86.20	–37.46	6.6E–07	51.94	88.95	1.4E–12	104.92*
Linear Quadratic in dose 100	I(dose100^2)	5.39	2.21	8.56	8.8E–04	51.94	88.95	1.4E−12	104.92

* Indicates model with lowest Akaike information criterion (AIC).
CI = confidence interval; I^2 = describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error; Tau = estimated standard deviation of the true underlying effect sizes across studies in the random-effects model meta-analysis.

Table 4-4. Benchmark Dose Estimates for DCHP and Fetal Testosterone in Rats

Analysis	Benchmark Response (BMR)	Benchmark Dose (BMD)	Confidence Interval, Lower Bound	Confidence Interval, Upper Bound
Linear in dose100	5%	23	18	33
Linear in dose100	10%	48	37	68
Linear in dose100	40%	231	178	329
LinearQuadratic in dose100*	5%	8.4	6.0	14
LinearQuadratic in dose100*	10%	17	12	29
LinearQuadratic in dose100*	40%	90	63	151
* Indicates model with lowest Akaike information criterion (AIC).				

4.3 Weight of Scientific Evidence

EPA concludes that the lowest HED of 2.4 (NOAEL of 10 mg/kg-day) supported by the high-quality study by Li et al. (2016) is appropriate for calculation for risk from acute, intermediate, and chronic durations. A total UF of 30 was selected for use as the benchmark margin of exposure based on an interspecies (UF_A) of 3, and an intraspecies (UF_H) of 10. Consistent with EPA guidance (2022, 2002b, 1993), EPA reduced the UF_A from a value of 10 to 3 because allometric body weight scaling to the three-quarter power was used to adjust the POD to obtain a HED (Appendix D).

EPA has *robust overall confidence in the selected POD* based on the following weight of scientific evidence:

- EPA has previously developed a weight of scientific evidence analysis and concluded that oral exposure to DCHP can induce effects on the developing male reproductive system consistent with a disruption of androgen action (see EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b)). Notably, EPA's conclusion was supported by the SACC (U.S. EPA, 2023c).
- DCHP exposure resulted in treatment related effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in nine studies of rats (Section 3.1). Observed effects included reduced mRNA and protein expression of INSL3 in fetal testes; reduced mRNA and protein expression of genes related to steroidogenesis in fetal testes; reduced fetal testicular testosterone; increased MNGs in fetal testes; fetal Leydig cell effects (increased fetal Leydig cell aggregation, decreased Leydig cell size, and decreased Leydig cell cytoplasmic and nuclear size); increased testicular histopathology in fetal, pre-pubertal, pubertal, and adult rats; reduced male pup AGD; increased male pup nipple retention; delayed preputial separation; increased reproductive tract malformations (*i.e.*, hypospadias); sperm abnormalities in adults; and decreased sperm count in adults.
- Of the nine oral studies considered in detail by EPA, five medium-to-high quality studies support PODs that are similar to the most-sensitive POD from the high-quality study by Li et al. (2016). Specifically, four studies of varying design that were conducted by several different research groups support a developmental LOAEL ranging from 20 to 33 mg/kg-day (HED = 4.7 to 7.8 mg/kg-day) (Ahbab et al., 2017; Ahbab and Barlas, 2015; Furr et al., 2014; Ahbab and Barlas,

2013), while a two-generation study of reproduction in rats that adhered to OECD TG 416, supports a developmental NOAEL of 17 mg/kg-day (HED of 4.0 mg/kg-day) (Hoshino et al., 2005). Across these five studies (Ahbab et al., 2017; Ahbab and Barlas, 2015; Furr et al., 2014; Ahbab and Barlas, 2013; Hoshino et al., 2005), effects on the developing male reproductive system consistent with a disruption of androgen action and phthalate syndrome were observed, as were other developmental and reproductive effects (*e.g.*, decreased fetal weight, increased resorptions, delayed skeletal ossification). However, the individual limitations and uncertainties of each of these studies (discussed in Section 4.1) precluded EPA from selecting any as the POD. Therefore, the NOAEL of 10 mg/kg-day from Li et al. (2016) is the most robust and appropriate.

- Meta-analysis and BMD modeling of fetal testicular testosterone data from three studies from two publications (Gray et al., 2021; Furr et al., 2014) supports a BMD_5 and $BMDL_5$ values of 8.5 and 6.0 mg/kg-day, respectively. Further, BMD modeling of fetal testicular testosterone data from individual studies supports similar BMD_5 and $BMDL_5$ values of 9.0 and 5.2 mg/kg-day from Furr et al. and 13.7 and 10 mg/kg-day from Gray et al. Although the analysis was limited by the lack of data in the low-end of the dose-response curve (*i.e.*, lowest data point for fetal testosterone was 33 mg/kg-day and the lowest dose-to-BMDL ratio ranged from 6 to 10), the derived $BMDL_5$ estimates ranging from 5.2 to 10 mg/kg-day are consistent with the selected NOAEL of 10 mg/kg-day.
- EPA's selected POD of 10 mg/kg/day is consistent with other regulatory and authoritative bodies that have also concluded that DCHP is a developmental toxicant and that developmental effects are relevant for estimating human risk (Health Canada, 2020; EC/HC, 2015; CPSC, 2014; ECHA, 2014; CPSC, 2011). Most recently, Health Canada used the same POD of 10 mg/kg-day to quantify risk from exposures to DCHP (Health Canada, 2020).
- EPA considers effects on the developing male reproductive system consistent with a disruption of androgen action to be relevant for setting a POD for acute duration exposures. This is based on studies of the toxicologically similar phthalate dibutyl phthalate (DBP), which have demonstrated that a single exposure during the critical window of development can disrupt expression of steroidogenic genes and decrease fetal testes testosterone.

4.4 Route-to-Route Extrapolation

EPA did not identify any studies conducted via the dermal or inhalation exposure routes that are relevant for determining human health risk. Therefore, EPA is using the oral HED of 2.4 mg/kg DCHP to extrapolate risk for the dermal and inhalation routes. When conducting route-to-route extrapolations, the preferred approach is to use validated physiologically-based pharmacokinetic (PBPK) models or chemical-specific pharmacokinetic data to account for potential route-specific differences in toxicokinetics (IGHRC, 2006; U.S. EPA, 1994). For DCHP, no PBPK model is available to support route-to-route extrapolation. Therefore, EPA used a combination of empirical absorption data, and default assumptions regarding potential route-specific differences in metabolism. As discussed further below, the available data accounting for differential absorption across routes (oral, dermal, inhalation) and similarities in metabolism indicate that the hazard derivation from different routes of exposures is reasonably supported.

Dermal Route

EPA has accounted for differences in absorption between the oral and dermal exposures routes. As discussed in Section 2.1, available data indicate 100 percent absorption of DCHP through the gastrointestinal tract following oral exposure, while EPA estimated steady-state dermal flux values for

DCHP to estimate dermal exposure (Section 2.3). However, potential route-specific differences in metabolism were not accounted for. Following oral exposure, phthalate diesters (including DCHP) are metabolized to a monoester metabolite (*e.g.*, MCHP) by esterases in the intestines or liver. Further oxidative metabolism or phase two conjugation reactions (*e.g.*, glucuronidation) may also occur in the liver prior to systemic circulation. Esterases are also present in the skin, and therefore metabolism of DCHP to its monoester metabolite MCHP also likely occurs via the dermal route prior to systemic circulation. For example, as discussed in the non-cancer human health hazard assessments of DBP (U.S. EPA, 2025i), DEHP (U.S. EPA, 2025k), and BBP (U.S. EPA, 2025h), dermal absorption studies with metabolically active human or rat skin demonstrate metabolism of DBP, DEHP, and BBP to their respective monoester metabolites MBP, MEHP, and MBzP, as well as other oxidative metabolites. No dermal absorption studies of DCHP with metabolically active skin are available, however, EPA considers it reasonable to assume that DCHP would undergo similar metabolism to MCHP and other oxidative metabolites in the skin before being absorbed and undergoing systemic circulation.

Use of the oral HED to extrapolate risk for the dermal route carries an inherent assumption of similar metabolism between the oral and dermal exposure routes, however, empirical data demonstrating this are not available and this is a source of uncertainty. However, EPA is confident that its human health risk characterization from the dermal route for DCHP is health protective.

Inhalation Route

For the inhalation route, EPA extrapolated the daily oral HED to an inhalation HEC using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest (see Appendix D for further details). EPA assumes similar absorption for the oral and inhalation routes, and no adjustment was made when extrapolating to the inhalation route. As discussed above, available data indicate 100 percent absorption of DCHP through the gastrointestinal tract following oral exposure (Section 2.1); although no inhalation toxicokinetic study of DCHP is available, studies of other phthalates (*i.e.*, DEHP, DIDP, and DINP) indicate phthalates are nearly completely absorbed through the respiratory tract, and 100 percent absorption is assumed for DCHP. Similar to the oral route of exposure, metabolism of DCHP to its monoester metabolite MCHP is expected to occur in the lung, however, the rate of metabolism in the lung may be slower than in the gastrointestinal tract and liver. For example, Ito et al. (2005) report lipase activity in rat liver and lung homogenate; however, lipase activity was approximately 12.6 times higher in the liver compared to the lung. Similarly, Choi et al. (2012) demonstrate metabolism of DEHP to MEHP in human small intestine, liver, and lung tissue samples, however, the metabolic rate of MEHP formation was highest in the small intestine and liver compared to the lung. Although no studies of DCHP metabolism in the lung are available, EPA considers it reasonable to assume that DCHP is metabolized to MCHP in the lung, due to the presence of lipases. However, when extrapolating the inhalation HEC from the oral HED EPA did not account for differences in rates of metabolism of DCHP (or any other phthalate) between exposure routes. Despite some remaining uncertainty, EPA is confident that its human health risk characterization via the inhalation route for DCHP is health protective.

5 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE

5.1 Hazard Considerations for Aggregate Exposure

For use in the risk evaluation and assessing risks from other exposure routes, EPA conducted route-to-route extrapolation of the toxicity values from the oral studies for use in the dermal and inhalation exposure routes and scenarios. Health outcomes that serve as the basis for acute, intermediate, and chronic hazard values are systemic and assumed to be consistent across routes of exposure. EPA therefore concludes that for consideration of aggregate exposures, it is reasonable to assume that exposures and risks across oral, dermal, and inhalation routes may be additive for the selected PODs in Section 6.

5.2 PESS Based on Greater Susceptibility

In this section, EPA addresses subpopulations likely to be more susceptible to DCHP exposure than other populations. Table 5-1 presents the data sources that were used in the PESS analysis evaluating susceptible subpopulations and identifies whether and how the subpopulation was addressed quantitatively in the risk evaluation of DCHP.

Although ample human epidemiologic data are reasonably available on health effects of DCHP (See Section 3.1.1), EPA was unable to identify direct evidence of differences in susceptibility among human populations. Animal studies demonstrating effects on male reproductive development and other developmental outcomes provide direct evidence that gestation is a particularly sensitive lifestage. Evidence from animal studies also suggests that the liver may also be a target organ, although the liver effects observed across reasonably available studies are generally not indicative of an adverse response (discussed further in Section 3.3). EPA is quantifying risks including those for PESS based on reproductive and developmental toxicity in the DCHP risk evaluation.

As summarized in Table 5-1, EPA identified a range of factors that may have the potential to increase biological susceptibility to DCHP—including life stage, chronic liver disease, pre-existing diseases, physical activity, diet, stress, and co-exposures to other environmental stressors that contribute to related health outcomes. Because the effect of these factors on susceptibility to health effects of DCHP is not known; EPA is uncertain about the direction and magnitude of any possible increased risk from effects associated with DCHP exposure for relevant subpopulations.

For non-cancer endpoints, EPA used a default value of 10 for human variability (UF_H) to account for increased susceptibility when quantifying risks from exposure to DCHP. The Risk Assessment Forum, in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), discussed some of the evidence for choosing the default UF of 10 when data are lacking and describe the types of populations that may be more susceptible, including different life stages (*e.g.*, children, elderly). Although U.S. EPA (2002b) did not discuss all the factors presented in Table 5-1, EPA considers the POD selected for use in characterizing risk from exposure to DCHP to be protective of susceptible life stages because it is based on effects on the developing male reproductive system consistent with phthalate syndrome in humans.

As discussed in U.S. EPA (2023b), exposure to DCHP and other toxicologically similar phthalates (*i.e.*, DEHP, DBP, BBP, DIBP, and DINP) that disrupt androgen action during the development of the male reproductive system cause dose additive effects. Cumulative effects from exposure to DCHP and other

toxicologically similar phthalates will be considered as part of U.S. EPA's forthcoming cumulative risk assessment of phthalates.

Table 5-1. PESS Evidence Crosswalk for Biological Susceptibility Considerations

Suscept -ibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DCHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DCHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	Direct quantitative animal evidence for developmental toxicity (*e.g.*, increased resorptions, decreased fetal body weight, skeletal retardation and delayed ossification). There is direct quantitative animal evidence for effects on the developing male reproductive system consistent with a disruption of androgen action.	(U.S. EPA, 2023b, c; Gray et al., 2021; Ahbab et al., 2017; Li et al., 2016; Ahbab and Barlas, 2015, 2013; Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DCHP is based on developmental toxicity and is considered protective of effects on the fetus and offspring.
	Pregnancy/ lactating status	Rodent dams not particularly susceptible during pregnancy and lactation, except for effects related to reduced maternal weight gain, food consumption, and increased absolute/relative liver weight.	(Ahbab et al., 2017; Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DCHP based on developmental toxicity is considered protective of effects on dams
	Males of reproductive age	One two-generation study of DCHP has shown effects on the male reproductive system, including testicular atrophy and decreased sperm counts in adult F1 males.	(Hoshino et al., 2005)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DCHP based on developmental toxicity is considered protective of adult male reproductive effects. Use of default 10× UF_H
	Children	Reduced offspring body weight gain has been observed in one gestational exposure study and in F1 and F2 offspring in one two-generation study of reproduction.	(Yamasaki et al., 2009; Hoshino et al., 2005)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DCHP based on developmental toxicity is considered protective of effects of offspring bodyweight gain. Use of default 10x UF_H
	Elderly	No direct evidence identified				Use of default 10x UF_H

Suscept -ibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DCHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DCHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Pre-existing disease or disorder	Health outcome/ target organs	No direct evidence identified		Several preexisting conditions may contribute to adverse developmental outcomes (*e.g.*, diabetes, high blood pressure, certain viruses). Individuals with chronic liver disease may be more susceptible to effects on these target organs. Viruses such as viral hepatitis can cause liver damage.	CDC (2023e) CDC (2023g)	Use of default 10x UF_H
	Toxicokinetics	No direct evidence identified		Chronic liver disease is associated with impaired metabolism and clearance (altered expression of phase 1 and phase 2 enzymes, impaired clearance), which may enhance exposure duration and concentration of DCHP.		Use of default 10x UF_H

Suscept -ibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DCHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DCHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Lifestyle activities	Smoking	No direct evidence identified		Smoking during pregnancy may increase susceptibility for developmental outcomes (*e.g.*, early delivery and stillbirths).	CDC (2023f)	Qualitative discussion in Section 5.2 and this table
	Alcohol consumption	No direct evidence identified		Alcohol use during pregnancy can cause developmental outcomes (*e.g.*, fetal alcohol spectrum disorders). Heavy alcohol use may affect susceptibility to liver disease.	CDC (2023d) CDC (2023a)	Qualitative discussion in Section 5.2 and this table
	Physical activity	No direct evidence identified		Insufficient activity may increase susceptibility to multiple health outcomes. Overly strenuous activity may also increase susceptibility.	CDC (2022)	Qualitative discussion in Section 5.2 and this table

Suscept -ibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DCHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DCHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Sociodemo -graphic status	Race/ethnicity	No direct evidence identified (*e.g.*, no information on polymorphisms in DCHP metabolic pathways or diseases associated race/ethnicity that would lead to increased susceptibility to effects of DCHP by any individual group).				Qualitative discussion in Section 5.2 and this table
	Socioeconomic status	No direct evidence identified		Individuals with lower incomes may have worse health outcomes due to social needs that are not met, environmental concerns, and barriers to health care access.	ODPHP (2023b)	Qualitative discussion in Section 5.2 and this table
	Sex/gender	No direct evidence identified				Use of default 10x UF_H

Suscept -ibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DCHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DCHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Nutrition	Diet	No direct evidence identified		Poor diets can lead to chronic illnesses such as heart disease, type 2 diabetes, and obesity, which may contribute to adverse developmental outcomes. Additionally, diet can be a risk factor for fatty liver, which could be a pre-existing condition to enhance susceptibility to DCHP-induced liver toxicity.	CDC (2023e) CDC (2023b)	Qualitative discussion in Section 5.2 and this table
	Malnutrition	No direct evidence identified		Micronutrient malnutrition can lead to multiple conditions that include birth defects, maternal and infant deaths, preterm birth, low birth weight, poor fetal growth, childhood blindness, undeveloped cognitive ability. Thus, malnutrition may increase susceptibility to some developmental outcomes associated with DCHP.	CDC (2021) CDC (2023b)	Qualitative discussion in Section 5.2 and this table

Suscept -ibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DCHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DCHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citation(s)	Description of Interaction	Key Citation(s)	
Genetics/ epigenetics	Target organs	No direct evidence identified		Polymorphisms in genes may increase susceptibility to liver or developmental toxicity.		Use of default 10x UF_H
	Toxicokinetics	No direct evidence identified		Polymorphisms in genes encoding enzymes (*e.g.*, esterases) involved in metabolism of DCHP may influence metabolism and excretion of DCHP.		Use of default 10x UF_H
Other chemical and nonchemical stressors	Built environment	No direct evidence identified		Poor-quality housing is associated with a variety of negative health outcomes.	ODPHP (2023a)	Qualitative discussion in Section 5.2 and this table
	Social environment	No direct evidence identified		Social isolation and other social determinants (*e.g.*, decreased social capital, stress) can lead to negative health outcomes.	CDC (2023c) ODPHP (2023c)	Qualitative discussion in Section 5.2 and this table
	Chemical co-exposures	Studies have demonstrated that co-exposure to DCHP and other toxicologically similar phthalates (*e.g.*, DEHP, DBP, BBP, DIBP, DINP) and other classes of antiandrogenic chemicals (*e.g.*, certain pesticides and pharmaceuticals – discussed more in (U.S. EPA, 2023b)) can induce effects on the developing male reproductive system in a dose-additive manner.	See (U.S. EPA, 2023b, c)			Qualitative discussion in Section 5.2 and this table and will be quantitatively addressed as part of the phthalate cumulative risk assessment.

6 POINTS OF DEPARTURE USED TO ESTIMATE RISKS FROM DCHP EXPOSURE, AND CONCLUSIONS

After considering hazard identification and evidence integration, dose-response evaluation, and weight of scientific evidence of POD candidates, EPA chose one non-cancer endpoint for use in determining the risk from acute, intermediate, and chronic exposure scenarios (Table 6-1). The critical effect is disruption to androgen action during the critical window of male reproductive development (*i.e.*, during gestation), leading to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. EPA has robust overall confidence in the selected POD of 10 mg/kg-day (HED = 2.4 mg/kg-day) for acute, intermediate, and chronic durations. There are no studies conducted via the dermal and inhalation route relevant for extrapolating human health risk. In the absence of inhalation studies, EPA performed route-to-route extrapolation to convert the oral HED to an inhalation HEC of 13 mg/m^3 (0.95 ppm). The Agency is also using the oral HED to extrapolate to the dermal route. HECs are based on daily continuous (24-hour) exposure and HEDs are daily values. The HECs are based on daily continuous (24-hour) exposure, and HEDs are daily values.

The POD of 10 mg/kg-day (HED = 2.4 mg/kg-day) will be used in the *Risk Evaluation for Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025n) to estimate acute, intermediate, and chronic non-cancer risk. EPA summarizes the cancer hazards of DCHP in a separate technical support document, *Cancer Human Health Hazard Assessment for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP), Butyl Benzyl Phthalate (BBP) and Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025a).

Table 6-1. Non-cancer HECs and HEDs Used to Estimate Risks

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HED[a] (mg/kg-day)	HEC[a] (mg/m^3) [ppm]	Benchmark MOE[b]	Reference (TSCA Study Quality Rating)
Acute, intermediate, chronic	Developmental toxicity	Rat	10 days during gestation	NOAEL[c] = 10	Phthalate syndrome-related effects (*e.g.*, ↓ fetal testicular testosterone; ↓AGD; Leydig cell effects; ↓ mRNA and/or protein expression of steroidogenic genes; ↓INSL3)	2.4	13 [0.95]	UF_A= 3 UF_H=10 *Total UF=30*	(Li et al., 2016) (Medium)

HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor

[a] HED and HEC values were calculated based on the most sensitive NOAEL of 10 mg/kg-day.

[b] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011c), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics. EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations.

[c] Statistically significant effects at 10 mg/kg-day are limited to fetal Leydig cell effects, decreased expression of genes and proteins involved in steroidogenesis, and decreased protein expression of INSL3 (all of which are not considered adverse in isolation). The remaining effects listed reached statistical significance at higher doses.

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APPENDICES

Appendix A EXISTING ASSESSMENTS FROM OTHER REGULATORY AGENCIES OF DCHP

The available existing assessments of DCHP are summarized in Table_Apx A-1, which includes details regarding external peer review, public consultation, and systematic review protocols that were used.

Table_Apx A-1. Summary of Peer-review, Public Comments, and Systematic Review for Existing Assessments of DCHP

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
U.S. CPSC	*Toxicity Review of Dicyclohexyl Phthalate (DCHP)* (CPSC, 2011) *Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (*CPSC, 2014*)*	Yes	Yes	No	- Peer-reviewed by panel of four experts. Peer-review report available at: https://www.cpsc.gov/s3fs-public/Peer-Review-Report-Comments.pdf - Public comments available at: https://www.cpsc.gov/chap - No formal systematic review protocol employed. - Details regarding CPSC's strategy for identifying new information and literature are provided on page 12 of (CPSC, 2014)
Health Canada	*State of the Science Report: Phthalate Substance Grouping: Medium-Chain Phthalate Esters: Chemical Abstracts Service Registry Numbers: 84-61-7; 84-64-0; 84-69-5; 523-31-9; 5334-09-8;16883-83-3; 27215-22-1; 27987-25-3; 68515-40-2; 71888-89-6* (EC/HC, 2015) *Supporting Documentation: Evaluation of Epidemiologic Studies on Phthalate Compounds and Their Metabolites for Hormonal Effects, Growth and Development and Reproductive Parameters* (Health Canada, 2018b)	Yes	Yes	No (Animal studies) Yes (Epidemiologic studies)	- Ecological and human health portions of the screening assessment report (Health Canada, 2020) were subject to external review and/or consultation. See page 2 of (Health Canada, 2020) for additional details. - State of the science report (EC/HC, 2015) and draft screening assessment report for the phthalate substance group subjected to 60-day public comment periods. Summaries of received public comments available at: https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative/phthalate.html#a1

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	Supporting Documentation: Evaluation of Epidemiologic Studies on Phthalate Compounds and Their Metabolites for Effects on Behaviour and Neurodevelopment, Allergies, Cardiovascular Function, Oxidative Stress, Breast Cancer, Obesity, and Metabolic Disorders (Health Canada, 2018a) *Screening Assessment – Phthalate Substance Grouping* (Health Canada, 2020)				- No formal systematic review protocol employed to identify or evaluate experimental animal toxicology studies. - Details regarding Health Canada's strategy for identifying new information and literature are provided in Section 1 of (EC/HC, 2015) and (Health Canada, 2020) - Human epidemiologic studies evaluated using Downs and Black Method (Health Canada, 2018a, b)
ECHA	*Committee for Risk Assessment RAC Opinion Proposing Harmonised Classification and Labelling at EU Level of Dicyclohexyl Phthalate, EC Number: 201-545-9, CAS Number: 84-61-7* (ECHA, 2014)	Yes	Yes	No	- Proposed classification was subject to a 60-day consultation in which interested parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions - Proposed classification was reviewed by ECHA's Committee for Risk Assessment (RAC)
NICNAS	*Phthalates Hazard Compendium: A Summary of Physicochemical And Human Health Hazard Data for 24 Ortho-Phthalate Chemicals* (NICNAS, 2008) *C4-6 Side Chain Transitional Phthalates: Human Health Tier II Assessment* (NICNAS, 2016)	Unknown	Unknown	Unknown	- No indication is provided that either assessment by NICNAS was subject to external peer-review, public consultation, or employed a systematic review protocol.

Appendix B SUMMARIES OF DEVELOPMENTAL AND REPRODUCTIVE STUDIES OF DCHP

This Appendix contains more detailed information on the available studies described in the developmental and reproductive toxicity hazard identification (Section 3.1), including information on individual study design and data tables.

In a two-generation reproductive toxicity study that adhered to OECD Test Guideline 416 (Hoshino et al., 2005), SD rats were administered DCHP through diet at 0, 240, 1,200, or 6,000 ppm (equivalent to a mean achieved intake over both generations of 0/0, 17/21, 85/106, and 430/523 mg/kg-day in males/females, respectively; Table_Apx B-1) continuously for two generations. Body weight gain decreased in both parental generations (F0 and F1) in both sexes. Additionally, slight decreases in food consumption occurred at 6,000 ppm in F0 females and at 1,200 and 6,000 ppm in F1 males. Offspring body weights were decreased at 6,000 ppm in the F1 males and females throughout the lactation period (PND 0 through PND 21) and in the F2 males and females at the end of lactation period (PND 21).

Treatment with DCHP had no effect on time to copulation, mating index, fertility index, gestation length, gestation index, birth index, total number of offspring at birth, number of offspring born alive, or sex ratio for either generation. Similarly, treatment with DCHP had no significant effect on serum hormone levels at PND 21 in F0 and F1 adult male (*i.e.*, serum testosterone, follicle stimulating hormone, luteinizing hormone) and female rats (*i.e.*, serum estradiol, follicle stimulating hormone, luteinizing hormone). No effect on F1 or F2 offspring viability was observed on PND 0, 4, or 21, nor did treatment with DCHP have any significant effects on any developmental landmarks in F1 or F2 offspring (*i.e.*, age of incisor eruption and eye opening, age at preputial separation, age at vaginal opening).

Histopathologic examination was conducted on liver, thyroid, and testes of F0 and F1 adult rats. Thyroid follicular cell hyperplasia was noted in the F0 males at 1,200 ppm and in both sexes of both generations at 6,000 ppm (Table_Apx B-2). Diffuse hepatocellular hypertrophy was observed in the F0 males and females at 1,200 ppm and in both sexes of both generations at 6,000 ppm. Furthermore, at 6,000 ppm, absolute and relative (to body weight) thyroid and liver weights were increased in the F0 males and females, and absolute and relative prostate weights were decreased in the F1 males. Several other statistically significant differences were noted in organ weights but were attributed to decreased body weight and/or were not corroborated by other findings indicative of an effect of treatment (*e.g.*, histopathology).

In the F1 males, sperm count was decreased by 15 percent at 1,200 ppm and 24 percent at 6,000 ppm when compared to controls; incidences of seminiferous tubule atrophy were increased at 1200 ppm (2/20) and 6,000 ppm (9/22) compared to controls (1/20). Absolute and adjusted (for body weight) anogenital distance was reduced 7 to 9 percent in the F2 males at 1,200 ppm and in F1 and F2 males at 6,000 ppm on PND 4. Males with nipple development (presence of areole mammae with no nipple) were noted at 1,200 ppm in the F2 males (18.4 percent of litters) and at 6,000 ppm in the F1 (16.1 percent of litters) and F2 (63.2 percent litters) generations (Table_Apx B-3).

Table_Apx B-1. Achieved Doses (mg/kg-day) in Two-Generation Study by Hoshino et al. (2005)

Group	Dose Group (ppm)			
	0	**240**	**1,200**	**6,000**
F0 Males Achieved Dose (mg/kg-day)	0	16	80	402
F1 Males Achieved Dose (mg/kg-day)	0	18	90	457
Average Male Achieved Dose (mg/kg-day)	**0**	**17**	**85**	**430**
F0 Females Achieved Dose (mg/kg-day)	0	21	104	511
F1 Females Achieved Dose (mg/kg-day)	0	21	107	534
Average Female Achieved Dose (mg/kg-day)	**0**	**21**	**106**	**523**

Table_Apx B-2. Histopathological Findings for F0/F1 Adults in Two-Generation Study by Hoshino et al. (2005) [a]

Organ/Histopathologic Finding	F0/F1 Adults	Dose Group (ppm)			
		0	**240**	**1,200**	**6,000**
Liver Diffuse Hepatocellular Hypertrophy (Slight)	F0 Adult Males	0/24[b]	0/24	4/24	16/24
	F0 Adult Females	0/24	0/24	3/24	12/24
	F1 Adult Males	0/20	0/23	0/20	14/22
	F1 Adult Females	0/20	0/23	0/20	9/22
Thyroid Follicular Cell Hypertrophy (Slight)	F0 Adult Males	0/24	0/24	3/24	7/24
	F0 Adult Females	0/24	0/24	0/24	6/24
	F1 Adult Males	0/20	0/23	0/20	7/22
	F1 Adult Females	0/20	0/23	0/20	6/22
Testis – Diffuse Seminiferous Tubule Atrophy (Severe)	F1 Adult Males	0/20	0/23	0/20	3/22
Testis – Focal Seminiferous Tubule Atrophy (Slight)	F1 Adult Males	1/20	0/23	2/20	6/22
Testis – Focal Seminiferous Tubule Atrophy (total)	F1 Adult Males	1/20	0/23	2/20	9/22

[a] Incidence data not evaluated for statistical significance by study authors.
[b] Indicates number of animals affected / number of animals examined.

Table_Apx B-3. Sperm Count, Anogential Distance, and Nipple Development in Males by Hoshino et al. (2005)

Outcome	Dose Group (ppm)			
	0	240	1,200	6,000
Testicular Spermatid count ($\times 10^6$/g) – F0 adult males	110.6 ± 9.02	–	–	104.8 ± 9.34
Testicular Spermatid count ($\times 10^6$/g) – F1 adult males	104.0 ± 12.66	93.4 ± 10.27	88.6 ± 10.32 (↓15%)*	79.2 ± 30.29 (↓24%)**
Anogenital distance (AGD) in F1 and F2 male offspring on PND 4				
F1 AGD (mm)	4.683 ± 0.522	4.860 ± 0.491	4.757 ± 0.448	4.373 ± 0.354** (↓7%)
F1 AGD/$BW^{1/3}$	2.171 ± 0.216	2.162 ± 0.213	2.107 ± 0.148	2.003 ± 0.151* (↓8%)
F2 AGD (mm)	4.618 ± 0.314	4.494 ± 0.300	4.281 ± 0.365** (↓7%)	4.191 ± 0.387** (↓9%)
F2 AGD/$BW^{1/3}$	2.072 ± 0.152	2.020 ± 0.152	1.932 ± 0.158** (↓7%)	1.882 ± 0.129** (↓9%)
Nipple development – %litters with males with presence of areole mammae with no nipple on PND 12 or PND 14				
F1 male offspring	0	0	0	16.1**
F2 male offspring	0	0	18.4	63.2**

* Indicates significantly different from the control value as reported by original study authors (* indicates $p < 0.05$; ** indicates $p < 0.001$).

Furr et al. (2014) conducted an *in vivo* fetal phthalate screen in which groups of pregnant Harlan SD rats were administered DCHP in corn oil via gavage from GD 14 through 18. Dams were sacrificed, and *ex vivo* fetal testicular testosterone production was measured by radioimmunoassay on GD 18. In dams that were dosed with 0, 100, 300, 600, and 900 mg/kg/day (Block 23; n = 2–3 per treatment), *ex vivo* fetal testosterone production decreased by 45 to 80 percent ($p < 0.01$) in male fetuses from all treated groups (≥100 mg/kg/day). A NOAEL was not established (Table_Apx B-4). In a subsequent experiment (Block 33; n = 3–4 per treatment), dams were dosed with 0, 33, 100, and 300 mg/kg-day DCHP, resulting in dose-dependently decreased *ex vivo* fetal testicular testosterone production in all treated groups, with decreases of 25 to 69 percent attaining significance ($p < 0.01$) at 100 mg/kg-day (55 percent decrease) and 300 mg/kg/day (69 percent decrease). Although the decrease at 33 mg/kg-day was not statistically significant, the decrease was considered treatment-related due to its magnitude (25% decrease) and dose-dependency; furthermore, the lack of statistical significance was attributed to the limited statistical power associated with the small sample size (n = 4). Therefore, EPA identified a LOAEL of 33 mg/kg-day for this study based on decreased fetal testosterone, and a NOAEL was not established.

Gray et. al (2021) conducted *in vivo* studies with a design similar to the previously described fetal phthalate screen by Furr et al. (2014). Groups of pregnant rats were administered DCHP at 0, 33 (Harlan rats only), 100, 300, 600, and 900 mg/kg/day in corn oil via gavage from GD 14 through 18. Dams were sacrificed, and *ex vivo* fetal testicular testosterone production was measured by radioimmunoassay on GD 18. In addition to fetal testosterone production, mRNA in the fetal rat testis was measured for a custom panel of 89 genes associated with sex determination, steroid and peptide hormone synthesis and transport, and PPAR activation using targeted RT-qPCR 96-well gene arrays. *Ex vivo* fetal testicular testosterone production in fetal testes was decreased 41 to 88 percent when compared to control across all doses (Table_Apx B-4). Additionally, mRNA expression significantly decreased ($p < 0.01$ compared to control) for key genes in the adverse outcome pathway for phthalate syndrome. This began at 100

mg/kg/day for the majority of the 14 genes reported in both rat strains. Exceptions included Nr0b1 (attained significance beginning at 600 mg/kg/day in Charles River SD rats), Cyp11a1, and Dhcr7 (attained significance beginning at 300 mg/kg/day in Charles River SD rats). Additionally, Rhbox 10 and Wnt7a did not change significantly in either strain and Cyp11b2 did not change in Harlan SD rats.

Table_Apx B-4. *Ex vivo* Fetal Testicular Testosterone Production in Male Fetuses in Furr et al. (2014) and Gray et al. (2021)

Reference	Mean, SEM, N	0 mg/kg-day	33 mg/kg-day	100 mg/kg-day	300 mg/kg-day	600 mg/kg-day	900 mg/kg-day
Furr (2014) (Block 23)	Mean ± SEM (% decrease)	9.87 ± 0.58	–	3.1 ± 0.40** (↓69%)	2.2 ± 0.43** (↓78%)	2 ± 0.31** (↓80%)	5.39 ± 0.80** (↓45%)
	N	3	–	3	2	3	3
Furr (2014) (Block 33)	Mean ± SEM (% decrease)	13.25 ± 1.57	9.89 ± 1.15 (↓25%)	5.92 ± 1.66** (↓55%)	4.10 ± 0.46** (↓69%)	–	–
	N	4	4	4	3	–	–
Gray (2021) (Block 148)	Mean ± SEM (% decrease)	9.43 ± 1.07	–	5.59 ± 0.71 (↓41%)	2.65 ± 0.26 (↓72%)	1.62 ± 0.33 (↓83%)	1.09 ± 0.39 (↓88%)
	N	3	–	3	3	3	3

In an extended developmental toxicity study by Yamasaki et al. (2009), pregnant CD (SD) IGS rats (n = 10 per treatment) were administered DCHP at 0, 20, 100, or 500 mg/kg-day by oral gavage from GD 6 through PND 20. AGD was measured at PND 4, and offspring were examined for retention of thoracic and abdominal nipples at PND 13. Dams were sacrificed the day after weaning on PND 21. Among the surviving offspring, two rats/sex/dam/dose group were randomly selected and mated (avoiding sibling matings) at 12 weeks of age and subjected to cesarean section examination on GD 13. The remaining offspring were terminated at 10 weeks of age and examined for changes in organ weights and abnormalities in reproductive organs and tissues. At 500 mg/kg-day, one dam died after showing signs of dystocia. Offspring viability index at PND 4 was slightly but significantly ($p < 0.05$) decreased at 500 mg/kg-day (97.8%) compared to controls (100%). The authors reported that offspring body weights were significantly decreased at this dose in both sexes on PND 14 and/or 21. Additionally at 500 mg/kg-day, the following treatment-related effects were observed in male offspring, indicating androgen insufficiency: hypospadias in two males, one sacrificed at 7 weeks of age in poor general condition; delayed preputial separation (45.6 vs. 43.5 days in controls); decreased absolute AGD (3.59 mm vs. 4.23 mm in controls) and decreased AGD adjusted for body weight (1.66 vs. 1.90 mm in controls); higher mean number of rats with areola/nipple retention (2.7 vs. 0 in controls), along with higher incidence of areola/nipple retention (67.6% vs. 0% in controls); decreased weights of the ventral prostate (28% decrease) and LABC muscle (12% decrease); and decreased testicular germ cells in "some male rats in this group (no data shown)." No treatment-related effects were observed at 100 mg/kg-day. No treatment-related effects were observed at any dose for reproductive parameters examined in the offspring mated and terminated at cesarean section. EPA identified a NOAEL of 100 mg/kg-day based on the developmental effects observed at 500 mg/kg-day.

In a developmental toxicity study by Saillenfait et al., (2009), pregnant SD rats (n = 22–25 per treatment) were administered DCHP via gavage at 0, 250, 500, or 750 mg/kg-day on GDs 6 through 20.

Dams were sacrificed on GD 21. AGD in male fetuses was dose-dependently decreased by 8 to 17 percent ($p < 0.01$) at 250, 500, and 750 mg/kg-day compared to controls. Additionally, at 750 mg/kg-day, fetal body weights were decreased compared to controls. Maternal effects included dose-dependent increases of 75 to 108 percent ($p < 0.01$) in hepatic palmitoyl CoA oxidase activity in all dose groups; increased absolute and relative liver weights at 500 and 750 mg/kg-day; increased ($p < 0.01$) serum ALT and AST at 750 mg/kg-day; and decreased body weight gain and food consumption at 750 mg/kg-day. Body weight gain and food consumption were also decreased at 500 mg/kg-day; however, these decreases only occurred at the initiation of treatment (GD 6–9), did not impact overall body weight gain, and were therefore considered transient and not adverse. EPA identified a LOAEL of 250 mg/kg-day (no NOAEL identified) based on the treatment-related decrease in male AGD observed in all dose groups.

In a developmental toxicity study by Ahbab et al. (2013), pregnant albino Wistar rats (n = 10 per treatment) were administered DCHP in corn oil by gavage at 0, 20, 100, and 500 mg/kg-day from GD 6 through GD 19. Dams were allowed to deliver naturally. Male offspring were examined at pre-pubertal (PND 20), pubertal (PND 32), and adult (PND 90) stages for hormone and enzyme levels (testosterone, estradiol, FSH, LH, inhibin B, and MIS/AMH), histopathology of reproductive organs (testes, epididymis, prostate, and seminal vesicles), immunohistochemical examination of testes (3β-HSD and MIS/AMH), sperm count and morphology, and morphometric measurements of seminiferous tubules and epididymis. No dose-related differences were noted in weights of the testes, epididymis, or prostate/seminal vesicles in the males at PND 22. Absolute and relative testes weights were decreased at 100 and 500 mg/kg-day on PND 32. At 500 mg/kg-day, absolute and relative prostate weights were increased on PND 32, and absolute epididymis and prostate weights were increased on PND 90. Several significant differences from controls were noted in testosterone and MIS levels at different stages; however, these differences were unrelated to dose. Inhibin B was decreased in the 100 and 500 mg/kg-day males at PND 90. The percentage of abnormal sperm was higher in all dose groups (23 to 27% abnormal sperm) compared to controls (11 percent abnormal sperm) at PND 90, and the abnormalities were evident across the spermatozoan (*e.g.*, head, neck, and tail defects). Histopathology examination indicated findings (*e.g.,* tubular atrophy) in the testes, epididymis, and prostate in all treated groups compared to no incidences in controls (see Table_Apx B-5). Seminiferous tubule diameter was smaller ($p < 0.05$) in all treated groups compared to controls.

Table_Apx B-5. Histopathology Data in Testes, Epididymis, and Prostate by Ahbab et al. (2013)

Tissue/Histopathologic Finding	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
Testes – prepubertal (n)	10	10	10	10
Tubular atrophy	0	6*	5*	8*
Germinal cell debris	0	3	6*	9*
Picnotic cells	0	4*	7*	9*
Increase in apoptotic cells	0	3	7*	10*
Atrophic and damaged tubules	0	5*	7*	9*
Tubules without lumen	0	0	2	4*
Testes – pubertal (n)	10	8	10	10
Tubular atrophy	0	3	8*	10*
Germinal cell debris	0	3	10*	10*
Edema	0	3	8*	6*

Tissue/Histopathologic Finding	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
Increase in apoptotic cells	0	2	6*	10*
Atrophic and damaged tubules	0	3	6*	10*
Attached seminiferous tubules	0	0	7*	4*
Testes – adult (n)	10	10	10	10
Sertoli cell vacuolization	0	6*	4*	8*
Picnotic cells	0	2	0	5*
Attached seminiferous tubules	0	10*	10*	10*
Epididymis – prepubertal (n)	10	10	10	10
Spermatogenic cells in lumen	0	4*	6*	8*
Atrophic tubules	0	3	6*	9*
Decreased lumen size	0	0	3	4*
Epididymis – pubertal (n)	10	8	10	10
Spermatogenic cells in lumen	0	8*	10*	10*
Atrophic tubules	0	8*	7*	7*
Decreased lumen size	0	1	4*	5*
Epididymis – adults (n)	10	10	10	10
Spermatogenic cells in lumen	0	2	5*	8*
Atrophic tubules	0	6*	4*	6*
Decreased lumen size	0	4*	0	4*
Decreased sperm number in lumen	0	4*	7*	10*
Tubules without sperm	0	2	5*	4*
Prostate gland – prepubertal (n)	10	10	10	10
Atrophic tubules	0	7*	9*	5*
Prostatic intraepithelial neoplasia	0	7*	9*	5*
Prostate gland – pubertal (n)	10	8	10	10
Atrophic tubules	0	5*	10*	10*
Prostatic intraepithelial neoplasia	0	3	10*	10*
Prostate gland – adult (n)	10	10	10	10
Atrophic tubules	0	5*	8*	10*
Prostatic intraepithelial neoplasia	0	5*	8*	8*
* Statistically different from control (vehicle) group, $P < 0.05$ (Fisher's exact test), as calculated by original study authors.				

In a second developmental toxicity study by Ahbab et al. (2015), pregnant albino Wistar rats (n = 10 per treatment group) were administered DCHP in corn oil by gavage at 0, 20, 100, and 500 mg/kg-day from GD 6 through 19. Dams were terminated on GD 20, and fetuses were removed by cesarean section, terminated by decapitation, and male fetal trunk blood was collected for plasma hormone analyses (testosterone and FSH). Fetal testes were collected for immunohistochemical staining for 3β-HSD,

MIS/AMH, AR, and PCNA. A higher number of dams had litters with resorptions at 20 mg/kg-day (8/10 litters), 100 mg/kg-day (9/10 litters), and 500 mg/kg-day (10/10 litters) compared to controls (2/10 litters), and the percentage of the litters with resorptions was higher in the treated groups (26 to 33 percent) compared to controls (3 percent) (Table_Apx B-6). Absolute and adjusted (for body weight) anogenital distance was shorter ($p < 0.05$) in the male fetuses in all treated groups compared to controls. Additionally in all treated groups, MIS was significantly ($p < 0.05$) decreased, and inhibin B was significantly ($p < 0.05$) increased, but FSH/inhibin B was significantly ($p < 0.05$) decreased. The authors stated that MIS/AMH is responsible for regression of the mullerian ducts, and the decreased fetal levels of this hormone suggest that Sertoli cell function is disrupted, although there were no mullerian duct remnants in the DCHP-treated male fetuses in this study. Fetal blood testosterone levels were dose-dependently decreased in all treated groups, with decreases of 12 to 42 percent attaining significance ($p < 0.05$) at 100 mg/kg-day (39 percent decrease) and 500 mg/kg/day (42 percent decrease). The number of Leydig cell clusters per surface area of testes was significantly ($p < 0.05$) decreased in all treated groups compared to controls. The percent of Leydig cell clusters that were small was dose-dependently decreased ($p < 0.05$) in all treated groups, and the percent of Leydig cell clusters that were medium or large were each dose-dependently increased in all treated groups compared to controls. Immunohistochemistry analyses of the testes showed significantly ($p < 0.05$) decreased expression of: 3β-HSD and AR in all treated groups; MIS/AMH at 100 and 500 mg/kg-day; and PCNA at 500 mg/kg-day. The authors reported that 3β-HSD is an enzyme responsible for testosterone production and is highly specific for Leydig cells in the testis and that the decreased expression of 3β-HSD corresponded with decreased testosterone levels in male fetuses in this study. Histopathologic findings were observed in the testes at 20 mg/kg-day DCHP above. Observed findings included atrophic and small seminiferous tubules, decreased germ cells in tubules, Sertoli cell only tubules, detached cells from tubular wall, and MNGs (Table_Apx B-6).

Table_Apx B-6. Histopathology Data in Testes and Resorption Data by Ahbab et al. (2015)

Ahbab (2015) – fetal testes histopath (n = 10)	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
Atrophic and small seminiferous tubules	0	8***	10***	10***
Decreased germ cells in tubules	0	5*	7**	8***
Sertoli cell only tubules	0	3	5*	7**
Detached cells from tubular wall	0	6*	8***	10***
MNG	0	2	5*	9***
Resorptions				
number of dams	2/10	8/10*	9/10*	10/10*
percent of litter	3 ± 2.2	33 ± 7.6*	31 ± 7.1*	26 ± 5.1*

* Significantly different from control (vehicle) group ($P < 0.05$) as calculated by original study authors.
** Significantly different from control (vehicle) group ($P < 0.01$) as calculated by original study authors.
*** Significantly different from control (vehicle) group ($P < 0.001$) as calculated by original study authors.

In a subsequent publication, Ahbab et al. (2017) reported additional data from their 2015 study (Ahbab and Barlas, 2015), including examination of hematology, placentae (one per litter), AGD in female fetuses, and skeletal examination of both the male and female fetuses (Table_Apx B-7). Absolute and adjusted (for body weight) anogenital distance was generally decreased ($p < 0.05$) in all treated groups for female fetuses; however, these decreases were not dose dependent. The authors presented images of skeletal staining of the fetuses with Alizarin red (for bone)/Alcian blue (for cartilage) and qualitatively reported wavy ribs and unossified skull bones and scapulae in the treated groups; however, fetal and litter incidences were not reported. The relative integrated density of fetal skeletal staining with Alizarin red was significantly lower in the treated groups compared to controls, indicating delayed ossification of the skeleton in the treated fetuses. Bone biometric analyses corroborated these findings, with decreased absolute and relative length of the skull in the male fetuses from all treated groups and the scapulae at 100 and 500 mg/kg-day. Additional decreases ($p < 0.05$) in relative (to body) length of the humerus and ulna in males from all treated groups and in the radius, femur, fibula, and tibia at 500 mg/kg-day were observed but may be attributed to the higher ($p < 0.05$) body (crown-rump) length in males from all treated groups compared to controls. Similarly in the female fetuses, decreases were observed in absolute length of the skull and scapulae in all treated groups and more consistently across the skeleton with increasing dose. Body length was increased over controls at 20 and 100 mg/kg-day but was comparable to controls at 500 mg/kg-day; therefore, the decreases in relative length of bones throughout the skeleton at 500 mg/kg-day can be attributed to treatment and were unrelated to larger body length.

Hematology analysis generally indicated increases in leukocytes (specifically increased lymphocytes and monocytes but decreased neutrophil granulocytes) and decreases in MCH, MCHC, and hemoglobin in the DCHP-treated animals. The authors attributed these differences in hematology parameters to decreased bone marrow development associated with skeletal retardation and anemia.

Placental measurements indicated significantly ($p < 0.05$) decreased diameter along the x-axis, increased diameter along the y-axis, increased placental weight and placental index, and decreased placental thickness generally in all treated groups compared to controls. Placental histopathology indicated significantly ($p < 0.05$) increased incidences of microscopic lesions in all treated groups compared to controls. Of these, the most sensitive changes occurred in the spongiotrophoblast (hemorrhage, decreased and irregular vessel formation) and the basal zone (hemorrhage, edema) at 20 mg/kg-day, with polymorphisms in the nucleus and degeneration in the cytoplasm in trophoblastic giant cells observed at 500 mg/kg-day. Relative integrated immunodensities of proliferating cell nuclear antigen (PCNA), peroxisome proliferator-activated receptor (PPAR)γ, estrogen receptor (ER)α, ERβ, and androgen receptor (AR) were significantly ($p < 0.05$) lower than controls in all treated groups, although these decreases were only dose-dependent for AR.

Table_Apx B-7. Anogenital Distance and Placenta Histopathology by Ahbab et al. (2017)

Parameter	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
AGD (mm) – female fetuses	2.14 ± 0.02	1.18 ± 0.12* (↓45%)	1.26 ± 0.11* (↓41%)	1.63 ± 0.06* (↓24%)
AGD/BW – female fetuses	0.42 ± 0.01	0.22 ± 0.03* (↓48%)	0.26 ± 0.02* (↓38%)	0.44 ± 0.03
AGD/BW$^{1/3}$ – female fetuses	1.25 ± 0.01	0.71 ± 0.08* (↓43%)	0.74 ± 0.06* (↓41%)	1.05 ± 0.05* (↓16%)
Number of placentae examined (litters)	105 (10)	58 (10)	65 (10)	72 (10)
Diameter – x-axis (mm)	13.9 ± 0.1	12.8 ± 0.2* (↓8%)	11.4 ± 0.3* (↓18%)	12.1 ± 0.2* (↓13%)
Diameter – y-axis (mm)	13.8 ± 0.3	15.5 ± 0.3* (↑12%)	13.9 ± 0.2	14.6 ± 0.2* (↑6%)

Parameter	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
Thickness of placenta (mm)	4.0 ± 0.1	3.6 ± 0.2*(↓10%)	3.9 ± 0.5	3.5 ± 0.1* (↓13%)
Weight of placenta (g)	0.46 ± 0.01	0.64 ± 0.04* (↑39%)	0.53 ± 0.02* (↑15%)	0.59 ± 0.02* (↑28%)
Placental index	0.28 ± 0.01	0.36 ± 0.02* (↑29%)	0.32 ± 0.01* (↑14%)	0.37 ± 0.01* (↑32%)
Placental – histopathology (n)	10	10	10	10
Trophoblastic giant cells				
Polymorphism in nucleus	0	3	1	9***
Degeneration in cytoplasm	0	2	1	5*
Spongiotrophoblast				
Degeneration	0	3	7**	9***
Hemorrhage	0	5*	4	10***
Decreased & irregular vessel formation	0	8***	9***	10***
Basal zone				
Hemorrhage	2	5	10***	10***
Edema	0	8***	10***	10***
Significantly different from controls at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***) as calculated by original study authors.				

In a developmental toxicity study by Li et al. (2016), pregnant SD rats (n = 6 per treatment) were administered DCHP in corn oil by gavage at 0, 10, 100, and 500 mg/kg-day from GD 12 through 21. The authors stated that this exposure window was selected because it corresponds to fetal Leydig cells emergence at GD 12 through expected parturition. Dams were allowed to deliver naturally on GD 21.5 (PND 1), and pups were terminated by asphyxiation with CO_2 on PND1 after measuring male pup body weight length, weight, and AGD. Randomly selected fetal testes (≥1 per litter; 6/dose group) were removed, frozen in liquid nitrogen, and stored at −80°C for analysis of cell distribution, Leydig cell-specific mRNA levels, and testicular testosterone; other testes were fixed in Bouin's solution for histochemical staining and examined for testes dysgenesis, Leydig cell morphological changes, and semiquantitative analysis of Leydig cell specific protein levels. In all treated groups (≥10 mg/kg-day), male pup body weights were 16 to 17 percent lower ($p < 0.001$) than controls on PND 1; however, this effect was not dose-related and is inconsistent with other studies, which do not observe decreases in fetal bodyweight until doses of 85 to 750 mg/kg-day (Saillenfait et al., 2009; Yamasaki et al., 2009; Hoshino et al., 2005) (Table_Apx B-8). The following dose-dependent differences were observed in all treated groups, attaining statistical significance ($p < 0.05$) at 100 and 500 mg/kg-day: decreased absolute AGD in male pups on PND 1; increased number MNGs per seminiferous tubule; and decreased testicular testosterone (Table_Apx B-8). Leydig cell aggregation (as measured by the mean number of fetal Leydig cells per cluster) dose-dependently increased ($p < 0.001$) in all treated groups (≥10 mg/kg-day), and decreases ($p < 0.001$) were noted in the size of the fetal Leydig cells, cytoplasm, nucleus, and cytoplasm/nucleus ratio at all doses. Real-time PCR analysis of mRNA levels in testes on PND 1 indicated decreases ($p < 0.05$) in *Star*, *Hsd3b1*, and *Hsd17b3* at ≥10 mg/kg-day; dose-dependent decreases in *Insl3*, attaining statistical significance at 100 and 500 mg/kg-day; and dose-dependent decreases in *Scarb1*, attaining significance at 500 mg/kg-day. Semiquantitative analysis of immunohistochemical staining of INSL3 or HSD3B1 showed reduced ($p < 0.05$) protein expression in all treated groups, confirming the decreased mRNA levels of these genes.

Table_Apx B-8. Developmental and Reproductive Toxicity Data by Li et al. (2016)

Parameter	0 mg/kg-day	10 mg/kg-day	100 mg/kg-day	500 mg/kg-day
Body weight – male pups PND 1	7.7 ± 0.7	6.5 ± 0.4*** (↓16%)	6.4 ± 0.9*** (↓17%)	6.5 ± 0.7*** (↓16%)
AGD (mm) – male pups PND 1	3.3 ± 0.3	3.0 ± 0.5 (↓9%)	2.7 ± 0.2* (↓18%)	2.6 ± 0.2* (↓21%)
Testes dysgenesis	0/6	0/6	1/6	3/6
MNGs#/Tubule (%)	0.37 ± 0.24	2.08 ± 0.46	15.67 ± 2.70***	27.06 ± 2.90***
Testicular testosterone (ng/mg)	1.90 ± 0.25	1.71 ± 0.35 (↓10%)	1.18 ± 0.23* (↓38%)	0.62 ± 0.14** (↓67%)
Mean number fetal Leydig cells per cluster	3 ± 0	5 ± 1***	11 ± 3***	13 ± 2***
Significantly different from controls at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***), as calculated by original study authors.				

Lv et al. (2019) studied the effects of DCHP on Leydig cell regeneration. Adult male Sprague-Dawley rats (n = 6 per treatment) were given an intraperitoneal injection of ethane dimethane sulfone (EDS) to eliminate all Leydig cells in the testes and were then administered DCHP in corn oil daily via oral gavage at 1, 10, 100, or 1,000 mg/kg-day DCHP from post-EDS day 7 to day 21 or 28, at which point the animals were terminated. Blood was collected to measure serum testosterone, LH, and FSH. Leydig cell number, size, and gene and protein expression were also measured. There were no effects of treatment on body weight or weights of the testes or epididymis. At 21 days post-EDS, serum testosterone levels were significantly ($p < 0.05$) increased at 10 and 100 mg/kg-day but significantly decreased ($p < 0.05$) at 1,000 mg/kg-day compared to controls. Additionally, the authors presented images of immunohistochemical staining and graphical data on Leydig cell number (CYP11 A1-positive), size (cells, cytoplasm, and nucleus), and labeling index that showed that at post-EDS day 21, Leydig cell number and labeling index were increased ($p < 0.05$) at 10 and 100 mg/kg-day, indicating regeneration and corroborating the increased testosterone levels at these lower doses. By 28 days post-EDS, (1) testosterone levels were comparable to controls at 10 and 100 mg/kg-day but remained decreased ($p < 0.05$) at 1,000 mg/kg-day; and (2) Leydig cell size and cytoplasm size were decreased ($p < 0.05$) at 100 and 1,000 mg/kg-day, along with decreased ($p < 0.05$) cell number (stained for CYP11 A1 and HSD11B1) and labeling index at 1,000 mg/kg-day. Serum FSH was lower ($p < 0.05$) than controls at 1000 mg/kg-day at both time points. Gene and protein expression corroborated the Leydig cell regeneration at 10 and 100 mg/kg-day at post-EDS day 21, with mRNA levels of *Lhcgr*, *Scarb1*, *Star*, *Cyp11a1*, *Hsd3b1*, *Cyp17a1*, *Hsd17b3*, *Hsd11b1*, and *Insl3* generally decreased at all dose levels at post-EDS day 28, but either comparable to controls or only decreased at 1,000 mg/kg-day at post-EDS day 21.

Appendix C FETAL TESTICULAR TESTOSTERONE AS AN ACUTE EFFECT

No studies of experimental animal models are available that investigate the antiandrogenic effects of DCHP following single dose, acute exposures. However, there are studies of dibutyl phthalate (DBP) available that indicate a single acute exposure during the critical window of development (*i.e.*, GD14–19) can reduce fetal testicular testosterone production and disrupt testicular steroidogenic gene expression. Two studies were identified that demonstrate single doses of 500 mg/kg DBP can reduce fetal testicular testosterone and steroidogenic gene expression. Johnson et al. (2012; 2011) gavaged pregnant SD rats with a single dose of 500 mg/kg DBP on GD 19 and observed reductions in steroidogenic gene expression in the fetal testes three (*Cyp17a1*) to six (*Cyp11a1, StAR*) hours post-exposure, while fetal testicular testosterone was reduced starting 18 hours post-exposure. Similarly, Thompson et al. (2005) reported a 50 percent reduction in fetal testicular testosterone 1 hour after pregnant SD rats were gavaged with a single dose of 500 mg/kg DBP on GD 19, while changes in steroidogenic gene expression occurred 3 (*StAR*) to 6 (*Cyp11a1, Cyp17a1, Scarb1*) hours post-exposure, and protein levels of these genes were reduced 6 to 12 hours post-exposure. Additionally, studies by Carruthers et al. (2005) further demonstrate that exposure to as few as two oral doses of 500 mg/kg DBP on successive days between GDs 15 to 20 can reduce male pup AGD, cause permanent nipple retention, and increase the frequency of reproductive tract malformations and testicular pathology in adult rats that received two doses of DBP during the critical window.

In summary, studies of DBP provide evidence to support use of effects on fetal testosterone and the developing male reproductive system consistent with phthalate syndrome as an acute effect. However, the database is limited to just a few studies of DBP that test relatively high (500 mg/kg) single doses of DBP. Although there are no single dose studies of DCHP that evaluate antiandrogenic effects on the developing male reproductive system, there are two studies that have evaluated effects on fetal testicular testosterone production and steroidogenic gene expression following daily gavage doses of 33 to 900 mg/kg-day DCHP on GDs 14 to 18 (5 total doses) (Gray et al., 2021; Furr et al., 2014). Across available studies, statistically significant reductions in fetal testicular testosterone production are consistently observed at the lowest doses tested in each study.

Appendix D CALCULATING DAILY ORAL HUMAN EQUIVALENT DOSES AND HUMAN EQUIVALENT CONCENTRATIONS

For DCHP, all data considered for PODs are obtained from oral animal toxicity studies in rats. Because toxicity values for DCHP are from oral animal studies, EPA must use an extrapolation method to estimate HEDs. The preferred method would be to use chemical-specific information for such an extrapolation. However, no PBPK models or chemical-specific information was identified for DCHP to support a quantitative extrapolation. In the absence of such data, EPA relied on the guidance from U.S. EPA (2011c), which recommends scaling allometrically across species using the three-quarter power of body weight ($BW^{3/4}$) for oral data. Allometric scaling accounts for differences in physiological and biochemical processes, mostly related to kinetics.

For application of allometric scaling in risk evaluations, EPA uses dosimetric adjustment factors (DAFs), which can be calculated using Equation_Apx D-1.

Equation_Apx D-1. Dosimetric Adjustment Factor

$$DAF = \left(\frac{BW_A}{BW_H}\right)^{1/4}$$

Where:

DAF	=	Dosimetric adjustment factor (unitless)
BW_A	=	Body weight of species used in toxicity study (kg)
BW_H	=	Body weight of adult human (kg)

U.S. EPA (2011c), presents DAFs for extrapolation to humans from several species. However, because those DAFs used a human body weight of 70 kg, the Agency has updated the DAFs using a human body weight of 80 kg for the DCHP risk evaluation (U.S. EPA, 2011a). EPA used a bodyweight of 0.25 kg for rats, as presented in U.S. EPA (2011c). The resulting DAF for rats is 0.236.

Use of allometric scaling for oral animal toxicity data to account for differences among species allows EPA to decrease the default intraspecies UF (UF_A) used to set the benchmark MOE; the default value of 10 can be decreased to 3, which accounts for any toxicodynamic differences that are not covered by use of $BW^{3/4}$. Using the appropriate DAF from Equation_Apx D-1, EPA adjusts the POD to obtain the HED using Equation_Apx D-2:

Equation_Apx D-2. Daily Oral Human Equivalent Dose

$$HED_{Daily} = POD_{Daily} \times DAF$$

Where:

HED_{Daily}	=	Human equivalent dose assuming daily doses (mg/kg-day)
POD_{Daily}	=	Oral POD assuming daily doses (mg/kg-day)
DAF	=	Dosimetric adjustment factor (unitless)

For this risk evaluation, differences in dermal and oral absorption are corrected for in the dermal exposure assessment, allowing the same HED to be used for both oral and dermal routes. EPA assumes

similar absorption for the oral and inhalation routes, and no adjustment was made when extrapolating to the inhalation route. For the inhalation route, the Agency extrapolated the daily oral HEDs to inhalation HECs using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest, as follows:

Equation_Apx D-3. Extrapolating from Oral HED to Inhalation HEC

$$HEC_{Daily,continuous} = HED_{Daily} \times \left(\frac{BW_H}{IR_R * ED_C}\right)$$

Where:

$HEC_{Daily,continuous}$	=	Inhalation HEC based on continuous daily exposure (mg/m^3)
HED_{Daily}	=	Oral HED based on daily exposure (mg/kg-day)
BW_H	=	Body weight of adult humans (kg) = 80
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

Based on information from U.S. EPA (2011a), EPA assumes an at rest breathing rate of 0.6125 m^3/h. Adjustments for different breathing rates required for individual exposure scenarios were made in the exposure calculations, as needed.

It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in studies and the default units for different OPPT models. Therefore, EPA presents all PODs in equivalents of both units to avoid confusion and errors. Equation_Apx D-4 presents the conversion of the HEC from mg/m^3 to ppm.

Equation_Apx D-4. Converting Units for HECs (mg/m3 to ppm)

$$X\ ppm = Y\ \frac{mg}{m^3} \times \frac{24.45}{MW}$$

Where:

24.45	=	Molar volume of a gas at standard temperature and pressure (L/mol), default
MW	=	Molecular weight of the chemical (MW of DCHP = 330.43 g/mol)

D.1 DCHP Non-cancer HED and HEC Calculations for Acute, Intermediate, and Chronic Duration Exposures

The acute, intermediate, and chronic duration non-cancer POD is based on a NOAEL of 10 mg/kg-day; the critical effect is male phthalate syndrome-related effects. This non-cancer POD is considered protective of effects observed following acute, intermediate, and chronic duration exposures to DCHP. EPA used Equation_Apx D-1 to determine a DAF specific to rats (0.236), which was in turn used in the following calculation of the daily HED using Equation_Apx D-2:

$$2.4\ \frac{mg}{kg-day} = 10\frac{mg}{kg-day} \times 0.236$$

EPA then calculated the continuous HEC for an individual at rest using Equation_Apx D-3:

$$13\ \frac{mg}{m^3} = 2.4\ \frac{mg}{kg-day} \times \left(\frac{80\ kg}{0.6125\frac{m^3}{hr} * 24\ hr}\right)$$

Equation_Apx D-4 was used to convert the HEC from mg/m^3 to ppm:

$$0.96\ ppm = 13\ \frac{mg}{m^3}\ \times \frac{24.45}{330.43}$$

Appendix E Considerations for Benchmark Response (BMR) Selection for Reduced Fetal Testicular Testosterone

E.1 Purpose

EPA has conducted an updated meta-analysis and benchmark dose modeling (BMD) analysis of decreased fetal rat testicular testosterone (U.S. EPA, 2025g). During the July 2024 Science Advisory Committee on Chemicals (SACC) peer-review meeting of the draft risk evaluation of diisodecyl phthalate (DIDP) and draft human health hazard assessments for diisononyl phthalate (DINP), the SACC recommended that EPA should clearly state its rationale for selection of benchmark response (BMR) levels evaluated for decreases in fetal testicular testosterone relevant to the single chemical assessments (U.S. EPA, 2024b). This appendix describes EPA's rationale for evaluating BMRs of 5, 10, and 40 percent for decreases in fetal testicular testosterone. (*Note: EPA will assess the relevant BMR for deriving relative potency factors to be used in the cumulative risk assessment separately from this analysis*.)

E.2 Methods

As described in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), "Selecting a BMR(s) involves making judgments about the statistical and biological characteristics of the data set and about the applications for which the resulting BMDs/BMDLs will be used." For the updated meta-analysis and BMD modeling analysis of fetal rat testicular testosterone, EPA evaluated BMR values of 5, 10, and 40 percent based on both statistical and biological considerations (U.S. EPA, 2025g).

In 2017, NASEM (2017) modeled BMRs of 5 and 40 percent for decreases in fetal testicular testosterone. NASEM did not provide explicit justification for selection of a BMR of 5 percent. However, justification for the BMR of 5 can be found elsewhere. As discussed in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a BMR of 5 percent is supported in most developmental and reproductive studies. Comparative analyses of a large database of developmental toxicity studies demonstrated that developmental NOAELs are approximately equal to the $BMDL_5$ (Allen et al., 1994a, b; Faustman et al., 1994).

EPA also evaluated a BMR of 10 percent as part of the updated BMD analysis. BMD modeling of fetal testosterone conducted by NASEM (2017) indicated that BMD_5 estimates are below the lowest dose with empirical testosterone data for several of the phthalates (*e.g.*, DIBP). As discussed in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) "For some data sets the observations may correspond to response levels far in excess of a selected BMR and extrapolation sufficiently below the observable range may be too uncertain to reliably estimate BMDs/BMDLs for the selected BMR." Therefore, EPA modeled a BMR of 10 percent because data sets for some of the phthalates may not include sufficiently low doses to support modeling of a 5 percent response level.

NASEM (2017) also modeled a BMR of 40 percent using the following justification: "previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40% (Gray et al., 2016; Howdeshell et al., 2015)."

Further description of methods and results for the updated meta-analysis and BMD modeling analysis that evaluated BMRs of 5, 10, and 40 percent for decreased fetal testicular testosterone are provided in EPA's *Meta-Analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), and Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025g).

E.3 Results

BMD estimates, as well as 95 percent upper and lower confidence limits, for decreased fetal testicular testosterone for the evaluated BMRs of 5, 10, and 40 percent are shown in Table_Apx E-1. BMD_5 estimates ranged from 8.4 to 74 mg/kg-day for DEHP, DBP, DCHP, and DINP, however, a BMD_5 estimate could not be derived for BBP or DIBP. Similarly, BMD_{10} estimates ranged from 17 to 152 for DEHP, DBP, DCHP, DIBP and DINP, however, a BMD_{10} estimate could not be derived for BBP. BMD_{40} estimates were derived for all phthalates (*i.e.*, DEHP, DBP, DCHP, DIBP, BBP, DINP) and ranged from 90 to 699 mg/kg-day.

In the mode of action (MOA) for phthalate syndrome, which is described elsewhere (U.S. EPA, 2023b) and in Section 3.1.2 of this document, decreased fetal testicular testosterone is an early, upstream event in the MOA that precedes downstream apical outcomes such as male nipple retention, decrease anogenital distance, and reproductive tract malformations. Decreased fetal testicular testosterone should occur at lower or equal doses than downstream apical outcomes associated with a disruption of androgen action. Because the lower 95 percent confidence limit on the BMD, or BMDL, is used for deriving a point of departure (POD), EPA compared BMDL estimates at the 5, 10, and 40 percent response levels for each phthalate (DEHP, DBP, DCHP, DIBP, BBP, DINP) to the lowest identified apical outcomes associated with phthalate syndrome to determine which response level is protective of downstream apical outcomes.

Table_Apx E-1 provides a comparison of BMD and BMDL estimates for decreased fetal testicular testosterone at BMRs of 5, 10, and 40 percent, the lowest LOAEL(s) for apical outcomes associated with phthalate syndrome, and the POD selected for each phthalate for use in risk characterization. As can be seen from Table_Apx E-1, $BMDL_{40}$ values for DEHP, DBP, DIBP, BBP, DCHP, and DINP are all well above the PODs selected for use in risk characterization for each phthalate by 3X (for BBP) to 25.4X (for DEHP). Further, $BMDL_{40}$ values for DEHP, DBP, DIBP, BBP, and DCHP, but not DINP, are above the lowest LOAELs identified for apical outcomes on the developing male reproductive system. These results clearly demonstrate that a BMR of 40 percent is not appropriate for use in human health risk assessment.

As can be seen from Table_Apx E-1, $BMDL_{10}$ values for DBP ($BMDL_{10}$, POD, LOAEL = 20, 9, 30 mg/kg-day, respectively) and DCHP ($BMDL_{10}$, POD, LOAEL = 12, 10, 20 mg/kg-day, respectively) are slightly higher than the PODs selected for use in risk characterization and slightly less than the lowest LOAELs identified based on apical outcomes associated with the developing male reproductive system. This indicates that a BMR of 10% may be protective of apical outcomes evaluated in available studies for both DBP and DCHP. $BMDL_{10}$ values could not be derived for DIBP or BBP (Table_Apx E-1). Therefore, no comparisons to the POD or lowest LOAEL for apical outcomes could be made for either of these phthalates at the 10 percent response level.

For DEHP, the $BMDL_{10}$ is greater than the POD selected for use in risk characterization by 5X ($BMDL_{10}$ and POD = 24 and 4.8 mg/kg-day, respectively) and is greater than the lowest LOAEL identified for apical outcomes on the developing male reproductive system by 2.4X ($BMDL_{10}$ and LOAEL = 24 and 10 mg/kg-day, respectively). *This indicates that a BMR of 10 percent for decreased fetal testicular testosterone is not health protective for DEHP*. For DEHP, the $BMDL_5$ (11 mg/kg-day) is similar to the selected POD (NOAEL of 4.8 mg/kg-day) and the lowest LOAEL identified for apical outcomes on the developing male reproductive system (10 mg/kg-day).

E.4 Weight of Scientific Evidence Conclusion

As discussed elsewhere (U.S. EPA, 2023b), DEHP, DBP, BBP, DIBP, DCHP, and DINP are toxicologically similar and induce effects on the developing male reproductive system consistent with a disruption of androgen action. Because these phthalates are toxicologically similar, it is more appropriate to select a single BMR for decreased fetal testicular testosterone to provide a consistent basis for dose response analysis and for deriving PODs relevant to the single chemical assessments. *EPA has reached the conclusion that a BMR of 5 percent is the most appropriate and health protective response level for evaluating decreased fetal testicular testosterone* when sufficient dose-response data are available to support modeling of fetal testicular testosterone in the low-end range of the dose-response curve. This conclusion is supported by the following weight of scientific evidence considerations.

- For DEHP, the $BMDL_{10}$ estimate is greater than the POD selected for use in risk characterization by 5X and is greater than the lowest LOAEL identified for apical outcomes on the developing male reproductive system by 2.4X. This indicates that a BMR of 10 percent is not protective for DEHP.
- The $BMDL_5$ estimate for DEHP is similar to the selected POD and lowest LOAEL for apical outcomes on the developing male reproductive system.
- $BMDL_{10}$ estimates for DBP ($BMDL_{10}$, POD, LOAEL = 20, 9, 30 mg/kg-day, respectively) and DCHP ($BMDL_{10}$, POD, LOAEL = 12, 10, 20 mg/kg-day, respectively) are slightly higher than the PODs selected for use in risk characterization and slightly less than the lowest LOAELs identified based on apical outcomes associated with the developing male reproductive system. This indicates that a BMR of 10 percent may be protective of apical outcomes evaluated in available studies for both DBP and DCHP. However, this may be a reflection of the larger database of studies and wider range of endpoints evaluated for DEHP, compared to DBP and DCHP.
- NASEM (2017) modeled a BMR of 40 percent using the following justification: "previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40% *(*Gray et al., 2016*;* Howdeshell et al., 2015*)*." However, publications supporting a 40 percent response level are relatively narrow in scope and assessed the link between reduced fetal testicular testosterone in SD rats on GD 18 and later life reproductive tract malformations in F1 males. More specifically, Howdeshell et al. (2015) found reproductive tract malformations in 17 to 100 percent of F1 males when fetal testosterone on GD 18 was reduced by approximately 25 to 72 percent, while Gray et al. (2016) found dose-related reproductive alterations in F1 males treated with dipentyl phthalate (a phthalate not currently being evaluated under TSCA) when fetal testosterone was reduced by about 45 percent on GD 18. Although NASEM modeled a BMR of 40 percent based on biological considerations, there is no scientific consensus on the biologically significant response level and no other authoritative or regulatory agencies have endorsed the 40 percent response level as biologically significant for reductions in fetal testosterone.
- $BMDL_{40}$ values for DEHP, DBP, DIBP, BBP, DCHP, and DINP are above the PODs selected for use in risk characterization for each phthalate by 3X to 25.4X (Table_Apx E-1). $BMDL_{40}$ values for DEHP, DBP, DIBP, BBP, and DCHP, but not DINP, are above the lowest LOAELs identified for apical outcomes on the developing male reproductive system. These results clearly demonstrate that a BMR of 40 percent is not health protective.

Table_Apx E-1. Comparison of BMD/BMDL Values Across BMRs of 5%, 10%, and 40% with PODs and LOAELs for Apical Outcomes for DEHP, DBP, DIBP, BBP, DCHP, and DINP

Phthalate	POD (mg/kg-day) Selected for use in Risk Characterization (Effect)	Lowest LOAEL(s) (mg/kg-day) for Apical Effects on the Male Reproductive System	BMD_5 Estimate [a] (mg/kg-day) [95% CI]	BMD_{10} Estimate [a] (mg/kg-day) [95% CI]	BMD_{40} Estimate [a] (mg/kg-day) [95% CI]	Reference For Further Details on the Selected POD and Lowest Identified LOAEL,
DEHP	NOAEL = 4.8 (↑ male RTM in F1 and F2 males)	10 to 15 (NR, ↓ AGD, RTMs)	17 [11, 31]	35 [24, 63]	178 [122, 284]	(U.S. EPA, 2025k)
DBP	$BMDL_5$ = 9 (↓ fetal testicular testosterone)	30 (↑ Testicular Pathology)	14 [9, 27]	29 [20, 54]	149 [101, 247]	(U.S. EPA, 2025i)
DIBP	$BMDL_5$ = 24 (↓ fetal testicular testosterone)	125 (↑ Testicular Pathology)	–[b]	55 [NA, 266][b]	279 [136, 517]	(U.S. EPA, 2025l)
BBP	NOAEL = 50 (phthalate syndrome-related effects)	100 (↓ AGD)	–[b]	–[b]	284 [150, 481]	(U.S. EPA, 2025h)
DCHP	NOAEL = 10 (phthalate syndrome-related effects)	20 (↑ Testicular Pathology)	8.4 [6.0, 14]	17 [12, 29]	90 [63, 151]	(U.S. EPA, 2025j)
DINP	$BMDL_5$ = 49 (↓ fetal testicular testosterone)	600 (↓ sperm motility)	74 [47, 158]	152 [97, 278]	699 [539, 858]	(U.S. EPA, 2025m)

AGD = anogenital distance; BMD = benchmark dose; BMDL = lower 95% confidence limit on BMD; CI = 95% confidence interval; LOAEL = lowest observable-adverse-effect level; NOAEL = no observable-adverse-effect level; POD = point of departure; RTM = reproductive tract malformations

[a] The linear-quadratic model provided the best fit (based on lowest AIC) for DEHP, DBP, DIBP, BBP, DCHP, and DINP.

[b] BMD and/or BMDL estimate could not be derived.

Appendix F BENCHMARK DOSE MODELING OF TESTOSTERONE DATA FROM LI ET AL. (2016), AHBAB ET AL. (2015), FURR ET AL. (2014), AND GRAY ET AL. (2021)

EPA conducted benchmark dose (BMD) modeling of *ex vivo* fetal testicular testosterone production data from three studies reported in two publications (Gray et al., 2021; Furr et al., 2014) and testicular testosterone content and serum testosterone data from two gestational exposure studies of DCHP (Li et al., 2016; Ahbab and Barlas, 2015).

BMD modeling for continuous testosterone data was conducted with EPA's BMD software (BMDS) Online (https://bmdsonline.epa.gov/). All standard BMDS continuous models that use maximum likelihood (MLE) optimization and profile likelihood-based confidence intervals were used in this analysis. Standard forms of these models (defined below) were run so that auto-generated model selection recommendations accurately reflect current EPA model selection procedures EPA's benchmark Dose Technical Guidance (U.S. EPA, 2012a). BMDS models that use Bayesian fitting procedures and Bayesian model averaging were not applied in this work.

Standard BMDS Models Applied to Continuous Endpoints:

- Exponential 3-restricted (exp3-r)
- Exponential 5-restricted (exp5-r)
- Hill-restricted (hil-r)
- Polynomial Degree 3-restricted (ply3-r
- Polynomial Degree 2-restricted (ply2-r)
- Power-restricted (pow-r)
- Linear-unrestricted (lin-ur)

EPA evaluated benchmark response (BMR) levels of 5, 10, and 40 percent relative deviation. BMRs of 5, 10, and 40% relative deviation were included for consistency with EPA's meta-analysis and benchmark dose analysis of fetal testicular testosterone. However, as described in Appendix E, EPA considers a BMR of 5 percent to be the most appropriate and health protective response level for evaluating decreased fetal testicular testosterone for POD determination. Model fit was judged consistent with EPA's benchmark Dose Technical Guidance (U.S. EPA, 2012a). An adequate fit was judged based on the $\chi 2$ 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 [note: this is a change from previous versions of BMDS, which required variance p-value > 0.10 for adequate fit]), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected (*i.e.*, 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 did not adequately fit the data (*i.e.*, Test 3; p-value < 0.05), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different adequately fitting models varied >3-fold; otherwise, the BMDL from the model with the lowest AIC was selected.

Table_Apx F-1 summarizes BMD modeling results for reduced testicular testosterone and serum testosterone, while more detailed BMD model results are provided in Appendices F.1 through F.5.

Table_Apx F-1. Summary of BMD Model Results for Decreased Testosterone

Data set	BMR	Best-Fit Model (Variance)	BMD_5 (mg/kg-day)	$BMDL_5$ (mg/kg-day)	Notes	Appendix Containing Results
Testicular Testosterone Content on PND 1 (Li et al., 2016)	5%	Hill (Constant)	6.9	1.2	Lowest dose/BMDL ratio > 3.0 BMD/BMDL ratio > 3.0	F.1
Fetal Serum Testosterone (Ahbab and Barlas, 2015)	5%	–	–	–	No models adequately fit the data set	F.2
Ex vivo Fetal Testis Testosterone Production (Block 23) (Furr et al., 2014)	5%	–	–	–	No models adequately fit the data set	F.3
Ex vivo Fetal Testis Testosterone Production (Block 33) (Furr et al., 2014)	5%	Exponential 3 (Constant)	9.0	5.2	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMD ratio > 3.0	F.4
Ex vivo Fetal Testis Testosterone Production (Gray et al., 2021)	5%	Exponential 3 (Constant)	13.7	10.0	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMD ratio > 3.0	F.5

F.1 BMD Model Results – Testicular Testosterone (Li et al. 2016)

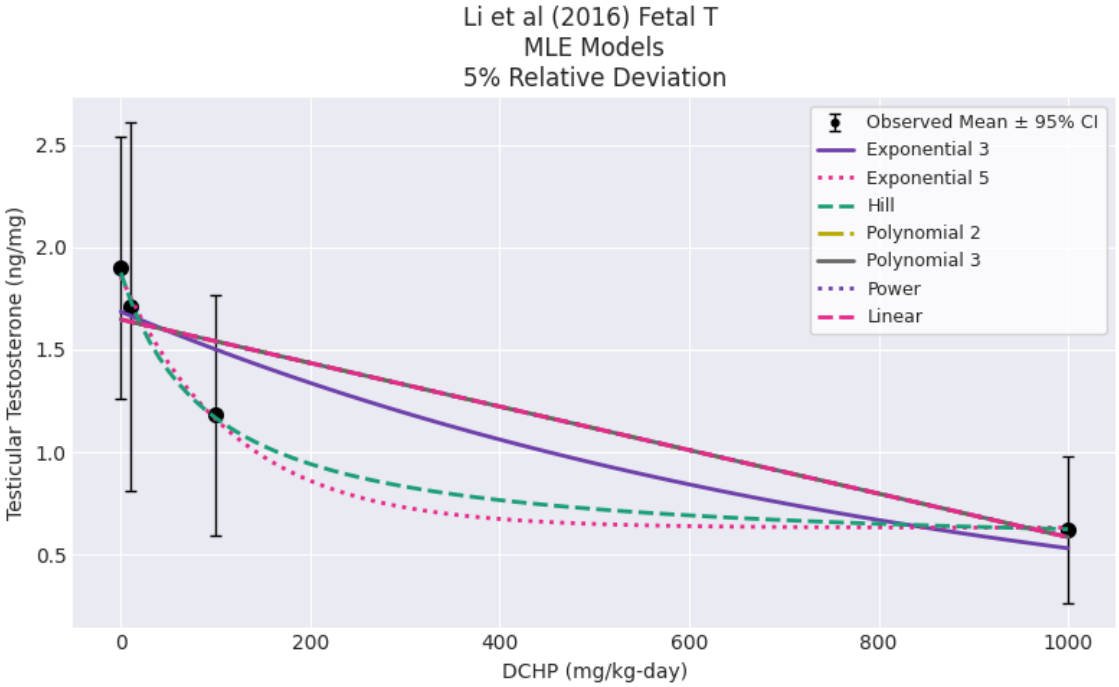
Table_Apx F-2. Testis Testosterone Content Data (Li et al. 2016)

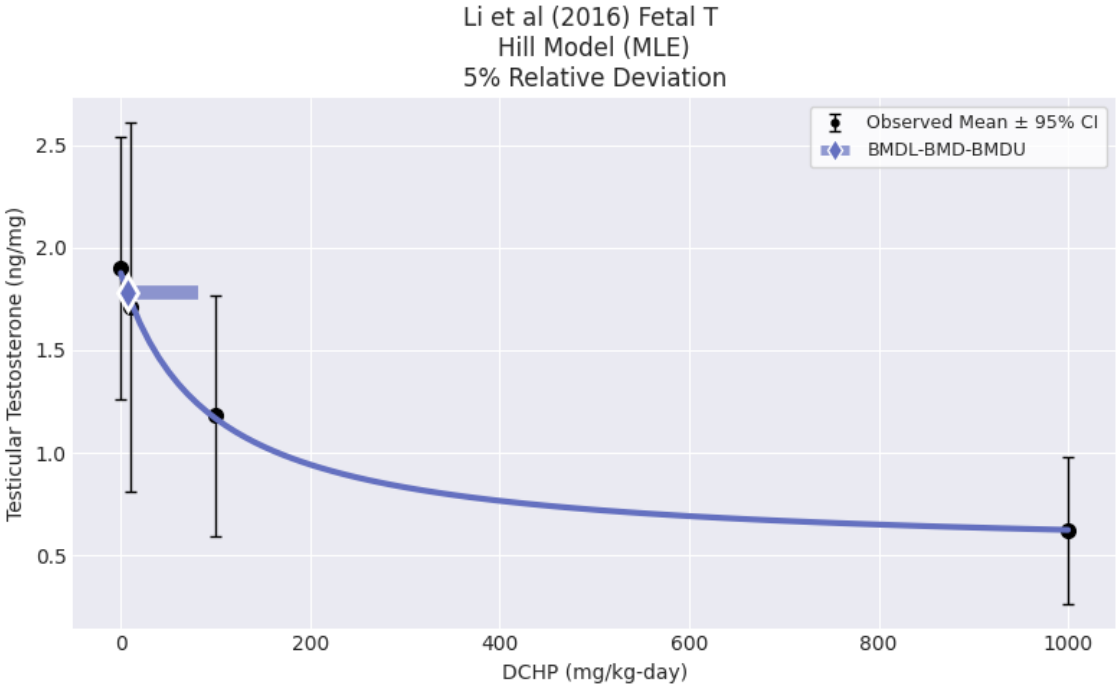
Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg)	Standard Deviation	Notes
0	6	1.9	0.61	Data from Table 2 in Li et al. (2016)
10	6	1.71	0.86	
100	6	1.18	0.56	
1000	6	0.62	0.34	

Table_Apx F-3. BMD Model Results – Testicular Testosterone (Li et al. 2016)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	44.368	15.423	91.135	31.681	441.855	153.601	0.246	49.646	Viable	
Exponential 5	Restricted	Constant	9.39	2.416	19.584	5.081	110.997	10.959	0.768	48.926	Viable	Lowest dose/BMDL ratio > 3.0 BMD/BMDL ratio > 3.0
Hill	**Restricted**	**Constant**	**6.852**	**1.177**	14.792	2.597	113.013	24.096	**0.852**	**48.874**	**Viable - Lowest BMDL**	**Lowest dose/BMDL ratio > 3.0 BMD/BMDL ratio > 3.0**
Polynomial Degree 2	Restricted	Constant	77.493	55.475	154.987	110.95	619.948	443.797	0.175	50.327	Viable	
Polynomial Degree 3	Restricted	Constant	77.537	55.473	155.074	110.948	620.296	443.785	0.175	50.327	Viable	
Power	Restricted	Constant	77.503	55.475	155.007	110.949	620.026	443.797	0.175	50.327	Viable	
Linear	Unrestricted	Constant	77.503	55.474	155.007	110.949	620.026	443.792	0.175	50.327	Viable	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.


Li et al (2016) Fetal T
MLE Models
5% Relative Deviation
Observed Mean ± 95% CI
Exponential 3
Exponential 5
Hill
Polynomial 2
Polynomial 3
Power
Linear
Testicular Testosterone (ng/mg)
DCHP (mg/kg-day)


Li et al (2016) Fetal T
Hill Model (MLE)
5% Relative Deviation
Observed Mean ± 95% CI
BMDL-BMD-BMDU
Testicular Testosterone (ng/mg)
DCHP (mg/kg-day)

Hill Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

BMR	5% Relative Deviation
Distribution	Normal + Constant variance
Modeling Direction	Down (↓)
Confidence Level (one sided)	0.95
Modeling Approach	MLE

Parameter Settings:

Parameter	Initial	Min	Max
g	0	-100	100
v	0	-100	100
k	0	0	5
n	1	1	18
alpha	0	-18	18

Modeling Summary:

BMD	6.85213
BMDL	1.17685
BMDU	82.1322
AIC	48.8738
Log-Likelihood	-20.4369
P-Value	0.852138
Model d.f.	1

Model Parameters:

Variable	Estimate	On Bound	Std Error
g	1.87589	no	0.193286
v	-1.36879	no	0.345523
k	93.1448	no	96.5853
n	1	yes	Not Reported
alpha	0.321486	no	0.0298353

Standard errors estimates are not generated for parameters estimated on corresponding bounds, although sampling error is present for all parameters, as a rule. Standard error estimates may not be reliable as a basis for confidence intervals or tests when one or more parameters are on bounds.

Goodness of Fit:

Dose	N	Sample Mean	Model Fitted Mean	Scaled Residual
0	6	1.9	1.87589	0.104162
10	6	1.71	1.74318	-0.143354
100	6	1.18	1.1672	0.0552932
1000	6	0.62	0.623727	-0.0161014

Dose	N	Sample SD	Model Fitted SD
0	6	0.61	0.566997
10	6	0.86	0.566997
100	6	0.56	0.566997
1000	6	0.34	0.566997

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-20.4195	5	50.8391
A2	-18.0442	8	52.0884
A3	-20.4195	5	50.8391
fitted	-20.4369	4	48.8738
reduced	-27.2983	2	58.5967

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	18.5083	6	0.00507982
Test 2	4.7507	3	0.190989
Test 3	4.7507	3	0.190989
Test 4	0.0347418	1	0.852138

Test 1: Test the null hypothesis that responses and variances don't differ among dose levels (A2 vs R). If this test fails to reject the null hypothesis (p-value > 0.05), there may not be a dose-response.

Test 2: Test the null hypothesis that variances are homogenous (A1 vs A2). If this test fails to reject the null hypothesis (p-value > 0.05), the simpler constant variance model may be appropriate.

Test 3: Test the null hypothesis that the variances are adequately modeled (A3 vs A2). If this test fails to reject the null hypothesis (p-value > 0.05), it may be inferred that the variances have been modeled appropriately.

Test 4: Test the null hypothesis that the model for the mean fits the data (Fitted vs A3). If this test fails to reject the null hypothesis (p-value > 0.1), the user has support for use of the selected model.

F.2 BMD Model Results – Fetal Serum Testosterone (Ahbab et al. 2015)

Table_Apx F-4. Fetal Serum Testosterone Content Data (Ahbab et al. 2015)

Dose (mg/kg-day)	N (# of litters)	Mean Serum Testosterone (pg/ml)	Standard Deviation	Notes
0	10	638	136	Data from Table 2 in Ahbab et al. (2015)
20	10	564	72	
100	10	388	40	
500	10	373	50	

Table_Apx F-5. BMD Model Results – Fetal Serum Testosterone (Ahbab et al. 2015)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	50.171	35.41	103.055	72.735	499.645	352.647	<0.001	493.949	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	9.984	3.968	17.593	8.492	110.789	21.548	-	473.132	Questionable	Lowest dose/BMDL ratio > 3.0 Zero degrees of freedom; saturated model Constant variance test failed (Test 2 p-value < 0.05)
Hill	Restricted	Constant	12.779	9.104	18.348	13.078	120.492	27.104	-	473.132	Questionable	Zero degrees of freedom; saturated model Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 2	Restricted	Constant	67.364	51.57	134.728	103.14	538.914	412.562	<0.001	495.71	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 3	Restricted	Constant	67.484	51.569	134.968	103.138	539.872	412.552	<0.001	495.71	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	67.313	51.571	134.626	103.141	538.503	412.566	<0.001	495.71	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	67.313	51.571	134.626	103.141	538.503	412.566	<0.001	495.71	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Non-constant	49.473	62.145	127.65	101.619	618.895	492.685	<0.001	487.856	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	3.16	7.876	14.283	6.73	93.652	21.631	-	459.31	Questionable	Lowest dose/BMDL ratio > 3.0 Zero degrees of freedom; saturated model
Hill	Restricted	Non-constant	8.846	11.457	16.203	12.54	89.339	29.652	-	459.31	Questionable	Zero degrees of freedom; saturated model
Polynomial Degree 2	Restricted	Non-constant	0	75.727	151.454	127.252	605.815	509.006	<0.001	488.847	Unusable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 BMDL does not exist
Polynomial Degree 3	Restricted	Non-constant	79.612	81.278	162.478	125.038	648.07	500.274	<0.001	491.37	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	63.626	75.727	151.454	127.252	605.815	509.007	<0.001	488.847	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	0	75.727	151.454	127.252	605.815	509.006	<0.001	488.847	Unusable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 BMDL does not exist

AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.

F.3 BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Block 23 Rats) (Furr et al. 2014)

Table_Apx F-6. *Ex Vivo* Fetal Testis Testosterone Production (Block 23) (Furr et al. 2014)

Dose (mg/kg-day)	N (# of litters)	*Ex Vivo* Fetal Testis Testosterone Production (ng/testis)	Standard Deviation	Notes
0	3	9.87	1.00	Data from Table 2 in Furr et al. (2014)
100	3	3.1	0.69	
300	2	2.2	0.61	
600	3	2	0.54	
900	3	5.39	1.39	

Table_Apx F-7. BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Block 23 – All Dose Groups included) (Furr et al. 2014)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	5.315	1.682	10.918	3.455	52.932	16.749	<0.001	74.993	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0
Exponential 5	Restricted	Constant	3.577	3.323	4.762	4.424	9.334	8.671	<0.001	59.667	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Goodness of fit p-value < 0.1
Hill	Restricted	Constant	5.577	3.179	5.847	5.776	6.658	6.498	<0.001	59.667	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	98.264	50.871	196.528	101.742	786.112	406.968	<0.001	75.459	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 3	Restricted	Constant	98	50.871	196	101.742	784	406.969	<0.001	75.459	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Constant	98.167	50.871	196.335	101.742	785.339	406.969	<0.001	75.459	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Linear	Unrestricted	Constant	98.167	50.871	196.335	101.742	785.339	406.969	<0.001	75.459	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Exponential 3	Restricted	Non-constant	362.386	2.806	744.371	5.764	3608.976	27.945	<0.001	73.442	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 5	Restricted	Non-constant	2.229	2.179	3.33	3.254	8.558	8.363	<0.001	59.667	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Goodness of fit p-value < 0.1
Hill	Restricted	Non-constant	69.962	65.016	73.376	71.71	83.576	7.775	<0.001	60.11	Questionable	Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	345.904	135.379	691.809	148.237	2767.235	519.838	<0.001	75.528	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 3	Restricted	Non-constant	379.63	128.824	759.261	130.606	3037.045	522.424	<0.001	75.504	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Non-constant	170.044	60.57	340.087	121.14	1360.349	484.559	<0.001	75.922	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Linear	Unrestricted	Non-constant	379.63	161.152	759.261	130.7	3037.045	522.424	<0.001	75.504	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.												

Table_Apx F-8. BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Block 23 – High Dose Removed) (Furr et al. 2014)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	5.263	3.2	10.811	6.574	52.414	31.872	<0.001	44.959	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	3.234	2.008	6.694	4.162	34.91	21.985	0.739	27.943	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0
Hill	Restricted	Constant	3.164	0.494	5.737	1.036	26.011	6.759	-	29.832	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	34.754	25.328	69.508	50.656	278.033	202.624	<0.001	56.464	Questionable	|Residual near BMD| > 2.0 Lowest dose/BMDL ratio > 3.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 3	Restricted	Constant	34.777	25.328	69.553	50.656	278.213	202.623	<0.001	56.464	Questionable	|Residual near BMD| > 2.0 Lowest dose/BMDL ratio > 3.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Constant	34.756	25.328	69.512	50.657	278.046	202.626	<0.001	56.464	Questionable	|Residual near BMD| > 2.0 lowest dose/BMDL ratio > 3.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Linear	Unrestricted	Constant	34.756	25.328	69.512	50.656	278.046	202.626	<0.001	56.464	Questionable	|Residual near BMD| > 2.0 lowest dose/BMDL ratio > 3.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Non-constant	5.263	3.2	10.811	6.574	52.414	31.872	<0.001	44.959	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	23.837	2.251	32.301	4.662	64.39	24.53	-	30.551	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Hill	Restricted	Non-constant	3.449	0.57	6.162	1.23	26.912	8.316	-	30.326	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Non-constant	48.754	40.349	97.508	82.352	390.031	309.163	<0.001	49.999	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 3	Restricted	Non-constant	48.718	38.648	97.435	77.296	389.741	309.183	<0.001	49.999	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Non-constant	48.042	38.614	96.084	77.229	384.336	308.916	<0.001	50.005	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Linear	Unrestricted	Non-constant	48.754	41.963	97.508	77.291	390.031	308.061	<0.001	49.999	Questionable	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.												

F.4 BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Block 33 Rats) (Furr et al. 2014)

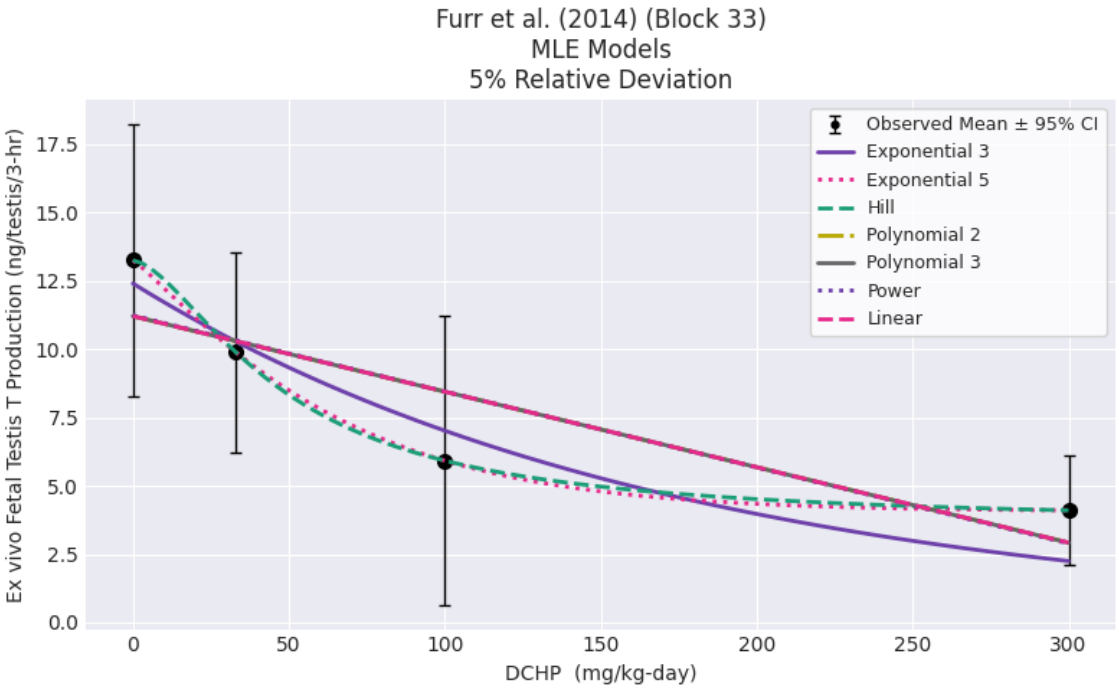
Table_Apx F-9. *Ex Vivo* Fetal Testis Testosterone Production (Block 33) (Furr et al. 2014)

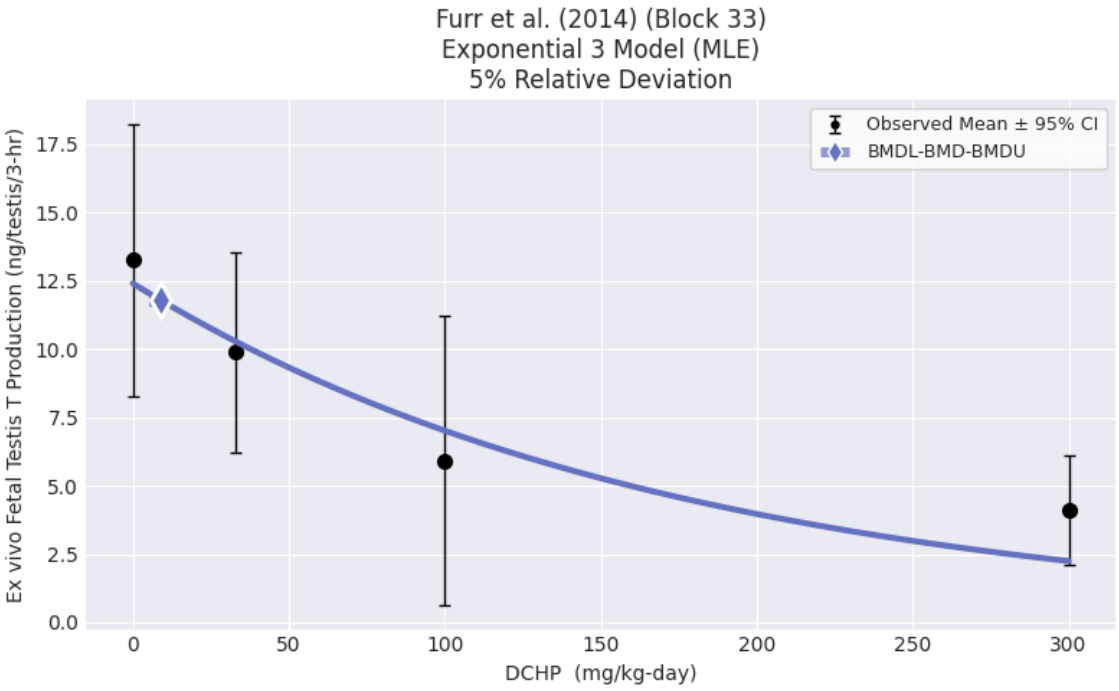
Dose (mg/kg-day)	N (# of litters)	*Ex Vivo* Fetal Testis Testosterone Production (ng/testis)	Standard Deviation	Notes
0	4	13.25	3.14	Data from Table 2 in Furr et al. (2014)
33	4	9.89	2.30	
100	4	5.92	3.32	
300	3	4.1	0.80	

Table_Apx F-10. BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Block 33) (Furr et al. 2014)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	**Restricted**	**Constant**	**9.002**	**5.186**	**18.492**	**10.653**	**89.654**	**51.65**	**0.369**	**76.79**	**Viable - Lowest AIC**	**Lowest dose/BMDL ratio > 3.0** **Lowest dose/BMD ratio > 3.0**
Exponential 5	Restricted	Constant	6.707	2.659	12.805	5.553	57.857	31.636	–	77.64	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Zero degrees of freedom; saturated model
Hill	Restricted	Constant	9.6	1.74	15.512	3.81	55.185	28.966	–	77.64	Questionable	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	20.282	15.213	40.563	30.426	162.252	121.704	0.031	80.589	Questionable	Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Constant	20.275	15.213	40.55	30.427	162.199	121.707	0.031	80.589	Questionable	Goodness of fit p-value < 0.1
Power	Restricted	Constant	20.181	15.209	40.361	30.419	161.445	121.676	0.031	80.598	Questionable	Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	20.283	15.213	40.565	30.426	162.26	121.704	0.031	80.589	Questionable	Goodness of fit p-value < 0.1

AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.


Furr et al. (2014) (Block 33)
MLE Models
5% Relative Deviation
Observed Mean ± 95% CI
Exponential 3
Exponential 5
Hill
Polynomial 2
Polynomial 3
Power
Linear
Ex vivo Fetal Testis T Production (ng/testis/3-hr)
DCHP (mg/kg-day)


Furr et al. (2014) (Block 33)
Exponential 3 Model (MLE)
5% Relative Deviation
Observed Mean ± 95% CI
BMDL-BMD-BMDU
Ex vivo Fetal Testis T Production (ng/testis/3-hr)
DCHP (mg/kg-day)

Exponential 3 Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

BMR	5% Relative Deviation
Distribution	Normal + Constant variance
Modeling Direction	Down (↓)
Confidence Level (one sided)	0.95
Modeling Approach	MLE

Parameter Settings:

Parameter	Initial	Min	Max
a	0	0	100
b	0	0	100
c	0	-20	0
d	1	1	18
log-alpha	0	-18	18

Modeling Summary:

BMD	9.00235
BMDL	5.18632
BMDU	9.18868
AIC	76.79
Log-Likelihood	-35.395
P-Value	0.368996
Model d.f.	3

Model Parameters:

Variable	Estimate	On Bound	Std Error
a	12.4046	no	1.1291
b	0.00569777	no	0.00186004
d	1	yes	Not Reported
log-alpha	1.84773	no	0.359334

Standard errors estimates are not generated for parameters estimated on corresponding bounds, although sampling error is present for all parameters, as a rule. Standard error estimates may not be reliable as a basis for confidence intervals or tests when one or more parameters are on bounds.

Goodness of Fit:

Dose	N	Sample Mean	Model Fitted Mean	Scaled Residual
0	4	13.25	12.4046	0.671186
33	4	9.89	10.2784	-0.30837
100	4	5.92	7.0167	-0.870741
300	3	4.1	2.24508	1.27543

Dose	N	Sample SD	Model Fitted SD
0	4	3.14	2.51901
33	4	2.3	2.51901
100	4	3.32	2.51901
300	3	0.8	2.51901

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-33.8198	5	77.6395
A2	-30.9887	8	77.9775
A3	-33.8198	5	77.6395
fitted	-35.395	2	74.79
reduced	-42.748	2	89.4959

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	23.5184	6	0.000640203
Test 2	5.66202	3	0.129263
Test 3	5.66202	3	0.129263
Test 4	3.15051	3	0.368996

Test 1: Test the null hypothesis that responses and variances don't differ among dose levels (A2 vs R). If this test fails to reject the null hypothesis (p-value > 0.05), there may not be a dose-response.

Test 2: Test the null hypothesis that variances are homogenous (A1 vs A2). If this test fails to reject the null hypothesis (p-value > 0.05), the simpler constant variance model may be appropriate.

Test 3: Test the null hypothesis that the variances are adequately modeled (A3 vs A2). If this test fails to reject the null hypothesis (p-value > 0.05), it may be inferred that the variances have been modeled appropriately.

Test 4: Test the null hypothesis that the model for the mean fits the data (Fitted vs A3). If this test fails to reject the null hypothesis (p-value > 0.1), the user has support for use of the selected model.

F.5 BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Gray et al. 2021)

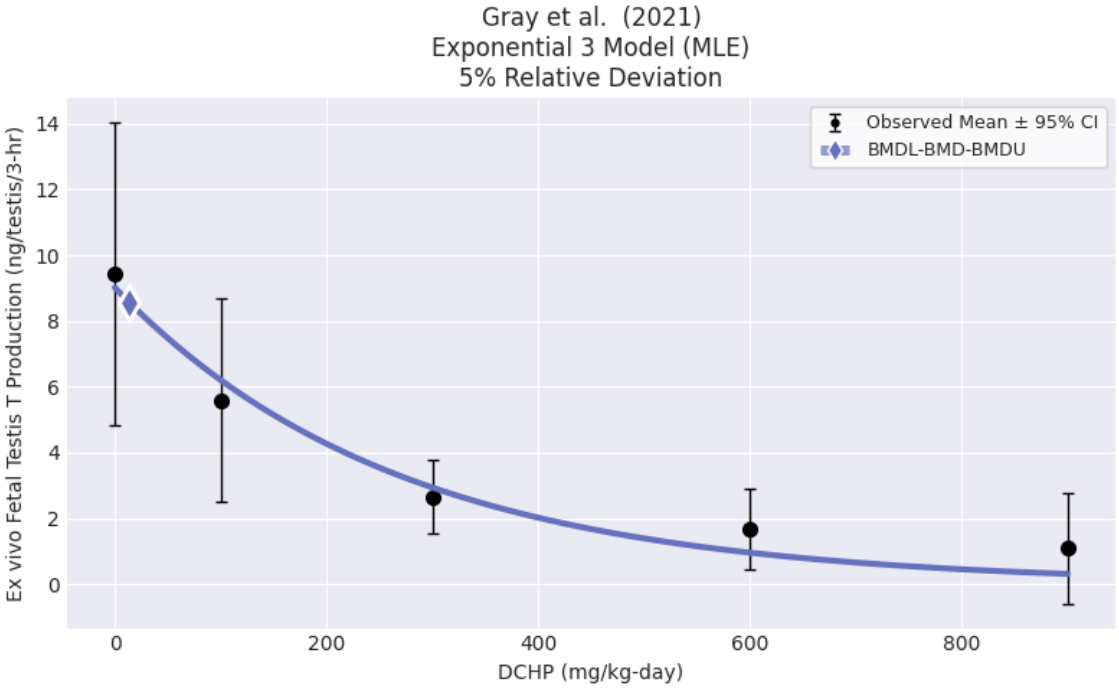
Table_Apx F-11. *Ex Vivo* Fetal Testis Testosterone Production (Gray et al. 2021)

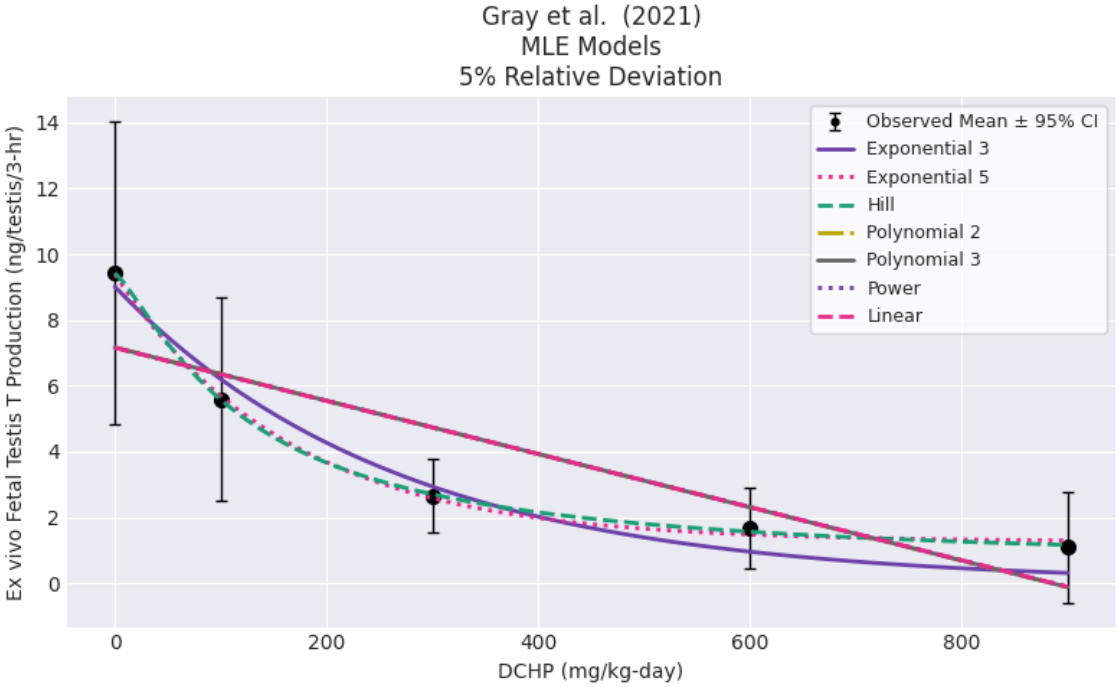
Dose (mg/kg-day)	N (# of litters)	*Ex Vivo* Fetal Testis Testosterone Production (ng/testis)	Standard Deviation	Notes
0	3	9.43	1.86	Data from Supplemental Excel File associated with Gray et al. (2021)
100	3	5.59	1.24	
300	3	2.65	0.45	
600	3	1.68	0.50	
900	3	1.09	0.68	

Table_Apx F-12. BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Gray et al. 2021)

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	**Restricted**	**Constant**	**13.715**	**10.045**	28.172	20.633	136.589	100.034	**0.237**	**50.535**	**Viable - Lowest AIC (BMR = 5%)**	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMD ratio > 3.0
Exponential 5	**Restricted**	**Constant**	9.818	7.09	**20.257**	**14.645**	**102.328**	**74.677**	**0.826**	**47.382**	**Questionable (BMR = 5%)** **Viable - Lowest AIC (BMR = 10% & 40%)**	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0 Lowest dose/BMD ratio > 10.0
Hill	Restricted	Constant	12.665	4.792	22.945	10.171	97.144	62.813	0.774	49.082	**Questionable**	Lowest dose/BMDL ratio > 3.0 Lowest dose/BMDL ratio > 10.0 Lowest dose/BMD ratio > 3.0
Polynomial Degree 2	Restricted	Constant	44.216	37.059	88.431	74.117	353.724	296.468	<0.001	65.894	**Questionable**	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Constant	44.242	37.058	88.484	74.116	353.935	296.462	<0.001	65.894	**Questionable**	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1
Power	Restricted	Constant	44.318	37.054	88.636	74.108	354.543	296.432	<0.001	65.894	**Questionable**	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	44.318	37.054	88.636	74.108	354.543	296.432	<0.001	65.894	**Questionable**	|Residual near BMD| > 2.0 Residual at control > 2.0 Goodness of fit p-value < 0.1

AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.


Gray et al. (2021)
Exponential 3 Model (MLE)
5% Relative Deviation
Observed Mean ± 95% CI
BMDL-BMD-BMDU
Ex vivo Fetal Testis T Production (ng/testis/3-hr)
DCHP (mg/kg-day)
0
2
4
6
8
10
12
14
0
200
400
600
800


Gray et al. (2021)
MLE Models
5% Relative Deviation
Observed Mean ± 95% CI
Exponential 3
Exponential 5
Hill
Polynomial 2
Polynomial 3
Power
Linear
Ex vivo Fetal Testis T Production (ng/testis/3-hr)
DCHP (mg/kg-day)
0
2
4
6
8
10
12
14
0
200
400
600
800

Exponential 3 Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

BMR	5% Relative Deviation
Distribution	Normal + Constant variance
Modeling Direction	Down (↓)
Confidence Level (one sided)	0.95
Modeling Approach	MLE

Parameter Settings:

Parameter	Initial	Min	Max
a	0	0	100
b	0	0	100
c	0	-20	0
d	1	1	18
log-alpha	0	-18	18

Modeling Summary:

BMD	13.7152
BMDL	10.0447
BMDU	21.9219
AIC	50.5351
Log-Likelihood	-22.2676
P-Value	0.236628
Model d.f.	4

Model Parameters:

Variable	Estimate	On Bound	Std Error
a	9.01781	no	0.589221
b	0.00373988	no	0.000668386
d	1	yes	Not Reported
log-alpha	0.131131	no	0.364392

Standard errors estimates are not generated for parameters estimated on corresponding bounds, although sampling error is present for all parameters, as a rule. Standard error estimates may not be reliable as a basis for confidence intervals or tests when one or more parameters are on bounds.

Goodness of Fit:

Dose	N	Sample Mean	Model Fitted Mean	Scaled Residual
0	3	9.43	9.01781	0.668626
100	3	5.59	6.20412	-0.996187
300	3	2.65	2.93656	-0.464838
600	3	1.68	0.956262	1.174
900	3	1.09	0.311397	1.263

Dose	N	Sample SD	Model Fitted SD
0	3	1.86	1.06776
100	3	1.24	1.06776
300	3	0.45	1.06776
600	3	0.5	1.06776
900	3	0.68	1.06776

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-19.4998	6	50.9996
A2	-15.1182	10	50.2364
A3	-19.4998	6	50.9996
fitted	-22.2676	2	48.5351
reduced	-38.7881	2	81.5761

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	47.3397	8	1.32085e-07
Test 2	8.76322	4	0.067298
Test 3	8.76322	4	0.067298
Test 4	5.53549	4	0.236628

Test 1: Test the null hypothesis that responses and variances don't differ among dose levels (A2 vs R). If this test fails to reject the null hypothesis (p-value > 0.05), there may not be a dose-response.

Test 2: Test the null hypothesis that variances are homogenous (A1 vs A2). If this test fails to reject the null hypothesis (p-value > 0.05), the simpler constant variance model may be appropriate.

Test 3: Test the null hypothesis that the variances are adequately modeled (A3 vs A2). If this test fails to reject the null hypothesis (p-value > 0.05), it may be inferred that the variances have been modeled appropriately.

Test 4: Test the null hypothesis that the model for the mean fits the data (Fitted vs A3). If this test fails to reject the null hypothesis (p-value > 0.1), the user has support for use of the selected model.