



United States  
Environmental Protection Agency

EPA Document# EPA-740-R-25-036

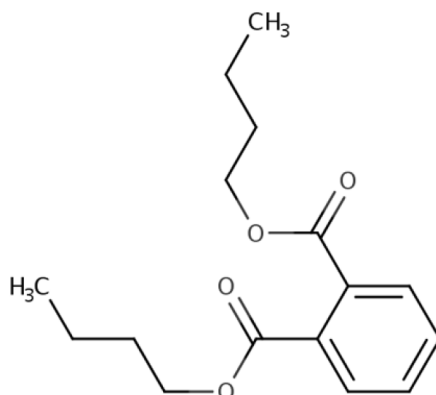
December 2025

Office of Chemical Safety and  
Pollution Prevention

# Non-Cancer Human Health Hazard Assessment for Dibutyl Phthalate (DBP)

## Technical Support Document for the Risk Evaluation

CASRN: 84-74-2



*December 2025*

# TABLE OF CONTENTS

<b>SUMMARY .....</b>	<b>9</b>
<b>1 INTRODUCTION .....</b>	<b>12</b>
1.1 Human Epidemiologic Data: Approach and Conclusions .....	12
1.2 Laboratory Animal Findings: Summary of Existing Assessments, Approach, and Methodology .....	14
1.2.1 Existing Assessments of DBP .....	14
1.2.2 Approach to Identifying and Integrating Laboratory Animal Data .....	17
1.2.3 Literature Identified and Hazards of Focus for DBP .....	19
<b>2 TOXICOKINETICS.....</b>	<b>21</b>
2.1 Oral Route.....	21
2.2 Inhalation Route.....	23
2.3 Dermal Route .....	24
2.4 Additional Toxicokinetic Considerations .....	26
2.5 Summary.....	27
<b>3 NON-CANCER HAZARD IDENTIFICATION .....</b>	<b>28</b>
3.1 Effects on the Developing Male Reproductive System .....	28
3.1.1 Summary of Available Epidemiological Studies.....	28
3.1.1.1 Previous Epidemiology Assessment (Conducted in 2019 or earlier) .....	28
3.1.1.1.1 Health Canada (2018b).....	29
3.1.1.1.2 Radke et al. (2019b; 2018) .....	30
3.1.1.1.3 NASEM report (2017) .....	33
3.1.1.1.4 Summary of the Existing Assessments of Male Reproductive Effects .....	33
3.1.1.2 Summary of Studies Identified by EPA (2018 through 2019 or Identified Through Public Comment) .....	34
3.1.2 Summary of Laboratory Animals Studies .....	35
3.1.2.1 Developing Male Reproductive System .....	36
3.1.2.2 Other Developmental and Reproductive Outcomes .....	37
3.1.3 Mode of Action for Phthalate Syndrome .....	54
3.2 Literature Considered for Non-Cancer Hazard Identification .....	58
3.3 Summary .....	58
<b>4 DOSE-REPOSE ASSESSMENT.....</b>	<b>60</b>
4.1 Selection of Studies and Endpoints for Non-Cancer Health Effects .....	60
4.2 Non-Cancer Oral Points of Departure for Acute, Intermediate, and Chronic Exposures.....	61
4.2.1 Studies Considered for Dose-Response Assessment.....	62
4.2.2 Benchmark Dose Modeling of Testosterone and Anogenital Distance Data .....	65
4.2.3 Selection of the Non-Cancer Oral Point of Departure .....	67
4.3 Weight of Scientific Evidence: POD for Acute, Intermediate, and Chronic Durations .....	76
4.4 Route-to-Route Extrapolation.....	77
<b>5 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE.....</b>	<b>79</b>
5.1 Hazard Considerations for Aggregate Exposure .....	79
5.2 PESS Based on Greater Susceptibility .....	79
<b>6 POINTS OF DEPARTURE USED TO ESTIMATE RISKS FROM DBP EXPOSURE, AND CONCLUSIONS .....</b>	<b>87</b>
<b>REFERENCES.....</b>	<b>89</b>

<b>APPENDICES .....</b>	<b>107</b>
<b>Appendix A Existing Assessments from Other Regulatory Agencies of DBP .....</b>	<b>107</b>
<b>Appendix B Literature Considered for Non-Cancer Hazards.....</b>	<b>111</b>
B.1 Reproductive and Developmental Effects .....	111
B.2 Neurotoxicity .....	117
B.3 Metabolic/Nutritional .....	121
B.4 Cardiovascular Health Effects .....	123
B.5 Immune adjuvant effects.....	124
<b>Appendix C Fetal Testicular Testosterone as an Acute Effect.....</b>	<b>127</b>
<b>Appendix D Calculating Daily Oral Human Equivalent Doses and Human Equivalent Concentrations .....</b>	<b>128</b>
D.1 DBP Non-Cancer HED and HEC Calculations for Acute, Intermediate, and Chronic Duration Exposures.....	129
<b>Appendix E Considerations for Benchmark Response (BMR) Selection for Reduced Fetal Testicular Testosterone .....</b>	<b>131</b>
E.1 Purpose .....	131
E.2 Methods .....	131
E.3 Results.....	132
E.4 Weight of Scientific Evidence Conclusion.....	133
<b>Appendix F Benchmark Dose (BMD) Modeling of Fetal Testicular Testosterone Data from Individual Gestational Exposures Studies of DBP.....</b>	<b>135</b>
F.1 BMD Model Results – Fetal Testicular Concentration (Martino-Andrade et al., 2008).....	138
F.2 BMD Model Results – Fetal Testicular Concentration (Kuhl et al., 2007) .....	143
F.3 BMD Model Results – Fetal Testicular Concentration (Struve et al., 2009) .....	148
F.3.1 4-Hour Post-Exposure .....	148
F.3.2 24-Hour Post-Exposure .....	154
F.4 BMD Model Results – Fetal Testicular Concentration (Johnson et al., 2007).....	157
F.4.1 1-Hour Post-Exposure .....	157
F.4.2 3-Hour Post-Exposure .....	160
F.4.3 6-Hour Post-Exposure .....	163
F.5 BMD Model Results – <i>Ex vivo</i> Fetal Testis Testosterone Production (Howdeshell et al., 2008).....	168
F.6 BMD Model Results – <i>Ex vivo</i> Fetal Testis Testosterone Production (Gray et al., 2021) .....	173
F.6.1 Block 70 Rat Data.....	173
F.6.2 Block 71 Rat Data.....	180
F.7 BMD Model Results – <i>Ex vivo</i> Fetal Testis Testosterone Production (Furr et al., 2014) .....	188
F.7.1 Block 18 Rat Data.....	188
F.7.2 Block 22 Rat Data.....	191
F.7.3 Block 26 Rat Data.....	194
<b>Appendix G Benchmark Dose (BMD) Analysis of Male Pup Nipple Retention (Mylchreest et al., 2000) .....</b>	<b>197</b>
G.1 BMD Model Results – Nipple/Areolae Retention (Frequentist, Rao-Scott Transformed Data).....	199
G.1.1 BMD Model Results for BMR of 10% .....	199
G.1.2 BMD Model Results for BMR of 5% .....	202
G.2 BMD Model Results – Nipple/Areolae Retention (Bayesian Model Averaging, Rao-Scott Transformed Data).....	205

G.2.1	BMD Results for BMR of 10% .....	205
G.2.1	BMD Results for BMR of 5% .....	207
G.3	BMD Model Results – Nipple/Areolae Retention (Frequentist, Original Data) .....	209
G.3.1	BMD Model Results for BMR of 10% .....	209
G.3.2	BMD Model Results for BMR of 5% .....	211
G.4	BMD Model Results – Nipple/Areolae Retention (Bayesian Model Averaging, Original Data) .....	213
G.4.1	BMD Model Results for BMR of 10% .....	213
G.4.2	BMD Model Results for BMR of 5% .....	215

## LIST OF TABLES

---

Table 1-1.	Summary of DBP Non-Cancer PODs Selected for Use by Other Regulatory Organizations.	15
Table 2-1.	Metabolites of DBP Identified in Urine from Rats and Humans after Oral Administration...	22
Table 3-1.	Summary of Scope and Methods Used in Previous Assessments to Evaluate the Association Between DBP Exposure and Male Reproductive Outcomes .....	29
Table 3-2.	Summary of Epidemiologic Evidence of Male Reproductive Effects Associated with Exposure to DBP (Radke et al., 2018) .....	31
Table 3-3.	Summary of Studies Evaluating Effects on the Developing Male Reproductive System Following <i>In Utero</i> Exposures to DBP .....	38
Table 3-4.	Summary of Studies Evaluating Effects on the Developing Male Reproductive System following Prepubertal and Pubertal Exposure to DBP .....	52
Table 4-1.	Dose-Response Analysis of Selected Studies Considered for Deriving the Non-Cancer POD .....	68
Table 4-2.	Summary of Effects of Gestational Exposure to DBP on Fetal Testicular Testosterone Content and <i>Ex Vivo</i> Fetal Testicular Testosterone Production Across Select Studies ....	72
Table 4-3.	Overall Analyses and Sensitivity Analyses of Rat Studies of DBP and Fetal Testicular Testosterone Content and <i>Ex Vivo</i> Fetal Testicular Testosterone Production (Updated Analysis Conducted by EPA) <sup>a</sup> .....	74
Table 4-4.	Benchmark Dose Estimates for DBP and Fetal Testicular Testosterone Content and <i>Ex Vivo</i> Fetal Testicular Testosterone Production in Rats .....	75
Table 5-1.	PESS Evidence Crosswalk for Biological Susceptibility Considerations .....	81
Table 6-1.	Non-Cancer HECs and HEDs Used to Estimate Risks for Acute, Intermediate, and Chronic Exposure Scenarios .....	87

## LIST OF FIGURES

---

Figure 1-1.	Overview of DBP Human Health Hazard Assessment Approach .....	18
Figure 2-1.	Proposed Metabolic Pathway of DBP Following Oral Exposure (From (ECJRC, 2004)) ....	23
Figure 3-1.	Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure .....	54

## LIST OF APPENDIX TABLES

---

Table_Apx A-1. Summary of Peer-review, Public Comments, and Systematic Review for Existing Assessments of DBP .....	107
Table_Apx B-1. Summary of Animal Toxicology Studies Evaluating Effects on the Developmental and Reproductive System Following Exposure to DBP .....	114
Table_Apx B-2. Summary of Animal Toxicology Studies Evaluating Effects on the Nervous System Following Exposure to DBP .....	119
Table_Apx B-3. Summary of Animal Toxicology Studies Evaluating Effects on Metabolism Following Exposure to DBP .....	122
Table_Apx B-4. Summary of Animal Toxicology Study Evaluating Effects on the Cardiovascular System Following Exposure to DBP .....	123
Table_Apx B-5. Summary of DBP Studies Evaluating Effects on the Immune System .....	125
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 ..	134
Table_Apx F-1. Summary of BMD Model Results for Decreased Fetal Testicular Testosterone Content and <i>Ex Vivo</i> Fetal Testicular Testosterone Production .....	136
Table_Apx F-2. Fetal Testis Testosterone Content Data (Martino-Andrade et al. 2009) .....	138
Table_Apx F-3. BMD Model Results – Fetal Testicular Testosterone Content (Martino-Andrade et al. 2009) .....	139
Table_Apx F-4. Fetal Testis Testosterone Content Data (Kuhl et al. 2007) .....	143
Table_Apx F-5. BMD Model Results – Fetal Testicular Testosterone Content (Kuhl et al. 2007) .....	144
Table_Apx F-6. Fetal Testis Testosterone Content Data (4-Hr Post-Exposure) (Struve et al. 2009) ....	148
Table_Apx F-7. BMD Model Results – Fetal Testicular Testosterone Content (4-Hr) (Struve et al. 2009) .....	149
Table_Apx F-8. Fetal Testis Testosterone Content Data (4-Hr Post-Exposure) (Struve et al. 2009) ....	154
Table_Apx F-9. BMD Model Results – Fetal Testicular Testosterone Content (24-Hr) (Struve et al. 2009) .....	155
Table_Apx F-10. Fetal Testis Testosterone Content Data (1-Hr) (Johnson et al. 2007) .....	157
Table_Apx F-11. BMD Model Results – Fetal Testicular Testosterone Content (1-Hr) (Johnson et al. 2007) .....	158
Table_Apx F-12. Fetal Testis Testosterone Content Data (3-Hr) (Johnson et al. 2007) .....	160
Table_Apx F-13. BMD Model Results – Fetal Testicular Testosterone Content (3-Hr) (Johnson et al. 2007) .....	161
Table_Apx F-14. Fetal Testis Testosterone Content Data (6-Hr) (Johnson et al. 2007) .....	163
Table_Apx F-15. BMD Model Results – Fetal Testicular Testosterone Content (6-Hr) (Johnson et al. 2007) .....	164
Table_Apx F-16. <i>Ex Vivo</i> Fetal Testis Testosterone Production Data (Howdeshell et al. 2008) .....	168
Table_Apx F-17. BMD Model Results – <i>Ex Vivo</i> Fetal Testis Testosterone Production (Howdeshell et al. 2008) .....	169
Table_Apx F-18. <i>Ex Vivo</i> Fetal Testis Testosterone Production Data (Block 70) (Gray et al. 2021) ....	173
Table_Apx F-19. <i>Ex Vivo</i> Fetal Testis Testosterone Production (Block 70) (Gray et al. 2021) .....	174
Table_Apx F-20. <i>Ex Vivo</i> Fetal Testis Testosterone Production Data (Block 71) (Gray et al. 2021) ....	180
Table_Apx F-21. <i>Ex Vivo</i> Fetal Testis Testosterone Production (Block 71) (Gray et al. 2021) .....	181
Table_Apx F-22. <i>Ex Vivo</i> Fetal Testis Testosterone Production Data (Block 18) (Furr et al. 2014) ....	188
Table_Apx F-23. <i>Ex Vivo</i> Fetal Testis Testosterone Production (Block 18) (Furr et al. 2014) .....	189
Table_Apx F-24. <i>Ex Vivo</i> Fetal Testis Testosterone Production Data (Block 22) (Gray et al. 2021) ....	191
Table_Apx F-25. <i>Ex Vivo</i> Fetal Testis Testosterone Production (Block 22) (Furr et al. 2014) .....	192
Table_Apx F-26. <i>Ex Vivo</i> Fetal Testis Testosterone Production Data (Block 26) (Gray et al. 2021) ....	194

Table_Apx F-27. <i>Ex Vivo</i> Fetal Testis Testosterone Production (Block 26) (Furr et al. 2014) .....	195
Table_Apx G-1. Original and Rao-Scott Adjusted Incidence of Nipples and/or Areolae in F1 Male Rats (Mylchreest et al. 2000) .....	197
Table_Apx G-2. Summary of BMD Model Results for F1 Male Nipple/Areolae Retention (Mylchreest et al. 2000) .....	198

## KEY ABBREVIATIONS AND ACRONYMS

---

ACE	Angiotensin converting enzyme
ADME	Absorption, distribution, metabolism and excretion
AGD	Anogenital distance
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
CASRN	Chemical Abstracts Service Registry Number
CI	Confidence Interval
CPSC	Consumer Product Safety Commission (U.S.)
BMD	Benchmark Dose
BMDL	Benchmark dose (lower confidence limit)
BBP	Butyl-benzyl-phthalate
DBP	Dibutyl phthalate
DEHP	Di-ethylhexyl phthalate
DIBP	Di-isobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Di-isononyl phthalate
E2	$\beta$ -estradiol
ECB	European Chemicals Bureau
ECP	Eosinophil Cationic Protein
ECHA	European Chemicals Agency
EDSP	Endocrine Disrupting Screening Program
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency (U.S.)
EPM	Elevated Plus Maze
F344	Fischer 344 rat
FSH	Follicle Stimulating Hormone
FST	Forced Swim Test
GD	Gestation Day
GLP	Good Laboratory Practice
GSH	Glutathione
HEC	Human equivalent concentration
HED	Human equivalent dose
Ig	Immunoglobulin
LH	Luteinizing Hormone
IHC	Immunohistochemistry
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MNG	Multinucleated gonocytes
MOA	Mode of action
MOE	Margin of exposure
NASEM	National Academies of Sciences, Engineering, and Medicine
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NTP	National Toxicology Program
NTP-CERHR	National Toxicology Program Center for the Evaluation of Risks to Human Reproduction

OFT	Open Field Test
OCSP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-operation and Development
OPPT	Office of Pollution Prevention and Toxics
OR	Odds Ratio
PBPK	Physiologically based pharmacokinetic
PND	Post-natal day
PECO	Population, exposure, comparator, and outcome
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal Day
PNW	Postnatal Week
POD	Point of departure
PPAR $\alpha$	Peroxisome proliferator activated receptor alpha
SACC	Science Advisory Committee on Chemicals
SD	Sprague-Dawley
TSCA	Toxic Substances Control Act
TST	Tail Suspension Test
UF	Uncertainty factor
U.S.	United States



## SUMMARY

This technical support document is in support of the TSCA *Risk Evaluation for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025n](#)). This document describes the use of reasonably available information to identify the non-cancer hazards associated with exposure to DBP and the points of departure (PODs) to be used to estimate risks from DBP exposures in the risk evaluation of DBP. EPA summarizes the cancer and genotoxicity hazards associated with exposure to DBP 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](#)).

EPA identified effects on the developing male reproductive system as the most sensitive and robust non-cancer hazard associated with oral exposure to DBP in experimental animal models (Section 3.1). Effects on the developing male reproductive system were also identified as the most sensitive and robust non-cancer effect following oral exposure to DBP by existing assessments of DBP, including those by the U.S. Consumer Product Safety Commission ([CPSC, 2014, 2010](#)), Health Canada ([Health Canada, 2020](#)), European Chemicals Agency ([2017a, b, 2010](#); [ECJRC, 2004](#)), The European Food Safety Authority ([2019, 2005](#)), the Australian National Industrial Chemicals Notification and Assessment Scheme ([NICNAS, 2013](#)), the NTP, ([NTP, 2003](#)) the California EPA ([OEHHA, 2007](#)) and in other assessments ([NASEM, 2017](#)). EPA also considered epidemiologic evidence qualitatively as part of hazard identification and characterization. However, epidemiologic evidence for DBP 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 assessment by Health Canada and U.S. CPSC.

As discussed further in Section 3.1, EPA identified 38 oral exposure studies (35 of rats, 3 of mice) that investigated the developmental and reproductive effects of DBP following gestational and/or perinatal exposure to DBP, including multi-generational studies of reproduction ([Wine et al., 1997](#); [NTP, 1995](#)). However, there are limited data that evaluate the effects of DBP following inhalation or dermal exposures. Data that evaluate chronic exposures via any route are limited to one study ([NTP, 2021](#)). Across available studies, the most sensitive developmental effects identified by EPA include effects on the developing male reproductive system consistent with a disruption of androgen action and development of phthalate syndrome.

EPA selected a point of departure (POD) of 9 mg/kg-day (human equivalent dose [HED] of 2.1 mg/kg-day) based on phthalate syndrome-related effects on the developing male reproductive system (decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production) to estimate non-cancer risks from oral exposure to DBP for acute, intermediate, and chronic durations of exposure in the risk evaluation of DBP. The POD was derived from EPA's updated meta-analysis originally conducted by the NASEM ([NASEM, 2017](#)) and subsequent benchmark dose (BMD) modeling of decreased fetal testicular testosterone (*ex vivo* testicular testosterone production or testicular testosterone content) in eight studies of rats exposed to DBP during gestation ([Gray et al., 2021](#); [Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)). The BMDL<sub>5</sub> of 9 mg/kg-day (HED 2.1 mg/kg-day) is within the range of PODs (*i.e.*, 1 to 10 mg/kg-day) identified from other studies based on antiandrogenic effects on the developing male reproductive system ([Furr et al., 2014](#); [Moody et al., 2013](#); [Boekelheide et al., 2009](#); [Lee et al., 2004](#)). These studies support the selection of the BMDL<sub>5</sub> of 9 mg/kg-day for the acute, intermediate, and chronic duration PODs. The sole chronic study identified by EPA does not offer a more sensitive chronic POD; the NTP ([2021](#)) identified a POD of 510 mg/kg-day (based on LOAEL; HED = 130 mg/kg-day).

The Agency performed  $\frac{3}{4}$  body weight scaling to yield the HED and applied the animal to human extrapolation factor (*i.e.*, interspecies extrapolation;  $UF_A$ ) of  $3\times$  and a within human variability extrapolation factor (*i.e.*, intraspecies extrapolation;  $UF_H$ ) of  $10\times$ . Thus, a total uncertainty factor (UF) of  $30\times$  was applied for use as the benchmark margin of exposure (MOE). Based on the strengths, limitations, and uncertainties discussed Section 4.3, EPA reviewed the weight of the scientific evidence and has robust overall confidence in the selected POD based on decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production for use in characterizing risk from exposure to DBP 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 4.2 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. Use of this POD based on sensitive male reproductive effects are expected to be protective of effects in other age groups (*e.g.*, older children, adult males, and women above reproductive age) and appropriate for a screening level assessment for these other age groups.

No data are reasonably available for the dermal or inhalation routes that are suitable for deriving route-specific PODs. Therefore, EPA used the acute/intermediate/chronic oral PODs to evaluate risks from dermal exposure to DBP. Differences in absorption are accounted for in dermal exposure estimates in the risk evaluation for DBP. For the inhalation route, EPA extrapolated the oral HED to an inhalation human equivalent concentration (HEC) using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest ([U.S. EPA, 1994](#)). The oral HED and inhalation HEC values selected by EPA to estimate non-cancer risk from acute/intermediate/chronic exposure to DBP in the risk evaluation of DBP are summarized in Table ES-1 and Section 6.

This non-cancer human health hazard assessment for DBP 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 HED and HEC Used to Estimate Risks**

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HED (mg/kg-day)	HEC (mg/m <sup>3</sup> ) [ppm]	Benchmark MOE	References (TSCA Quality Rating)
Acute, intermediate, chronic	Effects on the developing reproductive system	Rat	5 to 14 day throughout gestation	BMDL <sub>5</sub> = 9	↓ fetal testicular testosterone content and <i>ex vivo</i> fetal testicular testosterone production	2.1	11.6 [1.02]	UF <sub>A</sub> = 3 <sup>a</sup> UF <sub>H</sub> = 10 <i>Total UF</i> = 30	( <a href="#">Gray et al., 2021</a> ; <a href="#">NASEM, 2017</a> ) <sup>b</sup> ( <a href="#">U.S. EPA, 2025g</a> ) <sup>c, d</sup>

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

<sup>a</sup> EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance ([U.S. EPA, 2011b](#)), the UF<sub>A</sub> was reduced from 10 to 3.

<sup>b</sup> EPA conducted an updated BMD analysis of the meta-regression and BMD modeling of DBP and *ex vivo* fetal testicular testosterone production and fetal testicular testosterone content data in rats published by NASEM ([2017](#)). The updated analysis included eight total studies: seven studies from NASEM ([Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)), in addition to a more recent study by Gray et al. ([2021](#)).

<sup>c</sup> The updated 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](#)).

<sup>d</sup> TSCA Study Quality Ratings: *High confidence* for ([Gray et al., 2021](#); [Furr et al., 2014](#); [Howdeshell et al., 2008](#)) *Medium confidence* for ([Johnson et al., 2011](#); [Struve et al., 2009](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#)) and *Low confidence* for ([Kuhl et al., 2007](#)).

# 1 INTRODUCTION

---

In December 2019, the United States Environmental Protection Agency (U.S. EPA or the Agency) designated dibutyl phthalate (DBP) 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 702) ([U.S. EPA, 2019](#)). EPA published the draft and final scope documents for DBP 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 DBP and conduct a dose-response assessment to determine the toxicity values to be used to estimate risks from DBP exposures. This technical support document for DBP summarizes the non-cancer hazards associated with exposure to DBP and proposes non-cancer toxicity values to be used to estimate risks from DBP exposures. Cancer human health hazards associated with exposure to DBP 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 DBP have been reviewed by several regulatory and authoritative agencies, including the: U.S. Consumer Product Safety Commission (U.S. CPSC); U.S. Agency for Toxic Substances and Disease Registry (ATSDR); U.S. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR); The National Academies of Sciences, Engineering, and Medicine (NASEM); Health Canada; European Chemicals Bureau (ECB); European Chemicals Agency (ECHA); European Food Safety Authority (EFSA); and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS). EPA relied on information published in existing assessments by these regulatory and authoritative agencies as a starting point for its human health hazard assessment of DBP. Additionally, EPA considered literature published since the most recent existing assessments of DBP to determine if newer information might support the identification of new human health hazards or lower PODs for use in estimating human risk. EPA's process for considering and incorporating DBP literature is described in the *Systematic Review Protocol for Dibutyl Phthalate (DBP)* ([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, respectively.

## 1.1 Human Epidemiologic Data: Approach and Conclusions

---

To identify and integrate human epidemiologic data into the risk evaluation of DBP, EPA first reviewed the conclusions of existing assessments of DBP conducted by regulatory and authoritative agencies, as well as several systematic reviews of epidemiologic studies of DBP published by Radke et al.; authors are affiliated with the U.S. EPA's Center for Public Health and Environmental Assessment. Existing assessments reviewed by EPA are listed below. As described further in Appendix A, most of these epidemiologic 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](#));
- *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](#));
- *Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence* ([Radke et al., 2018](#));

- *Phthalate exposure and female reproductive and developmental outcomes: A systematic review of the human epidemiological evidence* ([Radke et al., 2019b](#));
- *Phthalate exposure and metabolic effects: A systematic review of the human epidemiological evidence* ([Radke et al., 2019a](#));
- *Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence* ([Radke et al., 2020a](#)); and
- *Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals* ([NASEM, 2017](#)).

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

EPA relied on conclusions from Health Canada ([2018a, b](#)) and systematic review publications in the open literature from authors affiliated with EPA's Center for Public Health and Environmental Assessment ([2020a](#); [2019b](#); [2019a](#); [Radke et al., 2018](#)) for interpretation of epidemiological studies published prior to publication of those assessments. EPA also considered the conclusions from NASEM ([2017](#)). OPPT reviewed literature to evaluate whether data alter conclusions of these previous assessments. To do this, EPA identified population, exposure, comparator, and outcome (PECO)-relevant literature published since the most recent existing assessment of DBP. PECO-relevant literature published since the most recent existing assessment(s) of DBP was identified by applying a literature inclusion cutoff date from existing assessments of DBP. For DBP, 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 the assessments provided the most robust and recent evaluation of human epidemiologic data for DBP. 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 DBP metabolites and health outcomes. PECO-relevant literature published between 2018 to 2019 that was identified through the literature search conducted by EPA in 2019, as well as references published between 2018 to 2025 that were submitted with public comments to the DBP Docket ([EPA-HQ-OPPT-2018-0503](#)), were 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 new studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Dibutyl Phthalate* ([U.S. EPA, 2025e](#)).

As described further in the *Systematic Review Protocol for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025g](#)), 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., 2020b](#)), 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 focused its epidemiologic evaluation on urinary biomonitoring data; epidemiologic studies that examined DBP metabolites in matrices other than urine were considered supplemental and not evaluated for data quality.



The Agency used epidemiologic studies of DBP qualitatively due to the low confidence in the level of evidence for association between urinary metabolites of DBP and health outcomes. This is consistent with the conclusions of Health Canada, U.S. CPSC, ECHA, EFSA, and Australia NICNAS. EPA reviewed the conclusions from Health Canada ([2018a, b](#)) and U.S. EPA systematic review articles ([Radke et al., 2020a](#); [Radke et al., 2019b](#); [Radke et al., 2019a](#); [Radke et al., 2018](#)) and used the conclusions as a starting point for its human health hazard assessment. The Agency also evaluated and summarized epidemiologic studies identified by EPA's systematic review process to use qualitatively during evidence integration to inform hazard identification and the weight of evidence.

The Agency did not use epidemiology studies quantitatively for dose-response assessment, primarily due to uncertainty associated with exposure characterization. Primary sources of uncertainty include 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 due to rapid elimination kinetics may not be representative of average urinary concentrations that are collected over a longer term or calculated using pooled samples. Additionally, the majority of epidemiological studies examine one phthalate and one exposure period at a time such that they are treated as if they occur in isolation, which contributes additional uncertainty due to co-exposure that may confound results for the majority of epidemiologic studies ([Shin et al., 2019](#); [Aylward et al., 2016](#)).

Following release of the draft non-cancer human health hazard assessment of DBP in December 2024, EPA updated the literature considered as part of the DBP human health hazard assessment. As described further in the DBP 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 DBP**

---

The human health hazards of DBP have been evaluated in existing assessments by U.S. EPA ([1987](#)), U.S. CPSC ([2014, 2010](#)), ATSDR ([2001](#)); NTP-CERHR ([2003](#)); NASEM ([2017](#)), California OEHHA ([2007](#)), Health Canada ([Health Canada, 2020](#); [EC/HC, 2015](#)); ECB ([2004](#)), ECHA ([2017a, b, 2010](#)), EFSA ([2019, 2005](#)), and Australia NICNAS ([2013](#)). These assessments have consistently identified male reproductive development as the most sensitive outcome for use in estimating human risk from exposure to DBP. The PODs from these assessments are shown in Table 1-1.

**Table 1-1. Summary of DBP Non-Cancer PODs Selected for Use by Other Regulatory Organizations**

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	(ECHA, 2017a)	(EFSA, 2019)	(Health Canada, 2020)	(ATSDR, 2001)	(CPSC, 2014)	(NICNAS, 2013)	(OEHA, 2007)
Pregnant rats (6–8/group) exposed to 0, 20, 200, 2000, or 10,000 mg/kg DBP via diet from GD-15–PND21 (equivalent to 0, 1.5–3, 14–29, 148–291, 712–1372 mg/kg-day). F1 evaluated at PND 2, PND14, PND21, and PNW 8–11 and PNW20 ( <a href="#">Lee et al., 2004</a> )	ND/2	↓ spermatocyte development on PND 21 and ↑ incidence of vacuolar degeneration of mammary gland alveolar cells in PNW 11 males	✓ <sup>a</sup>	✓ <sup>b</sup>					✓ <sup>h</sup>
Pregnant SD rats (5–7/dose) gavaged with 0, 0.1, 1, 10, 30, 50, 100, 500 mg/kg-day DBP on GD 12–19. ( <a href="#">Lehmann et al., 2004</a> ) <sup>c</sup>	10/50	↓ fetal testis testosterone content on GD19			✓ <sup>c</sup>			✓ <sup>g</sup>	
Pregnant albino rats (6–9/group) were exposed to 0, 2, 10, or 50 mg/kg-day DBP from GD14 – parturition. Endpoints evaluated in F1 from PND 1–PND 75 ( <a href="#">Ahmad et al., 2014</a> )	10/50	↓ sperm count & percent motile sperm, ↑ percent abnormal sperm in adult F1			✓ <sup>c</sup>				
Pregnant SD rats (4–10 litters/group) gavaged with 0 0.1, 1, 10, 30, 50, 100, 500 mg/kg-day DBP on GD 12–21 ( <a href="#">Boekelheide et al., 2009</a> ).	10/30	↑ testicular pathology (↓ testicular cell number; disorganized seminiferous tubules)			✓ <sup>c</sup>				
Pregnant SD rats (19–20 or 11 (high-dose) per dose) gavaged with 0, 0.5, 5, 50, 100, 500 mg/kg-day DBP on GDs 12–21 ( <a href="#">Mylchreest et al., 2000</a> )	50/100	↑ males with nipples and/or areolae on PND 14				✓ <sup>e</sup>	✓ <sup>f</sup>		
Pregnant SD rats (20/group) gavaged with 0, 50, 250, 500 mg/kg-day DBP on GD 1–PND 21 ( <a href="#">Zhang et al., 2004</a> )	50/250	↓ AGD and ↑ nipple retention					✓ <sup>f</sup>		
Male and female C57BL/6 mice (≤ 6/group) gavaged with 0, 1, 10, 50, 100, 250, or 500 mg/kg-day DBP from PND 4–PND 14 ( <a href="#">Moody et al., 2013</a> )	1/10 (LOEL)	Delayed spermatogenesis, reduced absolute AGD (relative to BW at higher dose) in mice (PND 4–14)			✓ <sup>d</sup>				
Male SD rats (8/group) gavaged with 0, 250, 500, 1,000, or 2,000 mg/kg-day DBP from PND 35–PND 65 ( <a href="#">Xiao-Feng et al., 2009</a> )	ND/250 (LOEL)	↓ Leydig cell number			✓ <sup>d</sup>				

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	<a href="#">(ECHA, 2017a)</a>	<a href="#">(EFSA, 2019)</a>	<a href="#">(Health Canada, 2020)</a>	<a href="#">(ATSDR, 2001)</a>	<a href="#">(CPSC, 2014)</a>	<a href="#">(NICNAS, 2013)</a>	<a href="#">(OEHHA, 2007)</a>
Wistar rats (PND35, prepubertal) gavaged with 0, 250, 500, or 1,000 mg/kg-day DBP for 15 days <a href="#">(Srivastava et al., 1990)</a>	ND/250 (LOEL)	Defective spermatogenesis and reproductive tract histopathology ( <i>i.e.</i> , shrunken tubules with spongy appearance).			✓ <sup>d</sup>				
<p><i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEL = No observed adverse effect level; LOAEL = lowest observed adverse effect level; GD = gestation day; PND = postnatal day; PNW = postnatal week; F1 = first-generation offspring; AGD = anogenital distance; BW = body weight.</p> <p><sup>a</sup> LOAEL from Lee et al. <a href="#">(2004)</a> used by ECHA to calculate derived no effect levels (DNELs) (see Section B 4.2.2 of <a href="#">(ECHA, 2017a)</a>)</p> <p><sup>b</sup> LOAEL from Lee et al. <a href="#">(2004)</a> used by EFSA to derive a stand-alone tolerable daily intake (TDI) for DBP based on reproductive and developmental toxicity (see Table 24 and Section 5.1 in <a href="#">(EFSA, 2019)</a>).</p> <p><sup>c</sup> Health Canada selected a NOAEL of 10 mg/kg-day from 3 co critical studies (<a href="#">Ahmad et al., 2014</a>; <a href="#">Boekelheide et al., 2009</a>; <a href="#">Lehmann et al., 2004</a>) to calculate hazard quotients for pregnant women and women of childbearing age and infants as part of its phthalate cumulative risk assessment (see Table F-6 of <a href="#">(Health Canada, 2020)</a>). Health Canada listed the doses for Lehmann et al. <a href="#">(2004)</a> as 0, 0.1, 1, 10, 50, 100, and 500, but was missing the 30 mg/kg-day dose that was included for the testosterone radioimmunoassay (RIA) only. All other endpoints in this study do not have a 30 mg/kg-day group.</p> <p><sup>d</sup> Health Canada selected a LOEL of 10-50 mg/kg-day based on delayed spermatogenesis in male mice (<a href="#">Moody et al., 2013</a>), and two studies in rats (<a href="#">Xiao-Feng et al., 2009</a>; <a href="#">Srivastava et al., 1990</a>) to calculate hazard quotients for children (prepubertal) as part of its phthalate cumulative risk assessment (see Table F-6 of <a href="#">(Health Canada, 2020)</a>).</p> <p><sup>e</sup> NOAEL used to derive an acute-duration oral MRL for developmental effects in the offspring of rats exposed to DBP during gestation (see Section 3.12.2, pp 76 of <a href="#">(ATSDR, 2001)</a>). Neither a chronic-duration oral nor intermediate-duration oral MRL was derived.</p> <p><sup>f</sup> NOAELs from antiandrogenic endpoints (<i>i.e.</i>, nipple retention, <i>ex vivo</i> fetal testicular testosterone production, AGD) across two studies (<a href="#">Zhang et al., 2004</a>; <a href="#">Mylchreest et al., 2000</a>) were used by U.S. CPSC to assign a NOAEL for developmental toxicity of 50 mg/kg-day based on antiandrogenic endpoints (see p. 205 of <a href="#">(CPSC, 2014)</a>).</p> <p><sup>g</sup> NOAEL from Lehmann et al. <a href="#">(2004)</a> used by Australia's NICNAS to calculate MOE for reproductive toxicity.</p> <p><sup>h</sup> LOAEL from Lee et al. <a href="#">(2004)</a> used by The California EPA to calculate a Maximum Allowable Dose Level (MADL) (See pp 22 in <a href="#">(OEHHA, 2007)</a>).</p>									

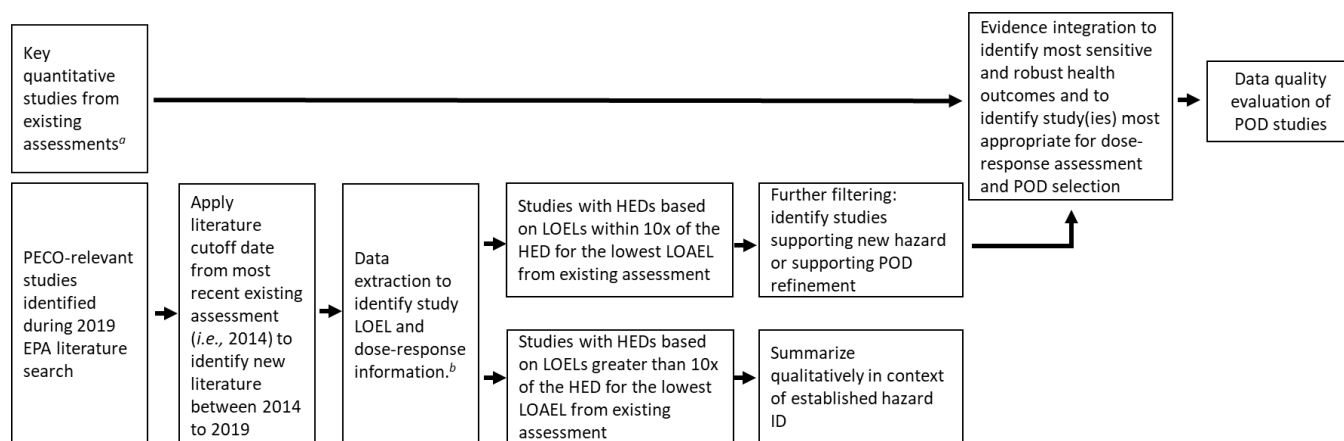


### 1.2.2 Approach to Identifying and Integrating Laboratory Animal Data

---

Figure 1-1 provides an overview of EPA's approach to identifying and integrating laboratory animal data into the DBP Risk Evaluation. EPA first reviewed existing assessments of DBP conducted by various regulatory and authoritative agencies. Existing assessments reviewed by EPA are listed below. The purpose of this review was to identify sensitive and human relevant hazard outcomes associated with exposure to DBP, and identify key studies used 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.

- *Integrated Risk Information System (IRIS), chemical assessment summary, dibutyl phthalate; CASRN 84-74-2* ([U.S. EPA, 1987](#));
- *Toxicity review of di-n-butyl phthalate (DBP)* ([CPSC, 2010](#));
- *Chronic Hazard Advisory Panel on phthalates and phthalate alternatives* ([CPSC, 2014](#));
- *Toxicological profile for di-b-phthalate* ([ATSDR, 2001](#));
- *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Butyl Phthalate (DBP)* ([NTP, 2003](#));
- *Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals* ([NASEM, 2017](#));
- *Proposition 65 Maximum Allowable Dose Level (MADL) for reproductive toxicity for di(n-butyl)phthalate (DBP)* ([OEHHHA, 2007](#));
- *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: Carcinogenicity of phthalates - mode of action and human relevance* ([Health Canada, 2015](#));
- *Screening assessment - Phthalate substance grouping* ([Health Canada, 2020](#));
- *European Union Risk Assessment Report: Dibutyl phthalate with addendum to the environmental section* ([ECJRC, 2004](#));
- *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH): Review of new available information for dibutyl phthalate (DBP) CAS No 84-74-2 Einecs No 201-557-4* ([ECHA, 2010](#));
- *Opinion on an Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP)* ([ECHA, 2017b](#));
- *Annex to the Background document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP)* ([ECHA, 2017a](#));
- *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to di-Butylphthalate (DBP) for use in food contact materials* ([EFSA, 2005](#));
- *Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials* ([EFSA, 2019](#)); and
- *Priority existing chemical assessment report no. 36: Dibutyl phthalate* ([NICNAS, 2013](#)).



**Figure 1-1. Overview of DBP Human Health Hazard Assessment Approach**

<sup>a</sup> Any study that was considered for dose-response assessment, not necessarily limited to the study used for POD selection.

<sup>b</sup> 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 DBP, EPA conducted literature searches and updates at three different timepoints, including 2014 through 2019, 2022, and 2025. These literature updates are described further below.

Similar to the epidemiological analysis, EPA used the 2015 Health Canada assessment ([EC/HC, 2015](#)) as a starting point. EPA identified key quantitative studies used to support dose-response analysis in other recent assessments and selected these key studies to inform evidence integration and dose-response analysis in this hazard assessment. EPA assumes that previous assessments effectively identified relevant key studies published prior to publication. EPA used systematic review to identify additional studies for consideration in the assessment as detailed in the *DBP Systematic Review Protocol* ([U.S. EPA, 2025q](#)). 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) across all durations (*i.e.*, acute, intermediate (>1 to 30 days), subchronic (>30 to 90 days), chronic) and routes of exposure (*i.e.*, oral, dermal, inhalation). Therefore, EPA considered additional literature published between 2014 to 2019 further as shown in Figure 1-1. EPA first screened titles and abstracts and then full texts for relevancy using PECO screening criteria described in the *Systematic Review Protocol for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025q](#)).

In development of the *Draft Non-Cancer Human Health Hazard Assessment for Dibutyl Phthalate (DBP)*, which was reviewed by the SACC during the August 4 through 8, 2025 meeting ([U.S. EPA, 2025o](#)), EPA considered PECO relevant studies identified through literature searches and updates between 2014 and 2022 and extracted key study information as described in the *DBP Systematic Review Protocol* ([U.S. EPA, 2025q](#)). Extracted information included: 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-observed-effect level (LOEL) Figure 1-1.

Information identified between 2019 and 2022 for DBP identified through systematic review was primarily limited to oral exposure studies. Study LOELs were converted to HEDs based on LOELs by

scaling allometrically across species using the three-quarter power of body weight ( $BW^{3/4}$ ) for oral data, which is the approach recommended by U.S. EPA when physiologically based pharmacokinetic (PBPK) models or other information to support a chemical-specific quantitative extrapolation is absent ([U.S. EPA, 2011b](#)). EPA's use of allometric body weight scaling is described further in Appendix D.

EPA conducted data quality evaluations for studies with HEDs based on LOELs that were within an order of magnitude of the lowest HED based on the lowest-observed-adverse-effect level (LOAEL) across existing assessments. Studies with HEDs for LOELs 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 provide information that supports a human health hazard not identified in previous assessments or to determine if they contain sufficient dose-response information to support a potentially lower POD than identified in existing assessments of DBP. Mechanistic studies and 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 and characterization process but did not undergo TSCA study quality evaluations. Instead, as discussed further in the Systematic Review protocol for DBP ([U.S. EPA, 2025q](#)), 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 final DBP human health hazard assessment. As described further in the DBP 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 DBP.

Data quality evaluations for DBP animal toxicity studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025d](#)).

### **1.2.3 Literature Identified and Hazards of Focus for DBP**

As described in the Systematic Review Protocol for DBP ([U.S. EPA, 2025q](#)), EPA identified 63 PECO-relevant animal toxicology 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, 2023a](#)), and one PECO-relevant study from its 2025 literature update. These studies (collectively identified from literatures searches and updates from 2014 to 2025) provided information pertaining to various primary hazard outcomes, including: reproduction/development, neurological, metabolic/nutritional, cardiovascular, and the immune system. Further details regarding EPA's handling of information provided in these studies are provided below, as well as in the Systematic Review Protocol for DBP ([U.S. EPA, 2025q](#)), in the supplemental file *Summary of Human Health Hazard Animal Toxicology Studies for Dibutyl Phthalate (DBP) - Literature Published from 2014 to 2019* ([U.S. EPA, 2025p](#)), and in 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, 2023a](#)).

- **Reproductive/Developmental.** EPA identified eight studies from 2014 to 2025 evaluating reproductive/developmental outcomes that provided potentially sensitive LOAELs ([Li et al., 2023](#); [Xie et al., 2019](#); [Zhang et al., 2018a](#); [Xie et al., 2016](#); [Ahmad et al., 2015](#); [de Jesus et al., 2015](#); [Sen et al., 2015](#); [Ahmad et al., 2014](#)). These studies of DBP are discussed further in

Section B.1. Of the eight, only Ahmad et al. (2014), de Jesus et al. (2015), and Li et al. (2023) evaluated endpoints relevant to phthalate syndrome (*i.e.*, histopathology, hormone levels, and/or organ weights of the male reproductive system, anogenital distance). The others evaluated a range of endpoints including changes in the estrus cycle or serum estradiol, progesterone, FSH, LH, number of ovarian follicles, reproductive organ weights (*i.e.*, ovary and or uterus), and/or pup body weights (Xie et al., 2019; Zhang et al., 2018a; Xie et al., 2016; Ahmad et al., 2015; Sen et al., 2015).

- **Neurological.** EPA identified three studies evaluating neurotoxicity that provided potentially sensitive LOAELs (Farzanehfar et al., 2016; Yan et al., 2016; Zuo et al., 2014). These studies of DBP are discussed further in Section B.2.
- **Nutritional/metabolic.** EPA identified three studies evaluating nutritional and/or metabolic outcomes that provided potentially sensitive LOAELs (Majeed et al., 2017; Ahmad et al., 2015; de Jesus et al., 2015). These studies of DBP are discussed further in Section B.3.
- **Cardiovascular.** EPA identified one study evaluating cardiovascular outcomes that provided potentially sensitive LOAELs (Xie et al., 2019). This study is discussed further in Section B.4.
- **Immune.** EPA identified two studies evaluating the immune adjuvant properties of DBP that provided potentially sensitive LOAELs (Li et al., 2014; Zuo et al., 2014). These studies of DBP are discussed further in Section B.5.
- **Chronic Toxicity/Cancer.** As discussed further 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), no two-year bioassays were identified outside of EPA's 2019 literature searches or through EPA's review of existing assessments of DBP. However, the Division of Translational Toxicology (DTT) more recently published a technical report (*i.e.*, two-year bioassays in mice and rats) (NTP, 2021), which was also considered by EPA in the development of this non-cancer TSD, and in the DBP cancer assessment (U.S. EPA, 2025a).

The most sensitive and robust PODs selected from existing hazard assessments of DBP are based on effects on the developing male reproductive system (EFSA, 2019; ECHA, 2017a; OEHHHA, 2007). Existing assessments have consistently shown that effects on other health outcomes (*i.e.*, female reproduction, neurological, cardiovascular, metabolic) are generally observed at higher dose levels than developmental effects on male reproduction or are not supported by as robust databases of studies. This is further supported by the more recent literature published from 2014 to 2025, as some of the lowest LOAELs were identified for reproductive and developmental effects (Table\_Apx B-1). Therefore, the Agency focused its non-cancer human health hazard assessment on toxicity to the male reproductive system following developmental exposures (Section 3.1).

Genotoxicity and carcinogenicity data for DBP 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

EPA identified several animal studies and 3 human studies ([Koch et al., 2012](#); [Seckin et al., 2009](#); [Anderson et al., 2001](#)) that evaluated the absorption, distribution, metabolism, and/or excretion (ADME) of DBP following oral exposure. In humans and rodents, DBP undergoes hydrolysis to the bioactive metabolite, mono-n-butyl phthalate (MBP) by non-specific lipases and esterases in the gastrointestinal tract ([Takahashi and Tanaka, 1989](#); [White et al., 1980](#); [Lake et al., 1977](#); [Rowland et al., 1977](#)), and a relatively small amount of the parent (DBP) reaches circulation ([White et al., 1980](#)). Human and rodent lipases show similar rates of conversion of DBP to MBP ([Lake et al., 1977](#)). MBP is rapidly absorbed and broadly distributed throughout the body with minimal bioaccumulation ([Fennell et al., 2004](#); [Foster et al., 1983](#); [Tanaka et al., 1978](#)). MBP can be excreted unchanged or undergo further oxidation to produce more hydrophilic oxidative products. Alternatively, MBP can undergo phase II biotransformation by glucuronosyltransferase, whereby MBP reacts with glucuronic acid to form glucuronide conjugates, namely MBP-glucuronide (MBP-G). In rat serum, 80 to 90 percent of the total MBP is free monobutyl phthalate and the remainder is MBP-G ([ATSDR, 2001](#); [Albro and Moore, 1974](#)). This differs from humans, where 25 to 30 percent of the total MBP in serum is free MBP and the remainder is MBP-G ([Silva et al., 2003](#)).

MBP can also be metabolized further through hydrolysis to phthalic acid, or through oxidation to produce 3-hydroxybutyl phthalate (3OH-MBP), 4-hydroxybutyl phthalate (4OH-MBP), 3-ketobutyl phthalate, or 4-carboxypropyl phthalate. Mono-carboxy-propyl phthalate (MCP) has also been detected in humans and rats exposed to DBP but is a minor metabolite (Table 2-1; Figure 2-1). In animals and humans, MBP and MBP-glucuronide are the primary metabolites of DBP ([ATSDR, 2001](#)). MBP and MBP-glucuronide are eliminated primarily in urine ([ATSDR, 2001](#); [Foster et al., 1983](#)), and to a smaller extent in feces ([Chang et al., 2013](#); [Fennell et al., 2004](#); [Saillenfait et al., 1998](#)). Enterohepatic circulation has been reported ([Tanaka et al., 1978](#)). A summary of different metabolites found in human and rat urine after oral administration of DBP is presented in Table 2-1.

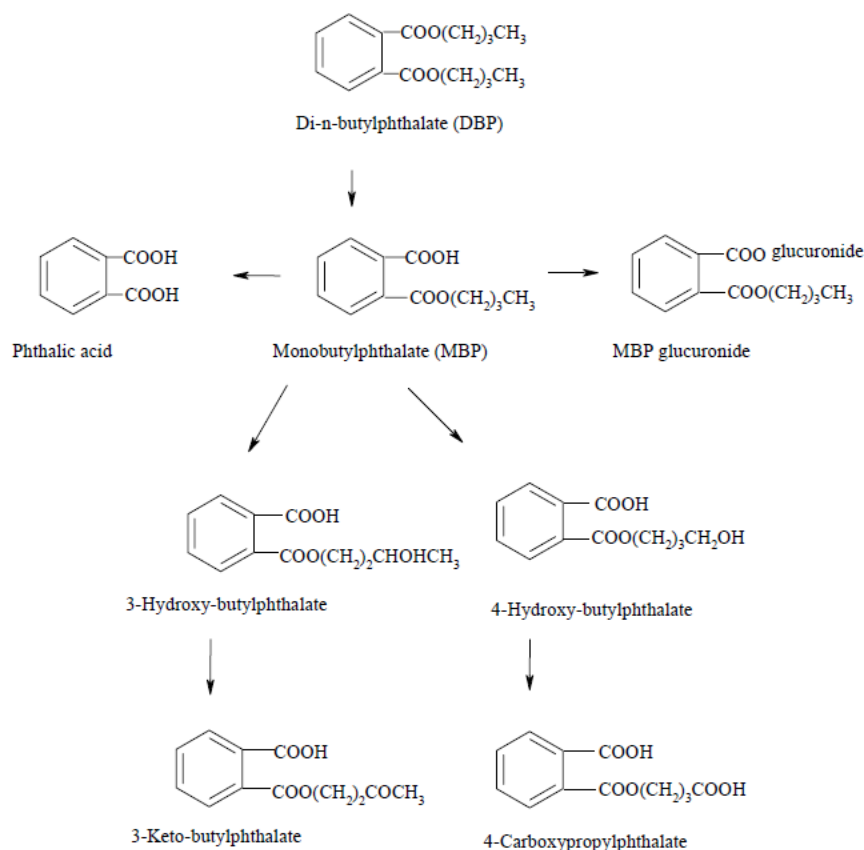
The elimination of various metabolites of DBP have been described in animals and humans. Since DBP does not bioaccumulate, excretion may also be an indicator for absorption. Studies in pregnant and non-pregnant animals have demonstrated that most (67 to 97 percent) of the administered dose is excreted in urine within 24 hours ([Chang et al., 2013](#); [Saillenfait et al., 1998](#); [Foster et al., 1983](#); [Tanaka et al., 1978](#)). The elimination half time has been estimated to be approximately 3-hours in plasma of pregnant rats given oral doses of 50 to 250 mg/kg-day DBP ([Fennell et al., 2004](#)). Similarly, it has been reported to be approximately 3.6 hours in rats given an intravenous injection of 30 mg/kg-day DBP ([Chang et al., 2013](#)). In humans, three separate studies suggest elimination of DBP between 73 and 92.2 percent within 24 hours ([Koch et al., 2012](#); [Seckin et al., 2009](#); [Anderson et al., 2001](#)). In Anderson et al. (2001), 24 volunteers consumed DBP (255 or 510 µg) administered in margarine spread on toast, and 73 percent of the administered dose was excreted in 24 hours. In Seckin et al. (2009), 17 volunteers ingested a capsule containing 3,600 µg DBP, and 78 percent of the administered dose was excreted within 24 hours. In Koch et al. (2012), one male volunteer ingested 60 µg/kg body weight of DBP, and 92.2 percent of the dose was eliminated within 24 hours. Koch et al. (2012) and Seckin et al. (2009) also provide data on elimination kinetics. In Koch et al., the elimination half-time for MBP was 2.6 hours, and approximately 6 hours for other metabolites, such as 3OH-MBP and 4OH-MBP ([Koch et al., 2012](#)). Seckin et al. reported a urinary elimination half-time for MBP of 6 hours in one individual who consumed a tablet containing 3,600 µg DBP ([Seckin et al., 2009](#)).

There are species differences in some aspects of DBP biotransformation such as excretion of MBP or MBP-G, which suggest differences in metabolism of MBP. Indeed, the proportion of MBP-G:MBP excreted differed in rats (1:1), guinea pigs (1.5:1), and hamsters (2.3:1) ([Tanaka et al., 1978](#)). Moreover, an *in vitro* study demonstrated that rates of MBP glucuronidation in pooled liver microsomes differ across species, where microsomes from mice and rats had slower rates of MBP-G formation from MBP substrate than human liver microsomes from rats with humanized livers ([Miura et al., 2019](#)). The data suggest that human liver microsomes have a faster rate of glucuronidation of MBP than mouse or rat microsomes. Additionally, human biomonitoring studies (*e.g.*, NHANES ([Silva et al., 2003](#))) and one human ingestion study ([Seckin et al., 2009](#)) have demonstrated that MBP in human urine and plasma is conjugated with glucuronide. In contrast, most MBP is mostly unconjugated in rodents.

**Table 2-1. Metabolites of DBP Identified in Urine from Rats and Humans after Oral Administration**

Urinary Metabolite	Abbreviation	Rat	Human <sup>a</sup>	Reference(s)
Mono-n-butyl phthalate	MBP	✓	✓	( <a href="#">Koch et al., 2012</a> ) (human) ( <a href="#">Seckin et al., 2009</a> ) (human) ( <a href="#">Anderson et al., 2001</a> ) (human) ( <a href="#">Silva et al., 2003</a> ) (human) <sup>a</sup> ( <a href="#">Foster et al., 1983</a> ) (rat) ( <a href="#">Albro and Moore, 1974</a> ) (rat) ( <a href="#">Tanaka et al., 1978</a> ) (rat, hamster) ( <a href="#">Clewell et al., 2009</a> ) (rat) <sup>b</sup> ( <a href="#">Fennell et al., 2004</a> ) (rat) <sup>c</sup>
Mono-n-butyl phthalate glucuronide	MBP-G	✓	✓	( <a href="#">Seckin et al., 2009</a> ) (human) ( <a href="#">Silva et al., 2003</a> ) (human) <sup>a</sup> ( <a href="#">Foster et al., 1983</a> ) (rat)
mono-carboxy-propyl phthalate	MCP	✓	✓	( <a href="#">Koch et al., 2012</a> ) (human) ( <a href="#">Calafat et al., 2006</a> ) (human <sup>a</sup> , rat) ( <a href="#">Albro and Moore, 1974</a> ) (rat)
3-hydroxybutyl phthalate	3OH-MBP	✓	✓	( <a href="#">Koch et al., 2012</a> ) (human) ( <a href="#">Albro and Moore, 1974</a> ) (rat)
4-hydroxybutyl phthalate	4OH-MBP	✓	✓	( <a href="#">Albro and Moore, 1974</a> ) (rat) ( <a href="#">Tanaka et al., 1978</a> ) (rat, hamster)
3-ketobutyl phthalate	–	✓	ND	( <a href="#">Albro and Moore, 1974</a> ) (rat) ( <a href="#">Tanaka et al., 1978</a> ) (rat)
4-carboxypropyl phthalate	–	✓	ND	( <a href="#">Albro and Moore, 1974</a> ) (rat) ( <a href="#">Tanaka et al., 1978</a> ) (rat)
Monobutanoic phthalic acid	–	✓	ND	( <a href="#">Fennell et al., 2004</a> ; <a href="#">General Motors, 1983a</a> ) (rat) <sup>c</sup>
Mono-n-hydroxybutylphthalate	–	✓	ND	( <a href="#">Fennell et al., 2004</a> ; <a href="#">General Motors, 1983a</a> ) (rat)
mono-1-hydroxybutan-2-one phthalic acid glucuronide	–	✓	ND	( <a href="#">Fennell et al., 2004</a> ) (rat)
Phthalic acid	PA	✓	ND	( <a href="#">Fennell et al., 2004</a> ; <a href="#">General Motors, 1983a</a> ) (rat) ( <a href="#">Albro and Moore, 1974</a> ) (rat)
ND = no data available				

Urinary Metabolite	Abbreviation	Rat	Human <sup>a</sup>	Reference(s)
<sup>a</sup> Metabolites detected as part of human urinary biomonitoring studies ( <a href="#">Calafat et al., 2006</a> ; <a href="#">Silva et al., 2003</a> ) not controlled exposure studies. Although biomonitoring studies do not distinguish between routes or pathways of exposure, urinary metabolites are shown for comparison to urinary metabolites detected in rodent models. <sup>b</sup> Reflects pup plasma concentrations on PND2 following exposure of dams from GD12 – PND14. <sup>c</sup> Reflect maternal urine concentrations 24 hours after a single dose of 100 mg/kg DBP on GD20 in CD rats.				



**Figure 2-1. Proposed Metabolic Pathway of DBP Following Oral Exposure (From ([ECJRC, 2004](#)))**

Note that metabolism of OH-MnBP into MCPP has been reported to occur in humans ([Koch et al., 2012](#)) and rats ([Calafat et al., 2006](#)) but is not shown in the figure.

Based on the reasonably available data, which indicate DBP is readily absorbed and most of the administered dose is eliminated in the urine within 24 hours following oral exposure in humans and rats, EPA assumes an oral absorption of 100 percent for the risk evaluation of DBP. This is consistent with assumptions used for adults and children in other existing assessments of DBP ([EFSA, 2019](#); [ECHA, 2017a](#); [NICNAS, 2013](#)).

## 2.2 Inhalation Route

EPA identified two inhalation studies of rats, but each had several uncertainties that preclude their use to inform the ADME of DBP following inhalation exposure in animals. However, inhalation studies from other phthalates (*i.e.*, DEHP and DIDP) exist and may be informative. No studies in humans were available that evaluated the ADME properties of DBP for the inhalation route.

Kawano (1980) and Walseth (1984) each provide data that DBP is absorbed and distributes to other tissues following inhalation exposure. Kawano (1980) exposed male Wistar rats to aerosolized DBP via a non-continuous whole body inhalation exposure. The target concentration was 50 mg/m<sup>3</sup>, and the actual concentration of DBP ranged from approximately 45 to 60 mg/m<sup>3</sup> over the course of 100 days, as verified by gas chromatography. Animals were exposed 6 hours/day on weekdays, 3 hours/day on Saturday, and rest day every Sunday. Changes in body and organ weight were observed, as well as changes in liver enzyme levels and changes in white blood cell counts. Walseth et al. (1984) exposed male Sprague Dawley rats to 0.5, 2.5, or 7 ppm DBP aerosols 6 hours/day for 5 days (equivalent to 5.7, 28.4, or 79.5 mg/m<sup>3</sup>). Rats were exposed via whole body inhalation in chambers, and exposure concentrations were verified via gas chromatograph, but the data for the aerosol concentrations during the experimental period were not provided. Changes in the activities of cytochrome P450 enzymes were observed in liver and lung samples. No data are available on DBP metabolism following inhalation exposure. CYPs and glucuronosyltransferases are included among the xenobiotic metabolizing enzymes found in the respiratory tract, so it is feasible that metabolism of DBP to MBP and MBP-G occurs in the lung. No data are available for elimination following inhalation exposure. Neither study characterized the particle size distribution (*e.g.*, no reporting of mass median aerodynamic diameter [MMAD] or geometric standard deviation [GSD]), which is an important limitation. The systemic effects observed by Kawano (1980) and Walseth (1984) may indicate that some absorption occurs in the lung. However, these data are difficult to interpret due to the whole-body inhalation exposure method in both available studies, including the potential for DBP deposited on the fur during whole body exposure and subsequent grooming resulting in oral exposure. The aforementioned limitations (*i.e.*, exposure method, lack of presentation of MMAD or GSD) limit the ability to quantify the achieved dose from these two whole-body inhalation studies.

Inhalation studies for other phthalates such as DIDP and DEHP exist (1991, 1983b), which may provide some insight into the toxicokinetic properties of DBP. In the DIDP exposure study, rats were exposed to aerosolized <sup>14</sup>C-DIDP (target concentration was 91 mg/m<sup>3</sup>; MMAD was 0.98 µm) via head-only inhalation for 6 hours/day, 5 days/week for 2 weeks. In the DEHP exposure study, rats were exposed to aerosolized <sup>14</sup>C-DEHP (target concentration was 100 mg/m<sup>3</sup>; MMAD was 0.6 µm) via head-only inhalation for 6 hours/day, 5 days/week for 2 weeks. In the DIDP study, absorption through the lung was approximately 73 percent over 72 hours. In the DEHP study, absorption through the lung was approximately 92 percent after 72 hours. Collectively, these studies of structurally similar phthalates provide some indication that DBP can be expected to be readily absorbed through the lung.

No data from animal models are reasonably available for the inhalation route that are suitable for deriving a route-specific POD. Therefore, EPA extrapolated the inhalation POD from the oral POD, assuming similar absorption for the oral and inhalation routes, and no adjustment was made when extrapolating between exposure routes. EPA assumes an inhalation absorption of 100 percent for the risk evaluation of DBP. This is consistent with assumptions used in existing assessments (NICNAS, 2013).

## 2.3 Dermal Route

EPA identified two *in vivo* studies (Doan et al., 2010; Elsisi et al., 1989) and three *in vitro/ex vivo* studies (Sugino et al., 2017; Beydon et al., 2010; Scott et al., 1987) that evaluated the ADME properties of DBP following dermal application. An additional study in humans was identified that provided data following dermal application of skin cream containing several phthalates, including DBP (Janjua et al., 2008).



Elsisi et al. (1989) provided data on the dermal absorption of eight phthalate diesters including DBP by measuring the percentage of dose excreted in the urine and feces daily over the 7-day exposure. Radiolabeled DBP ( $^{14}\text{C}$ -DBP) (5 to 8 mg/cm<sup>2</sup>) was applied to a circular area 1.3 centimeters in diameter (1.3 cm<sup>2</sup>) on the shaved skin on the backs of male F344 rats, and the application site was covered with a perforated circular plastic cap for seven days. Low levels (less than one percent for combined tissues) of  $^{14}\text{C}$  were found in adipose tissue, muscle, skin, and other tissues (*i.e.*, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord, and blood), suggesting DBP or its metabolites were systemically distributed. In the first 24 hours, 11 percent of the administered dose of DBP was excreted in urine and 1 percent was excreted in the feces. DBP was excreted at a near constant rate of 10 to 12 percent every 24 hours. After 7 days of exposure, approximately 61 percent of the applied dose was recovered in urine or feces. Based on the amount of radioactivity recovered from urine, feces, and other tissues, study authors estimated that approximately 66 percent of the applied dose of  $^{14}\text{C}$ -DBP was absorbed over seven days. The total recovery of the applied dose was 100 percent. Most of the applied dose was recovered in the urine and feces, and 33 percent of the applied dose was recovered from skin at the application site. DBP had a fast rate of excretion relative to other phthalates tested (*i.e.*, DEHP, DIBP, BBP, DIDP, DEP, DMP, and DHP), which may be related to its relatively low molecular weight and branched structure.

A more recent study in hairless guinea pigs (Doan et al., 2010) also reported high dermal absorption of DBP. Following a single dermal application via covered patch (3 x 3-centimeter square area; 9 cm<sup>2</sup>) of an emulsion containing 1 mg/cm<sup>2</sup> DBP, *in vivo* dermal absorption of DBP was estimated to be approximately 62 percent of the applied dose after 24 hours (Doan et al., 2010). The percent total recovery was 92.9 percent after 24 hours. The major strength of the *in vivo* part of this study was that the outcomes assessment method mostly agreed with guideline OECD 427 (OECD, 2004a). The study also included an *ex vivo* experiment, where skin was excised from the guinea pigs (anatomical site of the tissue collections was not specified) and radiolabeled DBP (1 mg/m<sup>2</sup>) was applied to a skin preparation. A total of 56.3 percent of the administered dose was absorbed after 6 hours, and the percent total recovery was 96.3 percent of the administered dose. Strengths of the *ex vivo* part of this study include that the test system was un-occluded, the skin was washed prior to application, and overall, the study complies with OECD guideline 428 (OECD, 2004b).

Scott et al. (1987) used epidermal membranes prepared from human abdominal skin and dorsal rat skin to compare percutaneous absorption rates of four phthalates, including DBP and DEHP. The authors also compared the permeability of human skin compared to rat skin. DBP is much more readily absorbed in rat skin than in human skin (steady state absorption rate:  $2.40 \pm 0.63 \mu\text{g}/\text{cm}^2/\text{hr}$  [human],  $93.35 \pm 0.94 \mu\text{g}/\text{cm}^2/\text{hr}$  [rat]), which is related to the relatively higher permeability of rat skin (permeability constant:  $0.23 \pm 0.06 \times 10^{-5} \text{ cm}/\text{hr}$  [human],  $8.95 \pm 0.09 \times 10^{-5} \text{ cm}/\text{hr}$  [rat]). A more recent *ex vivo* study by Sugino et al. (2017) also noted species differences between humans and rats. That study applied DBP to mounted skin membranes prepared from hairless rats or from human skin and evaluated the permeability of the skin. After dermal application of DBP, esterases in the skin hydrolyze DBP to MBP, which subsequently permeates the skin. The steady state permeability coefficients for MBP across stripped skin ( $K_p$ ) were  $6.8 \times 10^{-5} \pm 2.2 \times 10^{-5} \text{ cm}/\text{sec}$  in rats and  $7.2 \times 10^{-6} \pm 1.1 \times 10^{-6} \text{ cm}/\text{sec}$  in humans. This is equivalent to 0.245 cm/hr in rats and 0.026 cm/hr in humans, when adjusting for the BP metabolite. These values reflect faster rates for the metabolite of DBP (*i.e.*, MBP) than the rates for the parent chemical described by Scott et al. (1987).

An *ex vivo* study of dermal absorption by Beydon et al. (2010) evaluated percutaneous absorption and metabolism of DBP by skin esterases in skin samples from humans, rats, rabbits, guinea-pigs, and mice. DBP was hydrolyzed by carboxylesterases in the skin of all species evaluated; therefore, carboxylesterase activity was measured in addition to skin thickness and flux to determine the

relationship between DBP absorption flux and enzymatic activity across species. Beydon et al. (2010) reported differences in skin thickness across species, as well as fluxes. DBP fluxes for rats were 40 to 90 times higher for rats than humans ( $24.0 \pm 5.2 \mu\text{g}/\text{cm}^2/\text{hr}$  [Hairy rats] and  $48.9 \pm 17.7 \mu\text{g}/\text{cm}^2/\text{hr}$  [Hairless rats] compared to  $0.59 \pm 0.25 \mu\text{g}/\text{cm}^2/\text{hr}$  [human skin]), which is a similar finding to the aforementioned results of Scott et al. (1987). Of the species examined in Beydon et al. (2010), guinea-pig skin had the most comparable DBP flux to human skin. Nevertheless, DBP flux of guinea pig skin was approximately ten times higher than the DBP flux of human skin (Humans:  $0.59 \pm 0.25 \mu\text{g}/\text{cm}^2/\text{hr}$ ; Guinea pigs:  $5.39 \pm 0.88 \mu\text{g}/\text{cm}^2/\text{hr}$ ). Human and guinea-pig skin thickness ( $1.38 \pm 0.17 \text{ mm}$ ,  $1.31 \pm 0.05 \text{ mm}$ ) and epidermis and dermis carboxylesterase activities were comparable.

A study in humans provided data consistent with low dermal absorption of DBP following application of a skin cream formulation (Janjua et al., 2008). In that study, the authors applied  $2 \text{ mg}/\text{cm}^2$  of a control cream (no added phthalates) or a cream with 2 percent (weight-to-weight) DBP (and other phthalates) to the skin of participants (whole body topical application) for daily for 5 consecutive days. Urine was collected via a 24-hour pooled collection method, and concentration of MBP was analyzed to estimate absorption of DBP. The maximum dermal absorption in human participants in that study corresponded to approximately 6 percent of the applied dose of DBP. However, this study had significant limitations, including very large inter-individual variability in absorption values and daily variations in values for the same individual.

Although specific data on DBP dermal absorption in humans is limited to two *in vitro* studies (Beydon et al., 2010; Scott et al., 1987), several regulatory agencies (e.g., Danish EPA, ECHA, NICNAS) recognize that absorption of phthalates would likely be lower in human skin than through rat skin. This observation is based on data from *in vitro* migration studies conducted with DEHP and other phthalates. Notably, other regulatory agencies (e.g., Australia NICNAS, ECHA) have reached similar conclusions regarding the low dermal absorption of DBP (ECHA, 2013; NICNAS, 2012).

Details of the approach used by EPA to estimate exposure via the dermal exposure route for occupational, consumer, and general population exposure assessments can be found in the *Environmental Release and Occupational Exposure Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2025f) and *Consumer and Indoor Dust Exposure Assessment for Dibutyl Phthalate (DBP)* (U.S. EPA, 2025b). In sum, EPA used DBP dermal absorption data from the Beydon et al. (2010) study to estimate dermal absorption of liquid products and liquid formulations of DBP because this study was determined to be the most suitable dermal absorption study. Using Beydon et al. (2010), EPA derived an estimate of the steady-state flux of neat DBP of  $5.9 \times 10^{-4} \text{ mg}/\text{cm}^2/\text{h}$ .

## 2.4 Additional Toxicokinetic Considerations

---

### *Transfer across the placenta*

DBP and its metabolites can be transferred across the placenta to the fetus during gestation, including to the fetal testis, which is the target organ of toxicity (Section 3.1) (Clewett et al., 2009; Kremer et al., 2005; Fennell et al., 2004; Saillenfait et al., 1998). In pregnant Sprague-Dawley rats given a single oral dose of 0.5 or 1.5 g/kg radiolabeled DBP (di-n-butyl[carboxyl- $^{14}\text{C}$ ]phthalate) on GD14, radiolabel was detected in the plasma, placenta, embryo, and amniotic fluid within a half hour (Saillenfait et al., 1998). MBP was the major metabolite in plasma and MBP-glucuronide was the minor metabolite from both pregnant rats and the fetus. These findings were supported by additional studies, including that of Fennell et al. (2004), who reported MBP and MBP-glucuronide in plasma of the dams exposed to DBP, as well as the amniotic fluid and plasma of the fetus. Following exposure to 50 or 100 mg/kg DBP, the time to reach maximum plasma concentration ( $T_{\text{max}}$ ) in the maternal plasma for MBP and MBP-glucuronide is 0.5 hour and 1 hour, respectively. The  $T_{\text{max}}$  for fetal plasma is 1 and 4 hours, respectively. Kremer et al.

([2005](#)) provide data that further support transfer of DBP metabolite across the placenta. Briefly, 50 mg/kg MBP was administered to pregnant rats via intravenous injection on GD19. Levels of MBP-G were higher in fetal plasma than maternal plasma 24 hours after dosing, which may imply that MBP-G is diffusion limited from fetus to dam.

Clewell et al. ([2009](#)) provide data that demonstrate MBP is found in the fetal testes in addition to fetal plasma and embryonic tissue. In this study, pregnant rats were administered 0, 50, 100, or 500 mg/kg-day DBP via gavage from GD 12 through GD 19. Testes samples were collected from pooled (by litter) fetal testes to measure DBP metabolites. Testes samples from the low dose group (50 mg/kg-day) were below the limit of quantification for MBP, but at 100 and 500 mg/kg-day MBP was measured in testes for at least 4 hours after the final dose.

### ***Inter-individual and intra- species considerations***

Inter-individual and intra-species differences exist across various toxicokinetic parameters which may in turn impact toxicity. Interspecies differences in DBP toxicity, including to the male reproductive system, have been demonstrated ([Gray et al., 1982](#)), which may reflect species-specific differences in toxicokinetics. Some studies have demonstrated differences in toxicokinetics across species. For instance,  $\beta$ -glucuronidase activity in testicular tissue was shown to be higher in rats than in hamsters ([Foster et al., 1983](#)). For dermal exposures to DBP, data from Scott et al. ([1987](#)), Beydon et al. ([2010](#)), and Sugino et al. ([2017](#)) demonstrate that there are large differences in the absorption rates of DBP between human and rodent skin. These are important when considering the dermal absorption data provided by Elsis et al. where 10 to 12 percent of DBP applied to rodent skin is absorbed and excreted every 24 hours. There are also inter-individual ADME differences to account for, including age-related differences in metabolism of DBP in humans. For instance, the activity of glucuronosyltransferase differs between adults and infants, where adult activity is higher and achieved at 6 to 18 months of age ([Leeder and Kearns, 1997](#)). Additionally, toxicokinetic differences exist between males and females in general, and the paucity of data comparing toxicokinetic parameters across the sexes may also be considered. Toxicokinetic factors that modify susceptibility to DBP are further discussed in Section 5.

## **2.5 Summary**

---

The majority of data pertaining to the absorption, distribution, metabolism, and excretion of DBP are of oral exposure studies. Following oral exposure, DBP is hydrolyzed in the gut to the bioactive phthalate monoester, MBP, and rapidly absorbed in the gastrointestinal tract. MBP is broadly distributed throughout the body, and minimal bioaccumulation occurs. MBP and MBP-G are the predominant metabolites in humans and rodents. Most of the administered dose of DBP is excreted in urine within 24 hours, and a small proportion is also eliminated in the feces. DBP and its metabolites can cross the placenta to the developing fetus.

The reasonably available data on other routes of exposure are sparse, especially for inhalation. Studies that do exist for dermal routes of exposure suggest dermal absorption of approximately 11 percent and indicate a prerequisite for maximal absorption is hydrolysis of DBP to MBP by serine esterases in the skin ([Sugino et al., 2017](#)). Inter-individual and intra-species differences exist across various toxicokinetic parameters (e.g., species differences in skin thickness affect dermal absorption; differences in metabolism) which may in turn impact toxicity.

Given the toxicokinetic information available for DBP, EPA assumes an oral absorption of 100 percent and an inhalation absorption of 100 percent for the risk evaluation. The approach EPA used to estimate exposure via dermal routes of exposure is covered in the *Environmental Release and Occupational Exposure Assessment for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025f](#)) and *Consumer and Indoor Dust Exposure Assessment for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025b](#)).

### 3 NON-CANCER HAZARD IDENTIFICATION

---

As was stated in Section 1.2.3, EPA focused its hazard identification on effects on the developing male reproductive system. EPA evaluated non-cancer effects across studies cited in existing assessments and identified from 2014 to 2025 literature searches and/or updates, as described in Section 1.2.2. Other hazards considered by EPA but not used for point of departure derivation, such as neurotoxicity, metabolic effects, cardiovascular toxicity, and immune adjuvant effects that were evaluated as part of EPA's further filtering process are presented in Section 3.1.3.

The sections below focus on hazard identification, characterization, and weight of evidence analysis of on effects associated with the developing male reproductive system (3.1.2.1), which are the most sensitive human health hazard outcomes associated with oral exposure to DBP in laboratory animals. Several studies have also evaluated the effects of DBP exposure on the nervous system, cardiovascular system, immune system, and metabolism. Although the data on the health effects on animals following developmental exposures is abundant, data following chronic exposure durations to adult animals is limited to one well-conducted NTP technical report ([NTP, 2021](#)) with 2-year studies in mice and rats that provide far less sensitive LOAELs (*i.e.*, above 500 mg/kg-day) than the developmental studies. In the risk evaluation of DBP, effects on the developing male reproductive system form the basis of the POD used for acute, intermediate, and chronic exposure scenarios.

#### 3.1 Effects on the Developing Male Reproductive System

---

##### 3.1.1 Summary of Available Epidemiological Studies

---

###### 3.1.1.1 Previous Epidemiology Assessment (Conducted in 2019 or earlier)

---

EPA reviewed and summarized conclusions from previous assessments conducted by Health Canada ([2018b](#)) and NASEM ([2017](#)) as well as systematic review articles by Radke et al. ([2019b](#); [2018](#)) that investigated the association between exposure to DBP metabolites and male and female developmental and reproductive outcomes. As can be seen from Table 3-1, epidemiologic assessments by Health Canada ([2018b](#)), NASEM ([2017](#)), and systematic review articles by Radke et al., ([2019b](#); [2018](#)) varied in scope and considered different developmental and reproductive outcomes. Further, these assessments used different approaches to evaluate epidemiologic studies for data quality and risk of bias in determining the level of confidence in the association between phthalate exposure and evaluated health outcomes (Table 3-1). Sections 3.1.1.1.1, 3.1.1.1.2, and 3.1.1.1.3 provide further details on previous assessments of DBP by Health Canada ([2018b](#)), Radke et al., ([2019b](#); [2018](#)) and NASEM ([2017](#)), respectively, including conclusions related to exposure to DBP and health outcomes. Additionally, EPA also evaluated epidemiologic studies published after the Health Canada ([2018b](#)) assessment (*i.e.*, published between 2018 and 2019, or identified during the public comment period between the draft and final *Non-Cancer Human Health Hazard Assessment* technical support document) to determine if more recent epidemiologic studies would change the conclusions of existing epidemiologic assessments or provide useful information for evaluating exposure-response relationship. (Section 3.1.1.2).

**Table 3-1. Summary of Scope and Methods Used in Previous Assessments to Evaluate the Association Between DBP Exposure and Male Reproductive Outcomes**

Previous Assessment	Outcomes Evaluated	Method Used for Study Quality Evaluation
Health Canada ( <a href="#">2018b</a> )	<b>Hormonal effects:</b> <ul style="list-style-type: none"> <li>Sex hormone levels (<i>e.g.</i>, testosterone)</li> </ul> <b>Growth &amp; Development:</b> <ul style="list-style-type: none"> <li>AGD</li> <li>Birth measures (<i>e.g.</i>, low birth weight)</li> <li>Male infant genitalia (<i>e.g.</i>, hypospadias/cryptorchidism)</li> <li>Placental development and gene expression</li> <li>Preterm birth and gestational age</li> <li>Postnatal growth</li> <li>DNA methylation</li> </ul> <b>Reproductive:</b> <ul style="list-style-type: none"> <li>Altered male puberty</li> <li>Gynecomastia (<i>i.e.</i>, the increase of male breast glands in pubescent boys)</li> <li>Changes in semen parameters</li> <li>Sexual dysfunction (males)</li> <li>Sex ratio</li> </ul>	Downs and Black ( <a href="#">1998</a> )
Radke et al. ( <a href="#">2018</a> )	<ul style="list-style-type: none"> <li>AGD</li> <li>Hypospadias/cryptorchidism</li> <li>Pubertal development</li> <li>Semen parameters</li> <li>Time to pregnancy</li> <li>Testosterone</li> <li>Timing of pubertal development</li> </ul>	Approach included study sensitivity as well as risk of bias assessment consistent with the study evaluation methods described in ( <a href="#">U.S. EPA, 2022</a> )
Radke et al. ( <a href="#">2019b</a> )	<ul style="list-style-type: none"> <li>Pubertal development</li> <li>Time to pregnancy (Fecundity)</li> <li>Preterm birth</li> <li>Spontaneous abortion</li> </ul>	ROBINS-I ( <a href="#">Sterne et al., 2016</a> )
NASEM ( <a href="#">2017</a> )	<ul style="list-style-type: none"> <li>AGD</li> <li>Hypospadias (incidence, prevalence, and severity/grade)</li> <li>Testosterone concentrations (measured at gestation or delivery).</li> </ul>	OHAT (based on GRADE) ( <a href="#">NTP, 2015</a> )
<i>Abbreviations:</i> AGD = anogenital distance; ROBINS-I= Risk of Bias in Non-randomized Studies of Interventions; OHAT = National Toxicology Program’s Office of Health Assessment and Translation; GRADE = Grading of Recommendations, Assessment, Development and Evaluation.		

#### 3.1.1.1.1 Health Canada ([2018b](#))

Health Canada ([2018b](#)) considered 83 studies that evaluated the association between DBP and its metabolites (MBP/MnBP) and reproductive outcomes. The outcomes that were evaluated are listed in Table 3-1. Female reproductive outcomes were also evaluated by Health Canada (*e.g.*, altered female



puberty, pregnancy complications and loss, altered fertility and time to pregnancy, endometriosis and adenomyosis, uterine leiomyoma, sexual dysfunction in females, polycystic ovary syndrome, age at menopause). Health Canada considered associations with prenatal, perinatal, and adult exposures.

Health Canada evaluated studies that looked at individual phthalates (or their metabolites) and health outcomes, due to the challenging nature of interpreting results for the sum of several phthalates. To evaluate the quality of individual studies and risk of bias, Health Canada (2018b) used the Downs and Black evaluation criteria (Downs and Black, 1998) which is based on the quality of the epidemiology studies and the strength and consistency of the relationship between a phthalate and each health outcome. The level of evidence for association of a phthalate and each health outcome was established based on the quality of the epidemiology studies and the strength and consistency of the association.

There was limited evidence<sup>1</sup> for the association between DBP and its metabolites and sperm DNA damage/apoptosis, uterine leiomyoma, and sex ratio at birth. There was inadequate evidence for the association between DBP and its metabolites and sexual dysfunction in males and females, polycystic ovary syndrome, and age at menopause. The level of evidence could not be established for the association between DBP and its metabolites and altered fertility. There was no evidence for the association between exposure to DBP and its metabolites and endometriosis and adenomyosis. All other reproductive outcomes (*i.e.*, altered male or female puberty, gynecomastia, pregnancy complication and loss) did not have reported evidence of association with DBP and/or its metabolites.

Sixty-five studies were assessed by Health Canada (2018b) to evaluate the association between exposure to DBP and growth and developmental outcomes. These studies evaluated outcomes such as AGD, birth measures, male infant genitalia, placental development and gene expression, preterm birth and gestational age, as well as postnatal growth and DNA methylation. There was inadequate evidence of association for DBP and its metabolites and the following outcomes: birth measures, placental development, preterm birth and gestational age, postnatal growth and postnatal DNA methylation. There was no evidence of association for DBP and its metabolites and AGD. Health Canada (2018b) did not report evidence of an association between exposure to DBP and altered development of male infant genitalia (*e.g.*, hypospadias and cryptorchidism).

The relationship between DBP and its metabolites and the human endocrine system was investigated in 48 studies by Health Canada (2018b). Effects on thyroid-related hormones, sex hormones, and other hormones were the three categories used to evaluate the hormonal effects. The authors found that there was limited evidence for association between MBP/MnBP with sex hormone levels (*i.e.*, follicle stimulating hormone, luteinizing hormone, testosterone, estradiol, prolactin, inhibin B, anti-Mullerian hormone, androstenedione). There was inadequate evidence for association between MBP/MnBP and thyroid-related hormones or growth hormone homeostasis.

#### 3.1.1.1.2 Radke et al. (2019b; 2018)

Systematic reviews conducted by Radke et al. used in this assessment include male (2018) and female (2019b) developmental and reproductive outcomes. Radke et al. (2018) evaluated the associations between DBP or its metabolite (MBP) and male reproductive outcomes, including AGD and

---

<sup>1</sup> Health Canada defines **limited evidence** as “evidence is suggestive of an association between exposure to a phthalate or its metabolite and a health outcome; however, chance, bias or confounding could not be ruled out with reasonable confidence.” Health Canada defines **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 defines **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.”

hypospadias/cryptorchidism following *in utero* exposures; pubertal development following *in utero* or childhood exposures, and semen parameters, time to pregnancy (following male exposure), and testosterone following adult exposures (Table 3-2).

Data quality evaluation criteria and methodology used by Radke et al. (2018) were qualitatively similar to those used by NASEM (2017) (*i.e.*, OHAT methods) and Health Canada (2018b). Similar to NASEM (2017) and Health Canada (2018b), most studies reviewed by Radke et al. (2018) relied on phthalate metabolite biomarkers for exposure evaluation. Therefore, different criteria were developed for short-chain (DEP, DBP, DIBP, BBP) and long-chain (DEHP, DINP) phthalates due to better reliability of single measures for short-chain phthalates. Radke et al. (2018) used data quality evaluations to inform overall study confidence classifications, and ultimately evidence conclusions of “Robust,” “Moderate,” “Slight,” “Indeterminate,” or “Compelling evidence of no effect.” “Robust” and “Moderate” evidence of an association is distinguished by the amount and caliber of data that can be used to rule out other possible causes for the findings. “Slight” and “Indeterminate” describe evidence for which uncertainties prevent drawing a causal conclusion in either direction.

Radke et al. found the strongest inverse relationship between AGD and urinary MBP in a study reported by Bornehag et al. (2014). Inverse associations were also observed by Swan et al. (2015) and Swan (2008), the latter of which was statistically significant. An additional two birth cohort studies by Suzuki et al. (2012) and Jensen et al. (2016) reported no association. Overall, Radke et al. (2018) concluded that there was moderate evidence of an association between AGD and DBP exposure based on these five studies. Inverse associations were found between exposure to DBP and its metabolites and sperm parameters, including sperm concentration (8 of 12 studies) and sperm morphology (7 of 12 studies). Three of the studies (Wang et al., 2015; Liu et al., 2012; Hauser et al., 2006), found statistically significant and monotonic dose-response associations with sperm concentration, while two found statistically significant inverse associations with sperm motility (Axelsson et al., 2015a; Hauser et al., 2006). The results for semen parameters were noted throughout the entire spectrum of exposures noted in the research. The studies with lower exposure levels were more likely to indicate an association than those with higher levels. Ten studies assessed sperm morphology, six of which support an association with DBP. Biological plausibility for the association between exposure to DBP and semen parameters is provided by Jurewicz et al. (2013), who showed increased sperm aneuploidy with higher DBP exposure. However, not all studies reported associations for all sperm parameters. Indeed, one investigation spanning two studies found no association between DBP exposure and sperm apoptosis (Wang et al., 2016; You et al., 2015). Overall, the evidence of an association between higher DBP exposure and lower semen quality, specifically sperm concentration, was robust because it is consistent across many medium confidence studies and shows dose-response associations.

**Table 3-2. Summary of Epidemiologic Evidence of Male Reproductive Effects Associated with Exposure to DBP (Radke et al., 2018)**

Timing of Exposure	Outcome	Level of Confidence in Association
<i>In utero</i>	Anogenital distance	Moderate
	Hypospadias/cryptorchidism	Slight
<i>In utero</i> or childhood	Pubertal development	Indeterminate
Adult	Semen parameters	Robust
	Time to pregnancy	Moderate

Timing of Exposure	Outcome	Level of Confidence in Association
	Testosterone	Slight
Male Reproductive Outcomes Overall		Robust
Data for DBP are taken directly from Figure 3 in Radke et al. (2018)		

Time to pregnancy following male exposure to DBP was evaluated by one study ([Buck Louis et al., 2014](#)), which reported statistically significant associations between higher exposure to the DBP metabolite, MBP, and either a longer time to pregnancy or a lower fecundity. The evidence is deemed moderate due to the high degree of confidence in the study and its coherence with semen parameters. Ten studies ([Axelsson et al., 2015b](#); [Chang et al., 2015](#); [Den Hond et al., 2015](#); [Pan et al., 2015](#); [Wang et al., 2015](#); [Han et al., 2014](#); [Meeker and Ferguson, 2014](#); [Jurewicz et al., 2013](#); [Meeker et al., 2009a](#); [Pan et al., 2006](#)) are used to evaluate the relationship between exposure to DBP as measured by MBP and testosterone. Results from five studies ([Pan et al., 2015](#); [Wang et al., 2015](#); [Meeker and Ferguson, 2014](#); [Meeker et al., 2009a](#); [Pan et al., 2006](#)) show that higher exposure to DBP is associated with lower testosterone levels. Only Pan et al. (2015), found a statistically significant association with DBP and testosterone. There was no discernible pattern linking the observed relationships to the range or intensity of exposure. Thus, Radke et al. (2018) regarded the evidence for the association between DBP and testosterone as slight.

Radke et al. (2019b) also evaluated the association between DBP and its metabolite (MBP) and female reproductive and developmental outcomes. Four studies (two using childhood exposure measurements, two using prenatal exposure measurements) examined the association between pubertal development and DBP. Later age at pubarche (for at least one measure) was reported by two studies ([Wolff et al., 2014](#); [Mouritsen et al., 2013](#)) following childhood exposure to DBP and its metabolites, but the findings were inconsistent among the measure. One study ([Watkins et al., 2017](#)) found inconsistent results for *in utero* exposure in terms of age of menarche (a later age with higher MBP exposure) and pubic hair stages (an earlier age with higher exposure), the latter of which also disagreed with the findings for exposure during childhood. Overall, there is indeterminate evidence about the association between DBP exposure on pubertal development due to inconsistencies and lack of coherence among associated measures of puberty. In four studies ([Machtinger et al., 2018](#); [Wu et al., 2017](#); [Hauser et al., 2016](#); [Messerlian et al., 2015](#)), there were decreases in outcomes related to time to pregnancy in women undergoing *in vitro* fertilization in at least one secondary outcome related to DBP. However, because there was no association found for the primary fecundity outcomes (time to pregnancy and rate of clinical pregnancy), evidence of a relationship between fecundity and exposure to DBP is deemed indeterminate. Five studies serve as the basis for evaluating the evidence of an association between spontaneous abortion and DBP exposure. A high confidence study by Jukic et al. (2016) reported slightly higher odds ratios between MBP exposure levels and early pregnancy loss (tertile 2 OR [95% CI] = 1.1 [0.47, 2.58]; tertile 3 OR [95% CI] = 1.12 [0.46, 2.74]). Toft et al. (2012), reported an inverse association between exposure and clinical pregnancy loss and a monotonic increase in OR for early loss. A case-control study by Mu et al. (2015) found an inverse relationship between quartiles 2 and 3 and quartile 1, but an increased OR for clinical loss for quartile 4 compared to quartile 1. The associations that were reported were not statistically significant. Neither Yi et al. (2016) nor the high-confidence study by Messerlian et al. (2016), found an association between exposure to DBP and spontaneous abortion. The effect estimates for early loss were modest and not statistically significant, while the results for clinical loss were inconsistent. Overall, due to the inconsistency among the high



confidence studies, Radke et al. (2019b) concluded that there is slight evidence of association between early spontaneous abortion and DBP exposure.

Radke et al. (2019b) also evaluated six pregnancy cohort studies (two being nested cohort studies within a case-control design) that provided information on the associations between preterm birth and exposure to DBP and its metabolites. Two studies examined gestational duration (Polanska et al., 2016; Watkins et al., 2016) and the remaining studies examined preterm birth (Smarr et al., 2015; Ferguson et al., 2014; Meeker et al., 2009b). Three studies (Casas et al., 2016; Ferguson et al., 2014; Meeker et al., 2009b) found increased odds of preterm birth with increasing DBP exposure, including two high confidence studies. Meeker et al. (2009b) reported high OR and both Ferguson et al. (2014) and Meeker et al. (2009b) reported statistically significant results. Overall, Radke et al. (2019b) found moderate evidence of an association between DBP exposure and preterm birth, despite some inconsistencies across studies.

#### **3.1.1.1.3 NASEM report (2017)**

NASEM (2017) also evaluated the associations between *in utero* exposure to DBP and male reproductive outcomes. NASEM (2017) included a systematic review of the epidemiological evidence of the associations between exposure to various phthalates or their monoester or oxidative metabolites including DBP, and the following male reproductive outcomes (1) AGD measurements, 2) incidence, prevalence, and severity/grade of hypospadias, and 3) testosterone concentrations measured at gestation or delivery). In contrast to Health Canada (2018b), and Radke et al. (2018), NASEM (2017) relied on methodological guidance from the National Toxicology Program's Office of Health Assessment and Translation (OHAT) to assign confidence ratings and determine the certainty of the evidence to ultimately draw hazard conclusions (NTP, 2015).

NASEM (2017) concluded that there was inadequate evidence to establish an association between prenatal exposure to DBP and hypospadias due to the limited number of studies and dissimilar matrices utilized to evaluate them (urine and amniotic fluid). NASEM also concluded that there is inadequate evidence to determine whether fetal exposure to DBP is associated with a decrease in fetal testosterone in males, given the various different matrices used to measure testosterone (amniotic fluid, maternal serum, or cord blood), the differences in timing of exposure (during pregnancy or at delivery), and the limited number of studies. However, consistent with the conclusions of Radke et al. (2018) NASEM also concluded that there was moderate evidence of association between DBP and AGD. The AGD effect estimates in the meta-analysis NASEM (2017) (% change [95% CI] = -3.13 [-5.63, -0.64] [p = 0.04]) are slope estimates based on the assumption that exposure and effect have a monotonic dose-response relationship.

#### **3.1.1.1.4 Summary of the Existing Assessments of Male Reproductive Effects**

Each of the three assessments discussed above provided qualitative support as part of the weight of scientific evidence for the link between DBP exposure and male reproductive outcomes. Radke et al. (2018), and NASEM (2017) concluded that there was an association between exposure to DBP and decreased AGD, while Health Canada (2018b) did not. The scope and purpose of the assessments by Health Canada (2018b), and systematic review articles by Radke et al. (2018), and NASEM (2017) differ from that of Health Canada related to their moderate confidence conclusions drawn for AGD, which may be related to the different conclusions. Health Canada (2018b) was the most comprehensive review, considering pre and perinatal exposures, as well as peripubertal exposures and multiple different outcomes. NASEM (2017) evaluated fewer epidemiological outcomes than Health Canada (2018b) and systematic review articles by Radke et al. (2018), but also conducted a second systematic review of the animal literature (discussed further in 4.2). The results of the animal and epidemiological systematic reviews were considered together by NASEM (2017) to draw hazard conclusions. Each of the existing

assessments covered above considered a different number of epidemiological outcomes and used different data quality evaluation methods for risk of bias. Despite these differences, each assessment provides qualitative support as part of the weight of scientific evidence.

### 3.1.1.2 Summary of Studies Identified by EPA (2018 through 2019 or Identified Through Public Comment)

EPA also evaluated epidemiologic studies published after the Health Canada (2018b) assessment as part of its literature search (*i.e.*, published between 2018 and 2019 or identified through public comment in 2025). EPA identified 45 developmental (26 studies) and reproductive (19 studies) epidemiology studies published between 2018 to 2019. Fourteen of those studies were female reproductive outcomes (1 high confidence, 11 medium confidence, 1 low confidence and 1 uninformative) and 5 medium studies were male reproductive outcomes. Of the forty-five studies, five medium confidence studies evaluated male reproductive outcomes and 26 studies evaluated male developmental outcomes (2 high confidence, 18 medium confidence and 6 low confidence). Thirty-three studies found no association between exposure to DBP or its metabolites and developmental and reproductive outcomes. In contrast, two medium confidence male reproductive studies found a significant association between exposure to DBP or its metabolites while seven male developmental studies (1 high confidence; 3 medium confidence; 3 low confidence) found a significant association between exposure to DBP or its metabolites. Studies reporting an association are discussed further below.

Further information (*i.e.*, data quality evaluations and data extractions) on the studies identified by EPA can be found in:

- *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025e](#))
- *Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Dibutyl Phthalate (DBP)* ([U.S. EPA, 2025c](#)).

In text below, EPA discusses the evaluation of the studies by outcome that contribute to the weight of scientific evidence.

**Developmental Outcomes for Males.** A *medium* confidence study Arbuckle et al. (2018) reported a significant positive association between prenatal first trimester urinary MBP and anopenile distance in Canadian male infants at birth (beta [95% confidence interval] for the change in anopenile distance (millimeters) per ln- unit increase in MBP: 1.1689 [0.0207, 2.317]). One *medium* confidence study by Zhang et al. (2018b) reported a significant positive association between maternal urinary MBP during first, second and third trimesters and birth weight in male infants in the normal birth weight group (beta [95% CI] for change in birth weight per unit increase in MBP = 10.438 [0.502, 2.0374]). Another *medium* confidence study by Burns et al. (2022) reported a significant positive association between pre-pubertal urinary MBP and pubertal onset (as measured by pubic hair development) in boys in the fourth quartile of compared to the first quartile of MBP [beta (95% CI) for Q4 vs. Q1= 9.3 (1.5, 17.1)]; associations were positive but not significant for other quartiles, although the trend test was significant (p-value = 0.03). No other indicators of pubertal development had significant results.

**Developmental Outcomes for Females.** A *high* confidence study by Bloom et al. (2019) reported a significant negative association between maternal urinary MBP at gestational weeks 18 through 22 and 24 through 32 and odds of low birth weight in female infants only. A *medium* confidence study by Arbuckle et al. (2018) reported a significant positive association between prenatal first trimester urinary MBP and right-hand digit ratio (ratio of the lengths of the second and fourth finger digits of the right

hand) in female infants at six months (beta [95% confidence interval] for the change in hand digit ratio per ln-unit increase in MBP: 0.0122 [0.0018, 0.0227]). Another *medium* confidence study, Bloom et al. (2019) considered MIBP to be a metabolite of DBP rather than DIBP. The study reported a significant positive association between maternal urinary MiBP at gestational weeks 24–32 and odds of small for gestational age (OR [95% CI] per ln-unit increase maternal urinary DBP = 2.82 [1.21, 6.56]). Results for MBP were not statistically significant. No significant findings were found for other female reproductive outcomes such as anthropometric measures of female reproductive organs, fecundity/increased time to pregnancy, female reproductive hormones and uterine fibroids.

**Other Developmental Outcomes.** A *low* confidence study by Amin et al. (2018) reported significant positive associations between urinary MBP and BMI z-score (beta = 0.22; p-value < 0.001) and waist circumference (beta = 0.29; p-value < 0.001) in Iranian children and adolescents. Another *low* confidence study by Durmaz et al. (2018) reported significant positive correlations between urinary MBP and weight (Spearman correlation coefficient = 0.550; p-value < 0.01) and BMI (Spearman correlation coefficient = 0.611; p-value < 0.01) in 4- to 8-year-old Turkish girls. A *medium* confidence study by Boss et al. (2018) reported a significant positive association between maternal urinary MBP throughout pregnancy and gestational age at delivery (HR [95% CI] for change in gestational age per IQR increase in urinary MBP = 1.17 (1.05, 1.29)). No significant associations were observed for risk of preterm birth in this study. No significant findings for birth measures (placental). No significant findings were found for fetal loss.

**Reproductive Outcomes for Males.** Another *medium* confidence study by Tian et al. (2018) reported a significant positive association between urinary MBP and urinary androstenedione levels among healthy reproductive-age men in Xiamen, China (beta [95% confidence interval] for the change in ln-androstenedione per ln-unit increase in MBP: 0.35 [0.11, 0.60]). No significant findings were found for other male reproductive outcomes such as sperm quality parameters and biomarkers of prostate health.

EPA concurs with the conclusions of Health Canada (2018b) systematic review articles published by Radke et al. (2018) and NASEM (2017) that there is some evidence of association but not enough to conclude a causal relationship between DBP exposure and developmental and reproductive outcomes. Moreover, studies identified by EPA from 2018 to 2019 do not alter the previous conclusions from Health Canada (2018b), NASEM (2017), and systematic review articles published by Radke et al. (2018). Although there is moderate level of confidence in the association between DBP and health outcomes such as AGD and time to pregnancy, discussed above, causality was not established.

Therefore, EPA concludes that the existing epidemiological studies do not support quantitative exposure-response assessment due to uncertainty associated exposure characterization of individual phthalates, including source or exposure and timing of exposure as well as co-exposure confounding with other phthalates, discussed in 1.1. The epidemiological studies provide qualitative support as part of the weight of scientific evidence.

### **3.1.2 Summary of Laboratory Animals Studies**

---

For the initial literature search (*i.e.*, 2014 – 2019) EPA considered 52 studies across 39 publications that examined effects on the developing male reproductive system following oral exposure to DBP, including prenatal and perinatal exposure studies, and multi-generational studies of reproduction (Table 3-3; Table 3-4). All 52 of these studies were identified because they were key studies considered in dose-response analyses in previous assessments and the endpoints are consistent with phthalate syndrome. While EPA identified additional studies through systematic review, none were included for further analysis in the *Non-Cancer Human Health Hazard Assessment for Dibutyl Phthalate* due to

study limitations (see discussion in Sections 1.2.3 and 3.1.3). Following a review of literature submitted via public comment and by the SACC, EPA identified one PECO-relevant study examining effects on the developing male reproductive system which was included for further analysis. No studies evaluating effects on the developing male reproductive system following exposure to DBP are available for the dermal or inhalation exposure routes. Studies that have evaluated male reproductive outcomes following developmental exposure to DBP are discussed in Section 3.1.2.1. Other developmental and reproductive outcomes, such as changes in fetal body weight or reproductive organ weight, post-implantation loss, resorptions, or skeletal variations, are discussed in Section 3.1.2.2. Data from chronic studies of DBP are limited in sensitivity compared to the database of developmental exposure studies. Data from chronic studies of DBP are limited to one well-conducted NTP technical report ([NTP, 2021](#)) with 2-year studies in mice and rats that provide far less sensitive LOAELs (*i.e.*, above 500 mg/kg-day) than the developmental studies compared to the database of developmental exposure studies. Indeed, there is one study available: the technical report by the NTP ([2021](#)) that evaluated the toxicity of DBP in mice and rats exposed for up to 2 years. In rats exposed to 510 mg/kg-day, NTP reported increased gross findings (cryptorchidism, agenesis, small testis), increased microscopic findings in the testes (*e.g.*, seminiferous tubule dysgenesis, Leydig cell hyperplasia, and hypospermia), increased incidence of hepatocyte alteration in the liver of males and females, and increased incidence of hypertrophy in the pars distalis in males.

#### **3.1.2.1 Developing Male Reproductive System**

As part of the *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act*, EPA has previously considered the weight of evidence and concluded that oral exposure to DBP 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, 2023a](#))). Notably, EPA's conclusion was supported by the Science Advisory Committee on Chemicals (SACC) ([U.S. EPA, 2023b](#)). A summary of the MOA for phthalate syndrome and data available for DBP supporting this MOA is provided in 3.1.3. Readers are also 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, 2023a](#)) for a more thorough discussion of DBP'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.

Three studies evaluated effects on the developing male reproductive system following prepubertal or pubertal exposures to DBP ([Moody et al., 2013](#); [Xiao-Feng et al., 2009](#); [Srivastava et al., 1990](#)). Of these, only Moody et al. ([2013](#)) was considered for dose-response analysis in Section 4 because of methodological limitations in the latter two (*i.e.*, qualitative histopathological assessment of testes). Additionally, Srivastava et al. ([1990](#)) received an uninformative study evaluation rating.

There is a robust database showing adverse effects on the male reproductive system following developmental exposure to DBP in rats. Adverse effects include decreased *ex vivo* fetal testicular testosterone production and/or fetal testicular testosterone content, histopathological alterations in the testis, decreased anogenital distance, increased male nipple retention gross malformations of the male reproductive tract (*e.g.*, undescended testes, hypospadias, etc.), and sperm parameters. EPA identified 41 publications of oral exposure studies that have evaluated at least one of these effects, 37 of which are oral exposure studies following *in utero* exposure to DBP (34 studies of rats; 3 in mice; one in primates; Table 3-3), and four of which follow pubertal exposures (2 in rats, 1 in mice; Table 3-4). One publication in rabbits evaluated *in utero* and prepubertal exposure to DBP in two separate experiments.

Studies considered by EPA are summarized in Table 3-3 and Table 3-4, including study findings and limitations. Effects on the developing male reproductive system in the context of the mode of action for phthalate syndrome is further discussed in 3.1.3.

#### **3.1.2.2 Other Developmental and Reproductive Outcomes**

In addition to effects on the developing male reproductive system, developmental exposure to DBP has been associated with other developmental and reproductive effects in experimental animals. These include decreases in litter size, changes in sex ratio, increases in pup mortality, decreases in fetal weight, resorptions, post-implantation loss, and increase in skeletal variations (Table 3-3; Table 3-4). These effects generally, but not exclusively, occur at higher doses than those that elicit effects on the developing male reproductive system. Indeed, the majority of studies reviewed by EPA that observed developmental effects other than those on the male reproductive system observed them at doses ranging from 500 to 712 mg/kg-day or higher ([Giribabu et al., 2014](#); [Kim et al., 2010](#); [Drake et al., 2009](#); [Li et al., 2009](#); [Jiang et al., 2007](#); [Lee et al., 2004](#); [Ema et al., 1998](#); [Mylchreest et al., 1998](#)).

Nevertheless, there are two studies that reported decreased pup body weights at lowest doses of around 250 to 400 mg/kg-day. A multigeneration study by the NTP (reported by ([Wine et al., 1997](#))) exposed pregnant rats to dietary concentrations of DBP for equivalent to 52, 256, 509 mg/kg-day [males] or 80, 385, or 794 mg/kg-day [females]. The bodyweights of F1 pups (both absolute and adjusted for litter size) from exposed females were decreased in the mid and high dose groups (LOAEL = 385 mg/kg-day). The body weights of female F1 pups from the high dose group were decreased (10 to 15 percent) on PND0, 14, or 21. F2 pup body weights were significantly decreased at birth in all exposure groups, and 6 percent decreased from controls at the low dose (equivalent to 80 mg/kg-day). Zhang et al. ([2004](#)) also reported decreased pup body weights. In that study, pregnant SD rats were exposed to 0, 50, 250, or 500 mg/kg-day DBP via gavage from GD1 to PND21. Pup body weight at birth was decreased in the 250 mg/kg-day dose group, which coincided with decreased male AGD on PND4 as well as reductions in sperm motility and absolute epididymis weight in PND70 adults. No changes in PND70 body weight were observed, indicating that the decrease in pup body weight at birth was not permanent. Other unaffected outcomes included sex ratio and pup survival to weaning.



**Table 3-3. Summary of Studies Evaluating Effects on the Developing Male Reproductive System Following *In Utero* Exposures to DBP**

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Lee et al., 2004</a> ) (Medium)	Pregnant rats (6–8 dams/group) were exposed to 0, 20, 200, 2000, or 10,000 ppm DBP via diet from GD15 – PND21 (equivalent to 0, 1.5–3, 14–29, 148–291, 712 – 1372 mg/kg-day). Male and female F1 offspring were evaluated at PND 2, PND14, PND21, and PNW 8–11 and PNW20.	ND / 3	↓ spermatocyte development on PND 21 and ↑ vacuolar degeneration of alveolar cells and alveolar atrophy of mammary gland in PNW 11 males	<u>Maternal Effects</u> - ↓ BW gain on GDs 15–20 (712 mg/kg-day) <u>Other Developmental Effects</u> - ↓ male:female ratio (712 mg/kg-day) - ↓ absolute AGD (males) on PND2 & ↑ male NR on PND 14 (712 mg/kg-day) - ↑ relative liver (both sexes) & ↓ testes weight on PND21 (712 mg/kg-day) - Testicular pathology on PND 21 (aggregated foci of Leydig cells and decreased epididymal duct cross section at ≥148 mg/kg-day); Testicular pathology on PNW 11 (loss of germ cell development at ≥148 mg/kg-day) <u>Unaffected Outcomes</u> - Dam BW gain on PND 2 – PND 21; food consumption; # live offspring; offspring BW on PND 2; F1 relative kidney, adrenal, epididymis, ovary, uterus weight on PND 21; PPS; vaginal opening; estrous cyclicity; testicular pathology on PNW 20 <u>Limitations:</u> - Individual animal was the statistical unit, not the litter; Small sample size; Insufficient methodological details provided regarding histopathology; outcome measure timing concerns ( <i>i.e.</i> , male rats just beginning to develop spermatocytes around PND21)
( <a href="#">Li et al., 2023</a> ) (Medium) <sup>d</sup>	Pregnant C57BL/6 mice (4 dams/group) gavaged with 0, 0.5, 5, or 75 mg/kg-day DBP on GD 5–19	5 / 75	↓sperm density & motility; ↑ testicular pathology (changes in spermatogenic cell layer number, Sertoli cells, spermatogenesis, and local lumen enlargement) ↓serum testosterone; ↓serum LH; ↑serum FSH	<u>Unaffected Outcomes</u> - Sperm malformation rate <u>Limitations:</u> - Low sample size - Unclear if litter was the statistical unit for data analyses - Qualitative histopathology (no incidence data provided)
( <a href="#">Boekelheide et al., 2009</a> ) (Medium)	Pregnant SD rats (4–10 litters/group) gavaged with 0 0.1, 1, 10, 30, 50,	10 / 30	↑ testicular pathology (↓ testicular cell number; disorganized seminiferous tubules)	<u>Developmental Effects</u> - ↓ number of tubular cross sections (50, 100, 500 mg/kg-day) - ↓ cell proliferation on GD20 & GD21 (500 mg/kg-day) - ↑ number of MNGs (≥100 mg/kg-day)

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	100, 500 mg/kg-day DBP on GD 12–21.			<u>Limitations:</u> - Qualitative histopathology (no incidence data provided)
(Mahood et al., 2007) (Medium) <sup>d</sup>	Pregnant Wistar rats (4– 6 litters/group) gavaged with 0, 4, 20, 100, 500 mg/kg-day DBP on GD 13.5–20.5 (fetal tissue, for endpoints of testicular testosterone, MNGs, LC distribution) or GD 13.5–21.5 (postnatal tissue for endpoints of infertility, cryptorchidism, testis weights).	20 / 100	↓ fetal testicular testosterone content, ↑ MNGs, ↑ Leydig cell aggregation	<u>Developmental Effects</u> - ↓ testicular testosterone content on GD 21.5 (≥100 mg/kg-day) - ↑ MNGs on GD 21.5 (≥100 mg/kg-day) - Changes in Leydig cell distribution ( <i>i.e.</i> , ↓ # of total Leydig cell clusters, ↑ occurrence of medium (100 mg/kg-day) and large (500 mg/kg-day) Leydig cell clusters)- increased dysgenic areas (not statistically significant)
	Pregnant Wistar rats gavaged with 0, 4, 20, 100, 500 mg/kg-day DBP on GD 13.5–21.5.	100 / 500	↑ infertility, cryptorchidism, ↓ testis weight	<u>Developmental Effects</u> - ↑ incidence of infertility ( <i>i.e.</i> , male produce offspring with untreated females) and cryptorchidism on PND 90 (500 mg/kg-day) - ↑ incidence of Sertoli cell only tubules (SCO) in cryptorchid testes (≥100 mg/kg-day; 11/11 animals at 500 mg/kg-day) and increased incidence of SCO tubules in scrotal testes (≥20 mg/kg-day; flat dose- response) - ↓ absolute testis weight on GD 21.5 and PND 90 (500 mg/kg-day)
(Furr et al., 2014) <sup>b</sup> (High)	Pregnant Harlan SD rats (3–4/dose) gavaged with 0, 1, 10, 100 mg/kg-day DBP on GDs 14–18. Dams sacrificed on GD 18. (Block 22)	10 / 100	↓ <i>ex vivo</i> fetal testicular testosterone production (36%)	<u>Unaffected Outcomes</u> - Dam weight gain; fetal viability
	Pregnant Harlan SD rats (2–3/dose) gavaged with 0, 33, 50, 100, 300 mg/kg-day DBP on GDs 14–18. Dams sacrificed on GD 18. (Block 18)	50 / 100	↓ <i>ex vivo</i> fetal testicular testosterone production (35%)	<u>Unaffected Outcomes</u> - Dam weight gain; fetal viability

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	Pregnant Harlan SD rats (3–4/dose) gavaged with 0, 1, 10, 100 mg/kg-day DBP on GDs 14–18. Dams sacrificed on GD 18. (Block 26)	100 / ND	NA, no LOAEL identified	<u>Unaffected Outcomes</u> - Dam weight gain; fetal viability; <i>ex vivo</i> fetal testicular testosterone production
	Pregnant Harlan SD rats (3–4/dose) gavaged with 0, 750 mg/kg-day DBP on GDs 14–18. Dams sacrificed on GD 18. (Block 34)	ND / 750	↓ <i>ex vivo</i> fetal testicular testosterone production (89%)	<u>Unaffected Outcomes</u> - Dam weight gain; fetal viability
( <a href="#">Lehmann et al., 2004</a> ) (Uninformative)	Pregnant SD rats (5–7/dose) gavaged with 0, 0.1, 1, 10, 30, 50, 100, 500 mg/kg-day DBP on GD 12–19.	30 / 50	↓ fetal testicular testosterone content	<u>Maternal Effects</u> - Not reported <u>Developmental Effects</u> - ↓ testicular mRNA & protein expression of genes involved in steroidogenesis ( <i>e.g.</i> , <i>StAR</i> , <i>P450scc</i> , <i>CYP17</i> ) (≥50 mg/kg-day) and testis descent ( <i>Ins13</i> ) (≥500 mg/kg-day) - ↓ fetal testicular testosterone content on GD 19 (≥50 mg/kg-day) <u>Limitations/Uncertainties</u> - Authors state that the study was repeated, and a 30-mg/kg/day dose group was included for the testosterone radioimmunoassay (RIA). For other endpoints in this study, the 30 mg/kg-day dose group was not included.
( <a href="#">Mylchreest et al., 2000</a> ) (High)	Pregnant SD rats (19–20 or 11 (high-dose) per dose) gavaged with 0, 0.5, 5, 50, 100, 500 mg/kg-day DBP on GDs 12–21.	50 / 100	↑ males with nipples and/or areolae on PND 14	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ absolute AGD on PND 1 (500 mg/kg-day) - ↓ absolute epididymal, dorsal prostate, LABC weight on PND 110 (500 mg/kg-day) - ↑ hypospadias, absent or partial epididymis, vas deferens, SV and prostate on PND 110 (500 mg/kg-day) - ↑ Seminiferous tubule degeneration, interstitial cell hyperplasia, interstitial cell adenoma (500 mg/kg-day) <u>Unaffected Outcomes</u>



Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- Dam BW and food consumption; live pups per litter; sex ratio; birth weight; survival to weaning; absolute liver, kidney, adrenal, testis, vas deferens, SV, ventral prostate weight on PND 110; PPS; age at vaginal opening
(MacLeod et al., 2010) (Medium) <sup>d</sup>	Pregnant Wistar rats (at least 3 dams/dose) gavaged with 0 or 500 mg/kg-day DBP on GD 13.5–16.5 and sacrificed on GD17.5.	ND / 500	↓ fetal testicular testosterone content	<u>Developmental Effects</u> - ↓ fetal testicular testosterone content on GD17.5 (500 mg/kg-day)
	Pregnant Wistar rats (at least 3 dams/dose) gavaged with 0 or 500 mg/kg-day DBP on GD 13.5–20.5 and sacrificed on GD21.5.	ND/500	↓ fetal testicular testosterone content and ↓ AGD	<u>Developmental Effects</u> - ↓ fetal testicular testosterone content on GD21.5 (500 mg/kg-day) - ↓ absolute AGD (male) on GD21.5 (500 mg/kg-day)
	Pregnant Wistar rats (at least 3 dams/dose) gavaged with 0, 100, or 500 mg/kg-day DBP on GD 13.5–21.5 and sacrificed on PND 25.	ND / 100	↓ ventral prostate weight on PND25	<u>Developmental Effects</u> - ↓ absolute SV and testis (500 mg/kg-day) and ventral prostate weight (≥100 mg/kg-day) on PND 25 - ↓ penis length and absolute AGD on PND 25 (500 mg/kg-day)
	Pregnant Wistar rats (at least 3 dams/dose) gavaged with 0 and 500 mg/kg-day DBP on GD 13.5 – PND 15 and sacrificed on PND 25.	ND / 500	↓ male AGD and penis length	<u>Developmental Effects</u> - ↓ male absolute AGD and penis length on PND 25 (500 mg/kg-day)
(Zhang et al., 2004) (Medium)	Pregnant SD rats (20/group) gavaged with 0, 50, 250, 500 mg/kg-day DBP on GD 1–PND 21	50 / 250	↓ pup birth weight (12% [males]; 9.8% [females]; ↓ male AGD on PND 4 (absolute and BW normalized), ↓ absolute epididymis weight on PND 70; ↓ sperm motility and	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ live pups per litter (500 mg/kg-day) - ↓ sperm number on PND 70 (500 mg/kg-day) - Testicular pathology ( <i>i.e.</i> , small diameter tubules, degeneration or exfoliation of the germinal epithelium of the seminiferous tubules

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			total sperm heads per testis on PND 70	[250 mg/kg-day]; degeneration of seminiferous tubules, depletion of germ cells [500 mg/kg-day] <u>Unaffected Outcomes</u> - Dam BW during pregnancy or lactation; gestation length; sex ratio; pup survival to weaning; F1 male BW on PND 70; absolute testis, prostate, pituitary weight on PND 70 <u>Limitations</u> Qualitative histopathology ( <i>i.e.</i> , no incidence data)
( <a href="#">Giribabu et al., 2014</a> ) <sup>b</sup> (Medium) <sup>d</sup>	Pregnant Albino Wistar rats (6/group) were gavaged with 0, 100, or 500 mg/kg DBP on GD 1, 7, and 14 (equivalent to 0, 21, or 107 mg/kg-day for 3 doses). PND100 F1 males (8/group) were mated with unexposed females to evaluate reproductive performance.	ND / 100	↓ sperm count; sperm motility; ↑ percent abnormal sperm; ↑ serum FSH & LH in F1 at PND100; ↓ serum testosterone in F1 at PND100; ↓ levels of 17-hydroxysteroid dehydrogenase & 3-hydroxysteroid dehydrogenase in F1 at PND100; Abnormal testis histopathology ( <i>i.e.</i> , disorganized seminiferous tubules & ↑ interstitial spaces and ruptured epithelium) ↓ No. of pups ↓ relative weight of the seminal vesicle at PND100 in F1	<u>Maternal Effects</u> - ↓ number of pups delivered, mean number of live F2 fetuses (≥100 mg/kg) - ↑ number of resorptions in F2 on GD6 (≥100 mg/kg) <u>Developmental Effects</u> - ↓ No. of pups (500 mg/kg) - ↓ relative weight of the seminal vesicle at PND100 in F1 500 mg/kg) - ↓ sperm viability (500 mg/kg) <u>Unaffected Outcomes</u> - Fertility index; number of corpora lutea in F2 on GD6; Developmental landmarks ( <i>e.g.</i> , pinna unfolding, eye opening) of F1; survival rate of F1 on PND4 and PND21; body weights & most organ weights in F1; skeletal system & external anomalies in F2 on GD18 <u>Limitations:</u> -Qualitative histopathology (incidence data not provided) -Did not use litter as the statistical unit
( <a href="#">TherImmune Research Corporation, 2002</a> ; <a href="#">Wine et al., 1997</a> ; <a href="#">NTP, 1995</a> ) (Medium)	Continuous breeding protocol. Pregnant VAF Crl:CD BR outbred Sprague-Dawley albino rats (20/sex/group; 40/sex for controls) exposed to 0, 0.1, 0.5, or	ND / 80	- F2: ↓ live pup weight (all doses; not dose-dependent); - F1: ↓ live pups per litter (dose-dependent)	<u>Maternal Effects:</u> - ↓ body weight gain (11%) in P1 females at week 17 (794 mg/kg-day) <u>Developmental Effects:</u> - F1: ↓ number of live pups per litter (≥385 mg/kg-day) - F1: ↓ live pup weight (high dose [794 mg/kg-day] female x unexposed male)

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	1% DBP via diet starting 10 weeks prior to mating and throughout gestation and lactation periods continuously for 2 generations (equivalent to 52, 256, 509 mg/kg-day [males]; 80, 385, or 794 mg/kg-day [females]).			<p>- F1: Testicular pathology (<i>i.e.</i>, degeneration of seminiferous tubules [385 mg/kg-day]; interstitial cell hyperplasia and underdeveloped epididymis [794 mg/kg-day]; sperm content reduction [794 mg/kg-day]).</p> <p>- F2: ↓ mating index, fertility index, pregnancy index (794 mg/kg-day only)</p> <p><u>Other Outcomes:</u></p> <p>F1: ↓ terminal BW (13%) in females only (794 mg/kg-day)</p> <p>F2: ↓ seminal vesicle weight (794 mg/kg-day only); ↑ relative liver weight (males; 794 mg/kg-day); ↑ relative kidney (males; ≥385 mg/kg-day)</p> <p>F2: ↓ terminal BW (males [7.9%] and females [13%]; 794 mg/kg-day)</p> <p><u>Unaffected Outcomes:</u></p> <p>-F1 fertility &amp; average number of litters per pair; F1: live pup weight (high dose male x unexposed female)</p>
( <a href="#">Li et al., 2009</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (9–10/dose) fed diets from GD 6 – PND 28; diets contained 0, 0.037, 0.111, 0.333, 1% DBP (31, 94, 291, 797 mg/kg-day on GD 6–21; 55; 165, 486, 1,484 mg/kg-day on PND 0–15; 47, 140, 433, 1,283 mg/kg-day on PND 16–28) .	94 / 291	↓ male absolute AGD on PND1	<p><u>Developmental Effects</u></p> <p>- ↑ gestation length (797 mg/kg-day)</p> <p>- ↓ male and female BW on PND 0, 7, 14, 21, 28 (797 mg/kg-day)</p> <p>- ↑ relative liver weight (both sexes) and ↓ relative testes weight (797 mg/kg-day)</p> <p><u>Unaffected Outcomes</u></p> <p>- Live pups per litter; dam BW on GD 6–20; sex ratio; pinna detachment; incisor eruption; eye opening</p>
( <a href="#">Mylchreest et al., 1999</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (10/dose) gavaged with 0, 100, 250, 500 mg/kg-day DBP on GD 12–21.	100 / 250	↓ AGD on PND 1; ↑ NR on PND 14; epididymal dysgenesis/ agenesis, cryptorchidism, and degeneration of seminiferous epithelium in F1 males on PND 100–105	<p><u>Maternal Effects</u></p> <p>- None</p> <p><u>Developmental Effects</u></p> <p>- ↑ age at PPS (at 100 and 500, but not 250 mg/kg-day)</p> <p>- ↑ hypospadias and prostate agenesis (≥500 mg/kg-day)</p> <p>- ↑ Interstitial cell hyperplasia or adenoma (≥500 mg/kg-day)</p> <p>- ↓ absolute kidney, testis, epididymis, SV weight in F1 offspring on PND 100–105 (≥500 mg/kg-day)</p> <p><u>Unaffected Outcomes</u></p>

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- BW gain GD 0–21; BW during dosing; litter size; live pups per litter; sex ratio; live pup weight on PND 1; offspring BW, absolute liver, adrenal, vas deferens, prostate weight on PND 100–105
( <a href="#">Howdeshell et al., 2008</a> ) (High)	Pregnant SD rats (3–4/dose) gavaged with 0, 33, 50, 100, 300, 600 mg/kg-day DBP on GDs 8–18. Dams sacrificed on GD 18.	100 / 300	↓ <i>ex vivo</i> fetal testicular testosterone production	<u>Maternal Effects</u> - None <u>Unaffected Outcomes</u> - # of dams with whole litter loss; maternal body weight gain; # of implantations; # of live/dead fetuses; resorptions; fetal mortality
( <a href="#">Gray et al., 2021</a> ) <sup>c</sup> (High)	Pregnant Sprague-Dawley rats (3–4 litters group) exposed GD 14–18 via gavage to 0, 300, 600, or 900 mg/kg-day DBP (based on block 70 and 71 experiments)	ND / 300	↓ <i>ex vivo</i> fetal testicular testosterone production (62% [block 70; 47% [block 71]])	<u>Other effects:</u> - Dose-dependent reduction in genes involved in cholesterol absorption ( <i>CYP461a</i> ), cholesterol homeostasis ( <i>Ldlr</i> ), cholesterol biosynthesis ( <i>Cyp51</i> , <i>Dhcr24</i> , <i>Dhcr7</i> , <i>Ebp</i> , <i>Hmgcr</i> , <i>Hmgcs1</i> , <i>Idi1</i> , <i>Mvd</i> , <i>Nsdhl</i> , <i>RGD1564999</i> , & <i>Tm7sf2</i> ), or other functions in cholesterol metabolism ( <i>Cyp11a1</i> , <i>Insig1</i> ) (≥300 mg/kg-day) <u>Notes</u> - Fetal testicular testosterone data for additional blocks of animals are presented in Furr et al. ( <a href="#">2014</a> ).
( <a href="#">Li et al., 2015</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (2–5/dose) gavaged with 0, 100, 300, 900 mg/kg-day DBP on GD 12.5–20.5	100 / 300	↓ Fetal testicular testosterone content, Leydig cell aggregation, ↓ AGD, hypospadias, ↓ testis weight	<u>Developmental Effects</u> - ↓ testicular testosterone content on GD17.5 (≥300 mg/kg-day), GD19.5 (900 mg/kg-day), GD21.5 (900 mg/kg-day) - ↑ Leydig cell aggregation on GD19.5 and GD20.5 (≥300 mg/kg-day) - ↓ absolute AGD (males) on PND2, PND21, PND63 (≥300 mg/kg-day) - ↑ hypospadias (≥300 mg/kg-day) and cryptorchidism (900) on PND 63 - ↓ absolute testis weight on GD17.5 (≥300), GD19.5 (900 mg/kg-day), GD21.5 (900 mg/kg-day)
( <a href="#">Martino-Andrade et al., 2008</a> ) (Medium)	Pregnant Wistar rats (7–8/dose) gavaged with 0, 100, 500 mg/kg-day DBP on GDs 13–21. Dams terminated on GD21 (fetal study)	ND / 100	↓ male AGD	<u>Developmental Effects</u> - ↓ fetal testicular testosterone content (63%) on GD 21 (500 mg/kg-day) - ↑ MNGs, seminiferous cord diameter, Leydig cell aggregates on GD 21 (500 mg/kg-day) - ↓ absolute AGD (males) (500 mg/kg-day) and AGD normalized to cube root of BW (≥100 mg/kg-day) on GD 21

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				<u>Unaffected Outcomes</u> - Dam BW gain GD 12–21; implantation sites, post-implantation loss
	Pregnant Wistar rats (4–7/dose) gavaged with 0, 100, 500 mg/kg-day DBP on GDs 13–21. Dams allowed to deliver, and offspring examined up to PND90.	100 / 500	↑ male offspring NR on PND13	<u>Developmental Effects</u> - ↑ male pup NR on PND 13 (500 mg/kg-day) <u>Unaffected Outcomes</u> - Dam BW gain GD 12–21; male F1 BW on PND 90; absolute testis, epididymis, prostate, LABC, SV weight on PND 90; # of spermatids per testis on PND 90; reproductive tract malformations; PPS
( <a href="#">Kuhl et al., 2007</a> ) (Low)	Pregnant SD rats (10/dose) gavaged with 0, 100, 500 mg/kg DBP on GD 18 and sacrificed 24-hours later on GD 19.	100 / 500	↓ fetal testicular testosterone content (67%)	<u>Developmental Effects</u> - ↓ fetal testicular mRNA levels of <i>Star</i> , <i>SR-B1</i> , <i>Cyp11a1</i> , <i>CYP17</i> (≥100 mg/kg-day) Not considered adverse at 100 mg/kg-day in absence of decreases in fetal testosterone content at this dose.
( <a href="#">Drake et al., 2009</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (13–15 dams/dose) gavaged with 0, 100, 500 mg/kg-day DBP from GD 13.5–21.5 and reproductive outcomes evaluated in offspring at birth and throughout adulthood.	100 / 500	↓ AGD during adulthood; ↓ penis length, ↑ hypospadias & cryptorchidism, ↓ absolute testis and ventral prostate weight	<u>Maternal Effects</u> - Maternal effects not evaluated <u>Developmental Effects</u> - ↓ birth weight (8%; 500 mg/kg-day)
	Pregnant Wistar rats (8–17 litters/dose) gavaged with 0, 500 mg/kg-day DBP from GD 13.5–16.5 & sacrificed on GD 17.5.	ND / 500	↓ fetal intratesticular testosterone content, ↓ testicular <i>Star</i> and <i>Cyp11a1</i> mRNA	<u>Maternal Effects</u> Not reported
( <a href="#">Barlow et al., 2004</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (10–11/dose) gavaged with 0, 100, 500 mg/kg-day DBP on GDs 12–21.	ND / 100	↑ F1 males with NR on PND13	<u>Developmental Effects</u> - ↓ absolute AGD (male) on PND 1 and PND 180 (500 mg/kg-day) - ↑ males with areolae on PND 13 (≥100 mg/kg-day) and nipples on PND 180 (500 mg/kg-day) - ↑ incidence of gross lesions in testes (atrophied, enlarged, or absent), epididymides (agenesis), vas deferens (absent), SVs (mall or absent),

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				prostate (small or absent), penis (hypospadias) on PND 180, PND 370, PND 540 (500 mg/kg-day) - ↑ testicular pathology ( <i>e.g.</i> , unilateral and/or bilateral testicular dysgenesis and germ cell degeneration) on PND 180, PND 370, PND 540 (500 mg/kg-day)
( <a href="#">Scarano et al., 2010</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (5/group) gavaged with 0 or 100 mg/kg-day DBP from GD 12 – PND21.	ND /100	Histopathological abnormalities of fetal testis ( <i>e.g.</i> , Leydig-cell clusters, presence of MNGs, ↑ interstitial tissue area relative to tubular area)	<u>Maternal Effects</u> - Maternal effects not evaluated <u>Developmental Effects</u> - ↓ male AGD on PND4 (not statistically significant; 3.5 ± 0.2 mm vs. 3.1 ± 0.4 mm) <u>Unaffected Outcomes</u> - Serum testosterone levels in PND90 adults & <i>in vitro</i> testicular testosterone from PND90 animals; male F1 body weight at PND1 and PND90; sperm morphology and motility
( <a href="#">Struve et al., 2009</a> ) (Medium)	Pregnant SD rats (7–9/dose) fed diets containing 0, 100, 500 ppm (equivalent to 112, 582 mg/kg-day) DBP on GDs 12–19. Dams sacrificed on GD 19 or 20 (4- or 24-hours post-DBP exposure).	ND / 112	↓ fetal testicular testosterone content (71%) on GD 20; ↑ Leydig cell aggregates and seminiferous cord diameter on GD 19 and 20	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ absolute AGD (males) on GD 19 and 20 (500 mg/kg-day) - ↓ fetal testicular testosterone content on GD 19 (500 mg/kg-day) and GD 20 (≥100 mg/kg-day) - ↓ fetal testis mRNA levels for <i>Star</i> , <i>Scarb1</i> , <i>Cyp17a1</i> , <i>P450scc</i> / <i>Cyp11a</i> on GD 19 (≥100) and GD 20 (500 mg/kg-day) - ↑ MNGS (500 mg/kg-day) <u>Unaffected Outcomes</u> - Dam BW; litter size; sex ratio; fetal survival; fetal weights
( <a href="#">Ema et al., 1998</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (11/dose) fed diets containing 0, 0.5, 1.0 2.0% (equivalent to 331, 555, 661 mg/kg-day) DBP on GDs 11–21.	331 / 555	↓ AGD (absolute and BW normalized) of male fetuses on GD21; ↑ incidence of undescended testes	<u>Maternal Effects</u> - ↓ BW gain and food consumption on GDs 11–21 (≥555 mg/kg-day) <u>Developmental Effects</u> - ↓ fetal weight (661 mg/kg-day) - ↑ incidence of cleft palate, fusion of sternebrae, fusion of ribs (661 mg/kg-day) <u>Unaffected Outcomes</u> - Resorptions; post-implantation loss; # live fetuses per litter; sex ratio;



Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Gaido et al., 2007</a> ) (Medium) <sup>d</sup>	Pregnant C57BL/6 mice gavaged with 250 mg/kg DBP on GD 16, 17, and 18.	ND / 250	↓ seminiferous cord formation and ↑ MNGs (quantitative histopathology); ↑ seminiferous cord diameter, MNGs per cord, & nuclei/MNG	<u>Maternal Effects</u> - Not evaluated for 250 mg/kg-day experiment; authors report evidence of maternal toxicity at 1500 mg/kg-day in a preliminary experiment. <u>Developmental Effects</u> - Changes in gene expression (↑ <i>Btg2</i> , <i>Ctgf</i> , <i>Fos</i> , <i>Ier3</i> , <i>Nr4a</i> , <i>Pawr</i> , <i>Tnfrsf12a</i> & ↓ <i>Hsd11b2</i> , <i>Tk1</i> at 4- & 8-hour timepoints (500 mg/kg DBP on GD18); <u>Unaffected Outcomes</u> - fetal testicular testosterone content <u>Limitations:</u> Qualitative histopathology for some endpoints Insufficient information available to determine maternal toxicity
( <a href="#">Mylchreest et al., 1998</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (10/dose) gavaged with 0, 250, 500, 750 mg/kg-day DBP on GD 3 – PND 20	ND / 250	Reproductive tract malformations (hypospadias, non-scrotal testes, epididymal dysgenesis/ agenesis on PND 100)	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ male pup absolute AGD on PND 1 (≥500 mg/kg-day) - SV dysgenesis on PND 100 (≥500 mg/kg-day) - ↓ absolute testis and SV weight (≥500) and epididymis and prostate weight (750 mg/kg-day) - ↓ live pups per litter and pup survival to weaning (750 mg/kg-day) <u>Unaffected Outcomes</u> - Dam BW and food consumption during pregnancy and lactation; Dam absolute liver, kidney, adrenal, ovary, uterus weight on PND 21; pup sex ratio; offspring BW on PND 1, 21, 100 (both sexes); age at vaginal opening; age at first estrus; length of estrous cycle
( <a href="#">Jiang et al., 2007</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (10 dams/dose) gavaged with 0, 250, 500, 750, 1000 mg/kg-day DBP on GD 14–18. Dams allowed to deliver pups naturally.	ND / 250	↑ cryptorchidism	<u>Maternal Effects</u> - ↓ maternal BW gain on GDs 14–18 and 18–20 (≥750 mg/kg-day) <u>Developmental Effects</u> - ↓ live pups (≥750 mg/kg-day) - ↓ BW normalized AGD (males) on PND 1 (≥500 mg/kg-day) - ↑ hypospadias (≥500) and cryptorchidism (≥250 mg/kg-day) on PND 70 - ↓ BW, ↓ relative liver, kidney, prostate, testis, epididymis, adrenal, pituitary weight on PND 70 (≥500 mg/kg-day) <u>Unaffected Outcomes</u>

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- Maternal mortality; relative heart and spleen weight on PND 70
( <a href="#">Kim et al., 2010</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (minimum of 3 dams/dose) gavaged with 0, 250, 500, 700 mg/kg-day DBP on GD 10–19 and allowed to deliver naturally.	ND / 250	Delayed PPS	<u>Developmental Effects</u> - ↓ BW and absolute testes, epididymis, ventral prostate, SV, Cowper's gland, glans penis weight on PND 31 (700 mg/kg-day); ↓ absolute LABC weight on PND 31 (≥500 mg/kg-day) - ↑ incidence of cryptorchidism and hypospadias on PND 11 (700 mg/kg-day) - ↑ incidence of degeneration of seminiferous epithelium (700 mg/kg-day) - ↓ BW normalized AGD on PND 11 and ↑ F1 male NR (≥500 mg/kg-day) - ↓ Serum DHT and total testosterone on PND 31 (700 mg/kg-day)
( <a href="#">Mylchreest et al., 2002</a> ) (Medium) <sup>d</sup>	Pregnant SD rats gavaged with 0 and 500 mg/kg-day DBP on GD 12–21.	ND / 500	↓ fetal testis testosterone content on GD 18 and GD 21, testicular pathology (Leydig cell hyperplasia, testis atrophy, MNGs)	<u>Developmental Effects</u> - Leydig cell hyperplasia on GDs 16, 18, 21; testis atrophy on GD 18 and 21; MNGs on GD 21
( <a href="#">Howdeshell et al., 2007</a> ) <sup>c</sup> (Medium) <sup>d</sup>	Pregnant SD rats (6/dose) gavaged with 0 or 500 mg/kg-day DBP on GDs 14–18 and allowed to deliver pups naturally.	ND / 500	↓ AGD, ↓ LABC weight, ↓ <i>ex vivo</i> fetal testicular testosterone production (34%), testicular degeneration	<u>Developmental Effects</u> - ↓ absolute AGD (males) on PND 3 - ↓ absolute LABC weight at 7–11 months of age - Low incidence of testicular malformations (not statistically significant) - ↓ <i>ex vivo</i> testicular testosterone production and mRNA for <i>StAR</i> on GD 18 <u>Unaffected Outcomes</u> - Maternal BW gain on GDs 14–18; litter size; fetal and neonatal mortality; F1 BW on PND 3 (both sexes); # areolae per PND 14 male; # nipples per adult male
( <a href="#">Ferrara et al., 2006</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats gavaged with 0 or 500 mg/kg-day DBP on GD 15.5–21.5.	ND / 500	↑ MNGs and effects on germ cell numbers	<u>Developmental Effects</u> - ↑ incidence of MNGs in seminiferous cords on e19.5, e21.5, and PND 4 - ↑ incidence of apoptotic gonocytes on e15.5, 17.5 - ↓ germ cell # per testis on e21.5, PND 4, PND8, PND15, PND25 - ↓ germ cell proliferation index on PND 6 and PND 25

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Johnson et al., 2011</a> ) (Medium)	Pregnant SD rats (4/group) were gavaged with 0 or 500 mg/kg DBP from GD-12 – GD20 and evaluated at GD 20.	ND / 500	↓ fetal testicular testosterone content (34%); ↓ absolute male AGD; ↑ percentage of seminiferous cords with one or more MNGs	<u>Maternal Effects</u> - None <u>Unaffected Outcomes</u> - Maternal body weights (qualitative statement in text)
( <a href="#">Johnson et al., 2007</a> ) (Medium)	Pregnant SD rats gavaged with a single dose of 1, 10, 100, or 500 mg/kg-day DBP on GD19 and evaluated 1 hour after dosing.	ND / 500	↓ fetal intratesticular testosterone content (62%) on GD 19, 1 hour after dosing	<u>Maternal Effects</u> - Not reported <u>Developmental Effects</u> - Altered gene expression of <i>Erg1</i> , <i>Fos</i> , <i>Thbs1</i> , <i>Cxcl10</i> , <i>Nr4a1</i> , <i>Stc1</i> , <i>Edn1</i> , <i>Tnfrsf12a</i> , and <i>ler3</i> from interstitial cells, Sertoli cells, and/or peritubular myoid cells.
( <a href="#">Higuchi et al., 2003</a> ) (Medium) <sup>d</sup>	Pregnant rabbits (8 litters/group) were exposed to 0 or 400 mg/kg-day DBP from GD15–29 and male offspring were evaluated at PNW 6, 12, and 25.	ND / 400	↓ ejaculated sperm (43%); ↑ abnormal sperm; ↓ weight of testes (23%) and accessory sex glands (36%) on PNW12; ↓ serum testosterone at PNW6 (32%); Histopathological alterations in the seminiferous tubule epithelium and interstitium of the testes ( <i>e.g.</i> , desquamated premature germ cells)	<u>Maternal effects:</u> - None <u>Other effects:</u> - Hypospadias, hypoplastic prostate, and cryptorchid testes with carcinoma <i>in situ</i> -like cells in one male. <u>Unaffected Outcomes:</u> - Weight of epididymides (PNW12 and PNW25); weight of thyroid, liver, or testes (PNW25); hypothalamic content of GnRH (PNW12 or PNW25)
( <a href="#">van den Driesche et al., 2012</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (3–7 litters/group) were gavaged with 0, 500, or 750 mg/kg-day DBP from GD 13.5 – 20.5 and AGD and intratesticular testosterone content was evaluated at GD21.5.	ND / 500	↓ intratesticular testosterone content on GD 21.5; ↓ absolute AGD (males) on PND8	<u>Maternal Effects</u> - Not reported <u>Developmental Effects</u> - ↓ Intratesticular testosterone content on GD 21.5 (750 mg/kg-day) - ↑ Focal testicular dysgenesis (↑ percentage of large & small Leydig cell aggregates at PND8)

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	Pregnant Wistar rats (3–7 litters/group) were gavaged with 0, 500, or 750 mg/kg-day DBP from GD 19.5 – 20.5 and AGD and intratesticular testosterone content was evaluated at GD21.5.	ND / 500	↓ intratesticular testosterone content on GD 21.5; ↑ germ cell aggregation on GD21.5	<u>Maternal Effects</u> - Not evaluated <u>Developmental Effects</u> - ↓ intratesticular testosterone content on GD 21.5 ( 750 mg/kg-day) - focal testicular dysgenesis (↑ percentage of large & small Leydig cell aggregates at PND8) <u>Unaffected Outcomes</u> - Male AGD (absolute) on PND8; focal testicular dysgenesis (percentage of large & small Leydig cell aggregates at PND8)
( <a href="#">McKinnell et al., 2009</a> ) (Medium) <sup>d</sup>	Pregnant marmoset monkeys were exposed from gestational week 7–15 with 500 mg/kg-day MBP, and male offspring (11 offspring from 9 mothers) were evaluated at birth (n=6) or later in adulthood (n=5).	ND / 500	- Histopathological alterations in the testes (unusual clusters of undifferentiated germ cells)	<u>Unaffected Outcomes</u> - Gross testicular morphology; reproductive tract development; testosterone levels at birth; germ cell number and proliferation, Sertoli cell number, germ:Sertoli cell ratio
( <a href="#">Spade et al., 2018</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (3–6/dose) gavaged with 0 and 750 mg/kg-day DBP on GDs 17–21	ND / 750	↓ <i>ex vivo</i> fetal testicular testosterone production, ↑ incidence of MNGs	<u>Unaffected Outcomes</u> - Litter size; resorptions; fetal loss; terminal maternal BW
( <a href="#">Wilson et al., 2004</a> ) (Medium) <sup>d</sup>	Pregnant SD rats (3/dose) gavaged with 0 and 1,000 mg/kg-day DBP on GDs 14–18. Dams sacrificed on GD 18.	ND / 1,000	↓ fetal testicular testosterone production and testicular <i>Ins13</i> mRNA	<u>Unaffected Outcomes</u> - Testis progesterone production
( <a href="#">Ema et al., 2000</a> ) (Medium) <sup>d</sup>	Pregnant Wistar rats (10–13/dose) gavaged with 0, 1000, 1500 mg/kg DBP on GDs 12–14.	ND / 1000	↓ absolute AGD in male pups on GD21; ↓ fetal body weight (both sexes); ↓ maternal body weight gain and food consumption	<u>Maternal Effects</u> - ↓ maternal body weight gain and food consumption (≥1000 mg/kg-day) <u>Developmental Effects</u> - ↑ total litter resorptions (1500 mg/kg-day) - ↓ # live fetuses per litter (1500 mg/kg-day)

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			↓ fetal body weight (both sexes) (≥1000 mg/kg-day) ↓ absolute AGD (males) (≥1000 mg/kg-day)	- ↑ fetuses with undescended testes (1500 mg/kg-day) <u>Unaffected Outcomes</u> - Sex ratio; AGD (females) <u>Considerations:</u> - Decreased fetal body weights may be attributed to decreased maternal body weight gain and decreased food consumption.
	Pregnant Wistar rats (10/dose) gavaged with 0, 1000, 1500 mg/kg DBP on GDs 18–20.	ND / 1000	↓ fetal BW and ↓ AGD (males) on GD21; ↓ maternal BW gain	<u>Maternal Effects</u> - ↓ maternal BW gain (≥1000 mg/kg-day) and food consumption (1500 mg/kg-day) <u>Developmental Effects</u> - ↓ fetal weight (both sexes) (≥1000 mg/kg-day) - ↓ absolute AGD (males) (≥1000 mg/kg-day) - ↑ fetuses with undescended testes (1500 mg/kg-day) <u>Unaffected Outcomes</u> - Sex ratio; total litter resorptions; # of dead and live fetuses; # of fetuses with undescended testes; AGD in females
	Pregnant Wistar rats (10/dose) gavaged with 0, 500, 1000, 1500 mg/kg DBP on GDs 15–17.	ND / 500	↑ fetuses with undescended testes and ↓ AGD on GD21 (absolute and BW normalized)	<u>Maternal Effects</u> - ↓ maternal BW gain and food consumption (≥1,000 mg/kg-day) <u>Developmental Effects</u> - ↑ # of resorptions per litter (1500) mg/kg-day - ↓ # live fetuses per litter (1,500 mg/kg-day) - ↓ fetal weight (both sexes) (1,500 mg/kg-day) <u>Unaffected Outcomes</u> - Sex ratio; total litter resorptions; AGD (females)

**Abbreviations:** ↓ = statistically significant decrease; ↑ = statistically significant increase; ND = NOAEL or LOAEL not established; NOAEL = No observed adverse effect level; LOAEL = lowest observed adverse effect level; GD = gestation day; PND = postnatal day; PNW = postnatal week; F1 = first-generation offspring; F2 = second-generation offspring; AGD = anogenital distance; BW = body weight; LABC = levator ani plus bulbocavernosus muscles; MNGs = multinucleated gonocytes; LC = Leydig cell; NR = nipple retention; PPS = preputial separation; SV = seminal vesicle; DHT = dihydrotestosterone; FSH = follicle stimulating hormone; LH = luteinizing hormone; IHC = immunohistochemistry; StAR = steroidogenic acute regulatory protein; P450scc/ *Cyp11a1* = cytochrome P450 family 11, subfamily a, polypeptide 1; CYP17 = cytochrome P450 family 17; *Ins13* = insulin-like hormone 3; *SR-B1/Scarb1* = scavenger receptor class B member 1.

<sup>a</sup> TSCA Study Quality Evaluation was completed for all references that were considered for quantitative dose-response assessment in Section 4.1.

<sup>b</sup> These studies were conducted by EPA's Office of Research and Development (ORD).

<sup>c</sup> Time-weighted doses calculated for 3 doses spanning 14 days (e.g., GD1, GD7, and GD14 = 3 doses; GD1-GD14 = 14 days of dosing; 100 mg/kg x 3 doses = 300 mg/kg/ 14 days = 21.4 mg/kg-day). Effects considered after a single dose for acute POD listed for the LOAEL.

Reference (TSCA Study Quality Rating) <sup>a</sup>	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
<sup>d</sup> As discussed in the Systematic Review protocol for DBP ( <a href="#">U.S. EPA, 2025q</a> ) and consistent with Office of Pesticide Programs <i>Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment</i> ( <a href="#">U.S. EPA, 2012b</a> ), 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.				

**Table 3-4. Summary of Studies Evaluating Effects on the Developing Male Reproductive System following Prepubertal and Pubertal Exposure to DBP**

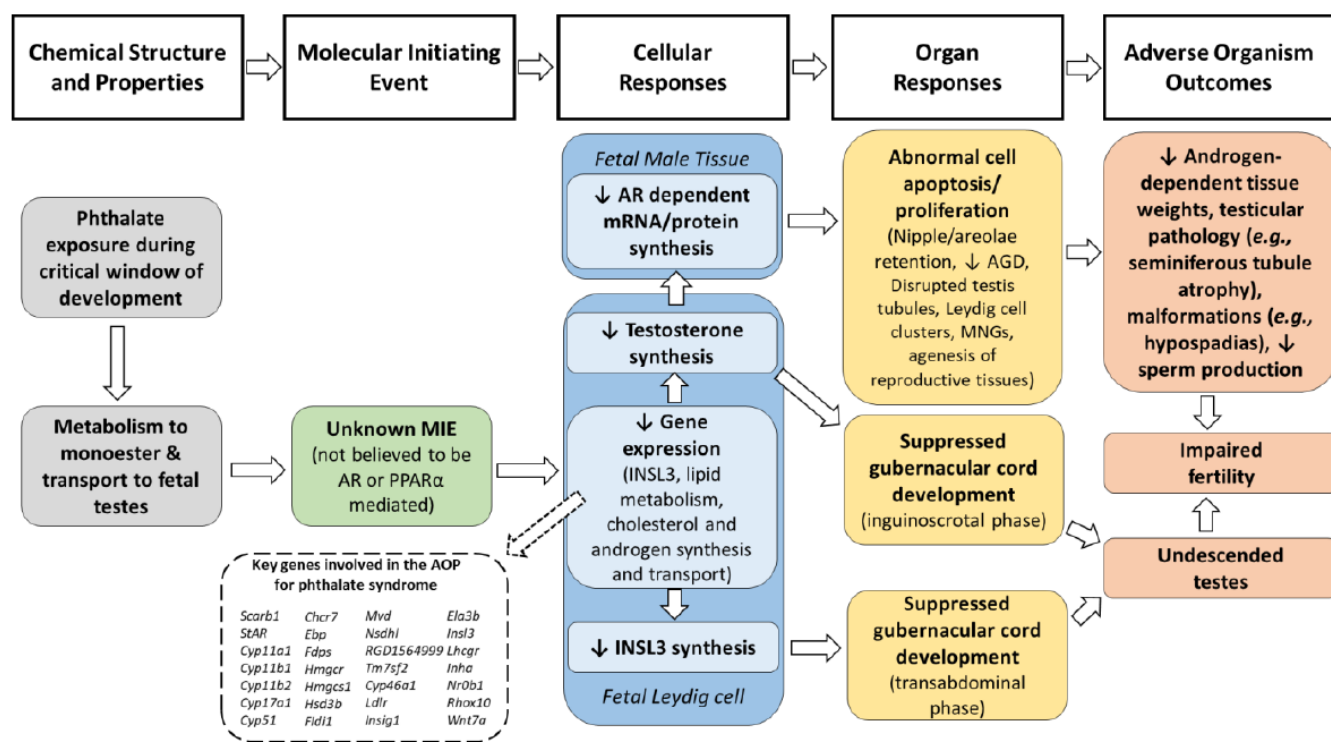
Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Xiao-Feng et al., 2009</a> ) (Medium) <sup>a</sup>	Male SD rats (8/group) gavaged with 0, 250, 500, 1,000, or 2,000 mg/kg-day DBP from PND35-PND65. An additional recovery group was maintained for 15 additional days after cessation of DBP exposure.	ND / 250 (LOEL)	↓ Leydig cell number (not considered adverse)	<u>Other Effects</u> <ul style="list-style-type: none"> <li>- ↓ serum testosterone (≥500 mg/kg-day)</li> <li>- ↑ serum glucocorticoid hormone (≥1000 mg/kg-day)</li> <li>- Histopathological changes in the testes (≥500 mg/kg-day)</li> <li>- ↑ gene expression of <i>11β-HSD1</i> &amp; <i>Glucocorticoid Receptor</i>; ↓ <i>StAR</i> (≥1000 mg/kg-day)</li> <li>- ↓ relative weight of testes (≤28%; ≥500 mg/kg-day) &amp; epididymis weight (absolute weight not reported)</li> </ul> <u>Limitations:</u> <ul style="list-style-type: none"> <li>- Qualitative histopathology (no incidence data provided)</li> </ul> <u>Unaffected outcomes</u> <ul style="list-style-type: none"> <li>- Body weight; relative adrenal weight</li> </ul>
( <a href="#">Moody et al., 2013</a> ) (Medium) <sup>a</sup>	Male and female C57BL/6 mice (≤ 6/group) gavaged with 0, 1, 10, 50, 100, 250, or 500 mg/kg-day DBP from PND4–14	ND / 1	Defective spermatogenesis (↑ incidence of partial spermatogenesis); ↓ AGD relative to body weight at adulthood; ↓ AGD relative to trunk length at PND14 & adulthood	<u>Other Effects</u> <ul style="list-style-type: none"> <li>- Delayed spermatogenesis (↓ cords containing pachytene spermatocytes [≥10 mg/kg-day])</li> <li>- ↓ Serum testosterone (PND14; 500 mg/kg-day); ↑ serum inhibin alpha subunit (PND14; 500 mg/kg-day)</li> <li>- Immature Sertoli cell and disorganization (100 mg/kg-day)</li> <li>- ↓ AGD relative to BW at PND 14 (500 mg/kg-day)</li> <li>- ↑ relative heart weight (PND3; 500 mg/kg-day)</li> </ul> <u>Limitations:</u> <ul style="list-style-type: none"> <li>- No dose-response in AGD (absolute or normalized to BW on PND14)</li> </ul>
( <a href="#">Srivastava et al., 1990</a> ) (Medium) <sup>a</sup>	Male Wistar albino rats gavaged with 0, 250, 500, or 1,000 mg/kg-day DBP from PNW5 – 7 (15 days).	ND / 250	↑ testes histopathology ( <i>i.e.</i> , defective spermatogenesis;	<u>Other effects:</u> <ul style="list-style-type: none"> <li>- ↑ activity of enzymes in testes, including lactate dehydrogenase (dose-responsive, beginning at 250 mg/kg-day), gamma-glutamyl transpeptidase (500,</li> </ul>



Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			shrunk tubules in testes); ↑ activity of enzymes in testes, lactate dehydrogenase, acid phosphatase, and glucose-6-phosphate dehydrogenase; ↑ activity of enzymes in testes, including lactate dehydrogenase	1,000 mg/kg-day), acid phosphatase, and glucose-6-phosphate dehydrogenase (dose-responsive, beginning at 250 mg/kg-day) - ↓ absolute and relative testes weight (500, 1,000 mg/kg-day) - ↓ terminal BW (500 [19% change], 1,000 mg/kg-day [36% change]) <u>Limitations:</u> - Qualitative histopathology
( <a href="#">Higuchi et al., 2003</a> ) (Medium) <sup>a</sup>	Pregnant rabbits (8 litters/group) were exposed to 0 or 400 mg/kg-day DBP from PNW 4–12 and male offspring were evaluated at PNW 6, 12, and 25.	ND / 400	-Hypothalamic content of GnRH (PNW12 or PNW25) - weight of accessory sex organs at PNW12 - ↑ abnormal sperm	<u>Other effects:</u> - Hypospadias, hypoplastic prostate, and cryptorchid testes with carcinoma <i>in situ</i> -like cells in one male <u>Unaffected Outcomes:</u> - Absolute organ weights including liver, kidney, thyroid, testes, and epididymides at PNW12 or PNW25.
<p><i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; ND = NOAEL or LOAEL not established; NOAEL = No observed adverse effect level; LOAEL = lowest observed adverse effect level; LOEL = lowest observed effect level; ND = no data; GD = gestation day; PND = postnatal day; PNW = postnatal week; AGD = anogenital distance; BW = body weight; FSH = follicle stimulating hormone; <i>11β-HSD1</i>=<i>11β-Hydroxysteroid dehydrogenase type 1</i>; StAR =Steroidogenic acute regulatory protein; GnRH = gonadotropin releasing hormone.</p> <p><sup>a</sup> As discussed in the Systematic Review protocol for DBP (<a href="#">U.S. EPA, 2025q</a>) and consistent with Office of Pesticide Programs <i>Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment</i> (<a href="#">U.S. EPA, 2012b</a>), 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.</p>				

### 3.1.3 Mode of Action for Phthalate Syndrome

EPA previously developed a weight of scientific evidence analysis and concluded that oral exposure to DBP can induce effects on the developing male reproductive system consistent with a disruption of androgen action. The proposed MOA for phthalate syndrome is shown in Figure 3-1, which explains the link between gestational and/or perinatal exposure to DBP and effects on the male reproductive system in rats. The MOA has been described in greater detail in 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, 2023a](#)) and is described briefly below. The MOA underlying phthalate syndrome has not been fully established; however, key events at the cellular-, organ-, and organism-level are generally understood (Figure 3-1).



**Figure 3-1. Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure**

Figure taken directly from ([U.S. EPA, 2023a](#)) 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.

#### Molecular Initiating Event

The molecular events (*i.e.*, the molecular initiating event) preceding cellular changes remain unknown. Several studies have provided evidence against the involvement of androgen receptor antagonism and peroxisome proliferator-activated receptor alpha (PPARα) activation ([Gray et al., 2021](#); [Foster, 2005](#); [Foster et al., 2001](#); [Parks et al., 2000](#)). Other studies have suggested depletion of zinc concentration in rodents ([Gray et al., 1982](#); [Foster et al., 1980](#)), which could perturb the function of zinc-containing proteins (*e.g.*, zinc-finger transcription factors or as an enzyme cofactor). Of note, *SF-1*, a transcription factor that regulates the INSL3 promoter, contains two zinc-finger motifs that are required for DNA binding. However, it is unclear if depletion is a consequence or a cause of decreased fetal testosterone synthesis and subsequent steps in the MOA shown in Figure 3-1.

## Cellular Responses

Cellular responses are more well understood. There is abundant evidence that DBP disrupts the production of fetal testicular testosterone in rodents. Disruption of testicular testosterone production during the masculinization programming window (*i.e.*, GDs 15.5 to 18.5 for rats; GDs 14 to 16 for mice; gestational weeks 8 to 14 for humans) can lead to antiandrogenic effects on the developing male reproductive system ([MacLeod et al., 2010](#); [Welsh et al., 2008](#); [Carruthers and Foster, 2005](#)). Consistent with the MOA outlined in Figure 3-1, many studies of DBP identified by EPA have demonstrated that oral exposure to DBP during the masculinization programming window can reduce testosterone synthesis in the fetal male Leydig cell and/or 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.

Testosterone production drives extratesticular male reproductive tract development and, together with INSL3, drives adverse organism-level outcomes, such as testicular descent. The vast majority of studies identified have found decreased fetal testicular testosterone (ranging from 34 to 85 percent) following exposures of pregnant rats to 500 mg/kg-day or higher (Table 3-3; Table 3-4). However, reductions in *ex vivo* fetal testicular testosterone production and/or fetal testicular testosterone content have also been observed at lower doses ranging from 50 to 112 mg/kg-day ([Gray et al., 2021](#); [Furr et al., 2014](#); [Struve et al., 2009](#); [Mahood et al., 2007](#); [Lehmann et al., 2004](#)). Furr et al. (2014) carried out several experiments in “blocks” conducted over 2 to 3 years, and observed decreased *ex vivo* fetal testicular testosterone production in male rats from multiple blocks at doses as low as 100 mg/kg-day, reflecting a 35 percent (Block 18) or 36 percent (Block 22) decrease in testosterone production. The data set from Mahood et al. (2007), demonstrates that a 14 percent decrease in testicular testosterone content coincides with other male reproductive effects including increased Leydig cell aggregation and increased incidence of MNGs, and are therefore biologically significant. In parallel with their observations of decreased fetal testicular testosterone content, Lehmann et al. (2004) reported reductions testicular mRNA and protein expression of genes involved in steroidogenesis (*e.g.*, StAR, P450scc, CYP17) at doses of 50 mg/kg-day and up, and testis descent (Insl3) at 500 mg/kg-day and up. Additionally, significant decreases in gene expression of *SR-B1*, *3 $\beta$ -HSD*, and *c-Kit* were observed at lower doses (0.1 or 1.0 mg/kg-day). Other studies of rats have also reported decreased fetal testicular testosterone production or content coinciding with decreased expression of genes involved in cholesterol transport and steroidogenesis (*e.g.*, see ([Gray et al., 2021](#); [Struve et al., 2009](#))). There is also evidence that key genes involved in testosterone biosynthesis are also downregulated in mice. For instance, decreased gene and protein expression of *StAR*, *P450scc*, and *3 $\beta$ -HSD* was reported in male C57BL/6 mice ([Li et al., 2023](#)). In that study, mice were exposed to 0, 0.5, 5, or 75 mg/kg-day DBP from GD5 through 19 via gavage and significant changes were only observed in the high dose group.

Moreover, several studies in rats have demonstrated that even a single exposure on a single day during the critical window (*i.e.*, GD 14 to 18) could elicit decreases in fetal testicular testosterone content and steroidogenic gene expression ([Johnson et al., 2012](#); [Johnson et al., 2011](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)). Kuhl et al. (2007) reported that fetal testicular mRNA levels of *StAR*, *SR-B1/Scarb1*, *P450scc/ Cyp11a1*, and *CYP17* were decreased in GD19 fetuses of pregnant rats exposed to doses as low as 100 mg/kg-day DBP on GD18. Fetal testicular testosterone content was decreased at 500 mg/kg-day. Another single exposure study reported decreased intratesticular testosterone on GD19 one hour after dosing with 500 mg/kg-day ([Johnson et al., 2007](#)). In later publications by the same authors ([Johnson et al., 2012](#); [Johnson et al., 2011](#)), reductions in steroidogenic gene expression was observed in the fetal testes 3 hours (*Cyp17a1*) to 6 hours (*P450scc/ Cyp11a1*, *StAR*) post-exposure in pregnant SD rats gavaged with a single dose of 500 mg/kg DBP on GD 19. Fetal testicular testosterone content was reduced starting at 18 hours post-exposure. Similarly, Thompson et al. (2005) reported a 50 percent

reduction in fetal testicular testosterone content 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 (*P450scc*/*Cyp11a1*, *Cyp17a1*, *Scarb1*) hours post-exposure, and protein levels of these genes were reduced 6 to 12 hours post-exposure. Altogether, these data support a mode of action where key changes in genes involved in steroidogenesis or testosterone transport precede cellular responses and subsequent organ-level responses.

### ***Organ-level Responses***

Organ-level responses in the reproductive system include Leydig cell aggregation or altered distribution of Leydig cells, reduced AGD, and increased nipple retention. Perturbations in Leydig cell morphology are indicative of disrupted androgen action. Leydig cells of the testes produce testosterone, INSL3, and dihydrotestosterone (DHT), which forms from its precursor, testosterone. Reduced AGD (which is an externally visible marker) stems from reduced production of testosterone by the Leydig cell during the masculinization programming window, as DHT functions to lengthen the perineum (*i.e.*, skin between the genitals and anus) of males. AGD is therefore a sensitive indicator of prenatal androgen exposure. Increased nipple retention also stems from reduced testosterone production, as DHT in peripheral tissues is necessary for apoptosis and regression of nipples in male rats. Each of these responses have been well documented in rodents exposed to DBP following gestational exposure. Indeed, three studies have reported increased incidences of Leydig cell aggregates at doses ranging from 100 mg/kg-day ([Scarano et al., 2010](#); [Struve et al., 2009](#); [Mahood et al., 2007](#)) to 300 mg/kg-day ([Li et al., 2015](#)). Aside from these studies, the majority of studies report histopathological alterations at doses above 100 mg/kg-day. At higher levels of exposure (*i.e.*, 250 to 750 mg/kg-day), Leydig cell hyperplasia has been observed ([Mylchreest et al., 2002](#)).

Many studies have demonstrated that oral exposure of rats to DBP during the masculinization programming window can reduce male pup AGD measured earlier in the postnatal window (*i.e.*, on PND1 through PND4) ([Li et al., 2015](#); [Jiang et al., 2007](#); [Barlow et al., 2004](#); [Lee et al., 2004](#); [Zhang et al., 2004](#); [Mylchreest et al., 2000](#); [Mylchreest et al., 1999](#); [Mylchreest et al., 1998](#)), after lactation on PND25 ([MacLeod et al., 2010](#)), or during adulthood ([Drake et al., 2009](#); [Barlow et al., 2004](#)). Similarly, increased male pup nipple retention (NR) around PND13 or PND14 has been consistently reported ([Kim et al., 2010](#); [Martino-Andrade et al., 2008](#); [Barlow et al., 2004](#); [Lee et al., 2004](#); [Mylchreest et al., 2000](#); [Mylchreest et al., 1999](#)). 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, as well as result in permanent NR, and increase the frequency of reproductive tract malformations and testicular pathology in adult rats that received two doses of DBP during the critical window (*i.e.*, GD 14 to 18). Reduced AGD has been reported in rats following exposures during the masculinization programming window at doses as low as 100 mg/kg-day ([Martino-Andrade et al., 2008](#)), but most commonly between 300 and 500 mg/kg-day (Table 3-3). Similarly, two studies have reported increased nipple retention at doses as low as 100 mg/kg-day ([Barlow et al., 2004](#); [Mylchreest et al., 2000](#)), but this effect is more commonly observed at doses of 250 mg/kg-day and higher (Table 3-3). Consistent with the animal literature, there is epidemiological evidence that supports an inverse association between *in utero* exposure to DBP and anogenital distance ([Radke et al., 2018](#)), which may reflect exposure or responsiveness to testosterone during fetal development.

Phthalates can also affect Sertoli cell function and development. Formation of lesions such as multinucleated gonocytes (MNGs) is one indication of perturbed Sertoli cell function and development. Increases in MNGs ([Spade et al., 2018](#); [Boekelheide et al., 2009](#); [Ferrara et al., 2006](#)) have been observed at higher levels of exposure to DBP (*i.e.*, 250 to 750 mg/kg-day). While MNGs are also observed in mice exposed to DBP during the critical window, decreased expression of genes involved in

steroidogenesis and cholesterol homeostasis that are observed in the testicular tissues of rats are not also found in mice, suggesting that altered formation of MNGs is not mechanistically related to decreased testosterone in mice as it is in rats ([Gaido et al., 2007](#)).

Additionally, 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, 2023a](#)), 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 DBP and its monoester metabolite, MBP, as well as mono-2-ethylhexyl phthalate (MEHP; a monoester metabolite of DEHP) in a human model. Generally, results from human explant and xenograft studies (*i.e.*, host serum testosterone production, host serum testosterone concentration, and MNG formation) suggest that human fetal testes are less sensitive to the antiandrogenic effects of phthalates, however, increased incidence of MNGs have been observed in two human xenograft studies of DBP ([van Den Driesche et al., 2015](#); [Spade et al., 2014](#); [Heger et al., 2012](#); [Mitchell et al., 2012](#)). As discussed in EPA's draft approach document ([U.S. EPA, 2023a](#)), the available human explant and xenograft studies have limitations and uncertainties, which preclude definitive conclusions related to species differences in sensitivity.

### ***Organism-level Responses***

Adverse outcomes at the organism-level have been observed following exposure to DBP during the masculinization programming window, including effects on androgen-dependent organ weights (*e.g.*, testes weight), testicular histopathology, seminiferous tubule atrophy, malformations (*e.g.*, hypospadias), cryptorchidism, or impaired fertility (Table 3-3).

Androgen-dependent testicular histopathology has been reported across a number of studies including degeneration of the seminiferous tissue ([Mylchreest et al., 1999](#)) or of the testicular tissues more generally ([Howdeshell et al., 2007](#)), or other perturbations ([van den Driesche et al., 2012](#); [Johnson et al., 2011](#); [McKinnell et al., 2009](#); [Zhang et al., 2004](#); [Higuchi et al., 2003](#)). Hypospadias and/or cryptorchidism following gestational exposure to DBP during the critical window has been reported in several rodent studies, some of which demonstrate lasting effects in adults that had been exposed *in utero* (LOAELs range 250 to 700 mg/kg-day), demonstrating the permanence of these effects ([Li et al., 2015](#); [Kim et al., 2010](#); [Jiang et al., 2007](#); [Mahood et al., 2007](#); [Mylchreest et al., 2000](#); [Mylchreest et al., 1998](#)). Reproductive tract malformations ([Mylchreest et al., 1998](#)) or delayed male puberty (*i.e.*, preputial separation) ([Kim et al., 2010](#)) have also been reported at doses of 250 mg/kg-day. Similarly, seminiferous tubule atrophy has been observed in adult rats that had been exposed to doses of DBP ranging from 250 to 500 mg/kg-day during the critical window of fetal development (*e.g.*, ([Barlow et al., 2004](#); [Mylchreest et al., 1999](#); [Wine et al., 1997](#); [NTP, 1995](#)), and others in Table 3-3). Epidemiological evidence is consistent with the findings of rodent studies. Indeed, the [Radke et al. \(2018\)](#) study determined that the level of evidence was *slight* for the association between *in utero* exposure to DBP and hypospadias and/or cryptorchidism ([Radke et al., 2018](#)).

Gestational exposure to DBP has also been associated with reductions in reproductive performance measures. In a multigenerational study with a continuous breeding protocol, decreased indices of mating, pregnancy, and fertility were observed in F1, but not F0 ([Wine et al., 1997](#); [NTP, 1995](#)) generation rats, indicating the heightened sensitivity of the F1 generation due to the gestational exposure. Mahood et al. ([2007](#)) reported increased incidence of infertility (approximately 75 percent of infertile/fertile animals per litter and overall) in adult rats exposed to 500 mg/kg-day DBP in utero (GD 13.5 to GD12.5). Although increased incidences were observed at the lower doses (*i.e.*, 4, 20, or 100



mg/kg-day), changes were not statistically significant and there was no dose-response (*i.e.*, the incidence of infertility across the 0, 4, 20, and 100 mg/kg-day groups was 1, 22, 14, and 33 percent, respectively). Increased incidence of cryptorchidism was observed in parallel with the increased incidence of infertility at 500 mg/kg-day, although cryptorchidism was observed in 1 of 19 animals in the 100 mg/kg-day group. Dose-responsive increases in the percent of seminiferous cords with MNGs (LOAEL = 100 mg/kg-day) and decreases in testis testosterone (LOAEL = 100 mg/kg-day) were also observed. Impaired fertility, reflected by reduced sperm count, reduced sperm motility, or increased percentages of abnormal sperm have also been reported in two studies following gestational exposures during the critical window ([Giribabu et al., 2014](#); [Zhang et al., 2004](#)). One study in rabbits also observed changes in post-puberty sperm parameters following gestational exposure to 400 mg/kg-day DBP, providing further evidence that the effects of DBP on fertility extend across species ([Higuchi et al., 2003](#)). This study also included a postnatal exposure, where fewer effects on fertility were observed compared to the gestational exposure. However, some studies that evaluated male fertility following DBP exposures during the critical window of up to 500 mg/kg-day did not observe any changes ([Scarano et al., 2010](#); [Martino-Andrade et al., 2008](#)). Further details on these studies are provided in Table 3-3 and Table 3-4. An important limitation of the majority of these studies is that histopathological evaluations were qualitative, which impacts the ability to interpret the results. Nevertheless, the few studies that provide quantitative histopathological data (*e.g.*, Mahood et al. (2007) and Mylchreest et al. (2000)) report similar findings to the qualitative findings (*e.g.*, Mylchreest et al. (1999)), and when considered together support that seminiferous tubule atrophy, MNG formation, and changes in Leydig cell morphology occur following exposure to DBP. In support of the animal data, there is epidemiological evidence that supports the association between exposure to DBP and indicators of fertility including semen parameters (*e.g.*, semen concentration, motility, and/or morphology), and time to pregnancy measured in adults.

### **3.2 Literature Considered for Non-Cancer Hazard Identification**

---

From the initial literature search (*i.e.*, 2014–2019) EPA identified 63 animal toxicology studies that provide data on PECO-relevant health effects following exposure to DBP. Of these, 12 studies provided LOAELs for PECO-relevant outcomes within an order of magnitude of the most sensitive PODs identified from prior assessments (*i.e.*, 20 mg/kg-day or lower). These studies evaluated reproductive and developmental outcomes (seven studies), neurological outcomes (three studies), nutritional/metabolic outcomes (three studies), cardiovascular outcomes (one study), and the immune adjuvant capacity of DBP (two studies). Limitations in most of these 12 studies impacted the interpretation of the results, and there was substantial uncertainty in the data. Therefore, EPA ultimately did not consider these animal toxicology studies further in Section 4. EPA did not conduct a full evidence integration for health outcomes other than those of the male reproductive system following developmental exposure (Section 3.1.2.1). Details and summaries of EPA's consideration of literature for Non-Cancer Hazard Identification are provided in Appendix B. Summarized study information on the remaining 51 studies is available in a supplemental file is ([U.S. EPA, 2025p](#)).

The 2025 literature update identified studies from public comments submitted to EPA or from the August 4 through 8, 2025 SACC meeting. One additional study ([Li et al., 2023](#)) was identified from the 2025 literature update and considered further for dose-response assessment in Section 4.

### **3.3 Summary**

---

Collectively, reasonably available studies consistently demonstrate that oral exposure to DBP during the masculinization programming window can disrupt androgen action, leading to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. Evidence from epidemiological studies indicates a *moderate* level of confidence in the association between DBP and health effects on the male reproductive system, such as AGD. Evidence from animal studies, including



the robust database of studies in rats, demonstrates adverse effects on the male reproductive system following developmental exposure to DBP. EPA's MOA analysis concluded that available studies consistently demonstrate that oral exposure to DBP during the masculinization programming window can disrupt androgen action, leading to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. As noted above, this conclusion was supported by the Science Advisory Committee on Chemicals (SACC) ([U.S. EPA, 2023b](#)) 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, 2023a](#)) for a more thorough discussion of DBP's effects on the developing male reproductive system and EPA's MOA analysis. EPA considered effects on the developing male reproductive system for dose-response analysis and for use in estimating risk to human health. The observed developmental effects are assumed to be relevant for extrapolating human risk. EPA further considered effects on the developing male reproductive system in Section 4.

## 4 DOSE-RESPONSE ASSESSMENT

---

EPA considered non-cancer hazard endpoints related to effects on the developing male reproductive system for dose-response analysis as described in the following sections. These hazard endpoints were selected for dose-response analysis because EPA has the highest confidence in these hazard endpoints for estimating non-cancer risk to human health and effects on the developing male reproductive system are the most sensitive based on available data. Other non-cancer hazard endpoints were therefore not considered for dose-response analysis or for estimating risk to human health.

For the DBP dose-response assessment, EPA first identified NOAEL and LOAEL values from 11 developmental toxicity studies (Table 4-1). Seven of these 11 developmental toxicity studies evaluated effects on the developing male reproductive system in mice and rats exposed to low doses of DBP (*i.e.*, at doses less than 100 mg/kg-day), and data from these 7 studies were further considered for benchmark dose (BMD) analysis ([Furr et al., 2014](#); [Moody et al., 2013](#); [Boekelheide et al., 2009](#); [Mahood et al., 2007](#); [Lee et al., 2004](#); [Lehmann et al., 2004](#); [Mylchreest et al., 2000](#)). One of the initial 11 studies did not test doses of DBP below 100 mg/kg-day, but as discussed further below, was included as part of EPA's meta-analysis and BMD analysis of fetal testosterone ([Martino-Andrade et al., 2008](#)). The remaining 3 of the initial 11 studies were not subjected to BMD analysis because they either evaluated only one dose level ([Clewett et al., 2009](#)) or because of data reporting limitation and/or because they were not very sensitive (*i.e.*, evaluated doses of 100 mg/kg-day or higher or did not identify sensitive phthalate-syndrome related effects for modeling) ([Barlow et al., 2004](#); [Wine et al., 1997](#)). For reduced fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production in rats, EPA conducted meta-analysis and benchmark dose modeling using the approach previously published by 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 eight publications was included in EPA's meta-analysis ([Gray et al., 2021](#); [Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)). Data from these individual studies were 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.

Acute, intermediate, and chronic non-cancer NOAEL/ LOAEL values identified by EPA are discussed further below in Section 4.2. EPA converted oral PODs derived from animal studies to HEDs using allometric body weight scaling to the three-quarters power ([U.S. EPA, 2011b](#)). Differences in dermal and oral absorption are corrected for as part of the dermal exposure assessment. In the absence of inhalation studies, EPA performed route-to-route extrapolation to convert oral HEDs to inhalation human equivalent concentrations (HECs) (Appendix D).

### 4.1 Selection of Studies and Endpoints for Non-Cancer Health Effects

---

EPA considered a 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, as described in Section 4.2.

EPA identified 39 studies that evaluated effects on the developing male reproductive system DBP exposure (Table 3-3; Table 3-4). In order to focus its dose-response assessment, EPA further considered the most sensitive studies of DBP supporting a LOAEL of 100 mg/kg-day or less in Section 4.2. Studies supporting a LOAEL of greater than 100 mg/kg-day are discussed in Section 3.1.2 as part of the non-

cancer hazard identification and characterization. EPA identified 11 studies investigating effects on the developing male reproductive system consistent with phthalate syndrome that support a LOAEL of 100 mg/kg-day or less and these studies are discussed further in Section 4.2 ([Furr et al., 2014](#); [Moody et al., 2013](#); [Boekelheide et al., 2009](#); [Clewell et al., 2009](#); [Martino-Andrade et al., 2008](#); [Mahood et al., 2007](#); [Barlow et al., 2004](#); [Lee et al., 2004](#); [Lehmann et al., 2004](#); [Mylchreest et al., 2000](#); [Wine et al., 1997](#)).

EPA considered the following factors during study and endpoint selection for POD determination from 11 studies with relevant non-cancer health effects based on the following considerations:

- Exposure duration;
- Dose range;
- Relevance (*i.e.*, considerations of species, direct vs. indirect effects, suitability of the endpoint as a biomarker or indicator of 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<sub>L</sub> applied).

The following sections 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**

---

EPA considered effects on the developing male reproductive system across 11 studies of rats with endpoints considered relevant to acute exposure duration ([U.S. EPA, 1996, 1991](#)), in addition to being relevant for intermediate and chronic durations ([Furr et al., 2014](#); [Moody et al., 2013](#); [Boekelheide et al., 2009](#); [Clewell et al., 2009](#); [Martino-Andrade et al., 2008](#); [Mahood et al., 2007](#); [Barlow et al., 2004](#); [Lee et al., 2004](#); [Lehmann et al., 2004](#); [Mylchreest et al., 2000](#); [Wine et al., 1997](#)). There is evidence that effects on the developing male reproductive system consistent with a disruption of androgen action can result from a single exposure during the critical window of development (*i.e.*, GD 14 to 18) (Appendix C). 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 review meeting of the DINP human health hazard assessment ([U.S. EPA, 2024](#)), as well as during the August 2025 peer review meeting of the human health hazard assessments of 5 phthalates, including DBP ([U.S. EPA, 2025o](#)).

These studies were previously discussed in Section 3.1.2.1 and are summarized in Table 4-1. The majority of studies in Table 4-1 entailed exposure durations that exceeded a single day but evaluated endpoints consistent with disruption of androgen action and included at least one dose during the critical window. Effects observed across these studies included testicular histopathology consistent with decreased spermatocyte development, decreased fetal testicular testosterone, content and *ex vivo* fetal testicular testosterone production, male mammary gland histopathology, decreased steroidogenic gene expression in the fetal testes, decreased male pup body weights, effects on fetal Leydig cells, increased incidence of MNGs, decreased anogenital distance, and increased nipple retention.

Studies in Table 4-1 were subjected to dose-response analysis to select the study and endpoint most appropriate to derive the POD for acute, intermediate, and chronic hazards. Candidate PODs range from

1 to 100 mg/kg-day based on antiandrogenic effects. Eight of these studies provided more sensitive candidate PODs of 50 mg/kg-day or less for effects on the developing male reproductive system, including decreased fetal testicular testosterone content and/or *ex vivo* fetal testicular testosterone production. EPA considers decreased fetal testicular testosterone (reported as *ex vivo* fetal testicular testosterone production or fetal testicular testosterone content) to be adverse and relevant to human health ([U.S. EPA, 2023a, b](#)).

#### 4.2.1 Studies Considered for Dose-Response Assessment

---

Eleven studies were considered by EPA that provided relatively sensitive candidate PODs based on antiandrogenic effects to the developing male reproductive system. Of these, four studies support LOAELs of 50 to 100 mg/kg-day based on decreases in fetal testicular testosterone ([Clewell et al., 2009](#)), decreases in number of live pups per litter in the first generation and decreases in live pup weight in the second generation offspring ([Wine et al., 1997](#)), decreased male AGD (mm/cube root BW) on GD21 ([Martino-Andrade et al., 2008](#)), and increased nipple retention in males on PND13 ([Barlow et al., 2004](#)). However, these studies are limited by poor dose-selection and did not test sufficiently low doses to establish a NOAEL. EPA considered BMD analysis of these studies to refine the identified LOAELs, however, BMD modeling of these studies was not conducted for various reasons. The [Clewell et al. \(2009\)](#) study only tested one dose (*i.e.*, the LOAEL of 50 mg/kg-day) in addition to the control and therefore is not amenable to BMD modeling. The most sensitive effect reported by Barlow et al. (2004) was increased male nipple retention on PND 13, supporting a LOAEL of 100 mg/kg-day. EPA did not attempt BMD analysis of nipple retention data from this study due to data reporting limitations (*e.g.*, data reported graphically only, sample size [# of litters evaluated on PND 13 not provided]). The most sensitive effect reported by Wine et al. (1997) was decreased number of live pups per litter in the first generation and decreased live pup weight in second generation offspring, supporting a LOAEL of 80 mg/kg-day. EPA did not attempt BMD analysis of data from this study due to uncertainties in the data. For example, the effect on number of live pups per litter was not consistently observed across generations (*i.e.*, observed in F1, but not F2), while the magnitude of response on F2 pup weight was small (5–6%) at the LOAEL. Finally, the most sensitive effect reported by Martino-Andrade et al. (2008) was reduced male AGD. EPA did not attempt to model AGD data from this study because as discussed further below in Section 4.2.2, NASEM (2017) conducted a meta-regression analysis and BMD analysis of decreased male rat AGD data from 16 studies (including data from Martino-Andrade et al.), which supports BMD<sub>5</sub>/BMDL<sub>5</sub> estimates of 153/115 mg/kg-day. Since this meta-analysis includes data from 16 studies, it is expected to provide more precise BMD<sub>5</sub>/BMDL<sub>5</sub> estimates and is therefore preferred over BMD analysis of data from individual studies. Given the limitations, EPA did not select these studies and endpoints for an acute/intermediate/chronic POD. Other studies tested lower doses that allowed for the identification of a NOAEL.

Five studies identified NOAELs ranging from 20 to 50 based on increased nipple retention in males in males on PND 14 ([Mylchreest et al., 2000](#)), increased incidence in testicular pathology ([Boekelheide et al., 2009](#)), or decreased fetal testicular testosterone content and/or *ex vivo* fetal testicular testosterone production ([Furr et al., 2014](#); [Mahood et al., 2007](#); [Lehmann et al., 2004](#)). The NOAEL of 20 mg/kg-day from Mahood et al. (2007) was based on increases in MNGs and Leydig cell aggregation at 100 mg/kg-day, in addition to decreased fetal testicular testosterone content. However, as described further below, each study contained limitations or areas of uncertainty that impacted the ability of EPA to interpret the results, and ultimately EPA did not select any of these candidate PODs.

Mylchreest et al. (2000) provided a POD of 11.8 mg/kg-day (HED) based on a NOAEL of 50 mg/kg-day. The POD was based on increased nipple retention in 31 percent of the males from the 100 mg/kg-day group on PND 14. Additionally, decreases in AGD at birth (PND1), decreased reproductive organ

weights, and histopathological lesions (*i.e.*, interstitial cell hyperplasia in the seminiferous epithelium) were observed in the high dose group (*i.e.*, 500 mg/kg-day). Because the study included a large sample size (*i.e.*, 20 litters/dose, per OECD TG 414 guidelines), an exposure period (GD 12–21) that encompassed the critical window of development, and evaluated a wide-range of dose groups (*i.e.*, 0.5, 5, 50, 100, or 500 mg/kg-day), EPA conducted additional BMD modeling of the most sensitive effect observed in the study (*i.e.*, nipple/areolae retention in F1 males). As described further in Appendix G, EPA evaluated BMRs of 5 and 10 percent using standard frequentist dichotomous models included in EPA's BMD Online software (Version 25.1), as well as Bayesian model averaging. BMD modeling results are summarized in Table\_Apx G-2. Based on the best-fitting Log-Probit model, BMD<sub>5</sub>/BMDL<sub>5</sub> and BMD<sub>10</sub>/BMDL<sub>10</sub> estimates are 51/29 mg/kg-day and 67/44 mg/kg-day, respectively, while Bayesian modeling averaging supports BMD<sub>5</sub>/BMDL<sub>5</sub> and BMD<sub>10</sub>/BMDL<sub>10</sub> estimates of 33/15 mg/kg-day and 59/30 mg/kg-day, respectively. Overall, based on the Bayesian model averaging approach, EPA considers this study to support a BMDL<sub>5</sub> of 15 mg/kg-day based on increased incidence of nipples/areolae in F1 males.

Although Mahood et al. (2007) and Lehman et al. (2004) identified sensitive candidate PODs of 4.7 mg/kg-day and 7.1 mg/kg-day (based on NOAELs of 20 and 30 mg/kg-day for reduced fetal testicular testosterone concentration), respectively, neither was considered further for benchmark dose analysis due to reporting deficiencies. For example, while both studies report mean and standard error information for all evaluated dose groups graphically, the sample size in both studies is reported as a range across dose groups, instead of a discrete value for each dose group. NASEM (2017) excluded both of these studies from their meta-analysis and BMD modeling analysis of fetal testosterone for similar data reporting deficiencies. Additionally, other studies provided more sensitive candidate PODs.

Furr et al. (2014) and Boekelheide et al. (2009) support candidate PODs based on NOAELs of 10 or 50 mg/kg-day. Furr et al. (2014) identified two candidate PODs based on NOAELs of 10 and 50 mg/kg-day (HED = 2.4 and 11.8 mg/kg-day, respectively) based on decreased *ex vivo* fetal testicular testosterone production at the next highest dose group (LOAEL = 100 mg/kg-day, for both studies). However, given the same LOAEL and large dose-spacing of the Block 22 data, the NOAEL of 10 mg/kg-day from Block 22 is likely an artifact of dose-selection, which decreases EPA's confidence in its utility as a POD. Additionally, there was no clear dose-response in Block 18 (*i.e.*, NOAEL = 50 mg/kg-day), and both blocks had low sample sizes. As discussed further in Section 4.2.2, EPA conducted BMD modeling of testosterone data from Furr et al. and included data from this study in its updated meta-analysis. Low sample size as also a limitation of Boekelheide et al. (2009). This study observed increased incidences of testicular pathology (*i.e.*, decreased testicular cell number and disorganized seminiferous tubules) in rats exposed to 30 mg/kg-day during gestation (NOAEL = 10 mg/kg-day; HED = 2.4 mg/kg-day). However, the adversity of this outcome is uncertain, which reduces EPA's confidence in this outcome as a POD. EPA considered BMD modeling of the most sensitive effect (*i.e.*, reduced testicular cell number) reported by Boekelheide et al., however, BMD modeling was not possible due to data reporting deficiencies (*i.e.*, study authors report mean, N, and a measure of variation graphically, but do not report whether the provided measure of variation is standard error or standard deviation]).

Two additional studies (Moody et al., 2013; Lee et al., 2004), identified the lowest candidate PODs reviewed by EPA (Table 4-1). Of note, these were below the BMDL<sub>5</sub> of 9 mg/kg-day (HED= 2.1 mg/kg-day) identified from the updated meta-analysis and BMD modeling conducted by EPA. Moody et al. (2013) and Lee et al. (2004) provided candidate PODs of 0.13 mg/kg-day (Moody et al., 2013) and 0.71 mg/kg-day (Lee et al., 2004), based on effects on the developing male reproductive system. Although the studies offer sensitive PODs and provide data from two different species (mice and rats) consistent with decreased spermatocyte development following gestational and/or postnatal exposure to



DBP, limitations related to insufficient methodological detail, study design and exposure timing, and evidence of maternal toxicity reduced EPA's confidence in the results.

Moody et al. (2013) offered a sensitive POD of 0.13 mg/kg-day (HED) based on delayed spermatogenesis in mice (LOAEL = 1 mg/kg-day). These data are inconsistent with the abundant literature indicating that the rat is more sensitive than the mouse to the antiandrogenic effects of phthalates. Nevertheless, the study represents an expansion of the data set to include additional sensitive lifestages and examine prepubertal exposure at the beginning of the first wave of spermatogenesis, as mice were exposed to DBP from PND4 to PND14. In adult animals exposed to 1 mg/kg-day, histopathological observations suggest defective spermatogenesis in addition to decreased AGD relative to body weight. However, these results are based on small sample sizes ( $n = 5-6$ ) and there are methodological limitations that hinder the interpretation of these results; data are presented as individual values rather than litter means, so it is unclear if the quantitative data are statistically analyzed correctly. In addition to these limitations, there is no clear dose-response for the reduction in AGD, which increases uncertainty in the data set. EPA considered BMD modeling of reduced AGD and increased incidence of partial spermatogenesis (the two most sensitive effects observed in the study). However, AGD was not modeled due to data reporting deficiencies (*i.e.*, reported graphically only and  $N$  not provided) and due to lack of clear dose-response relationship, while incidence of partial spermatogenesis was high across dose groups (ranging from 50–100%) compared to controls (incidence of 0%). EPA did not attempt to BMD model incidence of partial spermatogenesis 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%. Given the uncertainty regarding the permanence of effects and other limitations noted above, EPA did not consider this study further.

Lee et al. (2004) offered a POD of 0.71 mg/kg-day (HED; LOAEL = 3 mg/kg-day) based on increased incidence of reduced spermatocyte development in PND21 rats exposed to DBP from GD15 to PND21. EPA considered BMD modeling of reduced spermatocyte development incidence data, however, the prevalence of this lesion across dose groups ranged from 50 to 100 percent (incidence: 0/8, 4/8, 4/8, 8/8, 8/8 across control and dose groups). 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 percent. Additional limitations increased the uncertainty in the adversity of this endpoint, including the fact that the severity score was minimal to slight for the lowest two doses and severity did not increase to moderate until the two highest dose groups. Additional sources of uncertainty for this study include the age of outcome assessment being close to the beginning of spermatocyte development (which begins around PND21), which impacts the interpretation of the severity scores. Interpreting the histopathological data is further limited by insufficient methodological detail required to understand how the outcomes were assessed. Additionally, maternal weight gain during pregnancy was significantly decreased in the low dose group, which potentially confounds the observed effects on spermatocyte development.

Both Moody et al. (2013) and Lee et al. (2004) point to sensitive effects following exposure to DBP during a sensitive lifestage that is observed in both mice and rats. It is likely that species differences in sensitivity of these pubertal effects across the two studies is a function of study design, as in both cases, no NOAEL was identified (*i.e.*, lowest dose tested has an effect). However, the aforementioned limitations in each study impact the interpretation of the results and contribute uncertainty, and as a result EPA did not select either study for the POD for acute and/or intermediate exposures.

Data on chronic studies of DBP did not offer a more sensitive POD than the database of developmental exposure studies (Table 4-1). Moreover, NTP (2021) identified a LOAEL of 510 mg/kg-day



(HED = 120.6 mg/kg-day) based on increased gross findings in male rats (cryptorchidism, agenesis, small testis), increased microscopic findings in the testes (*e.g.*, seminiferous tubule dysgenesis, Leydig cell hyperplasia) and hypospermia), increased incidence of hepatocyte alteration in the liver of males and females, and increased incidence of hypertrophy in the pars distalis male rats. Because the scarce data that exist on chronic exposure durations of DBP ([NTP, 2021](#)) do not offer more sensitive PODs than those considered relevant for acute exposure durations (Table 4-1), EPA considered acute duration PODs for intermediate and chronic durations as well.

#### **4.2.2 Benchmark Dose Modeling of Testosterone and Anogenital Distance Data**

---

As part of the dose response analysis, EPA also reviewed a meta-regression analysis and benchmark dose (BMD) modeling analysis of decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production, as well as decreased male rat AGD data published by The National Academies of Sciences, Engineering, and Medicine (NASEM) ([2017](#)). Based on results from 12 studies of rats ([Li et al., 2015](#); [Furr et al., 2014](#); [van den Driesche et al., 2012](#); [Johnson et al., 2011](#); [Clewell et al., 2009](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#); [Mahood et al., 2007](#); [Lehmann et al., 2004](#)), NASEM found high confidence in the body of evidence and a high level of evidence that fetal exposure to DBP is associated with a reduction in fetal testosterone content and *ex vivo* fetal testicular testosterone production in rats. NASEM further conducted a meta-regression analysis and BMD modeling analysis on decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data from 7 studies of rats ([Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)). Five studies were excluded from this meta-analysis analysis due to deficiencies in data reporting (*i.e.*, sample sizes were not reported for each dose group) ([Li et al., 2015](#); [van den Driesche et al., 2012](#); [Clewell et al., 2009](#); [Mahood et al., 2007](#); [Lehmann et al., 2004](#)). Some of these studies were also reviewed for dose-response assessment by EPA (Table 4-1). NASEM found a statistically significant overall effect and linear trends in  $\log_{10}(\text{dose})$  and dose, with an overall large magnitude of effect (greater than 50 percent) in its meta-analysis for DBP. The linear-quadratic model provided the best fit (based on lowest AIC) (Table 4-4). BMD estimates from the linear-quadratic model were 12 mg/kg-day [95% confidence interval: 8, 22] for a 5 percent change (BMR = 5%) and 125 mg/kg-day [85, 205] for a 40 percent change (BMR = 40%) (Table 4-4).

NASEM ([2017](#)) also conducted a meta-regression analysis and BMD analysis of decreased male rat AGD. The analysis included AGD data from 16 rat studies ([Clewell et al., 2013](#); [Johnson et al., 2011](#); [Kim et al., 2010](#); [Scarano et al., 2010](#); [Drake et al., 2009](#); [Li et al., 2009](#); [Struve et al., 2009](#); [Martino-Andrade et al., 2008](#); [Jiang et al., 2007](#); [Barlow et al., 2004](#); [Lee et al., 2004](#); [Mylchreest et al., 2000](#); [Mylchreest et al., 1999](#); [Ema et al., 1998](#); [Mylchreest et al., 1998](#); [NTP, 1995](#)). NASEM found a statistically significant overall effect of a reduction in AGD ( $-6.88$  [95% CI:  $-8.94, -4.83$ ]) and linear trends in  $\log_{10}(\text{dose})$  ( $-4.14$  [95% CI:  $-5.63, -2.65$ ]) and dose ( $-2.42$  [95% CI:  $-2.80, -2.04$ ]). The results of the analysis were robust to sensitivity analysis (*i.e.*, leaving out results of individual studies, restricting the analysis to the high-dose group). The linear-quadratic model provided the best fit (based on lowest AIC), with statistically significant heterogeneity in all cases ( $I^2 > 75\%$ ) and a BMD<sub>5</sub> estimate of 153 mg/kg-day (95% CI: 115, 216). Overall, the meta-regression and BMD analyses conducted by NASEM demonstrate that decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production (BMD<sub>5</sub> estimate of 12 mg/kg-day) is a more sensitive endpoint than decreased male rat AGD (BMD<sub>5</sub> estimate of 153 mg/kg-day).

Since EPA identified new *ex vivo* fetal testicular testosterone production data ([Gray et al., 2021](#)) for DBP, an updated meta-analysis was conducted. EPA did not conduct an updated meta-analysis of decreased AGD, because this apical outcome, which is mechanistically linked to decreased fetal

testicular testosterone, is less sensitive than the fetal testicular testosterone endpoint. Using the publicly available R code provided by NASEM (<https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose>), EPA applied the same meta-analysis and BMD modeling approach used by NASEM, with the exception that the most recent Metafor package available at the time of EPA's updated analysis was used (*i.e.*, EPA used Metafor package Version 4.6.0, whereas NASEM used Version 2.0.0) and an additional BMR of 10 percent was modelled. Appendix E provides justification for the evaluated BMRs of 5, 10, and 40 percent. Fetal rat testosterone content and/or *ex vivo* fetal testicular testosterone production data from eight studies was included in the updated analysis, including new data from Gray et al. (2021) and data from the same 7 studies included in the 2017 NASEM analysis. Overall, the meta-analysis found a statistically significant overall effect and linear trends in  $\log_{10}(\text{dose})$  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 ( $I^2 > 90\%$ ). The statistical significance of these effects was robust to leaving out individual studies. The linear-quadratic model provided the best fit (based on lowest AIC) (Table 4-4). BMD estimates from the linear-quadratic model were 14 mg/kg-day [95% confidence interval: 9, 27] for a 5 percent change (BMR = 5%), 29 mg/kg-day [20, 54] for a 10 percent change (BMR = 10%), and 149 mg/kg-day [101, 247] for a 40 percent change (BMR = 40%) (Table 4-4). Notably, BMD<sub>5</sub> and BMD<sub>40</sub> estimates calculated by NASEM and as part of EPA's updated analysis are nearly identical (*i.e.*, BMD<sub>5</sub> values of 12 and 14 mg/kg-day; BMD<sub>40</sub> values of 125 and 140 mg/kg-day). Further methodological details and results (*e.g.*, forest plots, figures of BMD model fits) for the updated 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). EPA considered the BMDL<sub>5</sub> of 9 mg/kg-day further as a candidate POD.

EPA also conducted BMD modeling of the fetal testicular testosterone content and/or *ex vivo* fetal testicular testosterone production data from 7 of the 8 the individual studies included in the updated meta-analysis and BMD analysis of fetal testicular testosterone (Appendix F) (Gray et al., 2021; Furr et al., 2014; Struve et al., 2009; Howdeshell et al., 2008; Martino-Andrade et al., 2008; Johnson et al., 2007; Kuhl et al., 2007). Data from one study (Johnson et al., 2011) included in the updated meta-analysis was not subjected to BMD analysis because it only evaluated one dose group. The purpose of this BMD analysis was to determine if modeling testosterone data from individual studies using EPA's BMDS online (<https://bmdsonline.epa.gov/>) provides similar BMD<sub>5</sub> and BMDL<sub>5</sub> estimates compared to the updated meta-analysis. EPA's BMDS online includes additional continuous models (*i.e.*, exponential 3 and 5, Hill, polynomial degree 2 and 3, power, linear models) compared to the updated meta-analysis (*i.e.*, linear and linear-quadratic models). Appendix F summarizes EPA's methodology and results for this analysis. As can be seen from Table\_Apx F-1, data from 4 of the 7 publications was amenable to BMD modeling. BMD modeling of fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data supports BMD<sub>5</sub> and BMDL<sub>5</sub> values of 24 and 16 mg/kg-day based on the best-fitting Exponential 3 model (Martino-Andrade et al., 2008); BMD<sub>5</sub> and BMDL<sub>5</sub> values of 22 and 14 mg/kg-day based on the best-fitting exponential 3 model (Kuhl et al., 2007); BMD<sub>5</sub> and BMDL<sub>5</sub> values of 30 and 28 mg/kg-day based on the best-fitting linear model (Struve et al., 2009); and BMD<sub>5</sub> and BMDL<sub>5</sub> values of 49 and 39 mg/kg-day based on the best-fitting polynomial degree 3 model (Howdeshell et al., 2008) (Table\_Apx F-1). As can be seen from Table 4-1, this BMD analysis of fetal testicular testosterone data from individual studies provides several BMD<sub>5</sub> and BMDL<sub>5</sub> estimates (*e.g.*, BMD<sub>5</sub> and BMDL<sub>5</sub> values of 22 and 14 mg/kg-day from Kuhl et al. and 24 and 16 mg/kg-day from Martino-Andrade et al.) similar to the BMD<sub>5</sub> and BMDL<sub>5</sub> estimates from the updated meta-analysis (*i.e.*, BMD<sub>5</sub>/BMDL<sub>5</sub> values of 11 and 9 mg/kg-day). However, the meta-analysis of fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data reflects data from eight

studies and is expected to provide a more precise estimate and therefore is preferred over the BMD analysis of individual studies, which supports several slightly higher BMDL<sub>5</sub> estimates.

#### **4.2.3 Selection of the Non-Cancer Oral Point of Departure**

---

Ultimately, EPA selected the BMDL<sub>5</sub> of 9 mg/kg-day (HED of 2.1 mg/kg-day) based on the decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production from the meta-analysis and BMD analysis as the POD for assessing risks from acute, intermediate, and chronic durations of exposure. Numerous factors increase EPA's confidence in using the HED of 2.1 mg/kg-day based on the decreased fetal testicular testosterone. Notably, the BMDL<sub>5</sub> of 9 mg/kg-day falls within the narrow range of the NOAEL or LOAELs (*i.e.*, 1 to 10 mg/kg-day) identified in additional studies that evaluated effects on the developing male reproductive system ([Moody et al., 2013](#); [Boekelheide et al., 2009](#); [Mahood et al., 2007](#); [Lee et al., 2004](#); [Lehmann et al., 2004](#)), which provides support and confidence in both the effect and the dose at which it occurs. Additionally, BMD modeling of nipple/areolae retention data from Mylchreest et al. (2000) (supports BMDL<sub>5</sub> of 15 mg/kg-day) and fetal testicular testosterone content data from Martino-Andrade et al. (2008) and Kuhl et al. (2007) (supports BMDL<sub>5</sub> values of 14–16 mg/kg-day) provide similar BMDL<sub>5</sub> estimates of 14–16 mg/kg-day to the BMDL<sub>5</sub> of 9 mg/kg-day from the meta-analysis, which was selected as the POD. However, the meta-analysis of fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data reflects data from eight studies and is expected to provide a more precise estimate and therefore is preferred over the BMD analysis of individual studies, which support slightly higher BMDL<sub>5</sub> estimates. Additionally, the BMDL<sub>5</sub> is not constrained to one of the experimental doses within a given study, as a NOAEL or LOAEL would be, which may better define the POD ([U.S. EPA, 2012a](#)). Using allometric body weight scaling to the three-quarters power, EPA extrapolated an HED of 2.1 mg/kg-day. A total uncertainty factor of 30 was selected for use as the benchmark margin of exposure (based on an interspecies uncertainty factor (UF<sub>A</sub>) of 3 (see Appendix D for further discussion) and an intraspecies uncertainty factor (UF<sub>H</sub>) of 10).

EPA considered reducing the UF<sub>A</sub> further to a value of 1 based on lack of 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, 2023a](#)), 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 less sensitive than rat testes to the antiandrogenic effects of phthalates, however, effects on Sertoli cells and increased incidence of MNGs have been observed in four human xenograft studies of DBP ([van Den Driesche et al., 2015](#); [Spade et al., 2014](#); [Heger et al., 2012](#); [Mitchell et al., 2012](#)). As discussed in EPA's draft approach document ([U.S. EPA, 2023a](#)), the available human explant and xenograft studies have limitations and uncertainties, which preclude definitive conclusions related to species differences in sensitivity. For example, key limitations and uncertainties of the human explant and xenograft studies include: small sample size; human testis tissue was collected from donors of variable age and by variable non-standardized methods; and most of the testis tissue was taken from fetuses older than 14 weeks, which is outside of the critical window of development (*i.e.*, gestational weeks 8 to 14 in humans). Therefore, EPA did not reduce the UF<sub>A</sub>.

**Table 4-1. Dose-Response Analysis of Selected Studies Considered for Deriving the Non-Cancer POD**

Study Details (Reference) <sup>d</sup> (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors <sup>a b c</sup>	BMD Analysis Notes
Male C57BL/6J mice (n = 5–10/group) were fed corn oil with 0, 1, 10, 50, or 500 mg/kg-day DBP from PND4 – PND14 ( <a href="#">Moody et al., 2013</a> ) (Medium)	LOAEL = 1	Delayed spermatogenesis, reduced absolute AGD (relative to bodyweight at higher dose) in mice (PND 4–14)	0.13	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	- Study considered for BMD modeling, however, no modeling was conducted due to data reporting limitations and/or nature of response ( <i>i.e.</i> , all or nothing response) (Section 4.2.1)
Pregnant rats (6–8 dams/group) were exposed to 0, 20, 200, 2000, or 10,000 ppm DBP via diet from GD15 – PND21 (equivalent to 0, 1.5–3, 14–29, 148– 291, 712 – 1372 mg/kg-day) <sup>e</sup> ( <a href="#">Lee et al., 2004</a> ) (Medium)	LOAEL = 3	↓ spermatocyte development (PND 21), ↑ vacuolar degeneration of alveolar cells, alveolar atrophy of mammary gland (PNW 11 males)	0.71	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	- Study considered for BMD modeling, however, no modeling was conducted due to nature of response ( <i>i.e.</i> , all or nothing response) (Section 4.2.1)
Meta-regression and BMD modeling of fetal testicular testosterone in rats across 8 studies of rats exposed to 1–900 mg/kg-day DBP at various times during gestation (3 high-, 4 medium-, 1 low-confidence) <sup>k</sup>	BMDL <sub>5</sub> = 9	↓ Fetal testicular testosterone production and/or content	2.1	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- See ( <a href="#">U.S. EPA, 2025g</a> ) for BMD results
Pregnant SD rats (4–10 litters/group) gavaged with 0 0.1, 1, 10, 30, 50, 100, 500 mg/kg-day DBP on GD 12–21 ( <a href="#">Boekelheide et al., 2009</a> ) (Medium)	NOAEL = 10	↑ testicular pathology (↓ testicular cell number; disorganized seminiferous tubules)	2.36	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- Study considered for BMD modeling, however, no modeling was conducted due to data reporting limitations (Section 4.2.1)
Pregnant Harlan SD rats (3–4/dose) gavaged with 0, 1, 10, 100 mg/kg-day DBP on GDs 14–18 (Blocks 22 and 26) <sup>f, g</sup> ( <a href="#">Furr et al., 2014</a> ) (High)	NOAEL = 10	↓ <i>ex vivo</i> fetal testicular testosterone production	2.36	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- BMD modeling of fetal testosterone data from Blocks 22 and 26 attempted - No models adequately fit the data sets (Appendix F.7)
Pregnant SD rats (10/dose) gavaged with 0, 100, 500 mg/kg-day DBP on GD 18 and sacrificed 24-hours later on GD 19 ( <a href="#">Kuhl et al., 2007</a> ) (Low)	BMDL <sub>5</sub> = 14	↓ Fetal testicular testosterone content	3.31	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- See Appendix F.2 for BMD results

Study Details (Reference) <sup>d</sup> (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors <sup>a b c</sup>	BMD Analysis Notes
Pregnant SD rats (19–20 or 11 (high-dose) per dose) gavaged with 0, 0.5, 5, 50, 100, 500 mg/kg-day DBP on GDs 12–21 ( <a href="#">Mylchreest et al., 2000</a> ) (High)	BMDL <sub>5</sub> = 15	↑ males with nipples and/or areolae on PND 14	3.55	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- See Appendix G for BMD results
Pregnant Wistar rats (7–8/dose) gavaged with 0, 100, 500 mg/kg-day DBP on GDs 13–21 (fetal study) ( <a href="#">Martino-Andrade et al., 2008</a> ) <sup>g</sup> (Medium)	BMDL <sub>5</sub> = 16	↓ Fetal testicular testosterone content	3.78	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- See Appendix F.1 for BMD results
	LOAEL = 100	↓ male AGD (GD21)	23.64	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	
Pregnant Wistar rats gavaged with 0, 4, 20, 100, 500 mg/kg-day DBP on GD 13.5–20.5 ( <a href="#">Mahood et al., 2007</a> ) (Medium) <sup>f</sup>	NOAEL = 20	↓ fetal testicular testosterone content, ↑ MNGs, ↑ Leydig cell aggregation	4.73	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- Study considered for BMD modeling, however, no modeling was conducted due to data reporting limitations (Section 4.2.1)
Pregnant SD rats (7-9/dose) fed diets with 0, 112, 581 mg/kg-day DBP on GD 12-19 ( <a href="#">Struve et al., 2009</a> ) (Medium)	BMDL <sub>5</sub> = 28	↓ Fetal testicular testosterone content	6.62	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- See Appendix F.3 for BMD results
Pregnant SD rats (3–4 separate rat fetuses from 1–4 dams/group) gavaged with 0, 0.1, 1, 10, 30, 50, 100, 500 mg/kg-day DBP on GD 12–19 <sup>i</sup> ( <a href="#">Lehmann et al., 2004</a> ) (Uninformative)	NOAEL = 30	↓ fetal testis testosterone content on GD 19	7.09	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- Study considered for BMD modeling, however, no modeling was conducted due to data reporting limitations (Section 4.2.1)
Pregnant SD Rats (3-4/dose) gavaged with 0, 33, 50, 100, 300, 600 mg/kg-day DBP on GD 8-18 ( <a href="#">Howdeshell et al., 2008</a> ) (High)	BMDL <sub>5</sub> = 39	↓ Fetal testicular testosterone production	9.22	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- See Appendix F.5 for BMD results
Pregnant Harlan SD rats (2–3/dose) gavaged with 0, 33, 50, 100, 300 mg/kg-day DBP on GDs 14–18 (Block 18) <sup>f, g</sup> ( <a href="#">Furr et al., 2014</a> ) (High)	NOAEL = 50	↓ <i>ex vivo</i> fetal testicular testosterone production	11.82	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- BMD modeling of fetal testosterone data attempted - No models adequately fit the data set (Appendix F.7)



Study Details (Reference) <sup>d</sup> (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors <sup>a b c</sup>	BMD Analysis Notes
Pregnant SD rats (4 litters/dose) exposed to 0 or 50 mg/kg-day DBP from GD 12–19 via gavage, 12 hours after final dose ( <a href="#">Clewett et al., 2009</a> ) (Medium) <sup>f</sup>	LOAEL = 50 mg/kg-day	↓ fetal testicular testosterone concentration	11.82	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	- Study not amendable to BMD modeling (evaluated one dose level)
Continuous breeding protocol. Pregnant VAF Crl:CD BR outbred Sprague-Dawley albino rats (20/sex/group; 40/sex for controls) exposed to 0, 0.1, 0.5, or 1% DBP via diet starting 10 weeks prior to mating and throughout gestation and lactation periods continuously for 2 generations (equivalent to 52, 256, 509 mg/kg-day [males]; 80, 385, or 794 mg/kg-day [females]) ( <a href="#">Wine et al., 1997</a> ; <a href="#">NTP, 1995</a> ) (Low)	LOAEL = 80	F2: ↓ live pup weight; F1: ↓ live pups per litter	18.91	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	- Study not subjected to BMD analysis, as other studies evaluated lower doses and provided more sensitive outcomes for modeling (Section 4.2.1)
Pregnant SD rats (10/11/dose) gavaged with 0, 100, 500 mg/kg-day DBP on GDs 12–21 ( <a href="#">Barlow et al., 2004</a> ) (Medium) <sup>f</sup>	LOAEL = 100	↑ F1 males with NR (PND 13)	23.64	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	- Study not subjected to BMD analysis, as other studies evaluated lower doses and provided more sensitive outcomes for modeling (Section 4.2.1)
Pregnant SD rats (5 dams/group) gavaged with 0, 1, 10, 100, 500 mg/kg-day DBP on GD 19 ( <a href="#">Johnson et al., 2007</a> ) (Medium)	NOAEL = 100	↓ fetal testicular testosterone content	23.64	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 Total UF = 30	- BMD modeling of fetal testosterone data attempted No models adequately fit the data set (Appendix F.4)
Pregnant SD rats (3–4 dams/group) gavaged with 0, 300, 600, 900 mg/kg-day DBP on GD 14–18 (Block 70) ( <a href="#">Gray et al., 2021</a> ) (High)	LOAEL = 300	↓ <i>ex vivo</i> fetal testicular testosterone production	70.93	UF <sub>A</sub> = 3 UF <sub>H</sub> = 10 UF <sub>L</sub> = 10 Total UF = 300	- BMD modeling of fetal testosterone data from Block 70 and 71 rats was attempted, however, no BMD models adequately fit the data set (Appendix F.6)

EPA identified the above listed studies supporting derivation of candidate acute, intermediate, and chronic PODs.

*Abbreviations:* AGD = anogenital distance; GD = gestation day; HED = human equivalent; PND = postnatal day; LOAEL = lowest observed adverse effect level ; NOAEL = No-observed-adverse-effect level ; POD = point of departure; MNG= Multinucleated gonocytes; SD = Sprague Dawley; UF = uncertainty factor; UF<sub>A</sub>= interspecies uncertainty factor; UF<sub>H</sub> = intraspecies uncertainty factor; UF<sub>L</sub> = LOAEL-to-NOAEL uncertainty factor.

<sup>a</sup> EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance ([U.S. EPA, 2011b](#)), the interspecies uncertainty factor (UF<sub>A</sub>), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics.



Study Details (Reference) <sup>d</sup> (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors <sup>a b c</sup>	BMD Analysis Notes
<p><sup>b</sup> EPA used a default intraspecies (UF<sub>H</sub>) 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 DIBP.</p> <p><sup>c</sup> EPA used a LOAEL-to-NOAEL uncertainty factor (UF<sub>L</sub>) of 10 to account for the uncertainty inherent in extrapolating from the LOAEL to the NOAEL.</p> <p><sup>d</sup> Overall data quality determinations were not made for these studies because the acute POD was more sensitive than the acute/intermediate, or chronic candidate PODs, and these studies are not used quantitatively in the DBP risk evaluation.</p> <p><sup>e</sup> Equivalent doses provided by (NICNAS, 2008).</p> <p><sup>f</sup> Multiple time blocks in this experiment, which was carried out over 2–3 years, with each block consisting of 15 pregnant dams divided into 4–5 exposure groups.</p> <p><sup>g</sup> Considered in the metaanalysis of the effect of DBP on fetal testosterone by NASEM (2017).</p> <p><sup>h</sup> R code supporting NASEM’s meta-regression and BMD analysis of DBP is publicly available through GitHub (<a href="https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose">https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose</a>).”</p> <p><sup>i</sup> Authors state that the study was repeated, and a 30-mg/kg/day dose group was included for the testosterone radioimmunoassay (RIA). All other endpoints in this study do not have a 30 mg/kg-day group.</p> <p><sup>j</sup> Data from block 70 and 71 rats in Gray et al. (2021).</p> <p><sup>k</sup> The BMDL<sub>5</sub> was derived through meta-regression and BMD modeling of fetal testicular testosterone data from eight studies of DBP with rats (Gray et al., 2021; Furr et al., 2014; Johnson et al., 2011; Struve et al., 2009; Howdeshell et al., 2008; Martino-Andrade et al., 2008; Johnson et al., 2007; Kuhl et al., 2007).</p> <p><sup>l</sup> As discussed in the Systematic Review protocol for DBP (U.S. EPA, 2025q) and consistent with Office of Pesticide Programs <i>Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment</i> (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.</p>					

**Table 4-2. Summary of Effects of Gestational Exposure to DBP on Fetal Testicular Testosterone Content and *Ex Vivo* Fetal Testicular Testosterone Production Across Select Studies**

Study Details (Species, Duration, Exposure Route/ Method, Endpoint, Measurement timing, Reference; <i>TSCA Study Quality Rating</i> )	% of Control Testosterone Response by Dose (mg/kg-day) <sup>a</sup>											
	0	1	10	33	50	100	112	300	500	581	600	900
SD Rats (Block 18); GD 14–18; Oral/gavage; <i>ex vivo</i> fetal testicular testosterone production; GD 18 ( <a href="#">Furr et al., 2014</a> ) <sup>b</sup> <i>High</i>	100% (n = 3)	— <sup>d</sup>	—	32% (n=3)	86% (n=2)	<b>65%*</b> (n=3)	—	<b>23%*</b> (n=3)	—	—	—	—
SD Rats (Block 22); GD 14–18; Oral/gavage; <i>ex vivo</i> fetal testicular testosterone production; GD 18 ( <a href="#">Furr et al., 2014</a> ) <sup>b</sup> <i>High</i>	100% (n = 3)	88% (n=3)	80% (n=4)	—	—	<b>64%*</b> (n=4)	—	—	—	—	—	—
SD Rats; GD 19; Oral/gavage; testicular testosterone content; GD19 (1 hr post exposure) ( <a href="#">Johnson et al., 2007</a> ) <sup>b</sup> <i>Medium</i>	100% (n = 5)	—	109% (n=5)	67% (n=5)	—	84%* (n=5)	—	—	—	—	—	—
SD Rat; GD 8–18; Oral/gavage; <i>ex vivo</i> testicular testosterone production; GD 18 (2 hr incubation) ( <a href="#">Howdeshell et al., 2008</a> ) <sup>b</sup> <i>High</i>	100% (n = 3)	—	—	94% (n=4)	78% (n=4)	84% (n=4)	—	<b>66%*</b> (n=4)	—	—	<b>33%*</b> (n=4)	—
Wistar Rat; GD 13–21; Oral/gavage; testicular testosterone content; GD21 ( <a href="#">Martino-Andrade et al., 2008</a> ) <sup>b</sup> <i>Medium</i>	100% (n = 7)	—	—	—	—	71% (n=8)	—	—	<b>37%*</b> (n=7)	—	—	—
SD Rat; GD18; Oral/gavage; testicular testosterone content; GD19 ( <a href="#">Kuhl et al., 2007</a> ) <sup>b</sup> <i>Low</i>	100% (n = 10)	—	—	—	—	71% (n=10)	—	—	<b>33%*</b> (n=10)	—	—	—
SD Rat; GD12–19; Oral/diet; testicular testosterone content; GD19 (4 hr post exposure) ( <a href="#">Struve et al., 2009</a> ) <sup>b</sup> <i>Medium</i>	100% (n = 9)	—	—	—	—	—	56% (n=7)	—	—	<b>3.7%*</b> (n=7)	—	—

Study Details (Species, Duration, Exposure Route/ Method, Endpoint, Measurement timing, Reference; <i>TSCA Study Quality Rating</i> )	% of Control Testosterone Response by Dose (mg/kg-day) <sup>a</sup>											
	0	1	10	33	50	100	112	300	500	581	600	900
SD Rat; GD12–19; Oral/diet; testicular testosterone content; GD20 (24 hr post exposure) ( <a href="#">Struve et al., 2009</a> ) <sup>b</sup> <i>Medium</i>	100% (n = 9)	–	–	–	–	–	29%* (n = 7)	–	–	7.1%* (n = 7)	–	–
SD Rat; GD12–20; Oral/gavage; testicular testosterone content; GD20 ( <a href="#">Johnson et al., 2011</a> ) <sup>b</sup> <i>Medium</i>	100% n = 6)	–	–	–	–	–	–	–	15%* (n = 5)	–	–	–
SD Rats (Block 70); GD 14–18; Oral/gavage; <i>ex vivo</i> fetal testicular testosterone production; GD 18 ( <a href="#">Gray et al., 2021</a> ) <sup>c</sup> <i>High</i>	100% (n = 3)	–	–	–	–	–	–	62% (n = 4)	–	–	25% (n = 4)	16% (n = 4)
SD Rats (Block 71); GD 14–18; Oral/gavage; <i>ex vivo</i> fetal testicular testosterone production; GD 18 ( <a href="#">Gray et al., 2021</a> ) <sup>c</sup> <i>High</i>	100% (n = 4)	–	–	–	–	–	–	47% (n = 3)	–	–	22% (n = 4)	13% (n = 4)
SD = Sprague-Dawley; GD = Gestation Day; hr = hour The following studies reported fetal testicular testosterone data but are not represented in this table because the sample sizes were not reported for each dose group: ( <a href="#">Mahood et al., 2007</a> ); ( <a href="#">Lehmann et al., 2004</a> ); ( <a href="#">Clewett et al., 2009</a> ); ( <a href="#">Li et al., 2015</a> ); ( <a href="#">van den Driesche et al., 2012</a> ). <sup>a</sup> Effect on fetal testicular testosterone production reported as percent of control. Asterisks indicate statistically significant pairwise comparison to control, as reported by study authors. <sup>b</sup> Data used in meta-analysis and BMD modeling analysis of fetal testicular testosterone content and <i>ex vivo</i> fetal testicular testosterone production. <sup>c</sup> Data from Block 70 and 71 rats reported in supplemental information file associated with Gray et al. (2021). <i>Ex vivo</i> testosterone production data from Block 70 and 71 rats was not subjected to statistical analysis. <sup>d</sup> No data; dose not evaluated in this study.												

**Table 4-3. Overall Analyses and Sensitivity Analyses of Rat Studies of DBP and Fetal Testicular Testosterone Content and *Ex Vivo* Fetal Testicular Testosterone Production (Updated Analysis Conducted by EPA)<sup>a</sup>**

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P value	Tau	I <sup>2</sup>	P value for Heterogeneity	AICs
<b>Primary Analysis</b>									
Overall	intercept	-71.85	-95.76	-47.95	3.82E-09	67.01	95.60	2.74E-152	383.39
Trend in log10(dose)	log10(dose)	-62.44	-81.70	-43.19	2.08E-10	41.61	88.70	4.43E-50	349.26
Linear in dose100	dose100	-25.69	-31.55	-19.83	8.64E-18	57.78	94.26	3.38E-119	354.71
LinearQuadratic in dose100	dose100	-36.78	-54.53	-19.03	4.89E-05	54.79	93.26	1.72E-117	343.82*
LinearQuadratic in dose100	I(dose100^2)	1.70	-0.86	4.26	1.94E-01	54.79	93.26	1.72E-117	343.82
<b>Sensitivity Analysis</b>									
Overall minus Furr et al. 2014	intercept	-88.38	-117.31	-59.45	2.14E-09	67.21	93.19	2.16E-55	270.22
Overall minus Johnson et al. 2007	intercept	-76.78	-102.25	-51.31	3.47E-09	68.66	96.10	3.84E-153	350.04
Overall minus Howdeshell et al. 2008	intercept	-78.30	-105.70	-50.91	2.11E-08	70.83	95.72	3.63E-139	329.10
Overall minus Johnson et al. 2011	intercept	-69.59	-93.70	-45.48	1.53E-08	65.39	95.51	3.39E-148	359.45
Overall minus Kuhl et al. 2007	intercept	-72.06	-97.37	-46.75	2.39E-08	68.92	95.94	3.87E-152	362.13
Overall minus Martino-Andrade et al. 2009	intercept	-72.43	-97.80	-47.06	2.19E-08	69.11	95.94	1.74E-152	362.26
Overall minus Struve et al. 2009	intercept	-63.19	-86.77	-39.61	1.50E-07	62.87	95.50	2.53E-148	329.62
Overall minus Gray et al. 2021	intercept	-56.97	-80.64	-33.31	2.37E-06	59.25	94.78	3.05E-115	311.44
<sup>a</sup> ‘*’ Indicates lowest Akaike information criterion (AIC). CI = confidence interval; I <sup>2</sup> = 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 DBP and Fetal Testicular Testosterone Content and *Ex Vivo* Fetal Testicular Testosterone Production in Rats**

Analysis	BMR	BMD	CI, Lower Bound	CI, Upper Bound
<b>2017 NASEM Analysis for all strains of rats using Metafor Version 2.0.0 (as reported in Table C6–8 of NASEM, 2017)</b>				
Linear in dose100	5%	17	14	22
Linear in dose100	40%	174	143	222
LinearQuadratic in dose100*	5%	12	8	22
LinearQuadratic in dose100*	40%	125	85	205
<b>Updated Analysis using Metafor Version 4.6.0</b>				
Linear in dose100	5%	20	16	26
Linear in dose100	10%	41	33	53
Linear in dose100	40%	199	162	258
LinearQuadratic in dose100*	5%	14	9	27
LinearQuadratic in dose100*	10%	29	20	54
LinearQuadratic in dose100*	40%	149	101	247
* Indicates model with lowest AIC. BMD = benchmark dose; BMR = benchmark response; CI = confidence interval.				

### 4.3 Weight of Scientific Evidence: POD for Acute, Intermediate, and Chronic Durations

---

EPA concluded that the HED of 2.1 mg/kg-day (BMDL<sub>5</sub> of 9 mg/kg-day) is appropriate for calculation of risk from acute, intermediate, and chronic exposures to DBP. This POD is based on a meta-analysis and BMD modeling of decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production in eight studies of rats exposed to DBP during gestation. A total uncertainty factor of 30 was selected for use as the benchmark margin of exposure (based on an interspecies uncertainty factor (UF<sub>A</sub>) of 3 and an intraspecies uncertainty factor (UF<sub>H</sub>) of 10). Consistent with EPA guidance ([2022](#), [2002](#), [1993](#)), EPA reduced the UF<sub>A</sub> 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 for acute, intermediate, and chronic durations based on the following weight of the scientific evidence:

- EPA previously considered the weight of evidence and updated here and concluded that oral exposure to DBP 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, 2023a](#)). Notably, EPA's conclusion was supported by the SACC ([U.S. EPA, 2023b](#)).
  - EPA's conclusion was also supported by the SACC during the August 2025 peer review meeting for the human health hazard assessments of five phthalates including DBP ([U.S. EPA, 2025o](#)).
- DBP exposure resulted in effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in over 20 studies of rats (Section 3.1.2.1), 11 of which reported LOAELs at or below 100 mg/kg-day (Table 3-3). Observed effects in rats perinatally exposed to DBP included: disruption of testicular testosterone content and *ex vivo* fetal testicular testosterone production; reductions testicular mRNA and protein expression of genes involved in steroidogenesis (*e.g.*, StAR, P450scc, CYP17) and testis descent (Insl3); decreased AGD; increased NR; disrupted testis tubules; Leydig cell clusters; increased incidence of MNGs; changes in androgen-dependent organ weights (*e.g.*, testes weight); testicular histopathology; and/or malformations (*e.g.*, hypospadias).
- Alignment across epidemiological, animal toxicology, and mechanistic streams of evidence (Section 3.3).
- The POD is based on meta-regression analysis of fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data from eight studies of rats ([Gray et al., 2021](#); [Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)).
- Chronic studies do not offer a more sensitive chronic POD. The NTP ([2021](#)) identified a POD of 510 mg/kg-day (based on LOAEL in rats; HED = 130 mg/kg-day).
- The BMDL<sub>5</sub> of 9 mg/kg-day (HED 2.1 mg/kg-day) is within the range of PODs (*i.e.*, 1 to 15 mg/kg-day) identified from other studies based on antiandrogenic effects on the developing male reproductive system ([Furr et al., 2014](#); [Boekelheide et al., 2009](#)), including the BMDL<sub>5</sub> of 15 mg/kg-day identified from BMD analysis of male pup nipple retention from Mylchreest et al. ([2000](#)) (Appendix G). These studies support the selection of the BMDL<sub>5</sub> of 9 mg/kg-day for the acute, intermediate, and chronic duration PODs.



- Three developmental toxicity studies ([Furr et al., 2014](#); [Mahood et al., 2007](#); [Lehmann et al., 2004](#)) provide NOAEL values ranging from 10 to 30 mg/kg-day based on decreased fetal testicular testosterone content and/or *ex vivo* fetal testicular testosterone production.
- 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, based on studies of 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 content and/or *ex vivo* fetal testicular testosterone production.

## 4.4 Route-to-Route Extrapolation

---

EPA did not identify any reasonably available 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 2.1 mg/kg-day DBP 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 DBP, 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 DBP through the gastrointestinal tract following oral exposure, while EPA estimated steady-state dermal flux values for DBP to estimate dermal exposure (Section 2.3). However, potential route-specific differences in metabolism were not accounted for. Following oral exposure, phthalate diesters (including DBP) are metabolized to monoester metabolites (*e.g.*, MBP) 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 DBP to its monoester metabolite MBP also likely occurs via the dermal route prior to systemic circulation. For example, as discussed above in Section 2.3 (*e.g.*, studies by Beydon et al. ([2010](#)) and Sugino et al. ([2017](#))) and in the non-cancer human health hazard assessments of 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 MzBP, as well as other oxidative metabolites.

Despite some remaining uncertainty, EPA is confident that its human health risk characterization via the dermal route for DBP 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 DBP through the gastrointestinal tract following oral exposure and the lung following inhalation exposure (based on read-across to inhalation studies of DEHP and DIDP) (Sections

2.1 and 2.2). Similar to the oral route of exposure, metabolism of DBP to its monoester metabolite MBP 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 (measured by the rate of formation of mono(2-ethylhexyl) phthalate (MEHP) from DEHP) 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.

Despite some remaining uncertainty, EPA is confident that its human health risk characterization via the inhalation route for DBP 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 PODs in Section 6.

### 5.2 PESS Based on Greater Susceptibility

---

EPA addressed subpopulations expected to be more susceptible to DBP 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 DBP.

EPA did not identify direct evidence of differences in susceptibility among human populations. EPA identified indirect evidence for differences among human populations in ADME properties that may impact lifestage susceptibility to DBP. For instance, the activity of glucuronosyltransferase differs between adults and infants; adult activity is achieved at 6 to 18 months of age ([Leeder and Kearns, 1997](#)). Also, preexisting chronic liver or kidney disease may enhance susceptibility to DBP as a consequence of impaired metabolism and clearance (*i.e.*, altered functionality of phase I and phase II metabolic enzymes); impaired activity of UGTs can reduce metabolism of chemicals that rely on UGT conjugation to be excreted ([Sugatani, 2013](#)), including DBP (Section 2.1). Additional indirect evidence of differences among human populations that confer enhanced susceptibility to DBP, including other preexisting diseases, lifestyle factors, sociodemographic factors, genetic factors, and chemical co-exposures are presented in Table 5-1. Animal studies provide direct evidence of several factors that enhance susceptibility to DBP, including that gestation is a particularly sensitive lifestage for effects on male reproductive development to manifest. These, and other lines of evidence are summarized in Table 5-1. EPA is quantifying risks based on developmental toxicity in the DBP risk evaluation.

As summarized in Table 5-1, EPA identified a range of factors that may have the potential to increase biological susceptibility to DBP, including lifestage, chronic liver or kidney disease, pre-existing diseases, physical activity, diet, stress, and co-exposures to other environmental stressors that contribute to related health outcomes. The effect of these factors on susceptibility to health effects of DBP is not known. Therefore, EPA is uncertain about the magnitude of any possible increased risk from effects associated with DBP 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 DBP. The Risk Assessment Forum, in *A Review of the Reference Dose and Reference Concentration Processes* ([U.S. EPA, 2002](#)), discusses some of the evidence for choosing the default factor of 10 when data are lacking and describe the types of populations that may be more susceptible, including different lifestages (*e.g.*, of children and elderly). However, U.S. EPA ([2002](#)) did not discuss all the factors presented in Table 5-1. Although U.S. EPA ([2002](#)) did not discuss all the factors presented in Table 5-1, EPA considers the POD selected for use in characterizing risk from exposure to DBP to be protective of effects on the developing male reproductive system consistent with phthalate syndrome in humans. Thus, uncertainty remains whether

additional susceptibility factors would be covered by the default  $UF_H$  value of 10 chosen for use in the DBP risk evaluation.

As discussed in U.S. EPA ([2023a](#)), exposure to DBP and other toxicologically similar phthalates (*i.e.*, DEHP, DIBP, BBP, DCHP, DINP) that disrupt androgen action during the development of the male reproductive system cause dose additive effects. Cumulative effects from exposure to DBP and other toxicologically similar phthalates will be evaluated as part of U.S. EPA's cumulative risk assessment of phthalates.

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

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	<p>Direct quantitative animal evidence for developmental toxicity including multigenerational effects (<i>e.g.</i>, increased skeletal and visceral variations, decreased live births, decreased offspring body weight gain, and decreased offspring survival with increased severity in the second generation).</p> <p>There is direct quantitative animal evidence for effects on the developing male reproductive system consistent with a disruption of androgen action.</p>	<p>(<a href="#">Wine et al., 1997</a>)  (<a href="#">U.S. EPA, 2023a</a>)  (<a href="#">U.S. EPA, 2023b</a>)  (<a href="#">Lee et al., 2004</a>)  (<a href="#">Boekelheide et al., 2009</a>)  (<a href="#">Furr et al., 2014</a>)  (<a href="#">Mylchreest et al., 2000</a>)</p>			<p>POD for assessing risks from acute, intermediate, and chronic exposures to DBP is based on developmental toxicity (<i>i.e.</i>, reduced fetal testicular testosterone production) and is protective of effects on the fetus and offspring.</p>
	Pregnancy/ lactating status	<p>Rodent dams less susceptible than developing fetus during pregnancy and lactation during a continuous breeding multigenerational experiment. Dams reduction in body weight (14%) occurred at doses higher than those that caused developmental toxicity and pup weight changes observed in the absence of changes in maternal weight for other doses.</p>	<p>(<a href="#">Wine et al., 1997</a>)</p>			<p>POD for assessing risks from acute, intermediate, and chronic exposures to DBP is based on developmental toxicity (<i>i.e.</i>, reduced fetal testicular testosterone production) and is protective of effects in dams.</p>

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Males of reproductive age and adolescence	Crossover mating trial study by Wine et al. (1997) demonstrates that effects on F1 offspring are attributable to female (dam) exposure rather than male (sire) exposure. Nevertheless, three other studies of DBP exposure to males of reproductive age or adolescence suggest adverse metabolic effects (e.g., increased BW gain, BMI, serum glucose, and serum total cholesterol) (Majeed et al., 2017), adverse outcomes of the cardiovascular system (Xie et al., 2019), and neurobehavioral effects (Farzanehfar et al., 2016) in male rats and mice. Effects observed at doses ranging from 1 to 12.5 mg/kg-day DBP. See Section 3.1.3 for individual study details and Radke et al. summary of human evidence on adult male semen parameters.	(Wine et al., 1997) (Xie et al., 2019) (Majeed et al., 2017) (Farzanehfar et al., 2016) (Moody et al., 2013) (Lee et al., 2004)			POD for assessing risks from acute, intermediate, and chronic exposures to DBP based on developmental toxicity (i.e., reduced fetal testicular testosterone production) is protective of adult male reproductive effects.  Use of default 10x UF <sub>H</sub>
	Children	Reduced F1 and F2 rodent offspring bodyweight (live pup weight) was observed in a continuous breeding experiment. Decreased F2 live pup weight observed at lower dose.	(Wine et al., 1997)			POD for assessing risks from acute, intermediate, and chronic exposures to DBP is based on developmental toxicity (i.e., reduced fetal testicular testosterone production) and is protective of effects of offspring bodyweight gain.  Use of default 10x UF <sub>H</sub>



Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Elderly	Two cross sectional studies suggest associations with obesity in elderly populations and combined MBP and MiBP (80% is MBP) metabolites in serum and associations of DBP metabolites with adverse cognitive functioning in the elderly.	( <a href="#">Weng et al., 2022</a> ) ( <a href="#">Li et al., 2020</a> )			Use of default 10x UF <sub>H</sub>
	Toxicokinetics			The activity of enzymes involved in metabolism of DBP differ between adults and infants ( <i>e.g.</i> , glucuronosyltransferases, lipases, CYPs) and may result in abnormal toxicity.	( <a href="#">Leeder and Kearns, 1997</a> )	Use of default 10x UF <sub>H</sub>
Pre-existing disease or disorder	Health outcome/target organs	No direct evidence identified		Several preexisting conditions may contribute to adverse developmental outcomes ( <i>e.g.</i> , diabetes, high blood pressure, certain viruses).	CDC ( <a href="#">2023d</a> )	Use of default 10x UF <sub>H</sub>
	Toxicokinetics	No direct evidence identified		Chronic liver and kidney disease are 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 DBP.	( <a href="#">Sugatani, 2013</a> )	Use of default 10x UF <sub>H</sub>
Lifestyle activities	Smoking	No direct evidence identified		Smoking during pregnancy may increase susceptibility for developmental outcomes ( <i>e.g.</i> , early delivery and stillbirths).	CDC ( <a href="#">2023e</a> )	Qualitative discussion in Section 5.2 and this table
	Alcohol consumption	No direct evidence identified		Alcohol use during pregnancy can cause developmental outcomes ( <i>e.g.</i> , fetal alcohol spectrum disorders).	CDC ( <a href="#">2023c</a> )	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestyle activities	Physical activity	No direct evidence identified		Insufficient activity may increase susceptibility to multiple health outcomes.  Overly strenuous activity may also increase susceptibility.	CDC ( <a href="#">2022</a> )	Qualitative discussion in Section 5.2 and this table
Sociodemographic status	Race/ethnicity	No direct evidence identified ( <i>e.g.</i> , no information on polymorphisms in DBP metabolic pathways or diseases associated race/ethnicity that would lead to increased susceptibility to effects of DBP 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 ( <a href="#">2023b</a> )	
	Sex/gender	The key effect is male reproductive development.	<a href="#">(U.S. EPA, 2023a)</a>			Use of default 10x UF <sub>H</sub>
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 that impairs liver enzyme metabolism of DBP, thereby enhancing susceptibility to DBP toxicity.	CDC ( <a href="#">2023d</a> ) CDC ( <a href="#">2023a</a> )	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Nutrition	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 DBP.	CDC ( <a href="#">2021</a> ) CDC ( <a href="#">2023a</a> )	Qualitative discussion in Section 5.2 and this table
	Target organs	No direct evidence identified		Polymorphisms in genes may increase susceptibility to developmental toxicity, metabolic outcomes, or neurological effects.	( <a href="#">Cassina et al., 2012</a> ) ( <a href="#">Ingelman-Sundberg, 2004</a> )	Use of default 10x UF <sub>H</sub>
Genetics/epigenetics	Toxicokinetics	No direct evidence identified		Polymorphisms in genes encoding phase 1 or phase 2 metabolic enzymes ( <i>e.g.</i> , UGTs, CYPs) or other enzymes ( <i>e.g.</i> , lipases, esterases) involved in metabolism of DBP may influence metabolism and excretion of DBP		Use of default 10x UF <sub>H</sub>
Other chemical and nonchemical stressors	Built environment	No direct evidence identified		Poor-quality housing is associated with a variety of negative health outcomes.	ODPHP ( <a href="#">2023a</a> )	Qualitative discussion in Section 5.2 and this table
	Social environment	No direct evidence identified		Social isolation and other social determinants ( <i>e.g.</i> , decreased social capital, stress) can lead to negative health outcomes.	CDC ( <a href="#">2023b</a> ) ODPHP ( <a href="#">2023c</a> )	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Other chemical and nonchemical stressors	Chemical co-exposures	Studies have demonstrated that co-exposure to DBP and other toxicologically similar phthalates ( <i>e.g.</i> , DIBP, DEHP, DINP, BBP) and other classes of antiandrogenic chemicals ( <i>e.g.</i> , certain pesticides and pharmaceuticals – discussed more in (U.S. EPA, 2023a)) can induce effects on the developing male reproductive system in a dose-additive manner.	See (U.S. EPA, 2023a) and (U.S. EPA, 2023b)			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 DBP EXPOSURE, AND CONCLUSIONS

EPA considered the identified hazards, dose-response evaluation, and weight of the scientific evidence of POD candidates, and ultimately 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. Decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production was selected as the basis for the POD of 9 mg/kg-day (HED = 2.1 mg/kg-day) for acute, intermediate, and chronic durations. EPA has robust overall confidence in the selected POD 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 12 mg/m<sup>3</sup> (1.0 ppm). EPA 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.

**Table 6-1. Non-Cancer HECs and HEDs Used to Estimate Risks for Acute, Intermediate, and Chronic Exposure Scenarios**

Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HED <sup>a</sup> (mg/kg-day)	HEC (mg/m <sup>3</sup> ) [ppm]	Benchmark MOE	Reference (TSCA Quality Rating)
Development /Reproductive	Rat	5 to 14 days throughout gestation	BMDL <sub>5</sub> = 9	↓ fetal testicular testosterone content and <i>ex vivo</i> fetal testicular testosterone production	2.1	12 [1.0]	UF <sub>A</sub> = 3 UF <sub>H</sub> =10 <i>Total UF=30</i>	— <sup>b, c</sup>

**Abbreviations:** POD = Point of Departure; HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; UF = uncertainty factor BMDL<sub>5</sub> = Benchmark dose (lower confidence limit) associated with a 5% response level.

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

<sup>b</sup> The BMDL<sub>5</sub> was derived through meta-regression and BMD modeling of fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data from eight studies of DBP with rats ([Gray et al., 2021](#); [Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)).

<sup>c</sup> TSCA Study Quality Ratings: *High confidence* for ([Gray et al., 2021](#); [Furr et al., 2014](#); [Howdeshell et al., 2008](#)) *Medium confidence* for ([Johnson et al., 2011](#); [Struve et al., 2009](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#)) and *Low confidence* for ([Kuhl et al., 2007](#)).

The POD of 9 mg/kg-day (HED = 2.1 mg/kg-day) is used in the Risk Evaluation for DBP ([U.S. EPA, 2025n](#)) to estimate acute, intermediate, and chronic non-cancer risk. EPA summarizes the cancer hazards of DBP 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](#)).*



## REFERENCES

- [Ahmad, R; Gautam, AK; Verma, Y; Sedha, S; Kumar, S. \(2014\). Effects of in utero di-butyl phthalate and butyl benzyl phthalate exposure on offspring development and male reproduction of rat. Environ Sci Pollut Res Int 21: 3156-3165. <https://dx.doi.org/10.1007/s11356-013-2281-x>](#)
- [Ahmad, R; Verma, Y; Gautam, A; Kumar, S. \(2015\). Assessment of estrogenic potential of di-n-butyl phthalate and butyl benzyl phthalate in vivo. Toxicol Ind Health 31: 1296-1303. <https://dx.doi.org/10.1177/0748233713491803>](#)
- [Albro, PW; Moore, B. \(1974\). Identification of the metabolites of simple phthalate diesters in rat urine. J Chromatogr 94: 209-218. \[http://dx.doi.org/10.1016/S0021-9673\\(01\\)92368-4\]\(http://dx.doi.org/10.1016/S0021-9673\(01\)92368-4\)](#)
- [Allen, BC; Kavlock, RJ; Kimmel, CA; Faustman, EM. \(1994a\). Dose-response assessment for developmental toxicity II: Comparison of generic benchmark dose estimates with no observed adverse effect levels. Fundam Appl Toxicol 23: 487-495. <https://dx.doi.org/10.1006/faat.1994.1133>](#)
- [Allen, BC; Kavlock, RJ; Kimmel, CA; Faustman, EM. \(1994b\). Dose-response assessment for developmental toxicity III: statistical models. Fundam Appl Toxicol 23: 496-509. <https://dx.doi.org/10.1006/faat.1994.1134>](#)
- [Amin, MM; Parastar, S; Ebrahimpour, K; Shoshtari-Yeganeh, B; Hashemi, M; Mansourian, M; Kelishadi, R. \(2018\). Association of urinary phthalate metabolites concentrations with body mass index and waist circumference. Environ Sci Pollut Res Int 25: 11143-11151. <https://dx.doi.org/10.1007/s11356-018-1413-8>](#)
- [Anderson, WAC; Castle, L; Scotter, MJ; Massey, RC; Springall, C. \(2001\). A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Addit Contam 18: 1068-1074. <https://dx.doi.org/10.1080/02652030110050113>](#)
- [Arbuckle, TE; Agarwal, A; Macpherson, SH; Fraser, WD; Sathyanarayana, S; Ramsay, T; Dodds, L; Muckle, G; Fisher, M; Foster, W; Walker, M; Monnier, P. \(2018\). Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: The MIREC study. Environ Int 120: 572-583. <https://dx.doi.org/10.1016/j.envint.2018.08.034>](#)
- [ATSDR. \(2001\). Toxicological profile for di-n-butyl phthalate \(Update, September 2001\) \[ATSDR Tox Profile\]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=859&tid=167>](#)
- [Axelsson, J; Rylander, L; Rignell-Hydbom, A; Jönsson, BA; Lindh, CH; Giwercman, A. \(2015a\). Phthalate exposure and reproductive parameters in young men from the general Swedish population. Environ Int 85: 54-60. <http://dx.doi.org/10.1016/j.envint.2015.07.005>](#)
- [Axelsson, J; Rylander, L; Rignell-Hydbom, A; Lindh, CH; Jönsson, BA; Giwercman, A. \(2015b\). Prenatal phthalate exposure and reproductive function in young men. Environ Res 138C: 264-270. <http://dx.doi.org/10.1016/j.envres.2015.02.024>](#)
- [Aylward, LL; Hays, SM; Zidek, A. \(2016\). Variation in urinary spot sample, 24 h samples, and longer-term average urinary concentrations of short-lived environmental chemicals: implications for exposure assessment and reverse dosimetry. J Expo Sci Environ Epidemiol 27: 582-590. <https://dx.doi.org/10.1038/jes.2016.54>](#)
- [Barlow, NJ; McIntyre, BS; Foster, PMD. \(2004\). Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di\(n-butyl\) phthalate. Toxicol Pathol 32: 79-90. <http://dx.doi.org/10.1080/01926230490265894>](#)
- [Beydon, D; Payan, JP; Granclaude, MC. \(2010\). Comparison of percutaneous absorption and metabolism of di-n-butylphthalate in various species. Toxicol In Vitro 24: 71-78. <http://dx.doi.org/10.1016/j.tiv.2009.08.032>](#)
- [Bloom, MS; Wenzel, AG; Brock, JW; Kucklick, JR; Wineland, RJ; Cruze, L; Unal, ER; Yucel, RM; Jiyesova, A; Newman, RB. \(2019\). Racial disparity in maternal phthalates exposure;](#)

- Association with racial disparity in fetal growth and birth outcomes. *Environ Int* 127: 473-486. <https://dx.doi.org/10.1016/j.envint.2019.04.005>
- Boekelheide, K; Kleymenova, E; Liu, K; Swanson, C; Gaido, KW. (2009). Dose-dependent effects on cell proliferation, seminiferous tubules, and male germ cells in the fetal rat testis following exposure to di(n-butyl) phthalate. *Microsc Res Tech* 72: 629-638. <http://dx.doi.org/10.1002/jemt.20684>
- Bornehag, CG; Carlstedt, F; Jonsson, BAG; Lindh, CH; Jensen, TK; Bodin, A; Jonsson, C; Janson, S; Swan, SH. (2014). Prenatal phthalate exposures and anogenital distance in Swedish boys. *Environ Health Perspect* 123: 101-107. <http://dx.doi.org/10.1289/ehp.1408163>
- Boss, J; Zhai, J; Aung, MT; Ferguson, KK; Johns, LE; McElrath, TF; Meeker, JD; Mukherjee, B. (2018). Associations between mixtures of urinary phthalate metabolites with gestational age at delivery: a time to event analysis using summative phthalate risk scores. *Environ Health* 17: 56. <https://dx.doi.org/10.1186/s12940-018-0400-3>
- Buck Louis, GM; Sundaram, R; Sweeney, AM; Schisterman, EF; Maisog, J; Kannan, K. (2014). Urinary bisphenol A, phthalates, and couple fecundity: the Longitudinal Investigation of Fertility and the Environment (LIFE) study. *Fertil Steril* 101: 1359-1366. <http://dx.doi.org/10.1016/j.fertnstert.2014.01.022>
- Burns, JS; Sergeyev, O; Lee, MM; Williams, PL; Mínguez-Alarcón, L; Plaku-Alakbarova, B; Sokolov, S; Kovalev, S; Koch, HM; Lebedev, AT; Hauser, R; Korrick, SA; Russian Children's, S. (2022). Associations of prepubertal urinary phthalate metabolite concentrations with pubertal onset among a longitudinal cohort of boys. *Environ Res* 212: 113218. <https://dx.doi.org/10.1016/j.envres.2022.113218>
- Calafat, AM; Longnecker, MP; Koch, HM; Swan, SH; Hauser, R; Goldman, LR; Lanphear, BP; Rudel, RA; Engel, SM; Teitelbaum, SL; Whyatt, RM; Wolff, MS. (2015). Optimal exposure biomarkers for nonpersistent chemicals in environmental epidemiology. *Environ Health Perspect* 123: A166-A168. <https://dx.doi.org/10.1289/ehp.1510041>
- Calafat, AM; Silva, MJ; Reidy, JA; Earl, GL; Samandar, E; Preau, JL; Herbert, AR; Needham, LL. (2006). Mono-(3-carboxypropyl) phthalate, a metabolite of di-n-octyl phthalate. *J Toxicol Environ Health A* 69: 215-227. <http://dx.doi.org/10.1080/15287390500227381>
- Carruthers, CM; Foster, PMD. (2005). Critical window of male reproductive tract development in rats following gestational exposure to di-n-butyl phthalate. *Birth Defects Res B Dev Reprod Toxicol* 74: 277-285. <https://dx.doi.org/10.1002/bdrb.20050>
- Casas, M; Valvi, D; Ballesteros-Gomez, A; Gascon, M; Fernández, MF; Garcia-Esteban, R; Iñiguez, C; Martinez, D; Murcia, M; Monfort, N; Luque, N; Rubio, S; Ventura, R; Sunyer, J; Vrijheid, M. (2016). Exposure to bisphenol A and phthalates during pregnancy and ultrasound measures of fetal growth in the INMA-Sabadell cohort. *Environ Health Perspect* 124: 521-528. <http://dx.doi.org/10.1289/ehp.1409190>
- Cassina, M; Salviati, L; Di Gianantonio, E; Clementi, M. (2012). Genetic susceptibility to teratogens: State of the art [Review]. *Reprod Toxicol* 34: 186-191. <http://dx.doi.org/10.1016/j.reprotox.2012.05.004>
- CDC. (2021). CDC Health Topics A-Z: Micronutrients [Website]. [https://www.cdc.gov/nutrition/micronutrient-malnutrition/index.html?CDC\\_AA\\_refVal=https%3A%2F%2Fwww.cdc.gov%2Fimmpact%2Fin dex.html](https://www.cdc.gov/nutrition/micronutrient-malnutrition/index.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fimmpact%2Fin dex.html)
- CDC. (2022). CDC Health Topics A-Z: Physical activity [Website]. <https://www.cdc.gov/physicalactivity/index.html>
- CDC. (2023a). CDC Health Topics A-Z: Nutrition [Website]. <https://www.cdc.gov/nutrition/index.html>
- CDC. (2023b). CDC Health Topics A-Z: Stress at work [Website]. <https://www.cdc.gov/niosh/topics/stress/>

- CDC. (2023c). Fetal Alcohol Spectrum Disorders (FASDs): Alcohol use during pregnancy [Website]. <https://www.cdc.gov/ncbddd/fasd/alcohol-use.html>
- CDC. (2023d). Pregnancy: During pregnancy [Website]. <https://www.cdc.gov/pregnancy/during.html>
- CDC. (2023e). Smoking & Tobacco Use: Smoking during pregnancy - Health effects of smoking and secondhand smoke on pregnancies [Website]. [https://www.cdc.gov/tobacco/basic\\_information/health\\_effects/pregnancy/index.htm](https://www.cdc.gov/tobacco/basic_information/health_effects/pregnancy/index.htm)
- Chang, LW; Hou, ML; Tsai, TH. (2013). Pharmacokinetics of dibutyl phthalate (DBP) in the rat determined by UPLC-MS/MS. *International Journal of Molecular Sciences* 14: 836-849. <http://dx.doi.org/10.3390/ijms14010836>
- Chang, WH; Li, SS; Wu, MH; Pan, HA; Lee, CC. (2015). Phthalates might interfere with testicular function by reducing testosterone and insulin-like factor 3 levels. *Hum Reprod* 30: 2658-2670. <http://dx.doi.org/10.1093/humrep/dev225>
- Choi, K; Joo, H; Campbell, JL; Clewell, RA; Andersen, ME; Clewell, HJ. (2012). In vitro metabolism of di(2-ethylhexyl) phthalate (DEHP) by various tissues and cytochrome P450s of human and rat. *Toxicol In Vitro* 26: 315-322. <https://dx.doi.org/10.1016/j.tiv.2011.12.002>
- Clewell, RA; Kremer, JJ; Williams, CC; Campbell, JL; Sochaski, MA; Andersen, ME; Borghoff, SJ. (2009). Kinetics of selected di-n-butyl phthalate metabolites and fetal testosterone following repeated and single administration in pregnant rats. *Toxicology* 255: 80-90. <http://dx.doi.org/10.1016/j.tox.2008.10.010>
- Clewell, RA; Thomas, A; Willson, G; Creasy, DM; Andersen, ME. (2013). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. *Reprod Toxicol* 35: 70-80. <http://dx.doi.org/10.1016/j.reprotox.2012.07.008>
- Conley, JM; Lambright, CS; Evans, N; Cardon, M; Medlock-Kakaley, E; Wilson, VS; Gray, LE. (2021). A mixture of 15 phthalates and pesticides below individual chemical no observed adverse effect levels (NOAELs) produces reproductive tract malformations in the male rat. *Environ Int* 156: 106615. <https://dx.doi.org/10.1016/j.envint.2021.106615>
- CPSC. (2010). Toxicity review of di-n-butyl phthalate. In Toxicity review for di-n-butyl phthalate (Dibutyl phthalate or DBP). Bethesda, MD: U.S. Consumer Product Safety Commission, Directorate for Hazard Identification and Reduction. <https://web.archive.org/web/20190320060443/https://www.cpsc.gov/s3fs-public/ToxicityReviewOfDBP.pdf>
- CPSC. (2014). Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices). Bethesda, MD: U.S. Consumer Product Safety Commission, Directorate for Health Sciences. <https://www.cpsc.gov/s3fs-public/CHAP-REPORT-With-Appendices.pdf>
- de Jesus, MM; Negrin, AC; Taboga, SR; Pinto-Fochi, ME; Góes, RM. (2015). Histopathological alterations in the prostates of Mongolian gerbils exposed to a high-fat diet and di-n-butyl phthalate individually or in combination. *Reprod Toxicol* 52: 26-39. <http://dx.doi.org/10.1016/j.reprotox.2015.02.005>
- Den Hond, E; Tournaye, H; De Sutter, P; Ombelet, W; Baeyens, W; Covaci, A; Cox, B; Nawrot, TS; Van Larebeke, N; D'Hooghe, T. (2015). Human exposure to endocrine disrupting chemicals and fertility: A case-control study in male subfertility patients [Review]. *Environ Int* 84: 154-160. <http://dx.doi.org/10.1016/j.envint.2015.07.017>
- Doan, K; Bronaugh, RL; Yourick, JJ. (2010). In vivo and in vitro skin absorption of lipophilic compounds, dibutyl phthalate, farnesol and geraniol in the hairless guinea pig. *Food Chem Toxicol* 48: 18-23. <http://dx.doi.org/10.1016/j.fct.2009.09.002>
- Downs, SH; Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care

- interventions. *J Epidemiol Community Health* 52: 377-384.  
<https://dx.doi.org/10.1136/jech.52.6.377>
- Drake, AJ; van den Driesche, S; Scott, HM; Hutchison, GR; Seckl, JR; Sharpe, RM. (2009). Glucocorticoids amplify dibutyl phthalate-induced disruption of testosterone production and male reproductive development. *Endocrinology* 150: 5055-5064.  
<http://dx.doi.org/10.1210/en.2009-0700>
- Durmaz, E; Erkekoglu, P; Asci, A; Akçurum, S; Bircan, I; Kocer-Gumusel, B. (2018). Urinary phthalate metabolite concentrations in girls with premature thelarche. *Environ Toxicol Pharmacol* 59: 172-181. <https://dx.doi.org/10.1016/j.etap.2018.03.010>
- EC/HC. (2015). 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. Gatineau, Quebec: Environment Canada, Health Canada. [https://www.canada.ca/content/dam/ecccc/migration/ese-es/4d845198-761d-428b-a519-75481b25b3e5/sos\\_phthalates-20-medium-chain- en.pdf](https://www.canada.ca/content/dam/ecccc/migration/ese-es/4d845198-761d-428b-a519-75481b25b3e5/sos_phthalates-20-medium-chain- en.pdf)
- ECHA. (2010). Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH): Review of new available information for dibutyl phthalate (DBP) CAS No 84-74-2 Einescs No 201-557-4 (pp. 18).
- ECHA. (2013). Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006. Helsinki, Finland.  
<http://echa.europa.eu/documents/10162/31b4067e-de40-4044-93e8-9c9ff1960715>
- ECHA. (2017a). Annex to the Background document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP). (ECHA/RAC/RES-O-0000001412-86-140/F; ECHA/SEAC/RES-O-0000001412-86-154/F).  
<https://echa.europa.eu/documents/10162/1c33302c-7fba-a809-ff33-6bed9e4e87ca>
- ECHA. (2017b). Opinion on an Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP). (ECHA/RAC/RES-O-0000001412-86-140/F). Helsinki, Finland.  
<https://echa.europa.eu/documents/10162/e39983ad-1bf6-f402-7992-8a032b5b82aa>
- ECJRC. (2004). European Union Risk Assessment Report: Dibutyl phthalate with addendum to the environmental section - 2004. (EUR 19840 EN). Luxembourg: European Union, European Chemicals Bureau, Institute for Health and Consumer Protection.  
<https://echa.europa.eu/documents/10162/ba7f7c39-dab6-4dca-bc8e-dfab7ac53e37>
- EFSA. (2005). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to di-Butylphthalate (DBP) for use in food contact materials. 3: 242. <http://dx.doi.org/10.2903/j.efsa.2005.242>
- EFSA. (2019). Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials. *EFSA J* 17: ee05838.  
<https://dx.doi.org/10.2903/j.efsa.2019.5838>
- Elsisi, AE; Carter, DE; Sipes, IG. (1989). Dermal absorption of phthalate diesters in rats. *Fundam Appl Toxicol* 12: 70-77. [http://dx.doi.org/10.1016/0272-0590\(89\)90063-8](http://dx.doi.org/10.1016/0272-0590(89)90063-8)
- Ema, M; Miyawaki, E; Kawashima, K. (1998). Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. *Toxicol Lett* 98: 87-93.  
[http://dx.doi.org/10.1016/S0378-4274\(98\)00107-6](http://dx.doi.org/10.1016/S0378-4274(98)00107-6)
- Ema, M; Miyawaki, E; Kawashima, K. (2000). Critical period for adverse effects on development of reproductive system in male offspring of rats given di-n-butyl phthalate during late pregnancy. *Toxicol Lett* 111: 271-278. [http://dx.doi.org/10.1016/S0378-4274\(99\)00192-7](http://dx.doi.org/10.1016/S0378-4274(99)00192-7)
- Farzanehfard, V; Naderi, N; Kobarfard, F; Faizi, M. (2016). Determination of dibutyl phthalate neurobehavioral toxicity in mice. *Food Chem Toxicol* 94: 221-226.  
<http://dx.doi.org/10.1016/j.fct.2016.05.006>



- Faustman, EM; Allen, BC; Kavlock, RJ; Kimmel, CA. (1994). Dose-response assessment for developmental toxicity: I characterization of data base and determination of no observed adverse effect levels. *Fundam Appl Toxicol* 23: 478-486. <https://dx.doi.org/10.1006/faat.1994.1132>
- Fennell, TR; Krol, WL; Sumner, SCJ; Snyder, RW. (2004). Pharmacokinetics of dibutylphthalate in pregnant rats. *Toxicol Sci* 82: 407-418. <http://dx.doi.org/10.1093/toxsci/kfh294>
- Ferguson, KK; Mcelrath, TF; Meeker, JD. (2014). Environmental phthalate exposure and preterm birth. *JAMA Pediatr* 168: 61-67. <http://dx.doi.org/10.1001/jamapediatrics.2013.3699>
- Ferrara, D; Hallmark, N; Scott, H; Brown, R; Mckinnell, C; Mahood, IK; Sharpe, RM. (2006). Acute and long-term effects of in utero exposure of rats to di(n-butyl) phthalate on testicular germ cell development and proliferation. *Endocrinology* 147: 5352-5362. <http://dx.doi.org/10.1210/en.2006-0527>
- Foster, PMD. (2005). Mode of action: Impaired fetal Leydig cell function - Effects on male reproductive development produced by certain phthalate esters [Review]. *Crit Rev Toxicol* 35: 713-719. <https://dx.doi.org/10.1080/10408440591007395>
- Foster, PMD; Cook, MW; Thomas, LV; Walters, DG; Gangolli, SD. (1983). Differences in urinary metabolic profile from di-n-butyl phthalate-treated rats and hamsters. A possible explanation for species-differences in susceptibility to testicular atrophy. *Drug Metab Dispos* 11: 59-61.
- Foster, PMD; Mylchreest, E; Gaido, KW; Sar, M. (2001). Effects of phthalate esters on the developing reproductive tract of male rats [Review]. *Hum Reprod Update* 7: 231-235. <https://dx.doi.org/10.1093/humupd/7.3.231>
- Foster, PMD; Thomas, LV; Cook, MW; Gangolli, SD. (1980). Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. *Toxicol Appl Pharmacol* 54: 392-398. [http://dx.doi.org/10.1016/0041-008X\(80\)90165-9](http://dx.doi.org/10.1016/0041-008X(80)90165-9)
- Fox, JR; Hogan, KA; Davis, A. (2016). Dose-response modeling with summary data from developmental toxicity studies. *Risk Anal* 37: 905-917. <http://dx.doi.org/10.1111/risa.12667>
- Furr, JR; Lambright, CS; Wilson, VS; Foster, PM; Gray, LE, Jr. (2014). A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. *Toxicol Sci* 140: 403-424. <https://dx.doi.org/10.1093/toxsci/kfu081>
- Gaido, KW; Hensley, JB; Liu, D; Wallace, DG; Borghoff, S; Johnson, KJ; Hall, SJ; Boekelheide, K. (2007). Fetal mouse phthalate exposure shows that gonocyte multinucleation is not associated with decreased testicular testosterone. *Toxicol Sci* 97: 491-503. <http://dx.doi.org/10.1093/toxsci/kfm049>
- General Motors. (1983a). Effect of dose on di-isodecyl phthalate disposition in rats with cover letter. (OTS0206315).
- General Motors. (1983b). Toxicity and disposition of di-isodecyl phthalate following inhalation exposure in rats with cover letter [TSCA Submission]. (OTS0530340. 86-910000684. 86-910000684. TSCATS/414860). General Motors Co. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0530340.xhtml>
- General Motors. (1991). Disposition of di-2-ethylhexyl phthalate following inhalation and peroral exposure in rats with cover letter. (EPA/OTS; Doc #86-910000683). <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0530339.xhtml>
- Giribabu, N; Sainath, SB; Reddy, PS. (2014). Prenatal di-n-butyl phthalate exposure alters reproductive functions at adulthood in male rats. *Environ Toxicol* 29: 534-544. <http://dx.doi.org/10.1002/tox.21779>
- Goldman, JM; Murr, AS; Cooper, RL. (2007). The rodent estrous cycle: Characterization of vaginal cytology and its utility in toxicological studies [Review]. *Birth Defects Res B Dev Reprod Toxicol* 80: 84-97. <http://dx.doi.org/10.1002/bdrb.20106>

- [Gray, LE; Furr, J; Tatum-Gibbs, KR; Lambright, C; Sampson, H; Hannas, BR; Wilson, VS; Hotchkiss, A; Foster, PM.](#) (2016). Establishing the “Biological Relevance” of Dipentyl Phthalate Reductions in Fetal Rat Testosterone Production and Plasma and Testis Testosterone Levels. *Toxicol Sci* 149: 178-191. <https://dx.doi.org/10.1093/toxsci/kfv224>
- [Gray, LE, Jr.; Lambright, CS; Conley, JM; Evans, N; Furr, JR; Hannas, BR; Wilson, VS; Sampson, H; Foster, PM.](#) (2021). Genomic and hormonal biomarkers of phthalate-induced male rat reproductive developmental toxicity, Part II: A targeted RT-qPCR array approach that defines a unique adverse outcome pathway. *Toxicol Sci* 182: 195-214. <https://dx.doi.org/10.1093/toxsci/kfab053>
- [Gray, TJB; Rowland, IR; Foster, PM; Gangolli, SD.](#) (1982). Species differences in the testicular toxicity of phthalate esters. *Toxicol Lett* 11: 141-147. [https://dx.doi.org/10.1016/0378-4274\(82\)90119-9](https://dx.doi.org/10.1016/0378-4274(82)90119-9)
- [Hallmark, N; Walker, M; McKinnell, C; Mahood, IK; Scott, H; Bayne, R; Coutts, S; Anderson, RA; Greig, I; Morris, K; Sharpe, RM.](#) (2007). Effects of monobutyl and di(n-butyl) phthalate in vitro on steroidogenesis and Leydig cell aggregation in fetal testis explants from the rat: Comparison with effects in vivo in the fetal rat and neonatal marmoset and in vitro in the human. *Environ Health Perspect* 115: 390-396. <https://dx.doi.org/10.1289/ehp.9490>
- [Han, X; Cui, Z; Zhou, N; Ma, M; Li, L; Li, Y; Lin, H; Ao, L; Shu, W; Liu, J; Cao, J.](#) (2014). Urinary phthalate metabolites and male reproductive function parameters in Chongqing general population, China. *Int J Hyg Environ Health* 217: 271-278. <http://dx.doi.org/10.1016/j.ijheh.2013.06.006>
- [Hauser, R; Gaskins, AJ; Souter, I; Smith, KW; Dodge, LE; Ehrlich, S; Meeker, JD; Calafat, AM; Williams, PL.](#) (2016). Urinary phthalate metabolite concentrations and reproductive outcomes among women undergoing in vitro fertilization: results from the EARTH study. *Environ Health Perspect* 124: 831-839. <http://dx.doi.org/10.1289/ehp.1509760>
- [Hauser, R; Meeker, JD; Duty, S; Silva, MJ; Calafat, AM.](#) (2006). Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. *Epidemiology* 17: 682-691. <http://dx.doi.org/10.1097/01.ede.0000235996.89953.d7>
- [Health Canada.](#) (2015). Supporting documentation: Carcinogenicity of phthalates - mode of action and human relevance. In Supporting documentation for Phthalate Substance Grouping. Ottawa, ON.
- [Health Canada.](#) (2018a). 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. Ottawa, ON.
- [Health Canada.](#) (2018b). Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters. Ottawa, ON.
- [Health Canada.](#) (2020). Screening assessment - Phthalate substance grouping. (En14-393/2019E-PDF). Environment and Climate Change Canada. <https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/screening-assessment-phthalate-substance-grouping.html>
- [Heger, NE; Hall, SJ; Sandrof, MA; McDonnell, EV; Hensley, JB; McDowell, EN; Martin, KA; Gaido, KW; Johnson, KJ; Boekelheide, K.](#) (2012). Human fetal testis xenografts are resistant to phthalate-induced endocrine disruption. *Environ Health Perspect* 120: 1137-1143. <https://dx.doi.org/10.1289/ehp.1104711>
- [Higuchi, TT; Palmer, JS; Gray, LE, Jr; Veeramachaneni, DN.](#) (2003). Effects of dibutyl phthalate in male rabbits following in utero, adolescent, or postpubertal exposure. *Toxicol Sci* 72: 301-313. <http://dx.doi.org/10.1093/toxsci/kfg036>



- [Howdeshell, KL; Furr, J; Lambright, CR; Rider, CV; Wilson, VS; Gray, LE, Jr. \(2007\). Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: Altered fetal steroid hormones and genes. Toxicol Sci 99: 190-202. <http://dx.doi.org/10.1093/toxsci/kfm069>](#)
- [Howdeshell, KL; Hotchkiss, AK; Gray, LE. \(2016\). Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment \[Review\]. Int J Hyg Environ Health 220: 179-188. <https://dx.doi.org/10.1016/j.ijheh.2016.11.007>](#)
- [Howdeshell, KL; Rider, CV; Wilson, VS; Furr, JR; Lambright, CR; Gray, LE. \(2015\). Dose addition models based on biologically relevant reductions in fetal testosterone accurately predict postnatal reproductive tract alterations by a phthalate mixture in rats. Toxicol Sci 148: 488-502. <https://dx.doi.org/10.1093/toxsci/kfv196>](#)
- [Howdeshell, KL; Wilson, VS; Furr, J; Lambright, CR; Rider, CV; Blystone, CR; Hotchkiss, AK; Gray, LE, Jr. \(2008\). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicol Sci 105: 153-165. <https://dx.doi.org/10.1093/toxsci/kfn077>](#)
- [IGHRC. \(2006\). Guidelines on route-to-route extrapolation of toxicity data when assessing health risks of chemicals. Bedfordshire, UK: Institute of Environment and Health. \[http://www.iehconsulting.co.uk/IEH\\\_Consulting/IEHCPubs/IGHRC/cr12.pdf\]\(http://www.iehconsulting.co.uk/IEH\_Consulting/IEHCPubs/IGHRC/cr12.pdf\)](#)
- [Ingelman-Sundberg, M. \(2004\). Human drug metabolising cytochrome P450 enzymes: Properties and polymorphisms \[Review\]. Naunyn-Schmiedeberg's Arch Pharmacol 369: 89-104. <http://dx.doi.org/10.1007/s00210-003-0819-z>](#)
- [Ito, Y; Yokota, H; Wang, R; Yamanoshita, O; Ichihara, G; Wang, H; Kurata, Y; Takagi, K; Nakajima, T. \(2005\). Species differences in the metabolism of di\(2-ethylhexyl\) phthalate \(DEHP\) in several organs of mice, rats, and marmosets. Arch Toxicol 79: 147-154. <https://dx.doi.org/10.1007/s00204-004-0615-7>](#)
- [Janjua, NR; Frederiksen, H; Skakkebaek, NE; Wulf, HC; Andersson, AM. \(2008\). Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. Int J Androl 31: 118-130. <http://dx.doi.org/10.1111/j.1365-2605.2007.00841.x>](#)
- [Jensen, TK; Frederiksen, H; Kyhl, HB; Lassen, TH; Swan, SH; Bornehag, CG; Skakkebaek, NE; Main, KM; Lind, DV; Husby, S; Andersson, AM. \(2016\). Prenatal exposure to phthalates and anogenital distance in male infants from a low-exposed Danish cohort \(2010-2012\). Environ Health Perspect 124: 1107-1113. <http://dx.doi.org/10.1289/ehp.1509870>](#)
- [Jiang, J; Ma, L; Yuan, L; Wang, X; Zhang, W. \(2007\). Study on developmental abnormalities in hypospadiac male rats induced by maternal exposure to di-n-butyl phthalate \(DBP\). Toxicology 232: 286-293. <http://dx.doi.org/10.1016/j.tox.2007.01.018>](#)
- [Johnson, KJ; Heger, NE; Boekelheide, K. \(2012\). Of mice and men \(and rats\): phthalate-induced fetal testis endocrine disruption is species-dependent \[Review\]. Toxicol Sci 129: 235-248. <https://dx.doi.org/10.1093/toxsci/kfs206>](#)
- [Johnson, KJ; Hensley, JB; Kelso, MD; Wallace, DG; Gaido, KW. \(2007\). Mapping gene expression changes in the fetal rat testis following acute dibutyl phthalate exposure defines a complex temporal cascade of responding cell types. Biol Reprod 77: 978-989. <http://dx.doi.org/10.1095/biolreprod.107.062950>](#)
- [Johnson, KJ; McDowell, EN; Viereck, MP; Xia, JQ. \(2011\). Species-specific dibutyl phthalate fetal testis endocrine disruption correlates with inhibition of SREBP2-dependent gene expression pathways. Toxicol Sci 120: 460-474. <https://dx.doi.org/10.1093/toxsci/kfr020>](#)
- [Jukic, AM; Calafat, AM; McConnaughey, DR; Longnecker, MP; Hoppin, JA; Weinberg, CR; Wilcox, AJ; Baird, DD. \(2016\). Urinary concentrations of phthalate metabolites and bisphenol A and associations with follicular-phase length, luteal-phase length, fecundability, and early pregnancy loss. Environ Health Perspect 124: 321-328. <http://dx.doi.org/10.1289/ehp.1408164>](#)

- [Jurewicz, J; Radwan, M; Sobala, W; Ligocka, D; Radwan, P; Bochenek, M; Hawuła, W; Jakubowski, L; Hanke, W. \(2013\). Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones. \*Reprod Toxicol\* 42: 232-241. <http://dx.doi.org/10.1016/j.reprotox.2013.10.001>](#)
- [Kawano, M. \(1980\). \[Toxicological studies on phthalate esters: 1 inhalation effects of dibutyl phthalate \(DBP\) on rats\]. \*Nippon Eiseigaku Zasshi\* 35: 684-692.](#)
- [Keys, DA; Wallace, DG; Kepler, TB; Conolly, RB. \(2000\). Quantitative evaluation of alternative mechanisms of blood disposition of di\(n-butyl\) phthalate and mono\(n-butyl\) phthalate in rats. \*Toxicol Sci\* 53: 173-184. <http://dx.doi.org/10.1093/toxsci/53.2.173>](#)
- [Kim, TS; Jung, KK; Kim, SS; Kang, IH; Baek, JH; Nam, HS; Hong, SK; Lee, BM; Hong, JT; Oh, KW; Kim, HS; Han, SY; Kang, TS. \(2010\). Effects of in utero exposure to DI\(n-Butyl\) phthalate on development of male reproductive tracts in Sprague-Dawley rats. \*J Toxicol Environ Health A\* 73: 1544-1559. <http://dx.doi.org/10.1080/15287394.2010.511579>](#)
- [Koch, HM; Christensen, KLY; Harth, V; Lorber, M; Brüning, T. \(2012\). Di-n-butyl phthalate \(DnBP\) and diisobutyl phthalate \(DiBP\) metabolism in a human volunteer after single oral doses. \*Arch Toxicol\* 86: 1829-1839. <http://dx.doi.org/10.1007/s00204-012-0908-1>](#)
- [Kremer, JJ; Williams, CC; Parkinson, HD; Borghoff, SJ. \(2005\). Pharmacokinetics of monobutylphthalate, the active metabolite of di-n-butylphthalate, in pregnant rats. \*Toxicol Lett\* 159: 144-153. <http://dx.doi.org/10.1016/j.toxlet.2005.05.006>](#)
- [Kuhl, AJ; Ross, SM; Gaido, KW. \(2007\). CCAAT/enhancer binding protein beta, but not steroidogenic factor-1, modulates the phthalate-induced dysregulation of rat fetal testicular steroidogenesis. \*Endocrinology\* 148: 5851-5864. <http://dx.doi.org/10.1210/en.2007-0930>](#)
- [Lake, BG; Phillips, JC; Linnell, JC; Gangolli, SD. \(1977\). The in vitro hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. \*Toxicol Appl Pharmacol\* 39: 239-248. \[https://dx.doi.org/10.1016/0041-008X\\(77\\)90157-0\]\(https://dx.doi.org/10.1016/0041-008X\(77\)90157-0\)](#)
- [Lambrot, R; Muczynski, V; Lecureuil, C; Angenard, G; Coffigny, H; Pairault, C; Moison, D; Frydman, R; Habert, R; Rouiller-Fabre, V. \(2009\). Phthalates impair germ cell development in the human fetal testis in vitro without change in testosterone production. \*Environ Health Perspect\* 117: 32-37. <https://dx.doi.org/10.1289/ehp.11146>](#)
- [Lee, KY; Shibutani, M; Takagi, H; Kato, N; Takigami, S; Uneyama, C; Hirose, M. \(2004\). Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. \*Toxicology\* 203: 221-238. <http://dx.doi.org/10.1016/j.tox.2004.06.013>](#)
- [Leeder, JS; Kearns, GL. \(1997\). Pharmacogenetics in pediatrics: Implications for practice \[Review\]. \*Pediatr Clin North Am\* 44: 55-77. \[http://dx.doi.org/10.1016/S0031-3955\\(05\\)70463-6\]\(http://dx.doi.org/10.1016/S0031-3955\(05\)70463-6\)](#)
- [Lehmann, KP; Phillips, S; Sar, M; Foster, PMD; Gaido, KW. \(2004\). Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di \(n-butyl\) phthalate. \*Toxicol Sci\* 81: 60-68. <http://dx.doi.org/10.1093/toxsci/kfh169>](#)
- [Li, H; Jiang, Y; Liu, M; Yu, J; Feng, X; Xu, X; Wang, H; Zhang, J; Sun, X; Yu, Y. \(2023\). DNA methylation-mediated inhibition of MGARP is involved in impaired progeny testosterone synthesis in mice exposed to DBP in utero. \*Environ Toxicol\* 38: 914-925. <http://dx.doi.org/10.1002/tox.23734>](#)
- [Li, J; Li, L; Zuo, H; Ke, C; Yan, B; Wen, H; Zhang, Y; Yang, X. \(2014\). T-helper type-2 contact hypersensitivity of Balb/c mice aggravated by dibutyl phthalate via long-term dermal exposure. \*PLoS ONE\* 9: e87887. <http://dx.doi.org/10.1371/journal.pone.0087887>](#)
- [Li, N; Chen, X; Zhou, X; Zhang, W; Yuan, J; Feng, J. \(2015\). The mechanism underlying dibutyl phthalate induced shortened anogenital distance and hypospadias in rats. \*J Pediatr Surg\* 50: 2078-2083. <http://dx.doi.org/10.1016/j.jpedsurg.2015.08.046>](#)

- [Li, Y; Zhuang, M; Li, T; Shi, N. \(2009\). Neurobehavioral toxicity study of dibutyl phthalate on rats following in utero and lactational exposure. J Appl Toxicol 29: 603-611. <http://dx.doi.org/10.1002/jat.1447>](#)
- [Li, YL; Lv, J; Du, ZP; Feng, S; Sheng, J; Jin, ZX; Liu, KY; Gao, H; Li, XD; Cao, HJ; Yang, LS; Xu, DX; Tao, FB; Wang, QN. \(2020\). The levels of phthalate exposure and associations with obesity in an elderly population in China. Ecotoxicol Environ Saf 201: 110749. <http://dx.doi.org/10.1016/j.ecoenv.2020.110749>](#)
- [Liu, L; Bao, H; Liu, F; Zhang, J; Shen, H. \(2012\). Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality, a primary study. Environ Int 42: 78-83. <http://dx.doi.org/10.1016/j.envint.2011.04.005>](#)
- [Machtinger, R; Mansur, A; Baccarelli, AA; Calafat, AM; Gaskins, AJ; Racowsky, C; Adir, M; Hauser, R. \(2018\). Urinary concentrations of biomarkers of phthalates and phthalate alternatives and IVF outcomes. Environ Int 111: 23-31. <http://dx.doi.org/10.1016/j.envint.2017.11.011>](#)
- [MacLeod, DJ; Sharpe, RM; Welsh, M; Fisk, M; Scott, HM; Hutchison, GR; Drake, AJ; van Den Driesche, S. \(2010\). Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl 33: 279-287. <https://dx.doi.org/10.1111/j.1365-2605.2009.01005.x>](#)
- [Mahood, IK; Scott, HM; Brown, R; Hallmark, N; Walker, M; Sharpe, RM. \(2007\). In utero exposure to di\(n-butyl\) phthalate and testicular dysgenesis: Comparison of fetal and adult end points and their dose sensitivity. Environ Health Perspect 115: 55-61. <http://dx.doi.org/10.1289/ehp.9366>](#)
- [Majeed, KA; ur Rehman, H; Yousaf, MS; Zaneb, H; Rabbani, I; Tahir, SK; Rashid, MA. \(2017\). Sub-chronic exposure to low concentration of dibutyl phthalate affects anthropometric parameters and markers of obesity in rats. Environ Sci Pollut Res Int 24: 25462-25467. <http://dx.doi.org/10.1007/s11356-017-9952-y>](#)
- [Martino-Andrade, AJ; Morais, RN; Botelho, GG; Muller, G; Grande, SW; Carpentieri, GB; Leao, GM; Dalsenter, PR. \(2008\). Coadministration of active phthalates results in disruption of foetal testicular function in rats. Int J Androl 32: 704-712. <http://dx.doi.org/10.1111/j.1365-2605.2008.00939.x>](#)
- [McKinnell, C; Mitchell, RT; Walker, M; Morris, K; Kelnar, CJH; Wallace, WH; Sharpe, RM. \(2009\). Effect of fetal or neonatal exposure to monobutyl phthalate \(MBP\) on testicular development and function in the marmoset. Hum Reprod 24: 2244-2254. <http://dx.doi.org/10.1093/humrep/dep200>](#)
- [Meeker, JD; Calafat, AM; Hauser, R. \(2009a\). Urinary metabolites of di\(2-ethylhexyl\) phthalate are associated with decreased steroid hormone levels in adult men. J Androl 30: 287-297. <http://dx.doi.org/10.2164/jandrol.108.006403>](#)
- [Meeker, JD; Ferguson, KK. \(2014\). Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011-2012. J Clin Endocrinol Metab 99: 4346-4352. <http://dx.doi.org/10.1210/jc.2014-2555>](#)
- [Meeker, JD; Hu, H; Cantonwine, DE; Lamadrid-Figueroa, H; Calafat, AM; Ettinger, AS; Hernandez-Avila, M; Loch-Caruso, R; Tellez-Rojo, MM. \(2009b\). Urinary phthalate metabolites in relation to preterm birth in Mexico city. Environ Health Perspect 117: 1587-1592. <http://dx.doi.org/10.1289/ehp.0800522>](#)
- [Messerlian, C; Souter, I; Gaskins, AJ; Williams, PL; Ford, JB; Chiu, YH; Calafat, AM; Hauser, R; Team, ES. \(2015\). Urinary phthalate metabolites and ovarian reserve among women seeking infertility care. Hum Reprod 31: 75-83. <http://dx.doi.org/10.1093/humrep/dev292>](#)
- [Messerlian, C; Wylie, BJ; Minguez-Alarcon, L; Williams, PL; Ford, JB; Souter, IC; Calafat, AM; Hauser, R; Team, ES. \(2016\). Urinary Concentrations of Phthalate Metabolites and Pregnancy Loss among Women Conceiving with Medically Assisted Reproduction. Epidemiology 27: 879-888. <http://dx.doi.org/10.1097/EDE.0000000000000525>](#)

- Mitchell, RT; Childs, AJ; Anderson, RA; van Den Driesche, S; Saunders, PTK; McKinnell, C; Wallace, WHB; Kelnar, CJH; Sharpe, RM. (2012). Do phthalates affect steroidogenesis by the human fetal testis? Exposure of human fetal testis xenografts to di-n-butyl phthalate. *J Clin Endocrinol Metab* 97: E341-E348. <https://dx.doi.org/10.1210/jc.2011-2411>
- Miura, T; Uehara, S; Mizuno, S; Yoshizawa, M; Murayama, N; Kamiya, Y; Shimizu, M; Suemizu, H; Yamazaki, H. (2019). Steady-state human pharmacokinetics of monobutyl phthalate predicted by physiologically based pharmacokinetic modeling using single-dose data from humanized-liver mice orally administered with dibutyl phthalate. *Chem Res Toxicol* 32: 333-340. <http://dx.doi.org/10.1021/acs.chemrestox.8b00361>
- Moody, S; Goh, H; Bielanowicz, A; Rippon, P; Loveland, KL; Itman, C. (2013). Prepubertal mouse testis growth and maturation and androgen production are acutely sensitive to di-n-butyl phthalate. *Endocrinology* 154: 3460-3475. <http://dx.doi.org/10.1210/en.2012-2227>
- Mouritsen, A; Frederiksen, H; Sørensen, K; Aksglaede, L; Hagen, C; Skakkebaek, NE; Main, KM; Andersson, AM; Juul, A. (2013). Urinary phthalates from 168 girls and boys measured twice a year during a 5-year period: Associations with adrenal androgen levels and puberty. *J Clin Endocrinol Metab* 98: 3755-3764. <http://dx.doi.org/10.1210/jc.2013-1284>
- Mu, D; Gao, F; Fan, Z; Shen, H; Peng, H; Hu, J. (2015). Levels of phthalate metabolites in urine of pregnant women and risk of clinical pregnancy loss. *Environ Sci Technol* 49: 10651-10657. <http://dx.doi.org/10.1021/acs.est.5b02617>
- Mylchreest, E; Cattley, RC; Foster, PMD. (1998). Male reproductive tract malformations in rats following gestational and lactational exposure to di(n-butyl) phthalate: An antiandrogenic mechanism? *Toxicol Sci* 43: 47-60. <http://dx.doi.org/10.1006/toxs.1998.2436>
- Mylchreest, E; Sar, M; Cattley, RC; Foster, PMD. (1999). Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. *Toxicol Appl Pharmacol* 156: 81-95. <http://dx.doi.org/10.1006/taap.1999.8643>
- Mylchreest, E; Sar, M; Wallace, DG; Foster, PMD. (2002). Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. *Toxicol Sci* 64: 19-28.
- Mylchreest, E; Wallace, DG; Cattley, RC; Foster, PM. (2000). Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. *Toxicol Sci* 55: 143-151. <http://dx.doi.org/10.1093/toxsci/55.1.143>
- NASEM. (2017). Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals. In Consensus Study Report. Washington, D.C.: The National Academies Press. <https://dx.doi.org/10.17226/24758>
- NICNAS. (2008). Existing chemical hazard assessment report: Dibutyl phthalate. Sydney, Australia. <https://www.industrialchemicals.gov.au/sites/default/files/Dibutyl%20phthalate%20DBP.pdf>
- NICNAS. (2012). Priority existing chemical assessment report no. 35: Diisononyl phthalate. (PEC35 ISBN 9780980722185). Sydney, Australia: Australian Government Department of Health and Ageing. <https://www.industrialchemicals.gov.au/sites/default/files/PEC35-Diisononyl-phthalate-DINP.pdf>
- NICNAS. (2013). Priority existing chemical assessment report no. 36: Dibutyl phthalate (pp. 131). (PEC36). Sydney, Australia: Australian Department of Health, National Industrial Chemicals Notification and Assessment Scheme. <https://www.industrialchemicals.gov.au/sites/default/files/PEC36-Dibutyl-phthalate-DBP.pdf>
- NTP-CERHR. (2003). NTP-CERHR monograph on the potential human reproductive and developmental effects of di-isononyl phthalate (DINP) (pp. i-III90). (NIH Publication No. 03-4484). Research Triangle Park, NC: National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. [http://ntp.niehs.nih.gov/ntp/ohat/phthalates/dinp/dinp\\_monograph\\_final.pdf](http://ntp.niehs.nih.gov/ntp/ohat/phthalates/dinp/dinp_monograph_final.pdf)



- NTP. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice (pp. 1-G5). (ISSN 1521-4621 Toxicity Report Series Number 30; NIH Publication 95-3353). Research Triangle Park, NC: National Toxicology Program. <https://ntp.niehs.nih.gov/publications/reports/tox/000s/tox030>
- NTP. (2003). NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Butyl Phthalate (DBP). In Expert Panel Reports and NTP-CERHR Monographs (pp. 169). Research Triangle Park, NC: Center for the Evaluation of Risks to Human Reproduction/National Toxicology Program-National Institute of Environmental Health Sciences. <https://search.proquest.com/docview/733974278?accountid=171501>
- NTP. (2015). Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration. Research Triangle Park, NC: U.S. Department of Health and Human Services, National Toxicology Program, Office of Health Assessment and Translation. [https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015\\_508.pdf](https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf)
- NTP. (2021). NTP technical report on the toxicology and carcinogenesis studies of di-n-butyl phthalate (CASRN 84-74-2) administered in feed to Sprague Dawley (HSD: Sprague Dawley® SD®) rats and B6C3F1/n mice. (ISSN 0888-8051 Technical Report 600). Research Triangle Park, NC. <http://dx.doi.org/10.22427/NTP-TR-600>
- ODPHP. (2023a). Healthy People 2030 - Social determinants of health literature summaries: Neighborhood and built environment [Website]. <https://health.gov/healthypeople/priority-areas/social-determinants-health/literature-summaries#neighborhood>
- ODPHP. (2023b). Healthy People 2030 - Social determinants of health literature summaries: Poverty [Website]. <https://health.gov/healthypeople/priority-areas/social-determinants-health/literature-summaries/poverty>
- ODPHP. (2023c). Healthy People 2030 - Social determinants of health literature summaries: Social and community context [Website]. <https://health.gov/healthypeople/priority-areas/social-determinants-health/literature-summaries#social>
- OECD. (2004a). Test No. 427: Skin absorption: in vivo method. Paris, France.
- OECD. (2004b). Test No. 428: Skin absorption: In vitro method. Paris, France. <http://dx.doi.org/10.1787/9789264071087-en>
- OEHHA. (2007). Proposition 65 Maximum Allowable Dose Level (MADL) for reproductive toxicity for di(n-butyl)phthalate (DBP). California: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Reproductive and Cancer Hazard Assessment Section. <https://oehha.ca.gov/media/downloads/proposition-65/chemicals/dbpmadl062907.pdf>
- Pan, G; Hanaoka, T; Yoshimura, M; Zhang, S; Wang, P; Tsukino, H; Inoue, K; Nakazawa, H; Tsugane, S; Takahashi, K. (2006). Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China. *Environ Health Perspect* 114: 1643-1648. <http://dx.doi.org/10.1289/ehp.9016>
- Pan, Y; Jing, J; Dong, F; Yao, Q; Zhang, W; Zhang, H; Yao, B; Dai, J. (2015). Association between phthalate metabolites and biomarkers of reproductive function in 1066 Chinese men of reproductive age. *J Hazard Mater* 300: 729-736. <http://dx.doi.org/10.1016/j.jhazmat.2015.08.011>
- Parks, LG; Ostby, JS; Lambright, CR; Abbott, BD; Klinefelter, GR; Barlow, NJ; Gray, LE, Jr. (2000). The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol Sci* 58: 339-349. <https://dx.doi.org/10.1093/toxsci/58.2.339>
- Polańska, K; Ligocka, D; Sobala, W; Hanke, W. (2016). Effect of environmental phthalate exposure on pregnancy duration and birth outcomes. *Int J Occup Med Environ Health* 29: 683-697. <http://dx.doi.org/10.13075/ijomeh.1896.00691>

- Radke, EG; Braun, JM; Meeker, JD; Cooper, GS. (2018). Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence [Review]. *Environ Int* 121: 764-793. <https://dx.doi.org/10.1016/j.envint.2018.07.029>
- Radke, EG; Braun, JM; Nachman, RM; Cooper, GS. (2020a). Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence [Review]. *Environ Int* 137: 105408. <https://dx.doi.org/10.1016/j.envint.2019.105408>
- Radke, EG; Galizia, A; Thayer, KA; Cooper, GS. (2019a). Phthalate exposure and metabolic effects: A systematic review of the human epidemiological evidence [Review]. *Environ Int* 132: 104768. <https://dx.doi.org/10.1016/j.envint.2019.04.040>
- Radke, EG; Glenn, BS; Braun, JM; Cooper, GS. (2019b). Phthalate exposure and female reproductive and developmental outcomes: A systematic review of the human epidemiological evidence [Review]. *Environ Int* 130: 104580. <https://dx.doi.org/10.1016/j.envint.2019.02.003>
- Radke, EG; Yost, EE; Roth, N; Sathyanarayana, S; Whaley, P. (2020b). Application of US EPA IRIS systematic review methods to the health effects of phthalates: Lessons learned and path forward [Editorial]. *Environ Int* 145: 105820. <https://dx.doi.org/10.1016/j.envint.2020.105820>
- Rowland, IR; Cottrell, RC; Phillips, JC. (1977). Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat. *Food Chem Toxicol* 15: 17-21. [https://dx.doi.org/10.1016/s0015-6264\(77\)80257-5](https://dx.doi.org/10.1016/s0015-6264(77)80257-5)
- Saillenfait, AM; Payan, JP; Fabry, JP; Beydon, D; Langonne, I; Gallissot, F; Sabate, JP. (1998). Assessment of the developmental toxicity, metabolism, and placental transfer of di-n-butyl phthalate administered to pregnant rats. *Toxicol Sci* 45: 212-224. <http://dx.doi.org/10.1006/toxs.1998.2518>
- Scarano, WR; Toledo, FC; Guerra, MT; Pinheiro, PFF; Domeniconi, RF; Felisbino, SL; Campos, SG; Taboga, SR; Kempinas, WG. (2010). Functional and morphological reproductive aspects in male rats exposed to di-n-butyl phthalate (DBP) in utero and during lactation. *J Toxicol Environ Health A* 73: 972-984. <http://dx.doi.org/10.1080/15287391003751760>
- Schwartz, CL; Christiansen, S; Hass, U; Ramhøj, L; Axelstad, M; Löbl, NM; Svingen, T. (2021). On the use and interpretation of areola/nipple retention as a biomarker for anti-androgenic effects in rat toxicity studies [Review]. *Front Toxicol* 3: 730752. <https://dx.doi.org/10.3389/ftox.2021.730752>
- Scott, RC; Dugard, PH; Ramsey, JD; Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. *Environ Health Perspect* 74: 223-227. <http://dx.doi.org/10.2307/3430452>
- Seckin, E; Fromme, H; Völkel, W. (2009). Determination of total and free mono-n-butyl phthalate in human urine samples after medication of a di-n-butyl phthalate containing capsule. *Toxicol Lett* 188: 33-37. <http://dx.doi.org/10.1016/j.toxlet.2009.03.002>
- Sen, N; Liu, X; Craig, ZR. (2015). Short term exposure to di-n-butyl phthalate (DBP) disrupts ovarian function in young CD-1 mice. *Reprod Toxicol* 53: 15-22. <http://dx.doi.org/10.1016/j.reprotox.2015.02.012>
- Shin, HM; Bennett, DH; Barkoski, J; Ye, X; Calafat, AM; Tancredi, D; Hertz-Picciotto, I. (2019). Variability of urinary concentrations of phthalate metabolites during pregnancy in first morning voids and pooled samples. *Environ Int* 122: 222-230. <https://dx.doi.org/10.1016/j.envint.2018.11.012>
- Silva, MJ; Barr, DB; Reidy, JA; Kato, K; Malek, NA; Hodge, CC; Hurtz D, I; Calafat, AM; Needham, LL; Brock, JW. (2003). Glucuronidation patterns of common urinary and serum monoester phthalate metabolites. *Arch Toxicol* 77: 561-567. <http://dx.doi.org/10.1007/s00204-003-0486-3>
- Smarr, MM; Grantz, KL; Sundaram, R; Maisog, JM; Kannan, K; Louis, GM. (2015). Parental urinary biomarkers of preconception exposure to bisphenol A and phthalates in relation to birth outcomes. *Environ Health* 14: 73. <http://dx.doi.org/10.1186/s12940-015-0060-5>



- Spade, DJ; Bai, CY; Lambright, C; Conley, JM; Boekelheide, K; Gray, LE. (2018). Validation of an automated counting procedure for phthalate-induced testicular multinucleated germ cells. *Toxicol Lett* 290: 55-61. <https://dx.doi.org/10.1016/j.toxlet.2018.03.018>
- Spade, DJ; Hall, SJ; Saffarini, C; Huse, SM; McDonnell, EV; Boekelheide, K. (2014). Differential response to abiraterone acetate and di-n-butyl phthalate in an androgen-sensitive human fetal testis xenograft bioassay. *Toxicol Sci* 138: 148-160. <https://dx.doi.org/10.1093/toxsci/kft266>
- Srivastava, SP; Srivastava, S; Saxena, DK; Chandra, SV; Seth, PK. (1990). Testicular effects of di-n-butyl phthalate (DBP): Biochemical and histopathological alterations. *Arch Toxicol* 64: 148-152. <http://dx.doi.org/10.1007/BF01974401>
- Sterne, JAC; Hernán, MA; Reeves, BC; Savović, J; Berkman, ND; Viswanathan, M; Henry, D; Altman, DG; Ansari, MT; Boutron, I; Carpenter, JR; Chan, AW; Churchill, R; Deeks, JJ; Hróbjartsson, A; Kirkham, J; Jüni, P; Loke, YK; Pigott, TD; Ramsay, CR; Regidor, D; Rothstein, HR; Sandhu, L; Santaguida, PL; Schünemann, HJ; Shea, B; Shrier, I; Tugwell, P; Turner, L; Valentine, JC; Waddington, H; Waters, E; Wells, GA; Whiting, PF; Higgins, JPT. (2016). ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 355: i4919. <https://dx.doi.org/10.1136/bmj.i4919>
- Struve, MF; Gaido, KW; Hensley, JB; Lehmann, KP; Ross, SM; Sochaski, MA; Willson, GA; Dorman, DC. (2009). Reproductive toxicity and pharmacokinetics of di-n-butyl phthalate (DBP) following dietary exposure of pregnant rats. *Birth Defects Res B Dev Reprod Toxicol* 86: 345-354. <http://dx.doi.org/10.1002/bdrb.20199>
- Sugatani, J. (2013). Function, Genetic Polymorphism, and Transcriptional Regulation of Human UDP-glucuronosyltransferase (UGT) 1A1 [Review]. *Drug Metab Pharmacokinet* 28: 83-92. <http://dx.doi.org/10.2133/dmpk.DMPK-12-RV-096>
- Sugino, M; Hatanaka, T; Todo, H; Mashimo, Y; Suzuki, T; Kobayashi, M; Hosoya, O; Jinno, H; Juni, K; Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. *Toxicol Appl Pharmacol* 328: 10-17. <http://dx.doi.org/10.1016/j.taap.2017.05.009>
- Suzuki, Y; Yoshinaga, J; Mizumoto, Y; Serizawa, S; Shiraishi, H. (2012). Foetal exposure to phthalate esters and anogenital distance in male newborns. *Int J Androl* 35: 236-244. <http://dx.doi.org/10.1111/j.1365-2605.2011.01190.x>
- Swan, SH. (2008). Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans [Review]. *Environ Res* 108: 177-184. <http://dx.doi.org/10.1016/j.envres.2008.08.007>
- Swan, SH; Sathyanarayana, S; Barrett, ES; Janssen, S; Liu, F; Nguyen, RH; Redmon, JB; Team, TS. (2015). First trimester phthalate exposure and anogenital distance in newborns. *Hum Reprod* 30: 963-972. <http://dx.doi.org/10.1093/humrep/deu363>
- Takahashi, T; Tanaka, A. (1989). Biochemical studies on phthalic esters V. Comparative studies on in vitro hydrolysis of di-n-butyl phthalate isomers in rats. *Arch Toxicol* 63: 72-74. <http://dx.doi.org/10.1007/BF00334638>
- Tanaka, A; Matsumoto, A; Yamaha, T. (1978). Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. *Toxicology* 9: 109-123. [http://dx.doi.org/10.1016/0300-483X\(78\)90036-7](http://dx.doi.org/10.1016/0300-483X(78)90036-7)
- TherImmune Research Corporation. (2002). Dibutyl phthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet (Volumes 1 and 2) with redacted pathology report. (TherImmune No. 7244-201; NTP-RACB-97003). Research Triangle Park, NC: National Toxicology Program.
- Thompson, CJ; Ross, SM; Hensley, J; Liu, K; Heinze, SC; Young, SS; Gaido, KW. (2005). Differential steroidogenic gene expression in the fetal adrenal gland versus the testis and rapid and dynamic

- response of the fetal testis to di(n-butyl) phthalate. *Biol Reprod* 73: 908-917.  
<https://dx.doi.org/10.1095/biolreprod.105.042382>
- Tian, M; Liu, L; Wang, H; Wang, X; Martin, FL; Zhang, J, ie; Huang, Q; Shen, H. (2018). Phthalates induce androgenic effects at exposure levels that can be environmentally relevant in humans. *Environ Sci Technol Lett* 5: 232-236. <http://dx.doi.org/10.1021/acs.estlett.8b00138>
- Toft, G; Jönsson, BA; Lindh, CH; Jensen, TK; Hjollund, NH; Vested, A; Bonde, JP. (2012). Association between pregnancy loss and urinary phthalate levels around the time of conception. *Environ Health Perspect* 120: 458-463. <http://dx.doi.org/10.1289/ehp.1103552>
- U.S. EPA. (1987). Integrated Risk Information System (IRIS), chemical assessment summary, dibutyl phthalate; CASRN 84-74-2. Washington, DC: U.S. Environmental Protection Agency, National Center for Environmental Assessment.  
[https://cfpub.epa.gov/ncea/iris/iris\\_documents/documents/subst/0038\\_summary.pdf](https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0038_summary.pdf)
- U.S. EPA. (1991). Guidelines for developmental toxicity risk assessment. Fed Reg 56: 63798-63826.
- U.S. EPA. (1993). Reference Dose (RfD): description and use in health risk assessments background document 1A, March 15, 1993. Washington, DC: U.S. Environmental Protection Agency, Integrated Risk Information System. <https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments>
- U.S. EPA. (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [EPA Report]. (EPA600/890066F). Research Triangle Park, NC.  
<https://cfpub.epa.gov/ncea/risk/recorddisplay.cfm?deid=71993&CFID=51174829&CFTOKEN=25006317>
- U.S. EPA. (1996). Guidelines for reproductive toxicity risk assessment [EPA Report]. Fed Reg 61: 56274-56322.
- U.S. EPA. (2002). A review of the reference dose and reference concentration processes [EPA Report]. (EPA630P02002F). Washington, DC. <https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf>
- U.S. EPA. (2011a). Exposure factors handbook: 2011 edition [EPA Report]. (EPA/600/R-090/052F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment.  
<https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockkey=P100F2OS.txt>
- U.S. EPA. (2011b). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. (EPA100R110001). Washington, DC.  
<https://www.epa.gov/sites/production/files/2013-09/documents/recommended-use-of-bw34.pdf>
- U.S. EPA. (2012a). Benchmark dose technical guidance [EPA Report]. (EPA100R12001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.  
<https://www.epa.gov/risk/benchmark-dose-technical-guidance>
- U.S. EPA. (2012b). Guidance for considering and using open literature toxicity studies to support human health risk assessment. Washington, DC: U.S. Environmental Protection Agency, Office of Pesticide Programs. <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-considering-and-using-open-literature>
- U.S. EPA. (2019). Proposed designation of Dibutyl Phthalate (CASRN 84-74-2) as a high-priority substance for risk evaluation. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. [https://www.epa.gov/sites/production/files/2019-08/documents/dibutylphthalate\\_84-74-2\\_high-priority\\_proposeddesignation\\_082319.pdf](https://www.epa.gov/sites/production/files/2019-08/documents/dibutylphthalate_84-74-2_high-priority_proposeddesignation_082319.pdf)
- U.S. EPA. (2020a). Draft Scope of the risk evaluation for Dibutyl Phthalate (1,2-Benzenedicarboxylic acid, 1,2-dibutyl ester) CASRN 84-74-2 [EPA Report]. (EPA-740-D-20-016). Washington, DC.  
[https://www.epa.gov/sites/production/files/2020-04/documents/casrn-84-74-2\\_dibutyl\\_phthalate\\_draft\\_scope\\_4-15-2020\\_2.pdf](https://www.epa.gov/sites/production/files/2020-04/documents/casrn-84-74-2_dibutyl_phthalate_draft_scope_4-15-2020_2.pdf)

- [U.S. EPA.](#) (2020b). Final scope of the risk evaluation for dibutyl phthalate (1,2-benzenedicarboxylic acid, 1,2-dibutyl ester); CASRN 84-74-2 [EPA Report]. (EPA-740-R-20-016). Washington, DC: Office of Chemical Safety and Pollution Prevention.  
[https://www.epa.gov/sites/default/files/2020-09/documents/casrn\\_84-74-2\\_dibutyl\\_phthalate\\_final\\_scope\\_0.pdf](https://www.epa.gov/sites/default/files/2020-09/documents/casrn_84-74-2_dibutyl_phthalate_final_scope_0.pdf)
- [U.S. EPA.](#) (2021). Draft systematic review protocol supporting TSCA risk evaluations for chemical substances, Version 1.0: A generic TSCA systematic review protocol with chemical-specific methodologies. (EPA Document #EPA-D-20-031). Washington, DC: Office of Chemical Safety and Pollution Prevention. <https://www.regulations.gov/document/EPA-HQ-OPPT-2021-0414-0005>
- [U.S. EPA.](#) (2022). ORD staff handbook for developing IRIS assessments. (EPA600R22268). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Center for Public Health and Environmental Assessment.  
[https://cfpub.epa.gov/ncea/iris\\_drafts/recordisplay.cfm?deid=356370](https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=356370)
- [U.S. EPA.](#) (2023a). Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act. (EPA-740-P-23-002). Washington, DC: U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. <https://www.regulations.gov/document/EPA-HQ-OPPT-2022-0918-0009>
- [U.S. EPA.](#) (2023b). Science Advisory Committee on Chemicals meeting minutes and final report, No. 2023-01 - A set of scientific issues being considered by the Environmental Protection Agency regarding: Draft Proposed Principles of Cumulative Risk Assessment (CRA) under the Toxic Substances Control Act and a Draft Proposed Approach for CRA of High-Priority Phthalates and a Manufacturer-Requested Phthalate. (EPA-HQ-OPPT-2022-0918). Washington, DC: U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention.  
<https://www.regulations.gov/document/EPA-HQ-OPPT-2022-0918-0067>
- [U.S. EPA.](#) (2024). Science advisory committee on chemicals meeting minutes and final report No. 2024-2, docket ID: EPA-HQ-OPPT-2024-0073: For the draft risk evaluation for di-isodecyl phthalate (DIDP) and draft hazard assessments for di-isononyl phthalate (DINP). Washington, DC: U.S. Environmental Protection Agency, Science Advisory Committee on Chemicals.
- [U.S. EPA.](#) (2025a). Cancer Human Health Hazard Assessment for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), and Dicyclohexyl Phthalate (DCHP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA.](#) (2025b). Consumer and Indoor Exposure Assessment for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA.](#) (2025c). Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA.](#) (2025d). Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA.](#) (2025e). Data Quality Evaluation Information for Human Health Hazard Epidemiology for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA.](#) (2025f). Environmental Release and Occupational Exposure Assessment for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA.](#) (2025g). 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). Washington, DC: Office of Pollution Prevention and Toxics.

- [U.S. EPA](#). (2025h). Non-Cancer Human Health Hazard Assessment for Butyl Benzyl Phthalate (BBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025i). Non-Cancer Human Health Hazard Assessment for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025j). Non-Cancer Human Health Hazard Assessment for Dicyclohexyl Phthalate (DCHP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025k). Non-Cancer Human Health Hazard Assessment for Diethylhexyl Phthalate (DEHP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025l). Non-Cancer Human Health Hazard Assessment for Diisobutyl Phthalate (DIBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025m). Non-Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP). (EPA-740-R-25-009). Washington, DC: Office of Pollution Prevention and Toxics.  
<https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0436-0137>
- [U.S. EPA](#). (2025n). Risk Evaluation for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025o). Science Advisory Committee on Chemicals (SACC) meeting minutes and final report - Peer Review of the Draft Risk Evaluations of Dibutyl phthalate (DBP), Di(2-ethylhexyl) phthalate (DEHP), and Dicyclohexyl phthalate (DCHP), and the Technical Support Documents for Butylbenzyl phthalate (BBP) and Diisobutyl phthalate (DIBP). Washington, DC.  
<https://www.regulations.gov/docket/EPA-HQ-OPPT-2024-0551>
- [U.S. EPA](#). (2025p). Summary of Human Health Hazard Animal Toxicology Studies for Dibutyl Phthalate (DBP) - Literature Published from 2014 to 2019. Washington, DC: Office of Pollution Prevention and Toxics.
- [U.S. EPA](#). (2025q). Systematic Review Protocol for Dibutyl Phthalate (DBP). Washington, DC: Office of Pollution Prevention and Toxics.
- [van den Driesche, S; Kolovos, P; Platts, S; Drake, AJ; Sharpe, RM](#). (2012). Inter-relationship between testicular dysgenesis and Leydig cell function in the masculinization programming window in the rat. PLoS ONE 7: e30111. <http://dx.doi.org/10.1371/journal.pone.0030111>
- [van Den Driesche, S; McKinnell, C; Calarrão, A; Kennedy, L; Hutchison, GR; Hrabalkova, L; Jobling, MS; Macpherson, S; Anderson, RA; Sharpe, RM; Mitchell, RT](#). (2015). Comparative effects of di(n-butyl) phthalate exposure on fetal germ cell development in the rat and in human fetal testis xenografts. Environ Health Perspect 123: 223-230. <https://dx.doi.org/10.1289/ehp.1408248>
- [Walseth, F; Nilsen, OG](#). (1984). Phthalate esters II. Effects of inhaled dibutylphthalate on cytochrome P-450 mediated metabolism in rat liver and lung. Arch Toxicol 55: 132-136.  
<http://dx.doi.org/10.1007/BF00346052>
- [Wang, YX; You, L; Zeng, Q; Sun, Y; Huang, YH; Wang, C; Wang, P; Cao, WC; Yang, P; Li, YF; Lu, WQ](#). (2015). Phthalate exposure and human semen quality: Results from an infertility clinic in China - Supplementary material [Supplemental Data]. Environ Res 142.
- [Wang, YX; Zeng, Q; Sun, Y; Yang, P; Wang, P; Li, J; Huang, Z; You, L; Huang, YH; Wang, C; Li, YF; Lu, WQ](#). (2016). Semen phthalate metabolites, semen quality parameters and serum reproductive hormones: A cross-sectional study in China. Environ Pollut 211: 173-182.  
<http://dx.doi.org/10.1016/j.envpol.2015.12.052>
- [Watkins, DJ; Milewski, S; Domino, SE; Meeker, JD; Padmanabhan, V](#). (2016). Maternal phthalate exposure during early pregnancy and at delivery in relation to gestational age and size at birth: A preliminary analysis. Reprod Toxicol 65: 59-66.  
<http://dx.doi.org/10.1016/j.reprotox.2016.06.021>
- [Watkins, DJ; Sánchez, BN; Téllez-Rojo, MM; Lee, JM; Mercado-García, A; Blank-Goldenberg, C; Peterson, KE; Meeker, JD](#). (2017). Phthalate and bisphenol A exposure during in utero windows



- of susceptibility in relation to reproductive hormones and pubertal development in girls. *Environ Res* 159: 143-151. <http://dx.doi.org/10.1016/j.envres.2017.07.051>
- Welsh, M; Saunders, PTK; Fisk, M; Scott, HM; Hutchison, GR; Smith, LB; Sharpe, RM. (2008). Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest* 118: 1479-1490. <https://dx.doi.org/10.1172/jci34241>
- Weng, X; Tan, Y; Fei, Q; Yao, H; Fu, Y; Wu, X; Zeng, H; Yang, Z; Zeng, Z; Liang, H; Wu, Y; Wen, L; Jing, C. (2022). Association between mixed exposure of phthalates and cognitive function among the U.S. elderly from NHANES 2011-2014: Three statistical models. *Sci Total Environ* 828: 154362. <http://dx.doi.org/10.1016/j.scitotenv.2022.154362>
- White, RD; Carter, DE; Earnest, D; Mueller, J. (1980). Absorption and metabolism of three phthalate diesters by the rat small intestine. *Food Chem Toxicol* 18: 383-386. [http://dx.doi.org/10.1016/0015-6264\(80\)90194-7](http://dx.doi.org/10.1016/0015-6264(80)90194-7)
- Wilson, VS; Lambright, C; Furr, J; Ostby, J; Wood, C; Held, G; Gray, LE, Jr. (2004). Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. *Toxicol Lett* 146: 207-215. <https://dx.doi.org/10.1016/j.toxlet.2003.09.012>
- Wine, RN; Li, LH; Barnes, LH; Gulati, DK; Chapin, RE. (1997). Reproductive toxicity of di-n-butylphthalate in a continuous breeding protocol in Sprague-Dawley rats. *Environ Health Perspect* 105: 102-107. <http://dx.doi.org/10.1289/ehp.97105102>
- Wolff, MS; Teitelbaum, SL; McGovern, K; Windham, GC; Pinney, SM; Galvez, M; Calafat, AM; Kushi, LH; Biro, FM. (2014). Phthalate exposure and pubertal development in a longitudinal study of US girls. *Hum Reprod* 29: 1558-1566. <http://dx.doi.org/10.1093/humrep/deu081>
- Wu, H; Ashcraft, L; Whitcomb, BW; Rahil, T; Tougias, E; Sites, CK; Pilsner, JR. (2017). Parental contributions to early embryo development: Influences of urinary phthalate and phthalate alternatives among couples undergoing IVF treatment. *Hum Reprod* 32: 65-75. <http://dx.doi.org/10.1093/humrep/dew301>
- Xiao-Feng, Z; Nai-Qiang, Q; Jing, Z; Zi, L; Yang, Z. (2009). Di (n-butyl) phthalate inhibits testosterone synthesis through a glucocorticoid-mediated pathway in rats. *Int J Toxicol* 28: 448-456. <http://dx.doi.org/10.1177/1091581809342596>
- Xie, X; Deng, T; Duan, J; Ding, S; Yuan, J; Chen, M. (2019). Comparing the effects of diethylhexyl phthalate and dibutyl phthalate exposure on hypertension in mice. *Ecotoxicol Environ Saf* 174: 75-82. <http://dx.doi.org/10.1016/j.ecoenv.2019.02.067>
- Xie, Z; Wang, J; Dai, F; Jin, X; Wu, K; Chen, Q; Wang, Y. (2016). Effects of maternal exposure to di-n-butyl phthalate during pregnancy and breastfeeding on ovarian development and function of F1 female rats. *Environ Toxicol Pharmacol* 43: 38-43. <http://dx.doi.org/10.1016/j.etap.2016.01.022>
- Yan, B; Guo, J; Liu, X; Li, J; Yang, X; Ma, P; Wu, Y. (2016). Oxidative stress mediates dibutyl phthalate-induced anxiety-like behavior in Kunming mice. *Environ Toxicol Pharmacol* 45: 45-51. <http://dx.doi.org/10.1016/j.etap.2016.05.013>
- Yi, H; Gu, H; Zhou, T; Chen, Y; Wang, G; Jin, Y; Yuan, W; Zhao, H; Zhang, L. (2016). A pilot study on association between phthalate exposure and missed miscarriage. *Eur Rev Med Pharmacol Sci* 20: 1894-1902.
- You, L; Wang, Y; Zeng, Q; Li, M, in; Huang, Y; Hu, Y, u; Cao, W; Liu, A; Lu, W. (2015). Semen phthalate metabolites, spermatozoa apoptosis, and dna damage: a cross-sectional study in China. *Environ Sci Technol* 49: 3805-3812. <http://dx.doi.org/10.1021/acs.est.5b00588>
- Zhang, C; Gong, P; Ye, Y; Zhang, L; Chen, M; Hu, Y; Gu, A; Chen, S; Wang, Y. (2018a). NF- $\kappa$ B-vimentin is involved in steroidogenesis stimulated by di-n-butyl phthalate in prepubertal female rats. *Toxicology Research* 7: 826-833. <http://dx.doi.org/10.1039/c8tx00035b>

- Zhang, Y; Jiang, X; Chen, B. (2004). Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to di-n-butyl phthalate in utero and during lactation and determination of its NOAEL. *Reprod Toxicol* 18: 669-676. <http://dx.doi.org/10.1016/j.reprotox.2004.04.009>
- Zhang, YW; Gao, H; Mao, LJ; Tao, XY; Ge, X; Huang, K; Zhu, P; Hao, JH; Wang, QN; Xu, YY; Jin, ZX; Sheng, J; Xu, YQ; Yan, SQ; Tao, XG; Tao, FB. (2018b). Effects of the phthalate exposure during three gestation periods on birth weight and their gender differences: A birth cohort study in China. *Sci Total Environ* 613-614: 1573-1578. <http://dx.doi.org/10.1016/j.scitotenv.2017.08.319>
- Zuo, HX; Li, JQ; Han, B; Ke, CJ; Liu, XD; Zhang, YC; Li, L; Yang, X. (2014). Di-(n-butyl)-phthalate-induced Oxidative Stress and Depression-like Behavior in Mice with or without Ovalbumin Immunization. *Biomed Environ Sci* 27: 268-280. <http://dx.doi.org/10.3967/bes2014.001>



## APPENDICES

### Appendix A Existing Assessments from Other Regulatory Agencies of DBP

The available existing assessments of DBP 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 DBP**

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
U.S. EPA (IRIS Program)	<p><i>Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence</i> (<a href="#">Radke et al., 2018</a>)</p> <p><i>Phthalate exposure and female reproductive and developmental outcomes: A systematic review of the human epidemiological evidence</i> (<a href="#">Radke et al., 2019b</a>)</p> <p><i>Phthalate exposure and metabolic effects: A systematic review of the human epidemiological evidence</i> (<a href="#">Radke et al., 2019a</a>)</p> <p><i>Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence</i> (<a href="#">Radke et al., 2020a</a>).</p>	No	No	Yes	<p>- Publications were subjected to peer review prior to being published in a special issue of <i>Environment International</i></p> <p>- Publications employed a systematic review process that included literature search and screening, study evaluation, data extraction, and evidence synthesis. The full systematic review protocol is available as a supplemental file associated with each publication.</p>
ATSDR	<i>Toxicological profile for di-b-phthalate</i> ( <a href="#">ATSDR, 2001</a> )	Yes	Yes	No	- Draft reviewed by peer review panel of four experts (see p. xi of ( <a href="#">ATSDR, 2001</a> ) for more details).
U.S. CPSC	<i>Toxicity review of di-n-butyl phthalate (DBP)</i> ( <a href="#">CPSC, 2010</a> )	Yes	Yes	No	- Peer-reviewed by panel of four experts. Peer-review report available at:

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	<i>Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives</i> ( <a href="#">CPSC, 2014</a> )				<a href="https://www.cpsc.gov/s3fs-public/Peer-Review-Report-Comments.pdf">https://www.cpsc.gov/s3fs-public/Peer-Review-Report-Comments.pdf</a> -Public comments available at: <a href="https://www.cpsc.gov/chap">https://www.cpsc.gov/chap</a> - No formal systematic review protocol employed. - Details regarding CPSC's strategy for identifying new information and literature are provided on page 12 of ( <a href="#">CPSC, 2014</a> )
NASEM	<i>Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals</i> ( <a href="#">NASEM, 2017</a> )	Yes	No	Yes	- Draft report was reviewed by individuals chosen for their diverse perspectives and technical expertise in accordance with the National Academies peer review process. See Acknowledgements section of ( <a href="#">NASEM, 2017</a> ) for more details. - Employed NTP's Office of Health Assessment and Translation (OHAT) systematic review method
Health Canada	<i>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</i> ( <a href="#">EC/HC, 2015</a> )  <i>Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters</i> ( <a href="#">Health Canada, 2018b</a> )	Yes	Yes	No (Animal studies) Yes (Epidemiologic studies)	- Ecological and human health portions of the screening assessment report ( <a href="#">Health Canada, 2020</a> ) were subject to external review and/or consultation. See page 2 of ( <a href="#">Health Canada, 2020</a> ) for additional details. - State of the science report ( <a href="#">EC/HC, 2015</a> ) and draft screening assessment report for the phthalate substance group subjected to 60-day public comment periods. Summaries of received public comments available at: <a href="https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative/phthalate.html#a1">https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative/phthalate.html#a1</a>

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	<p><i>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</i> (<a href="#">Health Canada, 2018a</a>)</p> <p><i>Screening Assessment - Phthalate Substance Grouping</i> (<a href="#">Health Canada, 2020</a>)</p>				<p>- No formal systematic review protocol employed to identify or evaluate experimental animal toxicology studies.</p> <p>- Details regarding Health Canada's strategy for identifying new information and literature is provided in Section 1 of (<a href="#">EC/HC, 2015</a>) and (<a href="#">Health Canada, 2020</a>)</p> <p>- Human epidemiologic studies evaluated using Downs and Black Method (<a href="#">Health Canada, 2018a, b</a>)</p>
NICNAS	<p><i>Priority existing chemical assessment report no. 36: Dibutyl phthalate</i> (<a href="#">NICNAS, 2013</a>)</p>	No	Yes	No	<p>- NICNAS (<a href="#">2013</a>) states "The report has been subjected to internal peer review by NICNAS during all stages of preparation." However, a formal external peer review was not conducted.</p> <p>- NICNAS (<a href="#">2013</a>) states "Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made." See Preface of (<a href="#">NICNAS, 2013</a>) for more details.</p> <p>- No formal systematic review protocol employed.</p> <p>- Details regarding NICNAS's strategy for identifying new information and literature is provided in Section 1.3 of (<a href="#">NICNAS, 2013</a>)</p>
ECHA	<p><i>Opinion on an Annex XV dossier proposing restrictions on four phthalates</i></p>	Yes	Yes	No	<p>- Peer-reviewed by ECHA's Committee for Risk Assessment (RAC)</p>

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	<p>(DEHP, BBP, DBP, DIBP) (<a href="#">ECHA, 2017b</a>)</p> <p><i>Annex to the Background document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP) (<a href="#">ECHA, 2017a</a>)</i></p>				<ul style="list-style-type: none"> <li>- Subject to public consultation</li> <li>- No formal systematic review protocol employed.</li> </ul>
EFSA	<p><i>Update of the Risk Assessment of Di-butylphthalate (DBP), Butyl-benzyl-phthalate (BBP), Bis(2-ethylhexyl)phthalate (DEHP), Di-isononylphthalate (DINP) and Di-isodecylphthalate (DIDP) for Use in Food Contact Materials (<a href="#">EFSA, 2019</a>)</i></p>	No	Yes	No	<ul style="list-style-type: none"> <li>- Draft report subject to public consultation. Public comments and EFSA's response to comments are available at: <a href="https://doi.org/10.2903/sp.efsa.2019.EN-1747">https://doi.org/10.2903/sp.efsa.2019.EN-1747</a></li> <li>- No formal systematic review protocol employed.</li> <li>- Details regarding EFSA's strategy for identifying new information and literature are provided on page 18 and Appendix B of (<a href="#">EFSA, 2019</a>)</li> </ul>
NTP-CERHR	<p><i>NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Butyl Phthalate (DBP) (<a href="#">NTP, 2003</a>)</i></p>	No	Yes	No	<ul style="list-style-type: none"> <li>- Report prepared by NTP-CERHHR Phthalates Expert Panel and was reviewed by CERHR Core Committee (made up of representatives of NTP-participating agencies, CERHR staff scientists, member of phthalates expert panel)</li> <li>- Public comments summarized in Appendix III of (<a href="#">NTP-CERHR, 2003</a>)</li> <li>- No formal systematic review protocol employed.</li> </ul>

## Appendix B Literature Considered for Non-Cancer Hazards

---

### B.1 Reproductive and Developmental Effects

---

EPA evaluated seven studies that provide data on reproductive and developmental outcomes in rodents following oral exposure to DBP. The data set included 3 intermediate duration studies ([Zhang et al., 2018a](#); [Ahmad et al., 2015](#); [Sen et al., 2015](#)), 1 subchronic study ([Xie et al., 2019](#)), and 3 one-generation studies ([Xie et al., 2016](#); [de Jesus et al., 2015](#); [Ahmad et al., 2014](#)). These studies provided data on the effect of DBP exposure on reproductive hormone levels, the estrus cycle, reproductive organ weights, histopathological alterations of the uterus or ovary, and fertility, including evaluations of sperm. Developmental endpoints included measures of pup body weight. The effects that were most sensitive to DBP exposure (*i.e.*, the lowest LOELs) included decreases in the levels of 17- $\beta$ -estradiol (E2) at doses ranging from 0.01 to 1 mg/kg-day ([Xie et al., 2019](#); [Zhang et al., 2018a](#); [Sen et al., 2015](#)) and decreased pup body weight ([Ahmad et al., 2014](#)). However, each individual study had limitations that contributed uncertainty that impacted interpretation of the results and therefore none were considered further for dose response in Section 4. Detailed information on study designs is provided in Table\_Apx B-1.

In two rodent studies, ([Zhang et al., 2018a](#); [Sen et al., 2015](#)) decreased E2 was observed at doses ranging from 0.01 to 1 mg/kg-day, but these decreases did not consistently correspond with other reproductive health effects (*e.g.*, changes in histopathology or changes in estrus cyclicity). Zhang et al. ([2018a](#)) exposed adolescent (PND21) Sprague-Dawley rats to 0, 1, 10, or 500 mg/kg-day DBP via gavage from PND21 to 33 and observed increases in progesterone and relative uterus weight (non-dose-related) at 1 mg/kg-day. However, there were no measures of estrous cyclicity or attainment of puberty in this study. Vaginal opening, a marker of puberty, was not assessed. Female rats (SD) start cycling on average at PND 32, which is the first day of estrus. Without knowing if the females had begun to cycle, the data on uterine weight and hormones are difficult to interpret. Uterine weights are increased due to the effects of estradiol from growing follicles on the uterine epithelium. Uterine weight is highest on the day of proestrus when estrogen levels reach their peak in the estrus cycle ([Goldman et al., 2007](#)). The uncertainty of these measures without cycle day data limits any interpretation of any of the results as the variation in hormone levels and associated tissue changes are not aligned with puberty or cycle day. Similar results were reported in Sen et al. ([2015](#)), where adolescent (PND35) female CD-1 mice were orally exposed to 0.01, 0.1, or 1,000 mg/kg/day DBP for 10 days. Decreased E2 was observed in the 0.01 mg/kg-day dose group, but no other effects were reported at that dose. At the next highest dose group, 0.1 mg/kg-day, E2 was decreased with corresponding increases in serum FSH and LH, as well as decreased number of antral ovarian follicles. It is plausible that DBP acts on the ovary to elicit these effects, as E2 is produced by developing follicles. However, decreased E2 was also observed in the high-dose group (1,000 mg/kg-day DBP) but the average number of antral follicles was increased, albeit not at a statistically significant level. Moreover, similar to Zhang et al. ([2018a](#)), there is some uncertainty in the data set from Sen et al. ([2015](#)), including the hormone analysis, uterine weights and ovarian measures. Sen et al. ([2015](#)) did not measure the endpoints on the same day of diestrus (Di1 or Di2), which is problematic because E2 increases from Di1 to Di2, as the follicles grow and secrete more E2. In addition, the study was conducted immediately following the onset of puberty when cyclicity inconsistent and no evaluation of normal cyclicity can be determined or compared between dose groups. In addition to the aforementioned limitations, there were several factors that further increased uncertainty in the data set of studies of reproductive effects following DBP exposure including low sample size and the lack of an appropriate dose range (very low or very high, 0.01, 0.1 or 1000 mg/kg).

A study designed to evaluate cardiovascular outcomes (More information provided in Appendix B.4) also provided data on serum E2 levels following a 6-week gavage exposure to 0.1, 1, or 10 mg/kg-day

DBP in adult male mice. A non-monotonic, “U” shaped dose-response was observed in E2, with decreased E2 at the lowest dose tested (0.1 mg/kg-day), but increased E2 at higher dose levels (1 and 10 mg/kg-day) ([Xie et al., 2019](#)).

A one-generation study by Xie et al. ([2016](#)) also provided data on reproductive hormone levels following developmental exposures to DBP. Increased serum E2 was observed during specific phases of the estrus cycle in adult F1 offspring following in utero and lactational exposure (GD12 to PND21) to doses as low as 10 mg/kg-day. Specifically, increased serum E2 was observed during proestrus, diestrus, and metestrus in F1 females on PND63. These effects coincided with decreases in serum progesterone during proestrus, estrus, diestrus, and metestrus in F1 females PND63. There was no dose-response, and exposure to the mid and high doses (100 and 600 mg/kg-day) did not lead to significant increases in these hormones across multiple phases of the estrus cycle as was observed in the low-dose group. Furthermore, the changes in hormone levels at 10 mg/kg-day did not coincide with any functional changes such as those in estrus cyclicity, onset of vaginal opening, uterus weights or ovarian weights with doses tested up to 600 mg/kg-day. More data are needed to understand the impact of gestational and/or lactational DBP exposure on ovarian development and function in adult F1 offspring following maternal exposure.

Two additional studies provide data on reproductive and developmental effects in offspring following maternal exposure to DBP ([de Jesus et al., 2015](#); [Ahmad et al., 2014](#)). A one-generation reproductive study by de Jesus et al. ([2015](#)) reported reproductive effects in Mongolian gerbils at 5 mg/kg-day based on histopathological effects in the prostate of F1 offspring (*i.e.*, decreased epithelium height and decreased SMC thickness) and increased weight of the prostatic complex (seminal vesicle, coagulating gland, & dorsolateral, ventral & dorsal lobes) ([de Jesus et al., 2015](#)). In that study, pregnant gerbils were exposed to DBP from GD 0 to PND 28, then F1 (12 litters, 6 to 8 pups/litter) continued the same exposure through study termination at PNW14. This study contained several issues that limit the interpretation of results, including those related to chemical characterization (*e.g.*, drinking water exposure for a non-water-soluble phthalate), insufficient information on measures to reduce bias from the litter effect, dose-range issues, and only one dose other than the control.

In a gestational exposure study by Ahmad et al. ([2014](#)), pregnant albino rats were gavaged with 0, 2, 10, or 50 mg/kg-day DBP from GD14 to parturition, and endpoints were evaluated in F1 from PND1 to PND75. Decreased pup body weight was observed at doses as low as 2 mg/kg-day in PND21 males exposed to DBP from GD14 to parturition. The reduction in body weight was dose-dependent (other doses included 10 and 50 mg/kg-day). However, by adulthood (PND75), the effect was no longer dose-responsive and was significant at the high dose only. Of note, reduced pup body weight in this study was observed at a dose much lower dose than those which have been observed in the majority of other studies cited in existing assessments (Section 3.1.2.2). Indeed, the aforementioned study by Lee et al. ([2004](#)) (see Section 3.1.2.2) did not observe any change in pup body weight on PND21 following exposure to doses as low as 2 mg/kg (equivalent to 1.5 to 3 mg/kg-day) for a longer duration than Ahmad et al. ([2014](#)). Lee et al. included most of the critical window (*i.e.*, the critical window is GD 14 to 19 and the exposure range in Lee et al., was GD15 to PND21). Changes in pup body weight are not considered exposure-related.

A study by the same authors ([Ahmad et al., 2015](#)) evaluated the estrogenic effects of DBP in a 3-day uterotrophic assay and a 20-day pubertal assay, though several methodological limitations impact the ability to interpret results and draw conclusions from these studies. In the uterotrophic assay, PND20 female rats were exposed to 0, 10, or 100 mg/kg-day DBP once per day for 3 consecutive days via gavage. Decreased uterine wet weight was observed one day after exposure ended in the 100 mg/kg-day



group, but the effect is difficult to interpret as there was also an increase in body weight (over 10 percent) in this dose-group. In the pubertal assay, PND21 female rats were exposed to 0, 10, or 100 mg/kg-day DBP for 20 days via gavage, and animals were examined daily for body weights, vaginal opening (VO). The pubertal data are not conclusive; neither control nor DBP-exposed animals attained puberty (*i.e.*, first day of VO and the first day of estrus), although rats typically attain VO by PND32. The authors also reported significant decreases in uterine and ovarian wet weights. However, the females exposed to DBP had not yet begun to cycle, making it difficult to interpret the significance of the observed decreases in uterine and ovarian weights in DBP-exposed animals, as well as the lack of reporting of relative weights since there was a decrease in body weight. Altogether, these data do not suggest that DBP is an estrogen agonist, as it would have increased the uterine weight in the three-day uterotrophic assay.

Studies have provided data on developmental and reproductive health outcomes other than the male reproductive system following exposure to DBP. However, the data set still contains several limitations that increase uncertainty. Moreover, while decreased E2 ([Xie et al., 2019](#); [Zhang et al., 2018a](#); [Sen et al., 2015](#)) or decreased pup body weight ([Ahmad et al., 2014](#)) were observed at doses lower than some of the most sensitive PODs (*i.e.*, 2 mg/kg (equivalent to 1.5 to 3 mg/kg-day) in Lee et al. (2004)) identified in existing assessments (*e.g.*, ([EFSA, 2019](#); [ECHA, 2017a](#); [OEHHA, 2007](#))), the data set is not sufficiently robust given the amount of uncertainty resulting from the limitations in each individual study. Additionally, the increased E2 and progesterone observed in adult offspring following in utero and lactational exposure to 5 mg/kg-day do not coincide with other functional reproductive endpoints which makes it difficult to interpret the biological relevance of the changes in hormone levels ([Xie et al., 2016](#)). Concerns with other studies included evidence of a transient effect on body weight ([Ahmad et al., 2014](#)) and study design limitations ([de Jesus et al., 2015](#)). Therefore, EPA did not further consider these six studies on reproductive effects for POD selection (Section 4).

**Table\_Apx B-1. Summary of Animal Toxicology Studies Evaluating Effects on the Developmental and Reproductive System Following Exposure to DBP**

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Sen et al., 2015</a> )	Adolescent (PND35) female CD-1 (8/dose) were mice exposed to DBP at concentrations of 0.01, 0.1, or 1,000 mg/kg/day for 10 days via administered orally by placing a pipette tip containing the dosing solution into the mouth past the incisors and into the cheek pouch.	ND/0.01 (LOEL)	↓ serum E2	<u>Effects at 0.1 mg/kg-day</u> -↑ FSH; ↑ LH; ↓ E2 -↓ No. of antral ovarian follicles -↓ relative liver weight <u>Effects at 1,000 mg/kg-day</u> -↑ FSH; ↓ E2 -Changes in estrus cycle (↓ time in proestrus/estrus and ↑ time in metestrus/diestrus) -↓ No. of corpora lutea <u>Limitations</u> - Poorly designed study without adequate estrous cycle assessments - Large dose spacing; many effects non-monotonic or displayed flat D-R; small sample size (n =8)
( <a href="#">Xie et al., 2019</a> )	Male C57BL/6 mice (9/group) were exposed via gavage to 0.1, 1, or 10 mg/kg-day DBP for 6 weeks.	ND/0.1 (LOEL)	↓ serum E2;	<u>Effects at 1 mg/kg-day</u> -↑ serum E2 <u>Effects at 10 mg/kg-day</u> -↑ serum E2 <u>Limitations</u> -Study only included males
( <a href="#">Zhang et al., 2018a</a> )	Adolescent (PND21) Sprague-Dawley rats (10/group) were exposed to 0, 1, 10, or 500 mg/kg-day DBP via gavage from PND21–33.	ND/1	↑ serum progesterone ↓ serum E2; changes in ovarian histopathology; ↑ relative weight of uterus	<u>Effects at 10 mg/kg-day</u> - ↓ serum E2; ↑ progesterone <u>Effects at 500 mg/kg-day</u> -↓ serum E2; ↓ serum progesterone <u>Limitations</u> -Only evaluated females; Large dose spacing; Qualitative histopathology
( <a href="#">Ahmad et al., 2014</a> )	Pregnant albino rats (6–9/group) were gavaged with 0, 2, 10, or 50 mg/kg-day DBP from GD14 –	ND/2 (LOEL)	↓ pup body weight on PND21 (males)	<u>Maternal Effects</u> - ↓ maternal BW gain <u>Developmental Effects</u>

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	parturition Endpoints evaluated in F1 from PND1-PND75.			<ul style="list-style-type: none"> <li>- ↓ pup BW on PND1 (10 &amp; 50 mg/kg-day) &amp; PND21 (2, 10 and 50 mg/kg-day)</li> <li>- ↓ BW in F1 adults on PND75 (50 mg/kg-day)</li> </ul> <u>Reproductive Effects in adult F1</u> <ul style="list-style-type: none"> <li>- ↓ absolute weight of epididymis, testis, prostate, &amp; seminal vesicle in F1 adults on PND75 (50 mg/kg-day)</li> <li>- ↓ sperm count, ↓ percent motile sperm, ↑percent abnormal sperm (50 mg/kg-day)</li> </ul> <u>Other effects:</u> <ul style="list-style-type: none"> <li>- ↓ absolute weight of adrenal gland, liver &amp; kidney in F1 adults on PND75 (50 mg/kg-day)</li> </ul> <u>Unaffected Outcomes</u> <ul style="list-style-type: none"> <li>- Serum testosterone in F1 adults (PND75); Litter size, live/dead pups, sex ratio (PND1); Anogenital distance (PND5 &amp; PND25); Viability index (PND4); Weaning index (PND21)</li> </ul> <u>Limitations:</u> <ul style="list-style-type: none"> <li>- No statistical method to account for litter effects (<i>i.e.</i>, statistics on offspring presented as means of individual animals rather than litter means)</li> </ul>
<a href="#">(de Jesus et al., 2015)</a>	Pregnant Mongolian gerbils (12 dams /group) exposed via drinking water to 5 mg/kg-day DBP at concentrations of GD 0 to PND 28, then F1 (12 litters, 6–8 pups/litter) continued exposure through PNW14.	ND/5	Prostate histopathology in F1; ↑ wet weight of the prostatic complex (seminal vesicle, coagulating gland, & dorsolateral, ventral & dorsal lobes)	<u>Limitations</u> <ul style="list-style-type: none"> <li>- DBP administered in drinking water (solubility concerns)</li> <li>- Insufficient information on measures to reduce bias from the litter effect</li> <li>- Unconventional experimental animal used (gerbils)</li> <li>- Study only used one dose other than control</li> </ul>
<a href="#">(Xie et al., 2016)</a>	Pregnant Sprague-Dawley rats (8/group) were exposed to 0, 10, 1000, or 600 mg/kg-day DBP via gavage from GD12 – PND21, and F1 female evaluated on PND63.	ND/10	↑ serum E2 during proestrus, diestrus, & metestrus in F1 females (PND63); ↓ serum progesterone during proestrus, estrus,	<u>Effects at 100 mg/kg-day</u> <ul style="list-style-type: none"> <li>- ↓ serum progesterone during proestrus in F1 females</li> </ul> <u>Effects at 600 mg/kg-day</u> <ul style="list-style-type: none"> <li>- ↓ serum progesterone during proestrus in F1 females</li> </ul> <u>Limitations:</u> <ul style="list-style-type: none"> <li>- Large dose spacing</li> </ul>

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			diestrus, and metestrus in F1 females (PND63)	- Changes in hormone levels did not coincide with changes in other reproductive outcomes
<i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEL = no observed adverse effect level; LOAEL = Lowest-observed-adverse-effect level; LOEL = Lowest observed effect level; GD = Gestation Day; PND = Postnatal Day; PNW = Postnatal Week; F1 = First generation offspring; E2 = β-estradiol; FSH = follicle stimulating hormone; LH = Luteinizing Hormone; BW = body weight; ND = No data				

## B.2 Neurotoxicity

---

Three studies in male mice identified alterations in neurological health outcomes following DBP exposure ([Farzanehfar et al., 2016](#); [Yan et al., 2016](#); [Zuo et al., 2014](#)). These studies provided data on neurobehavioral effects, sometimes paired with brain histopathology. Behavioral alterations were observed at doses as low as 0.45 mg/kg-day ([Zuo et al., 2014](#)), albeit in a study with several limitations. Ultimately, no studies were considered further for dose response in Section 4. Detailed information on the study designs are provided in Table\_Apx B-2.

Zuo et al. ([2014](#)) observed reduced performance in the tail suspension test (TST; increased time spent immobile) in male BALB/c mice that had been exposed to 0.45 mg/kg-day DBP via gavage for 32 days. The TST is considered a proxy for depression-like behavior in mice. The test is conducted by suspending a mouse by its tail and recording the duration of time spent immobile or hanging passively. A normal mouse will be more mobile and try to free itself, but a mouse that exhibits depression-like behavior will not. The authors observed increased time spent immobile in the TST at the next highest dose level or 45 mg/kg-day as well. Effects at the high dose, but not the low dose, coincided with other neurobehavioral effects, such as reduced performance in the forced swim test (FST), specifically increased time spent immobile. Performance in the FST is another proxy for depression-like behavior in rodents. In the FST, normal mice will struggle to free themselves from the water to escape, but a mouse with depression-like behavior spends more time floating passively in the water without struggling. An important consideration of both the TST and FST is that they both involve a motor component, and correct interpretation relies on paring these tests with specific tests that evaluate motor function in the animals. Other limitations of this study include: subjective outcome measures for behavioral examinations; failure to state measures to reduce observer bias (*i.e.*, blinding); insufficient detail on the order in which neurobehavior tests were conducted; and restricting the experiment to male animals without justification. EPA is not considering neurological endpoints in Zuo et al. ([2014](#)) further for POD selection based on reporting deficiencies that compromise the ability to interpret results of the study.

Similar limitations were noted in Yan et al. ([2016](#)). Yan et al. reported reduced performance in the elevated plus maze (EPM; decreased time spent in the open arms) at in male Kunming mice exposed to 5 mg/kg-day DBP via gavage for 28 days. The EPM can be considered a proxy for anxiety-like behavior in rodents and is a type of maze that has sections that are open (with no top, just walls) and closed/dark (walls and a closed top). Mice that exhibit an anxiety-like behavior will spend more time in the closed arms than the open arms. Other dose tested included 25 and 125 mg/kg-day and a dose-responsive decrease in time spent in the open arms was observed. The majority of adverse neurobehavioral and functional effects were observed at 25 and 125 mg/kg-day, which are summarized in Table\_Apx B-2. Similar to the limitations in Zuo et al. ([2014](#)), Yan et al. ([2016](#)) only used male animals without providing an explanation, did not present information on animal body weight, provided qualitative histopathology data for the high dose only, and did not report measures used to account of observer bias in their tests.

Neurobehavioral effects were also observed in a study of male NMRI mice exposed to 0, 6.25, 12.5, 25, 50, 100, or 200 mg/kg-day DBP via gavage for 14 days ([Farzanehfar et al., 2016](#)). Reduced exploratory behavior in the open field test (OFT) was observed in mice exposed to 12.5 mg/kg-day DBP, reflected in decreased total distance traveled and decreased percent time spent central to the peripheral zone. These effects were also observed at the next highest dose level, in addition to decreased performance in the EPM, where the mice exposed to 25 mg/kg-day DBP or higher spent more time in the closed arms. A linear dose-dependent decrease in avoidance latency time in the passive avoidance test was observed,

beginning at 25 mg/kg-day. Avoidance latency is one outcome measured in the passive avoidance test, which is considered a proxy for long term memory in rodents. The test involves training mice to learn that one of two compartments will deliver an electric shock, which a mouse will normally learn to avoid. However, a mouse with a memory impairment may not avoid the room where they previously received the electric shock or may venture into that room after some time has elapsed. Neurobehavioral deficits observed at 25 mg/kg-day corresponded with histopathological changes in the granular cells of the dentate gyrus (decreased nuclei area and condensation). While the authors observed neurobehavioral effects at the 12.5 mg/kg-day dose (*i.e.*, decreased total distance movement), they do not present histopathological data for animals at this dose or the low dose of 6.25 mg/kg-day. No changes in rotarod performance or forelimb grip strength were observed at any dose level, suggesting that the reductions in performance in the passive avoidance test and OFT were not likely to be explained by deficits in motor function. Moreover, the increase in avoidance latency, paired with the locomotor data (*i.e.*, decreased exploratory behavior in OFT), yet exposed animals reenter dark compartment of the EPM), suggest that the memory of the negative stimulus delivered in the passive avoidance test is the result of impaired learning and memory. This study was more well designed than those of Zuo et al. ([2014](#)), Yan et al. ([2016](#)) and the methods were sufficiently detailed for neurobehavioral examinations, but several other limitations of this study exist, including only using males without an explanation, and the lack of histopathology data that correspond with the LOAEL. These data provide a LOAEL of 12.5 mg/kg-day based neurobehavioral effects following a 14-day exposure in adults. However, the LOAEL's identified for reproductive and developmental effects are more well supported by a robust database and are sometimes more sensitive. Although there is some evidence of neurotoxicity following exposure to DBP in experimental animals, EPA is not further considering these effects for dose-response assessment or for use in extrapolating human risk in Section 4. The database of experimental animal studies is not as robust as that of developmental and reproductive health outcomes, which remains the most sensitive and robust outcome from which to derive a POD.



**Table\_Apx B-2. Summary of Animal Toxicology Studies Evaluating Effects on the Nervous System Following Exposure to DBP**

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Zuo et al., 2014</a> )	Male BALB/c mice (8/group) were exposed to 0, 0.45, or 45 mg/kg-day DBP via gavage for 32 days. Neurobehavioral examinations conducted days 36, 37, and 39 prior to study termination on day 40.	ND/0.45	↑ immobile time in TST on day 37	<p><u>Effects at 45 mg/kg-day</u></p> <ul style="list-style-type: none"> <li>- ↑ immobile time in FST &amp; TST</li> </ul> <p><u>Unaffected Outcomes:</u></p> <ul style="list-style-type: none"> <li>- OFT endpoints (defecation numbers; distance in outer ring) on day 36; relative brain weight on day 40</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Only male animals were evaluated; Not guideline; Quantitative data for FST, authors do not state use of measures to reduce observer bias (<i>i.e.</i>, blinding); Insufficient detail on training period for behavior tests;</li> </ul>
( <a href="#">Yan et al., 2016</a> )	Male Kunming mice (9/group) exposed to 0, 5, 25, or 125 mg/kg-day DBP via gavage for 28 days.	ND/5	Neurobehavioral changes: ↓ percent time spent in open arms (EPM)	<p><u>Effects at 25 mg/kg-day</u></p> <ul style="list-style-type: none"> <li>- ↓ number of open arm entries (EPM); ↓ percent time spent in open arms (EPM); ↓ total distance traveled (OFT); ↑ percent distance in outer ring (OFT); ↑ defecations (OFT)</li> </ul> <p><u>Effects at 125 mg/kg-day</u></p> <ul style="list-style-type: none"> <li>- ↓ number of open arm entries (EPM); ↓ percent time spent in open arms (EPM); ↓ total distance traveled (OFT); ↑ percent distance in outer ring (OFT); ↑ defecations (OFT)</li> <li>- ↑ histopathological observations (damaged cells, hippocampal CA1 region)</li> <li>- ↓ relative brain weight (brain coefficient)</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Only male animals were evaluated; No data provided on animal body weight; Qualitative histopathology results provided only for high dose group; Authors do not state use of measures to reduce observer bias (<i>i.e.</i>, blinding)</li> </ul>
( <a href="#">Farzanehfar et al., 2016</a> )	Male NMRI mice (10/group) were exposed to 0, 6.26, 12.5, 25, 50, 100, or 200 mg/kg-day DBP for 14 days via gavage.	6.25/12.5	Neurobehavioral changes: ↓ total distance (OFT); ↓ percent time spent central to peripheral zone (OFT)	<p><u>Effects at 25 mg/kg-day</u></p> <ul style="list-style-type: none"> <li>- ↓ total distance (OFT); ↓ percent time spent central to peripheral zone (OFT); ↓ percent time spent in open arm (EPM); ↓ avoidance latency time</li> <li>- Histopathological findings in granular cells of dentate gyrus (↓ nuclei area and condensation)</li> </ul> <p><u>Effects at 50 mg/kg-day or higher</u></p> <ul style="list-style-type: none"> <li>- ↓ total distance (OFT); ↓ percent time spent central to peripheral zone (OFT); ↓ percent time spent in open arm (EPM); ↓ avoidance latency time</li> </ul>

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				<ul style="list-style-type: none"> <li>- Histopathological findings in granular cells of dentate gyrus (↓ nuclei area and condensation)</li> </ul> <u>Limitations:</u> <ul style="list-style-type: none"> <li>- Histopathology data were only provided for 25 and 100 mg/kg-day DBP groups</li> </ul>
<i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEL = no observed adverse effect level; LOAEL = Lowest-observed-adverse-effect level; LOEL = Lowest observed effect level; EPM = Elevated Plus Maze; TST = Tail suspension test; FST = Forced Swim test; OFT = open field test				

### B.3 Metabolic/Nutritional

---

Three studies were identified that provided data on nutritional or metabolic effects following exposure to DBP. The data set included one study ([Ahmad et al., 2015](#)) in female rats exposed to DBP for 20 days beginning on PND21, one 13-week study ([Majeed et al., 2017](#)), and one one-generation study ([de Jesus et al., 2015](#)). These studies reported effects at low doses ranging from 5 to 10 mg/kg-day. Detailed information on the study designs are provided in Table\_Apx B-3.

de Jesus et al. ([2015](#)) exposed pregnant Mongolian gerbils to 5 mg/kg-day DBP in drinking water from GD 0 to PND28. After weaning the F1 offspring continued the same exposure as their mothers until PNW14. Increased terminal body weight (approximately 8 percent) was observed in PNW14 offspring, which coincided with increased adiposity index (approximately 35 percent) as well as decreased total cholesterol, decreased serum LDL levels, and increased triglycerides. However, the results are difficult to interpret because the study contained serious flaws that limit its use for deriving a robust POD, including concerns regarding chemical administration in drinking water; DBP is not soluble in water. Other limitations include insufficient information on measures to reduce observer bias or control for intra litter correlations, and the study only used one dose other than control.

EPA identified a LOEL of 10 mg/kg-day in both Ahmad et al. ([2015](#)) and Majeed et al. ([2017](#)). Ahmad et al. ([2015](#)) exposed adolescent female rats to 0, 10, or 100 mg/kg-day DBP via gavage from PND21 to 42. Decreased body weight gain was reported at PND27 (7.29 percent), PND33 (10.1 percent), and PND43 (9.39 percent). Limitations of this study include the short exposure duration, low sample size (6/group) and large dose spacing. Majeed et al. ([2017](#)) exposed male and female albino rats to 0, 10, or 50 mg/kg-day DBP for 13 weeks via feed and reported increased body weight gain, increased AC/TC ratio, and decreased energy intake. This study was adequately designed (*e.g.*, reported feed consumption data, evaluated males and females, evaluated endpoints at several timepoints and in both sexes). However, it is difficult to reconcile the biological plausibility of increased body weight gain and increased body size (AC/TC ratio) given other known effects of DBP, namely decreased testosterone, which would more likely coincide with a decrease in body weight. Although it is possible that DBP acts through a different mechanism to elicit these effects. Nevertheless, even though these studies provide some evidence of metabolic effects following exposure to DBP in experimental animals, EPA is not further considering these effects for dose-response assessment or for use in extrapolating human risk. The database of experimental animal studies is not as robust as that of developmental and reproductive health outcomes, which remains the most sensitive *and* robust outcome from which to derive a POD.

**Table\_Apx B-3. Summary of Animal Toxicology Studies Evaluating Effects on Metabolism Following Exposure to DBP**

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
<a href="#">(de Jesus et al., 2015)</a>	Pregnant Mongolian gerbils (12 dams /group) exposed via drinking water to 5 mg/kg-day DBP at concentrations of GD 0 to PND 28, then F1 (12 litters, 6–8 pups/litter) continued the same exposure through PNW14 (study termination).	ND/5	↓ Total cholesterol; ↓ serum low density lipoprotein (LDL) levels; ↑ serum triglycerides ↑ terminal (PNW14) body weight (~8%) ↑ adiposity index (~35%)	<u>Limitations</u> - DBP administered in drinking water (solubility concerns) - Insufficient information on measures to reduce bias from the litter effect - Unconventional experimental animal used (gerbils) - Study only used one dose other than control
<a href="#">(Majeed et al., 2017)</a>	Male and female albino rats (24/sex/dose) were exposed to 0, 10, or 50 mg/kg-day DBP via diet for 13 weeks. Anthropometric measures recorded after 0, 45, or 90 ( <i>i.e.</i> , study termination) days of exposure.	ND/10	↑ BW gain (males) & ↑AC/TC ratio at study termination; ↓ energy intake (females)	<u>Effects at 50 mg/kg-day:</u> ↑ BW gain (males only); ↑ BMI (males only) ↑ energy intake (males); ↓ energy intake (females) ↑ Glucose (10 mg/kg-day) ↑ total serum cholesterol  <u>Other Effects</u> - Change in relative liver weight - ↑ ALP (females); ↑ ALT; ↑ albumin  <u>Limitations:</u> -Organ weight data and most serum chemistry presented as pooled values for both sexes, with result of analysis for sex by treatment effect provided & authors provide insufficient information to discern directionality and magnitude of the effect specific to each sex.
<a href="#">(Ahmad et al., 2015)</a>	Female rats (6/group) were exposed to 0, 10, or 100 mg/kg-day DBP via gavage from PND21 – 42.	ND/10	↓ BW at multiple timepoints (PND27, 33, & 42)	<u>Effects at 100 mg/kg-day</u> - ↓ BW at multiple timepoints (PND27, 33, & 42)
<p><i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEL = no observed adverse effect level; LOAEL = Lowest-observed-adverse-effect level; LOEL = Lowest observed effect level; AGD = anogenital distance; GD = gestation day; PND = postnatal day; AC/TC = abdominal circumference to thoracic circumference ratio; BW = body weight; ALP = Alkaline phosphatase; ALT = alanine aminotransferase; BMI = body mass index</p>				

## B.4 Cardiovascular Health Effects

EPA identified one subchronic study that was designed to evaluate cardiovascular outcomes ([Xie et al., 2019](#)). The study provided data on histopathological alterations in the heart and aorta of male C57BL/6 mice exposed to 0.1, 1, or 10 mg/kg-day DBP via gavage for 6 weeks (Table\_Apx B-4). At 0.1 mg/kg-day, the authors observed increased vascular wall thickness of the aortic vessels and increased ACE staining density in the thoracic aorta based on quantitative histopathology. Increased vascular wall thickness of aortic vessels was also observed at 10 mg/kg-day, but not 1 mg/kg-day. There were several limitations of the study including only including male animals and inconsistent reporting of results for mean blood pressure (in the figure, it is noted as a significant increase at 10 mg/kg-day, while the running text indicates no effect at this dose). The inconsistency reduces confidence in the study reporting overall. Despite the sensitive LOAEL for cardiovascular outcomes, the limitations of the single study available introduce enough uncertainty that EPA is not selecting a POD based on cardiovascular effects.

**Table\_Apx B-4. Summary of Animal Toxicology Study Evaluating Effects on the Cardiovascular System Following Exposure to DBP**

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
<a href="#">(Xie et al., 2019)</a>	Male C57BL/6 mice were exposed via gavage to 0.1, 1, or 10 mg/kg-day DBP for 6 weeks.	ND/0.1 (LOEL)	↑ vascular wall thickness of aortic vessels; ↑ ACE staining density in the thoracic aorta;	<u>Effects at 1 mg/kg-day</u> ↑ serum E2 <u>Effects at 10 mg/kg-day</u> ↑ serum E2; ↓ ACE staining density in the thoracic aorta <u>Limitations</u> -Study only included males -Inconsistent reporting of results for mean BP reported in text vs Figure2B
<i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEL = no observed adverse effect level; LOAEL = Lowest-observed-adverse-effect level; LOEL = Lowest observed effect level; E2 = 17β- estradiol; ACE = Angiotensin Converting Enzyme; BP = blood pressure				

## B.5 Immune adjuvant effects

---

EPA identified two studies that provide data on the immune adjuvant properties of DBP following intermediate duration exposure to DBP. LOELs based on immune adjuvant effects in male BALB/c mice in two studies ([Li et al., 2014](#); [Zuo et al., 2014](#)). Details on the study designs for each study are not provided in the text for brevity and are instead summarized in Table\_Apx B-5. The aforementioned study by Zuo et al. ([2014](#)) was designed to evaluate the relationship between atopic allergy and neurobehavioral effects in male mice exposed to DBP, and therefore included a second set of animals that were sensitized with ovalbumin (OVA) antigen in addition to exposure to DBP and challenged via OVA aerosol leading up to neurobehavioral testing. While exposure to DBP alone did not affect performance in the open field test, exposure to DBP and OVA (both 0.45 mg/kg-day and 45 mg/kg-day doses) resulted in changes in one parameter measured in the open field test, an increase in distance in the outer ring. This result implies OVA exposure exacerbated the reduction in performance in one parameter measured in the open field test. OVA exacerbated the reduction in performance in the TST and FST at the high-dose only. Serum IgE and IL-4 were increased in all groups exposed to OVA relative to their saline-controls (*i.e.*, groups that received no antigen). IgE was increased, and IL-4 was decreased in animals exposed to 45 mg/kg-day DBP (no OVA) relative to untreated controls (no OVA). A second study by Li et al. ([2014](#)) exposed mice for 40 days to DBP via dermal application in addition to sensitization with FITC via dermal application to their backs. Mice were challenged with FITC application to their right ear prior to behavioral testing. Dermal sensitization and immunological effects were observed in mice exposed to 4 mg/kg-day DBP + FITC relative to the comparator group (0 mg/kg-day DBP + FITC). Specifically, mice from the 4 mg/kg-day DBP + FITC group had increased ear swelling, increased bilateral ear weight, and histopathological changes in the ear such as an increased number of infiltrating inflammatory cells and degranulating mast cells. Other effects included an increase in cytokines and other molecules associated with inflammation in ear tissues (Table\_Apx B-5).

Although these studies provide some evidence for immune adjuvant effects of DBP in sensitized animals, EPA is not further considering these effects for dose-response assessment or for use in extrapolating human risk. Several sources of uncertainty reduce EPA's confidence in this outcome. First, the database of experimental animal studies that provide data on immune effects of DBP is limited to two studies, each in male mice of the same strain, so it is difficult to understand effects in other sexes, strains, or species. Second, available studies evaluate the adjuvant properties of DBP in experimental rodent models pre-sensitized by exposure to other compounds (*i.e.*, FITC, OVA). This co-exposure to DBP and other compounds is another source of uncertainty that further reduced EPA's confidence in this outcome. EPA is not further considering immune adjuvant effects for dose-response analysis or for use in estimating risk to human health.



**Table\_Apx B-5. Summary of DBP Studies Evaluating Effects on the Immune System**

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
( <a href="#">Zuo et al., 2014</a> )	Male BALB/c mice (8/group) exposed to 0, 0.45, 45 mg/kg-day DBP via gavage, 32 days with OVA sensitization via s.c. injection on days 7, 21, and 28. Mice challenged with aerosolized OVA for 30 mins each day on days 33–39. Neurobehavioral examinations conducted days 36, 37, 39 prior to study term. on day 40. Groups include: OVA + 0 mg/kg-day DBP (comparator), OVA + 0.45 mg/kg-day DBP, or OVA + 45 mg/kg-day DBP.	ND/0.45	↑ immobile time in FST (not dose-dependent) & TST (dose-dependent); ↑ distance in outer ring on day 36 (OFT); ↑ serum IgE & ↑IL-4 (neither are dose-dependent)	<p><u>Effects at 45 mg/kg-day</u></p> <p><i>Neurologic</i></p> <ul style="list-style-type: none"> <li>- ↑ immobile time in FST &amp; TST</li> <li>- ↑ distance in outer ring on day 36 (OFT)</li> </ul> <p><i>Immune</i></p> <ul style="list-style-type: none"> <li>- ↓ relative spleen weight</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Only male animals were evaluated</li> <li>- Not guideline; insufficient detail on recording equipment – data collection presumed to not have been automated.</li> <li>- Quantitative data for FST, TST, and OFT based on highly subjective, qualitative observations &amp; and authors do not state use of measures to reduce observer bias (<i>i.e.</i>, blinding) (observer bias away from null)</li> <li>- Insufficient detail on training period for behavior tests</li> </ul>
( <a href="#">Li et al., 2014</a> )	Male BALB/c mice (8/group) exposed to 0, 0.4, 4, 40 mg/kg-day DBP, 40 days via dermal application to their shaven backs. Mice were sensitized with FITC via dermal application to backs; on day 41 and 42 ( <i>i.e.</i> , after the exposure period); challenged with FITC (ear) on day 47. Groups: 0 mg/kg-day DBP + FITC (comparator), 0 mg/kg-day DBP + saline, 0.4 mg/kg-day DBP + FITC or saline, 4 mg/kg-day DBP + FITC or saline,	0.45/4	Dermal sensitization and immunological effects: ↑ ear swelling; ↑ bilateral ear weight; quantitative histopathological changes (↑ no. infiltrating inflammatory cells; ↑ degranulating mast cells in the ear); ↑ ECP & TSLP in ear tissues; ↑ cytokines IL-4, IL-5, IL-13, & IL-17A in ear tissue	<p><u>Effects at 40 mg/kg-day:</u></p> <ul style="list-style-type: none"> <li>- ↑ serum IgE 24 hours after FITC challenge</li> <li>- ↑ ear swelling; ↑ bilateral ear weight</li> <li>- ↑ quantitative histopathological changes in the ear</li> <li>- ↑ cytokines IL-4, IL-5, IL-13, &amp; IL-17A in ear tissue</li> <li>- ↑ ECP &amp; TSLP in ear tissue</li> </ul> <p><u>Limitations</u></p> <ul style="list-style-type: none"> <li>- Only male animals were evaluated</li> <li>- Study did not evaluate T cell subpopulations in primary or secondary immune organs (<i>i.e.</i>, spleen, thymus, lymph nodes)</li> </ul>

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	40 mg/kg-day DBP + FITC or saline.			
<i>Abbreviations:</i> ↓ = statistically significant decrease; ↑ = statistically significant increase; NOAEL = no observed adverse effect level; LOAEL = Lowest-observed-adverse-effect level; LOEL = Lowest observed effect level; OVA = ovalbumin; s.c. = subcutaneous; IL = interleukin; IgE = immunoglobulin E; OFT = open field test; FST = Forced Swim test; TST = tail suspension test; TSLP = thymic stromal lymphopoietin; ECP = eosinophil cationic protein; FITC = Fluorescein isothiocyanate				

## Appendix C Fetal Testicular Testosterone as an Acute Effect

---

Several studies of experimental animal models are available that investigate the antiandrogenic effects of DBP following single dose, acute exposures. Available studies indicate a single acute exposure during the critical window of development (*i.e.*, GD 14 to 18) 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 (*P450scc/ 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 (*P450scc/ 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 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, which contributes additionally uncertainty.

## Appendix D Calculating Daily Oral Human Equivalent Doses and Human Equivalent Concentrations

For DBP, all data considered for PODs are obtained from oral animal toxicity studies in rats or mice. Because toxicity values for DBP are from oral animal studies, EPA must use an extrapolation method to estimate human equivalent doses (HEDs). The preferred method would be to use chemical-specific information for such an extrapolation. EPA identified one study reporting a diffusion-limited, pH trapping PBPK model for DBP and MBP ([Keys et al., 2000](#)). However, the model was not fit for purpose (*i.e.*, the model was developed to predict blood concentrations of DBP and MBP following oral exposure in the rat, not to extrapolate HEDs between species). EPA relied on the guidance from U.S. EPA ([2011b](#)), 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 ([2011b](#)), presents DAFs for extrapolation to humans from several species. However, because those DAFs used a human body weight of 70 kg, EPA has updated the DAFs using a human body weight of 80 kg for the DBP risk evaluation ([U.S. EPA, 2011a](#)). EPA used the body weights of 0.025 kg for mice and 0.25 kg for rats, as presented in U.S. EPA ([2011b](#)). The resulting DAFs for mice and rats are 0.133 and 0.236, respectively.

Use of allometric scaling for oral animal toxicity data to account for differences among species allows EPA to decrease the default intraspecies uncertainty factor ( $UF_A$ ) 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, 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, EPA 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 <sup>3</sup> )
$HED_{Daily}$	=	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 <sup>3</sup> /hr) = 0.6125
$ED_C$	=	Exposure duration for a continuous exposure (hr/day) = 24

Based on information from U.S. EPA ([2011a](#)), EPA assumes an at rest breathing rate of 0.6125 m<sup>3</sup>/hr. Adjustments for different breathing rates required for individual exposure scenarios are made in the exposure calculations, as needed.

It is often necessary to convert between ppm and mg/m<sup>3</sup> 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<sup>3</sup> to ppm.

#### Equation\_Apx D-4. Converting Units for HECs (mg/m<sup>3</sup> to ppm)

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

Where:

	24.45	=	Molar volume of a gas at standard temperature and pressure (L/mol), default
MW	=		Molecular weight of the chemical (MW of DBP = 278.35 g/mol)

### D.1 DBP 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 BMDL<sub>5</sub> of 9 mg/kg-day, and the critical effect is decreased fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production. The BMDL<sub>5</sub> was derived by a meta-regression and BMD modeling of fetal testicular testosterone data from eight studies of DBP with rats ([Gray et al., 2021](#); [Furr et al., 2014](#); [Johnson et al., 2011](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)). R code supporting NASEM's original meta-regression and BMD analysis

of DBP (NASEM, 2017) is publicly available on GitHub (<https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose>). This non-cancer POD is considered protective of effects observed following all duration exposures to DBP.

EPA conducted meta-analysis and benchmark dose modeling using the approach previously published by 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).

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:

$$2.1 \frac{mg}{kg - day} = 9 \frac{mg}{kg - day} \times 0.236$$

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

$$12 \frac{mg}{m^3} = 2.1 \frac{mg}{kg - day} \times \left( \frac{80 kg}{0.6125 \frac{m^3}{hr} * 24 hr} \right)$$

Equation\_Apx D-4 was used to convert the HEC from mg/m<sup>3</sup> to ppm:

$$1.0 ppm = 12 \frac{mg}{m^3} \times \frac{24.45}{278.35}$$



## 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, 2024](#)). 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 dataset 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<sub>5</sub> ([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<sub>5</sub> 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 datasets 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 modelled 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<sub>5</sub> estimates ranged from 8.4 to 74 mg/kg-day for DEHP, DBP, DCHP, and DINP, however, a BMD<sub>5</sub> estimate could not be derived for BBP or DIBP. Similarly, BMD<sub>10</sub> estimates ranged from 17 to 152 for DEHP, DBP, DCHP, DIBP and DINP, however, a BMD<sub>10</sub> estimate could not be derived for BBP. BMD<sub>40</sub> 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, 2023a](#)) 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<sub>40</sub> 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<sub>40</sub> 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<sub>10</sub> values for DBP (BMDL<sub>10</sub>, POD, LOAEL = 20, 9, 30 mg/kg-day, respectively) and DCHP (BMDL<sub>10</sub>, 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<sub>10</sub> 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<sub>10</sub> is greater than the POD selected for use in risk characterization by 5X (BMDL<sub>10</sub> 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<sub>10</sub> 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<sub>5</sub> (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, 2023a](#)), 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<sub>10</sub> 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<sub>5</sub> estimate for DEHP is similar to the selected POD and lowest LOAEL for apical outcomes on the developing male reproductive system.
- BMDL<sub>10</sub> estimates for DBP (BMDL<sub>10</sub>, POD, LOAEL = 20, 9, 30 mg/kg-day, respectively) and DCHP (BMDL<sub>10</sub>, 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 reflect 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<sub>40</sub> 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<sub>40</sub> 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 <sub>5</sub> Estimate <sup>a</sup> (mg/kg-day) [95% CI]	BMD <sub>10</sub> Estimate <sup>a</sup> (mg/kg-day) [95% CI]	BMD <sub>40</sub> Estimate <sup>a</sup> (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]	( <a href="#">U.S. EPA, 2025k</a> )
DBP	BMDL <sub>5</sub> = 9 (↓ fetal testicular testosterone content and <i>ex vivo</i> fetal testicular testosterone production)	30 (↑ Testicular Pathology)	14 [9, 27]	29 [20, 54]	149 [101, 247]	( <a href="#">U.S. EPA, 2025i</a> )
DIBP	BMDL <sub>5</sub> = 24 (↓ <i>ex vivo</i> fetal testicular testosterone production)	125 (↑ Testicular Pathology)	— <sup>b</sup>	55 [NA, 266] <sup>b</sup>	279 [136, 517]	( <a href="#">U.S. EPA, 2025l</a> )
BBP	NOAEL = 50 (phthalate syndrome-related effects)	100 (↓ AGD)	— <sup>b</sup>	— <sup>b</sup>	284 [150, 481]	( <a href="#">U.S. EPA, 2025h</a> )
DCHP	NOAEL = 10 (phthalate syndrome-related effects)	20 (↑ Testicular Pathology)	8.4 [6.0, 14]	17 [12, 29]	90 [63, 151]	( <a href="#">U.S. EPA, 2025j</a> )
DINP	BMDL <sub>5</sub> = 49 (↓ fetal testicular testosterone content and <i>ex vivo</i> fetal testicular testosterone production)	600 (↓ Sperm motility)	74 [47, 158]	152 [97, 278]	699 [539, 858]	( <a href="#">U.S. EPA, 2025m</a> )
<p>Abbreviations: AGD = anogenital distance; BMD = benchmark dose; BMDL = lower 95% confidence limit on BMD; CI = 95% confidence interval; LOAEL = lowest observed-adverse-effect level; NOAEL = no observed-adverse-effect level; POD = point of departure; RTM = reproductive tract malformations</p> <p><sup>a</sup> The linear-quadratic model provided the best fit (based on lowest AIC) for DEHP, DBP, DIBP, BBP, DCHP, and DINP.</p> <p><sup>b</sup> BMD and/or BMDL estimate could not be derived.</p>						

## Appendix F    **Benchmark Dose (BMD) Modeling of Fetal Testicular Testosterone Data from Individual Gestational Exposures Studies of DBP**

---

EPA conducted benchmark dose (BMD) modeling of fetal testicular testosterone content and *ex vivo* fetal testicular testosterone production data from individual studies used in the meta-analysis and BMD analysis described in Section 4.2. EPA considered all eight gestational exposure studies of DBP ([Gray et al., 2021](#); [Furr et al., 2014](#); [Struve et al., 2009](#); [Howdeshell et al., 2008](#); [Martino-Andrade et al., 2008](#); [Johnson et al., 2007](#); [Kuhl et al., 2007](#)) for modeling, but data from one study ([Johnson et al., 2011](#)) was not subjected to BMD analysis because it only evaluated one dose group.

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 fetal testicular testosterone content and/or *ex vivo* testosterone production, while more detailed BMD model results are provided in Appendices F.1 through 0.

**Table\_Apx F-1. Summary of BMD Model Results for Decreased Fetal Testicular Testosterone Content and *Ex Vivo* Fetal Testicular Testosterone Production**

Data set	BMR	Best-Fit Model (Variance)	BMD (mg/kg-day)	BMDL (mg/kg-day)	Notes	Appendix Containing Results
Fetal Testicular Testosterone Content ( <a href="#">Martino-Andrade et al., 2008</a> )	5%	Exponential 3 (Constant)	24	16		F.1
Fetal Testicular Testosterone Content ( <a href="#">Kuhl et al., 2007</a> )	5%	Exponential 3 (Constant)	22	14		F.2
Fetal Testicular Testosterone Content (4-Hr) ( <a href="#">Struve et al., 2009</a> )	5%	Linear (Non-Constant)	30	28		F.3.1
Fetal Testicular Testosterone Content (24-Hr) ( <a href="#">Struve et al., 2009</a> )	5%	—	—	—	No models adequately fit the dataset	F.3.2
Fetal Testicular Testosterone Content (1, 3, 6-Hr) ( <a href="#">Johnson et al., 2007</a> )	5%	—	—	—	No models adequately fit the dataset	F.4
<i>Ex vivo</i> Fetal Testis Testosterone Production ( <a href="#">Howdeshell et al., 2008</a> )	5%	Polynomial Degree 3 (Constant)	49	39		F.5
<i>Ex vivo</i> Fetal Testis Testosterone Production (Block 70) ( <a href="#">Gray et al., 2021</a> )	5%	—	—	—	All model fits questionable for BMR of 5%	F.6.1



<b>Data set</b>	<b>BMR</b>	<b>Best-Fit Model (Variance)</b>	<b>BMD (mg/kg-day)</b>	<b>BMDL (mg/kg-day)</b>	<b>Notes</b>	<b>Appendix Containing Results</b>
<i>Ex vivo</i> Fetal Testis Testosterone Production (Block 71) ( <a href="#">Gray et al., 2021</a> )	5%	—	—	—	All model fits questionable for BMR of 5%	0
<i>Ex vivo</i> Fetal Testis Testosterone Production (Blocks 18, 22, 26) ( <a href="#">Furr et al., 2014</a> )	5%	—	—	—	All model fits questionable for BMR of 5%	0

## F.1 BMD Model Results – Fetal Testicular Concentration ([Martino-Andrade et al., 2008](#))

---

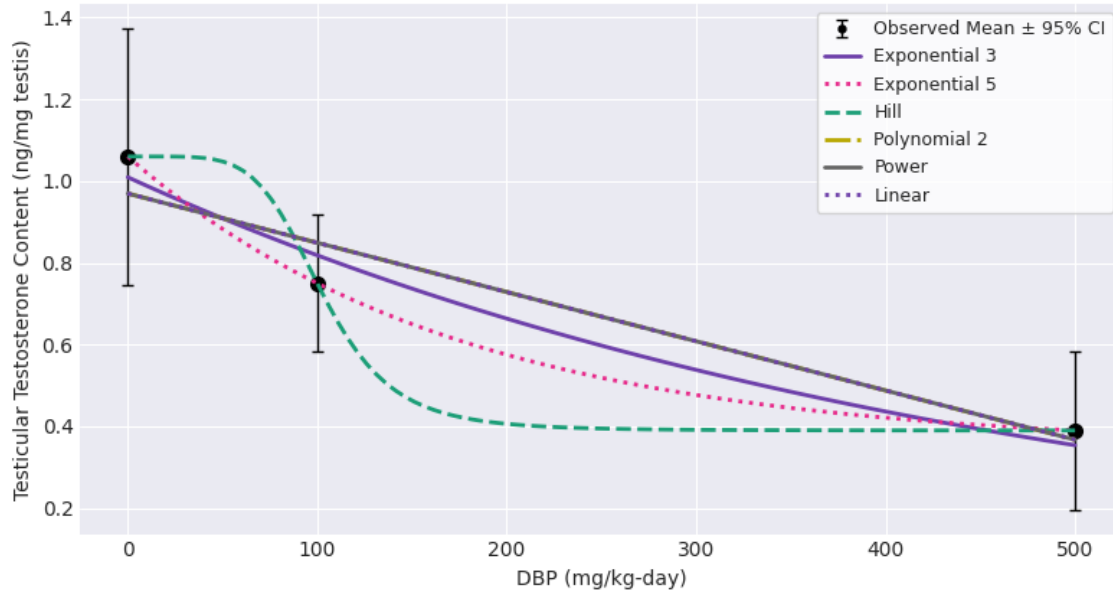
**Table\_Apx F-2. Fetal Testis Testosterone Content Data (Martino-Andrade et al. 2009)**

Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg testis)	Standard Deviation	Notes
0	7	1.06	0.34	Data extracted from Figure 1 in Martino-Andrade et al. (2009) by NASEM (2017) <sup>a</sup>
100	8	0.75	0.20	
500	7	0.39	0.21	
<sup>a</sup> <a href="https://hawcproject.org/ani/endpoint/23845/">https://hawcproject.org/ani/endpoint/23845/</a>				

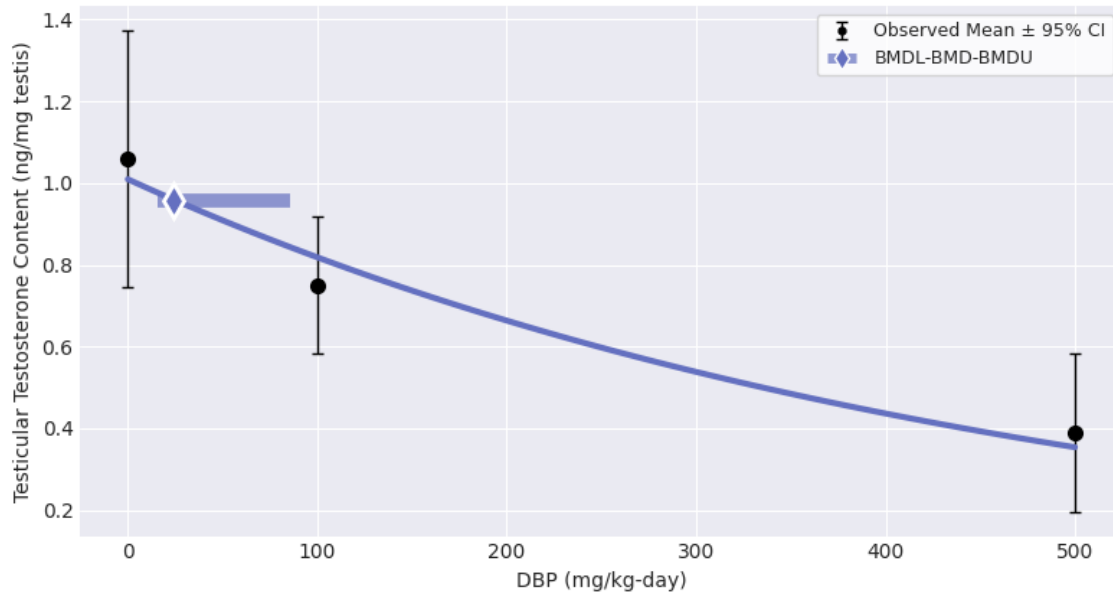
**Table\_Apx F-3. BMD Model Results – Fetal Testicular Testosterone Content (Martino-Andrade et al. 2009)**

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	24.468	15.67	50.259	32.188	243.675	156.057	0.572	6.25	Viable - Lowest AIC	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0
Exponential 5	Restricted	Constant	13.524	6.692	28.185	14.011	158.436	81.738	-	9.131	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	65.993	4.185	75.999	63.084	113.327	94.472	-	9.131	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	40.244	31.621	80.487	63.24	321.949	252.964	0.125	7.479	Viable	Lowest dose/BMDL ratio > 3.0
Power	Restricted	Constant	40.254	31.621	80.507	63.241	322.029	252.965	0.125	7.479	Viable	Lowest dose/BMDL ratio > 3.0
Linear	Unrestricted	Constant	40.254	31.621	80.507	63.24	322.029	252.963	0.125	7.479	Viable	Lowest dose/BMDL ratio > 3.0
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.												

Dataset #1  
MLE Models  
5% Relative Deviation



Dataset #1  
Exponential 3 Model (MLE)  
5% Relative Deviation



### 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	24.4681
BMDL	15.6701
BMDU	85.679
AIC	6.2497
Log-Likelihood	-0.12485
P-Value	0.571539
Model d.f.	2

#### Model Parameters:

Variable	Estimate	On Bound	Std Error
a	1.00928	no	0.0790989
b	0.00209634	no	0.000567643
d	1	yes	Not Reported
log-alpha	-2.82653	no	0.301509

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	7	1.06	1.00928	0.551407
100	8	0.75	0.818409	-0.795114
500	7	0.39	0.353834	0.393212

Dose	N	Sample SD	Model Fitted SD
0	7	0.34	0.243348
100	8	0.2	0.243348
500	7	0.21	0.243348

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	0.434573	4	7.13085
A2	1.74824	6	8.50352
A3	0.434573	4	7.13085
fitted	-0.12485	2	4.2497
reduced	-8.59012	2	21.1802

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	20.6767	4	0.000366995
Test 2	2.62733	2	0.268833
Test 3	2.62733	2	0.268833
Test 4	1.11885	2	0.571539

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 Testicular Concentration ([Kuhl et al., 2007](#))

---

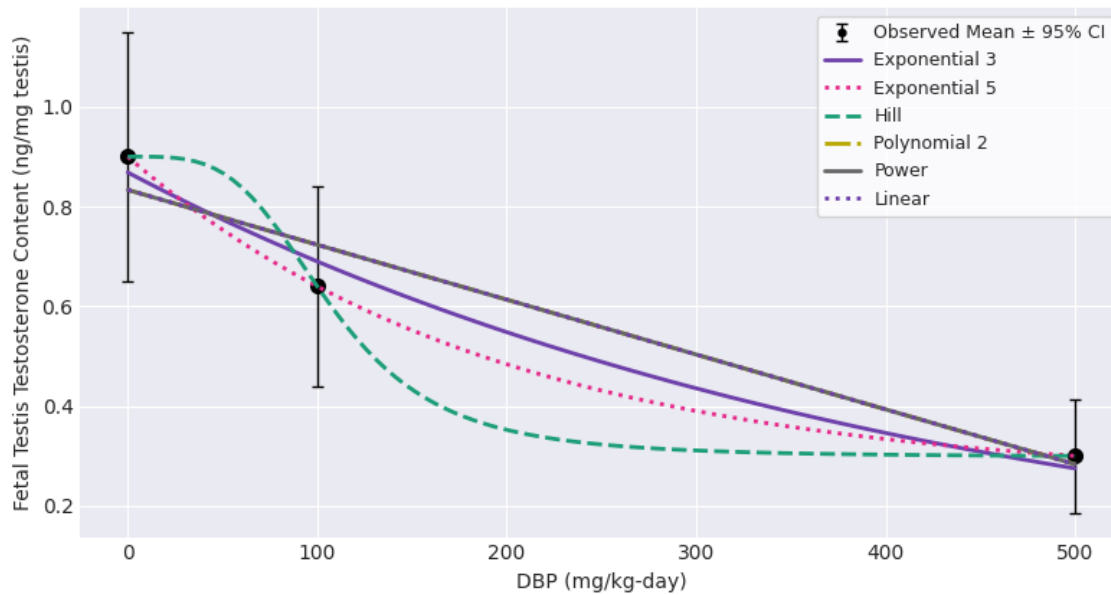
**Table\_Apx F-4. Fetal Testis Testosterone Content Data (Kuhl et al. 2007)**

Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg testis)	Standard Deviation	Notes
0	10	0.9	0.35	Data extracted from Figure 1 in Kuhl et al. (2007) by NASEM (2017) <sup>a</sup>
100	10	0.64	0.28	
500	10	0.3	0.16	
<sup>a</sup> <a href="https://hawcproject.org/ani/endpoint/23837/">https://hawcproject.org/ani/endpoint/23837/</a>				

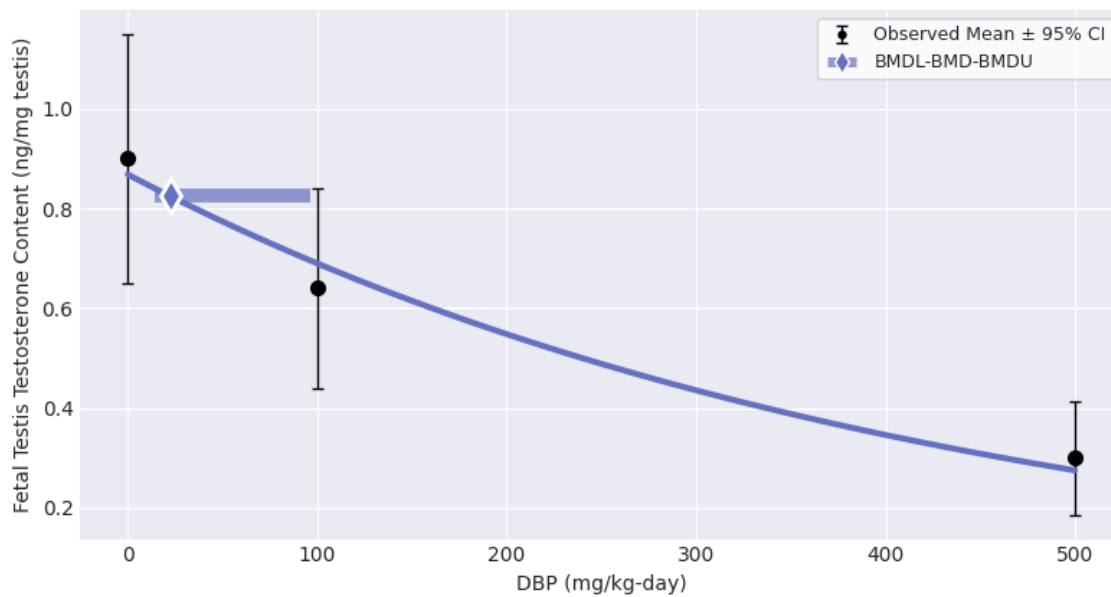
**Table\_Apx F-5. BMD Model Results – Fetal Testicular Testosterone Content (Kuhl et al. 2007)**

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMD L	BMD	BMDL	BMD	BMDL				
<b>Exponential 3</b>	<b>Restricted</b>	<b>Constant</b>	<b>22.304</b>	<b>13.988</b>	<b>45.813</b>	<b>28.734</b>	<b>222.12</b>	<b>139.31</b>	<b>0.741</b>	<b>11.067</b>	<b>Viable - Lowest AIC</b>	<b>Lowest dose/BMDL ratio &gt; 3.0 lowest dose/BMD ratio &gt; 3.0</b>
Exponential 5	Restricted	Constant	14.049	6.563	29.184	13.704	157.951	77.59	-	12.467	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	54.825	4.217	67.529	9.153	119.72	71.263	-	14.467	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	37.878	29.994	75.755	59.985	303.02	239.944	0.196	12.136	Viable	Lowest dose/BMDL ratio > 3.0
Power	Restricted	Constant	37.879	29.994	75.758	59.987	303.03	239.95	0.196	12.136	Viable	Lowest dose/BMDL ratio > 3.0
Linear	Unrestricted	Constant	37.879	29.994	75.758	59.985	303.03	239.944	0.196	12.136	Viable	Lowest dose/BMDL ratio > 3.0
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.												

Kuhl et al. (2007) - Fetal Testis Testosterone Content  
MLE Models  
5% Relative Deviation



Kuhl et al. (2007) - Fetal Testis Testosterone Content  
Exponential 3 Model (MLE)  
5% Relative Deviation



### 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	22.3036
BMDL	13.9885
BMDU	96.6493
AIC	11.0675
Log-Likelihood	-2.53373
P-Value	0.740582
Model d.f.	2

#### Model Parameters:

Variable	Estimate	On Bound	Std Error
a	0.868284	no	0.0730949
b	0.00229978	no	0.000644338
d	1	yes	Not Reported
log-alpha	-2.66896	no	0.258197

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	10	0.9	0.868284	0.380923
100	10	0.64	0.689896	-0.599274
500	10	0.3	0.274961	0.300723

Dose	N	Sample SD	Model Fitted SD
0	10	0.35	0.263295
100	10	0.28	0.263295
500	10	0.16	0.263295

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-2.23341	4	12.4668
A2	0.565944	6	10.8681
A3	-2.23341	4	12.4668
fitted	-2.53373	2	9.06746
reduced	-11.768	2	27.5361

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	24.668	4	5.86646e-05
Test 2	5.59871	2	0.0608494
Test 3	5.59871	2	0.0608494
Test 4	0.600639	2	0.740582

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.3 BMD Model Results – Fetal Testicular Concentration ([Struve et al., 2009](#))

---

#### F.3.1 4-Hour Post-Exposure

---

Table\_Apx F-6. Fetal Testis Testosterone Content Data (4-Hr Post-Exposure) (Struve et al. 2009)

Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg testis)	Standard Deviation	Notes
0	9	0.27	0.18	Data extracted from Figure 1 in Struve et al. (2009) by NASEM (2017) <sup>a</sup>
112.4	7	0.15	0.13	
582.1	7	0.01	0.03	
<sup>a</sup> <a href="https://hawcproject.org/ani/endpoint/23215/">https://hawcproject.org/ani/endpoint/23215/</a>				

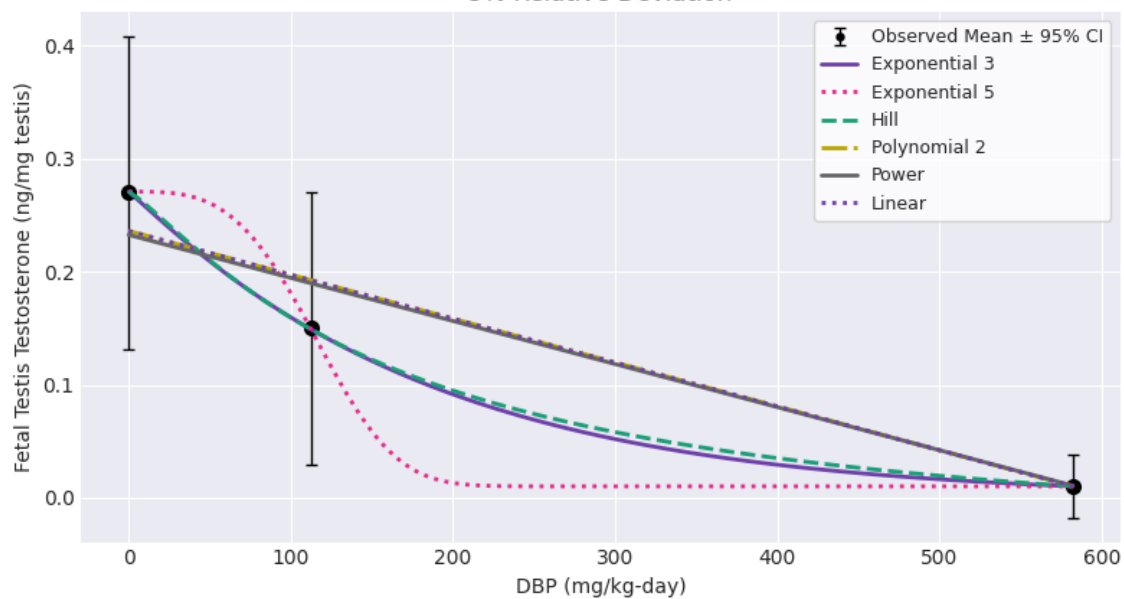


**Table\_Apx F-7. BMD Model Results – Fetal Testicular Testosterone Content (4-Hr) (Struve et al. 2009)**

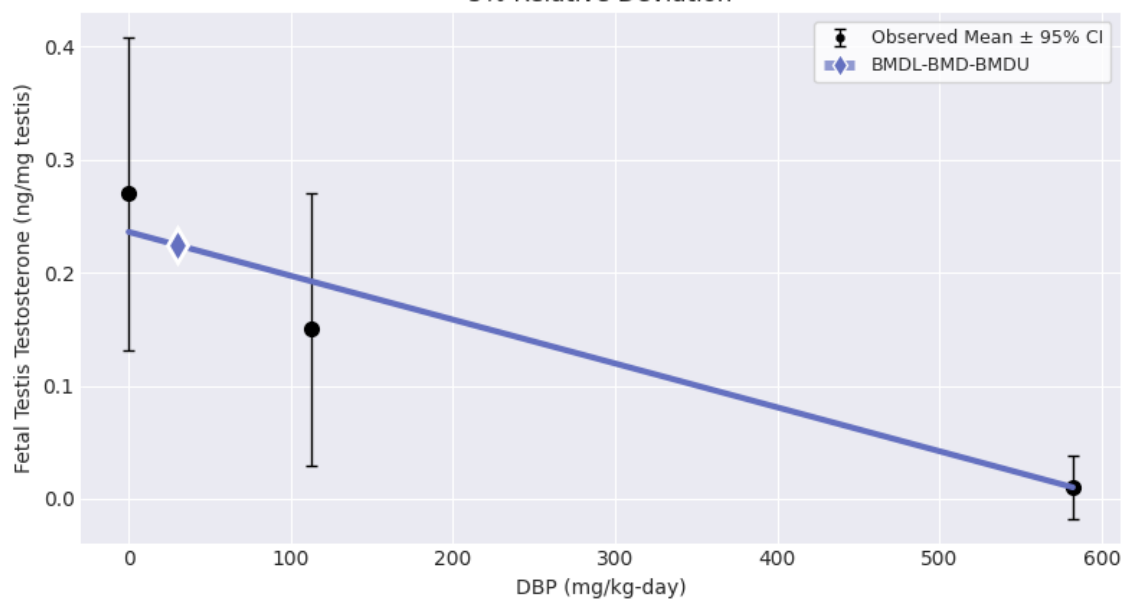
Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	10.976	4.175	21.809	8.575	98.317	41.576	–	–21.963	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0 lowest dose/BMD ratio > 10.0 Zero degrees of freedom; saturated model Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	15.553	3.507	27.837	7.249	100.222	37.097	–	–19.963	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Hill	Restricted	Constant	9.927	1.347	19.857	2.882	96.903	19.612	–	–19.963	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	29.256	21.776	58.512	43.55	234.049	174.201	0.246	–22.615	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	29.259	21.776	58.517	43.551	234.07	174.205	0.246	–22.615	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	29.259	21.776	58.517	43.55	234.07	174.203	0.246	–22.615	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-Constant	10.53	4.761	21.087	9.779	96.69	47.415	–	–36.466	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMD L	BMD	BMDL	BMD	BMDL				
												lowest dose/BMD ratio > 10.0 Zero degrees of freedom; saturated model
Exponential 5	Restricted	Non-Constant	54.474	4.754	67.31	9.772	107.289	47.431	–	–34.466	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Hill	Restricted	Non-Constant	12.333	4.985	22.929	11.652	96.449	46.399	–	–34.466	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
Polynomial Degree 2	Restricted	Non-Constant	30.417	27.93	60.835	55.86	243.341	223.442	0.229	–37.016	Viable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0
Power	Restricted	Non-Constant	30.52	27.862	61.04	55.644	244.161	223.297	0.227	–37.005	Viable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0
<b>Linear</b>	<b>Unrestricted</b>	<b>Non-Constant</b>	<b>30.417</b>	<b>27.93</b>	<b>60.835</b>	<b>55.86</b>	<b>243.341</b>	<b>223.443</b>	<b>0.229</b>	<b>–37.016</b>	<b>Recommended - Lowest AIC</b>	<b>Lowest dose/BMDL ratio &gt; 3.0 lowest dose/BMD ratio &gt; 3.0</b>
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = Not Applicable.												

Struve et al. (2009) - Fetal Testis Testosterone (4-hr)  
MLE Models  
5% Relative Deviation



Struve et al. (2009) - Fetal Testis Testosterone (4-hr)  
Linear Model (MLE)  
5% Relative Deviation



## Linear Model

Version: pybmds 25.1 (bmdscore 25.1)

### Input Summary:

BMR	5% Relative Deviation
Distribution	Normal + Nonconstant variance
Modeling Direction	Down (↓)
Confidence Level (one sided)	0.95
Modeling Approach	MLE
Degree	1

### Parameter Settings:

Parameter	Initial	Min	Max
g	0	0	1000
b1	0	-18	18
rho	0	0	18
alpha	0	-18	18

### Modeling Summary:

BMD	30.4174
BMDL	27.9304
BMDU	33.5311
AIC	-37.0156
Log-Likelihood	22.5078
P-Value	0.228544
Model d.f.	1

### Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.235985	no	0.0423332
b1	-0.000387907	no	7.57888e-05
rho	1.12313	no	0.430826
alpha	0.131449	no	0.0152065

Goodness of Fit:

Dose	N	Sample Mean	Model Fitted Mean	Scaled Residual
0	9	0.27	0.235985	0.633264
112.4	7	0.15	0.192384	-0.780468
582.1	7	0.01	0.0101839	-0.0176399

Dose	N	Sample SD	Model Fitted SD
0	9	0.18	0.161144
112.4	7	0.13	0.143679
582.1	7	0.03	0.0275864

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	14.9815	4	-21.963
A2	23.2341	6	-34.4683
A3	23.2327	5	-36.4655
fitted	22.5078	4	-37.0156
reduced	8.69291	2	-13.3858

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	29.0824	4	7.52167e-06
Test 2	16.5053	2	0.000260572
Test 3	0.00280791	1	0.95774
Test 4	1.4499	1	0.228544

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.3.2 24-Hour Post-Exposure

---

**Table\_Apx F-8. Fetal Testis Testosterone Content Data (4-Hr Post-Exposure) (Struve et al. 2009)**

Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg testis)	Standard Deviation	Notes
0	9	0.28	0.12	Data extracted from Figure 1 in Struve et al. (2009) by NASEM (2017) <sup>a</sup>
112.4	7	0.08	0.03	
582.1	7	0.02	0.03	
<sup>a</sup> <a href="https://hawcproject.org/ani/endpoint/23216/">https://hawcproject.org/ani/endpoint/23216/</a>				



**Table\_Apx F-9. BMD Model Results – Fetal Testicular Testosterone Content (24-Hr) (Struve et al. 2009)**

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	29.861	2.652	40.251	5.447	77.475	26.407	–	–45.984	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Exponential 5	Restricted	Constant	21.888	0.368	31.389	0.751	69.723	3.848	–	–44.489	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Hill	Restricted	Constant	3.323	0.019	6.532	0.036	33.221	0.156	–	–44.489	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Polynomial Degree 2	Restricted	Constant	29.273	22.717	58.546	45.434	234.185	181.74	<0.001	–34.874	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	29.274	22.718	58.547	45.435	234.19	181.74	<0.001	–34.874	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	29.274	22.717	58.547	45.434	234.19	181.738	<0.001	–34.874	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
												Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	4.799	2.699	9.858	5.544	47.797	26.879	0.004	-54.682	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	20.976	2.532	29.863	5.22	65.396	26.062	-	-58.812	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	3.67	0.726	6.703	3.081	29.01	9.537	-	-58.812	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	32.096	29.112	64.192	58.224	256.768	232.893	<0.001	-45.556	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	32.072	29.201	64.145	58.392	256.578	232.84	<0.001	-45.556	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	32.096	29.112	64.192	58.224	256.768	232.895	<0.001	-45.556	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = Not Applicable												

## F.4 BMD Model Results – Fetal Testicular Concentration ([Johnson et al., 2007](#))

---

### F.4.1 1-Hour Post-Exposure

---

Table\_Apx F-10. Fetal Testis Testosterone Content Data (1-Hr) (Johnson et al. 2007)

Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg testis)	Standard Deviation	Notes
0	5	0.238	0.12	Data extracted from Figure 1 in Johnson et al. (2007) by NASEM (2017) <sup>a</sup>
1	5	0.2	0.07	
10	5	0.16	0.04	
100	5	0.21	0.07	

<sup>a</sup> <https://hawcproject.org/ani/endpoint/23356/>

**Table\_Apx F-11. BMD Model Results – Fetal Testicular Testosterone Content (1-Hr) (Johnson et al. 2007)**

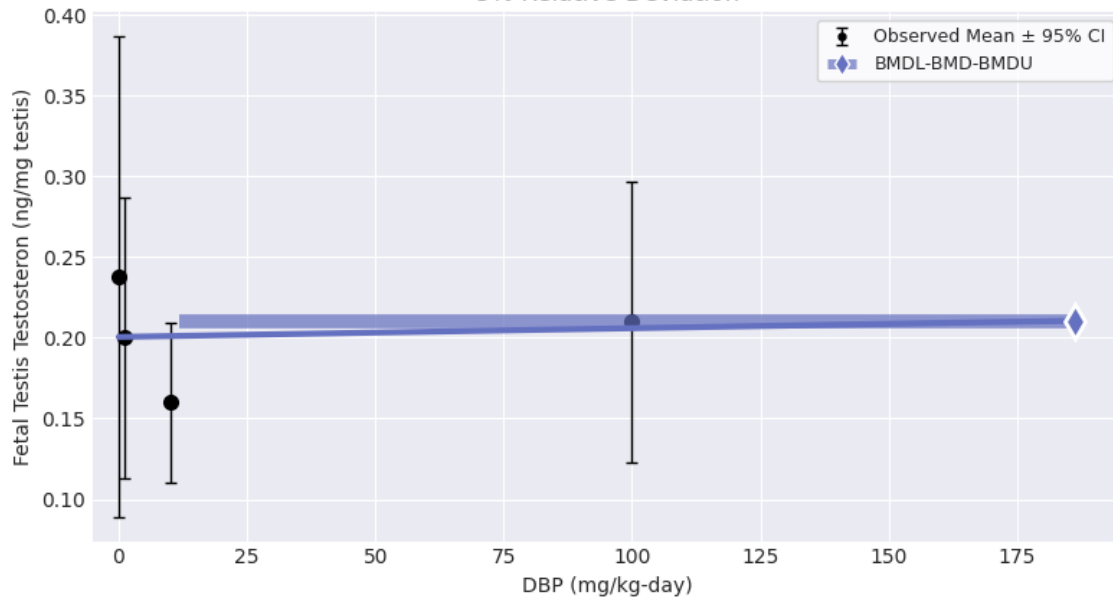
Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMD L	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	–	–	–	–	–	–	0.244	-37.757	Unusable	Did not successfully execute.
Exponential 5	Restricted	Constant	–	–	–	–	–	–	–	-35.757	Unusable	Did not successfully execute.
Hill	Restricted	Constant	–	–	–	–	–	–	–	-35.829	Unusable	Did not successfully execute.
Polynomial Degree 2	Restricted	Constant	–	–	–	–	–	–	0.42	-41.757	Unusable	Did not successfully execute.
Polynomial Degree 3	Restricted	Constant	–	–	–	–	–	–	0.42	-41.757	Unusable	Did not successfully execute.
Power	Restricted	Constant	–	–	–	–	–	–	0.097	-37.829	Unusable	Did not successfully execute.
<b>Linear<sup>a</sup></b>	<b>Unrestricted</b>	<b>Constant</b>	<b>186.203</b>	<b>11.858</b>	<b>372.409</b>	<b>23.716</b>	<b>1489.63</b>	<b>94.864</b>	<b>0.246</b>	<b>-39.774</b>	<b>Viable - Lowest AIC</b>	<b>BMD/highest dose ratio &gt; 1.0 BMD/BMDL ratio &gt; 3.0</b>

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

<sup>a</sup> Although the Linear model provided a BMDL<sub>5</sub> estimate of 12 mg/kg-day, the model had a poor visual fit and was not further considered.

# Johnson et al. (2007) - Fetal Testis Testosterone (1-Hr)

Linear Model (MLE)  
5% Relative Deviation



Linear Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

BMR	5% Relative Deviation
Distribution	Normal + Constant variance
Modeling Direction	Down ( $\downarrow$ )
Confidence Level (one sided)	0.95
Modeling Approach	MLE
Degree	1

Parameter Settings:

Parameter	Initial	Min	Max
g	0	-1e+06	1e+06
b1	0	-1e+06	1e+06
alpha	0	-18	18

Modeling Summary:

BMD	186.203
BMDL	11.8584
BMDU	-9999
AIC	-39.7738
Log-Likelihood	22.8869
P-Value	0.245975
Model d.f.	2

Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.200506	no	0.0206779
b1	5.38404e-05	no	0.000412007
alpha	0.00593691	no	1.1146e-05

Goodness of Fit:

Dose	N	Sample Mean	Model Fitted Mean	Scaled Residual
0	5	0.238	0.200506	1.0881
1	5	0.2	0.20056	-0.0162448
10	5	0.16	0.201044	-1.19113
100	5	0.21	0.20589	0.119275

Dose	N	Sample SD	Model Fitted SD
0	5	0.12	0.0770514
1	5	0.07	0.0770514
10	5	0.04	0.0770514
100	5	0.07	0.0770514

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	24.2894	5	-38.5788
A2	27.141	8	-38.2819
A3	24.2894	5	-38.5788
fitted	22.8869	3	-39.7738
reduced	22.8783	2	-41.7566

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	8.52528	6	0.202088
Test 2	5.70309	3	0.126984
Test 3	5.70309	3	0.126984
Test 4	2.80505	2	0.245975

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.4.2 3-Hour Post-Exposure

**Table\_Apx F-12. Fetal Testis Testosterone Content Data (3-Hr) (Johnson et al. 2007)**

Table 1. Mean Testis Testosterone Content Data (Table 1) (Johnson et al. 2007)				
Dose (mg/kg-day)	N (# of litters)	Mean Testis Testosterone (ng/mg testis)	Standard Deviation	Notes
0	5	0.238	0.12	Data extracted from Figure 1 in Johnson et al. (2007) by NASEM (2017) <sup>a</sup>
1	5	0.38	0.18	
10	5	0.39	0.07	
100	5	0.26	0.02	
<sup>a</sup> <a href="https://hawcproject.org/ani/endpoint/23357/">https://hawcproject.org/ani/endpoint/23357/</a>				

**Table\_Apx F-13. BMD Model Results – Fetal Testicular Testosterone Content (3-Hr) (Johnson et al. 2007)**

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	1353.772	26.046	1416.585	0	1542.932	103.728	0.024	-19.081	Questionable	Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05) BMD/highest dose ratio > 1.0 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Exponential 5	Restricted	Constant	–	–	–	–	–	–	–	-17.081	Unusable	Did not successfully execute.
Hill	Restricted	Constant	–	–	–	–	–	–	–	-18.568	Unusable	Did not successfully execute.
Polynomial Degree 2	Restricted	Constant	–	–	–	–	–	–	0.059	-23.081	Unusable	Did not successfully execute.
Polynomial Degree 3	Restricted	Constant	–	–	–	–	–	–	0.059	-23.081	Unusable	Did not successfully execute.
Power	Restricted	Constant	–	–	–	–	–	–	0.015	-20.568	Unusable	Did not successfully execute.
Linear	Unrestricted	Constant	24.065	10.121	48.13	20.24	192.521	80.962	0.043	-22.249	Questionable	Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1878.505	26.047	1965.822	50.881	2141.481	103.723	0.005	-17.081	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD/highest dose ratio > 1.0 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Exponential 5	Restricted	Non-constant	–	–	–	–	–	–	–	-15.081	Unusable	Did not successfully execute.
Hill	Restricted	Non-constant	–	–	–	–	–	–	–	-32.474	Unusable	Did not successfully execute.
Polynomial Degree 2	Restricted	Non-constant	–	–	–	–	–	–	0.015	-21.081	Unusable	Did not successfully execute.
Polynomial Degree 3	Restricted	Non-constant	–	–	–	–	–	–	0.015	-21.081	Unusable	Did not successfully execute.
Power	Restricted	Non-constant	–	–	–	–	–	–	1	-33.886	Unusable	Did not successfully execute.



Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P-Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Linear	Unrestricted	Non-constant	20.49	13.741	40.98	27.457	163.919	109.927	1	-35.94	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05)
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable												

#### F.4.3 6-Hour Post-Exposure

---

**Table\_Apx F-14. Fetal Testis Testosterone Content Data (6-Hr) (Johnson et al. 2007)**

<b>Dose (mg/kg-day)</b>	<b>N (# of litters)</b>	<b>Mean Testis Testosterone (ng/mg testis)</b>	<b>Standard Deviation</b>	<b>Notes</b>
0	5	0.238	0.12	Data extracted from Figure 1 in Johnson et al. (2007) by NASEM (2017) <sup>a</sup>
1	5	0.26	0.18	
10	5	0.16	0.07	
100	5	0.2	0.11	

<sup>a</sup> <https://hawcproject.org/ani/endpoint/23358/>

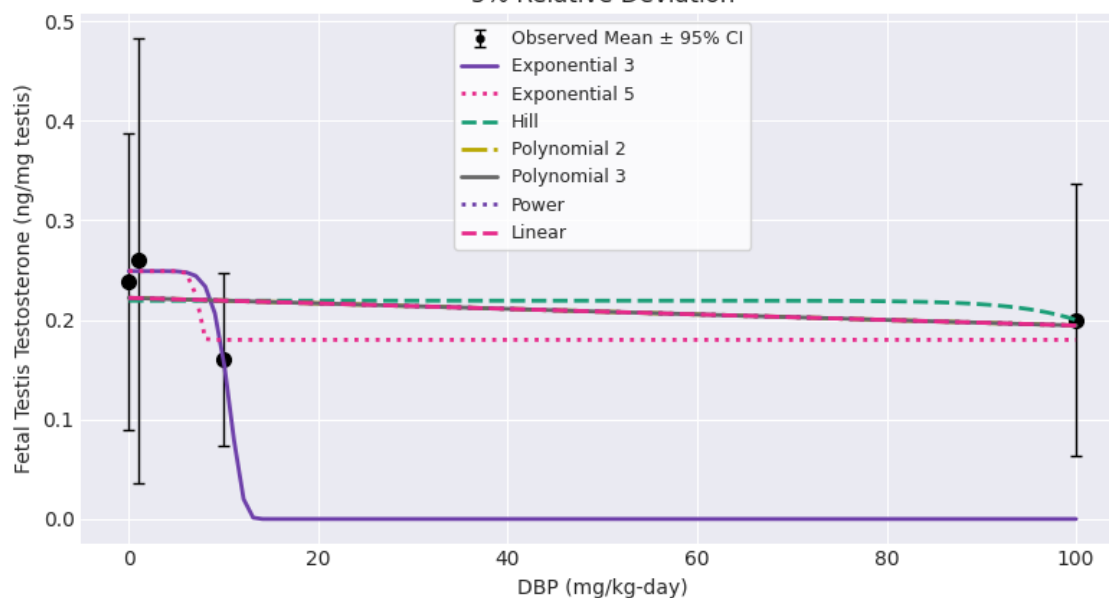
**Table\_Apx F-15. BMD Model Results – Fetal Testicular Testosterone Content (6-Hr) (Johnson et al. 2007)**

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	7.881	0.312	8.534	0.641	10.161	3.11	0.003	-10.844	Questionable	Lowest dose/BMDL ratio > 3.0 Goodness of fit p-value < 0.1 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Exponential 5	Restricted	Constant	6.779	0.178	7.102	0.379	–	–	–	-20.067	Questionable	Lowest dose/BMDL ratio > 3.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Hill	Restricted	Constant	96.51	0	100.794	0	109.944	0	–	-18.416	Unusable	Zero degrees of freedom; saturated model BMDL does not exist
Polynomial Degree 2	Restricted	Constant	40.01	9.077	80.021	18.152	320.083	72.613	0.375	-22.509	Viable	BMD/BMDL ratio > 3.0
Polynomial Degree 3	Restricted	Constant	40.005	9.077	80.01	18.153	320.039	72.613	0.375	-22.509	Viable	BMD/BMDL ratio > 3.0
<b>Power<sup>a</sup></b>	<b>Restricted</b>	<b>Constant</b>	<b>40.012</b>	<b>9.077</b>	80.023	18.154	320.092	72.617	<b>0.375</b>	<b>-22.509</b>	<b>Viable - Lowest AIC</b>	<b>BMD/BMDL ratio &gt; 3.0</b>
Linear	Unrestricted	Constant	40.012	9.077	80.023	18.153	320.092	72.611	0.375	-22.509	Viable	BMD/BMDL ratio > 3.0

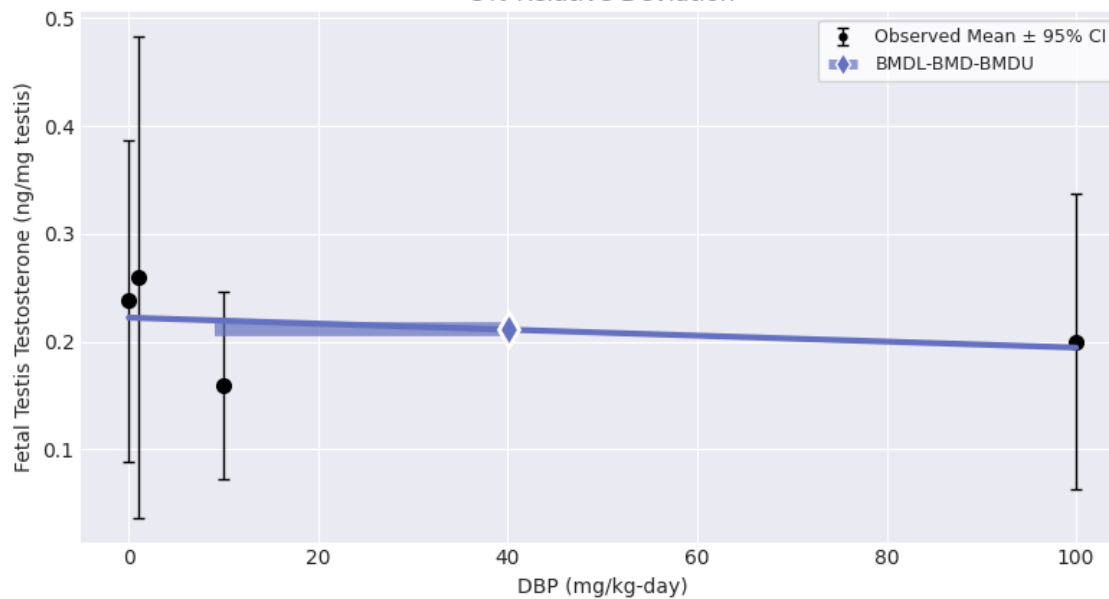
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = Not Applicable.

<sup>a</sup> Although the Power model provided a BMDL<sub>5</sub> estimate of 9.1 mg/kg-day, the model had a poor visual fit and was not further considered.

Johnson et al. (2007) - Fetal Testis Testosterone (6-Hr)  
MLE Models  
5% Relative Deviation



Johnson et al. (2007) - Fetal Testis Testosterone (6-Hr)  
Power Model (MLE)  
5% Relative Deviation



## Power 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
n	1	1	18
alpha	0	-18	18

### Modeling Summary:

BMD	40.0115
BMDL	9.07717
BMDU	-9999
AIC	-22.509
Log-Likelihood	14.2545
P-Value	0.374881
Model d.f.	2

### Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.222206	no	0.031821
v	-0.000277677	no	0.000633269
n	1	yes	Not Reported
alpha	0.0140754	no	6.26501e-05

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	5	0.238	0.222206	0.297687
1	5	0.26	0.221928	0.717566
10	5	0.16	0.219429	-1.12009
100	5	0.2	0.194438	0.104833

Dose	N	Sample SD	Model Fitted SD
0	5	0.12	0.11864
1	5	0.18	0.11864
10	5	0.07	0.11864
100	5	0.11	0.11864

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	15.2356	5	-20.4713
A2	17.3606	8	-18.7213
A3	15.2356	5	-20.4713
fitted	14.2545	3	-22.509
reduced	14.1588	2	-24.3176

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	6.40371	6	0.379517
Test 2	4.25004	3	0.235699
Test 3	4.25004	3	0.235699
Test 4	1.96229	2	0.374881

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 ([Howdeshell et al., 2008](#))

---

**Table\_Apx F-16. *Ex Vivo* Fetal Testis Testosterone Production Data (Howdeshell et al. 2008)**

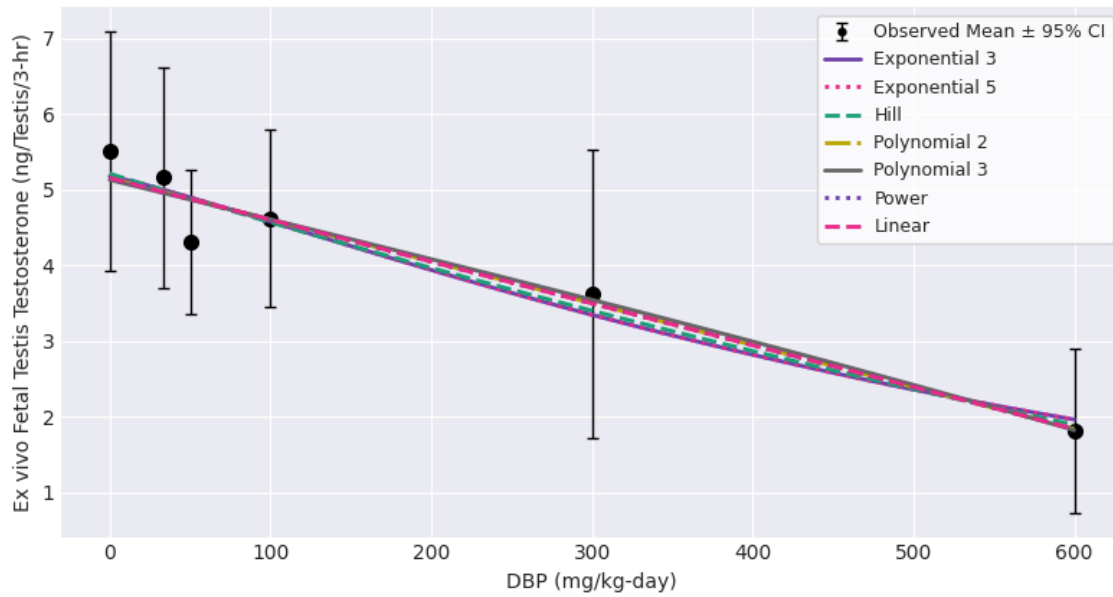
Dose (mg/kg-day)	N (# of litters)	Mean <i>Ex vivo</i> Fetal Testis Testosterone Production (ng/mg testis-2-hr)	Standard Deviation	Notes
0	3	5.51	0.64	Data extracted from Table 8 in Howdeshell et al. (2008)
33	4	5.16	0.92	
50	4	4.31	0.60	
100	4	4.62	0.74	
300	4	3.62	1.20	
600	4	1.81	0.68	



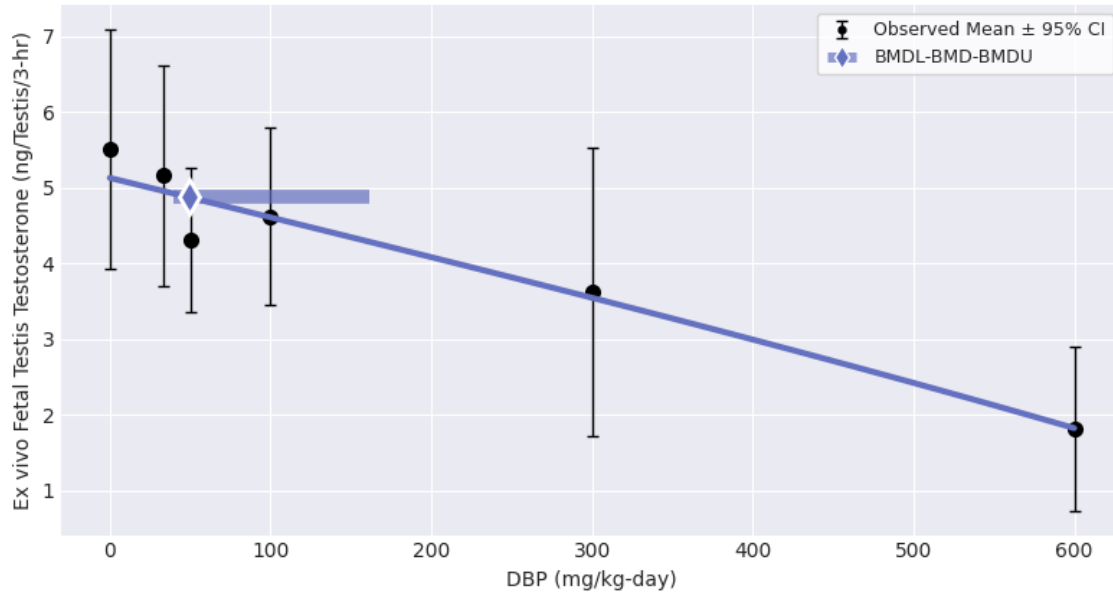
**Table\_Apx F-17. BMD Model Results – *Ex Vivo* Fetal Testis Testosterone Production (Howdeshell et al. 2008)**

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	46.96 3	24.328	87.628	49.963	344.131	242.292	0.411	61.86	Viable	
Exponential 5	Restricted	Constant	46.96 3	24.328	87.628	49.963	344.131	242.292	0.138	63.86	Viable	
Hill	Restricted	Constant	39.76	16.868	80.588	35.822	350.608	222.044	0.323	61.383	Viable	
Polynomial Degree 2	Restricted	Constant	46.91 1	39.274	93.772	78.551	373.883	314.049	0.493	59.297	Viable	
<b>Polynomial Degree 3</b>	<b>Restricted</b>	<b>Constant</b>	<b>49.26 2</b>	<b>39.289</b>	<b>98.405</b>	<b>78.582</b>	<b>384.842</b>	<b>314.281</b>	<b>0.495</b>	<b>59.289</b>	<b>Viable - Lowest AIC</b>	
Power	Restricted	Constant	46.62 7	39.234	93.254	78.467	373.015	313.868	0.491	59.31	Viable	
Linear	Unrestricted	Constant	46.62 3	39.247	93.246	78.494	372.985	313.977	0.493	59.297	Viable	
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.												

Howdeshell (2008) - Ex vivo Fetal Testis Testosterone  
MLE Models  
5% Relative Deviation



Howdeshell (2008) - Ex vivo Fetal Testis Testosterone  
Polynomial 3 Model (MLE)  
5% Relative Deviation



# Polynomial 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
Degree	3

## Parameter Settings:

Parameter	Initial	Min	Max
g	0	-1e+06	1e+06
b1	0	-1e+06	0
b2	0	-1e+06	0
b3	0	-1e+06	0
alpha	0	-18	18

## Modeling Summary:

BMD	49.2618
BMDL	39.2886
BMDU	161.263
AIC	59.2888
Log-Likelihood	-26.6444
P-Value	0.494755
Model d.f.	4

## Model Parameters:

Variable	Estimate	On Bound	Std Error
g	5.13032	no	0.255023
b1	-0.00520507	no	0.00215171
b2	-8.75533e-10	yes	Not Reported
b3	-8.57398e-10	yes	Not Reported
alpha	0.593958	no	0.104039

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	5.51	5.13032	0.853291
33	4	5.16	4.95852	0.522849
50	4	4.31	4.86996	-1.45315
100	4	4.62	4.60895	0.0286771
300	4	3.62	3.54557	0.193145
600	4	1.81	1.82177	-0.030535

Dose	N	Sample SD	Model Fitted SD
0	3	0.64	0.770687
33	4	0.92	0.770687
50	4	0.6	0.770687
100	4	0.74	0.770687
300	4	1.2	0.770687
600	4	0.68	0.770687

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-24.9492	7	63.8985
A2	-23.4171	12	70.8342
A3	-24.9492	7	63.8985
fitted	-26.6444	3	59.2888
reduced	-40.4555	2	84.911

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	34.0769	10	0.000179243
Test 2	3.06429	5	0.690075
Test 3	3.06429	5	0.690075
Test 4	3.39029	4	0.494755

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.6 BMD Model Results – *Ex vivo* Fetal Testis Testosterone Production ([Gray et al., 2021](#))

---

### F.6.1 Block 70 Rat Data

---

Table\_Apx F-18. *Ex Vivo* Fetal Testis Testosterone Production Data (Block 70) (Gray et al. 2021)

Dose (mg/kg-day)	N (# of litters)	Mean <i>Ex Vivo</i> Fetal Testis Testosterone Production (ng/mg testis)	Standard Deviation	Notes
0	3	8.450111	2.55	Data extracted from Supplemental Excel Files associated with Gray et al. (2021)
300	4	5.225583	1.81	
600	4	2.083	0.66	
900	4	1.348583	0.58	

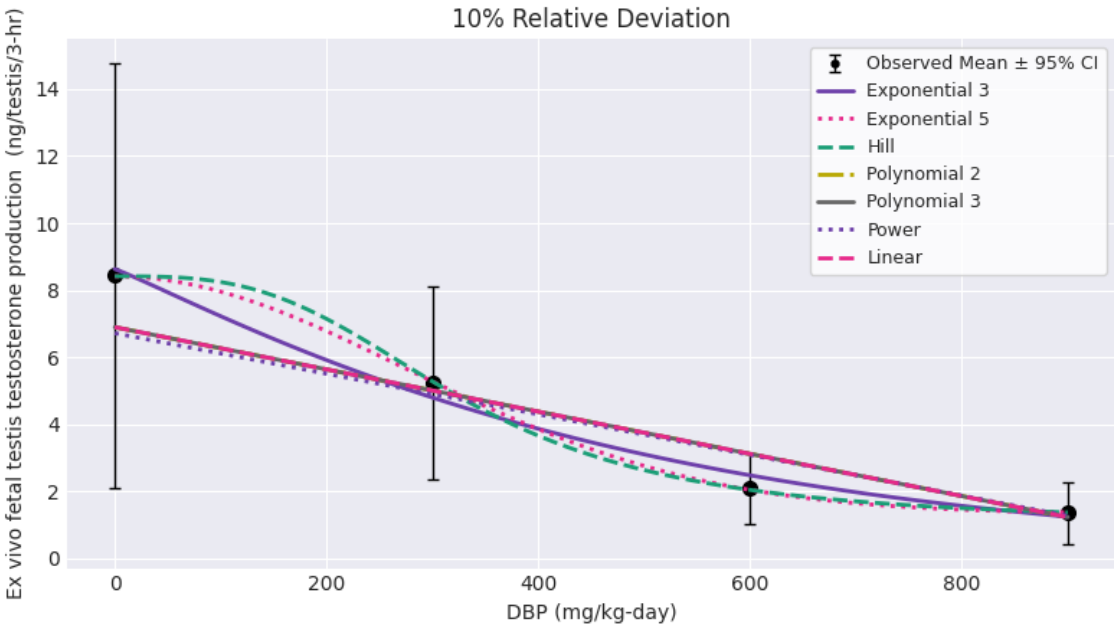
**Table\_Apx F-19. *Ex Vivo* Fetal Testis Testosterone Production (Block 70) (Gray et al. 2021)**

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	49.567	20.034	86.971	41.133	298.452	199.51	0.753	58.886	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	88.642	19.383	130.483	39.948	310.609	198.961	-	60.32	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Hill	Restricted	Constant	125.528	16.817	162.661	35.171	308.809	192.763	-	60.32	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 Zero degrees of freedom; saturated model Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	49.049	42.739	98.097	85.479	392.388	341.915	0.16	59.99	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 3	Restricted	Constant	49.156	42.733	98.312	85.466	393.247	341.864	0.159	59.992	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	49.005	42.742	98.01	85.485	392.039	341.938	0.16	59.99	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	49.005	42.742	98.01	85.484	392.039	341.938	0.16	59.99	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Constant variance test failed (Test 2 p-value < 0.05)

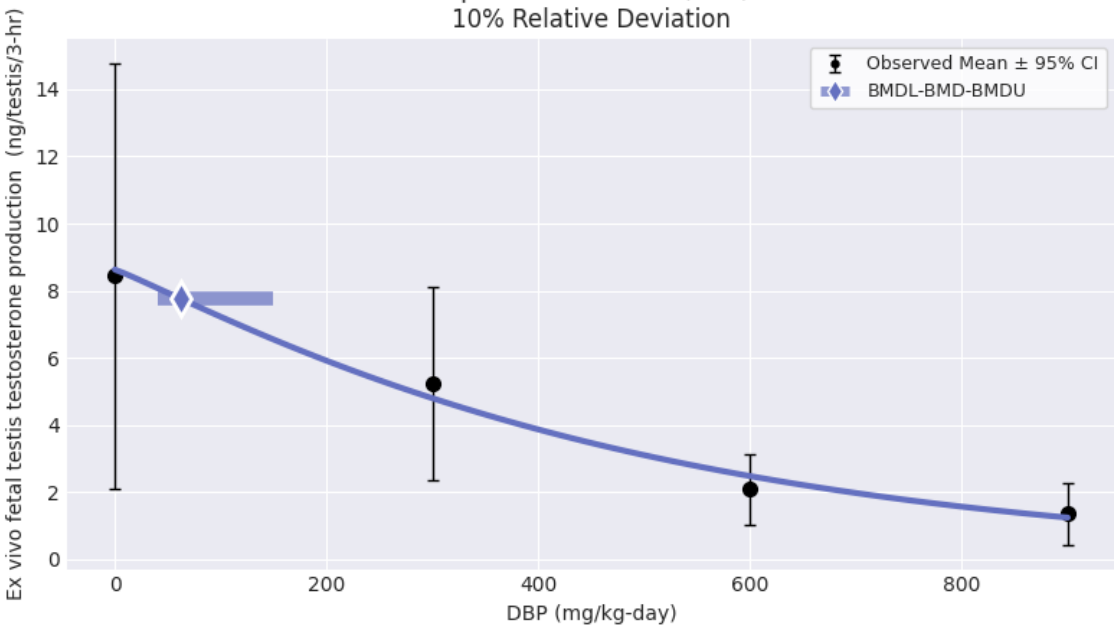
Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponentia 13	Restricted	Non-Constant	31.902	19.785	61.826	40.628	263.859	196.982	0.321	53.11	Questionable (BMR = 5%)  Viable - Lowest AIC (BMR = 10, 40%)	<u>BMR = 5%</u> Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0  <u>BMR = 5%</u> Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0  <u>BMR = 40%</u> No notes
Exponential 5	Restricted	Non-Constant	95.567	19.77	138.026	40.682	315.12	196.697	–	52.84	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	134.827	28.121	171.59	137.862	312.307	206.861	–	52.84	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Non-Constant	54.735	49.206	109.47	98.412	437.878	393.649	0.025	56.182	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Constant	54.733	49.206	109.466	98.413	437.865	393.65	0.025	56.182	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
Power	Restricted	Non-Constant	55.46	49.093	110.919	98.186	443.677	392.743	0.024	56.259	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-Constant	54.733	49.207	109.466	98.413	437.865	393.65	0.025	56.182	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1
AIC = Akaike information criterion; BMD = benchmark dose; BMDL =benchmark dose lower limit; NA = Not Applicable.												



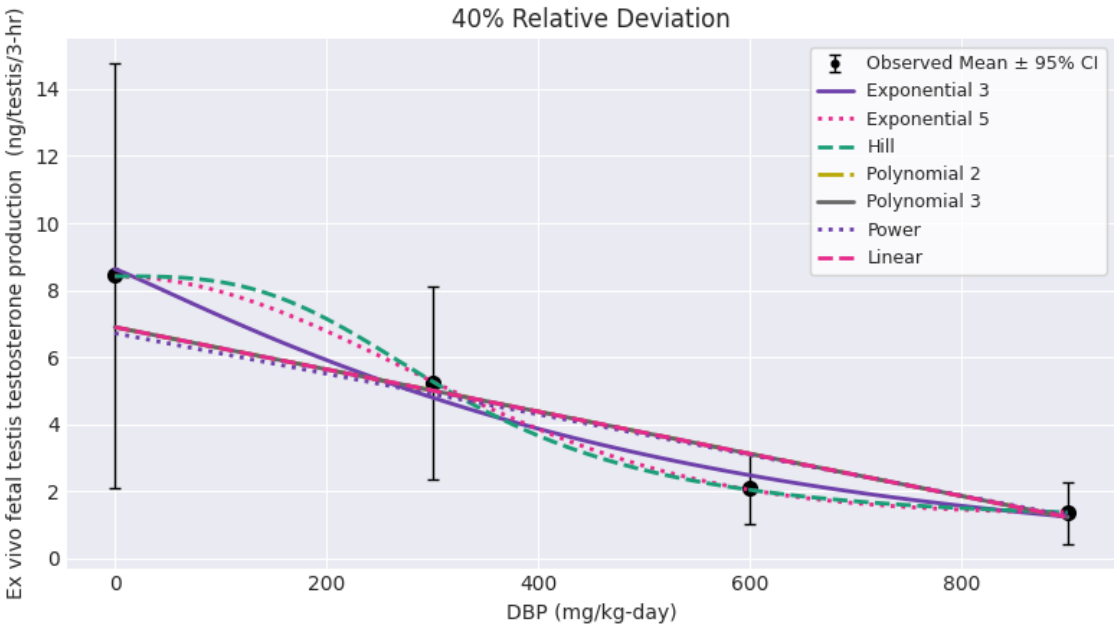
Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 70)  
MLE Models



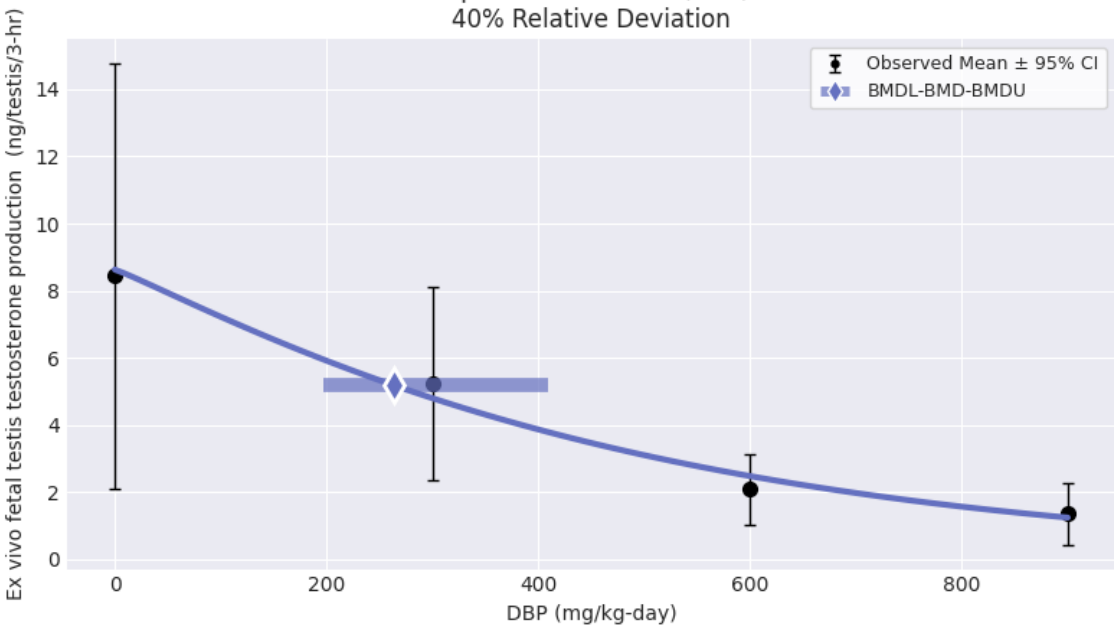
Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 70)  
Exponential 3 Model (MLE)



Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 70)  
MLE Models



Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 70)  
Exponential 3 Model (MLE)



### Exponential 3 Model

Version: pybmds 25.1 (bmdscore 25.1)

#### Input Summary:

BMR	40% Relative Deviation
Distribution	Normal + Nonconstant 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
rho	0	0	18
log-alpha	0	-18	18

#### Modeling Summary:

BMD	263.859
BMDL	196.982
BMDU	408.587
AIC	53.1101
Log-Likelihood	-21.555
P-Value	0.131931
Model d.f.	1

#### Model Parameters:

Variable	Estimate	On Bound	Std Error
a	8.62799	no	1.26185
b	0.00204396	no	0.000356143
d	1.0879	no	0.248186
rho	1.57704	no	0.593061
log-alpha	-1.77411	no	0.798655

Goodness of Fit:

Dose	N	Sample Mean	Model Fitted Mean	Scaled Residual
0	3	8.45011	8.62799	-0.136759
300	4	5.22558	4.79526	0.607062
600	4	2.083	2.4755	-0.932615
900	4	1.34858	1.23887	0.44995

Dose	N	Sample SD	Model Fitted SD
0	3	2.55	2.25288
300	4	1.81	1.41772
600	4	0.66	0.841718
900	4	0.58	0.487652

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-25.1599	5	60.3199
A2	-20.2904	8	56.5808
A3	-20.4202	6	52.8404
fitted	-21.555	5	53.1101
reduced	-37.6731	2	79.3463

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	34.7655	6	4.78461e-06
Test 2	9.73904	3	0.0209197
Test 3	0.259629	2	0.878258
Test 4	2.26963	1	0.131931

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.6.2 Block 71 Rat Data

---

**Table\_Apx F-20. *Ex Vivo* Fetal Testis Testosterone Production Data (Block 71) (Gray et al. 2021)**

<b>Dose (mg/kg-day)</b>	<b>N (# of litters)</b>	<b>Mean <i>Ex Vivo</i> Fetal Testis Testosterone Production (ng/mg testis-3-hr)</b>	<b>Standard Deviation</b>	<b>Notes</b>
0	4	9.925917	2.27	Data extracted from Supplemental Excel Files associated with Gray et al. (2021)
300	3	4.674222	0.93	
600	4	2.144167	0.43	
900	4	1.311	0.15	

**Table\_Apx F-21. *Ex Vivo* Fetal Testis Testosterone Production (Block 71) (Gray et al. 2021)**

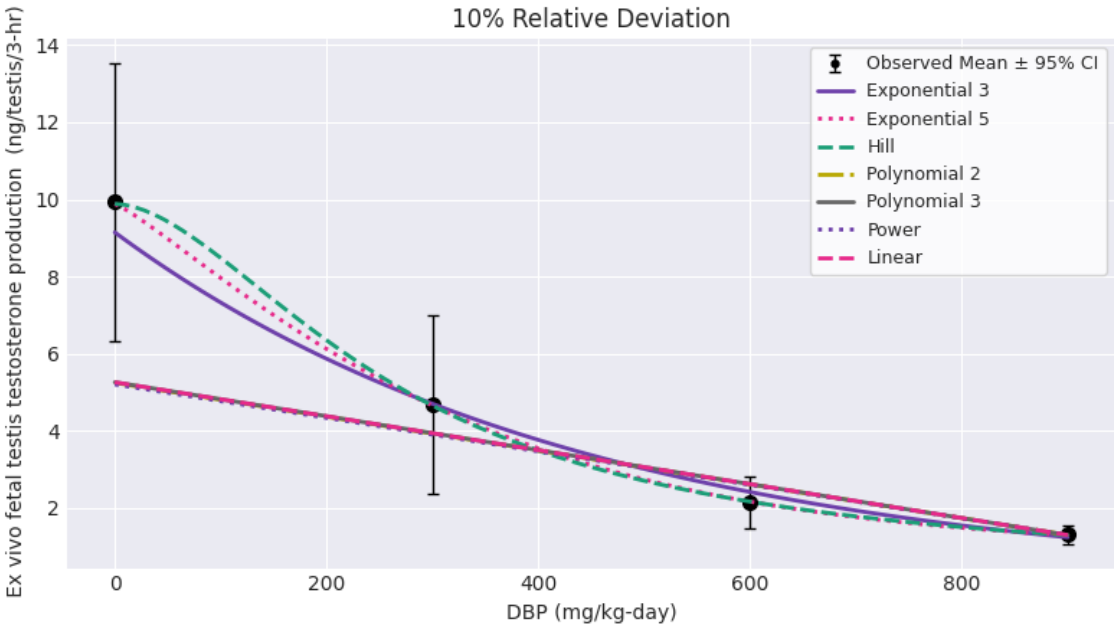
Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	20.846	16.929	42.818	34.773	207.599	168.594	0.972	51.378	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0 lowest dose/BMD ratio > 10.0 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	31.147	13.241	56.601	27.337	213.625	138.752	-	55.144	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 Constant variance test failed (Test 2 p-value < 0.05)
Hill	Restricted	Constant	56.672	9.116	84.713	19.276	224.296	114.273	-	55.144	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	46.501	41.234	93.002	82.467	372.006	329.87	0.005	61.63	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 3	Restricted	Constant	46.566	41.239	93.132	82.478	372.527	329.913	0.005	61.63	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	46.523	41.235	93.047	82.471	372.187	329.884	0.005	61.63	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1 Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	46.523	41.235	93.047	82.471	372.187	329.884	0.005	61.63	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 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	23.15	20.65	47.552	42.417	230.548	205.648	0.213	33.931	Questionable (BMR = 5%)  Viable – Lowest AIC (BMR = 10%, 40%)	<u>BMR = 5%</u> Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0 lowest dose/BMD ratio > 10.0  <u>BMR = 10%</u> Lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0  <u>BMR = 40%</u> None
Exponential 5	Restricted	Non-Constant	29.164	15.028	53.982	30.959	211.951	153.597	-	34.843	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
Hill	Restricted	Non-Constant	52.317	15.143	79.888	43.811	221.932	132.729	-	34.843	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	Non-Constant	59.896	55.812	119.793	108.792	479.171	435.168	<0.001	45.786	Questionable	Residual near BMD  > 2.0 lowest dose/BMDL ratio > 3.0 lowest dose/BMD 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	59.837	54.387	119.674	108.775	478.698	435.1	<0.001	45.786	Questionable	Residual near BMD  > 2.0 lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Non-Constant	60.064	54.429	120.127	108.906	480.509	435.404	<0.001	45.789	Questionable	Residual near BMD  > 2.0 lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 Residual at control > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5

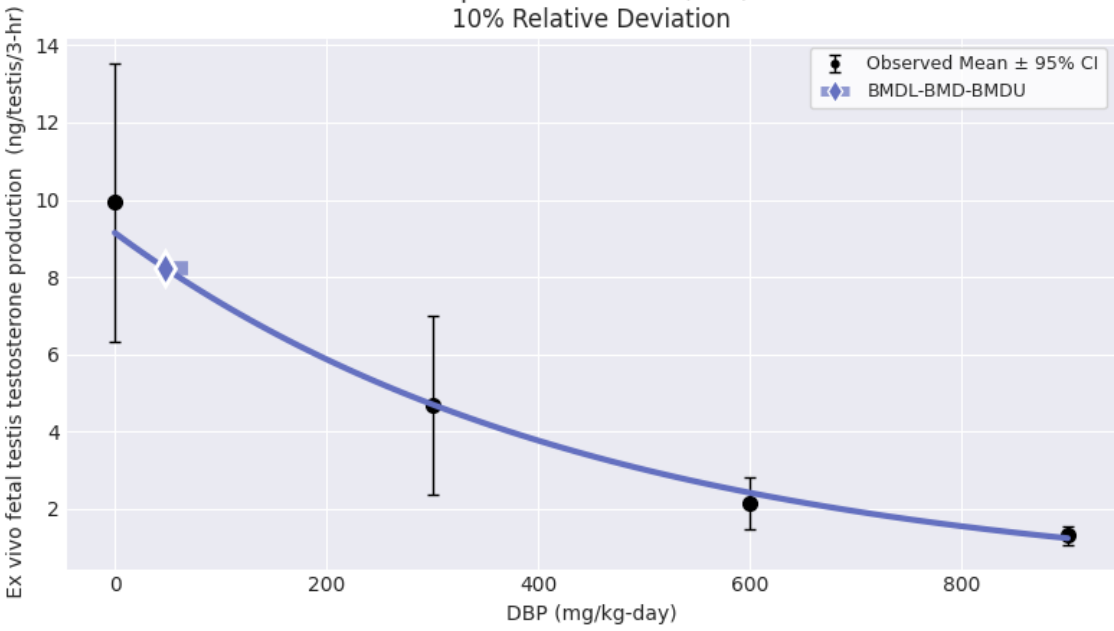


Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Linear	Unrestricted	Non-Constant	59.837	55.764	119.674	109.508	478.698	435.1	<0.001	45.786	Questionable	Residual near BMD  > 2.0 lowest dose/BMDL ratio > 3.0 lowest dose/BMD ratio > 3.0 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.												

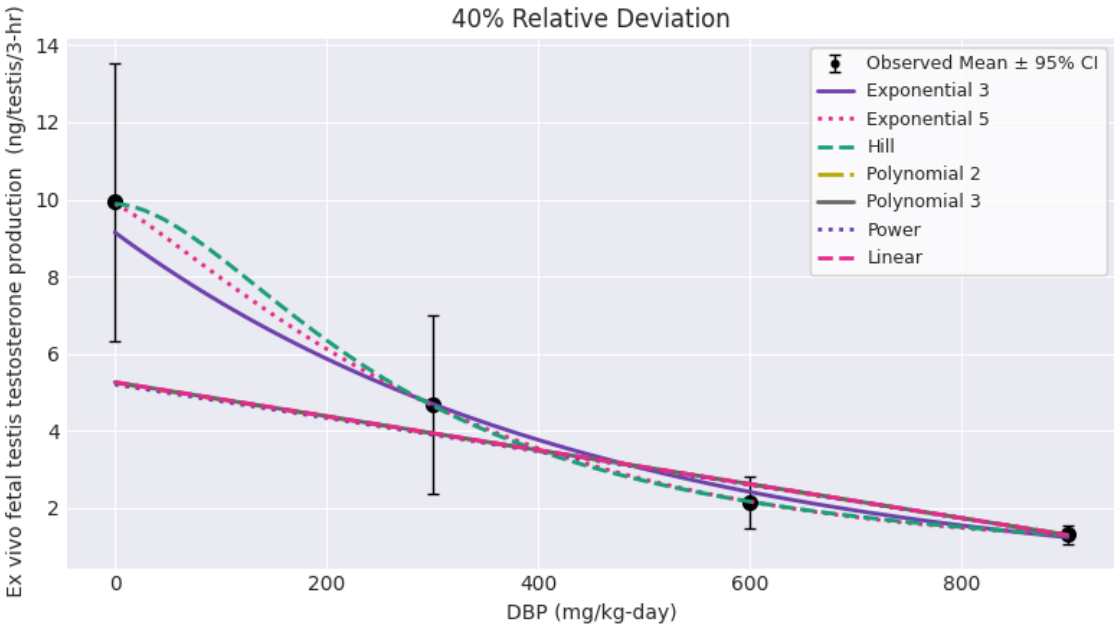
Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 71)  
MLE Models



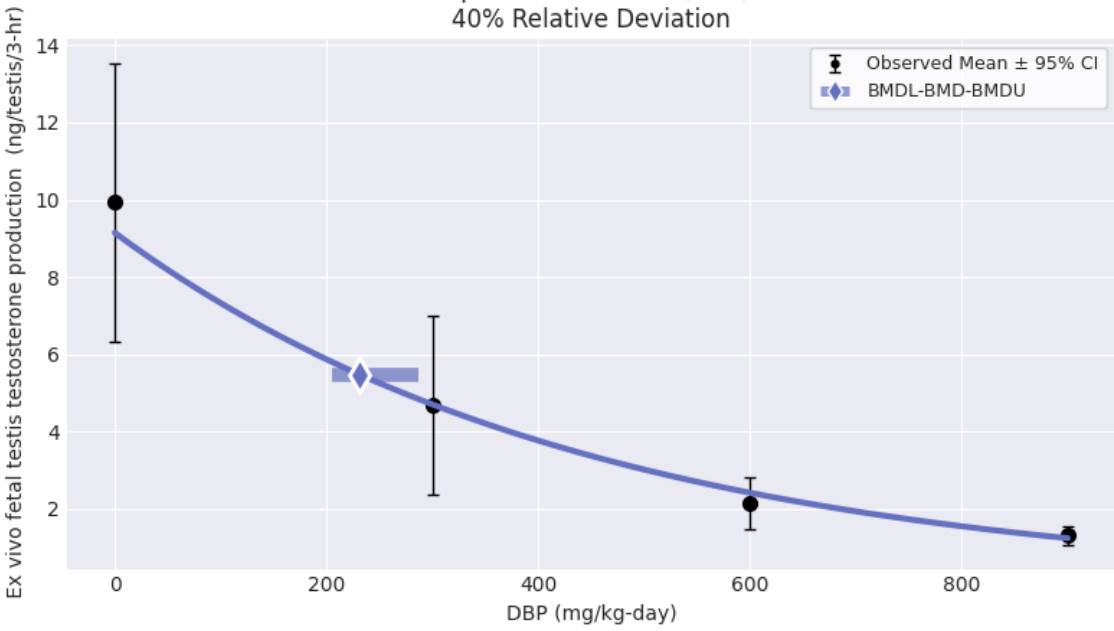
Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 71)  
Exponential 3 Model (MLE)



Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 71)  
MLE Models



Gray et al. (2021) Ex vivo fetal testis testosterone production (Block 71)  
Exponential 3 Model (MLE)



### Exponential 3 Model

Version: pybmds 25.1 (bmdscore 25.1)

#### Input Summary:

BMR	40% Relative Deviation
Distribution	Normal + Nonconstant 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
rho	0	0	18
log-alpha	0	-18	18

#### Modeling Summary:

BMD	230.548
BMDL	205.648
BMDU	286.707
AIC	33.9315
Log-Likelihood	-12.9657
P-Value	0.21349
Model d.f.	2

#### Model Parameters:

Variable	Estimate	On Bound	Std Error
a	9.14511	no	0.975202
b	0.00221571	no	0.000162934
d	1	yes	Not Reported
rho	2.56121	no	0.702311
log-alpha	-4.16819	no	0.884112

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	9.92592	9.14511	0.737532
300	3	4.67422	4.70444	-0.0579124
600	4	2.14417	2.42007	-1.43009
900	4	1.311	1.24494	0.802164

Dose	N	Sample SD	Model Fitted SD
0	4	2.27	2.11735
300	3	0.93	0.90387
600	4	0.43	0.385851
900	4	0.15	0.164715

Likelihoods:

Model	Log-Likelihood	# Params	AIC
A1	-22.5722	5	55.1444
A2	-11.0468	8	38.0937
A3	-11.4216	6	34.8431
fitted	-12.9657	4	33.9315
reduced	-40.6562	2	85.3125

Tests of Mean and Variance Fits:

Name	-2 * Log(Likelihood Ratio)	Test d.f.	P-Value
Test 1	59.2188	6	6.48555e-11
Test 2	23.0507	3	3.94117e-05
Test 3	0.749449	2	0.687479
Test 4	3.08833	2	0.21349

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.7 BMD Model Results – *Ex vivo* Fetal Testis Testosterone Production ([Furr et al., 2014](#))

---

### F.7.1 Block 18 Rat Data

---

Table\_Apx F-22. *Ex Vivo* Fetal Testis Testosterone Production Data (Block 18) (Furr et al. 2014)

Dose (mg/kg-day)	N (# of litters)	Mean <i>Ex Vivo</i> Fetal Testis Testosterone Production (ng/mg testis-3-hr)	Standard Deviation	Notes
0	3	9.9	0.68	Data extracted from Table 2 Furr et al. (2014)
33	3	3.13	1.35	
50	2	8.47	1.26	
100	3	6.46	2.70	
300	3	2.29	0.14	

**Table\_Apx F-23. *Ex Vivo* Fetal Testis Testosterone Production (Block 18) (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	12.483	5.817	25.642	11.948	124.32	57.93	<0.001	71.423	Questionable	Lowest dose/BMDL ratio > 3.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	10.131	3.266	20.941	6.923	107.703	31.31	<0.001	73.39	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Hill	Restricted	Constant	1.188	0.008	2.61	0.015	25.466	0.059	<0.001	72.484	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 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Polynomial Degree 2	Restricted	Constant	21.164	14.651	42.328	29.302	169.312	117.208	<0.001	71.66	Questionable	Residual near BMD  > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 3	Restricted	Constant	21.135	14.651	42.27	29.303	169.081	117.21	<0.001	71.66	Questionable	Residual near BMD  > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	21.142	14.651	42.283	29.302	169.133	117.21	<0.001	71.66	Questionable	Residual near BMD  > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 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				
Linear	Unrestricted	Constant	21.142	14.651	42.283	29.302	169.133	117.21	<0.001	71.66	Questionable	Residual near BMD  > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-Constant	58.138	13.692	85.023	27.051	195.688	119.58	0.04	59.78	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0
Exponential 5	Restricted	Non-Constant	58.138	13.691	85.023	27.051	195.688	119.58	0.011	61.78	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0
Hill	Restricted	Non-Constant	73.244	69.652	106.136	85.708	224.545	142.487	0.011	61.85	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 2	Restricted	Non-Constant	42.886	19.801	76.291	39.637	211.047	158.473	0.043	59.665	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 3	Restricted	Constant	38.967	19.816	75.205	41.167	220.543	158.806	0.099	57.628	Questionable	Residual near BMD  > 2.0 Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Non-Constant	64.105	19.723	96.979	62.316	221.945	157.779	0.037	59.934	Questionable	Nonconstant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0
Linear	Unrestricted	Non-Constant	21.237	19.805	42.473	40.532	169.894	157.431	0.075	58.262	Questionable	Residual near BMD  > 2.0 Nonconstant variance test failed (Test 3 p-value < 0.05) 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.7.2 Block 22 Rat Data

---

**Table\_Apx F-24. *Ex Vivo* Fetal Testis Testosterone Production Data (Block 22) (Gray et al. 2021)**

<b>Dose (mg/kg-day)</b>	<b>N (# of litters)</b>	<b>Mean <i>Ex Vivo</i> Fetal Testis Testosterone Production (ng/mg testis-3-hr)</b>	<b>Standard Deviation</b>	<b>Notes</b>
0	3	9.41	0.21	Data extracted from Table 2 Furr et al. (2014)
1	3	8.29	1.06	
10	4	7.49	1.40	
100	4	6.02	2.06	

**Table\_Apx F-25. *Ex Vivo* Fetal Testis Testosterone Production (Block 22) (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	14.334	8.503	29.443	17.466	142.748	84.679	0.247	54.166	Questionable	Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	2.157	0.183	4.74	0.403	–	–	0.367	54.184	Questionable	Lowest dose/BMDL ratio > 3.0 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Hill	Restricted	Constant	1.457	0.074	3.468	0.18	-97.319	–	0.408	54.056	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3.0
Polynomial Degree 2	Restricted	Constant	16.927	11.212	33.854	22.423	135.416	89.693	0.232	54.289	Questionable	Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 3	Restricted	Constant	16.937	11.211	33.874	22.423	135.496	89.692	0.232	54.289	Questionable	Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	16.932	11.212	33.863	22.423	135.454	89.693	0.232	54.289	Questionable	Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	16.932	11.212	33.863	22.423	135.454	89.693	0.232	54.289	Questionable	Control stdev. fit > 1.5 Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-Constant	14.345	8.507	29.466	17.475	142.86	84.725	0.021	54.16	Questionable	Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Exponential 5	Restricted	Non-Constant	2.157	0.183	4.74	0.403	-	-	0.008	54.184	Questionable	Lowest dose/BMDL ratio > 3.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0
Hill	Restricted	Non-Constant	0.255	0.067	0.633	0.184	-5.748	-	0.184	48.408	Questionable	Lowest dose/BMDL ratio > 3.0 lowest dose/BMDL ratio > 10.0 lowest dose/BMD ratio > 3.0 Control stdev. fit > 1.5 BMD/BMDL ratio > 3.0

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Polynomial Degree 2	Restricted	Non-Constant	16.932	11.212	33.864	22.423	135.455	89.692	0.009	54.289	Questionable	Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Polynomial Degree 3	Restricted	Constant	16.932	11.212	33.863	22.423	135.454	89.693	0.009	54.289	Questionable	Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Power	Restricted	Non-Constant	16.932	11.212	33.863	22.423	135.454	89.693	0.009	54.289	Questionable	Goodness of fit p-value < 0.1 Control stdev. fit > 1.5
Linear	Unrestricted	Non-Constant	16.932	11.212	33.863	22.423	135.454	89.693	0.009	54.289	Questionable	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.7.3 Block 26 Rat Data

---

**Table\_Apx F-26. *Ex Vivo* Fetal Testis Testosterone Production Data (Block 26) (Gray et al. 2021)**

<b>Dose (mg/kg-day)</b>	<b>N (# of litters)</b>	<b>Mean <i>ex vivo</i> Fetal Testis Testosterone Production (ng/mg testis/3-hr)</b>	<b>Standard Deviation</b>	<b>Notes</b>
0	3	4.75	0.83	Data extracted from Table 2 Furr et al. (2014)
1	4	7.6	0.52	
10	4	5.64	0.58	
100	3	3.58	1.30	

**Table\_Apx F-27. *Ex Vivo* Fetal Testis Testosterone Production (Block 26) (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	985.638	45.939	1023.668	89.744	1099.339	106.724	<0.001	61.328	Questionable	Residual near BMD  > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/highest dose ratio > 1.0 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Exponential 5	Restricted	Constant	–	–	–	–	–	–	–	63.328	Unusable	Did not successfully execute.
Hill	Restricted	Constant	–	–	–	–	–	–	–	55.071	Unusable	Did not successfully execute.
Polynomial Degree 2	Restricted	Constant	–	–	–	–	–	–	<0.001	57.328	Unusable	Did not successfully execute.
Polynomial Degree 3	Restricted	Constant	–	–	–	–	–	–	<0.001	57.328	Unusable	Did not successfully execute.
Power	Restricted	Constant	–	–	–	–	–	–	<0.001	51.652	Unusable	Did not successfully execute.
Linear	Unrestricted	Constant	11.669	7.904	23.338	15.808	93.351	–	<0.001	51.652	Questionable	Residual at control > 2.0 Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-Constant	14.497	1742.054	1813.065	28.32	1954.788	89.558	<0.001	63.328	Questionable	Residual near BMD  > 2.0 Goodness of fit p-value < 0.1 Control stdev. fit > 1.5 BMD/highest dose ratio > 1.0 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Exponential 5	Restricted	Non-Constant	13.032	1221.279	1294.405	10.301	–	–	–	65.328	Questionable	Residual near BMD  > 2.0 Zero degrees of freedom; saturated model Control stdev. fit > 1.5 BMD/highest dose ratio > 1.0 BMD/BMDL ratio > 3.0 BMD/BMDL ratio > 20.0
Hill	Restricted	Non-Constant	–	–	–	–	–	–	–	56.567	Unusable	Did not successfully execute.
Polynomial Degree 2	Restricted	Non-Constant	–	–	–	–	–	–	<0.001	59.328	Unusable	Did not successfully execute.
Polynomial Degree 3	Restricted	Constant	–	–	–	–	–	–	<0.001	59.328	Unusable	Did not successfully execute.

Models	Restriction	Variance	BMR = 5%		BMR = 10%		BMR = 40%		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL				
Power	Restricted	Non-Constant	–	-11.726	-23.452	-	-93.808	–	<0.001	53.4	Unusable	Did not successfully execute.
Linear	Unrestricted	Non-Constant	8.037	11.723	23.446	16.075	93.783	–	<0.001	53.4	Questionable	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.												

## Appendix G Benchmark Dose (BMD) Analysis of Male Pup Nipple Retention ([Mylchreest et al., 2000](#))

EPA conducted benchmark dose (BMD) modeling of the most sensitive effect observed in the gestational exposure study of DBP with Sprague-Dawley rats reported by Mylchreest et al. (2000). Briefly, F1 male rats gestationally exposed to DBP (*i.e.*, GDs 12–21) were analyzed for the incidence of nipple/areolae development and retention on PND 14 (see Figure 2 of Mylchreest et al.). Incidences of nipple retention were counted and aggregated across litters (Table\_Apx G-1). However, information of incidences at the individual or at the litter level was not reported by study authors. Therefore, the Rao-Scott (R-S) transformation of the incidences would be applied to account for intralitter correlation, as described by Fox et al. (2016). Table\_Apx G-1 presents the original incidence data, as well as the R-S transformed incidence data. The R-S transformation was done using EPA’s BMD Online (Version 25.1, <https://bmdsonline.epa.gov/>).

**Table\_Apx G-1. Original and Rao-Scott Adjusted Incidence of Nipples and/or Areolae in F1 Male Rats (Mylchreest et al. 2000)**

DBP Dose (mg/kg-d)	N (# pups examined)	Incidence of Nipples and/or Areolae	Fraction Affected	Design Effect (LS)	Design Effect (OR)	Design Effect (Average)	N (Rao-Scott Transformed) <sup>a</sup>	Incidence (Rao-Scott Transformed) <sup>a</sup>
0	134	9	0.067	2.348	2.437	2.393	56.007	3.762
0.5	119	8	0.067	2.349	2.438	2.393	49.722	3.343
5	103	13	0.126	2.894	3.075	2.985	34.511	4.356
50	120	12	0.1	2.679	2.822	2.751	43.626	4.363
100	141	44	0.312	3.904	4.295	4.1	34.392	10.732
500	58	52	0.897	5.537	6.34	5.939	9.767	8.756

<sup>a</sup> Rao-Scott transformation parameters: A = 1.685, b = 0.331, sigma = 0.125 (Least Squares Regression method); A = 1.833, b = 0.369, sigma = 0.109 (Orthogonal Regression method)

All BMD modeling was conducted using EPA’s BMD Online (Version 25.1). All standard dichotomous 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](#)). Bayesian model averaging was also applied in this work.

### Standard BMDS Online Version 25.1 Models Applied

- Dichotomous Hill – restricted
- Gamma – restricted
- Logistic – unrestricted
- Log-Logistic – restricted
- Log Probit – unrestricted
- Probit – unrestricted
- Multistage (degrees 1, 2, and 3) – restricted
- Quantal Linear – unrestricted



- Weibull – restricted

EPA evaluated benchmark response (BMR) levels of 5 percent extra risk (ER) and 10 percent ER. A BMR of 5 percent ER was selected for F1 male pup nipple retention because this effect is considered an adverse developmental effect that is mechanistically linked to decreased fetal testicular testosterone content and/or *ex vivo* fetal testosterone production (as outlined in Appendix E, EPA has also concluded that a BMR of 5% is the most appropriate response level for evaluated decreased fetal testicular testosterone content and/or *ex vivo* fetal testosterone production). The preferred model for the BMD derivations was chosen from the standard set of dichotomous models listed above. The modeling restrictions and model selection criteria facilitated in BMD Online Version 25.1, and defined in the BMD User Guide, were applied in accordance with EPA BMD Technical Guidance ([U.S. EPA, 2012a](#)).

Table\_Apx G-2 summarizes BMD modeling results for reduced fetal testicular testosterone content and/or *ex vivo* testosterone production, while more detailed BMD model results are reported in Appendices G.1 through G.4.

**Table\_Apx G-2. Summary of BMD Model Results for F1 Male Nipple/Areolae Retention (Mylchreest et al. 2000)**

Dataset	Best-fitting Model	BMR	BMD (mg/kg-day)	BMDL (mg/kg-day)	Appendix Containing Results
Rao-Scott Transformed Incidence Data	Log-Probit	5%	51	29	G.1
	Log-Probit	10%	67	44	
	Bayesian model average	5%	33	15	G.2
	Bayesian model average	10%	59	30	
Original Incidence Data	Log-Probit	5%	49	38	G.3
	Log-Probit	10%	66	53	
	Bayesian model average	5%	37	18	G.4
	Bayesian model average	10%	59	36	

## G.1 BMD Model Results – Nipple/Areolae Retention (Frequentist, Rao-Scott Transformed Data)

### G.1.1 BMD Model Results for BMR of 10%

#### Dataset

**Name:** Nipple retention in F1 male rats (Rao-Scott transformed)

Dose (mg/kg-day)	N	Incidence
0	56.007	3.762
0.5	49.722	3.343
5	34.511	4.356
50	43.626	4.363
100	34.392	10.732
500	9.767	8.756

#### Settings

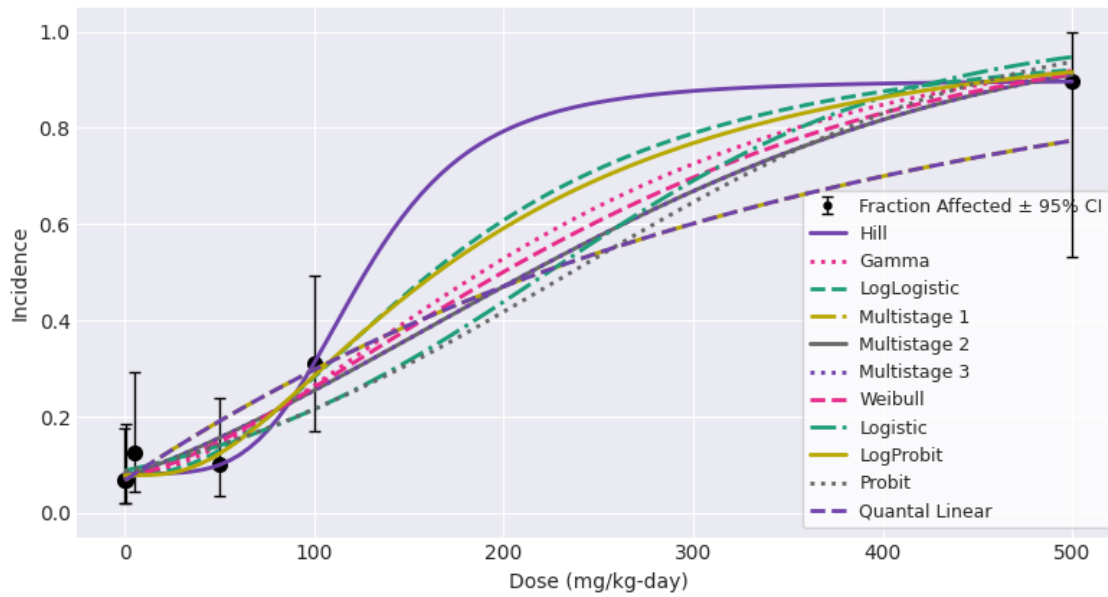
Setting	Value
BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

#### Maximum Likelihood Approach

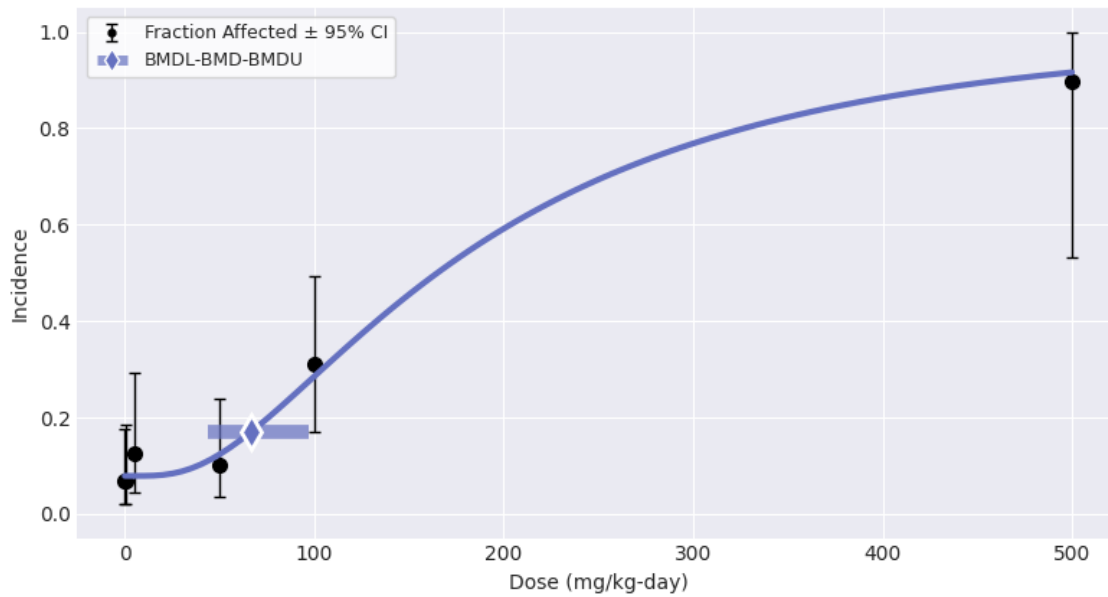
Model	BMDL	BMD	BMDU	P-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	43.649	75.877	98.575	0.547	164.918	-0.397	<0.001	Viable
Gamma	32.216	63.206	102.121	0.515	163.988	-0.294	-0.765	Viable
LogLogistic	40.534	65.731	98.211	0.604	163.524	-0.298	-0.591	Viable
Multistage 1	24.974	37.21	60.543	0.384	164.39	-0.017	-1.527	Viable
Multistage 2	29.434	55.718	107.136	0.445	164.465	-0.206	-1.025	Viable
Multistage 3	29.119	55.718	107.244	0.445	164.465	-0.206	-1.025	Viable
Weibull	30.907	59.798	104.236	0.479	164.217	-0.262	-0.899	Viable
Logistic	55.144	77.984	112.233	0.401	163.638	-0.553	1.374	Viable
LogProbit <sup>a</sup>	43.723	66.893	97.403	0.646	163.334	-0.323	-0.47	Recommended - Lowest AIC
Probit	56.998	75.976	104.662	0.437	163.424	-0.503	1.37	Viable
Quantal Linear	24.974	37.21	60.543	0.384	164.39	-0.017	-1.527	Viable

<sup>a</sup> BMDS recommended best fitting model

Nipple retention in F1 male rats (Rao-Scott transformed)  
MLE Models  
10% Extra Risk



Nipple retention in F1 male rats (Rao-Scott transformed)  
LogProbit Model (MLE)  
10% Extra Risk



# LogProbit Model

Version: pybmds 25.1 (bmdscore 25.1)

## Input Summary:

BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Modeling approach	frequentist_unrestricted

## Parameter Settings:

Parameter	Initial	Min	Max
g	0	-18	18
a	0	-18	18
b	0.0001	0.0001	18

## Modeling Summary:

BMD	66.8935
BMDL	43.7233
BMDU	97.4032
AIC	163.334
Log-Likelihood	-78.6671
P-Value	0.646447
Overall d.f.	3
Chi <sup>2</sup>	1.6574

## Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.078774	no	0.0186937
a	-6.74818	no	1.55883
b	1.30062	no	0.325412

## Goodness of Fit:

Dose	Size	Observed	Expected	Est Prob	Scaled Residual
0	56.0074	3.7617	4.41193	0.078774	-0.322528
0.5	49.7217	3.3426	3.91678	0.078774	-0.302272
5	34.5113	4.3558	2.71864	0.0787755	1.0345
50	43.6262	4.3626	5.38354	0.123402	-0.469967
100	34.392	10.7323	9.80747	0.285167	0.349287
500	9.7666	8.7563	8.94789	0.916173	-0.221221

## Analysis of Deviance:

Model	Log-Likelihood	# Params	Deviance	Test d.f.	P-Value
Full model	-77.9058	6	-	-	-
Fitted model	-78.6671	3	1.52261	3	0.677062
Reduced model	-98.2885	1	40.7654	5	1.04643e-07

## G.1.2 BMD Model Results for BMR of 5%

### Dataset

**Name:** Nipple retention in F1 male rats (Rao-Scott transformed)

Dose (mg/kg-day)	N	Incidence
0	56.007	3.762
0.5	49.722	3.343
5	34.511	4.356
50	43.626	4.363
100	34.392	10.732
500	9.767	8.756

### Settings

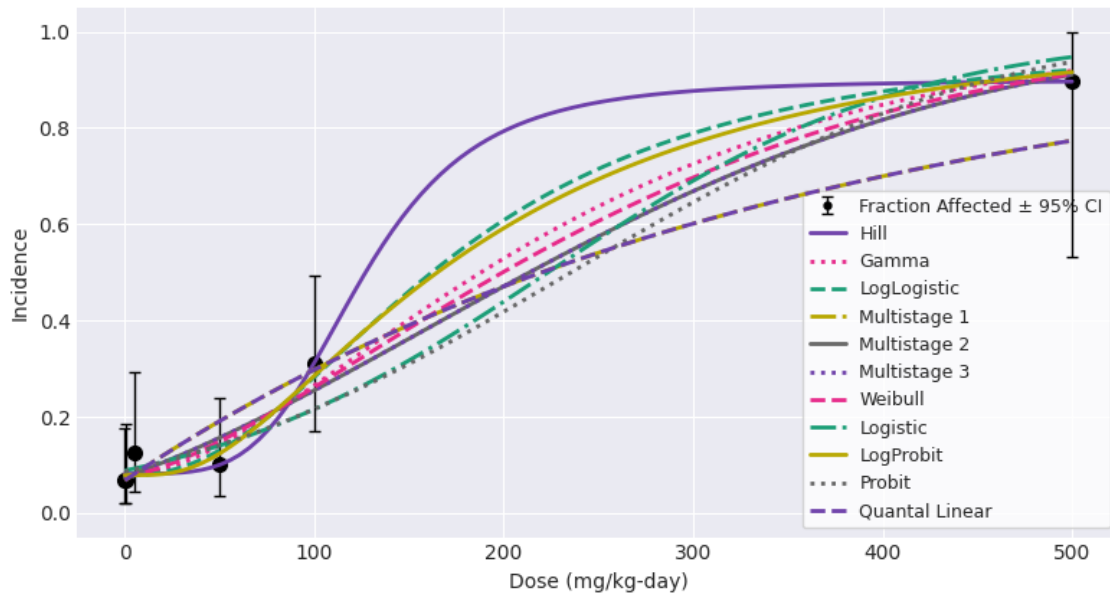
Setting	Value
BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

### Maximum Likelihood Approach

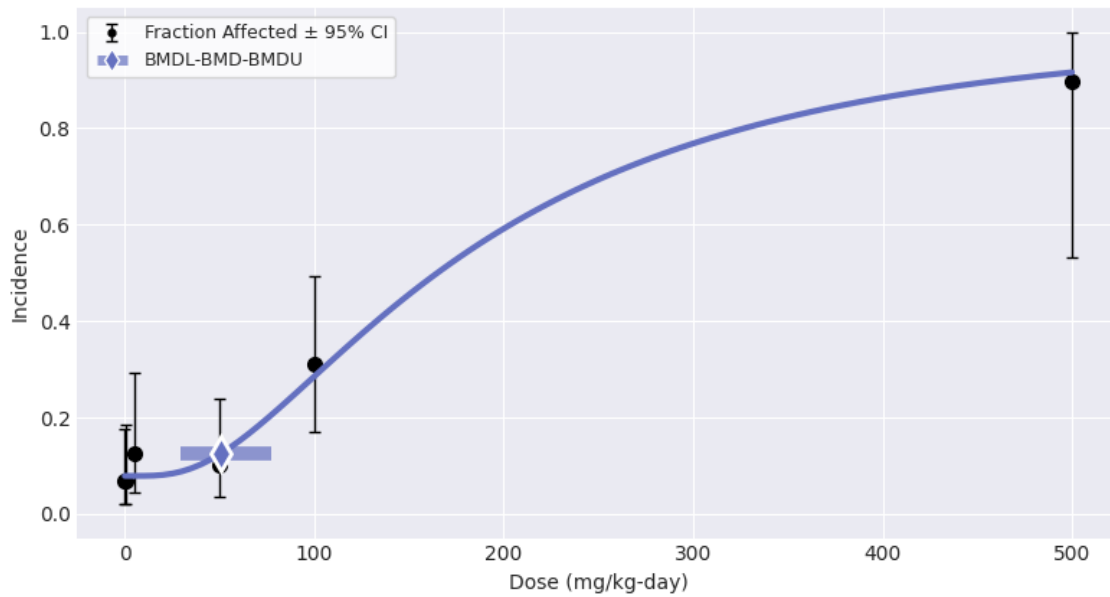
Model	BMDL	BMD	BMDU	P-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	26.739	63.119	92.737	0.547	164.918	-0.397	-0.	Viable
Gamma	15.731	41.824	75.601	0.515	163.988	-0.294	-0.765	Viable
LogLogistic	23.989	47.136	75.021	0.604	163.524	-0.298	-0.591	Viable
Multistage 1	12.158	18.115	29.475	0.384	164.39	-0.017	0.977	Viable
Multistage 2	14.383	29.691	74.518	0.445	164.465	-0.206	-1.025	Viable
Multistage 3	14.184	29.691	74.517	0.445	164.465	-0.206	-1.025	Viable
Weibull	15.053	36.553	72.144	0.479	164.217	-0.262	-0.899	Viable
Logistic	31.446	44.769	64.78	0.401	163.638	-0.553	-0.764	Viable
<b>LogProbit<sup>a</sup></b>	<b>29.105</b>	<b>50.591</b>	<b>77.498</b>	<b>0.646</b>	<b>163.334</b>	<b>-0.323</b>	<b>-0.47</b>	<b>Recommended - Lowest AIC</b>
Probit	31.677	42.675	59.064	0.437	163.424	-0.503	-0.778	Viable
Quantal Linear	12.158	18.115	29.475	0.384	164.39	-0.017	0.977	Viable

<sup>a</sup> BMDS recommended best fitting model

Nipple retention in F1 male rats (Rao-Scott transformed)  
MLE Models  
5% Extra Risk



Nipple retention in F1 male rats (Rao-Scott transformed)  
LogProbit Model (MLE)  
5% Extra Risk



## LogProbit Model

Version: pybmds 25.1 (bmdscore 25.1)

### Input Summary:

BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Modeling approach	frequentist_unrestricted

### Parameter Settings:

Parameter	Initial	Min	Max
g	0	-18	18
a	0	-18	18
b	0.0001	0.0001	18

### Modeling Summary:

BMD	50.5909
BMDL	29.1046
BMDU	77.498
AIC	163.334
Log-Likelihood	-78.6671
P-Value	0.646447
Overall d.f.	3
Chi <sup>2</sup>	1.6574

### Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.078774	no	0.0186937
a	-6.74818	no	1.55883
b	1.30062	no	0.325412

### Goodness of Fit:

Dose	Size	Observed	Expected	Est Prob	Scaled Residual
0	56.0074	3.7617	4.41193	0.078774	-0.322528
0.5	49.7217	3.3426	3.91678	0.078774	-0.302272
5	34.5113	4.3558	2.71864	0.0787755	1.0345
50	43.6262	4.3626	5.38354	0.123402	-0.469967
100	34.392	10.7323	9.80747	0.285167	0.349287
500	9.7666	8.7563	8.94789	0.916173	-0.221221

### Analysis of Deviance:

Model	Log-Likelihood	# Params	Deviance	Test d.f.	P-Value
Full model	-77.9058	6	-	-	-
Fitted model	-78.6671	3	1.52261	3	0.677062
Reduced model	-98.2885	1	40.7654	5	1.04643e-07

## G.2 BMD Model Results – Nipple/Areolae Retention (Bayesian Model Averaging, Rao-Scott Transformed Data)

### G.2.1 BMD Results for BMR of 10%

#### Dataset

**Name:** Nipple retention in F1 male rats (Rao-Scott transformed)

Dose (mg/kg-day)	N	Incidence
0	56.007	3.762
0.5	49.722	3.343
5	34.511	4.356
50	43.626	4.363
100	34.392	10.732
500	9.767	8.756

#### Settings

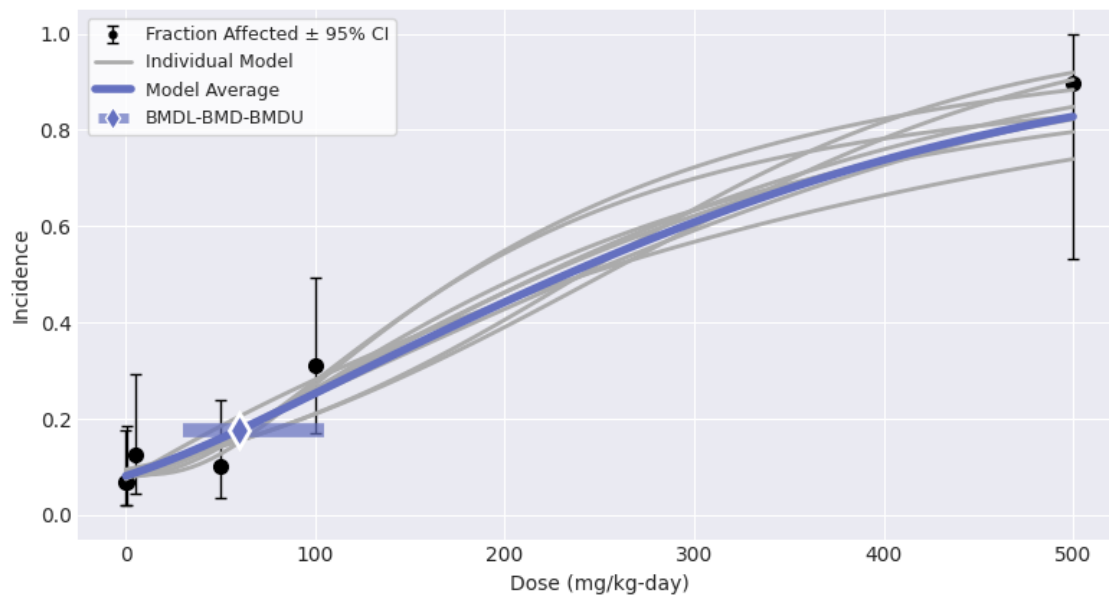
Setting	Value
BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

#### Bayesian Summary

Model	Prior Weights	Posterior Weights	BMDL	BMD	BMDU	Unnormalized Log Posterior Probability	Scaled Residual near BMD	Scaled Residual at Control
Hill	0.111	0.036	37.62	62.583	96.889	-88.774	-0.344	-0.779
Gamma	0.111	0.066	30.614	57.258	96.108	-78.333	-0.353	-1.039
Logistic	0.111	0.091	58.246	82.812	119.826	-79.826	-0.699	1.459
LogLogistic	0.111	0.056	34.64	62.036	103.614	-83.802	-0.397	-0.893
LogProbit	0.111	0.048	45.621	68.878	103.424	-82.213	-0.446	-0.534
Multistage 2	0.111	0.295	31.337	55.209	104.972	-83.721	-0.325	-1.111
Probit	0.111	0.117	60.047	80.726	113.408	-76.843	-0.617	1.471
Quantal Linear	0.111	0.221	27.483	41.525	68.982	-77.241	-0.199	-1.438
Weibull	0.111	0.07	29.402	58.178	103.611	-83.867	-0.356	-1.021
Model Average	-	-	30.209	59.44	104.946	-	-	-



Nipple retention in F1 male rats (Rao-Scott transformed)  
 Bayesian Model Average  
 10% Extra Risk



## G.2.1 BMD Results for BMR of 5%

### Dataset

**Name:** Nipple retention in F1 male rats (Rao-Scott transformed)

Dose (mg/kg-day)	N	Incidence
0	56.007	3.762
0.5	49.722	3.343
5	34.511	4.356
50	43.626	4.363
100	34.392	10.732
500	9.767	8.756

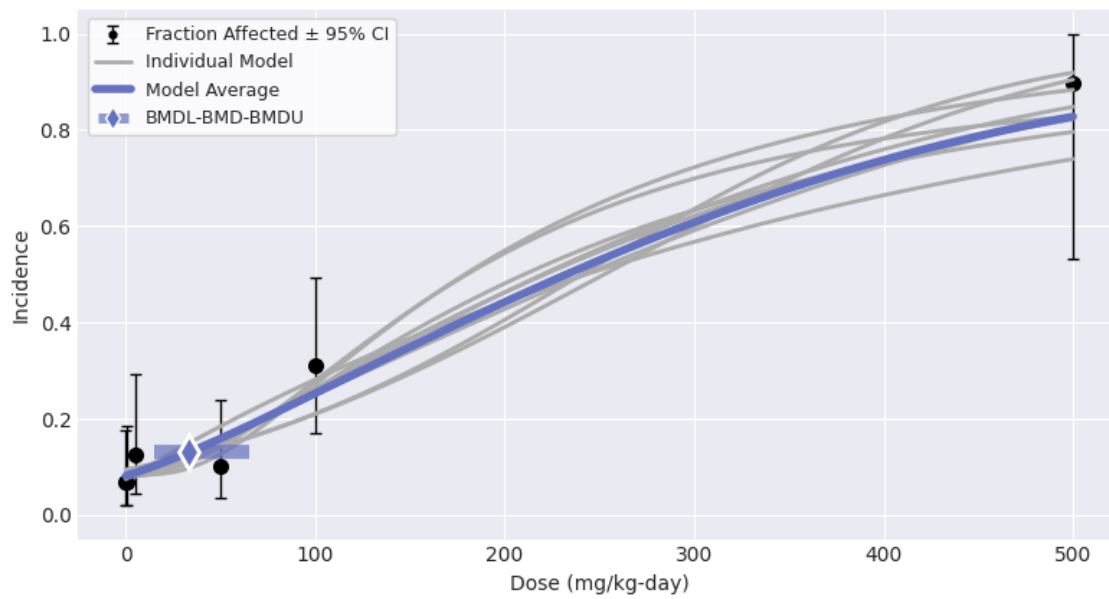
### Settings

Setting	Value
BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

### Bayesian Summary

Model	Prior Weights	Posterior Weights	BMDL	BMD	BMDU	Unnormalized Log Posterior Probability	Scaled Residual near BMD	Scaled Residual at Control
Hill	0.111	0.036	21.255	42.75	70.531	-88.774	-0.344	-0.779
Gamma	0.111	0.066	14.138	33.66	63.04	-78.333	-0.353	-1.039
Logistic	0.111	0.091	32.97	47.162	68.489	-79.826	-0.699	-0.811
LogLogistic	0.111	0.056	18.18	39.48	72.378	-83.802	-0.397	-0.893
LogProbit	0.111	0.048	30.755	51.161	80.562	-82.213	-0.446	-0.534
Multistage 2	0.111	0.295	15.364	28.184	64.011	-83.721	-0.325	-1.111
Probit	0.111	0.117	33.234	45.097	63.483	-76.843	-0.617	-0.796
Quantal Linear	0.111	0.221	13.38	20.216	33.583	-77.241	-0.199	0.848
Weibull	0.111	0.07	13.38	33.722	68.472	-83.867	-0.356	-1.021
Model Average	-	-	14.729	33.098	64.892	-	-	-

Nipple retention in F1 male rats (Rao-Scott transformed)  
 Bayesian Model Average  
 5% Extra Risk



### G.3 BMD Model Results – Nipple/Areolae Retention (Frequentist, Original Data)

---

#### G.3.1 BMD Model Results for BMR of 10%

---

##### Dataset

**Name:** Nipple retention in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	134	9
0.5	119	8
5	103	13
50	120	12
100	141	44
500	58	52

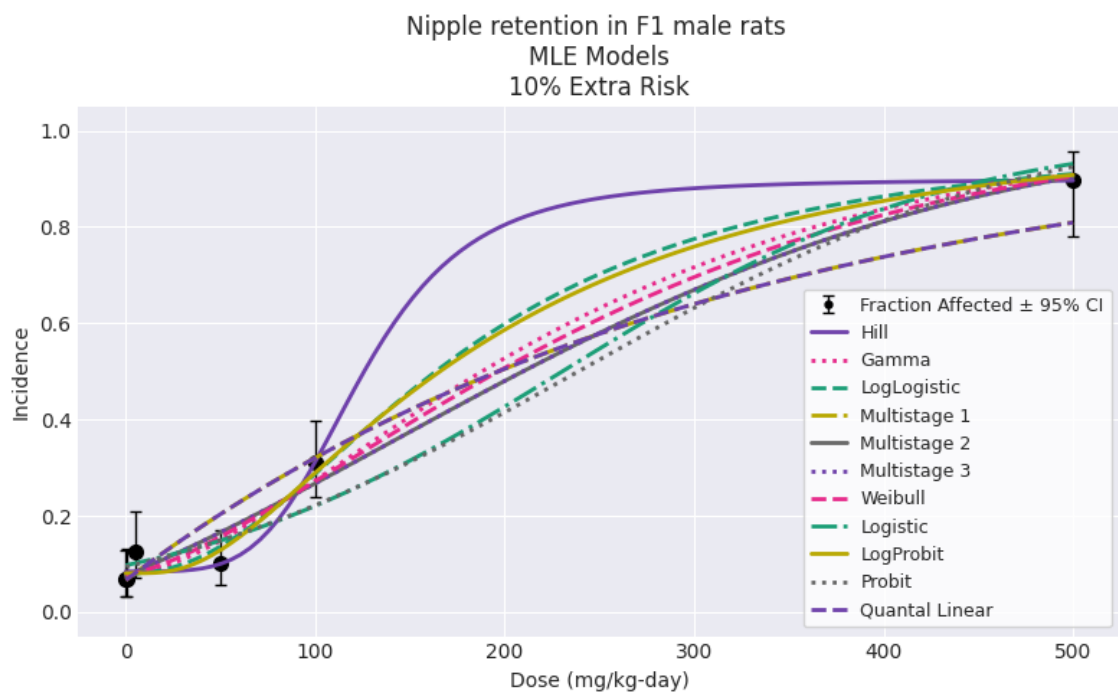
## Settings

Setting	Value
BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

## Maximum Likelihood Approach

Model	BMDL	BMD	BMDU	P-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	56.99	77.068	94.677	0.192	505.47	-0.713	<0.001	<b>Viable</b>
Gamma	43.417	60.043	78.709	0.078	507.032	-0.516	-1.491	<b>Questionable</b> Goodness of fit p-value < 0.1
LogLogistic	50.483	63.822	78.928	0.143	505.526	-0.526	-1.158	<b>Viable</b>
Multistage 1	27.382	33.17	40.844	0.008	513.517	0.042	-2.814	<b>Questionable</b>  Residual near BMD  > 2.0 Goodness of fit p-value < 0.1
Multistage 2	35.967	51.128	74.588	0.048	508.454	-0.354	-1.931	<b>Questionable</b> Goodness of fit p-value < 0.1
Multistage 3	35.326	51.127	74.589	0.048	508.454	-0.354	-1.931	<b>Questionable</b> Goodness of fit p-value < 0.1
Weibull	40.512	56.381	76.25	0.062	507.666	-0.459	-1.698	<b>Questionable</b> Goodness of fit p-value < 0.1
Logistic	67.113	78.842	92.56	0.009	511.684	-1.17	2.62	<b>Questionable</b>  Residual near BMD  > 2.0 Goodness of fit p-value < 0.1
LogProbit <sup>a</sup>	52.847	65.532	80.073	0.184	504.915	-0.57	-0.938	<b>Recommended - Lowest AIC</b>
Probit	66.35	75.978	87.317	0.013	510.801	-1.06	2.578	<b>Questionable</b>  Residual near BMD  > 2.0 Goodness of fit p-value < 0.1
Quantal Linear	27.382	33.17	40.843	0.008	513.517	0.042	-2.814	<b>Questionable</b>  Residual near BMD  > 2.0 Goodness of fit p-value < 0.1

<sup>a</sup> BMDS recommended best fitting model



### G.3.2 BMD Model Results for BMR of 5%

#### Dataset

**Name:** Nipple retention in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	134	9
0.5	119	8
5	103	13
50	120	12
100	141	44
500	58	52

## Settings

Setting	Value
BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

## Maximum Likelihood Approach

Model	BMDL	BMD	BMDU	P-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	39.201	64.665	88.726	0.192	505.47	-0.713	-0.	Viable
Gamma	24.908	38.808	54.909	0.078	507.032	-0.516	-1.491	Questionable Goodness of fit p-value < 0.1
LogLogistic	33.21	45.081	58.318	0.143	505.526	-0.526	-1.158	Viable
Multistage 1	13.33	16.148	19.884	0.008	513.517	0.042	1.683	Questionable Goodness of fit p-value < 0.1
Multistage 2	17.834	26.631	43.71	0.048	508.454	-0.354	1.565	Questionable Goodness of fit p-value < 0.1
Multistage 3	17.229	26.631	43.711	0.048	508.454	-0.354	1.565	Questionable Goodness of fit p-value < 0.1
Weibull	22.073	33.816	49.217	0.062	507.666	-0.459	-1.698	Questionable Goodness of fit p-value < 0.1
Logistic	37.825	44.765	52.965	0.009	511.684	-1.17	-1.495	Questionable Goodness of fit p-value < 0.1
LogProbit <sup>a</sup>	37.734	49.134	62.056	0.184	504.915	-0.57	-0.938	Recommended - Lowest AIC
Probit	36.633	42.311	48.999	0.013	510.801	-1.06	-1.502	Questionable Goodness of fit p-value < 0.1
Quantal Linear	13.33	16.148	19.884	0.008	513.517	0.042	1.683	Questionable Goodness of fit p-value < 0.1

<sup>a</sup> BMDS recommended best fitting model

## G.4 BMD Model Results – Nipple/Areolae Retention (Bayesian Model Averaging, Original Data)

### G.4.1 BMD Model Results for BMR of 10%

#### Dataset

**Name:** Nipple retention in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	134	9
0.5	119	8
5	103	13
50	120	12
100	141	44
500	58	52

#### Settings

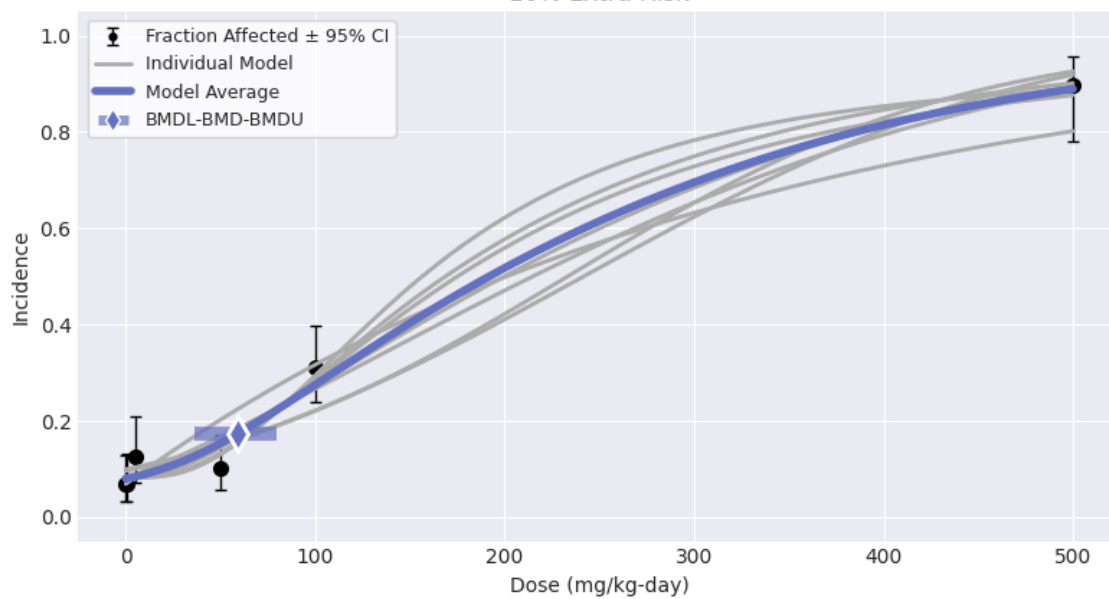
Setting	Value
BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

#### Bayesian Summary

Model	Prior Weights	Posterior Weights	BMDL	BMD	BMDU	Unnormalized Log Posterior Probability	Scaled Residual near BMD	Scaled Residual at Control
Hill	0.111	0.102	51.245	64.921	79.095	-258.082	-0.567	-1.058
Gamma	0.111	0.127	41.367	57.182	75.064	-248.807	-0.532	-1.675
Logistic	0.111	0.021	67.673	79.481	93.294	-252.37	-1.234	2.616
LogLogistic	0.111	0.095	48.026	61.881	78.033	-253.971	-0.583	-1.367
LogProbit	0.111	0.161	53.3	66.05	80.883	-251.248	-0.643	-0.974
Multistage 2	0.111	0.339	35.96	51.217	74.751	-254.463	-0.431	-1.969
Probit	0.111	0.036	66.93	76.682	88.2	-249.115	-1.11	2.591
Quantal Linear	0.111	0.029	28.06	34.045	42.002	-251.01	-0.066	-2.786
Weibull	0.111	0.091	40.138	55.966	75.799	-253.947	-0.517	-1.758
Model Average	-	-	35.905	59.167	79.87	-	-	-



Nipple retention in F1 male rats  
Bayesian Model Average  
10% Extra Risk



## G.4.2 BMD Model Results for BMR of 5%

### Dataset

**Name:** Nipple retention in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	134	9
0.5	119	8
5	103	13
50	120	12
100	141	44
500	58	52

### Settings

Setting	Value
BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

### Bayesian Summary

Model	Prior Weights	Posterior Weights	BMDL	BMD	BMDU	Unnormalized Log Posterior Probability	Scaled Residual near BMD	Scaled Residual at Control
Hill	0.111	0.102	34.022	47.187	61.825	-258.082	-0.567	-1.058
Gamma	0.111	0.127	22.978	35.743	50.701	-248.807	-0.532	-1.675
Logistic	0.111	0.021	38.063	45.029	53.254	-252.37	-1.234	-1.533
LogLogistic	0.111	0.095	30.379	42.122	55.744	-253.971	-0.583	-1.367
LogProbit	0.111	0.161	38.01	49.341	62.395	-251.248	-0.643	-0.974
Multistage 2	0.111	0.339	17.779	26.48	43.165	-254.463	-0.431	1.477
Probit	0.111	0.036	36.908	42.639	49.401	-249.115	-1.11	-1.525
Quantal Linear	0.111	0.029	13.66	16.574	20.448	-251.01	-0.066	1.594
Weibull	0.111	0.091	21.632	33.233	48.48	-253.947	-0.517	-1.758
Model Average	-	-	17.652	37.014	56.163	-	-	-

Nipple retention in F1 male rats  
Bayesian Model Average  
5% Extra Risk

