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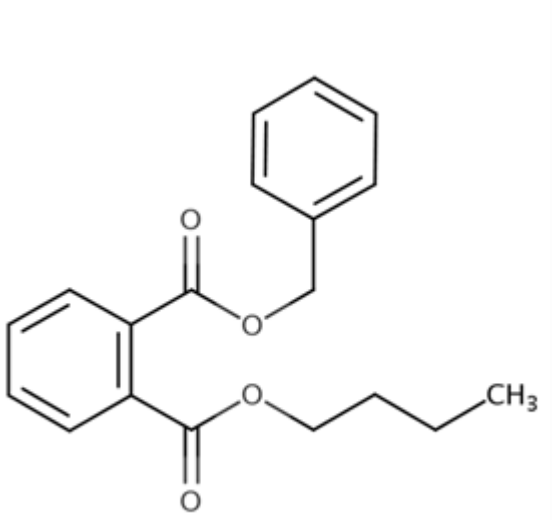
Non-cancer Human Health Hazard Assessment for Butyl Benzyl Phthalate (BBP)

Technical Support Document for the Risk Evaluation

CASRN 85-68-7



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KEY ABBREVIATIONS AND ACRONYMS

ADME	Absorption, distribution, metabolism, and excretion
AGD	Anogenital distance
BBP	Butyl benzyl phthalate
BMD	Benchmark dose
BMDL	Benchmark dose, lower confidence limit
BMR	Benchmark response
BW	Body weight
CASRN	Chemical abstracts service registry number
CD	Charles River Sprague-Dawley
CPSC	Consumer Product Safety Commission (U.S.)
DBP	Dibutyl phthalate
DCHP	Dicyclohexyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
ECB	European Chemicals Bureau
ECCCHC	Environment and Climate Change Canada Health Canada
ECHA	European Chemicals Agency
ECHC	Environment Canada Health Canada
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency (U.S.)
F344	Fischer 344 rat
GD	Gestation Day
HEC	Human equivalent concentration
HED	Human equivalent dose
hCG	Human chorionic gonadotropin
INSL3	Insulin-like factor 3
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MBP	Monobutyl phthalate
MBP ω-ox	Monobutyl phthalate ω-ox
MBzP	Monobenzyl phthalate
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
NTP-CERHR	National Toxicology Program Center for the Evaluation of Risks to Human Reproduction
OCSPP	Office of Chemical Safety and Pollution Prevention
OEHHA	Office of Environmental Health Hazard Assessment
OHAT	Office of Health Assessment and Translation
OPPT	Office of Pollution Prevention and Toxics
PBPK	Physiologically based pharmacokinetic
PECO	Population, exposure, comparator, and outcome
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal Day

POD	Point of departure
SACC	Science Advisory Committee on Chemicals
SD	Sprague-Dawley
TSCA	Toxic Substances Control Act
UF	Uncertainty factor
U.S.	United States
WOE	Weight of evidence

SUMMARY

This technical support document is in support of the Toxic Substances Control Act (TSCA) *Risk Evaluation for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025n). This document describes the use of reasonably available information to identify the non-cancer hazards associated with exposure to BBP and the points of departure (PODs) to be used to estimate risks from BBP exposures in the risk evaluation of BBP. Environmental Protection Agency (EPA, or the Agency) summarizes the cancer and genotoxicity hazards associated with exposure to BBP 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 BBP 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 BBP by existing assessments of the U.S. EPA (2002a), U.S. Consumer Product Safety Commission (CPSC) (2014, 2010), Health Canada (Health Canada, 2020; EC/HC, 2015a; 2015; EC/HC, 2000), European Chemical Agency (ECHA) (2017a, b, 2014a, 2010, 2008), the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (2015), European Chemicals Bureau (ECB) (2007), European Food Safety Authority (EFSA) (2019); California Office of Environmental Health Hazard Assessment (OEHHA) (2012), and the National Academies of Sciences, Engineering, and Medicine (NASEM) (2017). EPA also considered epidemiologic evidence qualitatively as part of hazard identification and characterization. However, epidemiologic evidence for BBP was not considered further for dose response analysis due to limitations and uncertainties in exposure characterization (discussed further in Sections 1.1 and 3.1.1). Use of epidemiologic evidence qualitatively is consistent with phthalates assessment by Health Canada, U.S. CPSC, NICNAS, ECHA, and NASEM.

As discussed further in Section 3.1.2, EPA identified 14 oral exposure studies (all in rats) that have investigated developing male reproductive system effects of BBP following gestational and/or perinatal exposure (Gray et al., 2021; Spade et al., 2018; Debartolo et al., 2016; Schmitt et al., 2016; Ahmad et al., 2014; Furr et al., 2014; Howdeshell et al., 2008; Aso et al., 2005; Tyl et al., 2004; Wilson et al., 2004; Ema et al., 2003; Ema and Miyawaki, 2002; Gray et al., 2000; Nagao et al., 2000). Three studies were multi-generation reproduction studies of BBP oral exposure (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000). Across available studies, the most sensitive 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 POD of 50 mg/kg-day (human equivalent dose [HED] of 12 mg/kg-day) based on phthalate syndrome-related effects on the developing male reproductive system (organ-level outcomes such as decreased anogenital distance (AGD); decreased fetal testicular testosterone; testicular histopathology) to estimate non-cancer risks from oral exposure to BBP for acute, intermediate, and chronic durations of exposure in the risk evaluation of BBP. The selected POD was derived from 4 co-critical prenatal exposure studies of BBP that support a no-observed-adverse-effect level (NOAEL) of 50 mg/kg-day (Tyl et al., 2004) and consensus lowest-observable-adverse-effect level (LOAEL) of 100 mg/kg-day (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005). Across the co-critical studies, 1 multi-generational study identified a NOAEL of 50 mg/kg-day based on decreased AGD (Tyl et al., 2004), and the other 3 studies support a LOAEL of 100 mg/kg-day based on decreased AGD, reduced *ex vivo* fetal testicular testosterone production, and slight increases in testicular pathology (*i.e.*, decreased epididymal and prostate weight, decreased sperm count and motility, decreased epididymal germ cells, and testes softening) (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005). The latter studies support the identified NOAEL.

The Agency performed ¾ body weight scaling to yield the HED and applied the animal-to-human extrapolation factor (*i.e.*, interspecies extrapolation; UF_A) of 3× and a within human variability extrapolation factor (*i.e.*, intraspecies extrapolation; UF_H) of 10×. Thus, a total UF of 30× is 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 scientific evidence and has **robust overall confidence in the selected POD based on decreased AGD and related phthalate syndrome effects for use in characterizing risk from exposure to BBP for acute, intermediate, and chronic exposure scenarios**. The applicability and relevance of this POD for all exposure durations (acute, intermediate, and chronic) is described in the introduction to Section 4 and additionally in 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 to assess risk for other age groups (*e.g.*, older children, adult males, and the elderly) is considered to be conservative and appropriate for a screening level assessment for these other age groups.

No data are available for the dermal or inhalation routes that are suitable for deriving route-specific PODs. Therefore, EPA used the acute/intermediate/chronic oral PODs to evaluate risks from dermal and inhalation exposure to BBP. For the dermal route, differences in absorption were accounted for in dermal exposure estimates in the risk evaluation for BBP. For the inhalation route, EPA extrapolated the oral HED to an inhalation human equivalent concentration (HEC) per EPA's *Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry* (U.S. EPA, 1994) using the updated human body weight and breathing rate relevant to continuous exposure of an individual at rest provided in EPA's *Exposure factors handbook: 2011 edition* (U.S. EPA, 2011b). Table ES-1 and Section 6 summarize EPA's selected oral HED and inhalation HEC values used to estimate non-cancer risk from acute/intermediate/chronic exposure to BBP in the risk evaluation of BBP.

This non-cancer human health hazard assessment for BBP was released for public comment and was peer-reviewed by the Science Advisory Committee on Chemicals (SACC) during the August 4 to 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	HEC[a] (mg/m^3) [ppm]	HED[a] (mg/ kg-day)	Benchmark MOE[b]	References[c] (TSCA Study Quality Rating)
Acute, Intermediate, Chronic	Developing male reproductive system	Rat	Multi-generational or 5-8 days during gestation	NOAEL = 50	Phthalate syndrome-related effects (*e.g.*, ↓AGD; ↓ fetal testicular testosterone; ↓ reproductive organ weights; Leydig cell effects; ↓ mRNA and/or protein expression of steroidogenic genes; ↓INSL3)	64.2 [5.03]	12	UF_A= 3 UF_H=10 *Total UF=30*	(Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005; Tyl et al., 2004)[d]

Abbreviations: AGD = Anogenital distance; HEC = Human equivalent concentration; HED = Human equivalent dose; INSL3: Insulin-like 3; MOE = Margin of exposure; NOAEL = No-observed-adverse-effect level; POD = Point of departure; UF = Uncertainty factor

[a] HED and HEC values were calculated based on the most sensitive NOAEL of 50 mg/kg-day.

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

[c] Tyl et al. (2004) support a NOAEL of 50 mg/kg-day based on decreased AGD and decreased reproductive organ weights in a multi-generational study at 250 mg/kg-day (LOAEL); the remaining effects listed reached statistical significance at higher doses (most of which are not considered adverse in isolation). Ahmad et al. (2014), Furr et al. (2014), and Aso et al. (2005) reflect supporting phthalate syndrome-related effects (*e.g.*, reduced *ex vivo* testicular testosterone production or testicular histopathological changes) at LOAEL = 100 mg/kg-day.

[d] TSCA Study Quality Ratings: *High confidence* for (Furr et al., 2014) and *Medium confidence* for (Ahmad et al., 2014; Aso et al., 2005; Tyl et al., 2004).

1 INTRODUCTION

In December 2019, EPA designated butyl benzyl phthalate (BBP) (CASRN 85-68-7) as a high-priority substance for risk evaluation following the prioritization process as required by Section 6(b) of the Toxic Substances Control Act (TSCA) and implementing regulations (40 CFR part 702) (U.S. EPA, 2019). Following publication of the draft and final scope documents for BBP in 2020 (U.S. EPA, 2020a, b), one of the next steps in the TSCA risk evaluation process is to identify and characterize the human health hazards of BBP, conduct a dose-response assessment, and to determine toxicity values, such as a point of departure (POD), to be used to estimate risks from BBP exposures. This technical support document for BBP summarizes the non-cancer hazards associated with exposure to BBP and summarizes the selected non-cancer toxicity values to be used to estimate risks from BBP exposures. Cancer human health hazards associated with exposure to BBP 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 BBP have been reviewed by several regulatory and authoritative agencies, including the: U.S. Consumer Product Safety Commission (U.S. CPSC); 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); Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS); and the California Office of Environmental Health Hazard Assessment (OEHHA). 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 BBP. Additionally, EPA considered literature published since the most recent existing assessments of BBP to determine if additional data might support the identification of new human health hazards or lower PODs for use in estimating human health risk. EPA's process for considering and incorporating BBP literature is described in the *Systematic Review Protocol for Butyl Benzyl Phthalate (BBP)* (also referred to as the BBP Systematic Review Protocol) (U.S. EPA, 2025p). EPA's approach and methodology for identifying and using human epidemiologic data and experimental laboratory animal data are described in Sections 1.1 and 1.2.

1.1 Human Epidemiologic Data: Approach and Conclusions

To identify and integrate human epidemiologic data into the BBP Risk Evaluation, EPA first reviewed existing assessments of BBP conducted by regulatory and authoritative agencies, as well as systematic reviews of epidemiological studies published by Radke et al. (2020b). Although the authors (*i.e.*, Radke et al.) are affiliated with the U.S. EPA's Center for Public Health and Environmental Assessment, the reviews do not reflect EPA policy. Existing assessments of BBP identified by EPA are listed below. As described further here and in Appendix A, most of these assessments have been subjected to peer-review and/or public comment periods and 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);

- *Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals* (NASEM, 2017);
- *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 US EPA IRIS systematic review methods to the health effects of phthalates: Lessons learned and path forward* (Radke et al., 2020b).

In developing the epidemiology human health hazard assessment for BBP, 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 ((Radke et al., 2020b; Radke et al., 2020a; Radke et al., 2019b; Radke et al., 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 BBP. PECO-relevant literature published since the most recent existing assessment(s) of BBP was identified by applying a literature inclusion cutoff date from existing assessments of BBP. For BBP, 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 BBP. 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 BBP metabolites and health outcomes. PECO-relevant literature published between 2018 to 2019 was identified through the literature search conducted by EPA in 2019, as well as references published between 2018 to 2023 that were submitted with public comments to the BBP docket (EPA-HQ-OPPT-2018-0501), and these studies 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 studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025e).

As described further in *the Systematic Review Protocol for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025p), 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 has focused its epidemiologic evaluation on urinary biomonitoring data; epidemiologic studies that examined BBP metabolites in matrices other than urine were considered supplemental and not evaluated for data quality.

The Agency used epidemiologic studies of BBP qualitatively. This approach is consistent with Health Canada, U.S. CPSC, ECHA, NICNAS, and other agencies. EPA 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. The majority of epidemiological studies introduced additional uncertainty by not considering BBP in isolation and failing to account for confounding effects from co-exposure to mixtures of multiple phthalates (Shin et al., 2019; Aylward et al., 2016). Conclusions from Health Canada (2018a, b) and systematic review articles (Radke et al., 2020a; Radke et al., 2019b; Radke et al., 2019a; Radke et al., 2018) regarding the level of evidence for association between urinary BBP metabolites and each health outcome were reviewed by EPA and used 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 scientific evidence (Shin et al., 2019; Aylward et al., 2016) (Section 3.1.1).

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

The human health hazards of BBP have been evaluated in existing assessments by U.S. EPA (2002a), U.S. CPSC (2014, 2010), Health Canada (Health Canada, 2020; EC/HC, 2015a; 2015; EC/HC, 2000), ECHA (2017a, b, 2014b, 2010, 2008), ECB (2007), EFSA (2019); OEHHA (2012), NTP (2003), NICNAS (2016, 2015), and NASEM (2017). These assessments consistently identified toxicity to the developing male reproductive system as the most sensitive outcomes for use in estimating human risk from exposure to BBP. The PODs from these assessments are shown in Table 1-1.

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

Brief Study Description (Reference) (TSCA Study Quality Rating)	**NOAEL/ LOAEL** (mg/kg-day)	**Critical Effect (s)**	**(CPSC, 2014)**	**(Health Canada, 2020)**	**(ECHA, 2017a)**	**(NICNAS, 2015)**	**(EFSA, 2019)**
Multigenerational Study: male and female CD rats (~20/dose) exposed via oral/diet to 0, 750, 3750, 11,250 ppm BBP (equivalent to 0, 50, 250, 750 mg/kg-day) continuously for two generations (Tyl et al., 2004) (Medium).	50/250	↓ AGD (PND 0) in F1 and F2 males and ↑ NR (PND 11-13) in F1and F2 males	✓[a]		✓[c]	✓[d]	✓[e]
Two-generation Study of Reproduction (Guideline not stated): Crj:CD(SD)IGS rats (24/dose) were exposed via oral/gavage to 0, 100, 200, 400 mg/kg-day continuously for two generations (Aso et al., 2005) (Medium).	None/100	↓ AGD (PND4) in F2 males		✓[b]	✓[c]	✓[d]	✓[e]
Two-generation Study of Reproduction (Guideline not stated): Male and female SD rats (20-24/group) were exposed continuously via oral gavage to 0, 20, 100, 500 mg/kg-day from 8-10 weeks of age for two-generations (Nagao et al., 2000) (Medium).	100/500	↓ AGD (PND 0) and ↓ serum testosterone (adult) in F1 males		✓[b]	✓[c]	✓[d]	✓[e]
Pregnant Harlan SD rats were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP (Block 36) via oral gavage from GD 14–18. Dams were sacrificed and	None/100	↓ *ex vivo* fetal testicular testosterone production		✓[b]			

Brief Study Description (Reference) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Critical Effect (s)	(CPSC, 2014)	(Health Canada, 2020)	(ECHA, 2017a)	(NICNAS, 2015)	(EFSA, 2019)
fetal tissue collected on GD 18 (Furr et al., 2014) (High).							
Pregnant Albino rats (≥6 dams/group) were exposed to 0, 4, 20, or 100 mg/kg-day BBP via oral gavage from GD 14–21. Dams were allowed to give birth naturally, and male offspring were sacrificed on PND 5, 25, or 75 (Ahmad et al., 2014) (Medium).	20/100	↓ Serum testosterone (PND 75), ↓ Epididymal and prostate weights (PND 75), and ↓ Sperm count/motility (PND 75)			✓[c]		

Abbreviations: ↓ = Statistically significant decrease; ↑ = Statistically significant increase; AGD = Anogenital distance; BMD = Benchmark dose; CD = Charles River Sprague-Dawley; LOAEL = Lowest-observed-adverse-effect level; NOAEL = No-observed-adverse-effect level; NR = Nipple retention; PND = Postnatal day; Sprague-Dawley.

[a] NOAEL of antiandrogenic endpoints (*i.e.,* AGD and NR) from (Tyl et al., 2004) used by U.S. CPSC to assign a NOAEL for developmental toxicity of 50 mg/kg-day based on AGD effects, as increased NR significantly occurred at 250 mg/kg-day in F1 and F2 generation (see p. 24, Table 2.1 and Appendix A-16 of (CPSC, 2014)).

[b] Health Canada selected a NOAEL of 50 mg/kg-day from three co-critical studies (Furr et al., 2014; Aso et al., 2005; Nagao et al., 2000) to calculate hazard quotients for pregnant women and women of childbearing age and infants (see Table 9-34 of (Health Canada, 2020)).

[c] NOAEL of 50 mg/kg-day supported by LOAELs of 100 mg/kg-day from four studies (Ahmad et al., 2014; Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000) was used to calculate derived no-effect-levels (DNELs) (see Section B 4.2.2 of (ECHA, 2017a).

[d] NOAEL of 50 mg/kg-day supported by three co-critical studies (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000) was used by Australia's NICNAS to calculate MOE for developmental effects.

[e] NOAEL of 50 mg/kg-day from Tyl et al. (2004) was selected by EFSA to derive a stand-alone tolerable daily intake (TDI) for BBP based on reproductive and developmental toxicity (see Table 22 and Section 4.7.6 and Table 24 in Section 5.1 in (EFSA, 2019); Tyl. et al (2004) was considered co-critical with two other studies (Aso et al., 2005; Nagao et al., 2000).

1.2.2 Approach to Identifying and Integrating Laboratory Animal Data

Table 1-1 provides an overview of EPA's approach to identifying and integrating laboratory animal data into the BBP Risk Evaluation. EPA first reviewed existing assessments of BBP conducted by 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 BBP, and identify key studies used to establish PODs for estimating human risk. Existing assessments reviewed by EPA are listed below. As described further in Appendix A, most of these assessments have been subjected to external peer-review and/or public comment periods.

- *Provisional Peer Reviewed Toxicity Values for butyl benzyl phthalate* (U.S. EPA, 2002a);
- *Chronic Hazard Advisory Panel on phthalates and phthalate alternatives* (CPSC, 2014);
- *Toxicity review for benzyl-n-butyl phthalate* (CPSC, 2010);
- *Supporting documentation: Carcinogenicity of phthalates - mode of action and human relevance* (Health Canada, 2015);
- *Canadian environmental protection act priority substances list assessment report: Butylbenzylphthalate* (EC/HC, 2000);
- *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, 2015a);
- *Screening assessment - Phthalate substance grouping* (Health Canada, 2020);
- *Substance name: Benzyl butyl phthalate, EC number: 201-622-7, CAS number: 85-68-7: Member state committee support documentation for identification of benzyl butyl phthalate (BBP) as a substance of very high concern* (ECHA, 2008);
- *Evaluation of new scientific evidence concerning the restriction contained in Annex XVII to regulation (EC) no. 1907/2006 (REACH): Review of new available information for benzyl butyl phthalate (BBP) CAS no. 85-68-7 Einecs no. 201-622-7* (ECHA, 2010);
- *Support document to the opinion of the member state committee for identification of benzyl butyl phthalate (BBP) as a substance of very high concern because of its endocrine disrupting properties which cause probable serious effects to human health and the environment which give rise to an equivalent level of concern to those of cmr1 and pbt/vpvb2 substances* (ECHA, 2014b);
- *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 on an Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP)* (ECHA, 2017b);
- *European union risk assessment report: Benzyl butyl phthalate (BBP)* (ECJRC, 2007);
- *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);
- *Safe Drinking Water and Toxic Enforcement Act of 1986 Proposition 65. Initial Statement of Reasons. Title 27, California Code of Regulations. Proposed amendment to Section 25805(b), Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity. Butyl benzyl phthalate (oral exposure)* (OEHHA, 2012);
- *NTP-CERHR monograph on the potential human reproductive and developmental effects of butyl benzyl phthalate (BBP)* (NTP, 2003);
- *Priority existing chemical assessment report no. 40: Butyl benzyl phthalate* (NICNAS, 2015);
- *C4-6 side chain transitional phthalates: Human health tier II assessment* (NICNAS, 2016); and

- *Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals* (NASEM, 2017).

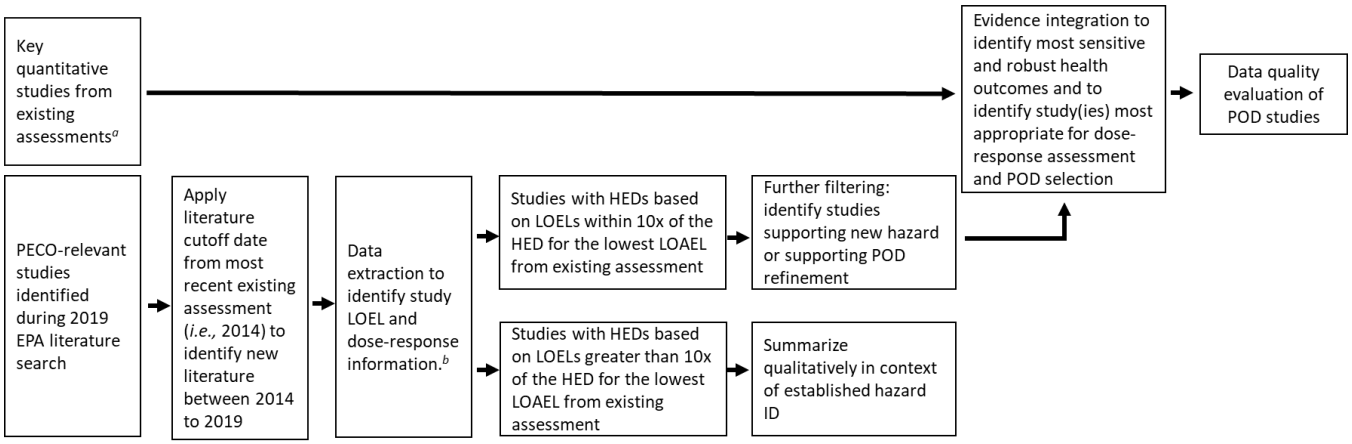

Figure 1-1. Overview of BBP Human Health Hazard Assessment Approach
Abbreviations: HED = Human equivalent dose; LOAEL = Lowest-observed-adverse-effect level; LOEL = Lowest-observed-effect level; PECO = Population, exposure, comparator, and outcome; POD = Point of departure.
[a] Any study that was considered for dose-response assessment, not necessarily limited to the study used for POD selection.
[b] Extracted information includes PECO relevance, species, exposure route and type, study duration, number of dose groups, target organ/systems evaluated, study-wide LOEL, and potentially exposed or susceptible subpopulations (PESS) categories

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

Similar to the epidemiological analysis, EPA used the 2015 Health Canada assessment (EC/HC, 2015a) as a starting point for the evaluation of animal data considered in this document. 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 *Systematic Review Protocol for Butyl Benzyl Phthalate* (U.S. EPA, 2025p). 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, subchronic, chronic) and routes of exposure (*i.e.*, oral, dermal, inhalation), as shown in Table 1-1 (literature evaluated from 2014-2019 is described below). Therefore, EPA considered literature published between 2014 to 2019 further as shown in Figure 1-1. For the BBP human health hazard assessment, EPA also considered literature related to effects on the developing male reproductive system identified through development of EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023a), which included a literature search in 2022. EPA first screened titles and abstracts and then full texts for relevancy using PECO screening criteria described in the *Systematic Review Protocol for Butyl Benzyl Phthalate* (U.S. EPA, 2025p).

In development of the draft human health hazard assessment for BBP, which was reviewed by the SACC in August 2025, EPA considered PECO relevant studies identified through this literature update published between 2014 and 2022 and extracted key study information as described in the *Systematic Review Protocol for Butyl Benzyl Phthalate* (U.S. EPA, 2025p). 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) (Table 1-1**)**.

Information identified between 2014 to 2022 for BBP 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 EPA when physiologically based pharmacokinetic (PBPK) models or other information to support a chemical-specific quantitative extrapolation is absent (U.S. EPA, 2011c). EPA's use of allometric body weight scaling is described further in Appendix D.

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 BBP. 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 BBP (U.S. EPA, 2025p), these studies were evaluated in a manner consistent with the Office of Pesticide Programs *Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment* (U.S. EPA, 2012b).

In 2025, EPA updated the literature considered as part of the BBP human health hazard assessment. As described further in the BBP Systematic Review Protocol (U.S. EPA, 2025p), 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 studies that support selection of a lower POD for BBP as described in greater detail in the next section.

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

1.2.3 Literature Identified and Hazards of Focus for BBP

As described in Section 1.2.2, and as described further in the *Systematic Review Protocol for Butyl Benzyl Phthalate* (U.S. EPA, 2025p), EPA reviewed literature published between 2014 to 2025 for information on sensitive human health hazards not previously identified in existing assessments, including information that may indicate a more sensitive POD. As described further in the *Systematic Review Protocol for Butyl Benzyl Phthalate* (U.S. EPA, 2025p), EPA identified 10 PECO-relevant animal toxicology studies published between 2014 to 2022, while no new PECO-relevant studies were identified from the 2025 literature update. The 10 studies provided information pertaining to various primary hazard outcomes, including: reproductive/developmental, neurotoxicity, immune adjuvant

effects, renal, and hepatic outcomes. Further details regarding EPA's handling of information provided in these 10 studies are provided below.

- ***Reproductive/Developmental***. EPA identified 7 studies evaluating reproductive/ developmental outcomes (Gray et al., 2021; Integrated Laboratory Systems, 2017; Debartolo et al., 2016; Schmitt et al., 2016; Ahmad et al., 2015; Alam and Kurohmaru, 2015; Ahmad et al., 2014). These studies of BBP are discussed further in Section 3.1. Of these, only 4 studies (Gray et al., 2021; Debartolo et al., 2016; Schmitt et al., 2016; Ahmad et al., 2014) evaluated endpoints relevant to phthalate syndrome outcomes from developing male exposure (*i.e*., histopathology and/or organ weights of the male reproductive system, anogenital distance). The other 3 studies evaluated a range of endpoints including changes in the estrus cycle or serum estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, number of ovarian follicles, reproductive organ weights (*i.e.,* ovary and/or uterus), pup body weights, or used a non-developmental exposure design (Integrated Laboratory Systems, 2017; Ahmad et al., 2015; Alam and Kurohmaru, 2015).
- ***Neurotoxicity***. EPA identified 3 studies evaluating neurological effects following BBP exposure (Debartolo et al., 2016; Schmitt et al., 2016; Min et al., 2014).
- ***Immune adjuvant effects***. EPA identified 1 study evaluating immunological effects following BBP exposure (Jahreis et al., 2018).
- ***Renal.*** EPA identified 2 studies evaluating renal effects following BBP exposure (Integrated Laboratory Systems, 2017; Nakagomi et al., 2017).
- ***Hepatic.*** EPA identified 1 study evaluating hepatic effects following BBP exposure (Nakagomi et al., 2017).

The most sensitive and robust PODs selected from existing hazard assessments of BBP have been based on effects on the developing male reproductive system (Health Canada, 2020; EFSA, 2019; ECHA, 2017a; NICNAS, 2015; CPSC, 2014). Existing assessments have consistently shown that effects on other health outcomes (*i.e.,* female reproduction, neurological, hepatic/renal, immune, and 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 literature published from 2014 to 2022, as some of the lowest NOAELs/LOAELs were identified for male 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). Literature relevant to developing male reproductive toxicity presenting phthalate syndrome-related effects were considered in non-cancer hazard assessment and are further discussed in Section 3.1.2. All other studies, as well as brief justification for their exclusion from further evaluation of dose-response and derivation of a POD for use in human health risk assessment, are discussed in Appendix B.

2 TOXICOKINETICS

2.1 Oral Route

EPA identified two animal studies available on the metabolism of BBP following oral exposure (Nativelle et al., 1999; Eigenberg et al., 1986), as well as one human oral exposure study (Anderson et al., 2001) and three human biomonitoring assessments identifying BBP metabolites in urine and feces (Apel et al., 2020; Frederiksen et al., 2011; Stahlhut et al., 2007). Based upon few experimental absorption, distribution, metabolism, and excretion (ADME) toxicokinetic assessments, orally administered BBP is readily absorbed through the gastrointestinal tract and mainly processed via first pass metabolism by intestinal and hepatic esterases (Anderson et al., 2001; Nativelle et al., 1999; Eigenberg et al., 1986). Following oral absorption in rats and humans, BBP is hydrolyzed into the diester monobutyl phthalate (MBP), followed by an appreciable amount of monobenzyl phthalate (MBzP) metabolite formation (Anderson et al., 2001; Nativelle et al., 1999; Eigenberg et al., 1986). However, identification of BBP metabolites in female Wistar rat urine has shown hippuric acid, generated from further hydrolyzation of MBP and MBzP to increase solubility for excretion, as the main recovered metabolite (Nativelle et al., 1999). Additional oxidized metabolites detected in urine, including phthalic acid, which has been observed in rats and humans (Anderson et al., 2001; Nativelle et al., 1999), as well as benzoic acid and monobutyl phthalate ω-ox (MBP ω-ox), are also observed in small quantities (Nativelle et al., 1999). A summary of BBP metabolite formation pathway of 6 different metabolites identified in rat and human samples (primarily urine and feces) after oral administration of BBP is presented in Table 2-1.

In an oral metabolism study, female Wistar rats were gavaged with 150, 475, 780, and 1500 mg/kg-day BBP for 3 consecutive days, followed by molecular characterization of urinary metabolites at 24, 48, and 72 hours post exposure (Nativelle et al., 1999). Here, Nativelle et al. (1999) identified 6 metabolites in urine collections, where the parent compound was not recovered. Hippuric acid represented the major recovered metabolite (51-56%), followed by MBP (29-34%), MBzP (7-12%), and small percentages (less than 3%) of MBP ω-ox and terminal acid hydrolysis products. It should also be noted that the study by Nativelle et al. (1999) observed a dose-dependent impact on metabolite excretion rate. Metabolite recovery over the three days at 150 to 1500 mg/kg-day showed a dose-dependent effect on metabolite quantity. Daily elimination analysis following administration of 475 mg/kg-day BBP resulted in a steady-state excretion rate over 72 hours post exposure, but 1500 mg/kg-day exposure resulted in increased relative MBP, MBzP, and hippuric acid metabolite levels at 72 hours. This toxicokinetic response indicates a time-dependent dose effect, where gastrointestinal absorption may be saturated and may shift to fecal elimination at excessive levels (Eigenberg et al., 1986). In sum, the proposed BBP degradation pathway based upon oral exposure data obtained from the Nativelle et al. (1999) study is shown in Figure 2-1.

Eigenberg et al. (1986) performed a single oral gavage exposure using radiolabeled BBP (^{14}C-BBP) at 2, 20, 200, and 2000 mg/kg in male F344 rats and made examinations at 24 and 96 hours post exposure. Here, 75 to 86 percent of the total dose was excreted within 24 hours in urine and feces, and 92 percent was recovered by the 96 hours post collection. For groups receiving 2 to 200 mg/kg, 75 percent of the dose was eliminated in urine vs. 20 percent in feces. However, in the high dose group of 2000 mg/kg, the predominant excretion route was feces (72%) and not urine (22%). In this same study, investigators also administered intravenous infusion of ^{14}C-BBP (20 mg/kg) through the tail vein to assess tissue distribution and toxicokinetic properties. In this kinetics assessment, blood BBP monoester metabolite levels peaked within 5 minutes of BBP administration. To determine the extent of biliary excretion, the bile ducts of rats were cannulated, and bile was collected over the course of 4 hours at regular time

intervals after dosing. After 4 hours, 55 percent of the dose was excreted in bile, whereas 34 percent was excreted in the urine, and larger quantities of BBP metabolites were found in the bile compared to the urine. Altogether, these data demonstrate that biliary excretion is the predominant route of excretion, and that reabsorption occurs (via enterohepatic circulation). In addition to biliary and enterohepatic recirculation for excretion, BBP metabolites rapidly distributed into multiple tissues, including brain, lung, liver, kidney, spleen, testes, small intestine, muscle (thigh), skin (abdominal), and adipose. Aside from urinary and fecal excretion levels, peak distribution levels (measured at 30 minutes) were observed in the small intestine, muscle, and skin. Half-lives of parent compound and monoester metabolites was approximately 6 hours across all tissues examined, with 84 percent of the total dose cleared within 24 hours of administration.

One controlled human BBP oral exposure study was identified (Anderson et al., 2001). In this study, participants (n = 13 volunteers, age and sex not specified) were orally exposed (ingestion) to the deuterated form of BBP (d_4-BBP) at a single low (253 µg) and high (506 µg) dose, followed by urinary metabolite measures 24 hours after dosing and at 2 and 6 days post exposure. MBzP was the predominant urinary metabolite (67% at low dose and 78% of the excretion fraction at high dose) in humans following oral d_4-BBP exposure when measured in urine 24 hours after dosing. MBP was identified as a minor urinary metabolite in humans, accounting for 6 percent of the excretion fraction at the high dose but was undetectable in the low dose group. No labeled phthalate monoester levels were found in urine when measured at 2 or 6 days following exposure, suggesting rapid uptake and excretion occurring within the first 24 hours. It should be noted participant sex was not reported in the Anderson et al. (2001) assessment, which may impact toxicokinetic assumptions, albeit variability of inter-individual excretion fractions was determined to be acceptable. Nevertheless, MBzP as such a major excretion fraction suggests this metabolite as a dominant biomarker of human exposure. Human biomonitoring assessments of multiple phthalates, including BBP, have consistently identified MBzP as the predominant metabolite (along with lesser amounts of MBP) in urine collections of multiple human sampling collections (Apel et al., 2020; Frederiksen et al., 2011; Stahlhut et al., 2007).

The available rodent studies (Nativelle et al., 1999; Eigenberg et al., 1986) established elimination profiles shifting from urine to feces at high doses of BBP (1500 to 2000 mg/kg-day), which indicates that oral absorption of BBP is saturable in rodents at high doses. However, elimination rates in urine were fairly constant across doses less than 200 mg/kg-day in rodents and in a low single dose human study (Anderson et al., 2001), which is within the range considered for dose response in this assessment. Given that approximately 75 percent BBP is excreted at lower doses in both rats (Eigenberg et al., 1986) and humans (Anderson et al., 2001) at 24 hours, no adjustment is needed to account for oral absorption between species. Therefore, based on the available data, and in accordance with prior agency assessments, **EPA will assume an oral absorption 100 percent for the risk evaluation of BBP**.

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

Urinary Metabolite	Abbreviation	Rat	Human[a]	Reference(s) (Species)
Monobutyl phthalate	MBP	✓	✓	(Eigenberg et al., 1986) (rat) (Nativelle et al., 1999) (rat) (Anderson et al., 2001) (human) (Frederiksen et al., 2011)[b] (human) (Apel et al., 2020) (human) (Stahlhut et al., 2007) (human)

Urinary Metabolite	Abbreviation	Rat	Human[a]	Reference(s) (Species)
Monobenzyl phthalate	MBzP	✓	✓	(Eigenberg et al., 1986) (rat) (Nativelle et al., 1999) (rat) (Anderson et al., 2001) (human) (Frederiksen et al., 2011) (human) (Apel et al., 2020) (human) (Stahlhut et al., 2007) (human)
Monobutyl phthalate ω-ox	MBP ω-ox	✓	ND	(Nativelle et al., 1999) (rat)
Hippuric acid	–	✓	ND	(Nativelle et al., 1999) (rat)
Phthalic acid	–	✓	ND	(Nativelle et al., 1999) (rat)
Benzoic acid	–	✓	ND	(Nativelle et al., 1999) (rat)

Abbreviations: ND = no data available

[a] Metabolites detected as part of human controlled experimental (Anderson et al., 2001) or biomonitoring/population data assessment studies (Apel et al., 2020; Frederiksen et al., 2011; Stahlhut et al., 2007). 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.

[b] Urinary MBP detection was reported as the sum of MBP and mono-iso-butyl phthalate isoforms due to chromatographic characterization limitations.

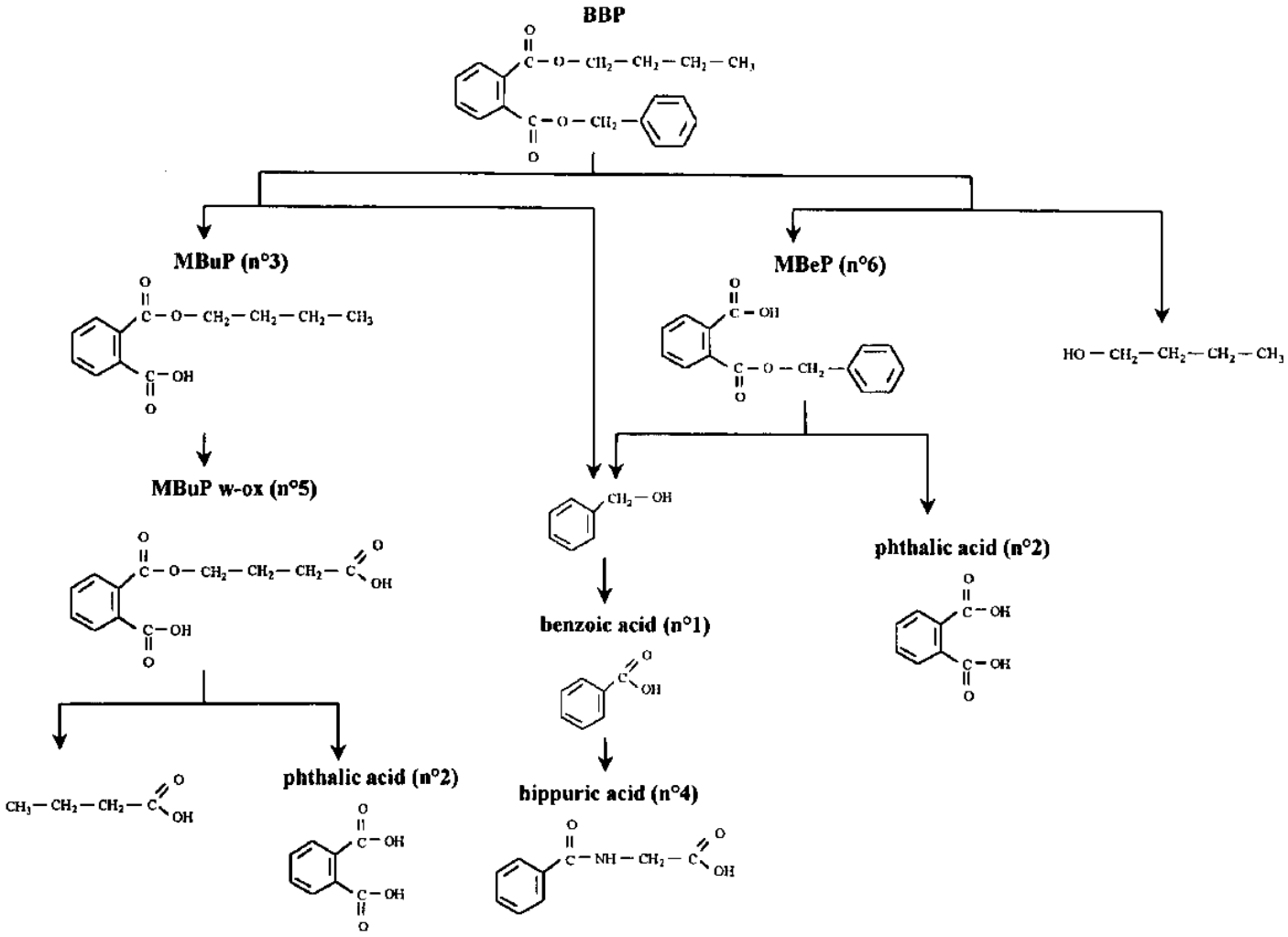

Figure 2-1. Proposed Metabolic Pathway of BBP Following Oral Exposure (Figure from Health Canada (EC/HC, 2015b))

Notes: Metabolic pathway is based upon data collected from oral administration of BBP in female Wistar rats. Original pathway is taken from Nativelle et al. (1999) and is also found in Health Canada (EC/HC, 2015b) report (Figure H-3). MBuP = Monobutyl phthalate (MBP); MBeP = Monobenzyl phthalate (MBzP); MBuP ω-ox = Oxidized monobutyl phthalate.

2.2 Inhalation Route

No controlled human exposure studies or *in vivo* animal studies are available that evaluate the ADME properties of BBP for the inhalation route. As discussed further in Sections 3 and 6, no data from experimental animal models are available for the inhalation route that are suitable for deriving a route-specific PODs. Therefore, EPA extrapolated the inhalation POD from the oral POD. For this risk evaluation, **EPA assumed similar absorption for the oral and inhalation routes (100% absorption)**, as done in previous assessments (NICNAS, 2015), and no adjustment was made when extrapolating to the inhalation route of exposure.

2.3 Dermal Route

EPA identified an *in vivo* rodent study and an *ex vivo* rodent study (Sugino et al., 2017; Elsisi et al., 1989) and three *in vitro* human studies evaluating ADME properties following dermal application of BBP (Sugino et al., 2017; DuPont, 2006a, b).

In the report by Elsisi et al. (1989), ADME properties of eight phthalates, including BBP, were analyzed through dermal application of radiolabeled parent compounds in male F344 rats. ^{14}C-BBP (5 to 8 mg/cm^2) was applied to shaved dorsal area skin (1.3 cm diameter application area) and covered with a plastic cap, and urine and feces were collected every 24 hours for the seven days. After 7 days, animals were sacrificed, and levels of ^{14}C-BBP were determined in organs. After 7 days, 86 percent of the applied dose was recovered, including approximately 30 percent of ^{14}C-BBP in urine and feces, 44.9 percent in skin at the site of application, 6.3 percent in the plastic cap, 4.6 percent in muscle, 0.17 percent in adipose tissue, 0.08 percent in skin, and less than 0.5 percent in other tissues (*i.e.*, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood). These results indicate that approximately 35 percent of the applied dose was absorbed over 7 days. However, combined urinary and fecal excretion was linear over 7 days, indicating approximately 5 percent adsorption per day for 7 days. Relative to other phthalates tested, BBP had a linear and intermediate excretion rate, with slower absorption and excretion likely being due to its higher molecular weight, as other medium-chain phthalates with a low molecular weight, such as dibutyl phthalate, showed rapid excretion. Elsisi et al. (1989) also observed low levels of distribution in muscle (4.6%), adipose (0.17%), and small amounts across brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord, and blood (summation of less than 0.5%). Thus, in addition to biliary excretion and enterohepatic recirculation, BBP metabolites may distribute into multiple non-circulatory or -hepatic compartments following dermal exposure. Oral exposure studies have noted relatively short BBP metabolite half-lives in both rats and humans (Anderson et al., 2001; Nativelle et al., 1999; Eigenberg et al., 1986). Because of the short metabolic half-life, it is assumed that BBP metabolites do not accumulate in tissues.

Sugino et al. (2017) used *in vitro* epidermal membranes (0.95 cm^2) prepared from abdominal excisions of male hairless rats (WBN/Ila-Ht) and human females to assess skin permeation properties of multiple phthalates, including BBP. Application of BBP to skin showed species-specific metabolite permeation outcomes, but no diffusion of the parent compound. In sections prepared from hairless rats, only the monoester metabolites MBP and MBzP diffused across dermal membranes, with more MBP metabolites relative to MBzP. Conversely, MBzP was the dominant metabolite recovered in human skin, and the permeability coefficient was markedly lower in human skin relative to the rat. An additional important finding of this *in vitro* assessment is that there also appeared to be metabolism-dependent processes impacting dermal uptake. Sugino et al. (2017) applied diisopropyl fluorophosphates (DFP), as serine-esterase inhibitor, to additional rat skin treatment groups, and noted both a shift toward MBzP metabolite production and impermeability of BBP metabolites following DFP application.

Lastly, Dupont et al. (2006a, b) conducted two independent assessments using *in vitro* human female cadaver abdominal skin sections (n = 3, 2 replicates from each donor). The first experiment utilized skin collections (468 to 487 µm thick) and exposed 0.64 cm^2 size sections to an infinite dermal load of 100 µL/cm^2 BBP for 8 hours, which was spiked with ^{14}C-BBP (for recovery estimate) into a non-radiolabeled formulation that was uniformly mixed. Recovery of the non-absorbed applied dose at the end of 8 hours was 96.4 percent, with a total estimated absorbed dose of 0.197 percent (165 µg BBP) (DuPont, 2006b). The second experiment by Dupont et al. (2006a) exposed 0.64 cm^2 abdominal skin sections (248 to 470 µm thick) to a BBP film matrix containing ^{14}C-BBP occluded parafilm directly placed on the skin for 8 hours. In this case, the total estimated exposure was 5958 µg BBP, and the total absorbed dose at the end of 8 hours was less than .01 percent (0.57 µg BBP).

Although human evidence is limited, multiple regulatory agencies assume that BBP dermal absorption is low, and that dermal migration is reportedly lower in human compared to rat skin for phthalates, including BBP (Sugino et al., 2017; Scott et al., 1987). This assumption, along with the lack of data and

uncertainty in available studies, has led several agency assessments to adopt a worst-case dermal bioavailability of 5 percent in humans (NICNAS, 2015; ECJRC, 2007).

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 *Environmental Release and Occupational Exposure Assessment for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025f) and *Consumer and Indoor Dust Exposure Assessment for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025b). Briefly, EPA used BBP dermal absorption data from Dupont (2006a) to estimate the flux-limited dermal absorption of BBP in solids. Using the Dupont (2006a) study estimate of 0.00057 mg over a 0.64 cm^2 area off BBP (0.0008906 mg/cm^2 of BBP) over an 8 hour period, the steady-state flux of neat BBP is estimated as 1.113×10^{-4} $mg/cm^2/hr$.

The Dupont (2006b) study serves as an upper bound of dermal exposure was used as a screening level assessment the Consumer Assessment for BBP (U.S. EPA, 2025b) to estimate flux-limited dermal absorptions from liquid formulations of BBP. Using the Dupont (2006b) study estimate of 0.165 mg on a 0.64 cm^2 area of BBP (0.258 mg/cm^2) over an 8 hour period, the steady-state flux of neat BBP is estimated as 3.22×10^{-2} $mg/cm^2/hr$. In the Occupational Assessment for BBP (U.S. EPA, 2025f), *in vitro* human skin dermal absorption data from Sugino et al. (2017), which provides important information on the importance of metabolically active skin to provide more accurate estimates of BBP dermal absorption, was used in refining the dermal assessment for BBP. The dermal flux value derived from Sugino et al. (2017) is estimated as 6.2×10^{-4} $mg/cm^2/h$ from *in vitro* human skin. EPA estimated the steady-state flux and assumed is equal to the average flux.

2.4 Summary

The majority of ADME information on BBP are obtained from rodent oral exposure studies. Following oral exposure, BBP rapidly undergoes esterase hydrolysis into MBP and MBzP, and then subsequent metabolism to the predominant urinary metabolite of hippuric acid (in rats), however, other minor urinary metabolites have been detected, including glucuronidated MBP and/or MBzP metabolites. Whereas hippuric acid is the major BBP urinary metabolite in rats, MBzP is the predominant metabolite detected in human urine following oral exposure. BBP is rapidly absorbed by the gastrointestinal tract and generally undergo hepatic metabolism and biliary excretion, along with some distribution throughout many bodily compartments. Reasonably available data suggest most of the administered dose of BBP is excreted through urine within 24 hours, albeit at excessively high doses, there is major fecal elimination potentially associated with saturated oral absorption.

ADME data on non-oral routes of exposure remains limited, with no quantitative inhalation studies currently available. The few *in vivo*/*in vitro* studies for dermal BBP exposure suggest a much lower dermal absorption rate compared to ingestion. However, there remain uncertainties in the data available on the toxicokinetics of BBP, particularly pertaining to inter-species and inter-individual factors and lack of comprehensive experimental data. In the Sugino et al. (2017) study using *ex vivo* epidermal membranes from rats and humans, dermal absorption and metabolite formation was reportedly impacted by serine esterase inhibitors, suggesting dermal rate-limiting enzymatic activity may be a consideration in inter-species differences or a source of uncertainty.

Although the exposure routes or conditions cannot be specified, human biomonitoring assessments have noted, in addition to urine metabolite detection, the presence of phthalate metabolites (including MBP and MBzP) in fetal serum, breast milk, and semen (Main et al., 2006; Lashley et al., 2004; Rozati et al., 2002). Given these findings, along with the observation of BBP metabolites in multiple tissues following dermal exposure (Elsisi et al., 1989) and intravenous infusion (Eigenberg et al., 1986), and comparative

analysis of phthalate kinetics in prior assessments (NICNAS, 2008), BBP metabolites are assumed to widely distribute after exposure, even being able to cross the placental barrier. However, metabolites appear to have short half-lives and there is no evidence for tissue accumulation.

Given the toxicokinetic information available for BBP, EPA assumes an oral absorption of 100 percent and an inhalation absorption of 100 percent for the risk evaluation. For dermal absorption, EPA is using a flux-limited absorption rate as described further in the *Consumer and Indoor Dust Exposure Assessment for Butyl benzyl phthalate (BBP)* (U.S. EPA, 2025b) and *Environmental Release and Occupational Exposure Assessment for Butyl benzyl phthalate (BBP)* (U.S. EPA, 2025f).

3 NON-CANCER HAZARD IDENTIFICATION

As discussed in Section 1.2, the effects on the developing male reproductive system has consistently been identified in existing assessments of BBP as the most sensitive effects associated with oral exposure to BBP in experimental animal models (Health Canada, 2020; EFSA, 2019; ECHA, 2017a, b; NASEM, 2017; NICNAS, 2016; EC/HC, 2015a; Health Canada, 2015; NICNAS, 2015; CPSC, 2014; OEHHA, 2012; CPSC, 2010; ECHA, 2010, 2008; ECJRC, 2007; NTP, 2003; U.S. EPA, 2002a; EC/HC, 2000). EPA identified no information through systematic review that would change this conclusion. Therefore, EPA focused its non-cancer hazard characterization on developing male reproductive toxicity, which is discussed in the sections below. Literature on non-cancer hazards identified by EPA in studies published between 2014 to 2019, but not used for POD derivation, are briefly presented in Section 3.1.2.2 and Appendix B.

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 BBP metabolites and male and female 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 BBP by Health Canada (2018b), Radke et al. (2019b; 2018), and NASEM (2017), respectively, including conclusions related to exposure to BBP and health outcomes. Additionally, 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) to determine if newer epidemiologic studies would change the conclusions of existing epidemiologic assessments or provide useful information for evaluating exposure-response relationship (Section 3.1.1.2). Overall, EPA considered there to be limitations in the epidemiological evidence for association between urinary metabolites of BBP and the developing male reproductive system. This stems from uncertainty associated with exposure characterization of individual phthalates, including source or exposure and timing of exposure as well as co-exposure confounding with other phthalates. Therefore, EPA considered epidemiologic studies of BBP qualitatively.

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

Previous Assessment	Outcomes Evaluated	Method Used for Study Quality Evaluation
Health Canada (2018b)	**Hormonal effects:** • Sex hormone levels (*e.g.*, testosterone) **Growth & Development:** • AGD • Birth measures • Male infant genitalia (*e.g.*, hypospadias/cryptorchidism) • Placental development and gene expression • Preterm birth and gestational age • Postnatal growth • DNA methylation **Reproductive:** • Altered male puberty • Gynecomastia • Changes in semen parameters • Sexual dysfunction (males) • Sex ratio	Downs and Black (Downs and Black, 1998)
Radke et al. (2018)	• AGD • Hypospadias/cryptorchidism • Pubertal development • Semen parameters • Time to pregnancy (male exposure) • Testosterone • Timing of pubertal development	Approach included study sensitivity as well as risk of bias assessment consistent with the study evaluation methods described in (U.S. EPA, 2022)
Radke et al. (2019b)	• Pubertal development • Time to pregnancy (Fecundity) • Preterm birth • Spontaneous abortion	ROBINS-I (Sterne et al., 2016)
NASEM (2017)	• AGD • Hypospadias (incidence, prevalence, and severity/grade) • Testosterone concentrations (measured at gestation or delivery).	OHAT (based on GRADE) (NTP, 2015)

Abbreviations: AGD = anogenital distance; GRADE = Grading of Recommendations, Assessment, Development and Evaluation; OHAT = National Toxicology Program's Office of Health Assessment and Translation; ROBINS-I= Risk of Bias in Non-randomized Studies of Interventions.

3.1.1.1.1 Health Canada (2018b)

Health Canada (2018b) considered 83 studies that evaluated the association between BBP and its metabolite (MBzP) and reproductive outcomes such as altered male puberty, altered female puberty, gynecomastia (*i.e.*, the increase of male breast glands in pubescent boys), changes in semen parameters, pregnancy complications and loss, altered fertility and time to pregnancy, endometriosis and adenomyosis, uterine leiomyoma, sexual dysfunction in males, sexual dysfunction in females, polycystic ovary syndrome, age at menopause, as well as sex ratio.

Data quality evaluation criteria and methodology used by Health Canada considered 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[1] for the association between BBP and its metabolites and decreased odds of polycystic ovary syndrome. There was also limited evidence for the association with infant sex ratio at birth (*i.e.*, male excess associated with maternal exposure to MBzP and/or MBP). There was inadequate evidence for the association between BBP and its metabolites and sexual dysfunction in males and females, changes in semen parameters and time to pregnancy. The level of evidence could not be established for the association between BBP and its metabolites and altered fertility. There was no evidence for the association between exposure to BBP and its metabolites and altered male puberty, gynecomastia, pregnancy loss, endometriosis and adenomyosis, and uterine leiomyoma. All other reproductive outcomes *(i.e.,* altered male or female puberty, gynecomastia, pregnancy complication and loss) did not have reported evidence of association with BBP and/or its metabolites.

Sixty-five studies were assessed by Health Canada (2018b) to evaluate the association between exposure to BBP and growth and developmental outcomes (outcomes listed in Table 3-1). There was limited evidence of association for BBP and its metabolites and postnatal growth in infants/children (with some variations regarding the direction of the associations) and altered placental gene expression. There was inadequate evidence of association for BBP and its metabolites and the following outcomes: birth measures, placental development, preterm birth and gestational age, postnatal DNA methylation, and sperm DNA damage/apoptosis. There was no evidence of association for BBP and its metabolites and AGD, as well as male infant genitalia (*e.g.*, hypospadias and cryptorchidism).

The relationship between BBP 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 MBzP with thyroid-related hormones and sex hormone levels (*i.e.*, follicle stimulating hormone, luteinizing hormone, testosterone, estradiol, prolactin,

[1] 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."

inhibin B, anti-Mullerian hormone, androstenedione). There was inadequate evidence for association between MBzP and 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 BBP or its metabolite (MBzP) and male reproductive outcomes, including AGD and 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.*, National Toxicology Program's Office of Health Assessment and Translation (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 (BBP, DEP, DBP, DIBP) 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, which contribute to 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. (2018) found that although it is difficult to determine whether phthalates cause male reproductive toxicity due to inconsistency across studies, the most consistent studies were those looking at semen parameters. Several medium quality studies contributed to the moderate level of evidence for the association between BBP exposure and a decline in the motility and overall quality of sperm. There is also moderate level of evidence from a single high confidence study that reported statistically significant associations between increased exposure to BBP and either longer time to pregnancy or reduced fecundability. Evidence for BBP exposure and testosterone, as well as pubertal development, was deemed indeterminate due to inconsistency in available studies. In five studies, three of which were medium confidence and reported a non-statistically significant inverse association, and two low confidence studies which reported no association, Radke et al. (2018) determined that there was slight evidence for an association between exposure to BBP and AGD which may be due to data availability and low exposure levels in the studies. Evidence for Hypospadias/cryptorchidism was considered to be slight and found in one low confidence study.

Table 3-2. Summary of Epidemiologic Evidence of Male Reproductive Effects Associated with Exposure to BBP[a]

Timing of Exposure	Outcome	Level of Confidence in Association
In utero	Anogenital distance	Slight
	Hypospadias/cryptorchidism	Slight
In utero or childhood	Pubertal development	Indeterminate

Timing of Exposure	Outcome	Level of Confidence in Association
Adult	Semen parameters	Moderate
	Time to pregnancy	Moderate
	Testosterone	Indeterminate
Male Reproductive Outcomes Overall		Moderate
[a] Table from Figure 3 in Radke et al. (2018).		

Radke et al. (2019b) evaluated the associations between BBP or its metabolite (MBzP) and female reproductive outcomes, including pubertal development (5 studies), time to pregnancy (3 studies), spontaneous abortion (5 studies) and preterm birth (6 studies). Radke et al. (2019b) determined the evidence for whether there is a relationship between BBP exposure and pubertal development is indeterminate[2] because the investigations reported conflicting results regarding the onset of puberty, pubic hair development, and breast development. The evidence for association between fecundity and spontaneous abortion and BBP exposure was also indeterminate. Finally, the authors determined that there was slight evidence of association between preterm birth and BBP exposure.

3.1.1.1.3 NASEM Report (2017)

NASEM (2017) evaluated the association between BBP exposure and the following outcomes: hypospadias, testosterone and AGD. 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 BBP, 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 OHAT to assign confidence ratings and determine the certainty of the evidence to ultimately draw hazard conclusions (NTP, 2015).

NASEM concluded that there was inadequate evidence to establish an association between prenatal exposure to BBP 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 BBP is associated with a decrease in fetal testosterone in males, given the various 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. NASEM concluded that the available studies (meta-analysis included three prospective cohort studies) do not support an association between BBP exposure and decreased AGD. However, NASEM found moderate confidence in the evidence of association between BBP (MBzP) and AGD. This finding is inconsistent with the conclusions of Radke et al. (2018), who found slight evidence of an association

[2] Radke et al. (2019b; 2018) define **moderate** evidence descriptors as "evidence that supports a hazard, differentiated by the quantity and quality of information available to rule out alternative explanations for the results." **Slight** and **indeterminant** evidence descriptors are defined as "evidence that could support a hazard or could support the absence of a hazard. These categories are generally limited in terms of quantity or confidence level of studies and serve to encourage additional research across the exposure range experienced by humans."

between exposure to BBP and AGD. The AGD effect estimates in NASEM (2017) for BBP (% change [95% CI] = -1.43 [-3.47, 0.61] [p = 0.17]) 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 BBP exposure and male reproductive outcomes. Radke et al. (2018) concluded that there was a slight level of confidence in the association between exposure to BBP and AGD, while Health Canada (2018b) and NASEM (2017) did not. Radke et al. (2018), also found a slight level of confidence in the association between exposure to BBP and cryptorchidism/hypospadias, but this association was not consistent with the findings of Health Canada (2018b) or NASEM (2017). The scope and purpose of the assessments by Health Canada (2018b), systematic review articles by Radke et al. (2018), and the report by NASEM (2017) differ from that of Health Canada and may be related to differences in confidence conclusions drawn for AGD. Health Canada (2018b) was the most comprehensive review, and considered 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 Section 4). 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 and limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.1.1.2 EPA Summary of Studies (2018- 2019)

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). EPA identified 24 new developmental and 16 new reproductive epidemiology studies published between 2018 to 2019. Eleven of those studies covered female reproductive outcomes (1 high confidence, 9 medium confidence, and 1 uninformative), and 5 medium confidence studies investigated male reproductive outcomes. Three medium confidence studies (Lee et al., 2020; Chin et al., 2019; Arbuckle et al., 2018) found significant associations with exposure to BBP and female reproductive outcomes, including associations with a slower rise in hCG (Chin et al., 2019), increased AGD at birth (Arbuckle et al., 2018), and increased uterine fibroids (Lee et al., 2020). However, there were no significant findings for male reproductive outcomes, aside from male pubertal outcomes. On the other hand, of the 24 male developmental studies, there were six studies (Burns et al., 2022; Bloom et al., 2019; Amin et al., 2018; Berger et al., 2018; Boss et al., 2018; Huang et al., 2018) with significant outcomes (1 high confidence, 2 medium confidence, 5 low confidence). Studies reporting an association are discussed further below.

In text below, EPA discussed the evaluation of the studies by outcome with significant results that contribute to the weight of scientific evidence. 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 Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025e);
- *Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025c).

Developmental Outcomes for Males

Twenty studies were evaluated for the association between BBP and developmental outcomes including birth measures, size trajectory, fetal loss, pubertal development, and gestational duration. Of those studied, two were *high* confidence, 12 were of medium confidence, and six were of *low* confidence. Two *medium* confidence studies looked at the associations between BBP and birth measures without significant results. In a *low* confidence study (Huang et al., 2018), mother-infant pairs from Wuhan, China reported a significant positive association between late pregnancy maternal urinary MBzP and birth length in boys [Beta (95% CI) per ln-ug/L increase in MBzP = 0.15 (0.01, 0.28)]. This study also reported significant associations with BBP metabolites and gestational age [beta (95% CI) per ln-ug/L increase in MBzP overall = 0.16 (0.03, 0.29); and in boys = 0.22 (0.04, 0.41)]. A second l*ow* confidence study reported associations between BBP and gestational duration (Boss et al., 2018) of mother-infant pairs from Boston, Massachusetts reported significant positive associations between prenatal urinary MBzP and gestational age [HR (95% CI) per interquartile range increase in MBzP averaged over three samples collected between 4.7 and 29.3 weeks gestation = 1.15 (1.03, 1.27); and for repeated measures of urinary MBzP collected at up to 38.3 weeks gestation = 1.13 (1.05, 1.22)].

Developmental Outcomes for Females

One *high* confidence study of mother-infant pairs from Charleston, South Carolina reported significant inverse associations across all tertiles of prenatal (18 to 22 weeks gestation) urinary MBzP and small for gestational age [OR (95% CI) = 0.30 (0.10, 0.85) for T2 *vs.* T1 and 0.29 (0.10, 0.81) for T3 *vs.* T1] (Bloom et al., 2019). One *medium* confidence study looked at the association between BBP and female reproductive hormones. This study (Arbuckle et al., 2018) reported a significant inverse association between prenatal first trimester urinary MBzP and anoclitoris distance in infant girls [Beta (95% CI) per ln-ug/L increase in MBzP = -1.2401 (-1.9080, -0.5723); p-value = 0.0004]. No significant results were reported for other anthropometric measurements in females.

Other Developmental Outcomes

Two l*ow* confidence studies reported associations between BBP and its metabolite and size trajectory. The first l*ow* confidence study (Amin et al., 2018) reported significant positive associations between MBzP exposure and body mass index z-score (Beta = 0.18, p-value = 0.002) and waist circumference (Beta = 0.22, p-value < 0.001) in Iranian children and adolescents aged 6 to18 years. The other l*ow* confidence study (Durmaz et al., 2018) reported a significant positive association between MBzP and body mass index in Turkish girls with premature thelarche (Spearman correlation coefficient = 0.375, p-value = 0.041). No significant results were found for fetal loss, anthropometric measures of female reproductive organs, polycystic ovary syndrome, or male reproductive outcome measures such as anthropometric measures of male reproductive organs, sperm parameters, prostate, and male reproductive hormones.

Reproductive Outcomes for Males

Two m*edium* confidence studies (Burns et al., 2022; Berger et al., 2018) reported associations between BBP and pubertal development. The first m*edium* confidence study (Burns et al., 2022) reported significant positive associations between prepubertal BBP exposure (measured between 8 and 13 years of age) and pubertal onset outcomes in Russian boys. These included testicular volume > 3 mL [mean shift in months (95% CI) = 5.6 (0.3, 11.0) for Q3 *vs.* Q1 ; 5.6, (0.6, 10.7) for Q4 *vs*. Q1; p-value for trend =0.006], Tanner Genitalia Stage ≥ 2 [Mean shift in months (95% CI) = 7.5, (1.1,13.8) for Q4 *vs.* Q1; p-value for trend =0.02], and Tanner Pubarche stage ≥ 2 [Mean shift in months (95% CI) = 15.1 (8.0, 22.2) for Q3 *vs*. Q1 and 14.2 (7.4, 21.0) for Q4 *vs*. Q1; p-value for trend < 0.001]. The other m*edium* confidence study (Berger et al., 2018) reported significant positive associations were between prenatal (mean 14.0 and 26.9 weeks' gestation) MBzP exposure and age at onset of thelarche in girls

[Mean shift in months (95% CI) = 1.9 (0.2, 3.6); for overweight/obese girls = 3.9 (1.2, 6.7)] and with pubarche onset in normal weight boys [Mean shift in months (95% CI) = 3.5, (0.4, 6.5)]. Significant inverse associations were observed for boys for onset of gonadarche [Mean shift in months (95% CI) = -3.1 (-5.2, - 0.9); for overweight/obese boys = -4.3 (-6.8, -1.8)] and for pubarche onset in overweight/obese boys [Mean shift in months (95% CI) = −3.6, (−5.7, −1.4)].

Reproductive Outcomes for Females

One *medium* confidence study looked at the association between BBP exposure and fecundity/increased time to pregnancy. This m*edium* confidence study (Chin et al., 2019) of North Carolina women without known fertility issues reported a significantly altered pattern of human chorionic gonadotropin (hCG) rise during the first 6 days after implantation among women with urinary MBzP levels above vs. below the median [p-value for the association between MBzP concentration above median and rate of hCG rise = 0.04]. No significant results were reported for fecundity outcomes (type of corpus luteum rescue, time from ovulation to implantation). One m*edium* confidence study (Lee et al., 2020) looked at the association between BBP and fibroids in adult premenopausal women in Korea. Significantly increased odds of uterine fibroids in quartile 2 (Q2) compared to quartile 1 (Q1) of urinary MBzP [OR (95% CI) for Q2 vs. Q1 = 4.82 (1.09-21.27)] were reported. Associations for quartiles 3 or 4 were positive but not statistically significant.

Conclusion

EPA considered the conclusions of Health Canada (2018b) and NASEM (2017) as well as Radke et al. (2018) and agrees that while there may be evidence of an association between BBP and male development and reproductive outcomes including sperm quality and AGD, it is not enough to conclude a causal relationship. Moreover, studies identified by EPA from 2018 to 2019 do not alter the previous conclusions from Health Canada (2018b) and NASEM (2017), and systematic review articles published by Radke et al. (2018) regarding developmental and reproductive outcomes. Although there is moderate evidence of association between BBP and AGD health outcomes 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 with exposure characterization of individual phthalates, including source or exposure and timing of exposure as well as co-exposure confounding with other phthalates, discussed in Section 1.1. The epidemiological studies provide qualitative support as part of the weight of scientific evidence.

3.1.2 Summary of Laboratory Animal Studies

EPA identified 14 oral exposure studies (all of rats) that have investigated the effects of BBP on the developing male reproductive system (Gray et al., 2021; Spade et al., 2018; Debartolo et al., 2016; Schmitt et al., 2016; Ahmad et al., 2014; Furr et al., 2014; Howdeshell et al., 2008; Aso et al., 2005; Tyl et al., 2004; Wilson et al., 2004; Ema et al., 2003; Ema and Miyawaki, 2002; Gray et al., 2000; Nagao et al., 2000). EPA identified ten of these through review of prior assessments as described in Section 1.2.1, and 4 of these were identified through systematic review of literature as described in Sections 1.2.2 and 1.2.3. No studies evaluating the developmental and/or reproductive toxicity of BBP are available for inhalation or dermal exposure routes.

There are numerous sources and assessments creating a robust data set demonstrating adverse male reproductive system effects following developmental exposure to BBP, which are summarized in Table 3-3, and include 3 multi-generational exposure assessments. These assessments include a variety of

endpoints to be used for evidence integration, including phenotypic changes, organ-level changes, and mechanistic outcomes. Importantly, all animal studies conducted exposures during the developmental masculinization programming window (*i.e.*, GD 15.5 to 18.5 for rats; GD 14 to 16 for mice; gestational weeks 8 to 14 for humans), which may disrupt cellular responses (*e.g.*, testicular testosterone production) and lead to antiandrogenic effects on the developing male reproductive system (MacLeod et al., 2010; Welsh et al., 2008; Carruthers and Foster, 2005). Available oral exposure studies of BBP evaluating developmental and reproductive outcomes are summarized in Table 3-3. Most of the available studies evaluate effects on the developing male reproductive system consistent with a disruption of androgen action following gestational, perinatal, pre-pubertal, or multi-generational oral exposures to BBP. However, several studies are available that evaluate other developmental outcomes (*e.g.*, post-implantation loss, resorptions, fetal body weight, female developmental effects, *etc.*). Effects on the developing male reproductive system (Sections 3.1.2.1 and 3.1.2.2) and other developmental and reproductive outcomes (Section 3.1.2.3) are discussed below.

Table 3-3. Summary of BBP Oral Exposure Studies Evaluating Effects on the Developing Male Reproductive System

Reference (TSCA Study Quality Rating)	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
(Ema et al., 2003) (Medium)[b]	Pregnant Wistar rats (16 dams/group) were exposed to 0, 167, 250, or 375 mg/kg-day MBP via oral gavage from GD 15–17. Dams were sacrificed, and fetal tissue collected on GD 21.	167/250	↓ AGD, Cryptorchidism	Developmental Outcomes - ↓ AGD - Cryptorchidism
(Howdeshell et al., 2008) (High)	Pregnant SD rats (4-9 dams/group) were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 8–18. Dams were sacrificed, and fetal tissue collected on GD 18.	100/300	↓ *ex vivo* fetal testicular testosterone production	Developmental Outcomes - ↓ *ex vivo* fetal testes testosterone production
(Furr et al., 2014) (High)	Pregnant Harlan SD rats were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP (Block 36) via oral gavage from GD 14–18. Dams were sacrificed, and fetal tissue collected on GD 18. (Block 36)	None/100	↓ *ex vivo* fetal testicular testosterone production	Developmental Outcomes - ↓ *ex vivo* fetal testicular testosterone production
	Pregnant Harlan SD rats were exposed to 0, 11, 33, or 100 mg/kg-day BBP (Block 37) via oral gavage from GD 14–18. Dams were sacrificed, and fetal tissue collected on GD 18. (Block 37)	100/None	None	Unaffected Outcomes - No effect on *ex vivo* fetal testicular testosterone production
(Gray et al., 2000) (Medium)[b]	Pregnant SD rats (5-10 dams/group) were exposed to 0 or 750 mg/kg/-day BBP via	None/750	↓ AGD (PND2), ↑ male NR (PND13), ↓ reproductive	Developmental Outcomes - ↓ AGD (PND 2)

Reference (TSCA Study Quality Rating)	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	oral gavage from GD 14–PND 3. Dams were allowed to give birth naturally and outcomes were evaluated in male offspring on PND 2, PND 3, PND 13, and at maturity (3–7 months of age).		organ weights, reproductive organ malformations	- ↑ NR (PND 13) - Permanent nipples (3–7 months) - ↓ absolute testes, LABC, SV, ventral prostate, glans penis, epididymis, cauda epididymis, caput-corpus epididymis weight (3–7 months) - Incomplete PPS due to genital malformations - Reproductive tract malformations (cleft phallus, hypospadias, vaginal pouch, SV and epididymal agenesis, fluid filled testis, small testis, testis absent, abnormal gubernaculum) (3–7 months) - Undescended testes (3–7 months) Unaffected outcomes - Mean age at PPS - Serum testosterone (3–7 months)
(Spade et al., 2018) (Medium)[b]	Pregnant SD rats (3-6 dams/group) were exposed to 0 or750 mg/kg-day BBP via oral gavage from GD 17–21. Dams were sacrificed, and fetal tissue collected on GD 21.	None/750	↓ *ex vivo* fetal testicular testosterone production, ↑ MNG incidence	Developmental Outcomes - ↓ *ex vivo* fetal testicular testosterone production - ↑ Incidence of MNGs
(Wilson et al., 2004) (Medium)[b]	Pregnant SD rats (3 dams/group) were exposed to 0 or 1000 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed, and fetal tissue collected on GD 18.	None/1000	↓ *ex vivo* fetal testicular testosterone production, ↓ testicular *Insl3* mRNA expression	Developmental Outcomes - ↓ *ex vivo* fetal testicular testosterone production Mechanistic Outcomes - ↓ Testicular *Insl3* mRNA Unaffected Outcomes - Testicular progesterone production
(Ema and Miyawaki, 2002) (Medium)[b]	Pregnant Wistar rats (16 dams/group) were exposed to 0, 250, 500, or 1000 mg/kg-day BBP via gastric intubation from GD 15-17. Dams were sacrificed and	250/500	↓ AGD, ↓ AGI, Cryptorchidism, ↑ Transabdominal testicular ascent	Developmental Outcomes - ↓ AGD - ↓ AGI - Cryptorchidism - ↑ Transabdominal testicular ascent Unaffected outcomes

Reference (TSCA Study Quality Rating)	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	fetal tissue collected on GD 21.			Fertility index, gestation indices
(Nagao et al., 2000) (Medium)	Two-generation Study of Reproduction (Guideline not stated): Male and female SD rats (20-24/group) were exposed continuously via oral gavage to 0, 20, 100, 500 mg/kg-day from 8-10 weeks of age for two-generations.	100/500	↓ AGD, ↓ serum testosterone, ↓ reproductive organ weights, testicular pathological changes, delayed PPS	Developmental Outcomes - ↓ AGD (F1 PND 0) - ↓ serum testosterone (F0 & F1 adults) - ↓ absolute testes & epididymis weight (F1 PND 22) - ↓ absolute testes, epididymis, ventral prostate weight (F1 adults) - Testicular pathology (↓ spermatocytes in seminiferous tubules (F1 PND 22); atrophy of seminiferous tubules (F1 adults); ↓ germ cells in seminiferous tubule (F1 adults); testicular edema (F1 adults); decreased sperm in epididymis, with cell debris (F1 adults) - Delayed PPS (F1) Unaffected Outcomes - Mating, fertility, delivery indices (F0, F1); gestation length (F0, F1); absolute reproductive organ weight (testes, epididymides, ventral prostate, SV; F0 adults); absolute SV weight (F1 adults); testicular pathology (F0); sperm motility and concentration (F0, F1 adults); serum testosterone (F1 PND 22); hypospadias (F1), cryptorchidism (F1)
(Aso et al., 2005) (Medium)	Two-generation Study of Reproduction (Guideline not stated): Crj:CD(SD)IGS rats (24/dose) were exposed via oral/gavage to 0, 100, 200, 400 mg/kg-day continuously for two generations.	None/100	↓ AGD, softening of testes ↓ spermatozoa in epididymis, ↓ germ cells in epididymal lumen	Developmental Outcomes - ↓ AGD (100-400/F2 PND 4) - Low rate for completed PPS (400/F1 males) - ↓ absolute epididymis weight (400/F0 adults; 200/F1 adults) & SV (400/F1 adults) - ↑ incidence of small testes (400/F1 adult), softening of testes (100/F1 adult); ↑ incidence of small or hypoplastic epididymides (400/F1 adult) - Testicular pathology (*e.g.*, Leydig cell hyperplasia (400/F0 & 400/F1 adults), diffuse atrophy of testicular seminiferous tubules (400/F1 adults); ↓ spermatozoa in epididymides (400/F0; 100/F1 adults), ↓ germ cells in epididymal lumen (F1 adults at 100), bilateral or unilateral partial aplasia or unilateral aplasia of epididymides (400/F1 adults) Unaffected outcomes

Reference (TSCA Study Quality Rating)	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- Mating index, days required for mating, gestation length, # implantations, fertility index, delivery index, gestation index, # of pups delivered, # of sperm in testis, epididymal sperm motility or morphology (F0 and F1 parents); serum hormones (FSH, LH, testosterone, estradiol (F0 and F1 parents); absolute testis and ventral prostate weight (F1 adults); AGD (F1 pups)
(Tyl et al., 2004) (Medium)	Two-generation Study of Reproduction (GLP-compliant and adhered to OPPTS 870.3800 [August 1998]): CD rats (~20/dose) were exposed via oral/diet to 0, 750, 3750, 11,250 ppm BBP (eq. 0, 50, 250, 750 mg/kg-day) continuously for two generations.	50/250	↓ AGD, ↓ absolute testes weight	Developmental Outcomes - ↓ AGD (F1 and F2 at PND 0) - ↓ absolute testes weight (F1 weanlings, PND 21) - ↓ Mating and fertility indices (750/F1) - ↓ epididymal sperm concentration & motility (750/F1 adults) - ↓ absolute testes, epididymis, prostate, SV weight (750/F1 adult) - ↓ absolute testes weight (750/F2 weanlings, PND 21) and ↓ epididymis weight (750/F1 weanlings, PND 21) - NR (750/F1 and F2, PND 11–13) - Delayed PPS (750/F1) - Undescended testes (750/F1 pups, PND 4) - Gross malformations (missing epididymis (whole or part), epididymis reduced in size, missing testes, testes reduced in size, and undescended testis(es) (750/F1 weanlings, PND 21) - Gross malformations (hypospadias, missing reproductive organ or portion(s) of organs and/or abnormal organ size and/or shape) (750/F1 adults) - Gross malformations (missing SVs, missing epididymides) (750/F2 pups, PND 4) - Testicular pathology (epididymal aspermia, testis dilation, seminiferous tubule degeneration & atrophy) (750/F1 adult) Unaffected Outcomes - Mating, fertility, gestation, pregnancy indices (F0); gestational and pregnancy indices (F1); absolute testes, epididymis, prostate, SV weight (F0); epididymal sperm concentration and motility (F0 adults)
Literature, as Identified in Section 1.2.3				

Reference (TSCA Study Quality Rating)	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
(Ahmad et al., 2014) (Medium)	Pregnant Albino rats (≥6 dams/group) were exposed to 0, 4, 20, or 100 mg/kg-day BBP via oral gavage from GD 14–21. Dams were allowed to give birth naturally, and male offspring were sacrificed on PND 5, 25, or 75. Endpoints evaluated in F1 from PND 1-PND 75.	20/100	↓ serum testosterone, ↓ absolute weight of epididymis and prostate, ↓ sperm count, ↓ percent motile sperm, ↑ percent abnormal sperm	Developmental Outcomes - ↓ serum testosterone (F1 adults, PND 75) - ↓ absolute epididymis and prostate weight (F1 adults, PND 75) - ↓ sperm count, ↓ percent motile sperm, ↑ percent abnormal sperm (F1 adults, PND 75) - ↓ pup body weight (4-100, F1, PND 1) - ↓ body weight (20 and 100, PND 75) Unaffected Outcomes - Litter size, live/dead pups, sex ratio (PND1); Anogenital distance (PND5 & PND25); testis descent; Viability index (PND4); Weaning index (PND21); testicular 17β-HSD activity (PND 75)
(Gray et al., 2021) (High)	Pregnant Harlan SD rats (3-4 dams/group) were exposed to 0, 11, 33, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed, and fetal tissue collected on GD 18.	11/33	↓ fetal testicular mRNA expression of steroidogenic genes, including *Insl3*	Developmental Outcomes - ↓ *ex vivo* fetal testes testosterone production (300) Mechanistic Outcomes - ↓ fetal testicular expression of *Insl3*, as well as steroidogenic genes (*Star* (100), *Cyp11a1*, *Cyp11b2*, *Cyp17a1* (300), *Dhcr7* (11), *Cyp11b1* (11), *Hsd3b* (100), and *Scarb1*) Additional Remarks Data are an expansion of previous dose response studies (Furr et al., 2014; Howdeshell et al., 2008)
	Pregnant Charles River SD rats (3-4 dams/group) were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18. (Block 78)	100/300	↓ *ex vivo* fetal testicular testosterone production	Developmental Outcomes - ↓ *ex vivo* fetal testes testosterone production Mechanistic Outcomes ↓ fetal testicular expression of *Insl3* (600) and steroidogenic genes (*Star* (600), *Cyp11a1* (600), *Cyp17a1* (600), *Dhcr7* (900), *Cyp11b1* (600), *Hsd3b* (900), *Scarb1* (600))
(Schmitt et al., 2016) (Medium)[b]	Female C57Bl/6J mice were gavaged with 0 or 500 mg/kg-day BBP on GD 9-16.	None/500	↓ AGD, ↓ Serum testosterone	Developmental Outcomes - ↓ AGD (10 and 20 weeks)

Reference (TSCA Study Quality Rating)	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	Dams were allowed to give birth naturally, and male pups were sacrificed at 4, 10, or 20 weeks of age.			- ↓ Serum testosterone (10 and 20 weeks)
(Debartolo et al., 2016) (Medium)[b]	Pregnant SD rats were exposed to 0 or 10 µg/mL-day BBP via spiked food pellet (solution pipetted onto pellet) during GD 5-7 through weaning on PND 23. Pups were necropsied on PND 23.	-[a]	↓ AGD, ↓ Relative body weight	Developmental Outcomes - ↓ AGD - ↓ Relative body weight

Abbreviations: ↓ = Statistically significant decrease; ↑ = Statistically significant increase; AGD = Anogenital distance; AGI = Anogenital index; BW = Body weight; CD = Charles River Sprague-Dawley; GD = Gestation day; LABC = Levator ani/bulbocavernosus muscles; LOAEL = Lowest-observed-adverse-effect level; MNGs = Multinucleated gonocytes; NOAEL = No-observed-adverse-effect level NR = Nipple retention; PND = Postnatal day; PPS = Preputial separation; SD = Sprague-Dawley; SV = Seminal vesicle.

[a] Achieved dose, including NOAEL/LOAEL dose, cannot be calculated in mg/kg-day, because dam body weight and food consumption were not reported (Debartolo et al., 2016).

[b] As discussed in the Systematic Review protocol for BBP (U.S. EPA, 2025p) and consistent with Office of Pesticide Programs *Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment* (U.S. EPA, 2012b), the study was of sufficient quality to be considered qualitatively as part of the weight of scientific evidence and was assigned a quality score of medium.

3.1.2.1 Developing Male Reproductive System

EPA previously developed a weight of scientific evidence analysis and concluded that oral exposure to BBP 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 brief summary of the MOA for phthalate syndrome and data available for BBP supporting this MOA is provided below. 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 BBP'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.

Mode of Action for Phthalate Syndrome

A MOA for phthalate syndrome is shown in Figure 3-1, which explains the link between gestational and/or perinatal exposure to BBP 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.

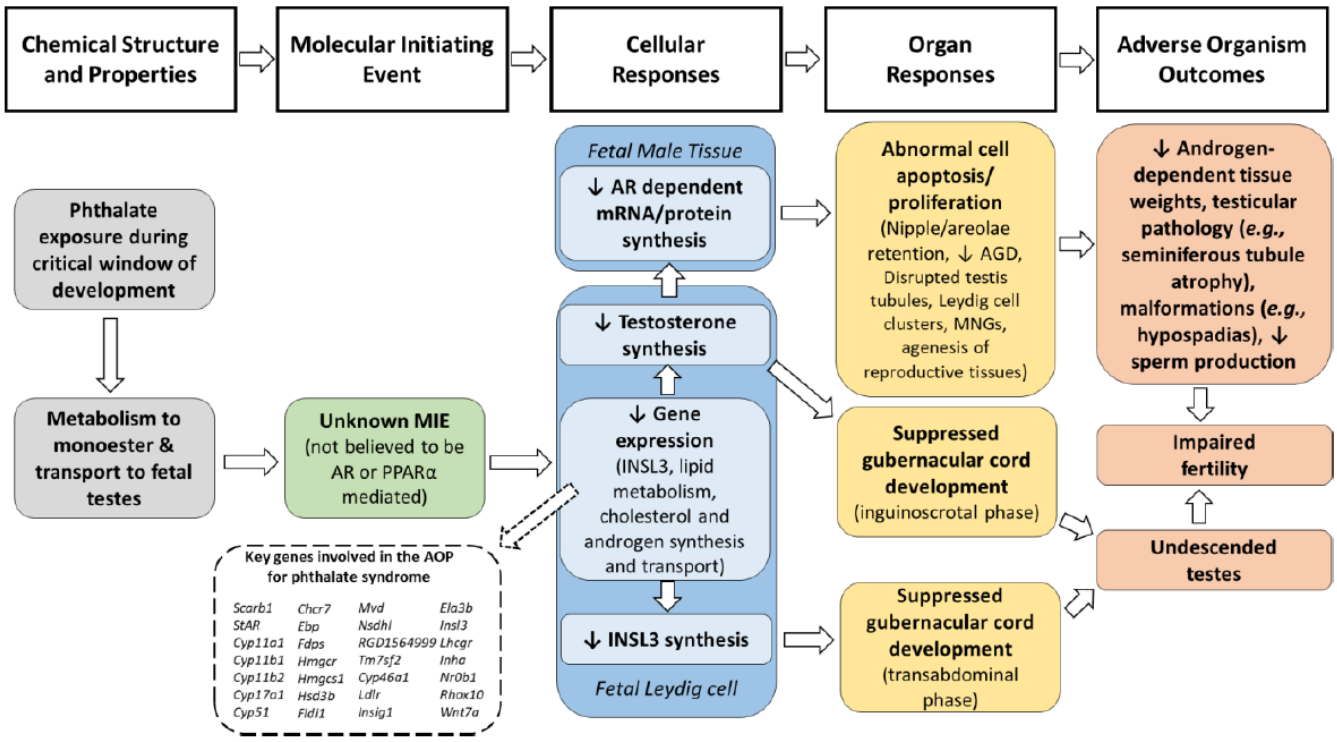

Figure 3-1. Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure

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.

Although the MOA underlying phthalate syndrome has not been fully established, key events at the cellular-, organ-, and organism-level are generally understood (Figure 3-1). In general, molecular events (*i.e.*, the molecular initiating event) of disrupted steroid and lipid metabolism cellular responses are critical in the phthalate syndrome MOA. Several studies have provided evidence against the direct impact of phthalates on androgen receptor and peroxisome proliferator-activated receptor alpha binding for transcriptional activity modulation (Gray et al., 2021; Foster, 2005; Foster et al., 2001; Parks et al., 2000). Other studies have suggested depletion of elemental zinc, which is essential in testicular function, could perturb function of zinc-containing proteins (*e.g.*, zinc-finger transcription factors or as an enzyme cofactor), conceivably resulting in adverse organ-level reactions (Gray et al., 1982; Foster et al., 1980). Of note, *SF-1*, a transcription factor that regulates the INSL3 (insulin-like factor 3) promoter, contains two zinc-finger motifs that are required for DNA binding. INSL3 is a small peptide hormone critical in Leydig cell steroidogenic machinery for cellular differentiation and testosterone production (Ivell et al., 2013). However, it is unclear if zinc depletion is a consequence or an upstream event preceding decreased fetal testosterone synthesis and subsequent steps in the MOA shown in Figure 3-1.

Exposure to BBP during the masculinization programming window of the gonads (*i.e.*, GDs 15.5 to 18.5 for rats; GDs 14 to 16 for mice; gestational weeks 8 to 14 for humans), in which androgen action drives development of the male reproductive system, can lead to antiandrogenic effects on the male reproductive system (MacLeod et al., 2010; Welsh et al., 2008; Carruthers and Foster, 2005). Consistent with the MOA outlined in Figure 3-1, there is experimental evidence of disrupted expression of key genes involved in lipid metabolism and cholesterol and androgen synthesis. In an early assessment by Wilson et al. (2004), pregnant SD rats were orally gavaged with 0 or 1000 mg/kg-day BBP during GD 14 to 18, and on GD 18, offspring were assessed for testicular effects. In this assessment, decreased testicular *Insl3* expression was noted at 1000 mg/kg-day, coinciding with decreased fetal testicular testosterone production. However, a clear limitation of this study was large dose spacing without dose-response assessment.

More recently, Gray et al. (2021) investigated the effects of *in utero* exposure to various phthalates, including BBP. In this multi-cohort study, pregnant Harlan SD rats were orally gavaged with 11, 33, 100, 300, 600, or 900 mg/kg-day BBP from GD 14 to 18, followed by assessment of relevant antiandrogenic genomic and hormonal biomarkers. In offspring, decreased fetal testicular expression of *Insl3* was noted at levels as low as 33 mg/kg-day, along with decreased expression of multiple steroidogenic genes at all levels of exposure, including steroidogenic acute regulatory protein, *Star*, and various cytochromes. This same assessment also included a group pregnant Charles River SD rats gavaged with 100 to 900 mg/kg-day BBP on GD 14 to 18. Analysis in these offspring found decreased testicular expression of *Insl3* occurring at 600 mg/kg-day, with additional steroidogenic genes expression disruption occurring at the same level or higher. Overall, available studies provide consistent evidence that gestational exposure to BBP disrupts mRNA expression of steroidogenic genes and *Insl3* in the fetal testes. Altogether, these data support a mode of action where changes in key genes involved in steroidogenesis or testosterone transport precede cellular responses and subsequent organ-level responses consistent with phthalate syndrome.

Within the MOA of phthalate syndrome, disrupted expression of key genes is suggested to impact fetal Leydig cell production of testosterone, which may contribute to organ-level adverse outcomes. Multiple studies indicate that gestational BBP exposure during the critical developmental window disrupts offspring testicular testosterone production (Gray et al., 2021; Spade et al., 2018; Furr et al., 2014; Howdeshell et al., 2008; Wilson et al., 2004). In addition to previously mentioned mechanistic outcomes, Wilson et al. (2004) noted decreased fetal testicular testosterone production in rats exposed to

1000 mg/kg-day BBP. Likewise, although there appeared to be slight strain-specific effects in BBP sensitivity, both SD cohorts in Gray et al. (2021) displayed decreased *ex vivo* testicular testosterone production, with Harlan SD showing 27 percent decrease in testosterone production at 100 mg/kg-day and Charles River SD showing 38 percent decrease in testosterone production at 300 mg/kg-day. Howdeshell et al. (2008) orally-gavaged SD dams to doses ranging from 100 to 900 mg/kg-day BBP during GD 8 to 18 and assessed *ex vivo* testicular testosterone production. Here, significant effects were noted in a dose-response fashion, with decreased testosterone levels noted at doses of 300 mg/kg-day and above (22% decrease in testosterone production at 300 mg/kg-day). Similarly, Spade et al. (2018) exposed SD dams to 750 mg/kg-day BBP during GD 17 to 21 and accordingly noted a higher dose effect of 69 percent decrease in testosterone production in BBP-exposed rats. Lastly, Furr et al. (2014) conducted a series of studies with BBP. In those studies, Harlan SD rats were orally gavaged with BBP at levels of 100 to 900 mg/kg-day (Block 36) or 11 to 100 mg/kg-day (Block 37) during GD 14 to 18, followed by *ex vivo* testosterone production assessment on GD 18. Interestingly, low-dose assessment in Block 37 did not indicate any significant BBP effects, even at 100 mg/kg-day. However, a dose-dependent decrease in *ex vivo* testicular testosterone production was observed at all levels of BBP exposure in Block 36 (100, 300, 600, and 900 mg/kg-day), with 100 mg/kg-day resulting in a 53 percent decrease and 900 mg/kg-day resulting in an 85 percent decrease in ex vivo fetal testicular testosterone production (Furr et al., 2014). Although a limitation of the Furr et al. (2014) assessment is the inconsistent findings of Blocks 36 and 37 at 100 mg/kg-day BBP, likely due to the variability within limited sample size of a small data set available for BBP, findings are generally consistent with effect-levels noted in other studies assessing BBP effects on testicular testosterone production (Gray et al., 2021; Howdeshell et al., 2008). Collectively, these studies demonstrate the ability of gestational BBP exposure to decrease testicular testosterone production.

In addition to BBP exposure-related decrements in testicular testosterone production and gene expression of steroidogenic genes, corroborating organ-level responses are also noted across multiple studies. As with effects commonly noted from other phthalates, sensitive organ-level responses include testicular histopathological changes, reproductive organ weight changes, and antiandrogenic-related abnormal growth and development effects, such as decreased anogenital distance (AGD) and nipple retention in male offspring. AGD, typically corrected for the cube root of body weight ratio, is regarded a sensitive hallmark of early-life reproductive and developmental androgen disruption from phthalate exposure (Schwartz et al., 2019).

Multiple studies have evaluated organ responses to perinatal BBP exposure. Ema et al. (2003) exposed pregnant Wistar rats to 0, 250, 500, or 1000 mg/kg-day BBP via gastric intubation during GD 15 to 17 and examined fetal offspring on GD 21. In this study, a significant increase in fetuses with cryptorchidism (*i.e.*, undescended testes) and male fetuses with decreased AGD was noted in groups that received 500 and 1000 mg/kg-day BBP. Ema et al. (2003) orally gavaged pregnant Wistar rats with either 0, 167, 250, or 375 mg/kg-day MBP, the major BBP metabolite. Dams were dosed from GD 15 to 17, and dams were sacrificed on GD 21 for fetal collection. In this study, effects of cryptorchidism incidence and significantly decreased AGD were noted at levels of 250 mg/kg-day and above. In other studies, many histopathological findings coincide with antiandrogenic effects, such as Spade et al. (2018) finding, in addition to decreased testosterone production at 750 mg/kg-day BBP, an increased incidence of multinuclear gonocytes (MNGs). In a study that tested a single dose level, Gray et al. (2000) orally gavaged pregnant SD rats with 0 or 750 mg/kg-day BBP from GD 14 to PND 3. Dams were allowed to give birth, and male offspring were sacrificed from PND 2 to mature adults, with evaluations occurring at PND 2, PND 13, and 3 to 7 months. Decreased AGD was noted at PND 2, and increased nipple retention was noted at PND 13 in BBP-exposed rats. Additionally, these early-life effects were accompanied by incomplete preputial separation, increased incidence of undescended testis,

and decreased testes and accessory sex organ weights (*e.g.*, absolute testes, seminal vesicles, LABC, ventral prostate, glans penis, paired epididymides, cauda epididymides, and caput-corpus epididymides) at 3 to 7 months of age. Reproductive tract malformations at 750 mg/kg-day BBP were also noted, including cleft phallus, hypospadias, epididymal agenesis, fluid filled or missing testis, and abnormal gubernaculum (Gray et al., 2000).

Three multi-generational studies of male reproductive and developmental outcomes were identified (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000). Although Tyl et al. (2004) was the only study to explicitly report adherence to multi-generation testing guidelines by U.S. EPA Office of Prevention, Pesticides, and Toxic Substances Guidelines, the other two-generation studies identified adhered to similar practices suggested by the Organisation for Economic Co-operation and Development two-generation reproduction toxicity testing guidelines (OECD, 2018).

Nagao et al. (2000) conducted a two-generation reproductive study in male and female SD rats using oral exposure doses of 0, 20, 100, and 500 mg/kg-day BBP from 8 to 10 weeks of age in the F0 generation, and female exposure continued during gestation and lactation until postpartum day 21. Dosing continued into F2 generation, and F1 rat observations were made on PND 0 and PND 21 at necropsy and in F2 rats on PND 0. In F1 offspring, multiple developmental outcomes were noted pertaining to hormonal, histopathological, and organ-level changes only at the highest level of exposure. In F1 adults, decreased circulating testosterone was noted in rats exposed to 500 mg/kg-day, along with decreases absolute testicular, epididymal, and ventral prostate weights. Testicular pathological incidence significantly increased at this same dose level and included decreased seminiferous tubule spermatocytes, seminiferous tubule atrophy, decreased seminiferous tubule germ cell, testicular edema, and decreased epididymal sperm. Importantly, 500 mg/kg-day BBP also resulted in delayed preputial separation, an androgen-sensitive sign of puberty, and decreased AGD in F1 and F2 offspring (Nagao et al., 2000).

Aso et al. (2005) conducted a similar multi-generational reproductive study in Charles River SD rats, in which rats were orally gavaged with 0, 100, 200, or 400 mg/kg-day BBP continuously for two generations, with exposure starting in the F0 parental generation at 5 weeks of age and at 3 weeks of age (*i.e.*, at weaning) for the F1 parental generation. In F1 offspring, the majority of BBP exposure-related effects occurred at 400 mg/kg-day, with a few effects also occurring at 100 mg/kg-day. Absolute organ weights were significantly decreased in F1 adults, including absolute epididymis weight (at 200 and 400 mg/kg-day) and seminal vesicle weight (at 400 mg/kg-day). In F1 adults, in addition to decreased germ cells in the epididymal lumen at 100 mg/kg-day and above, there was also an increased incidence of Leydig cell hyperplasia, diffuse atrophy of seminiferous tubules, decreased epididymal spermatozoa, and epididymal aplasia at 400 mg/kg-day. As expected, these histopathological changes in the F1 generation at 400 mg/kg-day were accompanied by the developmental outcome of lower rate of completed preputial separation. In F2 pups, reproductive and developmental assessment was largely limited to physical development analysis. Although AGD was not impacted by exposure in F1 male pups, F2 male pups showed significantly decreased AGD at BBP exposure levels of 100 mg/kg-day and above (Aso et al., 2005). These results by Aso et al. (2005) were also briefly summarized in the summary paper by Yamasaki et al. (2005).

Tyl et al. (2004) conducted a multi-generational study treating CD rats with BBP in the diet at 0, 750, 3750, and 11,250 ppm (equivalent to 0, 50, 250, and 750 mg/kg-day) continuously for two generations, with observations made in F1 weanlings (PND 21) and adults and F2 pups. In F1 pups, decreased absolute testicular and epididymal weight at levels 250 and 750 mg/kg-day were noted, which occurred in F1 adults as well at 750 mg/kg-day. Also, in F1 adults, decreased epididymal sperm concentration and

motility, as well as decreased absolute prostate and seminal vesicle weight, were noted at 750 mg/kg-day. In F1 weanling and adult examinations, a myriad of gross malformations (*e.g.*, reduced epididymal size, missing epididymis, hypospadias, and cryptorchidism) were noted at the highest BBP exposure level. Further, adverse developmental effects were noted in both F1 and F2 pups. At 750 mg/kg-day, delayed preputial separation was noted in F1 pups, and increased nipple retention was noted in F1 and F2 pups. However, in both F1 and F2 pups, the lower level of BBP exposure (at 250 mg/kg-day and above) resulted in significantly reduced AGD (Tyl et al., 2004).

In sum, these studies provide consistent evidence that oral BBP exposure in rats, particularly during the critical window of organogenesis and masculinization of the male reproductive system, can disrupt androgen action, leading to a cluster of anti-androgenic mechanistic-, cellular, and organ-level outcomes that are consistent with the MOA for phthalate syndrome (Figure 3-1). As previously noted, this conclusion was supported by the Science Advisory Committee on Chemicals (SACC) (U.S. EPA, 2023b), presented in EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023a), which provides a thorough discussion of BBP exposure effects on the developing male reproductive system and EPA's MOA analysis. Consistent with animal toxicology literature, epidemiological evaluations across several studies, as reported by Radke et al. (2018), provide some evidence that BBP exposure is associated with male reproductive toxicity. For example, Radke et al. (2018) found moderate evidence of an association between BBP and effects on semen parameters and increased time to pregnancy. These observed developmental effects are assumed to be relevant for extrapolating human risk, and thus EPA is considering developmental toxicity for dose-response analysis and use in estimating risk to human health. EPA's further consideration of developmental toxicity is discussed in Section 4.

3.1.2.2 Literature Considered for Non-Cancer Hazard Identification

EPA identified 10 animal toxicology studies that provide data on PECO-relevant health effects following exposure to BBP, as discussed in Section 1.2.3. Of these, 4 studies provided outcomes evaluating gestational exposure and relevant male reproductive and developmental effects (Table 3-3). For two of these studies, EPA identified several limitations or lack of sensitivity, including that of exposure dose uncertainty, lack of monotonic dose-response, or studies that only included relatively high doses) (Debartolo et al., 2016; Schmitt et al., 2016). Two studies identified potentially relevant NOAELs or LOAELs for adverse outcomes on developing male reproductive system (Gray et al., 2021; Ahmad et al., 2014), which are further considered in Section 4 for dose-response assessment. 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.

In a behavioral assessment by Schmitt et al. (2016), pregnant mice were exposed to 0 or 500 mg/kg-day BBP via gavage from GD 9 to 16, and pups were weaned and analyzed for behavioral physical activity levels and markers of endocrine disruption activity from postnatal week (PNW) 8 to PNW 20. Regarding relevant effects on developing male reproductive outcomes identified in this study, significantly decreased AGD and serum testosterone levels were noted at PNW 10 and 20. However, notable limitations of this study include that it only included a single high dose group, which precludes its use in understanding dose-response, and this study does not offer a more sensitive POD than those provided by earlier literature for effects of the developing male reproductive system; therefore, it was not considered further. Lastly, it was considered a limitation of the experimental design that BBP exposure ended on GD 16, which did not include the entire susceptible gestational exposure window of rats for BBP-related antiandrogenic effects (GD 15.5 to 18.5), as was done in other studies. This study is

shown in Table 3-3, and is included in Appendix B.1 discussion of literature presenting reproductive/developmental outcomes and Appendix B.2 discussion of literature depicting neurotoxicity outcomes.

A study by DeBartolo et al. (2016) provided data on neurobehavioral effects (*i.e.,* fear conditioning) and endocrine-disrupting outcomes following developmental exposure to BBP. Pregnant SD rats were exposed to 0 or 10 µg/mL BBP pipetted onto food pellets from GD 14 to PND 23. Regarding relevant effects on developing male reproductive outcomes identified in this study, significantly decreased relative body weight and AGD were noted in BBP-exposed male offspring when measured at PND 23. However, EPA identified substantial limitations of this study which impact the interpretation of the results and contribute to uncertainty in the data set. The largest limitation of this study includes substantial uncertainty regarding the achieved dose. BBP stock solution (10 µg/mL) was pipetted onto sweetened food pellets and fed to pregnant dams; however, the resulting concentration of the test diets were not determined, and maternal body weights and feed consumption were not reported, therefore achieved dose cannot be calculated. Also, treatment for the control and BBP-exposed groups was also stated to occur ~5 to 7 days during gestation, meaning not all exposed animals may have had the same exposure time duration. This study is shown in Table 3-3, and is presented in Appendix B.1 discussion of literature on reproductive/developmental outcomes and Appendix B.2 discussion of literature on neurotoxicity outcomes.

Two studies considered relevant to BBP dose-response assessment and POD derivation were identified (Gray et al., 2021; Ahmad et al., 2014). In the study by Ahmad et al. (2014), albino rats were gavaged with 0, 4, 20, or 100 mg/kg-day BBP from GD 14 to 21, and male offspring were sacrificed for analysis on PND, 5, 25, or 75. In this study, authors reported a LOAEL of 100 mg/kg-day (NOAEL of 20 mg/kg-day) based upon decreased serum testosterone, decreased epididymal and prostate weights, and sperm quality effects in F1 adults measured at PND 75. In Gray et al. (2021), two experiments were performed in SD rats. In the first experiment, pregnant Harlan SD rats were gavaged with 0, 11, 33, 100, 300, 600, or 900 mg/kg-day BBP from GD 14-18, with *ex vivo* fetal testicular testosterone and steroidogenic gene expression measurement on GD 18. This study yielded a NOAEL of 11 mg/kg-day BBP based on decreased *Insl3* expression at 33 mg/kg-day; significantly reduced *ex vivo* fetal testicular testosterone occurred at 300 mg/kg-day BBP levels and higher. The second experiment was conducted with pregnant Charles River SD rats gavaged with 0, 100, 300, 600, or 900 mg/kg-day from GD 14 to 18, with *ex vivo* fetal testicular testosterone and steroidogenic gene expression measured at GD 18. This study yielded a NOAEL of 100 mg/kg-day BBP based on decreased *ex vivo* fetal testicular testosterone occurring at levels of 300 mg/kg-day BBP and higher. These studies are shown in Table 3-3 and are presented in Appendix B.1 discussion of literature on reproductive/developmental outcomes. Further, these studies were among literature considered for dose-response assessment and POD derivation based upon potentially identified more sensitive effect levels for BBP-related developing male reproductive toxicity effects and thus are further discussed in Section 4.

3.1.2.3 Other Developmental and Reproductive Outcomes

In addition to effects on the developing male reproductive system, other developmental effects (*e.g.,* decreased fetal weight, resorptions, decreased mating and fertility index, and effects on female reproductive outcomes) have been observed in experimental animal models following oral exposure to BBP. However, these effects generally occur at equal or higher doses than those that result in effects on the developing male reproductive system and frequently coincide with maternal toxicity (Table 3-3). Data supporting other developmental effects of BBP are discussed below.

In the two-generation reproduction oral exposure studies, Aso et al. (2005) noted developmental toxicity outcomes in addition to effects on the developing male reproductive system. In SD rats orally gavaged with 0, 100, 200, or 400 mg/kg-day BBP, Aso et al. (2005) noted decreased AGD in F1 female offspring at 100 mg/kg-day and above and a reduced fertility index in the F1 generation at 400 mg/kg-day.

In another two-generation study, Nagao et al. (2000) gavaged SD rats to BBP doses of 0, 20, 100, and 500 mg/kg-day from 8 to 10 weeks of age in the F0 generation, and female exposure continued during gestation and lactation until postpartum day 21. F1 rat observations were made on PND 0 and PND 21 at necropsy. Here, Nagao et al. (2000) reported decreased male and female F1 pup weight at 100 and 500 mg/kg-day. Further developmental female toxicity was also noted in F1 offspring, where decreased AGD was reported in F1 pups at 500 mg/kg-day.

In the two-generation study by Tyl et al. (2004), CD rats were exposed to 0, 50, 250, and 750 mg/kg-day via the diet and numerous developmental and reproductive outcomes in F1 and F2 male and female offspring were examined. Authors reported numerous effects at the highest dose tested of 750 mg/kg-day across both sexes, including decreased uterine and ovarian weights in F1 and F2 offspring, decreased mating and fertility index in F1 generation, decreased implantations in F2 generation, decreased number of F2 live pups, and decreased fetal body weight in F1 male and female offspring.

Lastly, Howdeshell et al. (2008) gavaged SD rats with 0, 100, 300, 600, or 900 mg/kg-day BBP from GD 8 to 18 and examined fetal outcomes on GD 18. Authors reported developmental toxicity occurring at 600 mg/kg-day and above through decreased live fetuses, increased resorption, and increased fetal mortality. Further, in a single dose-level study conducted by Gray et al. (2000), SD rats were gavaged with 0 or 750 mg/kg-day BBP from GD 14 to PND 3. In this study, authors reported decreased mean pup weight at birth when assessed on PND 3 (Gray et al., 2000).

Collectively, available studies provide consistent evidence that gestational exposure to BBP can result in a spectrum of developmental effects in addition to those of the developing male reproductive system. However, effects on the developing male reproductive system (Section 3.1.2.1) occur at much lower doses than the aforementioned other developmental effects. Specifically, the lowest LOAELs for effects on the developing male reproductive system occur around 100 mg/kg-day, while the lowest LOAELs for other developmental outcomes discussed here range from 100 to 750 mg/kg-day, with most effects occurring at or above 200 mg/kg-day (Table 3-3). Therefore, effects on the developing male reproductive system are as sensitive and often robust than other endpoints to BBP exposure and are consistent with a disruption of androgen action and phthalate syndrome.

4 DOSE RESPONSE ASSESSMENT

EPA focused its dose-response analysis on developmental and reproductive toxicity, particularly effects relevant to phthalate syndrome in male rats. These effects are consistently observed across different strains of rat, varying exposure durations including single and multi-generations, and occur in a dose-related manner.

EPA identified evidence of other non-cancer hazard endpoints (*i.e.*, liver and kidney toxicity), but did not perform dose-response analysis of these endpoints because endpoints associated with developing male reproductive effects are supported by the most robust data set and available information that indicates male reproductive effects are at least as or more sensitive as other reported effects, increasing EPA's confidence in using these endpoints for estimating risk to human health. According to previous assessments by U.S. CPSC (2010) and NICNAS (2015), there is evidence for systemic toxicity following BBP exposure, including liver and kidney weight effects. However, Health Canada (2015a) concluded BBP has lower systemic toxicity effects that occur only at much higher exposure levels than developmental reproductive effects based upon multiple repeated oral dose toxicity studies. For acute developmental oral exposure studies, LOAEL systemic toxicity effects (such as changes in liver weight) occurred at levels ≥200 mg/kg-day, where some systemic effects are observed in females at lower doses but are not accompanied by changes in clinical chemistry markers or histopathological effects (EC/HC, 2015a). Further, for intermediate exposure duration, the lowest LOAEL for repeated intermediate oral exposure was determined to be 313 mg/kg-day based on increased liver and kidney weights, accompanied by histopathological changes (EC/HC, 2015a). No studies indicating more extensive hepatic or renal effects, or a more sensitive POD, were identified through the TSCA systematic review process, and thus EPA considered the conclusions of previous assessments of male developmental reproductive health effects as most sensitive toxicity indicators as valid.

For the BBP dose-response assessment, EPA first identified NOAEL and LOAEL values from the 14 developmental and reproductive toxicity studies (reported in 12 publications) considered for dose-response assessment (Table 4-1). Nine of the 14 studies provided dose-response information and tested doses of 100 mg/kg-day or less (Gray et al., 2021; Ahmad et al., 2014; Furr et al., 2014; Howdeshell et al., 2008; Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000). These nine studies were considered further for benchmark dose (BMD) modeling using EPA's BMD Software to attempt to refine the identified NOAEL or LOAEL. The remaining 5 of the initial 14 studies were not subjected to BMD analysis as they either evaluated a single dose level of BBP (Spade et al., 2018; Wilson et al., 2004; Gray et al., 2000) or were not very sensitive (*e.g.*, evaluated doses greater than 100 mg/kg-day or higher (Ema et al., 2003; Ema and Miyawaki, 2002). For one hazard endpoint (*i.e.*, reduced fetal testicular testosterone in rats), EPA conducted BMD modeling as well as an updated meta-analysis and BMD 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 four studies reported in three publications was included in EPA's meta-analysis (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008). Testosterone data from all four of the individual studies was also subjected to BMD analysis using EPA's BMD Software, so that results between the two analyses could be compared (Appendix F). In subsequent sections below the extent to which BMD modeling was or was not conducted for each study is discussed further.

No dermal or inhalation studies were available that could be used for dose-response assessment. Acute, intermediate, and chronic non-cancer NOAEL/LOAEL values identified by EPA are discussed further in Section 4.2. As discussed further in Section 4.2, EPA considers effects on the developing male

reproductive system consistent with a disruption of androgen action relevant for setting a POD for acute exposure durations. However, because these acute effects are the most sensitive effects following exposure to BBP, they are also considered protective of intermediate and chronic duration exposures. As described in Appendix D, EPA converted oral PODs derived from animal studies to HEDs using allometric body weight scaling to the three-quarters power (U.S. EPA, 2011c). Differences in dermal and oral absorption are corrected for in the dermal exposure assessment, allowing the same HEDs to be used for both oral and dermal routes. In the absence of inhalation studies, EPA performed route-to-route extrapolation to convert oral HEDs to inhalation human equivalent concentrations (HECs) (Appendix D).

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

EPA considered the suite of oral animal toxicity studies primarily demonstrating 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 considered the following factors during study and endpoint selection for POD determination from relevant non-cancer health effects:

- Exposure duration;
- Dose range;
- Relevance (*e.g.*, considerations of species, whether the study directly assesses the effect, whether the endpoint the best marker for the toxicological outcome, etc.);
- Uncertainties not captured by the overall quality determination;
- Endpoint/POD sensitivity; and
- Total uncertainty factors (UFs). EPA considers the overall uncertainty with a preference for selecting studies that provide lower uncertainty (*e.g.*, lower benchmark MOE) because they provide higher confidence (*e.g.*, use of a NOAEL vs a LOAEL with additional UF_L applied).

The 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 14 developmental and reproductive toxicity studies across 12 publications (all rat studies) with endpoints relevant to acute, intermediate, and chronic exposure durations (U.S. EPA, 1996, 1991). These studies were previously discussed in Section 3.1.2 and are summarized in Table 4-1. Primary endpoints considered relevant to all exposure durations include effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of male reproductive development in rats and other developmental effects, such as resorptions and decreased body weight. Although single dose studies evaluating the effects of BBP on the developing male reproductive system are not available, Alam et al. (2015) conducted a single dose gavage study in male adolescent SD rats (briefly discussed in Appendix C). In this study, one high-dose oral exposure to 500 mg/kg BBP resulted in a spectrum of antiandrogenic outcomes, including increased seminiferous tubule spermatocyte cell apoptosis and decreased absolute testis weight. Regarding acute developmental exposures, studies of the toxicologically similar phthalate dibutyl phthalate (DBP) have demonstrated that a single exposure during the critical window of development can disrupt expression of steroidogenic genes and decrease fetal testicular testosterone (Johnson et al., 2012; Johnson et al., 2011; Thompson et al., 2005). Therefore, EPA considers effects on the developing male reproductive system consistent with a disruption of androgen action to be relevant for setting a non-cancer POD for acute, intermediate, and

chronic exposure durations (see Appendix C for further discussion). Notably, the 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 diisodecyl phthalate (DIDP) and diisononyl phthalate (DINP) human health hazard assessment (U.S. EPA, 2024).

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

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
Pregnant Harlan SD rats (n = 3-4 dams/group) were exposed to 0, 11, 33, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18 (Gray et al., 2021) (High).	NOEL = 11[c]	↓ Fetal testicular mRNA expression of steroidogenic genes (including *Insl3*)	2.6	UF_A= 3 UF_H=10 *Total UF=30*	- Outcome (gene expression) not considered for BMD analysis
Pregnant Albino rats (≥6 dams/group) were exposed to 0, 4, 20, or 100 mg/kg-day BBP via oral gavage from GD 14–21. Dams were allowed to give birth naturally and male offspring were sacrificed on PND 5, 25, or 75 (Ahmad et al., 2014) (Medium).	NOAEL = 20	↓ serum testosterone, ↓ absolute weight of epididymis and prostate, ↓ sperm count, ↓ percent motile sperm, ↑ percent abnormal sperm	4.72	UF_A= 3 UF_H=10 *Total UF=30*	- Study considered for BMD modeling, however, data for most sensitive effects was not considered amenable to modeling due data reporting limitations (Section 4.2.3)
CD rats (n ~20/group) exposed via oral/diet to 0, 750, 3750, 11,250 ppm (eq. 0, 50, 250, 750 mg/kg-day) continuously for 2 generations (Tyl et al., 2004) (Medium).	NOAEL = 50	↓ AGD (F1 and F2)	11.8	UF_A= 3 UF_H=10 *Total UF=30*	- Study considered for BMD modeling, however, no additional BMD analysis was conducted because the most sensitive effect (reduced AGD) was previously included in a meta-analysis of reduced AGD data from 4 studies, which supports a $BMDL_5$ of 164 mg/kg-day (NASEM, 2017). Since the meta-analysis includes data from 4 studies and a wider range of doses, it is expected to provide more precise BMD_5/$BMDL_5$ estimates and is therefore preferred over BMD analysis of individual studies (Section 4.2.2)
Crj:CD(SD)IGS rats (n = 24/dose) oral/gavage with 0, 100, 200, 400 mg/kg-day BBP continuously for 2 generations (Aso et al., 2005) (Medium).	LOAEL = 100	↓ AGD (F2); softening of testes; ↓ spermatozoa in epididymis; ↓ germ cells in epididymal lumen	23.6	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- See Appendix G for BMD results
	$BMDL_5$ = 55	↑ incidence of seminiferous tubule atrophy	13	UF_A= 3 UF_H=10*Total UF=30*	

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
Pregnant Harlan SD rats (n = 2-4/group) were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18 (Block 36) (Furr et al., 2014) (High).	LOAEL = 100	↓ *ex vivo* fetal testicular testosterone production	23.6	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- BMD modeling of fetal testosterone data attempted - No models adequately fit the data set (Appendix F.3)
SD rats were exposed via oral gavage from GD 8–18 to 0, 100, 300, 600, 900 mg/kg-day BBP. Dams were sacrificed and fetal tissue collected on GD 18 (Howdeshell et al., 2008) (High).	NOAEL = 100	↓ *ex vivo* fetal testicular testosterone production	23.6	UF_A= 3 UF_H=10 *Total UF=30*	- See Appendix F.1 for BMD results
	$BMDL_5$ = 81	↓ *ex vivo* fetal testicular testosterone production	19.2	UF_A= 3 UF_H=10 *Total UF=30*	
Pregnant Charles River SD rats (n = 3-4 dams/group) were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18 (Gray et al., 2021) (High).	NOAEL = 100	↓ *ex vivo* fetal testicular testosterone production	23.6	UF_A= 3 UF_H=10 *Total UF=30*	- BMD modeling of fetal testosterone data attempted - No models adequately fit the data set (Appendix F.2)
Pregnant Harlan SD rats (n = 2-4/group) were exposed to 0, 11, 33, or 100 mg/kg-day BBP via oral gavage from GD 14-18. Dams were sacrificed and fetal tissue collected on GD 18 (Block 37) (Furr et al., 2014) (High).	NOAEL = 100	↓ *ex vivo* fetal testicular testosterone production	23.6	UF_A= 3 UF_H=10 *Total UF=30*	- BMD modeling of fetal testosterone data attempted - No models adequately fit the data set (Appendix F.4)
Male and female SD rats (n = 20-24/group) dosed (gavage) from 8-10 weeks of age with 0, 20, 100, and 500 mg/kg-day BBP continuously for 2-generations (Nagao et al., 2000) (Medium).	NOAEL = 100	↓ AGD (F1), ↓ serum testosterone (F1), ↓ reproductive organ weights (F1), testicular pathological changes (F1; *e.g.*, ↓ spermatocytes in seminiferous tubules, atrophy of seminiferous tubules, ↓ germ cells in seminiferous tubule, testicular edema, decreased sperm in epididymis)	23.6	UF_A= 3 UF_H=10 *Total UF=30*	- Study considered for BMD modeling, however, data not considered amenable to modeling or not considered sensitive enough to warrant BMD modeling (Section 4.2.1)

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
Pregnant Wistar rats (16 dams/group) were orally gavaged with 0, 167, 250, 375 mg/kg-day MBP on GD 15–17. Dams sacrificed and fetal tissue collected on GD 21 (Ema et al., 2003). (Medium)[d]	NOAEL = 167	↓ AGD, cryptorchidism	39.4	UF_A= 3 UF_H=10 *Total UF=30*	- Study not subjected to BMD analysis, as other studies evaluated lower doses and provided more sensitive outcomes for modeling (Section 4.2.1)
Pregnant Wistar rats (16 dams/group) were exposed to 0, 250, 500, or 1000 mg/kg-day BBP via gastric intubation from GD 15-17. Dams were sacrificed and fetal tissue collected on GD 21 (Ema and Miyawaki, 2002). (Medium)[d]	NOAEL = 250	↓ AGD, ↓ AGI, cryptorchidism, ↑ transabdominal testicular ascent	59	UF_A= 3 UF_H=10 *Total UF=30*	- 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 (5-10 dams/group) were exposed to 0 or 750 mg/kg-day BBP via oral gavage from GD 14–PND 3. Dams were allowed to give birth naturally and male offspring were sacrificed between PND 2–mature adults (3–7 months of age) (Gray et al., 2000). (Medium)[d]	LOAEL = 750	↓ AGD, ↑ male NR, ↓ reproductive organ weights, reproductive organ malformations	177	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- Study not amendable to BMD modeling (evaluated one dose level)
Pregnant SD rats (3-6 dams/group) were exposed to 0 or 750 mg/kg-day BBP via oral gavage from GD 17–21. Dams were sacrificed and fetal tissue collected on GD 21 (Spade et al., 2018). (Medium)[d]	LOAEL = 750	↓ *ex vivo* fetal testicular testosterone production, ↑ MNG incidence	177	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- Study not amendable to BMD modeling (evaluated one dose level)
Pregnant SD rats (3 dams/group) were exposed to 0 or 1000 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18 (Wilson et al., 2004). (Medium)[d]	LOAEL = 1000	↓ *ex vivo* fetal testicular testosterone production, ↓ Testicular *Insl3* mRNA expression	236	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	- Study not amendable to BMD modeling (evaluated one dose level)

Abbreviations: ↓ = Statistically significant decrease; ↑ = Statistically significant increase; AGD = Anogenital distance; AGI = Anogenital index; $BMDL_5$ = Lower 95% confidence limit on benchmark dose; CD = Charles River Sprague-Dawley; GD = Gestation day; LOAEL = Lowest-observed-adverse-effect level; MNGs = Multinucleated gonocytes; NOAEL = No-observed-adverse-effect level; NOEL = No-observed-effect level; NR = Nipple retention; PND = Postnatal day; SD = Sprague-Dawley.

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

Brief Study Description (Reference) (TSCA Study Quality Rating)	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	Uncertainty Factors[a] [b]	BMD Analysis Notes
[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to BBP. EPA used a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 to account for the uncertainty inherent in extrapolating from the LOAEL to the NOAEL. [c] NOAEL of 11 mg/kg-day is limited to decreased fetal testicular expression of genes involved in steroidogenesis, including *Insl3* (which are effects not considered adverse in isolation). Statistically significant adverse effects, particularly decreased *ex vivo* fetal testicular testosterone production, reached statistical significance at higher doses, resulting in a LOAEL = 300 mg/kg-day based upon *ex vivo* fetal testicular testosterone production. [d] As discussed in the Systematic Review protocol for BBP (U.S. EPA, 2025p) and consistent with Office of Pesticide Programs *Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Hazard Assessment* (U.S. EPA, 2012b), the study was of sufficient quality to be considered qualitatively as part of the weight of scientific evidence and was assigned a quality score of medium.					

4.2.1 Studies with Lack of Dose-Response Sensitivity and Increased Uncertainty

Of the 14 studies considered and presented in Table 4-1, six were not further evaluated for quantitative dose response due to the reasons detailed below, including limitations of dose selection and effect level sensitivity that was determined to increase uncertainties (Spade et al., 2018; Wilson et al., 2004; Ema et al., 2003; Ema and Miyawaki, 2002; Gray et al., 2000; Nagao et al., 2000).

Three studies (Spade et al., 2018; Wilson et al., 2004; Gray et al., 2000) that only tested one relatively high-dose-level of BBP effects on male reproductive outcomes, including fetal testicular testosterone production, were not further considered for dose-response analysis. Gray et al. (2000) gavaged SD rats with 0 or 750 mg/kg-day BBP from GD 14 to PND 3 and assessed offspring effects from PND 2 into adulthood. In this assessment, authors noted decreased AGD, increased nipple retention, and various reproductive organ malformations at 750 mg/kg-day BBP (Gray et al., 2000). Spade et al. (2018) gavaged SD rats with 750 mg/kg-day BBP and noted 69 percent decrease in *ex vivo* fetal testicular testosterone production relative to control, as well as an increase in testicular MNG incidence. In SD rats gavaged with 1000 mg/kg-day BBP, Wilson et al. (2004) reported 12 percent *ex vivo* fetal testicular testosterone relative to control, which coincided with decrease testicular mRNA expression of *Insl3*. However, experiments in each of these studies only tested one high dose level in addition to vehicle controls, support LOAELs ranging from 750 to 1000 mg/kg-day BBP, which are therefore not sensitive. Furthermore, these studies do not allow for the identification of a NOAEL, which increases the uncertainty in the data sets use for POD derivation. These three studies were not amenable to BMD modeling, as each study only evaluated a single dose level. Ultimately, these studies were not selected in dose-response assessment because other developmental studies of BBP are available that test more than one dose level and support identification of more sensitive NOAELs.

Two studies (Ema et al., 2003; Ema and Miyawaki, 2002) were similarly not considered further for dose-response analysis because other studies provide more sensitive NOAELs. Ema et al. (2003) gavaged Wistar rats with 0, 167, 250, or 375 mg/kg-day MBP (a major BBP metabolite) from GD 15 to 17 and made observations on GD 21. Here, Ema et al. (2003) noted a NOAEL of 167 mg/kg-day MBP based on occurrence of cryptorchidism and decreased AGD. Ema et al. (2002) exposed Wistar rats with 0, 250, 500, or 1000 mg/kg-day BBP via gastric intubation from GD 15 to GD 17 and also made observations on GD 21. Ema et al. (2002) reported a NOAEL of 250 mg/kg-day BBP based upon decreased AGD (and decreased anogenital index, a standardized AGD value corrected for body weight), cryptorchidism, and increased transabdominal testicular ascent. However, the doses at which developmental effects were observed in these studies (LOAELs of 250 mg/kg-day and 500 mg/kg-day supporting NOAELs of 167 and 250 mg/kg-day, respectively) were higher than doses at which similar outcomes and sensitive effects of androgen insufficiency (*e.g.*, decreased fetal testicular testosterone) were observed in other studies (Table 4-1). Neither study was subjected to BMD analysis, as other studies evaluated lower doses and provided more sensitive outcomes for modeling. Therefore, EPA did not select these studies because they do not provide the most sensitive robust endpoint for a POD relevant to these durations.

Nagao et al. (2000) gavaged SD rats from 8 to 10 weeks of age and continuously through two generations with either 0, 20, 100, or 500 mg/kg-day BBP. Authors noted significantly decreased AGD in F1 offspring at 500 mg/kg-day, along with decreased reproductive organ weights and increased testicular pathological changes (*e.g.*, decreased spermatocytes in seminiferous tubules, atrophy of seminiferous tubules, decreased seminiferous germ cells, testicular edema, and decreased sperm in epididymis) also occurring at 500 mg/kg-day, resulting in a study LOAEL of 500 mg/kg-day and NOAEL of 100 mg/kg-day (Nagao et al., 2000). EPA considered conducting BMD modeling of several

phthalate syndrome-related outcomes, including increased incidence of testicular pathology in F1 males, but after further evaluation concluded the data were not amenable to modeling or were not sensitive enough to warrant BMD modeling. For example, testicular pathology lesions impacted in F1 weanlings (*i.e.*, decreased spermatocytes in seminiferous tubules) was observed in 90 percent of 500 mg/kg-day group vs. 0 percent of 0, 20, and 100 mg/kg-day groups, while testicular pathological lesions in F1 adults (*i.e.*, atrophy of seminiferous tubules, decreased seminiferous tubule germ cells, decreased epididymal sperm, edema of the interstitium) was observed in 40 to 60 percent of 500 mg/kg-day group vs. 0 percent of 0, 20, and 100 mg/kg-day groups. EPA did not attempt to BMD model this histopathology data as this type of response generally is not amenable to BMD modeling due to lack of data in the low-end range of the curve near a BMR of 5 or 10 percent. However, as discussed later (Section 4.2.3), multiple studies, including Tyl et al. (2004), support a considerably lower consensus LOAEL and NOAEL (*i.e.*, 100 mg/kg-day and 50 mg/kg-day respectively), providing more sensitive effect levels of BBP exposure-related effects on developing male reproductive outcomes. Additionally, the large dose range between the NOAEL of 100 mg/kg-day and LOAEL of 500 mg/kg-day represents an additional source of effect level uncertainty. Considering these factors, Nagao et al. (2000) was not further selected for dose-response assessment, as other studies identified more sensitive LOAELs and NOAELs.

4.2.2 Meta-analysis and BMD Modeling of Fetal Testicular Testosterone and AGD Data

Of the 14 studies considered and presented in Table 4-1, 4 studies across 3 publications (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008) were explored for their dose-related relationship to *ex vivo* fetal testicular testosterone production in accordance with prior NASEM (2017) analysis that used BMD modeling for sensitive BBP POD derivation. Prior NASEM (2017) analysis of decreased rat AGD data from 4 studies (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000; Ashby et al., 1997) is also discussed.

Four studies across 3 publications provided consistent evidence of dose-related reductions in *ex vivo* fetal testicular testosterone production (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008). Study details, including BBP dose-response effects on percent decreased *ex vivo* fetal testicular testosterone production, are shown in Table 4-2. It is notable that the magnitude of effect on *ex vivo* fetal testicular testosterone production was consistent at similar doses across all 4 studies (Table 4-2). In the lower dose range at 0 to 33 mg/kg-day BBP, Furr et al. (2014) identified no BBP exposure-related effect. However, a dose-dependent decrease in *ex vivo* testicular testosterone production was noted at BBP doses of 100 to 900 mg/kg-day across studies (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008). In highest tested dose of 900 mg/kg-day in these studies, there was a range of 85 to 93 percent decrease in *ex vivo* testicular testosterone production relative to control groups for each study (Table 4-2). However, only one study identified a statistically significant effect on *ex vivo* fetal testicular testosterone production at 100 mg/kg-day, with Furr et al. (2014) reporting 53 percent decreased *ex vivo* fetal testicular testosterone production in Block 36. This contrasts with the other 3 dose-response studies indicating no BBP-related effect on this endpoint at 100 mg/kg-day (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008), suggesting some uncertainty in the identified LOAEL of 100 mg/kg-day by Furr et al. (2014) (Block 36) based upon fetal testicular testosterone effects. It should also be noted these individual studies are generally limited by small sample sizes of only 2 to 3 dams per dose group for most of these studies, except for Howdeshell et al. (2008). Additionally, Gray et al. (2021) reported testicular mRNA expression changes in pertinent steroidogenic genes (as discussed in Section 3.1.2.1). These mRNA changes suggested a no-observed-effect-level of 11 mg/kg-day (Table 4-1); however, these gene effects are not considered adverse in isolation, where additional study effects of diminished *ex vivo* fetal testicular testosterone production did not occur until a considerably higher level of 300 mg/kg-day (Table 4-2). Given that effects on *ex vivo* fetal testicular testosterone production are

considered as a sensitive indicator of BBP exposure and critical in phthalate-syndrome related MOA (Figure 3-1), EPA reviewed previous benchmark dose (BMD) modeling of fetal testosterone production effects to inform current POD selection (NASEM, 2017), discussed below.

Table 4-2. Effect of BBP Exposure on Fetal Testicular Testosterone Production[a]

Reference (TSCA Study Quality Rating)	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	0 mg/kg-day	11 mg/kg-day	33 mg/kg-day	100 mg/kg-day	300 mg/kg-day	600 mg/kg-day	900 mg/kg-day
(Furr et al., 2014) (High)	Harlan SD rats; GD 14-18; oral/gavage; 0, 100, 300, 600, 900 (Block 36)	100% (n=3)	-[b]	-[b]	**47%*** (n=3)	**33%*** (n=3)	**24%*** (n=3)	**15%*** (n=3)
	Harlan SD rats; GD 14-18; oral/gavage; 0, 11, 33, 100 (Block 37)	100% (n=3)	111% (n=3)	91% (n=3)	88% (n=3)	-[b]	-[b]	-[b]
(Howdeshell et al., 2008) (High)	SD rats; GD 8-18; oral gavage; 0, 100, 300, 600, 900	100% (n=9)	-[b]	-[b]	106% (n=4)	**78%*** (n=5)	**34%*** (n=5)	**10%*** (n=5)
(Gray et al., 2021)[c] (High)	Charles River SD rats; GD 14-18; oral/gavage; 0, 100, 300, 600, 900 (Block 78)	100% (n=3)	-[b]	-[b]	107% (n=3)	**62%*** (n=3)	**37%*** (n=2)	**7%*** (n=2)

Abbreviations: *= Statistical significance; GD = Gestation day; SD = Sprague-Dawley.

[a] Effect on *ex vivo* fetal testicular testosterone production reported as percent of control. Asterisks indicate statistically significant pairwise comparison to control, as reported by study authors.

[b] Exposure level was not tested in study (or block) and thus is not shown for given study.

[c] Data from Block 78 rats reported in supplemental information file associated with Gray et al. (2021).

In the NASEM (2017) analysis, experimental animal model evidence for BBP *in utero* exposure effects on fetal testicular testosterone was assessed using the systematic review methodology developed by the National Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT). NASEM concluded a high rating in the confidence in the body of evidence and evidence of outcome that exposure to BBP during the gestational window of susceptibility decreased fetal testicular testosterone production in rats. At the time, NASEM used the two available prenatal BBP exposure rat studies (Furr et al., 2014; Howdeshell et al., 2008) to conduct a meta-regression analysis and BMD modeling analysis on fetal testicular testosterone. NASEM found a statistically significant overall effect in $\log_{10}$(dose) and dose, with an effect magnitude >50 percent (significant heterogeneity in all cases, $I^2 > 85\%$) (Table 4-3). The best-fit linear quadratic model estimated 23 mg/kg-day [95% CI: 13, 74] for a 5 percent change (BMD_5) (benchmark response, BMR = -5.1) and 228 mg/kg-day [95% CI: 140, 389] for a 40 percent change (BMD_{40}) (BMR = 51) (Table 4-3).

NASEM (2017) also conducted a meta-regression analysis and BMD analysis of decreased male rat AGD. The analysis included AGD data from 4 rat studies (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000; Ashby et al., 1997). NASEM found a statistically significant overall effect of a reduction in AGD (–5.34 [95% CI: –8.35, –2.33]) and linear trends in $\log_{10}$(dose) (-4.68 [95% CI: –7.09, –2.27]) and dose (–2.07 [95% CI: –2.50, –1.63]). 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_5 estimate of 252 mg/kg-day (95% CI: 164, 377).

Overall, the meta-regression and BMD analyses conducted by NASEM demonstrate that decreased fetal testicular testosterone is a more sensitive endpoint than decreased male rat AGD. This is consistent with the MOA for phthalate-syndrome, where reduced AGD is an apical outcome downstream of and mechanistically linked to reduced fetal testicular testosterone production.

Table 4-3. Summary of NASEM (2017) Meta-Analysis and BMD Modeling for Effects of BBP on Fetal Testosterone[a, b, c]

Database Supporting Outcome	Confidence in Evidence	Evidence of Outcome	Heterogeneity in Overall Effect	Model with Lowest AIC	BMD_5 mg/kg-day (95% CI)	BMD_{40} mg/kg-day (95% CI)
2 rat studies[d]	High	High	$I^2 > 85\%$	Linear Quadratic	23 (13, 74)	228 (140, 389)

Abbreviations: AIC = Akaike Information Criterion; BMD_5 = Benchmark dose associated with a 5% change; BMD_{40} = Benchmark dose associated with a 40% change; CI = Confidence interval.

[a] R code supporting NASEM's meta-regression and BMD analysis of BBP is publicly available through GitHub (https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose).

[b] NASEM (2017) calculated a BMD_{40} for this endpoint because "previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40%."

[c] Taken from Table C6-4 of NASEM (2017).

[d] Studies in assessment are Furr et al. (2014) and Howdeshell et al. (2008).

Since EPA identified new fetal testicular testosterone dose-response data (Gray et al., 2021) for BBP, an updated meta-analysis was conducted. EPA did not conduct an updated meta-analysis of decreased AGD because this apical outcome that 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 data from three studies was included in the analysis (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008).Overall, the meta-analysis found a statistically significant overall effect and linear trends in $\log_{10}$(dose) and dose, with an overall effect that is large in magnitude (>50% change) (Table 4-4). 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 (Table 4-4). The linear-quadratic model provided the best fit (based on lowest AIC) (Table 4-4). The BMD for a 40 percent change (BMD_{40}) under the best-fit linear quadratic model was 284 mg/kg-day [95% CI: 150, 481] (Table 4-4). BMD estimates could not be generated for a 5 or 10 percent change (BMR = 5% or 10%) (Table 4-5). 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).

Although the meta- and BMD modeling analyses conducted by NASEM and EPA provided similar results for BMD_{40} and benchmark dose (lower confidence limit) associated with a 40 percent response level ($BMDL_{40}$) estimates, analysis including updated available data did not allow for the derivation of a BMD_5 for effects of BBP exposure on fetal testosterone production. EPA did not further consider the $BMDL_{40}$ estimate as a candidate for deriving a POD because, as described in Appendix E, the 40 percent response level is not considered health protective, and other available studies of BBP provide more sensitive PODs.

Because no benchmark dose (lower confidence limit) associated with a 5 percent response level ($BMDL_5$) could be derived via the updated meta-analysis and BMD analysis, EPA modelled individual fetal testicular testosterone data from the three publications included in the updated meta-analysis (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008) using EPA's BMD Software (BMDS version 3.3.2). For this analysis, BMRs of 1 control standard deviation, and 5, 10, and 40 percent were modelled using the full suite of standard continuous models provided in BMDS (Exponential, Hill, Polynomial, Power, Linear). Further methodological details and BMD modeling results are presented in Appendix F. Of the modelled data sets, adequate BMD model fits were only obtained for fetal testicular testosterone data from Howdeshell et al. (2008). The best fitting exponential 3 model supports BMD_5 and $BMDL_5$ values of 138 and 81 mg/kg-day, respectively, as shown in Table 4-1 (outputs are shown in Appendix F.1). However, as discussed below in Section 4.2.3, the $BMDL_5$ = 81 mg/kg-day was found to be slightly less sensitive than other identified NOAEL and LOAEL co-critical effect level studies.

Table 4-4. Overall Analyses of Rat Studies of BBP and Fetal Testosterone (Updated Analysis Conducted by EPA using Metafor Version 4.6.0)[a]

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P value	Tau	I^2	P value for Heterogeneity	AICs
Primary Analysis									
Overall	intercept	−83.62	−127.17	−40.06	1.68E−04	83.98	98.20	4.78E−151	169.89
Trend in log10(dose)	log10(dose)	−120.36	−169.45	−71.28	1.54E−06	49.93	94.66	3.34E−36	149.12

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P value	Tau	I^2	P value for Heterogeneity	AICs
Linear in dose100	dose100	−22.98	−30.32	−15.63	8.69E−10	69.12	97.13	7.81E−82	153.33
LinearQuadratic in dose100	dose100	−15.00	−36.40	6.40	1.70E−01	50.89	93.85	8.24E−53	140.94*
LinearQuadratic in dose100	I(dose100^2)	−1.04	−3.78	1.69	4.54E−01	50.89	93.85	8.24E−53	140.94
Sensitivity Analysis									
Overall minus (Furr et al., 2014)	intercept	−90.83	−160.08	−21.59	1.01E−02	97.63	97.87	2.72E−33	91.46
Overall minus (Gray et al., 2021)	intercept	−78.47	−125.70	−31.24	1.13E−03	77.72	98.17	5.38E−125	122.09
Overall minus (Howdeshell et al., 2008)	intercept	−84.05	−134.86	−33.24	1.19E−03	84.27	98.27	8.30E−102	123.25

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

Table 4-5. Benchmark Dose Estimates for BBP and Fetal Testosterone in Rats

Analysis	BMR	BMD	CI, Lower Bound	CI, Upper Bound
2017 NASEM Analysis using Metafor Version 2.0.0 (as reported in Table C6-4 of NASEM (2017))				
Linear in dose100	5%	23	19	29
Linear in dose100	40%	231	192	290
LinearQuadratic in dose100*	5%	23	13	74
LinearQuadratic in dose100*	40%	228	140	389
Updated Analysis using Metafor Version 4.6.0				
Linear in dose100	5%	22	17	33
Linear in dose100	10%	46	35	67
Linear in dose100	40%	222	168	327
LinearQuadratic in dose100*	5%	NA [a]	NA [a]	236
LinearQuadratic in dose100*	10%	NA [a]	NA [a]	280
LinearQuadratic in dose100*	40%	284	150	481

Abbreviations: AIC = Akaike information criterion; BMD = Benchmark dose; BMR = Benchmark response; CI = Confidence interval.
* Indicates model with lowest AIC.
[a] BMD and BMDL estimates could not be derived.

4.2.3 Co-critical Studies Supporting a Consensus LOAEL of 100 mg/kg-day and NOAEL of 50 mg/kg-day

Of the 14 studies considered and presented in Table 4-1, 4 studies were considered co-critical in dose-response assessment of BBP, supporting either a consensus LOAEL of 100 mg/kg-day (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005) or NOAEL of 50 mg/kg-day (Tyl et al., 2004).

In the study by Ahmad et al. (2014) albino rats were gavaged with 0, 4, 20, or 100 mg/kg-day BBP from GD 14 to 21, and male offspring were evaluated on PND 1, 5, 25, or 75. A suggested LOAEL of 4 mg/kg-day was identified by authors based on significantly decreased offspring body weight on PND 1 and PND 21. However, EPA considers the results in the study to support a LOAEL of 100 mg/kg-day and NOAEL of 20 mg/kg-day considering that the decreases in body weight at PND 1, though

statistically significant, were minor (3–4% decrease) in addition to not exhibiting a strong dose-response (*i.e.*, not likely to be biologically significant). The body weight decreases at PND 21 were greater in magnitude (13–22% decrease compared to controls) but were also not dose-dependent. Furthermore, the effect of statistically decreased body weights at 4, 20, or 100 mg/kg-day at PND 1 was not accompanied by effects on other examined developmental landmarks (*e.g.*, pinnae unfolding, eye opening, fur development, or testes descent), and offspring body weight changes showed substantial recovery when measured in adulthood at PND 75. Finally, only at 100 mg/kg-day were consistent effects on other endpoints observed in adult F1 males, including decreased absolute prostate and epididymis weights, decreased serum testosterone levels, decreased sperm count and motility, and increased sperm abnormalities. It was also noted that no statistical methods were used to account for litter effects of this study (*i.e.*, statistics on offspring were presented as means of individual animal rather than litter means). EPA considered conducting BMD modeling of the effects of this study, most of which showed statistically significant treatment effects only at 100 mg/kg-day (*i.e.*, epididymis or prostate weights, sperm count, and serum testosterone). However, due to limitations in data reporting, EPA did not consider the most sensitive dose-related effects reported in this study amenable to BMD modeling. For example, organ weights and sperm parameters did not report number of F1 pups examined per endpoint and serum testosterone was presented graphically only and authors do not report sample size or variation data (*i.e.*, standard deviation or standard error). Regarding consistency of results from Ahmad et al. (2014), decreased offspring body weight gain is an effect that does not occur in other studies until higher dose levels, such as at 500 mg/kg-day in Nagao et al. (2000) and 750 mg/kg-day in Tyl et al. (2004) (Table 4-1). Overall, the effects on body weight in the study by Ahmad et al. (2014) had too much uncertainty in results at low doses tested to be considered for POD derivation using the identified NOAEL of 20 mg/kg-day, including the fact that body weight changes supporting the NOAEL in this study were transient. However, results at the LOAEL of 100 mg/kg-day had more confidence and were consistent with other consensus studies showing developing male reproductive effects at the same level (Furr et al., 2014; Aso et al., 2005).

Furr et al. (2014) (Block 36), gavaged Harlan SD rats with 0, 100, 300, 600, 900 mg/kg-day BBP from GD 14 to 18 and evaluated *ex vivo* testicular testosterone production on GD 18. Significantly decreased *ex vivo* testicular testosterone production was found in all treatment groups, which decreased in a dose-dependent fashion. Rats exposed to 100 mg/kg-day BBP showed a 53 percent decrease in *ex vivo* testicular testosterone production, and at the highest tested dose of 900 mg/kg-day was further decreased to an 85 percent reduction. No NOAEL was identified in this study. As discussed above in Section 4.2.2, EPA attempted to BMD model fetal testosterone data from this study, however, no BMD models adequately fit the data set.

Two multi-generational studies were determined to provide sensitive effect levels, including a LOAEL of 100 mg/kg-day in the study by Aso et al. (2005) and a NOAEL of 50 mg/kg-day in the study by Tyl et al. (2004) (Table 4-1). Both studies were two-generation studies that assessed BBP gestational exposure effects on AGD and associated male reproductive organ abnormalities and histopathological outcomes. As discussed in Section 3.1.2.1, decreased AGD is highly correlated with *in utero* anti-androgenic activity and is considered one of the most sensitive biomarkers for phthalate effects on the developing male reproductive system (Schwartz et al., 2019).

Aso et al. (2005) gavaged SD rats with 0, 100, 200, or 400 mg/kg-day BBP continuously for two generations. Although most developmental male reproductive effects occurred at levels above 100 mg/kg-day (*e,g.*, incomplete preputial separation, decreased epididymal weight, small testes, and Leydig cell hyperplasia at 400 mg/kg-day), a study-wide LOAEL of 100 mg/kg-day was determined based upon decreased AGD noted in F2 male offspring and histopathology findings in the testes (*i.e.*,

softening of testes; seminiferous tubule atrophy, decreased spermatozoa in epididymis, and decreased germ cells in epididymal lumen). However, 100 mg/kg-day BBP was the lowest dose tested, and thus the study by Aso et al. (2005) did not derive a NOAEL, increasing uncertainty associated with the use of this study alone for a POD based on the LOAEL with no NOAEL established. Because a NOAEL could not be derived, EPA conducted BMD modeling of several of the most sensitive effects reported by Aso et al. (2005). EPA considered modeling of the testicular histopathology findings of F1 rats and modeled the endpoints exhibiting the strongest dose-response relationship (*e.g.*, exhibiting highest dose-related increases in incidence across dose groups). This included BMD modeling of incidence of soft testes and seminiferous tubule atrophy. BMD modeling of decreased AGD was not conducted, since AGD data from Aso et al. (2005) was included in the meta-analysis of decreased male pup AGD by NASEM (2017), which supports a $BMDL_5$ of 164 mg/kg-day. As described further in Appendix G, EPA evaluated BMRs of 5 and 10 percent using all standard frequentist dichotomus 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-1. For incidence of soft testes, based on the best-fitting Multistage 3 model, BMD_5/$BMDL_5$ and BMD_{10}/$BMDL_{10}$ estimates are 120/66 mg/kg-day and 240/145 mg/kg-day, respectively, while Bayesian modeling averaging supports BMD_5/$BMDL_5$ and BMD_{10}/$BMDL_{10}$ estimates of 203/81 mg/kg-day and 324/171 mg/kg-day, respectively. For incidence of seminiferous tubule atrophy, based on the best-fitting Weibull model, BMD_5/$BMDL_5$ and BMD_{10}/$BMDL_{10}$ estimates are 161/54 mg/kg-day and 219/109 mg/kg-day, respectively, while Bayesian modeling averaging supports BMD_5/$BMDL_5$ and BMD_{10}/$BMDL_{10}$ estimates of 126/55 mg/kg-day and 198/110 mg/kg-day, respectively. Overall, based on the Bayesian modeling averaging approach, EPA considers this study to support a $BMDL_5$ of 55 mg/kg-day based on increased incidence of seminiferous tubule atrophy in F1 males.

Tyl et al. (2004) conducted a two-generation diet exposure study in CD rats exposed to 0, 50, 250, or 750 mg/kg-day BBP. Tyl et al. (2004) identified a LOAEL of 250 mg/kg-day based on decreased AGD in both F1 and F2 offspring, which provides a more sensitive NOAEL (50 mg/kg-day) than the NOAEL of 100 mg/kg-day observed in the two-generation study by Nagao et al. (2000). EPA considered conducting BMD modeling of the most sensitive effect observed in the study by Tyl et al. (2004) (*i.e.*, decreased F1 and F2 male AGD. AGD data from Tyl et al. (2004) was included in the meta-analysis of decreased male pup AGD by NASEM (2017), which supports a $BMDL_5$ of 164 mg/kg-day.. It should be noted additional and more extensive anti-androgenic effects (increased nipple retention, gross testicular/epididymal histopathology changes, and cryptorchidism) occurred at the higher dose of 750 mg/kg-day BBP. In sum, Tyl et al. (2004) identified the most sensitive NOAEL of 50 mg/kg-day BBP.

Using a NOAEL/LOAEL approach in the studies of Table 4-1 considered for BBP POD derivation, EPA selected a NOAEL of 50 mg/kg-day for the BBP POD identified in Tyl et al. (2004), with supporting co-critical studies suggesting a consensus LOAEL of 100 mg/kg-day based upon associated cluster of antiandrogenic outcomes, including reduced *ex vivo* testicular testosterone production and testicular histopathological changes (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005). Although BBP effects on *ex vivo* fetal testicular testosterone production has been used in prior assessments for BMD modeling and effect level estimates (NASEM, 2017), updated meta-analysis modeling data by the EPA could not derive a BMD_5 (Table 4-5). Of individually modelled *ex vivo* fetal testicular testosterone production data sets, an adequate BMD model fit was obtained for Howdeshell et al. (2008) at a $BMDL_5$ value of 81 mg/kg-day (Appendix F.1), which was less sensitive than the NOAEL of 50 mg/kg-day identified in Tyl et al. (2004). While there were inconsistencies in the dose at which fetal testosterone production was decreased across the studies, the lowest LOAEL for decreased fetal testosterone was 100 mg/kg-day, identified in Block 36 in the

study by Furr et al. (2014) (Table 4-2). Aso et al. (2005) identified a LOAEL of 100 mg/kg-day based upon decreased AGD and slight reproductive organ histopathological effects. Ahmad et al. (2014) also identified LOAEL of 100 mg/kg-day and was found to have substantial limitations and uncertainty at the low doses tested (4 and 20 mg/kg-day). Further, using the lowest LOAEL of 100 mg/kg-day instead of a NOAEL would create a considerably larger UF and lower confidence in risk characterization due to 100 mg/kg-day being the lowest study dose tested across Furr et al. (2014), Aso et al. (2005), and Ahmad et al. (2014). This would require a LOAEL-to-NOAEL UF (UF_L) of 10, which would make the benchmark MOE 300 as opposed to a benchmark MOE of 30 by using a NOAEL of 50 mg/kg-day from Tyl et al. (2004).

In summary, EPA considers these 4 co-critical studies (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005; Tyl et al., 2004) to support a NOAEL of 50 mg/kg-day and a LOAEL of 100 mg/kg-day based upon decreased *ex vivo* fetal testicular testosterone production, decreased AGD, and slight testicular histopathology. EPA selected a NOAEL of 50 mg/kg-day (HED = 12 mg/kg-day) as the POD for assessing risks from acute, intermediate, and chronic durations of exposure. A total uncertainty factor of 30 was selected for use as the benchmark MOE (based on an interspecies uncertainty factor [UF_A] of 3 and an intraspecies uncertainty factor [UF_H] of 10). EPA's POD selection of a NOAEL of 50 mg/kg-day BBP is in accordance with the POD selection of multiple existing assessments conducted by other regulatory authorities, including U.S. CPSC (2014), Health Canada (Health Canada, 2020), ECHA (2017a), NICNAS (2015), and EFSA (2019), where the majority of these assessments also explicitly used Tyl et al. (2004) and Aso et al. (2005) as critical dose-response studies (see Table 1-1).

EPA considered reducing the UF_A further to a value of 1 based on apparent differences in toxicodynamics between rats and humans. As discussed in Section 3.1.4 of EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 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_A.

4.2.4 Conclusions on Additional Benchmark Dose Analysis

As discussed above (Section 4.2.3), EPA considered 4 co-critical studies (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005; Tyl et al., 2004) to support a NOAEL of 50 mg/kg-day and a LOAEL of 100 mg/kg-day based upon decreased *ex vivo* fetal testicular testosterone production, decreased AGD, and slight testicular histopathology. In response to SACC and public comments, EPA considered conducting

additional targeted BMD analysis for BBP, including of these 4 co-critical studies, in an attempt to further refine the BBP dose-response analysis.

As discussed above (Section 4.2.3), Ahmad et al. (2014) supports a NOAEL/LOAEL of 20/100 mg/kg-day based on reduced (>50%) serum testosterone in adult F1 males, decreased reproductive organ weight (*i.e.*, 12–13% decrease in absolute epididymis and prostate weight), and sperm effects (*i.e.*, 10% decrease in sperm count, 6% decrease in sperm motility, 54% increase in sperm abnormalities). Due to data reporting limitations, BMD analysis of data from Ahmad et al. was not possible. Furr et al. (2014) supports a LOAEL of 100 mg/kg-day (no NOAEL identified) for reduced fetal testicular testosterone production (decreased 53%). No BMD models adequately fit the data set, and therefore the LOAEL of 100 mg/kg-day from Furr et al. could not be refined. Similarly, Aso et al. (2005) supports a LOAEL of 100 mg/kg-day (no NOAEL identified) based on reduced F2 AGD (8–12% decrease), softening of the testes (incidence across dose groups: 0/24, 1/24, 2/24, 4/24), and increased incidence of testicular pathology (incidence of decreased spermatozoa: 0/24, 1/24, 2/24, 3/24; diffuse seminiferous tubule atrophy: 1/24, 1/24, 3/24, 9/24). BMD analysis of data from this study supports a $BMDL_5$ of 55 mg/kg-day based on increased incidence of seminiferous tubule atrophy. Finally, Tyl et al. (2004) supports a NOAEL/LOAEL of 50/250 mg/kg-day based on reduced F1 and F2 male AGD (reduced 3–8% at LOAEL). EPA did not attempt further BMD modeling of AGD data, since a NASEM (2017) meta-analysis of reduced male AGD, which included data from Tyl et al., was found to support a $BMDL_5$ of 164 mg/kg-day. The $BMDL_5$ of 164 mg/kg-day for reduced male AGD from the NASEM meta-analysis and was not used for risk assessment as it is above the LOAEL of 100 mg/kg-day supported by 3 studies (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005).

Overall, three of the co-critical studies clearly support a LOAEL of 100 mg/kg-day based on effects on the developing male reproductive system consistent with phthalate syndrome (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005). Two studies support NOAELs of 20 and 50 mg/kg-day (Ahmad et al., 2014; Tyl et al., 2004), while BMD analysis of data from Aso et al. (2005) supports a $BMDL_5$ of 55 mg/kg-day. The $BMDL_5$ of 55 mg/kg-day is very similar to the NOAEL of 50 mg/kg-day from Tyl et al. (2004) that was selected for use as the POD, as such EPA did not revise its POD selected for use in risk characterization. Overall, additional BMD analysis conducted in response to SACC and public comments is considered to further support the selected NOAEL of 50 mg/kg-day for use as the acute/intermediate/chronic POD.

4.3 Weight of Scientific Evidence

EPA considers 4 co-critical studies that support a consensus LOAEL of 100 mg/kg-day (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005) and NOAEL of 50 mg/kg-day (Tyl et al., 2004) based on effects on the developing male reproductive system consistent with a disruption of androgen action and phthalate syndrome. EPA has concluded that the HED of 12 mg/kg-day (NOAEL of 50 mg/kg-day) based on decreased AGD and associated anti-androgenic effects (*e.g.*, decreased fetal testicular testosterone production) observed in gestational BBP exposure studies in rats. This is assumed appropriate for calculation of risk from acute, intermediate, and chronic durations. A total UF of 30 was selected for use as the benchmark MOE based upon an interspecies UF (UF_A) of 3 and an intraspecies UF (UF_H) of 10. It should be noted that due to the strength of existing studies and amount of data available for other phthalate non-cancer hazards, EPA did not deem it necessary to apply a database UF (UF_D). This is because for BBP, there exists multiple dose-response developmental and multi-generational studies. Further, for toxicologically similar phthalates (*i.e.*, DEHP, DBP, DCHP), there exists larger databases of animal toxicology studies including numerous well-conducted subchronic and chronic toxicity studies identifying effects on the developing male reproductive system consistent with a

disruption of androgen action, which has been identified by EPA as the most sensitive and well-characterized hazard in experimental animal models. Because a robust database of studies that have evaluated BBP for effects on the developing male reproductive system are available, EPA is confident that the selected POD for BBP is health protective and that a UF_D is not warranted. Consistent with EPA guidance (2022, 2002b, 1993), EPA reduced the UF_A from a value of 10 to 3 because allometric body weight scaling to the three-quarter power was used to adjust the POD to obtain a HED (Appendix D). EPA has **robust overall confidence in the selected POD** based on the following weight of scientific evidence:

- EPA has previously considered the weight of scientific evidence and concluded that oral exposure to BBP 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).
- BBP exposure resulted in treatment-related effects on the developing male reproductive system consistent with a disruption of androgen action and phthalate syndrome-related outcomes during the critical window of development in 14 studies in rats (Section 3.1.2). Observed effects in male offspring included: altered testicular mRNA expression of lipid metabolism and steriodogenic synthesis genes; reduced fetal testicular testosterone content and/or testosterone production; reduced AGD; nipple retention; reproductive tract malformations (*e.g.*, hypospadias, cryptorchidism, decreased reproductive tissue weights); delayed preputial separation; testicular pathology (*e.g.*, degeneration of seminiferous tubules, testes softening, Leydig cell hyperplasia, edema, multinucleated gonocytes); decreases epididymal and seminiferous tubule germ cells; decreased sperm concentration and motility.
- In human epidemiological studies (discussed in 3.1.1), authors such as Radke et al. (2018) found that there was a slight level of confidence in the association between exposure to BBP and AGD, as well as cryptorchidism/hypospadias; however, this association was not consistent with the findings from Health Canada (2018b) or NASEM (2017), leading the EPA to conclude causality was not established due to uncertainty associated with exposure characterization of individual phthalates, including source or exposure and timing of exposure as well as co-exposure confounding with other phthalates.
- There was some alignment across epidemiological, animal toxicology, and mechanistic streams of evidence. The epidemiological evidence provides qualitative support for the association between BBP exposure and male reproductive outcomes, including AGD.
- EPA's selected POD of 50 mg/kg-day (HED of 12 mg/kg-day) is consistent with other regulatory and authoritative bodies that have also concluded developmental male reproductive toxicity and anti-androgenic effects (*i.e.*, decreased AGD, reduced *ex vivo* testicular testosterone production, testicular pathology) is a sensitive indicator of BBP exposure and relevant for estimating human risk (Health Canada, 2020; EFSA, 2019; ECHA, 2017a; NICNAS, 2015; CPSC, 2014). These assessments have used a similar POD of NOAEL = 50 mg/kg-day to quantify risk from BBP exposure Table 1-1.
- The multi-generational study critical in POD derivation (Tyl et al., 2004) was reported to adhere to good laboratory practice guidelines of reproduction toxicity studies, specifically *Health effects test guidelines OPPTS 870.3800 reproduction and fertility effects* (U.S. EPA, 1998).
- In response to SACC and public comments, EPA considered conducting additional targeted BMD analysis for BBP, including of the 4 co-critical studies that inform the NOAEL/LOAEL

values of 50/100 mg/kg-day (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005; Tyl et al., 2004), in an attempt to further refine the BBP dose-response analysis. Due to data reporting limitations, data from Ahmad et al. (2014) could not be BMD modelled, while no BMD models adequately fit the fetal testosterone data reported by Furr et al. (2014). BMD analysis of data from Aso et al. (2005) supported a $BMDL_5$ of 55 mg/kg-day, based on increased incidence of seminiferous tubule atrophy. EPA did not attempt to BMD model the most sensitive effect (*i.e.*, reduced F1 and F2 male AGD) reported by Tyl et al. (2004), since a NASEM (2017) meta-analysis of reduced male AGD, which included data from Tyl et al., was found to support a $BMDL_5$ of 164 mg/kg-day. The $BMDL_5$ of 164 mg/kg-day for reduced male AGD from the NASEM meta-analysis is clearly not health-protective or appropriate for use in risk assessment, as it is above the LOAEL of 100 mg/kg-day supported by 3 studies (Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005) where adverse findings for reduced *ex vivo* fetal testicular testosterone production, testicular histopathology (*i.e.*, testis softening and decreased epididymal spermatozoa), and sperm alterations (*i.e.*, decreased sperm count, decreased motile sperm, and increased abnormal sperm) were observed.

- EPA considered effects on the developing male reproductive systemic consistent with the disruption of androgen action to be relevant for POD selection for acute, intermediate, and chronic exposure durations, where BBP exposure during gestationally susceptible windows in rats can elicit a spectrum of anti-androgenic effects related to the phthalate syndrome MOA.

4.4 Route-to Route Extrapolation

EPA did not identify any studies conducted via the dermal or inhalation exposure routes that are relevant for determining human health risk. Therefore, EPA is using the oral HED of 12 mg/kg-day BBP 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 route-specific differences in toxicokinetics (IGHRC, 2006; U.S. EPA, 1994). For BBP, 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 BBP through the gastrointestinal tract following oral exposure, while EPA estimated steady-state dermal flux values for BBP to estimate dermal exposure (Section 2.3). However, potential route-specific differences in metabolism were not accounted for. Following oral exposure, phthalate diesters (including BBP) are metabolized to a monoester metabolite (*e.g.*, MBzP) 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 BBP to its monoester metabolites also likely occurs via the dermal route prior to systemic circulation. For example, as discussed in the non-cancer human health hazard assessments of DBP (U.S. EPA, 2025i) and DEHP (U.S. EPA, 2025k) dermal absorption studies with metabolically active human skin demonstrate metabolism of DBP and DEHP to their respective monoester metabolites MBP and MEHP, as well as other oxidative metabolites. Further, as discussed in Section 2.3 of this document, studies of

BBP with metabolically active skin have also demonstrated metabolism of BBP to MBP and MBzP (Sugino et al., 2017).

Despite some remaining uncertainty, EPA is confident that its human health risk characterization via the dermal route for BBP 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 BBP through the gastrointestinal tract following oral exposure (Section2.1); although no inhalation toxicokinetic study of BBP is available, studies of other phthalates (*i.e.*, DEHP, DIDP, and DINP) indicate phthalates are nearly completely absorbed through the respiratory tract, and 100 percent absorption is assumed for BBP. Similar to the oral route of exposure, metabolism of BBP to its monoester metabolites MBP and MBzP is expected to occur in the lung, however, the rate of metabolism in the lung may be slower than in the gastrointestinal tract and liver. For example, Ito et al. (2005) report lipase activity in rat liver and lung homogenate; however, lipase activity was approximately 12.6 times higher in the liver compared to the lung. Similarly, Choi et al. (2012) demonstrate metabolism of DEHP to MEHP in human small intestine, liver, and lung tissue samples, however, the metabolic rate of MEHP formation was highest in the small intestine and liver compared to the lung. Although no studies of BBP metabolism in the lung are available, EPA considers it reasonable to assume that BBP is metabolized to monoester metabolites in the lung, due to the presence of lipases.

Despite some remaining uncertainty, EPA is confident that its human health risk characterization via the inhalation route for BBP is health protective (U.S. EPA, 2025f).

5 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE

5.1 Hazard Considerations for Aggregate Exposure

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

5.2 PESS Based on Greater Susceptibility

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

As summarized in Table 5-1, EPA identified a range of factors that may have the potential to increase biological susceptibility to BBP, 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 BBP is not known; therefore, EPA is uncertain about the direction and magnitude of any possible increased risk from effects associated with BBP exposure for relevant subpopulations.

Animal studies demonstrating effects on male reproductive development (as discussed in Section 3.1.2.1) and other developmental outcomes (as discussed in Section 3.1.2.3) provide direct evidence that gestation is a particularly sensitive lifestage. Evidence from animal studies also demonstrates that the liver and kidneys may be sensitive target organs although the liver and kidney effects observed across reasonably available studies are generally not indicative of an adverse response. EPA is quantifying risks, including those for PESS, based on developmental toxicity in the BBP risk evaluation.

As discussed throughout Section 3.1.1, EPA concluded epidemiological studies qualitatively contribute to the weight of scientific evidence of demonstrating BBP toxicity effects on male reproductive development and other developmental outcomes. Although there is uncertainty in the exposure characterizations of epidemiological evidence such that it cannot be used as quantitative or direct evidence (*i.e.*, source of exposure, timing of exposure, co-exposures), EPA is acknowledging epidemiological evidence that provide support to animal studies, including PESS considerations.

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 BBP. The Risk Assessment Forum, in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), 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). Although U.S. EPA (2002b) did not discuss all the factors presented in Table 5-1, EPA considers the POD selected for use in characterizing risk from exposure to BBP to be protective of effects on the developing male reproductive system consistent with phthalate syndrome in humans.

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

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to BBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to BBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	Direct quantitative animal evidence for developmental toxicity (*e.g.*, decreased live births, decreased offspring body weight gain, and decreased offspring survival with increased severity in the second generation) for BBP. There is direct quantitative animal evidence for effects on the developing male reproductive system consistent with a disruption of androgen action (*e.g.*, decreased *ex vivo* fetal testicular testosterone production, decreased anogenital distance, testicular histopathology).	(U.S. EPA, 2023a); (U.S. EPA, 2023b)	There is epidemiological evidence for *in utero* exposure effects on developing male reproductive system (*e.g.*, reduced anogenital distance, hypospadias/cryptorchidism).	(Radke et al., 2018)	POD selected for assessing risks from acute, intermediate, and chronic exposures to BBP is based on developmental toxicity (*i.e.*, phthalate syndrome-related effects) and is protective of effects on the fetus and offspring.
	Pregnancy/ lactating status	Rodent dams not particularly susceptible during pregnancy and lactation, except for effects related to reduced maternal weight gain, food consumption, decreased ovarian and uterine weights, and increased kidney weight, which occurred at doses higher than those that caused developmental toxicity.	(Nagao et al., 2000); (Aso et al., 2005); (Tyl et al., 2004)			POD selected for assessing risks from acute, intermediate, and chronic exposures to BBP is based on developmental toxicity (*i.e.*, phthalate syndrome-related effects) and is protective of effects in dams.
	Males of reproductive age	Reduced body weight gain, increased liver and kidney and decreased lung and left epididymal weights, and decreased serum testosterone. Effects observed at higher doses than those that caused developmental toxicity.	(Nagao et al., 2000); (Aso et al., 2005); (Tyl et al., 2004)	There is epidemiological evidence for adult male reproductive effects, including reduced male fecundability (*e.g.*, impacted semen parameters, time to pregnancy)	(Radke et al., 2018)	POD selected for assessing risks from acute, intermediate, and chronic exposures to BBP is based on developmental toxicity (*i.e.*, phthalate syndrome-related effects) and is protective of adult male reproductive effects. Use of default 10× UF_H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to BBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to BBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Children	Reduced rodent offspring bodyweight gain between PNDs 1 to 21 was observed in one and two-generation studies of reproduction.	(Nagao et al., 2000); (Aso et al., 2005); (Tyl et al., 2004)			POD selected for assessing risks from acute, intermediate, and chronic exposures to BBP is based on developmental toxicity (*i.e.*, phthalate syndrome-related effects) and is protective of effects of offspring bodyweight gain Use of default 10× UF_H
	Elderly	No direct evidence identified				Use of default 10× UF_H
Pre-existing disease or disorder	Health outcome/ target organs	No direct evidence identified		Several preexisting conditions may contribute to adverse developmental outcomes (*e.g.*, diabetes, high blood pressure, certain viruses). Individuals with chronic liver and kidney disease may be more susceptible to effects on these target organs. Viruses such as viral hepatitis can cause liver damage.	(CDC, 2023e); (CDC, 2023g)	Use of default 10× UF_H
	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 BBP.		Use of default 10× UF_H
Lifestyle activities	Smoking	No direct evidence identified		Smoking during pregnancy may increase susceptibility for developmental outcomes (*e.g.*, early delivery and stillbirths).	(CDC, 2023f)	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to BBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to BBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	Alcohol consumption	No direct evidence identified		Alcohol use during pregnancy can cause developmental outcomes (*e.g.*, fetal alcohol spectrum disorders). Heavy alcohol use may affect susceptibility to liver disease.	(CDC, 2023d); (CDC, 2023a)	Qualitative discussion in Section 5.2 and this table
	Physical activity	No direct evidence identified		Insufficient activity may increase susceptibility to multiple health outcomes. Overly strenuous activity may also increase susceptibility.	(CDC, 2022)	Qualitative discussion in Section 5.2 and this table
Sociodemo-graphic status	Race/ethnicity	No direct evidence identified (*e.g.*, no information on polymorphisms in BBP metabolic pathways or diseases associated race/ethnicity that would lead to increased susceptibility to effects of BBP by any individual group).				Qualitative discussion in Section 5.2 and this table
	Socioeconomic status	No direct evidence identified		Individuals with lower incomes may have worse health outcomes due to social needs that are not met, environmental concerns, and barriers to health care access.	(ODPHP, 2023b)	
	Sex/gender	No direct evidence identified				Use of default 10× UF_H
Nutrition	Diet	No direct evidence identified		Poor diets can lead to chronic illnesses such as heart disease, type 2 diabetes, and obesity, which may contribute to adverse developmental outcomes. Additionally, diet can be a risk factor for fatty liver, which could be a pre-existing condition to enhance susceptibility to BBP-induced liver toxicity.	(CDC, 2023e); (CDC, 2023b)	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to BBP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to BBP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
	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 BBP.	(CDC, 2021); (CDC, 2023b)	Qualitative discussion in Section 5.2 and this table
Genetics/ epigenetics	Target organs	No direct evidence identified		Polymorphisms in genes may increase susceptibility to liver, kidney, or developmental toxicity.		Use of default 10× UF_H
	Toxicokinetics	No direct evidence identified		Polymorphisms in genes encoding enzymes (*e.g.*, esterases) involved in metabolism of BBP may influence metabolism and excretion of BBP.		Use of default 10× UF_H
Other chemical and nonchemical stressors	Built environment	No direct evidence identified		Poor-quality housing is associated with a variety of negative health outcomes.	(ODPHP, 2023a)	Qualitative discussion in Section 5.2 and this table
	Social environment	No direct evidence identified		Social isolation and other social determinants (*e.g.*, decreased social capital, stress) can lead to negative health outcomes.	(CDC, 2023c); (ODPHP, 2023c)	Qualitative discussion in Section 5.2 and this table
	Chemical co-exposures	Studies have demonstrated that co-exposure to BBP and other toxicologically similar phthalates (*e.g.*, DEHP, DBP, DINP) and other classes of antiandrogenic chemicals (*e.g.*, 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.	(U.S. EPA, 2023a); (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 BBP EXPOSURE, AND CONCLUSIONS

EPA evaluated the non-cancer hazards of BBP and identified effects on the developing male reproductive system as the most sensitive health effects indicator of BBP exposure. Anti-androgenic effects from exposure occurring during the critical window of male reproductive system development (*e.g.*, gestational exposure) leads to a spectrum of adverse developing male reproductive system outcomes, consistent with the phthalate syndrome pathways. After considering hazard identification and evidence integration, dose-response evaluation, and weight of scientific evidence of POD candidates, EPA chose one non-cancer endpoints for the risk evaluation in acute, intermediate, and chronic exposure scenarios (Table 6-1). EPA considers the selected non-cancer POD (NOAEL of 50 mg/kg-day; HED = 12 mg/kg-day) protective of non-cancer developmental toxicity effects. 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 human equivalent concentration (HEC) of 64.2 mg/m^3 (5.03 ppm). EPA is also using the oral HED to extrapolate to the dermal route.

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

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HEC[a] (mg/m^3) [ppm]	HED[a] (mg/ kg-day)	Benchmark MOE[b]	References[c] (TSCA Study Quality Rating)
Acute, Intermediate, Chronic	Developing male reproductive toxicity	Rat	Multi-generational or 5-8 days during gestation	NOAEL = 50	Phthalate syndrome-related effects (*e.g.*, ↓AGD; ↓ fetal testicular testosterone; ↓ reproductive organ weights; Leydig cell effects; ↓ mRNA and/or protein expression of steroidogenic genes; ↓INSL3)	64.2 [5.03]	12	UF_A= 3 UF_H=10 *Total UF= 30*	(Ahmad et al., 2014; Furr et al., 2014; Aso et al., 2005; Tyl et al., 2004)[d]

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HEC[a] (mg/m^3) [ppm]	HED[a] (mg/ kg-day)	Benchmark MOE[b]	References[c] (TSCA Study Quality Rating)
Abbreviations: AGD = Anogenital distance; HEC = Human equivalent concentration; HED = Human equivalent dose; INSL3: Insulin-like 3; MOE = Margin of exposure; NOAEL = No-observed-adverse-effect level; POD = Point of departure; UF = Uncertainty factor [a] HED and HEC values were calculated based on the most sensitive NOAEL of 50 mg/kg-day. [b] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011c), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics. EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations. [c] Tyl et al. (2004) support a NOAEL of 50 mg/kg-day based on decreased AGD and decreased reproductive organ weights in a multi-generational study at 250 mg/kg-day (LOAEL); the remaining effects listed reached statistical significance at higher doses (most of which are not considered adverse in isolation). Ahmad et al. (2014), Furr et al. (2014), and Aso et al. (2005) reflect supporting phthalate syndrome-related effects (*e.g.*, reduced *ex vivo* testicular testosterone production or testicular histopathological changes) at LOAEL = 100 mg/kg-day. [d] TSCA Study Quality Ratings: *High confidence* for (Furr et al., 2014) and *Medium confidence* for (Ahmad et al., 2014; Aso et al., 2005; Tyl et al., 2004).								

The selected POD of 50 mg/kg-day (HED = 12 mg/kg-day) will be used in the *Risk Evaluation for Butyl Benzyl Phthalate (BBP)* (U.S. EPA, 2025n) to estimate acute, intermediate, and chronic non-cancer risk. EPA summarizes the cancer hazards of BBP 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).

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APPENDICES

Appendix A EXISTING ASSESSMENTS FROM OTHER REGULATORY AGENCIES OF BBP

The available existing assessments of BBP are summarized 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 BBP

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
U.S. EPA (IRIS Program)	*IRIS Assessment of: Butyl benzyl phthalate (CASRN 85-68-7* (U.S. EPA, 1989), *Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence* (Radke et al., 2018).	No	No	Yes	- Publications were subjected to peer-review prior to being published in a special issue of *Environment International*. - 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.
U.S. CPSC	*Toxicity review for benzyl-n-butyl phthalate* (CPSC, 2010), *Chronic Hazard Advisory Panel on phthalates and phthalate alternatives* (CPSC, 2014).	Yes	Yes	No	- Peer-reviewed by panel of four experts. Peer-review report available at: https://www.cpsc.gov/s3fs-public/Peer-Review-Report-Comments.pdf -Public comments available at: https://www.cpsc.gov/chap - No formal systematic review protocol employed. - Details regarding CPSC's strategy for identifying new information and literature are provided on page 12 of (CPSC, 2014).
NASEM	*Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals* (NASEM, 2017).	Yes	No	Yes	- Draft report was reviewed by individuals chosen for their diverse perspectives and technical expertise in accordances with the National Academies peer-review process. See

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
					Acknowledgements section of (NASEM, 2017) for more details. - Employed NTP's Office of Heath Assessment and Translation (OHAT) systematic review method.
California OEHHA	*Safe Drinking Water and Toxic Enforcement Act of 1986 Proposition 65. Initial Statement of Reasons. Title 27, California Code of Regulations. Proposed amendment to section 25805(b), Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity. Butyl benzyl phthalate (oral exposure)* (OEHHA, 2012).	No	No	No	- In a statement of reasons for this evaluation, OEHHA (2012) states "BBP was added to the Proposition 65 list, based on formal identification as causing reproductive toxicity (developmental endpoint) by the National Toxicology Program (NTP) and in a report by its Center for the Evaluation of Risks to Human Reproduction (CERHR)" (page 2). - No formal systematic review protocol employed, although human and animal study selection is explained
Health Canada	*State of the science report: Phthalate substance grouping: Medium-chain phthalate esters: Chemical Abstracts Service Registry Numbers: 84-61-7; 84-64-0; 84-69-5; 523-31-9; 5334-09-8;16883-83-3; 27215-22-1; 27987-25-3; 68515-40-2; 71888-89-6* (EC/HC, 2015a), *Canadian environmental protection act priority substances list assessment report: Butylbenzylphthalate* (EC/HC, 2000), *Supporting Documentation: Carcinogenicity of Phthalates - Mode of Action and Human Relevance* (Health Canada, 2015), *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and*	Yes	Yes	No (Animal studies) Yes (Epidemiologic studies)	- Ecological and human health portions of the screening assessment report (Health Canada, 2020) were subject to external review and/or consultation. See page 2 of (Health Canada, 2020) for additional details. - EC/HC (2000) provides a summary of information critical to assessment (page 7) and search strategies employed for identification of relevant data (page 57). - State of the science report (EC/HC, 2015a) and draft screening assessment report for the phthalate substance group subjected to 60-day public comment periods. Summaries of received public comments available at: https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative/phthalate.html#a1 - No formal systematic review protocol employed to identify or evaluate experimental animal toxicology studies.

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	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), *Screening Assessment - Phthalate Substance Grouping* (Health Canada, 2020).				- Details regarding Health Canada's strategy for identifying new information and literature are provided in Section 1 of (EC/HC, 2015a) and (Health Canada, 2020) - Human epidemiologic studies evaluated using Downs and Black Method (Health Canada, 2018a, b).
NICNAS	*Priority existing chemical assessment report no. 40: Butyl benzyl phthalate* (NICNAS, 2015). *C4-6 side chain transitional phthalates: Human health tier II assessment* (NICNAS, 2016).	No	Yes	No	- NICNAS (2015) states "On completing a PEC assessment, the Director of NICNAS, in accordance with the Act, causes a draft report of the assessment to be prepared and makes it available to the applicants for factual correction and to the publc (including applicants and other interested parties) for comments." See Preface of for more details. - No formal systematic review protocol employed. - Details regarding NICNAS's strategy for identifying new information and literature are provided in Section 1.3 of (NICNAS, 2015).
ECHA	*Substance name: Benzyl butyl phthalate, EC number: 201-622-7, CAS number: 85-68-7: Member state committee support documentation for identification of benzyl butyl phthalate (BBP) as a substance of very high concern* (ECHA, 2008), *Evaluation of new scientific evidence concerning the restriction contained in Annex XVII to regulation (EC) no. 1907/2006 (REACH): Review of new*	No	Yes	No	- ECHA (2017b) states "This document presents opinions adopted by RAC and SEAC. The Background Document, as a supportive document to both RAC and SEAC opinions and their justifications, gives the details of the Dossier Submitter's proposal, amended for further information obtained during the public consultation and other relevant information resulting from the opinion making process." See document for more details. - No formal systematic review protocol employed.

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	available information for benzyl butyl phthalate (BBP) CAS no. 85-68-7 Einecs no. 201-622-7 (ECHA, 2010), *Support document to the opinion of the member state committee for identification of benzyl butyl phthalate (BBP) as a substance of very high concern because of its endocrine disrupting properties which cause probable serious effects to human health and the environment which give rise to an equivalent level of concern to those of cmr1 and pbt/vpvb2 substances* (ECHA, 2014b), *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).				
ECB	*European union risk assessment report: Benzyl butyl phthalate (BBP)* (ECJRC, 2007).	Nov	No	No	- ECB (2007) states "The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion on the European Commission on the quality of the risk assessment." See Forward for more details. - No formal systematic review protocol employed.
EFSA	*Update of the Risk Assessment of Di-butylphthalate (DBP), Butyl-benzyl-phthalate (BBP), Bis(2-ethylhexyl)phthalate (DEHP), Di-isononylphthalate (DINP) and Di-*	No	Yes	No	- Draft report subject to public consultation. Public comments and EFSA's response to comments are available at: https://doi.org/10.2903/sp.efsa.2019.EN-1747

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	isodecylphthalate (DIDP) for Use in Food Contact Materials (EFSA, 2019).				- No formal systematic review protocol employed. - Details regarding EFSA's strategy for identifying new information and literature are provided on page 18 and Appendix B of (EFSA, 2019).
NTP-CERHR	*NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP)* (NTP, 2003).	No	Yes	No	- 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) - Public comments summarized in Appendix III of (NTP, 2003) - No formal systematic review protocol employed.

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 animals following exposure to BBP. Of these, four studies provided relevant information specifically on developmental male reproductive toxicity following oral BBP exposure (Gray et al., 2021; Debartolo et al., 2016; Schmitt et al., 2016; Ahmad et al., 2014). These studies are discussed above in Section 3.1.2.3 and summarized in Table 3-3 or are discussed below. The remaining three studies provide data on other reproductive and developmental outcomes, including changes in the estrus cycle or serum estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, number of ovarian follicles, reproductive organ weights (*i.e.*, ovary and/or uterus), pup body weights, or non-developmental exposure design (Integrated Laboratory Systems, 2017; Ahmad et al., 2015; Alam and Kurohmaru, 2015). LOAELs provided by the data set ranged from 20 to 1000 mg/kg-day. The lowest LOAEL identified from the literature for reproductive and developmental effects was 20 mg/kg-day based on effects on body weight gain and reproductive organ weights (Ahmad et al., 2015). The other LOAELs from the remaining studies were at least an order of magnitude higher and therefore did not offer more sensitive PODs than those discussed in Section 3.1.2.1. These studies are summarized in Table_Apx B-1, but only Ahmad et al. (2015), Integrated Laboratory Systems (2017), and Alam et al. (2015) are discussed in the text below. The remaining studies (Gray et al., 2021; Debartolo et al., 2016; Schmitt et al., 2016; Ahmad et al., 2014) considered for data are discussed in Section 3.1.2.2. For most of these studies, EPA identified several limitations, including that of exposure dose uncertainty, lack of linear dose-response, or other exposure design deficiencies (*e.g.*, single-dose studies or studies that only included relatively high doses).

Ahmad et al. (2015) evaluated the estrogenic effects of BBP 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 pubertal assay, PND 21 female rats were exposed daily to 0, 20, or 200 mg/kg-day BBP for 20 days via oral gavage, and animals were examined daily for body weights and vaginal opening. The pubertal data are not conclusive; neither control nor BBP-exposed animals attained puberty (*i.e.*, first day of vaginal opening and the first day of estrus), although rats typically attain vaginal opening by PND32. Nevertheless, a LOAEL of 20 mg/kg-day was identified based on significantly decreased body weight gain at PND 27, 33, and 42, compared to the control group in the pubertal assay. There was also a significant decrease in uterus weight at 20 mg/kg-day compared to the control. Ovary weight was slightly, but not significantly, decreased at 20 mg/kg-day compared to the control group. In the uterotrophic assay, PND20 female rats were exposed to 0, 20, or 200 mg/kg-day BBP once per day for 3 consecutive days via gavage. Decreased uterine wet weight was observed one day after exposure ended in the 200 mg/kg-day group. The data do not support that BBP is an estrogen agonist. Although this is a relatively sensitive LOAEL, the study was limited by a large dose spacing, small sample size, and the study design, which was a non-guideline female pubertal assay that did not justify the selected exposure duration or window (*i.e.*, PND 21 to 42), and was therefore not considered further.

In a Good Laboratory Practice (40 CFR part 160) study by Integrated Laboratory Systems (2017), female SD rats were exposed to 0, 250, 500, 750, or 1000 mg/kg-day BBP via oral gavage from PND 22 through 42 or 43. Beginning on PND 22, animals were examined weekly for body weights,0 and daily for vaginal opening, as well as for estrous cyclicity. Clinical and histopathological observations were made in female rats at the end of exposure on either PND 42 or 43. The majority of significant BBP-

related effects occurred at levels of 750 mg/kg-day or higher, with decreased ovarian weight occurring at 750 and 100 mg/kg-day and increased medium ovarian follicles observed at 1000 mg/kg-day. The most sensitive effect level (LOAEL) was observed at 250 mg/kg-day through a significantly decreased number of corpora lutea. Although these endpoints speak to possible BBP-related adverse reproductive effects in female rats, this exposure design and endpoint evaluation was not deemed specifically relevant to the current non-cancer hazard assessment of developmental and reproductive toxicity effects associated with phthalate syndrome outcomes. Moreover, BBP-related effects in this study were associated with exposure during postnatal and rodent adolescence, which are assumed to not produce effective as sensitive as the observations made during critical gestational windows of susceptibility, as noted in other studies discussed throughout.

In a multi-cohort study by Alam et al. (2015), three week old male SD rats in experiment one were orally gavaged with 0 or 500 mg/kg BBP and necropsied for reproductive effects assessment at 3, 12, or 24 hours post exposure. In experiment two, male SD rats were also exposed to 0 or 500 mg/kg BBP, and assessed at 2, 4, 6, 9, and 12 days after treatment. Briefly in experiment one, adverse histopathological changes in seminiferous tubules (*i.e.*, reduction and/or disappearance of tubular lumens) was noted at 3 hours post exposure and thinning seminiferous epithelia and wide tubular lumina were noted by 24 hours post exposure. Increased seminiferous tubule spermatocyte cell apoptosis was also increased at 3, 12, and 24 hours post dosing. In experiment two, authors reported decreased absolute testis weight at days 6, 9, and 12 post exposure. With regard to seminiferous tubule spermatocyte apoptosis, increased apoptotic spermatogenic cells were observed at 2, 4, and 6 days post exposure, which was an effect that dissipated by the ninth day observation. Although these results by Alam et al. (2015) provide limited evidence that a single oral exposure to a high dose of BBP (500 mg/kg), this study was not conducted in developmental model, and this did not provide data on BBP-related effects on the developing male reproductive system, which as discussed earlier is determined the most sensitive indicator of BBP-related effects. Further, this was a single dose-response study, and limitations were identified, including histopathology of the testes were not quantified (*i.e.*, incidence of pathological changes observed) and reporting deficiencies/selection bias was noted (*i.e.*, day 12 histopathology results not presented) (Alam and Kurohmaru, 2015).

Table_Apx B-1. Summary of Animal Toxicology Studies Evaluating Additional Effects on the Developmental and Reproductive System Following Exposure to BBP

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
(Ahmad et al., 2014)	Pregnant Albino rats (≥6 dams/group) were exposed to 0, 4, 20, or 100 mg/kg-day BBP via oral gavage from GD 14–21. Dams were allowed to give birth naturally, and male offspring were sacrificed on PND 5, 25, or 75. Endpoints evaluated in F1 from PND 1-PND 75.	20/100	↓ serum testosterone, ↓ absolute weight of epididymis and prostate, ↓ sperm count, ↓ percent motile sperm, ↑ percent abnormal sperm	Developmental Outcomes - ↓ serum testosterone (F1 adults, PND 75) - ↓ absolute epididymis and prostate weight (F1 adults, PND 75) - ↓ sperm count, ↓ percent motile sperm, ↑ percent abnormal sperm (F1 adults, PND 75) - ↓ pup body weight (4-100, F1, PND 1) - ↓ body weight (20 and 100, PND 75) Unaffected Outcomes - Litter size, live/dead pups, sex ratio (PND1); Anogenital distance (PND5 & PND25); testis descent; Viability index (PND4); Weaning index (PND21); testicular 17β-HSD activity (PND 75)
(Ahmad et al., 2015)	Female rats (6/group) were exposed to 20 or 200 mg/kg-day BBP via oral/gavage from PND21 – 42 (20-day pubertal onset assay).	None/20	↓ BW at multiple timepoints (PND 27, 33, & 42), ↓ uterus weight	Effects at 200 mg/kg-day -↓ BW at multiple timepoints (PND27, 33, & 42) -↓ uterus wet weight; ↓ ovary wet weight Considerations: - ↓ BW at multiple timepoints (PND27, 33, & 42) at 20 mg/kg-day - No changes in day of VO Limitations - Large dose spacing; organ weight decreases displayed flat D-R; organ weight decreases likely a reflection of BW changes; small sample size (n =6) - reporting deficiencies (*i.e.*, rat strain not reported, results not reported for all measured outcomes, including estrus cyclicity)

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
	Immature female rats (6/group; 20 days old) were exposed to 20 or 200 mg/kg-day BBP via oral/gavage and sacrificed on day 4 (3-day uterotrophic assay).	20/200	↓ uterus weight	Other effects: - slight decrease in ovary wet weight (did not attain statistical significance) Limitations - Large dose spacing; organ weight decreases displayed flat D-R; organ weight decreases likely a reflection of BW changes; small sample size (n =6)
(Gray et al., 2021)	Pregnant Harlan SD rats (3-4 dams/group) were exposed to 0, 11, 33, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18.	11/33	↓ fetal testicular mRNA expression of steroidogenic genes, including *Insl3*	Developmental Outcomes - ↓ *ex vivo* fetal testes testosterone production (300) Mechanistic Outcomes - ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (33) and steroidogenic genes (*Star* (100), *Cyp11a1* (33), *Cyp11b2* (33), *Cyp17a1* (300), *Dhcr7* (11), *Cyp11b1* (11), *Hsd3b* (100), *Scarb1* (33)) Additional Remarks Data are an expansion of previous dose response studies (Furr et al., 2014; Howdeshell et al., 2008)
	Pregnant Charles River SD rats (3-4 dams/group) were exposed to 0, 100, 300, 600, or 900 mg/kg-day BBP via oral gavage from GD 14–18. Dams were sacrificed and fetal tissue collected on GD 18. (Block 78)	100/300	↓ *ex vivo* fetal testicular testosterone production	Developmental Outcomes - ↓ *ex vivo* fetal testes testosterone production (300) Mechanistic Outcomes - ↓ fetal testicular expression of *Insl3* (600) and steroidogenic genes (*Star* (600), *Cyp11a1* (600), *Cyp17a1* (600), *Dhcr7* (900), *Cyp11b1* (600), *Hsd3b* (900), *Scarb1* (600))
(Integrated Laboratory Systems, 2017)	SD rats (16/group) were exposed to 0, 250, 500, 750, or 1000 mg/kg-day BBP via oral/gavage from PND22 to PND42 or PND43.	None/250	↓ number of corpora lutea	Effects at 250 mg/kg-day - ↓ corpora lutea Effects at 500 mg/kg-day - none Effects at 750 or 1000 mg/kg-day

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
				- ↓ ovary weights (11% at 750 mg/kg-day; 17% at 1,000 mg/kg-day) - ↑ number of medium ovarian follicles (1,000 mg/kg-day) Considerations: -GLP (40 CFR part 160) - Non-linear dose-response for number of corpora lutea (no change at other tested doses) - No exposure-related effects on age at VO, onset of first estrus, mean estrus cycle length, estrus cycle regularity, gross pathology, microscopic histology of the left ovary, uterus, or mammary gland. - No effect on serum TSH or T4
(Schmitt et al., 2016)	Pregnant C57BL/6J inbred mice (6/group) were exposed via oral/gavage to 0 or 500 mg/kg-day BBP from GD9-16. Serum testosterone and estradiol evaluated at 4, 10, and 20 weeks.	None/500	Males: ↓ AGD at 10 and 20 weeks in F1; ↓ serum testosterone concentration in F1 at 10 and 20 weeks; Females: ↑ serum testosterone & ↓ estradiol concentrations in F1 at 20 weeks; ↑ days to VO.	Limitations: - Large dose spacing; small sample size (n =6); single dose study -Exposure did not encompass the full critical window (*i.e.*, GD14-19) for male antiandrogenic effects
(Alam and Kurohmaru, 2015)	Experiment 1: Male SD rats (8/group) were exposed to a single dose of 0 or 500 mg/kg-day BBP via oral/gavage and outcomes evaluated 3, 12, or 24 hours after exposure.	None/500	Histopathology of seminiferous tubules (reduction and/or disappearance of tubular lumen by 3 hours, thin seminiferous epithelia and wide tubular lumina by 24 hours), ↑ spermatocyte cells apoptosis in seminiferous	Limitations: - Single dose study -Histopathology of testes not quantified (*i.e.*, incidence) -Reporting deficiencies identified (*e.g.*, day 12 histopathology not presented)

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			tubules at 3, 12, and 24 hours pose-dosing	
	Experiment 2: Male SD rats (4/group) were exposed to a single dose of 0 or 500 mg/kg-day BBP via oral/gavage and outcomes evaluated 2, 4, 6, 9, or 12 days after exposure.	None/500	↑ spermatocyte cell apoptosis in seminiferous tubules at 2, 4, and 6 days pose-dosing, ↓ absolute weight of testis at 6, 9, and 12 days post-dosing	
(Debartolo et al., 2016)	Pregnant SD rats (22 control dams, 29 BBP dams) exposed to 10 µg/ml BBP from GD14 – PND23.	-[a]	↓ mean body weight at PND 23, ↓ AGD at PND 23 in both males and females	Considerations: - Dam body weights not provided; dose (mg/kg-day) cannot be calculated. - No histopathology observed via Nissl staining in cortex, hippocampus, or cerebellum Limitations: -Substantial limitations in study design - Single dose study -Inadequate exposure characterization

Abbreviations: ↓ = statistically significant decrease; ↑ = statistically significant increase; AGD = Anogenital distance; BW = Body weight; E2 = β-estradiol; F1 = First generation offspring; FSH = follicle stimulating hormone; GD = Gestational Day; LH = Luteinizing Hormone; LOAEL = Lowest-observed-adverse-effect level; NOAEL = no observed adverse effect level; PND = Postnatal Day; PNW = Postnatal Week; SD = Sprague-Dawley; T4 = Thyroxine; TSH = Thyroid stimulating hormone; VO = Vaginal Opening.

[a] Achieved dose, including NOAEL/LOAEL dose, cannot be calculated in mg/kg-day, because dam body weight and food consumption were not reported (Debartolo et al., 2016).

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B.2 Neurotoxicity

EPA identified three studies (Debartolo et al., 2016; Schmitt et al., 2016; Min et al., 2014) that provided data on neurological outcomes following exposures to BBP. Each study did not offer a more sensitive POD than those discussed in Section 4 and/or had limitations that impacted the interpretation of the results and were therefore not considered further. Detailed information on the study designs is provided in Table_Apx B-2.

A study by DeBartolo et al. (2016) provided data on neurobehavioral effects (*i.e.*, fear conditioning) and brain histopathology following developmental exposure to BBP. Pregnant SD rats were exposed to 0 or 10 µg/mL BBP pipetted onto food pellets from GD 14 to PND 23. The authors qualitatively report no evidence of an effect of BBP on the neocortex, hippocampus, and cerebellum in experiment 1, visualized by Nissl staining. The authors report decreased duration of freezing after the conditional stimulus (*i.e.*, an audible tone that precedes and electric shock) in both sexes, which they attribute to learning and memory impairments due to BBP. However, substantial limitations of this study impact the interpretation of the results and contribute to uncertainty in the data set. Reporting deficiencies were identified for the histopathology data (*i.e.*, histopathological changes only reported qualitatively and insufficient details in methods). For the fear conditioning experiments, limitations in the experimental design further impact the interpretation of the results. The largest limitation of this study includes substantial uncertainty regarding internal dose exposure through lack of adequate exposure characterization, which is due to BBP stock solution (10 µg/mL) being pipetted onto sweetened food pellets fed to pregnant dams; however, it was not reported that exposure was according to dam body weight, and there was no measure of food consumption noted in methods. Treatment for the control and BBP-exposed groups was also stated to occur ~5 to 7 days during gestation, meaning not all exposed animals may have had the same exposure time duration.

In a behavioral assessment by Schmitt et al. (2016), pregnant mice were exposed to 0 or 500 mg/kg-day BBP via gavage from GD 9 to 16 and F1 offspring were subjected to running wheel testing from postnatal week 8 to 20. Authors reported reductions in voluntary physical activity (*i.e.*, running wheel activity) in a pre-and perinatal exposure study in mice, which may reflect locomotor deficits. Male and female mice that had been exposed to BBP ran significantly less distance by postnatal week 20 compared to controls. However, limitations of this study include that it was a single dose study, which precludes its use in understanding dose-response relationships. Additionally, the study does not offer a more sensitive POD than those provided for effects of the developing male reproductive system and was not considered further.

Min et al. (2014) reported evidence of alterations in neurological health outcomes in male mice administered 0, 50, 250, or 1250 mg/kg-day BBP via gavage for 14 days (age at exposure not specified). Following exposure, the mice underwent swim trials in the Morris Water Maze, as well as trials in the forced swim test and tail suspension test. Brain tissue was collected to measure neurotransmitter (5-HT) levels as well as measurements of oxidative stress and a histopathologic evaluation of the hippocampal region of the brain. Reactive oxygen species were also measured via the DCF-DA assay, along with glutathione content and phosphorylated CREB to provide indices of oxidative stress. In the Morris Water Maze, increased escape latency was observed in mice exposed to BBP at dosages of 250 mg/kg-day and higher. Decreased time spent swimming in the target quadrant were only observed at the highest dose (1250 mg/kg-day), suggesting impaired memory in mice from the highest exposure group. Increased time spent immobile in the tail suspension and forced swim tests was observed in mice from the highest exposure group, while mice from the 250 mg/kg-day group exhibited increased time spent immobile in the tail suspension test, implying affected motor function. A dose-dependent decrease

hippocampal levels of 5-HT and phosphorylated CREB was observed, including significant decreases in the endpoints at all tested BBP doses. Other findings were consistent with increased oxidative stress, including increased reactive oxygen species in the brain in parallel with decreased glutathione content in the brain in mice exposed to BBP at doses of 250 mg/kg-day BBP or higher. Altogether, data from Min et al. (2014) provide LOAEL values of 50 mg/kg-day based on neurological effects following short-term exposure to BBP in one sex of one strain of mice.

In sum, the current database supporting neurological effects of BBP is too limited, especially compared to that of more sensitive male reproductive and developmental effects. Additionally, given the aforementioned limitations of this study, EPA did not consider these effects or studies for dose-response assessment or for use in extrapolating human risk in Section 4.

Table_Apx B-2. Summary of Animal Toxicology Study Evaluating Effects on the Nervous System Following Exposure to BBP

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
(Min et al., 2014)	Male SPF Kunming mice (6/group) were exposed to 0, 50, 250, or 1250 mg/kg-day BBP via gavage for 14 days. Mice were trained for MWM from days 1 – 11 and trials conducted on day 14. FST and TST conducted on day 14. Mice sacrificed one day after exposure and brains collected for histopathological evaluation and measurements of oxidative stress.	None/50	↓5-HT, ↓pCREB	Effects at 250 mg/kg-day - ↑ Average escape latency for 11 days (MWM) - ↑ Immobile time in TST - ↓ Brain GSH content - ↑ROS (brain) Effects at 1250 mg/kg-day - ↓ Swimming time in target quadrant (MWM) concentration ↑ Immobile time in FST & TST - ↑ Average escape latency for 11 days (MWM) - ↓ Brain GSH content - ↑ROS (brain) Limitations -Qualitative histopathology - GSH level interpretation is difficult without GSH:GSSH ratio
(Schmitt et al., 2016)	Pregnant C57BL/6J inbred mice (6/group) were exposed via oral/gavage to 0 or 500 mg/kg-day BBP from GD9-16. Running Wheel activity monitored from PNW8 –20.	None/500	↓ Performance on voluntary wheel running (↓distance and duration of exercise) in male and female mice on PNW20	Unaffected Outcomes: -Running wheel speed Limitations: -Single dose study

Reference	Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
(Debartolo et al., 2016)	Pregnant SD rats (11 control dams, 12 BBP dams) exposed to 10 ug/ml BBP from GD14 – PND23.	-[a]	↓ Duration of freezing in males and females on PND65 after conditional stimulus (audible tone)	Considerations: - Dam body weights not provided so dose (mg/kg-day) cannot be calculated. - No histopathology observed via Nissl staining in cortex, hippocampus, or cerebellum Limitations: -Substantial limitations in study design - Single dose study -Inadequate exposure characterization

Abbreviations: ↓ = statistically significant decrease; ↑ = statistically significant increase; 5-HT = 5-hydroxytryptamine; FST = Forced Swim test; GD = Gestational Day; GSH = Glutathione; LOAEL = Lowest-observed-adverse-effect level; MWM = Morris Water Maze; NOAEL = No-observed-adverse-effect level; pCREB = Phosphorylated cyclic adenosine monophosphate (cAMP) response element binding protein (CREB); PND = Postnatal day; PNW = Postnatal week; ROS = Reactive oxygen species; SD = Sprague-Dawley; TST = Tail suspension test.

[a] Achieved dose, including NOAEL/LOAEL dose, cannot be calculated in mg/kg-day, because dam body weight and food consumption were not reported (Debartolo et al., 2016).

B.3 Immune adjuvant effects

EPA identified one study that provided data on immune adjuvant effects following exposure of adult female BALB/cByJ mice (4 to 6/group) to 0 or 3 µg/ml BBP via drinking water (Jahreis et al., 2018). Exposure began one week prior to mating and lasted through either delivery or until weaning of the F1 (PND 21). The F1offpsring were immunized with ovalbumin and adjuvant (alum and MgOH) via i.p. injection on days 1 and 14 prior to receiving intranasal administration of ovalbumin on days 14-16 and 21-23. Female F1 were mated to un-exposed males, and the immunization paradigm was repeated in the F2. Outcomes evaluated included measurement of cell numbers in BAL, lung histology, IgE levels, cytokine levels (*e.g.*, IFN-γ, IL-17, IL10, IL-4), differentially methylated regions of DNA, and a regulatory T cell suppression assay. However, there were substantial limitations that impact the interpretation of the results of this study, particularly dose characterization. Indeed, the authors estimated the dose ranged from 0.48 to 0.6 mg BBP/kg/body weight per day (assuming 4-5 mL/d drinking water intake containing 3 ug/mL BBP) but did not report water intake for this drinking water study. Additionally, this was a single dose study that used a small number of animals. Ultimately, the study was not considered further.

B.4 Renal

EPA identified two studies that provided more data for renal outcomes following exposure to BBP (Integrated Laboratory Systems, 2017; Nakagomi et al., 2017). In this study, male and female SD rats were exposed to 0 or 500 mg/kg-day BBP for 14 days via gavage. Necropsies were performed on male (6 to 9/group) and female (5 to 9/group) rats at the time of sacrifice and kidneys were collected for histopathological evaluation. The data are limited to a qualitative description by authors. The authors reported, “light histopathologic changes in kidneys and marked changes in the hormone status of rats

exposed to BBP at 500 mg kg-day". Kidney weights were not reported for BBP-exposed animals. Endpoints from this study were not considered further due to limitations that impact the interpretation of the results, namely qualitative reporting of histopathology data, small sample size, and use of a single, high dose of BBP. Additionally, the study by Integrated Laboratory Systems (2017) (described in Section B.1) reported decreased blood urea nitrogen at 250 mg/kg-day BBP and higher in SD rats (16/group) exposed to 0, 250, 500, 750, or 1000 mg/kg-day BBP via oral/gavage from PND 22 to PND 42. However, there was no dose-response above 500 mg/kg-day performed in the Nakagomi et al. (2017) assessment, and other clinical chemistry markers of renal dysfunction noted by Integrated Laboratory Systems (2017) were only observed at doses of 750 mg/kg-day and higher (*e.g.*, increased serum phosphorus).

B.5 Hepatic

EPA identified one study that provided more data for hepatic outcomes (liver weight and liver histopathology data) following exposure to BBP (Nakagomi et al., 2017), which was described above in Section B.4. Liver weights were significantly decreased in male rats from the BBP group compared to control (approximately 14% increase). No significant change was reported for female rats. No histopathological effects of the liver were observed for rats exposed to BBP, as reported qualitatively in the text with one representative micrograph showing a section of the liver from a control and exposed rat (sex not specified). Endpoints from this study were not considered further due to limitations that impact the interpretation of the results, namely qualitative reporting of histopathology data, small sample size, and use of a single, high dose of BBP.

Appendix C FETAL TESTICULAR TESTOSTERONE AS AN ACUTE EFFECT

One experimental animal model study is available that investigates the antiandrogenic effects of BBP following single dose, acute exposure. In a multi-cohort study by Alam et al. (2015), three week old male SD rats were orally gavaged with 0 or 500 mg/kg BBP and necropsied for reproductive effects assessment at various timepoints, including 3, 12, or 24 hours post exposure, and 2, 4, 6, 9, or 12 days after treatment. Briefly, adverse histopathological changes in seminiferous tubules (*i.e.*, reduction and/or disappearance of tubular lumens) was noted at 3 hours post exposure and thinning seminiferous epithelia and wide tubular lumina were noted by 24 hours post exposure. Increased seminiferous tubule spermatocyte cell apoptosis was also increased at 3, 12, and 24 hours post dosing. In experiment two, authors reported decreased absolute testis weight at days 6, 9, and 12 post exposure. With regard to seminiferous tubule spermatocyte apoptosis, increased apoptotic spermatogenic cells were observed at 2, 4, and 6 days post exposure, which was an effect that dissipated by the ninth day observation (Alam and Kurohmaru, 2015). Moreover, there are studies of dibutyl phthalate (DBP) available (toxicologically similar to BBP) that indicate a single acute exposure during the critical window of development (*i.e.*, GD 14 to 19) can reduce fetal testicular testosterone production and disrupt testicular steroidogenic gene expression. Two studies were identified that demonstrate single doses of 500 mg/kg DBP can reduce fetal testicular testosterone and steroidogenic gene expression. Johnson et al. (2012; 2011) gavaged pregnant SD rats with a single dose of 500 mg/kg DBP on GD 19 and observed reductions in steroidogenic gene expression in the fetal testes three (*Cyp17a1*) to six (*Cyp11a1, Star*) hours post-exposure, while fetal testicular testosterone was reduced starting 18 hours post-exposure. Similarly, Thompson et al. (2005) reported a 50 percent reduction in fetal testicular testosterone 1-hour after pregnant SD rats were gavaged with a single dose of 500 mg/kg DBP on GD 19, while changes in steroidogenic gene expression occurred 3 (*Star*) to 6 (*Cyp11a1, Cyp17a1, Scarb1*) hours post-exposure, and protein levels of these genes were reduced 6 to 12 hours post-exposure. Additionally, studies by Carruthers et al. (2005) further demonstrate that exposure to as few as two oral doses of 500 mg/kg DBP on successive days between GDs 15 to 20 can reduce male pup AGD, cause permanent nipple retention, and increase the frequency of reproductive tract malformations and testicular pathology in adult rats that received two doses of DBP during the critical window.

In summary, single dose acute studies of BBP (Alam and Kurohmaru, 2015) and DBP (Johnson et al., 2012; Johnson et al., 2011) provide evidence to support use of effects on the male reproductive system, specifically testicular histopathological changes and reduced fetal testosterone, as an acute effect. However, the database is limited to just a few studies that test relatively high (500 mg/kg) single doses of BBP or DBP.

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

For BBP, all data considered for PODs are obtained from oral animal toxicity studies in rats. Because toxicity values for BBP 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. However, EPA did not identify existing PBPK models for BBP or other BBP information that would be useful in conducting chemical-specific quantitative dose extrapolation. In the absence of such data, EPA relied on the guidance from U.S. EPA (2011c), which recommends scaling allometrically across species using the three-quarter power of body weight ($BW^{3/4}$) for oral data. Allometric scaling accounts for differences in physiological and biochemical processes, mostly related to kinetics.

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

Equation C-1. Dosimetric Adjustment Factor

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

Where:

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

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

Use of allometric scaling for oral animal toxicity data to account for differences among species allows EPA to decrease the default intraspecies 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 C-1, EPA adjusts the POD to obtain the HED using Equation C-2:

Equation C-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 using Equation C-3 as follows:

Equation C-3. Extrapolating from Oral HED to Inhalation HEC

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

Where:

$HEC_{Daily,continuous}$	=	Inhalation HEC based on continuous daily exposure (mg/m^3)
HED_{Daily}	=	Oral HED based on daily exposure (mg/kg-day)
BW_H	=	Body weight of adult humans (kg) = 80
IR_R	=	Inhalation rate for an individual at rest (m^3/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^3/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^3 due to variation in concentration reporting in studies and the default units for different OPPT models. Therefore, EPA presents all PODs in equivalents of both units to avoid confusion and errors. Equation C-4 presents the conversion of the HEC from mg/m^3 to ppm.

Equation C-4. Converting Units for HECs (mg/m^3 to ppm)

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

Where:

24.45	=	Molar volume of a gas at standard temperature and pressure (L/mol), default
MW	=	Molecular weight of the chemical (MW of BBP = 312.37 g/mol)

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

The acute, intermediate, and chronic duration non-cancer POD is based on a NOAEL = 50 mg/kg/day, and the critical effect is developmental toxicity (*i.e.*, decreased AGD) in gestationally exposed CD rats (Tyl et al., 2004). EPA used Equation C-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 C-2:

$$11.8\ \frac{mg}{kg-day} = 50\frac{mg}{kg-day} \times 0.236$$

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

$$64.2\ \frac{mg}{m^3} =\ 11.8\ \frac{mg}{kg-day} \times (\frac{80\ kg}{0.6125\frac{m^3}{hr} * 24\ hr})$$

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

$$5.03\ ppm = 64.2\ \frac{mg}{m^3}\ \times \frac{24.45}{312.37}$$

Appendix E CONSIDERATIONS FOR BENCHMARK RESPONSE (BMR) SELECTION FOR REDUCED FETAL TESTICULAR TESTOSTERONE

E.1 Purpose

EPA has conducted an updated meta-analysis and BMD analysis of decreased fetal rat testicular testosterone (U.S. EPA, 2025g). During the July 2024 SACC peer-review meeting of the draft risk DIDP and draft human health hazard assessments for DINP, the SACC recommended that EPA should clearly state its rational for selection of 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 $BMDL_5$ (Allen et al., 1994a, b; Faustman et al., 1994).

EPA also evaluated a BMR of 10 percent as part of the updated BMD analysis. BMD modeling of fetal testosterone conducted by NASEM (2017) indicated that BMD_5 estimates are below the lowest dose with empirical testosterone data for several of the phthalates (*e.g.*, DIBP). As discussed in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) "*For some 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_5 estimates ranged from 8.4 to 74 mg/kg-day for DEHP, DBP, DCHP, and DINP, however, a BMD_5 estimate could not be derived for BBP or DIBP. Similarly, BMD_{10} estimates ranged from 17 to 152 for DEHP, DBP, DCHP, DIBP and DINP, however, a BMD_{10} estimate could not be derived for BBP. BMD_{40} estimates were derived for all phthalates (*i.e.*, DEHP, DBP, DCHP, DIBP, BBP, DINP) and ranged from 90 to 699 mg/kg-day.

In the MOA for phthalate syndrome, which is described elsewhere (U.S. EPA, 2023a) and in Section 3.1.2.1 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 POD, EPA compared BMDL estimates at the 5, 10, and 40 percent response levels for each phthalate (DEHP, DBP, DCHP, DIBP, BBP, DINP) to the lowest identified apical outcomes associated with phthalate syndrome to determine which response level is protective of downstream apical outcomes.

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

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

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

E.4 Weight of Scientific Evidence Conclusion

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

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

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

Abbreviations: AGD = Anogenital distance; BMD = Benchmark dose; $BMDL_5$ = Lower 95% confidence limit on BMD; CI = Confidence interval; LOAEL = Lowest-observed-adverse-effect level; NOAEL = No-observed-adverse-effect level; POD = Point of departure; RTM = Reproductive tract malformations.
[a] The linear-quadratic model provided the best fit (based on lowest AIC) for DEHP, DBP, DIBP, BBP, DCHP, and DINP.
[b] BMD and/or BMDL estimate could not be derived.

Appendix F BENCHMARK DOSE MODELING OF FETAL TESTICULAR TESTOSTERONE DATA FROM GRAY ET AL. (2021), HOWDESHELL ET AL. (2008), FURR ET AL. (2014)

EPA conducted BMD modeling of *ex vivo* fetal testicular testosterone data from four gestational exposure studies of BBP reported in three publications (Gray et al., 2021; Furr et al., 2014; Howdeshell et al., 2008).

The BMD modeling for continuous data was conducted with the EPA's BMD software (BMDS 3.3.2). All standard BMDS 3.3.2 continuous models that use maximum likelihood 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 3.3.2 models that use Bayesian fitting procedures and Bayesian model averaging were not applied in this work.

Standard BMDS 3.3.2 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 BMR levels of 1 control standard deviation (1 SD) and 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 (Appendix E). A BMR of 1 SD was included per EPA's Benchmark Dose Technical Guidance (U.S. EPA, 2012a), which recommends that the BMD corresponding to one control SD always be presented for reporting purposes. 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 *ex vivo* fetal testicular testosterone data, while more detailed BMD model results are provided in Appendices F.1 through F.4.

Table_Apx F-1. Summary of BMD Model Results for Decreased *Ex Vivo* Fetal Testicular Testosterone

Data Set	BMR	Best-Fit Model (Variance)	BMD (mg/kg-day)	BMDL (mg/kg-day)	Notes	Appendix Containing Results
(Howdeshell et al., 2008)	5%	Exponential 3 (constant)	138	81		F.1
(Gray et al., 2021)	5%	–[a]	–[a]	–[a]	No models adequately fit the data set[a]	F.2
(Furr et al., 2014) (Block 36 rats)	–	–	–	–	No models adequately fit the data set	F.3
(Furr et al., 2014) (Block 37 rats)	–	–	–	–	No models adequately fit the data set	F.4

BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response

[a] Although the polynomial degree 2 model (non-constant variance) provided an adequate statistical fit and supported BMD_5 and $BMDL_5$ values of 48 and 47 mg/kg-day, respectively, the model provided a poor visual fit, particularly in the low end range of the dose-response curve. Therefore, EPA did not further consider the derived BMD and BMDL values.

F.1 BMD Model Results of Howdeshell et al. (2008)

Table_Apx F-2. *Ex Vivo* Fetal Rat Testicular Testosterone Data (Howdeshell et al., 2008)

Dose (mg/kg-day)	N (# of litters)	Mean	Standard Deviation	Notes
0	9	3.45	0.4500	Data from Table 6 in Howdeshell et al. (2008)
100	4	3.66	0.5200	
300	5	2.68	0.6037	
600	2	1.18	0.3394	
900	2	0.34	0.1980	

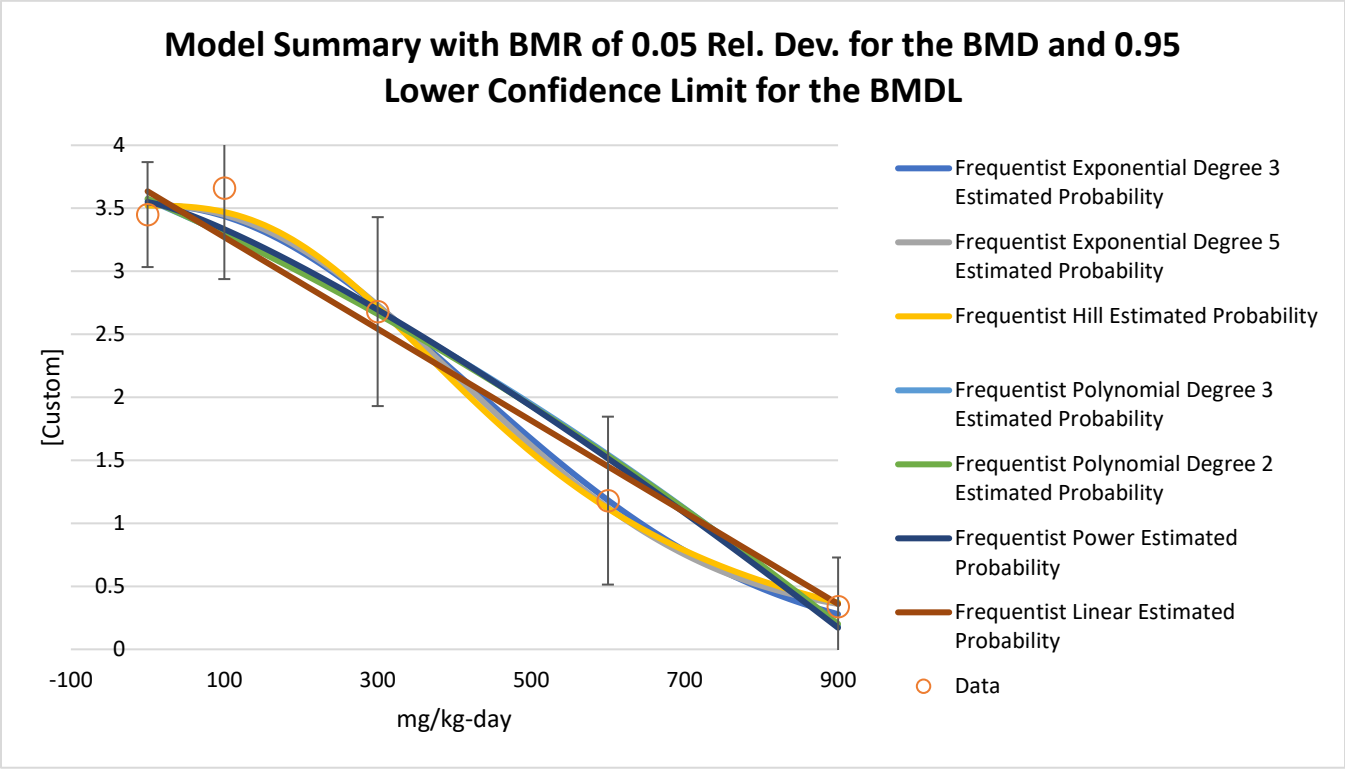
Table_Apx F-3. BMD Model Results *Ex Vivo* Fetal Testicular Testosterone (Howdeshell et al., 2008)

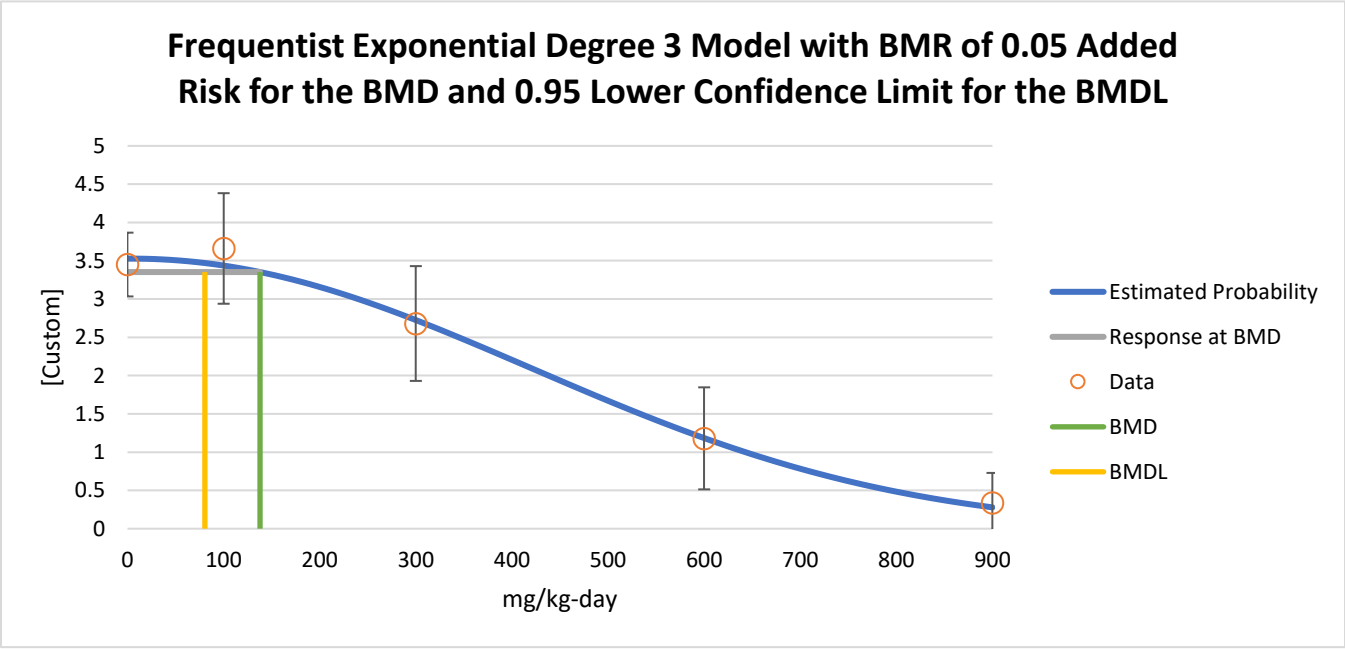
Models[a]	Restriction[b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	**Restricted**	**Constant**	**137.878**	**80.51109**	**194.8991**	**129.4477**	**416.3536**	**350.4237**	**218.8987**	**149.5615**	**0.4883665**	**34.60071824**	**Viable - Recommended**	**Lowest AIC**
Exponential 5	Restricted	Constant	146.2356	81.66467	201.768	130.8391	411.2116	345.5512	224.6711	150.9235	0.2511005	36.48451024	Viable - Alternate	
Hill	Restricted	Constant	159.9532	74.84564	210.1501	126.9003	401.1389	335.9305	230.161	149.4785	0.2959987	36.25947488	Viable - Alternate	
Polynomial Degree 3	Restricted	Constant	66.44438	44.68631	129.0148	89.37277	449.0893	357.795	169.5433	106.3084	0.0935472	37.90591815	Questionable	Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	64.35011	44.69158	125.49	89.38335	444.2024	357.4874	164.8979	106.3203	0.0935793	37.90523307	Questionable	Goodness of fit p-value < 0.1
Power	Restricted	Constant	85.29039	45.81249	148.472	91.65708	449.9177	366.6237	185.5577	111.5766	0.1375952	37.13421803	Viable - Alternate	
Linear	Unrestricted	Constant	49.94472	43.80531	99.88943	87.6111	399.5577	350.4423	133.5974	102.2819	0.1370596	36.69388381	Viable - Alternate	

Abbreviations: AIC = Akaike information criterion; BMD = Benchmark dose; BMDL = Benchmark dose lower limit; BMDS = Benchmark dose software; BMR = Benchmark response; SD = Standard deviation.

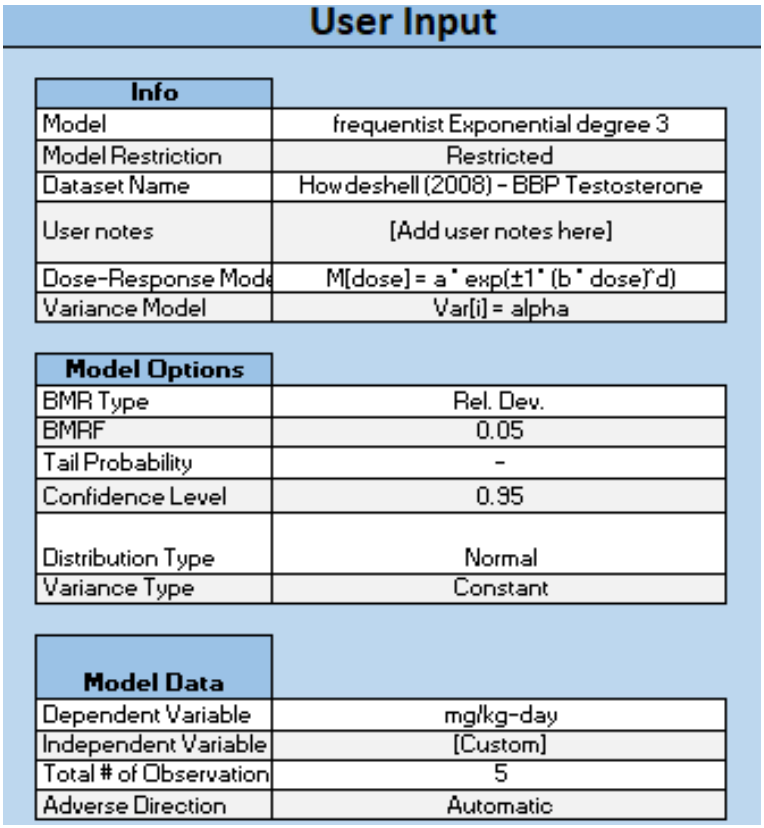
[a] Selected Model (bolded and shaded gray).

[b] Restrictions defined in the BMDS 3.3 User Guide.





Figure_Apx F-1. Frequentist Exponential Degree 3 Model of Howdeshell et al. (2008) data



User Input

Info	
Model	frequentist Exponential degree 3
Model Restriction	Restricted
Dataset Name	Howdeshell (2008) - BBP Testosterone
User notes	[Add user notes here]
Dose-Response Mode	M[dose] = a * exp(±1 * (b * dose)^d)
Variance Model	Var[i] = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	[Custom]
Total # of Observation	5
Adverse Direction	Automatic

Figure_Apx F-2. User Input of Frequentist Exponential Degree 3 Model of Howdeshell et al. (2008) Data

Model Results

Benchmark Dose	
BMD	137.8779888
BMDL	80.51109109
BMDU	214.4859617
AIC	34.60071824
Test 4 P-value	0.48836649
D.O.F.	2

Model Parameters				
# of Parameters	4			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	3.528232329	0.12965373	3.274116	3.782349
b	0.00173887	1.60E-04	0.001425	0.002053
d	2.079749845	4.18E-01	1.260538	2.898961
log-alpha	-1.628753523	3.02E-01	-2.2197	-1.03781

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	9	3.52823233	3.45	3.45	0.442915	0.45	0.45	-0.529891
100	4	3.43665147	3.66	3.66	0.442915	0.52	0.52	1.0085384
300	5	2.7248925	2.68	2.68	0.442915	0.6037	0.60374	-0.226641
600	2	1.18363214	1.18	1.18	0.442915	0.3394	0.33941	-0.011597
900	2	0.27875092	0.34	0.34	0.442915	0.198	0.19799	0.1955662

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-12.58366997	6	37.16734
A2	-10.44159567	10	40.88319
A3	-12.58366997	6	37.16734
fitted	-13.30035912	4	34.60072
R	-33.95385731	2	71.90771

* Includes additive constant of -20.21665. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	47.02452329	8	<0.0001
2	4.284148604	4	0.368914
3	4.284148604	4	0.368914
4	1.433378302	2	0.488366

Figure_Apx F-3. Model Results of Frequentist Exponential Degree 3 Model of Howdeshell et al. (2008) Data

F.2 BMD Model Results of Gray et al. (2021)

Table_Apx F-4. *Ex Vivo* Fetal Rat Testicular Testosterone Data (Gray et al., 2021)

Dose (mg/kg-day)	N (# of litters)	Mean	Standard Deviation	Notes
0	3	7.848667	1.202189	Data for Block 78 rats reported in Supplementary Data file associated with Gray et al. (2021)
100	3	8.409444	1.32299	
300	3	4.878667	0.634855	
600	3	2.921333	1.205674	
900	3	0.603111	0.223092	

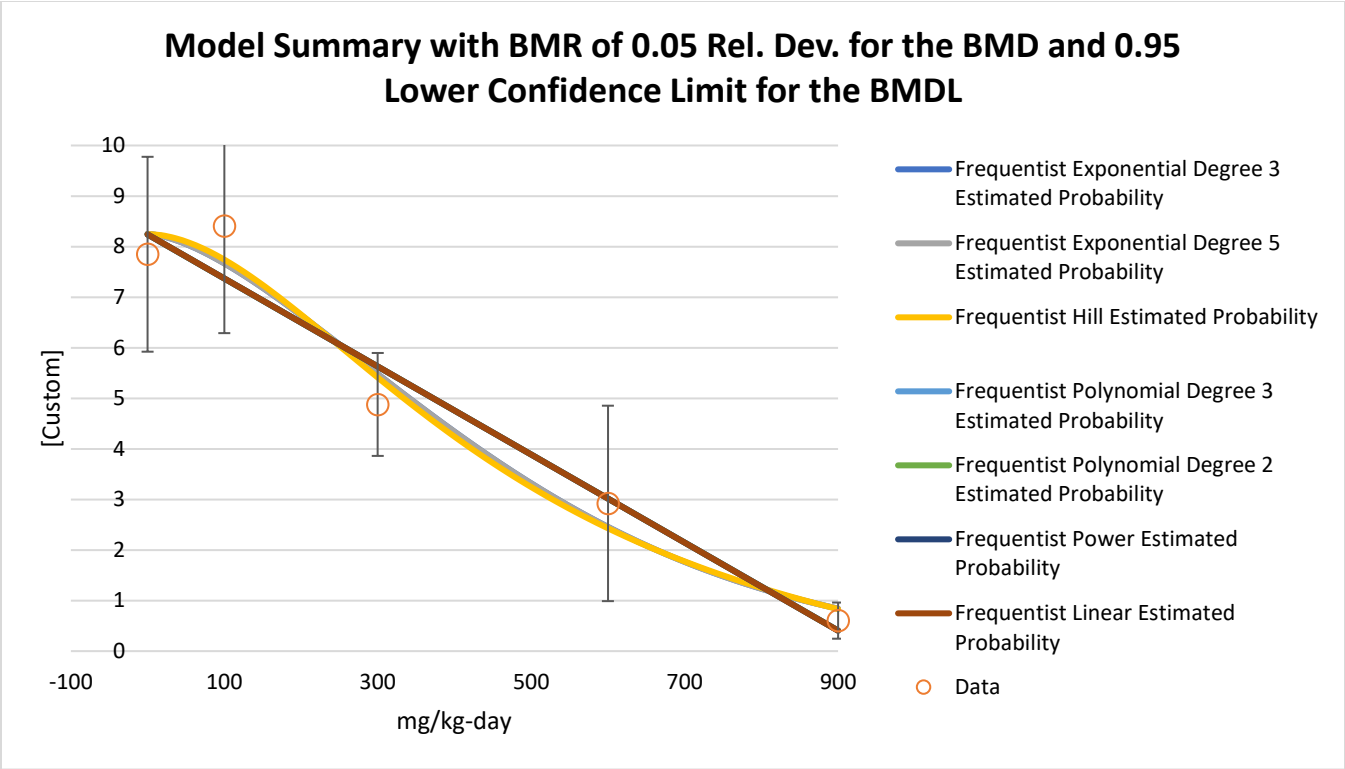
Table_Apx F-5. BMD Model Results *Ex Vivo* Fetal Testicular Testosterone (All Dose Groups) (Gray et al., 2021)

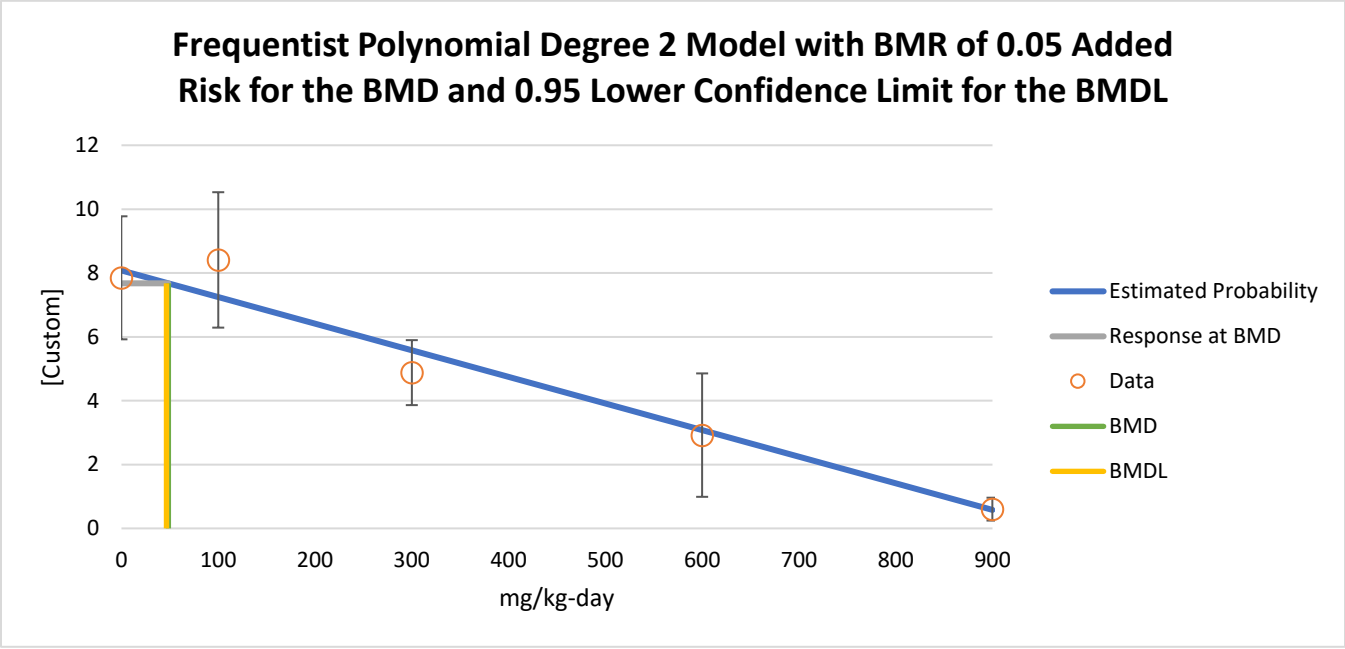
Models [a]	Restriction [b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	79.44	36.49647	125.8696	68.52385	345.3638	264.8549	140.8441	77.63085	0.0822269	49.78570739	Questionable	Goodness of fit p-value <0.1
Exponential 5	Restricted	Constant	79.44	36.49897	125.8696	68.52385	345.3638	264.8549	140.8441	77.63085	0.0253979	51.78570739	Questionable	Goodness of fit p-value <0.1
Hill	Restricted	Constant	88.91208	29.98603	133.0772	61.37096	338.0458	259.4567	145.5466	69.51906	0.0344126	51.26324531	Questionable	Goodness of fit p-value <0.1 BMDL 3x lower than lowest non-zero dose
Polynomial Degree 3	Restricted	Constant	47.34491	42.7451	94.68978	85.49016	378.7592	341.9607	117.7362	87.2974	0.0896355	49.28984585	Questionable	Goodness of fit p-value <0.1
Polynomial Degree 2	Restricted	Constant	47.33616	42.74652	94.67232	85.49304	378.6893	341.9722	117.7338	87.29664	0.0896369	49.28980913	Questionable	Goodness of fit p-value <0.1
Power	Restricted	Constant	47.32532	42.74833	94.65064	85.49666	378.6026	341.9866	117.6541	87.29526	0.0896377	49.28978915	Questionable	Goodness of fit p-value <0.1
Linear	Unrestricted	Constant	47.32533	42.74833	94.65064	85.49666	378.6025	341.9866	117.6541	87.29417	0.0896377	49.28978915	Questionable	Goodness of fit p-value <0.1
Exponential 3	Restricted	Non-Constant	132.0526	48.65299	188.3712	84.90216	410.5209	284.9006	262.7082	110.1942	0.0426644	48.1908878	Questionable	Goodness of fit p-value <0.1
Exponential 5	Restricted	Non-Constant	132.0526	48.65299	188.3711	84.90215	410.5208	284.9006	262.7082	110.1942	0.0120141	50.19088786	Questionable	Goodness of fit p-value <0.1
Hill	Restricted	Non-Constant	61.41628	27.89238	108.6075	55.80617	365.3485	263.4583	172.7294	84.31022	0.0447518	47.91007314	Questionable	Goodness of fit p-value <0.1 BMDL 3x lower than lowest non-zero dose
Polynomial Degree 3	Restricted	Non-Constant	48.38865	46.50351	96.77729	93.00702	387.1092	372.0281	170.7388	117.151	0.2048224	44.46722414	Viable - Alternate	
Polynomial Degree 2	**Restricted**	**Non-Constant**	**48.4699**	**46.5102**	**96.93983**	**93.02042**	**387.7593**	**372.0812**	**177.4433**	**117.4939**	**0.2086964**	**44.42271558**	**Viable - Recommended**	**Lowest AIC**
Power	Restricted	Non-Constant	49.9447	48.7917	99.20589	92.64517	391.4114	372.6303	189.1184	116.9643	0.0998621	46.49003582	Questionable	Goodness of fit p-value <0.1
Linear	Unrestricted	Non-Constant	48.4699	46.71352	96.93983	93.42708	387.7593	373.7082	177.4433	117.4921	0.2086964	44.42271558	Viable - Alternate	

Abbreviations: AIC = Akaike information criterion; BMD = Benchmark dose; BMDL = Benchmark dose lower limit; BMDS = Benchmark dose software; BMR = Benchmark response; SD = Standard deviation.

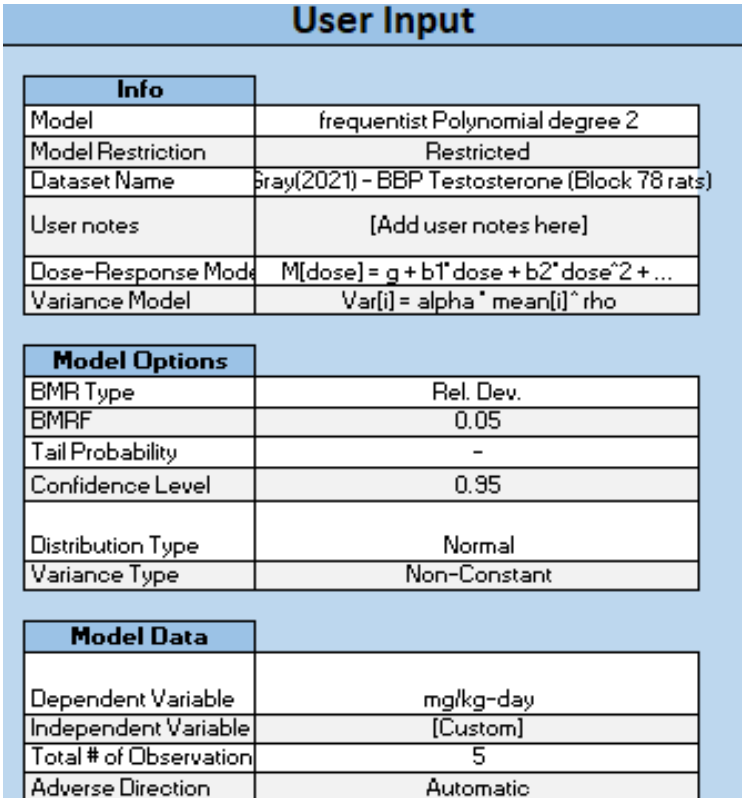
[a] Selected Model (bolded and shaded gray).

[b] Restrictions defined in the BMDS 3.3 User Guide.





Figure_Apx F-4. Frequentist Exponential Degree 3 Model of Gray et al. (2021) Data



User Input

Info	
Model	frequentist Polynomial degree 2
Model Restriction	Restricted
Dataset Name	Gray(2021) - BBP Testosterone (Block 78 rats)
User notes	[Add user notes here]
Dose-Response Mode	M[dose] = g + b1*dose + b2*dose^2 + ...
Variance Model	Var[i] = alpha * mean[i]^ rho

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Non-Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	[Custom]
Total # of Observation	5
Adverse Direction	Automatic

Figure_Apx F-5. User Input of Frequentist Exponential Degree 3 Model of Gray et al. (2021) Data

Model Results

Benchmark Dose	
BMD	48.46990407
BMDL	46.51019855
BMDU	77.34071876
AIC	44.42271558
Test 4 P-value	0.208696414
D.O.F.	3

Model Parameters				
# of Parameters	5			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	8.079313138	0.49034105	7.118262	9.040364
beta	-0.008334359	5.80E-04	-0.00947	-0.0072
beta2	Bounded	NA	NA	NA
rho	1.460822961	4.76E-01	0.527479	2.394166
alpha	0.103359443	7.75E-03	0.088165	0.118554

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	3	8.07931314	7.848667	7.848667	1.478876	1.2022	1.20219	-0.270132
100	3	7.24587722	8.409444	8.409444	1.365826	1.323	1.32299	1.4755594
300	3	5.57900537	4.878667	4.878667	1.128415	0.6349	0.63485	-1.074979
600	3	3.0786976	2.921333	2.921333	0.730944	1.2057	1.20567	-0.372892
900	3	0.57838984	0.603111	0.603111	0.215525	0.2231	0.22309	0.1986704

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-18.39458048	6	48.78916
A2	-14.33273648	10	48.66547
A3	-15.94105261	7	45.88211
fitted	-18.21135779	4	44.42272
R	-38.05514411	2	80.11029

* Includes additive constant of -13.78408. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	47.44481524	8	<0.0001
2	8.123687994	4	0.087151
3	3.216632255	3	0.359415
4	4.54061036	3	0.208696

Figure_Apx F-6. Model Results of Frequentist Exponential Degree 3 Model of Gray et al. (2021) Data

F.3 BMD Model Results of Furr et al. (2014) (Block 36 Rats)

Table_Apx F-6. *Ex Vivo* Fetal Rat Testicular Testosterone Data (Furr et al., 2014) (Block 36 Rats)

Dose (mg/kg-day)	N (# of litters)	Mean	Standard Deviation	Notes
0	3	11.63	0.225167	Data from Table 2 in Furr et al. (2014)
100	2	5.43	0.820244	
300	2	3.81	0.296985	
600	3	2.77	1.143154	
900	3	1.73	0.127279	

Table_Apx F-7. BMD Model Results *Ex Vivo* Fetal Testicular Testosterone (Block 36 – All Dose Groups) (Furr et al., 2014)

Models [a]	Restriction [b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	17.3487	11.35096	35.6356	23.31577	172.7742	113.0432	47.89558	28.74612	<0.0001	51.45915786	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 5	Restricted	Constant	6.53742	4.690565	13.53295	9.716502	70.61377	51.01258	8.614115	5.656828	0.0087808	37.33552192	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose BMD 10x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose Modeled control response std. dev. >|1.5| actual response std. dev.
Hill	Restricted	Constant	4.406264	3.14372	9.375719	6.362706	60.82678	42.65383	4.613014	2.955083	0.1345529	31.87673639	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose BMD 10x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial Degree 3	Restricted	Constant	48.39746	40.18862	96.79489	80.3777	387.1796	321.5089	223.096	154.1383	<0.0001	61.53466853	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

Models [a]	Restriction [b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
Polynomial Degree 2	Restricted	Constant	48.44536	40.18586	96.89071	80.37172	387.5628	321.4869	223.4798	154.1353	<0.0001	61.53482916	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Power	Restricted	Constant	49.1679	40.13384	98.3358	80.26768	393.3432	321.0707	222.1772	153.6257	<0.0001	61.59215522	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Linear	Unrestricted	Constant	48.37868	40.18983	96.75735	80.37966	387.0294	321.5167	222.9468	154.1385	<0.0001	61.53465443	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual for Dose Group Near BMD| > 2 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 3	Restricted	Non-Constant	28.63226	22.79934	58.81294	46.83166	285.1463	227.0567	269.7963	100.0567	0.0001714	45.85655362	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose Modeled control response std. dev. >|1.5| actual response std. dev.
Exponential 5	Restricted	Non-Constant	6.584692	4.690942	13.63099	9.717238	71.13498	51.02266	8.597824	5.658575	0.0095036	37.33137285	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose BMD 10x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose

Models [a]	Restriction [b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
														Modeled control response std. dev. >|1.5| actual response std. dev.
Hill	Restricted	Non-Constant	3.889547	2.960594	8.292334	6.225717	54.90352	43.90247	1.436185	0.693566	0.3308113	30.0887978	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose BMD 10x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose
Polynomial Degree 3	Restricted	Non-Constant	63.27256	56.91067	126.5451	113.8213	506.1804	455.2852	957.3279	467.3266	<0.0001	47.54806445	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Polynomial Degree 2	Restricted	Non-Constant	63.26384	56.91213	126.5277	113.8243	506.1108	455.2988	956.4118	467.3261	<0.0001	47.54806964	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Power	Restricted	Non-Constant	71.50585	56.69262	139.027	113.4702	525.5507	453.3178	1023.701	454.3581	<0.0001	49.78518364	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.
Linear	Unrestricted	Non-Constant	63.27256	60.30696	126.5451	120.6139	506.1804	455.2853	957.328	467.3295	<0.0001	47.54806445	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 |Residual at control| > 2 Modeled control response std. dev. >|1.5| actual response std. dev.

Abbreviations: AIC = Akaike information criterion; BMD = Benchmark dose; BMDL = Benchmark dose lower limit; BMDS = Benchmark dose software; BMR = Benchmark response; SD = Standard deviation.

[a] Selected Model (bolded and shaded gray).

[b] Restrictions defined in the BMDS 3.3 User Guide.

F.4 BMD Model Results of Furr et al. (2014) (Block 37 Rats)

Table_Apx F-8. *Ex Vivo* Fetal Rat Testicular Testosterone Data (Furr et al., 2014) (Block 37 Rats)

Dose (mg/kg-day)	N (# of litters)	Mean	Standard Deviation	Notes
0	4	10.94	3.24	Data from Table 2 in Furr et al. (2014)
11	3	12.17	0.536936	
33	4	10	3.3	
100	4	9.63	2.16	

Table_Apx F-9. BMD Model Results *Ex Vivo* Fetal Testicular Testosterone (Block 37 rats - All Dose Groups) (Furr et al., 2014)

Models [a]	Restriction [b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
Exponential 3	Restricted	Constant	30.10844	11.26244	61.84514	23.13395	299.8475	101.5943	139.0458	48.51259	0.5816642	74.52267227	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	30.43784	1.996375	32.10822	5.958151	-	-	-	-	0.485658	75.92507984	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3 BMDL 3x lower than lowest non-zero dose
Hill	Restricted	Constant	29.25549	0.12955	31.44254	0.157793	-	-	-	-	0.485658	75.92507985	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 20 BMD/BMDL ratio > 3 BMDL 3x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose
Polynomial Degree 3	Restricted	Constant	33.205	13.77656	65.87241	27.54961	250.9207	107.5624	136.5096	54.65605	0.2939821	76.54024317	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 2	Restricted	Constant	55.52211	13.56606	83.38946	27.12893	179.4662	107.9355	128.3908	53.79601	0.2641501	76.685824	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3
Power	Restricted	Constant	32.33752	13.78305	64.67505	27.56611	258.7002	101.476	136.8744	54.68127	0.5778416	74.53585932	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	32.32747	13.78304	64.65496	27.56616	258.6198	110.2644	136.6855	54.68155	0.5778512	74.53582612	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-Constant	-	-	-	-	-	-	-	-	-	-	Unusable	BMD computation failed
Exponential 5	Restricted	Non-Constant	30.43784	1.471662	32.10822	5.238397	-	-	-	-	0.0055921	77.92507984	Questionable	Goodness of fit p-value < 0.1 BMD/BMDL ratio > 20 BMD/BMDL ratio > 3 BMDL 3x lower than lowest non-zero dose
Hill	Restricted	Non-Constant	28.22122	8.46407	30.45435	9.456441	-	-	-	-	NA	79.91452333	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models [a]	Restriction [b]	Variance	BMR = 5%		BMR = 10%		BMR = 40%		BMR = 1 SD		P Value	AIC	BMDS Model Fit	BMDS Notes
			BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL				
Polynomial Degree 3	Restricted	Non-Constant	33.20414	14.27532	66.40826	28.55064	265.6328	109.4742	154.2012	56.69838	0.0185327	76.22425247	Questionable	Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-Constant	33.30109	14.27474	66.60218	28.54949	266.4087	111.7167	154.7987	56.70352	0.018533	76.22422372	Questionable	Goodness of fit p-value < 0.1
Power	Restricted	Non-Constant	33.3204	14.27492	66.64081	28.54985	266.5632	101.5496	154.8914	56.70377	0.018533	76.22422594	Questionable	Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-Constant	33.30108	14.27472	66.60217	28.54948	266.4087	114.1977	154.7986	56.7035	0.018533	76.22422372	Questionable	Goodness of fit p-value < 0.1

Abbreviations: AIC = Akaike information criterion; BMD = Benchmark dose; BMDL = Benchmark dose lower limit; BMDS = Benchmark dose software; BMR = Benchmark response; NA = Not Applicable; SD = Standard deviation.

[a] Selected Model (bolded and shaded gray). No models were selected.

[b] Restrictions defined in the BMDS 3.3 User Guide.

Appendix G BENCHMARK DOSE MODELING OF AUTOPSY FINDINGS AND TESTICULAR PATHOLOGY DATA FROM ASO ET AL. (2005)

EPA conducted benchmark dose (BMD) modeling of the most sensitive effects reported in the multi-generation study of reproduction of rats continuously exposed to BBP through the diet (Aso et al., 2005). Modelled effects include incidence of softening of the testis in F1 male rats, and incidence of testicular pathology, including diffuse atrophy of seminiferous tubules in F1 male rats. Since study authors appeared to evaluate only one adult F1 male rat from each litter, intralitter correlation cannot be accounted for. Therefore, dose-response analysis could only be performed on the original incidences.

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 because the evaluated effects are considered adverse developmental effects that are consistent with development of phthalate syndrome and may be mechanistically linked to decreased fetal testicular testosterone production (as outlined in 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). 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-1 summarizes BMD modeling results, while more detailed BMD model results are reported in Appendices G.1 through G.4.

Table_Apx G-1. Summary of BMD Model Results for Incidence of Soft Testes and Seminiferous Tubule Atrophy in F1 Adult Male Rats (Aso et al. 2005)

Dataset	Best-fitting Model	BMR	BMD (mg/kg-day)	BMDL (mg/kg-day)	Appendix Containing Results
Soft Testes	Multistage 3	5%	120	66	G.1
	Multistage 3	10%	240	145	
	Bayesian Model Average	5%	203	81	G.2
	Bayesian Model Average	10%	324	171	
Seminiferous Tubule Atrophy	Weibull	5%	161	54	G.3
	Weibull	10%	219	109	
	Bayesian Model Average	5%	126	55	G.4
	Bayesian Model Average	10%	198	110	

G.1 BMD Model Results – Incidence of Soft Testes (Frequentist)

G.1.1 BMD Model Results for BMR of 10%

Dataset

Name: Softening of testes in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	24	0
100	24	1
200	24	2
400	24	4

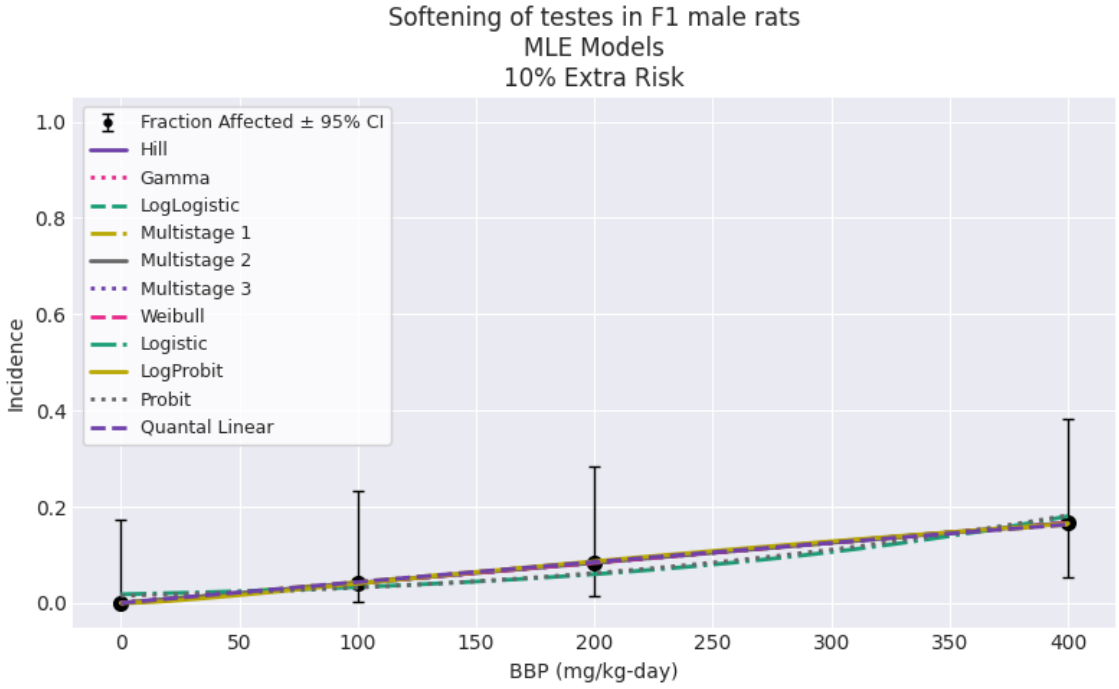
Settings

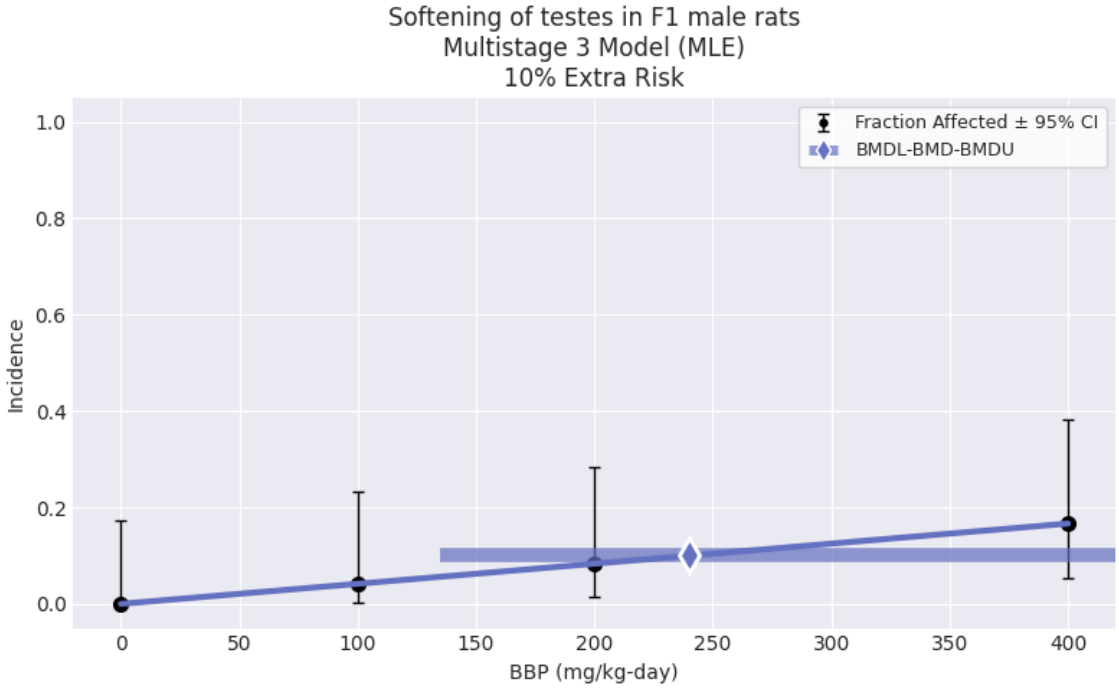
Setting	Value
BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

Maximum Likelihood Approach

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	0	236.322	596.031	1.	47.71	-0.001	-0.021	**Unusable** BMDL does not exist
Gamma	134.693	237.941	584.348	1.	47.709	-0.001	-0.012	**Viable**
LogLogistic	126.988	236.322	482.495	1.	47.71	-0.001	-0.021	**Viable**
Multistage 1	134.621	236.654	585.289	1.	45.714	-0.001	-0.033	**Viable**
Multistage 2	134.684	239.769	584.321	1.	45.709	-0.001	-0.001	**Viable**
Multistage 3[a]	134.686	239.995	584.304	1.	45.709	-0.001	-0.	**Recommended - Lowest AIC**
Weibull	134.692	238.119	584.33	1.	47.709	-0.001	-0.011	**Viable**
Logistic	236.395	316.934	682.71	0.683	48.878	-0.665	-0.176	**Viable**
LogProbit	0	230.865	-	0.997	47.714	-0.001	-0.058	**Unusable** BMDL does not exist
Probit	222.122	304.486	668.033	0.718	48.714	-0.608	-0.198	**Viable**
Quantal Linear	134.631	236.654	585.289	1.	45.714	-0.001	-0.033	**Viable**

[a] BMDS recommended best fitting model


Softening of testes in F1 male rats
MLE Models
10% Extra Risk
Fraction Affected ± 95% CI
Hill
Gamma
LogLogistic
Multistage 1
Multistage 2
Multistage 3
Weibull
Logistic
LogProbit
Probit
Quantal Linear
Incidence
BBP (mg/kg-day)


Softening of testes in F1 male rats
Multistage 3 Model (MLE)
10% Extra Risk
Fraction Affected ± 95% CI
BMDL-BMD-BMDU
Incidence
BBP (mg/kg-day)

Multistage 3 Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Modeling approach	frequentist_restricted
Degree	3

Parameter Settings:

Parameter	Initial	Min	Max
g	0	-18	18
b1	0	0	10000
b2	0	0	10000
b3	0	0	10000

Modeling Summary:

BMD	239.995
BMDL	134.686
BMDU	584.304
AIC	45.7089
Log-Likelihood	-21.8545
P-Value	1
Overall d.f.	3
Chi²	3.6552e-07

Model Parameters:

Variable	Estimate	On Bound	Std Error
g	1.523e-08	yes	Not Reported
b1	0.000416744	no	0.179409
b2	8.54816e-08	yes	Not Reported
b3	3.04226e-11	yes	Not Reported

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	Size	Observed	Expected	Est Prob	Scaled Residual
0	24	0	3.6552e-07	1.523e-08	-0.000604582
100	24	1	1	0.0416667	2.32534e-08
200	24	2	2	0.0833333	-3.51983e-08
400	24	4	4	0.166667	8.34343e-09

Analysis of Deviance:

Model	Log-Likelihood	# Params	Deviance	Test d.f.	P-Value
Full model	-21.8545	4	-	-	-
Fitted model	-21.8545	1	7.31039e-07	3	1
Reduced model	-25.0674	1	6.42592	3	0.0926302

G.1.2 BMD Model Results for BMR of 5%

Dataset

Name: Softening of testes in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	24	0
100	24	1
200	24	2
400	24	4

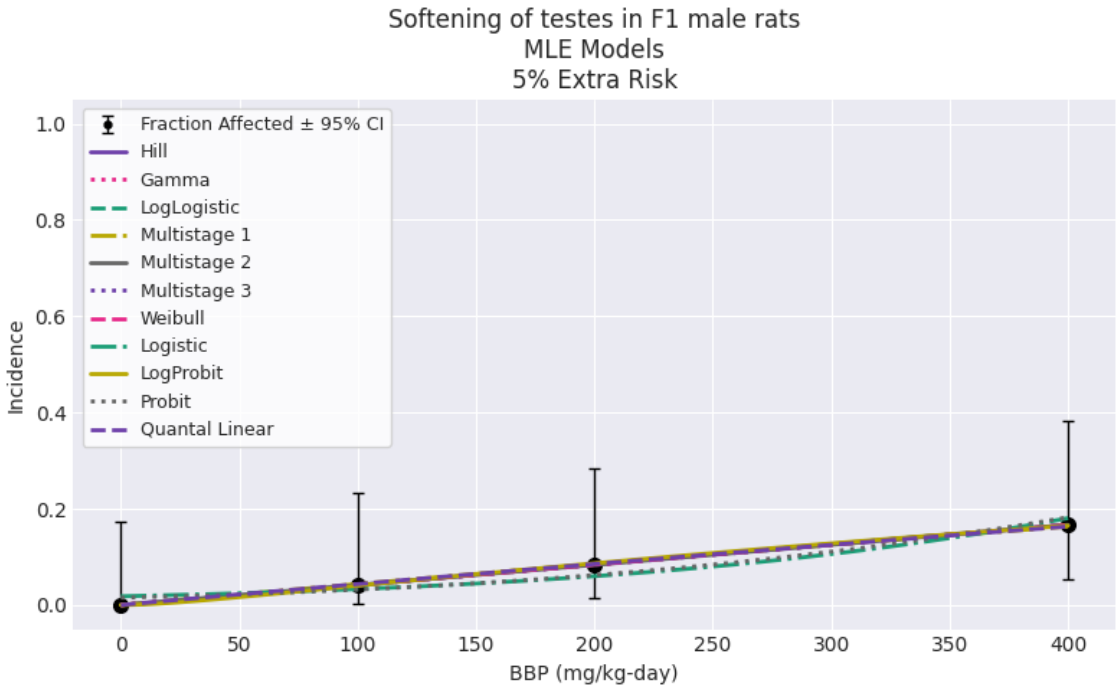
Settings

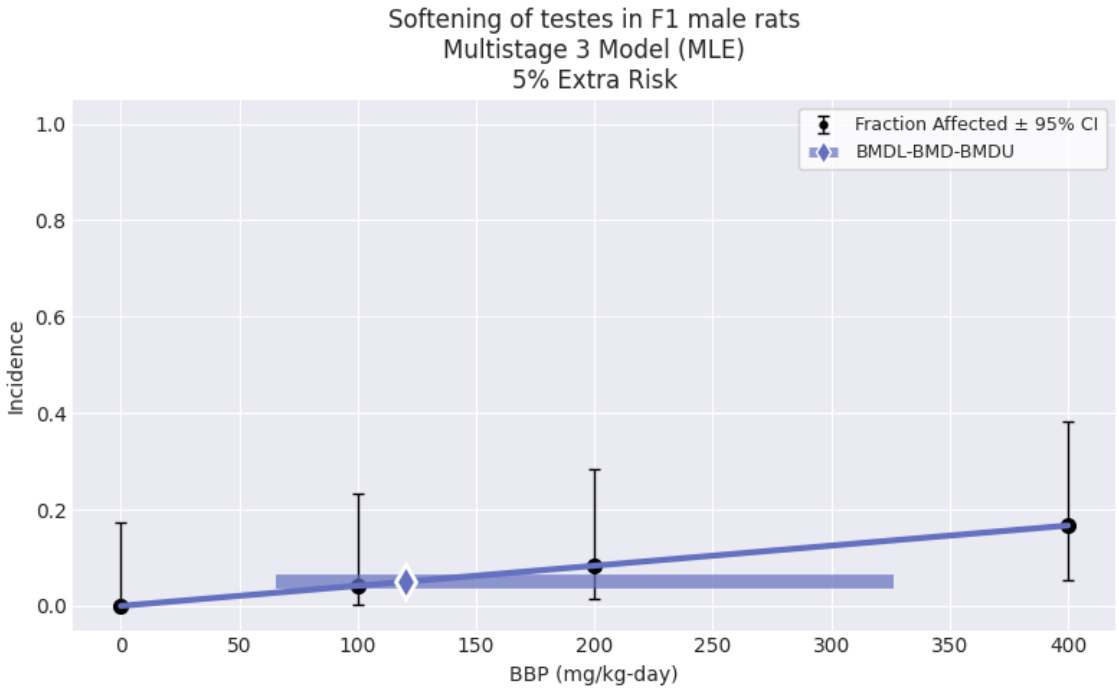
Setting	Value
BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

Maximum Likelihood Approach

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	0	120.446	237.475	1.	47.71	-0.001	0.015	**Unusable** BMDL does not exist
Gamma	65.574	120.245	237.381	1.	47.709	-0.001	0.008	**Viable**
LogLogistic	60.148	120.446	237.475	1.	47.71	-0.001	0.015	**Viable**
Multistage 1	65.541	115.212	284.944	1.	45.714	-0.001	-0.045	**Viable**
Multistage 2	65.573	120.059	316.09	1.	45.709	-0.001	<0.001	**Viable**
Multistage 3[a]	65.569	120.001	326.563	1.	45.709	-0.001	<0.001	**Recommended - Lowest AIC**
Weibull	65.573	120.247	238.732	1.	47.709	-0.001	0.008	**Viable**
Logistic	153.254	219.769	423.937	0.683	48.878	-0.665	0.484	**Viable**
LogProbit	0	120.406	230.441	0.997	47.714	-0.001	0.037	**Unusable** BMDL does not exist
Probit	141.272	205.544	403.207	0.718	48.714	-0.608	0.428	**Viable**
Quantal Linear	65.543	115.212	284.94	1.	45.714	-0.001	-0.045	**Viable**

[a] BMDS recommended best fitting model


Softening of testes in F1 male rats
MLE Models
5% Extra Risk
Fraction Affected ± 95% CI
Hill
Gamma
LogLogistic
Multistage 1
Multistage 2
Multistage 3
Weibull
Logistic
LogProbit
Probit
Quantal Linear
Incidence
BBP (mg/kg-day)


Softening of testes in F1 male rats
Multistage 3 Model (MLE)
5% Extra Risk
Fraction Affected ± 95% CI
BMDL-BMD-BMDU
Incidence
BBP (mg/kg-day)

Multistage 3 Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Modeling approach	frequentist_restricted
Degree	3

Parameter Settings:

Parameter	Initial	Min	Max
g	0	-18	18
b1	0	0	10000
b2	0	0	10000
b3	0	0	10000

Modeling Summary:

BMD	120.001
BMDL	65.5685
BMDU	326.563
AIC	45.7089
Log-Likelihood	-21.8545
P-Value	1
Overall d.f.	3
Chi²	3.6552e-07

Model Parameters:

Variable	Estimate	On Bound	Std Error
g	1.523e-08	yes	Not Reported
b1	0.000416744	no	0.179409
b2	8.54816e-08	yes	Not Reported
b3	3.04226e-11	yes	Not Reported

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	Size	Observed	Expected	Est Prob	Scaled Residual
0	24	0	3.6552e-07	1.523e-08	-0.000604582
100	24	1	1	0.0416667	2.32534e-08
200	24	2	2	0.0833333	-3.51983e-08
400	24	4	4	0.166667	8.34343e-09

Analysis of Deviance:

Model	Log-Likelihood	# Params	Deviance	Test d.f.	P-Value
Full model	-21.8545	4	-	-	-
Fitted model	-21.8545	1	7.31039e-07	3	1
Reduced model	-25.0674	1	6.42592	3	0.0926302

G.2 BMD Model Results – Incidence of Soft Testes (Bayesian Model Averaging)

G.2.1 BMD Model Results for BMR of 10%

Dataset

Name: Softening of testes in F1 male rats

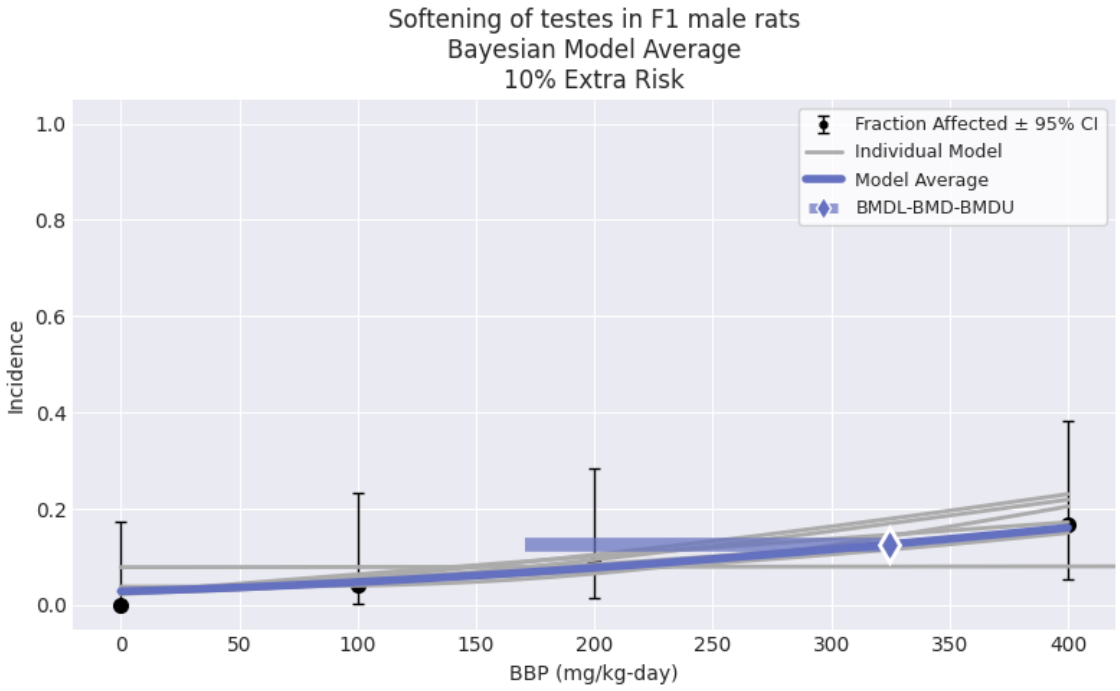
Dose (mg/kg-day)	N	Incidence
0	24	0
100	24	1
200	24	2
400	24	4

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	0.256	154.879	313.947	-	-33.606	-0.817	0.092
Gamma	0.111	0.071	178.826	325.564	917.752	-20.787	-0.899	0.071
Logistic	0.111	0	276.79	16331.345	-	-18.83	-1.431	1.559
LogLogistic	0.111	0.043	143.323	250.708	405.729	-30.023	-0.795	-0.179
LogProbit	0.111	0.043	193.7	313.645	511.571	-28.763	-0.978	-0.464
Multistage 2	0.111	<0.001	148.561	228.391	352.139	-25.511	-0.743	-0.34
Probit	0.111	0.456	229.724	348.951	-	-19.017	-0.839	0.229
Quantal Linear	0.111	0.077	141.276	257.414	609.704	-19.644	-0.779	-0.292
Weibull	0.111	0.053	160.323	326.302	-	-25.865	-0.876	0.166
Model Average	-	-	170.592	324.42	-	-	-	-


Softening of testes in F1 male rats
Bayesian Model Average
10% Extra Risk
Fraction Affected ± 95% CI
Individual Model
Model Average
BMDL-BMD-BMDU
Incidence
BBP (mg/kg-day)
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
400

G.2.2 BMD Model Results for BMR of 5%

Dataset

Name: Softening of testes in F1 male rats

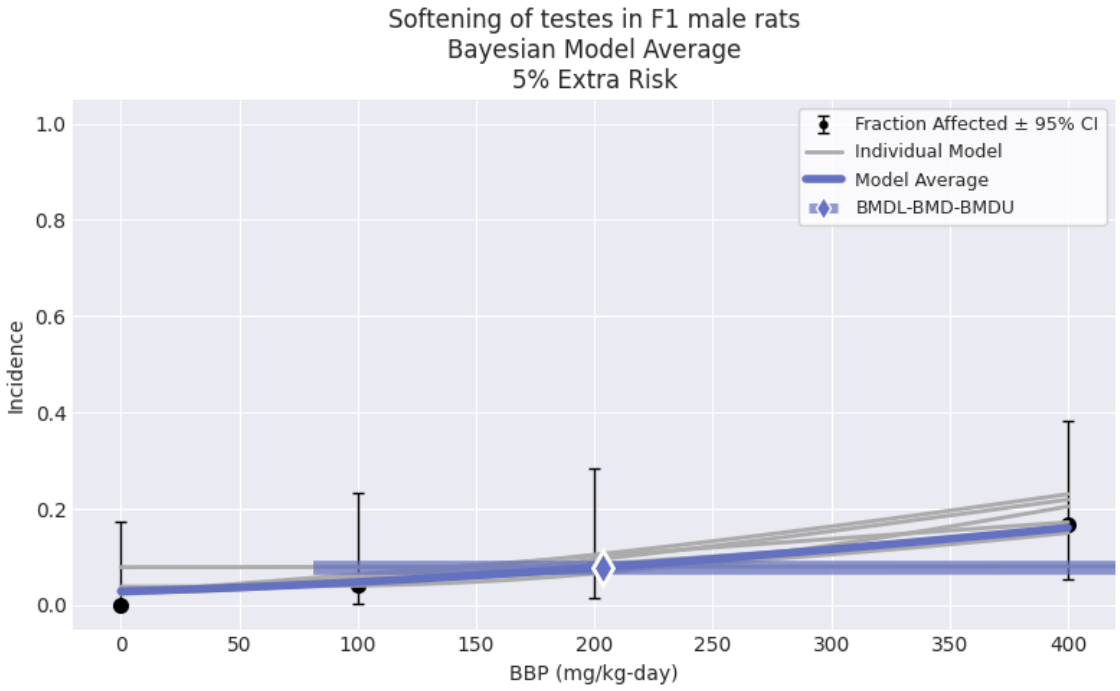
Dose (mg/kg-day)	N	Incidence
0	24	0
100	24	1
200	24	2
400	24	4

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	0.256	69.27	190.625	803.738	-33.606	-0.817	0.075
Gamma	0.111	0.071	94.957	201.867	604.13	-20.787	-0.899	0.054
Logistic	0.111	0	173.73	9500.123	-	-18.83	-1.431	1.559
LogLogistic	0.111	0.043	67.687	162.164	277.608	-30.023	-0.795	-0.179
LogProbit	0.111	0.043	125.361	241.829	391.451	-28.763	-0.978	0.357
Multistage 2	0.111	<0.001	78.063	133.148	216.094	-25.511	-0.743	-0.323
Probit	0.111	0.456	142.46	220.741	659.443	-19.017	-0.839	0.24
Quantal Linear	0.111	0.077	68.778	125.319	296.826	-19.644	-0.779	-0.443
Weibull	0.111	0.053	62.777	184.568	635.756	-25.865	-0.876	-0.02
Model Average	-	-	81.046	203.591	604.494	-	-	-


Softening of testes in F1 male rats
Bayesian Model Average
5% Extra Risk
Fraction Affected ± 95% CI
Individual Model
Model Average
BMDL-BMD-BMDU
Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
400
BBP (mg/kg-day)

G.3 BMD Model Results – Incidence of Seminiferous Tubule Atrophy (Frequentist)

G.3.1 BMD Model Results for BMR of 10%

Dataset

Name: Diffuse atrophy of seminiferous tubules in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	24	1
100	24	1
200	24	3
400	24	9

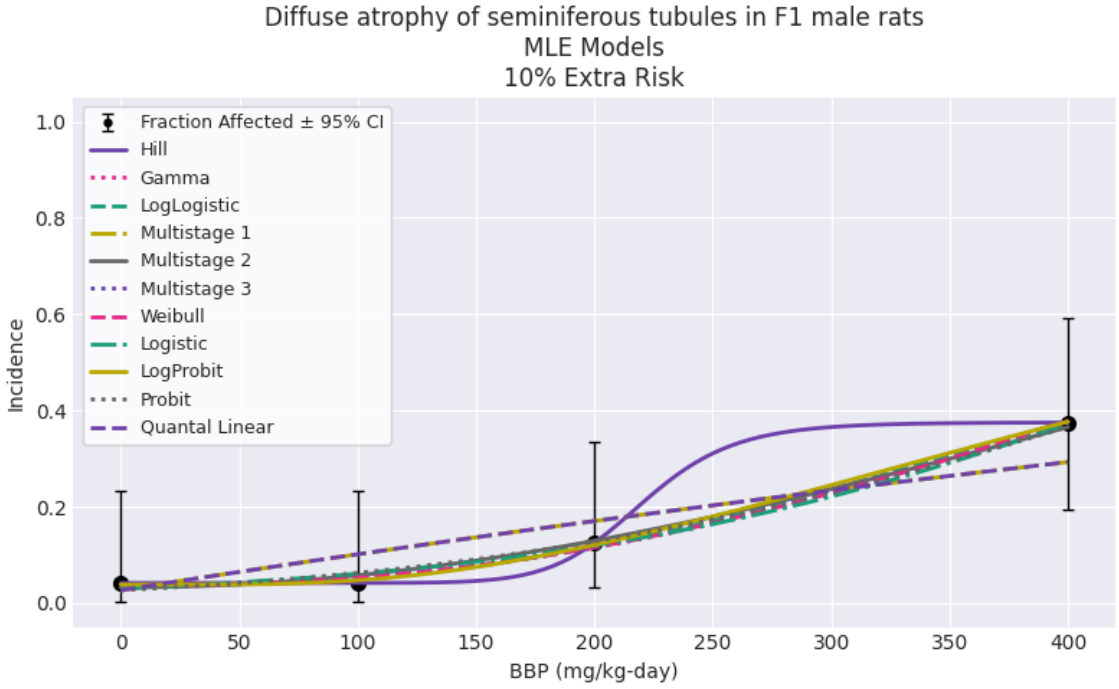
Settings

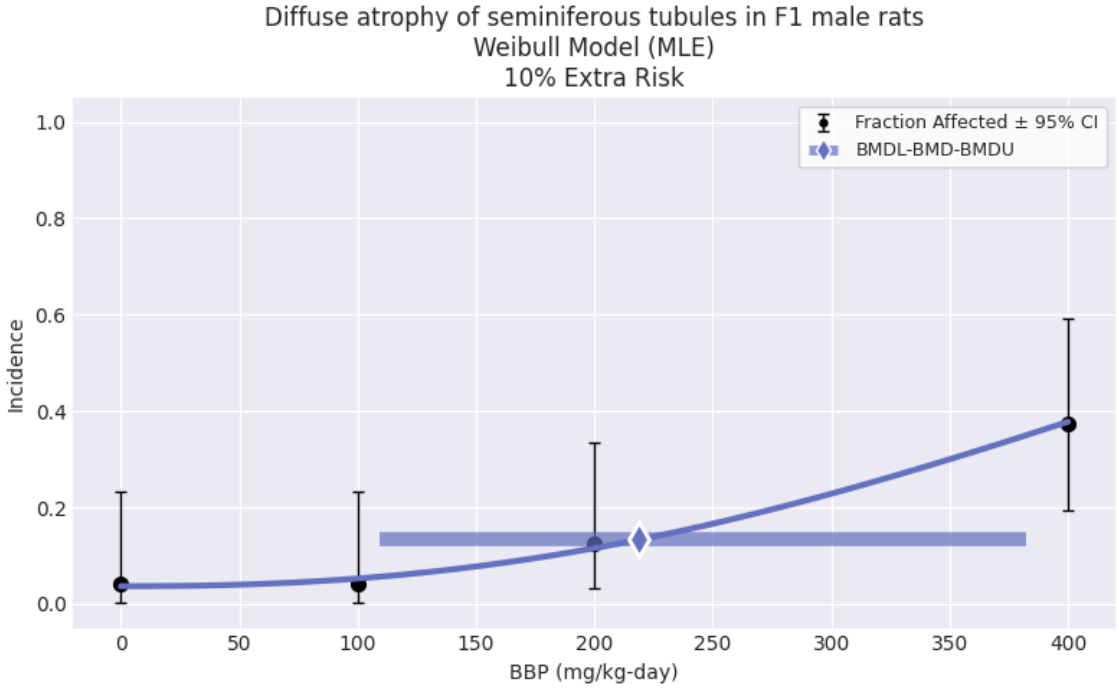
Setting	Value
BMR	10% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

Maximum Likelihood Approach

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	112.91	203.392	379.27	-	74.468	<0.001	<0.001	**Questionable** Zero degrees of freedom; saturated model
Gamma	110.33	215.956	352.296	0.802	72.532	0.11	0.114	**Viable**
LogLogistic	111.281	216.093	380.257	0.784	72.545	0.123	0.119	**Viable**
Multistage 1	81.645	131.784	264.16	0.32	72.951	0.464	-0.968	**Viable**
Multistage 2	107.007	199.84	284.469	0.909	70.665	0.255	-0.063	**Viable**
Multistage 3	107.924	219.147	315.39	0.945	70.585	0.141	0.15	**Viable**
Weibull[a]	109.486	218.617	382.417	0.953	70.567	0.129	0.144	**Recommended - Lowest AIC**
Logistic	161.046	209.45	271.948	0.872	70.748	0.334	0.083	**Viable**
LogProbit	113.629	212.356	389.05	0.867	72.496	0.086	0.063	**Viable**
Probit	149.064	195.281	261.984	0.821	70.854	0.455	-0.043	**Viable**
Quantal Linear	81.646	131.784	264.16	0.32	72.951	0.464	-0.968	**Viable**

[a] BMDS recommended best fitting model


Diffuse atrophy of seminiferous tubules in F1 male rats
MLE Models
10% Extra Risk
Fraction Affected ± 95% CI
Hill
Gamma
LogLogistic
Multistage 1
Multistage 2
Multistage 3
Weibull
Logistic
LogProbit
Probit
Quantal Linear
Incidence
BBP (mg/kg-day)


Diffuse atrophy of seminiferous tubules in F1 male rats
Weibull Model (MLE)
10% Extra Risk
Fraction Affected ± 95% CI
BMDL-BMD-BMDU
Incidence
BBP (mg/kg-day)

Weibull Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

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

Parameter Settings:

Parameter	Initial	Min	Max
g	0	-18	18
a	1	1	18
b	1e-06	1e-06	100

Modeling Summary:

BMD	218.617
BMDL	109.486
BMDU	382.417
AIC	70.5673
Log-Likelihood	-33.2836
P-Value	0.952925
Overall d.f.	2
Chi²	0.0964378

Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.0367089	no	0.0581535
a	2.35626	no	1.26532
b	3.23428e-07	yes	Not Reported

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	Size	Observed	Expected	Est Prob	Scaled Residual
0	24	1	0.881014	0.0367089	0.129159
100	24	1	1.26353	0.052647	-0.240867
200	24	3	2.77402	0.115584	0.144276
400	24	9	9.07217	0.378007	-0.0303809

Analysis of Deviance:

Model	Log-Likelihood	# Params	Deviance	Test d.f.	P-Value
Full model	-33.2339	4	-	-	-
Fitted model	-33.2836	2	0.0995556	2	0.951441
Reduced model	-39.8796	1	13.2916	3	0.00404664

G.3.2 BMD Model Results for BMR of 5%

Dataset

Name: Diffuse atrophy of seminiferous tubules in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	24	1
100	24	1
200	24	3
400	24	9

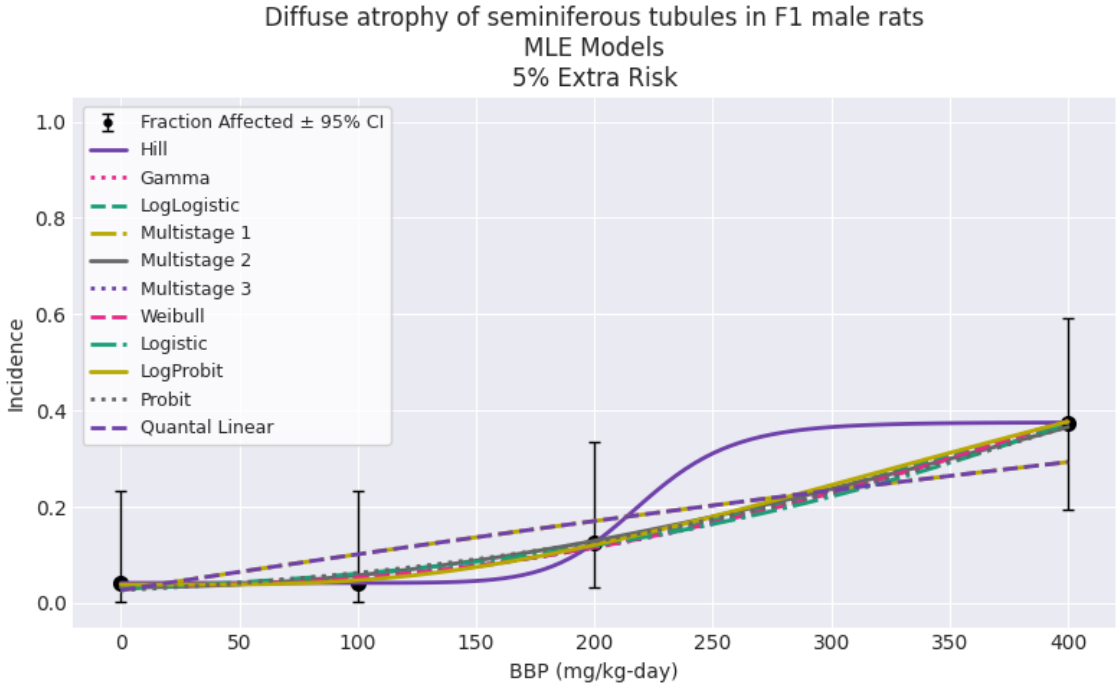
Settings

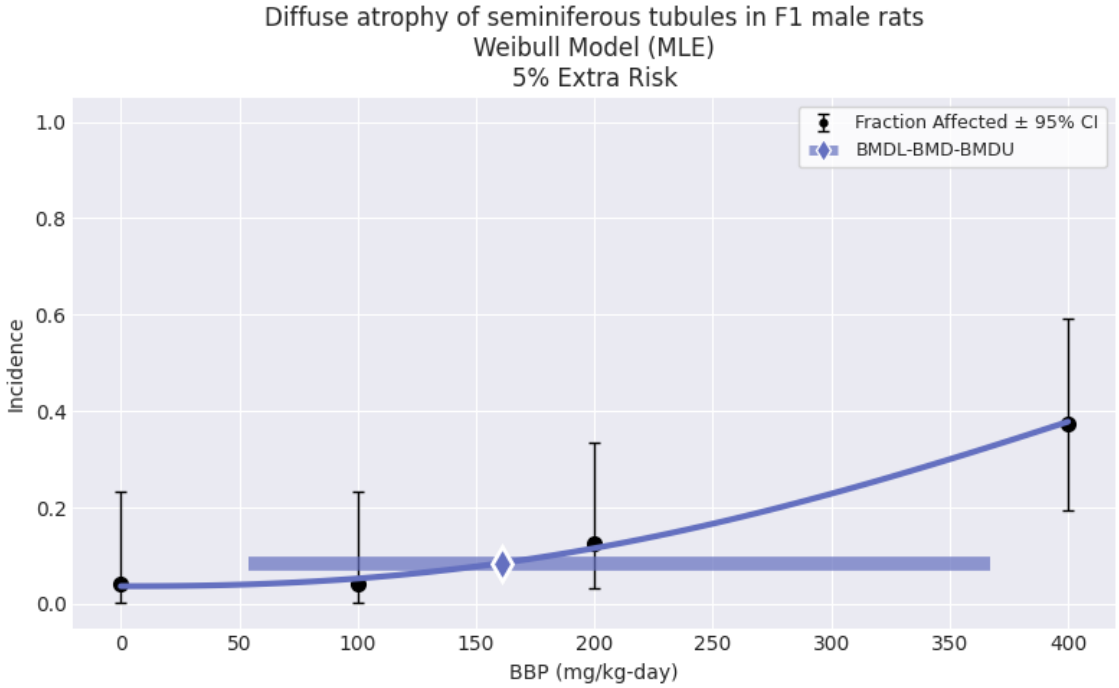
Setting	Value
BMR	5% Extra Risk
Confidence Level (one sided)	0.95
Maximum Multistage Degree	3

Maximum Likelihood Approach

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual at Control	Scaled Residual near BMD	Recommendation and Notes
Hill	58.556	188.257	203.825	-	74.468	<0.001	<0.001	**Questionable** Zero degrees of freedom; saturated model BMD/BMDL ratio > 3.0
Gamma	54.197	163.07	319.696	0.802	72.532	0.11	0.114	**Viable** BMD/BMDL ratio > 3.0
LogLogistic	57.083	161.977	365.023	0.784	72.545	0.123	0.119	**Viable**
Multistage 1	39.747	64.157	128.603	0.32	72.951	0.464	-0.968	**Viable**
Multistage 2	52.395	139.435	198.484	0.909	70.665	0.255	-0.335	**Viable**
Multistage 3	52.817	159.51	248.11	0.945	70.585	0.141	0.15	**Viable** BMD/BMDL ratio > 3.0
Weibull[a]	53.676	161.068	366.879	0.953	70.567	0.129	0.144	**Recommended - Lowest AIC** BMD/BMDL ratio > 3.0
Logistic	97.272	137.043	190.474	0.872	70.748	0.334	-0.393	**Viable**
LogProbit	64.239	164.552	382.861	0.867	72.496	0.086	0.063	**Viable**
Probit	88.388	124.398	174.137	0.821	70.854	0.455	-0.422	**Viable**
Quantal Linear	39.748	64.157	128.603	0.32	72.951	0.464	-0.968	**Viable**

[a] BMDS recommended best fitting model


Diffuse atrophy of seminiferous tubules in F1 male rats
MLE Models
5% Extra Risk
Fraction Affected ± 95% CI
Hill
Gamma
LogLogistic
Multistage 1
Multistage 2
Multistage 3
Weibull
Logistic
LogProbit
Probit
Quantal Linear
Incidence
BBP (mg/kg-day)


Diffuse atrophy of seminiferous tubules in F1 male rats
Weibull Model (MLE)
5% Extra Risk
Fraction Affected ± 95% CI
BMDL-BMD-BMDU
Incidence
BBP (mg/kg-day)

Weibull Model

Version: pybmds 25.1 (bmdscore 25.1)

Input Summary:

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

Parameter Settings:

Parameter	Initial	Min	Max
g	0	-18	18
a	1	1	18
b	1e-06	1e-06	100

Modeling Summary:

BMD	161.068
BMDL	53.676
BMDU	366.879
AIC	70.5673
Log-Likelihood	-33.2836
P-Value	0.952925
Overall d.f.	2
Chi²	0.0964378

Model Parameters:

Variable	Estimate	On Bound	Std Error
g	0.0367089	no	0.0581535
a	2.35626	no	1.26532
b	3.23428e-07	yes	Not Reported

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	Size	Observed	Expected	Est Prob	Scaled Residual
0	24	1	0.881014	0.0367089	0.129159
100	24	1	1.26353	0.052647	-0.240867
200	24	3	2.77402	0.115584	0.144276
400	24	9	9.07217	0.378007	-0.0303809

Analysis of Deviance:

Model	Log-Likelihood	# Params	Deviance	Test d.f.	P-Value
Full model	-33.2339	4	-	-	-
Fitted model	-33.2836	2	0.0995556	2	0.951441
Reduced model	-39.8796	1	13.2916	3	0.00404664

G.4 BMD Model Results – Incidence of Seminiferous Tubule Atrophy (Bayesian Model Averaging)

G.4.1 BMD Model Results for BMR of 10%

Dataset

Name: Diffuse atrophy of seminiferous tubules in F1 male rats

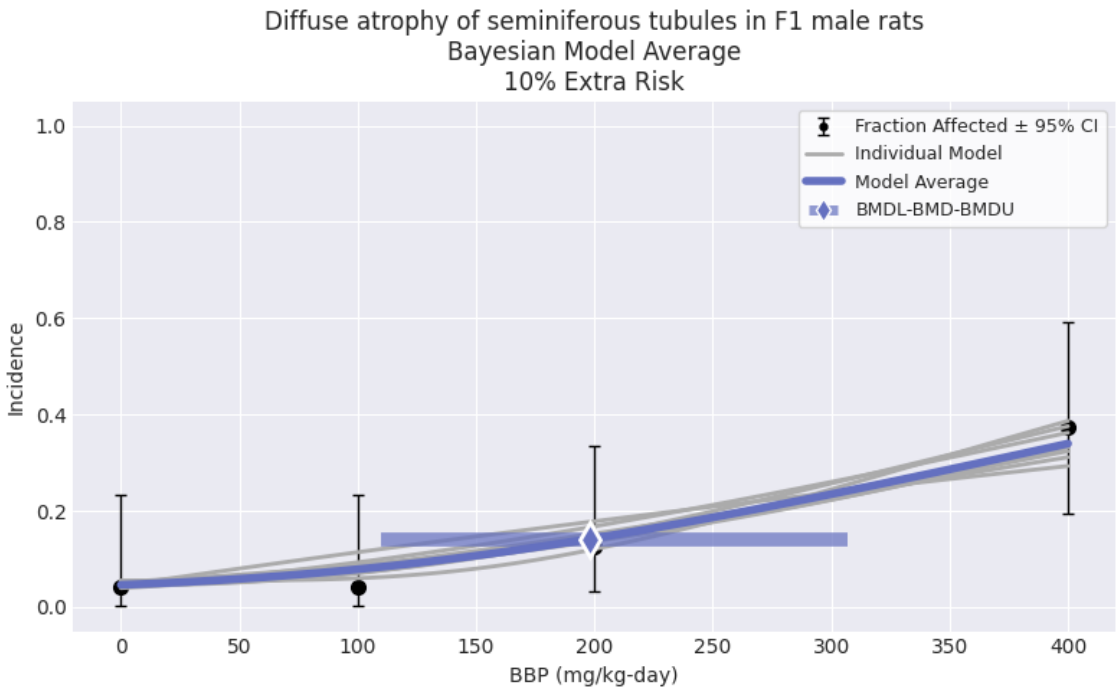
Dose (mg/kg-day)	N	Incidence
0	24	1
100	24	1
200	24	3
400	24	9

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	0.134	111.326	198.122	310.533	-44.044	-0.076	-0.239
Gamma	0.111	0.076	111.191	188.346	327.325	-32.075	-0.162	-0.375
Logistic	0.111	0.108	156.106	207.214	360.312	-33.354	-0.229	-0.238
LogLogistic	0.111	0.138	113.868	195.058	281.879	-39.4	-0.118	-0.302
LogProbit	0.111	0.109	147.436	227.154	320.226	-38.006	-0.289	0.072
Multistage 2	0.111	0.009	105.008	163.188	238.698	-35.892	-0.007	-0.56
Probit	0.111	0.269	149	198.227	302.851	-30.109	0.062	-0.165
Quantal Linear	0.111	0.081	85.909	139.945	276.782	-31.009	-0.068	-1.113
Weibull	0.111	0.076	105.257	197.585	321.601	-37.489	-0.172	-0.293
Model Average	-	-	109.781	197.766	306.789	-	-	-


Diffuse atrophy of seminiferous tubules in F1 male rats
Bayesian Model Average
10% Extra Risk
Fraction Affected ± 95% CI
Individual Model
Model Average
BMDL-BMD-BMDU
Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
400
BBP (mg/kg-day)

G.4.2 BMD Model Results for BMR of 5%

Dataset

Name: Diffuse atrophy of seminiferous tubules in F1 male rats

Dose (mg/kg-day)	N	Incidence
0	24	1
100	24	1
200	24	3
400	24	9

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	0.134	59.152	137.245	234.606	-44.044	-0.076	-0.55
Gamma	0.111	0.076	57.389	118.395	215.004	-32.075	-0.162	-0.767
Logistic	0.111	0.108	91.426	126.722	205.31	-33.354	-0.229	-0.788
LogLogistic	0.111	0.138	62.844	138.169	226.381	-39.4	-0.118	-0.56
LogProbit	0.111	0.109	101.923	180.711	278.306	-38.006	-0.289	0.072
Multistage 2	0.111	0.009	54.104	95.197	148.837	-35.892	-0.007	-0.864
Probit	0.111	0.269	86.745	121.079	175.608	-30.109	0.062	-0.644
Quantal Linear	0.111	0.081	41.824	68.13	134.748	-31.009	-0.068	-1.113
Weibull	0.111	0.076	48.244	128.234	228.392	-37.489	-0.172	-0.705
Model Average	-	-	54.865	126.362	225.801	-	-	-


Diffuse atrophy of seminiferous tubules in F1 male rats
Bayesian Model Average
5% Extra Risk
Fraction Affected ± 95% CI
Individual Model
Model Average
BMDL-BMD-BMDU
Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
400
BBP (mg/kg-day)