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Non-cancer Human Health Hazard Assessment for Diethylhexyl Phthalate (DEHP)

Technical Support Document for the Risk Evaluation

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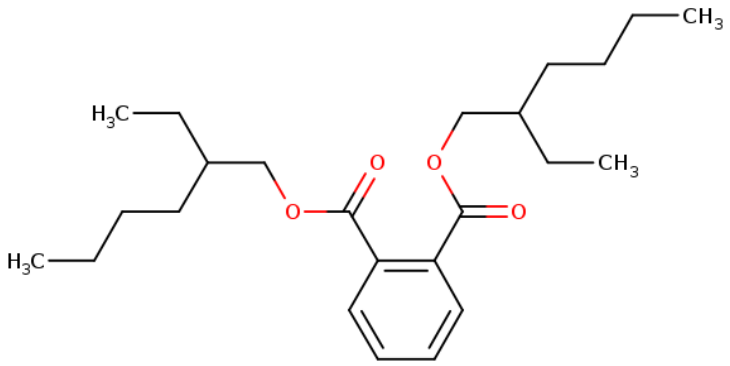

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KEY ABBREVIATIONS AND ACRONYMS

2-EH	2-Ethylhexanol
ACE	Angiotensin Converting Enzyme
ACEI	Angiotensin Converting Enzyme Inhibitor
ACR	Albumin-creatine ratio
ADME	Absorption, distribution, metabolism, and excretion
AGD	Anogenital distance
BK2R	Bradykinin B2 Receptor
BMD	Benchmark dose
BMDL	Benchmark dose lower bound
BMR	Benchmark response
BSID	Bayley Score for Infant Development
CASRN	Chemical abstracts service registry number
C_{max}	Maximum Concentration in nmol
CPSC	Consumer Product Safety Commission (U.S.)
CVD	Cardiovascular disease
DEHP	Diethylhexyl Phthalate
DBP	Dibutyl Phthalate
ECHA	European Chemicals Agency
eNOS	Endothelial Nitric Oxide Synthase
EPA	Environmental Protection Agency (U.S.)
FLC	Fetal Leydig Cell
FSH	Follicle stimulating hormone
GD	Gestational day
HEC	Human equivalent concentration
HED	Human equivalent dose
HOMA-IR	Homeostatic model assessment of insulin resistance
ICSI	Intracytoplasmic Sperm Injection
IUGR	Intrauterine Growth Retardation
IVF	*In vitro* Fertilization
LD	Lactation Day
LH	Luteinizing Hormone
LABC	Levator Ani plus Bulbocavernosus muscles
LOAEC	Lowest-observable-adverse-effect concentration
LOAEL	Lowest-observable-adverse-effect level
LOEL	Lowest-observable-effect level
MOA	Mode of action
MEHP	Mono-2-ethylhexyl phthalate
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
NASEM	National Academies of Sciences, Engineering, and Medicine
NHSII	Nurses' Health Study II
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NMDR	Non-monotonic dose response
NOAEC	No-observed-adverse-effect concentration
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPT	Office of Pollution Prevention and Toxics
PBPK	Physiologically based pharmacokinetic

PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal day
POD	Point of departure
SACC	Science Advisory Committee on Chemicals
SD	Sprague-Dawley (rat)
SHBG	Sex Hormone-Binding Globulin (nmol/mL or nmol/L)
SMI	Skeletal muscle index
TSCA	Toxic Substances Control Act
UF	Uncertainty factor
U.S.	United States
WISC	Wechsler Intelligence Scale for Children

SUMMARY

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

EPA identified developmental/reproductive toxicity as the most appropriate non-cancer hazard associated with oral exposure to DEHP in experimental animal models for use in human health risk assessment (Section 3.1). Existing assessments of DEHP—including by the Agency for Toxic Substances and Disease Registry (ATSDR, 2022), the U.S. Consumer Product Safety Commission (CPSC, 2014), Environment and Climate Change Canada/Health Canada (Health Canada, 2020), the European Chemicals Agency (ECHA, 2017a), and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2010)—also consistently identified developmental/reproductive toxicity as a sensitive and robust non-cancer effect following oral exposure to DEHP. In 2022, ATSDR also identified effects on the developing female reproductive tract and effects on glucose homeostasis following oral exposure, along with developmental/ reproductive toxicity following inhalation exposure.

EPA has selected a point of departure (POD) of 4.8 mg/kg-day (human equivalent dose [HED] of 1.1 mg/kg-day) to estimate non-cancer risks from oral exposure to DEHP for acute, intermediate, and chronic durations of exposure in the risk evaluation of DEHP. The selected POD is a no-observed-adverse-effect level (NOAEL) in rats and is further supported by three publications by Andrade and Grande (2006c; 2006a; 2006), which established a NOAEL of 5 mg/kg-day and 13 additional studies reporting effects on the developing male reproductive system consistent with disrupted androgen action and phthalate syndrome at LOAELs in a narrow range of 10 to 15 mg/kg-day.

The Agency has performed ¾-body weight scaling to yield the HED and is applying the animal to human uncertainty factor (*i.e.*, interspecies uncertainty factor; UF_A) of 3× and the within human variability uncertainty factor an (*i.e.*, intraspecies uncertainty factor; UF_H) of 10×. Thus, a total UF of 30× is applied for use as the benchmark MOE. Overall, based on the strengths, limitations, and uncertainties discussed in Section 4.3, *EPA has robust overall confidence in the selected POD based on effects on the developing male reproductive system. This POD will be used to characterize risk from exposure to DEHP for acute, intermediate, and chronic exposure scenarios.* For purposes of assessing non-cancer risks, the selected POD is considered most applicable to women of reproductive age, pregnant women, and infants. The selected POD is expected to be protective of other endpoints relevant to other age groups (*e.g.*, older children, adult males, and the elderly).

No reasonably available data were available for the dermal route that were suitable for deriving route-specific PODs. Therefore, EPA used the acute/intermediate/chronic oral POD to evaluate risks from dermal exposure to DEHP. Differences between oral and dermal absorption will be accounted for in dermal exposure estimates in the risk evaluation for DEHP. Although inhalation studies were available, EPA did not consider any of these studies to be suitable for quantitative derivation of a route-specific POD (see Section 3.8 for more detail). 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). The oral HED and inhalation HEC values selected by EPA to estimate non-cancer risk from acute/intermediate/ chronic exposure to DEHP in the risk evaluation of DEHP are summarized in Table ES-1 and Section 6.

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

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

Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HED[a] (mg/kg-day)	HEC (mg/m^3) [ppm]	Benchmark MOE	Reference(s) (TSCA Study Quality Rating)
Development /Reproductive	Rat	Continuous exposure for 3 generations	NOAEL = 4.8	↑total reproductive tract malformations in F1 and F2 males at 14 mg/kg-d	1.1	6.2 [0.39]	UF_A= 3 UF_H=10 *Total UF=30*	(Blystone et al., 2010; TherImmune Research Corporation, 2004) (High)

Abbreviations: POD = Point of Departure; HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; UF = uncertainty factor.

[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. EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations.

1 INTRODUCTION

In December 2019, the United States Environmental Protection Agency (EPA or the Agency) designated di-ethylhexyl phthalate (DEHP) 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) (U.S. EPA, 2019). EPA published the draft and final scope documents for DEHP in 2020 (U.S. EPA, 2020a, b). Following publication of the final scope document, one of the next steps in the TSCA risk evaluation process is to identify and characterize the human health hazards of DEHP and conduct a dose-response assessment to determine the toxicity values to be used to estimate risks from DEHP exposures. This technical support document for DEHP summarizes the non-cancer hazards associated with exposure to DEHP and summarizes the non-cancer toxicity values to be used to estimate risks from DEHP exposures. Cancer human health hazards associated with exposure to DEHP are summarized in EPA's *Cancer Human Health Hazard Assessment for Di-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 DEHP have been reviewed by several regulatory and authoritative agencies, including the U.S. Consumer Product Safety Commission (U.S. CPSC); U.S. Agency for Toxic Substances and Disease Registry (ATSDR); U.S. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR); National Academies of Sciences, Engineering, and Medicine (NASEM); Environment and Climate Change Canada/Health Canada (ECCC/HC); European Chemicals Bureau (ECB); European Chemicals Agency (ECHA); European Food Safety Authority (EFSA); and Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS). EPA relied on information published in existing assessments by these regulatory and authoritative agencies as a starting point for its human health hazard assessment of DEHP. EPA's approach and methodology for identifying and using human epidemiologic data and experimental laboratory animal data is described in Sections 1.1 and 1.2, respectively, as well as in the *Systematic Review Protocol for Diethylhexyl) Phthalate (DEHP)* (U.S. EPA, 2025p).

1.1 Human Epidemiologic Data: Approach and Conclusions

To identify and integrate human epidemiologic data into the *Risk Evaluation for Diethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025n), EPA first reviewed existing assessments of DEHP conducted by regulatory and authoritative agencies, as well as several systematic reviews of epidemiologic studies of DEHP published by researchers in U.S. EPA's Office of Research and Development, Center for Public Health and Environmental Assessment (CPHEA). Note: the CPHEA reviews do not reflect EPA policy. Existing assessments reviewed by EPA are listed below. As described further in Appendix A, most of these assessments have been subjected to peer review and/or public comment periods and have employed formal systematic review protocols:

- *Toxicological Profile for Di(2-Ethylhexyl)Phthalate (DEHP)* (ATSDR, 2022);
- *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters* (Health Canada, 2018b);
- *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for effects on behaviour and neurodevelopment, allergies, cardiovascular function, oxidative stress, breast cancer, obesity, and metabolic disorders* (Health Canada, 2018a);
- *Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence* (Radke et al., 2018);

- *Phthalate exposure and female reproductive and developmental outcomes: A systematic review of the human epidemiological evidence* (Radke et al., 2019b);
- *Phthalate exposure and metabolic effects: A systematic review of the human epidemiological evidence* (Radke et al., 2019a);
- *Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence* (Radke et al., 2020a); and
- *Application of Systematic Review Methods in an Overall Strategy for Evaluating Low-Dose Toxicity from Endocrine Active Chemicals* (NASEM, 2017).

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

EPA reviewed and summarized conclusions from previous assessments conducted by ATSDR (2022), 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 DEHP and specific health outcomes (Table 1-1). 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 1-1). Sections 3.1.1 and 3.1.1.2 (effects on the male and female Developmental and Reproductive Systems), Section 3.2.1 (Nutritional/Metabolic Effects on Glucose Homeostasis), Section 3.3.1 (Cardiovascular and Kidney Toxicity), Section 3.4.1 (Liver Toxicity), Section 3.5.1 (Neurotoxicity) Section 3.6.1 (Immunotoxicity) and Section 3.7.1 (Musculoskeletal Endpoints) provide further details on previous assessments of DEHP by ATSDR (2022), Health Canada (2018b), Radke et al. (2019b; 2018), and NASEM (2017), respectively, including conclusions related to exposure to DEHP and health outcomes. Conclusions of existing epidemiologic assessments were used to determine whether they would provide useful information for evaluating the exposure-response relationship of DEHP

Table 1-1. Summary of Scope and Methods Used in Previous Assessments to Evaluate the Association between DEHP and Health Outcomes

Previous Assessment	Outcomes Evaluated	Method Used for Study Quality Evaluation

ATSDR (2022)	• Body weight
	  ○ body mass index (BMI)
	  ○ waist circumference
	• Cardiovascular
	  ○ blood pressure
	• Hepatic
	  ○ serum lipids
	• Endocrine
	  ○ diabetes
	• Immunological
	  ○ allergy
	  ○ asthma
	• Neurological
	• Reproductive Effects
	• Developmental Effects
		Not Stated[1]

Health Canada (2018b)	**Hormonal effects:** • Sex hormone levels (*e.g.*, testosterone) **Growth & Development:** • Anogenital distance (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 • 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	ROBINS-I (Sterne et al., 2016)

[1] ATSDR provided a study inclusion criterion and a qualitative description of study evaluation, however, more formal data quality evaluation criteria were not described or provided.

Previous Assessment	Outcomes Evaluated	Method Used for Study Quality Evaluation
	• Time to pregnancy (Fecundity) • Preterm birth • Spontaneous abortion	
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; ROBINS-I= Risk of Bias in Non-randomized Studies of Interventions; OHAT = National Toxicology Program's Office of Health Assessment and Translation; GRADE = Grading of Recommendations, Assessment, Development and Evaluation.		

In the 2019 literature search, EPA identified and evaluated relevant studies using the systematic review process for epidemiology studies under TSCA. Further information (*i.e.*, data quality evaluations and data extractions) on the studies identified by EPA can be found in the *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Diethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025d) and *Data Extraction Information for Environmental Hazard and Human Health Hazard Animal Toxicology and Epidemiology for Diethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025c). To ensure thorough coverage of the important literature, recent assessments and systematic reviews of key health outcomes, when available, were utilized. ATSDR (2022) included literature up to June 2020; therefore, this represents the most recent available information and would have incorporated the studies identified by the EPA systematic review process under TSCA. Thus, EPA relied on ATSDR (2022) epidemiologic evaluations as a starting point in its evaluation of epidemiology studies, given that it provided the most robust and recent evaluation of human epidemiologic data for DEHP. Additionally, the Agency incorporated the work of other assessments such as those by Environment and Climate Change Canada/Health Canada (2018a, b), NASEM (2017) and EPA/CPHEA (Radke et al., 2020a; Radke et al., 2019b; Radke et al., 2019a; Radke et al., 2018). 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 DEHP metabolites and health outcomes. Similarly, publications by Radke et al. employed the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) in their study evaluation, while NASEM used the ROBIS, which is similar to the National Institute of Environmental Health Sciences (NIEHS) Office of Health Assessment and Translation (OHAT) method to evaluate epidemiologic study quality.

A thorough literature search was carried out by ATSDR (2022) to find epidemiological studies of DEHP and its metabolites. An extensive epidemiological database was developed through the literature search. As a result, for endpoints with a high number of epidemiological studies, a set of inclusion criteria was developed in order to focus the evaluation on studies that would be most helpful in identifying hazards. Only studies that satisfied the criteria, cited in Appendix B of ATSDR, were included in the Toxicological Profile. ATSDR (2022) concluded that the majority of the studies in the epidemiology database focus on the general public and their exposure to several phthalates or phthalate esters. However, DEHP has some of the same effects as other phthalates, in addition to having common urine metabolites (phthalic acid, for example, is a metabolite of various phthalate esters, such as butyl benzyl

phthalate and dibutyl phthalate). Therefore, definitive conclusions on cause and effect or dose-response for specific phthalate esters cannot be made based solely on human epidemiological research assessing potential negative effects from exposure to phthalates, such as DEHP. Thus, due to the number of issues mentioned, including incomplete dose-response data, exposure to various phthalate esters, lack of long-term exposure estimates, and unknown exposure route(s), human studies were not taken into consideration for Minimal Risk Levels (MRL) derivation.

As described further in the *Systematic Review Protocol for Di(2-ethylhexyl) Phthalate (DEHP)* (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 DEHP metabolites in matrices other than urine were considered supplemental and not evaluated for data quality.

The Agency is using epidemiologic studies of DEHP qualitatively. This approach is consistent with Health Canada, U.S. CPSC, ECHA, EFSA, and Australia NICNAS. Conclusions from ATSDR (2022), Environment and Climate Change Canada/ Health Canada (2018a, b), U.S. EPA systematic review articles (Radke et al., 2020a; Radke et al., 2019b; Radke et al., 2019a; Radke et al., 2018), and NASEM (2017) were reviewed by EPA and used as a starting point for its human health hazard assessment. The Agency did not use epidemiology studies quantitatively for dose-response assessment, primarily due to uncertainty associated with exposure characterization. Primary sources of uncertainty include the source(s) of exposure; timing of exposure assessment that may not be reflective of exposure during outcome measurements; and use of spot-urine samples, which due to rapid elimination kinetics may not be representative of average urinary concentrations that are collected over a longer term or calculated using pooled samples. Additionally, the majority of epidemiological studies examine one phthalate and one exposure period at a time such that they are treated as if they occur in isolation, which contributes additional uncertainty that may confound results for the majority of epidemiologic studies (Shin et al., 2019; Aylward et al., 2016).

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

The human health hazards of DEHP have been evaluated in existing assessments by U.S. EPA (1988), U.S. CPSC (2014, 2010a), ATSDR (2022); NTP-CERHR (2006); NASEM (2017), California OEHHA (2022), Environment and Climate Change Canada/ Health Canada (Health Canada, 2020; EC/HC,

2015); ECB (2008), ECHA (2017a, b, 2010), EFSA (2019, 2005), the Danish EPA (2011); and Australia NICNAS (NICNAS, 2010). The PODs used quantitively for risk characterization from these assessments are shown in Table 1-2.

With the exception of ATSDR (2022), these assessments have consistently identified the developing male reproductive tract as the most sensitive outcome for use in estimating human risk from exposure to DEHP and have identified the same endpoints and dose level. In 2010, Australia's NICNAS (2010) considered the no-observed-adverse-effect level (NOAEL) of 4.8 mg/kg-day from the three-generation reproduction study by TherImmune Research Corporation (2004) to be the most appropriate NOAEL to calculate risk estimates (*i.e.*, margins of exposure [MOE]) from reproductive toxicity to children and adults. In 2014, CPSC's Chronic Hazard Advisory Panel (CHAP) on phthalates considered this principal study along with several other developmental and reproductive studies with NOAELs ranging from 3 to 11 mg/kg-day (Blystone et al., 2010; Christiansen et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006; TherImmune Research Corporation, 2004) to determine a consensus NOAEL of 5 mg/kg-day as a recommendation to U.S. CPSC. U.S. CPSC selected NOAELs from antiandrogenic endpoints (*i.e.*, reproductive tract malformations, delayed vaginal opening, decreased spermatocytes and spermatids) across four studies (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006) and agreed with the consensus NOAEL for developmental toxicity of 5 mg/kg-day based on these effects on the developing male reproductive system for use in risk assessment (CPSC, 2014).

In 2017, ECHA calculated derived no effect levels (DNELs) using the NOAEL of 4.8 mg/kg-day from consideration of four co-critical studies (Christiansen et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004) (see Section B 4.2.1 of (ECHA, 2017a)). EFSA (2019) concurred with its prior opinion (EFSA, 2005) to derive a stand-alone tolerable daily intake (TDI) for DEHP based on the NOAEL from the study by TherImmune Research Corporation (2004) for reproductive and developmental toxicity (see Section 4.7.3 and Table 22 in Section 4.7.6 in EFSA (EFSA, 2019)).

Environment and Climate Change Canada/Health Canada derived age- and population-specific endpoints as part of its phthalate cumulative risk assessment (see Table F-6 of (Health Canada, 2020)) and selected a NOAEL of 4.8 mg/kg-day from the same five co-critical studies (Blystone et al., 2010; Christiansen et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004) to calculate hazard quotients for pregnant women, women of childbearing age, and infants. Additionally, Health Canada selected the NOAEL of 10 mg/kg-day based on decreased absolute and relative testis weight in rats exposed from PND5 to PND10 (Dostal et al., 1988) to calculate hazard quotients for children (prepubertal), although this endpoint was less sensitive than the increased incidences of reproductive tract malformations that Health Canada used to determine risk to pregnant women, women of childbearing age, and infants.

In summary, those five regulatory bodies identified the developing male reproductive tract as the most sensitive and robust outcome to use for human health risk assessment, and have consistently selected the same set of co-critical studies indicating a NOAEL of approximately 5 mg/kg-day and a lowest-observed-adverse-effect level (LOAEL) of approximately 15 mg/kg-day (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004), while several of these regulatory agencies also included the study by Christiansen et al. (2010), which had a similar NOAEL of 3 mg/kg-day and LOAEL of 10 mg/kg-day, but ultimately considered the NOAEL of 4.8 mg/kg-day from the three-generation reproduction study to be the most appropriate for POD selection (Blystone et al., 2010; TherImmune Research Corporation, 2004).

In 2022, ATSDR also identified potential hazards related to the developing female reproductive tract and glucose homeostasis following oral exposures. ATSDR derived a MRL for acute oral exposure of 3×10^{-3} mg/kg-day based on altered glucose homeostasis at the LOAEL of 1 mg/kg-day (Rajesh and Balasubramanian, 2014) and an MRL for intermediate duration oral exposure at 1×10^{-4} mg/kg-day based on delayed meiotic progression of germ cells in F1 female fetuses and accelerated folliculogenesis in F1 and F2 female offspring at the LOAEL of 0.04 mg/kg-day (Zhang et al., 2014). ATSDR also derived a MRL of 2×10^{-4} ppm for intermediate duration inhalation exposure based on reproductive effects observed at 0.3 ppm in inhalation studies in male rats (Kurahashi et al., 2005) and female rats (Ma et al., 2006).

Table 1-2. Summary of DEHP Non-cancer Oral PODs Selected for Use by Other Federal and International Regulatory Organizations

Brief Study Description	TSCA Data Quality	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	(ECHA, 2017a)	(EFSA, 2019)	(ATSDR, 2022)	(Health Canada, 2020)	(CPSC, 2014)	(NICNAS, 2010)
Sprague-Dawley (SD) rats; three-generation study of reproduction; 1.5, 10, 30, 100, 300, 1,000, 7,500, 10,000 ppm (0.1, 0.58, 1.7, 5.9, 17, 57, 447, 659 mg/kg-day) (Blystone et al., 2010; TherImmune Research Corporation, 2004)	High	4.8/14 (5.9/17 mean across 3 generations)	Significant ↑ total reproductive tract malformations in F1 & F2 males (testes, epididymis, seminal vesicles, prostate)	✓[a]	✓[b]		✓[e]	✓[g]	✓[h]
Wistar rats (6 dams/group); GD 9–21; oral/gavage; 0, 1, 10, or 100 mg/kg-day (Rajesh and Balasubramanian, 2014)	Medium	1.0 (LOAEL)	Altered glucose homeostasis in adult offspring (PND60) following fetal exposure			✓[c]			
CD-1 mice; GD 0.5–18.5; oral; 0 or 0.04 mg/kg-day (Zhang et al., 2014)	Low	0.04 (LOAEL)	Delayed meiotic progression of germ cells in GD 17.5 F_1 fetuses; accelerated folliculogenesis in F_1 & F_2 PND 21 offspring; ↓ E2 ♀			✓[d]			
Wistar rats; GD 6–21; oral/gavage; 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day (Andrade et al., 2006a)	Medium	5/15	Delayed preputial separation	✓[a]			✓[e]	✓[g]	
Wistar rats; GD to LD 21; oral/gavage; 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day (Andrade et al., 2006c)	Medium	5/15	↓ sperm production (19–25%); ↓ testis weight	✓[a]			✓[e]	✓[g]	
Wistar rats; GD 7 to LD 16; oral/gavage; 0, 10, 30, 100, 300, 600, 900 mg/kg-day (Christiansen et al., 2010)	High	3/10	↓ AGD, ↑ nipple retention	✓[a]			✓[e]		

Brief Study Description	TSCA Data Quality	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	(ECHA, 2017a)	(EFSA, 2019)	(ATSDR, 2022)	(Health Canada, 2020)	(CPSC, 2014)	(NICNAS, 2010)
Fischer 344 rats fed 0, 100, 500, 2,500, or 12,500 ppm DEHP in diet for up to 104 weeks (equivalent to 0, 5.8, 28.9, 146.6, and 789.0 mg/kg-day) (Dostal et al., 1988)	High	10/100	↓ testis weight				✓[f]		
Wistar rats; GD 6 to LD 21; oral/gavage; 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day (Grande et al., 2006)	Medium	5/15	Delayed vaginal opening					✓[g]	

↓ = statistically significant decrease; ↑ = statistically significant increase; AGD = anogenital distance; BW = body weight; F1 = first-generation offspring; F2 = second generation offspring; GD = gestation day; LD = lactational day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; PNW = postnatal week

[a] Four co-critical studies provided a NOAEL (Christiansen et al., 2010; 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004) used by ECHA to calculate derived no effect levels (DNELs) (see Section B 4.2.1 of (ECHA, 2017a)).

[b] EFSA (2019) concurred with its prior opinion (EFSA, 2005) to derive a stand-alone tolerable daily intake (TDI) for DEHP based on a NOAEL from TherImmune Research Corporation (2004) for reproductive and developmental toxicity (see Table 22 and Section 4.7.3 in (EFSA, 2019)).

[c] LOAEL used to derive an acute-duration oral MRL for developmental effects in the reproductive system of rats exposed to DEHP during gestation (see Appendix A, p. A-9–12 of (ATSDR, 2022)).

[d] LOAEL used to derive an intermediate-duration oral MRL for metabolic effects (see Appendix A, p. A-13–17 of (ATSDR, 2022). A chronic-duration oral MRL was not derived because the lowest identified candidate POD (*i.e.*, 5.8 mg/kg-day based on a NOAEL for decreased spermatogenesis at 28.9 mg/kg-day (LOAEL) in adult male rats exposed for 104 weeks (David et al., 2000)) was several orders of magnitude greater than the intermediate-duration MRL.

[e] Environment and Climate Change Canada/ Health Canada (ECCC/HC) selected a NOAEL of 4.8 mg/kg-day from 5 co-critical studies (Blystone et al., 2010; Christiansen et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004) to calculate hazard quotients for pregnant women and women of childbearing age and infants as part of its phthalate cumulative risk assessment (see Table F-6 of (Health Canada, 2020)).

[f] Health Canada selected a NOAEL of 10 mg/kg-day based on decreased absolute and relative testis weight in rats exposed from PND5 to PND10 (Dostal et al., 1988) to calculate hazard quotients for children (prepubertal) as part of its phthalate cumulative risk assessment (see Table F-6 of (Health Canada, 2020).

[g] NOAELs from antiandrogenic endpoints (*i.e.*, reproductive tract malformations, delayed vaginal opening, decreased spermatocytes and spermatids) across five studies (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006; TherImmune Research Corporation, 2004) were used by U.S. CPSC to assign a NOAEL for developmental toxicity of 5 mg/kg-day based on antiandrogenic endpoints (see Table 2.1 [p.24] and p. 205 of (CPSC, 2014)).

[h] NOAEL from (TherImmune Research Corporation, 2004) used by Australia's NICNAS to calculate MOEs for reproductive toxicity.

1.2.2 Approach to Identifying and Integrating Laboratory Animal Data

Figure 1-1 provides an overview of EPA's approach to identifying and integrating laboratory animal data into the DEHP risk evaluation. EPA first reviewed existing assessments of DEHP conducted by various regulatory and authoritative agencies. Existing assessments reviewed by the Agency are listed below. The purpose of this review was to identify sensitive and human relevant hazard outcomes associated with exposure to DEHP, and identify key studies used to establish PODs for estimating human risk. As described further in Appendix A, most of these assessments have been subjected to external peer review and/or public comment periods but have not employed formal systematic review protocols.

- *Toxicological Profile for Di(2-ethylhexyl)phthalate (DEHP)* (ATSDR, 2022);
- *Screening Assessment – Phthalate Substance Grouping* (Health Canada, 2020);
- *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);
- *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);
- *Application of Systematic Review Methods in an Overall Strategy for Evaluating Low-Dose Toxicity from Endocrine Active Chemicals* (NASEM, 2017);
- *Supporting Documentation: Carcinogenicity of Phthalates – Mode of Action and Human Relevance* (Health Canada, 2015);
- *State of the Science Report: Phthalate Substance Grouping: Medium-Chain Phthalate Esters: Chemical Abstracts Service Registry Numbers: 84-61-7; 84-64-0; 84-69-5; 523-31-9; 5334-09-8;16883-83-3; 27215-22-1; 27987-25-3; 68515-40-2; 71888-89-6* (EC/HC, 2015);
- *Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives (with Appendices)* (CPSC, 2014);
- *Technical Support Document for Cancer Potency Values, Appendix B: Chemical-Specific Summaries of the Information Used to Derive Unit Risk and Cancer Potency Values* (OEHHA, 2011);
- *Priority Existing Chemical Draft Assessment Report: Diethylhexyl Phthalate* (NICNAS, 2010);
- *European Union risk Assessment Report: Bis(2-ethylhexyl)phthalate (DEHP)* (ECJRC, 2008);
- *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of di(2-ethylhexyl) phthalate (DEHP)* (NTP-CERHR, 2006);
- *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials In Contact With Food (AFC) Related to Bis(2-ethylhexyl)phthalate (DEHP) for Use in Food Contact Materials* (EFSA, 2005);
- *Integrated Risk Information System (IRIS), Chemical Assessment Summary, di(2-ethylhexyl)phthalate (DEHP); CASRN 117-81-7* (U.S. EPA, 1988); and
- *Annex XV Restriction Report: Proposal for a Restriction, Version 2. Substance Name: bis(2-ehtylhexyl)phthlate (DEHP), Benzyl Butyl Phthalate (BBP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP)* (Danish EPA, 2011).

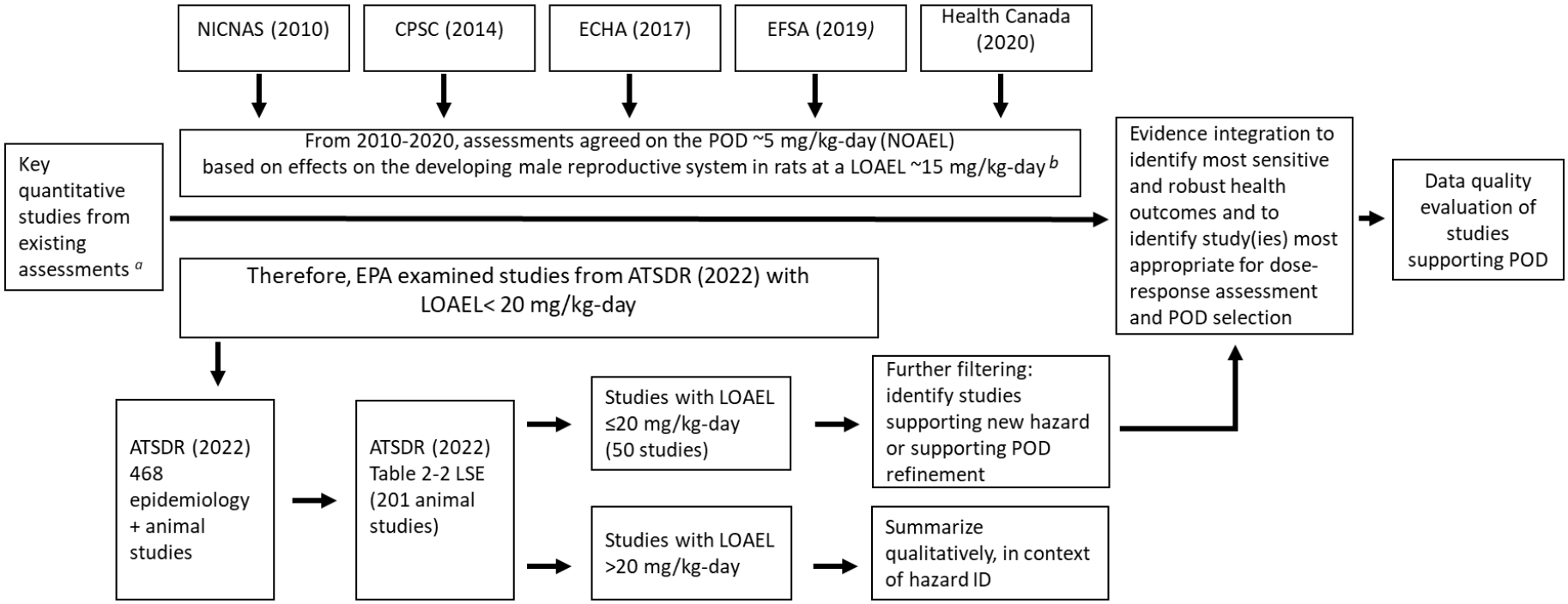


Figure 1-1. Overview of DEHP Human Health Hazard Assessment Approach

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

[b] POD based on NOAEL of 4.8 mg/kg-day and LOAEL of 14 mg/kg-day from three-generation reproductive study (Blystone et al., 2010; TherImmune Research Corporation, 2004) or co-critical with series of publications by Andrade and Grande et al. (2006c; 2006a; 2006) that established a NOAEL of 5 mg/kg-day and LOAEL of 15 mg/kg-day.

In developing the human health hazard assessment for DEHP, EPA conducted literature searches and updates at three different timepoints, including 2019, 2022 and 2025. The processes for identifying, including, synthesizing, and integrating the evidence from the literature search and updates are described further below.

EPA has used the ATSDR toxicological profile for DEHP (ATSDR, 2022) as a starting point for this non-cancer hazard assessment. Because ATSDR included literature through June 2020, and EPA's last literature search was conducted in 2019, the Agency considered the ATSDR assessment to be the most robust comprehensive assessment including the most the recent literature. The ATSDR assessment employed a systematic review process described in Appendix B.1 of the toxicological profile and included scientific literature up to June 2020 across 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, short-term, subchronic, and chronic) and routes of exposure (*i.e.*, oral, dermal, and inhalation).

ATSDR identified 468 studies regarding the health effects of DEHP, including epidemiology studies and animal toxicology studies. From among the animal toxicology studies, ATSDR developed selection criteria for studies considered for derivation of MRLs, and identified 201 animal toxicology studies, which are included as Levels of Significant Exposure (LSE) in Table 2-2 of the ATSDR toxicological profile (ATSDR, 2022). Briefly, ATSDR's selection criteria included (1) all chronic studies, primate studies, and study filling data gaps; (2) developmental and reproduction studies with at least one dose less than 100 mg/kg-day (given the extensive evidence base for developmental and reproductive toxicity at relatively low doses); and (3) studies with hazard other than developmental and reproductive toxicity with at least one dose less than 1,000 mg/kg-day; and (4) excluding studies with major design flaws and/or reporting deficiencies.

As described in Section 1.2.1, EPA surveyed the existing assessments of DEHP and found that the five national or international regulatory bodies that established hazard values for risk estimates prior to EPA's evaluation of DEHP (Health Canada, 2020; EFSA, 2019; ECHA, 2017a; CPSC, 2014; NICNAS, 2010) all consistently relied on the same suite of co-critical studies to select the NOAEL of approximately 5 mg/kg-day as the POD based on effects on the developing male reproductive tract at the LOAEL of approximately 15 mg/kg-day (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004). Given that all of the existing assessments prior to ATSDR selected the same POD for risk assessment—and the fact that ATSDR (2022) is the most recent comprehensive assessment of DEHP but identified other hazards (*e.g.*, effects on developing female reproductive system, glucose homeostasis, and inhalation hazards)—EPA focused on the 201 studies identified in ATSDR's Table 2-2 of LSEs to determine if any new hazards are identified or if there are more sensitive robust studies and endpoints appropriate for POD derivation for risk assessment compared to the POD identified in other existing assessments. Therefore, EPA considered the LOAEL of approximately 15 mg/kg-day from the prior existing assessments and decided to include all studies with effects (LOAEL) less than or equal to 20 mg/kg-day to identify sensitive studies and endpoints from ATSDR's LSE table.

Using this cut-off criterion of LOAEL less than or equal to 20 mg/kg-day, EPA identified a total of 50 animal toxicology studies from among the 201 studies in ATSDR's Table of LSE for further consideration in hazard identification and dose-response. All of the key studies used for derivation of PODs in existing assessments (presented in Table 1-2) are included among the 201 studies presented in ATSDR's LSE table. Importantly, with the exception of the study by Dostal et al. (1988), the studies presented in Table 1-2 were also included in the subset of 50 studies with LOAEL less than 20 mg/kg-

day selected by EPA for dose-response assessment. In the study by Dostal et al. (1988) treatment-related effects (on developing male reproductive tract) occurred at higher doses, with the LOAEL at 1,000 mg/kg-day and NOAEL at 100 mg/kg-day, well above the cut-off criterion for selecting studies with more sensitive endpoints.

For the DEHP 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.

In 2025, EPA updated the literature considered as part of the DEHP human health hazard assessment. As described further in the DEHP 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, included in this human health hazard assessment. Overall, EPA did not identify any studies that support selection of a lower POD for DEHP. EPA identified a dermal absorption study by Hopf et al. (2024) that reports dermal absorption rate of DEHP *in vitro* using human skin and *in vivo* with human subjects. This study is described in Section 2.1.2.

The principal and key studies identified by existing assessments were evaluated according to EPA's systematic review data quality evaluation criteria for TSCA, along with any study used quantitatively for derivation of the POD. Data quality evaluations for DEHP animal toxicity studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Diethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025p). Mechanistic studies and animal toxicology studies with LOAELs higher than 20 mg/kg-day 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 DEHP (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).

1.2.3 Scope of the DEHP Hazard Assessment

As described in Section 1.2.2, EPA further considered the 201 studies included in ATSDR's Table 2-2 of LSEs (ATSDR, 2022) to identify studies with sensitive endpoints (LOAEL <20 mg/kg-day) for information on human health hazards not previously identified in existing assessments— including information that may indicate a more sensitive POD than established by the regulatory bodies prior to the publication of ATSDR in 2022. One PECO-relevant study was identified based on the 2022 literature search, while no PECO-relevant studies were identified from the 2025 literature update. As described further in the *Systematic Review Protocol for Diethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025p), EPA identified 50 animal toxicology studies that provided information pertaining to hazard outcomes associated with exposure to less than or equal to 20 mg/kg/day, including: reproduction/development, metabolic/nutritional, cardiovascular/kidney, liver, neurological, immune, and musculoskeletal systems, in addition to hazards identified by the inhalation route. Further details regarding EPA's handling of this information are provided below.

- ***Reproductive/Developmental*.** EPA identified 25 studies evaluating reproductive/developmental outcomes that provided potentially sensitive LOAELs (Rajagopal et al., 2019b; Shao et al., 2019; Wang et al., 2017; Hsu et al., 2016; Zhang et al., 2014; Guo et al., 2013; Kitaoka et al., 2013; Li et al., 2012; Pocar et al., 2012; Blystone et al., 2010; Christiansen et al., 2010; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009b; Vo et al., 2009a; Lin et al., 2008; Ge et al., 2007; Andrade et

al., 2006b; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006; Akingbemi et al., 2004; TherImmune Research Corporation, 2004; Akingbemi et al., 2001; Ganning et al., 1990). These 25 studies of DEHP are discussed further in Section 3.1.

- ***Nutritional/metabolic.*** EPA identified 16 studies evaluating nutritional and/or metabolic outcomes (*e.g.*, effects on glucose and insulin homeostasis, lipid metabolism, etc.) that provided potentially sensitive LOAELs (Fan et al., 2020; Zhang et al., 2020b; Ding et al., 2019; Parsanathan et al., 2019; Rajagopal et al., 2019a, b; Li et al., 2018; Xu et al., 2018; Zhang et al., 2017; Gu et al., 2016; Venturelli et al., 2015; Mangala Priya et al., 2014; Rajesh and Balasubramanian, 2014; Rajesh et al., 2013; Schmidt et al., 2012; Lin et al., 2011b). These 16 studies of DEHP are discussed further in Section 3.1.3.
- ***Cardiovascular/Kidney.*** EPA identified four studies in animals that examined the effects of DEHP on the kidney and secondary effects on the cardiovascular system, such as changes in blood pressure, including three studies of mice (Deng et al., 2019; Xie et al., 2019; Kamijo et al., 2007) and one study of rats (Wei et al., 2012). These four studies of DEHP are discussed further in Section 3.3.
- ***Liver Toxicity.*** EPA identified 19 studies evaluating effects of DEHP on liver outcomes (*e.g.*, liver weight, histopathology, alterations in serum markers of liver toxicity, and peroxisome proliferation) in the subset of more sensitive studies (*i.e.*, LOAELs <20 mg/kg-day) subjected to detailed evaluation by EPA (Feng et al., 2020; Zhang et al., 2020b; Ding et al., 2019; Rajagopal et al., 2019a, b; Chiu et al., 2018; Li et al., 2018; Zhang et al., 2017; Pocar et al., 2012; Schmidt et al., 2012; Christiansen et al., 2010; Gray et al., 2009; Kamijo et al., 2007; Andrade et al., 2006c; Grande et al., 2006; Ma et al., 2006; TherImmune Research Corporation, 2004; Klimisch et al., 1992; Ganning et al., 1990). These 19 studies of DEHP are discussed further in Section 3.4.
- ***Neurological.*** Three neurotoxicity studies (Feng et al., 2020; Barakat et al., 2018; Tanida et al., 2009) were identified in the subset of more sensitive studies (*i.e.*, LOAELs less than or equal to 20 mg/kg-day). These three studies are discussed further in Section 3.5.
- ***Immune System.*** Three immunotoxicity studies (Han et al., 2014b; Guo et al., 2012; Yang et al., 2008) were identified in the subset of more sensitive studies (*i.e.*, LOAELs less than or equal to 20 mg/kg-day). These three studies are discussed further in Section 3.6.
- ***Musculoskeletal.*** EPA identified one study examining the effects of DEHP on musculoskeletal endpoints (Chiu et al., 2018) in ICR (CD-1) mice in the subset of more sensitive studies (*i.e.*, LOAELs less than or equal to 20 mg/kg-day). This study is discussed further in Section 3.7.
- ***Inhalation.*** EPA identified five studies (Larsen et al., 2007; Ma et al., 2006; Kurahashi et al., 2005; Klimisch et al., 1992; Merkle et al., 1988) that exposed laboratory animals to DEHP via the inhalation route, and these five studies are discussed further in Section 3.8.

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

2 TOXICOKINETICS

EPA has identified several human, primate, and rodent studies that characterized the absorption, distribution, metabolism, and excretion (ADME) properties of DEHP exposure that have been characterized in existing assessments (ATSDR, 2022; EFSA, 2019; CPSC, 2010b; NICNAS, 2010). These assessments reached similar conclusions regarding the toxicokinetic properties of DEHP. EPA has reviewed these assessments and through our systematic review, did find any additional studies that were not previously included.

2.1 Absorption

2.1.1 Oral and Inhalation Exposure Routes

EPA reviewed data from existing assessments on oral DEHP absorption which indicate absorption of 11 to 70 percent in humans and 30 to 78 percent in laboratory animals (ATSDR, 2022; EFSA, 2019). Similarly, NICNAS (2010) concluded that the extent of oral absorption in rats, non-human primates and humans has been estimated as 50 percent for doses up to 200 mg/kg-bw. Based on controlled oral exposure studies with human volunteers, the expectation is that greater than 70 percent of an oral dose of DEHP is absorbed (ATSDR, 2022; Kessler et al., 2012; Koch et al., 2005a). Other human studies reported lower oral absorption (11 to 47%); however, these studies have methodological limitations, such as analysis for a smaller number of urinary metabolites, use of non-radiolabeled DEHP, and lack of accounting for biliary excretion—all of which may underestimate absorption (Koch et al., 2004; Anderson et al., 2001; Schmid and Ch, 1985).

The inhalation route of exposure shows essentially complete absorption with approximately 98 percent of inhaled radiolabeled DEHP recovered in urine, feces, and tissues of male SD rats within 72 hours of inhalation exposure (ATSDR, 2022; General Motors, 1982).

2.1.2 Dermal Exposure Route

Dermal absorption (measured as percent absorption, permeability, or flux) was highly variable in animal studies, depending on several factors such as species, study design, and formulation, but primarily influenced by the loading dose, with percent dermal absorption inversely proportion to loading dose.

2.1.2.1 Study Summaries

In a study by Hopf et al. (2014), *in vitro* dermal absorption of neat DEHP, aqueous DEHP (166 µg/mL), or MEHP was tested using metabolically active viable human skin samples (1.77 cm^2 area) within 2 hours following surgical removal from abdominoplasty patients. Skin samples (n = 6) were dermatomed to 800 µm and dosed either with neat DEHP (2 mL), representing exposures among workers manufacturing DEHP, or 1.5 mL aqueous (emulsified in buffer solution) d4-DEHP (166 µg/mL) or MEHP (166 µg/mL), intended to represent exposure scenarios of aerosol deposition. Absorption of DEHP and the metabolite MEHP were measured in receptor fluid over the 24- or 72-hour exposure. The investigators reported that DEHP applied doses were calculated to be 1,114.1 mg/cm^2 for neat and 140.7 mg/cm^2 for emulsified DEHP. For aqueous DEHP, Kp was calculated to be 15.1×10^{-5} cm/hr, with a T_{lag} of 8 hours and a steady state flux at 0.025 µg/cm^2/hr. Neat DEHP had a longer T_{lag} of 30 hours and a lower Kp of 0.13×10^{-5} cm/hr and lower flux at 0.0013 µg/cm^2/hr. All of the absorbed DEHP was measured as MEHP (100% was metabolized). Tests with MEHP resulted in much higher permeability

(Kp = 436.1×10^{-5} cm/hr) and flux (0.724 µg/cm^2/hr), and human skin was further able to oxidize MEHP to 5-oxo-MEHP.

EPA also identified a subsequent dermal absorption study by Hopf et al. (2024) that reports fluxes of DEHP *in vitro* using human skin and *in vivo* with human subjects. For the *in vitro* studies, skin samples from abdominoplasty patients were dermatomed to a thickness of 800 µm and stored frozen (−20°C) until testing for dermal absorption, although storage duration was not reported. Dermal absorption of neat DEHP and aqueous emulsion with 1 percent (v/v) methanol were tested for 24 hours. Investigators only examined dermal absorption of metabolites of DEHP in the *in vitro* studies, even though the skin samples were previously frozen, and determined an *in vitro* dermal absorption rate of 0.0002±0.0001 µg/cm^2/hour for DEHP metabolites. For the *in vivo* experiments from Hopf et al. (2024), deuterated neat DEHP was applied to one forearm of human participants (n=5) at a rate of 10 µL/cm^2 to a 40 cm^2 area of one forearm for 6 hours. EPA estimated the dermal absorption rate from the cumulative urinary metabolites excreted over 30 hours and divided that mass by the surface area of application and the time, which resulted in an estimated dermal absorption rate of approximately 0.010 µg/cm^2/hour).

In a study by Chemical Manufacturers Association (1991), dermal absorption was evaluated in F344 rats following dermal application of a 15 cm^2 polyvinyl chloride (PVC) film containing 400 mg ^{14}C-DEHP (98.9% DEHP; 99.95% radiochemical purity)—equivalent to a 40.37 percent w/w film on shaved dorsal skin and adhered with a bandage of aluminum foil for 24 hours. For Experiment 1, the film was removed at the end of 24 hours, the skin was not washed, and the covering bandage was replaced, and absorption measured over 7 days. For Experiment II, the film was removed at the end of 24 hours, the skin washed, and the animals were immediately terminated. Urine and feces were collected at 12 hours, 24 hours, and daily thereafter. Investigators determined the amount of DEHP that was absorbed (in excreta + carcass), potentially absorbable (in skin at application site), and unabsorbed (skin wash). EPA focused its evaluation on the results of Experiment II, given that it reflects an exposure scenario in which an individual has contact with a solid containing DEHP for 24 hours and then washes. The results of Experiment II in this study indicated that only 0.126 percent total migrated out of the PVC film, with 0.0045 percent (17 µg DEHP) absorbed, 0.0183 percent potentially absorbable, and 0.1097 percent unabsorbed (and washed off), providing a mean absorption rate of 0.048 µg/cm^2/hour.

In a study by Barber (1992), full thickness rat skin and human stratum corneum were used to compare dermal absorption of undiluted neat DEHP between the species. Using Franz-type glass diffusion cells, skin samples from rat or human stratum corneum were placed over the donor side opening of the Franz cells. The diffusion cells were incubated with radiolabeled DEHP in the donor chamber for 32 hours. Permeability coefficient (Kp) in cm/hr and steady state flux (*e.g.*, absorption rate) in mg/cm^2/hr were calculated. Authors reported absorption rates of undiluted DEHP in rat and human stratum corneum at 0.42 µg/cm^2/hour and 0.10 µg/cm^2/hour, respectively, whereas the Kp is 4.31×10^{-7} cm/hour and 1.05×10^{-7} cm/hour in rats and humans, respectively; the resulting rat/human Kp ratio of 4.20 indicates that DEHP can penetrate full thickness rat skin 4 times more rapidly than in human stratum corneum. Tritiated water ($^3H_20$) was used to test skin integrity prior to and following tests with DEHP, and a damage ratio was calculated as the ratio of permeability to $^3H_20$ after treatment to that determined before testing with DEHP. Damage ratios indicated moderate damage to the rat skin (2.9, 6.9) with lower damage to human skin (2.6). Altogether, these data indicate that DEHP is more rapidly absorbed and results in higher damage to skin integrity in rat skin than in human stratum corneum.

In a similar *in vitro* study by Eastman Kodak (1989), the percutaneous absorption rates of DEHP through human stratum corneum and full thickness skin from Fischer 344 rats have been measured using Franz-type glass diffusion cells. Undiluted neat DEHP was put in the donor cell to expose skin samples

for a total of 32 hours, then the authors measured percutaneous absorption and determined absorption rates. Authors reported that the absorption of DEHP was found to be very slow for both species and followed a lag period of approximately 3 hours. As in the previous study, absorption through full thickness rat skin was found to be 4 times as fast as that through human stratum corneum. The absorption rates (mean ± SD) were determined to be 0.103 ± 0.020 µg/cm^2/hour in human stratum corneum compared to 0.418 ± 0.132 µg/cm^2/hour in rat skin after 32 hours of DEHP exposure. Additionally, undiluted DEHP led to moderate damage to human and rat skin after 32 hours. These data indicate that, while dermal absorption of DEHP is relatively slow, it is more rapidly absorbed through rat skin compared to human stratum corneum, which is observed in other dermal absorption studies (Deisinger et al., 1998; Elsisi et al., 1989).

A study by Sugino et al. (2017) measured skin permeation in full thickness abdominal skin from male hairless rats and abdominal skin (4 samples/individual) from two female Caucasians (aged 51 and 55 years) with undiluted neat DEHP for durations of 6 to 48 hours. To prepare skin from rats or humans for the experiments, they were tape stripped of the stratum corneum. Skin permeation was measured using side by side diffusion cells. Further, to examine whether esterase inhibition would affect skin permeation, a serine protease inhibitor, diisopropyl fluorophosphate was added to the receptor solutions in the diffusion cell. Authors measured esterase activity, DEHP skin permeation, concentrations, and metabolism in skin homogenates. They reported that neither DEHP nor MEHP, which were not metabolized by esterases, was transported through full thickness skin in rats or humans over the course of 48 hours. Tape stripping and esterase inhibition did not have an effect on skin permeation. Additionally, metabolism experiments indicate DEHP was not hydrolyzed to MEHP or phthalic acid in human or rat skin homogenates.

A study by Elsisi et al. (1989) investigated the absorption of phthalate diesters, including DEHP in male F344 rats, with DEHP at a loading dose of 5 to 8 mg/cm^2 (dissolved in 157 µmol/kg ethanol) applied to the rat's shaved back and left in place for 7 days. Results indicate that 6 percent of the applied dose was absorbed in the rats over the course of the 7 days, with 86 percent of the unabsorbed dose remained at the skin area of application 7 days following application.

Melnick et al. (1987) reported results of studies on DEHP conducted by the National Toxicology Program (NTP), including an *in vivo* study on dermal absorption of DEHP in male F344 rats. The fur was clipped from the middle of the back, and neat radiolabeled DEHP was dissolved in ethanol and applied to a 1.3 cm diameter area of the clipped skin at a dose of 30 mg/kg body weight. Excreta were collected at 24-hour intervals for 5 days. Total recovery was reported to be 105.8 percent, with 5.1 percent of the applied dose excreted in urine and feces, and a total of 1.8 percent remaining in tissues (primarily muscle tissue) after 5 days. The majority was not absorbed, with 86.74 percent remaining on the skin at the area of application, and an additional 12.16 percent on the plastic cap that was covering the application area.

In a dermal absorption study by Ng et al. (1992), female hairless guinea pigs had 13 µg/cm^2 DEHP (dissolved in 50 µl of acetone) applied to their dorsal skin, the estimated dermal absorption was approximately 53 percent of the applied dose after 24 hours. Further, to test percutaneous absorption *in vitro*, 200 µm sections of guinea pig skin, mounted on flow-through diffusion cells, were applied with 35.6, 153, or 313 nmol/cm^2 of radiolabeled DEHP (dissolved in 10 µl of acetone) and with or without (control group) esterase inhibitor, phenylmethylsulfonyl fluoride (174 mg/liter), and absorption of DEHP and metabolites were measured in receptor fluid in the diffusion cells for a total of 24 hours at 6-hour intervals, resulting in 6, 2.4, and 2.5 percent absorbed from lowest to highest dose, respectively (Ng et al., 1992). Results from the esterase inhibition study indicated that DEHP was metabolized to MEHP,

and the percent absorption of the total dose was 3.36 percent in the absence of an esterase inhibition compared to 2.67 percent of the dose in the presence of an esterase inhibitor at 24 hours (Ng et al., 1992). Further, the proportion of MEHP in the receptor fluid was decreased from 2.36 percent in the absence of an esterase inhibitor compared to 1.23 percent in the inhibitor treated group (Ng et al., 1992). These data suggest DEHP is absorbed into hairless guinea pig skin and metabolized to MEHP through esterases in the skin.

In another dermal absorption study (Chu et al., 1996), four female Hartley hairless guinea pigs were dermally exposed to 119, 107, 442, and 529 µg/cm^2 of DEHP (dissolved in acetone) applied to their dorsal region and sacrificed for skin harvesting at 6 hours, 24 hours, 7 days, and 14 days. Guinea pigs with a loading dose of 442 µg/cm^2 resulted in 19 percent of the applied dose dermally absorbed at 7 days post-treatment. The aim of the study was to see how the distribution of DEHP changes over time post-treatment and to examine the distribution of DEHP in the skin using autoradiographic analysis. The study applied DEHP at two application sites; one site was collected to determine the amount of DEHP that remained in the skin; the other site was used for autoradiographic analysis to determine the distribution of DEHP in the skin. Because the amount of DEHP that remained in the skin from the second application site was not taken into consideration for absorption measurements, the calculations of percent absorbed are not accurate. Therefore, data evaluation of this study through systematic review resulted in an overall quality determination of "uninformative."

In a study by Pan et al. (2014), *in vitro* dermal absorption of 5.4 mM DEHP was examined using full-thickness skin samples from pigs and nude mice and employed 40 percent ethanol in pH 7.4 buffer for both the donor chamber and receptor fluid. The investigators also conducted an *in vivo* study in nude mice by applying the same concentration of DEHP and diluent as the *in vitro* studies; however, dermal absorption was not measured in the *in vivo* study, which was conducted for physiological examination of the skin (transepidermal water loss, pH), immunohistology, and proteomic profiling. At the end of the 12-hour *in vitro* studies, DEHP was found in the skin of the mouse (12.0±4.82 nmol/mg) and pig (0.82±0.22 nmol/mg), with no DEHP absorbed into the receptor fluid. Additionally, examination of the hair follicles from *in vitro* studies in the nude mouse indicated follicular deposition of approximately 15 nmol DEHP per cm^2 skin.

Pelling et al. (1998) tested the *in vitro* dermal absorption of DEHP in rat skin, comparing two different receptor fluids, ethanol and phosphate-buffered saline (PBS) and two different skin sections (dermis and epidermis). Low dermal absorption of DEHP was observed with PBS as the receptor fluid, with less than 2 percent of the applied dose absorbed after one hour for both the epidermis and dermis sections. In contrast, when 50 percent ethanol was used as the receptor fluid, dermal absorption in the dermis was 5.6 percent of the applied dose, and absorption across the epidermis was considerably higher at 50.5 percent after one hour.

Scott et al. (1987) compared the *in vitro* dermal absorption of DEHP in rat and human skin after 72 hours, using 50 percent ethanol as the receptor fluid. Skin integrity was verified using tritiated water before and after the experiments. Steady state dermal absorption rate was higher in rat skin (2.24±0.23 µg/cm^2/hour) compared to human skin (1.06±0.23 µg/cm^2/hour), with a comparable lag time (3.1 to 3.9 hours).

2.1.2.2 Conclusions on the Selected Dermal Absorption Study

The Agency reviewed the dermal absorption studies of DEHP presented in Section 2.1.2.1 in order to select the most relevant and appropriate studies, parameters, and values to use in determining dermal

exposure in the occupational and consumer exposure assessments. EPA considered factors such as relevance of the test system, DEHP formulation, species, duration, loading dose, and whether the study was well conducted and had adequate reporting of data for use in risk assessment. EPA's rationale for the selection of the studies and parameters for use in risk assessment is described below.

EPA selected the study by Hopf et al. (2014) for determining dermal absorption of neat and aqueous DEHP because the study used metabolically-active human skin that was used within 2 hours of removal from patients undergoing abdominoplasty surgery, so that the skin retained esterase activity and metabolized DEHP to MEHP. Therefore, this study was considered to most closely approximate the dermal absorption of neat or aqueous DEHP in humans. A flux of 0.0013 µg/cm^2/hour was calculated for neat DEHP, and a flux of 0.025 µg/cm^2/hour was determined for aqueous DEHP. Data evaluation of this study through systematic review resulted in an overall quality determination of medium.

EPA also identified a subsequent dermal absorption study by Hopf et al. (2024) that reports fluxes of DEHP *in vitro* using human skin and *in vivo* with human subjects. *In vivo* experiments from Hopf et al. (2024) result in similar levels of estimated dermal uptake (approximately 0.010 µg/cm^2/hour) compared to *in vitro* results (0.025 µg/cm^2/hour) reported in metabolically active skin in the earlier study by Hopf et al. (2014); thereby adding to the weight of evidence supporting the selection of the dermal absorption rate from the *in vitro* studies using metabolically active human skin. EPA considered the *in vitro* data from Hopf et al. (2014) to have higher confidence than the value estimated from the *in vivo* study by Hopf et al. (2024) because the estimation from the *in vivo* study relies exclusively on the excreted DEHP and does not account for any DEHP that was absorbed but not excreted or DEHP that was excreted but was from other sources (*e.g.*, dietary exposure). Further, the *in vitro* experiments in the more recent study by Hopf et al. (2024) likely underestimate dermal absorption of DEHP because the investigators only measured for metabolites of DEHP but did not verify that the previously frozen skin samples were metabolically active, which is a reasonable explanation for the lower dermal absorption rate (0.0002 µg/cm^2/hour) noted in the more recent *in vitro* study by Hopf et al. (2024) compared to the previous *in vitro* study (0.0013 µg/cm^2/hour) using metabolically active human skin (Hopf et al., 2014).

The study by Hopf et al. (2014) was selected over consideration of other *in vitro* studies that were conducted using rat skin and human skin (Sugino et al., 2017; Eastman Kodak, 1989; Scott et al., 1987), *in vitro* studies using only human skin (Barber et al., 1992), rat skin (Pelling et al., 1998), or mouse and pig skin (Pan et al., 2014), *in vivo* dermal absorption studies in rodents (Chu et al., 1996; Elsisi et al., 1989), and a study comparing *in vitro* and *in vivo* dermal absorption in rodents (Ng et al., 1992). The studies by Barber et al. (1992) and Scott et al. (1987) had overall quality determinations of "medium"; however, these studies used skin from cadavers, instead of metabolically active skin, and these skin samples were immersed in a 60°C water bath to separate the epidermis and then refrozen until testing. Although the investigators tested the integrity of the skin samples prior to use, OECD 428 guidelines caution against using skin that has been refrozen (OECD, 2004). Furthermore, the variable storage conditions in the study by Barber et al. (1992) (4°C if used within 48 hours or −70°C if longer duration until testing) adds uncertainty to the resulting absorption measurements.

Similarly, the medium quality study by Eastman Kodak (1989) used human skin from donors from the National Donor Registry and immersed the skin in a 60 °C water bath to separate the stratum corneum for testing; and, in addition to the consideration of storage conditions of the skin prior to testing, this study had the limitation that the thickness and surface area of the skin samples were not reported; therefore, EPA could not calculate flux to use quantitatively for exposure assessment from these data.

While the studies by Elsisi et al. (1989), Melnick et al. (1987), and Ng et al. (1992) were *in vivo*, they were conducted on rodents as opposed to using human skin. The study by Elsisi et al. (1989) was generally well-conducted and received an overall data quality determination of "medium" via systematic review. However, the dermal flux value from this study was considered to have lower confidence than the value from the Hopf et al. (2014) study because EPA's calculation of a dermal flux value from this study (Elsisi et al., 1989) relied on multiplying the higher end of the loading dose range (8 mg/cm^2) by the fraction absorbed over the 7-day study and dividing by the 7-day duration to derive an average dermal flux.

Similarly, the dermal absorption study by Ng et al. (1992) on guinea pigs achieved an overall data quality determination of "medium" via systematic review. However, the wide discrepancy between the percent absorption observed in the *in vivo* study (53%) compared to the *in vitro* experiments (up to 6%) provided lower confidence compared to the flux values derived from the Hopf et al. (2014) study using metabolically active human skin.

The NTP study reported by Melnick et al. (1987) had an overall data quality determination of "low". Although it was reported that the rats were dermally dosed at 30 mg/kg body weight, the total mass (mg) and the body weights were not reported; therefore, the dose per surface area (mg/cm^2) could not be determined. Only three animals per group were tested, which fails to meet the minimum guideline requirement of four animals per group. Additional uncertainties were primarily due to reporting deficiencies in this peer-reviewed publication of the studies conducted by NTP.

Several dermal absorption studies were considered "uninformative" in their overall data quality determination due to substantial limitations and/or deficiencies. In the study by Sugino et al. (2017), the authors reported that neither DEHP nor MEHP was transported through full-thickness skin and that tape-stripping and esterase inhibition did not affect the skin permeation of DEHP. However, no quantitative data were reported regarding measurements of DEHP in the receptor fluid or skin samples from applications of DEHP. Similarly, the *in vivo* dermal absorption study by Chu et al. (1996) on guinea pigs for up to 14 days was uninformative because EPA was unable to accurately determine the fraction absorbed given that there were two applications. The study by Pan et al. (2014) did not conduct the standard tests to determine the validity, reliability, and quality of the experiments. Specifically: skin integrity was not tested prior to use; percent recovery was not determined; and inadequate data were provided in the results to demonstrate that the tests conformed to current standards or guidelines. Pelling et al. (1998) did not report doses or concentrations; only the volume applied, the specific activity, and the surface area of the skin sample were reported, resulting in a quality determination of "uninformative". Additionally in this study, skin integrity was only examined after 5 hours, and this study was conducted for 24 hours, so it is uncertain if the higher levels of DEHP in the ethanol receptor fluid was due to higher dermal absorption across the epidermal skin samples when this receptor fluid was used with DEHP, or if the ethanol compromised the skin integrity after durations longer than 5 hours.

For determining dermal absorption from contacts with solids containing DEHP, EPA used the study by Chemical Manufacturers Association (1991) because it was the only study identified that tested the dermal absorption of DEHP from a solid matrix (PVC film) and therefore provided relevant empirical data for these exposure scenarios, with a calculated flux of 0.025 µg/cm^2/hour. Data evaluation of this study through systematic review resulted in an overall quality determination of medium.

In conclusion, EPA used a flux value of 0.0013 µg/cm^2/hour for exposure scenarios involving neat DEHP and a flux of 0.025 µg/cm^2/hour for exposure scenarios involving aqueous DEHP, derived from

the study by Hopf et al. (2014). EPA used a flux of 0.048 µg/cm^2/hour for determining dermal absorption from contacts with solids containing DEHP, based on the study by Chemical Manufacturers Association (1991) See Section 2.4 of the *Environmental Release and Occupational Exposure Assessment for Diethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025f) and Section 2.3.1 of the *Consumer and Indoor Dust Exposure Assessment for Diethylhexyl Phthalate (DEHP*) (U.S. EPA, 2025b) for the description of the approach for determining dermal exposure from these values.

2.2 Distribution

Existing assessments did not identify any reliable studies that investigate the distribution of DEHP in humans following a quantified exposure. EPA also did not identify any such studies.

Numerous biomonitoring studies in humans have detected DEHP and its metabolites (MEHP, MEOHP, MEHHP, and MECPP) in human milk, including two studies in the United States (Hartle et al., 2018; Hines et al., 2009), one Canadian study (Zhu et al., 2006) and 10 studies from countries outside of North America (in Europe and Asia) (Kim et al., 2020; Kim et al., 2018; Guerranti et al., 2013; Zimmermann et al., 2012; Fromme et al., 2011; Lin et al., 2011a; Schlumpf et al., 2010; Latini et al., 2009; Hogberg et al., 2008; Main et al., 2006). However, these studies are not designed to identify exposure route or quantify relative distribution or speed of distribution into human milk. See the *Environmental Media and General Population and Environmental Exposure for Di-ethylhexyl Phthalate (DEHP)* for further details about concentrations of DEHP metabolites in human milk (U.S. EPA, 2025e). Animal studies show that lactating rats given an oral dose of DEHP (2,000 mg/kg) from lactational day (LD) 15 to 17 transferred DEHP and MEHP to their nursing pups via mammary milk (Dostal et al., 1987). Likewise, giving oral doses of DEHP (2,000 mg/kg) to nursing dams throughout the lactation period (LD 1 to 21), resulted in nursing pups with detectable levels of DEHP in their livers, suggesting DEHP in milk is bioavailable for oral absorption in nursing offspring (ATSDR, 2022; Parmar et al., 1985).

Additionally, DEHP has been detected in human adipose tissue in an autopsy, but it was potentially present due to possible contamination from plastics used in the handling and storage of tissues (Mes et al., 1974).

Reliable quantitative data are available from animal studies (*e.g.*, rodents, dogs, pigs, nonhuman primates) that characterize DEHP distribution (ATSDR, 2022; Kurata et al., 2012; Rhodes et al., 1986; General Motors, 1982; Ikeda et al., 1980; Tanaka et al., 1975). Following oral, intravenous, dermal, or inhalation routes of exposure in rats, DEHP was found in blood, liver, spleen, intestine, lungs, kidneys, heart, muscle, and adipose tissue within 4 hours, indicating that DEHP is quickly and widely distributed throughout the body, irrespective of route of exposure (General Motors, 1982). Specifically, for the inhalation route of exposure, male SD rats exposed to an aerosol (0.24 to 0.61 µm) of radiolabeled DEHP for 6 hours excreted 90 percent DEHP (50% in urine and 40% in feces) within 72 hours, with 7 percent remaining in the carcass, confirming systemic distribution and excretion following inhalation of DEHP (General Motors, 1982).

Tanaka et al. (1975) compared a time-course of distribution of radiolabeled DEHP following a single dose via oral (500 mg/kg) or intravenous (50 mg/kg) routes in rats, resulting in 53 percent dose in the liver, 20 percent in the spleen, 7.8 percent in the intestinal tissues and contents, 4.7 percent in the lungs, 3 percent in the kidneys, and 1.9 percent in the heart within 1 hour of intravenous administration. Following oral administration, the highest levels were noted 3 hours post-dosing, with 6.9 percent in the liver, 4.8 percent in the kidneys, 2.8 percent in the lungs, 2.4 percent in the spleen, 1.8 percent in the heart, and 1.2 percent in the muscle. Similarly, a time-course study of DEHP administered via

intravenous injection in SD rats showed distribution of radiolabeled chemical: to the lungs within 10 minutes; clearing from the lungs and distribution to the heart, liver, spleen, and proximal small intestine at one hour; and clearing from the lungs and liver and appearance of radiolabeled chemical throughout the intestinal tract at 24-hours post-injection (Wallin et al., 1974).

In a study by Rhodes et al. (1986), rats and marmosets were orally gavaged with 2,000 mg/kg of radiolabeled DEHP in single dose experiments and daily for 14 consecutive days. There were no substantive differences in the excretion profile following a single dose or multiple dose studies, indicating that repeated exposure did not alter the toxicokinetics of DEHP. However, the levels of DEHP or its metabolites in marmosets 24 hours after the 14th (final) dose were 10 to 20 percent of the levels in rats at the same time point, indicating lower absorption and distribution in marmosets, which the authors suggested was due to primates not hydrolyzing DEHP as readily as rodents.

In pregnant rats given an oral dose of radiolabeled DEHP (0–750 mg/kg-bw), radioactivity has been detected in the placenta, amniotic fluid, and fetal tissues (Clewell et al., 2010; Calafat et al., 2006; Stroheker et al., 2006; Singh et al., 1975).

Taken together, these studies indicate that regardless of exposure route, DEHP distributes rapidly (within 4 hours) and extensively systemically, and that maternal transfer of DEHP to offspring can occur through the placenta during gestation and through the milk during lactation.

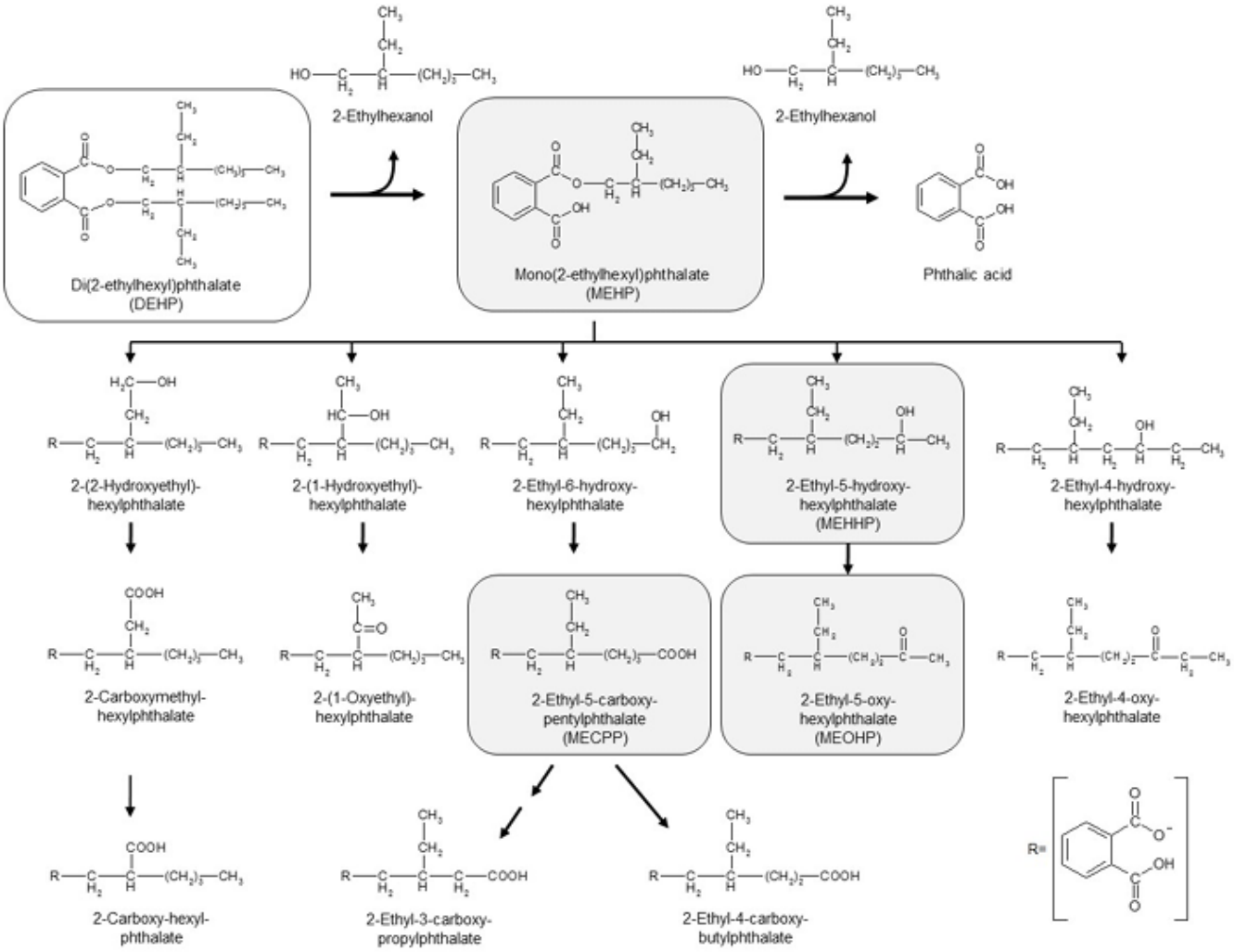
2.3 Metabolism

The metabolism of DEHP has been studied in humans, non-human primates, and rodents. Depiction of the metabolic pathway of DEHP is provided in Figure 2-1. The first step of metabolism for DEHP is a hydrolytic cleavage via hydrolases, including various carboxyesterases and lipases, into mono-2-ethylhexyl phthalate (MEHP) and 2-ethylhexanol (2-EH). DEHP hydrolase activity is present in multiple tissues throughout the body—including the liver, kidneys, lungs, skin, testes, and plasma—but is highest in the digestive system (White et al., 1980), where esterase activity in the pancreas and intestinal mucosa converts DEHP into MEHP (Barber et al., 1994; Rowland et al., 1977; Rowland, 1974). Additionally, based on data observing that most of DEHP is present in the plasma as MEHP after an oral dose, multiple studies suggest that DEHP is hydrolyzed during and following absorption in the digestive tract (Koo and Lee, 2007; Kessler et al., 2004). Hydrolysis of DEHP to MEHP is the rate-limiting step for absorption irrespective of route of exposure. Given that, and the fact that the majority of absorbed DEHP is present as MEHP, MEHP and other oxidative derivative monoester metabolites are expected to contribute to observed toxicity following exposure to DEHP. Subsequent steps of glucuronidation and excretion are expected to decrease toxicity.

The hydrolysis of DEHP on the second ester bond converts it from MEHP to phthalic acid (ATSDR, 2022). Following conversion to MEHP, the next step is ω- and ω-1 oxidation of MEHP via CYP2C1/2/19, then α or β oxidation via alcohol or aldehyde dehydrogenase into oxidized MEHP metabolites (Ito et al., 2005; Albro and Lavenhar, 1989).

Oxidized MEHP metabolites are then conjugated with glucuronic acid to form acyl glucuronides prior to urinary excretion. Primary metabolites of DEHP that are present in human urine are MEHP, MEHHP, MEOHP, MECPP, and the corresponding acyl-glucuronides (Zhao et al., 2018; Ito et al., 2014; Kurata et al., 2012; Anderson et al., 2011; Koch et al., 2005b; Koch et al., 2005a; Schmid and Ch, 1985; Albro et al., 1982). These urinary metabolites that are found in humans are observed in both monkey and rodent animal models, suggesting similar metabolism pathways for DEHP among mammalian species.

However, an oral administration study by Rhodes et al. (1986) indicated that marmosets have more extensive phase 2 conjugation than rats, with the majority of the metabolites in marmosets excreted in the urine in the conjugated forms, likely glucuronides. Additionally, biomonitoring studies in humans in the United States have detected in human milk a metabolite profile (MEHP, MEHHP, MEOHP, and MECPP) similar to the metabolites detected in urine (Hartle et al., 2018; Hines et al., 2009).



*Highlighted metabolites are measured in CDC's National Biomonitoring Program, (https://www.cdc.gov/biomonitoring/DEHP_BiomonitoringSummary.html).

Source: Adapted by permission from Macmillan Publishers Ltd: Lorber et al. (2010)

Figure 2-1. Metabolic Pathways for DEHP (Figure from ATSDR (2022))

2.4 Excretion

Excretion of DEHP and its metabolites have been studied in humans, monkeys, and rodents. Following oral exposure, metabolites of DEHP are excreted through urine, feces, respiration, and sweat (Genuis et al., 2012; Koch et al., 2005a; Koch et al., 2004; Anderson et al., 2001; Schmid and Ch, 1985; Lake et al., 1984; Daniel and Bratt, 1974). Studies with monkeys and rodents indicate that 30 to 50 percent of radiolabeled DEHP is excreted in urine within 24 to 168 hours following a single oral dose ranging from

85 to 2,000 mg/kg (Astill, 1989; Short et al., 1987; Astill et al., 1986; Rhodes et al., 1986; Lake et al., 1984; Daniel and Bratt, 1974).

DEHP has been detected in urine and feces following dermal exposure to radiolabeled DEHP ranging in duration from 24 to 168 hours (7 days) (Deisinger et al., 1998; Chu et al., 1996; Ng et al., 1992; Elsisi et al., 1989; Melnick et al., 1987). In humans, an estimated 11 to 74 percent of DEHP is excreted via urine between 8 and 24 hours (Koch et al., 2005a; Koch et al., 2004; Anderson et al., 2001; Schmid and Ch, 1985). Data on urinary excretion of DEHP and other phthalates is available from the U.S. Centers for Disease Control and Prevention's (CDC) National Health and Nutrition Examination Survey (NHANES) data set, which provides a relatively recent (data available from 2017 to 2018) and robust source of urinary biomonitoring data that is considered a national, statistically representative sample of the non-institutionalized, U.S. civilian population. Phthalates have elimination half-lives on the order of several hours and are quickly excreted from the body in urine and to some extent feces (ATSDR, 2022; EC/HC, 2015). Therefore, the presence of phthalate metabolites in NHANES urinary biomonitoring data indicates recent phthalate exposure. See the *Environmental Media and General Population and Environmental Exposure for Di-ethylhexyl Phthalate (DEHP)* for further details about concentrations of DEHP metabolites in human urine (U.S. EPA, 2025e).

Predominant pathways for excretion vary across species, route of exposure, and dose. Marmosets had a urinary:fecal excretion ratio of 2:1 after following an intravenous dose of 100 mg/kg of DEHP (Rhodes et al., 1986). Following an oral dose of DEHP (2.6 mg/kg), rats had a urinary:biliary ratio of 3:1 (Daniel and Bratt, 1974). Following dermal exposure to DEHP, rats had a urinary:fecal excretion ratio ranging from 1:1 to 3:1 (Deisinger et al., 1998); whereas hairless guinea pigs had ratios ranging from 4:1 to 5:1 ratio (Ng et al., 1992). Marmosets given a single oral dose of DEHP had ratios of urinary to fecal excretion ranging from 1:2 to 1:5 over a cumulative course of 7 days (Kurata et al., 2012).

Irrespective of pathway, the time course for excretion is fairly rapid, but is inversely proportional to dose. Following oral DEHP administration, the elimination half-life of DEHP and MEHP in blood, serum, and plasma is 2 to 4 hours in humans and marmosets (at an oral dose of 30 mg/kg) and 1.1 to 9.4 hours in rats (Kessler et al., 2012; Koo and Lee, 2007; Koch et al., 2005a; Kessler et al., 2004; Koch et al., 2004; Ljungvall et al., 2004; Oishi, 1990, 1989; Pollack et al., 1985; Sjöberg et al., 1985; Teirlynck and Belpaire, 1985). In rats, clearance of DEHP is decreased and the elimination half-life is increased with increasing dose of DEHP, including oral (4 to 2000 mg/kg) and intravenous (5 to 500 mg/kg) doses, indicating some degree of saturation of metabolism and excretion processes at higher doses (Koo and Lee, 2007; Oishi, 1990, 1989; Sjöberg et al., 1985). Overall, data indicate that DEHP is excreted within hours to days, primarily in the urine (⅔ to ¾), with biliary/feces excretion next (¼ to ⅓), and excretion via respiration and sweat accounting for much smaller proportion excreted. Furthermore, the data do not indicate substantive differences in the excretion pathways or time frame between rodents and humans.

2.5 Summary

The majority of data pertaining to the ADME properties of DEHP are from oral exposure studies in animals. ADME properties are qualitatively similar across species, particularly regarding phase 1 oxidation, although there are some quantitative differences in proportions of different metabolites. Data comparing marmosets to rats indicate that phase 2 conjugation (glucuronidation) may be more prominent in primates than in rodents, and repeated oral dosing of DEHP does not appear to influence the toxicokinetics (Rhodes et al., 1986). Regarding absorption, rat studies show greater than 98 percent absorption of DEHP following inhalation. In human intentional dose studies, at least 70 percent of the

oral dose was absorbed (Kessler et al., 2012; Koch et al., 2005a). In the animal studies described above, at least 30 percent of the oral dose of DEHP was absorbed. In contrast, for dermal exposure to DEHP, *in vitro* studies with human skin indicate that only 2 percent of the applied dose is absorbed through the skin, while rat studies indicate that approximately 6 percent is absorbed, and studies of hairless guinea pig studies indicate higher absorption at 19 to 50 percent of the dermal dose (ATSDR, 2022; Kessler et al., 2012; Koch et al., 2005a; Chu et al., 1996; Elsisi et al., 1989).

Regarding distribution, studies of rodents and monkeys orally and intravenously administered DEHP resulted in widespread distribution to the kidneys, liver, brain, spleen, adipose, lungs, and testes.

Regarding metabolism, DEHP is hydrolyzed in the gut to primary metabolites, such as MEHP, 2-EH, and phthalic acid (Koo and Lee, 2007; Kessler et al., 2004). MEHP is then oxidized by CYP2C1, CYP2C2, CYP2C19, and alcohol/aldehyde dehydrogenases to oxidized MEHP metabolites including MEHHP, 2-ethyl-5-oxyhexylphthalate; MEOHP, MECPP. Following oxidation, MEHP is conjugated with glucuronic acid and produces corresponding acyl-glucuronides, which vary in their respective amounts across species. DEHP is excreted primarily through urine and feces in humans, monkeys, and rodents, but also through inhalation and sweat, in addition to excretion via lactation (ATSDR, 2022; Genuis et al., 2012; NICNAS, 2010; Koch et al., 2005a; Koch et al., 2004; Anderson et al., 2001; Schmid and Ch, 1985; Daniel and Bratt, 1974). Metabolite excretion profiles observed in humans are similar to those that have been observed in other primates, in addition to rodents, although species differences in relative abundance of metabolites and glucuronide conjugates have been reported.

Given the toxicokinetic information available for DEHP, EPA is assuming an oral absorption of 100 percent and an inhalation absorption of 100 percent for the risk evaluation. In addition to absorption, DEHP showed a generally similar time-course and profile for distribution, metabolism, and excretion, regardless of whether the route of exposure was oral or inhalation. The approach EPA used to estimate exposure via dermal routes of exposure is covered in the *Environmental Release and Occupational Exposure Assessment for Di-ethylhexyl Phthalate (DEHP)* (U.S. EPA, 2025f).

3 NON-CANCER HAZARD IDENTIFICATION

As was stated in Section 1.2.3, EPA is focusing its hazard identification on developmental and reproductive toxicity indicated in previous assessments of DEHP (Health Canada, 2020; EFSA, 2019; ECHA, 2017a; CPSC, 2014; NICNAS, 2010). EPA considered the consensus LOAEL of approximately 15 mg/kg-day from these prior existing assessments and decided to include all studies with effects (LOAEL) less than or equal to 20 mg/kg-day to identify sensitive studies and endpoints from the more recent assessment by ATSDR (2022). Using this cut-off criterion of LOAEL less than or equal to 20 mg/kg-day, EPA identified a total of 50 animal toxicology studies from among the 201 studies in ATSDR's Table of LSE for further consideration in hazard identification and dose-response. These 50 studies included developmental and reproductive toxicity hazards presented in Section 3.1, but also included other hazards identified by EPA ultimately not used for point of departure derivation—such as metabolic effects, cardiovascular/kidney toxicity, neurotoxicity, immune adjuvant effects, musculoskeletal effects, and hazards identified by the inhalation route—which are presented in Sections 3.2 through 3.8. EPA evaluated non-cancer effects across epidemiological studies cited in existing assessments published between 2010 and 2022. More specifically, EPA reviewed the epidemiological conclusions from existing assessments and considered whether information from newer published literature would change those conclusions, since the ATSDR (2022) literature search through June 2020 is more recent than the 2019 TSCA literature search. In the risk evaluation of DEHP, developmental toxicity forms the basis of the POD used for acute, intermediate, and chronic exposure scenarios.

3.1 Developmental and Reproductive Toxicity

3.1.1 Summary of Epidemiological Studies

Epidemiologic studies investigating associations between urinary metabolites of DEHP and developmental and/or reproductive outcomes were identified by EPA and other organizations. The Agency reviewed and summarized the conclusions from previous assessments conducted by ATSDR (2022) and Health Canada (2018b), as well as systematic review publications by Radke et al. (2019b; 2018) and NASEM (2017) that investigated the association between DEHP exposure and male and female development and reproductive outcomes. Developmental and reproductive outcomes are summarized in Section 3.1.1.1 for males and Section 3.1.1.2 for females.

EPA concluded that the existing epidemiological studies do not support quantitative dose-response assessment due to uncertainty associated with exposure characterization of individual phthalates, which is discussed in Section 1.1. Thus, the epidemiological studies provide qualitative support as part of weight of scientific evidence.

3.1.1.1 Male Developmental and Reproductive Outcomes in Humans

Numerous epidemiological studies have assessed prenatal, early postnatal and/or pre-pubescent exposure to DEHP and developmental and reproductive outcomes in males. EPA considered ATSDR (2022) and Health Canada (2018a), Radke et al. (2019b; 2018) and NASEM (2017) as part of the weight of evidence regarding the associations between DEHP exposure and developmental and reproductive outcomes in males.

3.1.1.1.1 ATSDR (2022)

ATSDR (2022) evaluated a number of cross-sectional and cohort studies and assessed the relationships between urinary DEHP metabolites and sperm parameters, including concentration, count, motility, and morphology. The majority of studies (*i.e.*, 11 of 15), which included patients from fertility clinics and the general community, failed to find significant associations between DEHP metabolites and sperm count or concentration (Al-Saleh et al., 2019; Chang et al., 2017; Axelsson et al., 2015; Bloom et al., 2015a, b; Han et al., 2014a; Joensen et al., 2012; Jönsson et al., 2005). Four studies demonstrated significant associations but included males from subfertile couples. Of these, two (Mínguez-Alarcón et al., 2018; Chang et al., 2017) demonstrated negative associations between sperm count/concentration and urinary DEHP metabolites. The other two (Al-Saleh et al., 2019; Bloom et al., 2015a) demonstrated a positive association. Mínguez-Alarcón et al. (2018) also found a decreased percentage of normal sperm morphology with increasing levels of MEHP; no correlations with other urinary metabolites of DEHP were found in this investigation.

Studies of workers occupationally exposed to polyvinyl chloride (PVC) provide data on possible associations between inhalation exposure to DEHP and sperm parameters such as concentration, count, motility, and morphology. In a study of works in Taiwan, reduced sperm motility was associated with higher urinary levels of MEHP, MEHHP, and MEOHP in 47 PVC workers compared to 15 controls; no correlation was seen for sperm concentration or morphology (Huang et al., 2014). A study by Pan et al. (2006) found decreased free testosterone levels in male PVC workers in China who had higher levels of urinary MEHP; no associations were found with serum estradiol, luteinizing hormone (LH), or follicle stimulating hormone (FSH) (Pan et al., 2006). In a related study conducted in Taiwan by (Fong et al., 2015), no association was observed between urinary metabolites of DEHP and total testosterone, estradiol, LH, FSH, inhibin B, or sex hormone-binding globulin (SHBG); free testosterone was not assessed. In a study by Chang et al. (2015), male partners of infertile couples had higher levels of serum MEHP metabolites along with *higher* levels of total and free testosterone. However, in subsequent studies by the same authors, an association was found between *lower* testosterone levels and increases in other DEHP metabolites (*i.e.*, MEHHP, MEOHP, and MECPP) in an infertility clinic study by Chang et al. (2017). Other studies, such as the one by Woodward et al. (2020) found that decreased total and free testosterone was associated with increased ΣDEHP metabolites in males over age 60, but not in younger men. Human epidemiological research points to possible association between exposure to DEHP and lowered blood testosterone levels as well as poorer quality semen in adult males. Despite the paucity of research on the effects of DEHP exposure on human fertility, no study suggests an association between the two conditions.

Other birth size metrics assessed in epidemiological investigations of DEHP include birth length, birth weight, and head and chest circumference. In a case-control study of mother-infant pairs in China, Zhao et al. (2014) found that DEHP exposure increased the chances of intrauterine growth retardation (IUGR) across tertiles of maternal urine DEHP metabolites (42 infants with IUGR and 84 controls matched on maternal age). Additionally, a correlation was identified between lower birth weight and greater urine levels of MEHHP and MEOHP, particularly in male infants.

3.1.1.1.2 Health Canada (2018b)

Health Canada (2018b) evaluated the evidence of association[2] between DEHP and its metabolites and reproductive outcomes such as altered fertility or changes in semen parameters, time to pregnancy, and gestational age, and preterm birth (before 37 weeks). There was inadequate evidence for the association between DEHP and its metabolites and preterm birth and gestational age. The level of evidence could not be established for the association between DEHP and its metabolites and altered fertility. There was no evidence for the association between exposure to DEHP and its metabolites and time to pregnancy. According to Health Canada (2018a), there was inadequate evidence to support an association between male puberty endpoints (*i.e.*, pubic hair and age at testicular volume) and DEHP (MEHP, MEOHP, MEHHP, and MECPP). There was inadequate evidence of an association between DEHP and anogenital distance (AGD). Additionally, there was no evidence of association between DEHP (MEHP, MEOHP, MEHHP, MECPP) and gynecomastia (*i.e.*, an increase in breast glands in pubescent boys) or malformations of male infant genitalia (*e.g.*, hypospadias, cryptorchidism). Limited evidence was found to support a relationship between changes in sperm parameters (*e.g.*, concentration, motility, morphology) and DEHP (MEHP, MEOHP, MEHHP, MECPP, and MCMHP). Lastly, there was also limited evidence for association between increased sperm DNA damage/apoptosis and DEHP exposure (MCMHP, MECPP, MEHHP, MEOHP, MEHP). Overall, Health Canada found that the level of evidence could not be established for the association between DEHP and its metabolites and reproductive outcomes, such as altered fertility.

3.1.1.1.3 Radke et al. (2019b; 2018)

Radke et al. (2018) examined the association between DEHP and male reproductive outcomes such as AGD, semen parameters, time to pregnancy, testosterone, hypospadias/ cryptorchidism, and pubertal development. The evidence profile table from Radke et al. (2018) is summarized in Table 3-1 below. The authors found robust evidence for the association between DEHP metabolites and male reproductive effects overall, and moderate evidence specifically for adult exposure to DEHP metabolites and semen parameters and testosterone. Other levels of evidence are summarized in Table 3-1. The authors could not find clear evidence linking DEHP to hypospadias/cryptorchidism or pubertal development due to inconsistencies in the database and no clear pattern of evidence between observed associations and exposure level or range.

Radke et al. (2018) evaluated six studies (Jensen et al., 2016; Swan et al., 2015; Bornehag et al., 2014; Bustamante-Montes et al., 2013; Suzuki et al., 2012; Swan, 2008) for an association between exposure to DEHP and AGD. The strongest negative association between DEHP exposure and AGD was found in Bornehag et al. (2014), which reported the highest exposure levels for the sum of DEHP metabolites. Among the three medium confidence studies, Jensen et al. (2016) had the lowest exposure levels and the weakest association. Additionally, Swan et al. revealed statistically significant negative associations with MEHP (Swan et al., 2015; Swan, 2008) and total DEHP (Swan et al., 2015) and AGD; the findings from these studies were consistent and present a moderate level of confidence of an inverse association between exposure to DEHP and AGD. In terms of semen parameters, of all the studies that found an inverse association between sperm concentration and motility with increasing DEHP exposure, only the study by Bloom et al. (2015a) found a statistically significant association for sperm concentration. Two

[2] 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."

studies (Axelsson et al., 2015; Jurewicz et al., 2013) found a statistically significant association of sperm motility with MEOHP and MEHP. Moreover, Radke et al. (2018) found that there is a moderate to robust level of confidence in the association between increased DEHP and effects on sperm parameters. More specifically, there is a moderate level of evidence for the association between DEHP and lower-quality semen, especially as it relates to sperm concentration. Although the evidence was considered slight and the results were not statistically significant, there was some evidence that suggested that higher DEHP exposure in males is associated with increased time to pregnancy. Lower testosterone levels were associated with higher DEHP exposure in eight studies (Axelsson et al., 2015; Pan et al., 2015; Wang et al., 2015; Meeker and Ferguson, 2014; Specht et al., 2014; Jurewicz et al., 2013; Park et al., 2010; Meeker et al., 2009a). Two of these studies (Specht et al., 2014; Jurewicz et al., 2013) reported statistically significant associations. There was no discernible response pattern with increasing exposure level or exposure range in the association between DEHP exposure and decreased testosterone; however, medium confidence studies were more likely than low confidence studies to report an association, and low confidence studies typically had null, not conflicting, results. Although there was no clear response pattern of testosterone with increasing exposure to DEHP or exposure range, Radke et al. (2018) determined that there is moderate evidence for an association between exposure to DEHP and testosterone given the overall consistency of the association of decreased testosterone with increase DEHP exposure among the higher confidence studies. Radke et al. (2018) found robust evidence for the association between DEHP exposure and male reproductive outcomes.

Table 3-1. Summary of Epidemiologic Evidence of Male Reproductive Effects Associated with Exposure to DEHP (Radke et al., 2018)

Timing of Exposure	Outcome	Level of Confidence in Association
In utero	Anogenital distance	Moderate
	Hypospadias/cryptorchidism	Indeterminate
In utero or childhood	Pubertal development	Indeterminate
Adult	Semen parameters	Moderate
	Time to pregnancy	Slight
	Testosterone	Moderate
Male Reproductive Outcomes Overall		Robust
Data for DEHP taken directly from Figure 3 in Radke et al. (2018)		

3.1.1.1.4 NASEM report (2017)

NASEM (2017) evaluated five studies that evaluated the associations between *in utero* exposure to DEHP and AGD. They concluded that human studies provide a moderate degree of evidence for an association between fetal exposure to DEHP and decreases in AGD. This is consistent with findings by Radke et al. (2018). Although ATSDR found that there is some evidence of association between lower AGD and testicular decent in males with exposure to DEHP, Heath Canada found inadequate evidence of an association between AGD and DEHP.

EPA considered the conclusions on male development and reproductive parameters by ATSDR (2022), Health Canada (2018a), NASEM (2017) and Radke et al. (2019b; 2018). While some findings regarding

AGD are inconsistent across assessments, EPA agrees with the conclusions made by NASEM (2017) and Radke et al. (2018) that there is moderate evidence for the association between increased exposure to DEHP and decreased AGD as well as decreased testosterone and sperm parameters. However, EPA concludes that the existing epidemiological studies do not support quantitative exposure-response assessment due to uncertainty associated with exposure characterization of individual phthalates, which is discussed in Section 1.1. The epidemiological studies provide qualitative support as part of the weight of scientific evidence.

3.1.1.2 Female Developmental and Reproductive Outcomes in Humans

3.1.1.2.1 ATSDR (2022)

ATSDR (2022) reported that there is a paucity of epidemiological data evaluating the effects of DEHP exposure on female developmental and reproductive outcomes. This is due to either 1) the fact that urine samples were taken after the desired outcome and/or 2) exposure estimates were determined by a method other than using urinary metabolites. There were no associations found between urinary DEHP metabolites and serum estradiol in pregnant women, according to two additional cross-sectional investigations (Johns et al., 2015; Sathyanarayana et al., 2014). Furthermore, Johns et al. (2015) found no associations between DEHP exposure and progesterone or serum SHBG. Pregnant women with lower free testosterone had higher urine MECPP levels; no associations were observed with other DEHP metabolites, and there was no association between DEHP metabolites and total testosterone (Sathyanarayana et al., 2017). Sathyanarayana et al. (2014) found a relationship between lower levels of total and free serum testosterone and higher levels of DEHP metabolites in the urine of women giving birth to female infants. However, no such association was detected in women giving birth to male infants. Meeker and Ferguson (2014) conducted a cross-sectional investigation on women aged 20 to 80 who took part in the 2011 to 2012 NHANES survey. They found that there was no association for any specific metabolite of DEHP or age group, although higher urine metabolite levels were typically linked to lower blood total testosterone levels. Cross-sectional studies that examined whether exposure to DEHP affects women's reproductive hormones are limited and inconsistent. However, one study found that increased urinary levels of MEHP and MEOHP were associated with elevated blood levels of estrone and estradiol in 591 pregnant women, while no associations were found with the total amount of DEHP metabolites (Sathyanarayana et al., 2017).

There were no associations found between DEHP exposure and time to conception in three prospective cohort studies of couples who stopped taking birth control in order to become pregnant (Thomsen et al., 2017; Jukic et al., 2016; Buck Louis et al., 2014). Jukic et al. (2016) assessed the menstrual cycle and found that there was no association between altered luteal or follicular phase length and the majority of DEHP metabolites. One prospective cohort study of women undergoing *in vitro* fertilization (IVF), including but not limited to intracytoplasmic sperm injection (ICSI) therapy, found that higher maternal DEHP urine metabolites were associated with a lower fertilization rate (Machtinger et al., 2018). Along with higher maternal DEHP urine metabolites, two cohort studies in IVF patients also found decreased numbers of mature and total eggs and/or decreased top-quality embryos (Machtinger et al., 2018; Hauser et al., 2016). Lower ovarian antral follicle counts were associated with greater DEHP metabolite concentrations in urine samples taken prior to the determination of antral follicle counts, according to a different cohort study of women seeking examination for reproductive issues (Messerlian et al., 2015).

In several epidemiological studies, preterm birth was assessed using a categorical measure (<37 weeks of gestation). Six cohort studies (Zhang et al., 2020a; Bloom et al., 2019; Ferguson et al., 2019a; Gao et al., 2019) and three case-control studies (Ferguson et al., 2014c; Ferguson et al., 2014a; Meeker et al.,

2009a) reported that increased odds of preterm birth was associated with increased urinary DEHP metabolites. Increased odds were only observed in a portion of the study subjects for instance, white women, but not African American women, showed an association between increases in urinary MEHP and preterm birth (Bloom et al., 2019). Furthermore, Ferguson et al. (2019a) reported an interaction between increased preterm birth and the total of third trimester urine DEHP metabolites only among women who had experienced a stressful life event, such as a job loss, serious illness, family death, relationship issues, or legal or financial issues. Other cohort studies either reported no association between exposure and preterm birth (Hu et al., 2020; Ferguson et al., 2019b; Shoaff et al., 2016), or decreased odds of preterm birth with increasing exposure (Adibi et al., 2009). Increased maternal urine DEHP metabolite levels have been associated with an increased risk of post-term (>41 weeks) birth, according to two cohort studies (Gao et al., 2019; Adibi et al., 2009).

There was no discernible association between urine DEHP metabolite levels and gestational age at birth in trials where gestational age was a continuous variable. Out of the 10 studies that assessed gestational age, two (Adibi et al., 2009; Wolff et al., 2008) reported that higher gestational age at birth was associated with higher urinary DEHP metabolite levels, while one (Whyatt et al., 2009) reported that lower gestational age at birth was associated with higher metabolite levels. The other studies (Hu et al., 2020; Ferguson et al., 2019b; Gao et al., 2019; Gao et al., 2017; Casas et al., 2016; Shoaff et al., 2016; Su et al., 2014) found no association. Factors that may contribute to inconsistencies in the studies include the different times at which urine samples were collected, the validity of the outcome measurement, or the inclusion or exclusion of significant confounders. Crucially, a study's capacity to find an association may be significantly impacted by the time at which urine samples are collected.

Ferguson et al. (2019b; 2019a; 2014a) and Hu (2020) identified four studies that differentiated between causes of preterm birth, such as IUGR, preeclampsia, and other maternal complications, and spontaneous preterm delivery, which is defined as spontaneous labor or rupture of the membrane. Ferguson et al. (2019a; 2014a) reported associations between the total amount of DEHP metabolites in urine and spontaneous preterm birth in two cohorts. Associations were limited to third trimester urine levels for the Ferguson et al. (2019a) cohort; however, in their earlier study ((Ferguson et al., 2014a), this association showed an exposure-related trend across quartiles of exposure (geometric mean across three visits), and it also held true for three of the four individual metabolites measured (*i.e.*, MEHP, MEOHP, and MECPP).

Ferguson et al. (2014a; 2012) evaluated the associations between pro-inflammatory activities of DEHP and increased risk of preterm birth and found associations between DEHP exposure and systemic markers of inflammation and oxidative stress. The association was further supported by follow-up investigations of this cohort, which reported a positive correlation between maternal levels of DEHP (urinary metabolites) and urinary levels of 8-isoprostane, a biomarker of oxidative stress (Ferguson et al., 2015). Furthermore, the authors applied counterfactual mediation regression models to conclude that the relationship between urinary DEHP metabolites and spontaneous preterm birth was mediated by maternal urine levels of 8-isoprostane (Ferguson et al., 2017).

Pregnancy loss, or spontaneous abortion, and/or failed live birth were evaluated in four cohort studies of pregnant women (Machtinger et al., 2018; Jukic et al., 2016; Messerlian et al., 2016; Toft et al., 2012), two cohort studies of women receiving IVF/ICSI (Deng et al., 2020; Al-Saleh et al., 2019), and one case-control study that compared cases of spontaneous abortion and controls in China (Mu et al., 2015). When evaluating early (or biochemical) pregnancy loss, three studies reported increased risk of early pregnancy loss with an increase in urinary levels of one or more DEHP metabolites (Al-Saleh et al., 2019; Messerlian et al., 2016; Toft et al., 2012); one study observed decreased odds of early pregnancy

loss with increased urinary metabolite levels (Jukic et al., 2016); and one study observed no association (Deng et al., 2020). Regarding clinical pregnancy loss, only one study observed an association with exposure to DEHP (Al-Saleh et al., 2019).

3.1.1.2.2 Health Canada (2018a)

Health Canada (2018a) reported that there was limited evidence for the association between DEHP metabolites and altered female puberty (MECPP, MEHHP, MEOHP, and MEHP), as well as age at menopause (MEHHP and MEOHP). There was insufficient data to support a link between exposure to DEHP (MEHP, MEOHP, MEHHP) and polycystic ovarian syndrome (PCOS) or pregnancy loss. The degree of evidence supporting a relationship between altered fertility and exposure to DEHP (MEHP, MEHHP, MEOHP, and MECPP) could not be established. DEHP metabolites (MEHP, MEOHP, MEHHP, MECPP, MCMHP) were not shown to be associated with time to pregnancy or sex ratio.

3.1.1.2.3 Radke et al. (2019b)

Radke et al. (2019b) evaluated the relationship between exposure to DEHP and female developmental and reproductive outcomes such as pubertal development, fecundity, spontaneous abortion, and preterm birth depicted in Table 3-5.

Five studies evaluated the relationship between exposure to DEHP and pubertal development; three of which use prenatal exposure measurements (Su et al., 2014; Watkins et al., 2014; Hart et al., 2013), while two use childhood exposure measures (Wolff et al., 2014; Mouritsen et al., 2013). One study found a delayed onset of puberty in response to DEHP childhood exposure (Wolff et al., 2014). The results for prenatal exposure did not support that conclusion; two studies (Watkins et al., 2014; Hart et al., 2013) reported an earlier onset of puberty was associated with maternal urinary levels of DEHP metabolites, while one study (Su et al., 2014) found no association. Moreover, Watkins et al. (2016) found an inverse relationship between pubic hair development (which occurred earlier with increased exposure) and breast development (which occurred later with increasing exposure). Radke et al. (2019b) concluded that there is a lack of consistency and coherence across various measures of female pubertal development associated with exposure to DEHP. Ultimately, Radke et al. (2019b) determined that there is indeterminate evidence of an association between DHEP exposure and pubertal development.

The relationship between a woman's exposure to DEHP and her time to conception was investigated in three studies. Higher DEHP exposure does not appear to be associated with a longer time to conception. Given that exposure levels were generally low across the studies might have limited each study's ability to find associations between DEHP and time to pregnancy. In one study (Hauser et al., 2016), rates of clinical pregnancy in a population of couples undergoing fertility treatment were examined. Pregnant women who had higher exposure to DEHP had lower percentages of these outcomes (Q1: 0.57, 95% CI = 0.45–0.69, Q2: 0.46, 95% CI = 0.36–0.57, Q3: 0.49, 95% CI = 0.38–0.59, Q4: 0.38, 95% CI = 0.28–0.49*, p-trend = 0.04). Ultimately, Radke et al. (2019b) determined that there is slight evidence of an association between exposure to DEHP and time to pregnancy.

Additionally, three studies looked at related outcomes in women who underwent *in vitro* fertilization; two of the studies reported decreases in oocytes (Machtinger et al., 2018; Hauser et al., 2016); one reported no decrease in embryo quality (Wu et al., 2017); and the other two reported decreases in antral follicle count (Messerlian et al., 2015), embryo quality (Machtinger et al., 2018), and implantation (Hauser et al., 2016). Nonetheless, there was weak evidence of an association between female fecundity and DEHP exposure due to the paucity of supporting data from time-to-pregnancy studies, all of which

received high or medium confidence ratings. Five studies were used to evaluate the evidence for an association between spontaneous abortion and exposure to DEHP, three of which reported on early loss, three on clinical loss, and one on entire loss. Of the two high confidence investigations, Jukic et al. (2016) observed lower odds of early loss with increasing exposure, but Messerlian et al. (2016) indicated elevated risks of overall loss (early and clinical loss combined). Two low confidence investigations (Yi et al. (2016) for clinical loss and Toft et al. (2012) for early loss) found increased odds with increased exposure for both early and clinical loss; however, the impact estimates for one study (Mu et al., 2015; Toft et al., 2012) were imprecise. Ultimately, Radke et al. (2019b) determined that there is slight evidence of association between spontaneous abortion and DEHP exposure, and the degree of uncertainty stems from inconsistent results in the high confidence studies.

Radke et al. (2019b) evaluated the evidence of associations between exposure to DEHP and preterm birth across six studies that examined preterm birth as a dichotomous variable (Gao et al., 2017; Casas et al., 2016; Shoaff et al., 2016; Smarr et al., 2015; Ferguson et al., 2014b; Meeker et al., 2009b). Increased odds of preterm birth with higher DEHP exposure was reported in four of the six investigations on preterm birth, including two high confidence studies. Among them, Ferguson et al. (2014a) reached statistical significance, and the results of the other four studies were comparable. Both Ferguson et al. (2014a) (OR range: 1.23–2.17) and (Casas et al., 2016) found an exposure-response association, albeit one that was non-monotonic in the latter study. In addition to finding no association, the largest study, Gao et al. (2016), also found lower exposure levels than previous studies with Smarr et al. (2015) reporting lower levels of MEOHP, which can be a sign of decreased sensitivity. Given the constraints in examining gestational duration as a proxy for preterm birth, the two gestational duration studies did not find any association between the length of pregnancy and DEHP exposure; nonetheless, this is not regarded as incongruous with the preterm birth results. There is a lack of association in one high confidence study and the largest study, which may be partially explained by lower study sensitivity brought on by low exposure levels. However, there is consistency for preterm birth among multiple medium and high confidence studies in varied settings (*e.g.*, multiple different countries). Ultimately, Radke et al. (2019b) determined that there is moderate evidence that preterm birth is associated with DEHP exposure.

Table 3-2. Summary of Epidemiologic Evidence of Female Reproductive Effects Associated with Exposure to DEHP (Radke et al., 2019b)

Outcome	Level of Confidence in Association
Pubertal development	Indeterminate
Time to pregnancy	Slight
Spontaneous abortion	Slight
Preterm birth	Moderate
Data for DEHP are taken directly from Figure 4 in Radke et al. (2019b)	

3.1.1.3 Summary of the existing assessments of Developmental and Reproductive effects

Each of the assessments discussed above provide qualitative support as part of the weight of scientific evidence for the association between DEHP exposure and male and female developmental and reproductive outcomes. ATSDR (2022) found that adult males who are exposed to DEHP may

potentially have lower serum testosterone levels and lower-quality semen. ATSDR (2022), however found no association between DEHP exposure and infertility. Health Canada (2018b) found inadequate evidence to support an association between DEHP and AGD, while NASEM (2017) concluded that there is a moderate degree of evidence for an association between fetal exposure to DEHP and decreases in AGD. On the other hand, Radke et al. (2018) concluded that there was moderate evidence for the association between exposure to DEHP and AGD and robust evidence overall for the association between DEHP exposure and male reproductive outcomes. Radke et al. (2018), also found an indeterminate level of confidence in the association between exposure to DEHP and cryptorchidism/hypospadias, but this association was not consistent with the findings of Health Canada (2018b) or NASEM (2017). Health Canada (2018b) concluded that the lack of studies as well as the different matrices used to estimate fetal testis testosterone production (cord blood or amniotic fluid), and the variations in when testosterone is measured (during pregnancy or at deliver) make the data insufficient to draw inferences. 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 ATSDR (2022) and 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 regardless of the limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.1.1.4 EPA Conclusion

EPA considered the conclusions of ATSDR (2022), Health Canada (2018b), NASEM (2017) and systematic review publications by Radke et al. (2019b; 2018) and 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 however provide qualitative support as part of the weight of scientific evidence. Table 3-3 and Table 3-4 provide summaries of EPA's conclusions on male and female developmental and reproductive outcomes based on available epidemiologic literature and existing assessments by NASEM, Health Canada, ATSDR, and Radke et al.

Table 3-3. Summary of EPA Conclusions from Epidemiologic Studies on Male Developmental and Reproductive Outcomes

Male Developmental and Reproductive Outcome	EPA Conclusion	Reference(s)
Anogenital Distance (AGD)	Moderate evidence of decreased AGD with increased exposure to DEHP	NASEM (2017) Radke et al. (2018) Bornehag et al. (2014) Swan (2015; 2008) Jensen (2016)
Pubertal Development	Indeterminate evidence for association with DEHP exposure	Health Canada (2018a) Radke et al. (2018)
Hypospadias/Cryptorchidism	Indeterminate evidence for association with DEHP exposure	Radke et al. (2018)
Sperm Parameters - sperm count/concentration, sperm motility)	Moderate evidence of association with DEHP exposure affecting sperm concentration and motility. No significant association in majority of studies; negative association in subfertile males. Reduced motility associated with higher urinary MEHP, MEHHP, MEOHP in PVC workers.	Radke et al. (2018) Bloom (2015a) Axelsson et al. (2015) Jurewicz et al. (2013) Al-Saleh (2019) Chang et al. (2017) Minguez-Alarcon et al. (2018) Huang et al. (2014)
Testosterone Levels	Moderate evidence of decreased testosterone with increased DEHP exposure	Radke et al. (2018) Axelsson et al. (2015) Specht et al. (2014) Jurewicz et al. (2013) Park et al. (2010) Meeker et al. (2009a) Pan et al. (2015)

Table 3-4. Summary of EPA Conclusions from Epidemiologic Studies on Female Developmental and Reproductive Outcomes

Female Developmental and Reproductive Outcome	EPA Conclusion	Reference(s)
Pubertal Development	Indeterminate evidence of association with DEHP exposure	Radke et al. (2019b) Su et al. (2014) Watkins et al. (2014) Hart et al. (2013) Wolff et al. (2014) Mouritsen et al. (2013)
Time to Pregnancy (increased)	Slight evidence of association with DEHP exposure	Health Canada (2018a) Radke et al. (2019b) Hauser et al. (2016) Buck Louis et al. (2014) Jukic et al. (2016) Thomsen et al. (2017)
Spontaneous Abortion	Slight evidence of association with DEHP exposure	Jukic et al. (2016) Messerlian et al. (2016) Yi et al. (2016) Toft et al. (2012)
Preterm Birth	Moderate evidence of association with DEHP exposure	Radke et al. (2019b) Ferguson et al. (2014a) Smarr et al. (2015) Casas et al. (2016)

3.1.2 Summary of Laboratory Animal Studies

EPA has previously considered the weight of evidence and concluded that oral exposure to DEHP can induce effects on the developing male reproductive system consistent with a disruption of androgen action (see EPA's *Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023a)). Notably, EPA's conclusion was supported by the Science Advisory Committee on Chemicals (SACC) (U.S. EPA, 2023b). A summary of the mode of action (MOA) for phthalate syndrome and data available from the subset of more sensitive studies on DEHP (LOAEL <20 mg/kg-day) supporting this MOA is provided below. Readers are directed to see EPA's *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 DEHP's effects on the developing male reproductive system and EPA's MOA analysis and to the ATSDR's *Toxicological Profile for Di(2-Ethylhexyl)Phthalate (DEHP)* (ATSDR, 2022) for a complete description of this hazard, including the literature supporting effects at doses higher than considered by EPA in its focused scope for dose-response analysis. Effects on the developing male reproductive system are considered further for dose-response assessment in Section 4.

There is a robust database showing adverse effects on the male reproductive system following developmental exposure to DEHP in rats. Adverse effects include decreased fetal testis testosterone,

histopathological alterations in the testis (*e.g.*, seminiferous tubule atrophy, multinucleated gonocytes), decreased anogenital distance (AGD), increased male nipple retention, gross malformations of the male reproductive tract (*e.g.*, undescended testes and hypospadias), and sperm effects (*e.g.*, count, viability, motility, and morphology). In the subset of more sensitive studies (with LOAELs <20 mg/kg-day) examined in detail, EPA identified 12 oral exposure studies (including 10 studies of rats and 2 of mice) that evaluated the developmental effects on male offspring following *in utero* exposure to DEHP (Table 3-5). In addition to studies entailing *in utero* exposure, EPA also identified nine studies (eight of rats; one of mice) examining developmental and reproductive effects in male rodents exposed post-parturition, including four studies encompassing exposure from weaning through puberty or adulthood (Vo et al., 2009b; Ge et al., 2007; Akingbemi et al., 2004; Akingbemi et al., 2001) and five studies of adults (Hsu et al., 2016; Guo et al., 2013; Kitaoka et al., 2013; Li et al., 2012; Ganning et al., 1990). These are discussed in Section 3.1.2.2 and summarized in Table 3-6. While the majority of the developmental and reproductive studies examined effects on male reproductive system, EPA noted that developmental effects on the female reproductive tract are reported in three studies of rats (Shao et al., 2019; Andrade et al., 2006b; Grande et al., 2006) and two studies of mice (Zhang et al., 2014; Pocar et al., 2012), in addition to being examined in the three-generation reproductive toxicity study in rats (TherImmune Research Corporation, 2004); these studies are discussed below in Section 3.1.2.3 and summarized in Table 3-5.

3.1.2.1 Effects on Developing Male Reproductive System Following In Utero Exposure

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

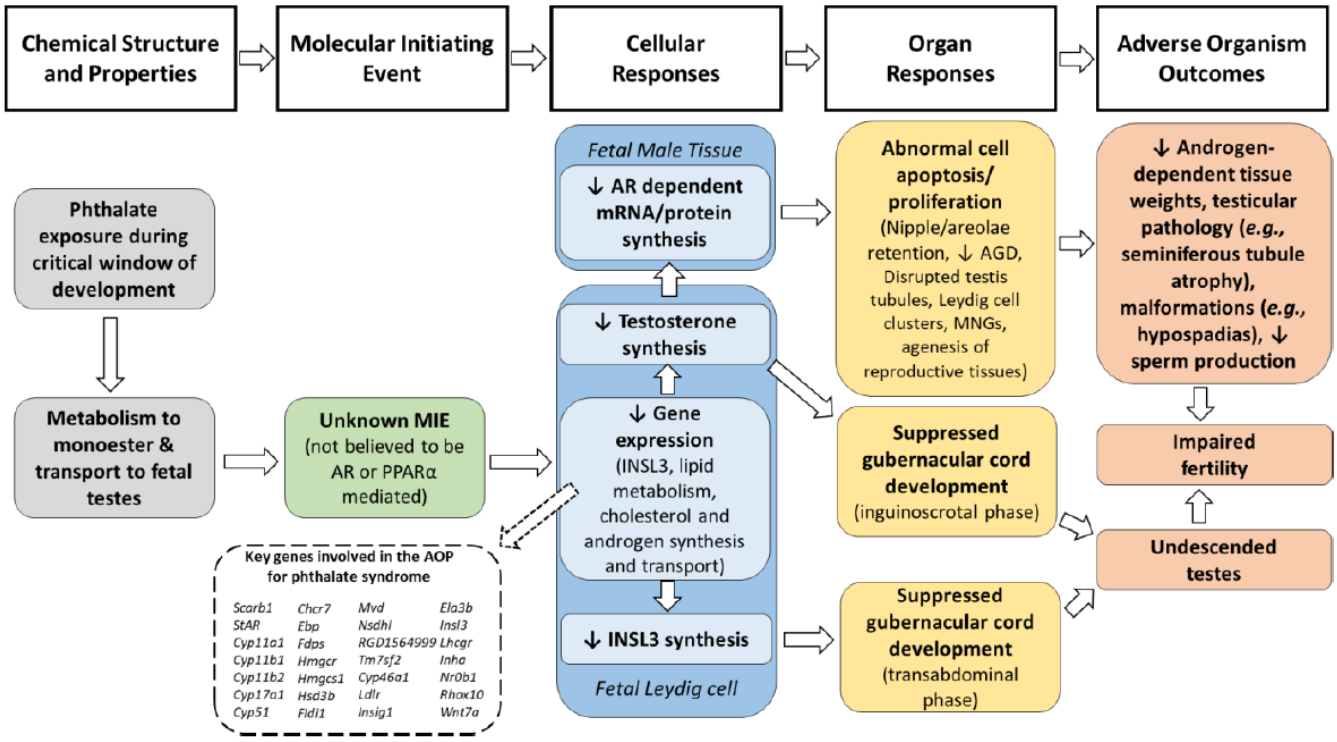


Figure 3-1. Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure
Figure taken directly from (U.S. EPA, 2023a) and adapted from (Conley et al., 2021; Gray et al., 2021; Schwartz et al., 2021; Howdeshell et al., 2016). Abbreviations: AR = androgen receptor; INSL3 = insulin-like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

The MOA underlying phthalate syndrome has not been fully established; however, key events at the cellular-, organ-, and organism-level are generally understood (Figure 3-1). Numerous studies evaluate the effects of DEHP on key events and adverse outcomes described in the proposed MOA, and results of those studies are largely consistent with the MOA, as described below.

Molecular Events
The molecular events (*i.e.*, the molecular initiating event) preceding cellular changes remain unknown. Several studies have provided evidence against the involvement of androgen receptor antagonism and peroxisome proliferator-activated receptor alpha (PPARα) activation (Gray et al., 2021; Foster, 2005; Foster et al., 2001; Parks et al., 2000).

Cellular Responses
Cellular responses are more well understood. There is abundant evidence that DEHP disrupts the production of fetal testicular testosterone in rodents. Disruption of testicular testosterone production during the masculinization programming window (*i.e.*, GDs 15.5–18.5 for rats; GDs 14–16 for mice; gestational weeks 8 to 14 for humans) can lead to antiandrogenic effects on the developing male reproductive system (MacLeod et al., 2010; Welsh et al., 2008; Carruthers and Foster, 2005). Consistent with the MOA outlined in Figure 3-1, many studies of DEHP identified by EPA have demonstrated that oral exposure to DEHP during the masculinization programming window can reduce testosterone synthesis in the fetal male Leydig cell and/or reduce expression (mRNA and/or protein) of insulin-like growth factor 3 (INSL3), as well as genes involved in steroidogenesis in the fetal testes of rats (U.S. EPA, 2023a).

Testosterone production drives extratesticular male reproductive tract development and, together with INSL3, drives organ-level outcomes, such as testicular descent. The vast majority of studies identified have found decreased testicular testosterone following exposures of pregnant rats to 100 mg/kg-day or higher, with decreases also observed at 10 mg/kg-day although less consistent as far as both directionality and persistence (Lin et al., 2009; Vo et al., 2009a; Lin et al., 2008; Akingbemi et al., 2001) Table 3-6. Serum testosterone and LH concentrations at 100 mg/kg-day were significantly lower than controls at PND 21 and PND 35 (comparable at PND 90) in F1 male Long-Evans rats exposed to DEHP from GD12 to 21 (Akingbemi et al., 2001). Similarly, *ex vivo* testosterone production in isolated Leydig cells was significantly decreased when examining both basal testosterone production (47% decrease) and LH-stimulated testosterone production (56% decrease) at 100 mg/kg-day compared to controls in this study. Lin et al. (2009) found serum testosterone levels significantly decreased at doses of 10 mg/kg-day and above on PND21 in F1 male Long-Evans rats exposed from GD12.5 to PND21.5, although by PND49, the decrease in testosterone only persisted at the higher dose of 750 mg/kg-day. Serum testosterone and LH were decreased by 63 to 66 percent at 500 mg/kg-day compared to controls on GD 21 in F1 male SD rats exposed to DEHP from GD11 to 21 (Vo et al., 2009a). Similarly, serum testosterone was significantly decreased at 500 and 750 mg/kg-day in F1 male offspring of pregnant CD-1 mice gavaged at 0, 0.2, 500, or 750 mg/kg-day from GD11 to PND0 (Barakat et al., 2018). Testicular testosterone was significantly decreased by 67 percent at 750 mg/kg-day compared to controls on GD21, although it was increased by 57 percent at 10 mg/kg-day in F1 male Long-Evans rats exposed to DEHP from GD2 to 20 (Lin et al., 2008).

In the aforementioned study by Lin et al. (2008) in which pregnant Long-Evans rats were administered DEHP in corn oil via oral gavage at 0, 10, 100, or 750 mg/kg-day from GD 2 to 20, the decreases in testosterone measured on GD 21 were accompanied by changes in testicular gene expression evaluated by examining a panel of 37 genes—including those that encode growth factors (*Igf1, Kitl*, *Lif*), growth factor receptors (*Igf1r*, *Kit*, *Lhcgr*, *Pdgfra*), cholesterol transporters (*Scarb1*, *Star*), and steroidogenic enzymes (*Cyp11a1*, *Cyp19*, *Sdr5a1*). Significant effects on gene expression included decreased expression of *Cyp11a* and *Lhcgr* at 100 and 750 mg/kg-day; decreased *Pdgfra*, *Scarb1*, *Star*, and *Insl* at 750 mg/kg-day; and increased *Srd5a1*, *Pdgfb*, and *Lif* at 750 mg/kg-day. Examination of levels of enzymes relevant for testosterone biosynthesis revealed decreased P450scc at 750 mg/kg-day, although 3βHSD, P450c17, and 17βHSD were not affected.

In a subsequent study by Lin et al. (2009), F1 male Long-Evans rats were exposed to 0, 10, or 750 mg/kg-day from GD 12.5 to PND 21.5, with subsets of male offspring killed at birth or at PND21 or PND49. At birth, gene expression analyses indicated reductions in genes associated with cholesterol transporters and steroidogenic enzymes, including *Scarb1*, *Star*, and *Hsd17b12* at 10 and 750 mg/kg-day. Additionally at 750 mg/kg-day, luteinizing hormone receptor gene (*Lhcgr*), testosterone biosynthetic enzymes *Cyp17a1* and *Hsd17b3*, testis descent gene *Insl3*, and cell junction gene *Gja1* were decreased. Sertoli cell genes, including *Kitl*, *Clu*, and *Fshr* were examined, with significant decreases in *Clu* and *Fshr* at 750 mg/kg-day. Examination of gene expression at PND21 indicated decreases in *Lhcgr*, *Kit*, *Scarb1*, *Hsd17b3*, *Srd5a1*, *Pcna*, *Gja1*, *Ar*, *Kitl*, and *Fshr* at 10 and 750 mg/kg-day; however, gene expression in the treated groups was comparable to controls at PND 49. Protein expression of P450c17 was decreased at 10 and 750 mg/kg-day at PND 21, and STAR, 3βHSD1, 17 βHSD3, and SRD5A were decreased at 750 mg/kg-day at PND1, with only SRD5A remaining decreased at PND49.

Similarly, Vo et al. (2009a) found significant down-regulation of testicular genes related to steroidogenesis (*StAR*, *Cyp11a1*, *Hsd3b1*) and alpha-actin cardiac 1 (*Actc1*) at 10 mg/kg-day on GD 21 in F1 male SD rats exposed to DEHP from GD11 to 21; whereas casein kinase 2 alpha 1 polypeptide

(*Csnk2a1*) was upregulated at this dose. At 500 mg/kg-day, stanniocalcin 1 (*Stc1*) and cysteine rich protein 61 (*cyr61*) expression were increased, and *Ard6* expression was decreased.

Organ Responses

Organ-level responses in the reproductive system include Leydig cell aggregation or altered distribution of Leydig cells, reduced AGD, and increased nipple retention. Perturbations in Leydig cell morphology are indicative of disrupted androgen action. Leydig cells in the testes produce testosterone, INSL3, and dihydrotestosterone (DHT), which forms from its precursor, testosterone. Reduced AGD stems from reduced production of testosterone by the Leydig cell, as DHT functions to lengthen the perineum (*i.e.*, skin between the genitals and anus) of males. Increased nipple retention also stems from reduced testosterone production, as DHT in peripheral tissues is necessary for apoptosis and regression of nipples in male rats. Each of these responses have been well documented in rodents exposed to DEHP following gestational exposure (see Section 3 of (U.S. EPA, 2023a) for further discussion). Two studies have reported increased incidences of Leydig cell aggregation at doses ranging from 10 to 750 mg/kg-day (Lin et al., 2009; Lin et al., 2008). The distribution of the number of fetal Leydig cells (FLCs) per cluster was significantly affected at 10 to 750 mg/kg-day on GD21 in F1 male Long-Evans rats exposed from GD2 to 20, with increased FLC per cluster at these doses, and decreased number of Leydig cells per testis and Leydig cell size (volume) at 100 and 750 mg/kg-day (Lin et al., 2008). In the follow up study by Lin et al. (2009), FLC aggregation was increased in F1 male Long-Evans rats via exposure to 0, 10, or 750 mg/kg-day from GD12.5 to PND21.5, with the average, median, and maximum numbers of Fetal Leydig Cells per cluster dose-dependently increased at 10 mg/kg-day and 750 mg/kg-day.

Many studies have demonstrated that oral exposure of rats to DEHP during the masculinization programming window can reduce male rat pup AGD. Effects on AGD were reported in 19 studies included in Table 3-8 of the *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023a), several of which (7) were included in the pool of 50 studies evaluated in the current assessment (Pocar et al., 2012; Christiansen et al., 2010; Gray et al., 2009; Vo et al., 2009a; Lin et al., 2008; Andrade et al., 2006a; TherImmune Research Corporation, 2004). Among these more sensitive studies (LOAEL <20 mg/kg-day), decreased AGD was reported at doses as low as 10 mg/kg-day in rats following gestational exposure, with measurements of AGD conducted late gestation just prior to parturition on GD 21 (Lin et al., 2008), early in the postnatal window (*i.e.*, on PND 1 through PND 2) (Christiansen et al., 2010; Gray et al., 2009; Lin et al., 2009), after lactation on PND 22 (Andrade et al., 2006a), during adulthood (Vo et al., 2009a), and in male offspring after continuous exposure in the multi-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004).

Similarly, four studies have reported increased nipple retention at doses ranging from 10 to 447 mg/kg-day (Blystone et al., 2010; Christiansen et al., 2010; Gray et al., 2009; Andrade et al., 2006a; TherImmune Research Corporation, 2004):

- Nipple retention was significantly increased at 10 mg/kg-day and above in F1 male Wistar rats exposed to DEHP from GD 7 to LD 16, with mean of 1.23 to 5.01 nipples per male in the treated groups compared to a mean of 0.22 nipples per male in controls (Study 1); although NR in the treated groups was comparable to controls in Study 2, and NR was significantly increased at 10 mg/kg-day and above when data from the two studies were combined (Christiansen et al., 2010).
- The percent of males with retained nipples was higher at 300 mg/kg-day (55%) compared to controls (11%) in F1 male SD rats exposed to DEHP from GD 8 to LD 17, with an increased number of areolae per male at this dose (2.9) compared to controls (0.7) (Gray et al., 2009).

- Incidences of nipple retention were significantly increased on PND 13 in F1 males exposed to 405 mg/kg-day throughout gestation (beginning at implantation) and lactation (Andrade et al., 2006a).
- Increased nipple retention in F3c males at 447 mg/kg-day and above in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004).

Phthalates can also affect Sertoli cell function and development. Formation of lesions such as multi-nucleated gonocytes (MNGs) is one indication of perturbed Sertoli cell function and development. Incidences of bi- and multi-nucleated gonocytes in the testes were increased in incidence and severity in F1 males exposed beginning at implantation (GD 6) and continuing throughout the remainder of gestation and lactation (Andrade et al., 2006a). As discussed in Section 3.1.3.7 of the *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023a), DEHP has been shown to cause MNG formation in at least seven studies of rats starting at doses as low as 100 mg/kg-day.

Adverse Organism Outcomes

Adverse outcomes at the organism-level have been observed following exposure to DEHP during the masculinization programming window, including effects on androgen-dependent reproductive or accessory sex organ weights (*e.g.*, testes, seminal vesicle, epididymis, Levator ani/bulbocavernosus [LABC], prostate weight) and histopathology, including reproductive tract malformations (Table 3-5). Androgen-dependent organ weights in male rat offspring were decreased following gestational exposure at doses as low as 10 mg/kg-day, as indicated in the following studies:

- Absolute weights of the ventral prostate and LABC were generally consistently decreased at 10 mg/kg-day and above in F1 male Wistar rats exposed to DEHP from GD 7 to LD 16 when examining combined data from two studies (Christiansen et al., 2010).
- Seminal vesicle weights were decreased at 100 mg/kg-day and above in F1 male SD rats exposed during gestation and lactation (GD 8 to PND 17) and examined at 7 months of age. A broader suite of reproductive organ weights (ventral prostate, seminal vesicles, LABC, Cowper's glands, epididymis, and testes) were decreased in the 7-month old F1 male rats exposed to 300 mg/kg-day DEHP from GD 8 to PND 17, in addition to F1 males exposed via the same maternal exposure window but then directly dosed via daily oral gavage from PND 18 to PND 63 (Gray et al., 2009).
- Absolute testes weights were significantly decreased at 100 and 750 mg/kg-day on GD 21 in F1 male Long-Evans rats exposed from GD 2 to 20 (Lin et al., 2008).
- Seminal vesicle weights were significantly decreased at 405 mg/kg-day in F1 males exposed to DEHP beginning at implantation (GD 6) and continuing throughout the remainder of gestation and lactation (Andrade et al., 2006c).
- Testes and epididymis weights were decreased at 447 mg/kg-day and above in F1, F2, and F3 males in the three-generation reproduction study in rats (Blystone et al., 2010; TherImmune Research Corporation, 2004).

Histopathology examination of androgen-dependent organs indicated effects on DEHP at doses as low as 11 mg/kg-day (Gray et al., 2009) but more routinely at doses 100 mg/kg-day and above (Blystone et al., 2010; Christiansen et al., 2010; TherImmune Research Corporation, 2004). In the study by Gray et al. (2009), maternal SD rats were dosed with DEHP during gestation and lactation (GD8 to PND17) and F1 male offspring were examined at 7 months of age (intrauterine cohort); while a subset of F1 offspring continued with direct dosing via oral gavage from PND 18 to PND 63 and terminated on PND 63 to PND 65 (puberty cohort). The following histopathology findings of the testes and epididymis were observed across both cohorts at 11, 33, and 100 mg/kg-day: retained nipples, fluid-filled flaccid testes,

hypoplastic (incompletely developed, similar to aplasia, but less severe) or malformed epididymis, epididymal granuloma with small testis, testicular seminiferous tubular degeneration (both moderate and mild severity, malformed seminal vesicles or coagulating glands, and true hermaphroditism, in one male, with uterine tissue and ovotestis. Males were assigned an ordinal classification regarding whether they exhibited effects of phthalate syndrome, and the incidences of phthalate syndrome were fairly consistent in the lower dose groups, with 8/71 (11.3%) at 11 mg/kg-day, 10/68 (11.6%) at 33 mg/kg-day, and 12/93 (12.9%) at 100 mg/kg-day and were significantly increased over controls (zero incidence), with higher significance and incidence at 300 mg/kg-day (38/74 males; 51.3%). In another study in which Wistar rats were exposed to DEHP during gestation and lactation (GD 7 to PND 16), incidences of mild external genital dysgenesis in F1 male Wistar rats were clearly dose-dependent and consistently statistically significant at doses at 100 mg/kg-day and above (Christiansen et al., 2010). In the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004), treatment-related effects were observed on histopathology of the testes at 447 mg/kg-day and above, including atrophy of seminiferous tubules characterized by loss of germ cells and the presence of Sertoli cell-only tubules, as well as occasional failure of sperm release in testes, and sloughed epithelial cells and residual bodies in the epididymis.

Reproductive performance measures were impaired at doses as low as 10 to 15 mg/kg-day with decreased sperm count and delayed sexual maturation in a few studies (Vo et al., 2009a; Andrade et al., 2006c; Andrade et al., 2006a), but more broadly across functional reproductive parameters at higher doses (300 mg/kg-day and above) (Blystone et al., 2010; Gray et al., 2009; TherImmune Research Corporation, 2004). Sperm count was decreased by 19 to 25 percent at 15 mg/kg-day and above in F1 males exposed beginning at implantation (GD 6) and continuing throughout the remainder of gestation and lactation, and these decreases were significant compared to both the concurrent and historical controls (Andrade et al., 2006c). In F1 male SD rats exposed to DEHP from GD11 to 21, sperm concentration was 24 percent lower than controls at 10 mg/kg-day and 53 percent lower at 500 mg/kg-day on PND63, with similar decreases in sperm viability at 10 mg/kg-day (14%) and 500 mg/kg-day (40%); and sperm motility was decreased by 13 to 47 percent in all dose groups (*e.g.*, 10 mg/kg-day and above) compared to controls (Vo et al., 2009a).

Preputial separation was significantly delayed at 15 mg/kg-day and above in F1 offspring exposed beginning at implantation (GD6) and continuing throughout the remainder of gestation and lactation, while body weight at criterion was comparable to controls at doses up to 405 mg/kg-day at which it was significantly decreased (Andrade et al., 2006a). Preputial separation was significantly delayed in F1 male SD rats exposed to 300 mg/kg-day DEHP from GD8 through PND63, with preputial separation occurring at mean of 49.1 days at 300 mg/kg-day compared to 45.7 days in controls (Gray et al., 2009).

Developmental toxicity was observed and reproductive performance was clearly compromised at 447 mg/kg-day and above in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004), with treatment-related decreases in: litter size; number of male pups; total number of pups per litter; terminal body weights of offspring; pup weights, unadjusted and adjusted for litter size; number of implantation sites; and mating, pregnancy, and fertility indices. Additionally at these doses, the following treatment-related effects were observed, including delayed: testes descent, vaginal opening, and preputial separation. None of the F1 mating pairs produced offspring at 659 mg/kg-day. Crossover matings were conducted at 447 and 659 mg/kg-day. When treated males were crossed with untreated females, there were decreased numbers of implantation sites and decreased mating, pregnancy, and fertility indices. When treated females were mated with untreated males at 447 mg/kg-day and above, pup weights were decreased in both sexes, and sperm count parameters were

decreased, including: density (sperm/mg cauda); sperm/cauda; spermatids/testis, and spermatids/mg testes.

Collectively, available studies consistently demonstrate that oral exposure to DEHP during the masculinization programming window in rats can disrupt androgen action, leading to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. As noted above, this conclusion was supported by the SACC (U.S. EPA, 2023b)

Table 3-5. Studies Evaluating Effects on the Developing Reproductive System (with LOAEL less than 20 mg/kg-day) Following *In Utero* Exposures to DEHP

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Female Wistar rats administered DEHP at 0, 3, 10, 30, 100, 300, 600, 900 mg/kg-d via oral gavage from GD 7–LD 16 (Gestation & Lactation) (Christiansen et al., 2010) (High)	3/10	↓ AGD, ↑ nipple retention, & ↓ LABC & ventral prostate weights in male pups.	Maternal Effects - None reported Developmental Effects - ↓ AGD, ↑ nipple retention, & ↓ LABC & ventral prostate weights in male pups at ≥10 mg/kg-d ↓ BW of F1 males at 300 mg/kg-d - Only effect at 3 mg/kg-d was mild external genitalia dysgenesis in 6/49 (12%) male pups from 4/14 (29%) litters; however, not consistently dose-related or statistically significant until ≥100 mg/kg-d. Unaffected Outcomes - Maternal clinical signs, body weight, & body weight gain - Gestation duration Limitations -Ventral prostate & LABC not subjected to histopathology.
Male and female SD rats administered DEHP in the diet at 1.5, 10, 30, 100, 300, 1000, 7500, 10,000 ppm (0.1, 0.58, 1.7, 5.9, 17, 57, 447, 659 mg/kg-d) continuously for 3 generations (3 litters per generation) (Blystone et al., 2010; TherImmune Research Corporation, 2004) (High)	4.8/14 (5.9/17 mean across 3gen)	↑ total malformations of reproductive tract (testes, epididymis, seminal vesicles, prostate), indicative of phthalate syndrome in F1 & F2 males (*e.g.*, epididymal agenesis, undescended testes, small fluid-filled testis, hypoplastic accessory sex organs, and hypospadias)	Parental Effects At ≥1000 ppm (57 mg/kg-d), ↑liver weights in parents & offspring, accompanied by hepatocellular hypertrophy. Dilation of the renal tubules and mineralization occasionally associated with chronic pyelonephritis in the kidney. Developmental/Reproductive Effects At ≥300 ppm (14 mg/kg-d in F1 & F2 offspring), ↑ total malformations of reproductive tract (testes, epididymis, seminal vesicles, prostate), indicative of phthalate syndrome in F1 & F2 males (*e.g.*, epididymal agenesis, undescended testes, small fluid-filled testis, hypoplastic accessory sex organs, and hypospadias).

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			At ≥7500 ppm (447 mg/kg-d), decreases in: litter size; number of male pups; total number of pups per litter; AGD; pup weights; number of implantation sites; mating, pregnancy, and fertility indices; sperm count; epididymis and testes weights. The following treatment-related effects were observed: delayed testes descent, vaginal opening, and preputial separation; ↑ nipple retention; ↑weights of liver, kidneys, and adrenals; cortical vacuolization of adrenals; and histopathology effects in testes, including atrophy of seminiferous tubules & occasional failure of sperm release in testes, & sloughed epithelial cells & residual bodies in epididymis. None of F1 mating pairs produced offspring at 10,000 ppm (659 mg/kg-d). Crossover matings at ≥447 mg/kg-d: when treated males crossed with untreated females, ↓ numbers of implantation sites; ↓ mating, pregnancy, and fertility indices. When treated females were mated with untreated males, ↓ AGD in male offspring, ↓ pup weights in both sexes, & ↓ sperm parameters, including: density (sperm/mg cauda); sperm/cauda; spermatids/testis, and spermatids/mg testes.
Female Wistar rats administered DEHP at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-d via oral gavage from GD 6–LD 21. (Gestation & Lactation) (Andrade et al., 2006a) (Medium)	5/15	Delayed preputial separation (PPS)	Maternal Effects - None reported Developmental Effects - Delayed preputial separation at 15 mg/kg-d -↑ absolute liver weights & ↑ MNG in testes at ≥135 mg/kg-d -↑ nipple retention, ↓ AGD, & ↓ F1 male body at PPS at 405 mg/kg-d Unaffected Outcomes - Maternal & offspring clinical signs, maternal weight gain, litter size, sex ratio, or number of viable pups
Female Wistar rats administered DEHP at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405	5/15	↓ (19–25%) sperm production on PND 144	Maternal Effects - None reported Developmental Effects

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
mg/kg-d via oral gavage from GD 6–LD 21. Offspring terminated for examination on PND 144 ± 7days. (Gestation & Lactation) (Andrade et al., 2006c) (Medium)			- ↓ sperm production on PND 144- ↓ seminal vesicle/coagulating gland weight at 405 mg/kg-d on PND 144. - Cryptorchism noted at one male each at 5, 135, & 405 mg/kg-d, but not dose-related. Unaffected Outcomes - Weights of liver, kidney, spleen, & thymus; sperm morphology; precoital interval, mating & pregnancy indices; litter size, fetal weight, and number of implantations, resorptions, and viable fetuses Limitations -↑ Serum testosterone, statistically significant only at 0.045, 0.45, & 405 mg/kg-day, but no difference from controls at 0.135, 1,215, 5, 15, 45, or 135 mg/kg-day, so unrelated to dose.
Female Long-Evans rats administered DEHP at 0, 10, 100, 750 mg/kg-d via oral gavage from GD 2–20. Examination at GD21 (Gestation only) (Lin et al., 2008) (Medium)	NE/10 (LOEL)	PND 1 male offspring: ↑ FLC/cluster and ↑ testicular testosterone (1.4 ng/mg vs. 0.89 ng/mg in controls)	Maternal Effects - None reported Developmental Effects - ↑ FLC/cluster and ↑ testicular testosterone (1.4 ng/mg vs. 0.89 ng/mg in controls) in male offspring on PND 1. - ↓ testes weight, FLC number & size at ≥100 mg/kg-d - ↓ testicular testosterone (0.29 ng/mg vs. 0.89 ng/mg in controls) & AGD at 750 mg/kg-d - ↑ expression of leukemia inhibitory factor (LIF) gene, growth factor produced by peritubular myoid cells, associated with ↑ FLC aggregation *in vitro*. Unaffected Outcomes - Maternal body weights; birth rate, litter size, offspring sex ratio, and male pup body weight
Female Long-Evans rats administered DEHP at 0,	NE/10	FLC aggregation & ↓steroidogenic & cholesterol	Maternal Effects - None reported

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
10, 750 mg/kg-d via oral gavage from GD 12.5–PND 21.5 (Gestation & Lactation) (Lin et al., 2009) (Low)		transporter gene expression (↓*Scarb1*, *Star*, *Hsd17b12*) at PND 1, ↓serum testosterone at PND 21	Developmental Effects - At ≥10 mg/kg-d, FLC aggregation & ↓ steroidogenic & cholesterol transporter gene expression (↓ *Scarb1*, *Star*, *Hsd17b12*) at PND 1, ↓ serum testosterone at PND 21 - Additionally at 750 mg/kg-d, ↓ AGD at PND 2, ↓ body weight at PND 2 & 35, ↓ luteinizing hormone receptor gene (*Lhcgr*), (testosterone biosynthetic enzymes *Cyp17a1* and *Hsd17b3*, testis descent gene *Insl3*, cell junction gene *Gja1*, & Sertoli cell genes, *Clu* and *Fshr* at PND 1. Unaffected Outcomes - Maternal body weights, birth rate, litter size, offspring sex ratio
Female Wistar rats administered DEHP at 0, 10, 100 mg/kg-d via oral gavage from GD 9 – LD 21. Examined effects in F1 adult male offspring at PND 80. (Gestation & Lactation) (Rajagopal et al., 2019b) (Medium)	NE/10	↓ serum testosterone & estradiol (E2) in F1 adult males	Maternal Effects - None reported Developmental Effect - ↓ serum testosterone & estradiol in F1 adult males at ≥10 mg/kg-d Limitations - ↓ T & E2 were significantly decreased at 10 & 100 mg/kg-d compared to controls; however, data were only depicted in bar graph, so unable to calculate a quantitative % decrease. Note: study primarily focused on glucose homeostasis (see Section 3.1.2.3) reporting the following effects: Increases in: fasting blood glucose, oral glucose tolerance, insulin tolerance, HOMA-IR (insulin resistance), ALT, AST, alkaline phosphatase, urea, creatinine, insulin, Glu-6P mRNA & activity, phosphoenolpyruvate carboxykinase mRNA & activity, interaction of Fox01with Glu-6P and phosphoenolpyruvate carboxykinase gene promoters. Decreases in: hepatic glycogen, glycogen synthase, IRβ, p-IRβ^{TYR1162}, IRS1, p-IRS1^{TYR632}, β-arrestin, c-SRC, Akt, p-Akt^{Ser473}, p-Akt^{Thr308,} p-Akt^{Tyr315}, proteins in liver.

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			Authors conclusions: DEHP impairs insulin signal transduction & alters glucose regulatory events that can lead to Type II diabetes in F1 male offspring.
Female SD rats administered DEHP at 0, 10, 100, 500 mg/kg-d via oral gavage from GD 11 – 21 (Gestation - Parturition) (Vo et al., 2009a) (Low)	NE/10	↓ sperm count, viability, & motility in F1 males at PND 63.	Maternal Effects - None reported. Developmental Effects - At ≥10 mg/kg-d, ↓ sperm count, viability, & motility in F1 males at PND 63 - Additionally at 500 mg/kg-d: ↓ body weight, LH, & testosterone in male fetuses on GD21 & ↑ nipple retention (9.06 ± 1.83 nipples/male vs. ND in other groups), hypospadias (100% males), & cryptorchidism (17.4% males) at PND 63. Unaffected Outcomes - Litter size, male offspring BW at PND63, AGD, weights of testes, epididymis, prostate, serum T and LH
Male CRL:CD (SD) rats administered DEHP at 0, 11, 33, 100, 300 mg/kg-d via oral gavage from GD 8–LD 17 (*in utero* cohort) or GD 8–PND 65 (puberty cohort) (Gestation & Lactation, or through puberty) (Gray et al., 2009) (Medium)	NE/11	↑ % F1 males in both cohorts with phthalate syndrome-related effects, such as: retained nipples, fluid-filled flaccid testes, hypoplastic or malformed epididymis, epididymal granuloma with small testis, testicular seminiferous tubular degeneration, malformed seminal vesicles or coagulating glands, and true hermaphroditism, in one male, with uterine tissue and ovotestis.	Maternal Effects - None reported. Developmental Effects - At ≥11 mg/kg-d, ↑ % F1 males in both cohorts with phthalate syndrome-related effects, such as nipple retention and malformations and histopathology findings in testes, epididymis, seminal vesicles, coagulating glands. - At ≥100 mg/kg-d ↓ absolute seminal vesicle weight in intrauterine cohort; ↑ liver weights in puberty cohort - At 300 mg/kg-d: ↓ AGD (↓16%) on PND2, ↑ nipple retention (55% vs. 11% in controls) on PND13 and PND 65 (1.22 nipples/male vs. 0 controls); ↓ absolute reproductive organ weights (prostate, seminal vesicles, LABC, Cowper's glands, epididymis) in both cohorts; and ↓ glans penis & testes in intrauterine cohort as adults. Unaffected Outcomes

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			- Maternal body weight & weight gain, female offspring body weight; serum testosterone and estradiol
Female CRL CD-1 mice administered DEHP via diet at 0, 0.2857, 28.57, 2857 ppm (0.05, 5, 500 mg/kg-d) from GD 0.5–PND 21. 3 F1 offspring per sex per litter continued on study post-treatment to PND 42. (Gestation & Lactation, subset continue to puberty) (Pocar et al., 2012) (Medium)[a]	NE/0.05	sperm count ↓ 51-53%, sperm viability ↓ 20%, ↓ body weight in both sexes, ↓ blastocyst rate *in vitro* with oocytes from untreated females fertilized from treated males. In females, ↓ body fat & % mature oocytes, ↑ ovary weight & degenerated oocytes.	Maternal Effects - ↑ relative liver weights by 11–18% at 0.05 & 5 mg/kg-d Developmental Effects - ↓ body weight in both sexes on PND21 & PND42 at ≥0.05 mg/kg-d - ↓ seminal vesicle weight at ≥0.05 mg/kg-d - ↓ sperm count & viability at ≥0.05 mg/kg-d - ↓ blastocyst rate *in vitro* with oocytes from untreated females fertilized from treated males at ≥0.05 mg/kg-d. - In females, ↓ body fat & % mature oocytes, ↑ ovary weight & degenerated oocytes at ≥0.05 mg/kg-d Unaffected Outcomes - Maternal ovary and uterus weight+ - Offspring clinical signs, sex ratio, viability index Limitations - Abortion in 9/10 dams at 500 mg/kg-d; therefore, offspring evaluation limited to 0.05 & 5 mg/kg-d groups vs. controls - Dose-response flat at 0.05 & 5 mg/kg-d for sperm count & sperm viability in males; and for % degenerated oocytes in adult female offspring. - Blastocyst rate decreased at 0.05 mg/kg-d in all three generations in subsequent study by Pocar (2017); however, the rate at 5 mg/kg-d was comparable to controls, indicating that the effect was not dose-related and lack of replicability between the two studies conducted in the same lab at the same doses.
Female rat administered DEHP at 0, 0.01, 0.1, 1 mg/kg-d via oral gavage	0.01/0.1	↑ Prostatic Intraepithelial Neoplasia (PIN) score & Gleason score at ≥0.1 mg/kg-↑	Maternal Effects - None reported

Brief Study Description (exposure window) (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
from GD7– PND21. Examined male offspring at PND 196. Silastic capsules with estradiol + testosterone implanted in subgroup on PND 90 & replaced PND 146. Sham-control group. Positive control injected with 17-estradiol-3-benzoate (EB) on PND 1, 3, & 5. (Gestation & Lactation) (Wang et al., 2017) (Medium)[a]		PIN score & Gleason score; however, not significant; ↓ absolute weights of prostate & testes & absolute & relative weights of epididymis	Developmental Effects -↑ PIN score & Gleason score at ≥0.1 mg/kg-d; however, not significant -↑ PSA at 1 mg/kg-d. -↓ absolute weights of prostate & testes & absolute & relative weights of epididymis at ≥0.1 mg/kg-d Unaffected Outcomes - None reported Limitations - PIN & Gleason scores were not statistically significant, organ weight decreases not corroborated by incidence or severity data for histopathology, and DEHP only resulted in cancer in longer term studies at much higher doses

Abbreviations: ↓ = statistically significant decrease; ↑ = statistically significant increase; AGD = anogenital distance; F1 = first-generation offspring; F2 = second-generation offspring; FLC = Fetal Leydig cell; GD = gestation day; LABC = levator ani plus bulbocavernosus muscles; LH = luteinizing hormone; LOAEL = lowest observed adverse effect level; MNGs = multinucleated gonocytes; NE = Not established because LOAEL was lowest (or only) dose group. NOAEL = No observed adverse effect level; NR = nipple retention; PND = postnatal day; PPS = preputial separation; StAR = steroidogenic acute regulatory protein; *SR-B1/Scarb1* = scavenger receptor class B member 1; SV = seminal vesicle

[a] As discussed in the Systematic Review protocol for DEHP (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.

3.1.2.2 Effects on Male Reproductive Tract Following Exposures Post-parturition

In addition to studies entailing *in utero* exposure, EPA also identified nine studies (eight of rats; one of mice) examining developmental and reproductive effects in male rodents exposed post-parturition, including four studies encompassing exposure from weaning through puberty or adulthood (Vo et al., 2009b; Ge et al., 2007; Akingbemi et al., 2004; Akingbemi et al., 2001) and five studies of adult rodents (Hsu et al., 2016; Guo et al., 2013; Kitaoka et al., 2013; Li et al., 2012; Ganning et al., 1990) which are summarized in Table 3-6. Although these studies entailed initiation of dosing after parturition, the majority of the hazards identified were related to effects on the male reproductive tract, similar to those described in studies that involved at least part of the exposure during the gestational window known to affect male reproductive development, as described in Section 3.1.2.1. These findings included changes in: sperm morphology (Hsu et al., 2016); testosterone and/or estradiol production (Li et al., 2012; Vo et al., 2009b; Ge et al., 2007; Akingbemi et al., 2004; Akingbemi et al., 2001); Leydig cell proliferation (Guo et al., 2013; Li et al., 2012; Akingbemi et al., 2004); sexual maturation in males(Ge et al., 2007); male reproductive organ weights (Vo et al., 2009b; Ge et al., 2007) and histopathology (Kitaoka et al., 2013; Vo et al., 2009b; Ganning et al., 1990); and decreased AGD (Vo et al., 2009b).

Table 3-6. Summary of Studies Evaluating Effects on the Male Reproductive System following Prepubertal, Pubertal, & Adult Exposure to DEHP

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Male Long-Evans rats administered DEHP at 0, 1, 10, 100, 200 mg/kg-d via oral gavage from PND 35-48 or PND 21-48 (Post weaning – puberty) (Akingbemi et al., 2001) (Medium)	1/10	↓ basal & LH-stimulated testosterone production on PND 49 after pre-pubertal (PND35-48) exposure, but ↑testosterone production with earlier & longer exposure (PND21-48)	Developmental/Reproductive Effects - In rats exposed PND21-34 (early 14-day exposure), ↓ Basal & LH-stimulated testicular testosterone production at 100 mg/kg-day following early exposure In rats exposed PND 35-48 (late 14-day exposure), ↓ Basal & LH-stimulated testicular testosterone production at ≥10 mg/kg-d, accompanied by ↓ steroidogenic enzymes (P450SCC, 3β-HSD, P45017α, and 17β-HSD) at ≥100 mg/kg-d. - In rats exposed PND 21-48 (28-day exposure) ↑ Serum testosterone (35–42%); ↑ interstitial fluid testosterone (41–45%); ↑ serum LH (59–86%), and ↑ basal and LH-stimulated testicular testosterone production at ≥10 mg/kg-d. Unaffected outcomes: - No effects on body weight, testis or seminal vesicle weights, or serum concentrations of LH or T at either age (PND 35 or PND 49)
Adult Male SD rats administered DEHP at 0, 0.03, 0.1, 0.3, 1 mg/kg-d via oral gavage from PND 42-105 (Adult exposure) (Hsu et al., 2016) (Medium)[a]	0.03/0.1	% sperm with bent tails was significantly higher at 0.1, 0.3, & 1 mg/kg-d (1.1–2.0%) vs. controls (0.3%), and % sperm with chromatin DNA damage (DFI%) was higher at these doses (4.8–6.4%) compared to controls (2.1%).	Developmental/Reproductive Effects Sperm abnormalities and chromatin DNA damage (DFI%) at ≥0.1 mg/kg-d Unaffected Outcomes: - Body weight and body weight gain from PND42-105, sperm count and sperm motility, relative weights of testes, epididymis, seminal vesicles, and kidneys. Notes: - Uncertainty regarding plausibility & replicability of the sperm abnormalities because: sperm abnormalities not observed in the three-generation reproduction study(TherImmune Research Corporation, 2004) in SD rats (3 litters/gen), with doses ranging from 10 ppm (0.10 mg/kg-day) to the highest doses of 10,000 ppm in diet in P gen (775 mg/kg-d), F1 gen (543 mg/kg-d), and 7500 ppm in F2 gen (359 mg/kg-d). Sperm count was decreased in F1, F2, & F3 males at 7500 ppm (≥359 mg/kg-d) and in parental generation at 10,000 ppm (775 mg/kg-d), but no effects on sperm morphology. However, DFI not examined in the three-generation reproduction study.

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Male Long-Evans rats administered DEHP at 0, 10, 100 mg/kg-d via oral gavage from PND 21-48, 21-90, or PND 21-120 (Post weaning – puberty or adult) (Akingbemi et al., 2004) (Medium)	NE/10	↑ serum estradiol (E2) & Leydig cell E2 production after PND 21–48 in males; ↑ serum testosterone & LH, ↓ Leydig cell testosterone & E2 production, Leydig cell proliferation after PND 21–90 in males; Leydig cell proliferation after PND 21–120.	Developmental/Reproductive Effects - In rats exposed PND21–48, ↑serum E2 & LH-stimulated Leydig cell E2 production at ≥10 mg/kg-d. ↑ basal Leydig cell E2 production & aromatase gene induction at 100 mg/kg-d - In rats exposed PND 21–90, ↑ serum LH & T; ↓ basal & LH-stimulated T production, & Leydig cell proliferation at ≥10 mg/kg-d. ↑ Gene expression of cell cycle proteins (Cyclin G1, p53, cyclin D3, and PCNA) generally at ≥10 mg/kg-d - In rats exposed PND 21–120, Leydig cell proliferation at ≥10 mg/kg-d; ↑ serum LH & T; ↓ basal & LH-stimulated T production but only significant ($p < 0.01$) at 100 mg/kg/day. Notes Some uncertainties regarding differing effects on T depending on timing and duration of dosing relevant to development; see Dose-Response Assessment in Section 4.2.2 for EPA's consideration of the studies by Akingbemi et.al (2004; 2001)
Male Long-Evans rats; administered DEHP at 0, 10, 500, 750 mg/kg-d via oral gavage from PND 21-49. Follow up study at 0, 10, and 500 mg/kg-d for shorter duration (PND21-34), not including 750 mg/kg-d (Post weaning – puberty) (Ge et al., 2007) (Medium)	NE/10	↓ time to PPS, ↑ serum T, & ↑ seminal vesicle weight at 10 mg/kg-d	Developmental/Reproductive Effects - In rats exposed from PND21-49: • At 10 mg/kg-d, ↓time to PPS (39.7 days vs. 41.5 days in controls); serum T (↑ 58%; $p < 0.01$), body weight (↑ 8%; $p < 0.05$), seminal vesicle weights (↑ 27%; $p < 0.05$). • At 750 mg/kg-d, ↑time to PPS (46.3 days vs. 41.5 days in controls), body weight (↓ 13%), testes weight (↓ 29%), prostate weight (↓ 45%), serum T (↓ 40%). - In rats exposed from PND21-34: • At 500 mg/kg-d, testes weights (↓ 22%, $p < 0.01$), serum T (↓ 78%; $p < 0.05$); cholesterol-stimulated T production (↓ 98%; $p < 0.01$). Unaffected outcomes - Gene expression of *Lhb* and *Ar* in pituitary glands

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			Limitations - Apparent "Biphasic" effect on sexual maturation likely due to differing effects on body weight at different doses & relationship between growth & development. -High dose of 750 mg/kg-d not evaluated in follow up study (PND21-34).
Male SD rats administered DEHP at 0, 10, 100, 500 mg/kg-d via oral gavage from PND 21-35. Terminal examination of rats on PND 36. (Post weaning – puberty) (Vo et al., 2009b) (Medium)[a]	NE/10	↓ serum testosterone; ↓ weights of prostate, seminal vesicles, epididymis; and testes histopathology at ≥10 mg/kg-d	Developmental/Reproductive Effects - At ≥10 mg/kg-d: ↓ serum T & ↓ absolute weights of prostate, seminal vesicles (NS at 100 mg/kg-d), epididymis (NS at 100 & 500 mg/kg-d) on PND 36. - Testes histopathology reported dilatation of the tubular lumen, degeneration of Leydig cells, and disorder of germ cells at 10 and 100 mg/kg-d. At 500 mg/kg-d: ↓ AGD & testes weights; testes histopathology reported stratification of germ cells, dilatation of the tubular lumen and stratification, and disorder of germ cells Unaffected Outcomes: - No effects on body weight or on steroidogenic genes (*StAR*, *Cyp11a1*, *Hsd3b1*), but ↑ LIM homeobox protein 1 (*Lhx1*) and phospholipase C, delta 1 (*Pldc1*) at 100 mg/kg-d and ↓ isochorismatase domain containing 1 (*Isoc1*) at 500 mg/kg-d Limitations: -Lack of dose-dependency for several decreases in repro organ weights, not explained by BW. - Histopathology of testes only presented in representative micrographs and described qualitatively with no quantitative incidence or severity data.
Adult male CRL Long-Evans 90-day old rats administered DEHP at 0, 10, 750 mg/kg-d for 7 days via oral gavage; 1 subgroup terminated at 7 days & another subgroup given i.p. injection of EDS to eliminate Leydig cells and	NE/10	Leydig cell numbers ↑ 20% after dosing 7 days (prior to EDS elimination of Leydig cells; Study 1).	Developmental/Reproductive Effects - Study 1: At ≥10 mg/kg-d, Leydig cell numbers ↑ 20% after dosing 7 days (prior to EDS elimination) - Study 2: ↑ Differentiation of stem cells to progenitor Leydig cells in adult male testes after EDS elimination of Leydig cells. Unaffected outcomes: - No deaths or clinical signs of toxicity and no effects on BW or food consumption

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
dosed DEHP for additional 4 days (Adult exposure) (Guo et al., 2013) (Medium)			Limitations: -EPA excluded Study 2 results from consideration for POD because Study 2 involved examination of response after EDS elimination of Leydig cells; however, included in weight of evidence -Only two dose levels included, with broad spacing between low and high dose.
Adult Male 90-day old Long-Evans rats administered DEHP at 0, 10, 750 mg/kg-d via oral gavage for 14-, 21-, and 35-days post-EDS elimination of Leydig cells (Adult exposure) (Li et al., 2012) (Medium)[a]	NE/10	Dose-dependent ↑ Leydig cell number at 14-, 21-, & 35-days post-EDS & ↑ Leydig cell proliferation (BrdU labeling index) at 14 & 21 days; ↑ serum LH at 10 mg/kg-d at 21 days; ↓serum testosterone at ≥10 mg/kg-day at 35 days.	Developmental/Reproductive Effects - At ≥10 mg/kg-d: ↑ Leydig cell number at 14-, 21-, & 35-days post-EDS; ↑ Leydig cell proliferation (BrdU labeling index) at 14 & 21 days; ↓ serum T at 35 days - Additionally at 750 mg/kg-d: ↓ Leydig cell specific genes (*Lhcgr*, *Cyp11a1*, *Hsd3b1*, and *Insl3*) beginning at 21 days post-EDS administration. Authors concluded that Leydig cell regeneration & proliferation after EDS, but gene expression & T remained decreased. Unaffected outcomes: - No effects on survival, clinical signs, or body weights. Limitations: - Exclude from consideration for POD but include in weight of evidence because entire study involved examination of response after EDS elimination of Leydig cells.
Adult Male A/J mice administered DEHP via diet at 0, 0.01, 0.1% (0, 12.3, 125 mg/kg-d) for 2, 4, & 8 weeks (Adult exposure) (Kitaoka et al., 2013) (Medium)	NE/12.3	↑ Sertoli cell vacuolation (dose- and time-dependent), germ cell sloughing in seminiferous tubules, lymphocytic infiltration in the testicular interstitium, & damage to the blood-testes-barrier at ≥12.3 mg/kg/day.	Developmental/Reproductive Effects - ↑ mean #Sertoli cell vacuoles per 100 seminiferous tubules at 12.3 mg/kg-d (14.5-20.0) and 125 mg/kg-d (16.3-22.7) compared to controls (1.0-1.3). - ↑ #lymphocytes per mm^2 testicular interstitium (*e.g.*, lymphocytic infiltration) at 12.3 mg/kg-d (19.2) & 125 mg/kg-d (22.6) compared to controls. - At ≥12.3 mg/kg-d, increased expression of IL-10 (in spermatids, endothelial cells, and interstitial cells) and IFN-γ (Sertoli and interstitial cells) in testes. - Horseradish peroxidase (HRP), used as a tracer, indicated blood-testes barrier compromised in DEHP-treated mice, with ↑ #seminiferous tubules infiltrated by HRP per 100 seminiferous tubules at 12.3 mg/kg-d (3.1 ± 0.8) and 125 mg/kg-d (2.4 ± 0.6) vs. no HRP inside the lumen of seminiferous tubules in controls.

Brief Study Description (TSCA Study Quality Rating)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			- "Degree of spermatogenic disturbance" in seminiferous tubules quantified by Johnson score (0=no cells in seminiferous tubules to 10=complete spermatogenesis). Johnson score lower at 125 mg/kg-d (8.8 ± 2.1) compared to controls (10 ± 0.0) at 8 weeks Unaffected outcomes: - No effects on body weights or testes weights. Limitations - Some quantification of histopathology (*e.g.*, scoring) included above; however, no incidence data.
Adult Male SD rats fed DEHP in diet at 0, 200, 2,000, or 20,000 ppm (0, 14, 140, and 1400 mg/kg-d) for 102 weeks. (Adult exposure) (Ganning et al., 1990) (Uninformative)	NE/14	Inhibition of spermatogenesis and general tubular atrophy in testes	Developmental/Reproductive Effects - At ≥14 mg/kg-d, inhibition of spermatogenesis & general tubular atrophy in testes Limitations - No quantitative (incidence or severity) data provided for histopathology. Overall data quality rating of "uninformative" in systematic review was due to qualitative reporting of data for effects on testes and spermatogenesis. Notes: Majority of treatment-related findings are related to liver toxicity; therefore, details reported in Section 3.4 on liver toxicity, and only effects on male reproductive system are described here.

NOAEL = No observed adverse effect level; LOAEL = lowest-observed-adverse-effect level; LOEL = lowest-observed-effect level; ND = no data; GD = gestation day; PND = postnatal day; AGD = anogenital distance; BW = body weight

[a] As discussed in the Systematic Review protocol for DEHP (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.

3.1.2.3 Effects on Developing Female Reproductive System

While the majority of the developmental and reproductive studies examined effects on male reproductive system, EPA noted that developmental effects on the female reproductive tract are reported in three studies of rats (Shao et al., 2019; Andrade et al., 2006b; Grande et al., 2006) and three studies of mice (Parra-Forero et al., 2019; Zhang et al., 2014; Pocar et al., 2012), in addition to being examined in the three-generation reproductive toxicity study in rats (TherImmune Research Corporation, 2004); these studies are discussed below.

In the study by Zhang et al. (2014), pregnant mice were administered DEHP in 0.1 percent DMSO at 0 or 0.04 mg/kg-day throughout gestation (GD 0.5 to GD18.5) and allowed to deliver naturally on GD19.5 (PND0), and F1 females were mated with wild-type (untreated) males. Maternal serum estradiol was measured at GD12.5. Meiosis-specific *Stra8* gene and protein expression were measured at GD13.5. Meiosis prophase 1 assay was measured in developing fetal oocytes collected from pregnant mice terminated on GD17.5. Folliculogenesis was evaluated at PND21 in F1 & F2 females, with follicles classified as: primordial; primary; secondary; or antral. On GD12.5, maternal serum estradiol at 0.04 mg/kg-day was lower than controls, and gene expression of *Cyp17a1*, *Cyp19a1*, *Aldhla1*, *ERα*, *FSHR*, *LHR*, *EGF*, and *EGFR* was significantly down-regulated in fetal mouse ovaries. On GD13.5, gene and protein expression of the meiosis specific *Stra8* gene was lower than controls, which the authors attributed to modifying methylation at the promoter, with significantly increased percent methylation in the treated group compared to controls. The authors reported delayed meiotic progression of female germ cells in fetal mouse ovary on GD17.5, with the percent of oocytes at leptotene (26.43%) and zygotene (60.17%) stages in treated group higher than controls (4.33% leptotene and 29.57% zygotene), and fewer treated animals in pachytene and diplotene stages. Examination of the follicle status in ovaries of F1 offspring at PND21 showed a decrease in the number of primary and increase in the number of secondary follicles, which the authors attributed to depletion of the primordial follicle pool through accelerated folliculogenesis, moderated by down-regulation of gene expression of folliculogenesis-related genes (*Cx43*, *Egr3*, *Tff1*, and *Ptgs2*). In the F2 females, the number of primordial follicles was significantly lower, and the number of secondary follicles was significantly higher, in the treated group compared to controls on PND21. One limitation of this study is that only a single dose level was tested; therefore, it is not possible to examine dose-response. Several other deficiencies and limitations were noted in this study, including: the fact that follicle staging in female offspring was evaluated at PND21, well before puberty (first estrous cyclicity with growing follicles); the study design employed a small sample size (n = 5 dams); intra-assay variability (QC) and sensitivity (LOD) were not reported for the ELISA for estradiol; and other reporting deficiencies (*e.g.*, sample size for fetal ovaries, and F1, and F2 offspring were not reported). Given these limitations, EPA did not consider this study useful quantitatively for derivation of a POD and did not consider it in dose-response analysis.

In a study by Parra-Forero et al. (2019), female CD-1 mice were dosed with 0, 0.02, 0.2, or 2 mg/kg-day in corn oil-free tocopherol via oral gavage for 30 days beginning at sexual maturation (vaginal opening). Following the 30-day dosing period, females in estrus were superovulated using intraperitoneal (i.p.) injection of pregnant mare serum gonadotropin (PMSG) followed by i.p. injection of human chorionic gonadotropin (hCG) 48 hours later and mated with unexposed males of proven fertility. Females were terminated (6/group/timepoint) at 24-, 48-, 72-, 84-, or 96- h post-mating for the evaluation of 1-, 2-, and 4-cell zygotes, morula, and blastocysts, respectively. Another group of unmated females (6/group) were terminated 16 hours after superovulation for evaluation of oocytes. Additional groups of animals were used to evaluate potential effects of DEHP on DNA replication during mitosis 18 hours after mating. The number of oocytes recovered was significantly decreased at 2 mg/kg/day in the superovulated females which were not mated. In mated animals, the number of unfertilized oocytes was significantly

increased over controls at 0.2 mg/kg-day and above; and in fertilized oocytes, an arrest in zygote development was observed at these doses, resulting in an overall significant decrease in preimplantation embryos. Specifically, at 48 hours, when zygotes are expected to be comprised of two cells, the percent of 1-cell zygotes was significantly increased at 2 mg/kg-day (25.12%) compared to controls (7.5%). At 72 hours (when a 4-cell zygote is expected), the percent of 1-cell zygotes was significantly increased at 2 mg/kg-day (14.6%) compared to controls (4.3%) and the percent of 2-cell zygotes was significantly increased at 0.2 mg/kg-day (12.4%) and 2 mg/kg-day (35.1%) compared to controls (5.5%). At 84 hours (morula expected), the percent of 1- and 2-cell zygotes at 0.2 mg/kg-day (4.3 and 33.8%, respectively) and 2 mg/kg-day (3.1 and 21.0%, respectively) were significantly increased over controls (1.4 and 4.6%, respectively). Similarly, at 96 hours (blastocyst expected), the percent of 1-cell zygotes, 2-cell zygotes, and morula at 0.2 mg/kg-day (6.8, 13.2, and 10.28%, respectively) and 2 mg/kg-day (13.7, 29.8, and 30.13%, respectively) were significantly increased over controls (3.1, 5.6, and 2.38%, respectively). The number of 1-cell zygotes with polar body extrusion was significantly lower at 2 mg/kg-day, indicating lack of successful oocytes maturation post-fertilization. The percentage of zygotes with abnormal morphology, characterized by fragmented blastomeres, was significantly increased at 0.2 mg/kg-day and above, and the number of zygotes with at least 10 percent of the inner side showing fragmentation, a cut-off suggestive of inability to implant, was significantly increased at 2 mg/kg-day. Study authors suggest arrested development may be due to impaired DNA replication during mitosis; in support, the numbers of 1-cell zygotes with DNA replication were significantly decreased at 0.2 mg/kg-day and above.

In the study by Shao et al. (2019), 15-day old female Wistar rats (12 per dose group) were administered DEHP via oral gavage at 0, 0.2, 1, or 5 mg/kg-day for 4 weeks. No acclimation period was reported, which would indicate that the animals had not be weaned at the time of study initiation. The following findings were noted at 0.2 mg/kg-day and above: decreased apoptosis of hypothalamic cells; increased GnRH in hypothalamus; and increased protein expression of IGF-1R, P13K, Akt, and GnRH. At 1 mg/kg-day and above: increased serum IGF-1 and GnRH; increased gene expression of IGF-1, mTOR, and GnRH; and increased protein expression of IGF-1 and mTOR were observed. At 5 mg/kg-day: increased Nissl staining and gene expression of IGF-1R & Akt were observed in the hypothalamus; and accelerated sexual maturation (decreased time to vaginal opening) was depicted in a bar graph, occurring approximately a week earlier than controls (approximately 28 vs. 35 days). The study authors proposed that DEHP may activate hypothalamic GnRH neurons prematurely through IGF-1 signaling pathway and promote GnRH release, leading to the accelerated sexual maturation observed in female rats at 5 mg/kg-day. However, as a potential adverse outcome pathway of phthalates on the hypothalamus is not well established, EPA did not consider the changes in gene and protein expression and decreased apoptosis in the hypothalamus at 0.2 and 1 mg/kg-day to be definitive evidence of an adverse response to the treatment with DEHP. Furthermore, the apparent effect on ***accelerated*** sexual maturation in the females treated with 5 mg/kg-day DEHP in this study is not replicated in other studies, therefore casting uncertainty on the attribution of this finding to treatment with DEHP. Specifically, under a different exposure paradigm targeting an earlier developmental stage, studies by Grande et al. (2006) reported that time to vaginal opening was ***delayed*** by 2 days in Wistar rats gavaged with 15 mg/kg-day DEHP from GD6 to LD21, with similar delays in preputial separation noted in the males (Andrade et al., 2006a), with body weights comparable to controls. Similarly, in the three-generation reproduction study of SD rats (Blystone et al., 2010; TherImmune Research Corporation, 2004), vaginal opening and preputial separation were delayed by up to a week in the F1c, F2c, and F3c pups starting at 7500 ppm (359 mg/kg-day), associated with decreased body weights in these animals. Finally, the fact that body weights were not reported in the study by Shao et al. (2019) precluded EPA from evaluating any relationship between growth and sexual development in that study.

In the study reported in a series of publications by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006), pregnant Wistar rats were administered DEHP in peanut oil by oral gavage at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, or 405 mg/kg-day from GD 6 to LD 21, and effects were examined in the F1 offspring. Grande et al. (2006) presented results on F1 female offspring from this study. Mean time to vaginal opening was significantly delayed in F1 females at 15 mg/kg-day and above (37.1 to 38.1 days) compared to controls (35.6 days). The age at first estrus was slightly delayed at 135 mg/kg-day and above (41.2 to 41.8 days) compared to controls (39.2 days), but the increased time to first estrus was not statistically significant. There were no dose-related effects on body weight at sexual maturation or body weight at first estrus. Liver weights were significantly increased in F1 females at 135 mg/kg-day and above.

Andrade et al. (2006b) reported results from the same study, specifically the examination of aromatase activity in the hypothalamic/preoptic area brain sections from a subset of F1 offspring. On PND 1, aromatase activity in the F1 males was significantly *decreased* at 0.135 and 0.405 mg/kg-day but *increased* at 15, 45, and 405 mg/kg-day; whereas, in the treated females at PND 1, aromatase activity was comparable to controls. On PND 22, aromatase activity in this area of the brain was increased in 0.405 mg/kg-day F1 males and in all treated groups in the F1 females except for the 0.045 and 5 mg/kg-day dose groups. The authors proposed a biphasic, non-monotonic effect of DEHP on aromatase activity in the hypothalamic/preoptic area that differed between males and females and at different ages.

The European Food Safety Authority (EFSA) developed an *Opinion on the impact of non-monotonic dose responses on EFSA's human health risk assessments*, which included DEHP as one of the two case studies examined (EFSA et al., 2021). EFSA noted that non-monotonic dose-response (NMDR) has been reported for aromatase activity in studies, and that changes in aromatase activity resulting in differences in testosterone metabolism are a possible mechanism to support the biological plausibility of the observed NMDR for postnatal testosterone with exposure to DEHP.

In a subsequent review of this evidence, Astuto et al. (2023) summarized the conclusions from that Opinion and applied an AOP framework to assess the biological plausibility DEHP's effect on testosterone, noting the effects on brain aromatase (CYP19) in the study by Andrade et al. (2006b). Astuto et al. (2023) noted that, because aromatase is responsible for the catalysis of testosterone to estradiol, a non-monotonic disruption of brain aromatase homeostasis could be directly caused by the disruption in testosterone homeostasis, or secondary to the hormonal imbalance caused by a disruption of testosterone homeostasis. However, in their overall assessment, the authors concluded that the "available evidence is inconclusive regarding the assessment of possible NMDR for testosterone levels" and cited the need for studies with sufficient number of doses and appropriate exposure windows.

EPA acknowledges that a NMDR for aromatase activity cannot be ruled out but does not consider the nonmonotonic statistically significant differences in aromatase reported in the study by Andrade et al. (2006b) to sufficiently explain the monotonic dose-related effects described in the other publications by Andrade and Grande et al. (2006c; 2006a; 2006) which were more definitively due to treatment with DEHP. Overall, EPA considers there to be too much scientific uncertainty associated with the apparent NMDR effects to use these endpoints quantitatively in risk characterization.

In a study by Pocar et al. (2012), CD-1 mice were administered DEHP in the diet at 0, 0.05, 5, and 500 mg/kg-day throughout gestation and lactation (GD0.5 to LD21). Abortion occurred in 9 out of 10 dams at 500 mg/kg-day; therefore, evaluation of effects in offspring was limited to the 0.05 and 5 mg/kg-day groups compared to controls. Effects on the female development and reproduction are reported below, while effects specific to male development and reproduction in this study are reported above in Section

3.1.2.1and Table 3-5. Body weights were measured in offspring on PND 42. Oocyte maturation was determined *in vitro* in oocytes from maternally-exposed female offspring, with oocytes categorized as: (1) not matured (germinal vesicle and metaphase I) = diffuse or slightly condensed chromatin or with clumped or strongly condensed chromatin with or without metaphase plate but no polar body; (2) mature MII oocytes = oocytes with metaphase plate and a polar body; or (3) degenerated = oocytes with no visible chromatin or with fragmented cytoplasm and/or abnormal chromatin patterns. Relative (to body weight) liver weights in the maternal animals were significantly increased by 11 to 18 percent over controls at 0.05 and 5 mg/kg-day. In the offspring at these doses: body weights were decreased by 18 to 24 percent on PND21 and by 6 to 14 percent on PND42 in both sexes; percent body fat was decreased by 29 to 42 percent in females; and absolute ovary weights were increased by 13 to 32 percent. While many of the differences in organ weight and percent body fat were not dose-dependent and/or may be related to decreased body weight at PND42, the increase in absolute ovary weight cannot be explained by decreased body weight. *In vitro* oocyte maturation tests showed a decrease in mature oocytes in the treated groups (80%) compared to controls (88%) and an increase in degenerated oocytes (18% treated vs. 8% controls). The dose- response is essentially flat for oocyte maturation and degeneration, even though the mid dose is 100× higher than the low dose.

In order to determine if the effects on body weights reported in the study by Pocar et al. (2012) were due to treatment with DEHP or if they were incidental, EPA considered the examination of the effects of DEHP on body weights in mouse studies evaluated by ATSDR (2022) and concluded that effects on body weights in mice exposed during gestation were inconsistent and, when present, usually only occur from treatment with DEHP at doses several orders of magnitude higher than the doses used in the study by Pocar et al. (2012), based on the following summary from page 232 of the ATSDR toxicological profile for DEHP (ATSDR, 2022):

- Gestational studies in mice showed more consistent effects, with decreased offspring body weights in most studies at ≥191 mg/kg-day, but generally not at doses ≤100 mg/kg-day (Ungewitter et al., 2017; Maranghi et al., 2010; RTI International, 1988; Tyl et al., 1988; Shiota and Nishimura, 1982; Shiota et al., 1980).
- One gestational study also reported decreased fetal body weight & crown-rump length at maternal doses ≥50 mg/kg-day during gestation (Shen et al., 2016).
- In contrast, increased F1 offspring body weight and visceral adipose tissue were reported in 1-generation studies at doses ≥0.05 mg/kg-day (Fan et al., 2020; Schmidt et al., 2012).
- However, other 1-generation studies report a lack of body weight effects in offspring at maternal doses up to 180.77 mg/kg-day (Bastos Sales et al., 2018; Tanaka, 2002).
- Similarly, no changes in body weight or visceral or inguinal adipose tissue were observed in postnatal week (PNW) 22 mouse offspring following maternal exposure to 0.05 or 500 mg/kg-day throughout gestation & lactation followed by high-fat diet consumption for 19 weeks, compared with unexposed high-fat diet controls (Hunt et al., 2017).

Other assessments (Health Canada, 2020; EFSA, 2019; ECHA, 2017a, b; CPSC, 2014) did not reference the study by Pocar et al. (2012). ATSDR characterized the effects on reproductive organ weights and sperm parameters in mice in this study as “potentially transient”, noting that the evidence for severe and permanent reproductive tract malformation and lesions in rat offspring occur at much higher maternal doses (3 to 10 mg/kg-day), and EPA concurs with this conclusion. Furthermore, the effects on sperm count and viability and oocyte maturation and degeneration observed in Pocar et al. (2012) did not manifest in functional deficits in reproductive performance in CD-1 mouse offspring exposed to DEHP at doses up to 95 mg/kg-day from GD1 to GD17 (RTI International, 1988).

Finally, in a subsequent study by Pocar et al. (2017), pregnant CRL CD-1 mice were fed test diets at comparable doses as in the previous study (0, 0.05, and 5 mg/kg-day) throughout gestation and lactation (GD0 to LD21). Female offspring were mated with untreated males through a total of three generations to determine transgenerational effects. The cleavage rate, blastocyst rate, and blastocyst/cleaved rate were decreased at 0.05 mg/kg-day in all three generations; however, the rates at 5 mg/kg-day were comparable to controls, indicating that the effect was not dose-related. In addition to being unrelated to dose, the lack of replicability between the two studies conducted in the same lab at the same doses increases uncertainty that these findings are due to treatment and reduces EPA's confidence in the use of this study for POD derivation.

3.1.3 Conclusions on Developmental and Reproductive Toxicology

3.1.3.1 Conclusions on Developing Reproductive System in Males

Dose-response and temporality:
EPA considered laboratory animal studies with LOAELs less than 20 mg/kg-day to identify any information that may indicate a more sensitive POD than the one established by regulatory bodies prior to the publication of ATSDR in 2022. In this subset of more sensitive studies evaluated by EPA, DEHP exposure resulted in treatment-related effects on the developing male reproductive system in numerous oral exposure studies in rodents, of which 15 studies (comprising 19 publications) were well-conducted and reported LOAELs in a narrow dose range of 10 to 15 mg/kg-day based on a suite of effects consistent with phthalate syndrome. EPA has previously considered the more complete database of studies including effects occurring at higher doses in studies which identified LOAELs higher than 20 mg/kg-day. The dose-response across that broader range of doses is described 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), in which EPA examined the weight of evidence and concluded that oral exposure to DEHP can induce effects on the developing male reproductive system consistent with a disruption of androgen action. The epidemiology data, while providing moderate to robust evidence of effects on the developing male reproductive system, generally have uncertainties related to exposure characterization and temporality which could not be established due to the study design, thus the epidemiological evidence was deemed inadequate to be used in exposure-response analysis. More specifically, the cross-sectional nature of many of the epidemiological studies precluded EPA from establishing whether the exposure preceded the outcome in these studies.

Strength, consistency, and specificity:
In studies in laboratory animals, DEHP exposure resulted in treatment-related effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in numerous oral exposure studies in rodents, of which 15 studies (comprising 19 publications) were well-conducted and reported LOAELs at or below 20 mg/kg-day (Table 3-5 and Table 3-6). Rodents perinatally exposed to DEHP in these 15 studies showed treatment-related effects consistent with phthalate induced androgen insufficiency, including: altered testosterone production; decreased steroidogenic and cholesterol transporter gene expression (*Scarb1*, *Star*, *Hsd17b12)*; FLC aggregation, decreased AGD; increased NR; decreased male reproductive organ weights (prostate, seminal vesicles, epididymis, and LABC); delayed sexual maturation, decreased sperm production, count, viability, and motility; testes histopathology (*e.g.*, inhibition of spermatogenesis, tubular atrophy, Sertoli cell vacuolation, germ cell sloughing in seminiferous tubules, lymphocytic infiltration in testicular interstitium), and reproductive tract malformations in males indicative of phthalate syndrome. Beyond the evidence provided by this subset of more sensitive studies, the strength, specificity, and

consistency of the effects of DEHP on the developing male reproductive system consistent with a disruption of androgen action is well described in EPA's consideration of the weight of evidence in the *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).

Although the epidemiological evidence for the association between exposure to DEHP and male developmental and reproductive outcomes varied across assessments, EPA notes some qualitative similarities in the findings from epidemiology and laboratory animal studies, such as decreases in testosterone, sperm parameters, and AGD; thereby underscoring the human relevance of these endpoints. ATSDR (2022) found that adult males who are exposed to DEHP may potentially have lower serum testosterone levels and lower-quality semen. These findings in humans may align with decreases in genes involved in steroidogenesis in the fetal testes of rats, along with decreases in testosterone, and effects on sperm parameters (*e.g.*, count, motility, and/or morphology) (U.S. EPA, 2023a; Vo et al., 2009a; Andrade et al., 2006c). Consistent with the conclusions from Health Canada (2018b), EPA agrees that the lack of sufficient studies on testosterone production in developing human males, as well as the different matrices used to estimate fetal testis testosterone production (cord blood or amniotic fluid), and the variations in when testosterone is measured (during pregnancy or at delivery) make the data insufficient to draw inferences.

Similarly, many laboratory studies have demonstrated that oral exposure of rats to DEHP during the masculinization programming window can reduce male rat pup AGD. Effects on AGD were reported in 19 studies included in Table 3-8 of the *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023a), several of which were included in the pool of 50 studies evaluated in the current assessment (Pocar et al., 2012; Christiansen et al., 2010; Gray et al., 2009; Vo et al., 2009a; Lin et al., 2008; Andrade et al., 2006a; TherImmune Research Corporation, 2004). Epidemiology assessments tend to support the decreased AGD noted in animal studies, with NASEM (2017) concluding that there is a moderate degree of evidence for an association between fetal exposure to DEHP and decreases in AGD, and Radke et al. (2018) also concluding that there was moderate evidence for the association between exposure to DEHP and AGD, in addition to robust evidence overall for the association between DEHP exposure and male reproductive outcomes. However, Health Canada (2018b) found inadequate evidence to support an association between DEHP and AGD. While some findings regarding AGD are inconsistent across assessments, particularly Health Canada (2018b), EPA agrees with the conclusions made by NASEM (2017) and Radke et al. (2018) that there is moderate evidence for the association between increased exposure to DEHP and decreased AGD, as well as decreased testosterone and sperm parameters.

Laboratory studies indicate DEHP can result in reproductive tract malformations in androgen-dependent organs (Blystone et al., 2010; Christiansen et al., 2010; Gray et al., 2009; TherImmune Research Corporation, 2004). In an epidemiology assessment, Radke et al. (2018) found an indeterminate level of confidence in the association between exposure to DEHP and cryptorchidism/hypospadias, but this association was not consistent with the findings of Health Canada (2018b) or NASEM (2017).

Biological plausibility and coherence[3]:
Animal data available from the subset of more sensitive studies (LOAEL less than 20 mg/kg-day) on DEHP supporting the MOA for phthalate syndrome indicated robust evidence across the key events in the adverse outcome pathway, including cellular responses (*e.g.*, decreases testosterone production and steroidogenic gene and protein expression), organ responses (Leydig cell aggregation, changes in Sertoli cell function and development, decreased AGD, increased nipple retention, changes in reproductive organ weights and histopathology), and organism-level effects on reproduction. Collectively, available studies consistently demonstrate that oral exposure to DEHP during the masculinization programming window in rats can disrupt androgen action, leading to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. As noted above, this conclusion was supported by the SACC (U.S. EPA, 2023b).

Additionally, several studies examined developmental and reproductive effects in male rodents exposed post-parturition. Although these studies entailed initiation of dosing after parturition, hazards to the developing male reproductive system, similar to those resulting from gestational exposure, were observed, including changes in: sperm morphology (Hsu et al., 2016); testosterone and/or estradiol production (Li et al., 2012; Vo et al., 2009b; Ge et al., 2007; Akingbemi et al., 2004; Akingbemi et al., 2001); Leydig cell proliferation (Guo et al., 2013; Li et al., 2012; Akingbemi et al., 2004); sexual maturation in males(Ge et al., 2007); male reproductive organ weights (Vo et al., 2009b; Ge et al., 2007) and histopathology (Kitaoka et al., 2013; Vo et al., 2009b; Ganning et al., 1990); and decreased AGD (Vo et al., 2009b).

Readers are directed to see EPA's Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act (U.S. EPA, 2023a) for a more thorough discussion of DEHP's effects on the developing male reproductive system and EPA's MOA analysis and to the ATSDR's Toxicological Profile for Di(2-Ethylhexyl)Phthalate (DEHP) (ATSDR, 2022) for a complete description of this hazard, including the literature supporting effects at doses higher than considered by EPA in its focused scope for dose-response analysis.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
EPA has previously considered the weight of evidence and concluded that oral exposure to DEHP 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)), and this conclusion was supported by the SACC (U.S. EPA, 2023b). In studies in laboratory animals, DEHP exposure resulted in treatment-related effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in numerous oral exposure studies in rodents, of which 15 studies (comprising 19 publications) were well-conducted and reported LOAELs within a narrow dose range of 10 to 15 mg/kg-day based on the suite of effects on the developing male reproductive system consistent with phthalate syndrome. While epidemiology studies for DEHP generally have uncertainties related to exposure characterization, available studies provide moderate to robust evidence of effects on the developing male reproductive system, including decreases

[3] As defined by the 2005 EPA Cancer Guidelines: *Biological plausibility*: an inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A lack of mechanistic data, however, is not a reason to reject causality. *Coherence*. An inference of causality may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. Information is considered from animal bioassays, toxicokinetic studies, and short-term studies. The absence of other lines of evidence, however, is not a reason to reject causality.

in AGD and testosterone and effects on sperm parameters. In conclusion, EPA considers the observed developmental effects in males to be relevant for human health risk assessment and therefore further evaluated developmental toxicity via dose-response analysis in Section 4.

3.1.3.2 Conclusions on Developing Reproductive System in Females

Dose-response and temporality:
In the study by Zhang et al. (2014), pregnant mice were administered DEHP in 0.1 percent DMSO at 0 or 0.04 mg/kg-day throughout gestation (GD 0.5 to GD18.5) and allowed to deliver naturally on GD19.5 (PND0), and F1 females were mated with wild-type (untreated) males. On GD12.5, maternal serum estradiol at 0.04 mg/kg-day was lower than controls, and gene expression of *Cyp17a1*, *Cyp19a1*, *Aldhla1*, *ERα*, *FSHR*, *LHR*, *EGF*, and *EGFR* was significantly down-regulated in fetal mouse ovaries. On GD13.5, gene and protein expression of the meiosis-specific *Stra8* gene was lower than controls. On GD17.5, delayed meiotic progression of female germ cells in fetal mouse ovary was characterized by the percent of oocytes at leptotene (26.43%) and zygotene (60.17%) stages in treated group higher than controls (4.33% leptotene and 29.57% zygotene), with fewer treated animals in pachytene and diplotene stages. On PND 21, there was a decrease in the number of primary and increase in the number of secondary follicles in ovaries of F1 offspring. In the F2 females, the number of primordial follicles was significantly lower, and the number of secondary follicles was significantly higher, in the treated group compared to controls on PND21. Although ATSDR selected this study as the principal study for derivation of the intermediate oral MRL, EPA considered the fact that only a single dose level was tested to be a substantive limitation, in that it is not possible to examine dose-response.

In discussing the effects of DEHP on female developmental and reproductive system, ATSDR (2022) reported that there is a paucity of epidemiological data evaluating the effects of DEHP exposure on female developmental and reproductive outcomes. This is due to either 1) the fact that urine samples were taken after the desired outcome and/or 2) exposure estimates were determined by a method other than using urinary metabolites. There were no associations found between DEHP exposure and time to conception in three prospective cohort studies of couples who stopped taking birth control in order to become pregnant (Thomsen et al., 2017; Jukic et al., 2016; Buck Louis et al., 2014). Jukic et al. (2016) assessed the menstrual cycle and found that there was no association between altered luteal or follicular phase length and the majority of DEHP metabolites. In several epidemiological studies, preterm birth was assessed using a categorical measure (<37 weeks of gestation). Health Canada (2018a) reported that there was limited evidence for the association between DEHP metabolites and altered female puberty (MECPP, MEHHP, MEOHP, and MEHP), as well as age at menopause (MEHHP and MEOHP). There was insufficient data to support a link between exposure to DEHP (MEHP, MEOHP, MEHHP) and polycystic ovarian syndrome (PCOS) or pregnancy loss. The degree of evidence supporting a relationship between altered fertility and exposure to DEHP (MEHP, MEHHP, MEOHP, and MECPP) could not be established. DEHP metabolites (MEHP, MEOHP, MEHHP, MECPP, MCMHP) were not shown to be associated with time to pregnancy or sex ratio. Radke et al. (2019b) determined that there is moderate evidence that preterm birth is associated with DEHP exposure. They also determined that there is slight evidence of association between spontaneous abortion and DEHP exposure, and the degree of uncertainty stems from inconsistent results in the high confidence studies. Finally, Radke et al. (2019b) found that there is indeterminate evidence of an association between DHEP exposure and pubertal development.

In a study by Pocar et al. (2012), CD-1 mice were administered DEHP in the diet at 0, 0.05, 5, and 500 mg/kg-day throughout gestation and lactation (GD0.5 to LD21). Abortion occurred in 9 out of 10 dams

at 500 mg/kg-day; therefore, evaluation of effects in offspring was limited to the 0.05 and 5 mg/kg-day groups compared to controls. *In vitro* oocyte maturation tests showed a decrease in mature oocytes in the treated groups (80%) compared to controls (88%) and an increase in degenerated oocytes (18% treated vs. 8% controls). The dose- response is essentially flat for oocyte maturation and degeneration, even though the mid dose is 100× higher than the low dose.

Strength, consistency, and specificity:
The effects on delayed meiotic progression of germ cells in fetal ovaries and accelerated folliculogenesis reported in the study by Zhang et al. (2014) were not examined in other oral studies in rodents. However, in a study of young sexually mature mice gavaged with DEHP for 30 days (Parra-Forero et al., 2019), significant differences in the number of oocytes and on zygote development were observed, with decreases in the numbers of oocytes and fertilized oocytes, increases in zygote fragmentation and arrested zygote development, and decreases in DNA replication at 2 mg/kg-day compared to controls.

In the study by Shao et al. (2019), in which 15-day old female Wistar rats were administered DEHP via oral gavage at 0, 0.2, 1, or 5 mg/kg-day for 4 weeks, the apparent effect on ***accelerated*** sexual maturation in the females treated with 5 mg/kg-day DEHP in this study is not replicated in other studies, therefore casting uncertainty on the attribution of this finding to treatment with DEHP. Specifically, under a different exposure paradigm targeting an earlier developmental stage, studies by Grande et al. (2006) reported that time to vaginal opening was ***delayed*** by 2 days in Wistar rats gavaged with 15 mg/kg-day DEHP from GD6 to LD21, with similar delays in preputial separation noted in the males (Andrade et al., 2006a), with body weights comparable to controls. The age at first estrus was slightly delayed at 135 mg/kg-day and above (41.2 to 41.8 days) compared to controls (39.2 days), but the increased time to first estrus was not statistically significant. There were no dose-related effects on body weight at sexual maturation or body weight at first estrus. Similarly, in the three-generation reproduction study of SD rats (Blystone et al., 2010; TherImmune Research Corporation, 2004), vaginal opening and preputial separation were delayed by up to a week in the F1c, F2c, and F3c pups starting at 7500 ppm (359 mg/kg-day), associated with decreased body weights in these animals. Finally, the fact that body weights were not reported in the study by Shao et al. (2019) precluded EPA from evaluating any relationship between growth and sexual development in that study.

In a study by Pocar et al. (2012), CD-1 mice were administered DEHP in the diet at 0, 0.05, 5, and 500 mg/kg-day throughout gestation and lactation (GD0.5 to LD21). Abortion occurred in 9 out of 10 dams at 500 mg/kg-day; therefore, evaluation of effects in offspring was limited to the 0.05 and 5 mg/kg-day groups compared to controls. While many of the differences in organ weight and percent body fat were not dose-dependent and/or may be related to decreased body weight at PND42, the increase in absolute ovary weight cannot be explained by decreased body weight. *In vitro* oocyte maturation tests showed a decrease in mature oocytes in the treated groups (80%) compared to controls (88%) and an increase in degenerated oocytes (18% treated vs. 8% controls). In order to determine if the effects on body weights reported in the study by Pocar et al. (2012) were due to treatment with DEHP or if they were incidental, EPA considered the examination of the effects of DEHP on body weights in mouse studies evaluated by ATSDR (2022) and concluded that effects on body weights in mice exposed during gestation were inconsistent and, when present, usually only occur from treatment with DEHP at doses several orders of magnitude higher than the doses used in the study by Pocar et al. (2012).

Finally, in a subsequent study by Pocar et al. (2017), pregnant CRL CD-1 mice were fed test diets at comparable doses as in the previous study (0, 0.05, and 5 mg/kg-day) throughout gestation and lactation (GD0 to LD21). Female offspring were mated with untreated males through a total of three generations to determine transgenerational effects. The cleavage rate, blastocyst rate, and blastocyst/cleaved rate

were decreased at 0.05 mg/kg-day in all three generations; however, the rates at 5 mg/kg-day were comparable to controls, indicating that the effect was not dose-related. In addition to being unrelated to dose, the lack of replicability between the two studies conducted in the same lab at the same doses increases uncertainty that these findings are due to treatment and reduces EPA's confidence in the use of this study for POD derivation.

Biological plausibility and coherence:
The studies examining effects on the female reproductive tract are limited in number, and in the species and doses tested, and the majority of the reported endpoints are not replicated across studies. Three studies in mice indicate that it may be possible that oral exposure to DEHP could delay meiotic progression of germ cells in fetal ovaries and accelerate folliculogenesis (Zhang et al., 2014), decrease oocyte maturation and increase oocyte degeneration (Pocar et al., 2012), and/or arrest zygote development in young sexually mature mice (Parra-Forero et al., 2019). However, there is no proposed adverse outcome pathway that establishes a mechanism through which these effects may occur, and these endpoints were either not examined in other oral studies in rodents or they were not consistently observed in studies in which they were examined. The apparent effects in these studies were noted at doses from 0.04 to 5 mg/kg-day (Pocar et al., 2012). However, no functional deficits in reproductive performance were observed in CD-1 mouse offspring exposed to DEHP at doses up to 95 mg/kg-day from GD1 to GD17 in a study by NTP (1988). Therefore, the persistence, biological relevance, and adversity were not established for effects on folliculogenesis, oocyte maturation and degeneration, and zygote development in mice orally exposed to DEHP.

The study by Shao et al. (2019) dosed 15-day old female Wistar rats with DEHP via oral gavage at 0, 0.2, 1, or 5 mg/kg-day for 4 weeks and proposed that DEHP may activate hypothalamic GnRH neurons prematurely through IGF-1 signaling pathway and promote GnRH release, leading to the accelerated sexual maturation observed in female rats at 5 mg/kg-day. The study demonstrated changes in gene and protein expression and decreased apoptosis in the hypothalamus at 0.2 and 1 mg/kg-day; however, EPA did not consider these differences to be definitive evidence of an adverse response to the treatment with DEHP. Furthermore, just was with the effects on folliculogenesis, a potential adverse outcome pathway of phthalates on the hypothalamus is not well established.

In the study reported in a series of publications by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006), pregnant Wistar rats were administered DEHP by oral gavage from implantation through weaning of offspring. Mean time to vaginal opening showed a small but significant delayed in F1 females at 15 mg/kg-day and above compared to controls, accompanied by a slight delay in the age at first estrus at 135 mg/kg-day and above which was not statistically significant. There were no dose-related effects on body weight at sexual maturation or body weight at first estrus. Given that sexual maturation was slightly delayed in the F1 female offspring at 15 mg/kg-day and above, but body weights were comparable to controls on the day of vaginal opening, it may be that DEHP delayed sexual maturation secondary to effects on growth, and animals matured when they reached a similar body weight. Regardless of the relationship between growth and development, the effects on vaginal opening at 15 mg/kg-day were not more sensitive than the effects on males at the same dose in the study by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006), indicating that females are not more sensitive than males.

Andrade et al. (2006b) reported results from the same study, specifically the examination of aromatase activity in the hypothalamic/preoptic area brain sections from a subset of F1 offspring. Although the authors proposed a biphasic, non-monotonic effect of DEHP on aromatase activity in the hypothalamic/preoptic area that differed between males and females and at different ages, none of these

statistically significant differences were dose related. Therefore, EPA does not consider the findings reported in this study to be sufficient to conclude that the differences in aromatase explain the treatment-related effect of delayed sexual maturation.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
Radke et al. (2019b) provided a summary of epidemiology evidence for effects of DEHP exposure on reproduction and development in females and found slight confidence in the association between DEHP exposure and time to pregnancy, slight confidence in the association with DEHP and increases in spontaneous abortion, and moderate confidence in the association between DEHP exposure and increases in preterm birth. EPA took into account the conclusions drawn by ATSDR (2022), Health Canada (2018b), NASEM (2017) and systematic review publications by Radke et al. (2019b; 2018) and agree that there is some evidence that DEHP exposure is associated with these reproductive outcomes in females.

There are several studies in rodents which indicate effects of DEHP exposure during gestation and/or lactation on the developing female reproductive tract. However, the studies showing effects on folliculogenesis, oocyte maturation and degeneration, and zygote development in mice orally exposed to DEHP at doses from 0.04 to 5 mg/kg-day (Parra-Forero et al., 2019; Zhang et al., 2014; Pocar et al., 2012) did not show dose-concordance and coherence with the developmental toxicity study in which no functional deficits in reproductive performance were observed in CD-1 mouse offspring exposed to DEHP at doses up to 95 mg/kg-day from GD1 to GD17 in a study by NTP (1988). Therefore, the persistence, biological relevance, and adversity were not established for these effects.

The study by Shao et al. (2019) on rats had several limitations, including the fact that body weights were not reported which precluded EPA from evaluating any relationship between growth and sexual maturation in that study. However, the primary limitation with the study by Shao et al. (2019) centers around the fact that a potential adverse outcome pathway of phthalates on the hypothalamus is not well established, so EPA did not consider the changes in gene and protein expression and decreased apoptosis in the hypothalamus at 0.2 and 1 mg/kg-day to be definitive evidence of an adverse response to the treatment with DEHP. Furthermore, the apparent effect on accelerated sexual maturation in the females treated with 5 mg/kg-day DEHP in this study is not replicated in other studies, in which delays (and not acceleration) in sexual maturation are observed (Blystone et al., 2010; Andrade et al., 2006a; Grande et al., 2006; TherImmune Research Corporation, 2004).

The study by Grande et al. (2006) indicating delayed vaginal opening in F1 females at 15 mg/kg-day and above corroborates similar findings on preputial separation in males from the companion study report (Andrade et al., 2006a) and therefore indicates that the developing female reproductive tract is not more sensitive than that of males. The lack of established mechanism underlying effects on sexual maturation further contributes to uncertainty around the plausibility of the effects. While a single study evaluated the potential for altered aromatase activity in the hypothalamic/preoptic area brain sections from a subset of F1 offspring in this study (Andrade et al., 2006b) to contribute to the effects on sexual maturation, the evidence in that study is not sufficient to define a clear MOA to explain the treatment-related effects described in the other publications by Andrade and Grande et al. (2006c; 2006a; 2006).

There are a growing number of studies examining the female reproductive tract as a target of DEHP toxicity. However, there is uncertainty given the limited strength, consistency, specificity, dose concordance, biological coherence, and established adversity associated with effects in many of these studies (Parra-Forero et al., 2019; Shao et al., 2019; Zhang et al., 2014; Pocar et al., 2012), or the fact

that they do not provide a sex-specific endpoint that is more sensitive than the well-established effects on developing male reproductive tract (Andrade et al., 2006a; Grande et al., 2006). EPA concluded that there is too much variability in the adverse outcomes in these studies to use them quantitatively for risk characterization. Notably, SACC ultimately agreed with this conclusion, noting that this variability may be due to differences in endocrine status (age, reproductive stage such as pubertal, adult, aging, menopausal) which "reinforces the difficulties in identifying a specific POD for adverse female reproductive system effects" (U.S. EPA, 2025o).

3.2 Nutritional/Metabolic Effects Related to Glucose/Insulin Homeostasis and Lipid Metabolism

3.2.1 Summary of Epidemiological Studies

The Agency reviewed and summarized the conclusions from previous assessments conducted by ATSDR (2022) and Health Canada (2018b), as well as a systematic review publication by Radke et al. (2019b) that investigated the association between urinary metabolites of DEHP and metabolic effects, including glucose homeostasis and lipid metabolism.

3.2.1.1.1 ATSDR (2022)

ATSDR (2022) reviewed many epidemiological studies, most of which had a cross-sectional design and investigated the relationships between urinary metabolites, an estimation of DEHP exposure, and anthropometric measures of body weight, including BMI, waist circumference, and risk of obesity or overweight. ATSDR concluded there was no consistent association found in the existing data assessing the effects of phthalate exposure on obesity outcomes or waist circumference or distribution of fat. Although the human epidemiological data indicate a possible link between adult obesity and DEHP exposure, ATSDR concluded this research was limited by their cross-sectional design and inconsistent confounder control, and EPA concurs.

3.2.1.1.2 Health Canada (2018a)

Health Canada (2018a) evaluated the epidemiological evidence of the data supporting the relationship between insulin resistance and glucose control biomarkers in children, adolescents, and adults and DEHP metabolites (MEHP, MEHHP, MEOHP, 6OH-MEHP, and MECPP) and found that the level of evidence of an association was inadequate for several DEHP metabolites (*i.e.,* 6OH-MEHP, MECPP), while there was limited evidence for association between DEHP metabolites (MEHP, MEHHP, MEHOP) and insulin resistance and glucose biomarkers. Health Canada also indicated that there was inadequate evidence for the association between exposure to DEHP metabolites (MEHHP, MEOHP, and MECPP) and adult-onset diabetes; and the additional DEHP metabolites (MEHP, MCMHP) and the diagnosis of diabetes was not supported by sufficient evidence. In individuals with Type 2 Diabetes (T2D), there was insufficient evidence to support a link between DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and eye conditions or retinopathy.

3.2.1.1.3 Radke et al. (2019a)

A systematic review by Radke et al. (2019a) assessed the evidence of an association between DEHP exposure and diabetes, insulin resistance, gestational diabetes, obesity, and renal effects across seven epidemiological studies. Radke et al. (2019a) found that the study conducted by Sun et al. (2014) showed a statistically significant strong positive association between the risk of diabetes and increased

exposure to DEHP. However, the results were not pooled, and the small non-significant positive associations were limited to participants from NHSII, a younger cohort with higher exposure levels and a more homogenous mean age of 46 vs. 66. Furthermore, the associations were more pronounced when controlling for BMI. Seven studies were used to evaluate the evidence for an association between adult insulin resistance and DEHP exposure. Higher levels of exposure were associated with higher levels of glucose, insulin, and homeostatic model assessment of insulin resistance (HOMA-IR), according to five studies, four of which were medium confidence studies. Four studies had results for at least one outcome that were statistically significant, and Huang et al. (2014) found exposure-response gradients for all three of the outcomes. However, some uncertainties exist as a result of the limitations of the study. Although this is somewhat mitigated by an analysis in women correcting for history of DM and finding consistent results, the positive result in Kim et al. (2013) is challenging to interpret because the investigation included patients with a history of diabetes diagnosis. Although the study by Dirinck et al. (2015) had high effect estimates, uncertainties were found in the study evaluation, selection bias (obese patients), as well as residual confounding by diet and other factors making the results difficult to interpret. Caloric intake was considered in two studies (Huang et al., 2014; Kim et al., 2013), but this may not be sufficient to account for variations in exposure levels by type of food (*e.g.*, packaged foods). Despite these drawbacks, the exposure-response gradient shown in a sensitive and well-conducted investigation and the general consistency in the direction of the association across studies boost confidence in the association. Out of the three studies, only one showed that increased exposure to DEHP is associated with increased insulin resistance in adolescents. According to three research conducted in children, there was no discernible association between DEHP exposure and insulin resistance in children (Carlsson et al., 2018; Kataria et al., 2017; Watkins et al., 2016).

Radke et al. (2019a) found that there was no conclusive evidence of an association between elevated blood glucose and increased exposure to DEHP phthalates among the four studies reviewed that looked at blood glucose and/or impaired glucose tolerance in pregnant women. In one study, there was coherence between diabetes and insulin resistance and consistency and an exposure-response gradient for DEHP exposure; however, the results for diabetes were not statistically significant. An earlier analysis by Thayer et al. (2012) found inconsistencies in the data on phthalate exposure and obesity. According to a single prospective investigation on adult weight increase by Song et al. (2014), there was no association between DEHP and weight gain over a ten-year period. The study employed the controls from the nested case-control study on type 2 diabetes previously reported by Song et al. (2014).

3.2.1.1.4 Summary of the existing assessments of Nutritional/Metabolic Effects on Glucose Homeostasis

Each of the assessments discussed above provided qualitative support as part of the weight of scientific evidence for the link between DEHP exposure and nutritional/metabolic effects on glucose homeostasis and lipid metabolism. ATSDR (2022) found that the available research evaluating the impact of phthalate exposure on obesity outcomes, waist circumference, or fat distribution did not consistently show any association. Even though epidemiological research suggests a potential connection between DEHP exposure and obesity, ATSDR (2022) came to the conclusion that the cross-sectional design and uneven confounder control were the study's limitations. Health Canada (2018a) found that there was insufficient evidence to support an association between DEHP metabolites and adult-onset diabetes, but there was an association between DEHP exposure and insulin resistance and glucose biomarkers. Radke et al. (2019a) found that exposure to DEHP has a slight but non-significant positive association with type 2 diabetes, however they indicated that interpreting results for insulin resistance is challenging since diet may still cause residual confounding. Radke et al. (2019a) also found that blood glucose levels did not appear to rise in response to increasing DEHP exposure and there was no association seen with

obesity. The scope and purpose of the assessments by ATSDR (2022), Health Canada (2018b), and systematic review articles by Radke et al. (2019a), were similar in conclusions draw. 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 regardless of the limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.2.1.1.5 EPA Conclusion

Overall, EPA took into account conclusions drawn by ATSDR (2022), ECCC/HC (2018a), Health Canada (2018a), and systematic review articles published by Radke et al. (2019a) and found that the cross-sectional design of many of the studies, the inconsistencies in controlling confounding, coherence and other uncertainties in the studies do not make clear whether there is a definitive association between DEHP and nutritional and metabolic effects. 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. Thus, the epidemiological studies provide qualitative support as part of weight of scientific evidence.

3.2.2 Summary of Laboratory Animal Studies

As discussed in Section 1.2.2, EPA considered laboratory animal studies with LOAELs less than 20 mg/kg-day to identify any information that may indicate a more sensitive POD than the one established by regulatory bodies prior to the publication of ATSDR in 2022. In the subset of studies that were evaluated by EPA, effects of DEHP on nutritional and metabolic effects were examined in four prenatal exposure studies (3 in mice and 1 in rats) (Fan et al., 2020; Gu et al., 2016; Rajesh and Balasubramanian, 2014; Schmidt et al., 2012); three perinatal exposure studies (1 in mice and 2 in rats) (Rajagopal et al., 2019a, b; Schmidt et al., 2012; Lin et al., 2011b); three lactational exposure studies in rats (Parsanathan et al., 2019; Venturelli et al., 2015; Mangala Priya et al., 2014); and seven studies in which rats or mice were directly exposed for durations of greater than one day (Zhang et al., 2020b; Ding et al., 2019; Li et al., 2018; Xu et al., 2018; Venturelli et al., 2015; Rajesh et al., 2013) or chronic (>90 days) duration (Zhang et al., 2017). No inhalation or dermal studies reporting effects of DEHP related to nutritional and metabolic health outcomes were available. Available studies are summarized in Table 3-7 and Appendix B.2. These studies are discussed further below.

3.2.2.1 Prenatal, Perinatal, and Lactational Exposure

Available prenatal (3 studies), perinatal (3 studies), and lactational (3 studies) exposure studies of DEHP in rats and mice report nutritional and metabolic effects that are related to glucose/insulin homeostasis and lipid metabolism. These effects are generally inconsistent across studies irrespective of exposure, species, and sex, have a limited number of supportive studies, or are of uncertain biological significance.

Gu et al. (Gu et al., 2016) studied the effects of gestational exposure to 0.05, and 500 mg/kg-day DEHP in male and female mice. Dams in the 500 mg/kg-day dose group exhibited a 100 percent abortion rate; therefore, no litters were available for examination at higher doses. No adverse reproductive effects occurred in the 0.05 mg/kg-day dose group; therefore, this group and the control mice were used for subsequent stages of the study. The following outcomes related to glucose/insulin homeostasis and lipid metabolism changed significantly in both male and female F1 mice in the 0.05 mg/kg-day dose group:

increased visceral fat weight, fasting blood glucose, serum leptin, triglycerides, and total cholesterol. Body weight and subcutaneous fat weight remained unchanged relative to control animals.

Fan et al. (2020), studied the effects of gestational exposure to 0.2, 2, and 20 mg/kg-day DEHP in male and female mice. Bodyweight increased at weeks 5 through 12 in F1 males in the lowest tested dose (0.2 mg/kg-day) but remained unchanged at higher doses in males and in all F1 females; therefore, only males from the 0.2 mg/kg-day dose group and the control group were used for subsequent stages of the study. The following outcomes related to glucose/insulin homeostasis and lipid metabolism changed significantly in F1 male mice in the 0.02 mg/kg-day group relative to control: increased fat mass, decreased energy expenditure, increased plasma glucose, increased serum lipids (total cholesterol, triglycerides, HDL, and LDL), and increased blood glucose AUC following the glucose tolerance test (GTT). Additionally, histological changes (white adipose adipocyte hypertrophy and lipid droplets in liver cells) were noted in F1 males in the 0.02 mg/kg-day dose group, although these results were not quantified or statistically analyzed.

Rajesh et al. (2014) studied the effects of gestational exposure to 1, 10, and 100 mg/kg-day DEHP in male and female rats. The following outcomes related to glucose/insulin homeostasis and lipid metabolism changed significantly and dose-dependently starting at the lowest tested dose (1 mg/kg-day) in both male and female F1 rats: decreased lean body weight, increased fasting glucose, decreased fasting insulin, decreased glycogen concentration in the gastrocnemius muscle, and decreased glucose uptake and oxidation in the gastrocnemius muscle. Additionally, fat weight increased dose-dependently starting at 10 mg/kg-day in both male and female rats. Glucose levels remained dose-dependently increased relative to control throughout the GTT and insulin tolerance test (ITT), although statistical significance varied across measurement timepoints, and overall AUC was not measured.

Schmidt et al. (2012) studied the effects of peri-natal exposure to 0.05, 5, and 500 mg/kg-day DEHP in male and female mice. Dams in the 500 mg/kg-day dose group exhibited a 100 percent abortion rate; therefore, no litters were available for examination at higher doses. DEHP exposure did not significantly alter the litter size in the other groups; therefore, these groups were used for subsequent stages of the study. Body weight increased significantly and dose-dependently in F1 males and females, and females were more sensitive than males. Specifically, on PND 21, bodyweight increased significantly starting at 0.05 mg/kg-day in females and 5 mg/kg-day in males. At PND 85, bodyweights were significantly and dose-dependently increased at 0.05 mg/kg-day for both sexes. Visceral fat weight also increased in males and females beginning at the low dose (0.05 mg/kg-day); however, the changes were only dose-dependent in females, as changes decreased back towards control levels at the highest dose in males.

Lin et al. (2011b) studied the effects of peri-natal exposure to 1.25 mg and 6.25 mg/kg-day DEHP in male and female rats. This study is limited because rats were only exposed to two low doses (1.25 and 6.25 mg/kg-day); however, it measured several endpoints related to glucose/insulin homeostasis across multiple timepoints, allowing the analysis of temporal concordance. The following outcomes remained significantly altered in the same direction for both doses across all timepoints measured (PND 21, PNW 15, and PNW 21): β-cell ultrastructural changes in F1 males and females including increased mitochondrial area, increased optical density, decreased filled granules, and increased immature and empty granules.

Importantly, several outcomes related to glucose/insulin homeostasis, although significantly altered in the same direction across both doses at a given time, were transient, or differed in the direction of change depending on the timepoint at which they were measured, as well as the sex of the animal. In both females and males, effects on insulin resistance (as measured by glucose levels after the ITT) were

transient and returned to control levels at PNW 27 in both sexes. Additional increases in fasting glucose and glucose tolerance (as measured by glucose levels after the GTT) and decreases in insulin production (indicated by decreased pancreatic insulin content, decreases in fasting insulin, and decreases in insulin levels after the GTT relative to control) were specific to females. Specifically, in females, fasting blood glucose and glucose tolerance temporarily decreased at PND 21 before approaching control levels at PNW 15 and increasing at PNW 27. The latency of increases in fasting glucose and glucose tolerance (PNW 27 following lactational exposure) increases the uncertainty about the dose- and time-concordance of these effects in female rats. The study authors hypothesized that these changes were likely due to a sex-specific effect of DEHP on impaired insulin secretion in females. In support of this hypothesis, pancreatic insulin content decreased alongside β -cell mass at PND 21 and remained decreased by PNW 27. Additionally, both fasting serum insulin and insulin levels after the GTT decreased in females at PND 21, increased at PNW 15, and decreased by PNW 27. Conversely, in males, changes in fasting blood glucose and glucose tolerance were transient and decreased at PND 21 and PNW 15 before returning to control levels at PNW 27. The study authors hypothesized that the lack of a consistent change in fasting glucose levels and glucose tolerance in males was likely due to an adaptive increase in the release of insulin in males. Accordingly, decreases in pancreatic insulin content and β -cell mass at PND 21 were transient in males and returned to control levels by PNW 27, and fasting serum insulin and insulin levels after the GTT decreased at PND 21, approached control levels at PNW 15, and ultimately increased at PNW 27.

Rajagopal et al. (2019a, b) studied the effects of peri-natal exposure to 10 and 100 mg/kg-day DEHP in male rats. The following outcomes related to glucose homeostasis changed significantly and dose-dependently starting at the lowest dose 10 mg/kg-day in F1 males: increased fasting serum insulin; increased fasting blood glucose, increased HOMA-IR score, increased hepatic glycogen concentration, increased activity of glycogen synthase, and decreased glucose uptake and glucose oxidation by hepatic cells. Additionally, blood glucose levels remained significantly and dose-dependently increased relative to control throughout the GTT and ITT starting at 10 mg/kg-day, although the overall AUC was not measured. Notably, this study is limited in that it only investigated male offspring; therefore, it is unclear whether any of the observed effects are sex specific.

Mangala Priya et al. (2014) and Parsanathan et al. (2019) studied the effects of lactational exposure to 1, 10, and 100 mg/kg-day DEHP in F1 female and male rats, respectively. Fasting blood glucose levels significantly increased across all 3 tested doses in F1 female rats (Mangala Priya et al., 2014). Glucose uptake (attaining significance beginning at 1 mg/kg-day in both studies) and glucose oxidation (attaining significance beginning at 1 and 10 mg/kg-day in males and females, respectively) also decreased dose-dependently in cardiac tissue (Parsanathan et al., 2019; Mangala Priya et al., 2014). Additionally, bodyweight decreased dose-dependently and significantly at PND 22 in F1 male rats starting at 1 mg/kg-day (Parsanathan et al., 2019).

Venturelli et al. (2015) studied the effects of lactational exposure to 7.5 and 75 mg/kg-day in F1 male rats. Insulin tolerance (as measured by decreased glucose decay rate) did not change at earlier timepoints (PND 22 and 60) but increased significantly and dose-dependently at PND 90 beginning at the lowest tested dose (7.5 mg/kg-day). The latency of this effect (PND 90 following lactational exposure) suggests that it may be spurious and not treatment related. Fasting serum glucose increased at the highest tested dose (75 mg/kg-day). Offspring body weights and fasting insulin in the treated groups were comparable to controls. Because this study only investigated male offspring, it is unclear whether the effect on insulin tolerance is sex-specific.

3.2.2.2 Direct Exposure of Adolescents and Adults

Available studies of short-term to subchronic duration (6 studies ranging from 3 to 15 weeks) and chronic duration (1 study) in rats and mice directly exposed to DEHP during adolescence and adulthood reported effects related to glucose/insulin homeostasis and lipid metabolism; however many of these findings are inconsistent across studies irrespective of exposure duration and species. Additionally, it is difficult to determine whether results in these studies are consistent across sexes because all but one study either only studied males or combined data for males and females in each dose group.

Zhang et al. (2017) studied the effects of exposure to 0.05, 5, and 500 mg/kg-day DEHP in adult male rats for 15 weeks. This study measured endpoints related to glucose/insulin homeostasis at multiple timepoints, allowing the analysis of temporal concordance. Fasting blood glucose and insulin levels in the treated groups were comparable to controls throughout the study (PNW 3, 5, and 15). Glucose tolerance (as measured by glucose levels after the GTT) increased over time, although results were not statistically analyzed. Specifically, although glucose remained unchanged when the GTT was performed at PNW 3, glucose increased dose-dependently at 5 mg/kg-day and above at PNW 5. By PNW 15, glucose increased dose-dependently in all treated groups. Insulin levels after the GTT were potentially indicative of an adaptive response to increasing glucose levels in DEHP-treated animals. Specifically, insulin levels after the GTT decreased at PNW 3; however, they approached control levels at 5 weeks and dose-dependently increased at 15 weeks. Terminal bodyweight decreased significantly at 500 mg/kg-day. Because this study only investigated males, it is unclear whether any of the observed effects are sex-specific.

Schmidt et al. (2012) studied the effects of exposure to 0.05, 5, and 500 mg/kg-day DEHP in female mice for 8 weeks. Adipocytes of all DEHP-exposed mice were larger (hypertrophied) than those of controls, and this was confirmed quantitatively by a statistically significant, dose-dependent decrease in the number of adipocytes per unit area starting at the lowest dose. Interestingly, although present across all doses, statistically significant increases in bodyweight were highest at 5 mg/kg-day and visceral fat percentage was highest at 0.5 mg/kg-day, and therefore did not show clear dose-response. Additionally, food intake also followed this trend, increasing significantly in all dose groups with the highest increase in the lower dose groups (0.05 and 5 mg/kg-day). This suggests that increased food intake in DEHP-treated animals may be a confounding factor that is responsible for the effects observed in this study. Because this study only investigated females, it is unclear whether any of the observed effects are sex-specific.

Li et al. (2018) studied the effects of exposure to 1, 10, 100, and 300 mg/kg-day DEHP male mice (starting at 5 to 6 weeks old) for 35 days. Dose-dependent decreases in terminal body weight reached significance starting at 100 mg/kg-day. Additionally, triglyceride levels increased dose-dependently at 1, 10, and 100 mg/kg-day; however, they dropped but remained significantly increased relative to control at 300 mg/kg-day. Histopathology indicated that lipid droplets accumulated in hearts of mice at higher (100 and 300 mg/kg-day) doses; however, these results were not statistically analyzed. Because this study only investigated males, it is unclear whether any of the observed effects are sex-specific.

Ding et al. (2019) studied the effects of exposure to 0.18, 1.8, 18, 180 mg/kg-day DEHP in 3-week-old male mice for 3 weeks. The following endpoints related to lipid metabolism changed significantly and dose-dependently: decreased hepatic lipase in the liver (starting at 1.8 mg/kg-day), increased total cholesterol levels (starting at 1.8 mg/kg-day), and decreased serum LCAT and HDL levels (starting at 18 mg/kg-day). Additionally, HbA1C levels, which are related to altered glucose homeostasis, increased significantly and dose-dependently starting at 1.8 mg/kg-day. Other endpoints related to lipid metabolism (increases in body weight gain, triglycerides, and LDL) and increased fasting blood glucose

occurred dose-dependently but did not reach significance until the highest dose tested (180 mg/kg-day). Insulin and C-peptide levels significantly increased at 1.8 and 18 mg/kg/day but were unchanged relative to control at 180 mg/kg-day. Terminal body weight, hepatic glycogen, and HOMA-IR remained unchanged at doses as high as 180 mg/kg-day. Because this study only investigated males, it is unclear whether any of the observed effects are sex-specific.

Zhang et al. (2020b) studied the effects of exposure to 5, 50, and 500 mg/kg-day DEHP in adolescent male and female rats for 8 weeks. At the highest dose tested (500 mg/kg-day), dose-dependent increases in serum total cholesterol and HDL reached statistical significance, and bodyweight significantly increased from 3 weeks through 8 weeks (bodyweight was not significantly altered in lower dose groups). The volume of adipocytes increased at 5 and 50 mg/kg-day, but not 500 mg/kg-day, therefore this increase was not dose-related. Further no statistical analyses were conducted on the adipocyte volume data. Serum triglycerides, LDL, and levels of triglyceride and total cholesterol in the liver and adipose tissue remained unchanged at doses as high as 500 mg/kg-day. Data were combined for males and females in this study, making it impossible to discern whether effects were consistent across both sexes.

Venturelli (2015) studied the effects of DEHP exposure to adolescent male rats administered DEHP at 7.5 and 75 mg/kg-day via oral gavage daily from PND 22 to 52. Dose-dependent increases in fasting serum glucose reached significance at the highest dose (75 mg/kg-day) in male rats. Notably, in this study, no effects were observed on the ITT, bodyweight, weight of fat deposits, fasting serum insulin, serum triglycerides, or serum cholesterol at doses as high as 75 mg/kg-day. Because this study only investigated males, it is unclear whether any of the observed effects are sex-specific.

Rajesh et al. (2013) studied the effects of exposure to 10 and 100 mg/kg-day DEHP in adult male rats for 30 days. The following outcomes related to altered glucose/insulin homeostasis and lipid metabolism changed significantly and dose-dependently starting at the lowest dose (10 mg/kg-day): decreased glycogen levels, glucose uptake, and glucose oxidation in adipose tissue; and increased hydroxyl radical production, hydrogen peroxide generation, and lipid peroxidation in adipose tissue. Because this study only investigated males, it is unclear whether any of the observed effects are sex-specific.

Xu et al. (2018) studied the effects of exposure to 5, 50, and 500 mg/kg-day DEHP in adolescent male and female rats for 28 days. The following outcomes related to glucose/insulin homeostasis changed dose-dependently but did not reach statistical significance until 50 mg/kg-day: increased fasting blood glucose, fasting serum insulin, fasting serum leptin, and HOMA-IR score. No effects on terminal body weights and body weight gain were observed at doses as high as 500 mg/kg-day. Data were combined for males and females in this study, making it impossible to discern whether dose-dependent effects were consistent across both sexes.

Table 3-7. Summary of Studies Evaluating Effects of DEHP on Glucose Homeostasis and Lipid Metabolism

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
Pre-natal exposure studies		
Female C57BL/6J Mice; GD 1–19; gavage; 0, 0.05, 500 mg/kg-d. (Gu et al., 2016) (Medium)[b]	NE/0.05	At 0.05 mg/kg/d (LOAEL): At PNW 9 in F1 males and females: ↑ visceral fat weight; ↑ serum leptin, ↑ insulin, ↑ fasting blood glucose levels, ↑ triglyceride levels; ↑ total cholesterol; Changes in mRNA expression (↑ Tbx15 in SC fat & ↑ Gpc4 in visceral fat) At 500 mg/kg-d: 100% abortion Unaffected outcomes: Food intake in dams; body weight in F1 males and females; subcutaneous fat weight in F1 males and females
Female ICR Mice; 28 days (7 days premating–PND 0); gavage; 0, 0.2, 2, & 20 mg/kg-d. Other than body weight and food consumption, authors only presented data from F1 males dosed at 0.2 mg/kg-d. (Fan et al., 2020) (Medium)[b]	NE/0.2	At 0.2 mg/kg-d in F1 males (higher doses were excluded by study authors): ↑ body weight at weeks 5–12 at the low dose only At PNW 12: ↑ fat mass; ↓ energy expenditure; ↑ plasma glucose; ↑ total cholesterol, triglyceride, HDL, and LDL; histological changes (white adipose adipocyte hypertrophy and lipid droplets in liver cells); ↑ blood glucose and AUC following the GTT; ↑ blood glucose (but not AUC) following the ITT; ↓ mRNA expression of thermogenic genes in the brown fat pads (*Ucp1*, *Cidea*, *Adbr3)* Unaffected outcomes: Body weight in F1 females (all tested doses); food intake in male and female offspring (all tested doses)
Female Wistar Rats; GD 9–21; gavage; 0, 1, 10, 100 mg/kg-d. (Rajesh and Balasubramanian, 2014) (Medium)	NE/1	At ≥1 mg/kg-d (PND 60): In F1 males and females: ↓ lean body weight; ↑ fasting glucose; ↓ fasting insulin; ↑ glucose levels after GTT and ITT; ↓ glycogen concentration in the gastrocnemius muscle; ↓ insulin binding in gastrocnemius muscle; ↓ glucose uptake and oxidation in gastrocnemius muscle; Changes in mRNA expression in gastrocnemius muscle: (↓ IR, AKT1, and GLUT4) Changes in protein expression and phosphorylation in gastrocnemius muscle of F1 males and females:

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
		↓ plasma membrane expression of IR; ↓ $IR^{TYR\ 1162/1163}$; ↓ IRS1 in females; ↓ $IRS1^{Tyr632}$ in males; ↓ $AKT^{Tyr315/316/312}$; ↓ AKT^{Ser473} in males, ↓ c-SRC, ↓ MTOR, ↓ $AS160^{Thr642}$ in males, ↓ ACTN4 in males, ↓GLUT4 in plasma membrane; ↓ GLUT4 in cytosol in males, ↓ GLUT4 immunofluorescence, ↓ nuclear MYOD in males, ↓ nuclear SREBP1c, ↓ binding of MYOD to GLUT4 promoter; ↑ expression of HDAC2 in cytosol, PTEN, binding of HDAC2 to GLUT4 promoter, Dnmt1 mRNA and protein, Dnmt3a/3b mRNA and protein; ↑ methylation At ≥10 mg/kg-d (PND 60): ↑ fat weight in F1 males and females; Changes in protein expression and phosphorylation in gastrocnemius muscle of F1 males and females ↓ IRS1 in males; ↓ $IRS1^{Tyr632\ in}$ females; ↓AKT^{Thr308}; ↓ β-arrestin 2; ↓ ACTN4 in females; ↓ RAB8A; ↓ GLU4 in the cytosol in females, ↓ nuclear MYOD in females; ↑ $GLUT4^{Ser488}$) At ≥100 mg/kg-d (PND 60): Changes in protein expression and phosphorylation in gastrocnemius muscle of F1 males and females (↑ $IRS1^{Ser636/639}$; ↓ total AKT; ↓ AKT^{ser473} in females; ↓$AS160^{Thr642}$ in females) Unaffected outcomes: IRS1 mRNA; PDK1; AS160; Dnmt 3l mRNA and protein
Peri-natal exposure studies		
Study2: Female C3H/N Mice; 8 weeks (1 week pre-mating–PND 21); diet; 0, 0.05, 5, 500 mg/kg-d. Dams terminated at PND 21 (weaning). F1 female offspring mated on PND 84 with unexposed males. F1 dams terminated 24 hours after mating, F2 embryos examined. (Schmidt et al., 2012) (Medium)[b]	NE/0.05	At ≥0.05 mg/kg-d: ↑ visceral fat in F1 males and females; ↑ body weight in F1 females on PND 21 and F1 males and females on PND 84 At ≥5 mg/kg-d: ↑ body weight in F1 males on PND 21 At 500 mg/kg-d: 100% abortion Unaffected outcomes: Preimplantation embryos in F1 females; % of degenerated blastocysts in F1 females

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
Female Wistar Rat; GD 0–PND 21; gavage; 0, 1.25, 6.25 mg/kg-d (Lin et al., 2011b) (Medium)[b]	NE/1.25	At ≥ 1.25 mg/kg-d: ↓ body weight in F1 males and females (PND 1-week 7 or 9, respectively) At PND21 (week 3): ↓ fasting blood glucose in F1 males and females; ↓ fasting serum insulin in F1 males and females; ↓ blood glucose and insulin after GTT (statistical significance varied across timepoints) in F1 males and females; ↓ glucose and insulin AUC in F1 males and females; ↓ adipocyte size and body fat percentage in F1 males and females; ↓ β-cell mass and pancreatic insulin content in F1 males and females; β-cell ultrastructural changes in F1 males and females; mitochondrial changes in F1 males and females; ↓ mRNA expression of Pdk-1 and insulin in F1 males and females; ↑ mRNA expression of genes involved in endoplasmic reticulum stress; ↑ Ucp2 mRNA expression in F1 females At week 15: ↑ fasting serum insulin in F1 females; ↑ insulin after GTT in F1 females; ↑ insulin AUC in F1 females; ↓ glucose AUC in F1 males At week 27: ↑ fasting blood glucose in F1 females; ↓ fasting serum insulin in F1 females; ↑ fasting serum insulin in F1 males; ↑ blood glucose levels after GTT in F1 females (significance varied); ↑ glucose AUC in F1 females; ↓ insulin levels in F1 females after GTT (significance varied); ↓ insulin AUC in F1 females; ↑ insulin levels after GTT in F1 males; ↑ insulin AUC in F1 males; β-cell ultrastructural changes in F1 males and females; mitochondrial changes in F1 males and females; ↑ pancreas weight in F1 females; ↓ β-cell mass and pancreatic insulin content in F1 females; ↓ *ex vivo* glucose-stimulated insulin secretion by islets isolated from F1 females; ↑ *ex vivo* glucose-stimulated insulin secretion by islets from F1 males At 6.25 mg/kg-d: ↓ body weight in F1 males and females (week 7 or 9 respectively through week 27) Unaffected outcomes: F0 dam body weight (GD 0–PND 21); fasting blood glucose and serum insulin in F0 dams at weaning; litter size; sex ratio in litters; cumulative food intake (when expressed relative to body weight) in F1 males and females; fasting serum glucagon in F1 males and females; fasting blood glucose levels in F1 males and females at week 15; fasting serum insulin F1 males at week 15; glucose levels and serum insulin levels in F1 males and glucose levels in F1 females after GTT at week 15; fasting blood glucose in F1 males at week 27;

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
		blood glucose levels in F1 males after GTT; blood glucose levels after ITT in F1 males and females on weeks 15 and 27; adipocyte size and body fat percentage in F1 males and females (week 27); pancreas weight and β-cell area in F1 males and females (week 3); glucagon mRNA expression
Female Wistar Rats; GD 9–PND21; gavage; 0, 10, 100 mg/kg-d. (Rajagopal et al., 2019a, b) (Medium)	NE/10	At ≥10 mg/kg-d (in F1 males): ↓ bodyweight (PND 1–PND 80) On PND 80: ↑ fasting blood glucose; ↑ fasting serum insulin; ↑ blood glucose after GTT; ↑ blood glucose after ITT, ↑ HOMA-IR score; ↑ hepatic glycogen concentration; ↑ activity of glycogen synthase; ↓ glucose uptake and glucose oxidation by hepatic cells; Changes in mRNA expression (↑*G-6-Pase* and *PEPCK*; ↓ *IRβ* and *GLUT2)*; ↑ activity of G-6-Pase and PEPCK; ↑ binding of FoxO1 to G-6-Pase and PEPCK promotors; ↓ testosterone and estradiol; ↑ AST, ALT, ALP, urea, and creatinine; Changes in protein expression and phosphorylation: ↓ cytosolic GLUT2; ↓ IRβ and IRβ^{Tyr1162}; ↓ IRS1 and IRS1^{Tyr632}; ↓ β-Arrestin; ↓ Akt and Akt^{Ser473}; ↑ GSK3β; ↓ GSK3β^{Ser9}; ↑ FoxO1; ↓ FoxO1^{Ser256} At 100 mg/kg-d: Changes in protein expression and phosphorylation (↑ c-Src; ↓ Akt^{Thr308}) Unaffected outcomes: Akt^{Tyr315}
Lactational exposure studies		
Female Wistar Rat; PND 1–21; gavage; 0, 1, 10, 100 mg/kg-d. (Mangala Priya et al., 2014) (Medium)[b]	NE/1	At ≥1 mg/kg-d in F1 females on PND 60: ↑ fasting blood glucose (PND 59); ↓ IR, IRS-1, IRS1^{Tyr632}, Akt^{ser473}, plasma membrane expression of glucose transporter 4, ↓ glucose uptake, ↓ glucose oxidation in cardiac muscle Unaffected outcomes: Akt expression in cardiac muscle; cytosol expression of glucose transporter 4 in cardiac muscle
Female Wistar Rats; PND 1–21; gavage; 0, 1, 10, 100 mg/kg-d.	NE/1	At ≥1 mg/kg-d in F1 males at PND22: ↓ Body weight (PND 9–22); ↓ heart weight; ↓ glucose uptake in cardiac tissue, ↓ IR-β protein expression in cardiac tissue; ↓ GLUT4 protein expression in cardiac tissue

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
(Parsanathan et al., 2019) (Medium)[b]		At ≥10 mg/kg-d: ↓ glucose oxidation, ↓ $IRS1^{Tyr632}$ At 100 mg/kg-d: ↑ fasting blood glucose, ↓ IRS1, ↓ Akt^{Ser473}, ↑ $Glut4^{Ser488}$ Unaffected outcomes: Protein expression of Akt and AS160 in cardiac tissue
Experiment1: Female Wistar Rats; PND 1–21; gavage; 0, 7.5, 75 mg/kg-d. (Venturelli et al., 2015) (Medium)[b]	NE/7.5	At ≥7.5 mg/kg-d in F1 males: ↓ Glucose decay rate during ITT on PND 90; ↓ serum triglyceride on PND 92 At 75 mg/kg-d: ↓ serum cholesterol and ↑ fasting glucose on PND 92; ↓ AUC during ITT on PND 90; ↓ Insulin secretion in isolated pancreatic islets stimulated with glucose Unaffected outcomes: Maternal or offspring body weights, food consumption, organ weights (liver, kidneys, adrenals, or fat deposits); fasting insulin in male offspring on PND 92; ITT on PND 22 or 60
Direct exposure of adolescents and adults		
Adult male SD rats; 15 weeks; gavage; 0, 0.05, 5, 500 mg/kg-d. (Zhang et al., 2017) (Medium)[b]	NE/0.05	At ≥ 0.05 mg/kg/d: Altered protein expression in the liver (↓ GLUT4, ↓ IR, ↑ PPAR gamma); Histology in the liver (vacuolar degeneration and accumulation of inflammatory factors) [not statistically analyzed]; ↑ blood glucose after GTT at PNW 15 [not statistically analyzed]; ↓ insulin after GTT at PNW3 [not statistically analyzed]; ↑ insulin after GTT at PNW 5 and 15 [not statistically analyzed] At ≥5 mg/kg/d: ↑ serum ALP; ↑ relative liver weight; ↓ SOD activity; ↑ lipid peroxidation After GTT, ↑ serum glucose at PNW5 [not statistically analyzed]

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
		At 500 mg/kg-d: ↓ terminal bodyweight at PNW 15; ↑ serum AST and ALT; Central necrosis in the liver at 500 mg/kg/day [not statistically analyzed] Unaffected outcomes: Fasting blood glucose and insulin prior to the GTT
Study1: Female C3H/N Mice; 8 weeks (7 weeks pre-mating – GD1); diet; 0, 0.05, 5, 500 mg/kg-d. (Schmidt et al., 2012) (Medium)[b]	NE/0.05	At ≥0.05 mg/kg-d in F0 dams: ↑ body weight; ↑ food consumption; ↑ visceral fat; ↑ adipocytes per unit area & adipocyte hypertrophy; Changes in mRNA expression (↑ leptin in visceral fat; ↓ adiponectin in visceral fat; ↑ *Fabp4* at 0.05 mg/kg-day only) At 500 mg/kg-d in F0 dams: ↑ plasma Leptin; Changes in mRNA expression (↑ *PPARα* & *PPARγ* in liver; ↓ *PPARα* in visceral fat) Unaffected outcomes: Preimplantation embryos; % of degenerated blastocysts
5 to 6-week-old male C57BL/6 mice; 35 days; gavage; 0, 1, 10, 100 or 300 mg/kg-d (Li et al., 2018) (Medium)[b]	NE/1	At ≥1 mg/kg-d: ↑ blood ALT and triglyceride levels; altered endogenous metabolites and metabolic pathways involved in fatty acid and glucose metabolism in cardiomyocytes At ≥10 mg/kg-d: ↑ CHE; ↑ relative (but not absolute) heart weight At ≥100 mg/kg-d: ↓ terminal bodyweight; ↑ CHO and T4; lipid droplets in cardiac papillary muscle cells [reported qualitatively]; altered Cytosol and mitochondrial Na+-K+ ATPase and Ca2+-Mg2+-ATPase activities At 300 mg/kg-d: ↑ blood glucose and CREA Unaffected outcomes:

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
		N/A
3-week-old male ICR mice; 3 weeks; oral administration in corn oil (feeding vs. gavage not specified); 0, 0.18, 1.8, 18, 180 mg/kg-d (Ding et al., 2019) (Medium)[b]	0.18/1.8	At ≥0.18 mg/kg-d (not adverse): ↓ hepatic lipase (HL) in liver; ↑ phosphorylated IRS1 and phosphorylated PI3K in liver At ≥1.8 mg/kg-d: ↑ serum glycated hemoglobin (HbA1C); ↑ serum insulin and C-peptide levels (1.8 and 18 mg/kg-day only); ↑ total cholesterol; ↑ *Cacna2d2* mRNA expression; ↑ phosphorylated mTOR (1.8 and 180 mg/kg-day only); ↑ SHC protein expression At ≥18 mg/kg-d: ↑ MDA in liver; ↓ serum LCAT and HDL; ↑ serum cTn1; ↓ mRNA levels of *Acsl6*, *Cpt1c*, and *Prkar2b*; expression of proteins related to glucose transport and uptake (↑ phosphorylated AKT and ↓ GLUT4); ↑ phosphorylated GSK-3β; ↑ phosphorylated SHC; ↑ phosphorylated ERK1/2 At 180 mg/kg-d: ↑ body weight gain; ↑ heart rate (10%) and mean blood pressure (29%); ↑ serum ALT and ALP; ↑ fasting blood glucose; ↓ liver G6PD activity; ↓ liver GCK levels; ↑ serum triglyceride; ↑ serum LDL; ↑ hs-CRP; ↓ *Slc2a3* mRNA expression; ↓ IR-β and IRS1 protein expression Unaffected outcomes: terminal body weight; absolute or relative organ weights (heart, liver, spleen, lung, kidney, brain, and testes); SBP; DBP; serum uric acid, urea, creatinine, AST, and total protein; Hepatic glycogen; HOMA-IR; *Rps6ka6* mRNA expression; PI3K, AKT, GSK-3β, mTOR, and ERK1/2 protein expression
Adolescent male/female Wistar rats; 8 weeks; gavage; 0, 5, 50, 500 mg/kg-d with normal diet (ND) or high-fat diet (HD) Data combined for males and females in each group. Results for HD not reported in this table.	NE/5	At ≥5 mg/kg-d: Structural abnormalities in the liver including disordered hepatocyte cords, vacuolar degeneration, and accumulation of inflammatory cytokines (quantitative data was not reported); ↑ volume of adipocytes (5 and 50 only, quantitative data not reported) Changes in protein expression: ↑ PDK4 in liver (Western Blot); ↑ phosphorylated JAK2, STAT5A, and phosphorylated STAT5A in adipose (Western Blot)

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
(Zhang et al., 2020b) (Medium)[b]		Changes in mRNA expression: ↓ Jak2 in liver; ↑ Fas in liver; ↑ Fas in adipose; ↑ Stat5b in adipose (only at 5) At ≥50 mg/kg-d: Changes in protein expression: ↑ Ap2 and Fas in liver (IHC); ↑ JAK2 in adipose (Western blot); Changes in mRNA expression: ↑ Stat5b mRNA in liver; ↑ Jak2 and Stat5a mRNA in adipose At 500 mg/kg-d: ↑ terminal body weight (8 weeks); ↑ serum total cholesterol & HDL; ↑ number of adipocytes (quantitative data not reported) Changes in protein expression: ↑ IHC staining for PDK4 in liver; ↑ P-STAT5A (Western blot) in liver; ↑ Fas in adipose (Western blot) Changes in mRNA expression: ↑ Ap2 in liver; ↑ Pdk4 mRNA in adipose Unaffected outcomes: serum levels of triglyceride, LDL, LEP, or ADP; levels of triglyceride and total cholesterol in the liver and adipose; Stat5a and Pdk4 mRNA in liver; JAK2, P-JAK2 STAT5A, and phosphorylated STAT5B in liver via Western blot; STAT5B and Ap2 in liver (Western Blot); Ap2 mRNA in adipose; STAT5B, phosphorylated STAT5B, and PDK4 in adipose (Western blot)
Experiment 2: Male Wistar Rats; PND 22–52; gavage; 0, 7.5, 75 mg/kg-d (Venturelli et al., 2015) (Medium)[b]	NE/7.5	At ≥ 7.5 mg/kg-d: ↓ androgen metabolites in feces on PND 49 At 75 mg/kg-d: ↑ Fasting serum glucose on PND 52 Unaffected outcomes: Body weight and eating behavior on PND 22–52; ITT on PND 50

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
		On PND 53: organ weights (liver, kidneys, adrenals, or fat deposits); fasting serum insulin; serum triglycerides; serum cholesterol
Adult male Wistar Rats; 30 days; gavage; 0, 10, 100 mg/kg-d Results from an additional antioxidant (Vitamin C+E) dose group not considered in the evaluation. (Rajesh et al., 2013) (Medium)[b]	NE/10	At ≥10 mg/kg-d in adipose tissue: ↑ H_2O_2, hydroxyl radicals, and lipid peroxidation in adipose tissue; ↓ glycogen; ↓ glucose uptake and oxidation Expression of insulin signaling molecules: ↓ IR mRNA and protein; ↓ IRS1 mRNA and protein; ↓ $IRS1^{Tyr\ 632}$; ↓ Akt^{Ser473}; ↓plasma membrane and cytosolic GLUT4 protein; ↓ nuclear SREBP-1c protein; ↑ GLUT4 mRNA (10 mg/kg-day only); ↑ $GLUT4^{Ser488}$ At 100 mg/kg-d: ↑ fasting blood glucose; ↓ β-arrestin2 protein in adipose tissue Unaffected outcomes: $IRS1^{Ser\ 636/639}$; Akt protein
Adolescent male/female Wistar rats; 28 days; gavage; 0, 5, 50, 500 mg/kg-d. Data combined for males and females in each group. (Xu et al., 2018) (Medium)[b]	5/50	At ≥5 mg/kg-d (not adverse): According to Western blot: ↓ Ob-R in liver (↓ 19%) and pancreas (↓ 25%). At ≥50 mg/kg-d: ↑ food consumption, fasting blood glucose (↑ 69–104%), fasting serum insulin (↑ 29%), fasting serum leptin (↑ 59%), insulin resistance index homeostasis model assessment (HOMA-IR, ↑ 99–177%) According to Western blot: ↑ JAK2 in liver; ↑ SOCS3 in liver According to immunohistochemistry: ↑ SOCS3 in liver and pancreas At 500 mg/kg-d: ↑ relative liver weight; ↑ SOCS3 mRNA expression in liver and pancreas According to Western Blot: ↓ IR in liver and pancreas; ↑ STAT3 in the liver and pancreas; ↑ SOCS3 in the pancreas

Brief Study Description (TSCA Study Quality Rating)	NOAEL/LOAEL[a]	Effects and Remarks
		According to immunohistochemistry: ↑ JAK2 in the liver, ↑ STAT3 in the liver; ↓ Ob-R in liver and pancreas Unaffected outcomes: Terminal body weights and body weight gain; relative pancreas weight; JAK2 protein expression in the pancreas (Western Blot); JAK2 and STAT3 protein expression in the pancreas (immunohistochemistry), mRNA expression of JAK2, STAT3, and Ob-R in liver and pancreas

[a] LOAEL/NOAEL is for glucose homeostasis and lipid metabolism endpoints and does not necessarily reflect the overall study LOAEL/NOAEL.

[b] As discussed in the Systematic Review protocol for DEHP (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.

NE = Not established; NOAEL = No observed adverse effect level; LOAEL = lowest observed adverse effect level; LOEL = lowest observed effect level; ND = no data; GD = gestation day; PND = postnatal day; PNW = postnatal week; BW = body weight; AUC = area under the curve; LDL = low density lipoprotein; HDL = high density lipoprotein ; GTT = glucose tolerance test; ITT = insulin tolerance test; HOMA-IR = homeostatic model assessment for insulin resistance

3.2.3 Conclusions on Nutritional/Metabolic Effects Related to Glucose/Insulin Homeostasis and Lipid Metabolism

Available laboratory animal studies of rats and mice provide evidence of nutritional and metabolic effects related to glucose/insulin homeostasis and lipid metabolism that are dose-dependent, but inconsistent across timepoints, inconsistent across studies in sensitivity and direction of change, or have a limited number of supportive studies. This is discussed in more detail below.

In an assessment of the epidemiological evidence, ATSDR (2022) found that the available research evaluating the impact of phthalate exposure on obesity outcomes, waist circumference, or fat distribution did not consistently show any association. Even though epidemiological research suggests a potential connection between DEHP exposure and obesity, ATSDR (2022) came to the conclusion that the cross-sectional design and uneven confounder control were the studies' limitations. Health Canada (2018a) found that there was insufficient evidence to support an association between DEHP metabolites and adult-onset diabetes, but there was an association between DEHP exposure and insulin resistance and glucose biomarkers. Radke et al. (2019a) found that exposure to DEHP has a slight but non-significant positive association with type 2 diabetes, however they indicated that interpreting results for insulin resistance is challenging since diet may still cause residual confounding. Radke et al. (2019a) also found that blood glucose levels did not appear to rise in response to increasing DEHP exposure and there was no association seen with obesity. Conclusions drawn by ATSDR (2022), ECCC/HC (2018a), Health Canada (2018a), and systematic review articles published by Radke et al. (2019a) found that the cross-sectional design of many of the studies, the inconsistencies in controlling confounding, coherence and other uncertainties in the studies do not make clear whether there is a definitive association between DEHP and nutritional and metabolic effects.

Dose-response:
In rodent studies, interpretation of the dose-response of findings by Gu et al. (2016) and Fan et al. (2020) in gestationally exposed mice is impossible because data were only available for a single dose. In the case of Gu et al. (2016), offspring only survived in the low dose group (0.05 mg/kg-day). In the case of Fan et al. (2020), the study authors presented data from F1 males in the low dose group (0.2 mg/kg-day) because body weight did not significantly increase in the higher dose groups (2 of 20 mg/kg-day) for males or in any dose group for females, and investigators focused the examination of effects on DEHP on glucose homeostasis in those animals showing increased body weight, even though not dose-related. Therefore, it is not possible to determine whether effects related to altered glucose/insulin homeostasis and lipid metabolism that occurred in DEHP-exposed mice were dose-dependent in these studies. Additionally, interpretation of the dose-response of findings in the peri-natal studies by Lin et al. (2011b) and Schmidt et al. (2012) is also limited because rats were only exposed to two dose groups (1.25 and 6.25 mg/kg-day) in the study by Lin et al. (2011b) or animals only survived at two dose groups (0.05 and 5 mg/kg-day) in the study by Schmidt et al. (2012).

As discussed in more detail above in Section 3.2.2, the remainder of laboratory animal studies that measured endpoints related to altered glucose/insulin homeostasis and lipid metabolism observed dose-dependent changes in multiple endpoints across a wide range of doses (1 to 100 mg/kg-day in pre-natal studies, 10 to 100 mg/kg-day in perinatal studies, 1 to 100 mg/kg-day in lactational studies, and 0.05 to 500 mg/kg-day in studies of directly exposed animals). Treatment-related effects observed in multiple studies included increased fasting serum glucose(Parsanathan et al., 2019; Rajagopal et al., 2019a, b; Venturelli et al., 2015; Mangala Priya et al., 2014; Rajesh and Balasubramanian, 2014; Lin et al., 2011b); changes in fasting insulin (Rajagopal et al., 2019a, b; Xu et al., 2018; Lin et al., 2011b); impaired glucose tolerance (as measured by increased glucose levels after the GTT) (Rajagopal et al.,

2019a, b; Zhang et al., 2017; Rajesh and Balasubramanian, 2014; Lin et al., 2011b); impaired insulin resistance (as measured by increased glucose levels after the ITT and HOMA-IR score) (Rajagopal et al., 2019a, b; Xu et al., 2018; Venturelli et al., 2015; Rajesh and Balasubramanian, 2014); increased serum leptin (Xu et al., 2018; Schmidt et al., 2012); altered glycogen concentration in tissues (Rajagopal et al., 2019a, b; Rajesh and Balasubramanian, 2014; Rajesh et al., 2013); decreased glucose uptake and oxidation in tissues (Parsanathan et al., 2019; Rajagopal et al., 2019a, b; Mangala Priya et al., 2014; Rajesh and Balasubramanian, 2014; Rajesh et al., 2013), increased fat mass or fat weight (Rajesh and Balasubramanian, 2014; Schmidt et al., 2012), changes in serum lipid levels (triglycerides, serum HDL, LDL, and total cholesterol) (Zhang et al., 2020b; Ding et al., 2019; Li et al., 2018; Venturelli et al., 2015).

Temporality:
Three studies (one peri-natal exposure study, one lactational study, and one in directly exposed animals) evaluated glucose/insulin homeostasis-related endpoints across multiple timepoints, allowing the analysis of temporal concordance for these endpoints (Zhang et al., 2017; Venturelli et al., 2015; Lin et al., 2011b). The endpoints measured include glucose tolerance, fasting glucose, insulin resistance, fasting insulin, and insulin after the GTT. These effects were generally either transient or spurious and potentially not treatment-related because they appeared suddenly at the latest timepoint tested.

Regarding glucose tolerance (as measured by increased glucose levels after the GTT), one chronic study in directly exposed male rats showed a consistent effect across two timepoints during treatment (PNWs 5 and 15) (Zhang et al., 2017), whereas a study in perinatally exposed rats showed inconsistent changes in males and females across three timepoints post-treatment (PND 21, PNW 15, and PNW 27) (Lin et al., 2011b). In the study by Zhang et al. (2017), although glucose remained unchanged when the GTT was performed at PNW 3, it increased starting at 5 mg/kg-day at PNW 5. By PNW 15, glucose increased starting at the lowest tested dose (0.05 mg/kg-day). In the study by Lin et al. (2011b), glucose changed in different directions depending on the sex of the animals and timepoint. In females, glucose levels did not increase until PNW 27, whereas in males, glucose levels never increased. The latency of the effect in females (PNW 27 following lactational exposure), in addition to the inconsistency in directionality between sexes and timepoint prior to PNW27, increases the uncertainty regarding time-concordance of the effects on glucose tolerance in this study.

Regarding insulin resistance (as measured by increased glucose levels after the ITT), one study in lactationally exposed male rats reported a dose-dependent increase at the final endpoint (PND 90) (Venturelli et al., 2015), whereas another study in perinatally exposed rats showed a transient decrease in males and females that approached control levels at PNW 27 (Lin et al., 2011b). In the study by Venturelli et al. (2015), this endpoint did not change at earlier timepoints (PND 22 and 60), but increased dose-dependently at PND 90 beginning at the lowest dose (7.5 mg/kg-day). The latency of this effect following lactational exposure (*i.e.*, not occurring immediately following weaning or at PND 60 but occurring at PND 90) suggests that it may be spurious as opposed to being related to treatment. In the study by Lin et al. (2011b), glucose levels after the ITT decreased in males and females, but returned to control levels by PNW 27 in both sexes.

Regarding insulin after the GTT, effects over time in directly exposed male rats (Zhang et al., 2017)and perinatally exposed male rats (Lin et al., 2011b) involved an initial decrease, followed by an increase at later time points, which may be indicative of an adaptive response to increased glucose tolerance due to DEHP treatment. In the study by Zhang et al. (2017), insulin levels after the GTT decreased at PNW 3; however, they approached control levels at 5 weeks and dose-dependently increased at 15 weeks. In the study by Lin et al. (2011b), insulin levels after the GTT decreased at PND 21, approached control levels

at PNW 15, and ultimately increased at PNW 27 in males. Notably, this study also observed a potentially spurious and non-treatment-related pattern of effect in females, in which insulin levels after the GTT decreased at PND 21, increased at PNW 15, and decreased by PNW 27.

Fasting glucose and insulin levels in directly exposed male rats (Zhang et al., 2017)and perinatally exposed male rats (Lin et al., 2011b) either did not change or changed transiently. In the study by Zhang et al. (2017), fasting blood glucose and insulin levels in the treated groups were comparable to controls throughout the study (PNW 3, 5, and 15). In the study by Lin et al. (2011b), changes in fasting blood glucose were transient in males, with decreases at PND 21 and PNW 15 before returning to control levels at PNW 27. Similarly, changes in fasting insulin were more sporadic, with decreases in males at PND 21, approaching control levels at PNW 15, and ultimately increasing at PNW 27. Notably, this study also observed sporadic changes in fasting glucose and fasting insulin in females. Specifically, fasting blood glucose temporarily decreased at PND 21 before approaching control levels at PNW 15 and increasing at PNW 27. Additionally, fasting serum insulin decreased in females at PND 21, increased at PNW 15, and decreased by PNW 27. The latency of this increase in fasting blood glucose (PNW 27 following lactational exposure), in addition to the inconsistency in directionality between sexes and timepoint prior to PNW27, increases the uncertainty about the dose- and time-concordance of effects in females in this study.

In summary, the temporal response of glucose/insulin homeostasis-related effects in laboratory animals is uncertain due to only three studies that measure these effects across multiple timepoints. Effects on glucose tolerance, insulin resistance, and fasting glucose and insulin levels, although consistent across doses tested at earlier timepoints, were either transient or spurious and potentially not treatment-related because they appeared suddenly at the latest timepoint tested. One exception is glucose tolerance, which increased dose-dependently in chronically exposed male rats across PNWs 5 and 15 (Zhang et al., 2017), although this effect was not consistent over time in perinatally exposed males or females (Lin et al., 2011b).

Strength, consistency, and specificity:
Of the endpoints related to altered glucose/insulin homeostasis and lipid metabolism that occurred within the cutoff of 20 mg/kg-day selected by EPA, several could not be evaluated for consistency across studies because they were supported by a single study. These include: histopathological changes in the pancreas such as decreased β-cell mass and pancreatic insulin content (Lin et al., 2011b); increased glycogen synthase activity (2019a, b); decreased hepatic lipase, decreased serum LCAT levels, and increased HbA1C levels (Ding et al., 2019); and increased hydroxyl radical production, hydrogen peroxide generation, and lipid peroxidation in adipose tissue (Rajesh et al., 2013).

When looking across the studies that EPA is considering for a given effect, the following dose-dependent effects related to altered glucose/insulin homeostasis and lipid metabolism emerge as **inconsistent** across studies in terms of sensitivity (*i.e.,* the dose at which effects begin) and/or the direction of change. These are described below.

Although glycogen concentration changed consistently beginning at lowest tested dose (ranging from 1 to 10 mg/kg-day) across studies, the direction of effect is inconsistent across studies, potentially due to differences in the target tissue. Specifically, glycogen increased in the livers of male rats in a lactational exposure study (Rajagopal et al., 2019a, b) and decreased in the gastrocnemius muscle of male and female rats in a gestational exposure study (Rajesh and Balasubramanian, 2014). In directly exposed animals, glycogen decreased in the adipose tissue of adult male rats (Rajesh et al., 2013) and did not change in the livers of adolescent male mice (Ding et al., 2019).

Serum leptin increased consistently, but with varying sensitivity, across studies. Specifically, serum leptin increased starting at the lowest tested dose of 0.05 mg/kg-day in prenatally exposed male and female mice (Gu et al., 2016). In directly exposed animals, serum leptin did not increase at similar doses, with increases not occurring until 50 mg/kg-day and higher in male and female rats (Xu et al., 2018) and only at 500 mg/kg-day in female mice (Schmidt et al., 2012). Because of the limited number of studies, it is unclear whether the timing of exposure vs. other effects are responsible for this difference.

Effects on bodyweight are inconsistent across studies in terms of sensitivity and direction of effect, with no clear effect of exposure timing, species, or sex. Specifically, in perinatal exposure studies, bodyweight increased starting at the lowest tested dose of 0.05 mg/kg-day in a study of male and female mice (Schmidt et al., 2012), decreased starting at the lowest tested dose of 1.25 mg/kg-day in male and female rats in the study by Lin et al. (2011b), and decreased starting at the lowest tested dose of 10 mg/kg-day in male rats in a different study(Rajagopal et al., 2019a, b). In male rats exposed via lactation, bodyweight decreased starting at 1 mg/kg-day in one study (Parsanathan et al., 2019) but remained unchanged at 75 mg/kg-day in another study (Venturelli et al., 2015). In directly exposed animals, one study reported increased bodyweight in female mice starting at 0.05 mg/kg-day (Schmidt et al., 2012), while a study in rats indicated increased body weight in males and females only at 500 mg/kg-day (Zhang et al., 2020b). Two additional studies found decreased bodyweight did not change significantly until 100 mg/kg-day in male mice (Li et al., 2018) and 500 mg/kg-day in male rats (Zhang et al., 2017). Two studies found no change in bodyweight in rats at doses up to 75 mg/kg-day (Venturelli et al., 2015) or 500 mg/kg-day (Xu et al., 2018) or in mice at doses as high as 180 mg/kg-day (Ding et al., 2019).

Effects on adiposity (as measured by fat mass, visceral fat weight, and body fat percentage) are inconsistent across studies in terms of sensitivity, with no clear effect of exposure timing, species, or sex. In prenatal exposure studies, fat mass or visceral fat weight increased in male and female mice at LOAELs of 0.05 and 0.2 mg/kg-day (Fan et al., 2020; Gu et al., 2016), and in male and female rats at 10 mg/kg-day (Rajesh and Balasubramanian, 2014). Notably, the studies by Fan et. (2020) and Gu et al. (2016) are limited in that they only examine a single dose group; therefore, the dose-responsiveness of this endpoint is uncertain and supported by a single prenatal study (Rajesh and Balasubramanian, 2014). In one perinatal exposure study, body fat percentage and adipocyte size remained unchanged relative to control in male and female rats, although the highest dose was only 6.25 mg/kg-day (Lin et al., 2011b). Similarly, in one lactational exposure study, the weight of fat deposits remained unchanged relative to control at the highest dose of 75 mg/kg-day in male rats (Venturelli et al., 2015). Effects on adiposity similarly varied across studies in directly exposed animals. Specifically, although Schmidt et al. (2012) observed increased visceral fat mass alongside increased adipocytes per unit area and adipocyte hypertrophy in adult female mice starting at the lowest tested dose of 0.05 mg/kg-day, Venturelli et al. (2015) found no effect on the weight of fat deposits in male rats at the highest dose of 74 mg/kg-day.

Effects on serum lipids (triglycerides, total cholesterol, HDL, and LDL) are inconsistent across studies in terms of sensitivity and direction of effect, with no clear effect of exposure timing, species, or sex. Specifically, in two prenatal exposure studies, total cholesterol and triglycerides increased in male and female mice starting at the lowest tested doses (0.05 mg/kg-day and 0.2 mg/kg-day, respectively) (Fan et al., 2020; Gu et al., 2016). Furthermore, one of these studies additionally measured HDL and LDL and found that it increased at the lowest dose (0.2 mg/kg-day) male mice (Fan et al., 2020). Notably, the studies by Fan et. (2020) and Gu et al. (2016) are limited in that they only examine or test a single dose group; therefore, the dose-responsiveness of this endpoint is uncertain. In one lactational exposure study,

total triglycerides decreased starting at the lowest dose of 7.5 mg/kg-day and total cholesterol did not decrease until 75 mg/kg-day in male rats (Venturelli et al., 2015). In directly exposed animals, triglyceride levels increased at the lowest dose of 1 mg/kg-day in 5 to 6-week-old male mice exposed for 35 days (Li et al., 2018), but did not increase until 180 mg/kg-day in another study of 3-week-old male mice exposed for 3 weeks in mice (Ding et al., 2019). In directly exposed adolescent rats, triglyceride levels did not change at doses as high as 75 and 500 mg/kg-day after 30 days and 8 weeks of exposure, respectively (Zhang et al., 2020b; Venturelli et al., 2015). Although cholesterol levels increased in mice exposed for 3 weeks starting at 1.8 mg/kg-day (Ding et al., 2019), they did not increase until 500 mg/kg-day in combined data from male and female and rats exposed for 8 weeks (Zhang et al., 2020b) and did not change in male rats at doses as high as 500 mg/kg-day in another study (Venturelli et al., 2015). Similarly, HDL increased in rats at 500 mg/kg-day (Zhang et al., 2020b) but decreased in mice starting at 18 mg/kg-day (Ding et al., 2019), and LDL increased in mice starting at 180 mg/kg-day (Ding et al., 2019) but remained unaltered in rats (Zhang et al., 2020b) at 500 mg/kg-day.

Effects on fasting insulin levels, which indicate impaired insulin production if decreased, are inconsistent across studies in terms of sensitivity and direction of effect, with no clear pattern due to exposure timing, species, or sex. Specifically, in gestational exposure studies, fasting insulin levels increased in male and female mice beginning at the lowest tested dose (0.05 mg/kg-day) in (Gu et al., 2016), but decreased beginning at the lowest tested dose (1mg/kg-day) in F1 male and female rats (Rajesh and Balasubramanian, 2014). In one peri-natal study, fasting insulin decreased beginning at the lowest tested dose (1.25 mg/kg-day) in male and female rats at PND 21, and remained decreased in females but increased in males by PNW 27 (Lin et al., 2011b). Conversely, in an additional peri-natal study in male rats, fasting insulin levels increased beginning at the lowest tested dose (10 mg/kg-day) (Rajagopal et al., 2019a, b). In one lactational exposure study, fasting insulin levels remained unchanged at doses as high as 75 mg/kg-day in F1 male rats (Venturelli et al., 2015). In directly exposed animals, fasting insulin levels did not increase until 50 mg/kg-day in one study of rats (Xu et al., 2018) and remained unchanged at doses up to 500 mg/kg-day in several additional studies of rats (Zhang et al., 2017; Venturelli et al., 2015; Lin et al., 2011b).

The two studies that measured insulin levels after the GTT suggest that insulin levels decrease transiently in DEHP-treated animals before returning to normal or increase to maintain glucose homeostasis with age. In one peri-natal study, insulin levels measured after the GTT decreased beginning at the lowest tested dose (1.25 mg/kg-day) in male and female rats at PND 21. By PNW 27, insulin after the GTT remained decreased in females but increased in males (Lin et al., 2011b). In one study in directly exposed male rats, insulin decreased starting at the lowest tested dose of 0.05 mg/kg-day when the GTT was performed at PNW 3; however, by PNW 15, insulin was increased in all treated groups.

When looking across the studies that EPA is considering for a given effect related to altered glucose/insulin homeostasis, the following dose-dependent effects emerge as **consistent** across most available studies (in the case of abnormal glucose tolerance) or consistent across a subset of studies from specific windows of developmental exposure (in the case of increased fasting glucose and insulin resistance) in terms of sensitivity (*i.e.*, the dose at which effects begin) and direction of change. However, limitations regarding dose range and latency of effect in several of these studies ultimately reduced the number of studies supporting a robust effect for these endpoints.

The development of abnormal glucose tolerance (which is measured by increased glucose levels after the GTT) occurred consistently and with similar sensitivity across species, sex, and timing of exposure. Specifically, in prenatal studies, glucose levels increased following the GTT starting at the lowest tested

dose of 0.2 mg/kg-day in male mice (Fan et al., 2020) and the lowest tested dose of 1 mg/kg-day in prenatally exposed male and female rats (Rajesh and Balasubramanian, 2014). Notably, authors excluded data from animals tested at doses higher than 0.2 mg/kg-day in the study by Fan et al. (Fan et al., 2020); therefore, it is uncertain whether this effect was dose-dependent and treatment related. In one perinatal study, glucose tolerance increased starting at the lowest tested dose 10 mg/kg-day in male rats (Rajagopal et al., 2019a, b). In another perinatal study, glucose levels decreased starting at the lowest tested dose of 1.25 in male and female rats at PND 21 before returning to control levels in males at PNW 27, but increasing in females at PNW 27 (Lin et al., 2011b). Notably, the latency of this effect (PNW 27 following lactational exposure), in addition to the inconsistency in directionality between sexes and timepoint prior to PNW27, suggests that this effect may be spurious at lower doses in prenatally exposed animals. In directly exposed adult male rats, glucose tolerance increased starting at 5 mg/kg-day at PNW 5 and starting at the lowest tested dose of 0.05 mg/kg-day at PNW 27 (Zhang et al., 2017).

Although fasting glucose levels increased in most studies, this endpoint was more sensitive in prenatal and perinatal exposure studies relative to lactational exposure studies and studies in directly exposed adults. In prenatal studies, fasting glucose consistently increased in male and female rats and mice starting at the lowest doses tested, ranging from 0.05 to 1 mg/kg-day (Fan et al., 2020; Gu et al., 2016; Rajesh and Balasubramanian, 2014). Notably, authors excluded data from animals tested a doses higher than 0.2 mg/kg-day in the study by Fan et al. (Fan et al., 2020) and data were not available for doses higher than 0.05 in the study by Gu et al. (2016) due to spontaneous abortions in dams treated at the higher dose group; therefore, it is uncertain whether this effect was dose-dependent and treatment related in these studies. In one perinatal study with a limited dose range (1.25 and 6.25 mg/kg-day), fasting glucose levels returned to normal by PNW 27 in male rats and did not increase until PNW 27 in female rats (Lin et al., 2011b). Notably, the latency of this effect (PNW 27 following lactational exposure), in addition to the inconsistency in directionality between sexes and timepoint prior to PNW27, suggests that this effect may be spurious at lower doses in prenatally exposed animals. In a perinatal study that tested higher doses, fasting glucose increased in male rats starting at lowest tested dose of 10 mg/kg-day at PND 80 (Rajagopal et al., 2019a, b). In lactational exposure studies, although fasting blood glucose levels increased significantly starting at the lowest tested dose of 1 mg/kg-day in female rats (Mangala Priya et al., 2014), this endpoint did not increase until 75 and 100 mg/kg-day in two other studies of male rats exposed during lactation (Parsanathan et al., 2019; Venturelli et al., 2015). In directly exposed rats and mice, fasting blood glucose remained unchanged relative to control until 75 mg/kg-day in adolescent male rats, until 180 mg/kg-day in 3-week-old mice, until 300 mg/kg-day in 5 to 6 week-old male mice, and until 500 mg/kg-day in adolescent male and female rats (Ding et al., 2019; Li et al., 2018; Zhang et al., 2017).

Although insulin resistance increased in most studies, this endpoint was more sensitive in prenatal, perinatal, and lactational exposure studies relative to studies in directly exposed adolescent animals. In prenatal studies, glucose levels following the ITT and/or HOMA-IR increased starting at the lowest tested dose of 0.2 to 10 mg/kg-day in male mice (Fan et al., 2020) and starting at the lowest tested dose of 1 mg/lg-day in male and female rats (Rajesh and Balasubramanian, 2014). Notably, authors excluded data from animals tested at doses higher than 0.2 mg/kg-day in the study by Fan et al. (Fan et al., 2020); therefore, it is uncertain whether this effect was dose-dependent and treatment related. In one perinatal study with a limited dose range, insulin decreased transiently at 1.25 and 6.25 mg/kg-day in male and female F1 rats before returning to control levels at PNW 27 in both sexes (Lin et al., 2011b). However, in a perinatal study that tested higher doses, insulin resistance increased starting at the lowest tested dose of 10 mg/kg-day at PND 80 in male rats (Rajagopal et al., 2019a, b). In a lactational exposure study, insulin resistance increased on PND 90 in male rats starting at the lowest tested dose of 7.5 mg/kg-day

(Venturelli et al., 2015). Notably, insulin resistance did not change at earlier timepoints (PND 22 and 60); therefore, the latency of this effect (PND 90 following lactational exposure) suggests that it may be spurious and not treatment-related. In directly exposed animals, insulin resistance did not increase significantly until 50 mg/kg-day in male and female rats in one study (Xu et al., 2018), and no changes in insulin resistance were observed at doses as high as 75 mg/kg-day in adolescent male rats in another study (Venturelli et al., 2015) and 180 mg/kg-day in adolescent male mice (Ding et al., 2019).

In summary, the following endpoints related to glucose/insulin homeostasis changed consistently across studies in rodents beginning at doses less than or equal to the 20 mg/kg-day cutoff selected by EPA: abnormal glucose tolerance, increased fasting glucose levels, and increased insulin resistance. However, limitations regarding dose range and latency of effect in several of these studies ultimately reduced the number of studies supporting a robust effect for these endpoints. Additionally, although serum leptin and adiposity increased consistently across studies, these changes lacked more than one study supporting their sensitivity at LOAELs below the 20 mg/kg-day cutoff selected by EPA. Finally, other endpoints related to altered glucose/insulin homeostasis and lipid metabolism, including fasting insulin, glycogen concentration, bodyweight, and serum lipids, were inconsistent across studies in terms of the direction of effect and /or began at doses higher than doses at which effects on the developing male reproductive tract are observed.

Biological plausibility and coherence:
Mechanistic data from studies in developmentally and directly exposed laboratory animals support a biologically plausible mechanism for the effects of DEHP on glucose/insulin homeostasis involving decreased insulin signaling and decreased glucose uptake and oxidation. Specifically, downregulation, dysregulated phosphorylation, and epigenetic silencing of genes and proteins involved in insulin signaling and/or decreased insulin binding were observed alongside decreased glucose uptake and oxidation in the gastrocnemius muscle, liver, and cardiac muscle in developmentally exposed rats (Parsanathan et al., 2019; Rajagopal et al., 2019a, b; Mangala Priya et al., 2014; Rajesh and Balasubramanian, 2014). Similarly, in adult rats, changes in protein expression and phosphorylation that suggest decreased insulin signaling and glucose uptake were consistent in the livers of mice (Ding et al., 2019) and in the pancreas (Xu et al., 2018), liver (Xu et al., 2018; Zhang et al., 2017), and adipose tissue (Rajesh et al., 2013) of rats. One of these studies also reported corresponding downstream effects including decreased glucose uptake and oxidation (Rajesh et al., 2013).

Available human epidemiologic studies show some limited evidence of an association between exposure to DEHP and clinical outcomes related to the nutritional and metabolic effects observed in laboratory animal studies, such as metabolic syndrome, diabetes, altered glucose metabolism, altered insulin metabolism, and adiposity. However, there are limitations associated with the available epidemiological studies related to exposure misclassification due to use of a single spot urine sample in several studies, periods of heightened susceptibility and timing of exposure assessment, and phthalate mixture effects. Until these limitations are addressed, results from the available epidemiological studies of DEHP should be interpreted with caution.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
Available evidence from 16 studies in developmentally and directly exposed rats and mice suggests that DEHP can elicit dose-dependent effects related to altered glucose/insulin homeostasis and/or effects on lipid metabolism at doses lower than 20 mg/kg-day. These effects are supported by a biologically plausible mechanism involving decreased insulin signaling and decreased glucose uptake and oxidation across developmentally and directly exposed animals. However, these effects are inconsistent when

measured across multiple timepoints, inconsistent across studies in sensitivity and direction of change, or have a limited number of supportive studies. A subset of effects on glucose-insulin homeostasis (including impaired glucose tolerance, increased fasting glucose levels, and impaired insulin resistance) were consistent and sensitive across studies; however, an adverse outcome pathway demonstrating effects of DEHP or other phthalates on glucose homeostasis is not well established, and the largely mechanistic endpoints measured in these studies did not manifest themselves in adverse apical outcomes in the animals in these studies (*e.g.*, no clinical signs of toxicity such as lethargy, polyuria, etc.). Finally, the human-relevance of these effects is difficult to determine given the lack of robust epidemiological evidence supporting effects of DEHP on diseases related to glucose homeostasis, such as diabetes, altered glucose metabolism, altered insulin metabolism, adiposity, and metabolic syndrome. Due to these limitations and uncertainties, EPA is not further considering effects on glucose/insulin homeostasis and lipid metabolism for dose-response analysis or for use in estimating risk to human health. Notably, during the August 2025 peer-review meeting, SACC agreed with EPA and was in consensus that that the current evidence of effects on DEHP on glucose/insulin homeostasis and lipid metabolism is insufficiently clear and consistent to support or conduct a quantitative dose response assessment and to develop a POD (U.S. EPA, 2025o).

3.3 Cardiovascular and Kidney Toxicity

3.3.1 Summary of Epidemiological Studies

The Agency reviewed and summarized the conclusions from previous assessments conducted by ATSDR (2022), Health Canada (2018a), as well as a systematic review publication by Radke et al. (2019a) that investigated the association between urinary metabolites of DEHP and renal outcomes, in addition to cardiovascular outcomes (*e.g.*, high blood pressure) that may be secondary to the effects on the renin-angiotensin-aldosterone system (RAAS) or other effects on the kidneys.

3.3.1.1 ATSDR (2022)

A small number of cross-sectional epidemiological studies by ATSDR (2022) assessed renal clinical chemistry and/or urinalysis parameters in populations exposed to DEHP. Cross-sectional epidemiological studies found no differences in the levels of serum urea or creatinine among workers exposed to DEHP (Wang et al., 2014) or children exposed to DEHP through tainted food (Chang et al., 2020; Wu et al., 2013). However, two studies (Tsai et al., 2016; Trasande et al., 2014) indicate that elevated levels of DEHP metabolites in urine are correlated with increases in the albumin to creatinine (ACR) ratio in urine.

The epidemiological studies of cardiovascular effects presented by ATSDR (2022) included cohort, cross-sectional, and case-control studies of blood pressure as well as a single cross-sectional study of subclinical atherosclerosis. Seven cross-sectional studies in the general population and three pregnancy cohort studies (one assessing blood pressure in mothers, two evaluating blood pressure in children) evaluated the possible association between DEHP exposure and high blood pressure. DEHP urine metabolite levels were associated with elevated blood pressure in four of the seven cross-sectional investigations (James-Todd et al., 2016b; Trasande and Attina, 2015; Shiue and Hristova, 2014; Trasande et al., 2013a) that employed NHANES data. No associations between DEHP exposure and high blood pressure were found in the other three cross-sectional investigations (Lin et al., 2020; Ko et al., 2019; Lin et al., 2016) in Taiwan. Another cross-sectional study conducted on Taiwanese adolescents and young adults aged 12 to 30 years old assessed the possible association between subclinical atherosclerosis and DEHP exposure (Lin et al., 2020). Urinary MEHP levels were found to

be positively associated with carotid intima-media thickness. There was no association seen between urine MEHHP and MEOHP. The use of single urine tests to determine exposure and the inability to demonstrate timing between exposure and outcome are the limitations of these cross-sectional research. According to Werner et al. (2015) and Vafeiadi et al. (2018), there was no association between the concentration of DEHP metabolites in the urine of pregnant women and their blood pressure, pregnancy-induced hypertensive disorders, or the blood pressure of their 4- to 6-year-old offspring. Another cohort found that 10-year-old female offspring of a mother with a DEHP metabolite in her urine had lower systolic and diastolic blood pressure; no such relationship was found in the male offspring (Sol et al., 2020). In the overall conclusion, ATSDR (2022) found that while there is a dearth of reliable human evidence on the effects of exposure to DEHP and adverse outcomes on the kidneys, the available data are inconsistent, thus an association between exposure to DEHP and associated cardiovascular and kidney effects could not be established.

3.3.1.2 Health Canada (2018a)

Health Canada (2018a) evaluated several cross-sectional and cohort studies that evaluated the relationship between DEHP and cardiovascular function and risk factors such as cardiovascular disease (CVD), blood pressure, blood lipids and albumin/creatine ratio in adults, children, pregnant women, and newborns. The assessment covered the following cardiovascular functions: albuminuria, diastolic and systolic blood pressure, blood glucose, cholesterol, HDL and LDL cholesterol, albumin-creatine ratio (ACR), and cholesterol. The study population comprised adults, older people over 70, and children and adolescents. The evidence for the association between DEHP metabolites (MEHP, MEOHP, MEHHP, MECPP, and MCMHP) to elevated blood pressure and CVD in adults was insufficient. Additionally, there was insufficient data to support an association between blood lipids and DEHP metabolites (MEHP and MCMHP). In children and adolescents, there was insufficient data to support a relationship between DEHP (MEHP, MEOHP, MEHHP, and MECPP) and Albumin-Creatine Ratio (ACR). Health Canada also determined that there was inadequate evidence for an association between exposure to DEHP and its metabolites (MEHP, MEHHP, and MEOHP), and renal injury biomarkers.

3.3.1.3 Radke et al. (2019a)

Several epidemiological studies identified by Radke et al. (2019a) evaluated the relationship between DEHP and renal effects; however, the evidence was deemed insufficient because of serious flaws in the exposure measurement, low confidence studies and inconsistent results.

3.3.1.4 Summary of the existing assessments of Cardiovascular and Kidney Toxicity

The scope and purpose of the assessments by ATSDR (2022), Health Canada (2018a), and systematic review by Radke et al. (2019a), were similar and came to the same conclusions regarding the association between exposure to DEHP and cardiovascular and kidney effects. ATSDR (2022) found that the available data are scare and inconsistent, thus an association between exposure to DEHP and associated cardiovascular and kidney effects could not be established. Health Canada (2018a) found that there was insufficient data to support an association between blood lipids, ACR and DEHP. Similarly, Radke et al. (2019a) found that there was insufficient evidence for the association between exposure to DEHP and cardiovascular and kidney effects because of serious flaws in the exposure measurement, low confidence studies and inconsistent results. Meanwhile, Health Canada (2018a) found that there was inadequate evidence for the association between exposure to DEHP and renal injury biomarker. 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 regardless of the

limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.3.1.5 EPA conclusion

EPA took into account the conclusions of ATSDR (2022), Health Canada (2018a), and systematic review publications by Radke et al. (2019a) and determined that the lack of data, the limitations of the cross-sectional studies and the inability to demonstrate whether exposure occurred before outcome, makes it difficult to draw a conclusion on whether exposure to DEHP is associated with cardiovascular function and risk factors. 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. Thus, the epidemiological studies provide qualitative support as part of the weight of scientific evidence.

3.3.2 Summary of Animal Studies

EPA identified four studies in animals that examined the effects of DEHP on the kidney and secondary effects on the cardiovascular system, such as changes in blood pressure, including three studies of mice (Deng et al., 2019; Xie et al., 2019; Kamijo et al., 2007) and one study of rats (Wei et al., 2012). In the study by Deng (2019), C57/BL6 male mice were gavaged with DEHP in saline for 6 weeks at 0, 0.1, 1, or 10 mg/kg-day in addition to an angiotensin converting enzyme inhibitor (ACEI) group and a group dosed with 10 mg/kg-day DEHP+ACEI. At 0.1 mg/kg-day and above, systolic blood pressure and ventricular wall thickness were increased. Additionally at 1 mg/kg-day and above, heart rate and ACE levels in heart tissue were increased, and bradykinin levels, BK2R, and endothelial nitric oxide synthase (eNOS) were decreased. Co-treatment with ACEI and 10 mg/kg-day DEHP resulted in the majority of these endpoints being comparable to saline controls, leading the investigators to conclude that DEHP may increase blood pressure by activating ACE levels and inhibiting the bradykinin-NO pathway, resulting in increased systolic blood pressure and heart rate and ventricular wall thickening.

A similar study by Xie et al. (2019) used mice of the same sex and strain and employed the same dose levels and duration as the study by Deng et al. (2019), but also included additional groups injected with estradiol receptor inhibitor ICI182780 and estradiol receptor inhibitor+10 mg/kg-day DEHP, in addition to the groups tested with ACEI and co-treatment of ACEI+10 mg/kg/-day DEHP. Results were also similar to those in the study by Deng et al. (2019), with increases in mean and systolic blood pressure, vascular wall thickness of the aorta, and levels of ACE, AngII, AT1R. and eNOS (in aorta) at 0.1 mg/kg-day and above. Diastolic blood pressure was increased at 1 mg/kg-day and above, and authors reported histopathology evidence of hypertensive renal injury and immune cell infiltration around the blood vessels and glomeruli starting at 1 mg/kg/day and above; however, no quantitative data were provided. The estradiol and estradiol inhibitor groups indicated that these effects are not modulated by estradiol but instead affirmed that DEHP may increase blood pressure in mice through the RAAS.

The study by Kamijo (2007) used PPAR-null (Sv/129 x C57BL/6N chimeras) and wild-type mice fed diets containing DEHP at concentrations of 0, 100, or 500 ppm (equivalent to 0, 9.5, and 48.5 mg/kg-day) for 22 months. EPA is only considering the effects in wild-type mice quantitatively in hazard identification, and the effects in PPAR-null mice is included in the discussion only to inform the mechanism of action of kidney toxicity. Wild-type mice fed diets containing DEHP had elevated systolic blood pressure, likely secondary to renal effects of mild glomerulonephritis, cell proliferation, and proteinuria. Systolic blood pressure was increased at 9.5 mg/kg-day and above, but only at 22

months (not at 12 months). The glomerular lesions in wild-type mice were mild, with only slight increases in markers of cell proliferation and fibrosis or oxidative stress in glomeruli, along with increases in cell proliferation and mesangial expansion indices compared with controls. These findings were substantially higher in incidence and severity, and often with earlier onset, in PPAR-null mice, leading authors to conclude that PPAR-alpha is protective of the nephrotoxic effects of chronic DEHP exposure.

In the study by Wei et al. (2012) pregnant Wistar rats were given DEHP in corn oil at 0, 0.25, or 6.25 mg/kg-day via oral gavage daily from GD 0 to LD 21; and blood pressure, renal histopathology and function, and renal development gene expression were measured in the offspring. Offspring body weights were significantly decreased at 6.25 mg/kg-day at all time points reported: PND0; PND21; 15 weeks; and 21 weeks in both sexes. At 6.25 mg/kg-day, absolute kidney weight was decreased in females at 15 weeks but increased in males at 21 weeks, and relative (to body weight) kidney weights were increased at PND0 and PND21 in the combined sexes and at 15 and 21 weeks in males. At 15 and 21 weeks, systolic and diastolic blood pressure in the DEHP-treated groups was comparable to controls. However, at 33 weeks, systolic blood pressure at 0.25 and 6.25 mg/kg-day was significantly higher than controls; and diastolic blood pressure was elevated at 0.25 mg/kg-day but was comparable to controls at 6.25 mg/kg-day. Similarly, heart rate was decreased in the 6.25 mg/kg-day males at 15 and 21 weeks but was comparable to controls at 33 weeks and in the females at all time points.

The number of glomeruli per kidney was significantly lower in the 0.25 and 6.25 mg/kg-day males and females at PND 21 and week 33. The mean individual glomerular volume was significantly increased in the males at 0.25 and 6.25 mg/kg-day at PND 21 and week 33 but only increased in the females at 6.25 mg/kg-day at PND 21. The total glomerular volume was significantly decreased in the 0.25 and 6.25 mg/kg-day males and females at week 33. In renal function measurements at week 21: creatinine clearance was significantly decreased in the 0.25 and 6.25 mg/kg-day males and females; serum urea nitrogen was significantly increased in the 0.25 and 6.25 mg/kg-day males; and urinary total protein was significantly increased in the 0.25 and 6.25 mg/kg-day females and the 6.25 mg/kg-day males. Intrarenal AngII expression was decreased in offspring at 6.25 mg/kg-day at birth; whereas intrarenal renin expression is significantly increased in the offspring at 0.25 mg/kg-day, but not at 6.25 mg/kg-day. Serum levels of renin angiotensin system (RAS), endothelin-1 (ET-1), and nitric oxide (NO) were measured at 21 weeks. DEHP exposure did not induce any alterations in RAS or ET-1 but significantly reduced NO levels at 0.25 and 6.25 mg/kg-day. PPARα was higher than controls at 6.25 mg/kg-day at birth and at 0.25 and 6.25 mg/kg-day at weaning. Nephron pathway-related genes (Foxd4, Gdnf, Pax2, and Wnt1) showed significantly decreased expression at 0.25 and 6.25 mg/kg-day, while nephron structure related genes: (Cdh11, Calm1, and Ywhab) were increased. These data indicate that gestational DEHP exposure may affect renal development and increase blood pressure later in life in rats.

3.3.3 Conclusions on Cardiovascular and Kidney Health Effects

Dose-response and temporality
In an assessment of the epidemiology evidence, ATSDR (2022) found that the available data are scare and inconsistent, thus an association between exposure to DEHP and associated cardiovascular and kidney effects could not be established. Health Canada (2018a) found that there was insufficient data to support an association between blood lipids, ACR and DEHP. Similarly, Radke et al. (2019a) found that there was insufficient evidence for the association between exposure to DEHP and cardiovascular and kidney effects because of serious flaws in the exposure measurement, low confidence studies and inconsistent results. Meanwhile, Health Canada (2018a) found that there was inadequate evidence for

the association between exposure to DEHP and renal injury biomarker. The limitations of the cross-sectional studies and the inability to demonstrate whether exposure occurred before outcome, makes it difficult to draw a conclusion on whether exposure to DEHP is associated with cardiovascular function and risk factors.

In animal studies, the two 6-week studies of male mice found similar effects, including increases in blood pressure, ventricular wall thickness, heart rate and ACE levels in heart tissue, and decreases in bradykinin levels, BK2R, and endothelial nitric oxide synthase (eNOS) after 6 weeks of exposure via oral gavage at doses ranging from 0.1 to 10 mg/kg-day (Deng et al., 2019; Xie et al., 2019). In the chronic study by Kamijo et al. (2007), DEHP was fed to mice at concentrations of 0, 100, or 500 ppm (equivalent to 0, 9.5, and 48.5 mg/kg-day) for 22 months, and wild-type mice fed diets containing DEHP had elevated systolic blood pressure at 9.5 and 48.5 mg/kg-day, likely secondary to renal effects of mild glomerulonephritis, cell proliferation, and proteinuria. It is not possible to determine if the effects seen at lower doses (0.1 to 1 mg/kg-day) in the shorter-term studies by Deng et al. (2019) and Xie et al. (2019) are replicated in the chronic study, given that 9.5 mg/kg-day was the lowest dose tested by Kamijo et al. (2007); however, the studies do not demonstrate a lack of dose-concordance. However, there is a lack of temporality related to dose, given that the effects on systolic blood pressure are only observed after 22 months (and not at 12 months) in the chronic study, while the effects were noted after only 6 weeks even at lower doses in the studies by Deng et al. (2019) and Xie et al. (2019).

In the study by Wei et al. (2012) in which pregnant Wistar rats were gavaged with DEHP throughout gestation and lactation, the increases in blood pressure and effects on clinical chemistry and histopathology indicating an effect on the kidney were often inconsistent in dose-response and between sexes or were transient or exhibited an implausible latency. Systolic and diastolic blood pressure in the DEHP-treated groups was comparable to controls at 15 and 21 weeks. However, at 33 weeks, systolic blood pressure was significantly higher than controls at 0.25 and 6.25 mg/kg-day, although diastolic blood pressure was only increased at 0.25 mg/kg-day but was comparable to controls at 6.25 mg/kg-day. Several factors increase the uncertainty that the effects on blood pressure in offspring are due to treatment with DEHP, including the inconsistent dose-relationship and the questionable plausibility regarding the latency (occurring at 33 weeks with no effects at 15 weeks or 21 weeks). Similarly, heart rate was decreased in the 6.25 mg/kg-day males at 15 and 21 weeks but was comparable to controls at 33 weeks and in the females at all time points, so the fact that these findings were only observed in males and were transient indicates that the findings are not adverse and may even be unrelated to treatment.

Evaluation of effects on the kidneys in the study by Wei et al. (2012) were more consistent than effects on blood pressure, heart rate, and clinical chemistry but did not result in consistent secondary effects on cardiovascular outcomes. Mean individual glomerular volume was significantly increased in the males at 0.25 and 6.25 mg/kg-day at PND 21 and week 33 but only increased in the females at 6.25 mg/kg-day at PND 21. Therefore, this finding was transient in females, although the total glomerular volume was significantly decreased at both doses in both sexes at week 33. In renal function measurements at week 21: creatinine clearance was decreased at both doses in both sexes; serum urea nitrogen was increased at both doses in males; and urinary total protein was increased both doses in females and at 6.25 mg/kg-day in males. Intrarenal AngII expression was decreased in offspring at 6.25 mg/kg-day at birth; however, intrarenal renin expression was increased in the offspring at 0.25 mg/kg-day, but not at 6.25 mg/kg-day, again indicating a lack of dose-response. There were no effects on serum renin angiotensin system (RAS) or endothelin-1 (ET-1) measurements at 21 weeks, although serum nitric oxide (NO) levels were decreased at both dose levels. Expression of nephron pathway-related genes (Foxd4, Gdnf,

Pax2, and Wnt1) were decreased, while nephron structure related genes: (Cdh11, Calm1, and Ywhab) were increased in both dose groups.

Strength, consistency, and specificity:
The database of studies in experimental animals that have evaluated cardiovascular toxicity and associated risk factors following exposure to DEHP is limited, and findings related to the sensitivity and timing of some endpoints varied across study design and species. In the study by Wei et al. (2012) in which pregnant Wistar rats were gavaged with DEHP throughout gestation and lactation, the increases in blood pressure and effects on clinical chemistry and histopathology indicating an effect on the kidney were often inconsistent in dose-response and between sexes or were transient or exhibited an implausible latency (occurring at 33 weeks with no effects at 15 weeks or 21 weeks). Similarly, heart rate was decreased in the 6.25 mg/kg-day males at 15 and 21 weeks but was comparable to controls at 33 weeks and in the females at all time points, so the fact that these findings were only observed in males and were transient indicates that the findings are not adverse and may even be unrelated to treatment.

Two 6-week studies were available that were specifically designed to evaluate cardiotoxicity (Deng et al., 2019; Xie et al., 2019). Limitations of these studies include only being conducted in a single sex (males) of a single strain and species (mice), in addition to reporting deficiencies, including the qualitative reporting of histopathology data. Nevertheless, the consistency across endpoints within these two studies—including increased heart rate, blood pressure, and vascular wall thickening of the ventricles or aorta—suggest that DEHP may affect the cardiovascular system. Again, it is not possible to determine if the effects seen at lower doses (0.1 to 1 mg/kg-day) in these 6-week studies in mice (Deng et al., 2019; Xie et al., 2019) are replicated in the chronic study, given that 9.5 mg/kg-day was the lowest dose tested by Kamijo et al. (2007). However, these studies lack consistency when looking at time and dose concordance, given that the effects on systolic blood pressure are only observed after 22 months (and not at 12 months) in the chronic study, while the effects were noted after only 6 weeks even at lower doses in the studies by Deng et al. (2019) and Xie et al. (2019).

Biological plausibility and coherence:
Mechanistic data from these two studies support a biologically plausible mechanism for these effects involving the ACE pathway (Deng et al., 2019; Xie et al., 2019). In the study by Deng et al. (2019), increases in systolic blood pressure, ventricular wall thickness, heart rate, and ACE levels in heart tissue and decreases in bradykinin levels, BK2R, and endothelial nitric oxide synthase (eNOS) were observed in mice gavaged with DEHP. The observation that co-treatment with ACEI and 10 mg/kg-day DEHP resulted in the majority of these endpoints being comparable to saline controls, supports the conclusion that DEHP may increase blood pressure by activating ACE levels and inhibiting the bradykinin-NO pathway, resulting in increased systolic blood pressure and heart rate and ventricular wall thickening.

The chronic dietary study by Kamijo et al. (2007) used a similar strain of wild-type mice (C57BL/6N) fed test diets for up to 22 months, and the wild-type mice fed diets containing DEHP had elevated systolic blood pressure, likely secondary to renal effects of mild glomerulonephritis, cell proliferation, and proteinuria. While these findings seem to align with those reported in the intermediate duration studies by Deng et al. (2019) and Xie et al. (2019), it is important to note that the increases in systolic blood pressure were not observed at 12 months, but only after 22 months in the study by Kamijo et al. (2007). The latter finding is inconsistent with the effects on blood pressure occurring after 6 weeks of dosing in the studies by Deng et al. (2019) and Xie et al. (2019). In addition to the inconsistencies between these studies regarding the dose and duration at which effects occur, the glomerular lesions in wild-type mice were mild, and these minor effects may not be unexpected for mice at 22 months of age.

It is important to determine the extent and consistency of evidence that any effects noted in animal studies are conserved across species and observed in humans. ATSDR (2022), Health Canada (2018a) and Radke et al. (2019a) identified several epidemiologic studies investigating the association between urinary metabolites of DEHP and renal outcomes, in addition to cardiovascular outcomes (*e.g.*, high blood pressure) that may be secondary to effects on the kidney. DEHP urine metabolite levels were associated with elevated blood pressure in four of the seven cross-sectional investigations (James-Todd et al., 2016b; Trasande and Attina, 2015; Shiue and Hristova, 2014; Trasande et al., 2013b) that employed NHANES data, although no associations between DEHP exposure and high blood pressure were found in the three cross-sectional investigations in Taiwan (Lin et al., 2020; Ko et al., 2019; Lin et al., 2016). While there was no association between the concentration of DEHP metabolites in the urine of pregnant women and their blood pressure, pregnancy-induced hypertensive disorders, or the blood pressure of their 4- to 6-year-old offspring (Vafeiadi et al., 2018; Werner et al., 2015), another cohort found that 10-year-old female offspring of a mother with a DEHP metabolite in her urine had lower systolic and diastolic blood pressure—although no such relationship was found in the male offspring (Sol et al., 2020). EPA determined that the inconsistency in both the presence of the association and the directionality of the effect on blood pressure increase uncertainty and reduce the Agency's confidence that DEHP exposure in humans is associated with increased blood pressure.

Similarly, Health Canada (2018a) determined that the evidence for the association between DEHP metabolites and elevated blood pressure and CVD in adults was insufficient, and there was insufficient data to support an association between DEHP metabolites and blood lipids, albumin-creatine ratio (ACR), or renal injury biomarkers. Several epidemiological studies identified by Radke et al. (2019a) evaluated the relationship between DEHP and renal effects; however, the evidence was deemed insufficient because of serious flaws in the exposure measurement, low confidence in the studies, and inconsistent results. EPA agrees with the conclusions of ATSDR (2022), Health Canada (2018a), and systematic review publications by Radke et al. (2019a) regarding the limitations of the cross-sectional studies and the inconsistent findings in the cohort studies regarding any potential effects of DEHP on blood pressure or CVD.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment.

There is limited evidence that DEHP can elicit effects on the kidney and secondary effects on the cardiovascular system, such as changes in blood pressure, in experimental laboratory animals. The data were limited to four studies, including the three studies in mice and one study in rats. The intermediate-duration studies were only conducted in a single sex (males) of a single strain and species and had reporting deficiencies, including the qualitative reporting of histopathology data (Deng et al., 2019; Xie et al., 2019). The chronic study in mice (Kamijo et al., 2007) indicated elevated systolic blood pressure, likely secondary to renal effects of mild glomerulonephritis, cell proliferation, and proteinuria in mice fed diets containing DEHP; however, the increased blood pressure was only noted after 22 months and was not observed at 12 months; whereas, this finding was evident at 6 weeks in the other two studies of mice. In addition to the inconsistencies between these studies regarding the dose and duration at which effects occur, the glomerular lesions in wild-type mice were mild, and these minor effects may simply be associated with aging mice (22 months old). Finally, in the study by Wei et al. (2012) in which pregnant Wistar rats were gavaged with DEHP throughout gestation and lactation, the increases in blood pressure and effects on clinical chemistry and histopathology indicating an effect on the kidney were often inconsistent in dose-response and between sexes or were transient or exhibited an implausible latency.

In addition to the uncertainties within the animal studies themselves, there is lack of evidence indicating that the effects on the kidneys and secondary cardiovascular effects on blood pressure occur in humans. Studies on humans have yielded inconsistent findings about the association between exposure to DEHP and increased blood pressure and other adverse cardiovascular outcomes. Due to these limitations and uncertainty, EPA is not further considering effects on the kidneys or cardiovascular outcomes for dose-response analysis or for use in estimating risk to human health.

3.4 Liver Toxicity

3.4.1 Summary of Epidemiological Studies

ATSDR (2022) and Health Canada (2018a) assessments identified several epidemiologic studies investigating the association between urinary metabolites of DEHP and liver outcomes.

3.4.1.1 ATSDR (2022)

ATSDR (2022) found that there was limited human studies on the effects of DEHP exposure on the liver. The assessment of clinical chemistry markers, such as blood enzymes and lipid and cholesterol assessments, is the only human data on the hepatic effects of DEHP. The few epidemiologic data evaluated by ATSDR (2022) on the hepatic effects of DEHP indicate that occupational exposure levels may be linked to elevated blood liver enzyme levels and reduced plasma cholinesterase activity. Urinary DEHP metabolite levels were generally not consistently related with changes in cholesterol or triglyceride levels in studies of exposures from the general population. Studies investigating additional liver endpoints in people exposed to DEHP in consumer goods or the environment were lacking. According to one study, people in China who were exposed at work had higher serum enzyme levels [(Alanine amino-transferase (ALT)], (Wang et al., 2014). In the available cohort (Vafeiadi et al., 2018; Perng et al., 2017) and cross-sectional (2020; Ko et al., 2019; James-Todd et al., 2016a; Lin et al., 2016; Trasande and Attina, 2015; Yaghjyan et al., 2015a, b; Trasande et al., 2014) studies, there was no consistent associations between the levels of cholesterol or serum triglycerides in humans.

Although ATSDR (2022) concluded that epidemiological data on hepatotoxicity is few and yields inconsistent results, Health Canda (2018a) found that the evidence for association between renal/hepatic injury and DEHP itself and its metabolites (MEHP, MEHHP, MEOHP) and biomarkers of liver injury was inadequate.

3.4.1.2 Summary of Liver Effects

ATSDR (2022) and Health Canda (2018a) were the only previous assessments that looked at exposure to DEHP and liver effects. The scope and purpose of the assessments by ATSDR (2022) and Health Canada (2018a) were similar and drew the same conclusions that the data on DEHP exposure and liver effects was inconsistent and inadequate. 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 regardless of the limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.4.1.3 EPA Summary

EPA took into account the conclusions drawn by ATSDR (2022) and Health Canada (2018a) which looked at the data and determined that there is limited information available on how exposure to DEHP

affects the liver in humans, thus the existing epidemiological studies were inadequate and do not support quantitative dose-response assessment. The EPA also 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 however qualitative support as part of the weight of scientific evidence.

3.4.2 Summary of Animal Studies

There is consistent evidence of dose-related liver toxicity in animal toxicology studies following subchronic and chronic oral exposure to DEHP, comprising the following effects: increases in relative liver weights; increases in serum markers of liver toxicity (*e.g.*, ALT, AST, ALP, GGT); and non-cancer histopathologic findings (*e.g*., hepatocellular hypertrophy, focal necrosis). Further, there is evidence that, DEHP and other phthalates can activate PPARα, which is mechanistically linked to most of the observed non-cancer liver effects. EPA summarizes the cancer hazards of DEHP 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). The reader is directed to that document for a more complete weight of evidence evaluation of the effects on the liver, including those effects modulated through the PPARα mechanisms of action that progress from non-cancer to cancer.

The liver has consistently been identified as a hazard endpoint in existing human health hazard assessments of DEHP, although generally at doses higher than doses which affect the male reproductive system (ATSDR, 2022; OEHHA, 2022; Health Canada, 2020; EFSA, 2019; ECHA, 2017a, b; NASEM, 2017; EC/HC, 2015; CPSC, 2014, 2010a; ECHA, 2010; NICNAS, 2010; ECJRC, 2008; NTP-CERHR, 2006; EFSA, 2005; U.S. EPA, 1988). Most recently, ATSDR (2022) concluded that liver toxicity is among one of the primary non-cancer health effects in laboratory animals following exposure to DEHP. Adverse liver effects (centrilobular necrosis and inflammation, hepatocyte cytoplasmic eosinophilia, bile duct lesions, altered foci, sinusoidal or vacuolar degeneration) observed in rodents tend to occur at relatively high doses (generally ≥100 mg/kg-day in rats). At lower dose levels, the adversity of liver effects are unclear, with the predominant effects observed in laboratory animals including elevated liver weight, hypertrophy, and peroxisome proliferation, which may be adaptive responses.

EPA concurs with ATSDR's conclusion on hepatotoxicity of DEHP. As discussed in Section 1.2.2, EPA considered laboratory animal studies with LOAELs less than 20 mg/kg-day in the following section in order to identify any information on liver effects that may indicate a more sensitive POD than the one established by regulatory bodies prior to the publication of ATSDR in 2022. In the subset of studies with LOAELs less than 20 mg/kg-day evaluated by EPA, 14 intermediate and 4 chronic exposure studies measured liver effects. Available studies include five oral exposure studies of short-term to subchronic duration (3 to 8 weeks) (Feng et al., 2020; Zhang et al., 2020b; Ding et al., 2019; Chiu et al., 2018; Li et al., 2018), three chronic oral exposure studies (Zhang et al., 2017; Kamijo et al., 2007; Ganning et al., 1990), one prenatal developmental study (Schmidt et al., 2012), seven perinatal developmental studies (Rajagopal et al., 2019a, b; Pocar et al., 2012; Christiansen et al., 2010; Gray et al., 2009; Andrade et al., 2006c; Grande et al., 2006), and one three-generation reproductive study (TherImmune Research Corporation, 2004) in rats and mice. Available studies are summarized in Table 3-8 and Appendix B. These studies are discussed further below.

Considerations for Interpretation of Hepatic Effects

Consistent with previous guidance (Hall et al., 2012; U.S. EPA, 2002a), EPA considered hepatocellular hypertrophy and corresponding increases in liver size and weight to be adaptive non-adverse responses, unless accompanied by treatment-related, biologically significant changes (*i.e.*, 2- to 3-fold) in clinical markers of liver toxicity: that is, decreased albumin; or increased ALT, AST, ALP, gamma glutamyltransferase (GGT), bilirubin, cholesterol; and/or histopathology indicative of an adverse response (*e.g.*, hyperplasia, degeneration, necrosis, inflammation). Furthermore, it is well documented that phthalates, including DEHP, can induce peroxisome proliferation in the livers of mice and rats, and there is evidence supporting a role for peroxisome-proliferator-activated receptor alpha (PPARα) activation in peroxisome-induced hepatic effects of DEHP (U.S. EPA, 2025a). For purposes of identifying study NOAEL and LOAEL values, effects consistent with peroxisome proliferation and PPARα activation were also considered relevant for setting the NOAEL and LOAEL.

In the subset of laboratory animal studies considered by EPA (with LOAELs <20 mg/kg-day), adverse liver effects generally occurred at higher doses, consistent with ATSDR's findings. Specifically, 9 studies listed in Table 3-8 included liver effects among the effects reported at LOAELs or LOELs less than 20 mg/kg-day (Zhang et al., 2020b; Rajagopal et al., 2019a, b; Chiu et al., 2018; Li et al., 2018; Zhang et al., 2017; Pocar et al., 2012; Kamijo et al., 2007; Ganning et al., 1990). The remaining 8 studies listed in Table 3-8 indicated liver effects at doses higher than 20 mg/kg-day, and the hazards occurring at doses lower than 20 mg/kg-day in those studies were associated with hazards other than liver toxicity, such as the developmental and reproductive hazards described in Section 3.1.

In a study by Pocar et al. (2012), CD-1 mice were administered DEHP in the diet at 0, 0.05, 5, and 500 mg/kg-day throughout gestation and lactation (GD 0.5–LD 21). Evaluation of effects in offspring was limited to the 0.05 and 5 mg/kg-day groups because abortion occurred in 9/10 dams at 500 mg/kg-day. Relative (to body weight) liver weights in the maternal animals were significantly increased by 11 to 18 percent over controls at 0.05 and 5 mg/kg-day. However, absolute liver weight and maternal body weight were not reported; therefore, it is not possible to determine if the increased relative liver weights reflect decreased maternal body weight. Furthermore, clinical chemistry and histopathology examination of the liver were not performed, so it is not possible to discern if the increased relative liver weight observed in this study is adverse.

Zhang et al. (2017) exposed adult male SD rats (n = 10 per group) to 0, 0.05, 5 or 500 mg/kg-day DEHP via gavage for 15 weeks. Terminal body weights were significantly lower (9%) in the 500 mg/kg/day dose group compared to control. Significant increases were observed in serum ALP at 5 mg/kg-day (↑120%) and 500 mg/kg-day (↑145%), and in AST (↑70%) and ALT (↑100%) at 500 mg/kg-day. Relative liver weight significantly increased at 5 mg/kg-day (↑26%), and at 500 mg/kg/day (↑49%). The study authors reported that the liver architecture was disrupted with disordered hepatocyte cord, accumulation of inflammatory factors and vacuolar degeneration in treated groups that progressed to central necrosis in the 500 mg/kg-day group. However, no quantitative data were reported for incidence or severity for these histopathology findings. PPARγ was significantly and dose-dependently increased across all dose groups. Oxidative stress was indicated by dose-dependent decreases in SOD activity and increases in lipid peroxidation, reaching significance at 5 and 500 mg/kg-day. The results of this study indicate clear adverse effects on the liver at 500 mg/kg-day. However, the effects reported at 5 mg/kg-day are limited to increased relative liver weight and ALP, therefore it is not possible to fully evaluate whether the effects at this dose are adaptive or adverse without quantitative data on the incidence and severity of the histopathology findings in the liver.

In a study by Li et al. (2018), male C57BL/6 mice (17 per group) were administered DEHP in 5 percent PEG at dose levels of 0, 1, 10, 100 or 300 mg/kg-day via oral gavage daily for 35 days. Terminal body weights were significantly decreased by 9 percent in the 100 and 300 mg/kg/day groups compared to control. ALT (↑46–83%) and triglycerides (↑17–88%) were increased at 1 mg/kg-day and above, and total cholesterol was increased at 100 mg/kg-day and above (↑83–92%). The increases in ALT and triglycerides at 1 mg/kg-day were of a smaller magnitude than at higher doses, and EPA was not able to fully evaluate the adversity of effects on the liver because the study authors only reported organ weights and histopathology evaluation of the heart in this study and not the liver.

In a study to determine the effects of DEHP on lipid metabolism, Zhang et al. (2020b) gavaged 21-day-old adolescent Wistar rats (n = 10 per sex per group) with 0, 5, 50, or 500 mg/kg/day DEHP daily for 8 weeks. In addition to normal rats, the authors also studied a cohort of animals fed a high fat diet (results not included here). The authors reported structural abnormalities in the liver including incidences of disordered hepatocyte cords, vacuolar degeneration, and accumulation of inflammatory cytokines in all dose groups. However, quantitative data were not reported for incidence or severity of these findings, as the histopathology data were only presented in representative micrographs. In the adipose tissue, the volume of adipocytes was increased at 5 and 50 mg/kg/day and the number of adipocytes was increased at 500 mg/kg/day; however, no quantitative data were reported. Additionally at 500 mg/kg-day, increases in terminal body weights, serum total cholesterol, and HDL were observed. Again, the lack of quantitative histopathology data precluded EPA from determining the magnitude or severity of the effects on the liver.

In a study by Chui et al. (2018), ICR (CD-1) mice were treated with 0 (corn oil), 1, 10, or 100 mg/kg-day (n = 12/group) of DEHP by oral gavage for 8 weeks. There were no changes in body weights in mice exposed to DEHP for 8 weeks; however, liver to body ratio was significantly increased in mice exposed to 10 and 100 mg/kg-day. Given that the purpose of this study was to test the potential effects of DEHP on bone development, the investigators did not examine the liver for histopathology changes or evaluate clinical chemistry parameters (*e.g.*, ALT) to determine if the increased liver weight was adaptive or adverse. Further details regarding results related to bone structure and development during the *in vivo* and *in vitro* of this study are included in Section 3.7 and Appendix B.3.4.

A study conducted by Kamijo et al. (2007) PPAR-null and wild-type mice (n = 20–34/group) were administered DEHP in the diet at concentrations of 0, 100, or 500 ppm (equivalent to 0, 9.5, and 48.5 mg/kg-day, estimated based on food consumption rate of 3.1 g/day) for 22 months. Body weights in the treated wild-type and PPAR-null mice were comparable to controls at 22-months. However, relative liver weight was decreased by 7 to 8 percent in wild-type mice at 9.5 and 48.5 mg/kg-day. As this study was designed to examine effects of PPAR-α in mediating toxicity of DEHP to the kidney, the study authors did not evaluate clinical chemistry or histopathology of the liver. Furthermore, the relative liver weights were minor, and liver toxicity from DEHP would be expected to result in an increase in organ weight instead of a decrease. Therefore, these minor decreases in liver weight were not considered adverse.

Rajagopal et al. (2019a, b) gavaged pregnant Wistar rats (n = 6 per group) to 0, 10, or 100 mg/kg-day DEHP daily from GD 9 to PND 21.Although the primary purpose of this study was to examine the effects of DEHP on hepatic insulin signaling and glucose homeostasis in male offspring, endpoints evaluated at study termination on PND 80 also included clinical chemistry measurements of liver and kidney function. Serum AST, ALT, and ALP were significantly and dose-dependently increased at 10 and 100 mg/kg-day. However, as these data were depicted in a bar graph, the magnitude of the increases

over controls were not presented. Given this fact, and the lack of histopathology data, EPA was unable to fully evaluate the adversity of these effects on the liver.

In a study by Ganning et al. (1990), DEHP was administered in the diet at concentrations of 0, 200, 2,000, or 20,000 ppm (equivalent to 0, 14, 140, and 1,400 mg/kg-day) for 102 weeks. Body weights were significantly decreased at 140 and 1,400 mg/kg-day beginning at Week 18 and continuing throughout the remainder of the study, and the authors reported that body weights were decreased by 10 percent at 140 mg/kg-ay and decreased by 20 percent at 1,400 mg/kg-day compared to controls. The protein content of the mitochondrial fraction from the liver was dose-dependently increased at 140 and 1,400 mg/kg-day. Peroxisomal palmitoyl-CoA dehydrogenase activity was increased over controls as follows: at 14 mg/kg-day, a continuous, slow moderate increase was observed with a doubling of activity by 2 years; the 140 mg/kg-day group had continuously increasing activity with an 8-fold increase after 2 years; and the 1,400 mg/kg-day group showed an 8-fold increase after 4 weeks and plateaued after 40 weeks with a 12-fold increase compared to controls. Peroxisomal catalase activity was dose-dependently increased at 140 and 1,400 mg/kg-day, attained statistical significance beginning at Week 33 and continuing through Week 73 and then returned to control levels by Week 102. Peroxisomal urate oxidase activity was dose-dependently decreased at 140 and 1400 mg/kg-day throughout the study and at all doses (greater than or equal to 14 mg/kg-day) beginning at Week 57. Mitochondrial carnitine acetyltransferase activity was dose-dependently increased over controls at 14 mg/kg-day and above, reaching a maximum at 1,400 mg/kg-day after approximately 20 weeks of treatment and increased more slowly at 140 mg/kg-day, although the levels at 140 and 1,400 mg/kg-day were similar at the end of the 2-year study. Microsomal NADH-cytochrome c reductase activity was unaffected by treatment. NADH-cytochrome c reductase was not affected, but NADPH-cytochrome c reductase and cytochrome P450 were increased in the first 24 weeks and then decreased to a level still higher than controls. A recovery group treated for one year and then taken off test diets showed a return toward control levels. EPA determined that the LOAEL is 14 mg/kg-day based on changes in liver enzymes, including: increased peroxisomal palmitoyl-CoA dehydrogenase activity; decreased peroxisomal urate oxidase activity; and increased mitochondrial carnitine acetyltransferase activity. Additionally, qualitative reporting of effects on the testes in this study at 14 mg/kg-day and above are described in dose-response Section 4.2.2 and in the study summaries in Appendix B.1.

Table 3-8. Summary of Studies Evaluating Effects of DEHP on the Liver

Brief Study Description (TSCA Study Quality Rating)	NOEL/LOEL for Liver Effects (mg/kg-day) [a]	Liver Effects and Remarks
Female CD-1 mice; GD 0.5–PND 21; diet; 0, 0.05, 5, and 500 mg/kg-d (Pocar et al., 2012) (Medium)[b]	NE/0.05	At ≥ 0.05 mg/kg-d: ↑ Relative liver weight in F0 dams (11–18%; 500 mg/kg-day group not analyzed). Note: Histopathology and clinical chemistry parameters indicative of adverse effects on the liver (*e.g.*, ALT) were not examined..
Adult male SD Rats; 15 weeks; gavage; 0, 0.05, 5, 500 mg/kg-d. (Zhang et al., 2017) (Medium)[b]	NE/0.05	At ≥ 0.05 mg/kg-d: ↑ PPARγ protein expression; histology in the liver (vacuolar degeneration and accumulation of inflammatory factors) [not statistically analyzed] At ≥5 mg/kg-d: ↑ serum ALP (145%); ↑ relative liver weight; ↓ SOD activity; ↑ lipid peroxidation At 500 mg/kg-d: ↑ serum AST (70%) and ALT (100%); Central necrosis in the liver at 500 mg/kg/day [not statistically analyzed]
5- to 6-week-old male C57BL/6 mice; 35 days; gavage; 0, 1, 10, 100, or 300 mg/kg-d (Li et al., 2018) (Medium)[b]	NE/1	At ≥1 mg/kg-d: ALT (↑ 46–83%) and triglycerides (↑17–88%)
Adolescent male/female Wistar rats; 8 weeks; gavage; 0, 5, 50, or 500 mg/kg-d with normal diet (ND) or high-fat diet (HD). Data combined for males and females in each group. Results for HD not reported in this table. (Zhang et al., 2020b) (Medium)[b]	NE/5	At ≥5 mg/kg-d: Structural abnormalities in the liver including disordered hepatocyte cords, vacuolar degeneration, and accumulation of inflammatory cytokines (quantitative data was not reported)
Adult male ICR (CD-1) mice; 8 weeks; gavage; 0, 1, 10, or 100 mg/kg-d. (Chiu et al., 2018) (Medium)[b]	1/10	At ≥ 10 mg/kg-d: ↑ relative liver weight Note: Histopathology and clinical chemistry parameters indicative of adverse effects on the liver (*e.g.*, ALT) were not examined.
Male PPAR-null and wild-type SV-129 mice; 22 months; diet; 0, 9.5, and 48.5 mg/kg-d. Results	NE/9.5	At ≥ 9.5 mg/kg-d: ↓ Relative liver weight in wild-type mice at 22 months.

Brief Study Description (TSCA Study Quality Rating)	NOEL/LOEL for Liver Effects (mg/kg-day) [a]	Liver Effects and Remarks
only presented for wild-type mice in this table. (Kamijo et al., 2007) (Medium)[b]		
Female Wistar Rats; GD 9–PND 21; gavage; 0, 10, 100 mg/kg-d. (Rajagopal et al., 2019a, b) (Medium)[b]	NE/10	At ≥10 mg/kg-d: ↑ serum AST (200%), ALT (116%), ALP (34%) in F1 males (PND 80)
Adult male SD rats; 102 weeks; diet; 0, 14, 140, and 1,400 mg/kg-d. (Ganning et al., 1990) (Medium)[b]	NE/14 (LOAEL)	At ≥14 mg/kg-d: ↑ Peroxisomal palmitoyl-CoA dehydrogenase activity; ↓ Peroxisomal urate oxidase activity beginning at week 57; ↑ Mitochondrial carnitine acetyltransferase activity; ↑ NADPH-cytochrome c reductase and cytochrome P450 (returned to control after 30 weeks) At ≥140 mg/kg-d: ↑ Peroxisomal catalase activity (weeks 33–73, then returned to control levels during the second year) At 1,400 mg/kg-d: Extensive peroxisomal proliferation after 1 week Unaffected outcomes: Mitochondrial cytochrome oxidase activity; microsomal NADH-cytochrome c reductase activity; according to electron microscopy, no changes in rough and smooth endoplasmic reticulum, no indication of cell damage or of damage to membrane of organelles; mitochondrial membranes appeared intact. Note: Histopathology and clinical chemistry parameters indicative of adverse effects on the liver (*e.g.*, ALT) were not examined.
Female SD rats; GD 8–PND 63; gavage; 0, 11, 33, 100, or 300 mg/kg-d. (Gray et al., 2009) (Medium)[b]	33/100	At ≥100 mg/kg-d: ↑ relative liver weight (>20%) in F1 males on PND 64 (bodyweight was used as a covariate)
Female Wistar rats; GD 6–PND 21; gavage; 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, or 405 mg/kg-d (Andrade et al., 2006c) (Medium)[b]	45/135	At ≥135 mg/kg-d: ↑ relative liver weight (9–13%) on PND 1 in F1 males (bodyweight was used as a covariate) Unaffected outcomes:

Brief Study Description (TSCA Study Quality Rating)	NOEL/LOEL for Liver Effects (mg/kg-day) [a]	Liver Effects and Remarks
		Liver weight on PND 22
Female Wistar rats; GD 6–LD 21; gavage; 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, or 405 mg/kg-d (Grande et al., 2006) (Medium)[b]	45/135	At ≥135 mg/kg-d: ↑ Liver weights (17%) on PND 1 in F1 females (bodyweight was used as a covariate) At 405 mg/kg-d: ↑ Liver weights in F0 dams Unaffected outcomes: Liver weight on PND 22
Pubertal male normal and pubertal type 2 diabetes mellitus ICR mice; 3 weeks; gavage; 0, 0.18, 1.8, 18 and 180 mg/kg-d. Results only presented for normal mice in this table. (Feng et al., 2020) (Medium)[b]	180/NE	Unaffected outcomes: Relative liver weight in normal mice
3-week-old male ICR mice; 3 weeks; oral administration in corn oil (feeding vs. gavage not specified); 0, 0.18, 1.8, 18, or 180 mg/kg-d. (Ding et al., 2019) (Medium)[b]	18/180	At 180 mg/kg-d: ↑ serum ALT and ALP Unaffected outcomes: Absolute or relative organ weights (heart, liver, spleen, lung, kidney, brain, and testes); AST
Male/female SD rats; 3 generations starting 5 weeks prior to mating; diet; 1.5 (control), 10, 30, 100, 300, 1,000, 7,500, and 10,000 ppm. See Table_Apx B-1for achieved doses for each generation. The control dose level was reported as 1.5 ppm because that was the concentration DEHP measured in the control diet. (TherImmune Research Corporation, 2004) (Medium)[b]	300 ppm (14 mg/kg-d)/ 1,000 ppm (57 mg/kg-d)	At ≥ 1,000 ppm (57 mg/kg-d): ↑ relative (and absolute in some dose groups) liver weight in F1 males and F2 females (F2 females returned to control at 10,000); hepatocellular hypertrophy in F1 males At ≥ 7,500 ppm (447 mg/kg-d): ↑ relative (and absolute in some dose groups) liver weight in F0 males and females, F1 females, and F1 males; hepatocellular hypertrophy (not statistically analyzed) in F0 males and females, F1 females, and F2 males
Female Wistar rats; GD 7–PND 16; gavage; 0, 10, 30, 100, 300, 600, or 900 mg/kg-d (Christiansen et al., 2010) (Medium)[b]	100/300	At ≥300 mg/kg-d: ↑ liver weight in F1 males at PND 16 (bodyweight was used as a covariate)

Brief Study Description (TSCA Study Quality Rating)	NOEL/LOEL for Liver Effects (mg/kg-day) [a]	Liver Effects and Remarks
Female C3H/N Mice; 8 weeks (7 weeks pre-mating – GD1); diet; 0, 0.05, 5, 500 mg/kg-d. (Schmidt et al., 2012)	5/500	At 500 mg/kg-d: ↑ *PPARα* & *PPARγ* mRNA in liver in F0 dams

[a] Increased liver weight, induction of hepatic enzymes, and peroxisome proliferation were considered non-adverse, adaptive responses unless accompanied by histopathology and/or clinical chemistry. In this case, these are NOELs/ LOELs.

[b] As discussed in the Systematic Review protocol for DEHP (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.

3.4.3 Conclusions on Liver Effects

The liver has consistently been identified as a hazard in existing human health hazard assessments of DEHP (ATSDR, 2022; OEHHA, 2022; Health Canada, 2020; EFSA, 2019; ECHA, 2017a, b; NASEM, 2017; EC/HC, 2015; CPSC, 2014, 2010a; ECHA, 2010; NICNAS, 2010; ECJRC, 2008; NTP-CERHR, 2006; EFSA, 2005; U.S. EPA, 1988). The evidence of DEHP effects on the liver in animal toxicology studies following subchronic and chronic exposure is well established, with non-cancer effects including increases in liver weights; serum markers of liver toxicity (*e.g.*, ALT, AST, ALP, GGT); and non-cancer histopathologic findings (*e.g.*, hepatocellular hypertrophy, focal necrosis). Further, there is evidence that DEHP and other phthalates can activate PPARα, which is mechanistically linked to most of these observed non-cancer liver effects, which can progress to cancer in a dose- and time-dependent manner (U.S. EPA, 2025a). EPA summarizes the cancer hazards of DEHP 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), and the reader is directed to that document for a more complete weight of evidence evaluation of the effects on the liver, describing those effects modulated through the PPARα mechanisms of action, and including a wider dose range than reflected in the subset of studies closely evaluated in this non-cancer hazard assessment.

EPA is focusing this section on determining whether the effects on the liver at these lower doses are supported by sufficient evidence to be considered adverse instead of adaptive, and whether they may be more sensitive than the POD consistently selected by regulatory bodies prior to the hazard assessment by ATSDR (2022).

ATSDR (2022) concluded that adverse liver effects (centrilobular necrosis and inflammation, hepatocyte cytoplasmic eosinophilia, bile duct lesions, altered foci, sinusoidal or vacuolar degeneration) observed in rodents tend to occur at relatively high doses (generally ≥100 mg/kg/day in rats). At lower dose levels, the adversity and human relevance of liver effects are unclear, with the predominant effects observed in laboratory animals including increased liver weight, hypertrophy, peroxisome proliferation, and/or enzyme induction which may be adaptive responses.

After a closer examination of the nine laboratory animal studies with liver effects included among the findings occurring at doses less than 20 mg/kg-day (Table 3-8), EPA concurs with ATSDR that adverse liver effects generally occurred at higher doses. These nine studies include studies in both rats and mice, with doses ranging from 0.05 to 1400 mg/kg-day and cover exposure durations ranging from those inclusive of gestation and lactation up to chronic studies of 22 months. Several studies indicated changes in liver weights (Chiu et al., 2018; Pocar et al., 2012; Kamijo et al., 2007) or increased enzyme activity in the liver indicative of peroxisome proliferation (Ganning et al., 1990), but the lack of serum clinical chemistry and histopathology examination of the liver in these studies precluded EPA from making a determination about whether these increased liver weights and enzyme activity were adverse or adaptive at these doses. Other studies reported increases in clinical chemistry parameters of magnitude which would not be considered biologically relevant and adverse in isolation (Li et al., 2018; Zhang et al., 2017), or the magnitude was not reported because the data were only depicted in bar graphs (Rajagopal et al., 2019a, b); and these studies either only included a qualitative description of histopathology findings in the liver (Li et al., 2018; Zhang et al., 2017) or did not examine the liver for histopathology changes (Rajagopal et al., 2019a, b). Therefore, it was not possible for EPA to fully evaluate whether the effects noted in those studies were adaptive or adverse without quantitative data on the incidence and/or severity of the histopathology findings in the liver. And other studies only reported a qualitative description of histopathology findings in the liver without any corroborating findings in organ weights

or clinical chemistry (Zhang et al., 2020b); again, the lack of quantitative histopathology data precluded EPA from determining the magnitude or severity of the effects on the liver. In contrast, adverse effects on the liver were established at higher doses in this subset of studies evaluated by EPA, such as the increased AST (70%) and ALT (100%) corroborated by central necrosis in the liver at 500 mg/kg/day in the study by Zhang et al. (2017).

Epidemiologic studies evaluating liver effects of DEHP exposure are inconsistent and limited in number. One study (Wang et al., 2014) suggests that occupational exposure may be associated with increased serum liver enzyme levels and decreased plasma cholinesterase activity. In studies of general population exposures, urinary metabolite levels were not consistently associated with changes in triglyceride or cholesterol levels (ATSDR, 2022).

Given the limitations in the existing epidemiological data and adverse effects on liver observed in laboratory animals are generally reported at dose levels at or above levels associated with male reproductive effects, EPA is not further considering liver effects for dose-response analysis or for use in estimating DEHP risk to human health.

3.5 Neurotoxicity

3.5.1 Summary of Epidemiological Studies

Numerous epidemiological research evaluated data on exposure to DEHP and neurological outcomes, which were limited to children and infants; however, there is a lack of reasonably available epidemiological data in adults. EPA looked at the assessments by ATSDR (2022), ECCC/HC (2018a) and Radke et al. (2020a) for qualitative support for weight of evidence for the association between DEHP exposure and neurological outcomes.

3.5.1.1 ATSDR (2022)

The majority of the epidemiological information assessed by ATSDR (2022) about the neurological effects of DEHP comes from studies where exposure occurred either before or soon after birth. Using NHANES data, five cross-sectional studies assessed different neurological consequences in adults, while one cohort assessed depression in elderly patients. The Bayley Score for Infant Development (BSID) was commonly used for children under 3 years old, while the Wechsler Intelligence Scale for Children (WISC) was used for older children in these studies. Standard instruments were also employed to assess development. However, due to variations in the instruments used to assess development, ages at assessment, gestational timing of maternal urine collection, nature and quantity of covariates considered in the analyses, differences in study populations, and specific DEHP metabolites measured in urine, the available studies measuring these endpoints are not strictly comparable. Numerous validated measures of general behavioral development, social behavior (including screening for social impairments related to Autism Spectrum Disorder [ASD]), gender-related play, and measures of attentiveness (including screening for Attention Deficit Hyperactivity Disorder [ADHD]) were evaluated for epidemiological studies of behavior and attention. The neurological status of the infant, cognitive, mental, and psychomotor development, behavior and emotional development, social development and autism spectrum disorders, and gender-related behaviors are among the neurodevelopmental consequences that have been examined by ATSDR (2022). There were 26 studies of 13 birth cohorts in the database for epidemiological studies of cognitive/mental and psychomotor development, and 13 studies of 9 birth cohorts in the database for epidemiological studies of behavior and attention that was assessed by ATSDR (2022). Numerous cohorts were designed longitudinally to assess the development of

psychomotor, cognitive, and mental skills over a range of ages; however, due to the lack of substantive epidemiological data particularly on adults a conclusion on the association between DEHP and neurological outcomes could not be reached.

3.5.1.2 Health Canada (2018a)

According to Health Canada (2018a), there is insufficient evidence to associate DEHP metabolites (MEOHP, MEHHP, and MEHP) to changes in behavioral and cognitive functioning as well as impaired mental and psychomotor neurodevelopment. Additionally, there was insufficient data to support an association between the other DEHP metabolite (MECPP), modified behavioral and cognitive performance, and changed mental and psychomotor neurodevelopment.

3.5.1.3 Radke et al. (2020a)

The evaluation of the relationship between exposure to DEHP and cognition by Radke et al. (2020a) is based on 11 included studies, with a focus on the 10 medium and 1 high confidence studies that revealed no discernible trend of greater association in studies with wider ranges or higher exposure levels. Eight studies are used to evaluate the relationship between exposure to DEHP and motor effects, with an emphasis on the six medium- and high-confidence investigations. Each of these studies focused on the motor impact in young children (≤4 years), with the exception of one that updated Whyatt et al. (2012) and assessed impacts at 11 years old (Balalian et al. (2019)). Similar to cognition, there was some evidence of the sex of the child altering the effect, but the direction varied between the research studies. Tellez-Rojo et al. (2013) found that results in girls drove the inverse association; however, Kim et al. (2011) found that the association was stronger in boys. Overall, there were some studies that have shown indications of a relationship between DEHP exposure and neurodevelopmental outcomes; nevertheless, because of the inconsistent results across the literature, the evidence for the association between DEHP exposure and cognition is deemed weak.

3.5.1.4 Summary of existing assessments of Neurotoxicity

The scope and purpose of the assessments by ATSDR (2022), Health Canada (2018a), and systematic review by Radke et al. (2020a), draw similar conclusions. ATSDR (2022) found that because of variations in the instruments used to assess development, ages at assessment, gestational timing of maternal urine collection, nature and quantity of covariates considered in the analyses and differences in study populations, and specific DEHP metabolites measured in urine, the available studies measuring these endpoints are not strictly comparable. Therefore, due to the lack of substantive epidemiological data particularly on adults a conclusion on the association between DEHP and neurological outcomes could not be reached. Health Canada (2018a), found that there is insufficient evidence to associate DEHP metabolites (MEOHP, MEHHP, and MEHP) to changes in behavioral and cognitive functioning as well as impaired mental and psychomotor neurodevelopment. Finally, Radke et al. (2020a) found that because of the inconsistent results across the literature, the evidence for the association between DEHP exposure and cognition is weak. 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 regardless of the limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.5.1.5 EPA Conclusion

EPA took into account conclusions drawn by ATSDR (2022), Health Canada (2018a), and systematic review publications by Radke et al. (2020a) and determined that due to the inconsistent results among studies and inconclusive results the existing epidemiological studies do not support quantitative exposure-response assessment. 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 however qualitative support as part of the weight of scientific evidence.

3.5.2 Summary of Animal Studies

Three neurotoxicity studies (Feng et al., 2020; Barakat et al., 2018; Tanida et al., 2009) were identified in the subset of more sensitive studies (LOAEL less than 20 mg/kg-day) subjected to detailed evaluation by EPA to determine if a more sensitive POD would be provided by these studies compared to the POD of 4.8 mg/kg-day based on the effects on male reproductive tract observed in the three-generation reproduction toxicity study (Blystone et al., 2010; TherImmune Research Corporation, 2004) selected by most other regulatory agencies for risk assessment.

In a neurobehavioral study by Barakat et al. (2018), pregnant CD-1 mice (n = 4–7/group) were administered DEHP in corn oil at 0, 0.2, 500, or 750 mg/kg-day from GD 11 to PND 0 (birth) to investigate the effects of prenatal exposure on neurobehavior and recognition memory in male offspring, including examination of the possible mechanism of oxidative damage in the hippocampus. Neurobehavioral parameters were measured in the offspring at ages of 16 to 22 months. Elevated plus maze (EPM) and open field tests (OFT) were used to measure anxiety levels. Y-maze and novel object recognition (NOR) tests were employed to measure memory function. Authors also measured serum levels of testosterone, brain weight, and collected tissue for histology and immunohistochemistry (IHC). Oxidative damage in the hippocampus was measured by the levels of oxidative DNA damage and by spatial light interference microscopic counting of hippocampal neurons.

Effects in the study by Barakat et al. (2018) that could be attributed to treatment because they were dose-related and statistically significant only occurred at higher doses of 500 and 750 mg/kg-day and were not necessarily specific to neurotoxicity. The EPM test showed that mice in the 750 mg/kg-day DEHP group took significantly more time before making entries into open arms, which the authors attributed to increased anxiety. During the NOR test, mice prenatally exposed to 500 and 750 mg/kg-day displayed significantly less time (seconds) exploring the new object when compared to the control group, which the authors attributed to impaired short-term recognition memory. However, when expressed as a percentage of time spent exploring objects (new object + past object), the treated groups were comparable to controls. Using computerized microscopy (SLIM) on the hippocampus, the number of pyramidal neurons in the different regions of the hippocampus were significantly lower than controls in the dentate gyrus (DG) and CA2 region at 500 and 750 mg/kg-day and in CA1 region at 750 mg/kg-day. Serum testosterone was significantly decreased in the 500 and 750 mg/kg-day male offspring. The study authors reported that DEHP-treated mice “remarkably decreased [androgen receptor] AR expression in the pyramidal neurons” in the brain of the offspring at 750 mg/kg-day; however, they acknowledged that this assertion was based on visual observation of the immunohistochemistry and that quantitative measurements of AR expression were not conducted. The study authors reported that prenatal DEHP exposure in mice resulted in stronger immunostaining for OHdG and TG (DNA oxidation markers) compared to controls, with increased OHdG in regions

CA2, CA3, and DG, and increased TG in CA2 and DG. However, these data were only reported qualitatively in text and presented as representative micrographs in figures. Finally, the interpretation of dose-response in this study was challenging, given the broad dose-spacing between the low dose (0.2 mg/kg-day) and the higher two doses (500 and 750 mg/kg-day).

The investigators attributed numerous other findings in this study to effects of DEHP on learning and memory in mice; however, EPA determined that these findings were likely unrelated to treatment with DEHP because they were either not statistically significant or were unrelated to dose, including the following:

1. increased time in the OFT for DEHP-treated mice to go to the center region (dose-related but high variation and lack of statistical significance);
2. lower number of entries into the center region at 0.2 mg/kg-day and above compared to controls (statistically significant, but not dose-dependent, with greatest difference from control occurring at the low dose and least difference from control at the high dose);
3. significantly lower alteration behavior (*i.e.*, rather than entering the next arm, these animals tended to enter the arm just visited) and significantly fewer arm entries in the Y-maze test at 0.2 mg/kg-day, which the authors attributed to impaired spatial memory or locomotion; however, these endpoints were only significantly decreased at the low dose;
4. significantly fewer pyramidal neurons in CA1 and CA2/3 subregions of the hippocampus at 0.2 and 750 mg/kg-day, indicated by manual counting following Nissl and hematoxylin & eosin staining; however, this finding was not dose-related, as the 500 mg/kg-day group was comparable to controls;
5. significantly higher COX-2 positive neurons in the 0.2 and 750 mg/kg-day groups by IHC, which the authors attributed to neuronal inflammation in these subregions of the hippocampus; but again, the 500 mg/kg-day group was comparable to controls; and
6. decreased brain weight in DEHP-treated mice, although not dose-dependent or statistically significant.

In a neurotoxicity study by Feng et al. (2020), pubertal normal (P-normal) and pubertal type 2 diabetes mellitus (P-T2DM) ICR mice (n = 10/group) were administered DEHP in corn oil at 0, 0.18, 1.8, 18 and 180 mg/kg-day via oral gavage daily for 3 weeks. To test neurobehavioral effects, authors conducted an OFT and Morris water maze test (MWM). At study termination, the animals were killed, and the brain was weighed, and enzyme activity of superoxide dismutase (SOD), acetylcholinesterase (AChE), and glutathione peroxidase (GSH-Px) were measured, along with gene expression of *Slc6a4*, *Tph2*, *Fgf17*, *Gabrr1*, *Avp*, and *Pax8* (related to regulating serotonergic synapses, GABAergic synapses, phospholipase D, and thyroid hormone synthesis) by RT-PCR, protein expression by Western blot, and determination of levels of the neurotransmitters 5-hydroxytyptamine (5-HT) and γ-aminobutyric acid (GABA) and Ca^{2+} and cAMP by ELISA. Additionally, select other organs were weighed, including heart, liver, spleen, lungs, and kidneys. In the OFT, normal mice had significant decreases in clockwise rotation count at 1.8 mg/kg-day and above and in total distance at 18 mg/kg-day and above; and significantly increased time in the central area at 1.8 mg/kg-day and above.

Both treated and control P-T2DM mice exhibited the same changes in these parameters compared to normal mice. In P-T2DM treated mice, significant differences compared to the P-T2DM controls were noted at 1.8 mg/kg-day and above for decreased total distance; 0.18 mg/kg-day and above for decreased clockwise rotation; and increased time in central area at 18 mg/kg-day and higher. For the MWM test, in the learning phase of the test, a significant decrease in swimming speed and a significant increase in latency in locating the platform were observed in the P-normal mice exposed

to DEHP, P-T2DM control group, and P-T2DM mice exposed to any dose of DEHP when compared to the P-normal control group. DEHP exposed P-T2DM mice had the most pronounced effects out of all the groups, with authors suggesting DEHP may impair locomotion and learning of mice. During the memory phase of the test (*e.g.*, referred to as space exploration in the study report), decreases in swimming speed, time (stops) in the original platform quadrant, and residence time in the target quadrant were all decreased at 0.18 mg/kg-day and above in both normal and P-T2DM mice, with the DEHP exposed P-T2DM mice having the most dramatic decreases. The authors suggested that these data indicate DEHP impairs spatial learning and memory. Real time PCR data revealed significant reductions in *Slc6a4*, *Tph2*, *Gabrr1*, and *Pax8* when compared to the P-normal control group. In contrast, there was significantly increased expression of *Avp* and *Fgf17* in the P-T2DM control group and at all doses of DEHP. When measuring enzyme activity in the brains of these mice, decreases were observed in AChE and SOD in normal mice treated with DEHP at 0.18 mg/kg-day and above and in GSH-Px at 1.8 mg/kg-day and above compared to normal controls. AChE, GSH-Px, and SOD in the P-T2DM control group were lower than normal controls, with GSH-Px and SOD in P-T2DM mice lower than P-T2DM controls at doses of 1.8 mg/kg-day and above and AChE in P-T2DM mice lower than P-T2DM controls at 18 mg/kg-day and above.

Similarly, all mice exposed to DEHP had significantly reduced neurotransmitters 5-HT and GABA when compared to the P-normal control group. Furthermore, the P-T2DM groups exposed to DEHP had even more pronounced decreases in both 5-HT and GABA content when compared to the P-T2DM control group. Brain calcium content was significantly increased in the P-T2DM control group and in all DEHP-treated groups, with P-T2DM exposed mice having a more significant increase. Additionally, cAMP levels in brain tissue were significantly reduced in P-T2DM mice and all DEHP administered groups when compared to the P-normal control group. This already significant reduction was exacerbated in P-T2DM mice at 18 and 180 mg/kg-day when compared to the P-Normal mice at the same doses. When measuring protein expression of the calcium signaling pathway, authors reported that DEHP exposure did not alter the total protein expression of CaMKII but did significantly increase protein expression of CaM and p-CaMKII in both P-normal and P-T2DM mice groups at 1.8 mg/kg-day and above when compared to the P-normal control group. Likewise, P-T2DM mice exposed to DEHP had a more significant increase in CaM and p-CaMKII levels at 1.8 mg/kg-day and above. When authors evaluated GPCRs–cAMP–PKA–ERK–CREB signaling pathway, both unphosphorylated and phosphorylated PKA, ERK1/2, and CREB protein expression significantly decreased with increasing doses of DEHP when compared to P-normal controls. These changes in expression were more noticeable in the P-T2DM. Relative (to body weight) testes weights were significantly decreased at 180 mg/kg-day in P-normal mice.

Overall, any adverse effects were potentiated in P-T2DM mice exposed to DEHP, suggesting that these mice are more sensitive to the effects of DEHP in this study. The study authors concluded that these data indicate DEHP causes neurotoxicity via cAMP–PKA–ERK1/2–CREB signaling pathway and calcium signaling. EPA determined that the vast majority of these findings were most pronounced in the pubertal type 2 diabetes mellitus (P-T2DM) ICR mice; whereas the P-normal mice, while showing some statistically significant effects, were much more similar to controls and not reaching a level of adversity compared to the pubertal type 2 diabetes mellitus (P-T2DM) ICR mice.

In a neurotoxicity study by Tanida et al. (2009), pregnant ICR mice (n = 6–7/group) were administered DEHP at 1 mg/kg-day in sesame oil daily via oral gavage from GD 8 to 17; male offspring were administered the same dosage as their dam via gavage from PND 3 to 7. Offspring were sacrificed at PNW 2, 4, and 6 for evaluation of body weight, brain weight, and tyrosine hydroxylase and Fos immunoreactivity in the midbrain dopaminergic nuclei (tyrosine hydroxylase is a marker for

biosynthetic activity of dopamine; Fos is a marker of neuronal activation). The following findings were noted in the treated group compared to controls. Body weight was significantly decreased by 6 to 13 percent at all ages (PNW 2, 4, and 6 weeks). Absolute brain weight was slightly, but significantly, decreased by 4 percent at PNW 6, but comparable at other time-points. Relative brain weight was significantly increased by 15 percent at PNW 2 and by 8 percent at PNW 4. Immunohistochemistry findings in the mouse midbrains dopaminergic nuclei revealed that the number of tyrosine hydroxylase- and Fos-immunoreactive neurons was significantly decreased at PNW 4 and 6, indicating a decrease in dopaminergic neurons. The intensity of tyrosine hydroxylase immunoreactivity was reported as 50 to 80 percent of control at PNW 2 and 6.

3.5.3 Conclusions on Neurotoxic Health Effects

Dose-response and temporality:
In the study by Barakat et al. (2018) examining neurobehavioral effects at 16 to 22 months in male mice following maternal exposure during gestation, the investigators attributed numerous findings in this study to effects of DEHP on learning and memory. However, EPA determined that the findings at 0.2 mg/kg were likely unrelated to treatment with DEHP because they were not dose-related and/or were not statistically significant, including: increased time to enter the center region in the open-field test; lower number of entries into the center region; lower alteration behavior and significantly fewer arm entries in the Y-maze test; fewer pyramidal neurons in CA1 and CA2/3 subregions of the hippocampus; higher COX-2 positive neurons by IHC; and decreased brain weight. It is important to note that the interpretation of dose-response in this study is hindered by the broad dose-spacing between the low dose (0.2 mg/kg-day) and the higher two doses (500 and 750 mg/kg-day).

Effects in the study by Barakat et al. (2018) that could be attributed to treatment because they were dose-related and statistically significant only occurred at higher doses of 500 and 750 mg/kg-day and were not necessarily specific to neurotoxicity, including: longer time before making entries into open arms at 750 mg/kg-day; less time exploring the new object at 500 and 750 mg/kg-day; lower number of pyramidal neurons in the dentate gyrus (DG) and CA2 region at 500 and 750 mg/kg-day and in CA1 region of the hippocampus at 750 mg/kg-day. The study authors reported that prenatal DEHP exposure in mice resulted in stronger immunostaining for OHdG and TG (DNA oxidation markers) compared to controls, with increased OHdG in regions CA2, CA3, and DG, and increased TG in CA2 and DG. However, the fact that these data were only reported qualitatively in text and presented as representative micrographs in figures is an additional limitation in the study.

Given that the treatment-related findings in the study by Barakat (2018) only occur at 500 and 750 mg/kg-day, and the findings at 0.2 mg/kg-day were not dose-dependent and/or not statistically significant, EPA does not consider the endpoints in this study to be as robust or sensitive as the POD of 4.8 mg/kg-day based on the effects on male reproductive tract in the three-generation reproduction toxicity study (Blystone et al., 2010; TherImmune Research Corporation, 2004).

Interpretation of the findings in the perinatal neurotoxicity study by Tanida et al. (2009) is limited by fact there was only one dose level tested, so it was not possible to examine a dose-relationship for incidence or severity of the immunohistochemistry effects on dopaminergic neurons in the midbrain or the relationship of the differences in body weight and brain weight.

In order to further put the findings in these three more sensitive mouse studies into context of the larger evidence base of animal toxicology studies, EPA referred to ATSDR's summary of the studies

evaluating neurological function in rodents following oral exposure to DEHP (ATSDR, 2022). There were no effects on FOB or motor activity in F344 rats dosed up to 1,500 mg/kg-day for 10-14 days (Moser et al., 2003; Moser et al., 1995) or on FOB in rats dosed up to 1,000 mg/kg-day for 9 weeks or 10,000 mg/kg-day for 4 weeks (Dalgaard et al., 2000). Increased anxiety in elevated plus maze and open field tests was reported in rats after 30 days exposure to 500 mg/kg-day, with no changes in motor activity. In the Morris water maze test, spatial leaning was impaired in rats following 5 months of treatment at doses of 100 mg/kg-day and above, although spatial memory and swimming speed were unaffected at doses up to 500 mg/kg-day (Ran et al., 2019). More specifically in studies in mice exposed orally to DEHP, no changes in exploratory behavior were noted in F0 mice assessed for behavior after three weeks of exposure at doses up to 180.77 mg/kg-day in a one-generation reproductive toxicity study (Tanaka, 2002), and much higher doses of 6,922 mg/kg-day and above resulted in clinical signs of toxicity (*e.g.*, hunched posture, hypoactivity) after 28 days exposure (Hazleton, 1992). Citing their table of levels of significant exposure, ATSDR also noted that there were no effects of DEHP on brain weights or histopathology of the brain, spinal cord, or peripheral nerve in numerous rodent studies of acute duration up to 1100 mg/kg-day, intermediate duration up to 10,000 mg/kg-day, or chronic duration up to 1,821 mg/kg-day.

Strength, consistency, and specificity:
Many of the effects in the study by Barakat et al. (2018) that could be attributed to treatment were not necessarily specific to neurotoxicity and only occurred at higher doses of 500 and 750 mg/kg-day. During the NOR test, mice prenatally exposed to 500 and 750 mg/kg-day displayed significantly less time exploring the new object when compared to the control group, which the authors attributed to impaired short-term recognition memory. However, when expressed as a percentage of time spent exploring objects (new object + past object), the treated groups were comparable to controls; therefore, EPA considers it is plausible that the offspring at 500 and 750 mg/kg-day spent less time exploring objects in general. The EPM test showed that mice in the 750 mg/kg-day DEHP group took significantly more time before making entries into open arms, which the authors attributed to increased anxiety. Both of these findings could have been due to reduced general condition in these animals.

In the study by Tanida et al. (2009), it is reasonable that the slight decrease in absolute brain weight and increase in relative brain weight in male offspring correspond to their decreased body weight and not a specific neurotoxic effect, given that these are young mice at study termination (2–6 weeks old) in an active stage of growth and development. EPA is unable to determine the implications of the apparent decrease in dopaminergic neuron activity in the midbrain detected by immunohistochemistry, with respect to whether it results in a permanent or adverse effect on neurological development and function.

In the study by Feng et al. (2020), investigators examined the potential neurotoxic effects of DEHP on pubertal normal (P-normal) and pubertal type 2 diabetes mellitus (P-T2DM) ICR mice administered DEHP via gavage at 0, 0.18, 1.8, 18 and 180 mg/kg-day for 3 weeks. Neurobehavioral effects were examined using an OFT and Morris water maze test (MWM). In the OFT, normal mice had significant decreases in clockwise rotation count at 1.8 mg/kg-day and above and in total distance at 18 mg/kg-day and above; and significantly increased time in the central area at 1.8 mg/kg-day and above. However, these findings are not specific to a neurotoxic effect and may indicate general decreased activity. For the MWM test, in the learning phase of the test, a significant decrease in swimming speed and a significant increase in latency in locating the platform were observed in the P-normal mice exposed to DEHP, P-T2DM control group, and P-T2DM mice exposed to any dose of DEHP when compared to the P-normal control group. DEHP exposed P-T2DM mice had the most

pronounced effects out of all the groups. During the memory phase of the test (*e.g.*, referred to as space exploration in the study report), decreases in swimming speed, time (stops) in the original platform quadrant, and residence time in the target quadrant were all decreased at 0.18 mg/kg-day and above in both normal and P-T2DM mice, with the DEHP exposed P-T2DM mice having the most dramatic decreases. The authors suggested that these data indicate DEHP impairs locomotion and spatial learning and memory. Again, EPA notes that the effects on decreased swimming speed and increased time to locate the platform may be due to decreased general condition and therefore slower swimming and increased time instead of impaired learning and memory, given that the researchers did not report the distance of the swim path.

In an assessment of the epidemiology evidence, ATSDR (2022) found that because of variations in the instruments used to assess development, ages at assessment, gestational timing of maternal urine collection, nature and quantity of covariates considered in the analyses and differences in study populations, and specific DEHP metabolites measured in urine, the available studies measuring these endpoints are not strictly comparable. Therefore, due to the lack of substantive epidemiological data, particularly on adults, a conclusion on the association between DEHP and neurological outcomes could not be reached. Health Canada (2018a) found that there is insufficient evidence to associate DEHP metabolites (MEOHP, MEHHP, and MEHP) to changes in behavioral and cognitive functioning as well as impaired mental and psychomotor neurodevelopment. Finally, Radke et al. (2020a) found that because of the inconsistent results across the literature, the evidence for the association between DEHP exposure and cognition is weak. The inconsistent results among studies and inconclusive results the existing epidemiological studies do not support quantitative exposure-response assessment.

Biological plausibility and coherence:
In the study by Feng et al. (2020), the authors proposed that DEHP "could possibly impair blood glucose control and thereby predispose to T2DM", and that T2DM is associated with cognitive decline in learning and memory. In addition to the effects observed in the OFT and MWM described above, which may be due to non-specific effects on activity level, differences in gene expression and reductions in enzyme activity and neurotransmitters in the brains of these mice were noted, along with increased protein expression of the calcium signaling pathway. The study authors concluded that these data indicate DEHP causes neurotoxicity via cAMP–PKA–ERK1/2–CREB signaling pathway and calcium signaling and concluded that T2DM mice are more sensitive to the effects of DEHP. EPA determined that the vast majority of these findings were most pronounced in the pubertal type 2 diabetes mellitus (P-T2DM) ICR mice; whereas the P-normal mice, while showing some statistically significant effects, were much more similar to controls and not reaching a level of adversity compared to the pubertal type 2 diabetes mellitus (P-T2DM) ICR mice. EPA notes that evidence supporting effects of DEHP on this pathway is limited to this study examining neurotoxic effects associated with T2DM, and a more in-depth discussion of potential effects of DEHP on glucose homeostasis more generally can be found in Section 3.2.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
Overall, EPA considers these three studies on neurotoxicity to have too much uncertainty regarding the limitations in the individual studies and the clinical relevance of the findings for human health to consider them further in dose-response for derivation of an oral POD. All three studies were in mice, with no studies of rats or other species.

EPA determined that the vast majority of the findings in the study by Feng et al. (2020) were most pronounced in the pubertal type 2 diabetes mellitus (P-T2DM) ICR mice; whereas the P-normal mice, while showing some statistically significant effects, were much more similar to controls and not reaching a level of adversity compared to the pubertal type 2 diabetes mellitus (P-T2DM) ICR mice.

In the study by Tanida et al. (2009), it is reasonable that the slight decrease in absolute brain weight and increase in relative brain weight in male offspring correspond to their decreased body weight, given that these are young mice at study termination (2–6 weeks old) in an active stage of growth and development. EPA is unable to determine the implications of the apparent decrease in dopaminergic neuron activity in the midbrain detected by immunohistochemistry, with respect to whether it results in a permanent or adverse effect on neurological development and function. Interpretation of the findings in this study is further limited by fact there was only one dose level tested, so it was not possible to examine a dose-relationship for incidence or severity of the immunohistochemistry effects on dopaminergic neurons in the midbrain.

Given the fact that studies examined by ATSDR indicate that neurobehavioral effects are not observed in rats at doses lower than 100 mg/kg-day or in other studies in mice at doses lower than 6922 mg/kg-day, and none of the oral studies in rodents identified any effects on brain weight or histopathology of tissues of the nervous system (ATSDR, 2022), EPA considers the effects in the three low-dose neurotoxicity studies of mice (Feng et al., 2020; Barakat et al., 2018; Tanida et al., 2009) to be inconsistent with dose-response for neurotoxic endpoints in other studies.

Finally, EPA examined the epidemiological assessments by ATSDR (2022), Health Canada (2018a) and Radke et al. (2020a). Although ATSDR (2022) assessed 26 studies of 13 birth cohorts examining cognitive/mental and psychomotor development and 13 studies of 9 birth cohorts evaluating behavior and attention, a conclusion on the association between DEHP and neurological outcomes could not be reached due to the lack of substantive epidemiological data particularly on adults. The evaluation of the relationship between exposure to DEHP and cognition by Radke et al. (2020a) based on 11 medium to high quality studies revealed no discernible trend of greater association in studies with wider ranges or higher exposure levels. Health Canada (2018a) determined there is insufficient evidence to associate DEHP metabolites to changes in behavioral, cognitive functioning, or impaired mental and psychomotor neurodevelopment. Overall, EPA determined that the evidence of association of DEHP exposure with neurological outcomes were inconsistent among studies or inconclusive. Therefore, EPA will not further consider the neurotoxicity studies (Feng et al., 2020; Barakat et al., 2018; Tanida et al., 2009) in dose-response analysis.

3.6 Immunotoxicity

3.6.1 Summary of Epidemiological Studies

ATSDR (2022) and Health Canada (2018a) has identified several epidemiologic studies investigating the association between urinary metabolites of DEHP and immunological outcomes.

3.6.1.1 ATSDR (2022)

According to ATSDR (2022), there are conflicting data about possible associations between human allergy and asthma risk and DEHP exposure. Several epidemiological studies found no association between adult or pediatric DEHP exposure and measures of allergy or asthma. A few human epidemiological studies on children; however, point to a possible association between exposure to

DEHP and allergies, asthma, wheeze, or airway inflammation (Franken et al. 2017; Gascon et al. 2015; Kim et al. 2018), as well as allergies (Ku et al. 2015; Podlecka et al. 2020; Wang et al. 2014). The forced expiratory volume in one second (FEV1/forced vital capacity, or FVC) ratio was found to be improved in a study of thirty community service workers (mean age 46 years) who had been exposed to DEHP along with other air, liquid, or solid pollutants for an average of 7.9 years (men) and 5.6 years (women) during waste and recycle processing or loading. Additionally, higher urinary MEHP levels (median 5.94 ng/mL) were also observed. General population research yields inconsistent results. Increased DEHP metabolite (MEHHP and MEOHP) levels in urine were associated with worse pulmonary function test scores (FEV1/FVC and forced expiratory flow at 25 to 75 percent of FVC [FEF25–75]) in a panel study involving 418 Korean adults over 60 years of age. However, this association was only seen in people with particular genetic polymorphisms in the catalase (*CAT*), superoxide dismutase (*SOD2*), and myeloperoxidase (MPO) genes (GC-GC in CAT, TC-TC in SOD2, and Ag-AG in MPO) (Park et al. 2013). The authors of the study hypothesized that gene-environment interactions could modify how exposure to DEHP affects lung function. There were no studies that examined the dermal effects of oral or inhalation exposure to DEHP in humans. In an early patch test study, 23 volunteers had undiluted DEHP (dose not specified) applied to their backs under occluded conditions for 7 days, followed by a challenge application 10 days later, with no reports of dermal irritation or skin sensitization (Shaffer et al. 1945). Studies assessing possible associations between prenatal exposure and elevated IgE levels or a child's risk of wheeze had conflicting results. ATSDR (2022) found that the information currently available on humans does not point to the respiratory system as a vulnerable area for DEHP toxicity.

3.6.1.2 Health Canada (2018a)

Studies evaluated by Health Canada (2018a) assessed the relationship between phthalate exposure and skin allergy reactions, such as conjunctivitis, rhinoconjunctivitis, allergic rhinitis, and asthma and wheeze. Several of the studies reported an association between higher skin allergy responses and exposure to DEHP. Nevertheless, there was insufficient data to support a link between skin allergies and DEHP metabolites (MECPP, MEHHP, MEOHP, and MEHP). The seven studies also discovered insufficient evidence linking DEHP metabolites (MECPP, MEHHP, MEOHP, and MEHP) to allergic rhinitis, conjunctivitis, or rhinoconjunctivitis, and minimal evidence linking them to asthma and/or wheezing. The relationship between DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and respiratory tract infections, skin allergies, and allergic rhinitis, conjunctivitis, or rhinoconjunctivitis was not well supported by the available data. There was inadequate evidence for the association between DEHP metabolites (MEHP, MEHHP, MEOHP) to asthma, wheeze, and/or decreased pulmonary function.

3.6.1.3 Summary of the Immune Effects discussed in existing assessments

The scope and purpose of the assessments by ATSDR (2022) and Health Canada (2018a) were similar in conclusions drawn for the association between exposure to DEHP and immunological outcomes in humans. ATSDR (2022) found that the information currently available on humans do not support the respiratory system as a vulnerable area for DEHP toxicity. Health Canada (2018a) found that the relationship between DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and respiratory tract infections, skin allergies, and allergic rhinitis, conjunctivitis, or rhinoconjunctivitis was not well supported by the available data and there was inadequate evidence for the association between DEHP metabolites (MEHP, MEHHP, MEOHP) to asthma, wheeze, and/or decreased pulmonary function. 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

regardless of the limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.6.1.4 EPA Conclusion

EPA took into account the conclusions drawn by ATSDR (2022) and Health Canada (2018a)and determined that there was inadequate evidence of association between DEHP exposure and immunotoxicity. 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 however qualitative support as part of the weight of scientific evidence.

3.6.2 Summary of Animal Studies

NICNAS (2010) concluded that DEHP is not a skin sensitizer in animals and limited data indicated no sensitization occurs in humans. Similarly, ECHA (2017a) determined that the evidence from three dermal sensitization studies is not suggestive for adjuvant effects in mice following dermal application of DEHP. No discussion of dermal sensitization was included in the toxicological profile of DEHP by ATSDR (2022).

Although there are no established guideline methods for evaluating respiratory sensitization *in vivo*, several mechanistic studies by of DEHP have investigated this outcome. Three immunotoxicity studies (Han et al., 2014b; Guo et al., 2012; Yang et al., 2008) were identified in the subset of more sensitive studies (LOAEL <20 mg/kg-day) subjected to detailed evaluation by EPA to determine if a more sensitive POD would be provided by these studies compared to the POD of 4.8 mg/kg-day based on the effects on male reproductive tract observed in the three-generation reproduction toxicity study (Blystone et al., 2010; TherImmune Research Corporation, 2004) selected by most other regulatory agencies for risk assessment.

In an allergic asthma model study by Guo et al. (2012) to test whether DEHP has adjuvant effects, Balb/c mice were gavaged with 0 (saline), 0.03, 0.3, or 3 mg/kg-day of DEHP with and without subcutaneous injections of ovalbumin (OVA) for 52 days (n = 8/group). OVA was used as the sensitizer for this allergic asthma model. To evaluate whether long term DEHP exposure on pulmonary inflammation and immune response, authors measured airway hyperresponsiveness, immune cells in BALF, serum IgE, and cytokine levels in lung tissue. DEHP exposure alone did not increase airway hyperresponsiveness; however, OVA+DEHP caused significant airway resistance when compared to the OVA alone group. In the OVA+DEHP 3 mg/kg-day group, there was high resistance and low compliance. The study authors stated that the highest dose of DEHP and OVA promoted airway hyperresponsiveness. The ratio of eosinophils to total cells in BALF did not significantly change with DEHP alone. However, this ratio is significantly higher in mice exposed to both OVA and DEHP at all concentrations when compared to the saline control group. Serum total IgE levels were not altered in the DEHP-only exposed groups, but with OVA added to any dose of DEHP, the serum total IgE was significantly increased by 80 percent over saline controls. When measuring cytokines in lung tissue, the levels of Th1 cytokine, IFNγ, were not affected by OVA only, but the highest dose of DEHP+OVA significantly increased its levels. Further, levels of IL-4, a Th2 cytokine, were significantly increased in all DEHP+OVA treatment groups when compared to the saline controls. However, only the highest DEHP dose+OVA induced a significant increase in IL-4 when compared to the OVA only group. Similarly, the IFNγ/IL-4 ratio was significantly increased in

all DEHP+OVA treatment groups compared to the saline controls. The highest dose of DEHP+OVA showed the greatest increase in the IFNγ/IL-4 ratio when compared to the OVA-only group. These data indicate that DEHP may promote and may potentiate allergic asthma by adjuvant effect.

In an immunotoxicity study by Han et al. (2014b), weanling BALB/c mice were divided into eight groups (n = 8/group) and administered 0.03, 0.3, or 3 mg/kg DEHP with OVA (sensitizer) or saline for 28 days. Authors measured serum OVA-specific immunoglobulin, germinal center formation in the spleen, lymphocyte surface markers and nuclear transcription factors, and intracellular cytokines and both gene and protein expression in Tfh cells. DEHP treatment alone did not increase serum OVA-specific immunoglobulin levels; however, with OVA sensitization, DEHP treatment induced significant increases of 45 to 75 percent in serum IgE and IgG1 levels when compared to the corn oil+OVA control group. Similarly, when measuring germinal center formation using immunofluorescence, DEHP treatment alone did not elicit any germinal center reactions, but in mice at 300 µg/kg and above, there was a significant increase in mean fluorescence intensity of PNA+ germinal center when compared to the corn oil+OVA control group. Using flow cytometry to test the humoral immune response, authors revealed DEHP treatment alone did not stimulate an increase in cell quantity of Tfh and plasma cells. In the OVA-sensitized mice treated with DEHP, the authors reported that DEHP stimulates "the expansion of CD4+CXCR5+ICOS +/CD4+CXCR5+PD-1+Tfh cells and CD19+CD138+GL7+plasma cells." To further elucidate why there was an altered humoral immune response, investigators performed "an adoptive transfer of mixed Th cells and B cells from either DEHP-exposed or normal mice into SCID mice." There was a significant increase in IgE and IgG1 antibody production when Tfh cells or B cells from DEHP treated mice were co-transferred with B cells from normal or DEHP treated mice when compared to the control group. Further, IL-4 and IL-21 were significantly increased in Tfh cells from mice exposed to DEHP and sensitized with OVA when compared to the corn oil OVA control group. Gene expression and protein production of *Bcl-6* and *c-Maf*, genes and proteins related to Tfh differentiation, were measured. OVA sensitized animals treated with DEHP had significant increases in both mRNA and protein expression of Bcl-6 and *c-Maf* when compared to the corn oil OVA control group. Altogether, these data indicate that DEHP may act as an adjuvant when administered via oral gavage by inducing toxic effects in Tfh cells.

In an asthma-like OVA-immunized rat model study by Yang et al. (2008), male Wistar rats were divided into five groups (8 per group): saline (control), ovalbumin (OVA), DEHP 0.7mg/kg-day+OVA, DEHP 70 mg/kg-day+OVA, and DEHP 70 mg/kg-day. To test whether DEHP has an adjuvant effect on OVA-immunized rats, animals were given DEHP by oral gavage for 30 days. On days 19 to 27 of the exposure duration, rats were given a hypodermal injection of saline or OVA (1 mg). On days 31 to 37 animals were exposed to either aerosolized saline or OVA. Authors measured airway hyperresponsiveness (AHR), BAL cell counts, and lung histology. Results show OVA alone induced AHR, and DEHP significantly increased AHR in OVA-immunized rats in a dose-dependent manner. DEHP alone caused a slightly higher AHR when compared to the negative control groups, but it was lower than the DEHP+OVA groups. Histological examination of lungs revealed OVA induced increased mucus secretion, inflammatory cells infiltration, and airway wall thickness. DEHP was shown to aggravate these effects in OVA-immunized mice, but DEHP alone did not cause any alteration in these animals. Lastly, OVA exposed animals had significantly increased eosinophils in the BAL, an indicator or allergic asthma. Further, DEHP exposure in OVA-immunized mice significantly increased total cell counts and eosinophils in a dose dependent manner. In contrast, DEHP alone did not cause any significant differences in the BAL cell counts when compared to the control group. These data indicate DEHP acts as an adjuvant in an OVA-immunized asthma rat model by as indicated by aggravated AHR and effects on lung histology.

3.6.3 Conclusions on Health Effects on Immune System

Dose-response and temporality:
Although there are no established guideline methods for evaluating respiratory sensitizers *in vivo*, several mechanistic studies by of DEHP have investigated this outcome. The studies by Guo et al. (2012) and Han et al. (2014b) used the same dose levels of 0 (saline control), 0.03, 0.3, or 3 mg/kg-day of DEHP with and without subcutaneous injections of OVA. The study by Guo et al. (2012)had a duration of 52 days, whereas the study by Han et al. (2014b) was for 28 days. Similar immune responses were noted at these doses in both studies, including increased airway hyperresponsiveness, infiltration of eosinophils in the BALF, serum IgE, IL-4, and IFNγ/IL-4 in the study by Guo et al. (2012), and similar findings indicative of an increased humoral immune response as demonstrated by flow cytometry and an examination of the response following co-transferred B cells. Importantly, DEHP-exposure alone did not elicit an immune response in either study. Yang et al. (2008) tested whether DEHP has an adjuvant effect on OVA-immunized male Wistar rats by administering DEHP by oral gavage for 30 days. The low dose in the study by Yang et al. (2008) (0.7 mg/kg-day) was similar to the mid-dose in the studies by Guo et al. (2012) and Han et al. (2014b) (0.3 mg/kg-day), with the study by Yang et al. (2008) including a high dose 2 orders of magnitude higher (70 mg/kg-day). Similar effects on the immune system were noted at 0.7 and 70 mg/kg-day in the study by Yang et al. (2008), including airway hyperresponsiveness and histological observations of increased mucus secretion, inflammatory cells infiltration, and airway wall thickness. OVA exposed animals had significantly increased eosinophils in the BAL, an indicator or allergic asthma, and DEHP exposure in OVA-immunized rats significantly increased total cell counts and eosinophils in a dose dependent manner. Aside from a slight increase in airway responsiveness in the absence of OVA sensitization, DEHP alone did not cause any alteration in these animals but instead aggravated these effects in OVA-immunized rats.

Strength, consistency, and specificity:
In addition to comparable dose levels, the immunotoxicity studies by Guo et al. (2012) and Han et al. (2014b) used the same sensitization model (OVA), species (mouse), strain (BALB/c), and sample size (n = 8). These three immunotoxicity studies examined the effects of DEHP as an adjuvant to promote or potentiate an allergic response to the allergen, ovalbumin (OVA). The study by Guo et al. (2012) employed an allergic asthma model in Balb/c mice that indicated that DEHP promotes and potentiates allergic asthma to OVA through adjuvant effects, but importantly, there were no effects of treatment with DEHP alone on airway hyperresponsiveness, the ratio of eosinophils to total cells in bronchoalveolar lavage fluid (BALF), or serum total IgE levels. Similarly, the immunotoxicity study by Han (2014b), also in Balb/c mice, indicated that DEHP acts as an adjuvant when administered via oral gavage by inducing toxic effects in T follicular helper (Tfh) cells; but again, DEHP treatment alone did not increase serum OVA-specific immunoglobulin levels; elicit any germinal center reactions when measuring germinal center formation using immunofluorescence; or stimulate an increase in cell quantity of Tfh and plasma cells when humoral immune response was examined using flow cytometry. Similarly, Yang et al. (2008) employed an asthma-like OVA-immunized rat model to examine airway hyperresponsiveness (AHR), BAL cell counts, and lung histology. While DEHP alone caused a slightly higher AHR when compared to the negative control groups, the response was lower than the DEHP+OVA groups; and DEHP alone did not cause any of the effects on lung histology (increased mucus secretion, inflammatory cells infiltration, or airway wall thickness) observed in those treated with

OVA or co-treated with OVA+DEHP, nor did treatment with DEHP alone result in any significant differences in the BALF cell counts when compared to the control group.

In an assessment of the epidemiology evidence, ATSDR (2022) found that the information currently available on humans do not support the respiratory system as a vulnerable area for DEHP toxicity. Health Canada (2018a) found that the relationship between DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and respiratory tract infections, skin allergies, and allergic rhinitis, conjunctivitis, or rhinoconjunctivitis was not well supported by the available data, and there was inadequate evidence for the association between DEHP metabolites (MEHP, MEHHP, MEOHP) to asthma, wheeze, and/or decreased pulmonary function. The assessments were similar in conclusions drawn for the association between exposure to DEHP and immunological outcomes, and overall they determined that there was inadequate evidence of association between DEHP exposure and immunotoxicity.

Biological plausibility and coherence:
ATSDR (2022) summarized the evidence supporting a mechanism for the immune adjuvant effects of DEHP and stated that the effects on the humoral immune response are mediated by cytokines released from hyperfunctioning T follicular helper cells (CD4+ Th cell subset), which synthesize increased levels of IL-21 and IL-4, resulting in increased secretion of the immunoglobulins IgE and IgG1 related to allergic response. EPA considers the evidence in these three immunotoxicity studies (Han et al., 2014b; Guo et al., 2012; Yang et al., 2008) to support this mechanism of enhanced humoral immune response from DEHP acting as an adjuvant in animals sensitized to OVA. However, it is important to reaffirm that, aside from a slight increase in airway responsiveness in the absence of OVA sensitization in the study in rats (Yang et al., 2008), DEHP exposure alone did not result in sensitization or effects on other immune endpoints examined but instead acted as an adjuvant to exacerbate these effects in OVA-sensitized rodents.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
In conclusion, there is conflicting evidence in humans about possible associations between allergy and asthma risk and DEHP exposure. Several epidemiological studies found no association between adult or pediatric DEHP exposure and measures of allergy or asthma. A few human epidemiological studies on children point to a possible association between exposure to DEHP and allergies, asthma, wheeze, or airway inflammation (ATSDR, 2022). In animal studies, DEHP alone did not elicit any treatment-related effects on the immune system, with the exception of a minor increase in AHR (Yang et al., 2008), but instead only elicited effects on the immune system when acting as an adjuvant to exacerbate an allergic response to OVA. Given that those results are inherently testing the effects of an interaction of chemicals, they are therefore considered inappropriate for derivation of an oral POD, and EPA is not considering the three immunotoxicity studies (Han et al., 2014b; Guo et al., 2012; Yang et al., 2008) further in dose-response analysis.

3.7 Musculoskeletal Endpoints

3.7.1 Summary of Epidemiological Studies

ATSDR (2022) and Health Canada (2018a) assessments identified a few epidemiologic studies investigating the association between urinary metabolites of DEHP and musculoskeletal outcomes.

3.7.1.1 ATSDR (2022)

There is only one cohort study by Lee et al. (2020) of mother-child pairings that has epidemiological data for DEHP exposure and the musculoskeletal outcome. Reduced skeletal muscle index (SMI) in 6-year-old girls, but not in boys, was associated with maternal urine DEHP metabolite levels in a cohort study of 481 mother-child pairs assessed by ATSDR (2022). There were no associations found between the percentage of reduced skeletal muscle (% SM) in mothers or the percentage of SM in children's urine when it came to DEHP metabolite levels. Regarding the impact of DEHP exposure on human musculoskeletal systems, no further studies could be found.

3.7.1.2 Health Canada (2018a)

According to Health Canada (2018a), there is inadequate evidence supporting the majority of DEHP metabolites (MEHP, MEHHP, and MEOHP), osteoporosis, and bone mineral density. It was also decided that there was insufficient evidence for vitamin D and the other DEHP metabolites—MEOHP and MECPP. There was no evidence that DEHP metabolite (MECPP) and osteoporosis or bone mineral density were related. Two DEHP metabolites (MEHP, MEHHP) and total serum 25(OH)D and/or vitamin D deficiency in adults and pregnant women were found to be associated; however, the evidence for this relationship was weak.

3.7.1.3 Summary of the existing assessments of Musculoskeletal Endpoints

The scope and purpose of the assessments by ATSDR (2022) and Health Canada (2018a) were similar in conclusions drawn. ATSDR (2022) found only one study that looked at the effect of DEHP exposure on musculoskeletal system. This study found that there was no association between DEHP exposure in mother-child pairs and reduced skeletal muscle. Health Canada (2018a) found that there is inadequate evidence supporting the majority of DEHP metabolites (MEHP, MEHHP, and MEOHP), osteoporosis, and bone mineral density. 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 regardless of the limitations of the epidemiological data, each assessment provides qualitative support as part of the weight of scientific evidence.

3.7.1.4 EPA Conclusion

EPA took in to account the conclusions drawn by ATSDR (2022) and Health Canada (2018a) and determined that there was inadequate evidence to support the association between DEHP and musculoskeletal endpoints. 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 however qualitative support as part of the weight of scientific evidence.

3.7.2 Summary of Animal Studies

EPA identified one study examining the effects of DEHP on musculoskeletal endpoints (Chiu et al., 2018) in which groups of ICR (CD-1) mice (12 per group) were treated with 0 (corn oil), 1, 10, or 100 mg/kg-day of DEHP by oral gavage for 8 weeks for the *in vivo* portion of the study. Next, harvested bone marrow stromal cells (BMSCs) from untreated and DEHP-treated mice were isolated and treated with 0, 10, 25, 50, 100, or 125 mM of DEHP or 0, 5, 10, 25, 50, or 100 mM of DEHP's

major metabolite, MEHP, to conduct *in vitro* studies. BMSCs were cultured in osteo-blast differentiation medium with or without DEHP or MEHP (0–100 mM) for 7, 14, or 21 days.

There were no changes in body weights in mice exposed to DEHP for 8 weeks; however, liver to body ratio was significantly increased in mice exposed to 10 and 100 mg/kg-day. When measuring bone microstructure and bone morphometric parameters from mice exposed to 10 or 100 mg/kg-day DEHP for 8 weeks, authors reported significant decreases in bone mineral density, bone volume density (BV/TV) of trabecular bone (decreased 17%), thickness, and the number of trabecular bones when compared to the control group. In contrast, DEHP treatment did not alter trabecular separation, nor have an effect cortical bone BMD and other microstructure parameters.

DEHP and MEHP treatment significantly and dose-dependently inhibited osteoblast mineralization (25 µM and above for DEHP and 10 µM and higher for MEHP) at day 21 and alkaline phosphatase (ALP) activity at day 7. Additionally, BMSCs treated with 10 or 100 µM of DEHP or MEHP had significant decreases in expression of osteogenic genes *Runx2*, *ALP*, and *OCN* when compared to the controls. Similarly, *Wnt-1* and *β-catenin* gene expression was significantly decreased following treatment with either 10 or 100 µM of DEHP or MEHP; in contrast, both DEHP and MEHP significantly increased the ratios of phosphorylated β-catenin and β-catenin in BMSCs during osteoblast differentiation. DEHP and MEHP upregulated *Erα* protein expression as well. Further, when measuring adipogenesis in BMSCs, DEHP did not alter adipogenesis; however, MEHP treatment (1, 5, and 10 mM) significantly and dose-dependently increased adipocyte differentiation and *PPARγ* during adipogenesis when compared to control cells.

BMSCs had significantly increased adipocyte differentiation from 1, 10, and 100 mg/kg DEHP-treated mice when compared to the controls. Similarly, *PPARγ* mRNA expression was significantly increased in harvested BMSCs from DEHP treated mice when compared to the controls. *ALP* activity and mineralization was significantly decreased in BMSCs isolated from mice exposed to 10 and 100 mg/kg of DEHP. Likewise, *Runx2*, *Wnt1*, and *β-catenin* mRNA expression significantly decreased in BMSCs from DEHP treated mice at the same concentrations. Study authors concluded that these data indicate that DEHP and MEHP inhibit osteoblastogenesis, promote adipogenesis in BMSCs, and negatively alter bone microstructure possibly through the *Wnt/β-catenin* and *PPARγ* pathways.

3.7.3 Conclusions on Musculoskeletal Endpoints

Dose-response and temporality:
In the single animal study EPA identified examining the effects of DEHP on musculoskeletal endpoints (Chiu et al., 2018), mice were administered DEHP via oral gavage at 0 (corn oil), 1, 10, or 100 mg/kg-day for 8 weeks for the *in vivo* portion of the study. Treatment-related effects at 10 and 100 mg/kg-day supported a dose-response within this study, with increased relative liver weights and decreased bone mineral density, bone volume density (BV/TV) of trabecular bone (decreased 17%), thickness, and the number of trabecular bones when compared to the control group. However, DEHP treatment did not alter trabecular separation, nor have an effect cortical bone BMD and other microstructure parameters. The majority of the findings reported in this study (Chiu et al., 2018) are from the *ex vivo* portion of the study associated with concentrations *in vitro* (*e.g.*, µmol or mmol); therefore, it challenging to equate these *in vitro* concentrations to *in vivo* doses to the animal.

Although EPA focused its review of studies with LOAELs less than 20 mg/kg-day for dose-response, EPA reviewed ATSDR's (2022) consideration of a musculoskeletal hazard related to DEHP

exposure. ATSDR reported that no adverse musculoskeletal effects were observed following acute, intermediate, or chronic exposure oral exposure at doses up to 3000 mg/kg-day in nine studies of rats or following intermediate or chronic exposure at doses up to 2600 mg/kg-day in six studies of mice (ATSDR, 2022).

In an assessment of the epidemiology evidence, ATSDR (2022) identified only one epidemiology study that investigated the effects of DEHP exposure on the musculoskeletal system. This study found that there was no association between DEHP exposure in mother-child pairs and reduced skeletal muscle. Health Canada (2018a) found that there is inadequate evidence supporting the majority of DEHP metabolites (MEHP, MEHHP, and MEOHP), osteoporosis, and bone mineral density. EPA concluded that there was inadequate evidence to support the association between DEHP and musculoskeletal endpoints.

Strength, consistency, and specificity:
The one animal study by Chiu et al. (2018) with sensitive (LOAEL<20 mg/kg-day) reported decreased bone mineral density and bone volume fraction, accompanied by decreased osteoblastogenesis and mineralization of bone marrow stromal cells in mice exposed to DEHP for 8 weeks at 10 mg/kg-day and above. There are very few epidemiology studies examining the relationship between exposure to DEHP and musculoskeletal outcomes to determine if similar outcomes are observed in humans exposed to DEHP. Health Canada examined the association of DEHP exposure with osteoporosis, bone mineral density, and the relationship to Vitamin D, and determined that there is inadequate evidence supporting an association between the majority of DEHP metabolites (MEHP, MEHHP, and MEOHP) and osteoporosis or bone mineral density and no proof of an association of MECPP with these outcomes. Two DEHP metabolites (MEHP, MEHHP) have a weak association with low vitamin D levels in pregnant women and adults in general, but there is insufficient evidence for an association of other DEHP metabolites (MEOHP and MECPP) with vitamin D. The one cohort study by Lee et al. (2020) examined by ATSDR reported reduced skeletal muscle index (SMI) in 6-year-old girls, but not in boys, associated with maternal urine DEHP metabolite levels in a cohort study of 481 mother-child pairs. However, there were no associations found between the percentage of skeletal muscle (% SM) in mothers or the percentage of SM in children's urine when it came to DEHP metabolite levels. Furthermore, this association was related to skeletal muscle and not bone development, so it is challenging to determine the relevance of these findings in humans to the findings in the one animal study in mice by Chiu et al. (2018).

Biological plausibility and coherence:
There is very limited evidence among the few epidemiology studies and the single animal study with sensitive effects on musculoskeletal endpoints to provide a basis for EPA to make any determination regarding biological plausibility of effects of DEHP exposure on bone development or other musculoskeletal endpoints. The one study in mice showing effects at 10 and 100 mg/kg-day was not corroborated by findings in other studies in rats and mice at much higher doses. Furthermore, the epidemiological evidence demonstrates that there was inadequate evidence to support the association between DEHP and musculoskeletal endpoints; therefore there is substantial uncertainty about any relevance to humans of the effects noted in the single study in mice (Chiu et al., 2018).

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
EPA concluded that: (1) effects on bone development and structure were only noted in a single animal study in the pool of studies identifying more sensitive hazards (LOAEL <20 mg/kg-day); (2) no adverse musculoskeletal effects were observed following intermediate or chronic exposure oral

exposure at doses up to 3000 mg/kg-day in nine studies of rats or at doses up to 2600 mg/kg-day in six other studies of mice (ATSDR, 2022); and (3) the epidemiology assessment indicated insufficient evidence of an effect on musculoskeletal development. Additionally, the majority of the findings in the one animal study (Chiu et al., 2018) are from the *ex vivo* portion of the study associated with concentrations *in vitro* (*e.g.,* µmol or mmol); therefore, it challenging to equate these *in vitro* concentrations to *in vivo* doses to the animal. Finally, the *in vivo* effects (liver weight, bone microstructure and morphometric parameters) in this study were noted at 10 mg/kg-day and above—equivalent to LOAELs observed in many of the developmental and reproductive toxicity studies. Therefore, this study does not provide a more sensitive LOAEL than the consensus indicated by numerous developmental and reproductive outcomes. Therefore, EPA determined that the evidence for DEHP resulting in effects on this endpoint was minimal and decided not to consider the effects of DEHP on musculoskeletal endpoints in the study by Chui et al. (2018) further in dose-response.

3.8 Hazards Identified by Inhalation Route

3.8.1 Summary of Epidemiological Studies

EPA did not identify any specific epidemiological studies that looked at inhalation exposure to DEHP and any health outcome. However, there are studies that looked at aggregate exposure through urinary metabolites levels that represents all routes of exposure. There are limited epidemiological evidence regarding exposure to DEHP and respiratory effects.

ATSDR (2022) noted that in a study of 30 community service workers (mean age 46 years) who were exposed to DEHP along with other air, liquid, or solid pollutants for an average of 7.9 years (men) and 5.6 years (women) during waste and recycle processing or loading, Kolena et al. (2014) found improved pulmonary function (ratio of forced expiratory volume in 1 second [FEV1]/forced vital capacity [FVC]) with higher urinary MEHP levels (median 5.94 ng/mL). In a later study by the same author, Kolena et al. (2020), observed that 32 male firemen (mean age 38 years) exposed to DEHP and other air pollutants had enhanced pulmonary function (FEV1/FVC) and higher urinary MEHP, MEHHP, MEOHP, and MECPP levels. ATSDR (2022) concluded that small sample size restricts the interpretation of studies with increased pulmonary function, and they did not find other inhalation studies that evaluated lung function in workers after being exposed to DEHP. EPA agrees with the limitations noted by ATSDR regarding these studies, and add that the Health Canada (2018a) similarly noted that humans are concurrently exposed to various phthalates from multiple sources and through multiple pathways, adding to the lack of clarity.

3.8.2 Summary of Animal Studies

EPA identified five studies (Larsen et al., 2007; Ma et al., 2006; Kurahashi et al., 2005; Klimisch et al., 1992; Merkle et al., 1988) that exposed laboratory animals to DEHP via the inhalation route (see Table 3-9). Detailed study summaries for these inhalation studies are included in Appendix B.4.

The studies by Kurahashi et al. (2005) of male rats and Ma et al. (2006) of female rats were considered co-critical studies by ATSDR (2022) for POD departure selection for deriving a MRL for short-term inhalation exposure. Both studies exposed post-weaning Wistar rats via whole-body inhalation 6 hours per day, 5 days per week to DEHP at 0, 5, or 25 mg/m^3 and identified a LOAEC of 5 mg/m^3, with no NOAEC established. There are several limitations with these studies that increase uncertainty and reduce EPA's confidence in using the studies quantitatively to derive an inhalation POD. One of these areas of uncertainty is related to confidence in the exposure characterization. Test atmospheres were

generated by vaporizing DEHP (99.0–99.9%) contained in a flask immersed in oil at 90°C for the low-concentration or 130°C for the high concentration, and DEHP was measured once daily in the exposure chambers with a gas chromatograph with a column temperature of 220°C. Test atmosphere concentrations in the exposure chambers indicated that target concentrations were achieved and maintained within narrow limits in the Kurahashi et al. (2005) study (98–102% target) and in the Ma et al. (2006) study in Experiment 2 (91–104% target)—although concentrations were consistent, but lower, in Experiment 1 (79–82% target). However, because the exposures were via whole-body inhalation, it is uncertain to extent to which dermal absorption and oral exposure through ingestion associated with grooming may have contributed a dose to the animals that was not via inhalation.

There are several areas of uncertainty related to the findings in the studies that lower EPA's confidence in using these endpoints quantitatively to derive an inhalation POD for use in risk assessment. In the study of male rats (Kurahashi et al., 2005), relative (to body weight) seminal vesicle weights were significantly increased by 30 to 31 percent over controls in the 5 and 25 mg/m^3 animals at 8 weeks (absolute weight not reported) with body weights comparable to controls. If seminal vesicle weight was increased due to an androgen effect, then it would be expected to also occur following the 4-week exposure from PND 28 to PND 56. One would also expect to see increases in other androgen-dependent tissues, such as the prostate. However, seminal vesicle weights were comparable to controls at 4 weeks, and there were no treatment-related effects on weights of testes, epididymis, or ventral prostate in this study at either time point. Serum testosterone in the 5 and 25 mg/m^3 groups was significantly increased over controls at 8 weeks. However, serum testosterone was also increased over controls at 4 weeks, but in a manner that was not concentration-dependent, only attaining significance at 5 mg/m^3 and not at the high concentration of 25 mg/m^3. In this inhalation study (Kurahashi et al., 2005), there were no effects of treatment on plasma FSH or LH; gene expression of enzymes involved in testosterone biosynthesis in the testes (P450scc, 3β-HSD, CYP17, and CYP19), or testes histopathology, casting further doubt that the increases in serum testosterone and seminal vesicle weight are treatment-related.

In the study of female rats (Ma et al., 2006), body weights were significantly decreased at 25 mg/m^3 from exposure day 24 to 63 in Experiment 1 (exposed PND 22–42); however, body weights were comparable to controls in Experiment 2 (exposed PND 22–84). Sexual maturation and age at first estrous were accelerated at 5 and 25 mg/m^3 in both experiments. Mean age at vaginal opening was significantly earlier at 5 mg/m^3 (29.2 days, 30.3 days) and 25 mg/m^3 (29.5 days, 29.7 days) compared to controls (31.8 days, 32.0 days). Similarly, mean age at first estrous was significantly earlier at 5 mg/m^3 (30.6 days, 31.0 days) and 25 mg/m^3 (29.8 days, 30.6 days) compared to controls (32.7 days, 33.4 days). In Experiment 1, serum FSH, LH, and estradiol levels in the treated groups were comparable to controls. In Experiment 2, serum estradiol and LH levels at 25 mg/m^3 were significantly higher than controls. Total cholesterol was significantly lower than controls in Experiment 1 (18–21% lower) but significantly higher than controls in Experiment 2 (19–25% higher). Furthermore, the ***accelerated*** sexual maturation, while statistically significant, is a relatively small shift and is not replicated in other studies, therefore casting uncertainty on the attribution of this finding to treatment with DEHP. Specifically, oral studies by Grande et al. (2006) reported that time to vaginal opening was ***delayed*** by 2 days in Wistar rats gavaged with 15 mg/kg-day DEHP from GD6 to LD21, with similar delays in preputial separation noted in the males (Andrade et al., 2006a), with body weights comparable to controls. Similarly, in the three-generation reproduction study of SD rats (Blystone et al., 2010; TherImmune Research Corporation, 2004), vaginal opening and preputial separation were delayed by up to a week in the F1c, F2c, and F3c pups starting at 7500 ppm (359 mg/kg-day), associated with decreased body weights in these animals.

There are additional uncertainties in this study regarding the analysis of the estrous cycle data and the serum hormone data collected from females on diestrus. For the extended dosing to PND 84 with a collection on diestrus, the usefulness of the data is limited by the fact that there is a substantial difference between Diestrus 1 (Di1) and Diestrus 2 (Di2), with estrogen starting to increase on Di2 and then reaching its peak on Proestrus after Di2. Therefore, hormone measurements can vary widely when taken during Di1 (very little estrogen as antral follicle starting to grow) and also on Di2 (the follicles that are growing produce estrogen). EPA considers the lack of consistency between the two experiments and the challenges with the way the researchers measured and presented the estrous cycle and hormone data to reduce the Agency's confidence in considering the studies quantitatively for derivation of an inhalation POD.

The studies conducted by Merkle et al. (1988) and Klimisch et al. (1992) used similar test concentrations of DEHP**;** both studies tested at 10 and 50 mg/m^3, with the high concentration of 300 mg/m^3 in the developmental toxicity study by Merkle (1988) and 1,000 mg/m^3 in the 4-week inhalation exposure study conducted by Klimisch et al. (1992). Both studies were conducted in accordance with OECD guidelines (OECD 412 and OECD 414, respectively) and employed nose-only exposures, ensuring that exposure occurred only via inhalation. This also eliminates concerns associated with whole-body exposure in which dermal absorption and oral exposure through ingestion associated with grooming can occur. Furthermore, both studies measured analytical concentrations of DEHP in the air in the test chamber, with analytical measurements indicating that target concentrations were achieved and maintained with minimal variation (90–110% target), and particle size distribution analysis indicated acceptable MMAD less than 1.2 µm and GSD (2.9–9.5 µm).

However, EPA considered the effects reported in the studies by Merkle et al. (1988) and Klimisch et al. (1992) to be minor, transient, and not adverse. Specifically, in the development toxicity inhalation study (Merkle et al., 1988), the decrease in maternal body weight in the animals exposed to the high concentration, while statistically significant, was minor (<10% difference from controls). Similarly, the incidences of visceral variations noted in the fetuses at this concentration (26% fetuses in 56% litters) were significantly increased over controls (6% fetuses in 17% litters). However, the study authors reported that the majority of these visceral variations were renal pelvis dilatation, which is common in this strain of rats and within the range of historical control incidence for this performing laboratory. Furthermore, no data were provided on the specific types of visceral variations or any quantitative data on the incidence of renal pelvis dilatation in this study. Finally, renal pelvis dilatation is often associated with decreased fetal body weights and delayed development, and no effects on fetal weight were reported. The study authors further reported that there were no differences in offspring development in the satellite group that continued on study throughout the lactation period.

Similarly, in the 4-week study by Klimisch et al. (1992), treatment-related findings were limited to the high concentration and were minor (≤10%) increases in clinical chemistry (albumin, inorganic phosphorus) and weights of the liver and lungs. However, there were no findings in histopathology or electron microscopy (*e.g.*, peroxisome proliferation) of the liver to corroborate and an adverse effect. The minor increase in relative lung weights in males was corroborated by a slight increase in semi-quantitative grading of foam-cell content and alveolar septal thickening in lungs. All of these changes were reversible (comparable to controls) after 8 weeks of recovery; and in Satellite group II, there were no effects on fertility index or on pre- or post-implantation loss on GD 14 in untreated females mated with treated males.

Larsen et al. (2007) conducted inhalation studies using BALB/cJ mice to determine any effects of exposure to DEHP on respiratory irritation, inflammation, or sensitization. In the first experiment, mice

(n = 8/group) were exposed to 3.7, 18.4, 31.6, or 300 mg/m^3 DEHP aerosol in acetone for 60 minutes, and ventilatory parameters measured were before and after exposure using whole-body plethysmographs, so each animal served as its own control. The only finding following this acute exposure was rapid shallow breathing, indicated by increased respiratory rate and decreased tidal volume, at the highest concentration of 300 mg/m^3; however, it is uncertain whether this observation was a clinical sign due to acute exposure to DEHP given that it could be attributed to a behavioral reflex to avoid inhalation of the acetone (<1,900 ppm) carrier. The second experiment measured airway inflammation markers in BALF up to 48 hours following the 60-minute exposure to 300 mg/m^3 DEHP. Examination of BALF indicated no inflammatory response. In the third experiment, designed to determine respiratory sensitization from repeated exposure to DEHP, mice (10 per group) were exposed to: ovalbumin (OVA) control; OVA+DEHP; or OVA+$Al(OH)_3$ for 20 minutes per day, 5 days per week for 2 weeks, then 20 minutes weekly for 12 weeks. DEHP concentrations were 0.022, 0.094, 1.7, or 13 mg/m^3, and OVA concentration was 13 mg/m^3. Given that this study is testing the adjuvant effects of DEHP to enhance respiratory sensitization from OVA, it is confounded by co-exposure and not useful quantitatively for determining an inhalation POD.

Table 3-9. Dose-Response Analysis of Animal Toxicity Studies on DEHP via Inhalation

Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	Reference(s) (TSCA Study Quality Rating)
28-d old male Wistar rats exposed via whole-body inhalation 6 h/d, 5 d/wk at 0, 5, or 25 mg/m^3 (n = 12) for up to 8 wk; 6 rats/group killed at 4 wk, and remaining 6 rats/group at 8 wks.	5 mg/m^3 = LOAEC	↑ Serum testosterone and relative seminal vesicle weight weights ↑ 30–31% at ≥5 mg/m^3 at 8 wks, with body weights comparable to controls No effects on: body weight; weights of testes, epididymis, or ventral prostate; plasma FSH or LH; gene expression of enzymes involved in testosterone biosynthesis (P450scc, 3β-HSD, CYP17, and CYP19), or testes histopathology.	(Kurahashi et al., 2005) (Medium)
21-day old female Wistar-Imamichi rats exposed via whole-body inhalation 6 h/d, 5 d/wk at 0, 5, or 25 mg/m^3 from PND22-42 (Experiment 1) or PND22-84 (Experiment 2). In Experiment 2, rats were evaluated for changes in estrous cyclicity from PND 49 to PND 84.	5 mg/m^3 = LOAEC	↓ Body weight at 25 mg/m^3 from day 24–63 in Exp1, but comparable to controls in Exp2. Sexual maturation and age at first estrous accelerated at ≥5 mg/m^3 in both experiments. ↑Irregular estrous cycles at 25 mg/m^3 (29%) vs. controls (14%) in Exp2. In Exp1, serum FSH, LH, and estradiol comparable to controls, but in Exp2, ↑serum estradiol & LH at 25 mg/m^3. Total cholesterol ↓ 18-21% in Exp1, but ↑ 19–25% in Exp2. In Exp1, mRNA levels of aromatase ↑ 145% over controls, but in Exp2, there were no changes in mRNA expression of genes involved in estradiol biosynthesis.	(Ma et al., 2006) (Medium)
Pregnant Wistar rats (n = 25/group) head-nose exposure to aerosols at 0, 0.01, 0.05, 0.3 mg/L (0, 10, 50, 300 mg/m^3) for 6 hr/d from GD 6–15; 20/group terminated on GD2 0; remaining 5/group delivered and killed on PND 21. OECD 414 guideline for teratogenicity	50 mg/m^3 = NOAEC	Maternal body weight ↓ 9% at 0.3 mg/L on LD21. ↑ Visceral retardations at 0.3 mg/L (25.94% fetuses, 56.25% litters) over controls (6.94% fetuses, 16.67% litters), reported to be mostly renal pelvis dilatation, common in this strain of rats & high incidence in historical controls. During the post exposure and lactation periods, there were no differences in offspring development.	(Merkle et al., 1988) (Medium)[a]
M/F Wistar rats head-nose exposure to aerosols at 0, 0.01, 0.05, or 1.0 mg/L (0, 10, 50, 1,000 mg/m^3) for 6 hr/d, 5 d/wk for 4 wks. OECD 412 guideline (with additional measurements of fertility and electron microscopy). 15 males/group mated with untreated females (2–5/group) 2 and 6 wks after exposure, and untreated females killed on GD 14 to examine uterine contents.	50 mg/m^3 = NOAEC	At 1.0 mg/L: albumin ↑ 6–7% in ♂&♀; inorganic phosphorus ↑ 10% in ♂; absolute liver weight ↑ 9% in ♀; and relative liver weight ↑ 8% ♂ & 5% in ♀; but no findings in liver histopathology or electron microscopy (*e.g.*, peroxisome proliferation) to corroborate and an adverse effect. Relative lung weights ↑ 6% in ♂, corroborated by slight ↑ semi-quantitative grading of foam-cell content & alveolar septal thickening in lungs. All of these changes were reversible (comparable to controls) after 8 weeks of recovery. Satellite group II, no effects on fertility index or on pre- or post-implantation loss on GD 14 in untreated♀	(Klimisch et al., 1992) (Medium)[a]

Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	Reference(s) (TSCA Study Quality Rating)
BALB/cJ mice DEHP aerosol in acetone to determine: (1) irritation. Mice (n = 8) exposed to 3.7, 18.4, 31.6 or 300 mg/m^3 for 60 min; respiratory parameters measured before and after using body plethysmographs, so each animal served as its own control.	31.6 mg/m^3= NOAEC	DEHP did not cause sensory irritation in the upper respiratory tract as indicated by normal TB and comparable TP values in all exposure groups; however, rapid shallow breathing was observed at the highest concentration of 300 mg/m^3, indicating respiratory irritation, with decreased tidal volume up to 35% and increased respiratory rate of 15% of pre-exposure values by the end of the exposure.	(Larsen et al., 2007) (Medium)[a]
(2) The 2nd experiment measured airway inflammation response by bronchoalveolar lavage (BAL) in mice exposed for 60 min to 300 mg/m^3. BAL was collected 0, 6, 16, 24 and 48 h after end of exposure (n = 7/group).	300 mg/m^3 = NOAEC	No significant alterations in macrophage cell numbers over time, therefore authors suggested that even at the highest concentration, DEHP does not induce inflammation.	(Larsen et al., 2007) (Medium)[a]
(3) adjuvant effect/allergic airway inflammation from repeated exposure to DEHP, the third experiment, mice (n = 10/group) were exposed to: OVA control; OVA+ DEHP; or OVA+ Al(OH)3 for 20 min/day, 5 days/week for 2 wks, then 20 min weekly for 12 wks. DEHP at 0.022, 0.094, 1.7, or 13 mg/m^3, and OVA = 13 mg/m^3.	1.7 mg/m^3= NOAEC	IgG1 levels were significantly increased in the highest concentration (13 mg/m^3) when compared to the OVA control group. Furthermore, the numbers of eosinophils, neutrophils, and lymphocytes were significantly increased and the number of alveolar macrophages was significantly decreased in the Al(OH)^3 control group but was significantly increased in the 13 mg/m^3 DEHP group compared to OVA controls. IL-5 and IL-10 cytokine production from mediastinal lymph nodes was highest in the Al(OH)3 and 13 mg/m^3 DEHP group when compared to the OVA group. This same trend was seen in superficial and deep cervical lymph nodes. All DEHP concentrations increased INFγ secretion in MLNs. INFγ levels were less in the SLNs and DLNs. These results indicate DEHP inhalation increases inflammatory cells in the BAL and increased IgG1 levels at high concentrations, but lower doses of DEHP do not have an adjuvant effect nor induce pulmonary inflammation in this model	(Larsen et al., 2007) (Medium)[a]

[a] As discussed in the Systematic Review protocol for DEHP (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.

3.8.3 Conclusions on Hazards Identified by Inhalation Route

Dose-response and temporality:
The studies by Kurahashi et al. (2005) of male rats and Ma et al. (2006) of female rats exposed post-weaning Wistar rats via whole-body inhalation 6 hours per day, 5 days per week to DEHP aerosols at 0, 5, or 25 mg/m^3 and identified a LOAEC of 5 mg/m^3, with no NOAEC established. Serum testosterone in the 5 and 25 mg/m^3 groups was significantly increased over controls at 8 weeks. However, serum testosterone was also increased over controls at 4 weeks, but in a manner that was not concentration-dependent, only attaining significance at 5 mg/m^3 and not at the high concentration of 25 mg/m^3. Therefore, there is a lack of a definitive concentration-dependent relationship with testosterone at different time points. Furthermore, the studies conducted by Merkle et al. (1988) and Klimisch et al. (1992) used higher test concentrations of DEHP; both studies tested at 10 and 50 mg/m^3, with the high concentration of 300 mg/m^3 in the developmental toxicity study by Merkle (1988) and 1,000 mg/m^3 in the 4-week inhalation exposure study conducted by Klimisch et al. (1992). However, the findings in the studies by Merkle et al. (1988) and Klimisch et al. (1992) were considered to be minor, transient, and not adverse. Notably, the study by Klimisch et al. (1992) exposed rats for 4 weeks, so this study duration can be directly compared to the 4-week time point in the studies by Kurahashi et al. (2005) and Ma et al. (2006).

Strength, consistency, and specificity:
In the study of female rats (Ma et al., 2006), body weights were significantly decreased at 25 mg/m^3 from exposure day 24 to 63 in Experiment 1 (exposed PND 22–42); however, body weights were comparable to controls in Experiment 2 (exposed PND 22–84). Sexual maturation and age at first estrous were accelerated at 5 and 25 mg/m^3 in both experiments. In Experiment 1, serum FSH, LH, and estradiol levels in the treated groups were comparable to controls. In Experiment 2, serum estradiol and LH levels at 25 mg/m^3 were significantly higher than controls. Total cholesterol was significantly lower than controls in Experiment 1 but significantly higher than controls in Experiment 2. The lack of consistency in the results between the two experiments may reflect real differences in the nature of the effect over different exposure durations but introduces uncertainty and complicates interpretation of results.

Biological plausibility and coherence:
In the study of male rats (Kurahashi et al., 2005), relative (to body weight) seminal vesicle weights were significantly increased by 30 to 31 percent over controls in the 5 and 25 mg/m^3 animals at 8 weeks (absolute weight not reported) with body weights comparable to controls. If seminal vesicle weight was increased due to an androgen effect, then it would be expected to also occur following the 4-week exposure from PND 28 to PND 56. One would also expect to see increases in other androgen-dependent tissues, such as the prostate. However, seminal vesicle weights were comparable to controls at 4 weeks, and there were no treatment-related effects on weights of testes, epididymis, or ventral prostate in this study at either time point. As stated above, serum testosterone was increased at both concentrations at 8 weeks, although at 4 weeks, it was only significantly increased at the low concentration. There were no effects of treatment on plasma FSH or LH; gene expression of enzymes involved in testosterone biosynthesis in the testes (P450scc, 3β-HSD, CYP17, and CYP19), or testes histopathology, casting further doubt that the increases in serum testosterone and seminal vesicle weight are treatment-related.

In the study of female rats (Ma et al., 2006), the *accelerated* sexual maturation, while statistically significant, is a relatively small shift, and is not replicated in other studies, therefore casting uncertainty on the attribution of this finding to treatment with DEHP. Specifically, oral studies by Grande et al.

(2006) and the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004) delays in vaginal opening instead of accelerated sexual maturation.

Overall conclusions, statement of areas of confidence and uncertainty, and recommendations for risk assessment:
In conclusion, EPA did not consider any of the five inhalation studies in animals (Larsen et al., 2007; Ma et al., 2006; Kurahashi et al., 2005; Klimisch et al., 1992; Merkle et al., 1988) to be suitable for quantitative derivation of a POD. Although the studies by Kurahashi et al. (2005) of male rats and Ma et al. (2006) of female rats were considered co-critical studies by ATSDR (2022) for POD departure selection for deriving a MRL, both of these studies had limitations and uncertainties described above in Section 3.8.2, with one of the primary areas of uncertainty related to exposure characterization. While the test atmosphere concentrations were measured by gas chromatography, the authors did not state whether they determined particle size distribution, and no measurements of MMAD or GSD were reported, so it is not possible to discern the degree of deposition in the lungs which hampers the ability to quantify dose. Furthermore, because the exposures were via whole-body inhalation, it is uncertain to extent to which dermal absorption and oral exposure through ingestion associated with grooming may have contributed a dose to the animals that was not via inhalation. Uncertainties regarding whether the effects observed in the study of female rats by Ma et al. (2006) were due to DEHP are related to the inconsistency in the occurrence, temporality, and directionality of many of the effects on reproductive hormones, organ weights, and sexual maturation. There are additional uncertainties in this study regarding the analysis of the estrous cycle data and the serum hormone data collected from females on diestrus. For the extended dosing to PND 84 with a collection on diestrus, the usefulness of the data is limited by the fact that there is a substantial difference between Diestrus 1 (Di1) and Diestrus 2 (Di2), with estrogen starting to increase on Di2 and then reaching its peak on Proestrus after Di2. Therefore, hormone measurements can vary widely when taken during Di1 (very little estrogen as antral follicle starting to grow) and also on Di2 (the follicles that are growing produce estrogen). EPA considers the lack of consistency between the two experiments and the challenges with the way the researchers measured and presented the estrous cycle and hormone data to reduce the Agency's confidence in considering the studies quantitatively for derivation of an inhalation POD.

The studies conducted by Merkle et al. (1988) and Klimisch et al. (1992) employed nose-only exposures, ensuring that exposure occurred only via inhalation, and both studies measured acceptable analytical concentrations of DEHP in the air in the test chamber, in addition to acceptable particle size distribution. However, EPA considered the effects reported in these studies to be minor, transient, and not adverse. Furthermore, the fact that the studies by Merkle et al. (1988) and Klimisch et al. (1992) tested at higher concentrations than the studies by Kurahashi et al. (2005) and Ma et al. (2006) and did not observe adverse effects casts doubt that the findings observed in the latter studies were due to treatment with DEHP.

Finally, the study by Larsen et al. (2007) conducted using BALB/cJ mice to determine any effects of exposure to DEHP on respiratory irritation, inflammation, or sensitization only resulted in rapid shallow breathing following acute exposure at the highest concentration, and it was unclear if this finding was due to DEHP or a reflexive behavior to avoid inhalation of the acetone carrier. The second experiment by Larsen et al. (2007) tested the adjuvant effects of DEHP to enhance respiratory sensitization from OVA and was therefore confounded by co-exposure and not useful quantitatively for determining an inhalation POD.

EPA did not identify any specific epidemiological studies that looked at inhalation exposure to DEHP and any health outcome. However, ATSDR (2022) noted two studies that examined respiratory

outcomes associated with urinary metabolites levels representing an aggregation of all routes of exposure, including a study of workers performing waste and recycle processing or loading, (Kolena et al., 2014) and a study of male firefighters (Kolena et al., 2020). EPA agrees with the limitations noted by ATSDR regarding these studies, including the limited sample size and potential confounding factors of co-exposure to numerous other individual chemicals and mixtures in the job roles in these two studies, and adds that Health Canada (2018a) similarly noted that humans are concurrently exposed to various phthalates from multiple sources and through multiple pathways, adding to the lack of clarity regarding exposure characterization. Given the uncertainties associated with the epidemiology and animal toxicity studies via the inhalation route of exposure, EPA did not consider these studies quantitatively for determining an inhalation POD. During the August 2025 peer-review meeting, the SACC generally agreed with the uncertainties associated with the inhalation studies and supported the use of route-to-route extrapolation to address inhalation risk based on oral exposure studies, as the endpoints from the oral studies are the most robust (U.S. EPA, 2025o).

3.9 Weight of Evidence Conclusions: Hazard Identification

EPA identified 50 animal toxicology studies that provided information pertaining to hazard outcomes associated with exposure to less than or equal to 20 mg/kg/day, including: reproduction/development, metabolic/nutritional, cardiovascular/kidney, liver, neurological, immune, and musculoskeletal systems, in addition to hazards identified by the inhalation route.

For the metabolic hazard, a subset of effects on glucose-insulin homeostasis (including impaired glucose tolerance, increased fasting glucose levels, and impaired insulin resistance) was consistently observed across studies. However, an adverse outcome pathway demonstrating effects of DEHP on glucose homeostasis is not well established, and the largely mechanistic endpoints measured in these studies did not manifest themselves in clinical signs of toxicity such as lethargy, polyuria, etc. or other adverse apical outcomes. Further, the human-relevance of these effects is difficult to determine given the lack of robust epidemiological evidence supporting effects of DEHP on adverse clinical outcomes in humans associated with metabolic syndrome such as diabetes, high blood pressure, and high LDL cholesterol and the lack of human studies on glucose tolerance and insulin tolerance linked to exposure to DEHP. Due to these limitations and uncertainties, EPA is not further considering effects on glucose/insulin homeostasis and lipid metabolism for dose-response analysis or for use in estimating risk to human health.

For the cardiovascular/kidney hazard: in addition to the uncertainties within the animal studies themselves, there is lack of evidence indicating that the effects on the kidneys and secondary cardiovascular effects on blood pressure occur in humans. Studies on humans have yielded inconsistent findings about the association between exposure to DEHP and increased blood pressure and other adverse cardiovascular outcomes. Due to these limitations and uncertainty, EPA is not further considering effects on the kidneys or cardiovascular outcomes for dose-response analysis or for use in estimating risk to human health.

Similarly for the hazard to the liver, EPA is not further considering these effects for dose-response analysis or for use in estimating DEHP risk to human health, given the limitations in the existing epidemiological data and the fact that the adverse effects on liver observed in laboratory animals are generally reported at dose levels at or above levels associated with male reproductive effects.

For the neurotoxic hazard, EPA determined that the epidemiological evidence of association of DEHP exposure with neurological outcomes in humans was inconsistent among studies or inconclusive. EPA

considered the three studies on neurotoxicity studies in mice to have too much uncertainty regarding the limitations in the individual studies and the clinical relevance of the findings for human health to consider them further in dose-response for derivation of an oral POD.

Regarding immunotoxicity, there is conflicting evidence in humans about possible associations between allergy and asthma risk and DEHP exposure. In animal studies, DEHP alone did not elicit any treatment-related effects on the immune system, with the exception of a minor increase in AHR in one study (Yang et al., 2008), but instead only elicited effects on the immune system when acting as an adjuvant to exacerbate an allergic response to OVA. Given that those results are inherently testing the effects of an interaction of chemicals, they are therefore considered inappropriate for derivation of an oral POD, and EPA is not considering the three immunotoxicity studies further in dose-response analysis.

In the single study examined by EPA identifying a musculoskeletal hazard, EPA concluded that the effects on bone development and structure were only noted in this one animal study in the pool of studies identifying more sensitive hazards, and no adverse musculoskeletal effects were observed following intermediate or chronic exposure oral exposure at doses up to 3000 mg/kg-day in nine studies of rats or at doses up to 2600 mg/kg-day in six other studies of mice (ATSDR, 2022). Furthermore, the epidemiology assessment indicated insufficient evidence of an effect on musculoskeletal development. Therefore, EPA determined that the evidence for DEHP resulting in effects on this endpoint was minimal and decided not to consider the effects of DEHP on musculoskeletal endpoints in the study by Chui et al. (2018) further in dose-response.

EPA did not consider any of the five inhalation studies in animals (Larsen et al., 2007; Ma et al., 2006; Kurahashi et al., 2005; Klimisch et al., 1992; Merkle et al., 1988) to be suitable for quantitative derivation of a POD. Although the studies by Kurahashi et al. (2005) of male rats and Ma et al. (2006) of female rats were considered co-critical studies by ATSDR (2022) for POD selection for deriving an inhalation MRL, both of these studies had limitations and uncertainties described above in Section 3.8.2 3.8.1, including whether the effects observed in the study of female rats by Ma et al. (2006) were due to DEHP considering the inconsistency in the occurrence, temporality, and directionality of many of the effects on reproductive hormones, organ weights, and sexual maturation. EPA considered the effects reported in studies conducted by Merkle et al. (1988) and Klimisch et al. (1992) to be minor, transient, and not adverse. Furthermore, the fact that the studies by Merkle et al. (1988) and Klimisch et al. (1992) tested at higher concentrations than the studies by Kurahashi et al. (2005) and Ma et al. (2006) and did not observe adverse effects casts doubt that the findings observed in the latter studies were due to treatment with DEHP. Finally, the study by Larsen et al. (2007) conducted using BALB/cJ mice to measure respiratory irritation, inflammation, or sensitization only resulted in rapid shallow breathing following acute exposure at the highest concentration, and it was unclear if this finding was due to DEHP or a reflexive behavior to avoid inhalation of the acetone carrier. The second experiment by Larsen et al. (2007) tested the adjuvant effects of DEHP to enhance respiratory sensitization from OVA and was therefore confounded by co-exposure and not useful quantitatively for determining an inhalation POD.

Regarding the hazard to the female reproductive tract, EPA determined that epidemiological evidence indicated slight confidence in the association between DEHP exposure and time to pregnancy, slight confidence in the association with DEHP and increases in spontaneous abortion, and moderate confidence in the association between DEHP exposure and increases in preterm birth. In spite of this limited epidemiological evidence of an association of DEHP exposure with some adverse female reproductive outcomes in humans, the few animals studies examining endpoints related to developing female reproductive tract had substantial deficiencies, limitations, lack of replication, and uncertainties

(Shao et al., 2019; Zhang et al., 2014; Pocar et al., 2012), or did not provide a sex-specific endpoint that is more sensitive than the well-established effects on developing male reproductive tract (Andrade et al., 2006a; Grande et al., 2006). Although ATSDR derived a MRL for intermediate duration oral exposure based on delayed meiotic progression of germ cells and accelerated folliculogenesis in female offspring reported by Zhang et al. (2014), it is important to note that these endpoints were not examined in other oral studies in rodents, so it is not possible to determine replicability, and this study only tested a single dose level, so it is not possible to examine dose-response. Therefore, these studies indicating potential effects on the developing female reproductive tract will not be considered further by EPA in dose-response analysis to derive a POD for human health risk assessment.

EPA has concluded that oral studies on the developing male reproductive system comprise the most robust, well supported evidence base of all hazards identified from exposure to DEHP, and in addition to this hazard assessment, EPA previously considered the weight of evidence and concluded that oral exposure to DEHP can induce effects on the developing male reproductive system consistent with a disruption of androgen action (U.S. EPA, 2023a). In numerous oral exposure studies in rodents, DEHP exposure during the critical window of development for disruption of androgen action resulted in treatment-related effects on the developing male reproductive system. Fifteen of these studies (comprising 19 publications) were well-conducted and reported LOAELs within a narrow dose range of 10 to 15 mg/kg-day based on the suite of effects on the developing male reproductive system consistent with phthalate syndrome. Epidemiology studies provide moderate to robust evidence of effects on the developing male reproductive system associated with exposure to DEHP, including decreases in AGD and testosterone and effects on sperm parameters; thereby corroborating the findings in animal studies and supporting the well-established adverse outcome pathway. In conclusion, EPA considers the observed developmental effects in males to be relevant for human health risk assessment and therefore further evaluated developmental toxicity via dose-response analysis in Section 4.

4 DOSE-REPONSE ASSESSMENT

EPA considered non-cancer hazard endpoints from developmental and reproduction studies for dose-response analysis as described in the following sections. While the vast majority of these studies indicate effects on the developing male reproductive system consistent with phthalate syndrome (previously described in Section 3.1.2.1 and Section 3.1.2.2), EPA also examined effects on development and reproduction in females (described above in Section 3.1.2.3). Given the deficiencies and uncertainties associated with many of the studies on the developing female reproductive system (Shao et al., 2019; Zhang et al., 2014; Pocar et al., 2012), or the fact that they do not provide a sex-specific endpoint that is more sensitive than the well-established effects on developing male reproductive tract (Andrade et al., 2006a; Grande et al., 2006), the effects on the developing female reproductive tract will not be considered further by EPA in dose-response analysis to derive a POD for human health risk assessment. The studies indicating effects primarily on the developing male reproductive system were selected for dose-response analysis because EPA has the highest confidence in these hazard endpoints for estimating non-cancer risk to human health. As described in Section 3, other non-cancer hazard endpoints were not considered for dose-response analysis due to limitations and uncertainties that reduce EPA's confidence in using these endpoints for estimating risk to human health.

For most hazard endpoints, the Agency used a NOAEL/LOAEL approach for the dose-response analysis based on a subset of critical studies. EPA considered NOAEL and LOAEL values from oral toxicity studies in experimental animal models. The epidemiology data, while providing moderate to robust evidence of effects on the developing male reproductive system, generally have uncertainties related to exposure characterization and temporality; thus the available epidemiology studies are not suitable for exposure-response analysis. For one hazard endpoint (*i.e.*, reduced fetal testicular testosterone in rats), the Agency conducted an updated meta-analysis and benchmark dose modeling using the approach previously published by NASEM (2017), which is further described in EPA's *Meta-Analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), Dicyclohexyl Phthalate (DCHP), and Diisononyl Phthalate* (U.S. EPA, 2025g). Acute, intermediate, and chronic non-cancer NOAEL/LOAEL values identified by EPA are discussed further below in Section 4.2. The Agency converted oral PODs derived from animal studies to HEDs using allometric body weight scaling to the ¾-quarters power (U.S. EPA, 2011c). Differences in dermal and oral absorption are corrected for as part of the dermal exposure assessment. Although several inhalation studies were identified, they were not determined to be informative and appropriate for derivation of a POD because the observed effects were minor, transient, and not adverse (Klimisch et al., 1992; Merkle et al., 1988), associated with uncertainties regarding exposure characterization and achieved dose (Ma et al., 2006; Kurahashi et al., 2005), or were confounded by co-exposure to other test materials (Larsen et al., 2007), as explained in Section 3.8.2. In the absence of acceptable inhalation studies, EPA performed route-to-route extrapolation to convert oral HEDs to inhalation HECs (Appendix D).

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

As described in Section 1.2.2, EPA further considered the 201 studies included in ATSDR's Table 2-2 LSEs (ATSDR, 2022) to identify studies with sensitive endpoints (LOAEL <20 mg/kg-day) for information on human health hazards not previously identified in existing assessments, including information that may indicate a more sensitive POD than established by the regulatory bodies prior to the publication of ATSDR in 2022. Of the 50 animal toxicology studies that EPA identified with a LOAEL less than 20 mg/kg-day, 24 of these studies evaluated reproductive/developmental outcomes and primarily indicated effects on the developing male reproductive system consistent with phthalate

syndrome. EPA included these 24 studies in its dose-response assessment to derive non-cancer PODs for estimating risks for acute, intermediate (> 1 day up to 30 days), and chronic exposure scenarios, as described in Section 4.2.

EPA conducted a synthesis of relevant non-cancer health effects in these 24 studies based on the following considerations:

- 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

Among the 24 sensitive (LOAEL less than 20 mg/kg-day) developmental/reproduction studies, 5 studies (discussed in Section 4.2.1) reported effects of DEHP at lower doses than the NOAEL of 4.8 mg/kg-day identified in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004). However, as discussed in Section 4.2.1, each of these studies had substantial deficiencies, limitations, and lack of replication which decreased EPA's confidence and precluded their use quantitatively for derivation of a POD for use in risk assessment (Shao et al., 2019; Wang et al., 2017; Hsu et al., 2016; Zhang et al., 2014; Pocar et al., 2012).

Thirteen of the 24 developmental/reproduction studies (discussed in Section 4.2.2) indicated LOAELs in the narrow range of 10 to 14 mg/kg-day based on findings primarily corroborating effects on the developing male reproductive system. Of these 13 studies, the LOAEL was observed at the lowest dose tested in 11 studies (Rajagopal et al., 2019b; Guo et al., 2013; Kitaoka et al., 2013; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009b; Vo et al., 2009a; Lin et al., 2008; Ge et al., 2007; Akingbemi et al., 2004; Ganning et al., 1990), while 2 of the 13 studies resulted in a LOAEL of 10 mg/kg-day but included lower doses to establish a NOAEL of 1 mg/kg-day (Akingbemi et al., 2001) or 3 mg/kg-day (Christiansen et al., 2010).

Six of these 24 publications (discussed in Section 4.2.3) identified a LOAEL at 14 or 15 mg/kg-day based primarily on a effects on the developing male reproductive system consistent with a disruption of androgen action and development of phthalate syndrome, including the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004) and the series of publications from the study by Andrade and Grande (2006b; 2006c; 2006a; 2006). EPA considers these six studies co-critical to support a consensus NOAEL of 5 mg/kg-day.

Additionally, as part of the dose response analysis, EPA reviewed a meta-regression analysis and benchmark dose (BMD) modeling analysis of decreased fetal testicular testosterone data published by NASEM (2017). EPA identified new fetal testicular testosterone data for DEHP (Gray et al., 2021) and conducted an updated meta-analysis. The results of the initial NASEM meta-analysis and EPA's updated analysis are discussed in Section 4.2.4.

4.2.1 Studies with Substantial Deficiencies, Limitations, and Uncertainties

Several studies (Shao et al., 2019; Wang et al., 2017; Hsu et al., 2016; Zhang et al., 2014; Pocar et al., 2012) reported effects of DEHP at lower doses than the NOAEL of 4.8 mg/kg-day identified in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004). However, each of these studies had substantial deficiencies and limitations, described below, which decreased EPA's confidence and precluded their use quantitatively for derivation of a POD for use in risk assessment. Given the deficiencies and uncertainties associated with these studies, they will not be considered further by EPA for study and endpoint selection to derive a POD for risk assessment:

In the study by Wang et al. (2017), pregnant SD rats were administered DEHP in corn oil via oral gavage at dose levels of 0, 0.01, 0.1, and 1 mg/kg-day daily beginning at implantation and continuing through the remainder of gestation and lactation (GD 7–LD 21). The objective of this study was to determine if male offspring exposed to DEHP *in utero* and during lactation were more susceptible to developing prostate cancer. On PND 90, one group of F1 males (11 per dose group) was implanted with silastic capsules containing testosterone and estradiol, while another group of F1 males (11 per dose group) were implanted with empty silastic capsules; these capsules were replaced on PND 146. On PND 196, all rats were terminated, and blood was collected, along with testes, epididymis, and prostate. Additionally, positive control groups were included in which F1 males were treated with 25 µg/pup 17-estradiol-3-benzoate (EB) by injection in nape of the neck on PNDs 1, 3, and 5, with one group implanted with the silastic capsules containing testosterone and estradiol, and the other EB-treated group implanted with sham-control empty capsules. EPA only considered groups dosed with DEHP compared to vehicle controls quantitatively for dose-response analysis (excluding groups treated with testosterone and estradiol and/or EB). Prostatic Intraepithelial Neoplasia (PIN) score (used to assess precursor lesions to prostate carcinogenesis) and Gleason score (indicating prostate carcinogenesis) were increased over negative controls at 0.1 mg/kg-day and above; however, these increases were not statistically significant. Absolute weights of prostate and testes and absolute and relative weights of epididymis were increased over negative controls at 0.1 mg/kg-day and above; however, histopathology was only reported qualitatively and depicted in representative micrograph images in the publication, but no quantitative data were provided on incidence or severity. Prostate specific antigen (PSA) was significantly increased over negative controls at 1 mg/kg-day. Although this study may provide limited evidence of increased susceptibility to prostate cancer with early exposure to DEHP, EPA determined 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) that tumors only occur at much higher doses in the liver (147 mg/kg-day and above), pancreas (189 mg/kg-day and above), and testes (Leydig cell tumors at 300 mg/kg-day) in long-term rodent bioassays of DEHP. In describing the study by Wang et al. (2017), ATSDR (2022) stated that prostate cancer or precursor lesions were not increased in adult SD rat offspring at doses up to 1 mg/kg-day but noted that the small sample size may limit the power to detect these effects. EPA determined that the study by Wang et al. (2017) had substantial uncertainty, providing low confidence for quantitative use in risk assessment because the findings were not statistically significant, not corroborated by incidence or severity data for histopathology, and DEHP only resulted in cancer in longer term studies at much higher doses.

In a study by Hsu et al. (2016), male SD rats were administered DEHP via oral gavage at 0.03, 0.1, 0.3, or 1 mg/kg-day from PND42 through PND105. At study termination, body weights, and weights of testes, epididymis, seminal vesicles, and kidneys were measured, along with sperm parameters (count, motility, morphology, reactive oxygen species (ROS), and chromatin structure analyses). There were no effects of treatment on sperm count or motility. Normal sperm was significantly lower only at 1 mg/kg-day (94.0%) compared to controls (96.2%). However, percent sperm with bent tails was significantly higher at 0.1, 0.3, and 1 mg/kg-day (1.1–2.0%) compared to controls (0.3%), and the percent of sperm with chromatin DNA damage, as indicated by DNA Fragmentation Index (DFI %), was higher at these doses (4.8–6.4%) compared to controls (2.1%). However, EPA considered this study to have high uncertainty regarding the plausibility and replicability of the effects on sperm for the following reasons: (1) sperm abnormalities were not observed in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004), also in SD rats, with the highest dose tested at 10,000 ppm in diet in the first parental generation (775 mg/kg-day) and F1 (543 mg/kg-day) generations, and 7500 ppm in the F2 generation (359 mg/kg-day); and (2) sperm count was decreased in F1, F2, and F3 males at 7500 ppm (359 mg/kg-day and above) and in first parental generation at 10,000 ppm (775 mg/kg-day), and these doses are much higher than those reporting apparent effects on sperm morphology in the study by Hsu et al. (2016). Therefore, EPA is not considering the study by Hsu et al. (2016) further for quantitative derivation of a POD for risk assessment.

Additionally, several studies on the developing female reproductive system with LOAELs less than 20 mg/kg-day were identified by EPA (Shao et al., 2019; Zhang et al., 2014; Pocar et al., 2012); however, these studies had substantial deficiencies, limitations, and lack of replication, discussed above in Section 3.1.2.3, which increase uncertainty and decrease EPA's confidence in using these studies quantitatively for derivation of a POD. Additionally, the study by Grande et al. (2006), which indicated delayed vaginal opening in F1 females at 15 mg/kg-day and above`, corroborates similar findings on preputial separation in males from the companion study report (Andrade et al., 2006a), and therefore indicates that the developing female reproductive tract is not more sensitive than that of males.

4.2.2 Studies Supporting Consensus LOAEL of 10 mg/kg-day

In addition to the 6 studies described above, 13 of the 24 sensitive developmental/reproduction studies resulted in LOAELs ranging from 10 to 14 mg/kg-day based on similar findings primarily corroborating effects on the developing male reproductive system. EPA considers these 13 studies to support a consensus LOAEL of 10 mg/kg-day. While the 2 studies described below established NOAELs of 1 mg/kg-day (Akingbemi et al., 2001) or 3 mg/kg-day (Christiansen et al., 2010) and LOAELs at 10 mg/kg-day, the remaining 11 studies resulted in LOAELs in the narrow range from 10 to 14 mg/kg-day but did not establish a NOAEL, as treatment-related effects were observed at the lowest dose tested(Rajagopal et al., 2019b; Guo et al., 2013; Kitaoka et al., 2013; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009b; Vo et al., 2009a; Lin et al., 2008; Ge et al., 2007; Akingbemi et al., 2004; Ganning et al., 1990). These 11 studies evaluated effects on the developing male reproductive system consistent with a disruption of androgen action and phthalate syndrome in rats (with 1 study in mice) following oral exposure to DEHP, with about half of the studies entailing dosing during gestation and/or lactation (Rajagopal et al., 2019b; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009a; Lin et al., 2008) and the remaining involving post-weaning exposures (Guo et al., 2013; Kitaoka et al., 2013; Vo et al., 2009b; Ge et al., 2007; Akingbemi et al., 2004; Ganning et al., 1990). These 11 studies have previously been presented in Table 3-5 and Table 3-6, while Table 4-4 provides brief study descriptions including a description of the effects observed at the LOAEL. Detailed study summaries are included in Appendix B.1.

Although these 11 studies consistently support a LOAEL of 10 mg/kg-day for DEHP, they are limited by dose-selection and did not test sufficiently low doses to establish a NOAEL. Therefore, EPA did not select any of these studies for deriving the POD because other, more sensitive developmental studies are available that evaluated doses below 10 mg/kg-day and allowed for a developmental NOAEL to be established. Instead, these studies comprise a robust database indicating a consensus LOAEL of 10 mg/kg-day and serve to refine the threshold at which treatment-related effects of DEHP occur. The treatment-related effects that form the basis for the LOAELs of 10 to 14 mg/kg-day in these 11 studies comprised effects on the following: steroidogenic and cholesterol transporter gene expression; hormones (testosterone, estradiol); sperm parameters (count, motility, morphology); sexual maturation; and organ weights, gross pathology, and histopathology of male reproductive tract (testes, epididymis, seminal vesicles, prostate)—including malformations characterizing phthalate syndrome. Importantly, with the exception of decreased steroidogenic and cholesterol transporter gene expression (*Scarb1*, *Star*, and *Hsd17b12*) observed on PND 1 in the study by Lin et al. (2009), all of these endpoints were examined in the principal study and co-critical studies on which the NOAEL of approximately 5 mg/kg-day is based. Therefore, EPA is confident that the principal and co-critical studies were adequately sensitive and comprehensive to justify the selected NOAEL of 4.8 mg/kg-day as the POD.

Two studies by Akingbemi et al. (2004; 2001), which in spite of some uncertainties discussed below, support a NOAEL of 1 mg/kg-day and a LOAEL at 10 mg/kg-day. In the first study by Akingbemi et al. (2001), post-weanling Long-Evans rats were gavaged with DEHP at 0, 1, 10, 100, or 200 mg/kg-day for 14 days (from PND 21–34 or PND 35–48) or for 28 days (from PND 21–48), and young adult Long-Evans rats similarly exposed for 28 days (from PND 62–89). In rats exposed to DEHP for 14 days, there were no decreases in serum concentrations; however, basal and LH-stimulated testicular testosterone production were decreased following 14-day exposure, with decreases at 100 mg/kg-day and above following earlier exposure (PND 21–34) and decreases at 10 mg/kg-day and above following later exposure (PND 35–48), accompanied by significant increases in steroidogenic enzymes ($P450_{SCC}$, 3β-HSD, P45017α, and 17β-HSD) in rats exposed at 100 mg/kg-day and above from PND35 through 49. In male rats exposed for 28 days (PND 21 through 48), significant *increases* were observed in: serum testosterone concentration (35–42% increase); interstitial fluid testosterone concentration (41–45% increase); serum LH (59–86% increase), and basal and LH-stimulated testicular testosterone production at 10 mg/kg-day and above. The authors attributed the inhibition of Leydig cell testosterone production to two factors, (1) decreased pituitary LH secretion; and (2) decreased steroidogenic enzyme activity and proposed a compensatory mechanism via negative feedback loop to explain the apparent shift in directionality of testosterone production depending on the duration and timing of exposure, with decreased testosterone stimulating the pituitary gland to increase LH production, which in turn results in Leydig cells increasing testosterone production. It was reported that no treatment-related effects were observed in older rats exposed from PND 62 through PND 89.

In a second study by Akingbemi et al. (2004), Long-Evans male rats were gavaged with DEHP at 0, 10, or 100 mg/kg-day from weaning (PND 21) to PND 90 or 120 and measured serum LH and testosterone by radioimmunoassay and *ex vivo* Leydig cell testosterone production (Experiment I). A second set of animals was similarly administered DEHP at the same doses, duration, and age to determine Leydig cell proliferation, measured by: (1) expression of cell division cycle marker, (2) tritiated thymidine incorporation, and (3) changes in cell number (Experiment II). A third set of male rats were administered DEHP at similar doses from PND 21 through PND 90, and serum 17β-estradiol (E2), Leydig cell E2 production, and aromatase gene (*Cyp19*) expression in Leydig cells were measured at PND 48 and PND 90 (Experiment III). In rats exposed from PND 21 through PND 90, serum LH and testosterone concentrations were significantly increased, and basal and LH-stimulated testicular

testosterone production were significantly decreased at 10 mg/kg-day and higher. In rats exposed longer (PND 21–120), similar increases in serum LH and testosterone concentrations and decreases in basal and LH-stimulated testicular testosterone production were observed but were only significant at 100 mg/kg/day. Leydig cell proliferation was indicated at 10 mg/kg-day and above based on significant increases in all three criteria (described above for Experiment II) following DEHP treatment from PND 21 through 90. Gene expression of cell cycle proteins (Cyclin G1, p53, cyclin D3, and PCNA) were generally increased at 10 mg/kg-day and above following treatment from PND 21 through PND 90. The authors attributed the increased proliferative activity in Leydig cells to induction of cell cycle proteins. Serum E2 levels and LH-stimulated Leydig cell E2 production were significantly increased at 10 mg/kg-day and above, while basal Leydig cell E2 production and aromatase gene induction were noted at 100 mg/kg-day following treatment from PND 21 through PND 48.

Overall, EPA considers the two studies by Akingbemi et al. (2004; 2001) to support a NOAEL of 1 mg/kg-day and a LOAEL at 10 mg/kg-day.

Table 4-1. Summary of Patterns of Change in Serum Hormone Levels and Leydig Cell Steroidogenesis During DEHP Exposure (Akingbemi et al., 2004; Akingbemi et al., 2001)

Parameter	Exposure Period		
	PND 21–48	PND 21–90	PND 21–120
Serum hormones			
LH	↑	↑	↑
T	↑	↑	↑
E2	↑	–	ND
Leydig cell steroidogenesis			
T	↑	↓	↓
E2	↑	↓	ND
Number of Leydig Cells in the testis	ND	↑	↑

↑ statistically significant increase; ↓ statistically significant decrease; — = unchanged; PND = postnatal day; T = testosterone; E2= β-estradiol; ND = not determined
Taken directly from Table 1 in Akingbemi et al. (2004), which also summarizes data from Akingbemi et al. (2001).

NICNAS (2010) and ATSDR (2022) considered the two studies by Akingbemi to support a NOAEL of 1 mg/kg-day and a LOAEL of 10 mg/kg-day based on increased serum LH and testosterone in rats exposed PND 21 through 48, with younger rats more sensitive to effects of DEHP on steroidogenesis. For POD selection, NICNAS (2010) provided a synthesis of data from several studies supporting a NOAEL from 1 through 10 mg/kg-day for effects on fertility and development and ultimately concluded that the NOAEL of 4.8 mg/kg-day from the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004) to be the most appropriate POD for risk estimates in adults and children. Other than these two regulatory bodies, no other assessments referenced the studies by Akingbemi et al. (2004; 2001), including Health Canada (Health Canada, 2020), ECHA (2017a, b), EFSA (2019), or U.S. CPSC (2014). Overall, EPA agrees with NICNAS (2010) and ATSDR (2022) who considered the two studies by Akingbemi to support a NOAEL of 1 mg/kg-day and a LOAEL of 10 mg/kg-day. Although the studies by Akingbemi et al. (2004; 2001) provide a more sensitive candidate POD, with a NOAEL of 1 mg/kg-day, EPA also agrees with NICNAS (2010) that the POD from the three-generation reproduction study in rats (Blystone et al., 2010; TherImmune Research Corporation,

2004) is more robust. EPA also agrees that the more sensitive NOAEL of 1 mg/kg-day provided by the study by Akingbemi (2001) is a reflection of lower dose selection, whereas the slightly higher NOAEL around 5 mg/kg-day reflected in the co-critical studies provides a more robust NOAEL (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006; TherImmune Research Corporation, 2004). It is important to note that the co-critical studies forming the basis of the NOAEL of approximately 5 mg/kg-day do not all uniformly examine all of the same endpoints as those evaluated in the studies by Akingbemi et al. (2004; 2001). While the study by Andrade et al. (2006c) measured serum testosterone, the three-generation reproduction study did not examine testosterone levels or production, although FSH and estradiol were measured in females(Blystone et al., 2010; TherImmune Research Corporation, 2004).

In a study by Christiansen et al. (2010), pregnant Wistar rats were gavaged with DEHP at 0, 10, 30, 100, 300, 600, or 900 mg/kg-day (Study 1) and at doses of 0, 3, 10, 30, or 100 mg/kg-day (Study 2) from GD 7 to LD 16. Results from the two independent studies, conducted 8 months apart, were reported in this publication. In Study 1, absolute AGD was significantly decreased by 8 to 14 percent at 10 mg/kg-day and above compared to controls, with significant decreases in body weight only noted at 300 mg/kg-day and above. Nipple retention was significantly increased at 10 mg/kg-day and higher, with mean of 1.23 to 5.01 nipples per male in the treated groups compared to a mean of 0.22 nipples per male in controls. Incidences of mild external genital dysgenesis were significantly increased at 100 mg/kg-day and above (17–50%) compared to controls (2%). In contrast, in Study 2, absolute AGD was only significantly decreased by 4 percent compared to controls at 100 mg/kg-day; nipple retention in the treated groups was comparable to controls; and incidences of mild external genitalia dysgenesis were significantly increased by 12 to 15 percent at 3 and 100 mg/kg-day, with increases of 8 to 10 percent (not statistically significant) at 10 and 30 mg/kg-day.

When data from both studies were combined, AGD was significantly decreased and nipple retention was significantly increased at 10 mg/kg-day and above. It was apparent that the incidences of mild external genitalia dysgenesis were clearly dose-dependent and consistently statistically significant only at doses at 100 mg/kg-day and higher. The study authors did not consider the incidences of external genitalia dysgenesis at 3 mg/kg-day to support a LOAEL, and EPA agrees with the determination of the LOAEL at 10 mg/kg-day based on increased nipple retention and decreased AGD, with the NOAEL established at 3 mg/kg-day. Additionally, when examining the data from the combined studies, absolute weights of the ventral prostate and LABC were generally consistently significantly decreased at 10 mg/kg-day and above; however, these organs were not subjected to histopathological examination.

Given the inconsistencies between the two studies in the endpoints of AGD and nipple retention, EPA did not consider the NOAEL of 3 mg/kg-day in the study by Christiansen et al. (2010) as the POD. Again, the more sensitive NOAEL of 3 mg/kg-day provided by the study by Christiansen et al. (2010) is more of a reflection of lower dose selection. Instead, the study by Christiansen et al. (2010) supports the consensus NOAEL of 5 mg/kg-day (or 4.8 mg/kg-day) based on studies by Andrade and Grande (2006b; 2006c; 2006a; 2006) and the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004). Furthermore, the LOAEL of 10 mg/kg-day based on decreased AGD and increased nipple retention in the study by Christiansen et al. (2010) aligns with 11 other studies with LOAELs at the lowest dose tested in the narrow range from 10 to 14 mg/kg-day based on effects on the developing male reproductive system (Rajagopal et al., 2019b; Guo et al., 2013; Kitaoka et al., 2013; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009b; Vo et al., 2009a; Lin et al., 2008; Ge et al., 2007; Akingbemi et al., 2004; Ganning et al., 1990).

4.2.3 Principal and Co-critical Studies Supporting a Consensus NOAEL of 4.8 to 5 mg/kg-day (LOAEL 14 to 15 mg/kg-day)

Prior to the current assessment by EPA, five regulatory bodies (Health Canada, 2020; EFSA, 2019; ECHA, 2017a; CPSC, 2014; NICNAS, 2010) identified the developing male reproductive tract as the most sensitive and robust outcome to use for human health risk assessment, and have consistently selected the same set of co-critical studies indicating a NOAEL of approximately 5 mg/kg-day and a LOAEL of approximately 15 mg/kg-day (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004), while several of these regulatory agencies also included the study by Christiansen et al. (2010), which had a similar NOAEL of 3 mg/kg-day and LOAEL of 10 mg/kg-day, but ultimately considered the NOAEL of 4.8 mg/kg-day from the three-generation reproduction study to be the most appropriate for POD selection (Blystone et al., 2010; TherImmune Research Corporation, 2004).The six publications of developmental/reproduction studies supporting a NOAEL of 4.8 to 5 mg/kg-day and a LOAEL of 14 to 15 mg/kg-day are described below.

In a three-generation reproductive study conducted by TherImmune Research Corporation (2004), DEHP was administered in the diet at concentrations of 1.5 (control), 10, 30, 100, 300, 1,000, 7,500, and 10,000 ppm to SD rats starting 6 weeks prior to mating and continuously for three generations, with three litters per generation. One to two males per litter were used for breeding purposes for the subsequent generation, and for the third generation, the remaining males (up to 6 per litter) were not culled, but were instead maintained to adulthood for histological examination. The control dose level was reported as 1.5 ppm because that was the concentration DEHP measured in the control diet. Achieved doses averaged 0.1 (control), 0.58, 1.7, 5.9, 17, 57, 447, and 659 mg/kg-day across the three generations; achieved dose for each generation is shown in Table_Apx B-1. The 10,000 ppm animals only completed the F1 generation and were terminated after failing to produce any F2 litters. In the non-mating males selected from the F1 and F2 male pups, aplastic testes and epididymis, and small testes, seminal vesicles, and prostates were noted in 1 to 3 animals at 300 ppm (14 mg/kg-day). The investigators concluded that, although the incidence of these findings is low, they are consistent with the syndrome of effects seen with other phthalate-induced male reproductive toxicity, and the incidences of small testes exceeded the historical control incidence at TherImmune Research Corporation. The authors noted that these findings represent sampling of only a small number of animals (1 male/litter) and are potentially treatment-related.

Given the limited initial sampling of 1 male/litter from this three-generation reproduction study (TherImmune Research Corporation, 2004), Blystone et al. (2010) conducted further evaluation of the reproductive tract malformations to elucidate whether the incidences of reproductive tract malformations in the males at 300 ppm were treatment-related. Power analysis curves generated from Monte Carlo simulation demonstrated that there is a substantial increase in the ability to detect an increased incidence of 10 percent over controls when 3 pups/litter were examined (66% of the time) or 4 pups/litter were examined (86% of the time) compared to examining 1 pup/litter (5% of the time). Therefore, Blystone et al. (2010) examined all males (1 to 6 per litter) from the F1c and F2c litters for malformations of the testes, epididymides, prostate, and seminal vesicles, and any reproductive tract malformations (RTMs) were recorded as ordinal data (present or absent) and evaluated separately for each generation and pooled across generations. When F1 and F2 litters were combined, litter incidence of RTM consistent with phthalate syndrome *(e.g.*, aplastic testes and epididymis; small testes, seminal vesicles, and prostate) were significantly increased over controls at 300 ppm, with malformations in the testes, epididymides, and/or prostate affecting 5 of 86 males from 5 of 25 litters compared to zero incidences observed in the 93 control males comprising 24 litters (see Table_Apx B-1). Therefore, the LOAEL in the study based on the more in-depth examination of all male offspring for RTM is 300 ppm (equivalent to 14 mg/kg-day), with the NOAEL established at 100 ppm (equivalent to 4.9 mg/kg-day in the F1

generation and 4.8 mg/kg-day in the F2 generation). Details on effects occurring at higher doses (such as delayed testes descent, vaginal opening, and preputial separation; increased nipple retention; decreased mating, pregnancy, and fertility indices; and effects on sperm count and male reproductive organ weights and histopathology) are included in Appendix B.1.

Blystone et al. (2010) also conducted BMD modeling on the RTM data using EPA's BMD Software (BMDS Version 2.1.1). Briefly, incidence of male RTMs in F1 (# affected litters/total # of litters: 0/14, 1/13, 1/16, 0/15, 4/17, 2/15, 9/13*, 8/8*), F2 (# affected litters/total # of litters: 0/10, 0/10, 0/10, 0/8, 1/8, 2/15, 3/10, 9/9*), and F1 and F2 combined litters ((# affected litters/total # of litters: 0/24, 1/23, 1/26, 0/23, 5/25*, 5/25*, 18/22*) were modeled using dichotomous models (*i.e.*, Logistic, Log Logistic, Log Probit, Gamma, Probit, Weibull) and a BMR of 5 percent extra risk. For this analysis the number of affected litters per dose group was modelled (*i.e.*, the litter was the statistical unit; Table_Apx B-1). Blystone *et al*. provided a summary of BMD modeling results, expressed in terms of the dietary concentration, in the publication, with details of the modeling results included as supplementary data. EPA performed a linear regression (see Appendix F) on the dietary concentrations (ppm) and achieved intake (mg/kg-day) values reported in the publication, to derive the achieved intake values associated with the BMD_5 and $BMDL_5$ for the F1, F2, and combined F1/F2 generations (Table 4-2). BMD modeling of litter incidences of total RTM data supports BMD_5/$BMDL_5$ estimates of 11.6/7.0 mg/kg-day from the best-fitting Weibull model for the F1 generation, 10.4/2.2 mg/kg-day from the best-fitting Weibull model for the F2 generation, and 8.5/5.6 mg/kg-day from the best-fitting Weibull model for combined F1 and F2 generations (Table 4-2). BMD_5 estimates from this analysis range from 8.5 to 11.6 mg/kg-day and are slightly lower than the LOAEL of 14 mg/kg-day supported by this study. Similarly, the $BMDL_5$ for RTM in the F1 offspring (7.0 mg/kg-day) was slightly higher than the NOAEL of 4.8 mg/kg-day, the $BMDL_5$ for the F2 offspring (2.2 mg/kg-day) was slightly lower than this NOAEL, while the $BMDL_5$ for the combined F1 and F2 offspring RTM (5.6 mg/kg-day) was comparable to the NOAEL of 4.8 mg/kg-day.

Table 4-2. BMD Modeling of Reproductive Tract Malformations (RTM) in F1 and F2 Male Offspring in Three-generation Reproductive Toxicity Study (Blystone et al., 2010)

Generation	Best-Fitting Model	BMD_5 (ppm)[a]	$BMDL_5$ (ppm)[a]	BMD_5 (mg/kg-day)[b]	$BMDL_5$ (mg/kg-day)[b]
F1	Weibull	257	169	11.6	7.0
F2	Weibull	234	77	10.4	2.2
F1 and F2 combined	Weibull	198	142	8.5	5.6

[a] BMD and BMDL values provided in Blystone et al. (2010) were reported as ppm DEHP in the diet.
[b] EPA performed linear regression on the dietary concentrations (ppm) and achieved intake (mg/kg-day) values reported in Blystone et al. (2010) (Appendix F), to derive the achieved intake values (in mg/kg-day) associated with the BMD_5 and $BMDL_5$ (expressed as ppm) for RTM in the F1, F2, and combined F1/F2 offspring.

A study presented in a series of publications by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006) supports a LOAEL of 15 mg/kg-day and a NOAEL of 5 mg/kg-day, which align well with the NOAEL and LOAEL in the three-generation reproduction study described above (Blystone et al., 2010;

TherImmune Research Corporation, 2004). In the study reported by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006), pregnant Wistar rats were gavaged with 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, or 405 mg/kg-day DEHP from GD 6 to LD 21, and effects were examined in the F1 offspring. In male offspring, preputial separation was significantly delayed at 15 mg/kg-day and above, with the body weight at sexual maturation significantly decreased at 405 mg/kg-day (Andrade et al., 2006a). Sperm count was decreased by 19 to 25 percent at 15 mg/kg-day and above, and these decreases were significant compared to both the concurrent and historical controls; whereas the decreases noted at lower doses were smaller in magnitude (9 to 16%) and generally only decreased compared to concurrent (and not historical) controls (Andrade et al., 2006c). The authors considered this threshold of a 20 percent decrease to be biologically significant. EPA agrees with the determination that the effects on sperm count were adverse at 15 mg/kg-day and above, given the magnitude, increase over both concurrent and historical controls, and evidence of other effects occurring at that dose in this study (*e.g.*, delayed sexual maturation). It is also notable that in female offspring, mean time to vaginal opening was significantly delayed in F1 females at 15 mg/kg-day and above (37.1 to 38.1 days) compared to controls (35.6 days), with no dose-related effects on body weight at sexual maturation in the females (Grande et al., 2006). Details on effects occurring at higher doses (such as increased nipple retention and decreased AGD) are included in Appendix B.1.

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

As part of the dose-response analysis, EPA also reviewed a meta-regression analysis and BMD modeling analysis of decreased fetal testicular testosterone and male pup AGD data published by NASEM (2017). Based on results from 11 studies of rats (Furr et al., 2014; Saillenfait et al., 2013; Klinefelter et al., 2012; Hannas et al., 2011; Vo et al., 2009a; Culty et al., 2008; Howdeshell et al., 2008; Lin et al., 2008; Martino-Andrade et al., 2008; Borch et al., 2006; Borch et al., 2004), NASEM conducted a meta-regression analysis and BMD modeling analysis on decreased fetal testicular testosterone production data from seven studies of rats (Furr et al., 2014; Saillenfait et al., 2013; Hannas et al., 2011; Culty et al., 2008; Howdeshell et al., 2008; Lin et al., 2008; Martino-Andrade et al., 2008). Four studies were excluded from this meta-analysis analysis for various reasons. For example, three studies were excluded because sample sizes were not reported for each dose group (Vo et al., 2009a; Borch et al., 2006; Borch et al., 2004), while one study was excluded because testosterone was measured after stimulation of the testes with LH (Klinefelter et al., 2012). NASEM found a statistically significant overall effect and linear trends in $\log_{10}$(dose) and dose when data from all strains of rats was considered, with an overall large magnitude of effect (>50%) in its meta-analysis for DEHP. Sensitivity analysis indicated that the overall effect was robust to excluding individual studies. Overall, the linear-quadratic model provided the best fit (based on lowest AIC). BMD estimates from the linear-quadratic model were 15 mg/kg-day (95% confidence interval: 11, 24) for a 5 percent change (BMR = 5%) and 161 mg/kg-day (118, 236) for a 40 percent change (BMR = 40%) (Table 4-6).

NASEM (2017) also conducted a meta-regression analysis and BMD analysis of decreased male rat AGD. The analysis included AGD data from 13 rat studies (Li et al., 2013; Zhang et al., 2013; Christiansen et al., 2010; Christiansen et al., 2009; Gray et al., 2009; Lin et al., 2009; Culty et al., 2008; Lin et al., 2008; Martino-Andrade et al., 2008; Andrade et al., 2006c; Jarfelt et al., 2005; TherImmune Research Corporation, 2004; Moore et al., 2001). NASEM found a statistically significant overall effect of a reduction in AGD (–3.96 [95% CI: –5.07, –285]) and linear trends in $\log_{10}$(dose) (-1.97 [95% CI: –2.98, –0.96]) and dose (–1.55 [95% CI: –1.86, –1.24]) when data from all strains of rats (*i.e.*, Long Evans, SD, Wistar) was considered. 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 low heterogeneity (23%, p=0.12) and

a BMD_5 estimate of 270 mg/kg-day (95% CI: 180, 420). In subgroup analyses, SD rats appeared less sensitive than Wistar rats for decreased AGD, with a BMD_5 estimate of 290 mg/kg-day (95% CI: 170, >1,000) for SD rats, and a BMD_5 estimate of 150 mg/kg-day (95% CI: 100, 280) for Wistar rats.

Overall, the meta-regression and BMD analyses conducted by NASEM clearly demonstrate that decreased fetal testicular testosterone is a more sensitive endpoint than decreased male rat AGD.

Table 4-3. Summary of Studies Included in EPA's Meta-analysis and BMD Modeling Analysis for DEHP

Reference (TSCA Study Quality Rating)	Included in NASEM Meta-analysis and BMD Modeling Analysis?	Brief Study Description	Measured Outcome
(Lin et al., 2008) (Medium)	Yes	Pregnant Long-Evans rats (6-9 dams/group) gavaged with 0, 10, 100, 750 mg/kg-day DEHP on GD 2-20	Fetal testis testosterone content on GD 21
(Martino-Andrade et al., 2008) (Medium)	Yes	Pregnant Wistar rats (7 dams/group) gavaged with 0, 150 mg/kg-day DEHP on GD 13-21	Fetal testis testosterone content on GD 21
(Hannas et al., 2011) (Medium)	Yes	Pregnant Wistar rats (3-6 dams/group) gavaged with 0, 100, 300, 500, 625, 750, 875 mg/kg-day DEHP on GD 14-18	*Ex vivo* fetal testicular testosterone production (3-hour incubation) on GD 18
	Yes	Pregnant SD rats (3-6 dams/group) gavaged with 0, 100, 300, 500, 625, 750, 875 mg/kg-day DEHP on GD 14-18	
(Culty et al., 2008) (Medium)	Yes	Pregnant SD rats (3 dams/group) gavaged with 0, 117, 234, 469, 938 mg/kg-day DEHP on GD 14-20	*Ex vivo* fetal testicular testosterone production (24-hour incubation) on GD 21
(Furr et al., 2014) (High)	Yes	Pregnant SD rats (2-3 dams/group) gavaged with 0, 100, 300, 600, 900 mg/kg-day DEHP on GD 14-18 (Block 31)	*Ex vivo* fetal testicular testosterone production (3-hour incubation) on GD 18
	Yes	Pregnant SD rats (2-3 dams/group) gavaged with 0, 100, 300, 600, 900 mg/kg-day DEHP on GD 14-18 (Block 32)	
(Howdeshell et al., 2008) (High)	Yes	Pregnant SD rats (4 dams/group) gavaged with 0, 100, 300, 600, 900 mg/kg-day DEHP on GD 14-18	*Ex vivo* fetal testicular testosterone production (3-hour incubation) on GD 18

Reference (TSCA Study Quality Rating)	Included in NASEM Meta-analysis and BMD Modeling Analysis?	Brief Study Description	Measured Outcome
(Saillenfait et al., 2013) (High)	Yes	Pregnant SD rats (8-16 dams/group) gavaged with 0, 50, 625 mg/kg-day DEHP on GD 12-19	*Ex vivo* fetal testicular testosterone production (3-hour incubation) on GD 19
(Gray et al., 2021) (High)	No	Pregnant SD rats (2-3 dams/group) gavaged with 0, 100, 300, 600, 900 mg/kg-day DEHP on GD 14-18 (Block 76).	*Ex vivo* fetal testicular testosterone production (3-hour incubation) on GD 18
	No	Pregnant SD rats (3 dams/group) gavaged with 0, 100, 300, 600, 900 mg/kg-day DEHP on GD 14-18 (Block 77).	

Because EPA identified new fetal testicular testosterone data for DEHP (Gray et al., 2021), 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 [accessed October 15, 2024], 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 [2017] used Version 2.0.0) and an additional BMR of 10 percent was modeled. Appendix E provides justification for the selected BMRs of 5, 10, and 40 percent. Fetal rat testosterone data from eight studies was included in the updated analysis, including data from Gray et al. (2021) and data from the same seven studies included in the 2017 NASEM analysis. 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 4-5). There was substantial, statistically significant heterogeneity in all cases ($I^2 > 90\%$). The statistical significance of the overall effect was robust to leaving out individual studies (Table 4-5). The linear-quadratic model provided the best fit (based on lowest AIC) (Table 4-5). BMD estimates from the linear-quadratic model were 17 mg/kg-day (95% confidence interval: 11, 31) for a 5 percent change (BMR = 5%), 35 mg/kg-day [24, 63] for a 10 percent change (BMR = 10%), and 178 mg/kg-day (122, 284) for a 40 percent change (BMR = 40%) (Table 4-6). Notably, BMD5 and BMD40 estimates calculated by NASEM and as part of EPA's updated analysis are similar (*i.e.*, BMD5 values of 15 and 17 mg/kg-day; BMD40 values of 161 and 178 mg/kg-day). Further methodological details and results (*e.g.*, forest plots, figures of BMD model fits) for the updated meta-analysis and BMD modeling of fetal testicular testosterone data are provided in the *Meta-Analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), Dicyclohexyl Phthalate (DCHP), and Diisononyl Phthalate* (U.S. EPA, 2025g).

The $BMDL_5$ of 11 mg/kg-day (associated BMD_5 of 17 mg/kg-day) based on decreased fetal testicular testosterone production (Table 4-6) was not selected as the POD because it is not as sensitive as the POD provided by the NOAEL of 4.8 mg/kg-day from the three-generation reproduction study (Blystone

et al., 2010; TherImmune Research Corporation, 2004). Notably, BMD modeling of total RTM litter incidence data from Blystone *et al.* supports BMD_5/$BMDL_5$ estimates of 11.6/7.0 mg/kg-day for the F1 generation, 10.4/2.2 mg/kg-day for the F2 generation, and 8.5/5.6 mg/kg-day for combined F1 and F2 generations (Table 4-2). $BMDL_5$ estimates range from 2.2 to 7.0 mg/kg-day and are similar to the selected POD based on a NOAEL of 4.8 mg/kg-day, further supporting its selection for use in risk characterization.

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

Study Details (species, duration, exposure route/ method, doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors [a] [b]	Reference(s) (TSCA Study Quality Rating)
Principal and co-critical studies					
Male and female SD rats administered DEHP in the diet at 1.5, 10, 30, 100, 300, 1,000, 7,500, 10,000 ppm (mean achieved dose of 0.1, 0.58, 1.7, 5.9, 17, 57, 447, 659 mg/kg-d) continuously across 3 generations (3 litters per generation).	NOAEL = 4.8	↑ total reproductive tract malformations (testes, epididymis, seminal vesicles, prostate) in F1 and F2 males at 14 mg/kg-d	1.1	UF_A= 3 UF_H=10 *Total UF=30*	(Blystone et al., 2010; TherImmune Research Corporation, 2004) (High)
Female Wistar rats administered DEHP at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-d via oral gavage from GD 6–LD 21 (Gestation and Lactation)	NOAEL = 5	Delayed preputial separation at ≥15 mg/kg-d	1.2	UF_A= 3 UF_H=10 *Total UF=30*	(Andrade et al., 2006a) (Medium)
Female Wistar rats administered DEHP at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-d via oral gavage from GD 6–LD 21 (Gestation and Lactation)	NOAEL = 5	↓ 19–25% sperm production at ≥15 mg/kg-d	1.2	UF_A= 3 UF_H=10 *Total UF=30*	(Andrade et al., 2006c) (Medium)
Female Wistar rats administered DEHP at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-d via oral gavage from GD 6–LD 21 (Gestation and Lactation)	NOAEL = 5	Delayed vaginal opening at ≥15 mg/kg-d	1.2	UF_A= 3 UF_H=10 *Total UF=30*	(Grande et al., 2006) (Medium)
Studies supporting consensus LOAEL of 10 mg/kg-day					
Male Long-Evans rats administered DEHP at 0, 1, 10, 100, 200 mg/kg-d via oral gavage from PND 35–48 or PND 21–48 (Post Weaning – Puberty)	NOAEL = 1	↓ basal & LH-stimulated testosterone production on PND 49 after pre-pubertal (PND 35–48) exposure, but ↑ testosterone production with earlier and longer exposure (PND 21–48) at 10 mg/kg-d	0.2	UF_A= 3 UF_H=10 *Total UF=30*	(Akingbemi et al., 2001) (Medium)
Male Long-Evans rats administered DEHP at 0, 10, 100 mg/kg-d via oral gavage from PND 21–48, 21-90, or PND 21–120 (Post Weaning – Puberty or Adult)	LOAEL = 10	↑ serum estradiol (E2) & Leydig cell E2 production after PND 21–48 in males; ↑ serum testosterone and LH, ↓ Leydig cell testosterone and E2 production, Leydig cell proliferation after PND 21–90 in males; Leydig cell proliferation after PND 21–120.	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Akingbemi et al., 2004) (Medium)
Female Wistar rats administered DEHP at 0, 3, 10, 30, 100, 300, 600, 900 mg/kg-d via oral gavage from GD 7–LD 16 (Gestation and Lactation)	NOAEL = 3	↓ AGD, ↑ nipple retention, and ↓ LABC and ventral absolute prostate weights in male pups	0.7	UF_A= 3 UF_H=10 *Total UF=30*	(Christiansen et al., 2010) (High)

Study Details (species, duration, exposure route/ method, doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors [a] [b]	Reference(s) (TSCA Study Quality Rating)
Male Long-Evans rats; administered DEHP at 0, 10, 500, 750 mg/kg-d via oral gavage from PND 21-49. Follow up study at 0, 10, and 500 mg/kg-day for shorter duration (PND 21–34), not including 750 mg/kg-d (Post Weaning – Puberty)	LOAEL = 10	↓ time to preputial separation (39.7 days vs. 41.5 days in controls); ↑ serum testosterone (58%; $p<0.01$), ↑ body weight (8%), ↑ seminal vesicle weights (↑27%)) when dosed PND21-49	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Ge et al., 2007) (Medium)
Adult male CRL Long-Evans 90-day old rats administered DEHP at 0, 10, 750 mg/kg-d for 7 days via oral gavage; 1 subgroup terminated at 7 days and another subgroup given i.p. injection of EDS to eliminate Leydig cells and dosed DEHP for additional 4 days (Adult Exposure)	LOAEL = 10	↑ Leydig cell numbers (20%) after dosing 7 days (prior to EDS elimination of Leydig cells (Study 1)	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Guo et al., 2013) (Medium)
Female Long-Evans rats administered DEHP at 0, 10, 100, 750 mg/kg-d via oral gavage from GD 2–20 (Gestation Only)	LOAEL = 10	↑ FLC/cluster and ↑ testicular testosterone in F1 males on PND 1	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Lin et al., 2008) (Medium)
Female Long-Evans rats administered DEHP at 0, 10, 750 mg/kg-d via oral gavage from GD 12.5–PND 21.5 (Gestation and Lactation)	LOAEL = 10	↑ FLC aggregation and ↓ steroidogenic and cholesterol transporter gene expression (*Scarb1*, *Star*, *Hsd17b12*) at PND 1, ↓ serum testosterone at PND 21 in F1 males.	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Lin et al., 2009) (Low)
Female Wistar rats administered DEHP at 0, 10, 100 mg/kg-d via oral gavage from GD 9 – LD 21. Examined effects in F1 adult male offspring at PND 80 (Gestation and Lactation)	LOAEL = 10	↓ serum testosterone and estradiol (E2) in F1 adult males	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Rajagopal et al., 2019b) (Medium)
Female SD rats administered DEHP at 0, 10, 100, 500 mg/kg-d via oral gavage from GD 11–21 (Gestation – Parturition)	LOAEL = 10	↓ sperm count, viability, and motility at PND 63	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Vo et al., 2009a) (Low)
Male SD rats administered DEHP at 0, 10, 100, 500 mg/kg-d via oral gavage from PND 21–35 (Post Weaning – Puberty)	LOAEL = 10	↓ serum testosterone; ↓ absolute weights of prostate, seminal vesicles, epididymis; and testes histopathology	2.4	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Vo et al., 2009b) (Medium)[d]
Male CRL:CD (SD) rats administered DEHP at 0, 11, 33, 100, 300 mg/kg-d via oral gavage from GD 8–LD 17 (*in utero* cohort), GD 8–PND 65 (puberty cohort)	LOAEL = 11	↑ % of F1 males in both cohorts with "phthalate syndrome": retained nipples, fluid-filled flaccid testes, hypoplastic or malformed epididymis, epididymal	2.6	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Gray et al., 2009) (Medium)

Study Details (species, duration, exposure route/ method, doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	Uncertainty Factors [a] [b]	Reference(s) (TSCA Study Quality Rating)
(Gestation and Lactation, or through Puberty)		granuloma with small testis, testicular seminiferous tubular degeneration, malformed seminal vesicles or coagulating glands, and true hermaphroditism, in one male, with uterine tissue and ovotestis			
Adult Male A/J mice administered DEHP via diet at 0, 0.01, 0.1% (0, 12, 125 mg/kg-d) for 2, 4, and 8 weeks (Adult Exposure)	LOAEL = 12	↑ Sertoli cell vacuolation, germ cell sloughing in seminiferous tubules, lymphocytic infiltration in the testicular interstitium, and damage to the blood-testes-barrier	1.6	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Kitaoka et al., 2013) (Medium)
Adult Male SD rats fed DEHP in diet at 0, 200, 2000, or 20,000 ppm (0, 14, 140, and 1,400 mg/kg-d) for 102 weeks (Adult Exposure)	LOAEL=14	Inhibition of spermatogenesis and general tubular atrophy in testes	3.3	UF_A= 3 UF_H=10 UF_L=10 *Total UF=300*	(Ganning et al., 1990) (Medium)[d]
EPA Meta-analysis and BMD modeling of fetal testosterone					
Meta-regression and BMD modeling of fetal testicular testosterone in rats across eight studies of rats exposed to 1–600 mg/kg-day DEHP at various times during gestation	$BMDL_5$ = 11	↓ Fetal testicular testosterone	2.6	UF_A= 3 UF_H=10 *Total UF=30*	(U.S. EPA, 2025g)[c]

AGD = anogenital distance; BMDL = benchmark dose lower bound; EDS = ethane dimethanesulfonate; FLC = fetal Leydig cell; GD = gestation day; LABC = levator ani plus bulbocavernosus muscles; LD = lactation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = post-natal day; SD = Sprague-Dawley (rat); UF = uncertainty factor

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

[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to DIDP. 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] Meta-regression and BMD modeling of fetal testicular testosterone in 8 studies of rats exposed during gestation (Gray et al., 2021; Furr et al., 2014; Saillenfait et al., 2013; Hannas et al., 2011; Culty et al., 2008; Howdeshell et al., 2008; Lin et al., 2008; Martino-Andrade et al., 2008), including 4 high- and 4 medium-confidence studies.

[d] As discussed in the Systematic Review protocol for DEHP (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.

Table 4-5. Overall Meta-analyses and Sensitivity Analyses of Rat Studies of DEHP and Fetal Testosterone (Updated Analysis Conducted by EPA)[a]

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P-value	Tau	I^2	P value for Heterogeneity	AICs
Primary analysis									
Overall	Intercept	−103.69	−127.11	−80.27	4.04E−18	75.18	98.65	5.73E−270	477.69
Trend in log10(dose)	log10(dose)	−135.61	−170.18	−101.03	1.51E−14	46.35	96.47	2.53E−177	432.47
Linear in dose100	dose100	−21.92	−25.82	−18.02	3.46E−28	67.96	98.46	0.00E00[b]	448.00
LinearQuadratic in dose100	dose100	−30.88	−45.45	−16.31	3.26E−05	61.77	97.86	4.22E−238	435.16 *
LinearQuadratic in dose100	I(dose100 ^2)	1.21	−0.69	3.10	2.13E−01	61.77	97.86	4.22E−238	435.16
Sensitivity analysis									
Overall minus Lin et al. (2008)	Intercept	−108.89	−132.57	−85.22	1.95E−19	73.35	98.67	3.02E−264	441.10
Overall minus Saillenfait et al. (2013)	Intercept	−103.49	−127.52	−79.45	3.21E−17	75.21	98.61	4.86E−234	454.76
Overall minus Furr et al. (2014)	Intercept	−89.06	−112.06	−66.07	3.20E−14	66.18	98.48	3.72E−220	377.11
Overall minus Gray et al. (2021)	Intercept	−110.14	−136.73	−83.54	4.76E−16	76.76	98.49	1.55E−166	386.87
Overall minus Hannas et al. (2011)	Intercept	−106.48	−136.42	−76.55	3.13E−12	81.07	97.77	1.03E−181	343.54
Overall minus Howdeshell et al. (2008)	Intercept	−106.36	−131.60	−81.12	1.47E−16	77.33	98.83	6.46E−270	433.45
Overall minus Culty et al. (2008)	Intercept	−99.32	−124.00	−74.65	3.02E−15	75.33	98.75	1.25E−251	431.74
Overall minus Martino-Andrade et al. (2008)	Intercept	−105.35	−129.11	−81.59	3.64E−18	75.39	98.68	4.27E−270	466.34

[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.
[b] p-value too small to calculate and rounded to zero.

Table 4-6. Benchmark Dose Estimates for DEHP and Fetal Testosterone in Rats[a]

Analysis	BMR	BMD	CI, Lower Bound	CI, Upper Bound
2017 NASEM Analysis for all strains of rats using Metafor Version 2.0.0[b]				
Linear in dose100	5%	22	20	26
Linear in dose100	40%	222	195	258
LinearQuadratic in dose100*	5%	15	11	24
LinearQuadratic in dose100*	40%	161	118	236
Updated Analysis using Metafor Version 4.6.0				
Linear in dose100	5%	23	20	28
Linear in dose100	10%	48	41	58
Linear in dose100	40%	233	198	283
LinearQuadratic in dose100*	5%	17	11	31
LinearQuadratic in dose100*	10%	35	24	63
LinearQuadratic in dose100*	40%	178	122	284

[a] Indicates model with lowest Akaike information criterion (AIC). BMD = benchmark dose; BMR = benchmark response; CI = confidence interval.
[b] As reported in Table C5-8 and C5-9 of NASEM, 2017.

4.3 Weight of Scientific Evidence: Study Selection for POD

EPA has reached the conclusion that the HED of 1.1 mg/kg-day (NOAEL of 4.8 mg/kg-day) is appropriate for calculation of risk from acute, intermediate, and chronic exposures to DEHP. This POD is based on significant increases in total reproductive tract malformations (RTM) in testes, epididymis, seminal vesicles, prostate in F1 and F2 males at 14 mg/kg-day in the three-generation reproductive toxicity study (Blystone et al., 2010; TherImmune Research Corporation, 2004). EPA considers the study presented in a series of publications by Andrade and Grande et al. (2006c; 2006a; 2006) that established a LOAEL of 15 mg/kg-day and a NOAEL of 5 mg/kg-day, to align well with the NOAEL and LOAEL in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004) and are therefore considered these studies as co-critical. As the POD derived from both of these studies is based on a NOAEL, a total uncertainty factor of 30 was selected for use as the benchmark margin of exposure (based on an interspecies uncertainty factor [UF_A] of 3 and an intraspecies uncertainty factor [UF_H] of 10). Consistent with EPA guidance (2022, 2002b, 1993), EPA reduced the UF_A from a value of 10 to 3 because allometric body weight scaling to the three-quarter power was used to adjust the POD to obtain a HED (see also Appendix D).

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

EPA considers the selected POD to be relevant for all durations of exposure (acute, intermediate, and chronic). The selected POD is based on effects from continuous exposure throughout three generations in a reproductive toxicity study (Blystone et al., 2010; TherImmune Research Corporation, 2004) and is supported by co-critical studies by Andrade and Grande et al. (2006c; 2006a; 2006) in which maternal dosing was initiated at implantation and continued throughout the remainder of gestation and lactation periods through weaning of offspring. Therefore, the endpoints are relevant to both short-term and chronic endpoints and are more sensitive than supported by other chronic studies. Although single dose studies evaluating the effects DEHP on the developing male reproductive system are not available, studies of the toxicologically similar phthalate dibutyl phthalate (DBP) have demonstrated that a single exposure during the critical window of development can disrupt expression of steroidogenic genes and decrease fetal testes testosterone. Therefore, EPA considers effects on the developing male reproductive system consistent with a disruption of androgen action to be relevant for setting a POD for acute duration exposures (see Appendix C for further discussion). Notably, SACC agreed with EPA's decision to consider effects on the developing male reproductive system consistent with a disruption of androgen action to be relevant for setting a POD for acute durations during the July 2024 peer-review meeting of the DINP human health hazard assessment (U.S. EPA, 2024).

EPA has *robust overall confidence in the selected POD for acute, intermediate and chronic durations* based on the following weight of the scientific evidence:

- EPA has previously considered the weight of evidence and concluded that oral exposure to DEHP 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).
- Available epidemiology studies provide further evidence of male reproductive effects and underscore the human relevance of these endpoints. While epidemiology studies for DEHP generally have uncertainties related to exposure characterization, available studies were concluded to provide moderate to robust evidence of effects on the developing male reproductive system, including decreases in AGD and testosterone and effects on sperm parameters.
- DEHP exposure resulted in treatment-related effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in numerous oral exposure studies in rodents, of which 15 studies (comprising 19 publications) were well-conducted and reported LOAELs at or below 20 mg/kg-day (Table 4-4). Observed effects in rats perinatally exposed to DEHP in these 15 studies indicated effects in a narrow dose range of 10 to 15 mg/kg-day, and included altered testosterone production; decreased steroidogenic and cholesterol transporter gene expression (*Scarb1, Star, Hsd17b12)*; FLC aggregation, decreased AGD; increased NR; decreased male reproductive organ weights (prostate, seminal vesicles, epididymis, and LABC); delayed sexual maturation, decreased sperm production, count, viability, and motility; testes histopathology (*e.g.,* inhibition of spermatogenesis, tubular atrophy, Sertoli cell vacuolation, germ cell sloughing in seminiferous

tubules, lymphocytic infiltration in testicular interstitium), and reproductive tract malformations in males indicative of phthalate syndrome.

- The selected POD based on a NOAEL of 4.8 mg/kg-day is based on effects consistent with phthalate syndrome in two high quality studies, including a three-generation reproductive toxicity study in rats (TherImmune Research Corporation, 2004) and a follow up analysis which examined a larger number of pups from this study in order to have greater power to detect statistically significant increases in reproductive tract malformations (Blystone et al., 2010). Blystone et al. (2010) also conducted BMD modeling on the RTM data, which supports BMD_5/$BMDL_5$ estimates of 11.6/7.0 mg/kg-day for the F1 generation, 10.4/2.2 mg/kg-day for the F2 generation, and 8.5/5.6 mg/kg-day for combined F1 and F2 generations (Table 4-2). BMD_5 estimates from this analysis range from 8.5 to 11.6 mg/kg-day and are slightly lower than the LOAEL of 14 mg/kg-day supported by this study, while $BMDL_5$ estimates ranged from 2.2 to 7.0 mg/kg-day and are consistent with the NOAEL of 4.8 mg/kg-day, and support its selection as the POD.
- Furthermore, the medium-quality studies by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006), which exposed rats starting at implantation and throughout the remainder of gestation and lactation, established a LOAEL of 15 mg/kg-day and a NOAEL of 5 mg/kg-day, which are similar to the NOAEL (4.8 mg/kg-day) and LOAEL (14 mg/kg-day) in the three-generation reproduction study(Blystone et al., 2010; TherImmune Research Corporation, 2004). Therefore, consideration of these studies as co-critical studies provides additional strength and confidence in the selected POD, in both the outcomes and the dose at which they occur.
- In addition to the principal and co-critical studies (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006; TherImmune Research Corporation, 2004), 13 other studies indicated similar effects on the developing reproductive system in a narrow dose range supporting LOAELs of 10 to 14 mg/kg-day. Eleven of the 13 studies did not test low enough doses to establish a NOAEL (Rajagopal et al., 2019b; Guo et al., 2013; Kitaoka et al., 2013; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009b; Vo et al., 2009a; Lin et al., 2008; Ge et al., 2007; Akingbemi et al., 2004; Ganning et al., 1990). The two remaining studies support NOAELs of 1 and 3 mg/kg-day (Christiansen et al., 2010; Akingbemi et al., 2001). Although these NOAELs are lower than the selected POD (NOAEL of 4.8 mg/kg-day), this is merely a reflection of dose-selection, and EPA has higher confidence in the POD (NOAEL of 4.8 mg/kg-day) as a robust consensus NOAEL based on a high quality three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004) co-critical with the studies by Andrade and Grande et al. (2006b; 2006c; 2006a; 2006).
- The $BMDL_5$ of 11 mg/kg-day from EPA's updated meta-regression analysis and BMD modeling of decreased fetal testicular testosterone production data from eight studies of rats (Gray et al., 2021; Furr et al., 2014; Saillenfait et al., 2013; Hannas et al., 2011; Culty et al., 2008; Howdeshell et al., 2008; Lin et al., 2008; Martino-Andrade et al., 2008) was not selected as the POD because it is not as sensitive as the POD provided by the NOAEL of 4.8 mg/kg-day from the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004). However, the $BMDL_5$ of 11 mg/kg-day adds further strength and confidence to EPA's POD selection, given that the effect of decreased fetal testosterone production is a hallmark step in the adverse outcome pathway indicating effects on the developing male reproductive system consistent with phthalate syndrome on which EPA's POD is based.
- Beyond the BMD modeling supporting EPA's update to NASEM's meta-regression analysis of decreased fetal testosterone production data, EPA considers the NOAEL-LOAEL approach for POD selection from the evidence base for DEHP to provide greater confidence than any potential

POD based on individual BMD modeling, given the extensive database indicating a narrow threshold of effects. Selection of the NOAEL of 4.8 mg/kg-day from the three-generation reproduction (Blystone et al., 2010; TherImmune Research Corporation, 2004), supported by a NOAEL of 5 mg/kg-day in the co-critical studies by Andrade and Grande (2006c; 2006a; 2006), provides a robust NOAEL at 4.8 mg/kg-day with even greater confidence imparted by the fact that there are 15 studies providing LOAELs in the narrow range of 10 to 15 mg/kg-day based on effects on the developing male reproductive tract, resulting in a consensus LOAEL at 10 mg/kg-day. This narrow threshold (NOAEL of 4.8; LOAEL of 10 mg/kg-day) is based on all of the effects representing key events across the AOP and across all 15 studies. EPA acknowledges that, within a given study, BMD modeling of effects is generally preferred when deriving a POD because a BMD is unaffected by dose-selection, and the model takes into account considerations such as variability and sample size. However, in consideration of the extensive evidence for DEHP, it is unlikely that further BMD modeling would better refine the already narrow threshold supported by these studies. The fact that the BMD modeling conducted by Blystone et al. (2010) resulted in $BMDL_5$ for RTM ranging from 2.2 to 7.0 mg/kg-day (BMD_5 estimates ranged from 8.5 to 11.6 mg/kg-day), thus wrapping the NOAEL of 4.8 mg/kg-day, indicates as much. Therefore, EPA has robust confidence that using the entire body of evidence represents the best available science, compared to BMD modeling of any individual endpoint within an individual study.

- Similar to EPA, five regulatory bodies (Health Canada, 2020; EFSA, 2019; ECHA, 2017a; CPSC, 2014; NICNAS, 2010) identified the developing male reproductive tract as the most sensitive and robust outcome to use for human health risk assessment, and have consistently selected the same set of co-critical studies indicating a NOAEL of approximately 5 mg/kg-day and a LOAEL of approximately 15 mg/kg-day (Blystone et al., 2010; Andrade et al., 2006c; Andrade et al., 2006a; TherImmune Research Corporation, 2004), while several of these regulatory agencies also included the study by Christiansen et al. (2010), which had a similar NOAEL of 3 mg/kg-day and LOAEL of 10 mg/kg-day, but ultimately considered the NOAEL of 4.8 mg/kg-day from the three-generation reproduction study to be the most appropriate for POD selection (Blystone et al., 2010; TherImmune Research Corporation, 2004).
- Several studies (Shao et al., 2019; Wang et al., 2017; Hsu et al., 2016; Zhang et al., 2014; Pocar et al., 2012) reported effects of DEHP at lower doses than the NOAEL of 4.8 mg/kg-day identified in the three-generation reproduction study (Blystone et al., 2010; TherImmune Research Corporation, 2004). However, the dose-response data in each of these studies was less clear, and the studies generally had substantial deficiencies and limitations which decreased EPA's confidence and precluded their use quantitatively for derivation of a POD for use in risk assessment.

4.4 Route-to-Route Extrapolation

EPA did not identify any studies conducted via the dermal or inhalation exposure routes that are appropriate for directly determining human health risk. Therefore, EPA is using the oral HED of 1.1 mg/kg DEHP to extrapolate risk for the dermal and inhalation routes.

When conducting route-to-route extrapolations, the preferred approach is to use validated physiologically-based pharmacokinetic (PBPK) models or chemical-specific pharmacokinetic data to account for potential route-specific differences in toxicokinetics (IGHRC, 2006; U.S. EPA, 1994). Although several PBPK models have been developed for DEHP (Martínez et al., 2018; Sharma et al., 2018; Adachi et al., 2015; Lorber et al., 2010; Cahill et al., 2003; Keys et al., 1999) these models are not

fit for purpose and have not been validated to support route-to-route extrapolation for regulatory risk assessment. A detailed description of each of these models and their limitations is included in Section 3.1.5 of the *Toxicological Profile for DEHP* (ATSDR, 2022). Of the available PBPK models, the models described by Keys et al. (1999) are the most fully developed; however, these models have limitations that preclude their use in dosimetry predictions, including the fact that the model attributes all elimination of MEHP to liver metabolism, which does not account for the extensive urinary excretion (ATSDR, 2022). Given the limitations and lack of validation of the available PBPK models that preclude their use in regulatory risk assessment, EPA conducted route-to-route extrapolation using 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 2.1.1, available data indicate an assumption of 100 percent absorption of DEHP through the gastrointestinal tract following oral exposure is reasonable. Based on controlled oral exposure studies with human volunteers, the expectation is that greater than 70 percent of an oral dose of DEHP is absorbed (ATSDR, 2022; Kessler et al., 2012; Koch et al., 2005a).

As discussed in Section 2.1.2, dermal absorption of DEHP and/or its monoester metabolite MEHP have been evaluated extensively in 3 *in vivo* studies of rats, 2 *in vivo* studies of guinea pigs, 6 *in vitro* studies with human skin, 5 *in vitro* studies with rat skin, and 1 *in vitro* study each of mouse and pig skin. Overall, EPA selected the *in vitro* dermal absorption study by Hopf et al. (2014) of neat aqueous DEHP using metabolically active viable human skin samples to estimate steady-state dermal flux values for DEHP to estimate dermal exposure to aqueous DEHP (Section 2.1.2). EPA also selected the *in vivo* dermal exposure study of DEHP from solid PVC film with rats by Chemical Manufacturers Association (1991) to estimate the dermal absorption from contact with solids containing DEHP. Given the extensive database of DEHP and/or MEHP dermal absorption studies, EPA has a strong understanding of the dermal absorption kinetics of DEHP and has robust confidence in its dermal absorption approach.

It is important to account for potential route-specific differences in metabolism. Following oral exposure, phthalate diesters (including DEHP) are metabolized to monoester metabolites (*e.g.,* MEHP) 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 DEHP to its monoester metabolite MEHP also occurs via the dermal route prior to systemic circulation. For example, as discussed above in Section 2.1.2 (Hopf et al., 2014) and in the non-cancer human health hazard assessments of DBP (U.S. EPA, 2025i) and BBP (U.S. EPA, 2025h), dermal absorption studies with metabolically active human or rat skin demonstrate metabolism of DEHP, DBP, and BBP to their respective monoester metabolites MEHP, MBP, and MzBP, as well as other oxidative metabolites. Given that the study by Hopf et al. (2014) was the only study that EPA identified which used metabolically-active human skin, EPA used these data to determine dermal absorption of liquid formulations of DEHP as described in Section 2.1.2. Therefore, EPA is confident that its human health risk characterization via the dermal route for DEHP is health protective

Inhalation Route

EPA identified five studies (Larsen et al., 2007; Ma et al., 2006; Kurahashi et al., 2005; Klimisch et al., 1992; Merkle et al., 1988) that exposed laboratory animals to DEHP via the inhalation route (see Table

3-9). EPA did not consider any of the five inhalation studies in animals to be suitable for quantitative derivation of a POD. Although the studies by Kurahashi et al. (2005) of male rats and Ma et al. (2006) of female rats were considered co-critical studies by ATSDR (2022) for POD selection for deriving an inhalation MRL, both of these studies had limitations and uncertainties described above in Section 3.8.2. SACC agreed with EPA that the uncertainties regarding the biological data in these studies (Ma et al., 2006; Kurahashi et al., 2005) and their inconsistency with the experimental data from other investigators make their use in determining an inhalation POD problematic (U.S. EPA, 2025o).

Therefore, 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 DEHP through the gastrointestinal tract following oral exposure and the lung following inhalation exposure (Section 2.1.1). The rapid absorption and distribution and similar metabolism and excretion profiles, regardless of route of exposure, supports the use of route-to-route extrapolation of oral studies to determine an inhalation POD. Reliable quantitative data are available from animal studies (*e.g.*, rodents, dogs, pigs, nonhuman primates) that characterize DEHP distribution (ATSDR, 2022; Kurata et al., 2012; Rhodes et al., 1986; General Motors, 1982; Ikeda et al., 1980; Tanaka et al., 1975). Following oral, intravenous, dermal, or inhalation routes of exposure in rats, DEHP was found in blood, liver, spleen, intestine, lungs, kidneys, heart, muscle, and adipose tissue within 4 hours, indicating that DEHP is quickly and widely distributed throughout the body, irrespective of route of exposure (General Motors, 1982). Specifically, for the inhalation route of exposure, male SD rats exposed to an aerosol (0.24 to 0.61 µm) of radiolabeled DEHP for 6 hours excreted 90 percent DEHP (50% in urine and 40% in feces) within 72 hours, with 7 percent remaining in the carcass, confirming systemic distribution and excretion following inhalation of DEHP (General Motors, 1982). Similar to the oral route of exposure, metabolism of DEHP to its monoester metabolite MEHP 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. Regardless of the differences in metabolism in the lungs compared to the gastrointestinal tract, the evidence that DEHP is quickly absorbed and distributed to the liver and gastrointestinal tract regardless of route of exposure indicates that the overall toxicokinetic processes are similar in both the time-course and outcome (General Motors, 1982; Tanaka et al., 1975; Wallin et al., 1974). Therefore, EPA has a strong understanding of the absorption kinetics for the inhalation route and has robust confident that its human health risk characterization via the inhalation route for DEHP is health protective.

5 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE

5.1 Hazard Considerations for Aggregate Exposure

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

5.2 PESS Based on Greater Susceptibility

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

Several factors may increase biological susceptibility to the effects of DEHP. Animal studies provide direct evidence of several factors that enhance susceptibility to DEHP, including that gestation is a particularly sensitive lifestage for effects on male and female reproductive development to manifest. These and other lines of evidence are summarized in Table 5-1. EPA is quantifying risks based on developmental toxicity in the DEHP risk evaluation.

EPA identified indirect evidence for differences among human populations in ADME properties that may impact lifestage susceptibility to DEHP. For instance, the activity of glucuronosyltransferase differs between adults and infants; adult activity is achieved at 6 to 18 months of age (Leeder and Kearns, 1997). Also, preexisting chronic liver or kidney disease may enhance susceptibility to DEHP as a consequence of impaired metabolism and clearance (*i.e.*, altered functionality of phase I and phase II metabolic enzymes); impaired activity of uridine diphosphate (UDP)-glucuronosyltransferases (UGTs) can reduce metabolism of chemicals that rely on UGT conjugation to be excreted (Sugatani, 2013), including DEHP (Section 2.3). Additional indirect evidence of differences among human populations that confer enhanced susceptibility to DEHP, such as other preexisting diseases, lifestyle factors, sociodemographic factors, genetic factors, and chemical co-exposures are presented in Table 5-1. The effect of these factors on susceptibility to health effects of DEHP is not known. Therefore, EPA is uncertain about the magnitude of any possible increased risk from effects associated with DEHP exposure for relevant subpopulations.

For non-cancer endpoints, EPA used a default value of 10 for human variability (UF_H) to account for increased susceptibility when quantifying risks from exposure to DEHP. 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 UF of 10 when data are lacking and describe the types of populations that may be more susceptible, including different lifestages (*e.g.*, of children and elderly). However, U.S. EPA (2002b) did not discuss all the factors presented in Table 5-1. Thus, uncertainty remains whether additional susceptibility factors would be covered by the default UF_H value of 10 chosen for use in the DEHP risk evaluation.

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

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

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
Lifestage	Embryos/ fetuses/infants	Direct quantitative animal evidence for developmental toxicity (*e.g.*, decreased fetal body weight). There is direct quantitative animal evidence for effects on the developing male reproductive system consistent with a disruption of androgen action. There is direct quantitative animal evidence for effects on the developing female reproductive system. There is direct quantitative animal evidence of nutritional/metabolic effects on glucose homeostasis and lipid metabolism after gestational, lactational, and peripubertal exposure. However, there is uncertainty regarding whether these subclinical effects are adverse and if they correspond to adverse clinical outcomes in humans (*e.g.*, Type II diabetes). There is direct quantitative animal evidence of effects on offspring bodyweight after gestational, lactational, and peripubertal exposure. However, the direction of effect varies across studies, with no clear pattern depending on exposure duration, exposure timing, sex, or species.	(U.S. EPA, 2023a, b; Fan et al., 2020; Parsanathan et al., 2019; Rajagopal et al., 2019a, b; Shao et al., 2019; Wang et al., 2017; Gu et al., 2016; Venturelli et al., 2015; Mangala Priya et al., 2014; Rajesh and Balasubramanian, 2014; Zhang et al., 2014; Pocar et al., 2012; Schmidt et al., 2012; Lin et al., 2011b; Blystone et al., 2010; Christiansen et al., 2010; Gray et al., 2009; Lin et al., 2009; Vo et al., 2009a; Lin et al., 2008; Andrade et al., 2006b; Andrade et al., 2006c; Andrade et al., 2006a; Grande et al., 2006; TherImmune Research Corporation, 2004)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DEHP is based on developmental toxicity (*i.e.*, reproductive tract malformations in F1 and F2 males consistent with a disruption of androgen action and phthalate syndrome) and is protective of effects on the fetus and offspring.

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
Lifestage	Pregnancy/ lactating status	Rodent dams not particularly susceptible during pregnancy and lactation, except for effects related to increased maternal weight and body fat, increased absolute/relative liver and kidney weight, and decreased maternal serum estradiol.	(Zhang et al., 2014; Pocar et al., 2012; Schmidt et al., 2012)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DEHP is based on developmental toxicity (*i.e.*, reproductive tract malformations in F1 and F2 males) and is protective of effects in dams.
	Males of reproductive age	There is direct quantitative animal evidence of effects on the male reproductive tract in male rodents exposed during adolescence and adulthood.	(Hsu et al., 2016; Guo et al., 2013; Kitaoka et al., 2013; Li et al., 2012; Vo et al., 2009b; Ge et al., 2007; Akingbemi et al., 2004; Akingbemi et al., 2001; Ganning et al., 1990)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DEHP based on developmental toxicity (*i.e.*, reproductive tract malformations in F1 and F2 males) is protective of adult male reproductive effects. Use of default 10x UF_H
	Children	There is direct quantitative animal evidence of effects on the male reproductive tract in male rodents exposed during adolescence (key citations mentioned above). There is direct quantitative animal evidence of nutritional/metabolic effects on glucose homeostasis and lipid metabolism in rodents exposed during adolescence. However, there is uncertainty regarding whether these subclinical effects are adverse and if they correspond to adverse clinical outcomes in humans (*e.g.*, Type II diabetes).	(Zhang et al., 2020b; Xu et al., 2018; Venturelli et al., 2015)			POD selected for assessing risks from acute, intermediate, and chronic exposures to DEHP is based on developmental toxicity (*i.e.*, reproductive tract malformations in F1 and F2 males) and is protective of effects on children. Use of default 10x UF_H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
		There is direct quantitative animal evidence of effects on bodyweight in rodents exposed during adolescence. However, the direction of effect varies across studies, with no clear pattern depending on exposure duration, exposure timing, sex, or species.				
Lifestage	Elderly	No direct evidence identified				Use of default 10x UF_H
	Toxicokinetics			In rats, oral absorption of DEHP appears to be greater in immature animals compared with mature animals, but no age-related differences in oral absorption were seen in marmosets. Young children might convert DEHP to MEHP more efficiently than older children or adults due to higher gastric lipase activity. In addition, compared to adults, children generally have a reduced capacity to metabolize compounds via glucuronidation, which could result in delayed excretion of DEHP or its metabolites. The MEHP metabolite of DEHP also undergoes glucuronidation and has been shown to interfere with bilirubin conjugation, possibly as a competitive inhibitor of glucuronidase.	ATSDR (2022)	Use of default 10x UF_H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
Preexisting disease or disorder	Health outcome/ target organs	There is direct quantitative animal evidence for greater neurotoxicity sensitivity in a mouse diabetes model following pubertal exposure. There is direct quantitative animal evidence for greater sensitivity to endocrine and metabolic toxicity in a mouse diabetes model following exposure during adolescence.	(Feng et al., 2020; Ding et al., 2019)	Several preexisting conditions may contribute to adverse developmental outcomes (*e.g.*, diabetes, high blood pressure, certain viruses). Individuals with chronic liver disease may be more susceptible to effects on these target organs. Viruses such as viral hepatitis can cause liver damage.	CDC (2023e) CDC (2023g)	Use of default 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 DEHP.	(Sugatani, 2013)	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
	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

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
Sociodemo-graphic status	Race/ethnicity	No direct evidence identified (*e.g.*, no information on polymorphisms in DEHP metabolic pathways or diseases associated race/ethnicity that would lead to increased susceptibility to effects of DEHP by any individual group).				Qualitative discussion in Section 5.2 and this table
	Socioeconomic status	No direct evidence identified		Individuals with lower incomes may have worse health outcomes due to social needs that are not met, environmental concerns, and barriers to health care access.	ODPHP (2023b)	Qualitative discussion in Section 5.2 and this table
	Sex/gender	See "life-stage" section above regarding direct quantitative animal evidence for effects on the developing male reproductive system consistent with a disruption of androgen action.				POD selected for assessing risks from acute, intermediate, and chronic exposures to DEHP based on developmental toxicity (*i.e.*, reproductive tract malformations in F1 and F2 males) Use of default 10x 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 that impairs liver enzyme metabolism of DEHP, thereby enhancing susceptibility to DEHP toxicity.	CDC (2023e) CDC (2023b)	Qualitative discussion in Section 5.2 and this table
	Malnutrition	No direct evidence identified		Micronutrient malnutrition can lead to multiple conditions that include birth defects, maternal and infant deaths, preterm birth, low birth weight, poor fetal growth,	CDC (2021) CDC (2023b)	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
				childhood blindness, undeveloped cognitive ability. Thus, malnutrition may increase susceptibility to some developmental outcomes associated with DEHP.		
Genetics/ epigenetics	Target organs	There is direct quantitative epidemiological evidence for genetic polymorphisms associated with greater sensitivity to immune responses.	(Park et al., 2013)	Polymorphisms in genes may increase susceptibility to developmental toxicity, metabolic outcomes, or neurological effects. Epidemiological studies report the following potential associations: Enhanced association between MEHP levels in meconium and low birth weight or short birth length in infants exhibiting e paraoxonase-2 148AG/GG (PON-2 A148AG/GG) genotype; Urinary DEHP metabolites were associated with greater decreases in lung function in elderly Koreans with certain polymorphisms in oxidative stress-related genes (CAT, MPO, and SOD2); Urinary DEHP metabolites were associated with poor attentional performance in children with the dopamine receptor D4 (DRD4) gene 4/4 variant, but not in children without the DRD4 4/4 genotype; Urinary MEHP and odds of leiomyoma or adenomyosis in individuals with GSTM1 null-type polymorphisms and not in those with wild-type GSTM1.	(ATSDR, 2022; Cassina et al., 2012; Ingelman-Sundberg, 2004)	Use of default 10x UF_H
	Toxicokinetics	No direct evidence identified		Polymorphisms in genes encoding phase 1 or phase 2 metabolic		Use of default 10x UF_H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
				enzymes (*e.g.*, UGTs, CYPs) or other enzymes (*e.g.*, lipases, esterases) involved in metabolism of DEHP may influence metabolism and excretion of DEHP.		
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. Interaction with increased preterm birth associated with total third trimester DEHP urinary metabolites, but only among women who had experienced a stressful life event (SLE), such as a job loss, serious illness, family death, relationship issues, or legal or financial issues: Odds Ratio for total DEHP metabolites in 3rd trimester for preterm birth was 1.44 (1.06, 1.95) and spontaneous preterm birth 1.47 (1.04, 2.08). When asked about SLE, 24 preterm births among 281 mothers who reported SLE (8.5%), and 34 preterm births among 429 mothers who reported no SLE during pregnancy (7.9%).	CDC (2023c) ODPHP (2023c) Ferguson (2019a)	Qualitative discussion in Section 5.2 and this table Qualitative discussion in Section 3.1.1.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DEHP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DEHP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citations	
Other chemical and nonchemical stressors	Chemical co-exposures	Studies have demonstrated that co-exposure to DEHP and other toxicologically similar phthalates (*e.g.*, DBP, DIBP, DINP, BBP) 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.	See (U.S. EPA, 2023a) and (U.S. EPA, 2023b)			Qualitative discussion in Section 5.2 and this table and will be quantitatively addressed as part of the phthalate cumulative risk assessment.

6 PODS USED TO ESTIMATE RISKS FROM DEHP EXPOSURE, AND CONCLUSIONS

After considering hazard identification and evidence integration, dose-response evaluation, and weight of scientific evidence of POD candidates, EPA chose one non-cancer endpoint for use in determining the risk from acute, intermediate, and chronic exposure scenarios (Table 6-1). The critical effect is disruption to androgen action during the critical window of male reproductive development (*i.e.*, during gestation), leading to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. Reproductive tract malformations characteristic of phthalate syndrome was selected as the basis for the POD of 4.8 mg/kg-day (HED = 1.1 mg/kg-day) for acute, intermediate, and chronic durations. EPA has robust overall confidence in the selected POD for acute, intermediate, and chronic durations. There are no studies conducted via the dermal and inhalation route relevant for extrapolating human health risk. In the absence of suitable inhalation studies, the Agency performed route-to-route extrapolation to convert the oral HED to an inhalation HEC of 6.2 mg/m^3 (0.39 ppm). EPA is also using the oral HED to extrapolate to the dermal route. HECs are based on daily continuous (24-hour) exposure, and HEDs are daily values.

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

Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HED[a] (mg/kg-day)	HEC (mg/m^3) [ppm]	Benchmark MOE	Reference (TSCA Study Quality Rating)
Development /Reproductive	Rat	Continuous exposure for generations	NOAEL = 4.8	↑ total reproductive tract malformations in F1 and F2 males at 14 mg/kg-d	1.1	6.2 [0.39]	UF_A= 3 UF_H=10 *Total UF=30*	(Blystone et al., 2010; TherImmune Research Corporation, 2004) (High)

POD = point of departure; HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; UF = uncertainty factor

[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. EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations.

The POD of 4.8 mg/kg-day (HED = 1.1 mg/kg-day) will be used in the *Risk Evaluation for Di(2-ethylhexyl) Phthalate (DEHP)* (U.S. EPA, 2025n) to estimate acute, intermediate, and chronic non-cancer risk. EPA summarizes the cancer hazards of DBP in a separate technical support document, *Cancer Human Health Hazard Assessment for Di(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP), Butyl Benzyl Phthalate (BBP) and Dicyclohexyl Phthalate (DCHP)* (U.S. EPA, 2025a).

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APPENDICES

Appendix A EXISTING ASSESSMENTS FROM OTHER REGULATORY AGENCIES OF DEHP

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

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

Agency	Assessment(s) (Reference)	External Peer Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
ATSDR	*Toxicological profile for di(2 ethylhexyl)phthalate (DEHP)* (ATSDR, 2022)	Yes	Yes	Partial [a]	- Draft reviewed by peer-review panel of three experts (see p. vi of (ATSDR, 2022) for more details). - "Partial" systematic review is explained in the footnote below.
U.S. EPA (IRIS Program)	*Integrated Risk Information System (IRIS), Chemical Assessment Summary, Di(2-ethylhexyl)phthalate (DEHP); CASRN 117-81-7* (U.S. EPA, 1988)	Yes	Yes	No	
	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).	No	No	Yes	- Publications were subject to peer review prior to publication 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

Agency	Assessment(s) (Reference)	External Peer Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
U.S. CPSC	*Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)* (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 accordance with the National Academies peer-review process. See Acknowledgements section of (NASEM, 2017) for more details. - Employed NTP's Office of Heath Assessment and Translation (OHAT) systematic review method
Health Canada	*State of the Science Report: Phthalate Substance Grouping: Medium-Chain Phthalate Esters: Chemical Abstracts Service Registry Numbers: 84-61-7; 84-64-0; 84-69-5; 523-31-9; 5334-09-8;16883-83-3; 27215-22-1; 27987-25-3; 68515-40-2; 71888-89-6* (EC/HC, 2015) *Supporting Documentation: Evaluation of Epidemiologic Studies on Phthalate Compounds and their Metabolites for Hormonal Effects, Growth and Development and Reproductive Parameters* (Health Canada, 2018b) *Supporting Documentation: Evaluation of Epidemiologic Studies on Phthalate Compounds and their Metabolites for Effects on Behaviour and Neurodevelopment,*	Yes	Yes	No (Animal studies) Yes (Epidemiologic studies)	- Ecological and human health portions of the screening assessment report (Health Canada, 2020) were subject to external review and/or consultation. See page 2 of (Health Canada, 2020) for additional details. - State of the science report (EC/HC, 2015) and draft screening assessment report for the phthalate substance group subjected to 60-day public comment periods. Summaries of received public comments available at: https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative/phthalate.html#a1 - 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
	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 is provided in Section 1 of (EC/HC, 2015) and (Health Canada, 2020) - Human epidemiologic studies evaluated using Downs and Black Method (Health Canada, 2018a, b)
NICNAS	*Priority Existing Chemical Draft Assessment Report: Diethylhexyl Phthalate* (NICNAS, 2010)	No	Yes	No	- NICNAS (2010) states "The report has been subjected to internal peer review by NICNAS during all stages of preparation," and note that the "human health hazard sections were also reviewed by an external expert". However, a formal external peer review was not conducted. - NICNAS (2010) states "Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made." See Preface of (NICNAS, 2010) for more details. - No formal systematic review protocol employed. - Details regarding NICNAS's strategy for identifying new information and literature is provided in Section 1.3 of (NICNAS, 2010)
ECHA/ECJRC	*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)	Yes	Yes	No	- Peer reviewed by ECHA's Committee for Risk Assessment (RAC) - Subject to public consultation - No formal systematic review protocol employed.

Agency	Assessment(s) (Reference)	External Peer Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	European Union Risk Assessment Report: Bis(2-ethylhexyl)phthalate (DEHP) (ECJRC, 2008)				
EFSA	*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) *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) Related to Bis(2-ethylhexyl)phthalate (DEHP) for Use in Food Contact Materials* (EFSA, 2005)	No	Yes (February to April 2019)	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 - 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 Di(2-ethylhexyl) Phthalate (DEHP)* (NTP-CERHR, 2006)	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-CERHR, 2006) - No formal systematic review protocol employed.

[a] From among the animal toxicology studies, ATSDR developed selection criteria for studies considered for derivation of MRLs, and identified 201 animal toxicology studies, which are included as Levels of Significant Exposure (LSE) in Table 2-2 of the ATSDR toxicological profile (ATSDR, 2022). Briefly, ATSDR's selection criteria included (1) all chronic studies, primate studies, and study filling data gaps; (2) developmental and reproduction studies with at least one dose <100 mg/kg-day (given the extensive evidence base for developmental and reproductive toxicity at relatively low doses); and (3) studies with hazard other than developmental and reproductive toxicity with at least one dose <1,000 mg/kg-day; and (4) excluding studies with major design flaws and/or reporting deficiencies. Although ATSDR stated that they exclude studies with major deficiencies, no formal systematic review with defined metrics and rated criteria for data quality evaluation were reported.

Appendix B SUMMARIES OF IDENTIFIED HAZARDS OF DEHP

B.1 Summaries of Developmental and Reproductive Studies of DEHP

In a three-generation reproductive study conducted by **TherImmune Research Corporation (**2004**),** DEHP was administered in the diet at concentrations of 1.5 (control), 10, 30, 100, 300, 1,000, 7,500, and 10,000 ppm to SD rats (17/sex/group) starting 6 weeks prior to mating and continuously for three generations, with three litters per generation (achieved doses in mg/kg-day provided in Table_Apx B-1). The control dose level was reported as 1.5 ppm because that was the concentration DEHP measured in the control diet. The 10,000 ppm animals only completed the F1 generation and were terminated after failing to produce any F2 litters. The first two litters (F1a and F1b) were counted and weighed at PND 1 and then were terminated on PND 1 without being subjected to necropsy. The third litter born (F1c) was reared (without culling) until weaning on PND 21. On PND 16, up to six males and two females were randomly selected from each litter to be maintained to adulthood for histopathology. One to two males in the litter were selected to be parents of the F2 generation (avoiding sibling matings), with the additional nonbreeding males maintained until necropsy as sexually mature adults. Similar methods were employed for the F2 generation, resulting in 3 litters with F2c litters were selected for breeding to produce the F3 litters. With the exception of F3c litters (in which 1 to 2 males/litter terminated at PND 63/64 and not evaluated for reproductive tract malformations), animals from all control litters (14 litters for F1 and 10 litters for F2) and 8 to 17 litters per dose level were subjected to gross necropsy on PND 194 to PND 263.

In the non-mating males selected from the F1 and F2 male pups, aplastic testes and epididymis, and small testes, seminal vesicles, and prostates were noted in 1 to 3 animals at 300 ppm (Table_Apx B-1). The investigators concluded that, although the incidence of these findings is low, they are consistent with the syndrome of effects seen with other phthalate-induced male reproductive toxicity, and the incidences of small testes exceeds the historical control incidence at TherImmune Research Corporation. The authors noted that these findings represent sampling of only a small number of animals (1 male/litter) and are potentially treatment-related.

Given the limited sampling of 1 male/litter from the TherImmune Research Corporation study, **Blystone et al, (**2010**)** conducted further evaluation of the reproductive tract malformations to elucidate whether the incidences of reproductive tract malformations in the males at 300 ppm were treatment-related. Power analysis curves generated from Monte Carlo simulation demonstrated that there is a substantial increase in the ability to detect an increased incidence of 10 percent over controls when 3 pups/litter are examined (66% of the time) or 4 pups/litter are examined (86% of the time) compared to examining 1 pup/litter (5% of the time). Therefore, all males from the F1c and F2c litters (1 to 6 males per litter) were examined for malformations of the testes, epididymides, prostate, and seminal vesicles, and any reproductive tract malformations (RTMs) were recorded as ordinal data (present or absent) and evaluated separately for each generation and pooled across generations. When F1 and F2 litters were combined, RTM consistent with phthalate syndrome were significantly increased over controls at 300 ppm, with malformations in the testes, epididymides, and prostate affecting 5 of 86 males from 5 of 25 litters compared to no incidences observed in the 93 control males comprising 24 litters (Table_Apx B-1). *Therefore, the LOAEL in the study based on the more in-depth examination of offspring for RTMs is 300 ppm (equivalent to 14 mg/kg-day), with the NOAEL established at 100 ppm (equivalent to 4.9 mg/kg-day in the F1 generation and 4.8 mg/kg-day in the F2 generation).*

Table_Apx B-1. Achieved Dose and Incidences of Reproductive Tract Malformations (RTMs) in F1 and F2 Offspring Administered DEHP in the Diet via Continuous Exposure for Three Generations [a]

(TherImmune Research Corporation, 2004)	1.5 ppm (Control)	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm	7,500 ppm	10,000 ppm
Achieved dose (mg/kg-day)								
P1 (F0) generation	0.12	0.78	2.4	7.9	23	77	592	775
P2 (F1) generation	0.09	0.48	1.4	4.9	14	48	391	543
P3 (F2) generation	0.10	0.47	1.4	**4.8**	**14**	46	359	–
Mean	0.10	0.58	1.7	5.9	17	57	447	659
Target	0.10	0.50	1.5	5.0	15	50	400	–
Incidence of reproductive tract malformations (RTM) from macroscopic observations								
F1 generation								
No. litters (male pups)	14 (56)	13 (46)	16 (49)	15 (51)	17 (55)	15 (52)	13 (40)	8 (31)
Testes	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	9*** (17)	8*** (31)
Epididymis	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)	0 (0)	1 (1)	8*** (21)
Seminal vesicles	0 (0)	1 (1)	0 (0)	0 (0)	2 (2)	0 (0)	1 (1)	1 (1)
Prostate	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	2 (4)	2 (2)	1 (1)
Total RTM [b]	0 (0)	1 (1)	1 (1)	0 (0)	**4 (4)**	**2 (4)**	**9*** (18)**	**8*** (31)**
F2 generation								
No. litters (male pups)	10 (37)	10 (35)	10 (35)	8 (31)	8 (31)	10 (35)	9 (30)	–
Testes	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	3 (3)	**9*** (20)**	–
Epididymis	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	3 (3)	**9*** (16)**	–
Seminal vesicles	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–
Prostate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–
Total RTM [b]	0 (0)	0 (0)	0 (0)	0 (0)	**1 (1)**	**3 (3)**	**9*** (20)**	–
Total RTM (F1+F2)[b]	0/24 (0/93)	1/23 (1/81)	1/26 (1/84)	0/23 (0/82)	**5/25* (5/86)**	**5/25* (7/87)**	**18/22*** (38/70)**	–

[a] Data from Blystone et al. (2010)
[b] The number of affected litters per dose group was subjected to BMD analysis by Blystone et al. using EPA's BMD Software Version 2.1.1.

Additionally, the following treatment-related effects were observed at higher doses:

- At 1,000 ppm (57 mg/kg-d) and above, hepatocellular hypertrophy was noted in the liver, and dilation of the renal tubules and mineralization occasionally associated with chronic pyelonephritis was observed in the kidney.
- At 7,500 ppm (447 mg/kg-day) and above: decreased litter size; decreased number of male pups across all litters combined (F1a + 1b + 1c) and at 10,000 ppm in F1a litter; decreased total number of pups per litter in F1a at 7,500 and 10,000 and decreased across all litters combined (F1a + 1b + 1c) at 7,500 ppm; decreased AGD at 7,500 ppm in the F1a, F1b, F2a, F2c, and F3a males and at 10,000 ppm in the F1a, F1b, and F1c males; decreased terminal body weights in F1

and F2 males at 7,500 ppm and in both sexes in the F0 and F1 generation at 10,000 ppm; delayed testes descent, vaginal opening, and preputial separation were delayed at 10,000 ppm and at 7,500 in the F1c offspring; decreased pup weights, unadjusted and adjusted for litter size, at 7,500 ppm in the F2c litter and combined F2a, b, c litters, with decreases continuing at 7,500 ppm throughout the lactation period (PND 1–21) for the F2c males and females; decreased number of implantation sites; *decreased* mating, pregnancy, and fertility indices; decreased sperm count; decreased epididymis and testes weights, increased weights of liver, kidneys, and adrenals; increased nipple retention in F3c male pups at 7,500 ppm; and histopathology effects in testes, including atrophy of seminiferous tubules characterized by loss of germ cells and the presence of Sertoli cell-only tubules, as well as occasional failure of sperm release in testes, and sloughed epithelial cells and residual bodies in the epididymis. Cortical vacuolization in the adrenal gland was increased at 7,500 ppm.

- At 10,000 ppm, pup weights, unadjusted and/or adjusted for litter size, were decreased in both sexes in the F1a and F1b litters on PND 1 and in the F1c litters on PND 1, 4, 7, 14, and 21. None of the F1 mating pairs produced offspring.

In a study presented in a series of publications by **Andrade and Grande (**2006b**;** 2006c**;** 2006a**;** 2006**)**, pregnant Wistar rats were administered DEHP in peanut oil by oral gavage at 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, or 405 mg/kg-day from GD 6 to LD 21, and effects were examined in the F1 offspring, which support *a LOAEL of 15 mg/kg-day and a NOAEL of 5 mg/kg-day*.

In the first publication by Andrade et al. (2006a), preputial separation was significantly delayed at 15 mg/kg-day and above, with the body weight at criterion significantly decreased by 9 percent at 405 mg/kg-day compared to controls. At 135 mg/kg-day and above, absolute liver weights were increased by 9 to 13 percent over controls at on PND 1. Bi- and multi-nucleated gonocytes in the testes were increased in incidence and severity, affecting 5 of 6 F1 males at 135 mg/kg-day (very slight to slight severity) and 6 of 6 F1 males at 405 mg/kg-day (very slight/moderate/severe) compared to 0 out of 6 controls. Additionally at 405 mg/kg-day, incidences of nipple retention were significantly higher than controls on PND 13, affecting 13 out of 41 males from 5 out of 12 litters compared to 0 incidences in controls (and any other treated group). AGD was significantly decreased at 405 mg/kg-day compared to controls on PND 22. There were no treatment-related effects on maternal clinical signs, body weight, litter size, sex ratio, or viability, and no incidences of hypospadias or incomplete testes descent in F1 males or effects of treatment on testes or epididymis weights.

In a second publication by Andrade et al. (2006b) reporting results from the same study, researchers examined aromatase activity in the hypothalamic/preoptic area brain sections from a subset of F1 offspring. On PND 1, aromatase activity in the F1 males was significantly *decreased* at 0.135 and 0.405 mg/kg-day but *increased* at 15, 45, and 405 mg/kg-day; whereas, in the treated females at PND 1, aromatase activity was comparable to controls. On PND 22, aromatase activity in this area of the brain was increased in 0.405 mg/kg-day F1 males and in all treated groups in the F1 females except for the 0.045 and 5 mg/kg-day dose groups. None of these statistically significant differences were dose-related, and they were inconsistent between sexes and time points. However, the authors proposed a biphasic, non-monotonic effect of DEHP on aromatase activity in the hypothalamic/preoptic area that differed between males and females and at different ages.

In a third publication by Andrade et al. (2006c), again reporting results from the same study, sperm count was decreased by 19 to 25 percent at 15 mg/kg-day and above, and these decreases were significant compared to both the concurrent and historical controls; whereas the decreases noted at lower doses were smaller in magnitude (9–16%) and generally only decreased compared to concurrent (and

not historical) controls. The authors considered this threshold of a 20 percent decrease to be biologically significant. Absolute seminal vesicle weights were significantly decreased by 10 percent at 405 mg/kg-day compared to controls. Serum testosterone was significantly increased at 0.045, 0.405, and 405 mg/kg-day; however, these differences were unrelated to dose. There were no effects on sperm morphology.

Grande et al. (2006) presented results on F1 female offspring from the same study. Mean time to vaginal opening was significantly delayed in F1 females at 15 mg/kg-day and above (37.1–38.1 days) compared to controls (35.6 days). The age at first estrus was delayed at 135 mg/kg-day and above (41.2 –41.8 days) compared to controls (39.2 days); however, the differences in time to first estrus were not statistically significant. There were no dose-related effects on body weight at sexual maturation or body weight at first estrus. Liver weights were significantly increased by 17 percent at 135 mg/kg-day and above compared to controls.

In a study by **Christiansen et al. (**2010**)**, pregnant Wistar rats were administered DEHP in corn oil by oral gavage at 0, 10, 30, 100, 300, 600, or 900 mg/kg-day (Study 1) and doses of 0, 3, 10, 30, or 100 mg/kg-day (Study 2) from GD 7 to LD 16. On PND 1, F1 offspring were weighed, sexed, and anogenital distance was measured. On PND 12, F1 animals were examined for nipple retention. On PND 16, external genitalia were examined in the F1 males, and any signs of demasculinization were scored on a 3-point scale, with Score 0 denoting no effect and Scores 1, 2, and 3 indicating mild, moderate, and severe dysgenesis of the external genitalia, respectively. The scoring criteria were well described, and the investigators were blind to the treatment status of the animals. Male offspring were terminated on PND 16 and subjected to a gross necropsy. Organ weights were determined for liver, kidneys, adrenals, and reproductive organs (testes, epididymis, prostate, bulbourethral glands, and LABC muscles), and histopathology and immunohistochemistry analyses were performed on the testes, along with examination of gene expression in the ventral prostate. Results from the two independent studies, conducted 8 months apart, were reported in this publication. In Study 1, absolute AGD was significantly decreased by 8 to 14 percent at 10 mg/kg-day and above compared to controls, with significant decreases in body weight of 9 to 13 percent noted at 300 mg/kg-day and higher. Nipple retention was also significantly increased at 10 mg/kg-day and above, with mean of 1.23 to 5.01 nipples per male in the treated groups compared to a mean of 0.22 nipples per male in controls. Incidences of mild external genital dysgenesis were significantly increased at 100 mg/kg-day and above (17–50%) compared to controls (2%).

In contrast in Study 2: absolute AGD was only significantly decreased by 4 percent compared to controls at 100 mg/kg-day; nipple retention in the treated groups was comparable to controls; and incidences of mild external genitalia dysgenesis were significantly increased by 12 to 15 percent at 3 and 100 mg/kg-day, with increases of 8 to 10 percent (not significant) at 10 and 30 mg/kg-day. When data from the two studies were combined: anogenital distance was significantly decreased and nipple retention was significantly increased at 10 mg/kg-day and above; and it was apparent that the incidences of mild external genitalia dysgenesis were clearly dose-dependent and consistently statistically significant only at doses at 100 mg/kg-day and above. Additionally, when examining the data from the combined studies, absolute weights of the ventral prostate and LABC were generally consistently significantly decreased at 10 mg/kg-day and above; however, these organs were not subjected to histopathological examination. The study authors did not consider the incidences of external genitalia dysgenesis at 3 mg/kg-day to support a LOAEL, and EPA agrees with the determination of *the LOAEL at 10 mg/kg-day based on increased nipple retention and decreased AGD, with the NOAEL established at 3 mg/kg-day.*

In a study by **Akingbemi et al. (**2001**)**, pregnant Long-Evans rats were administered DEHP via oral gavage in corn oil at a dose level of 0 (vehicle control) or 100 mg/kg-day from GD 12 through GD 21, and male offspring were examined at weaning (PND 21), puberty (PND 35), and adult (PND 90) stages. In this study, serum testosterone and LH concentrations at 100 mg/kg-day were significantly lower than controls at PND 21 and PND 35 but were comparable at PND 90. Similarly, *ex vivo* testosterone production in isolated Leydig cells was significantly decreased when examining both basal testosterone production (47% decrease) and LH-stimulated testosterone production (56% decrease) at 100 mg/kg-day compared to controls.

Akingbemi et al. (2001**)** also examined effects of direct administration of DEHP on post-weanling Long-Evans rats dosed with DEHP via oral gavage in corn oil at 0, 1, 10, 100, or 200 mg/kg-day for 14 days (from PND 21–34 or PND 35–48) or for 28 days (from PND 21–48), and young adult Long-Evans rats were similarly exposed for 28 days (from PND 62–89). In rats exposed to DEHP for 14 days, there were no effects of treatment on body weight, testis or seminal vesicle weights, or serum concentrations of LH or testosterone at either age (PND 35 or PND 49). Although there were no decreases in serum concentrations, basal and LH-stimulated testicular testosterone production were decreased following 14-day exposure, with decreases at 100 mg/kg-day and higher following earlier exposure (PND 21–PND 34) and decreases at 10 mg/kg-day and higher following later exposure (PND 35–48), accompanied by significant increases in steroidogenic enzymes ($P450_{SCC}$, 3β-HSD, P45017α, and 17β-HSD) in rats exposed at 100 mg/kg-day and above from PND35 through 49. Interestingly, in male rats exposed for the full 28 days (PND 21–48), significant *increases* were observed in: serum testosterone concentration (35–42% increase); interstitial fluid testosterone concentration (41–45% increase); serum LH (59–86% increase), and basal and LH-stimulated testicular testosterone production at 10 mg/kg-day and above. In rats exposed from PND 21 through PND 48, significant increases over controls were noted. The authors attributed the inhibition of Leydig cell testosterone production to two factors: (1) decreased pituitary LH secretion; and (2) decreased steroidogenic enzyme activity and proposed a compensatory mechanism via negative feedback loop to explain the apparent shift in directionality, with decreased testosterone stimulating the pituitary gland to increase LH production, which in turn results in Leydig cells increasing testosterone production. It was reported that no treatment-related effects were observed in older rats exposed from PND 62 through PND 89. In this study, the *NOAEL was not established, and the LOAEL is 10 mg/kg-day based on decreased basal and LH-stimulated testosterone production on PND 49 after pre-pubertal (PND 35–48) exposure, with increased testosterone production with earlier and longer exposure (PND 21–48).*

In a second study by **Akingbemi et al. (**2004**)**, Long-Evans male rats were administered DEHP via oral gavage in corn oil at dose levels of 0, 10, or 100 mg/kg-day from weaning (PND 21) to PND 90 or PND 120 and measured serum LH and testosterone by radioimmunoassay and *ex vivo* Leydig cell testosterone production (Experiment I). A second set of animals was similarly administered DEHP at the same doses, duration, and age to determine Leydig cell proliferation, measured by the following: (1) expression of cell division cycle marker, (2) tritiated thymidine incorporation, and (3) changes in cell number (Experiment II). A third set of male rats were administered DEHP at similar doses from PND 21 through PND 90, and serum 17β-estradiol (E2), Leydig cell E2 production, and aromatase gene (Cyp19) expression in Leydig cells were measured at PND 48 and PND 90 (Experiment III). In rats exposed from PND 21 through 90, serum LH and testosterone concentrations were significantly increased, and basal and LH-stimulated testosterone production were significantly decreased, at 10 mg/kg-day and above. In rats exposed longer (PND 21–120), similar increases in serum LH and testosterone concentrations and decreases in basal and LH-stimulated testosterone production were observed but were only significant at 100 mg/kg/day. Leydig cell proliferation was indicated at 10 mg/kg-day and above based on significant increases in all three criteria following DEHP treatment from PND21 through 90. Gene expression of

cell cycle proteins (Cyclin G1, p53, cyclin D3, and PCNA) were generally significantly increased at 10 mg/kg-day and above following treatment from PND 21 through 90, and the authors attributed the increased proliferative activity in Leydig cells to induction of cell cycle proteins. Serum E2 levels and LH-stimulated Leydig cell E2 production were significantly increased at 10 mg/kg-day and above, while basal Leydig cell E2 production and aromatase gene induction were noted at 100 mg/kg-day following treatment from PND 21 through 48. *The NOAEL was not established, and the LOAEL is 10 mg/kg-day based on the following effects in males: increased serum estradiol (E2) and Leydig cell E2 production after PND 21 through 48; increased serum testosterone and LH, decreased Leydig cell testosterone and E2 production with increased Leydig cell proliferation after PND 21 through 90; and Leydig cell proliferation after PND 21 through 120.*

In a study by **Gray et al. (**2009**)**, pregnant SD rats were administered DEHP via oral gavage at 0, 11, 33, 100, or 300 mg/kg-day from GD 8 to LD17 (*in utero* cohort), with a subset of male offspring continuing exposure until PND 63 (puberty cohort). AGD was measured at PND 2, and nipple retention was assessed at PND 13. Male offspring from both cohorts were subjected to a gross necropsy on PND 63 to PND 65 after reaching maturity, and the adrenals, liver, kidneys, and reproductive organs were weighed. Histopathology examination was performed on the testes and epididymis, and the following lesions were observed at the lower doses (11, 33, and 100 mg/kg-day): retained nipples, fluid-filled flaccid testes, hypoplastic (incompletely developed, similar to aplasia, but less severe) or malformed epididymis, epididymal granuloma with small testis, testicular seminiferous tubular degeneration (both moderate and mild severity, malformed seminal vesicles or coagulating glands, and true hermaphroditism, in one male, with uterine tissue and ovotestis. Males were assigned an ordinal classification regarding whether they exhibited these effects of phthalate syndrome. Incidences of phthalate syndrome were fairly consistent in the lower dose groups, with 8 of 71 males (11.3%) at 11 mg/kg-day, 10 of 68 males (11.6%) at 33 mg/kg-day, and 12 of 93 males (12.9%) at 100 mg/kg-day and were significantly increased over controls (zero incidence), with higher significance and incidence at 300 mg/kg-day (38 of 74 males; 51.3%). At 100 mg/kg-day and above, liver weights were significantly increased in the puberty cohort at PND 64, and absolute seminal vesicle weights were significantly decreased in the *in utero* cohort. Additionally at 300 mg/kg-day, absolute AGD was significantly decreased by 16 percent compared to controls, with minor decreases in body weight in males on PND2 (7%).

The percent of males with retained nipples was higher at 300 mg/kg-day (55%) compared to controls (11%), with an increased number of areolae per male at this dose (2.9) compared to controls (0.7). Additionally at 300 mg/kg-day, the reproductive organ weights (ventral prostate, seminal vesicles, LABC, Cowper's glands, epididymis, and testes) were decreased in the F1 males from both cohorts. Sexual maturation was significantly delayed in the puberty cohort, with preputial separation occurring at mean of 49.1 days at 300 mg/kg-day compared to 45.7 days in controls. Serum testosterone and E2 were unaffected in either cohort at necropsy at PND 63 through 65. *The results of this study support a LOAEL at 11 mg/kg-day based on increased incidence of histopathology findings indicative of phthalate syndrome, with the NOAEL not established.*

In a study by **Ge et al. (**2007**)**, male Long-Evans rats were administered DEHP via oral gavage in corn oil at 0, 10, 500, or 750 mg/kg-day for 28 days after weaning (PND 21–49). At 10 mg/kg-day, sexual maturation was accelerated, with significantly decreased time to preputial separation at this dose (39.7 ± 0.1 days) compared to controls (41.5 ± 0.1 days), along with significantly increased serum testosterone (58% increase), and significantly increased body weight (8% increase) and seminal vesicle weights (27% increase). At the much higher dose of 750 mg/kg-day, delayed sexual maturation was noted, with significantly increased time to preputial separation at this dose (46.3 ± 0.6 days), along with significantly decreased body weight (13% decrease), testes weight (29% decrease), prostate weight

(45% decrease), and serum testosterone (40% decrease). The authors also reported data showing that gene expression of *Lhb* and *Ar* in pituitary glands was unaffected by treatment. The investigators conducted a follow up study in which male Long-Evans rats were administered DEHP via oral gavage in corn oil at 0, 10, and 500 mg/kg-day for the shorter duration of 14 days (PND 21–PND 34), dropping the high dose of 750 mg/kg-day in the second experiment. After 14 days of treatment, testes weights were significantly decreased by 22 percent at 500 mg/kg-day compared to controls, along with decreased serum testosterone (78% decrease) and significantly decreased of 98 percent in cholesterol-stimulated testosterone production at this dose. *The NOAEL was not established, and the LOAEL is 10 mg/kg-day based on accelerated sexual maturation in males (decreased time to PPS), increased serum testosterone, and increased seminal vesicle weights.*

In a study by **Guo et al. (**2013**)** 90-day old male Long-Evans rats were administered DEHP via oral gavage in corn oil at 0, 10, or 750 mg/kg-day for 7 days; the authors reported that this duration was required for differentiation of stem into progenitor Leydig cells. After 7 days, these animals were euthanized by CO_2 to collect testes and blood, and the number of Leydig cells per 100 seminiferous tubules were determined (Experiment 1). Immunohistochemistry evaluations were performed on Leydig cells, and cells labeled positive for 3β-HSD (3β-HSD^{pos}) were progenitor Leydig cells, and those labeled positive for 11β-HSD1 were immature and adult Leydig cells (generally developing at PND 28 or later). The number of 3β-HSD^{pos} Leydig cells was significantly increased by 20 percent in both the 10 mg/kg-day and 750 mg/kg-day groups compared to controls, indicating progenitor cells. A second experiment was conducted using the same exposure regimen as before, except that after 7 days of exposure, male rats were given an intraperitoneal injection of ethane dimethanesulfonate (EDS) to eliminate Leydig cells so that the investigators could examine regeneration. Serum concentration of testosterone was determined by radioimmunoassay, and Leydig cell gene expression was examined. Four days post-EDS administration, testosterone was undetected in the control rats, indicating effectiveness of the test system in eliminating Leydig cells. However, in rats treated with 10 and 750 mg/kg-day DEHP, low levels of testosterone remained (approximately 10% normal). Similarly, the number of 3β-HSD^{pos} Leydig cells was undetected in controls, while low levels remained in the 10 and 750 mg/kg-day DEHP groups.

Examination of Leydig cell gene expression showed upregulation of Leydig cell lineage markers (*Lhcgr*, *Cyp11a1*, *Hsd3b1*, and *Cyp17a1*), while gene expression associated with immature and adult Leydig cells (*Hsd11b1*, *Insl13*, and *Hsd17b3*) were undetectable. *Nes* levels in the treated groups were comparable to normal levels but decreased compared to controls. Because EDS eliminates more advanced (immature and adult) Leydig cells without eliminating newly formed progenitor cells, the authors reported that the increased number of Leydig cells was not caused by the proliferation of adult Leydig cells, but possibly from the differentiation of existing putative stem cells into newly formed progenitor Leydig cells. *The NOAEL was not established, and the LOAEL is 10 mg/kg-day based on increased number of Leydig cell after dosing for one week (prior to EDS elimination of Leydig cells).*

In a study with a similar design conducted by **Li et al. (**2012**)**, adult (90-day old) male Long-Evans rats were given an intraperitoneal (i.p.) injection of EDS to eliminate mature Leydig cells and then administered DEHP in corn oil via oral gavage at dose levels of 0, 10, or 750 mg/kg-day for 35 days. Serum testosterone and LH levels were determined by radioimmunoassay; Leydig cell numbers and proliferation rate were measured; and Leydig cell gene expression were measured by qPCR. There were no effects of treatment on survival, clinical signs, or body weights. Leydig cell numbers were increased over controls at 10 and 750 mg/kg-day at 14-, 21-, and 35-days post-EDS elimination of Leydig cells, due to the significant increase in Leydig cell precursors from day 14 to day 21 after EDS elimination. However, serum testosterone levels remained significantly decreased at 35 days post-EDS in the 10 and 750 mg/kg-day groups compared to controls, despite the increased in Leydig cell number. The only

significant difference in LH was at 10 mg/kg-day 21 days post-EDS; however, this difference was transient and not dose-dependent, or Leydig cell-specific genes (*Lhcgr*, *Cyp11a1*, *Hsd3b1*, and *Insl3*) were significantly down-regulated in the 750 mg/kg-day group compared to controls beginning at 21 days post-EDS administration. The study authors considered these results to indicate that DEHP increases Leydig cell proliferation but inhibits differentiation during the regeneration of Leydig cells.

In a study by **Kitaoka et al. (**2013**)**, adult male A/J mice were fed diets containing DEHP at 0, 0.01, and 0.1 percent (equivalent to 0, 12.3, and 125 mg/kg-day) for 2 weeks (10 per group), 4 weeks (10 per group), and 8 weeks (15 per group). There were no effects of treatment on body weights or testes weights. The authors reported that histopathology evaluations showed a "few seminiferous tubules with germ cell sloughing at 2, 4, and 8 weeks in the 0.01 percent DEHP group. In the 0.1 percent DEHP group, there were also a few pathological changes at 2 weeks and foci of some seminiferous tubules with mild germ cell sloughing in the lumen, intermingled with normal seminiferous tubules at 4 and 8 weeks." However, no further data were provided regarding incidence or severity. The investigators evaluated at least 100 seminiferous tubules in each treatment group and determined the "degree of spermatogenic disturbance" according to Johnsen's scoring system, ranging from a score of 1 (no cells in the seminiferous tubules) to a score of 10 (complete spermatogenesis); the mean score was reported for each group. Johnsen's scores showed that there was no significant pathological changes in the DEHP-treated groups at Weeks 2 or 4; however, significant spermatogenic disturbance was observed at 125 mg/kg-day (8.8 ± 2.1), but not at 12.3 mg/kg-day (9.8 ± 0.5) compared to controls (10.0 ± 0.0) at 8 weeks.

Vacuolization of the cytoplasm in the Sertoli cells was significantly increased in both a dose- and time-dependent manner, with the mean number of Sertoli cell vacuoles per 100 seminiferous tubules higher at 12.3 mg/kg-day (14.5–20.0) and 125 mg/kg-day (16.3–22.7) compared to controls (1.0–1.3). The number of lymphocytes per mm^2 testicular interstitium (*e.g.*, lymphocytic infiltration) was dose-dependently and significantly increased at 12.3 mg/kg-day (19.2) and 125 mg/kg-day (22.6) compared to controls. At greater than or equal to 12.3 mg/kg-day, increased expression of IL-10 (in spermatids, endothelial cells, and interstitial cells) and IFN-γ (Sertoli and interstitial cells) were observed in the testes. Horseradish peroxidase (HRP), used as a tracer, demonstrated that the blood-testes barrier was compromised in the DEHP-treated animals, with no HRP detected inside the lumen of seminiferous tubules in the control animals; whereas the number of seminiferous tubules infiltrated by HRP beyond the blood-testes-barrier per 100 seminiferous tubules was increased at 12.3 mg/kg-day (3.1 ± 0.8) and 125 mg/kg-day (2.4 ± 0.6). *The NOAEL was not established, and the LOAEL is 12 mg/kg-day based on increased Sertoli cell vacuolation (dose- and time-dependent), germ cell sloughing in seminiferous tubules, lymphocytic infiltration in the testicular interstitium, and damage to the blood-testes-barrier.*

In a study by **Lin et al. (**2008**)**, pregnant Long-Evans rats were administered DEHP in corn oil via oral gavage at 0, 10, 100, or 750 mg/kg-day from GD 2 through 20. On GD 21, testosterone production, fetal Leydig cell (FLC) numbers and distribution, and testicular gene expression were evaluated. There were no effects of treatment on maternal body weights, birth rate, litter size, offspring sex ratio, or male pup body weights. The distribution of the number of FLC per cluster was significantly affected in all dose groups, with fewer FLC clusters containing one cell per cluster (4–10% in the treated groups vs. 20% in controls) and more FLC containing 6 to 30 FLC per cluster (35–56% in the treated groups compared to 29% in controls). However, the mean number of FLC per cluster was only significantly increased at 750 mg/kg-day (9 FLC per cluster) compared to controls (2 FLC per cluster)—largely driven by the significant increase at this dose in the clusters that contain more than 30 FLC (7 compared to 1 in controls). On GD 21, testicular testosterone was significantly increased by 57 percent over controls at 10 mg/kg-day but was decreased by 67 percent at 750 mg/kg-day. Absolute AGD was significantly

decreased by 9 percent at 750 mg/kg-day compared to controls. Absolute testes weight, absolute number of Leydig cells per testis, and Leydig cell size (volume) were significantly decreased at 100 and 750 mg/kg-day.

Testicular gene expression was evaluated by examining a panel of 37 genes, including those that encode growth factors (*Igf1*, *Kitl*, *Lif*), growth factor receptors (*Igf1r*, *Kit*, *Lhcgr*, *Pdgfra*), cholesterol transporters (*Scarb1*, *Star*), and steroidogenic enzymes (*Cyp11a1*, *Cyp19*, *Sdr5a1*). Effects on testicular gene expression were characterized by significantly: decreased expression of *Cyp11a* and *Lhcgr* at 100 and 750 mg/kg-day; decreased *Pdgfra*, *Scarb1*, *Star*, and *Insl* at 750 mg/kg-day; and increased *Srd5a1*, *Pdgfb*, and *Lif* at 750 mg/kg-day. Examination of levels of enzymes relevant for testosterone biosynthesis revealed decreased P450scc at 750 mg/kg-day, although 3βHSD, P450c17, and 17βHSD were not affected. Because Lif was increased at 750 mg/kg-day, and this dose was associated with larger-sized FLC clusters, the authors explored a potential causal relationship by examining the effects of LIF on FLCs *in vitro*. LIF at 1 ng/mL (IC50 for LH-stimulated testosterone production) and 10 mg/mL (concentration showing maximum inhibition) were tested *in vitro* and showed a concentration-dependent increase in FLC aggregation, with clusters containing two or more cells comprising 25 percent at 1 ng/mL and 39 percent at 10 mg/mL compared to 5 percent in controls. This causal relationship was further supported by the fact that treatment with LIF antibody antagonized the effects of LIF on FLC aggregation, with only 10 percent of the clusters containing two or more cells in the 10 ng/mL LIF + AB group. *The NOAEL was not established, and the LOAEL is 10 mg/kg-day based on Fetal Leydig cell aggregation (increased number of FLC per cluster) and increased testicular testosterone in F1 males on PND 1.*

In a subsequent study by **Lin et al. (**2009**)**, pregnant Long-Evans rats were administered DEHP in corn oil via oral gavage at 0, 10, or 750 mg/kg-day from GD 12.5 to PND 21.5. Subsets of male offspring were killed by inhalation of CO_2 at birth for FLC analysis or at PNDs 21 or 49 for Adult Leydig Cell (ALC) analysis and measurement of serum testosterone. Testes were removed, weighed, and subjected to immunohistochemical analysis of FLC distribution and real-time PCR analysis of Leydig cell mRNA levels. The body weights and AGD of male pups were measured on PND 2. There were no effects of treatment on maternal body weight or on birth rate or offspring sex ratio. The average, median, and maximum numbers of FLCs per cluster (presented in bar graphs) were dose-dependently and significantly increased at 10 mg/kg-day and 750 mg/kg-day. Additionally at 750 mg/kg-day, body weight in male offspring was significantly decreased by 16 percent on PND 2 and continued to be significantly decreased by 13 percent at PND 35; and absolute AGD was significantly decreased by 18 percent on PND 2. At birth, gene expression analyses indicated reductions in genes associated with cholesterol transporters and steroidogenic enzymes, including *Scarb1*, *Star*, and *Hsd17b12* at 10 and 750 mg/kg-day. Additionally at 750 mg/kg-day, luteinizing hormone receptor gene (*Lhcgr*), testosterone biosynthetic enzymes *Cyp17a1* and *Hsd17b3*, testis descent gene *Insl3*, and cell junction gene *Gja1* were decreased. Sertoli cell genes, including *Kitl*, *Clu*, and *Fshr* were examined, with significant decreases in *Clu* and *Fshr* at 750 mg/kg-day. The authors asserted that this suggests that Sertoli cells are less sensitive to DEHP exposure than FLC. The authors stated that progenitor Leydig cells differentiate into ALC around PND 49. Serum testosterone levels were significantly decreased at 10 and 750 mg/kg-day at PND 21 and remained decreased at 750 mg/kg-day at PND 49. Examination of gene expression at PND 21 indicated significant decreases in *Lhcgr*, *Kit*, *Scarb1*, *Hsd17b3*, *Srd5a1*, *Pcna*, *Gja1*, *Ar*, *Kitl*, and *Fshr* at 10 and 750 mg/kg-day; however, gene expression in the treated groups was comparable to controls at PND 49. Protein expression of P450c17 was significantly decreased at 10 and 750 mg/kg-day at PND 21, and STAR, 3βHSD1, 17 βHSD3, and SRD5A were decreased at 750 mg/kg-day at PND 1, with only SRD5A remaining decreased at PND 49. The NOAEL was not established, and *the LOAEL is*

10 mg/kg-day based on increased FLC per cluster, decreased gene expression of genes associated with cholesterol transport and steroidogenesis, and decreased serum testosterone.

In a study by **Vo et al. (**2009a**)** pregnant SD rats (n = 8) were administered DEHP in corn oil daily via oral gavage at doses of 0, 10, 100, or 500 mg/kg-day daily from GD 11 through 21. Similar groups of pregnant rats were administered testosterone propionate (TP) at 1 mg/kg-day to elicit androgenic effects or flutamide at 1, 10, or 50 mg/kg-day to examine the effects of this anti-androgen (androgen receptor antagonist); however, EPA is only discussing the results relevant to DEHP. On GD 21, 4 dams from each group were euthanized; the male fetuses were counted and weighed; blood was collected for measurement of serum testosterone and LH; and testis from four fetuses per dam were collected and fixed for immunohistochemical analysis, with the remaining testes pooled within a treatment group for RNA analysis. The surviving dams (4 per group) were allowed to deliver naturally, and male offspring were examined as follows: pups were counted, weighed, and sexed on PND 1 and weighed weekly thereafter. Male offspring were examined for nipple retention on PND 13. On PND 63 male offspring were terminated; AGD was measured; blood was collected for measurement of serum testosterone and LH; testes, epididymis, and prostate were weighed; the left testis was subjected to immunohistochemistry evaluation; and the right testis was used for determination of sperm count, motility, and viability. On GD 21, pup body weight was significantly decreased by 24 percent at 500 mg/kg-day compared to controls, and serum testosterone and LH were significantly decreased by 63 to 66 percent. On PND 63, AGD was significantly decreased by 20 percent at 100 mg/kg-day. Sperm concentration was significantly decreased by 24 percent at 10 mg/kg-day and by 53 percent at 500 mg/kg-day compared to controls, with similar significant decreases in sperm viability at 10 mg/kg-day (14%) and 500 mg/kg-day (40%); and sperm motility was significantly decreased by 13 to 47 percent in all dose groups (*e.g.*, 10 mg/kg-day and above) compared to controls.

Results of immunohistochemistry analysis of the testis were presented in micrographs in Figure 1 of the publication, and the authors reported that androgen receptor (AR) proteins were located in the interstitial cells, peritubular myoid cells, and undifferentiated epithelial cells, and " a few or no stained cells were detected following maternal DEHP treatment, whereas large numbers of cells that stained positive for AR proteins were observed in the Flu-treated groups." However, no quantitative data were provided. Real-time PCR confirmation of gene profiles from the microarray data from testes of male fetuses on GD21 indicated significant down-regulation of genes related to steroidogenesis (*StAR*, *Cyp11a1*, *Hsd3b1*) and alpha-actin cardiac 1 (*Actc1*) at 10 mg/kg-day compared to controls; whereas casein kinase 2 alpha 1 polypeptide (*Csnk2a1*) was significantly upregulated at this dose. At 500 mg/kg-day, stanniocalcin 1 (*Stc1*) and cysteine rich protein 61 (*cyr61*) expression were significantly increased, and *Ard6* expression was significantly decreased. *EPA determined that the LOAEL is 10 mg/kg-day based on decreases in sperm count, viability, and motility, and down-regulation of genes associated with steroidogenesis.*

In a subsequent study, **Vo et al. (**2009b**)** examined the effects of DEHP on male offspring, with dosing beginning at weaning, using the same dose levels as were examined in the previous gestational exposure study. In this study, male SD rats were administered DEHP in corn oil at 0, 10, 100, or 500 mg/kg-day via oral gavage daily from PND 21 through 35. The investigators also included similar groups of male rats administered testosterone propionate (TP) at 1 mg/kg-day to elicit androgenic effects or flutamide at 1, 10, or 50 mg/kg-day to examine the effects of the androgen receptor antagonist; again, EPA is only discussing the results relevant to DEHP. At termination on PND 36, blood was collected for measurement of serum testosterone and LH; weights of testes, epididymis, prostate, and seminal vesicles were recorded; and testis from four males per group were used for gene expression analysis, with remaining testis subjected to histopathology examination. Absolute organ weights were significantly

decreased compared to controls for the epididymis at 10 mg/kg-day, seminal vesicles at 10 and 500 mg/kg-day, testes at 500 mg/kg-day, and prostate in all DEHP-treated groups; while body weights in the treated groups were comparable to controls. AGD was decreased at 500 mg/kg-day. Serum testosterone was significantly decreased in all DEHP-treated groups, with LH showing a non-significant decreasing trend compared to controls.

Results of histopathology examination of the testes were presented in micrographs in Figures 3 and 4 of the publication, and these representative images indicated: dilatation of the tubular lumen, degeneration of Leydig cells, and disorder of germ cells at 10 and 100 mg/kg-day; and at 500 mg/kg-day, stratification of germ cells, dilatation of the tubular lumen and stratification, and disorder of germ cells were noted. However, no quantitative data were provided. There were no significant differences in expression of genes related to steroidogenesis (*StAR*, *Cyp11a1*, *Hsd3b1*) compared to controls. Real-time PCR confirmation of gene profiles from the microarray data from testes of males on PND 36 indicated significant up-regulation of LIM homeobox protein 1 (*Lhx1*) and phospholipase C, delta 1 (*Pldc1*) at 100 mg/kg-day and down-regulation of isochorismatase domain containing 1 (*Isoc1*) at 500 mg/kg-day. *EPA determined that the LOAEL is 10 mg/kg-day based on decreased absolute weights of the epididymis, seminal vesicles, and prostate, and decreased serum testosterone.*

In a study by **Ganning et al. (**1990**)**, DEHP was administered in the diet at concentrations of 0, 200, 2,000, or 20,000 ppm (equivalent to 0, 14, 140, and 1,400 mg/kg-day) for 102 weeks. There were no treatment-related clinical signs. The authors stated that there were no hyperplastic nodules or primary liver carcinomas. Body weights were significantly decreased at 140 and 1,400 mg/kg-day beginning at Week 18 and continuing throughout the remainder of the study, and the authors reported that body weights were decreased by 10 percent at 140 mg/kg-ay and decreased by 20 percent at 1,400 mg/kg-day compared to controls. Liver and testes were collected at termination and examined microscopically. The authors reported that all dose levels of DEHP exerted a "pronounced effect on the function of the testes after prolonged treatment, consisting of inhibition of spermatogenesis and general tubular atrophy" compared to controls, which had "normal histological structure and cell appearance"; however, no quantitative incidence or severity data were provided. The protein content of the mitochondrial fraction from the liver was dose-dependently increased at 140 and 1,400 mg/kg-day.

Peroxisomal palmitoyl-CoA dehydrogenase activity was increased over controls as follows: at 14 mg/kg-day, a continuous, slow moderate increase was observed with a doubling of activity by 2 years; the 140 mg/kg-day group had continuously increasing activity with an 8-fold increase after 2 years; and the 1,400 mg/kg-day group showed an 8-fold increase after 4 weeks and plateaued after 40 weeks with a 12-fold increase compared to controls. Peroxisomal catalase activity was dose-dependently increased at 140 and 1,400 mg/kg-day, attained statistical significance beginning at Week 33 and continuing through Week 73 and then returned to control levels by Week 102. Peroxisomal urate oxidase activity was dose-dependently decreased at 140 and 1400 mg/kg-day throughout the study and at all doses (greater than or equal to 14 mg/kg-day) beginning at Week 57. Mitochondrial carnitine acetyltransferase activity was dose-dependently increased over controls at 14 mg/kg-day and above, reaching a maximum at 1,400 mg/kg-day after approximately 20 weeks of treatment and increased more slowly at 140 mg/kg-day, although the levels at 140 and 1,400 mg/kg-day were similar at the end of the 2-year study. Microsomal NADH-cytochrome c reductase activity was unaffected by treatment. NADH-cytochrome c reductase was not affected, but NADPH-cytochrome c reductase and cytochrome P450 were increased in the first 24 weeks and then decreased to a still higher than control levels. A cessation of treatment experiment after treatment for 1 year showed a return toward control levels. EPA determined that the LOAEL is 14 mg/kg-day based on qualitative reporting of effects on the testes characterized as "inhibition of spermatogenesis and general tubular atrophy" compared to controls, which had "normal histological

structure and cell appearance"; and changes in liver enzymes, including: increased peroxisomal palmitoyl-CoA dehydrogenase activity; decreased peroxisomal urate oxidase activity; and increased mitochondrial carnitine acetyltransferase activity.

In the study by **Wang et al. (**2017**)**, pregnant SD rats were administered DEHP in corn oil via oral gavage at dose levels of 0, 0.01, 0.1, and 1 mg/kg-day daily beginning at implantation and continuing through the remainder of gestation and lactation (GD 7–LD 21). The objective of this study was to determine if male offspring exposed to DEHP *in utero* and during lactation were more susceptible to developing prostate cancer. On PND 90, 1 group of F1 males (11 per dose group) was implanted with silastic capsules containing testosterone and estradiol, while another group of F1 males (11 per dose group) were implanted with empty silastic capsules; these capsules were replaced on PND 146. On PND 196, all rats were terminated, and blood was collected, along with testes, epididymis, and prostate. Additionally, positive control groups were included in which F1 males were treated with 25 µg per pup 17-estradiol-3-benzoate (EB) by injection in nape of the neck on PNDs 1, 3, and 5, with one group implanted with the silastic capsules containing testosterone and estradiol, and the other EB-treated group implanted with sham-control empty capsules. EPA only considered groups dosed with DEHP compared to vehicle controls quantitatively for dose-response analysis (excluding groups treated with testosterone and estradiol and/or EB). Prostatic Intraepithelial Neoplasia (PIN) score (used to assess precursor lesions to prostate carcinogenesis) and Gleason score (indicating prostate carcinogenesis) were increased over negative controls at 0.1 mg/kg-day and above; however, these increases were not statistically significant. Absolute weights of prostate and testes and absolute and relative weights of epididymis were increased over negative controls at 0.1 mg/kg-day and above. However, histopathology was only reported qualitatively and depicted in representative micrograph images in the publication, but no quantitative data were provided on incidence or severity. PSA was significantly increased over negative controls at 1 mg/kg-day.

In a study by **Hsu et al. (**2016**)**, male SD rats were administered DEHP via oral gavage at 0.03, 0.1, 0.3, or 1 mg/kg-day from PND 42 through 105. At study termination, body weights, and weights of testes, epididymis, seminal vesicles, and kidneys were measured, along with sperm parameters (count, motility, morphology, reactive oxygen species (ROS), and chromatin structure analyses). There were no effects of treatment on sperm count or motility. Normal sperm was significantly lower only at 1 mg/kg-day (94.0%) compared to controls (96.2%). However, percent sperm with bent tails was significantly higher at 0.1, 0.3, and 1 mg/kg-day (1.1–2.0%) compared to controls (0.3%), and the percent of sperm with chromatin DNA damage, as indicated by DNA Fragmentation Index (DFI%), was higher at these doses (4.8–6.4%) compared to controls (2.1%).

In a study by **Shao et al. (**2019**)**, 15-day old female Wistar rats were administered DEHP via oral gavage at 0, 0.2, 1, or 5 mg/kg-day for 4 weeks. No acclimation period was reported, which would indicate that the animals had not be weaned at the time of study initiation. The following findings were noted at 0.2 mg/kg-day and higher: decreased apoptosis of hypothalamic cells; increased *GnRH* in hypothalamus; and increased protein expression of *IGF-1R*, *P13K*, *Akt*, and *GnRH*. At 1 mg/kg-day and above: increased serum *IGF-1* and *GnRH*; increased gene expression of *IGF-1*, *mTOR*, and *GnRH*; and increased protein expression of *IGF-1* and *mTOR* were observed. At 5 mg/kg-day: increased Nissl staining and gene expression of *IGF-1R* and *Akt* were observed in the hypothalamus; and accelerated sexual maturation (decreased time to vaginal opening) was depicted in a bar graph, occurring approximately a week earlier than controls (approximately 28 vs. 35 days). The study authors proposed that DEHP may activate hypothalamic GnRH neurons prematurely through *IGF-1* signaling pathway and promote *GnRH* release, leading to the accelerated sexual maturation observed in female rats at 5 mg/kg-day.

In a study by **Zhang et al. (**2014**)**, pregnant mice were administered DEHP in 0.1 percent DMSO at 0 or 0.04 mg/kg-day throughout gestation (GD 0.5–18.5) and allowed to deliver naturally on GD 19.5 (PND 0), and F1 females were mated with wild-type (untreated) males. Maternal serum estradiol was measured at GD 12.5. Meiosis-specific *Stra8* gene and protein expression were measured at GD 13.5. Meiosis prophase 1 assay was measured in developing fetal oocytes collected from pregnant mice terminated on GD 17.5. Folliculogenesis was evaluated at PND 21 in F1 and F2 females, with follicles classified as: primordial; primary; secondary; or antral. On GD 12.5, maternal serum estradiol at 0.04 mg/kg-day was lower than controls, and gene expression of *Cyp17a1*, *Cyp19a1*, *Aldhla1*, *ERα*, *FSHR*, *LHR*, *EGF*, and *EGFR* was significantly down-regulated in fetal mouse ovaries. On GD 13.5, gene and protein expression of the meiosis-specific *Stra8* gene was lower than controls, which the authors attributed to modifying methylation at the promoter, with significantly increased percent methylation in the treated group compared to controls. The authors reported delayed meiotic progression of female germ cells in fetal mouse ovary on GD 17.5, with the percent of oocytes at leptotene (26.43%) and zygotene (60.17%) stages in treated group higher than controls (4.33% leptotene and 29.57% zygotene), and fewer treated animals in pachytene and diplotene stages. Examination of the follicle status in ovaries of F1 offspring at PND21 showed a decrease in the number of primary and increase in the number of secondary follicles, which the authors attributed to depletion of the primordial follicle pool through accelerated folliculogenesis, moderated by down-regulation of gene expression of folliculogenesis-related genes (*Cx43*, *Egr3*, *Tff1*, and *Ptgs2*). In the F2 females, the number of primordial follicles was significantly lower and the number of secondary follicles was significantly higher in the treated group compared to controls on PND21.

In a study by **Pocar et al. (**2012**)**, CD-1 mice were administered DEHP in the diet at 0, 0.05, 5, and 500 mg/kg-day throughout gestation and lactation (GD 0.5–LD 21). Abortion occurred in 9/10 dams at 500 mg/kg-day; therefore, evaluation of effects in offspring was limited to the 0.05 and 5 mg/kg-day groups compared to controls. Body weights, AGD, sperm count, and sperm viability were measured in offspring on PND 42. Oocyte maturation was determined *in vitro* in oocytes from maternally-exposed female offspring, with oocytes categorized as (1) not matured (germinal vesicle and metaphase I) = diffuse or slightly condensed chromatin or with clumped or strongly condensed chromatin with or without metaphase plate but no polar body; (2) mature MII oocytes = oocytes with metaphase plate and a polar body; or (3) degenerated = oocytes with no visible chromatin or with fragmented cytoplasm and/or abnormal chromatin patterns. Male-specific effects were examined through *in vitro* fertilization using sperm from maternally-exposed males with oocytes from untreated females; cleavage was measured at 24 hours and blastocyst rate was determined at 96-hours post-fertilization. Relative (to body weight) liver weights in the maternal animals were significantly increased by 11 to 18 percent over controls at 0.05 and 5 mg/kg-day. In the offspring at these doses: body weights were decreased by 18 to 24 percent on PND 21 and by 6 to 14 percent on PND 42 in both sexes; percent body fat was decreased by 29 to 42 percent in females; absolute seminal vesicle weights were decreased by 20 to 26 percent; and absolute ovary weights were increased by 13 to 32 percent. Sperm count was decreased by 51 to 53 percent, and sperm viability decreased by 20 percent compared to controls. *In vitro* oocyte maturation tests showed a decrease in mature oocytes in the treated groups (80%) compared to controls (88%) and an increase in degenerated oocytes (18% treated vs. 8% controls). In maternally-treated male offspring (with oocytes from untreated females, the blastocyst rate was dose-dependently lower at 0.05 mg/kg-day (13.5%) and 5 mg/kg-day (4.4%) compared to controls (43.9%).

B.2 Summaries of Nutritional/Metabolic Studies on Effects Related to Glucose/Insulin Homeostasis and Lipid Metabolism

Gu et al. (2016**)** exposed pregnant C57BL/6J mice (n = 6–7 per treatment) to 0, 0.05, or 500 mg/kg-day DEHP via gavage from GD 1 through 19. Food intake was not significantly altered by DEHP exposure in dams. A 100 percent abortion rate occurred for dams exposed to 500 mg/kg-day DCHP, and therefore, the offspring of the 0 and 0.05 mg/kg-day group were used for subsequent stages of the study. Pups were sacrificed at nine weeks of age, blood and adipose tissue were collected, and the following endpoints were measured: serum leptin, insulin, total triglyceride, total cholesterol, and fasting glucose levels; weights of the inguinal (subcutaneous) and gonadal (visceral) fat pads; and mRNA expression levels of two developmental genes, T box 15 (*Tbx15*) and glypican 4 (*Gpc4*), in fat tissues. Serum leptin and insulin levels were significantly higher in DEHP-exposed F1 males and females relative to control. Although there were no significant treatment-related effects on body weights or subcutaneous fat weights in F1 offspring, visceral fat weights were significantly higher in F1 DEHP-exposed males and females relative to control. Consistent with this increase in visceral fat mass, serum total triglycerides, total cholesterol, and fasting glucose levels were significantly increased by approximately 8, 13, and 16 percent, respectively, in F1 males and females in the DEHP treatment group compared with control F1 offspring. *Tbx15* mRNA expression level in subcutaneous fat significantly increased in the DEHP-treated F1 offspring compared with the F1 control group, whereas no significant increase was observed in visceral fat. Compared with the F1 control group, *Gpc4* mRNA expression in visceral fat significantly increased in the F1 DEHP-exposed offspring, whereas no significant increase was detected in the subcutaneous fat. Notably, the authors did not separate male and female samples in their mRNA expression analysis.

Mangala Priya et al. (2014**)** exposed lactating Wistar albino rat dams (n = 3 per treatment) to 0, 1, 10, and 100 mg/kg-day DEHP via gavage from PND 1 to PND 21. On PND 58, F1 females were fasted overnight, and their blood was collected for glucose estimation on PND 59. On PND 60, F1 females were sacrificed, and cardiac muscle was isolated for protein expression analysis of insulin signaling molecules and analysis of glucose uptake and oxidation. Fasting blood glucose level significantly increased in all treated groups compared to control on PND 59. Protein expression of insulin receptor (IR), insulin receptor substrate 1 (IRS-1), and IRS-1^{Tyr632} decreased significantly and dose-dependently in all treatment groups compared to control. Although Akt protein expression was unaltered, Akt^{Ser473} significantly decreased across all treated groups. Additionally, protein expression of glucose transporter 4 (GLUT4), which is mandatory for the entry of glucose molecule, significantly and dose-dependently decreased in the plasma membrane in all treatment groups compared to control and remained unaltered in the cytosol. Furthermore, 14C-2-deoxyglucose uptake and 14C-glucose oxidation also decreased significantly and dose-dependently in all treatment groups compared to control.

Xu et al. (2018**)** exposed 21-day old adolescent Wistar rats (10/sex/group) to 0, 5, 50 or 500 mg/kg/day DEHP via gavage for 28 days. Animals were sacrificed after 28 days of exposure and were evaluated for the following endpoints: body weight, food intake, fasting blood glucose levels, serum insulin and leptin levels, organ weight (liver, pancreas) and gene and protein expression of Janus-activated kinase 2 (*JAK2*), signal transducer and activator of transcription 3 (*STAT3*), suppressor of cytokine signaling 3 (*SOCS3*), leptin receptor (Ob-R) and insulin receptor (*IR*) in the liver and pancreas. Data from males and females in each experimental group were combined for all endpoints.

Terminal body weights and body weight gains were not significantly different from control. Food intake was significantly higher (10 and 14%) in the 50 and 500 mg/kg/day group, respectively compared to control. Fasting blood glucose (69 and 104%), fasting serum insulin (19 and 29%), and fasting serum

leptin (22 and 59%) levels increased dose-dependently and reached significance at 50 and 500 mg/kg/day. Calculated insulin resistance index homeostasis model assessment (HOMA-IR) increased dose-dependently and reached significance in the 50 and 500 mg/kg/day groups (corresponding to a 2- and 2.7-fold increase in these groups, respectively). Relative liver weight was significantly increased (35%) in the 500 mg/kg/day group compared to control. No exposure-related changes in relative pancreas weight were seen.

According to Western Blot analysis of protein expression in the liver, *JAK2* increased dose-dependently and reached significance at 50 and 500 mg/kg-day; *STAT3* increased dose-dependently and reached significance at 500 mg/kg-day; and *SOCS3* increased significantly at 50 and 500 mg/kg-day. Protein expression of Ob-R in the liver decreased significantly at all dose groups, and IR decreased dose-dependently and reached significance at 500 mg/kg-day. In the pancreas, protein expression of *JAK2* was not significantly different from control; *STAT3* increased dose-dependently and reached significance at 500 mg/kg-day; and SOCS3 increased significantly at 500 mg/kg-day. Protein expression of *Ob-R* in the pancreas decreased significantly and dose-dependently at all doses, and *IR* decreased dose-dependently and reached significance at 500 mg/kg-day.

According to immunohistochemistry staining in the liver, *JAK2* and *STAT3* significantly increased at 500 mg/kg-day; *SOCS3* increased dose-dependently and reached significance at 50 mg/kg-day; *Ob-R* decreased dose-dependently and reached significance at 500 mg/kg-day. In the pancreas, *JAK2* and *STAT3* were not significantly different from controls; *SOCS3* increased dose-dependently and reached significance at 50 and 500 mg/kg-day; and *Ob-R* decreased significantly at 500 mg/kg-day.

In the liver and pancreas, mRNA expression of *JAK2*, *STAT3*, and *Ob-R* did not change significantly relative to control. *SOCS3* increased significantly at 500 mg/kg-day.

Venturelli et al. (2015**)** exposed lactating Wistar rat dams (n = 5 per treatment) to 0, 7.5, 75 mg/kg-day DEHP via gavage from PND 1 to 21. Dams were observed for clinical signs of toxicity, body weight, and food consumption. During weaning through PND 92, male offspring were evaluated for weight gain and food ingestion. Additionally, the age of preputial separation was measured, fecal samples were collected for analysis of fecal androgen metabolites from PND 22 to PND 88, and insulin tolerance tests (ITT) were performed on PND 22, 60, and 90. Male offspring were sacrificed on PND 92, and the following additional endpoints were evaluated: serum levels of glucose, insulin, triglycerides and cholesterol; organ weights (liver, kidneys, adrenal glands, testicles, epididymis, ventral prostate, seminal vesicles and retroperitoneal, epididymal and inguinal fat pads), and *ex vivo* insulin secretion from isolated pancreatic islets in response to glucose.

Body weight, food intake, and organ weights of DEHP-treated dams were not different than control (data not shown). In male offspring, no exposure-related effects were observed on body weight or eating behavior from weaning through PND 92, or on organ weights and isolated fat deposits (data not shown). Fasting serum glucose concentration increased significantly by 13 percent in the 75 mg/kg-day dose group compared to control. Fasting serum insulin concentrations did not differ significantly in any dose groups compared to control. In the ITT, a significant, dose-dependent decrease in glucose decay rate was seen on PND 90 in both dose groups and in area under the curve (AUC) in the 75 mg/kg-day group compared to control (data not shown), suggesting reduced insulin sensitivity. No exposure-related changes occurred in the ITT at PND 22 or 60. Pancreatic islets isolated from male offspring in the 75 mg/kg-day dose group released significantly less insulin compared to control after stimulation with glucose *ex vivo* (50 and 70% reduction when stimulated with 8.3 mM and 17.7 mM glucose, respectively). Serum triglyceride levels decreased significantly by 32 percent in both dose groups and

serum cholesterol decreased significantly by and 21 percent, in the 75 mg/kg-day dose group. The concentration of fecal androgen metabolites increased significantly only on PND 88 in the 7.5 mg/kg-day dose group, and no significant changes occurred at the higher dose or at any other timepoints. The mean age of preputial separation was not different between groups.

In a second experiment in the same publication by **Venturelli et al. (**2015**)**, prepubertal male Wistar rats (n = 15 per treatment) were exposed to the same doses of DEHP for 30 days (PND 22–52). Animals were evaluated for body weight gain and food ingestion throughout the treatment period. The age of preputial separation was measured, fecal samples were collected for analysis of fecal androgen metabolites from PND 28 to PND 49, and an insulin tolerance test (ITT) was performed on PND 50. Male offspring were sacrificed on PND 53, and the following additional endpoints were evaluated: serum levels of glucose, insulin, triglycerides and cholesterol and organ weights (liver, kidneys, adrenal glands, testicles, epididymis, ventral prostate, seminal vesicles and retroperitoneal, epididymal and inguinal fat pads).

No treatment-related effects were observed on body weight, eating behavior, organ weights (data not shown), fasting serum insulin concentrations, ITT test results, serum triglyceride and cholesterol levels, and mean age for preputial separation. Fasting serum glucose concentration increased significantly by 30 percent in males in the 75 mg/kg-day dose group compared to control. The concentration of fecal androgen metabolites decreased significantly on PND 49 in both dose groups; however, there was no dose-response, and the changes did not persist at any other timepoints.

Zhang et al. (2020b**)** treated 21-day-old adolescent Wistar rats (n = 10 per sex per group) with 0, 5, 50, 500 mg/kg/day DEHP by gavage for 8 weeks. Animals were weighed throughout the treatment period. At the end of the treatment period, rats were fasted overnight and sacrificed. The following endpoints were evaluated in blood: serum levels of triglyceride, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), leptin (LEP) and adiponectin (ADP). Liver and adipose were also evaluated for triglyceride and total cholesterol levels, histopathology, and expression (mRNA and protein) of key molecules involved in lipid metabolism in adipose and liver. In addition to normal rats, the authors also studied a cohort of animals fed a high fat diet (results not included here).

There were no significant differences in body weight, lipids, or hormones between males and females; therefore, authors combined the data from both sexes for analysis. Terminal body weights were significantly increased in the 500 mg/kg/day dose group compared to control. Serum total cholesterol and HDL increased significantly at 500 mg/kg/day, and there were no treatment-related effects on serum levels of triglycerides, LDL, LEP, or ADP. Levels of triglycerides and total cholesterol in the liver and adipose were not different from control. Structural abnormalities in the liver including disordered hepatocyte cords, vacuolar degeneration, and accumulation of inflammatory cytokines were seen in all dose groups (quantitative data was not reported). In the adipose tissue, the volume of adipocytes was increased at 5 and 50 mg/kg/day and the number of adipocytes were increased at 500 mg/kg/day compared to control (quantitative data was not reported).

DEHP exposure altered the mRNA and protein expression of key molecules involved in lipid metabolism, adipogenesis and lipid accumulation in both the liver and adipose tissue. These involve the JAK2/STAT5 and TYK2/STAT1 pathways. In liver tissue, Janus kinase 2 (*JAK2*) mRNA expression decreased significantly across all tested doses relative to control. *Fas* mRNA increased significantly and dose-dependently across all DEHP-exposed groups. Signal transducer and activator of transcription 5B (Stat5b) mRNA increased dose-dependently and achieved significance at 50 and 500 mg/kg-day compared to control. Activating enhancer binding protein 2 (Ap2) mRNA increased significantly at 500

mg/kg-day DEHP. Stat5a and pyruvate dehydrogenase kinase 4 (*PDK4*) mRNA did not change relative to control in the liver. Immunohistochemistry staining for *Ap2* and *Fas* protein expression dose-dependently increased and reached statistical significance at 50 and 500 mg/kg-day compared to control. IHC staining for PDK4 protein expression increased significantly at 500 mg/kg-day. Western blot analysis showed no significant treatment related effects on JAK2, phosphorylated *JAK2*, *STAT5A*, and phosphorylated *STAT5B* relative to control. Phosphorylated *STAT5A* significantly increased at 500 mg/kg-day compared to control, and *PDK4* increased significantly across all tested doses compared to control. Although *STAT5B* and *Ap2* expression changed significantly across all dose groups, there was no clear dose-related trend.

In adipose tissue, mRNA expression of *Jak2* and *Stat5a* increased dose-dependently and achieved statistical significance at 50 and 500 mg/kg-day. Additionally, *Fas* mRNA expression increased significantly and dose-dependently across all dose groups. *Pdk4* mRNA expression increased significantly at 500 mg/kg-day. *Stat5b* mRNA increased significantly at the lowest dose but remained similar to control at the higher doses. *Ap2* mRNA expression did not change significantly across any dose groups. Western blot analysis showed that JAK2 significantly increased at 50 and 500 mg/kg-day; phosphorylated JAK2 (*p-JAK2*) , *STAT5A*, and *p-STAT5A* significantly and dose-dependently increased at all doses, and Fas significantly increased at 500 mg/kg-day. Although *STAT5B*, *p-STAT5B*, and *PDK5* changed significantly in some dose groups, no clear dose-related trend was observed.

Rajesh et al. (2013**)** exposed adult male Wistar albino rats (n = 6 per treatment) to 0, 10, and 100 mg/kg-day DEHP via gavage for 30 days. After the completion of treatment, rats were fasted overnight and sacrificed. Blood was collected for serum glucose determination. Additionally, the following endpoints were measured in adipose tissue: lipid peroxidation, glucose uptake, glycogen content, and expression of insulin signaling genes and proteins.

Fasting blood glucose levels significantly increased in males dosed with 100 mg/kg-day DEHP relative to control. Hydroxyl radical production, hydrogen peroxide generation, and lipid peroxidation significantly and dose-dependently increased in adipose tissue of males from both dose groups compared to control. Glycogen levels, 14C-2-deoxyglucose uptake, and 14C-glucose oxidation in adipose tissue decreased significantly and dose-dependently in both dose groups relative to control.

Gene and protein expression analysis showed altered expression of insulin signaling molecules that could account for decreased glucose uptake in adipose tissue and increased serum glucose levels. Specifically, insulin receptor (*IR*) mRNA and protein expression significantly decreased in the adipose tissue of males dosed with 10 and 100 mg/kg-day DEHP relative to control. Insulin receptor substrate-1 (IRS-1) mRNA and protein levels in adipose tissue significantly decreased at 10 and 100 mg/kg-day; however, only changes in mRNA were dose-dependent. IRS-1$^{\text{Tyr 632}}$ decreased significantly and dose-dependently compared to control, whereas IRS-1$^{\text{Ser 636/639}}$ levels changed significantly but inconsistently across the two dose groups. β-arrestin2 protein expression significantly decreased at 100 mg/kg-day when compared to control. Although Akt protein expression was unaltered by DEHP treatment, phosphorylated Akt$^{\text{Ser473}}$ decreased significantly and dose-dependently. AS160 protein expression decreased significantly and dose-dependently across all groups. Glucose transporter-4 (*GLUT4*) mRNA expression increased significantly at 10 mg/kg-day but did not change significantly at 100 mg/kg-day relative to control. Cytosolic and plasma membrane *GLUT4* protein expression decreased significantly and dose-dependently in all treatment groups. *GLUT4*^{Ser488} level increased significantly and dose-dependently in all treated groups. Nuclear expression of the mature transcription factor SREBP-1c decreased significantly and dose-dependently. Additionally, co-treatment with antioxidant vitamins (C

and E) and 100 mg/kg-DEHP significantly reversed most effects seen in the 100 mg/kg-day DEHP group except for effects on β-arrestin2 and AS160 protein expression.

Lin et al. (2011b**)** exposed pregnant Wistar rats (n = 10–12 per treatment) to 0, 1.25, and 6.25 mg/kg-day DEHP via gavage from GD 0 to PND 21. Dam body weight was measured throughout the treatment period. Dams were allowed to deliver naturally, and litter size, sex ratio, and birth weights were recorded. Fasting blood glucose and serum insulin were measured in dams at weaning (PND 21). Offspring body weight was measured from PND 1 to postnatal week (PNW) 27. Energy intake was measured from PNW 11 to 20. Oral glucose and insulin tolerance tests (GTT and ITT) were administered on PNW 3, PNW 15, and PNW 26. Pancreatic insulin levels (PNW 3 and PNW 27), glucose-stimulated insulin secretion (PNW 27), electron microscopy (PNW 3 and 27), and gene expression were also analyzed.

Body weight of dams remained unchanged between the treatment and control groups throughout gestation or lactation, although data was not shown. At weaning (PND 21), fasting blood glucose and fasting serum insulin were not significantly different between the control or treatment groups in dams. No treatment-related effect on liter size or the proportion of females per litter was observed between any groups. Birth weight of F1 males and females was significantly lower than that of controls for both dose groups and remained so throughout the preweaning period (PND 1–21). After weaning, body weight of F1 males and females in the 1.25 mg/kg-day treatment group was significantly lower than that of controls until week 9 for females and until week 7 for males. Body weight of F1 males and females in the 6.25 mg/kg-day treatment group was significantly lower than controls at all measured timepoints (from weaning until week 27). Cumulative food intake from weeks 11 to 20 significantly decreased in F1 males and females in the 6.25 mg/kg-day dose group; however, when the data were expressed relative to body weight, there were no significant differences among all groups. No significant treatment-related effects on fasting serum glucagon levels were observed up to week 27 in F1 males and females (data was not shown).

At PNW 3, fasting blood glucose and serum insulin were significantly lower in F1 females and males in both dose groups compared with controls. In the GTT, blood glucose levels and insulin levels in F1 males and females in both DEHP dose groups were lower than control, although statistical significance varied across different doses and timepoints. Glucose and insulin AUC values were significantly lower in F1 males and females in both DEHP dose groups compared with controls.

At PNW 15, fasting serum insulin was significantly elevated in F1 females in both dose groups. There were no treatment-related effects on fasting serum insulin in F1 males or on fasting blood glucose levels in any group. In the GTT, glucose levels and serum insulin levels in F1 males and glucose levels in F1 females were not different from controls in either dose group. Insulin in F1 females increased significantly in both dose groups relative to control. Consistently, insulin AUC was significantly higher in F1 females at both doses relative to control, whereas it was unchanged relative to control in F1 males at both doses. Glucose AUC values were significantly decreased in F1 males at both doses relative to control, whereas it was unchanged relative to control in F1 females at both doses.

At PNW 27, fasting blood glucose was significantly elevated, but fasting serum insulin was significantly decreased, in F1 females for both dose groups relative to control. In F1 males, fasting blood glucose remained unchanged relative to control, whereas fasting serum insulin levels were significantly higher in both dose groups relative to control. In the GTT, blood glucose levels were elevated in both dose groups relative to control for F1 females, with significance varying depending on the dose and timepoint. Glucose AUC was significantly increased at both dose groups relative to control in F1 females. Insulin

levels were decreased in F1 females, with significance varying depending on the dose and timepoint. Insulin AUC was significantly decreased at both dose groups relative to control. Blood glucose levels were unaltered in DEHP-treated F1 males relative to control after glucose administration. Insulin levels were significantly increased in F1 males at both dose groups relative to control at 30, 60, and 120 minutes. Consistently, insulin AUC was higher in F1 males at both doses relative to control.

In the ITT conducted on PND 21, blood glucose levels were lower in F1 males and females relative to control, although statistical significance varied across different doses and sampling times. By weeks 15 (data not shown) and 27, no treatment-related effects were observed on blood glucose levels after insulin administration. Glucose AUC was not reported. The study authors concluded that DEHP exposure did not induce insulin resistance in offspring.

At PNW 3, adipocyte size and body fat percentage significantly decreased in F1 males and females in both dose groups relative to control; however, no treatment-related effects were observed in these two parameters at PNW 27.

At PNW 3, pancreas weight and β-cell area were unaffected in F1 males and females. β-cell mass and pancreatic insulin content significantly decreased in F1 males and females for both doses relative to control. β-cell ultrastructural changes including hypertrophic rough endoplasmic reticulum and swollen mitochondria with minimal cristae were qualitatively reported alongside significant increases in the average mitochondrial area, significantly increased optical density of the mitochondria, significantly increased percentage of immature granules, and significantly decreased percentage of filled granules in F1 females in both treatment groups compared with controls. Mitochondrial swelling was qualitatively reported alongside significantly increased average mitochondrial area in β-cells, significantly increased percentage of immature granules, and significantly decreased percentage of filled granules in β-cells from F1 males in both treatment groups compared with controls.

At PNW 27, pancreas weight significantly increased in F1 females in both dose groups. β-cell mass and pancreatic insulin content were significantly decreased in F1 females at both dose groups; however, these were no longer significantly decreased in F1 males. β-cell area significantly decreased in F1 females and increased in F1 males for both doses. Regarding β-cell ultrastructure, the changes noted at PNW 3 were maintained in F1 males and females, with the following additional effects: derangements in β-cells were much more prominent in F1 males and females in both treated groups relative to control. Additionally, remarkably swollen mitochondria, with essentially complete loss of defined structure within the membrane, were observed for F1 females in both treated groups relative to control. Furthermore, the optical density of the mitochondria increased significantly in F1 males in the 6.25 mg/kg-day group, and the percentage of empty granules was significantly increased in both treated groups relative to control for both sexes.

The authors also evaluated glucose-stimulated insulin secretion *ex vivo* at PNW 27. Islets from F1 females in both dose groups secreted significantly lower insulin levels relative to control in the presence of medium (5.8 mM) and high (16.7 mM) doses of glucose. Conversely, islets from F1 males in both dose groups secreted significantly higher insulin levels relative to control in the presence of low (3mM) and medium (5.8 mM) glucose.

Finally, expression of mRNA essential to the development of pancreas and β-cell function in offspring at weaning was measured. mRNA expression of pancreatic and duodenal homeobox-1 (*Pdx-1*) and insulin significantly decreased in F1 males and females in both dose groups relative to control. mRNA expression of genes involved in endoplasmic reticulum stress including activating transcription factor

4 (*Atf4*), *Atf6*, and binding immunoglobulin protein (*Bip*) increased significantly in F1 males and females in both dose groups relative to control. Additionally, mRNA expression of uncoupling protein 2 (*Ucp2*) increased in both sexes in both dose groups, although this reached significance in females only. No difference was observed in the mRNA expression glucagon among groups.

Parsanathan et al. (2019**)** exposed lactating Wistar rats (n = 3 per treatment) to 0, 1, 10, and 100 mg/kg-day DEHP via gavage for 3 weeks (PND 1–21). After the treatment period, male offspring were fasted overnight and were sacrificed on PND 22. Endpoints evaluated in male offspring included body weight, fasting blood glucose levels, organ weight (heart), glucose uptake and glucose oxidation in cardiac tissue, and cardiac protein levels of insulin signaling molecules (insulin receptor subunit b (IR-β), insulin receptor substrate 1 (IRS1), protein kinase B (Akt), Akt substrate (AS160) and glucose transporter type 4 (GLUT4).

Body weight decreased dose-dependently in DEHP-exposed male offspring relative to control from PND 9 to PND 22, although statistical comparisons were not provided until PND 22. At PND 22, terminal body weight and heart weight were decreased significantly and dose-dependently across all dose groups. Fasting blood glucose levels were increased in the 100 mg/kg-day dose group compared with control. In the cardiac muscle, 14C-2-deoxyglucose uptake decreased in all dose groups and 14C-glucose oxidation decreased in the 10 and 100 mg/kg-day groups compared to control.

DEHP exposure also significantly altered the signaling molecules related to glucometabolic activities in the cardiac tissue compared to control. Insulin receptor (InsR) protein expression significantly decreased in DEHP-exposed males at all dose groups compared to control. Insulin receptor substrate 1 (IRS1) protein expression significantly decreased in the 100-mg/kg-day group compared to control. Phosphorylated IRS1^{Tyr632} decreased in the 10 and 100 mg/kg-day treated groups compared to control. Akt and AS160 protein expression were unaltered; however, Akt^{Ser473} significantly decreased in the 100 mg/kg-day group compared to control. GLUT4 protein expression significantly decreased in across all treated groups compared to control. GLUT4^{Ser488} increased significantly in the100 mg/kg-day group compared to control.

Rajesh and Balasubramanian (2014**)** exposed pregnant Wistar rats (n = 6 per treatment) to 0, 1, 10, and 100 mg/kg-day DEHP via gavage from GD 9 to GD 21. On PND 60, oral glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were conducted in offspring after overnight fasting. Offspring were sacrificed on PND 60, and the following endpoints were measured: body and visceral adipose weights and serum glucose and insulin. Additionally, the following endpoints were measured in skeletal (gastrocnemius) muscle: expression of genes and proteins involved in insulin signaling, DNA methylation, and evaluation of insulin receptors and glucose uptake and oxidation.

Lean body weight and glycogen concentration in the gastrocnemius muscle decreased significantly and dose-dependently across all dose groups (4–21%). Fat weight increased (2–7%) dose-dependently and significance was achieved at 10 and 100 mg/kg-day doses for both male and female offspring. Fasting glucose levels increased (16–49%) and fasting insulin levels decreased (21–70%) significantly and dose-dependently in male and female offspring for all dose groups compared with controls. GTT and ITT results suggested that DEHP exposure impaired both glucose and insulin tolerance in males and females for all dose groups. Specifically, during both tests, blood glucose concentrations in DEHP exposed groups were dose-dependently higher than in the control group, although statistical significance varied across doses and timepoints. Additionally, insulin binding decreased significantly and dose-dependently (13–36%) in gastrocnemius muscle of male and female offspring relative to control. 14C-2-

deoxyglucose uptake and 14C-glucose oxidation also significantly and dose-dependently declined in the gastrocnemius muscle of both sexes compared to controls.

Several genes and proteins involved in insulin signaling were dysregulated in the gastrocnemius muscle of male and female offspring in response to DEHP exposure. Specifically, mRNA expression, protein expression in the plasma membrane, and tyrosine phosphorylation of insulin receptor (INSR $^{Tyr1162/1163}$) reduced significantly and dose-dependently in both male and female offspring. Although insulin receptor substrate 1 (Irs1) mRNA was unaltered, IRS1 protein levels decreased significantly across all doses in females and in males in the 10 and 100 mg/kg-day dose groups. IRS1^{Tyr632} was significantly reduced in males exposed in all dose groups and in females in the 10 and 100 mg/kg-day dose groups. Unlike tyrosine phosphorylation, phosphorylated IRS1$^{Ser636/639}$ was significantly increased in males and females in the100 mg/kg-day dose group. Histone deacetylase 2 (HDAC2) protein level in the cytosol increased dose-dependently in males and females relative to control.

Akt (Akt1) mRNA expression and Akt$^{Tyr315/316/312}$ decreased significantly and dose-dependently in males and females compared with control. Total AKT protein decreased significantly at 100 mg/kg-day in males and females. Akt^{Ser473} decreased significantly at all doses in males and at 100 mg/kg-day in F1 females. Akt^{Thr308} level was significantly decreased at 10 and 100 mg doses of DEHP treatment in both male and female offspring.

Phosphatase and tensin homolog (PTEN) protein expression increased in all dose groups for males and females compared to control. β-arrestin 2 protein expression significantly decreased in males and females in the10 and 100 mg/kg-day groups compared to control. Proto-oncogene tyrosine-protein kinase (c-SRC) and mammalian target of rapamycin (MTOR) expression significantly decreased in all dose groups in males and females compared to control, and these changes were dose-dependent for MTOR. Pyruvate dehydrogenase kinase 1 (PDK1) protein levels remained unaltered in all treated groups. Although Akt substrate of 160 (AS160) protein level remained unaltered in all dose groups when compared with the control group, the AS160^{Thr642} level was significantly and dose-dependently reduced in males and was dose-dependently reduced in females, attaining significance in the 100 mg/kg-day group. Actin alpha 4 (ACTN4) protein expression decreased significantly and dose-dependently in F1 males and decreased dose-dependently in F1 females, attaining significance in the 10 and 100 mg/kg-day dose group. Ras-related protein (RAB) 13 expression decreased dose-dependently in F1 males and females compared to control. RAB8A protein expression decreased significantly in males and females at 10 and 100 mg/kg-day. Although these changes were dose-dependent in males, there was no clear dose-response relationship in females.

Expression, post-translational modification, and localization of glucose transporter 4 (GLUT4) changed in response to *in utero* DEHP exposure. Glut4 mRNA expression decreased significantly and dose-dependently in male and female offspring compared with the control group. Plasma membrane expression of GLUT4 decreased significantly and dose-dependently in all experimental groups compared to control. Additionally, cytosolic expression of GLUT4 significantly decreased in all treatment groups in males and at 10 and 100 mg/kg-day in females compared to control. Additionally, the authors also qualitatively measured immunofluorescence staining intensity of GLUT4 protein, which decreased in a dose-dependent manner in the PM as well as cytosol region compared to control. Conversely, GLUT4^{Ser488} significantly increased at 10 and 100 mg/kg-day for both sexes.

Expression and binding of the transcriptional enhancer myoblast determination protein 1 (MYOD) and transcriptional repressor histone deacetylase 2 (HDAC2) towards GLUT4 also changed in response to in utero DEHP exposure. Nuclear MYOD protein expression dose-dependently decreased in male and

female offspring. Changes were significant at all doses in males and at 10 and 100 mg/kg-day in females. Nuclear sterol regulatory element binding protein-1c (SREBP1c) proteins decreased significantly in males and females at all doses. Conversely, HDAC2 expression increased significantly and dose-dependently in males and females. Chromatin Immunoprecipitation ChIP assay demonstrated a significant, dose-dependent increase in the binding of HDAC2 to the GLUT4 promoter region in all dose groups of both sexes compared to control. MYOD binding to the same promoter region decreased significantly and dose-dependently in males and decreased significantly in all treatment groups in females.

Global DNA methylation and methylation of the GLUT4 promoter region were also altered in gastrocnemius muscle following in utero DEHP exposure. Specifically, global methylation (as measured by 5-Methyl-20-deoxycytidine level) significantly increased in a dose-dependent manner in all treated male and female offspring compared with controls. Additionally, methylation increased in the GLUT4 promoter at all doses in males and females according to images of ethidium bromide-stained DNA gels, although this was not measured quantitatively or analyzed statistically.

Developmental DEHP exposure additionally up-regulated expression of DNA methyltransferases (DNMTs) in the gastrocnemius muscle. Dnmt1mRNA increased significantly in both sexes across all doses when compared with controls, and DNMT1 protein increased significantly and dose-dependently across all doses in both sexes. Dnmt3a/Dnmt3b mRNA and protein levels were increased significantly and dose-dependently across all doses in both sexes. Dnmt3l mRNA and protein levels were unaltered compared with the control group.

Schmidt et al. (2012**)** exposed female C3H/N mice (n = 25 per treatment) to 0, 0.05, 5, or 500 mg/kg-day DEHP in the diet for 8 weeks (7 weeks pre-mating through GD 1). Food intake and weight gain were measured throughout the treatment period in dams. Dams were sacrificed on GD 1, and the following endpoints were measured: visceral fat, expression of PPAR isoforms in liver and visceral fat, plasma concentration of leptin, leptin, adiponectin, and fatty acid protein 4 (FABP4) mRNA levels in visceral fat tissue, and F1 preimplantation embryos.

No clinical signs of toxicity were observed in dams. Average weekly food intake was significantly higher in all DEHP-exposed groups compared with controls. At the end of the 8-week treatment period, the body weights of DEHP-exposed dams from all three experiments combined were significantly higher than those of controls. Additionally, DEHP-treated dams had significantly more visceral fat tissue than controls, although this increase was not dose-dependent. Sections of visceral adipose tissue were subjected to histological examination. The adipocytes of all DEHP-exposed mice were larger (hypertrophied) than those of controls, and this was confirmed quantitatively by a statistically significant, dose-dependent decrease in adipocytes per unit area. PPARα and PPARγ mRNA expression in the liver increased significantly in the 500 mg/kg-day DEHP group. PPARα mRNA expression in visceral fat tissue decreased significantly in the 500 mg/kg-day DEHP group, whereas PPARγ expression was not altered by DEHP treatment. Plasma leptin concentration increased dose-dependently and was significantly higher in the 500 mg DEHP treatment group compared with controls. Leptin mRNA expression and fatty acid protein 4 (FABP4) mRNA expression in visceral fat significantly increased across all DEHP-exposed dams; however, the highest increase was in the lowest dose group for both genes. Adiponectin mRNA expression in visceral fat decreased significantly and dose-dependently in all DEHP treatment groups. No significant differences were observed in the average number of preimplantation embryos between treatment groups or in the percent of degenerated blastocysts; however, study authors did note that degenerated blastocysts increased from 14 percent in controls to 32 percent in the highest dose group.

In a second experiment in the same publication by **Schmidt et al. (**2012**),** exposed F0 dams (n = 15 per treatment group) to 0, 0.05, 5, or 500 mg/kg-day DEHP in the diet for 8 weeks (1 week pre-mating through PND 21). Dams were sacrificed at weaning (PND 21) and visceral fat was measured. F1 offspring were maintained until PND 84 without additional exposure. Bodyweights were measured at PND 21 and PND 84, and visceral fat was measured at PND 84. At PND 84, sexually mature F1 females were mated and sacrificed on GD 1, and the number of F2 preimplantation embryos was measured.

No clinical signs of toxicity were reported in F0 dams. The abortion rate was 100 percent in the 500 mg/kg-day dose group, and therefore, only the 0.05 and 5 mg/kg-day dose groups were used for subsequent analyses. The body weights of DEHP-exposed F1 males and females increased dose-dependently on PND 21 (30–50%) and PND 84 (10–15%). Increases were statistically significant in all dose groups compared to control except for F1 males in the 0.05 mg/kg-day dose group on PND 21. Visceral fat increased significantly in F0 dams, F1 males, and F1 females in both dose groups compared to control; furthermore, this increase was dose-dependent in F0 dams and F1 females. No significant differences were observed in the average number of preimplantation embryos in F1 females between treatment groups or in the percent of degenerated blastocysts; however, study authors did note that degenerated blastocysts increased from 8 percent in controls to 28 and 29 percent in the 0.05 mg/kg-day and 5 mg/kg-day dose groups, respectively.

Rajagopal (2019a**,** b**)** exposed pregnant Wistar rats (n = 6 per group) to 0, 10, or 100 mg/kg-day DEHP via oral gavage from GD 9 to PND 21 (including the lactational period). On PND 80, fasting blood glucose and insulin levels were measured from one cohort of male offspring. Another cohort of male offspring underwent an oral glucose tolerance test (GTT) and an insulin tolerance test (ITT) 2 days before sacrifice (PND80). Additional endpoints were evaluated at PND 80 including body weight and serum testosterone, estradiol (E), ALP, AST, ALT, urea, and creatine. In excised livers, the following were determined: glycogen levels, glucose uptake and oxidation, protein levels of glucose transporter 2 (GLUT2), insulin receptor (IR-β), transcriptional factors and signaling molecules, enzyme activities, and gene expression.

DEHP-exposed male offspring showed significantly lower birth weight compared to the control, and body weight in DEHP-exposed male rats continued to be significantly and dose-dependently lower compared to control through PND 80. The fasting blood glucose level was significantly higher in the male offspring in all dose groups compared to the control group. Additionally, fasting serum insulin concentration was significantly and dose-dependently increased in all dose groups. Additionally, impaired glucose and insulin tolerances were observed in both dose groups compared to the control group. Specifically, after the glucose challenge, the blood glucose level was significantly and dose-dependently elevated at 1 hour, 2 hour, and 3 hours post-challenge in DEHP-exposed animals relative to control. After insulin injection, blood glucose level was significantly and dose-dependently elevated from 15 through 90 minutes post-injection in DEHP-exposed animals compared to control. Additionally, according to the HOMA-IR (homeostasis model assessment for insulin resistance), insulin resistance increased dose-dependently in DEHP-exposed rats compared to control. Glucose uptake and glucose oxidation by hepatic cells also significantly decreased dose-dependently at both doses.

Serum liver (AST, ALT, and ALP) and kidney (urea and creatinine) function markers increased significantly and dose-dependently in all dose groups compared to the control group. The hepatic glycogen concentration and activity of glycogen synthase were significantly and dose-dependently decreased in all dose groups. Both testosterone and estradiol levels were significantly and dose-dependently decreased in all dose groups.

Perinatal DEHP exposure also altered the expression of genes and proteins involved in insulin signaling in the liver. Specifically, cytosolic GLUT2 protein levels were significantly decreased, and plasma membrane expression of IR-β and IR-β ^{Tyr1162} in the liver were significantly and dose-dependently decreased in all dose groups compared to the control. mRNA expression of IR-β and GLUT2 were also significantly reduced in both dose groups. Insulin receptor substrate 1 (IRS1) expression and IRS1^{Tyr632} were also reduced significantly in both treatment groups. β-Arrestin was significantly decreased in all dose groups, whereas proto-oncogene tyrosine-protein kinase (c-Src) protein was significantly declined only in rats from the 100 mg/kg-day dose group compared to the control. Akt protein and Akt^{Ser473} were significantly and dose-dependently reduced in DEHP-exposed groups. Akt^{Thr308} was significantly reduced at 100 mg/kg-day, and no change was observed in Akt^{Tyr315}. Glycogen synthase kinase-3 beta (GSK3β) was significantly and dose-dependently increased and GSK3β^{Ser9} was significantly and dose-dependently decreased in DEHP-exposed rats compared to the control. Forkhead box 1 (FoxO1), a transcription factor that initiates the transcription of gluconeogenic enzymes, was significantly and dose-dependently increased in DEHP-exposed groups compared to control, whereas FoxO1^{Ser256} was significantly and dose-dependently decreased in DEHP-exposed rats compared to control. mRNA expression of two important enzymes involved in gluconeogenesis, glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK), was significantly and dose-dependently increased in DEHP-treated groups, along with increased activities of these enzymes. Consistently, binding of the transcription factor FoxO1 to the G-6-Pase and PEPCK promoters increased significantly and dose-dependently in DEHP-treated rats compared to control.

Fan et al. (2020**)** exposed female ICR mice (n = 6 per treatment) to 0, 0.2, 2, or 20 mg/kg-day DEHP via gavage for 28 days (7 days before parental mating through PND 0). In male offspring, food intake was measured from post-natal week (PNW) 5 to 11 and bodyweight was measured from PNW 1 to PNW 12. Fecal matter was collected from PNW 4 to 12 for gut microbiota profiling. At PNW 12, body composition and metabolic rate were measured using MRI and metabolic chambers. Male offspring were sacrificed at PNW 12, and the following endpoints were measured: plasma total cholesterol, triglycerides, HDL, LDL, and glucose; intraperitoneal glucose tolerance test (GTT), intraperitoneal insulin tolerance test (ITT); fat and liver tissue histology; RNA sequencing analysis of liver tissue; and metabolomic profiling of liver tissue.

Body weight increased significantly from PNW 5 to 12 in male offspring in the lowest dose group (0.2 mg/kg-day) and remained unchanged for the other dose groups. No differences were seen in offspring bodyweights for females. Food intake was not different between the groups. For all other endpoints, authors only report data from males in the low (0.02 mg/kg-day) dose and control groups.

According to MRI, fat mass was significantly higher in male offspring in the 0.02 mg/kg-day dose group relative to control. Consistently, histological analysis showed white adipocyte hypertrophy and increased lipid deposits in the liver. Energy expenditure was significantly lower compared to control. Expression of several thermogenic genes (uncoupling protein 1 [Ucp1], cell death-inducing DNA fragmentation factor-like effector A [CIDEA], and adrenoceptor Beta 3 [Adrb3]) in the brown fat pads was significantly lower than in the control group. Prenatal low-dose DEHP exposure significantly elevated the levels of total cholesterol, triglycerides, HDL, LDL, and glucose in plasma. Furthermore, 16S rDNA gene amplicon sequencing of fecal samples showed dysbiosis of the microbiota in DEHP-exposed males relative to control.

Blood glucose levels were elevated relative to control levels throughout the GTT, and overall glucose AUC was significantly higher in the prenatal DEHP low dose exposed group relative to control. Blood

glucose levels were also elevated in DEHP-treated males relative to control during the ITT; however, overall glucose AUC was not statistically significantly higher relative to control.

RNA sequencing showed that prenatal low-dose DEHP exposure induced significant transcriptional changes in 1,726 differentially expressed genes in the liver, which were enriched for metabolism-related pathways and thiamine transport. Additionally, low-dose DEHP exposure significantly altered levels of hepatic metabolites, including N-acetylglutamic acid, D-glucuronic acid, thiamine, and glucose 6-phosphate. Metabolic pathway analysis showed that ascorbate and aldarate metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and thiamine metabolism were among the top significantly enriched pathways. mRNA expression of two thiamine transporters, solute carrier family 2 member 2 (Slc2a2) and solute carrier family 19 member 2 (Slc19a2), significantly increased and decreased, respectively, in the livers of DEHP-exposed males. Slc19a3 expression was unaltered.

Zhang et al. (2017**)** exposed adult male SD rats (n = 10 per group) to 0, 0.05, 5 or 500 mg/kg/day DEHP via gavage for 15 weeks. Oral GTT and insulin tolerance tests (ITT) were performed at 3, 5, and 15 weeks of exposure. Rats were sacrificed after 15 weeks of exposure and evaluated for body weight, liver serum chemistry (ALT, AST, and ALP), and liver weight and histopathology. Oxidative stress (superoxide dismutase [SOD] activity and lipid peroxidation) and protein expression of insulin receptor (IR-β), glucose transporter 4 (GLUT4), and peroxisome proliferator-activated receptor gamma (PPARγ) were also evaluated in the liver.

Terminal body weights were significantly lower (9%) in the 500 mg/kg/day dose group relative to control. Serum AST (approximately 70%) and ALT (approximately 100%) significantly increased in the 500 mg/kg-day dose group, and serum ALP significantly increased in the 5 and 500 mg/kg-day dose groups (approximately 120 and 145%, respectively). Relative liver weight increased significantly (26 and 49%) at 5 and 500 mg/kg/day, respectively from controls. Histologically, the liver architecture was disrupted with disordered hepatocyte cord, accumulation of inflammatory factors and vacuolar degeneration in treated groups that progressed to central necrosis in the 500 mg/kg/day group (quantitative data were not reported). Protein expression of GLUT4 and insulin receptor in the liver decreased significantly in all dose groups, and PPARγ increased significantly and dose-dependently across all dose groups. SOD activity decreased and lipid peroxidation increased dose-dependently and reached significance at 5 and 500 mg/kg-day compared to control.

Statistics were not presented for glucose homeostasis endpoints. No differences were noted for fasting blood glucose and insulin levels prior to the GTT and ITT tests. After glucose challenge, serum glucose levels were higher in the 5 and 500 mg/kg/day groups at week 5 and in all exposed groups at week 15 relative to controls. Serum insulin levels after glucose challenge were decreased at week 3 and increased at week 5 and 15 in all dose groups, suggesting development of insulin resistance in the exposed groups.

Ding et al. (2019) exposed 3-week-old male ICR mice (10/group) to 0, 0.18, 1.8, 18 or 180 mg/kg-day DEHP dissolved in corn oil for three weeks. The authors did not specify whether animals were exposed to DEHP via gavage or feeding. Fasting blood glucose and body mass were measured once a week. After the 3-week treatment period, mice were sacrificed, and the following additional endpoints were evaluated: systolic blood pressure, diastolic blood pressure, heart rate, body weight, serum clinical chemistry, serum levels of insulin, C-peptide, glycated hemoglobin (HbA1c), total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, lecithin-cholesterol acyltransferase (LCAT), hypersensitive C-reactive protein (hs-CRP) and cardiac troponin 1 (cTn1), blood biochemistry, liver levels of glucose-6-phosphate dehydrogenase (G6PD) activity, glucokinase (GCK), insulin receptor (IR-β), glycogen, hepatic lipase (HL) and malondialdehyde (MDA) levels,

organ weight (heart, liver, spleen, lung, kidney, brain, and testes) and the expression of glucose transport and uptake-related proteins and cell growth-related proteins in the liver. In addition to normal mice, the authors also studied the effects of DEHP in a type 2 diabetes mellitus model (results not included here).

Body weight gain significantly increased in the 180 mg/kg-day group relative to control; however, no exposure-related changes in terminal body weight were observed. Additionally, no significant changes in absolute or relative organ weights (heart, liver, spleen, lung, kidney, brain, and testes) were observed. Heart rate (10%) and mean blood pressure (29%) significantly increased in the 180 mg/kg-day group, compared to control; however, systolic blood pressure (SBP) and diastolic blood pressure (DBP) did not change in any dose groups. Regarding blood biochemical indexes, serum ALT and ALP levels significantly increased at 180 mg/kg/day compared to control; however, no changes in serum uric acid, urea, creatinine, AST or total protein were seen at any dose of DEHP.

Fasting blood glucose levels increased dose-dependently and reached significance at 180 mg/kg/day. HbA1C levels increased dose-dependently and achieved significance beginning with the 1.8 mg/kg-day dose group. Insulin and C-peptide levels significantly increased at 1.8 and 18 mg/kg/day but were unchanged relative to control at 180 mg/kg-day. Hepatic glycogen and HOMA-IR (insulin resistance index) were not different at any dose compared to control. Additionally, liver G6PD activity and GCK levels significantly decreased at 180 mg/kg/day.

DEHP exposure additionally altered lipid metabolism as measured by increased total cholesterol (significant at 1.8 mg/kg-day and above), triglycerides, and LDL (dose-dependent and significant at180 mg/kg-day), and MDA (dose dependent and significant at 18 mg/kg-day and above). Additionally, DEHP exposure decreased LCAT and HDL levels (dose dependent and significant at 18 mg/kg-day and above) and HL levels (significant and dose-dependent at 0.18 mg/kg-day and above) compared to control. DEHP also increased levels of the cardiovascular markers hs-CRP (dose dependent and significant at 180 mg/kg-day) and cTn1 (dose dependent and significant at 18 mg/kg-day and above) compared to controls.

DEHP exposure altered the expression of genes related to glucose metabolism, lipid metabolism, and protein metabolism in the liver. Specifically, DEHP significantly decreased mRNA expression of solute carrier family 2 member 3 (Slc2a3) at 180 mg/kg-day, Acsl6 (dose-dependent) at 18 mg/kg-day and above, carnitine palmitoyltransferase 1C (Cpt1c) at 18 mg/kg-day and above, and protein kinase cAMP-dependent type II regulatory subunit beta (Prkar2b) at 18 mg/kg-day and above. DEHP increased voltage-dependent calcium channel subunit alpha 2 delta-2 (Cacna2d2) at 1.8 mg/kg-day and above. Ribosomal protein S6 kinase A6 (Rps6ka6) increased significantly at 0.18 and 18 mg/kg-day but remained unaltered compared to control at other doses.

DEHP exposure altered the expression of proteins related to glucose transport and uptake. IR-β and IRS-1 significantly decreased in the 180 mg/kg-day dose group. Phosphorylated IRS and phosphorylated Phosphatidylinositol 3-kinase (PI3K) significantly increased in all dose groups, and these changes were dose-dependent in the case of phosphorylated PI3K. PI3K and AKT expression did not change compared to control. Phosphorylated AKT expression significantly increased in the 18 and 180 mg/kg-day dose groups, while GLUT4 expression significantly decreased in these dose groups.

Additionally, DEHP altered expression of proteins related to glycogen, fatty acid, and protein synthesis. Specifically, although GSK-3β expression was unaltered relative to control, phosphorylated GSK-3β increased significantly in the 18 and 180 mg/kg-day dose groups. mTOR expression was also unaltered relative to control, whereas phosphorylated mTOR increased in the 1.8 and 180 mg/kg-day dose groups.

Finally, DEHP exposure altered proteins related to cell growth. Specifically, SHC increased significantly starting at 1.8 mg/kg-day and phosphorylated SHC increased significantly and dose-dependently beginning at 18 mg/kg-day. While extracellular signal-regulated kinase (ERK) 1/2 expression was unaltered relative to control, phosphorylated ERK1/2 increased dose-dependently and reached significance beginning at 18 mg/kg-day.

Li et al. (2018**)** exposed five to 6-week-old male C57BL/6 mice (17 per group) to 0, 1, 10, 100 or 300 mg/kg/day DEHP in 5 percent PEG via oral gavage daily for 35 days. The following endpoints were measured post-treatment: terminal body weight, blood biochemistry (ALT, glucose, creatinine, total cholesterol, thyroxine [T4], triglycerides , and cholinesterase), heart weight, and heart histopathology. In the heart, mitochondria and cytosol ATP synthase activity, enzyme activity (acyl coenzyme A synthetase, carnitine palmitoyl transferase-1, pyruvate dehydrogenase, interleukin-1b, citrate synthase and lactate dehydrogenase), metabolomic profiling, and expression of genes involved in metabolism of fatty acids, glycolysis, and the TCA cycle were also measured.

Terminal body weights were significantly decreased by 9 percent in the 100 and 300 mg/kg/day groups compared to control. Plasma ALT and triglycerides (at 1 mg/kg-day and higher), cholinesterase (10 mg/kg-day and above), total cholesterol and T4 (100 mg/kg-day and above) and glucose and creatinine (300 mg/kg/day) were significantly increased over controls. Relative (to body weight) heart weight was dose-dependently increased by 10 to 14 percent over controls at 10 mg/kg-day and above. Histologically, lipid droplets were present in cardiac papillary muscle cells at doses 100 mg/kg-day and above (reported qualitatively). Na+-K+ ATPase and Ca2+-Mg2+-ATPase activities in cardiomyocytes were significantly increased in the cytosol and significantly decreased in the mitochondria at 100 mg/kg-day and above compared to control. Additionally, DEHP exposure also altered the activity of cardiac enzymes. Specifically, CPT1 activity decreased (100 mg/kg-day and above), PDH activity increased (starting at 1 mg/kg-day), and IL-1β increased (300 mg/kg-day). LDH activity decreased at all doses; however, there was no clear dose-response relationship. ACS and CS enzyme activities did not change significantly in any groups compared to control. DEHP exposure significantly increased the mRNA expression of genes related to the metabolism of fatty acids (cluster of differentiation 36 [CD36], PPARα, Ucp3, Acyl-CoA thioesterase 2 [Acot2], fatty acid binding protein 3 [Fabp3], acyl-CoA Oxidase 2 [Acox2], acyl-CoA Synthetase Long Chain 1 [Acs1], acyl-coenzyme A synthetase [Acsm], long-chain fatty acid transport protein 6 [Slc27a6], glycerol-3-phosphate dehydrogenase 2 [Gpd2]) and genes related to glycolysis and the TCA cycle (citrate synthase [Cs], hexokinase 2 [HK2], Glut4, glyceraldehyde-3-phosphate dehydrogenase [GAPDH], Enolase 1 [Eno1], protein kinase [Pk], dihydrolipoamide acetyltransferase [Dlat], dihydrolipoamide dehydrogenase [Dld], pyruvate dehydrogenase [Pdhb], and phosphoglycerate kinase 2 [Pgk2]), although statistical significance differed depending on the dose. DEHP exposure did not alter carnitine palmitoyltransferase I (Cpt1) or phosphoenolpyruvate carboxykinase 1 (Pck1) expression. Additionally, metabolomic profiling revealed that DEHP alters endogenous metabolites and metabolic pathways involved in fatty acid and glucose metabolism in cardiomyocytes at all doses. The authors concluded that DEHP inhibits β-oxidation of fatty acids and gluconeogenesis in cardiomyocytes, promotes glycolysis, and inhibits the TCA cycle and interferes with the synthesis and transport of fatty acids in mitochondria which inhibits the synthesis and metabolism of ATP.

B.3 Summaries of Other Hazard Studies of DEHP

B.3.1 Cardiovascular and Kidney Toxicity Study Summaries

In a cardiovascular toxicity study by **Deng et al. (**2019**),** C57/BL6 male mice (n = 8) were gavaged with DEHP in saline for 6 weeks at concentrations of 0 (control), 0.1, 1, or 10 mg/kg-day in addition to an angiotensin converting enzyme inhibitor (ACEI) group and a group dosed with 10 mg/kg-day DEHP and ACEI. Blood pressure, heart rate, immunohistochemistry, and tissue histopathology were measured following the treatment period. The study authors reported that systolic blood pressure and heart rate at 10 mg/kg-day were significantly increased over saline controls, with systolic blood pressure increased by 22 percent at 10 mg/kg-day (133.87 ± 2.2 mmHg) compared to the controls (109.7 ± 2.9 mmHg) and heart rate increased by 20 percent at 10 mg/kg-day (612.1 ± 20.67 beats/min) compared to controls (486.75 ± 30.69 beats/min). Although not described in the text, the bar graph depicting these results in Figure 2 of the publication indicated that systolic blood pressure was also significantly increased over saline controls at 0.1 and 1 mg/kg-day, and heart rate was significantly increased at 1 mg/kg-day. Systolic blood pressure and heart rate in the ACEI group and in the group co-treated with ACEI and 10 mg/kg-day DEHP were comparable to saline controls. Additionally, there was a dose-dependent significant increase in ventricular wall thickness in all groups treated with DEHP (0.1 mg/kg-day and above); and, as was observed with heart rate and systolic blood pressure, co-treatment with ACEI and 10 mg/kg-day DEHP resulted in ventricular wall thickness comparable to saline controls. Levels of ACE in heart tissue were dose-dependently and significantly increased over controls starting at 1 mg/kg-day, and co-treatment with ACEI returned ACE to levels comparable to saline controls. Bradykinin levels were significantly decreased at 1 and 10 mg/kg-day compared to saline controls, but co-treatment with ACEI and 10 mg/kg-day DEHP was comparable to treatment with 10 mg/kg-day DEHP alone, indicating that ACEI did not prevent decreased bradykinin. Immunohistochemistry of heart tissue indicated significant decreases in the optical density of BK2R at 1 and 10 mg/kg-day DEHP and in eNOS starting at 1 mg/kg-day; co-treatment with ACEI and 10 mg/kg-day DEHP resulted in BK2R comparable to saline controls but did not fully restore eNOS to control levels. Calcium levels in cytoplasm in heart tissue was significantly decreased at 1 and 10 mg/kg-day DEHP, and serum NO levels were dose-dependently and significantly decreased starting at 0.1 mg/kg-day; co-treatment with ACEI and DEHP resulted in levels of cytoplasm calcium and serum NO comparable to saline controls. The investigators concluded that these data indicate that DEHP may increase blood pressure by activating ACE levels and inhibiting the bradykinin-NO pathway, resulting in increased systolic blood pressure and heart rate and ventricular wall thickening.

A study conducted by **Kamijo et al. (**2007**)** PPAR-null and wild-type mice (n = 20–34/group) were administered DEHP in the diet at concentrations of 0, 100, or 500 ppm (equivalent to 0, 9.5, and 48.5 mg/kg-day, estimated based on food consumption rate of 3.1 g/day) for 22 months. Clinical parameters were examined at 0, 6, 12, and 22 months and included systolic blood pressure and serum levels of MEHP, urea nitrogen, and creatinine. At study termination, organ weights were determined, and the kidneys were evaluated microscopically. Systolic blood pressure was significantly increased over controls at starting at 100 ppm in the PPAR-null mice at 12 and 22 months and to a lesser extent in the wild-type mice but only at 22 months. Total urine protein excretion (mg/day) was significantly increased over controls at 100 ppm and above in PPAR-null mice and to a lesser extent in the wild-type mice at 12 and 22 months. Serum urea nitrogen and creatinine were significantly increased over controls starting at 100 ppm but only in PPAR-null mice at 22 months. Histopathology analyses of the glomeruli indicated a dose dependent increase at 100 ppm and above in cell proliferation and mesangial expansion in the glomeruli of wild-type mice and to a lesser extent in PPAR-null mice. Body weights and weights of the kidneys and testes in the

treated wild-type and PPAR-null mice were comparable to controls. However, liver weights were decreased in a dose-dependent manner at 100 ppm and above in wild-type mice at 22-months.

The study authors reported that approximately 25 percent of PPAR-null mice that were exposed to 500 ppm DEHP had inflammatory findings in the glomeruli, including mesangiolysis, mesangial edema, crescent formation, and macrophage infiltration; however, no quantitative data were provided. Immunoblot analyses of the glomeruli indicated significant dose-dependent increases in α-smooth muscle actin (α-SMA), proliferating cell nuclear antigen (PCNA), TGFβ1, and 4-HNE proteins at 100 ppm and above in the PPAR-null mice and to a lesser extent in wild-type mice. Additionally, in PPAR-null mice, DEHP induced oxidative stress in the glomeruli, as indicated by presence of 4HNE-modified proteins, 8-OHdG, and NADPH oxidase subunits, Nox4 and p47phox, in a dose-dependent manner. From these data, authors conclude that PPAR-alpha is protective of the nephrotoxic effects of chronic DEHP exposure.

In a study on cardiovascular toxicity by **Xie et al. (**2019**),** C57BL/6 male mice were gavaged with 0 (saline), 0.1, 1, or 10 mg/kg-day (n = 9/group) of DEHP for 45 days to determine the effect of DEHP on high blood pressure and the underlying mechanisms. Additional groups included mice injected with estradiol receptor inhibitor ICI182780, angiotensin converting enzyme inhibitor (ACEI) (Enalapril Maleate), estradiol receptor inhibitor + 10 mg/kg-day DEHP, and ACEI + 10 mg/kg/-day DEHP. Following 42 days of exposure, blood pressure was measured; however, after the 45 days, authors measured aortic vessel and kidney histopathology, immunohistochemical expression of ACE, Angiotensin II (AngII) and Angiotensin Type 1 Receptor (AT1R), estradiol levels, intracellular eNOS, and nitric oxide. Mean blood pressure and systolic blood pressure were significantly increased over saline controls in all DEHP-treated groups (0.1 mg/kg-day and above), and diastolic blood pressure was significantly increased over controls at 1 mg/kg-day and above. Vascular wall thickness of the aorta was significantly increased in all DEHP-treated groups (0.1 mg/kg-day and above), and the smooth muscle cells of the vascular artery wall were reported to be hypertrophied and disordered. DEHP significantly increased expression of ACE, AngII, and AT1R. Authors measured kidney histopathology in these mice following DEHP exposure and found evidence of hypertensive renal injury and immune cell infiltration around the blood vessels and glomeruli starting at 1 mg/kg/day; however, no quantitative data were provided. In contrast, estradiol levels were not altered with DEHP exposure when compared to the saline control group. Moreover, there was no change in blood pressure measurements between the 10 mg/kg-day DEHP alone group and with the estradiol receptor inhibitor. The authors reported significant decreases in eNOS expression in the aortas of mice exposed to increasing DEHP concentrations when compared to the control group. These results indicate that DEHP increases blood pressure in mice through the renin-angiotensin-aldosterone system (RAAS) in mice.

In a nephrotoxicity study by **Wei et al. (**2012**)** pregnant Wistar rats (30/group) were administered DEHP in corn oil at 0, 0.25, or 6.25 mg/kg-day via oral gavage daily from GD 0 to LD 21. Blood pressure, renal histopathology and function, and renal development gene expression were measured in the offspring. There were no effects of treatment on maternal body weights during lactation or on litter size, or sex ratio of offspring. Offspring body weights were significantly decreased at 0.25 mg/kg-day only at PND 21 but were significantly decreased at 6.25 mg/kg-day at all time points reported: birth (PND 0); weaning (PND 21); 15 weeks; and 21 weeks in both sexes. Absolute kidney weight at 6.25 mg/kg-day was significantly decreased in the females at 15 weeks but increased in the males at 21 weeks. Relative (to body weight) kidney weights were significantly increased at PND 0 and PND 21 in the combined sexes, and at 15 and 21 weeks in the males. At 15 and 21 weeks, systolic and diastolic blood pressure in the DEHP-treated groups was comparable to controls.

However at 33 weeks, systolic blood pressure at 0.25 and 6.25 mg/kg-day was significantly higher than controls, and diastolic blood pressure was elevated in the 0.25 mg/kg-day group, although diastolic blood pressure in the offspring at 6.25 mg/kg-day was comparable to controls. Heart rate was decreased in the 6.25 mg/kg-day males at 15 and 21 weeks but was comparable to controls at 33 weeks and in the females at all time points. When measuring renal development, authors reported the renal cortex appearing thinner and higher proportion of the cortex in the nephrogenic zone in each DEHP dose group. The number of glomeruli per kidney was significantly lower in the 0.25 and 6.25mg/kg-day males and females at PND21 and Week 33. The mean individual glomerular volume was significantly increased in the males at 0.25 and 6.25 mg/kg-day at PND21 and Week 33, but only increased in the females at 6.25 mg/kg-day at PND21. The total glomerular volume was significantly decreased in the 0.25 and 6.25 mg/kg-day males and females at Week 33. From birth to the conclusion of the study, both DEHP groups had decreased glomerular size, swelling, and reduction in Bowman's capsule. In renal function measurements at Week 21: creatinine clearance was significantly decreased in the 0.25 and 6.25 mg/kg-day males and females; serum urea nitrogen was significantly increased in the 0.25 and 6.25 mg/kg-day males; and urinary total protein was significantly increased in the 0.25 and 6.25 mg/kg-day females and the 6.25 mg/kg-day males. Intrarenal AngII expression was decreased in offspring exposed to DEHP-6.25 at birth; whereas intrarenal renin expression is significantly increased in the offspring at 0.25 mg/kg-day, but not at 6.25 mg/kg-day. Authors measured serum levels of renin angiotensin system (RAS), endothelin-1 (ET-1), and NO at 21 weeks. DEHP exposure did not induce any alterations in RAS or ET-1, but significantly reduced NO levels at 0.25 and 6.25 mg/kg-day. PPARα was higher than controls at 6.25 mg/kg-day at birth and at 0.25 and 6.25 mg/kg-day at weaning. Nephron pathway related genes (Foxd4, Gdnf, Pax2, and Wnt1) showed significantly decreased expression at 0.25 and 6.25 mg/kg-day, while nephron structure related genes: (Cdh11, Calm1, and Ywhab) were increased. These data indicate that gestational DEHP exposure causes may affect renal development and increase blood pressure later in life in rats.

B.3.2 Immunotoxicity Study Summaries

In an allergic asthma model study by **Guo et al. (**2012**)** to test whether DEHP has adjuvant effects, Balb/c mice were gavaged with 0 (saline), 0.03, 0.3, or 3 mg/kg-day of DEHP with and without subcutaneous injections of ovalbumin (OVA) for 52 days (n = 8/group). OVA was used as the sensitizer for this allergic asthma model. To evaluate whether long term DEHP exposure on pulmonary inflammation and immune response, authors measured airway hyperresponsiveness, immune cells in BALF, serum IgE, and cytokine levels in lung tissue. DEHP exposure alone did not increase airway hyperresponsiveness; however, OVA+DEHP caused significant airway resistance when compared to the OVA alone group. In the OVA+DEHP 3 mg/kg-day group, there was high resistance and low compliance. Authors stated that the highest dose of DEHP and OVA promoted airway hyperresponsiveness. The ratio of eosinophils to total cells in BALF did not significantly change with DEHP alone. However, this ratio is significantly higher in mice exposed to both OVA and DEHP at all concentrations when compared to the saline control group. Serum total IgE levels were not altered in the DEHP only exposed groups, but with OVA added to any dose of DEHP, the serum total IgE was significantly increased by 80 percent over saline controls. When measuring cytokines in lung tissue, the levels of Th1 cytokine, IFNγ, were not affected by OVA only, but the highest dose of DEHP+OVA significantly increased its levels. Further, levels of IL-4, a Th2 cytokine, were significantly increased in all DEHP+OVA treatment groups when compared to the saline controls. However, only the highest DEHP dose+OVA induced a significant increase in IL-4 when compared to the OVA only group. Similarly, the IFNγ/IL-4 ratio was significantly increased in all DEHP+OVA treatment groups compared to the saline controls. The highest dose of DEHP+OVA

showed the greatest increase in the IFNγ/IL-4 ratio when compared to the OVA only group. These data indicate that DEHP promotes and potentiates allergic asthma by adjuvant effect.

In an immunotoxicity study by **Han et al. (**2014b**),** weanling BALB/c mice were divided into 8 groups (8 per group) and administered 30, 300, or 3,000 µg/kg DEHP with OVA (sensitizer) or saline for 28 days. Authors measured serum OVA-specific immunoglobulin, germinal center formation in the spleen, lymphocyte surface markers and nuclear transcription factors, and intracellular cytokines and both gene and protein expression in T follicular helper (Tfh) cells. DEHP treatment alone did not increase serum OVA-specific immunoglobulin levels; however, with OVA sensitization, DEHP treatment induced significant increases of 45 to 75 percent in serum IgE and IgG1 levels when compared to the corn oil+OVA control group. Similarly, when measuring germinal center formation using immunofluorescence, DEHP treatment alone did not elicit any germinal center reactions, but in mice at 300 µg/kg and above, there was a significant increase in mean fluorescence intensity of PNA+ germinal center when compared to the corn oil+OVA control group. Using flow cytometry to test the humoral immune response, authors revealed DEHP treatment alone did not stimulate an increase in cell quantity of Tfh and plasma cells. In the OVA sensitized mice treated with DEHP, authors reported that DEHP stimulates "the expansion of CD4+CXCR5+ICOS +/CD4+CXCR5+PD-1+Tfh cells and CD19+CD138+GL7+plasma cells." To further elucidate why there was an altered humoral immune response, investigators conducted "an adoptive transfer of mixed Th cells and B cells from either DEHP-exposed or normal mice into SCID mice." There was a significant increase in IgE and IgG1 antibody production when Tfh cells or B cells from DEHP treated mice were co-transferred with B cells from normal or DEHP treated mice when compared to the control group. Further, IL-4 and IL-21 were significantly increased in Tfh cells from mice exposed to DEHP and sensitized with OVA when compared to the corn oil OVA control group. Gene expression and protein production of Bcl-6 and c-Maf, genes and proteins related to Tfh differentiation, were measured. OVA sensitized animals treated with DEHP had significant increases in both mRNA and protein expression of Bcl-6 and c-Maf when compared to the corn oil OVA control group. Altogether, these data indicate that DEHP acts as an adjuvant when administered via oral gavage by inducing toxic effects in Tfh cells.

In an asthma-like OVA-immunized rat model study by **Yang et al. (**2008**),** male Wistar rats were divided into 5 groups (n = 8/group): saline (control), ovalbumin (OVA), DEHP 0.7mg/kg-day+OVA, DEHP 70 mg/kg-day+OVA, and DEHP 70 mg/kg-day. To test whether DEHP has an adjuvant effect on OVA-immunized rats, animals were given DEHP by oral gavage for 30 days. On days 19 to 27 of the exposure duration, rats were given a hypodermal injection of saline or OVA (1 mg). On days 31 to 37 animals were exposed to either aerosolized saline or OVA. Authors measured airway hyperresponsiveness (AHR), BAL cell counts, and lung histology. Results show OVA alone induced AHR, and DEHP significantly increased AHR in OVA-immunized rats in a dose dependent manner. DEHP alone caused a slightly higher AHR when compared to the negative control groups, but it was lower than the DEHP+OVA groups. Histological examination of lungs revealed OVA induced increased mucus secretion, inflammatory cells infiltration, and airway wall thickness. DEHP was shown to aggravate these effects in OVA-immunized mice, but DEHP alone did not cause any alteration in these animals. Lastly, OVA exposed animals had significantly increased eosinophils in the BAL, an indicator or allergic asthma. Further, DEHP exposure in OVA-immunized mice significantly increased total cell counts and eosinophils in a dose dependent manner. In contrast, DEHP alone did not cause any significant differences in the BAL cell counts when compared to the control group. These data indicate DEHP acts as an adjuvant in an OVA-immunized asthma rat model by as indicated by aggravated AHR and effects on lung histology.

B.3.3 Neurotoxicity Study Summaries

In a neurobehavioral study by **Barakat et al. (**2018**),** pregnant CD-1 mice (n = 4–7/group) were administered DEHP in corn oil at 0, 0.2, 500, or 750 mg/kg-day from GD 11 to PND 0 (birth) to investigate the effects of prenatal exposure on neurobehavior and recognition memory in male offspring, including examination of the possible mechanism of oxidative damage in the hippocampus. Neurobehavioral parameters were measured in the offspring at ages of 16 to 22 months. Elevated plus maze (EPM) and open field tests (OFT) were used to measure anxiety levels. Y-maze and novel object recognition (NOR) tests were employed to measure memory function. Authors also measured serum levels of testosterone, brain weight, and collected tissue for histology and immunohistochemistry (IHC). Oxidative damage in the hippocampus was measured by the levels of oxidative DNA damage and by spatial light interference microscopic counting of hippocampal neurons. In the OFT, mice exposed to DEHP tended to take more time to go to the center region when compared to controls, but this difference did not reach statistical significance. However, all DEHP-treated groups (0.2 mg/kg-day and above) had significantly lower number of entries into the central region, although the decreases were not dose-dependent. The EPM test showed that mice in the 750 mg/kg-day DEHP group took significantly more time before making entries into open arms, which the authors attributed to increased anxiety. Prenatal DEHP exposure did not change the numbers of entries into the open arms or the time spent in open arms.

In the Y-maze test, mice exposed to the lowest dose (0.2 mg/kg-day) displayed significantly lower alteration behavior (*i.e.*, rather than entering the next arm, these animals tended to enter the arm just visited) and had significantly fewer arm entries, which the authors attributed to impaired spatial memory or locomotion. However, these findings were unrelated to dose, in that the percent of alternation and number of arm entries at 500 and 750 mg/kg-day were comparable to controls. During the NOR test, mice prenatally exposed to 500 and 750 mg/kg-day displayed significantly less time (seconds) exploring the new object when compared to the control group, which the authors attributed to impaired short-term recognition memory. However, when expressed as a percentage of time spent exploring objects (new object + past object), the treated groups were comparable to controls; therefore, EPA considers it is plausible that the offspring at 500 and 750 mg/kg-day spent less time exploring objects in general.

Histological examination of the hippocampus of offspring at 22 months old indicated that mice exposed to 0.2 and 750 mg/kg-day DEHP during gestation had significantly fewer pyramidal neurons in CA1 and CA2/3 subregions of the hippocampus, indicated by manual counting following Nissl and hematoxylin and eosin staining; however, this finding was not dose-related, given that the number of neurons in these regions of the hippocampus at 500 mg/kg-day was comparable to controls. Using computerized microscopy (SLIM) on the hippocampus, the number of pyramidal neurons in the different regions of the hippocampus were comparable to controls at 0.2 mg/kg-day but were significantly lower than controls in the dentate gyrus (DG) and CA2 region at 500 and 750 mg/kg-day and in CA1 region at 750 mg/kg-day. IHC indicated DEHP exposure increased COX-2 immunoreactivity in subregions CA2 and CA3 in the mice, with significantly higher COX-2 positive neurons in the 0.2 and 750 mg/kg-day groups compared to the controls, which the authors attributed to neuronal inflammation in these subregions of the hippocampus. Again, these differences were not dose-related, in the percent of COX-2 positive neurons at 500 mg/kg-day was comparable to controls. The study authors reported that the mean brain weight of DEHP-treated mice was lower than controls; however, these decreases were not dose-dependent or statistically significant. Serum testosterone was significantly decreased in the 500 and 750 mg/kg-day male offspring.

The study authors reported that DEHP-treated mice has a "remarkably decreased AR expression in the pyramidal neurons" in the brain of the offspring at 750 mg/kg-day; however, they acknowledged that this assertion was based on visual observation of the immunohistochemistry and that quantitative measurements of AR expression were not conducted. The study authors reported that prenatal DEHP exposure in mice resulted in stronger immunostaining for OHdG and TG (DNA oxidation markers) compared to controls, with increased OHdG in regions CA2, CA3, and DG, and increased TG in CA2 and DG. However, these data were only reported qualitatively in text and presented as representative micrographs in figures.

In a neurotoxicity study by **Feng et al. (**2020**),** pubertal normal (P-normal) and pubertal type 2 diabetes mellitus (P-T2DM) ICR mice (n = 10/group) were administered DEHP in corn oil at 0, 0.18, 1.8, 18 and 180 mg/kg-day via oral gavage daily for 3 weeks. To test neurobehavioral effects, authors conducted an open field test (OFT) and Morris water maze test (MWM). At study termination, the animals were killed, and the brain was weighed, and enzyme activity of superoxide dismutase (SOD), acetylcholinesterase (AChE), and glutathione peroxidase (GSH-Px) were measured, along with gene expression of *Slc6a4, Tph2*, *Fgf17*, *Gabrr1*, *Avp* and *Pax8* (related to regulating serotonergic synapses, GABAergic synapses, phospholipase D, and thyroid hormone synthesis) by RT-PCR, protein expression by Western blot, and determination of levels of the neurotransmitters 5-hydroxytyptamine (5-HT) and γ-aminobutyric acid (GABA) and Ca^{2+} and cAMP by ELISA. Additionally, select other organs were weighed, including heart, liver, spleen, lungs, and kidneys.

In the OFT, normal mice had: significant decreases in clockwise rotation count at 1.8 mg/kg-day and above and in total distance at 18 mg/kg-day and above; and significantly increased time in the central area at 1.8 mg/kg-day and above. P-T2DM mice exhibited the same changes in these parameters, including in the controls, compared to normal mice, with significant differences compared to the P-T2DM controls at: 1.8 mg/kg-day and above for decreased total distance; 0.18 mg/kg-day and above for decreased clockwise rotation; and increased time in central area at 18 mg/kg-day and above. For the MWM test, in the learning phase of the test, a significant decrease in swimming speed and a significant increase in latency in locating the platform were observed in the P-normal mice exposed to DEHP, P-T2DM control group, and P-T2DM mice exposed to any dose of DEHP when compared to the P-normal control group. DEHP exposed P-T2DM mice had the most pronounced effects out of all the groups, with authors suggesting DEHP may impair locomotion and learning of mice. During the memory phase of the test (*e.g.*, referred to as space exploration in the study report), decreases in swimming speed, time (stops) in the original platform quadrant, and residence time in the target quadrant were all decreased at 0.18 mg/kg-day and above DEHP in both normal and P-T2DM mice, with the DEHP exposed P-T2DM mice having the most dramatic decreases. The authors suggested that these data indicate DEHP impairs spatial learning and memory.

Real time PCR data revealed significant reductions in Slc6a4, Tph2, Gabrr1 and Pax8 when compared to the P-normal control group. In contrast, there was significantly increased expression of Avp and Fgf17 in the P-T2DM control group and at all doses of DEHP. When measuring enzyme activity in the brains of these mice, decreases were observed in AChE and SOD in normal mice treated with DEHP at 0.18 mg/kg-day and above and in GSH-Px at 1.8 mg/kg-day and above compared to normal controls. AChE, GSH-Px, and SOD in the P-T2DM control group were lower than normal controls, with GSH-Px and SOD in P-T2DM mice at 1.8 mg/kg-day and above lower than P-T2DM controls and AChE in P-T2DM mice at 18 mg/kg-day and above lower than P-T2DM controls. Similarly, all mice exposed to DEHP had significantly reduced neurotransmitters 5-HT and GABA when compared to the P-normal control group. Furthermore, the P-T2DM groups exposed to DEHP had even more pronounced significant decreases in both 5-HT and GABA content when

compared to the P-T2DM control group. Brain calcium content was significantly increased in the P-T2DM control group and in all DEHP-treated groups, with P-T2DM exposed mice having a more significant increase. Additionally, cAMP levels in brain tissue were significantly reduced in P-T2DM mice and all DEHP administered groups when compared to the P-normal control group. This already significant reduction was exacerbated in P-T2DM mice at 18 and 180 mg/kg-day when compared to the P-Normal mice at the same doses. When measuring protein expression of the calcium signaling pathway, authors reported that DEHP exposure did not alter the total protein expression of CaMKII but did significantly increase protein expression of CaM and p-CaMKII in both P-normal and P-T2DM mice groups at 1.8 mg/kg-day and above when compared to the P-normal control group. Likewise, P-T2DM mice exposed to DEHP had a more significant increase in CaM and p-CaMKII levels at 1.8 mg/kg-day and above. When authors evaluated GPCRs–cAMP–PKA–ERK–CREB signaling pathway, both unphosphorylated and phosphorylated PKA, ERK1/2 and CREB protein expression significantly decreased with increasing doses of DEHP when compared to P-normal controls. These changes in expression were more noticeable in the P-T2DM. Relative (to body weight) testes weights were significantly decreased at 180 mg/kg-day in P-normal mice. Overall, any adverse effects were potentiated in P-T2DM mice exposed to DEHP, suggesting that these mice are more sensitive to the effects of DEHP in this study. The study authors concluded that these data indicate DEHP causes neurotoxicity via cAMP–PKA–ERK1/2–CREB signaling pathway and calcium signaling.

In a neurotoxicity study by **Tanida et al. (**2009**),** pregnant ICR mice (6 or 7 per group) were orally treated with 1 mg/kg-day of DEHP from PND 3 to 7 in the sole administration experiment. In the mixed administration experiment, BPA (5 mg/kg-day) and DEHP (1 mg/kg-day) were co-administered to male pups. At 2-, 4-, and 6-weeks post-birth, authors measured body and brain weights, conducted immunohistochemistry on TH-ir, Fos-ir, and Double-ir cells in the midbrain. Body weight was significantly decreased by 6 to 9 percent at all time points when compared to the controls. At 6 weeks, absolute brain weight was significantly reduced compared to controls, and relative brain weight was significantly decreased at 2- and 4-weeks post birth. Immunohistochemistry findings in the mouse midbrains dopaminergic nuclei revealed TH-immunoreactivity (rate-limiting step of dopamine synthesis) in the perikarya of the neurons, and within axons and dendrites. When authors measured TH-immunoreactivity intensity in the A8 , A9, and A10 sections of the midbrain dopaminergic nuclei, sole DEHP administration was shown to decrease TH-immunoreactivity intensity in A9 at 2 weeks and 6 weeks, with intensity being 50 to 80 percent of controls. Additionally, at 6 weeks, DEHP exposure caused A10 to have only 20 to 50 percent of the intensity compared to controls. A8 exhibited TH-immunoreactivity intensity at 50 to 80 percent of controls at 2 weeks and 6 weeks, indicating decreased activity of dopaminergic neurons. The number of TH-ir neurons was significantly decreased following sole DEHP administration in the A9 area at week 6. Following DEHP administration, from 2 to 4 weeks, the mean number of Fos-ir perikarya were increased, then decreased from 4 to 6 weeks. In A8, DEHP exposure significantly reduced the number of Fos-ir neurons at 4 weeks when compared to the control group. Altogether, these data indicate that DEHP alone can cause irreversible changes in neurodevelopment and decrease function in midbrain dopaminergic neurons.

B.3.4 Musculoskeletal Toxicity Study Summaries

In a study by **Chui et al. (**2018**),** ICR (CD-1) mice were treated with 0 (corn oil), 1, 10, or 100 mg/kg-day (n = 12/group) of DEHP by oral gavage for 8 weeks for *in vivo* studies. Next, harvested bone marrow stromal cells (BMSCs) from untreated and DEHP-treated mice were isolated and treated with 0, 10, 25, 50, 100 or 125 mM of DEHP or 0, 5, 10, 25, 50, or 100 mM of DEHP's major

metabolite, MEHP, to conduct *in vitro* studies. BMSCs were cultured in osteo-blast differentiation medium with or without DEHP or MEHP (0 to 100 mM) for 7, 14 or 21 days. DEHP and MEHP treatment significantly and dose-dependently inhibited osteoblast mineralization (25 µM and above for DEHP and 10 µM and above for MEHP) at day 21 and alkaline phosphatase (ALP) activity at day 7. Additionally, BMSCs treated with 10 or 100 µM of DEHP or MEHP had significant decreases in expression of osteogenic genes Runx2, ALP, and OCN when compared to the controls. Similarly, Wnt-1 and β-catenin gene expression was significantly decreased following treatment with either 10 or 100 µM of DEHP or MEHP; in contrast, both DEHP and MEHP significantly increased the ratios of phosphorylated β-catenin and β-catenin in BMSCs during osteoblast differentiation. DEHP and MEHP upregulated Erα protein expression as well. Further, when measuring adipogenesis in BMSCs, DEHP did not alter adipogenesis; however, MEHP treatment (1, 5, and 10 mM) significantly and dose-dependently increased adipocyte differentiation and PPARγ during adipogenesis when compared to control cells. BMSCs had significantly increased adipocyte differentiation from 1, 10, and 100 mg/kg DEHP-treated mice when compared to the controls. Similarly, PPARγ mRNA expression was significantly increased in harvested BMSCs from DEHP treated mice when compared to the controls. ALP activity and mineralization was significantly decreased in BMSCs isolated from mice exposed to 10 and 100 mg/kg of DEHP. Likewise, Runx2, Wnt1, and β-catenin mRNA expression significantly decreased in BMSCs from DEHP treated mice at the same concentrations. There were no changes in body weights in mice exposed to DEHP for 8 weeks; however, liver to body ratio was significantly increased in mice exposed to 10 and 100 mg/kg-day. When measuring bone microstructure and bone morphometric parameters from mice exposed to 10 or 100 mg/kg-day DEHP for 8 weeks, authors reported significant decreases in bone mineral density, bone volume density (BV/TV) of trabecular bone (decreased 17%), thickness, and the number of trabecular bones when compared to the control group. In contrast, DEHP treatment did not alter trabecular separation, nor have an effect cortical bone mineral density and other microstructure parameters. The study authors concluded that these data indicate that DEHP and MEHP inhibit osteoblastogenesis, promote adipogenesis in BMSCs, and negatively alter bone microstructure possibly through the Wnt/β-catenin and PPARγ pathways.

B.4 Summaries of Inhalation Studies for DEHP

In an inhalation study by **Kurahashi et al. (**2005**),** prepubertal (28-day old) male Wistar rats were exposed to 0 (control), 5, or 25 mg/m^3 of DEHP (12 per group) 6 hours per day, 5 days per week for up to 8 weeks. Six rats per concentration were terminated after 4 weeks of exposure, and the remaining 6 rats per concentration were terminated after 8 weeks of exposure. Test atmosphere concentrations in the exposure chambers were measured daily by gas chromatograph and averaged 5.1 ± 1.3 mg/m^3 and 24.6 ± 5.2 mg/m^3 for the 5 and 25 mg/m^3 groups, respectively. Plasma was collected to measure testosterone, FSH, and LH after 4 and 8 weeks of exposure. Similarly, body weight, testes, seminal vesicles, epididymis, and ventral prostate were weighed at 4 and 8 weeks. One testis was used for histopathology and measuring expression of steroidogenesis genes. Relative (to body weight) seminal vesicle weights were significantly increased by 30 to 31 percent over controls in the 5 and 25 mg/m^3 animals at 8 weeks. Absolute seminal vesicle weights were not reported; however, body weights comparable to controls. Serum testosterone in the 5 and 25 mg/m^3 groups were significantly increased over controls at 8 weeks. Serum testosterone was also increased over controls at 4 weeks, with significant increase at 5 mg/m^3, although the increase at 25 mg/m^3 was not significant at this time point. There were no treatment-related effects on body weight; weights of testes, epididymis, or ventral prostate; plasma FSH or LH; gene expression of enzymes involved in testosterone biosynthesis (P450scc, 3β-HSD, CYP17, and CYP19), or testes histopathology. When measuring histopathology of the testis, germ cell degeneration was the main effect following any DEHP exposure level. However, the study authors did not regard this as

pathologic as this effect is common in immature seminiferous tubules. When they measured how many immature seminiferous tubules among rats at the 4-week timepoint, they concluded the results were varied with no dose-response relationship, and at 8 weeks, all the animals had matured seminiferous tubules. These data indicate that DEHP increased plasma testosterone in prepubertal rats, suggesting they are more sensitive to inhalation compared to oral dosing.

In an inhalation study by **Ma et al. (**2006**),** 21-day old female Wistar-Imamichi rats were randomly assigned, stratified by body weight, to the treatment groups (Experiment 1; n = 10) or randomly-assigned, stratified by body weight and litter, to the treatment groups (Experiment 2; n = 12) and exposed via whole-body inhalation 6 hours per day, 5 days per week to DEHP at concentrations of 0, 5, or 25 mg/m^3 from PND 22 through PND 42 (Experiment 1) or PND 22 through PND 84 (Experiment 2). In Experiment 2, rats were evaluated for changes in estrous cyclicity from PND 49 to 84. The authors stated that detection of treatment-related effects in serum hormone concentrations could be best detected by sampling during the diestrous stage; therefore, animals were terminated during PND 85 through 88 for these measurements. The investigators measured pubertal development, organ weights, estrous cyclicity, serum concentrations of FSH, LH, testosterone, estradiol, cholesterol, and gene expression of estradiol biosynthetic enzymes in the ovaries. Test atmosphere concentrations in the exposure chambers were measured daily by gas chromatography and averaged 4.10 ± 1.96 mg/m^3 and 19.78 ± 3.69 mg/m^3 in Experiment 1 and 5.21 ± 2.73 mg/m^3 and 22.72 ± 7.59 mg/m^3 in Experiment 2 for the 5 and 25 mg/m^3 groups, respectively.

None of the animals exposed to DEHP showed any visible signs of toxicity, nor any significant differences in relative or absolute weight of liver, kidney, lung, ovary, or uterus in either experiment. Body weights were significantly decreased at 25 mg/m^3 from exposure Day 24 to 63 in Experiment 1; however, body weights were comparable to controls in Experiment 2. Sexual maturation and age at first estrous were accelerated at 5 and 25 mg/m^3 in both experiments. Mean age at vaginal opening was earlier at 5 mg/m^3 (29.2 days, 30.3 days) and 25 mg/m^3 (29.5 days, 29.7 days) compared to controls (31.8 days, 32.0 days). Similarly, mean age at first estrous was earlier at 5 mg/m^3 (30.6 days, 31.0 days) and 25 mg/m^3 (29.8 days, 30.6 days) compared to controls (32.7 days, 33.4 days). Irregular estrous cycles were significantly more prevalent in animals exposed to 25 mg/m^3 (29%) compared to the 5 mg/m^3 group (12%) and controls (14%) in experiment 2. In Experiment 1, serum FSH, LH, and estradiol levels in the treated groups were comparable to controls. In Experiment 2, serum estradiol and LH levels at 25 mg/m^3 were significantly higher than controls. Total cholesterol was significantly decreased by 18 to 21 percent compared to controls in Experiment 1, but significantly increased by 19 to 25 percent over controls in Experiment 2. In experiment 1, 25 mg/m^3 of DEHP increased mRNA levels of aromatase 145 percent over controls. In contrast, in experiment 2, there were no changes in mRNA expression of genes involved in estradiol biosynthesis. The study authors concluded that inhalation of DEHP advances the onset of puberty and alters post-pubertal reproductive function.

In a developmental study conducted by **Merkle et al. (**1988**),** which was conducted according to OECD 414 guideline for teratogenicity studies, pregnant Wistar rats (25 per group) were nose-only exposed to aerosolized DEHP at target concentrations of 0, 0.01, 0.05 and 0.3 mg/L for 6 hours per day from GD 6 to 15. DEHP aerosol concentrations were collected from the breathing zone of the animals and measured by gas chromatography for concentration verification, with reported analytical concentrations of 0.011 ± 0.0015, 0.048 ± 0.0082, and 0.30 ± 0.020 mg/L; however, the frequency of measurements was not reported. The particle size of the aerosol was determined using a 7-stage cascade impactor and measuring the different fractions using gas chromatography to determine particle size distribution and mass median aerodynamic diameter (MMAD), resulting in MMAD less than 1.2µm across all concentrations; geometric standard deviation (GSD) was not reported. Maternal animals (20 per group)

were terminated on GD 20 and subjected to a cesarean section for examination of uterine contents, and fetuses were examined for external, visceral, and skeletal malformations and variations. The remaining five dams/group were allowed to litter, and the offspring were examined for: survival (viability and lactation indices); reflexes, including righting (PND 6), grip strength (PND 13), pupillary response (PND 20), and auditory startle (PND 21); and developmental landmarks (eye/ear auricle opening, incisor eruption, and fur growth).

There were no clinical signs of toxicity and no effects of treatment on maternal body weight or body weight gain during gestation. Maternal body weight was significantly decreased by 9 percent in the 0.3 mg/L group compared to controls at the end of the lactation period (LD 21). Post-implantation loss (reported as percent dead implantations) was significantly increased at 0.05 mg/L (19.98%) compared to controls (7.63%), resulting in lower number of live fetuses per dam at this concentration (10.59) compared to controls (12.00). However, these findings were considered unrelated to treatment because the highest concentration was comparable to controls. There were no treatment-related effects on external or skeletal malformations, variations, or retardations. The incidence of visceral retardations (determined by Barrow/Taylor method) at 0.3 mg/L (25.94% of fetuses, 56.25% of litters) was increased over controls (6.94% of fetuses, 16.67% of litters), and the increase in litter incidence was statistically significant. The study authors reported that these visceral retardations were mostly dilatation of the renal pelvis, which they considered unrelated to treatment because it is common in this strain of rats and observed at a high incidence in historical controls. During the post exposure lactation period, there were no differences in offspring development.

In an inhalation toxicity study by **Klimisch et al. (**1992**),** conducted according to OECD 412 guideline (with additional measurements of fertility and electron microscopy), male and female Wistar rats were nose-only exposed to aerosolized DEHP at concentrations of 0, 0.01, 0.05, or 1.0 mg/L for 6 hours per day, 5 days per week for 4 weeks. These concentrations were equivalent to an achieved dose of 0, 2.3, 11, and 230 mg/kg-day in males and 0, 3.6, 18, and 360 mg/kg-day in females, assuming 100 percent deposition and absorption. DEHP aerosol concentrations were collected from the breathing zone of the animals approximately hourly during exposure and measured by gas chromatography for concentration verification, with reported analytical concentrations of 0.011 ± 0.0045, 0.049 ± 0.007, and 0.94 ± 0.13 mg/L. The particle size of the aerosol was determined using a cascade impactor and measuring the different fractions using gas chromatography to determine particle size distribution and mass median aerodynamic diameter (MMAD), resulting in MMAD less than 1.2 µm across all concentrations and geometric standard deviation (GSD) of 2.9 to 9.5 µm. Ten animals per sex per group comprised the main study and, along with an additional two per sex per group in satellite group I, were terminated at the end of the exposure period, with the main study animals subjected to hematology, clinical chemistry, and pathology analyses, and the animals in satellite group I evaluated specifically for liver pathology. To examine reversibility and effects on fertility, an additional 15 males per group comprised satellite group II, and were mated with untreated females (2 to 5 per group) at 2 weeks and 6 weeks after the end of exposure (corresponding to two spermatogenic cycles), and the untreated females were killed on GD 14 to examine uterine contents (*e.g.,* corpora lutea, implantations, resorptions).

There were no mortalities or clinical signs of toxicity and no effects of treatment on body weights, body weight gain, or hematology. At 28 days in the 1.0 mg/L group: serum albumin was significantly increased by 6 percent over controls in males and 7 percent over controls in females; inorganic phosphate was significantly increased by 10 percent over controls in males; absolute liver weight was significantly increased by 9 percent in females; and relative liver weight was significantly increased by 8 percent in males and 5 percent in females; however, there were no findings in liver histopathology or electron microscopy (*e.g.,* peroxisome proliferation) to corroborate and an adverse effect of treatment.

Relative lung weights were significantly increased by 6 percent over controls in the males, which was corroborated by slight increases in semi-quantitative grading of foam-cell content and alveolar septal thickening in the lungs in this group. All of these changes were reversible so that they were comparable to controls after 8 weeks of recovery. In satellite group II, there were no effects on fertility index or on pre- or post-implantation losses on GD 14 in untreated females mated with treated males. The study authors concluded that the no observed effect level (NOEL) in this study is 0.05 mg/L (equivalent to 11 mg/kg-day in males and 18 mg/kg-day in females).

Lastly, in a study by **Larsen et al. (**2007**),** BALB/cJ mice were exposed to aerosolized DEHP in acetone during three different experiments to determine: irritation; inflammation; and adjuvant effect/allergic airway inflammation. To measure acute irritation effects of DEHP, the first experiment exposed mice to 3.7, 18.4, 31.6 or 300 mg/m^3 (8 per group) for 60 minutes, and respiratory parameters were measured using body plethysmographs before and after exposure, so that each animal served as its own control. Respiratory parameters included respiratory frequency (breaths/min), time of inspiration, time of expiration, time from end of inspiration until beginning of expiration (time of brake, TB), time from end of expiration until beginning of next inspiration (time of pause, TP), tidal volume (VT), and mid-expiratory flow rate (mL/s). The second experiment measured the airway inflammation response by bronchoalveolar lavage (BAL) in mice exposed for 60 minutes to 300 mg/m^3. BAL was collected 0, 6, 16, 24 and 48 h after end of exposure (n = 7/group). To determine respiratory sensitization from repeated exposure to DEHP, in the third experiment, mice (n = 10/group) were exposed to: OVA control; OVA+ DEHP; or OVA+ $Al(OH)_3$ for 20 minutes per day, 5 days per week for 2 weeks, then 20 min weekly for 12 weeks. DEHP concentrations were 0.022, 0.094, 1.7, or 13 mg/m^3, and OVA concentration was 13 mg/m^3. Aerosols collected from the breathing zone of the mice indicated DEHP concentrations in the exposure chamber were 0.022 ± 0.0029, 0.094 ± 0.0032, 1.7 ± 0.64, and 13 ± 3.2 mg/m^3, for the 0.022, 0.094, 1.7, or 13 mg/m^3, respectively; and OVA concentrations were 0.14 ± 0.04 and 70 ± 11 mg/m^3 for the low- and high-concentrations. By weight, 92 percent of the DEHP particles had an aerodynamic diameter of less than 3.5 µm, 57 percent were less than 1.55 µm, 22 percent were less than 0.93 µm, and 8 percent were less than 0.52 µm. Based on the particle size distribution, the authors concluded that DEHP was able to reach all levels of the respiratory tract.

In experiment 1, the authors reported that DEHP did not cause sensory irritation in the upper respiratory tract as indicated by normal TB and comparable TP values in all exposure groups; however, rapid shallow breathing was observed at the highest concentration of 300 mg/m^3, indicating respiratory irritation, with decreased tidal volume up to 35 percent and increased respiratory rate of 15 percent of pre-exposure values by the end of the exposure. In experiment 2, there were no significant alterations in macrophage cell numbers over time, therefore authors suggested that even at the highest concentration, DEHP does not induce inflammation. In experiment 3, liver weights and body weights of the treated groups were comparable to controls following repeated exposure to DEHP. DEHP did not have any effect on IgE serum levels; however, IgG1 levels were significantly increased in the highest concentration (13 mg/m^3) when compared to the OVA control group. Furthermore, the numbers of eosinophils, neutrophils, and lymphocytes were significantly increased, and the number of alveolar macrophages was significantly decreased in the 13 mg/m^3 DEHP group compared to OVA controls. Different lymph nodes were excised from 2 to 3 mice, such as superficial cervical (SLN), deep cervical (DLNs) and mediastinal (MLN) lymph nodes. Cytokines and the number of lymphocytes were measured in these lymph nodes following *ex vivo* cultures and stimulation for 5 days with medium or OVA (1 mg/mL). Neither DEHP nor $Al(OH)_3$ had an effect on the number of lymphocytes in the DLNs. IL-5 and IL-10 cytokine production from MLNs was highest in the $Al(OH)_3$ and 13 mg/m^3 DEHP group when compared to the OVA group. This same trend was seen in SLNs and DLNs. All DEHP concentrations increased INFγ secretion from groups were observed in MLNs. INFγ levels were less in the SLNs and

DLNs. These results indicate DEHP inhalation increases inflammatory cells in the BAL and increased IgG1 levels at high concentrations, but lower doses of DEHP do not have an adjuvant effect nor induce pulmonary inflammation in this model.

Appendix C FETAL TESTICULAR TESTOSTERONE AS AN ACUTE EFFECT

No studies of experimental animal models are available that investigate the antiandrogenic effects of DEHP following single dose, acute exposures. However, there are studies of DBP available that indicate a single acute exposure during the critical window of development (*i.e.*, GD 14–19) can reduce fetal testicular testosterone production and disrupt testicular steroidogenic gene expression. Two studies were identified that demonstrate single doses of 500 mg/kg DBP can reduce fetal testicular testosterone and steroidogenic gene expression. Johnson et al. (2012; 2011) gavaged pregnant SD rats with a single dose of 500 mg/kg DBP on GD 19 and observed reductions in steroidogenic gene expression in the fetal testes three (*Cyp17a1*) to six (*Cyp11a1, StAR*) hours post-exposure, while fetal testicular testosterone was reduced starting 18 hours post-exposure. Similarly, Thompson et al. (2005) reported a 50 percent reduction in fetal testicular testosterone 1-hour after pregnant SD rats were gavaged with a single dose of 500 mg/kg DBP on GD 19, while changes in steroidogenic gene expression occurred 3 (*StAR*) to 6 (*Cyp11a1, Cyp17a1, Scarb1*) hours post-exposure, and protein levels of these genes were reduced 6 to 12 hours post-exposure. Additionally, studies by Carruthers et al. (2005) further demonstrate that exposure to as few as two oral doses of 500 mg/kg DBP on successive days between GDs 15 to 20 can reduce male pup AGD, cause permanent nipple retention, and increase the frequency of reproductive tract malformations and testicular pathology in adult rats that received two doses of DBP during the critical window.

In summary, studies of DBP provide evidence to support use of effects on fetal testosterone as an acute effect. However, the database is limited to just a few studies of DBP that test relatively high (500 mg/kg) single doses of DBP. Although there are no single dose studies of DEHP that evaluate antiandrogenic effects on the developing male reproductive system, there are four studies that have evaluated effects of DEHP on fetal testicular testosterone production following daily gavage doses of 100 to 900 mg/kg-day DEHP on GDs 14 to 18 (5 total doses) with *ex vivo* fetal testicular testosterone production examined on GD 18 (Gray et al., 2021; Furr et al., 2014; Hannas et al., 2011; Howdeshell et al., 2008)—all of which consistently report decreased fetal testosterone production at doses as low as 100 to 300 mg/kg-day.

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

For DEHP, all data considered for PODs are obtained from oral animal toxicity studies, including 17 studies in rats and one study in mice (see Table 4-4). Because toxicity values for DEHP are from oral animal studies, EPA must use an extrapolation method to estimate HEDs. The preferred method would be to use chemical-specific information for such an extrapolation. However, no suitable fit-for-purpose PBPK models (*i.e.*, those specifically designed to extrapolate across species) have been validated for regulatory use in risk assessment, and chemical-specific information was not identified for DEHP to support a derivation of a data derived extrapolation factor (DDEF) (U.S. EPA, 2014). In the absence of such data, EPA relied on the guidance from U.S. EPA (2011c), which recommends scaling allometrically across species using the three-quarter power of body weight ($BW^{3/4}$) for oral data. Allometric scaling accounts for differences in physiological and biochemical processes, mostly related to kinetics.

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

Equation_Apx D-1. Dosimetric Adjustment Factor

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

Where:

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

U.S. EPA (2011c), presents DAFs for extrapolation to humans from several species. However, because those DAFs used a human body weight of 70 kg, EPA has updated the DAFs using a human body weight of 80 kg for the DEHP risk evaluation (U.S. EPA, 2011a). EPA used a bodyweight of 0.25 kg for rats and 0.025 kg for mice, as presented in U.S. EPA (2011c). The resulting DAF is 0.236 for rats and 0.133 for mice.

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

Equation_Apx D-2. Daily Oral Human Equivalent Dose

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

Where:

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

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

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

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

Where:

$HEC_{Daily,continuous}$	=	Inhalation HEC based on continuous daily exposure (mg/m^3)
HED_{Daily}	=	Oral HED based on daily exposure (mg/kg-day)
BW_H	=	Body weight of adult humans (kg) = 80
IR_R	=	Inhalation rate for an individual at rest (m^3/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_Apx D-4 presents the conversion of the HEC from mg/m^3 to ppm.

Equation_Apx D-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 DEHP = 390.56 g/mol)

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

The acute, intermediate, and chronic duration non-cancer POD is based on a NOAEL of 4.8 mg/kg-day, and the critical effect is male phthalate syndrome-related effects (*i.e.*, increased incidence of reproductive tract malformations in the F2 generation of SD rats following dietary exposure to DEHP for three-generations (Blystone et al., 2010; TherImmune Research Corporation, 2004). This non-cancer POD is considered protective of effects observed following acute, intermediate, and chronic duration exposures to DEHP. EPA used Equation_Apx D-1 to determine a DAF specific to rats (0.236), which was in turn used in the following calculation of the daily HED using Equation_Apx D-2:

$$1.1\ \frac{mg}{kg-day} = 4.8 \frac{mg}{kg-day} \times 0.236$$

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

$$6.2\ \frac{mg}{m^3} = 1.1\ \frac{mg}{kg-day} \times \left(\frac{80\ kg}{0.6125 \frac{m^3}{hr} * 24\ hr}\right)$$

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

$$0.39\ ppm = 6.2\ \frac{mg}{m^3}\ \times \frac{24.45}{390.56}$$

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

E.1 Purpose

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

E.2 Methods

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

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

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

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

Further description of methods and results for the updated meta-analysis and BMD modeling analysis that evaluated BMRs of 5, 10, and 40 percent for decreased fetal testicular testosterone are provided in EPA's *Meta-Analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-*

ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), and Dicyclohexyl Phthalate (DCHP) (U.S. EPA, 2025g).

E.3 Results

BMD estimates, as well as 95 percent upper and lower confidence limits, for decreased fetal testicular testosterone for the evaluated BMRs of 5, 10, and 40 percent are shown in Table_Apx E-1. BMD5 estimates ranged from 8.4 to 74 mg/kg-day for DEHP, DBP, DCHP, and DINP; however, a BMD5 estimate could not be derived for BBP or DIBP. Similarly, BMD10 estimates ranged from 17 to 152 for DEHP, DBP, DCHP, DIBP and DINP; however, a BMD10 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) as well as in Section 3.1.2 of this document, decreased fetal testicular testosterone is an early, upstream event in the MOA that precedes downstream apical outcomes such as male nipple retention, decrease anogenital distance, and reproductive tract malformations. Decreased fetal testicular testosterone should occur at lower or equal doses than downstream apical outcomes associated with a disruption of androgen action. Because the lower 95 percent confidence limit on the BMD, or BMDL, is used for deriving a 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, BMDL40 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 3× (for BBP) to 25.4× (for DEHP). Further, BMDL40 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 (BMDL10, 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 5× ($BMDL_{10}$ 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 2.4× ($BMDL_{10}$ 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 5× and is greater than the lowest LOAEL identified for apical outcomes on the developing male reproductive system by 2.4×. *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.
- BMDL10 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 reflect the larger database of studies and wider range of endpoints evaluated for DEHP, compared to DBP and DCHP.
- NASEM (2017) modeled a BMR of 40 percent using the following justification: "previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40 percent (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 3× to 25.4× (Table_Apx E-1). BMDL40 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.

During 2024 peer-review meeting of DINP (U.S. EPA, 2024) and the 2025 peer-review meeting of DEHP, DBP, BBP, DIBP, and DCHP (U.S. EPA, 2025o), SACC supported EPA's use of a BMR of 5 percent for evaluating decreased fetal testicular testosterone for establishing a POD for use in risk assessment, as well as the use of a BMR of 40 percent for establishing relative potency factors (RPFs) for use in the cumulative risk assessment (CRA) of phthalates.

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

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

AGD = anogenital distance; BMD = benchmark dose; BMDL = lower 95% confidence limit on BMD; CI = 95% confidence interval; LOAEL = lowest observable-adverse-effect level; NOAEL = no-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 Regression Analysis of Dietary Concentration (ppm) and Achieved Intake (mg/kg-day) in Blystone et al. (2010) to Convert $BMDL_5$ for Reproductive Tract Malformations from Dietary Concentration to Achieved Intake

Generation	ppm in diet	Achieved Dose (mg/kg-day)
F1	1.5	0.09
F1	10	0.48
F1	30	1.4
F1	100	4.9
F1	300	14
F1	1000	48
F1	7500	391
F1	10000	543
F2	1.5	0.1
F2	10	0.47
F2	30	1.4
F2	100	4.8
F2	300	14
F2	1000	46
F2	7500	359

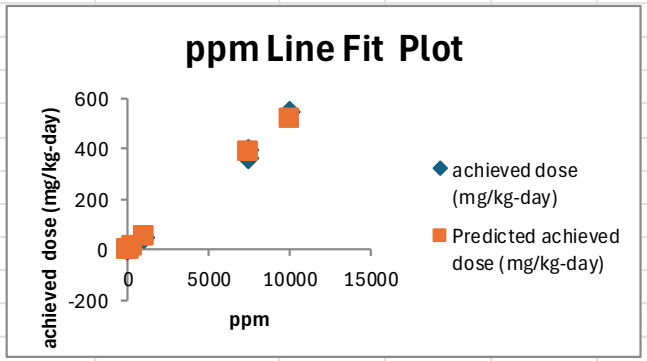
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.998301959
R Square	0.996606802
Adjusted R Square	0.996345787
Standard Error	10.78230037
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	443895.6021	443895.6021	3818.194	1.93297E-17
Residual	13	1511.354017	116.2580013		
Total	14	445406.9561			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	-1.800839488	3.196403993	-0.563395457	0.5827535	-8.706250487	5.104572	-8.70625	5.104572
ppm	0.052205738	0.000844869	61.79153657	1.933E-17	0.05038051	0.054031	0.050381	0.054031



RESIDUAL OUTPUT

Observation	*Predicted achieved dose (mg/kg-day)*	*Residuals*	*Standard Residuals*
1	-1.722530881	1.812530881	0.174448135
2	-1.278782108	1.758782108	0.169275051
3	-0.234667349	1.634667349	0.157329551
4	3.419734311	1.480265689	0.14246907
5	13.86088191	0.139118092	0.013389505
6	50.4048985	-2.4048985	-0.231460916
7	389.7421954	1.257804577	0.121058165
8	520.2565404	22.74345961	2.188958074
9	-1.722530881	1.822530881	0.175410591
10	-1.278782108	1.748782108	0.168312595
11	-0.234667349	1.634667349	0.157329551
12	3.419734311	1.380265689	0.132844509
13	13.86088191	0.139118092	0.013389505
14	50.4048985	-4.4048985	-0.42395213
15	389.7421954	-30.74219542	-2.958801257