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Pollution Prevention

Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act

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Docket

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ABBREVIATIONS AND ACRONYMS

AGD	Anogenital distance
ATSDR	Agency for Toxic Substances and Disease Registry
BBP	Butyl benzyl phthalate
BMD	Benchmark dose
CASRN	Chemical Abstracts Service Registry Number
CDC	U.S. Centers for Disease Control and Prevention
CDR	Chemical Data Reporting (Database)
CHAP	Chronic Hazard Advisory Panel
CI	Confidence interval
COU	Condition of use
CPHEA	Center for Public Health and Environmental Assessment
CPSC	U.S. Consumer Product Safety Commission
CRA	Cumulative risk assessment
DBP	Dibutyl phthalate
DCHP	Dicyclohexyl phthalate
DEHP	Di-ethylhexyl phthalate
DEP	Diethyl phthalate
DHT	Dihydrotestosterone
DI	Dietary intake
DIBP	Di-isobutyl phthalate
DIDP	Di-isodecyl phthalate
DINP	Di-isononyl phthalate
DMP	Dimethyl phthalate
DMR	Discharge Monitoring Report
DNEL	Derived no effect level
DPP	Dipentyl phthalate
ECHA	European Chemicals Agency
ECRAD	Existing Chemical Risk Assessment Division
ED50	Effective dose (causing a 50 percent response)
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
ESD	Emission Scenario Documents
EU	European Union
FDA	U.S. Food and Drug Administration
FHSA	Federal Hazardous Substances Act
GD	Gestational day
GS	Generic scenario
HI	Hazard index
HQ	Hazard quotient
IC	Index chemical
INSL3	Insulin-like Growth factor 3
IRIS	Integrated Risk Information System
LABC	Levator Ani/bulbocavernosus
LOAEL	Lowest-observed-adverse-effect-level
LOEL	Lowest-observed-effect-level
MBP	Monobutyl phthalate
MEHP	Mono-2-ethylhexyl phthalate
MIE	Molecular initiating event

MNG	Multinucleated gonocyte
MOA	Mode of action
MOE	Margin of exposure
NAS	National Academy of Sciences (now National Academies of Sciences, Engineering, and Medicine [NASEM])
NHANES	National Health and Nutrition Evaluation Surveys
NEI	National Emissions Inventory
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH HHE	National Institute of Occupational Safety and Health: Health Hazard Evaluation
NPDES	National Pollutant Discharge Elimination System
NR	Nipple retention
NRC	National Research Council (now NASEM)
NTP	National Toxicology Program
OCSPP	EPA's Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-operation and Development
OHAT	NTP's Office of Health Assessment and Translation
OLEM	EPA's Office of Land and Emergency Management
OPP	EPA' Office of Pesticide Programs
OPPT	EPA's Office of Pollution Prevention and Toxics
ONU	Occupational non-user
ORD	EPA's Office of Research and Development
OSHA CEHD	Occupational Safety and Health Administration: Chemical Exposure Health Data
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal day
PNW	Postnatal week
POD	Point of departure
POTW	Publicly owned treatment work
PPS	Preputial separation
RCR	Risk characterization ratio
RCRA	Resource Conservation and Recovery Act
RPF	Relative potency factor
RfV	Reference Value
SACC	Science Advisory Committee on Chemicals
SAR	Structure-activity relationship
SD	Sprague-Dawley (rats)
SDS	Safety Data Sheet
StAR	Steroidogenic acute regulatory protein
SV	Seminal vesicle
TD	Tolerable daily intake
TP	Testicular pathology
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
WWTP	Wastewater treatment plant

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA or the Agency) is currently conducting risk evaluations for five phthalates designated as high-priority substances under the Toxic Substances Control Act (TSCA)—di-ethylhexyl phthalate (DEHP), butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), di-isobutyl phthalate (DIBP), and dicyclohexyl phthalate (DCHP)—as well as two phthalates subject to manufacturer-requested risk evaluation: di-isononyl phthalate (DINP) and di-isodecyl phthalate (DIDP).

Phthalates are a group of ubiquitous environmental chemicals that are used in many industrial and consumer products, including cosmetics, building and construction materials, and polyvinyl chloride products, to make plastics more flexible and durable. Some phthalates are used in food contact materials and have been measured in food. Studies investigating human exposure to phthalates have demonstrated widespread exposure to some phthalates and that humans may become co-exposed to multiple phthalates at the same time. Further, some phthalates have been shown to cause common adverse effects on the developing male reproductive system, sometimes referred to as "phthalate syndrome." Because humans are co-exposed to some phthalates and because some phthalates can cause common adverse effects on the developing male reproductive system, EPA believes that the best approach to assess risk to human health may be to look at the combined risk to health from exposure to multiple phthalates.

As one of the first steps in the risk evaluation process, EPA published the final scope documents for the seven phthalates between 2020 and 2021. During the public comment periods for the draft scope documents, EPA received comments from multiple stakeholders urging the Agency to assess phthalates for cumulative risk to human health because humans are co-exposed to multiple phthalates and because some phthalates can cause common adverse effects. The next step in the risk evaluation process is to conduct individual risk evaluations for DEHP, BBP, DBP, DIBP, DCHP, DINP, and DIDP, which will characterize risk from their conditions of use (COUs). EPA's Office of Pollution Prevention and Toxics (OPPT) has not yet conducted a cumulative risk assessment (CRA) under TSCA, as it is still developing the methods and approaches for conducting CRA under TSCA. Moreover, the results of the individual phthalate risk evaluations are important inputs into the CRA and the development of individual risk evaluations is still ongoing.

This draft document provides a description of a proposed approach to conduct a CRA on the phthalates, but is not itself a CRA as no risk estimates are presented nor has any work on risk evaluation been completed. This draft document, along with the *Draft Proposed Principles of Cumulative Risk Assessment under the Toxic Substances Control Act* (hereafter referred to as Draft Proposed Principles of CRA under TSCA), will be released for public comments and reviewed by the Science Advisory Committee on Chemicals (SACC) in 2023. EPA will then use the peer review and public input to guide the development of the CRA for phthalates. Although EPA is required to draft individual risk determinations for each individual phthalate risk evaluation, the phthalate CRA will not contain a risk determination. Instead, results from the CRA are anticipated to inform EPA's individual phthalate risk determinations, pending completion of the CRA in parallel with individual phthalate risk evaluations.

TSCA does not expressly require EPA to conduct CRAs. However, TSCA does require that EPA, when conducting TSCA risk evaluations in 3 to 3.5 years [15 U.S.C. § 2605(b)(4)(G)], consider the reasonably available information, consistent with the best available science, and make decisions based on the weight of scientific evidence [15 U.S.C. § 2625(h), (i), (k)]. EPA is also required to conduct the risk evaluations in consideration of potentially exposed or susceptible subpopulations (PESS) [15 U.S.C. § 2605(b)(4)] and, among other requirements at 15 U.S.C. § 2605(b)(4)(F), "integrate and assess available information on hazards and exposures for the conditions of use of the chemical substance, including

information that is relevant to specific risks of injury..." EPA recognizes that for some chemical substances undergoing risk evaluation, the best available science may indicate that the development of a CRA is appropriate to ensure that any risks to human health are adequately characterized. To support CRA of chemical substances under TSCA, and as noted above, EPA has developed the Draft Proposed Principles of CRA under TSCA, which describes the proposed principles of CRA as potentially conducted in support of TSCA risk evaluations and relies heavily on long-standing EPA practice and guidance documents for mixtures risk assessment. The draft principles document lays the foundation for EPA's proposed approach for CRA of chemical substances undergoing risk evaluation under TSCA section 6(b).

EPA has conducted a preliminary review of stakeholder comments received during the phthalate scoping process, previous phthalate CRAs conducted by other regulatory agencies (ECCC/HC, 2020; EFSA, 2019; NICNAS, 2015a, 2014a, b; U.S. CPSC, 2014; NICNAS, 2013, 2012; ECHA, 2011), and recommendations of the National Research Council (NRC) (2008). Based in part on this information, EPA believes that the best available science indicates that several phthalates undergoing risk evaluation should be assessed for cumulative risk to human health. This draft document describes EPA's proposed approach for assessing these high-priority and manufacturer-requested phthalates for cumulative risk to human health under TSCA. Text Box ES-1 provides a high-level summary of EPA's proposed approach for CRA.

Individual phthalate risk evaluations are required to consider exposures from the COUs of a single phthalate and will include evaluation of all observed hazards, consideration of all age groups and lifestages, and assessment of aggregate exposures. In contrast, the scope and purpose of CRAs are more focused on the shared toxicological properties and relevant lifestages. In addition, cumulative exposure assessment is more complicated due to combining exposures across multiple phthalates.

Text Box ES-1. Summary of EPA's Proposed Approach for CRA of High-Priority and Manufacturer-Requested Phthalates

EPA proposes to:

- Group DEHP, BBP, DBP, DIBP, DCHP and DINP, but not DIDP, for CRA under TSCA.
- Address phthalate syndrome by focusing on the most sensitive effect (versus addressing the syndrome as a whole).
- Assess DEHP, BBP, DBP, DIBP, DCHP and DINP for cumulative risk to human health under an assumption of dose addition.
- Use a relative potency factor approach for the phthalate CRA conducted in support of TSCA.
- Focus its CRA efforts on PESS susceptible to phthalate syndrome (*i.e.*, pregnant women, women of reproductive age, male infants, male toddlers, male children).
- Consider exposures from TSCA COUs, as well as non-attributable and non-TSCA exposures.
- Use a scenario-building approach to estimate cumulative exposure for susceptible populations who may also be workers, consumers, and members of the general population (*e.g.*, fenceline communities).
- Use biomonitoring data when available to support exposure assessment.

EPA has developed a conceptual model to outline its proposed approach for estimating cumulative risk to phthalates within the cumulative chemical group. EPA's draft conceptual model, which is shown in Figure 2-1 and described in Section 2, outlines 10 proposed steps for conducting a phthalate CRA under TSCA. A brief description and summary of the outcome of each step follows:

Step 1 in EPA's draft conceptual model is to determine which high-priority and manufacturer-requested phthalates to include in the cumulative chemical group. As described in EPA's Draft Proposed Principles of CRA under TSCA document (and in Section 3 of this document), chemicals included in a cumulative chemical group should be toxicologically similar and there should be evidence

of co-exposure to the chemicals over a relevant timeframe (*e.g.*, exposed to multiple phthalates during a known sensitive lifestage).

To determine which high-priority and manufacturer-requested phthalates are toxicologically similar, EPA reviewed data for seven key outcomes associated with phthalate syndrome; that is, decreased fetal testicular gene expression and testosterone production, decreased male pup anogenital distance, nipple/areolae retention in male pups, hypospadias, seminiferous tubule atrophy, and multinucleated gonocyte formation (Sections 3.1.3.1 to 3.1.3.7). These key outcomes were selected based on EPA's current understanding of phthalate syndrome and its underlying mode of action. Notably, many of the key outcomes have also been selected as the critical effect (or co-critical effect) in previous phthalate CRAs (Table 3-1). Based on the weight of evidence, EPA proposes that DEHP, BBP, DBP, DIBP, DCHP, and DINP, but not DIDP, are toxicologically similar and induce effects on the developing male reproductive system consistent with phthalate syndrome (Section 3.1.7). Of note, the TSCA Work Plan includes one additional phthalate (*i.e.*, di-n-octyl phthalate) that is not currently prioritized for risk evaluation. However, Environment Canada/Health Canada (EC/HC, 2015e) concluded that di-n-octyl phthalate does not induce effects on the developing male reproductive system consistent with phthalate syndrome (EC/HC, 2015e). Di-n-octyl phthalate is not discussed further in this document.

When considering phthalates for grouping, EPA also considered how to address phthalate syndrome, which is currently identified as the common adverse effect, as part of a CRA. EPA is proposing to focus on the most sensitive effect(s) (as opposed to assessing the syndrome as a whole) (Section 4.1). As described in Section 4.2, empirical evidence from *in vivo* phthalate mixture studies indicate that phthalates induce effects on the developing male reproductive system in a manner consistent with dose addition. Therefore, EPA is proposing to assess DEHP, BBP, DBP, DIBP, DCHP, and DINP for cumulative risk to human health under an assumption of dose addition, which is consistent with the recommendations of the NRC (2008). EPA is considering the applicability of two component-based, dose additive approaches, including the hazard index (HI) and relative potency factor (RPF) approaches. EPA considers there to be sufficient data available to support RPF derivation for DEHP, BBP, DBP, DIBP, DCHP, and DINP (Section 4.3.3) and is proposing to use an RPF approach to assess these phthalates for cumulative risk. EPA has identified six potential options that are being considered for deriving RPFs for phthalates, which are described in Section 4.4.2.

To determine if the U.S. population is co-exposed to multiple high-priority and manufacturer-requested phthalates, EPA conducted a high-level review of National Health and Nutrition Evaluation Surveys (NHANES) urinary biomonitoring data (Section 3.2). Available NHANES data demonstrate that the U.S. population is co-exposed to multiple phthalates, including DEHP, BBP, DBP, DIBP, DINP, and DIDP. Recent NHANES data are not available for DCHP. However, DCHP has been identified to be used in various industrial, commercial, and consumer uses covered under TSCA. Based on exposure to DCHP through identified TSCA uses, EPA anticipates there will be co-exposure to DCHP and other high-priority and manufacturer-requested phthalates for certain populations and exposure scenarios (Section 3.2). These data qualitatively demonstrate that humans are co-exposed to DEHP, BBP, DBP, DIBP, DCHP, DINP, and DIDP. EPA's proposed approach for quantifying phthalate co-exposure is outlined in Section 6.

Because the weight of evidence indicates that DEHP, BBP, DBP, DIBP, DCHP and DINP (but not DIDP) are toxicologically similar and that the U.S. population is co-exposed to these phthalates over a relevant timeframe, EPA is proposing to group these phthalates for CRA under TSCA.

Step 2 in EPA's draft conceptual model (Figure 2-1) is to identify populations with potentially increased susceptibility to phthalate syndrome. As part of the individual phthlate risk evaluations, EPA will conduct consumer, occupational, and general population exposure assessments. Within these populations, potentially exposed or susceptible subpopulations (PESS) with greater susceptibility to the developmental and reproductive effects associated with phthalate syndrome, include pregnant women, women of reproductive age, male infants, male toddlers, and male children. These PESS are proposed to be the focus of EPA's approach for CRA of DEHP, BBP, DBP, DIBP, DCHP and DINP (Section 5).

Step 3 in EPA's draft conceptual model (Figure 2-1) is to identify TSCA COUs[1] and other potential sources of exposure. Sources of exposure including TSCA COUs, non-attributable, and non-TSCA sources relevant to cumulative exposure and release will be identified using conceptual models in individual phthalate scopes and literature reviews.

Step 4 in EPA's draft conceptual model (Figure 2-1) is exposure scenario-building for individual phthalates for TSCA COUs. For identified TSCA COUs and populations, specific routes of exposure and pathways for each exposure source are identified. Prior to the development of the phthalate CRA, exposure scenarios for individual TSCA COUs and estimates of exposure will be completed in the individual risk evaluations. Determination of co-exposure to multiple TSCA COUs or multiple phthalates in a single TSCA COU will be completed in Step 7 of the conceptual model for consumers (Section 6.4.1), workers (6.4.2), and the general population (Section 6.4.3).

Step 5 in EPA's draft conceptual model (Figure 2-1) is to build exposure scenarios of individual phthalates for non-attributable and non-TSCA sources. EPA is proposing to include both non-attributable and non-TSCA exposures as part of the phthalate CRA because certain non-TSCA (*e.g.*, dietary) and non-attributable (*e.g.*, household dust) exposure pathways are anticipated to be major contributors to phthalate exposure leading to cumulative risk (discussed further in Section 6.2.2). The Agency is considering two approaches for estimating non-attributable and non-TSCA phthalate exposure, including a scenario-based approach (Section 6.3.2.1) and a reverse dosimetry-based approach (Section 6.3.2.2). Because the reverse dosimetry approach, using biomonitoring data such as NHANES, does not distinguish between routes or pathways of exposure and does not allow for source apportionment, it provides an estimate of total non-attributable phthalate exposure. NHANES data may reflect exposure from TSCA, non-attributable, and other non-TSCA sources, but exposures from TSCA COUs cannot necessarily be source apportioned. As described in Section 6.3.2.5, EPA is proposing to estimate non-attributable and non-TSCA exposures for DEHP, BBP, DBP, DIBP, DCHP, and DINP from major exposure pathways using a scenario-based approach. The reverse dosimetry approach, which does not allow for source apportionment, may be used to help characterize phthalate exposure and serve as a comparator for scenario-based intake estimates (*i.e.*, help contextualize whether scenario-based estimates are an over- or underestimation of total exposure).

Steps 6 and 7 in EPA's draft conceptual model (Figure 2-1) are to identify major pathways of exposure (Step 6) and determine the likelihood of phthalate co-exposure (Step 7). As shown in EPA's draft conceptual model (Figure 2-1), EPA is proposing to assess PESS who are consumers (Section 6.4.1), workers (Section 6.4.2), and fenceline communities as part of the general population

[1] Condition of use (COU) (15 U.S.C. § 2602(4)): "means the circumstances, as determined by the Administrator, under which a chemical substance is intended, known, or reasonably foreseen to be manufactured, processed, distributed in commerce, used, or disposed of."

(Section 6.4.3) for cumulative risk from exposure to DEHP, BBP, DBP, DIBP, DCHP, and DINP through TSCA COUs. EPA proposes to identify major pathways of exposure and likelihood of co-exposure to these phthalates through various pathways for combining to estimate cumulative exposure to identified PESS (**Steps 6 to 7** in conceptual model).

- Major pathways of exposure for individual phthalates are combined to estimate aggregate exposure and can be considered exposures attributable to TSCA COUs, non-attributable, or non-TSCA.
- To estimate cumulative exposure to consumers (Section 6.4.1), EPA proposes to combine the non-attributable and non-TSCA exposures across phthalates with exposure from individual consumer COUs, as reasonable. Determining reasonable cumulative exposure scenarios may involve considering the likelihood of co-exposure, the possibility of double counting, and of over- or under-estimating exposures.
- To estimate cumulative exposure to workers (Section 6.4.2), EPA proposes to combine the non-attributable and non-TSCA exposure with cumulative occupational exposure from TSCA COUs in a work setting, as reasonable.
- For cumulative exposure to the general populations, specifically fenceline communities (Section 6.4.3), EPA proposes estimating cumulative exposures from single or multiple facility releases to ambient air and/or water and combining with non-attributable and non-TSCA exposure, as reasonable.
- EPA recognizes that some individuals may be part of multiple populations and may require additional combinations of exposures. For example, combining occupational exposures with consumer exposures and fenceline exposures for workers who use consumer products at home and who live near the fenceline of a facility with phthalate releases.

Steps 8 to 10 in EPA's draft conceptual model (Figure 2-1) are to convert individual phthalate exposure estimates to index chemical equivalents using RPFs (Step 8), and then to combine exposures to estimate cumulative exposure (Step 9) and cumulative risk (Step 10). Because EPA is proposing to use an RPF approach (Section 4.3.3), exposure from individual phthalates for each exposure scenario will be scaled to the potency of an index chemical and expressed as index chemical equivalents (**Step 8** in conceptual model), which will then be summed to estimate cumulative exposure for each exposure scenario (expressed as index chemical equivalents) (**Step 9** in conceptual model). Cumulative risk may then be estimated using a margin of exposure (MOE) approach (Section 4.3.3) (**Step 10** in conceptual model).

EPA is soliciting comments from the SACC on charge questions and comments from the public for the SACC meeting scheduled on May 8–11, 2023.

1 BACKGROUND

In December 2019, the U.S. Environmental Protection Agency (EPA or the Agency) designated butyl benzyl phthalate (BBP, Chemical Abstracts Service Registry Number [CASRN] 85-68-7), dibutyl phthalate (DBP, CASRN 84-74-2), dicyclohexyl phthalate (DCHP, CASRN 84-61-7), di-ethylhexyl phthalate (DEHP, 117-81-7), and di-isobutyl phthalate (DIBP, CASRN 85-69-5) as high-priority substances for risk evaluation under the Toxic Substances Control Act (TSCA) (U.S. EPA, 2019b, c, d, e, f). Additionally, on May 24, 2019, EPA received requests from industry, pursuant to 40 CFR 702.37, to conduct risk evaluations for di-isodecyl phthalate (DIDP, CASRNs 26761-40-0 and 68515-49-1) (ACC HPP, 2019a) and di-isononyl phthalate (DINP, CASRNs 28553-12-0 and 68515-48-0) (ACC HPP, 2019b). The Agency determined that the requests met the applicable regulatory criteria and requirements, as prescribed under 40 CFR 702.37, and granted the manufacturer-requested risk evaluations for DIDP and DINP on December 2, 2019. As one of the first steps in the risk evaluation process, EPA published the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), and DIBP (U.S. EPA, 2020c) in August 2020, fulfilling TSCA requirements under TSCA section 6(b)(4)(D) and as described in 40 CFR 702.41(c)(8). In August 2021, EPA published the final scope documents for DIDP (U.S. EPA, 2021b) and DINP (U.S. EPA, 2021c).

During the public comment periods for the draft scope documents for the high-priority phthalates and phthalates subject to manufacturer-requested risk evaluation, EPA received comments from multiple stakeholders urging the Agency to assess phthalates for cumulative risk to human health.[2,3] Recognizing that human exposure to phthalates is widespread and that multiple phthalates can disrupt development of the male reproductive system in laboratory animals at potentially human relevant doses, in 2007 EPA asked the National Research Council (NRC) of the National Academy of Sciences (NAS; now National Academies of Sciences, Engineering, and Medicine [NASEM]) to form a committee to review the health effects of phthalates and determine whether a cumulative risk assessment (CRA) of phthalates is appropriate. Additionally, EPA asked the NRC to provide recommendations on specific approaches that could be used to assess phthalates for cumulative risk. NRC published their findings and recommendations to EPA in a 2008 report *Phthalates and Cumulative Risk Assessment: The Tasks Ahead* (NRC, 2008). Ultimately, the NRC concluded that "sufficient data are available to proceed with the cumulative risk assessment of phthalates…" [p. 10 of (NRC, 2008)].

In 2010, and in response to the NRC recommendations, EPA's Office of Research and Development's Integrated Risk Information System (IRIS) Program convened a 2-day peer consultation workshop to discuss and evaluate the NRC recommendations. As summarized in the final workshop report (U.S. EPA, 2011), there was broad support by both expert panelists and stakeholders to continue developing a cumulative hazard assessment.

Other regulatory agencies have assessed phthalates for cumulative risk since NRC published their recommendations (NRC, 2008)—including the Chronic Hazard Advisory Panel (CHAP) of the U.S.

[2] For example, see comments submitted to the DEHP Docket (EPA-HQ-OPPT-2018-0433) received from the Environmental Defense Fund (EPA-HQ-OPPT-2018-0433-0033), Environmental Protection Network (EPA-HQ-OPPT-2018-0433-0028), Project TENDR (EPA-HQ-OPPT-2018-0433-0045); and University of California, San Francisco Program on Reproductive Health and the Environment (EPA-HQ-OPPT-2018-0433-0013).

[3] For example, see comments submitted to the DINP Docket (EPA-HQ-OPPT-2018-0436) received from University of California, San Francisco Program on Reproductive Health and the Environment (EPA-HQ-OPPT-2018-0436-0009); Environmental Protection Network (EPA-HQ-OPPT-2018-0436-0026); Earthjustice (EPA-HQ-OPPT-2018-0436-0028, EPA-HQ-OPPT-2018-0436-0033); and Defend Our Health, Black Women for Wellness, Alaska Community Action on Toxics and Breast Cancer Prevention Partners (EPA-HQ-OPPT-2018-0436-0042).

Consumer Product Safety Commission (U.S. CPSC, 2014); Environment and Climate Change Canada, Health Canada (ECCC/HC, 2020); the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) of Australia (NICNAS, 2015a, 2014a, b, 2013, 2012); the European Food Safety Authority (EFSA, 2019), and the Danish EPA (ECHA, 2011). Although the phthalate CRAs conducted by these regulatory agencies vary in scope and regulatory purpose, they generally adhere to NRC recommendations (NRC, 2008). For example, the CRAs primarily focus on assessing phthalates based on their shared ability to disrupt development of the male reproductive system through a disruption of androgen action (*i.e.*, cause phthalate syndrome), and have all relied upon an assumption of dose addition (see Appendices A.1 to A.5 for summaries of phthalate CRAs conducted by these agencies).

1.1 What Is EPA Proposing in this Work?

As required under section 6(b)(4) of TSCA, EPA issued a final rule, *Procedures for Chemical Risk Evaluation Under the Amended Toxic Substances Control Act* (82 FR 33726) (hereinafter "Risk Evaluation Rule"), in July 2017, which provides the procedural requirements for EPA's risk evaluations, including for chemicals designated as High-Priority Substances and chemical substances subject to a Manufacturer-Requested Risk Evaluation. To date, EPA's Office of Pollution Prevention and Toxics (OPPT) has focused risk evaluations on individual chemical substances, not the evaluation of multiple chemical substances for cumulative risk to human health. TSCA does not define cumulative risk nor explicitly require EPA to conduct CRAs. However, TSCA does require EPA, when conducting TSCA risk evaluations, to (1) consider the reasonably available information, (2) use the best available science, and (3) make decisions based on the weight of the scientific evidence [15 U.S.C. § 2625(h), (i), (k)]. EPA is also required to conduct the risk evaluations in consideration of potentially exposed or susceptible subpopulations (PESS) [15 U.S.C. § 2605(b)(4)] and, among other requirements at 15 U.S.C. § 2605(b)(4)(F), "integrate and assess available information on hazards and exposures for the conditions of use of the chemical substance, including information that is relevant to specific risks of injury..." EPA recognizes that for some chemical substances undergoing risk evaluation, the best available science may indicate that the development of a CRA is appropriate to ensure that risks of injury to human health and the environment are adequately characterized. Although EPA is required to draft individual risk determinations for each individual phthalate risk evaluation, the phthalate CRA will not contain a risk determination. Instead, results from the CRA are anticipated to inform EPA's individual phthalate risk determinations, pending completion of the CRA in parallel with individual phthalate risk evaluations. To support CRA of chemical substances undergoing TSCA section 6(b) risk evaluations, EPA has developed a document titled *Draft Proposed Principles of Cumulative Risk Assessment under the Toxic Substances Control Act* (hereafter referred to as Draft Proposed Principles of CRA under TSCA). EPA's Draft Proposed Principles of CRA under TSCA document describes the proposed principles of CRA, which form the underpinning of EPA's draft approach for CRA of high-priority and manufacturer-requested phthalates.

The Agency has reviewed the recommendations of the NRC (2008), comments received from stakeholders on the draft scope documents (see footnotes in Section 1), and CRAs conducted by other regulatory agencies (see Appendices A.1 to A.5). Based on this information, EPA believes the best available science indicates that several high-priority and manufacturer-requested phthalates should be assessed for cumulative risk to human health.

As part of conducting a risk evaluation under TSCA section 6(b), EPA must "determine whether a chemical substance presents unreasonable risk of injury to health . . . including an unreasonable risk to a potentially exposed or susceptible subpopulation [(PESS)] identified as relevant to the risk evaluation by [EPA] . . ." [15 U.S.C. 2605(b)(4)(A)]. EPA has identified phthalate syndrome as a specific risk from a number of the phthalates undergoing risk evaluation. The Agency has also identified a number of PESS

that have a greater susceptibility to phthalate toxicity—including pregnant women/women of reproductive age, male infants, male toddlers, and male children (discussed in Section 5). Due to toxicological similarity, shared ability to elicit key markers of phthalate syndrome, and co-exposures to multiple phthalates to the aforementioned PESS (one of the factors laid out in Section 3.4 of the Draft Proposed Principles of CRA under TSCA), EPA is proposing that a subset of the phthalates undergoing risk evaluation represent a cumulative chemical group, and that a cumulative risk assessment is necessary to ensure that individual risk evaluations on the phthalates in the cumulative chemical group have considered the reasonably available information, are consistent with the best available science, and based on the weight of the scientific evidence (15 U.S.C. 2625(h), (i), & (k)).

This draft document describes EPA's proposed approach for evaluating the phthalates in the cumulative chemical group for cumulative risk to human health under TSCA. The phthalates included in OPPT's proposed CRA are limited, at this time, to those undergoing risk evaluation under TSCA and are inclusive of the phthalates that have been most commonly considered for CRA by other agencies (see Appendix A).

This document describes EPA's draft proposed approach for assessing high-priority and manufacturer-requested phthalates for cumulative risk to human health under TSCA based on the principles of CRA described in the Draft Proposed Principles of CRA under TSCA. The proposed approach described in this document follows many of the recommendations made by NRC (2008). Individual phthalate risk evaluations will consider exposures from a single phthalate and will include evaluation of all observed hazards, consideration of more age groups and lifestages, and assessment of aggregate exposures. In contrast, the scope and purpose of CRAs are more focused on the shared toxicological properties and relevant lifestages. In addition, cumulative exposure assessment is more complicated due to combining exposures across multiple phthalates.

At the date of publication of this document, EPA has not yet completed all the expected systematic review or data quality evaluation for the individual high-priority and manufacturer-requested phthalates. Although this document is not reflective of complete systematic review, EPA has reviewed several key documents prepared by various authoritative bodies and regulatory agencies along with numerous studies and databases of toxicological and exposure information. As appropriate, EPA's proposed approach may be revised based on any new information that is identified through the systematic review process. Some key documents used to develop this proposed approach include

- *Phthalates and Cumulative Risk Assessment: The Tasks Ahead* (NRC, 2008)
- *Application of Systematic Review Methods in an Overall Strategy for Evaluating Low-Dose Toxicity from Endocrine Active Chemicals* (NASEM, 2017)
- *Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives* (U.S. CPSC, 2014) and supporting toxicity reviews of DEHP (U.S. CPSC, 2010c), BBP (U.S. CPSC, 2010a), DBP (U.S. CPSC, 2010b), DIBP (U.S. CPSC, 2011), DCHP (U.S. CPSC, 2010e), DINP (U.S. CPSC, 2010f), and DIDP (U.S. CPSC, 2010d)
- *Screening Assessment, Phthalate Substance Grouping* (ECCC/HC, 2020) and supporting reports (EC/HC, 2015a, b, c, e; Health Canada, 2015)
- Existing Chemical Hazard Assessment Reports for DIBP (NICNAS, 2008b) and DEHP (NICNAS, 2008a) and Priority Existing Chemical Assessment Reports for BBP (NICNAS, 2015a), DBP (NICNAS, 2013), DINP (NICNAS, 2012), DIDP (NICNAS, 2015b)

- *Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and diisodecylphthalate (DIDP) for use in food contact materials* (EFSA, 2019)

This draft document, along with the Draft Proposed Principles of CRA under TSCA, will be reviewed by the Science Advisory Committee on Chemicals (SACC) and receive public comments in 2023. EPA will use the peer review and public input to guide the subsequent development of the CRA for phthalates.

2 KEY CONCEPTS AND PROPOSED CONCEPTUAL MODEL

Individual phthalate risk evaluations will consider exposures from a single phthalate and will include evaluation of all observed hazards, consideration of all age groups and lifestages, and assessment of aggregate exposures. In contrast, the scope and purpose of CRAs are more focused on the shared toxicological properties and relevant lifestages. In addition, cumulative exposure assessment is more complicated due to combining exposures across multiple phthalates. Therefore, EPA has provided some definitions to key concepts relevant to CRAs in Section 2.1 and developed a draft conceptual model described in Section 2.2 and shown in Figure 2-1 to outline its proposed approach for estimating cumulative risk to several of high-priority and manufacturer-requested phthalates.

2.1 Key Concepts

- **Cumulative chemical group:** A group of chemical substances included in a CRA. As discussed in EPA's Draft Proposed Principles of CRA under TSCA, the cumulative chemical group is developed based on evidence of toxicologic similarity and co-exposure over a relevant timeframe.
- **Co-exposure:** Characterizing co-exposure requires consideration of the source of chemical exposure, populations impacted by exposure, and the possible varying routes and pathways of exposure. Additionally, the magnitude, frequency, and duration of exposure to multiple chemical substances influence the potential for co-exposure to occur within a given period of time (*e.g.*, 24 hours, 1 year, a lifetime); where the magnitude of exposure is the level of exposure dictated by the physical and chemical properties of the chemical substance and exposure scenario, frequency is the number of exposure events over a given time, and duration is the length of exposure time per event (OECD, 2018; U.S. EPA, 2001).
- **Relevant timeframe of exposure:** Timeframes in which exposure duration or frequency is relevant to effects of concern. This can include, but may not be limited to, exposure to multiple chemicals at the same time, exposure to persistent chemicals at different times that may bioaccumulate in the body or having persistent effects from exposure to multiple chemicals at different times. Relevant timeframes of exposure can vary by factors including, but not limited to, chemical properties, lifestages, or effect. Relevant timeframes of exposure for phthalates will be determined through the risk evaluation process.
- **Relative potency factor:** A numerical quantity used to scale the dose of one chemical to an equitoxic dose of another chemical based on differences in potencies. The latter chemical is typically termed the "index chemical" and is usually the chemical in the cumulative chemical group with the most robust toxicological database and/or is considered to be the most representative of the type of toxicity caused by other chemicals in the cumulative chemical group (U.S. EPA, 2000).
- **Scenario-based evaluation:**[4] Estimates that use available information on concentrations of chemicals in the exposure medium, and information about when, where, and how individuals might contact the exposure medium—activities that can lead to transfer of the agent from the exposure medium to the individual. Approach develops specific exposure scenarios and then uses data, a series of exposure factors, and models to estimate exposure within the scenario (U.S. EPA, 2019a).

[4] Referred to as indirect estimation in EPA's *Guidelines for Human Exposure Assessment* (U.S. EPA, 2019a).

- **Reverse dosimetry:** Estimates chemical intake using empirical biomonitoring data and information about chemical absorption, distribution, metabolism, and excretion rates (U.S. EPA, 2019a).
- **TSCA COU exposure:**[5] Exposure that can be attributed to a specific TSCA COU (*e.g.*, inhalation exposure during consumer use of an adhesive). Note that exposure scenarios for TSCA COUs will be completed in individual phthalate risk evaluations and evaluated for different populations such as consumers, workers, and general population.
- **Non-attributable exposure:** Exposure from pathways that cannot be attributed to a specific TSCA COU or another specific source. Household dust or human milk are a few examples in which phthalate concentrations measured in those media may result from multiple sources of phthalates that may nor may not be attributed to a TSCA COU or another specific source.
- **Non-TSCA exposure:**[6] Exposure that can be attributed to specific activities that are excluded from the TSCA definition of "chemical substance," under TSCA section 3(2), such as a pesticide, food, food additive, drug, cosmetic, or medical device.

2.2 Proposed Conceptual Model

EPA has developed a conceptual model to outline its proposed approach for estimating cumulative risk to several of the high-priority and manufacturer-requested phthalates. EPA's draft conceptual model, which is shown in Figure 2-1, outlines 10 proposed steps for conducting a phthalate CRA under TSCA using a scenario-based approach. The conceptual model provides illustrative steps that may not be inclusive of all details, such as all populations or all pathways of exposure, to be considered in an actual cumulative assessment. The remainder of this document is structured around this draft conceptual model. Some steps are described in greater detail in the document while others require risk evaluation work to be conducted to be developed further.

The steps included in the conceptual model are provided below:

- **Step 1. Identifying the Cumulative Chemical Group:** Identified based on a shared ability to elicit key markers of phthalate syndrome and evidence of human co-exposure. EPA's proposed cumulative chemical group includes DEHP, BBP, DBP, DIBP, DCHP, and DINP (Section 3.3).
- **Step 2. Populations:** EPA will conduct consumer, occupational, and general population (*e.g.*, fenceline) exposure assessments for each individual phthalate. The key human populations considered in these exposure assessments include consumers, workers, and the general population. Within these groups, there are PESS with increased susceptibility to the developmental and reproductive effects associated with phthalate syndrome, including pregnant women/women of reproductive age, male infants, male toddlers, and male children (described further in Section 5).

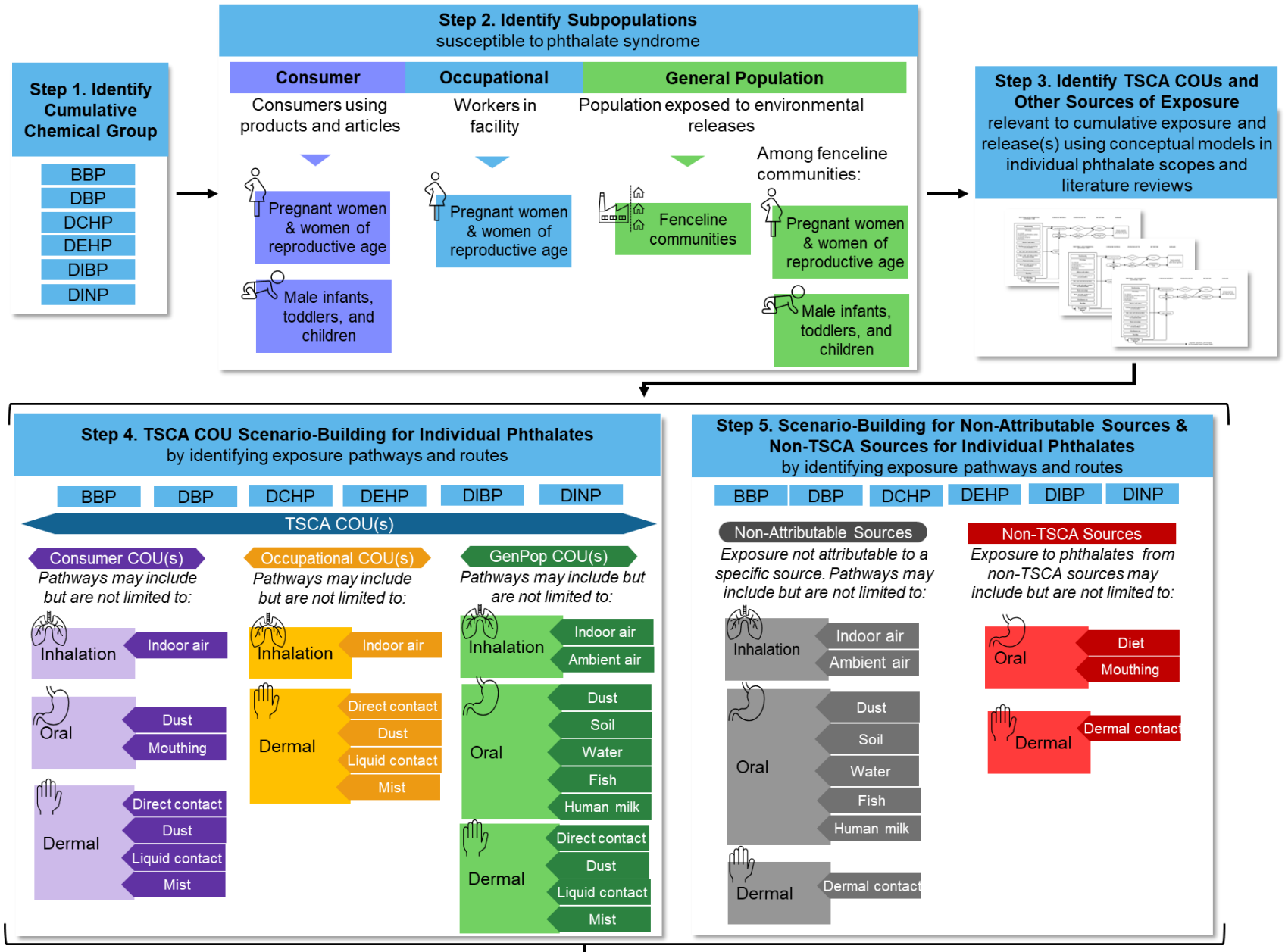
[5] Condition of use (COU) (40 CFR § 702.33): "means the circumstances, as determined by the Administrator, under which a chemical substance is intended, known, or reasonably foreseen to be manufactured, processed, distributed in commerce, used, or disposed of."

[6] TSCA section 3(2) also excludes from the definition of "chemical substance" "any food, food additive, drug, cosmetic, or device (as such terms are defined in section 201 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321]) when manufactured, processed, or distributed in commerce for use as a food, food additive, drug, cosmetic, or device" as well as "any pesticide (as defined in the Federal Insecticide, Fungicide, and Rodenticide Act [7 U.S.C. 136 et seq.]) when manufactured, processed, or distributed in commerce for use as a pesticide." Section 2.2.2 of each final scope document for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c) outline the uses of each phthalate that EPA has determined to be non-TSCA uses.

- **Step 3. Identify TSCA COUs and Other Sources of Exposure:** After gathering the specific COUs for each phthalate from their individual risk evaluation scope documents, the cross-chemical comparisons are used to establish the COUs likely to result in co-exposure to multiple phthalates under TSCA (Section 6.2.1). Other sources of exposure that are not considered TSCA COUs may also be identified as major sources of exposure for the identified populations through a review of the literature.
- **Step 4. Exposure Scenario-Building for Individual Phthalates for TSCA COUs:** For TSCA COUs and populations, specific routes of exposure and pathways for each exposure source are identified. Exposure scenarios for individual TSCA COUs and estimates of exposure will be completed in the individual risk evaluations. Determinations on the likelihood of co-exposure to multiple phthalates in multiple TSCA COUs or multiple phthalates in a single TSCA COU will be completed in Step 7 of the conceptual model for consumers (Section 6.4.1), workers (6.4.2), and the general population, specifically fenceline communities (Section 6.4.3).
- **Step 5. Exposure Scenario-Building for Individual Phthalates for Non-Attributable and Non-TSCA Sources:** For identified sources of exposure (non-attributable or non-TSCA) and populations, specific routes of exposure and pathways for each exposure source are considered. Exposure scenarios are considered for major sources of exposure and exposure is estimated for the various pathways of exposure. Scenario-building to estimate non-attributable and non-TSCA exposures is discussed in Section 6.3.2.1.
- **Steps 6 to 9. Determining Cumulative Exposure Estimates:** Cumulative exposure potentially assessed under TSCA may be estimated by combining exposures from major exposure pathways from TSCA COUs, non-attributable, and non-TSCA sources that may lead to co-exposure over a relevant timeframe, which can mean exposure to multiple chemicals at the same time, exposure to persistent chemicals at different times that may bioaccumulate in the body, or having persistent effects from exposure to multiple chemicals at different times. This process involves:
    - **Step 6. Identifying Major Pathways of Exposure:** Determining the major pathways of exposure from TSCA COUs (completed in individual risk evaluations), non-attributable, and non-TSCA sources for each phthalate. Different pathways of exposure may be relevant for different populations and for different phthalates. For example, the human milk and formula-fed pathways are most relevant for infant scenario-building, while mouthing may be most relevant to infants and toddlers. Major pathways of exposure for individual phthalates may be combined at this step to estimate aggregate exposure.
    - **Step 7. Determining Co-exposure:** Determining likelihood of co-exposure across TSCA COUs, non-attributable sources, and non-TSCA sources for the various phthalates. Estimating the exposure associated with the consumer (Section 6.4.1), occupational (Section 6.4.2), and general population (fenceline) (Section 6.4.3) TSCA COU exposures and adding these exposures across COUs and across phthalates if reasonable. Determining reasonable cumulative exposure scenarios may involve considering the likelihood of co-exposure, the possibility of double counting, and of over- or under-estimating exposures
    - **Step 8. Convert Exposures to Index Chemical Equivalents:** Because EPA is proposing to use an RPF approach (Section 4.3.3), phthalate exposure from each individual phthalate will be scaled to the potency of an index chemical and expressed in units of index chemical equivalents.
    - **Step 9. Estimating Cumulative Exposure:** Combining the TSCA COU or release cumulative exposure, the relevant non-attributable TSCA cumulative exposure, and the

non-TSCA cumulative exposure to estimate cumulative exposure in a reasonable manner for consumer (Section 6.4.1), occupational (Section 6.4.2), and general population (Section 6.4.3).

- **Step 10. Estimate Cumulative Risk:** To estimate cumulative risk for each specific exposure scenario, an MOE (ratio of index chemical point of departure [POD] to cumulative exposure estimate expressed in index chemical equivalents [Step 9]) is calculated for comparison to the benchmark MOE (*i.e.*, the total uncertainty factor associated with the assessment) (Section 4.3.2). The lower the MOE (margin between the toxicity effect level and the exposure dose), the more likely a chemical is to pose a risk.



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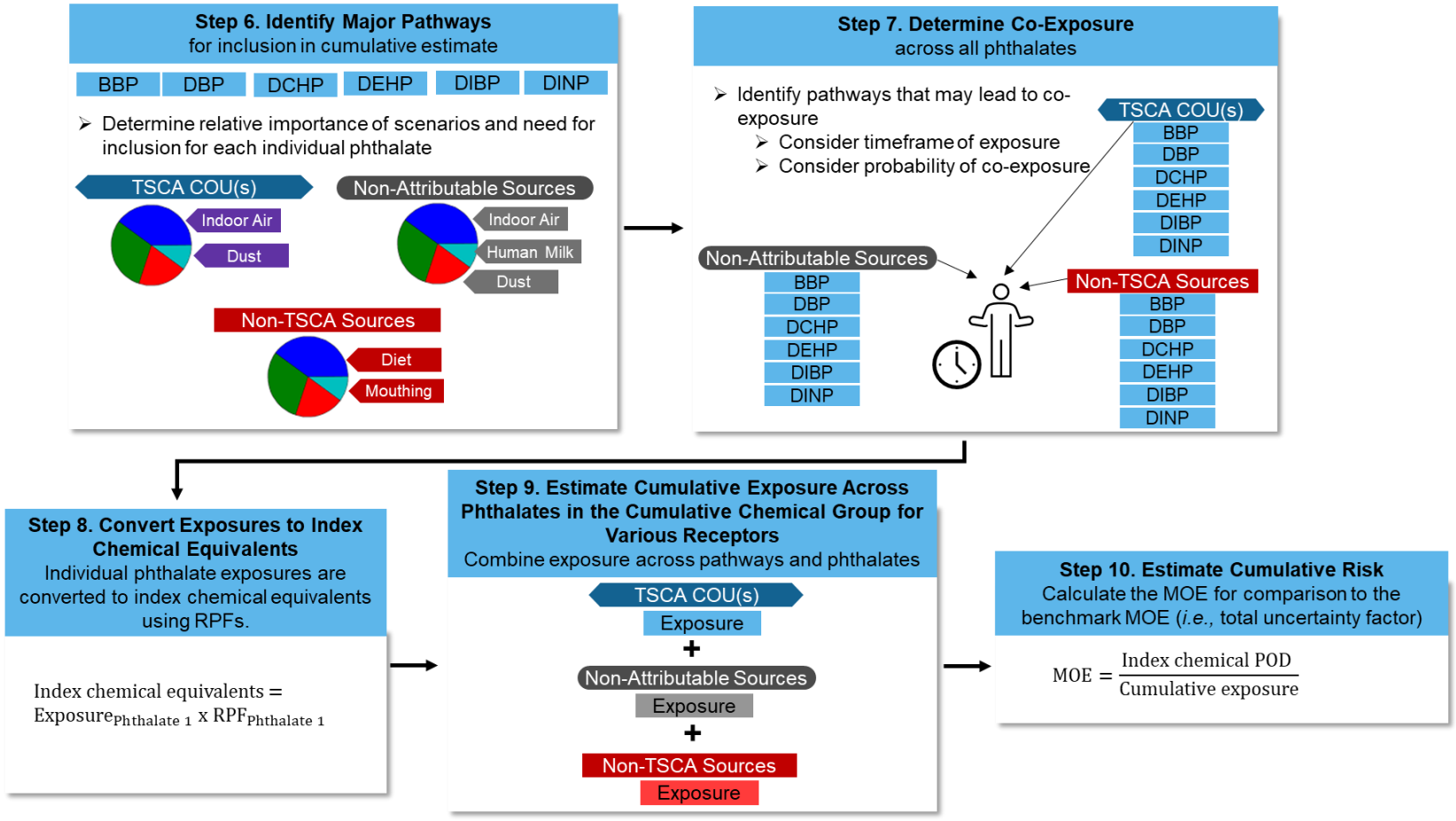

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858 **Figure 2-1. Cumulative Risk Assessment Conceptual Model**

3 CONSIDERATIONS FOR GROUPING PHTHALATES FOR CRA: STEP 1 IN CONCEPTUAL MODEL (Figure 2-1)

As described in EPA's Draft Proposed Principles of CRA under TSCA, there are two primary considerations for grouping chemicals for inclusion in a CRA, including (1) toxicologic similarity, and (2) evidence of co-exposure over a relevant timeframe. Figure 3-1 presents a decision tree for determining which of the high-priority (DEHP, BBP, DBP, DIBP, DCHP) and manufacturer-requested (DINP, DIDP) phthalates currently undergoing risk evaluation to group for CRA. The establishment of cumulative chemical group(s) for purposes of CRA is developed using a weight of evidence narrative that clearly characterizes the strengths and uncertainties of the evidence of toxicological similarity and potential co-exposure for each chemical considered. Evidence supporting the toxicologic similarity of the high-priority and manufacturer-requested phthalates is discussed in Section 3.1, evidence demonstrating co-exposure of humans to the high-priority and manufacturer-requested phthalates is discussed in Section 3.2, and EPA's proposed chemical substance grouping for CRA is summarized in Section 3.3.

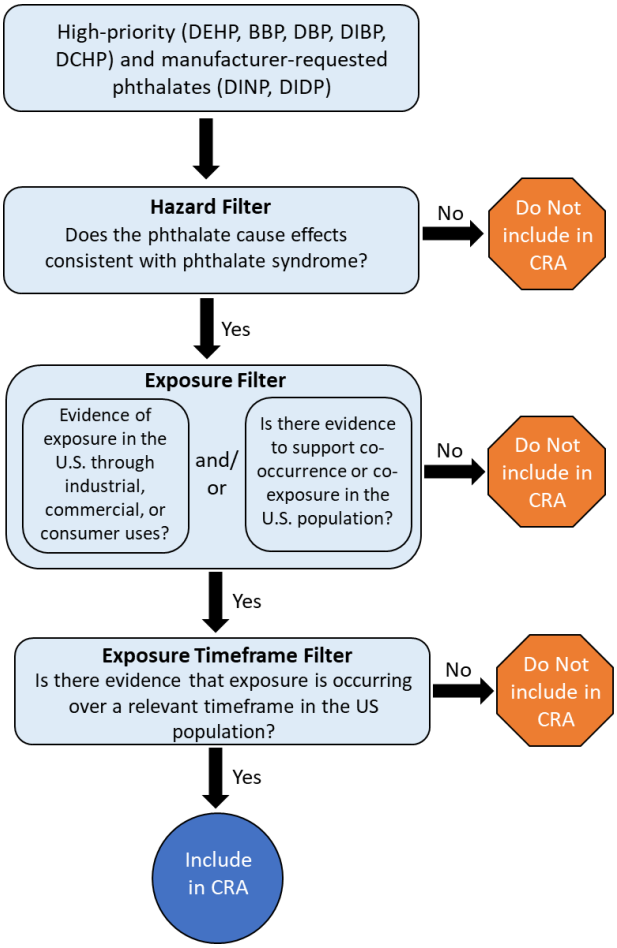

Figure 3-1. Decision Tree for Grouping Phthalates for CRA
Adapted from (EC/HC, 2015a).

3.1 Evidence of Toxicologic Similarity

As described in EPA's Draft Proposed Principles of CRA under TSCA, evidence for toxicological similarity exists along a continuum and includes (from most to least informative/restrictive):

- identical toxicodynamics (*i.e.*, same mode of action [MOA]) (same molecular initiating event [MIE], downstream key events, and apical outcome));
- similar toxicodynamics (*e.g.*, similar MOA [different MIE, convergent toxicodynamic pathways leading to a common downstream effect, and same apical outcome]);
- shared syndrome;
- shared apical outcome (MIE and other key events unknown);
- effect on the same target organ;
- structural similarity; and
- similarly shaped dose-response curves in comparable toxicity studies.

In considering which chemicals to include in a CRA, the NRC (2008) concluded that "…the effects that make up the androgen-insufficiency syndrome" should be included regardless of mechanism of action or chemical structure. In part, NRC's recommendation was based on the availability of *in vivo* mixture studies of phthalates and other antiandrogens with mixed MOAs that provide empirical evidence demonstrating the applicability of dose additive models (NRC, 2008). However, NRC also emphasized that mechanism of action data is still desirable for defining critical pathways, determining human relevance of observed effects, and reducing uncertainty in risk estimates.

Although NRC (2008) focused on the antiandrogenic effects of phthalates, the committee acknowledged that other health effects of phthalates may also be important. For example, liver toxicity, female reproductive toxicity, and neurodevelopmental outcomes have also been observed following exposure to some phthalates (as discussed in (ATSDR, 2022; EFSA, 2019; U.S. CPSC, 2014)). Further, stakeholders have urged EPA to consider assessing phthalates for cumulative risk based on not just their antiandrogenic effects on the male reproductive system, but also on the growing epidemiologic evidence of adverse neurodevelopmental outcomes (see Project TENDR and EarthJustice comments cited in footnotes in Section 1). EPA will consider these and the other health effects of phthalates as part of the individual phthalate risk evaluations. However, for these health effects, data appear more limited across the high-priority and manufacturer-requested phthalates and effects tend to occur at higher doses than observed for antiandrogenic effects. For example, with the potential exceptions of DIDP and DINP, recent phthalate risk assessments have concluded that the developing male reproductive system is more sensitive than the liver for most phthalate diesters (EFSA, 2019). This is further supported by a recent systematic review of DIBP animal toxicology studies conducted by EPA researchers in the Center for Public Health and Environmental Assessment (CPHEA), who found only slight evidence for female reproductive toxicity and liver toxicity but robust evidence for DIBP-induced male reproductive toxicity (*i.e.*, phthalate syndrome) (Yost et al., 2019). Additionally, the Agency for Toxic Substances and Disease Registry (ATSDR) recently identified neurodevelopmental effects in rodent models as a sensitive outcome following acute developmental exposures to DEHP (ATSDR, 2022). However, ATSDR also identified inconsistencies in the toxicological database and refrained from using this health outcome as the basis of a minimal risk level due to uncertainty in the database (see Appendix A [p. A-9] of (ATSDR, 2022) for further details).

Additionally, EPA CPHEA researchers recently conducted a systematic review and meta-analysis of epidemiologic studies of five phthalates (*i.e.*, DEHP, DINP, DBP, DIBP, BBP), which are also undergoing TSCA risk evaluation, and concluded that there is limited evidence supporting an association between prenatal phthalate exposure and neurodevelopmental outcomes such as cognition, motor effects, behavior (*e.g.*, attention-deficit/hyperactivity disorder [ADHD]), infant behavior, and social behavior (*e.g.*, autism spectrum disorder) (Radke et al., 2020).

Given the limitations and uncertainties discussed above, EPA believes that the most robust reasonably available dataset to support conducting a human health CRA is based on phthalate syndrome. Other health effects of the high-priority and manufacturer-requested phthalates will be evaluated as part of the individual phthalate risk evaluations. Following completion of systematic review for the individual phthalates, EPA may consider whether any new information would change this conclusion. Notably, EPA's proposal to focus on the shared ability of phthalates to disrupt androgen action and cause a common syndrome (*i.e.*, phthalate syndrome) is consistent with the recommendations of the NRC (2008) and with how other regulatory agencies (*i.e.*, U.S. CPSC, Australia NICNAS, EFSA, Danish EPA, and Health Canada) have evaluated phthalates for cumulative risk to human health (see Appendix A).
The remainder of Section 3.1 is organized as follows:

- Section 3.1.1, Phthalate Syndrome Mode of Action (MOA), provides a summary of the current state of the science regarding the proposed MOA for phthalate syndrome.
- Section 3.1.2, Key Outcomes for Grouping High-Priority and Manufacturer-Requested Phthalates for CRA, provides a description of the key outcomes assessed by EPA to support the proposed cumulative chemical group for CRA.
- Section 3.1.3, Key Outcomes Data, provides a summary of data available for each of the high-priority and manufacturer-requested phthalates underlying the key outcomes that EPA is evaluating to support the proposed cumulative chemical group for CRA.
- Section 3.1.4, Phthalate Syndrome in Humans, provides a summary of mechanistic explant and xenograft studies investigating phthalate syndrome in human fetal testis tissue and outlines several recent systematic reviews of human epidemiologic studies examining effects on the male reproductive system.
- Section 3.1.5, Species Differences in Sensitivity, provides a summary of differences in species sensitivity to phthalate-induced male reproductive toxicity.
- Section 3.1.6, Data Integration and Weight of Evidence Analysis, provides EPA's weight of evidence narrative to support development of a cumulative chemical group for CRA.
- Section 3.1.7, Proposed Conclusions on Toxicologic Similarity, summarizes EPA's proposed conclusions on the toxicological similarity of the high-priority and manufacturer-requested phthalates.

3.1.1 Phthalate Syndrome Mode of Action (MOA)

As can be seen from Figure 3-2, DEHP, DBP, BBP, DIBP, DCHP, DINP, and DIDP are structurally-related ortho phthalate diesters with varying length linear or branched alkyl or aryl ester chains. Gestational and/or postnatal exposure to certain structurally-related phthalates can lead to a spectrum of effects on the developing male reproductive system, known as phthalate syndrome. Phthalate syndrome is characterized by both androgen-dependent and -independent effects on the male reproductive system. The MOA for rat phthalate syndrome has been discussed by various organizations (NASEM, 2017; NRC, 2008), regulatory agencies (Health Canada, 2015; U.S. CPSC, 2014), and other research groups (Gray et al., 2021; Arzuaga et al., 2020; Howdeshell et al., 2017). To date, the MOA underlying phthalate syndrome has not been fully established; however, key cellular-, organ-, and organism-level effects are generally understood (Figure 3-3). Nevertheless, the molecular events preceding cellular changes remain unknown. Although androgen receptor antagonism and peroxisome proliferator-activated receptor alpha activation have been hypothesized to play a role, studies have generally ruled out the involvement of these receptors (Foster, 2005; Foster et al., 2001; Parks et al., 2000).

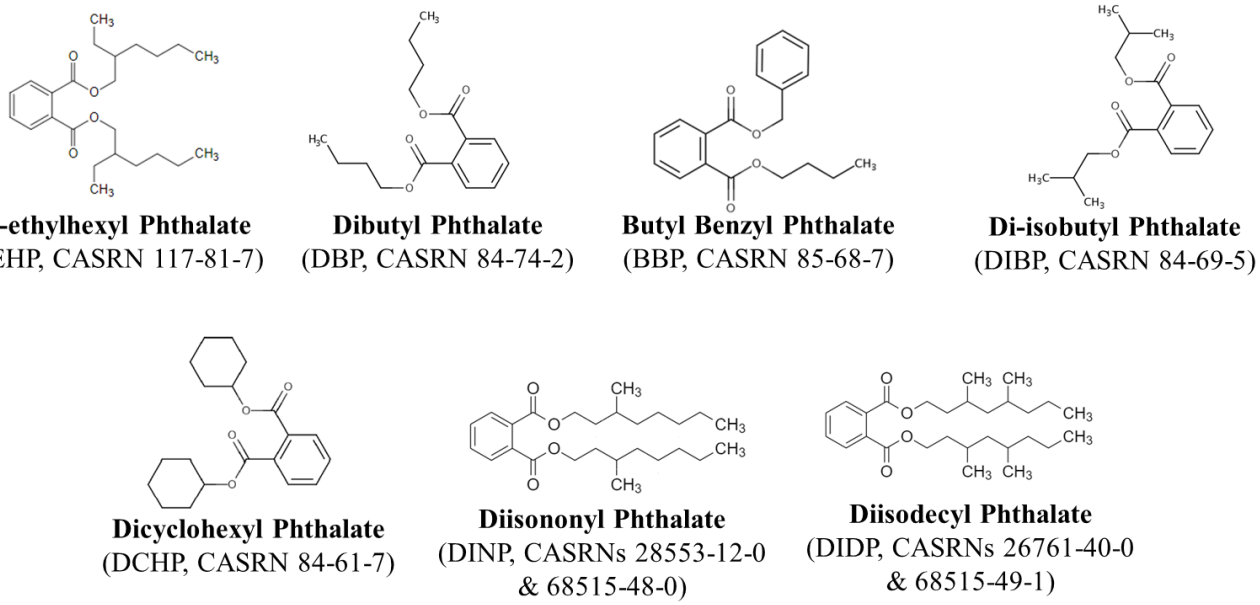

Figure 3-2. Chemical Structures of Phthalates Being Evaluated under TSCA
Representative structures are shown for DIDP and DINP, which are isomeric mixtures with branched ester carbon backbones varying in length (discussed further in Section 3.1.2.1).

Studies have demonstrated that gestational exposure to certain phthalate diesters, and their subsequent hydrolysis to monoester metabolites, which occur during a critical window of development (*i.e.*, the masculinization programming window) can lead to antiandrogenic effects on the developing male reproductive system (NRC, 2008). In rats, the masculinization programming window in which androgen action drives development of the male reproductive system occurs between days 15.5 to 18.5 of gestation, while the mouse critical window corresponds to gestational days 14 to 16, and the human masculinization programming window is between gestational weeks 8 to 14 (MacLeod et al., 2010; Welsh et al., 2008; Carruthers and Foster, 2005).

In vivo pharmacokinetic studies with rats have demonstrated that the monoester metabolites of DEHP, DBP, BBP, and DINP can cross the placenta and be delivered to the target tissue, the fetal testes (Clewell et al., 2013a; Clewell et al., 2010). *In utero* phthalate exposure can affect both Leydig and Sertoli cell function in the fetal testes. Histologic effects observed following phthalate exposure include Leydig cell aggregation and/or altered tissue distribution, as well as reductions in Leydig cell numbers. Functional effects on Leydig cells have also been reported. Leydig cells are responsible for producing hormones required for proper development of the male reproductive system, including insulin-like growth factor 3 (INSL3), testosterone, and dihydrotestosterone (DHT) (Scott et al., 2009). Phthalate exposure during the critical window reduces mRNA and/or protein levels of INSL3, as well as genes involved in steroidogenesis, sterol synthesis, and steroid and sterol transport (Figure 3-3) (Gray et al., 2021; Hannas et al., 2012).

Gene array experiments have demonstrated that phthalates known to disrupt testicular testosterone production alter a distinct cluster of genes (Gray et al., 2021). Key genes in this cluster are depicted in Figure 3-3 and include reductions in mRNA for proteins involved in steroid hormone and sterol transport (*Scarb1*, *StAR*); testis steroid hormone biosynthesis (*Cyp11A1*, *Hsd3b*, *Cyp17A1*, *Dhcr7*); testicular testosterone and peptide hormone INSL3 syntheses (*Insl3*); pituitary stimulation of Leydig cell testosterone synthesis (*Lhcgr*); testis development (*Inha*); and mRNA for enzymes involved in adrenal hormone synthesis (*e.g.*, *Cyp11b1*, *Cyp11b2*). Decreased steroidogenic mRNA expression leads to decreased fetal testicular testosterone production, as well as reductions in DHT levels, which is

produced from testosterone by 5α-reductase in the peripheral tissues. Because DHT is required for growth and differentiation of the perineum and for normal apoptosis of nipple anlage in male rats, reduced DHT levels can lead to phenotypic changes (*i.e.*, nipple/areolae retention [NR] and reduced anogenital distance [AGD] in males) indicative of reduced Leydig cell function and androgen action.

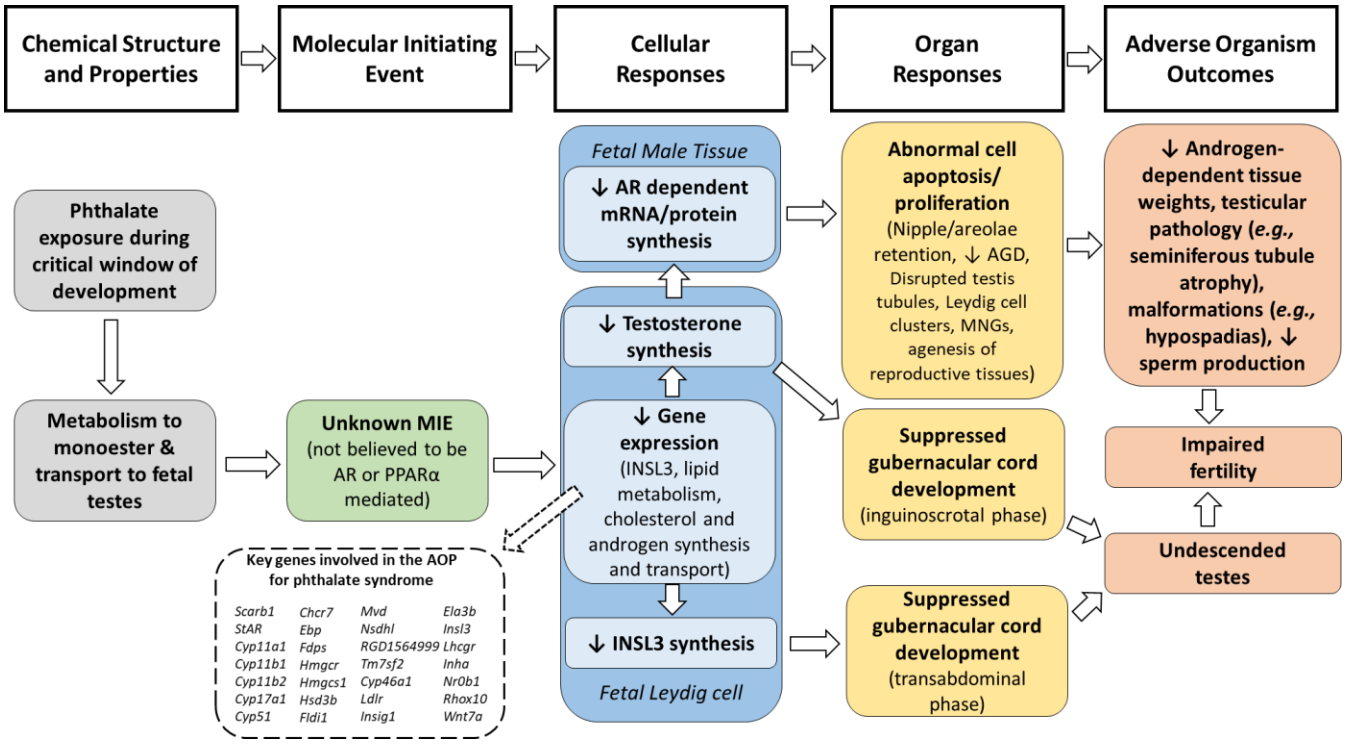

Figure 3-3. Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure
Figure adapted from (Conley et al., 2021; Gray et al., 2021; Schwartz et al., 2021; Howdeshell et al., 2017).
AR = androgen receptor; INSL3 = insulin-like growth factor 3; MNG = multinucleated gonocyte; PPARα = peroxisome proliferator-activated receptor alpha.

Gestational exposure to certain phthalate diesters can also affect Sertoli cell function, development, and interactions with germ cells contributing to seminiferous tubule degeneration (Boekelheide et al., 2009). Immature Sertoli cells secrete Anti-Müllerian hormone and play an essential role in gonadal development (Lucas-Herald and Mitchell, 2022). Reported Sertoli cell effects include decreased Sertoli cell numbers, changes in mRNA and/or protein levels of genes involved in Sertoli cell function, and altered cellular development and Sertoli-germ cell interactions. Because proper Sertoli cell function is necessary for germ cell proliferation and development, altered Sertoli cell function can contribute to increased germ cell death, decreased germ cell numbers, and increased formation of multinucleated gonocytes (MNGs) (Arzuaga et al., 2020).

At the organ level, a disruption of androgen action can lead to reduced testes and accessory sex gland (*e.g.*, epididymis, seminal vesicle [SV], prostate, etc.) weight; agenesis of accessory organs; delayed preputial separation (PPS); testicular pathology (*e.g.*, interstitial cell hyperplasia); and severe reproductive tract malformations such as hypospadias. INSL3 is crucial for gubernacular cord development and the initial transabdominal descent of the testes to the inguinal region (Adham et al., 2000), while androgen action is required for the inguinoscrotal phase of testicular descent. Thus, reduced INSL3 and testosterone levels following gestational phthalate exposure can prevent gubernaculum development and testicular descent into the scrotum. Collectively, these effects can lead

to reduced spermatogenesis, increased sperm abnormalities, and reduced fertility and reproductive function (Gray et al., 2021; Arzuaga et al., 2020; Howdeshell et al., 2017; NASEM, 2017; NRC, 2008).

Postnatal exposure of male pups to phthalate diesters that cause phthalate syndrome following exposure during the critical window of development can also lead to a disruption of Leydig and Sertoli cell function when exposure occurs at the peripubertal lifestage. The MOA for postnatal effects on male reproduction is similar to the MOA for gestational effects, with some distinctions. EPA researchers in CPHEA recently reviewed the MOA for DBP-induced male reproductive effects following postnatal exposure (Arzuaga et al., 2020). Briefly, cellular effects observed following peripubertal phthalate exposure include altered Leydig cell development and function and reduced steroidogenic enzyme expression and/or activity in the testes leading to reductions in testicular and/or serum testosterone levels. In the seminiferous cord, effects on Sertoli and germ cells have also been observed—including altered Sertoli cell development and function, altered interactions between Sertoli and germ cells, and disrupted germ cell development. The molecular events preceding these cellular changes have not been established. At the organ level, effects include incomplete development and/or reduced testes and accessory sex gland weight, as well as a disruption (*e.g.*, decreased organ weight, altered hormone levels) of the hypothalamic-pituitary-gonadal axis, which plays an important role in the development and function of the male reproductive system. Collectively, these effects can lead to decreased spermatogenesis and male fertility (Arzuaga et al., 2020).

3.1.2 Key Outcomes for Grouping High-Priority and Manufacturer-Requested Phthalates for CRA

To determine which high-priority and manufacturer-requested phthalates are toxicologically similar and appropriate for grouping for inclusion in a CRA, EPA reviewed studies that addressed seven key outcomes associated with phthalate syndrome.[7] The selected outcomes are not comprehensive of all the effects associated with phthalate syndrome, but instead were selected to inform EPA's cumulative chemical grouping for CRA based on EPA's current understanding of phthalate syndrome and its underlying MOA. Notably, many of the key outcomes have also been selected as the critical effect (or co-critical effect) in previous phthalate CRAs (Table 3-1). Key outcomes examined to support phthalate grouping based on toxicologic similarity include

1) Effects on fetal testicular expression of genes involved in steroidogenesis and Insl3 (Section 3.1.3.1). Reduced mRNA expression of cholesterol transport and steroidogenesis genes is believed to play an early role in the development of phthalate syndrome. Reduced expression of steroidogenic genes in the fetal testes leads to reduced testosterone production. *Insl3* expression was also selected to inform EPA's approach as it represents an androgen-independent mechanism that contributes to the development of phthalate syndrome. INSL3 is crucial for gubernacular cord development and the initial transabdominal descent of the testes to the inguinal region (Adham et al., 2000).

2) Effects on fetal testicular *testosterone* (Section 3.1.3.2). Testosterone is an androgen produced by fetal Leydig cells that is required for the proper development of the male reproductive system.

3) Effects on anogenital distance (AGD) (Section 3.1.3.3). Under the Organisation for Economic Co-operation and Development (OECD) guidance, decreased male AGD is considered a hallmark of antiandrogenic substances and should be considered an adverse effect relevant for

[7] The TSCA Work Plan includes one additional phthalate (*i.e.*, di-n-octyl phthalate) that is not currently prioritized for risk evaluation. However, Environment Canada/Health Canada (EC/HC, 2015e) concluded that di-n-octyl phthalate does not induce effects on the developing male reproductive system consistent with phthalate syndrome. Di-n-octyl phthalate is not further discussed in this document.

setting the NOAEL (OECD, 2013). DHT is an androgen derived from testosterone by the enzyme 5α-reductase. DHT functions to lengthen the perineum in fetal males relative to females. Reduced AGD in males at birth is indicative of a disruption of androgen action during development.

4) Nipple/areolae retention (NR) (Section 3.1.3.4). NR in male rats is a biomarker of disrupted androgen action during fetal development. During development, DHT, derived from testosterone produced by Leydig cells, is required for the normal regression of nipple anlage in male rats. Disrupted fetal testicular testosterone production is believed to contribute to NR in male pups by reducing DHT levels (Schwartz et al., 2021). Under OECD guidance NR in male pups is considered an adverse effect of exposure and should be considered relevant for setting the NOAEL (OECD, 2013).

5) Hypospadias (Section 3.1.3.5). Hypospadias is a malformation of the external male genitalia in which the urethra does not open on the tip of the penis. As discussed in NASEM (2017), mechanistic studies conducted with rats provide evidence that the formation of hypospadias (and other male reproductive tract malformations) is linked with reduced testosterone production by fetal Leydig cells (Howdeshell et al., 2015).

6) Seminiferous tubule atrophy/degeneration (Section 3.1.3.6). Germ cells develop into spermatozoa in close proximity to Sertoli cells in seminiferous tubules. Seminiferous tubule atrophy/degeneration is a pathologic lesion associated with phthalate syndrome frequently reported following *in utero* exposure to certain phthalates. Although there is uncertainty underlying the MOA associated with phthalate-induced effects on the seminiferous cord, seminiferous tubule atrophy was selected to serve as a key outcome because it is a sensitive adverse effect frequently reported by board-certified pathologists.

7) Multinucleated gonocytes (MNGs) (Section 3.1.3.7). Phthalates can affect Sertoli cell function, development, and interactions with germ cells. Proper Sertoli cell function is necessary for germ cell proliferation and development and altered Sertoli cell function contributes to increased germ cell death, decreased germ cell numbers, and increased formation of MNGs. Although there is uncertainty underlying the MOA associated with MNG formation, induction of MNGs is a sensitive indicator of exposure to a number of phthalates, and may serve as an indicator of altered Sertoli-germ cell interactions (Spade et al., 2018; Spade et al., 2014).

EPA's decision to focus its review on seven key outcomes associated with phthalate syndrome for purposes of grouping phthalates for CRA under TSCA is consistent with the approach used by Health Canada (EC/HC, 2015a; Health Canada, 2015). Health Canada developed a chemical category of phthalates for effects on the developing male reproductive system based on a structure-activity relationship (SAR) analysis. The SARs analysis focused on three key outcomes associated with the phthalate syndrome MOA, including effects on (1) steroidogenic gene expression, (2) fetal testicular testosterone production, and (3) AGD. The chemical category was then assessed for cumulative risk to human health (ECCC/HC, 2020). EPA's current approach expands on Health Canada's approach by assessing several additional key outcomes associated with phthalate syndrome, including testicular INSL3 mRNA expression, NR, hypospadias, seminiferous tubule atrophy, and MNG formation. Through EPA's systematic review of the individual phthalates additional key outcomes may be identified and EPA will assess these additional outcomes for relevance for inclusion in the CRA.

Table 3-1. Summary of Critical Effects Selected for Use in Previous Phthalate CRAs[a] [b]

Regulatory Agency	DEHP	BBP	DBP	DIBP	DCHP	DINP
Danish EPA (ECHA, 2011)	↓ testes weight, seminiferous tubule atrophy	↓ AGD	↓ spermatocyte development	↓ AGD, NR	–	–
Australia NICNAS (2015a, 2013, 2012)	↓ testes weight, seminiferous tubule atrophy	↓ testicular testosterone	↓ testicular testosterone	–	–	↓ testicular testosterone
Health Canada (ECCC/HC, 2020)	Small and/or aplastic epididymis, TP (including tubular atrophy), other rat phthalate syndrome effects	↓ AGD	↓ testicular testosterone, fertility effects, ↓ tubular & interstitial cell #, altered seminiferous tubule structure, effects on spermatocyte development	↓ AGD, NR, ↓ testicular testosterone, effects on fertility	↓ AGD, TP, ↑ resorption	MNGs, Leydig cell aggregation
EFSA (2019)	↓ testes weight, seminiferous tubule atrophy	↓ AGD	↓ spermatocyte development	–	–	↓ testicular testosterone, MNGs
U.S. CPSC (2014)[c]	↓ Spermatocytes & spermatids, reproductive tract malformations, delayed vaginal opening	↓AGD, NR	↓AGD, NR	↓AGD	–	NR

[a] Effects highlighted in gray indicate overlap with key outcomes selected for review by EPA in this document.
[b] DIDP is not shown in this table because it has not been included in previous phthalate CRAs. Studies have demonstrated that gestational exposure to DIDP does not disrupt development of the male reproductive system in a manner consistent with phthalate syndrome.
[c] Case 3 point of departures identified by U.S. CPSC's *de novo* literature review are shown.
AGD = anogenital distance; CPSC = Consumer Product Safety Commission; EFSA = European Food Safety Authority; MNG = multinucleated gonocytes; NR = nipple retention; TP = testicular pathology

3.1.2.1 Study Selection Strategy

As of the publication of this document, EPA has not completed its systematic review or data quality evaluation for the high-priority and manufacturer-requested phthalates. Therefore, a systematic review protocol was not employed by EPA to identify studies supporting the seven key outcomes assessed in this document. Instead, EPA conducted targeted literature searches and reviewed several documents prepared by various authoritative bodies and regulatory agencies (see list of documents in Section 1.1) to identify studies that support each of the seven key outcomes.

EPA focused its review on studies that were conducted using *in vivo* models and included an exposure that at a minimum covered the critical window of development (*i.e.*, GDs 15.5 to 18.5 in rats; GDs 14 to 16 in mice). This included both guideline and non-guideline studies and may include prenatal exposure studies, perinatal exposure studies, and single or multi-generation reproductive studies. Although, oral, dermal, and inhalation exposure studies were considered, the only studies identified that covered the

critical window were oral exposure studies. Lack of inhalation and dermal exposure studies is considered a data gap and is discussed further in Section 3.1.6.5. The majority of studies identified were conducted using rat models, however, studies conducted with other species (*i.e.*, mouse, rabbit, and primate) were also considered.

Finally, while DEHP, DIBP, DBP, BBP, and DCHP are discrete chemical substances, DIDP and DINP are isomeric mixtures with multiple CASRNs. DIDP (CASRNs 26761-40-0 and 68515-49-1) is an isomeric mixture with branched ester carbon backbones composed of 7 (approximately 0 to 10 percent) or ≥8 (approximately 70 to 90 percent) carbons (ECHA, 2013). Two different isomeric mixtures of DINP are commercially available, including DINP-1 (CASRN 68515-48-0) and DINP-2 (CASRN 28553-12-0), which contain linear and branched ester carbon backbones composed of 6 (5 to 10 percent for DINP-1 and -2), 7 (45 to 55 percent for DINP-1; 40 to 45 percent for DINP-2), or ≥8 (20 to 45 percent for DINP-1; 35 to 50 percent for DINP-2) carbons (ECHA, 2013). In the final scope documents for DINP (U.S. EPA, 2021c) and DIDP (U.S. EPA, 2021b), EPA determined that the two CASRNs for DINP and DIDP should be treated as categories of chemical substances as defined in 15 U.S.C § 2625(c). Therefore, EPA considered studies of both CASRNs for DINP and DIDP relevant for informing toxicological similarity.

3.1.2.2 Availability of Studies to Inform Key Outcomes

EPA reviewed the toxicology studies available for the high-priority and manufacturer-requested phthalates. While the amount of available data varies for each phthalate, data for all the proposed key outcomes were available for DEHP, BBP, DBP, DIBP, DCHP, DINP and DIDP, except data for MNGs for DIDP (Table 3-2). Additionally, although EPA's review focused on studies that assessed seven key outcomes, EPA extracted data for all phthalate syndrome-related effects reported in each reviewed study. Tables summarizing all observed phthalate syndrome-related effects for each study and phthalate can be found in Appendices B.2 (DEHP), B.3 (BBP), B.4 (DBP), B.5 (DIBP), B.6 (DCHP), B.7 (DINP), and B.8 (DIDP).

Table 3-2. Summary of Studies Supporting the Proposed Key Outcomes

Key Outcome	DEHP	BBP	DBP	DIBP	DCHP	DINP	DIDP
↓ Steroidogenic gene and *Insl3* expression (Section 3.1.3.1)	✓	✓	✓	✓	✓	✓	*x*
↓ Fetal testicular testosterone (Section 3.1.3.2)	✓	✓	✓	✓	✓	✓	*x*
↓ Anogenital distance (Section 3.1.3.3)	✓	✓	✓	✓	✓	✓	*x*
Nipple retention (Section 3.1.3.4	✓	✓	✓	✓	✓	✓	*x*
↑ Hypospadias (Section 3.1.3.5)	✓	✓	✓	✓	✓	*x*	*x*
Seminiferous tubule atrophy (Section 3.1.3.6)	✓	✓	✓	✓	✓	✓	*x*
↑ Multinucleated gonocytes (Section 3.1.3.7)	✓	✓	✓	✓	✓	✓	–

✓ Studies available, effects observed
x Studies available, no effects were observed

3.1.3 Key Outcomes Data

3.1.3.1 Fetal Testicular Gene Expression

3.1.3.1.1 Cholesterol Transport and Steroidogenesis

An early step in the hypothesized MOA for phthalate syndrome is a disruption of expression of cholesterol transport and steroidogenesis genes in the fetal testes. The molecular events preceding these cellular changes are unknown. The testicular steroidogenesis pathway is depicted in Figure 3-4. The scavenger receptor class B member 1 gene (*scarb1*) encodes the SR-B1 protein, which transports cholesterol into Leydig cells. The steroidogenic acute regulatory protein (encoded by *StAR* gene) transports cholesterol across the mitochondrial membrane, which is the rate-limiting step in testicular steroidogenesis (Petrescu et al., 2001). Cytochrome P450 family 11 subfamily A member (CYP11A1, also referred to as P450 side-chain cleavage enzyme [P450scc]) catalyzes the conversion of cholesterol to pregnenolone, which is next converted to progesterone by 3-beta-hydroxysteroid dehydrogenase (3β-HSD). Progesterone is then converted to androstenedione by CYP17A1, and then to testosterone by 17β-HSD.

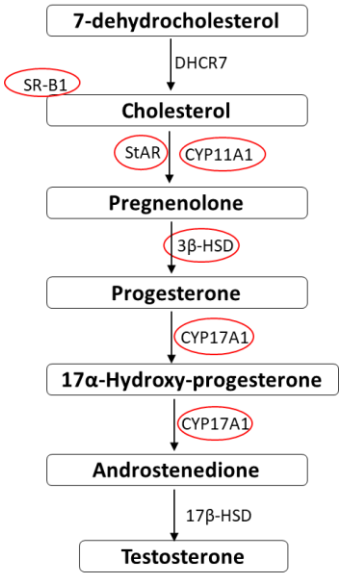

Figure 3-4. Testicular Steroidogenesis Pathway
Adapted from Hannas et al. (2012).
Red circles indicate genes assessed as part of the fetal testicular gene expression key outcome.

EPA identified 20 *in vivo* experimental studies published by multiple research groups that evaluated fetal testicular expression of key cholesterol transport (*i.e.*, *Scarb1*, *StAR*) and steroidogenesis (*i.e.*, *Cyp11a1*, *3bHSD*, *Cyp17A1*) genes following exposure during the critical window of development (Table 3-3). Identified studies were primarily conducted using rat models (18 rat studies and 2 mouse studies). Studies evaluating steroidogenesis were available for DEHP (six rat studies), BBP (one rat study), DBP (seven rat studies and one mouse study), DIBP (five rat studies and one mouse study), DCHP (two rat studies), DINP (five rat studies), and DIDP (two rat studies).

Across available rat studies (conducted with multiple strains, including Sprague-Dawley [SD], Wistar, and Long-Evans) of DEHP, BBP, DBP, DIBP, and DCHP, consistent dose-dependent decreases in mRNA expression of cholesterol transport and steroidogenesis genes in fetal testes were observed (Table 3-3; Figure_Apx B-1). In a study by Hannas et al. (2011), SD and Wistar rats were orally

exposed to DEHP during the critical window and then *StAR* and *Cyp11a1* mRNA was measured in the fetal testis. Similar dose-dependent decreases in mRNA expression of both genes were observed for both rat strains (Figure 3-5).

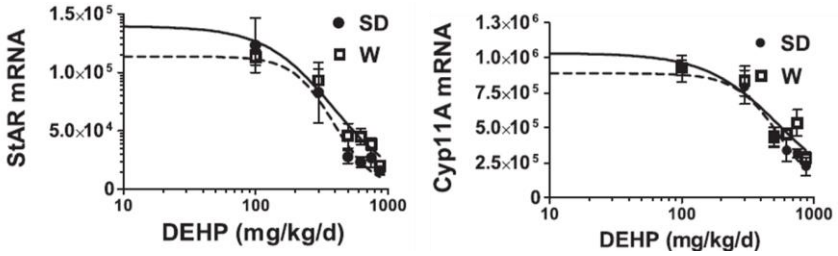

Figure 3-5. *CYP11A* and *StAR* mRNA Expression in SD and Wistar Rats
Adapted from (Hannas et al., 2011).

Time course experiments conducted with SD rats have demonstrated that changes in mRNA expression in the fetal testes occur rapidly following phthalate exposure (Johnson et al., 2012; Thompson et al., 2005). Thompson et al. observed reduced mRNA levels of *StAR* as early as 3-hours after a single gavage dose of 500 mg/kg DBP, while *Scarb1*, *Cyp11a1*, and *Cyp17a1* mRNA was reduced starting 6 hours post-exposure. Nearly identical results were obtained by Johnson et al. (*i.e.*, *Cyp17a1* mRNA was reduced 3 hours after a single gavage dose of 500 mg/kg DBP, while *StAR* and *Cyp11a1* mRNA was reduced starting 6 hours post-exposure).

The two available mouse studies (one for DIBP and DBP) provide somewhat contrasting results. Gestational exposure (GD 0 to 21) of ICR mice to 450 mg/kg DIBP resulted in reduced testicular expression of cholesterol transport and steroidogenesis genes, and the effect was more pronounced when exposure to dams continued through PND 21 resulting in ongoing lactational exposure to DIBP for the pups (Wang et al., 2017). However, this study measured gene expression on PND 21, and it is unclear if gene expression was disrupted during the critical window. In contrast, Gaido et al. (2007) conducted a series of microarray experiments in which CD-1 mice were exposed to a single acute dose (500 mg/kg on GD 18) or repeated doses (250 mg/kg/day on GDs 14 to 17) of DBP and found no effect on cholesterol transport or steroidogenesis gene expression. The doses of DBP administered to mice by Gaido et al. were greater than those shown to affect steroidogenic gene expression in rats, indicating mice are less sensitive than rats.

For DINP, dose-dependent decreases in mRNA expression of cholesterol transport and steroidogenesis genes were observed in four out of five studies (all conducted with SD rats) starting at doses as low as 100 mg/kg/day (Table 3-3; Figure_Apx B-1). In the one study where no effect was reported, treatment of SD rats with up to 750 mg/kg/day DINP throughout the critical window had no effect on testicular mRNA expression of *StAR*, *Cyp11a1*, or *3bHSD* (Adamsson et al., 2009).

For DIDP, two studies in which SD rats were gavaged with up to 1,500 mg/kg/day throughout the critical developmental window consistently found no effect on fetal testicular mRNA expression of cholesterol transport or steroidogenic genes (Gray et al., 2021; Hannas et al., 2012).

Gray et al. (2021) conducted a series of dose-response studies in which SD rats were gavaged with each of the five high-priority and two manufacturer-requested phthalates throughout the critical window of development (*i.e.*, GDs 14 to 18) and then evaluated fetal testicular mRNA expression of cholesterol transport and steroidogenesis genes. Dose-response data from this study are shown in Figure_Apx B-1. To compare phthalate potency at reducing mRNA expression, EPA calculated ED50 (the effective dose

that caused a 50 percent response) values for cholesterol transport and steroidogenesis genes for each phthalate (except DIDP) (Table 3-4). As can be seen in Table 3-4, estimated 95 percent confidence intervals overlap for most genes across phthalates, which limits the comparisons that can be made. However, several trends in the dataset are apparent. First, DCHP, DEHP, BBP, and DBP appear to be consistently more potent than DIBP at reducing fetal testicular mRNA expression, while DINP is consistently the least potent phthalate.

3.1.3.1.2 *Insl3* mRNA Expression

INSL3 is crucial for gubernacular cord development and the initial transabdominal descent of the testes to the inguinal region (Adham et al., 2000), while androgen action is required for the inguinoscrotal phase of testicular descent. Reduced INSL3 and testosterone levels following gestational phthalate exposure can prevent gubernaculum development and testicular descent into the scrotum. EPA identified 12 *in vivo* experimental studies published by multiple research groups that evaluated fetal testicular expression of *Insl3* mRNA following exposure to the high-priority and manufacturer-requested phthalates (Table 3-3). All identified studies were conducted using rat models. Studies evaluating *Insl3* mRNA were available for DEHP (6 rat studies), BBP (2 rat study), DBP (3 rat studies), DIBP (3 rat studies), DCHP (2 rat studies), DINP (4 rat studies), and DIDP (2 rat studies).

Consistent, dose-dependent reductions in *Insl3* mRNA were observed for DEHP, BBP, DBP, DIBP and DCHP across the available rat studies, regardless of strain tested (Table 3-3 and Figure_Apx B-1). In a study by Hannas et al. (2011), SD and Wistar rats were orally exposed to DEHP during the critical window and then *Insl3* mRNA was measured. A similar dose-response was observed for both strains, indicating no strain-specific differences (Figure 3-6).

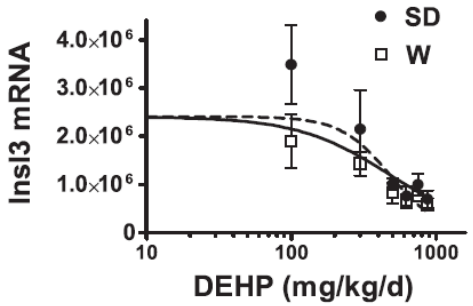

Figure 3-6. *Insl3* mRNA Expression in SD and Wistar Rats
Adapted from (Hannas et al., 2011).

For DINP, three out of four studies (all conducted with SD rats) demonstrate that DINP can reduce fetal testicular mRNA expression of *Insl3* in a dose-dependent manner at doses as low as 10 mg/kg/day. In the one study that did not report reduced *Insl3* mRNA expression, treatment of SD rats with 750 mg/kg/day DINP throughout the critical window of development caused a slight, but statistically significant, increase in *Insl3* mRNA (Adamsson et al., 2009). For DIDP, two studies in which SD rats were gavaged with up to 1,500 mg/kg/day throughout the critical window of development consistently found no effect on fetal testicular expression of *Insl3* mRNA (Gray et al., 2021; Hannas et al., 2012).

To support relative potency comparisons, ED50 values were calculated for reduced *Insl3* mRNA using dose-response data from Gray et al. (2021) (Figure_Apx B-1). As can be seen in Table 3-4, estimated 95 percent confidence intervals overlap for some phthalates, which limits the comparisons that can be made. However, from the available data, DCHP, DEHP, BBP, and DBP appear to be slightly more potent than DIBP at reducing fetal testicular *Insl3* mRNA expression, while DINP is the least potent of the phthalates.

1274 **Table 3-3. Studies Evaluating Fetal Testicular Steroidogenic Gene and *Ins3* mRNA Expression**

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	*Scarb1* (NOEL/ LOEL, mg/kg/d) [c]	*StAR* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp11a1* (NOEL/ LOEL, mg/kg/d) [c]	*3bHSD* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp17a1* (NOEL/ LOEL, mg/kg/d) [c]	*Insl3* (NOEL/ LOEL, mg/kg/d) [c]
DEHP	(Gray et al., 2021)	Rat (HSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	100/300	100/300	100/300	100/300	100/300	100/300
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	300/600	100/300	300/600	300/600	300/600	300/600
	(Hannas et al., 2011)	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 500, 625, 750, 875	–	300/500	300/500	–	–	500/625
		Rat (W)	GD 14–18 (GD 18)	0, 100, 300, 500, 625, 750, 875	–	300/500	300/500	–	–	300/500
	(Wilson et al., 2004)	Rat (SD)	GD 14–18 (GD 18)	0, 750	–	–	–	–	–	None/750
	(Saillenfait et al., 2013)	Rat (SD)	GD 12–19 (GD 19)	0, 50, 625	None/50	None/50	50/625	50/625	50/625	–
	(Borch et al., 2006b)	Rat (W)	GD 7–21 (GD 21)	0, 10, 30, 100, 300	100/300	30/100	100/300	–	NE [a]	100/300
	(Culty et al., 2008)	Rat (SD)	GD 14–19 (GD 19)	0, 234, 469, 938	–	NE	234/469	–	234/469	234/469
	(Lin et al., 2008)	Rat (LE)	GD 2–20 (GD 21)	0, 10, 100, 750	100/750	100/750	10/100	–	–	100/750
BBP	(Gray et al., 2021)	Rat (HSD)	GD 14–18 (GD 18)	0, 11, 33, 100, 300, 600, 900	11/33	33/100	11/33	33/100	100/300	11/33
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	300/600	300/600	300/600	600/900	300/600	300/600
	(Wilson et al., 2004)	Rat (SD)	GD 14–18 (GD 18)	0, 750	–	–	–	–	–	None/750
DBP	(Gray et al., 2021)	Rat (HSD)	GD 14–18 (GD 18)	0, 1, 10, 33, 50, 100, 300, 750	50/100	50/100	100/300	50/100	50/100	50/100

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	*Scarb1* (NOEL/ LOEL, mg/kg/d) [c]	*StAR* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp11a1* (NOEL/ LOEL, mg/kg/d) [c]	*3bHSD* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp17a1* (NOEL/ LOEL, mg/kg/d) [c]	*Insl3* (NOEL/ LOEL, mg/kg/d) [c]
DBP	(Wilson et al., 2004)	Rat (SD)	GD 14–18 (GD 18)	0, 750	–	–	–	–	–	None/750
	(Lehmann et al., 2004)	Rat (SD)	GD 12–19 (GD 19)	0, 0.1, 1, 10, 50, 100, 500	0.1/1	10/50	10/50	None/0.1	100/500	100/500
	(Struve et al., 2009)	Rat (SD)	GD 12–19 (GD 19)	0, 112, 582	None/112	None/112	None/112	–	None/112	–
			GD 12–19 (GD 20)	0, 112, 582	112/582	NE	112/582	–	112/582	–
	(Kuhl et al., 2007)	Rat (SD)	GD 18 (GD 19)	0, 100, 500	None/100	None/100	None/100	–	None/100	–
	(Drake et al., 2009)	Rat (W)	e13.5–16.5 (e17.5)	0, 500	–	None/500	None/500	–	–	–
	(Johnson et al., 2012)	Rat (SD)	GD 19 (1 h post dose)	0, 500	–	NE	NE	–	NE	–
			GD 19 (3 h)	0, 500	–	NE	NE	–	None/500	–
			GD 19 (6 and 18 h)	0, 500	–	None/500	None/500	–	None/500	–
	(Thompson et al., 2005)	Rat (SD)	GD 19 (0.5, 1, and 2 h post dose)	0, 500	NE	NE	NE	–	NE	–
			GD 19 (3 h)	0, 500	NE	None/500	NE	–	NE	–
			GD 19 (6, 12, 18, and 24 h)	0, 500	None/500	None/500	None/500	–	None/500	–
	(Gaido et al., 2007)	Mouse (CD-1)	GD 18 (2, 4, 8 hours after final dose)	0, 500	NE	NE	NE	–	NE	–
			GD 14–17 (2 hours after final dose)	0, 250	NE	NE	NE	–	NE	–
DIBP	(Gray et al., 2021)	Rat (HSD)	GD 14–18 (GD 18)	0, 100, 200, 300, 500, 600, 750, 900	100/200	100/200	200/300	100/200	100/200	200/300
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	–	–	–	–	–	–

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	*Scarb1* (NOEL/ LOEL, mg/kg/d) [c]	*StAR* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp11a1* (NOEL/ LOEL, mg/kg/d) [c]	*3bHSD* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp17a1* (NOEL/ LOEL, mg/kg/d) [c]	*Insl3* (NOEL/ LOEL, mg/kg/d) [c]
DIBP	(Hannas et al., 2011)	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	–	100/300	None/100	–	–	–
	(Hannas et al., 2012)	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	100/300	100/300	100/300	100/300	100/300	100/300
	(Saillenfait et al., 2017)	Rat (SD)	GD 13–19 (GD 19)	0, 250	None/250	None/250	NE	NE	None/250	–
	(Boberg et al., 2008)	Rat (W)	GD 17–21 (GD 19 or 21)	0, 600	None/600	None/600	None/600	–	None/600	None/600
	(Wang et al., 2017)	Mouse (ICR)	GD 0–21 (PND 21)	0, 450	–	NE	None/450	NE	NE	–
			GD 0–PND 21 (PND 21)	0, 450	–	NE	None/450	None/450	None/450	–
DCHP	(Gray et al., 2021)	Rat (HSD)	GD 14–18 (GD18)	0, 33, 100, 300, 600, 900	33/100	33/100	33/100	33/100	33/100	33/100
		Rat (CRSD)	GD 14–18 (GD18)	0, 100, 300, 600, 900	–	–	–	–	–	–
	(Li et al., 2016)	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500, 1,000	100/500	None/10	NE [a]	None/10	NE [a]	10/100
DINP	(Gray et al., 2021)	Rat (HSD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	None/500	None/500	None/500	None/500	None/500	None/500
	(Hannas et al., 2011)	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	–	750/1,000	750/1,000	–	–	–
	(Hannas et al., 2012)	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	None/500	None/500	None/500	None/500	None/500	None/500
	(Li et al., 2015a)	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500, 1000	NE	100/500	10/100	10/100	10/100	None/10
	(Adamsson et al., 2009)	Rat (SD)	e13.5–17.5 (e19.5)	0, 250, 750	–	NE	NE	NE	–	250/750 [b]
DIDP	(Gray et al., 2021)	Rat (CRSD)	GD 14–18 (GD 18)	0, 300, 750, 1,000, 1,500	NE	NE	NE	NE	NE	NE

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	*Scarb1* (NOEL/ LOEL, mg/kg/d) [c]	*StAR* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp11a1* (NOEL/ LOEL, mg/kg/d) [c]	*3bHSD* (NOEL/ LOEL, mg/kg/d) [c]	*Cyp17a1* (NOEL/ LOEL, mg/kg/d) [c]	*Insl3* (NOEL/ LOEL, mg/kg/d) [c]
	(Hannas et al., 2012)	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	NE	NE	NE	NE	NE	NE

[a] Apparent dose-related decrease in gene expression, however, statistical significance was not achieved.
[b] Adamsson et al. (2009) report a slight, but statistically significant, increase in mRNA expression of *Insl3* at the highest dose tested.
[c] NOEL/LOEL values reflect study authors statistical analysis (*i.e.*, the LOEL is the lowest value where a statistically significant effect was observed).
"–" = gene was not measured in the study; CRSD = Charles River Sprague-Dawley; e = embryonic day; GD = gestational day; HSD = Harlan Sprague-Dawley; LE = Long Evans; NE = no effect; LOEL = lowest observed effect level; NOEL = no observed effect level; PND = postnatal day; SD = Sprague-Dawley; W = Wistar

Table 3-4. ED50 Values (mg/kg/day) for Reduced mRNA Expression of Steroidogenic Genes and *Insl3*

Phthalate	Star ED50 (95% CI)	*Scarb1* ED50 (95% CI)	*Cyp11a1* ED50 (95% CI)	*Cyp17a1* ED50 (95% CI)	*3bHSD* ED50 (95% CI)	*Insl3* ED50 (95% CI)
DCHP	99 (48, 202)	62 (40, 96)	129 (49, 338)	53 (30, 92)	95 (37, 244)	162 (97, 270)
DEHP	109 (33, 196)	120 (62, 178)	173 (102, 249)	134 (101, 168)	242 (80, 503)	158 (104, 215)
BBP	77 (46, 129)	50 (20, 121)	126 (59, 266)	180 (129, 251)	164 (72, 372)	167 (65, 434)
DBP	247 (74, 824)	295 (111, 779)	367 (170, 793)	285 (186, 437)	530 (288, 974)	237 (149, 376)
DIBP	324 (201, 523)	287 (159, 519)	407 (253, 654)	371 (219, 626)	595 (325, 1,089)	414 (261, 656)
DINP	592 (493, 709)	594 (440, 802)	1148 (862, 1,530)	802 (698, 921)	1,016 (750, 1,376)	1,537 (730, 3,236)

ED50 values and 95% confidence intervals (95% CI) were estimated using data from dose-response experiments conducted by Gray et al. (2021) with Harlan SD rats (Figure_Apx B-1). ED50s were calculated using methods described in Gray et al. (2021).

3.1.3.2 Fetal Testicular Testosterone

Testosterone is necessary for the proper development of the male reproductive system and a disruption of testosterone levels during the masculinization programming window (*i.e.*, GDs 15.5 to 18.5 in rats (Welsh et al., 2008)) contributes to the spectrum of effects that make up phthalate syndrome. EPA identified a large amount of *in vivo* experimental data (38 studies from multiple laboratories) that support this key outcome (Table 3-5). Available studies have primarily been conducted using rat models (34 rat and 4 mouse studies identified). DEHP (13 rat studies and 2 mouse studies), DBP (16 rat studies and 1 mouse study) and DINP (9 rat studies) have the largest amount of data available, while fewer studies are available for DIBP (5 rat studies and 1 mouse study), BBP (5 rat studies), DCHP (3 rat studies) and DIDP (3 rat studies).

As can be seen in Table 3-5, available rat studies (conducted with Wistar, SD, and Long-Evans strains) of DEHP, BBP, DBP, DIBP, and DCHP provide consistent evidence that gestational exposure during the critical window of development leads to reduced fetal testicular testosterone and/or *ex vivo* fetal testicular testosterone production. Notably, the effect on fetal testicular testosterone consistently occurred in a dose-dependent manner (see Figure_Apx B-1, which presents dose-response data for the five high-priority and two manufacturer-requested phthalates (Gray et al., 2021)) and was large in magnitude at the lowest LOEL identified for DEHP (28 percent decrease at 50 mg/kg/d (Saillenfait et al., 2013)), BBP (53 percent decrease at 100 mg/kg/d (Furr et al., 2014)), DBP (40 percent decrease at 50 mg/kg/d (Lehmann et al., 2004)), DIBP (55 percent decrease at 250 mg/kg/d (Saillenfait et al., 2017)) and DCHP (25 percent decrease at 33 mg/kg/d (Gray et al., 2021)). Time course experiments conducted with SD rats have demonstrated rapid reductions in fetal testicular testosterone following phthalate exposure (Johnson et al., 2012; Thompson et al., 2005). Thompson et al. reported a 50 percent reduction in fetal testicular testosterone as early as 1 hour after a single gavage dose to 500 mg/kg DBP, while Johnson et al. reported an approximate 60 percent reduction in testosterone starting 18 hours after a single gavage dose of 500 mg/kg DBP.

The four available mouse studies (one each of DBP and DIBP and two of DEHP) provide somewhat contrasting results. Gestational exposure during the critical window to 450 mg/kg/day DIBP reduced postnatal testicular testosterone and *ex vivo* testicular testosterone production in ICR mice on PND 21 (Wang et al., 2017); however, effects on testicular testosterone were not evaluated during the fetal lifestage in this study. In contrast, exposure to 1,000 to 1,500 mg/kg/day MBP or DBP during the critical window did not affect fetal testicular testosterone in C57B1/6J mice (Gaido et al., 2007). Similarly, gestational exposure of CD-1 mice to doses of up to 500 mg/kg/day DEHP (Do et al., 2012) or C57B1/6J mice to doses of 500 to 1,000 mg/kg/day MEHP did not affect testicular testosterone (Gaido et al., 2007).

For DINP, effects on testosterone from the nine available rat studies were slightly less consistent. In 7 out of 9 studies, gestational exposure to DINP throughout the critical window of development dose-dependently reduced fetal testicular testosterone levels and/or *ex vivo* testosterone production (Table 3-5 and Figure_Apx B-1). Two studies (Clewell et al., 2013b; Adamsson et al., 2009) did not report an effect on fetal testicular testosterone following gestational exposure at doses that caused an effect in other studies (*i.e.*, 720 to 750 mg/kg/day DINP). Inconsistencies may be due to differences in phthalate potency (*i.e.*, DINP is less potent than other phthalates at disrupting steroidogenic gene expression (Table 3-4) and testosterone (Table 3-6)), as well as timing of when testosterone was measured. For example, Clewell et al. (2013a) gavaged rats with up to 750 mg/kg/day DINP on GDs 12 to 19 and measured testicular testosterone levels at 2 and 24 hours after the final dose. Testicular testosterone

levels were reduced 50 to 65 percent in the two highest treatment groups two hours, but not 24 hours, after the final dose indicating a transient effect on testosterone.

For DIDP, three studies (all conducted with SD rats) were identified that investigated effects on fetal testicular testosterone production. All three studies consistently found that exposure to DIDP throughout the critical window had no effect on *ex vivo* fetal testicular testosterone production at doses as high as 1,500 mg/kg/day (Table 3-5). This is consistent with studies showing DIDP not affecting mRNA expression of steroidogenic genes (Section 3.1.3.1.1).

Differences in the potency of the high-priority and manufacturer-requested phthalates to reduce fetal testicular testosterone in rats are apparent. ED50 values for reduced *ex vivo* fetal testicular testosterone production for these phthalates are reported by Furr et al. (2014) and shown in Table 3-6. Similarly, Gray et al. (2021) report dose-response studies evaluating *ex vivo* fetal testicular testosterone production in rats for the high-priority and manufacturer-requested phthalates. EPA used this dose-response data (Figure_Apx B-1) to calculate ED50 values for reduced *ex vivo* fetal testicular testosterone production. As can be seen from Table 3-6, estimated 95 percent confidence intervals overlap for some phthalates. However, data from both Furr et al. and Gray et al. indicate that DCHP, DEHP, and DBP are slightly more potent than BBP and DIBP at reducing fetal testicular testosterone production, while DINP is clearly the least potent.

EPA's findings are consistent with a recent systematic review and meta-analysis conducted by NASEM (2017). NASEM assessed experimental animal evidence for effects on fetal testicular testosterone following *in utero* exposure to DEHP, BBP, DBP, DIBP, and DINP (DCHP not included in analysis) using the systematic review methodology developed by the National Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT). NASEM found high confidence in the body of evidence and a high level of evidence that fetal exposure to DEHP, BBP, DBP, DIBP, and DINP is associated with a reduction in fetal testosterone in rats. Furthermore, NASEM found a statistically significant overall effect and linear trends in $\log_{10}$(dose) and dose, with an overall large magnitude of effect (>50 percent), for DEHP, BBP, DBP, DIBP, and DINP in their respective meta-analyses. For DEHP, NASEM found that data were amenable to conducting separate subgroup analyses of SD and Wistar rat strains. Meta-analysis found that SD rats were slightly more sensitive to DEHP than Wistar rats (Table 3-7). Benchmark dose (BMD) values based on benchmark response (BMR) values of 5 and 40 percent were calculated by NASEM and are shown in Table 3-7. A comparison of BMD values indicates similar trends in potency as was observed based on ED50 values calculated by EPA.

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Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	Dose-Response Observed?	NOEL (mg/kg/d) [f]	LOEL (mg/kg/d) [f]	% Decrease from Control at LOEL
DEHP	(Saillenfait et al., 2013) [b]	Rat (SD)	GD 12–19 (GD 19)	0, 50, 625	Yes	None	50	28%
	(Furr et al., 2014) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	None	100	21–63% [c]
	(Gray et al., 2021) [a]	Rat (HSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	None	100	38%
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	29%
	(Culty et al., 2008) [a]	Rat (SD)	GD 14–20 (GD 20)	0, 117, 234, 469, 938	Yes	None	117	60% [d]
	(Hannas et al., 2011) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 500, 625, 750, 875	Yes	100	300	39%
		Rat (W)	GD 14–18 (GD 18)	0, 100, 300, 500, 625, 750, 875	Yes	100	300	50%
	(Howdeshell et al., 2008) [a]	Rat (SD)	GD 8–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	42%
	(Borch et al., 2006b) [a b]	Rat (W)	GD 7–21 (GD 21)	0, 10, 30, 100, 300	Yes	100	300	60–80% [a b d]
	(Borch et al., 2004) [a b]	Rat (W)	GD 7–21 (GD 21)	0, 300, 750	Yes	None	300	70% [a b d]
	(Lin et al., 2008) [b]	Rat (LE)	GD 2–20 (GD 21)	0, 10, 100, 750	Yes	100	750	67%
	(Parks et al., 2000) [a]	Rat (SD)	GD 14–17 (GD17)	0, 750	–	None	750	54%
			GD 14–18 (GD 18)	0, 750	–	None	750	59%
			GD 14–20 (GD 20)	0, 750	–	None	750	57%
			GD 14–PND 2 (PND 2)	0, 750	–	None	750	42%
	(Spade et al., 2018) [a]	Rat (SD)	GD 17–21 (GD 21)	0, 750	–	None	750	62%
	(Wilson et al., 2004) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 750	–	None	750	50% [d]
	(Martino-Andrade et al., 2008) [b]	Rat (W)	GD 13–21 (GD 21)	0, 150	–	150	None	–

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	Dose-Response Observed?	NOEL (mg/kg/d) [f]	LOEL (mg/kg/d) [f]	% Decrease from Control at LOEL
	(Do et al., 2012) [b]	Mouse (CD-1)	GD 9–18 (GD 18)	0, 0.0005, 0.001, 0.005, 0.5, 50, 500	No	500	None	–
	(Gaido et al., 2007) [b]	Mouse (C57B1/6J)	GD 14–16 (GD 17)	0, 500 (MEHP)	No	500	None	–
			GD 15–17 (GD 17)	0, 1000 (MEHP)	No	1000	None	–
BBP	(Furr et al., 2014) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	None	100	53%
				0, 11, 33, 100	No	100	None	–
	(Howdeshell et al., 2008) [a]	Rat (SD)	GD 8–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	22%
	(Gray et al., 2021) [a]	Rat (HSD)	GD 14–18 (GD 18)	0, 11, 33, 100, 300, 600, 900	Yes	33	100	27%
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	38%
	(Spade et al., 2018) [a]	Rat (SD)	GD 17–21 (GD 21)	0, 750	–	None	750	69%
	(Wilson et al., 2004) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 750	–	None	750	80% [d]
DBP	(Lehmann et al., 2004) [b]	Rat (SD)	GD 12–19 (GD 19)	0, 0.1, 1, 10, 50, 100, 500	Yes	10	50	40% [d]
	(Furr et al., 2014) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 33, 50, 100, 300	Yes	50	100	35%
	(Gray et al., 2021) [a]	Rat (HSD)	GD 14–18 (GD 18)	0, 1, 10, 33, 50, 100, 300, 750	Yes	50	100	32%
	(Mahood et al., 2007) [b]	Rat (W)	GD 13.5–20.5 (GD 21.5)	0, 4, 20, 100, 500	Yes	20	100	14% [d]
	(Struve et al., 2009) [b]	Rat (SD)	GD 12–19 (GD 20)	0, 112, 582	Yes	None	112	70%
	(Howdeshell et al., 2008) [a]	Rat (SD)	GD 8–18 (GD 18)	0, 33, 50, 100, 300, 600	Yes	100	300	34%
	(Li et al., 2015b) [b]	Rat (W)	e12.5–20.5 (e17.5)	0, 100, 300, 900	Yes	100	300	75% [d]
			e12.5–20.5 (e19.5)	0, 100, 300, 900	Yes	300	900	50% [d]
			e12.5–20.5 (e21.5)	0, 100, 300, 900	Yes	300	900	40% [d]

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	Dose-Response Observed?	NOEL (mg/kg/d) [f]	LOEL (mg/kg/d) [f]	% Decrease from Control at LOEL
DBP	(Martino-Andrade et al., 2008) [b]	Rat (W)	GD 13–21 (GD 21)	0, 100, 500	Yes	100	500	63%
	(Kuhl et al., 2007) [b]	Rat (SD)	GD 18 (GD 19)	0, 100, 500	Yes	100	500	85%
	(Mylchreest et al., 2002) [b]	Rat (SD)	GD 12–21 (GD 18)	0, 500	–	None	500	66%
			GD 12–21 (GD 21)	0, 500	–	None	500	74%
	(MacLeod et al., 2010) [b]	Rat (W)	e13.5–21.5 (e20.5)	0, 500	–	None	500	–
	(Drake et al., 2009) [b]	Rat (W)	e13.5–16.5 (e17.5)	0, 500	–	None	500	40% [d]
	(Johnson et al., 2012) [b]	Rat (SD)	GD 19 (1, 3, and 6 hours post-dose)	0, 500	–	500	None	–
			GD 19 (18 hours)	0, 500	–	None	500	60% [d]
	(Thompson et al., 2005) [b]	Rat (SD)	GD 19 (0.5 hours post-dose)	0, 500	–	500	None	–
			GD 19 (1, 2, 3, 6 hours)	0, 500	–	None	500	50% [d]
			GD 19 (12, 18, 24 hours)	0, 500	–	None	500	75% [d]
	(van den Driesche et al., 2012) [b]	Rat (W)	e13.5–20.5 (e21.5)	0, 500, 750	Yes	None	500	70% [d]
			e13.5–20.5 (e21.5)	0, 750	–	None	750	35% [d]
	(Spade et al., 2018) [a]	Rat (SD)	GD 17–21 (GD 21)	0, 750	–	None	750	75%
	(Wilson et al., 2004) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 1000	–	None	1000	85% [d]
	(Gaido et al., 2007) [b]	Mouse (C57B1/6J)	GD 14–16 (GD 17)	0, 1000 (MBP)	–	1000	None	–
			GD 14–16 (GD 17)	0, 1500 (DBP)	–	1500	None	–
			GD 15–17 (8 hours post-final dose)	0, 1000 (MBP)	–	1000	None	–
DIBP	(Saillenfait et al., 2017) [a]	Rat (SD)	GD 13–19 (GD 19)	0, 250	–	None	250	55%
	(Gray et al., 2021) [a]	Rat (HSD)	GD 14–18 (GD 18)	0, 100, 200, 300, 500, 600, 750, 900	Yes	200	300	66%

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	Dose-Response Observed?	NOEL (mg/kg/d) [f]	LOEL (mg/kg/d) [f]	% Decrease from Control at LOEL
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	34%
	(Hannas et al., 2011) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	66%
	(Howdeshell et al., 2008) [a]	Rat (SD)	GD 8–18 (GD 18)	0, 100, 300, 600, 900	Yes	100	300	40%
	(Borch et al., 2006a) [a b]	Rat (W)	GD 7–20/21 (GD 20/21)	0, 600	–	None	600	90% [a b d]
	(Wang et al., 2017) [b]	Mouse (ICR)	GD 0–21 (PND 21)	0, 450	–	None	450	50% [b d]
			GD 0–PND 21 (PND 21)	0, 450	–	None	450	50% [b d]
DCHP	(Gray et al., 2021) [a]	Rat (HSD)	GD 14–18 (GD 18)	0, 33, 100, 300, 600, 900	Yes	None	33	25%
		Rat (CRSD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	None	100	41%
	(Furr et al., 2014) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 100, 300, 600, 900	Yes	33	100	69%
				0, 33, 100, 300	Yes	33	100	55%
	(Li et al., 2016) [b]	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500	Yes	10	100	38%
DINP	(Clewell et al., 2013a) [b]	Rat (SD)	GD 12–19 (2 hours post final dose)	0, 50, 250, 750	Yes	50	250	50%
			GD 12–19 (24 hours post final dose)	0, 50, 250, 750	No	750	None	–
	(Boberg et al., 2011) [a b]	Rat (W)	GD 7–PND 17 (GD 21)	0, 300, 600, 750, 900	–[e]	300	600	50% [b d]
	(Gray et al., 2021) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	Yes	None	500	29%

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	Dose-Response Observed?	NOEL (mg/kg/d) [f]	LOEL (mg/kg/d) [f]	% Decrease from Control at LOEL
	(Hannas et al., 2011) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	Yes	None	500	30%
	(Furr et al., 2014) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 750	–	None	750	24-50% [c]
	(Borch et al., 2004) [a b]	Rat (W)	GD 7–21 (GD 21)	0, 750	–	None	750	65% [a d] 75% [b d]
	(Li et al., 2015a) [b]	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500, 1,000	Yes	500	1000	57%
	(Adamsson et al., 2009) [b]	Rat (SD)	e13.5–17.5 (e19.5)	0, 250, 750	No	750	None	–
	(Clewell et al., 2013b) [b]	Rat (SD)	GD 12–PND 14 (PND 49–50)	0, 56, 288, 720	No	720	None	–
DIDP	(Hannas et al., 2012) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	No	1500	None	–
	(Furr et al., 2014) [a]	Rat (SD)	GD 14–18 (GD 18)	0, 500, 750, 1,000, 1,500	No	1500	None	–
	(Gray et al., 2021) [a]	Rat (CRSD)	GD 14–18 (GD 18)	0, 300, 750, 1,000, 1,500	No	1500	None	–

[a] *Ex vivo* fetal testicular testosterone production measured.
[b] Testes testosterone level measured.
[c] Range reflects results from multiple studies conducted using the same doses and methods reported within the publication (Blocks 31–32 for DEHP; Blocks 1, 5, and 7 for DINP).
[d] Value estimated based on graphical presentation of data.
[e] Fetal testicular testosterone was significantly reduced at 600 mg/kg/d DINP and appear reduced at higher doses, however, the effect at higher doses was not statistically significant. Testicular testosterone production appeared reduced by ≥50% at doses ≥300 mg/kg/d DINP, however, the effect was not statistically significant due to variability in the control samples (Boberg et al., 2011).
[f] NOEL/LOEL values reflect study authors statistical analysis (*i.e.*, the LOEL is the lowest value where a statistically significant effect was observed).
CRSD = Charles River Sprague-Dawley; e = embryonic day; GD = gestational day; HSD = Harlan Sprague-Dawley; LE = Long Evans; LOEL = lowest-observed-effect-level; NOEL = no-observed-effect-level; PND = postnatal day; SD = Sprague-Dawley; W = Wistar

Table 3-6. ED50 Values for Reduced *ex vivo* Fetal Testicular Testosterone Production

Phthalate	ED50 (95% CI) (mg/kg/day) (Furr et al., 2014)	ED50 (95% CI) (mg/kg/day) (Gray et al., 2021)[a]
DCHP	62 (40, 96)	91 (46, 180)
DEHP	121 (92, 160)	143 (132, 156)
DBP	158 (101, 248)	154 (88, 268)
BBP	172 (116, 257)	228 (150, 347)
DIBP	288 (248, 335)	275 (226, 334)
DINP	738 (617, 884)	918 (780, 1,081)

[a] ED50 values and 95% confidence intervals (95% CI) were estimated using data from dose-response experiments conducted by Gray et al. (2021) with Harlan Sprague-Dawley rats (Figure_Apx B-1). ED50s calculated using methods described in Gray et al. (2021).

Table 3-7. Summary of NASEM (2017) Systematic Review and Meta-Analysis Results for Effects on Fetal Testosterone

Phthalate	Database Supporting Outcome	Confidence in Evidence	Evidence of Outcome	Heterogeneity	Model with Lowest AIC	BMD_5 mg/kg/day (95% CI)	BMD_{40} [c] mg/kg/day (95% CI)
DEHP[a]	11 rat studies & 1 mouse study	High	High	I^2 > 90% (combined)	Linear quadratic	15 (11, 24)	160 (120, 240)
				I^2 > 95% (SD)	Linear quadratic	13 (9, 23)	140 (100, 230)
				I^2 = 21% (W)	Linear quadratic	23 (21, 24)	230 (210, 240)
BBP	2 rat studies	High	High	I^2 > 85%	Linear quadratic	23 (13, 74)	230 (140, 390)
DBP	12 rat studies	High	High	I^2 > 80%	Linear quadratic	12 (8, 22)	130 (85, 210)
DIBP	2 rat studies	High	High	I^2 > 60%	Linear	ND[b]	270 (225, 340)

Phthalate	Database Supporting Outcome	Confidence in Evidence	Evidence of Outcome	Heterogeneity	Model with Lowest AIC	BMD_5 mg/kg/day (95% CI)	BMD_{40} [c] mg/kg/day (95% CI)
DINP	4 rat studies	High	High	I^2 > 20%	Linear quadratic	76 (49, 145)	701 (552, 847)

[a] Meta-analyses were conducted for combined strain data, as well as individual Wistar (W) and Sprague-Dawley (SD) data.
[b] The 5% change was well below the range of the data (NASEM, 2017).
[c] NASEM (2017) calculated BMD40s for this endpoint because "previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40%."

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3.1.3.3 Anogenital Distance (AGD)

DHT is an androgen derived from testosterone by the enzyme 5α-reductase. In rodents, DHT functions to lengthen the perineum in males relative to females. Reduced AGD in males at birth is indicative of a disruption of testosterone production by Leydig cells and is considered a biomarker of disrupted androgen action during development. Compared to rodent models, the role of androgen action on AGD is less well established in humans. However, observational human data are consistent with androgen action during gestation playing a role in lengthening the perineum in humans (reviewed in (Thankamony et al., 2016)). This is consistent with the conclusions of NASEM (2017). After reviewing available mechanistic information, NASEM concluded that "androgen-dependent development of the male reproductive tract and androgen-dependent AGD appear to be well conserved across mammalian species (including humans)."

EPA identified a large number of *in vivo* experimental studies (55 studies total from multiple research groups) that evaluated AGD following gestational exposure to the high-priority and manufacturer-requested phthalates (Table 3-8). Available studies were of varying design (*i.e.*, gestational, perinatal, and multi-generation exposure studies) and were primarily conducted using rat models (51 rat and 4 mouse studies). DEHP (16 rat and 3 mouse studies) and DBP (18 rat studies) have the largest amount of data available. Fewer studies investigating AGD are available for BBP (five rat studies), DIBP (three rat studies and one mouse study), DCHP (five rat studies), DINP (six rat studies), and DIDP (one rat study).

Available experimental rat studies (conducted with Wistar, SD, and Long-Evans strains) of DEHP, BBP, DBP, DIBP, and DCHP provide consistent evidence that gestational exposure during the critical window leads to a dose-dependent reduction in male pup AGD (Table 3-8). Importantly, statistically significant reductions in AGD were consistently observed for both absolute AGD (*i.e.*, measured in mm) and body weight normalized AGD (*i.e.*, mm/body weight or mm/cube root of body weight) for DEHP, BBP, DBP, DIBP, and DCHP—indicating that the effect on AGD was not due to differences in pup size or body weight. One out of 14 rat studies of DEHP reported no effect on AGD (Martino-Andrade et al., 2008), however, this study included only a single dose group (*i.e.*, 150 mg/kg/day) at a level that inconsistently reduced AGD across the other available studies of DEHP (Table 3-8).

Effects on AGD are less consistent across the four available mouse studies of DEHP and DIBP. One study in which C57BL/6 mice were gavaged with 100 to 500 mg/kg/day DEHP on embryonic days 12 to 17 reported a dose-dependent decrease in absolute fetal male AGD starting at the lowest dose (Liu et al., 2008). In contrast, AGD was not reduced in CD-1 mice gestationally exposed to up to 500 mg/kg/day DEHP via gavage (Do et al., 2012) or 5 mg/kg/day DEHP via diet (Pocar et al., 2012) or to ICR mice exposed to 450 mg/kg/day DIBP via diet (Wang et al., 2017). However, in the study of DIBP, AGD was evaluated on PND 21, which is considered a less sensitive timepoint for AGD evaluation because AGD can be affected by growth and changes in body weight (OECD guidance recommends AGD be measured between PND 0 to PND 4 (OECD, 2013)). Studies evaluating AGD in mice for other phthalates were not identified, and it is unclear whether inconsistencies across mouse studies are due to strain differences in sensitivity or some other factor.

For DINP, there is inconsistent evidence of an effect on male pup AGD following exposure during the critical window (Table 3-8). Two out of six rat studies reported reduced AGD following exposure to DINP. Boberg et al. (2011) dosed Wistar rats with 300 to 900 mg/kg/day DINP on GD 7 through PND 17 and reported a dose-dependent reduction in both absolute and bodyweight normalized AGD on PND 13 in the highest treatment group; however, the effect was no longer apparent at PND 90. In a second study conducted by Clewell et al. (2013b), SD rats were dosed with 56 to 720 mg/kg/day DINP from

GD 12 through PND 14. Absolute and bodyweight normalized AGD was reduced in a dose-dependent manner at PND 14, but not at PNDs 2 or 49. Four additional studies conducted with SD rats found no effect on AGD following exposure during the critical window to doses of DINP ranging from 750 to 1,165 mg/kg/day (Li et al., 2015a; Clewell et al., 2013a; Masutomi et al., 2003; Gray et al., 2000). Inconsistent effects on AGD are consistent with DINP being a less potent antiandrogen, as demonstrated by potency comparisons for effects on gene expression (Table 3-4) and testosterone (Table 3-6).

For DIDP, AGD has only been evaluated in one study, a two-generation reproduction study of SD rats (Hushka et al., 2001). Absolute AGD was unaffected in male pups of both the F1 and F2 generations when exposed to 300 to 400 mg/kg/day DIDP (highest dose tested). This is consistent with DIDP having no effect on fetal testicular expression of steroidogenic genes (Section 3.1.3.1) or fetal testosterone (Section 3.1.3.2).

To support relative potency comparisons, EPA conducted preliminary dose-response modeling of data from studies that reported reduced male pup AGD following gestational exposure to each of the high-priority and manufacturer-requested phthalates. For this preliminary analysis, data for DEHP, DBP, BBP, DIBP, and DCHP were modeled to estimate an ED50 value for each phthalate. DINP was not included in the initial dose-response analysis because effects on AGD were generally not large enough in magnitude to support an accurate ED50 prediction. As can be seen from Table 3-9, 95 percent confidence intervals for ED50 estimates generally overlapped, which prohibits direct potency comparisons. A comparison of ED50 values for reduced AGD with those for changes in testosterone and gene expression indicate AGD is a less sensitive outcome.

EPA's findings are consistent with a recent systematic review and meta-analysis conducted by NASEM (2017) (summarized in Table 3-10). NASEM evaluated experimental animal evidence for effects on AGD following *in utero* exposure to DEHP, BBP, DBP, and DINP (DIBP, DCHP, and DIDP were not included) using the systematic review methodology developed by NTP's OHAT. NASEM found high confidence in the body of evidence and a high level of evidence that fetal exposure to DEHP, BBP, and that DBP is associated with reduced AGD in male rats. For DINP, NASEM had very low confidence in the body of evidence and determined that there was inadequate evidence to support an association. Meta-analyses found statistically significant overall effects and linear trends in $\log_{10}$(dose) and dose for DEHP, BBP, and DBP. Additional meta-analyses of mouse as well as Wistar and SD rat data were conducted for DEHP. Wistar rats were found to be more sensitive than SD rats, which is in contrast to what was observed for effects on testosterone (*i.e.*, SD rats were slightly more sensitive than Wistar rats). For mice, the overall effect was not statistically significant; however, significant linear trends in $\log_{10}$(dose) and dose were reported and mice were found to be similarly sensitive to DEHP-induced effects on AGD as SD rats.

1453 **Table 3-8. Studies Evaluating Anogenital Distance in Male Pups[a b]**

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [h]	LOAEL (mg/kg/d) [h]	Dose-Response Data
DEHP	(Christiansen et al., 2010)	Rat (W)	GD 7–PND 16 (PND 1)	0, 10, 30, 100, 300, 600, 900	None	10 [c g]	3.7±0.1, 3.4±0.1, 3.4±0.1, 3.4±0.1, 3.4±0.1, 3.3±0.1, 3.2±0.1 (mm, x̄±SE)
		Rat (W)	GD 7–PND 16 (PND 1)	0, 3, 10, 30, 100	30	100 [c g]	3.40±0.1, 3.4±0.1, 3.4±0.1, 3.4±0.1, 3.25±0.1 (mm, x̄±SE)
	(Vo et al., 2009)	Rat (SD)	GD 11–21 (PND 63)	0, 10, 100, 500	10	100 [c]	38±1.3, 37±0.9, 31±1.2, 36±2.5 (mm, x̄±SE)
	(Liu et al., 2008)	Mouse (C57BL/6)	e12–17 (e19)	0, 100, 200, 500	None	100 [c]	0.208±0.01, 0.198±0.01, 0.193±0.01, 0.181±0.12 (mm, x̄±SD)
	(Gray et al., 2009)	Rat (SD)	GD 8–PND 17 (PND 2)	0, 11, 33, 100, 300	100	300 [c]	3.3±0.11, 3.2±0.05, 3.2±0.09, 3.2±0.05, 2.7±0.08 (mm, x̄±SE)
	(TherImmune Research Corporation, 2004) [f]	Rat (SD)	GD 0–21 (PND 1)	0.12, 0.78, 2.4, 7.9, 23, 77, 592, 775 (F1)	77 (F1)	592 (F1) [c d]	–[a]
				0.09, 0.48, 1.4, 4.9, 14, 48, 391, 543 (F2)	48 (F2)	391 (F2) [c d]	–[a]
				0.1, 0.47, 1.4, 4.8, 14, 46, 359 (F3)	46 (F3)	359 (F3) [c d]	–[a]
	(Jarfelt et al., 2005)	Rat (W)	GD 7–PND 17 (PND 3)	0, 300, 750	None	300 [c]	4.6±0.1, 4.0±0.6, 3.8±0.4 (mm, x̄±SE)
	(Andrade et al., 2006b)	Rat (W)	GD 6–21 (PND 22)	0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405	135	405 [c]	–[a]
	(Li et al., 2013)	Rat (SD)	GD 12–19 (PND 1)	0, 500, 750, 1,000	500	750 [e]	0.63±0.12, 0.61±0.12, 0.55±0.06, 0.56±0.03 (mm/bw, x̄±SE)
	(Saillenfait et al., 2009a)	Rat (SD)	GD 12–21 (PND 1)	0, 500	None	500 [c e]	1.32±0.08, 1.08±0.05 (mm/$\sqrt[3]{bw}$, x̄±SD)
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (PND 3)	0, 500	None	500 [c]	–[a]

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [h]	LOAEL (mg/kg/d) [h]	Dose-Response Data
DEHP	(Moore et al., 2001)	Rat (SD)	GD 9–PND 21 (PND 1)	0, 375, 750, 1,500	375	750 [c e]	–[a]
	(Lin et al., 2008)	Rat (LE)	GD 2–20 (GD 21)	0, 10, 100, 750	100	750 [c]	4.5±0.1, 4.3±0.1, 4.8±0.1, 4.1±0.1 (mm, x̄±SE)
	(Parks et al., 2000)	Rat (SD)	GD 14–PND 2 (PND 2)	0, 750	None	750 [c]	–[a]
	(Borch et al., 2004)	Rat (W)	GD 7–21 (PND 3)	0, 750	None	750 [c]	–[a]
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 2)	0, 750	None	750 [c]	3.42±0.08, 2.41±0.08 (mm, x̄±SE)
	(Culty et al., 2008)	Rat (SD)	GD 14–PND 0 (PND 60)	0, 234, 469, 700, 750, 938, 1,250	938	1,250 [c]	–[a]
	(Martino-Andrade et al., 2008)	Rat (W)	GD 3–21 (GD 21)	0, 150	150	None [c e]	–
	(Do et al., 2012)	Mouse (CD-1)	GD 9–18 (GD 18)	0, 0.0005, 0.001, 0.005, 0.5, 50, 500	500	None [c]	–
	(Pocar et al., 2012)	Mouse (CD-1)	GD 0.5–PND 21 (PND 42)	0, 0.05, 5	5	None [e]	–
BBP	(Aso et al., 2005) [f]	Rat (SD)	GD 0–21 (PND 4)	0, 100, 200, 400 (F1, F2)	400 (F1)	None (F1) [c e]	–
					None (F2)	100 (F2) [c e]	2.12±0.16, 1.96±0.11, 1.94±0.16, 1.87±0.21 (mm/∛bw, x̄±SE)
	(Ema et al., 2003)	Rat (W)	GD 15–17 (GD 21)	0 , 167, 250, 375	167	250 [c e]	–[a]
	(Tyl et al., 2004) [f]	Rat (SD)	GD 0–21 (PND 0)	0, 50, 250, 750 (F1)	50	250 [c]	2.06±0.03, 2.01±0.04, 1.89±0.02, 1.71±0.03 (mm, x̄±SE)
				0, 50, 250, 750 (F2)	50	250 [c]	2.05±0.01, 2.05±0.02, 1.99±0.01, 1.77±0.03 (mm, x̄±SE)
	(Nagao et al., 2000) [f]	Rat (SD)	GD 0–21 (PND 0)	0, 20, 100, 500 (F1)	100 (F1)	500 (F1) [c]	2.6±0.2, 2.6±0.2, 2.5±0.1, 2.4±0.3 (mm, x̄±SD)
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 2)	0, 750	None	750 [c]	3.42±0.08, 2.53±0.09 (mm, x̄±SE)
DBP	(Mylchreest et al., 1999)	Rat (SD)	GD 3–21 (PND 1)	0, 100, 250, 500, 750	250	500 [c]	–[a]
			GD 12–21 (PND 1)	0, 100, 250, 500	100	250 [c]	–[a]
	(Zhang et al., 2004)	Rat (SD)	GD 1–PND 21 (PND 4)	0, 50, 250, 500	50	250 [c d]	–[a]

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [h]	LOAEL (mg/kg/d) [h]	Dose-Response Data
DBP	(Li et al., 2009)	Rat (W)	GD 6–PND 1 (PND 1)	0, 31, 94, 291, 797	94	291 [c]	3.80±0.15, 3.67±0.13, 3.72±0.20, 3.59±0.22, 2.78±0.16 (mm, x̄±SD)
	(Li et al., 2015b)	Rat (W)	e12.5–20.5 (PND 2)	0, 100, 300, 900	100	300 [c]	3.0±0.3, 2.9±0.2, 2.5±0.3, 2.2±0.2 (mm, x̄±not specified)
	(Mylchreest et al., 1998)	Rat (SD)	GD 3–PND 20 (PND 1)	0, 250, 500, 750	250	500 [c]	–[a]
	(Jiang et al., 2007)	Rat (SD)	GD 14–18 (PND 1)	0, 250, 500, 750	250	500 [d]	0.65±0.08, 0.64±0.08, 0.61±0.05, 0.59±0.03 (mm/bw, x̄±SD)
	(Kim et al., 2010)	Rat (SD)	GD 10–19 (PND 11)	0, 250, 500, 700	250	500 [d]	–[a]
	(Drake et al., 2009)	Rat (W)	e13.5–21.5 (>12 weeks)	0, 100, 500	100	500 [c]	–[a]
	(Mylchreest et al., 2000)	Rat (SD)	GD 12–21 (PND 1)	0, 0.5, 5, 50, 100, 500	100	500 [c]	–[a]
	(Struve et al., 2009)	Rat (SD)	GD 12–19 (GD 20)	0, 100, 500	100	500 [c]	1.95±0.28, 1.90±0.2, 1.67±0.18 (mm, x̄±SE)
	(Martino-Andrade et al., 2008)	Rat (W)	GD 12–21 (GD 21)	0, 100, 500	None	100 [e]	2.04±0.03, 1.88±0.04, 1.79±0.04 (mm/∛bw, x̄±SE)
	(Barlow et al., 2004)	Rat (SD)	GD 12–21 (PND 1)	0, 100, 500	100	500 [c]	–[a]
			GD 12–21 (PND 180)	0, 100, 500	100	500 [c]	–[a]
	(MacLeod et al., 2010)	Rat (W)	e13.5–20.5 (e21.5)	0, 500	None	500 [c]	–[a]
			e13.5–21.5 (PND 25)	0, 100, 500	100	500 [c]	–[a]
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (PND 3)	0, 500	None	500 [c]	–[a]
	(van den Driesche et al., 2012) [a]	Rat (W)	e13.5–20.5 (e21.5)	0, 500, 750	None	500 [c]	–[a]
		Rat (W)	e19.5–20.5 (e21.5)	0, 500, 750	750	None [c]	–
	(Ema et al., 1998)	Rat (W)	GD 11–21 (GD 21)	0, 331, 555, 661	331	555 [c d]	–[a]
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 2)	0, 642	None	642 [c e]	2.27±0.04, 2.04±0.04 (mm/∛bw, x̄±SE)
			GD 12–PND 14 (PND 14)	0, 642	None	642 [c e]	3.40±0.04, 3.11±0.04 (mm/∛bw, x̄±SE)

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [h]	LOAEL (mg/kg/d) [h]	Dose-Response Data
			GD 12–PND 14 (PND 50)	0, 642	642	None	–
	(Lee et al., 2004)	Rat (SD)	GD 15–PND 21 (PND 2)	0, 2, 14, 148, 712	148	712 [c]	3.7±0.2, 3.9±0.2, 3.8±0.3, 3.8±0.2, 3.0±0.1 (mm, x̄±SD)
DIBP	(Saillenfait et al., 2008)	Rat (SD)	GD 12–21 (PND 1)	0, 125, 250, 500, 625	125	250 [c]	2.55±0.17, 2.44±0.15, 2.28±0.30, 2.02±0.13, 1.98±0.16 (mm, x̄±SD)
	(Saillenfait et al., 2017)	Rat (SD)	GD 13–19 (GD 19)	0, 250	None	250 [e]	1.77±0.07, 1.68±0.07 (mm/∛bw, x̄±SD)
	(Borch et al., 2006a)	Rat (W)	GD 7–20/21 (GD 20/21)	0, 600	None	600 [c d]	–[a]
	(Wang et al., 2017)	Mouse (ICR)	GD 0–21 (PND 21)	0, 450	450	None	–
			GD 0–PND 21 (PND 21)	0, 450	450	None	–
DCHP	(Ahbab and Barlas, 2015)	Rat (W)	GD 6–19 (GD 20)	0, 20, 100, 500	None	20 [c d e]	–[a]
	(Li et al., 2016)	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500	10	100 [c]	3.3±0.3, 3.0±0.5, 2.7±0.2, 2.6±0.2 (mm, x̄±SE)
	(Saillenfait et al., 2009b)	Rat (SD)	GD 6–20 (GD 21)	0, 250, 500, 750	None	250 [e]	1.66±0.07, 1.52±0.09, 1.47±0.09, 1.43±0.08 (mm/∛bw, x̄±SD)
	(Yamasaki et al., 2009)	Rat (SD)	GD 6–PND 20 (PND 4)	0, 20, 100, 500	100	500 [e]	1.90±0.15,–, –, 1.66±0.11 (mm/∛bw, x̄±SD) [b]
	(Hoshino et al., 2005) [f]	Rat (SD)	GD 0–21 (PND 4)	0, 21, 104, 511 (F1)	104 (F1)	511 (F1) [e]	2.2±0.22, 2.2±0.21, 2.1±0.15, 2.0±0.15 (mm/∛bw, x̄±SD)
				0, 21, 107, 534 (F2)	21 (F2)	107 (F2) [e]	2.1±0.15, 2.0±0.13, 1.9±0.16, 1.9±0.13 (mm/∛bw, x̄±SD)
DINP	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 2)	0, 56, 288, 720	720	None [c e]	–
			GD 12–PND 14 (PND 14)	0, 56, 288, 720	288	720 [c e]	–[a]
			GD 12–PND 14 (PND 49)	0, 56, 288, 720	720	None [c e]	–
	(Boberg et al., 2011)	Rat (W)	GD 7–PND 17 (PND 13)	0, 300, 600, 750, 900	750	900 [e]	11.6±1.0, 11.4±0.8, 11.3±0.2, 11.3±0.8, 11.0±0.9 (mm/∛bw, x̄±SD)

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [h]	LOAEL (mg/kg/d) [h]	Dose-Response Data
			GD 7–PND 17 (PND 90)	0, 300, 600, 750, 900	900	None [e]	–
	(Masutomi et al., 2003)	Rat (SD)	GD 15–PND 2 (PND 2)	0, 30, 307, 1,165	1,165	None [c]	–
	(Li et al., 2015a)	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500, 1,000	1,000	None [c e]	–
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 2)	0, 750	750	None [c d]	–
	(Clewell et al., 2013a)	Rat (SD)	GD 12–PND 14 (GD 20)	0, 50, 250, 750	750	None [c e]	–
DIDP	(Hushka et al., 2001)	Rat (SD)	GD 0–21 (PND 0)	0, 15, 50, 165, 300–400	300–400 (F1, F2)	None [c]	–

[a] Dose-response observed, but data not extracted because data was only presented graphically or, in some cases, data was reported at the pup level and was not extracted for the purposes of this document (*e.g.*, see (TherImmune Research Corporation, 2004)).
[b] AGD not reported for all dose groups.
[c] AGD reporting metric: mm
[d] AGD reporting metric: mm/bodyweight
[e] AGD reporting metric: mm/$\sqrt[3]{\text{bodyweight}}$
[f] Multi-generation reproduction study. F1 and F2 indicates pups produced by F0 and F1 parental generations, respectively.
[g] Statistical analysis of combined data from both studies indicates a significant effect at 10 mg/mg/day (Christiansen et al., 2010).
[h] NOAEL/LOAEL values reflect study authors statistical analysis (*i.e.*, the LOAEL is the lowest value where a statistically significant effect was observed).
e = embryonic day; GD = gestational day; LOAEL = lowest-observed-adverse-effect-level; NOAEL = no-observed-adverse-effect-level; PND = postnatal day; SD = Sprague-Dawley; W = Wistar

Table 3-9. Summary of ED50 Values for Reduced (% Control) Male AGD

Phthalate	ED50 (95% CI) (mg/kg/day)
DCHP	1,128 (825, 2,042)
DEHP	1,314 (1068, 1,846)
DBP	920 (775, 1,149)
BBP	813 (685, 1,002)
DIBP	777 (594, 1,177)
ED50 value indicates the dose at which male pup AGD was reduced to 50% of the control value. A description of the methodology used to estimate the ED50 values is provided in Appendix C.	

Table 3-10. Summary of NASEM (2017) Systematic Review and Meta-Analysis Results for Effects on AGD

Phthalate	Database	Confidence in Evidence	Evidence of Outcome	Heterogeneity	Model with Lowest AIC	BMD_5 (mg/kg/day) (95% CI)
DEHP[a]	16 rat studies & 3 mouse study	High	High	$I^2 > 20\%$	Linear quadratic	270 (180, 420) (combined)
						290 (170, >1,000) (SD)
						150 (100, 280) (W)
BBP	6 rat studies	High	High	$I^2 > 75\%$	Linear quadratic	250 (160, 380)
DBP	22 rat studies	High	High	$I^2 > 75\%$	Linear quadratic	150 (120, 220)
DINP[b]	4 rat studies	Very Low	Inadequate	–	–	–

[a] Meta-analyses were conducted for combined strain data, as well as individual Wistar (W) and Sprague-Dawley data.
[b] NASEM did not conduct a meta-analysis for DINP due to their conclusion of inadequate evidence for this outcome.

3.1.3.4 Nipple Retention

DHT is an androgen derived from testosterone by the enzyme 5α-reductase. DHT is necessary for proper apoptosis and regression of nipple anlagen in male rats. Because phthalate exposure reduces fetal testicular testosterone production, DHT levels in peripheral tissues are also reduced leading to retained nipples/areolas (NR). EPA identified 26 *in vivo* experimental animal studies from multiple research groups that evaluated NR in male pups following phthalate exposure during the critical window (Table 3-11). Available studies were of varying design (*i.e.*, gestational, perinatal, multi-generation exposure studies), but were all conducted using either SD or Wistar strains. DEHP (12 rat studies) and DBP (eight rat studies) have the largest amount of data available. Fewer studies investigating NR are available for BBP (two rat studies), DIBP (one rat study), DCHP (two rat studies), DINP (three rat studies), and DIDP (one rat study).

As noted in Table 3-11, there is variability in how publications report NR (*e.g.*, NR is reported as mean number of nipples/areolas per male, incidence of males with NR, or mean percent of litters with males with NR, etc.). Furthermore, publications may or may not distinguish between retained areolas versus retained nipples. These discrepancies in data reporting can make comparisons between studies difficult. However, across available studies a consistent dose-dependent increase in NR was observed for male pups gestationally exposed to DEHP, BBP, DBP, DIBP, or DCHP when evaluated between PNDs 11 to 14, which is consistent with OECD recommendations for timing of when evaluation of this outcome should occur (OECD, 2013). For one study of DEHP (Martino-Andrade et al., 2008), retained nipples in male pups was not observed, however, the study tested a single dose level (*i.e.*, 150 mg/kg/day), which produced inconsistent effects on NR across the other available studies of DEHP. For DINP, rat studies are somewhat inconsistent. Two studies conducted with Wistar and SD rats demonstrate a dose-related increase in male NR at doses ranging from 750 to 900 mg/kg/day, while a third study found no increase in NR at a high dose of 720 mg/kg/day.

Several studies have examined whether or not NR is a permanent malformation in adult male rats that were gestationally or perinatally exposed to phthalates. Available studies consistently report permanent nipples in adult male rats exposed to DEHP (Gray et al., 2009; Saillenfait et al., 2009a; Howdeshell et al., 2007; Gray et al., 2000), BBP (Gray et al., 2000), DBP (Clewell et al., 2013b; Howdeshell et al., 2007; Barlow et al., 2004) and DIBP (Saillenfait et al., 2008). No studies were identified that evaluated permanent nipples in adult male rats exposed to DCHP. For DINP, there is inconsistent evidence of permanent nipples. Boberg et al. (2011) found that Wistar rats exposed to doses of DINP ≥750 mg/kg/day had increased NR at PND 13, but permanent nipples were not observed at PND 90, while Gray et al. (2000) reported permanent nipples in two out of 52 adult (3 to 7 months of age) males perinatally exposed to 750 mg/kg/day DINP.

For DIDP, NR has only been evaluated in one study—a two-generation reproduction study of SD rats (Hushka et al., 2001). No increase in NR was reported in F1 or F2 male pups following exposure to up to 300 to 400 mg/kg/day DIDP (highest dose tested). This is consistent with DIDP having no effect on fetal testicular expression of steroidogenic genes (Section 3.1.3.1), fetal testicular testosterone (Section 3.1.3.2), and AGD (Section 3.1.3.3).

To support relative potency comparisons, EPA conducted preliminary dose-response modeling of data from studies that reported NR for males as the percent of males per litter showing any retained nipples/areolas. For this preliminary analysis, data for DEHP, DBP, BBP, DIBP, and DCHP were modeled to estimate the ED50 for each phthalate. DINP was not included in this preliminary analysis because the two available studies either did not report data as percent of males per litter showing any

retained nipples/areolas (*i.e.*, Boberg et al. (2011) reported data as the number of nipples per male) or only tested one dose level (Gray et al., 2000) and do not support ED50 predictions. As can be seen from Table 3-11, 95 percent confidence intervals overlapped for some ED50 estimates; however, based on this initial analysis DEHP and DBP appeared to be more potent than DCHP, DIBP, and BBP at inducing male pup NR.

1515 **Table 3-11. Studies Evaluating Nipple Retention in Male Pups**

Phthalate	Refence	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [k]	LOAEL (mg/kg/d) [k]	Dose-Response Data [a]
DEHP	(Christiansen et al., 2010)	Rat (W)	GD 7–PND 16 (PND 12)	0, 10, 30, 100, 300, 600, 900	3	10 [i]	0.22±0.08, 3.14±0.94, 1.81±0.82, 1.23±0.68, 5.21±1.25, 4.63±1.72, 5.01±1.36 [b]
				0, 3, 10, 30, 100	100	None [i]	0.38±0.92, 0.59±0.99, 1.13±1.26, 0.31±0.40, 0.86±1.23 [b]
	(Gray et al., 2009)	Rat (SD)	GD 8–PND 17 (PND 13)	0, 11, 33, 100, 300	100	300	0.7±0.4, 0.8±0.3, 0.3±0.1, 0.7±0.3, 2.9±0.6 [b] 11±5.5, 21±8.9, 10±4.7, 16±6.7, 55%±10.1 [c]
			GD 8–PND 17 (PNM 7)	0, 11, 33, 100, 300	100	300	0±0, 0.08±0.08, 0±0, 0.15±0.12, 1.22±0.41 [b]
	(Jarfelt et al., 2005)	Rat (W)	GD 7–PND 17 (PND 13)	0, 300, 750	None	300	0.1±0.2, 3.9±2.7, 5.2±1.7 [b]
	(Andrade et al., 2006b)	Rat (W)	GD 6–PND 21 (PND 13)	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405	135	405	0/60, 0/45, 0/46, 0/54, 0/58, 0/63, 0/42, 0/50, 0/41, 0/56, 13/41 [d]
	(Vo et al., 2009)	Rat (SD)	GD 11–21 (PND 13)	0, 10, 100, 500	100	500	0, 0, 0, 9.06±1.83 [b] (±SD)
	(Saillenfait et al., 2009a)	Rat (SD)	GD 12–21 (PND 12–14)	0, 500	None	500	0/43, 42/56 [d]
			GD 12–21 (PND 70–120)	0, 500	None	500	0/42, 25/54 [d]
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (PND 14)	0, 500	None	500	6.3±6.3, 55.8±16.4% [c]
			GD 14–18 (PNM 7–11)	0, 500	None	500	0, 41.3±16.7% [c]
	(Moore et al., 2001)	Rat (SD)	GD 9–PND 21 (PND 14)	0, 375, 750, 1500	None	375	– [f g]
	(Borch et al., 2004)	Rat (W)	GD 7–21 (PND 13)	0, 750	None	750	– [g]
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 13)	0, 750	None	750	0, 6.3±1.1 [b]; 0, 86.9±5% [c]
			GD 14–PND 3 (PNM 3–7)	0, 750	None	750	– [h]
	(Martino-Andrade et al., 2008)	Rat (W)	GD 13–21 (PND 13)	0, 150	150	None	2/18, 5/26 [d]

Phthalate	Refence	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [k]	LOAEL (mg/kg/d) [k]	Dose-Response Data [a]
	(TherImmune Research Corporation, 2004)	Rat (SD)	GD 0–PND 13 (PND 13)	0.1, 0.47, 1.4, 4.8, 14, 46, 359 (F2)	46 (F3)	359 (F3)	0±1, 0±0, 0±0, 0±1, 0±0, 0±0, 11±7% [e j]
BBP	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 13)	0, 750	None	750	0, 5.1±0.9 [b]; 0, 70±11% [c]
			GD 14–PND 3 (PNM 3–7)	0, 750	None	750	–[h]
	(Tyl et al., 2004)	Rat (SD)	GD 0–PND 13 (PND 11–13)	0, 50, 250, 750	250 (F1, F2)	750 (F1, F2)	0.07±0.04, 0.00±0.00, 0.02±0.00, 1.29±0.33 (F1) [b j]
							0.05±0.03, 0.12±0.04, 0.19±0.08, 3.14±0.50 (F2) [b j]
DBP	(Mylchreest et al., 2000)	Rat (SD)	GD 12–21 (PND 14)	0, 0.5, 5, 50, 100, 500	50	100	9/134, 8/119, 13/103, 12/120, 44/141, 52/58 [d]
	(Barlow et al., 2004)	Rat (SD)	GD 12–21 (PND 13)	0, 100, 500	None	100	–[g]
			GD 12–21 (PND 180)	0, 100, 500	100	500	–[g]
	(Mylchreest et al., 1999)	Rat (SD)	GD 12–21 (PND 14)	0, 100, 250, 500	100	250	0/57, 0/58, 35/62, 47/54 [d]
	(Kim et al., 2010)	Rat (SD)	GD 10–19 (PND 11)	0, 250, 500, 700	250	500	0/201, 0/53, 3/36, 31/55 [d]
	(Martino-Andrade et al., 2008)	Rat (W)	GD 13–21 (PND 13)	0, 100, 500	100	500	2/18, 5/31, 7/8 [d]
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (PND 14)	0, 500	None	500	6.3±6.3, 41.3±18.7% [c]
			GD 14–18 (PNM 7–11)	0, 500	500	None	0±0, 21.8±13.4% [c]
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 14)	0, 642	None	642	1.8±0.4, 5.8±0.8 [b]
			GD 12–PND 14 (PND 50)	0, 642	None	642	0.6±0.4, 2.5±0.5 [b]
	(Lee et al., 2004)	Rat (SD)	GD 15–PND 21 (PND 14)	0, 2, 14, 148, 712	148	712	0, 4, 13, 15, 100% [c]
DIBP	(Saillenfait et al., 2008)	Rat (SD)	GD 12–21 (PND 12–14)	0, 125, 250, 500, 625	125	250	0/76, 0/78, 8/96, 47/79, 56/76 [d]
			GD 12–21 (PNW 11–21 or 16–17)	0, 125, 250, 500, 625	125	250	0/46, 0/40, 4/55, 24/44, 29/38 [d]

Phthalate	Refence	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [k]	LOAEL (mg/kg/d) [k]	Dose-Response Data [a]
DCHP	(Yamasaki et al., 2009)	Rat (SD)	GD 6–PND 20 (PND 13)	0, 20, 100, 500	100	500	–[g]
	(Hoshino et al., 2005)	Rat (SD)	GD 0–PND 14 (PND 14)	0, 14, 70, 349 (F1)	70	349	0, 0, 0, 16% (F1) [f,j]
			GD 0–PND 12 (PND 12)	0, 14, 72, 351 (F2)	72	351	0, 0, 18, 63% (F2) [f,j]
DINP	(Boberg et al., 2011)	Rat (W)	GD 7–PND 17 (PND 13)	0, 300, 600, 750, 900	600	750	1.98±0.83, 2.00±0.64, 2.91±0.69, 3.14±1.21, 3.17±0.92 [b (±SD)]
			GD 7–PND 17 (PND 90)	0, 300, 600, 750, 900	900	None	–
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 13)	0, 750	None	750	0, 0.11±0.09[b]; 0, 22.4±8.9% [c]
			GD 14–PND 3 (PNM 3–7)	0, 750	None	750	0, 2/52 [d]
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 14)	0, 56, 288, 720	720	none	–[b]
			GD 12–PND 14 (PND 49)	0, 56, 288, 720	720	none	–[b]
DIDP	(Hushka et al., 2001)	Rat (SD)	GD 0–PND 14 (PND 12–14)	0, 15, 50, 165, 300–400 (F1, F2)	300–400 (F1, F2)	None (F1, F2)	0±0, 0±0, 0±0, 0±0, 0±0 [b (±SD)]

[a] Response data is provided for each respective treatment group included in the study, starting with the control response.
[b] Mean number of nipples/areolas per male. Unless otherwise indicated, variation is reported as ± SEM.
[c] Mean (± SEM) percent of males with nipples/areolas.
[d] Incidence of males with nipples/areolas to total number of examined animals.
[e] Mean (± SEM) percent of male pups per litter with retained nipples/areolas.
[f] Mean percent of litters with males with retained areolas.
[g] Dose-response observed, but data not extracted because data was only presented graphically.
[h] Study authors reported that most DEHP and BBP exposed adult males had permanently retained nipples, however, the effect is not quantified (Gray et al., 2000).
[i] Statistical analysis of combined data from both studies indicates a significant effect at 10 mg/mg/day (Christiansen et al., 2010).
[j] Multi-generation reproduction study. F1 and F2 indicate pups produced by F0 and F1 parental generations, respectively.
[k] NOAEL/LOAEL values reflect study authors statistical analysis (*i.e.*, the LOAEL is the lowest value where a statistically significant effect was observed).
GD = gestational day; LOAEL = lowest-observed-adverse-effect-level; NOAEL = no-observed-adverse-effect-level; PND = postnatal day; PNM = postnatal month; PNW = postnatal week; SD = Sprague-Dawley; W = Wistar

Table 3-12. Summary of ED50 Values for Percent Males per Litter with Retained Nipples/Areolas

Phthalate	ED50 (95% CI) (mg/kg/day)
DCHP	588 (324, 1,067)
DEHP	368 (275, 491)
DBP	331 (240, 463)
BBP	749 (551, 2,020)
DIBP	479 (366, 628)

ED50 values indicate the dose at which 50% of males per litter had retained nipples/areolas. A description of the methodology used to estimate the ED50 values is provided in Appendix C.

3.1.3.5 Hypospadias

As discussed by NASEM (2017), mechanistic studies conducted with rats provide evidence that link the formation of hypospadias (and other male reproductive tract malformations) with reduced fetal testosterone production by fetal Leydig cells (Howdeshell et al., 2015). EPA identified 27 *in vivo* experimental studies conducted by multiple research groups that evaluated hypospadias in experimental models. Available studies have primarily been conducted using rats (24 rat studies and 1 study conducted each with mice, rabbits, and marmosets). DEHP (9 rat studies and 1 mouse study) and DBP (9 rat studies, 1 rabbit study, and 1 marmoset study) have the most available data, while fewer studies are available for BBP (3 rat studies), DIBP (1 rat study), DCHP (1 rat study), DINP (3 rat studies), and DIDP (1 rat study).

For DEHP, available data are suggestive of a strain-specific difference in sensitivity. Across the six available studies conducted with SD rats, consistent dose-related increases in hypospadias were observed starting at doses as low as 100 mg/kg/day DEHP (Table 3-13). In contrast, no hypospadias were observed in two studies in which Wistar rats were exposed to up to 405 mg/kg/day (Andrade et al., 2006b) or 900 mg/kg/day DEHP (Christiansen et al., 2010). In a third study, a slight (3 percent) increase in hypospadias was observed in Wistar rats administered 300, but not 750 mg/kg/day DEHP (Jarfelt et al., 2005). For DBP, consistent dose-related increases in hypospadias were observed across all available studies of SD (6 studies) and Wistar (2 studies) rats. Furthermore, hypospadias were observed starting at comparable levels of exposure to DBP across strains (*i.e.*, the lowest LOAELs were 250 and 300 mg/kg/day for SD and Wistar rats, respectively) (Table 3-13). Presently, it is unclear why strain-specific differences in sensitivity exist for DEHP, but not DBP, for hypospadias.

Sufficient studies are not available to assess whether or not strain differences in sensitivity exist for BBP (three SD rat studies), DIBP (one SD rat study), or DCHP (one SD rat study). Regardless, the available studies of BBP, DIBP and DCHP report consistent dose-related increases in hypospadias following gestational exposure throughout the critical window (Table 3-13). In one two-generation study of BBP (Nagao et al., 2000), hypospadias were not observed at the highest dose tested (*i.e.*, 500 mg/kg/day), however, this dose is lower than that shown to induce hypospadias in other studies of BBP (*i.e.*, 750 mg/kg/day), including a two-generation study (Tyl et al., 2004).

In the one available mouse study, doses of DEHP ranging from 100 to 500 mg/kg/day caused a dose-dependent increase in the hypospadias (Liu et al., 2008). Similarly, in the one available rabbit study of DBP, hypospadias were observed in 1 out of 17 male pups (representing eight litters) exposed to 400

mg/kg/day (only dose level tested) (Higuchi et al., 2003). In contrast, no hypospadias were reported in 11 male offspring originating from 9 pregnant marmosets treated with 500 mg/kg/day MBP from weeks 7 through 15 of gestation (McKinnell et al., 2009).

No significant increases in hypospadias were observed in any of the three rat studies (one with Wistar and two with SD rats) of DINP at doses as high as 720 to 900 mg/kg/day. This is consistent with findings for steroidogenic gene expression, fetal testicular testosterone, AGD, and NR results, all of which indicate DINP is a less potent antiandrogen than other phthalates.

For DIDP, no hypospadias were reported in the two available two-generation studies of SD rats at doses as high as 600 mg/kg/day (Hushka et al., 2001), which is consistent with DIDP not disrupting fetal testicular steroidogenesis or causing reduced AGD and NR.

EPA's findings are consistent with a recent systematic review conducted by NASEM (2017). NASEM evaluated experimental animal evidence of hypospadias following *in utero* exposure to DEHP, BBP, and DBP (DINP, DIBP and DCHP were not included) using the systematic review methodology developed by NTP's OHAT. For both DEHP (8 rat studies and 1 mouse study) and BBP (two rat studies), NASEM concluded that there is moderate confidence in the body of evidence and a moderate level of evidence that gestational exposure to DEHP and BBP are associated with an increase in hypospadias in male rats. In part, NRC downgraded confidence in the body of evidence for DEHP due to unexplained inconsistency in response across rat strains (*i.e.,* SD rats were more sensitive than Wistar rats). For DBP (eight studies in rats), NRC concluded that there is high confidence in the body of evidence and a high level of evidence that gestational exposure to DBP is associated with hypospadias in male rats. NASEM did not conduct a meta-analysis of incidence data for hypospadias.

To support relative potency comparisons, EPA conducted preliminary dose-response modeling of data from studies reporting increased incidence of hypospadias in adult F1 males following gestational or perinatal exposure. For this preliminary analysis, data for DEHP, DBP, BBP, DIBP, and DCHP was modeled to estimate the ED50 for each phthalate. DINP was not included in this preliminary analysis because statistically significant increases in hypospadias have not been reported in male rats following gestational exposure to DINP. As can be seen from Table 3-14, 95 percent confidence intervals overlapped for some ED50 estimates; however, based on this initial analysis, DIBP and DCHP appeared more potent than DEHP, BBP, and DBP.

1587 **Table 3-13. Studies Evaluating Incidence of Hypospadias**

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [f]	LOAEL (mg/kg/d) [f]	Response (% Males Affected) [a]
DEHP	(Gray et al., 2009)	Rat (SD)	GD 8–PND 17 or 63 (PND 63–65)	0, 11, 33, 100, 300	33	100	0, 0, 0, 1.1, 1.4% [b]
	(Liu et al., 2008)	Mouse (C57BL/6)	e12–17 (e19)	0, 100, 200, 500	None	100	0, 7.1, 14, 76%
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (PNM 7)	0, 500	None	500	0, 1.9%
	(Li et al., 2013)	Rat (SD)	GD 12–19 (PND 1)	0, 500, 750, 1000	None	500	0, 11, 31, 37%
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PNM 3–7)	0, 750	None	750	0, 42%
	(Vo et al., 2009)	Rat (SD)	GD 11–21 (PND 63)	0, 10, 100, 500	100	500	0, 0, 0, 100%
	(Saillenfait et al., 2009a)	Rat (SD)	GD 12–21 (PND 70–120)	0, 500	None	500	0, 14.8%
			GD 12–21 (PND 70–84)	0, 625	None	625	0, 37%
	(Jarfelt et al., 2005)	Rat (W)	GD 7–PND 17 (PND 22)	0, 300, 750	None	300	0, 3, 0%
	(Andrade et al., 2006b)	Rat (W)	GD 6–PND 21 (PND 33)	0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405	405	None	–
	(Christiansen et al., 2010)	Rat (W)	GD 7–PND 16 (PND 16)	0, 10, 30, 100, 300, 600, 900	900 [g]	None	–
				0, 10, 30, 100	100 [g]	None	–
BBP	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PNM 3–7)	0, 750	None	750	0, 29%
	(Tyl et al., 2004) [c]	Rat (SD)	GD 0–21 (PND 4)	0, 50, 250, 750	250 (F2)	750 (F2)	0, 0, 0, 7%
	(Nagao et al., 2000) [c]	Rat (SD)	GD 0–21 (PND 21–22)	0, 20, 100, 500	500 (F1, F2)	None	–
DBP	(Mylchreest et al., 1998)	Rat (SD)	GD 3–PND 20 (PND 100)	0, 250, 500, 750	None	250	0, 3, 21, 43%
	(Li et al., 2015b)	Rat (W)	GD 12.5–20.5 (PND 63)	0, 100, 300, 900	100	300	0, 0, 23, 44%
	(Mylchreest et al., 1999)	Rat (SD)	GD 12–21 (PND 100–105)	0, 100, 250, 500	250	500	0, 0, 0, 40%
	(Jiang et al., 2007)	Rat (SD)	GD 14–18 (PND 1)	0, 250, 500, 750	250	500	0, 0, 7, 41%

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [f]	LOAEL (mg/kg/d) [f]	Response (% Males Affected) [a]
DBP	(Mylchreest et al., 2000)	Rat (SD)	GD 12–21 (PND 110–120)	0, 0.5, 5, 50, 100, 500	100	500	0, 0, 0, 0, 0, 9%
	(Barlow et al., 2004)	Rat (SD)	GD 12–21 (PND 180)	0, 100, 500	100	500	0, 0, 16%
			GD 12–21 (PND 370)	0, 100, 500	100	500	0, 0, 22%
			GD 12–21 (PND 540)	0, 100, 500	100	500	0, 0, 26%
	(Drake et al., 2009)	Rat (W)	e13.5–21.5 (>12 weeks)	0, 100, 500	100	500	0, 0, 31%
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 49–50)	0, 642	None	642	0.1, 11%
	(Kim et al., 2010)	Rat (SD)	GD 10–19 (PND 11)	0, 250, 500, 700	500	700	0, 0, 0, 47%
	(Higuchi et al., 2003)	Rabbit (Dutch-Belted)	GD 15–29 (PNW 12)	0, 400	None	400	0, 5.9% [d]
	(McKinnell et al., 2009)	Marmoset	GW 7–15 (PND 1–5)	0, 500 (MBP)	500	None	–
			GW 7–15 (PNM 18–21)	0, 500 (MBP)	500	None	–
DIBP	(Saillenfait et al., 2008)	Rat (SD)	GD 12–21 (PND 76–122)	0, 125, 250, 500, 625	250	500	0, 0, 0, 11, 56%
DCHP	(Yamasaki et al., 2009)	Rat (SD)	GD 6–PND 20 (PND 70)	0, 20, 100, 500	100	500	0, 0, 0, 12.5%
DINP	(Boberg et al., 2011)	Rat (W)	GD 7–PND 17 (PND 90)	0, 300, 600, 750, 900	900	None	–
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PNM 3–7)	0, 750	750	None	–
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 49)	0, 56, 288, 720	720	None	0.9, 0, 0, 2.4% [e]
DIDP	(Hushka et al., 2001) [c]	Rat (SD)	GD 0–21 (PND 0)	0, 165, 300–400, 600	600 (F1, F2) [c]	None	–
				0, 15, 50, 165, 300–400	300–400 (F1, F2) [c]	None	–

[a] Response data is provided for each respective treatment group included in the study, starting with the control response.
[b] Combined data from PUB (exposed from GD 8–PND 65) and IUL (exposed from GD 8–PND 17) cohorts (Table 6 of (Gray et al., 2009)).
[c] Multi-generation reproduction study. F1 indicates male pups produced by F0 mating pairs, while F2 indicates male pups produced by F1 mating pairs.
[d] One out of 17 male pups (representing 8 litters) manifested hypospadias.

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL (mg/kg/d) [f]	LOAEL (mg/kg/d) [f]	Response (% Males Affected) [a]
[e] One out of 111 control (0.9%) and two out of 84 high-dose (2.4%) male pups manifested hypospadias described as mild/slight. The effect was not significant. [f] NOAEL/LOAEL values reflect study authors statistical analysis (*i.e.*, the LOAEL is the lowest value where a statistically significant effect was observed). In some cases statistical analyses were not reported. In these cases, the NOAEL reflects the lowest dose where no hypospadias were observed. [g] Study authors report mild dysgenesis of the external genitalia in all dose groups. Moderate to severe dysgenesis of the external genitalia, which includes hypospadias, was not reported. EPA interpreted this to indicate that no hypospadias were observed at any dose in the study (Christiansen et al., 2010). e = embryonic day; GD = gestational day; NOAEL = no-observed-adverse-effect-level; PND = postnatal day; PNM = postnatal month; SD = Sprague-Dawley; W = Wistar							

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Table 3-14. Summary of ED50 Values for Hypospadias

Phthalate	ED50 (95% CI) (mg/kg/day)
DCHP	699 (631, 825)
DEHP	846 (804, 904)
DBP	958 (919, 999)
BBP	878 (829, 948)
DIBP	626 (603, 653)
ED50 values indicate the dose at which 50% of males exhibited hypospadias. A description of the methodology used to estimate the ED50 values is provided in Appendix C.	

3.1.3.6 Seminiferous Tubule Atrophy

Seminiferous tubule atrophy/degeneration is a pathologic lesion frequently reported in adult animals following *in utero* exposure to certain phthalates. Although there is uncertainty underlying the mechanisms associated with phthalate-induced effects on the seminiferous cord, seminiferous tubule atrophy was selected to serve as a key outcome because it is a sensitive, adverse effect frequently reported by board certified pathologists. EPA identified 22 *in vivo* experimental studies that evaluated testicular pathology and reported seminiferous tubule atrophy following gestational exposure to the high-priority and manufacturer-requested phthalates. All studies were conducted using rat models. Data were available for DEHP (three studies), BBP (three studies), DBP (eight studies), DIBP (one study), DCHP (two studies), DINP (five studies), and DIDP (one study).

As can be seen from Table 3-15, available studies consistently demonstrate that exposure to DEHP, BBP, DBP, DIBP, and DCHP lead to a dose-dependent increase in incidence of seminiferous tubule atrophy. Studies reporting seminiferous tubule atrophy are of varying design, and increased incidence of seminiferous tubule atrophy has been reported consistently across studies utilizing different exposure paradigms (*i.e.*, gestational, perinatal, and one or two-generation continuous exposure studies). Notably, studies have demonstrated that gestational exposure to DEHP on GDs 14 to 18 (Howdeshell et al., 2007); DBP on GDs 14 to 18 (Hotchkiss et al., 2010; Howdeshell et al., 2007) or GDs 12 to 21 (Barlow et al., 2004; Mylchreest et al., 2000; Mylchreest et al., 1999); and DIBP on GDs 12 to 21 (Saillenfait et al., 2008) is sufficient to cause increased seminiferous tubule atrophy in adults. This demonstrates that exposure during gestation is sufficient to cause tubular atrophy later in life, well after cessation of exposure.

For DINP, effects on seminiferous tubule atrophy are less consistent. Three studies reported no significant increase in seminiferous tubule atrophy at doses ranging from 577 to 1,165 mg/kg/day DINP (Clewell et al., 2013b; Masutomi et al., 2003; Waterman et al., 2000). Boberg et al. (2011) reported that a "few animals had small areas of tubular degeneration in areas of focal Leydig cell hyperplasia." However, the dose levels at which tubular degeneration was observed were not reported. A fifth study reported low incidence of tubular atrophy in adult rats exposed to 750 mg/kg/day DINP on GD 14 through PND 3 (Gray et al., 2000). For DIDP, no seminiferous tubule atrophy was reported in available two-generation reproductive studies at doses as high as 600 mg/kg/day.

To support relative potency comparisons, EPA conducted preliminary dose-response modeling of data for incidence of seminiferous tubule atrophy in adult F1 male rats following gestational or perinatal phthalate exposure. For this preliminary analysis, data for DEHP, DBP, BBP, DIBP, and DCHP was

modeled to estimate the ED50 for each phthalate. DINP was not included in this preliminary analysis, as tubular atrophy was reported qualitatively and at a low incidence in the one available study in which this pathologic lesion was observed. As can be seen from Table 3-16, 95 percent confidence intervals overlapped for some ED50 estimates. However, based on this initial analysis, DIBP and DCHP appear to be slightly more potent than DEHP and BBP, while DBP appears to be the least potent.

1630 **Table 3-15. Studies Reporting Seminiferous Tubule Atrophy**

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL[g] (mg/kg/d)	LOAEL[g] (mg/kg/d)	Seminiferous Tubule Atrophy (# Affected Males/Total Males Examined or % Males Affected) (Severity, if Reported)
DEHP	(TherImmune Research Corporation, 2004)	Rat (SD)	–[a] (F1 Adults)	0.09, 0.48, 1.4, 4.9, 14, 48, 391, 543	48	391	0/10, 0/10, 0/10, 1/10, 1/10, 0/10, 10/10, 10/10
	(Gray et al., 2009)	Rat (SD)	GD 8–PND 17 (PNM 7)	0, 11, 33, 100, 300	–[c]	–[c]	–[b]
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (7–11 months)	0, 500	None	500	0, 33%
BBP	(Aso et al., 2005)	Rat (SD)	–[a] (F1 Adults)	0, 100, 200, 400	200	400	1/24, 1/24, 3/24, 9/24
	(Nagao et al., 2000)	Rat (SD)	–[a] (F1 Adults)	0, 20, 100, 500	100	500	0/10, 0/10, 0/10, 6/10
	(Tyl et al., 2004)	Rat (SD)	–[a] (F1 Adults)	0, 50, 250, 750	250	750	3/30, 0/29, 4/28, 23/28
DBP	(Mylchreest et al., 1999)	Rat (SD)	GD 12–21 (PND 100–105)	0, 100, 250, 500	100	250	5/51, 0/51, 3/55, 11/45 (Grade 1) [h] 1/51, 1/51, 0/55, 1/45 (Grade 2) 0/51, 0/51, 1/55, 2/45 (Grade 3) 2/51, 0/51, 5/55, 19/45 (Grade 4)
	(Mylchreest et al., 1998)	Rat (SD)	GD 3–PND 20 (PND 100)	0, 250, 500, 750	None	250	–[b]
	(Wine et al., 1997)	Rat (SD)	–[a] (F1 Adults)	0, 256–385, 509–794	None	256–385	1/10, 3/10, 8/10
	(Hotchkiss et al., 2010)	Rat (SD)	GD 14–18 (PND 123–135)	0, 250, 500, 750, 1,000	250	500	1/15, 0/4, 2/7, 5/5, 6/6
	(Mylchreest et al., 2000)	Rat (SD)	GD 12–21 (PND 110–120)	0, 0.5, 5, 50, 100, 500	100	500	5/134, 6/118, 3/103, 3/120, 5/140, 4/58 (Grade 1) [h] 0/134, 1/118, 0/103, 0/120, 1/140, 2/58 (Grade 2) 0/134, 0/118, 0/103, 0/120, 0/140, 0/58 (Grade 3) 0/134, 0/118, 0/103, 0/120, 1/140, 25/58 (Grade 4)
	(Barlow et al., 2004)[j]		GD 12–21 (PND 180)	0, 100, 500	100	500	0/60, 0/65, 22/45

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL[g] (mg/kg/d)	LOAEL[g] (mg/kg/d)	Seminiferous Tubule Atrophy (# Affected Males/Total Males Examined or % Males Affected) (Severity, if Reported)
		Rat (SD)	GD 12–21 (PND 370)	0, 100, 500	100	500	2/61, 0/61, 20/74
			GD 12–21 (PND 540)	0, 100, 500	100	500	0/45, 0/49, 20/35
	(Howdeshell et al., 2007)	Rat (SD)	GD 14–18 (PNM 7–11)	0, 500	None	500	0, 14%
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 49–50)	0, 642	None	642	2/24, 6/26
DIBP	(Saillenfait et al., 2008)	Rat (SD)	GDs 12–21 (PNW 11–12)	0, 125, 250, 500, 625	125	250	2/24, 0/20, 1/28, 3/22, 1/20 (Grade 1) [i] 0/24, 1/20, 1/28, 1/22, 0/20 (Grade 2) 0/24, 0/20, 2/28, 0/22, 2/20 (Grade 3) 0/24, 0/20, 1/28, 4/22, 0/20 (Grade 4) 0/24, 1/20, 2/28, 8/22, 17/20 (Grade 5)
DCHP	(Ahbab and Barlas, 2015)	Rat (SD)	GD 6–19 (GD 20)	0, 20, 100, 500	None	20	0/10, 8/10, 10/10, 10/10
	(Hoshino et al., 2005)	Rat (SD)	–[a] (F1 Adults)	0, 18, 90, 457	90	457	1/20, 0/23, 2/20, 6/22 (slight) 0/20, 0/23, 0/20, 3/22 (severe)
DINP	(Gray et al., 2000)	Rat (SD	GD 14–PND 3 (PNM 3–7)	0, 750	None	750	–[b]
	(Masutomi et al., 2003)	Rat (SD)	GD 15–PND 10 (PNW 11)	0, 30, 307, 1,165	1165	None	NA [e]
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 49–50)	0, 56, 288, 720	720	None	2/24, 1/20, 0/20, 1/20 [d]
	(Boberg et al., 2011)	Rat (W)	GD 7–PND 17 (PND 90)	0, 300, 600, 750, 900	900	None [f]	NA
	(Waterman et al., 2000)	Rat (SD)	–[a] (F1 Adults)	0, 133–153, 271–307, 543–577	577	None	NA [d]
DIDP	(Hushka et al., 2001)	Rat (SD)	–[a] (F1 Adults)	0, 15, 50, 165, 300–400	300–400	None	NA [d]
				0, 165, 300–400, 600	600	None	NA [d]

[b] Incidence of lesion reported qualitatively.
[c] Gray et al. (2009) report mild to moderate testicular seminiferous tubular degeneration, however, doses at which lesions were observed are not stated.
[d] Histologic examination of testes revealed no significant increase in seminiferous tubule atrophy or any other testicular pathologies.

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Doses (mg/kg/d)	NOAEL[g] (mg/kg/d)	LOAEL[g] (mg/kg/d)	Seminiferous Tubule Atrophy (# Affected Males/Total Males Examined or % Males Affected) (Severity, if Reported)

[e] Tubule atrophy not reported. Testicular pathology limited to degeneration of meiotic spermatocytes and vacuolar degeneration of Sertoli cells in high dose males.
[f] Boberg et al. (2011) report "Testicular histology at PND 90 appeared unaffected although a few animals had small areas of tubular degeneration in areas of focal Leydig cell hyperplasia." However, the doses at which this effect was observed were not reported.
[g] NOAEL/LOAEL values as reported by study authors.
[h] Severity grades reflect the percentage of degenerated tubules (Grade 1 = less than 5%; Grade 2 = 6–20%; Grade 3 = 21–50%; Grade 4 = greater than 50%).
[i] Severity grades reflect the percentage of degenerated tubules (Grade 1 = less than 5%; Grade 2 = 5–25%; Grade 3 = 26–45%; Grade 4 = 46–85%; Grade 5 = 86–100%).
[j] Reported as unilateral testicular dysgenesis, which is defined as "areas of aberrant or immature seminiferous tubules associated with proliferative Leydig cells."
GD = gestational day; LOAEL = lowest observed adverse effect level; NOAEL = no observed adverse effect level; PND = postnatal day; PNM = postnatal month; PNW = postnatal week; SD = Sprague-Dawley; W = Wistar

Table 3-16. Summary of ED50 Values for Incidence of Seminiferous Tubule Atrophy

Phthalate	ED50 (95% CI) (mg/kg/day)
DCHP	380 (350, 412)
DEHP	472 (438, 508)
DBP	628 (576, 683)
BBP	417 (392, 444)
DIBP	344 (313, 377)

[a] ED50 values indicate the dose at which a 50% incidence of seminiferous tubule atrophy was observed. A description of the methodology used to estimate ED50 values is provided in Appendix C.

3.1.3.7 Multinucleated Gonocyte (MNG) Formation

Phthalates can affect Sertoli cell function, development, and interactions with germ cells. Proper Sertoli cell function is necessary for germ cell proliferation and development and altered Sertoli cell function contributes to increased germ cell death, decreased germ cell numbers, and increased formation of MNGs. There is uncertainty underlying the mechanisms associated with MNG formation; however, it may serve as a biomarker of altered Sertoli-germ cell interactions (Spade et al., 2018; Spade et al., 2014). EPA identified 24 *in vivo* experimental studies that evaluated MNG formation following gestational exposure to the high-priority and manufacturer-requested phthalates. The majority of available studies were conducted using rat models (22 rat studies, 1 mouse studies, and 1 marmoset study). The most data was available for DEHP (seven rat studies) and DBP (nine rat studies, one mouse study, and one marmoset study), while less data was available for BBP (one rat study), DIBP (one rat study), DCHP (two rat studies) and DINP (four rat studies). No studies were available for DIDP.

As can be seen from Table 3-17, there is variability in how publications report MNGs, which makes comparisons across studies difficult (*e.g.*, this outcome may be reported as MNGs per testis or seminiferous cross-section, incidence of animals with MNGs in testes, percentage of total germ cells multinucleated, average number of nuclei per germ cell, etc.). However, the available rat studies (conducted with both SD and Wistar rats) consistently demonstrate that gestational exposure to DEHP, DBP, DCHP, and DINP can increase MNG formation in a dose-dependent manner (Table 3-17). One rat study investigating MNGs is available each for BBP (Spade et al., 2018) and DIBP (Borch et al., 2006a), and these studies only tested a single dose level (*i.e.*, 600 mg/kg/day DIBP; 750 mg/kg/day BBP). However, both studies reported marked increases in MNGs following gestational exposure to BBP or DIBP. In one mouse study of DBP, a dose-dependent increase in the number of MNGs per seminiferous cord cross-section was reported at all dose-levels tested. In contrast to the results observed in rats and mice, MNGs were not observed in marmosets gestationally exposed to 500 mg/kg/day MBP (a dose that causes MNGs in mice and rats), however, unusual clusters of undifferentiated germ cells were found in two of six MBP-exposed animals (McKinnell et al., 2009).

Table 3-17. Studies Reporting on the Incidence of MNGs

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Dose (mg/kg/d)	NOEL (mg/kg/d) [i]	LOEL (mg/kg/d) [i]	Dose-Response Data [a]
DEHP	(Borch et al., 2006b)	Rat (W)	GD 7–21 (GD 21)	0, 10, 30, 100, 300	30	100	–[b]
	(Nardelli et al., 2017)	Rat (SD)	GD 8–PND 21 (PND 3)	0, 30, 300	30	300	–[c f]
	(Andrade et al., 2006a)	Rat (W)	GD 6–PND 21 (PND 144)	0, 5, 15, 45, 135, 405	135	405	–[b]
	(Parks et al., 2000)	Rat (SD)	GD 14–PND 2 (PND 2)	0, 750	None	750	–[b]
	(Gray et al., 2000)	Rat (SD)	GD 14–PND 3 (PND 3)	0, 750	None	750	–[b]
	(Spade et al., 2018)	Rat (SD)	GD 17–21 (GD 21)	0, 750	None	750	2, 76 [d]
	(Martino-Andrade et al., 2008)	Rat (W)	GD 13–21 (GD 21)	0, 150	150	None	None
BBP	(Spade et al., 2018)	Rat (SD)	GD 17–21 (GD 21)	0, 750	None	750	2, 64 [d]
DBP	(Boekelheide et al., 2009)	Rat (SD)	GD 12–20 (GD 21)	0.1, 1, 10, 30, 50, 100, 500	50	100[i]	–[c]
	(Mahood et al., 2007)	Rat (W)	GD 13.5–20.5 (GD 21.5)	0, 4, 20, 100, 500	20	100 [f]	–[c]
	(Struve et al., 2009)	Rat (SD)	GD 12–19 (GD 19)	0, 112, 582	None	112	0/8, 1/6, 1/6 [e]
			GD 12–19 (GD 20)	0, 112, 582	None	112	0/9, 2/7, 6/7 [e]
	(Gaido et al., 2007)	Mouse (C57B16)	GD 16–18 (GD 19)	0, 250, 500	None	250	–[c h]
	(Mylchreest et al., 2002)	Rat (SD)	GD 12–21 (GD 21)	0, 500	None	500	–[b]
	(van Den Driesche et al., 2015)	Rat (W)	e13.5–21.5 (e21.5)	0, 500	None	500	0, 3.9% [g]
	(Martino-Andrade et al., 2008)	Rat (W)	GD 13–21 (GD 21)	0, 100, 500	100	500	–[c f]
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 2)	0, 642	None	642	1/24, 21/21 [e]
	(Ferrara et al., 2006)	Rat (W)	e13.5–17.5 (e17.5)	0, 500	500	None	None
			e13.5–19.5 (e19.5)	0, 500	None	500	–[c f]
			e13.5–20.5 (e21.5)	0, 500	None	500	–[c f]
			e13.5–21.5 (e21.5)	0, 500	None	500	–[c f]

Phthalate	Reference	Species (Strain)	Exposure Window (Time of Outcome Evaluation)	Dose (mg/kg/d)	NOEL (mg/kg/d) [i]	LOEL (mg/kg/d) [i]	Dose-Response Data [a]
			e13.5–21.5 (PND 4)	0, 500	None	500	–[c f]
			e19.5–20.5 (e21.5)	0, 500	None	500	–[c f]
	(Spade et al., 2018)	Rat (SD)	GD 17–21 (GD 21)	0, 750	None	750	2, 60 [d]
	(McKinnell et al., 2009)	Marmoset	GW 7–15 (PND 1–5 or 18–21 months)	0, 500	500	None	None
DIBP	(Borch et al., 2006a)	Rat (W)	GD 7–20/21 (GD 21)	0, 600	None	600	1/10, 10/16 [e]
DCHP	(Ahbab and Barlas, 2015)	Rat (W)	GD 6–19 (GD 20)	0, 20, 100, 500	20	100	0/10, 2/10, 5/10, 9/10 [e]
	(Li et al., 2016)	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500	10	100	0.4, 2, 16, 27% [f]
DINP	(Li et al., 2015a)	Rat (SD)	GD 12–21 (GD 21.5)	0, 10, 100, 500, 1,000	10	100	–[c f]
	(Clewell et al., 2013a)	Rat (SD)	GD 12–19 (GD 20)	0, 50, 250, 750	50	250	0, 0 ,0.75, 1.25 [d]
	(Clewell et al., 2013b)	Rat (SD)	GD 12–PND 14 (PND 2)	0, 56, 288, 720	56	288	1/24, 2/20, 7/20, 18/19 [e]
	(Boberg et al., 2011)	Rat (W)	GD 7–21 (GD 21)	0, 300, 600, 750, 900	300	600	0/7, 2/8, 3/5, 6/7, 6/6 [e]

[a] Response data is provided for each respective treatment group included in the study, starting with the control response.
[b] MNGs reported qualitatively in text.
[c] Dose-response observed, but data not extracted because data was only presented graphically.
[d] MNGs per testis cross-section.
[e] Incidence of animals with MNGs in testes.
[f] Percent seminiferous cords with MNGs.
[g] Expressed as a percentage of all germ cells.
[h] MNGs per seminiferous cord cross-section.
[i] NOEL/LOEL values reflect study authors statistical analysis (*i.e.*, the LOEL is the lowest value where a statistically significant effect was observed).
e = embryonic day; GD = gestational day; LOEL = lowest-observed-effect-level; NOEL = no-observed-effect-level; PND = postnatal day; SD = Sprague-Dawley; W = Wistar

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3.1.4 Phthalate Syndrome in Humans

As discussed by NRC (2008) and NASEM (2017), rat phthalate syndrome shows similarities with the hypothesized human testicular dysgenesis syndrome (Wohlfahrt-Veje et al., 2009). However, the etiology of the human syndrome is unknown, and it is unclear if endocrine disrupting chemicals such as phthalates play a role.

To help inform EPA's understanding of the human relevance of phthalate syndrome, EPA reviewed two types of studies, (1) mechanistic explant and xenograft studies of human fetal testis tissue (discussed in Section 3.1.4.1), and (2) human epidemiologic studies evaluating associations between phthalate exposure and effects on the male reproductive system (discussed in Section 3.1.4.2). Several recent systematic reviews of human epidemiology studies have been conducted by NASEM (2017) and EPA CPHEA scientists (Radke et al., 2018). These reviews include five of the high-priority and manufacturer-requested phthalates, including DEHP, BBP, DBP, DIBP, and DINP; results from these systematic reviews are the focus of discussion in Section 3.1.4.2. Neither review included DCHP, so EPA further reviewed several risk assessments conducted by other regulatory agencies to identify epidemiologic studies of DCHP (ECCC/HC, 2020; ECHA, 2014; U.S. CPSC, 2014, 2010e); however, no epidemiologic studies of DCHP and male reproductive outcomes were identified.

3.1.4.1 Human Explant and Xenograft Studies

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. Hallmark et al. (2007) dosed human fetal testis explants (obtained from four donors during gestational weeks 15 to 20) with DBP or MBP for 24 to 48 hours and observed no effect on basal, human chorionic gonadotropin (hCG) stimulated, or 22R-hydroxy-cholesterol (22-R-CHO) stimulated testosterone production. In contrast, MBP reduced hCG (but not 22-R-CHO) stimulated testosterone production and caused a slight but significant increase in Leydig cell aggregate size in fetal testes explants obtained from Wistar rats at GD 19.5. Similarly, Lambrot et al. (2009) observed no effect on basal or luteinizing hormone stimulated testosterone production or expression of *Insl3* and steroidogenic genes (*P450c17*, *P450scc*, *StAR*) in human fetal testes explants (obtained from donors between gestational weeks 7 to 12) exposed to MEHP for 3 days. However, the researchers did observe decreased germ cell numbers and an increase in the number of apoptotic germ cells.

Two separate research groups have developed xenograft protocols to evaluate the effects of phthalates on human fetal testis tissue. Mitchell et al. (2012) grafted human fetal testis tissue (obtained from 12 donors at 14 to 20 weeks of gestation) into castrate male CD-1 nude mice, which were then gavaged with 500 mg/kg/day DBP or MBP for up to 21 days. Treatment with DBP had no effect on host serum testosterone or seminal vesicle weight after 21 days of exposure, while MBP had no effect on host seminal vesicle weight. Treatment with MBP appeared to reduce host serum testosterone by around 50 percent; however, this effect was highly variable and was not statistically significant. Concurrent studies in which Wistar rat fetal testis tissue (obtained on GD 17.5) was grafted into castrate male mice were also conducted to help validate human results. After 4 days of oral exposure to 500 mg/kg/day DBP, a trend in reduced host serum testosterone level, reduced host seminal vesicle weight, and reduced mRNA expression of *StAR* and *Cyp11a1* in retrieved rat xenografts was observed. These affects are consistent with a disruption of androgen action. In a subsequent study by the same research group, the effects of DBP on Sertoli and germ cells in human xenografts was investigated (van Den Driesche et al., 2015). Briefly, human fetal testis tissue (obtained from seven donors between gestational weeks 14 to 20) was

grafted into male CD-1 nude mice, which were then gavaged with 500 mg/kg/day DBP for 21 days. In retrieved xenografts, DBP was found to reduce germ cell numbers, increase the incidence of MNGs, while immunostaining demonstrated a disruption of Sertoli cell cytoplasm distribution in xenografts with germ cell aggregation.

Concomitantly with the study by Mitchell et al. (2012), Heger et al. (2012) grafted human (obtained at 10 to 24 weeks of gestation), Fischer rat (obtained on GD 16), and CD-1 mouse (obtained on GD 15) fetal testis tissue into male Crl:NIH-Foxn1^{rnu} nude rats. Hosts were then gavaged with 100, 250, or 500 mg/kg/day DBP for 1 to 3 days and effects on steroidogenesis were measured in retrieved xenografts. For human xenografts, testosterone production could not be accurately measured and was only reported to be "highly variable" by study authors, while no effect of DBP was observed on steroidogenic gene expression. Similarly, DBP had no effect on testosterone production or steroidogenic gene expression in mouse grafts; however, an increased incidence of MNGs was observed in both human and mouse grafts following exposure to DBP. In contrast, a reduction in testosterone production and steroidogenic gene expression as well as an increase in MNGs were observed in rat xenografts following exposure to DBP. In a second study by the same research group, Spade et al. (2014) grafted human fetal testis tissue (obtained from six donors at 16 to 22 weeks of gestation) into adult castrated male nude mice. Hosts were then gavaged with 500 mg/kg/day DBP or 75 mg/kg/day abiraterone acetate (CYP17A1 inhibitor) for 14 days. Treatment with DBP had no effect on host serum testosterone or progesterone levels, host seminal vesicle, prostate or LABC weight, and microarray analysis indicated no widespread impact on steroidogenic gene expression. In contrast, abiraterone reduced host serum testosterone levels as well as SV, LABC, and prostate weight.

Collectively, human explant and xenograft studies suggest that human fetal testis tissue is not sensitive to the antiandrogenic effects of MEHP, DBP, or MBP. These results call into question the relevance of the rat model for use in human health risk assessment. However, there are limitations associated with these studies, which have been discussed extensively (Arzuaga et al., 2020; ECHA, 2017; EC/HC, 2015c; Albert and Jégou, 2014; Habert et al., 2014; U.S. CPSC, 2014). First, the majority of human fetal testis tissue used in xenograft and explant studies was obtained from fetuses older than 14 weeks of gestational age. Male programming of the testes occurs during gestational weeks 8 to 14 in humans (MacLeod et al., 2010); therefore, it is possible that effects on testosterone and steroidogenic gene expression were not observed due to the age of the fetal material. However, in the only study that utilized human fetal testis tissue obtained from donors between gestational weeks 7 to 12 (Lambrot et al., 2009), no effect on testosterone production or steroidogenic gene expression was observed in explants following exposure to MEHP, which would seem to argue against this possibility. Additionally, Hallmark et al. (2007) and Spade et al. (2014) exposed human fetal testis explants and xenografts, respectively, to CYP17 inhibitors (*i.e.,* ketoconazole and abiraterone) known to disrupt testicular steroidogenesis and observed reductions in testosterone. These findings indicate that steroidogenesis can be disrupted in human explants and xenografts, regardless of fetal age, at least through certain mechanisms.

Secondly, compared to rat explant and xenograft studies in which fetal testis tissue was obtained using a standard protocol and at a consistent gestational age, human fetal testis tissue was obtained from donors of variable age and by more variable methods, which likely contributed to the observed variability. Other potential issues raised with the human fetal testis explant studies (Lambrot et al., 2009; Hallmark et al., 2007) include the short phthalate exposure durations (*i.e.,* 1 to 3 days) that were necessary because explants were only viable *in vitro* for a few days. This raises the possibility that longer exposure durations that are more reflective of *in utero* phthalate exposure in humans might have resulted in an effect on steroidogenesis (Albert and Jégou, 2014). Further, other hormonal effects (*e.g.,* the

hypothalamic-pituitary-gonadal axis) that are known to play a role in testis development cannot be accounted for using *in vitro* explant models (ECHA, 2017; EC/HC, 2015c; Habert et al., 2014). Another potential issue that has been raised with human xenograft studies is variability in the testosterone assays. For example, in the study by Mitchell et al. (2012) there appeared to be a 50 percent reduction in host serum testosterone after 21 days of exposure to MBP; however, this result did not reach statistical significance due to variability and small sample size (N = 3–4). Similarly, results from the testosterone assay by Heger et al. (2012) was also reported to be highly variable and study authors did not quantitatively report results. Assay variability is likely in part due to small sample sizes and inherent biological variability in human tissue, as well as due to use of pooled results from human fetal testis tissue of varying ages.

NASEM (2017) attempted to address the human tissue sample size issue by conducting a meta-analysis of human xenograft studies of DBP and MBP and serum testosterone (Spade et al., 2014; Mitchell et al., 2012). Overall, NASEM observed a trend toward decreased serum testosterone (−14.5 percent [95 percent CI: −40.4, 22.6]); however, this effect was not statistically significant due to the low precision of the estimate (see Figure 3-17 in NASEM (2017)).

Generally, results from human explant and xenograft studies suggest that human fetal testes are not sensitive to the antiandrogenic effects of phthalates, which has led some to conclude that rats are not an appropriate model for use in human health risk assessment. However, as discussed above, human explant and xenograft studies have limitations, and therefore the human relevancy of antiandrogenic effects of phthalates should not be ruled out. Notably, other authoritative agencies have drawn similar conclusions regarding the human explant and xenograft studies, and concluded that the rat is an appropriate model for use in human health risk assessment (ECHA, 2017; NASEM, 2017; EC/HC, 2015c; U.S. CPSC, 2014).

3.1.4.2 Epidemiologic Studies

Two recent systematic reviews investigating associations between phthalate exposure and effects on the male reproductive system were evaluated (Radke et al., 2018; NASEM, 2017). NASEM employed NTP's OHAT systematic review methodology to evaluate the relationship between gestational exposure to metabolites of DEHP, BBP, DBP, DIBP, and DINP (DCHP and DIDP not included) and several outcomes, including decreased AGD, hypospadias, and testosterone concentrations during gestation or at birth. For hypospadias and testosterone, NASEM identified a limited number of epidemiologic studies and identified a number of confounding factors within the available studies, which led NASEM to conclude that there was inadequate evidence to determine if fetal exposure to DEHP, DBP, DIBP, BBP, or DINP is associated with hypospadias or a reduction in fetal testosterone. In contrast, NASEM identified a number of prospective cohort studies investigating the effects of gestational phthalate exposure on AGD at birth. NASEM found moderate confidence in the body of evidence for DEHP, BBP, DBP, DIBP, and DINP and conducted further meta-analyses of each phthalate. Although the meta-analyses found no statistically significant overall effect to support gestational exposure to BBP, DIBP, and DINP being associated with reduced AGD, they found statistically significant estimates of 4 and 3 percent decreases in AGD for DEHP and DBP, respectively, per $\log_{10}$ increase in exposure. These findings led NASEM to conclude that there is a moderate level of evidence to support an association between gestational exposure to DEHP and DBP and reduced AGD (Table 3-18).

Table 3-18. Summary of NASEM (2017) Systematic Review and Meta-Analysis for Epidemiologic Studies of AGD

Phthalate	# of Studies	Confidence in Evidence	Heterogeneity (I^2)	Summary Estimate (% Change) (95% CI)	Meta-Analysis Conclusion	Evidence of Outcome
DEHP	6 prospective	Moderate	0%	−4.07 (−6.49, −1.66) (p = 0.001)	Evidence of effect	Moderate
BBP	4 prospective	Moderate	0%	−1.43 (−3.47, 0.61) (p = 0.17)	No evidence of effect	Inadequate
DBP	4 prospective	Moderate	0%	−3.13 (−5.63, −0.64) (p = 0.014)	Evidence of effect	Moderate
DIBP	3 prospective	Moderate	0%	−2.23 (−5.15, 0.70) (p = 0.13)	No evidence of effect	Inadequate
DINP	3 prospective	Moderate	58%	−0.96 (−4.17, 2.25) (p = 0.56)	No evidence of effect	Inadequate

In a second systematic review of human epidemiologic studies, Radke et al. (2018) evaluated the strength of evidence supporting an association between phthalate exposure and male reproductive effects. The review included DEHP, BBP, DBP, DIBP, and DINP (but not DCHP or DIDP) and focused on outcomes such as AGD, semen parameters (*i.e.*, concentration, motility, and morphology), time to pregnancy (male exposure), testosterone, timing of pubertal development, hypospadias, and cryptorchidism. Notably, both DEHP and DBP showed moderate evidence of an association between gestational exposure and reduced AGD, while evidence of an association was slight for other evaluated phthalates, which is consistent with the results of NASEM (2017). For other outcomes (*i.e.*, hypospadias/cryptorchidism, testosterone in infants, and timing of pubertal development) associated with gestational and/or childhood phthalate exposure, the study authors identified a limited number of studies and concluded that there was slight or inadequate evidence to support an association (Table 3-19).

For outcomes associated with adult phthalate exposure, Radke et al. found (1) moderate evidence of postnatal exposure to DBP and BBP and time to pregnancy; (2) moderate evidence of postnatal exposure to DEHP, DINP, and DIBP and reduced testosterone levels in adults; and (3) moderate to robust evidence of an association between DEHP, DINP, DBP, and BBP with effects on semen parameters such as concentration, motility, and morphology (Table 3-19). As noted by the study authors, because DEHP and DBP tended to have the most available studies and higher exposure levels compared to some of the other evaluated phthalates, it may explain the difference in confidence in the strength of associations for certain outcomes. Regardless, Radke et al. generally concluded that there is robust evidence of an association between exposure to DEHP and DBP with male reproductive effects and moderate evidence of an association for DINP, DIBP, and BBP.

Table 3-19. Summary of Epidemiologic Evidence of Male Reproductive Effects Associated with Phthalates[a]

Timing of Exposure	Outcome	DEHP	DINP	DBP	DIBP	BBP
In utero	↓ AGD	**M**[b] (0/3/3)[c]	S (0/3/0	**M** (0/3/2)	S (0/2/1)	S (0/3/2)
	Hypospadias/ cryptorchidism	I (0/2/2)	S (0/2/1)	S (0/2/1)	S (0/2/1)	S (0/2/1)
In utero or childhood	Testosterone in infants	I (0/0/1)	I (0/0/1)	I (0/0/1)	I (0/0/1)	I (0/0/1)
	Timing of pubertal development	I (0/1/2)	I (0/0/1)	I (0/1/1)	I (0/0/1)	I (0/1/2)
Adult	Semen parameters	**M** (0/12/2)	**M** (0/3/0)	**R** (0/11/1)	S (0/4/0)	**M** (0/9/1)
	Time to pregnancy	S (1/0/0)	I (1/0/0)	**M** (1/0/0)	S (1/0/0)	**M** (1/0/0)
	Testosterone in adults	**M** (0/9/4)	**M** (0/5/0)	S (0/7/3)	**M** (0/2/2)	I (0/6/2)
Overall Evidence		**R**	**M**	**R**	**M**	**M**

[a] Table adapted from Radke et al. (2018).
[b] Strength of evidence descriptors: R = robust (bolded and cell shaded dark gray); M = moderate (bolded and light gray); S = slight; I = indeterminant. Robust and moderate descriptors indicate evidence supports a hazard based on the quantity and quality of available information, which rules out alternative explanations for the results. Slight and indeterminant descriptors indicate that evidence could support the presence or absence of a hazard and is typically limited by quantity or confidence level in available studies.
[c] Numbers in parentheses indicate the number of high, medium, and low confidence studies, respectively, used as part of the overall strength of evidence evaluation.

3.1.5 Species Differences in Sensitivity

Differences in species sensitivity to testicular toxicity of phthalate diesters has been recognized for decades (Gray et al., 1982) and has been discussed extensively by various authoritative agencies (*e.g.*, see (NASEM, 2017)), regulatory bodies (*e.g.*, see (ECHA, 2017; U.S. CPSC, 2014)), and research groups (*e.g.*, see (Arzuaga et al., 2020; Johnson et al., 2012)). As discussed in Sections 3.1.3.1 to 3.1.3.6, the majority of *in vivo* studies investigating the effects of gestational phthalate exposure have been conducted using rat models. Available rat data provide consistent evidence that gestational exposure to certain phthalates during the critical window of development can lead to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. Studies that investigated phthalate syndrome following gestational exposure during the critical window are available for mice (Wang et al., 2017; Do et al., 2012; Pocar et al., 2012; Liu et al., 2008; Gaido et al., 2007), rabbits, (Higuchi et al., 2003), and marmosets (McKinnell et al., 2009). Results from mouse and marmoset studies are inconsistent with findings from rat studies and indicate species differences in sensitivity. Mouse, rabbit, and marmoset data are discussed below, while available rat data are discussed further as part of the data integration and weight of evidence analysis in Section 3.1.6.

Consistent with findings from rat studies, gestational exposure of Dutch-Belted rabbits to 400 mg/kg DBP on GDs 15 to 29 caused numerous effects consistent with phthalate syndrome (Higuchi et al., 2003). Observed effects included (1) reduced absolute paired testis weight at postnatal week (PNW) 12 (but not at PNW 25) and accessory sex gland weight at PNWs 12 and 25; (2) sperm effects (*i.e.*, reduced ejaculate volume, sperm concentration and total sperm per ejaculate; morphologically abnormal sperm with acrosomal and nuclear defects); (3) pathological changes of the seminiferous epithelium; and (4)

reduced serum testosterone at PNW 6 (but not at PNWs 12 and 25). Additionally, 1 out of 17 male pups exposed to DBP *in utero* exhibited gross malformations of the reproductive tract, including undescended testes, malformed prepuce, hypospadias, hypoplastic seminal vesicle and prostate, and agenesis of the bulbourethral gland. This study indicates that like rats, rabbits are also sensitive to phthalate-induced effects on the developing male reproductive system.

In contrast to rats and rabbits, no effects on testicular morphology or development (*i.e.*, no hypospadias, cryptorchidism, small testes, impaired spermatogenesis, or testicular dysgenesis), serum testosterone, germ cell number or proliferation, or Sertoli cell number were noted at birth in marmosets dosed with 500 mg/kg/day MBP between gestational weeks 7 through 15 (McKinnell et al., 2009). Large clumps of undifferentiated germ cells were found in two of six marmosets exposed to MBP; however, the significance of this finding is unclear.

In vivo mouse studies investigating phthalate syndrome provide inconsistent results. Gaido et al. (2007) observed (1) no effect on fetal testicular testosterone in mice exposed to DBP, MBP, or MEHP at doses ranging from 500 to 1,500 mg/kg/day; and (2) no effect on expression of steroidogenic genes in mice exposed to a single dose of 500 mg/kg DBP on GD 18 or multiple doses of 250 mg/kg DBP on GDs 14 to 17. Similarly, Do et al. (2012) found no reduction in fetal testicular testosterone in mice exposed up to 500 mg/kg/day DEHP on GDs 9 to 18. Furthermore, as discussed in Section 3.1.4.1, xenograft studies of mouse fetal testis tissue found no effect of DBP on testosterone production or steroidogenic gene expression in grafts retrieved from exposed hosts (Heger et al., 2012). For DBP, MBP, DEHP, and MEHP, results consistently indicate that gestational phthalate exposure does not disrupt steroidogenesis during the critical window in *in vivo* mouse models, which is inconsistent with rat models.

In contrast to results for DBP and DEHP, Wang et al. (2017) observed a disruption of testicular steroidogenesis in ICR mice exposed to 450 mg/kg/day DIBP from GD 0 to 21 or GD 0 through PND 21. Observed effects include reduced testicular mRNA and protein expression of cholesterol transport and steroidogenic genes in offspring at PND 21 and PND 80 and reduced serum and testis testosterone levels in offspring at PND 21 and PND 80. These results indicate a persistent disruption of testicular steroidogenesis following gestational and/or perinatal exposure to DIBP. However, the study authors did not measure testosterone or steroidogenic gene expression in fetal testis, and it is unclear if steroidogenesis was disrupted during the critical window following exposure to DIBP (Wang et al., 2017).

Although studies indicate that steroidogenesis is not disrupted during the critical window in mouse models following gestational exposure to DEHP or DBP, other effects consistent with phthalate syndrome have been observed in mice. One study in which mice were gavaged with 100 to 500 mg/kg DEHP on embryonic days 12 to 17 reported a dose-dependent reduction in AGD (Liu et al., 2008); however, three other studies in which mice were exposed to up to 500 mg/kg/day DEHP (Do et al., 2012), 5 mg/kg/day DEHP (Pocar et al., 2012) or 450 mg/kg/day DIBP (Wang et al., 2017) throughout the critical window found no effect on AGD. Effects on male reproductive organ and accessory gland weight have been observed following gestational exposure to DEHP and DIBP in mouse models. For example, Do et al. reported a dose-dependent reduction in absolute testis weight in mice gestationally exposed to 50 mg/kg/day or more of DEHP; Pocar et al. (2012) reported a dose related decrease in absolute seminal vesicle, but not testis, weight at low doses of DEHP (≥0.05 mg/kg/day); and Wang et al. (2017) reported decreased absolute testis, but not epididymis, weight in mice exposed to 450 mg/kg/day DIBP. Other notable effects consistent with the development of phthalate syndrome after gestational and/or perinatal exposure to phthalates have been reported, including: (1) hypospadias and reduced anterior urethra length at doses of 100 mg/kg/day or greater of DEHP (Liu et al., 2008); (2)

decreased sperm concentration and viability after low dose (*i.e.*, 0.05 and 5 mg/kg/day) exposure to DEHP (Pocar et al., 2012); (3) decreased sperm concentration and motility after perinatal exposure to 450 mg/kg DIBP (Wang et al., 2017); and (4) dose-related increases in seminiferous cord diameter and MNG formation at dose of 250 to 500 mg/kg/day DBP (Gaido et al., 2007). Presumably, these effects on the male reproductive system are occurring in the absence of a disruption of fetal testicular steroidogenesis during the critical window in mouse models, which is inconsistent with rat models.

Finally, and as discussed in Section 3.1.4.1, mechanistic explant and xenograft studies conducted with human fetal testis tissue generally indicate that the human fetal testis may be less sensitive to phthalate-induced disruptions of steroidogenesis. However, these studies have limitations and their results should be interpreted cautiously. For example, testosterone results were highly variable in the xenograft studies conducted by both Mitchell et al. (2012) and Heger et al. (2012). Variability may be attributable to small sample size, variable methods by which the human tissue was obtained, variable age of fetal material, and/or the fact that most studies were conducted using testis obtained from fetuses outside of the male programming window.

Notably, scientists from EPA's CPHEA recently conducted a species concordance analysis for DBP that incorporated additional study types (*i.e.*, mechanistic studies conducted using *in vitro* cell culture models and *ex vivo* tissue culture models) and exposure periods (*i.e.*, postnatal/peripubertal exposures) (Arzuaga et al., 2020). The study authors draw several notable conclusions based on the totality of data for DBP. First, fetal rats appear to be more sensitive than other mammalian species to the antiandrogenic effects of DBP. Second, effects on the seminiferous cord and germ cells (*e.g.*, decreased Sertoli cell numbers, altered interactions between germ and Sertoli cells, impaired germ cell development, increased germ cell apoptosis) appear to be conserved across most mammalian species, including human xenografts. Third, that antiandrogenic effects, as well as effects on Sertoli cells and germ cells, appear to be conserved across most mammalian species, including human xenografts, following postnatal exposure to DBP.

3.1.5.1 Species Difference in Metabolism and Toxicokinetics

Species differences in phthalate metabolism and toxicokinetics have been reported, and discussed extensively by various agencies (*e.g.*, see (ATSDR, 2022; NASEM, 2017)) and regulatory bodies (*e.g.*, see (ECHA, 2017; U.S. CPSC, 2014)). Most recently, ATSDR (2022) summarized available toxicokinetic data for DEHP and reached several notable conclusions based on the totality of available information across species and exposure routes. First, ATSDR concluded that DEHP can be absorbed via the (1) oral (>70 percent for humans; ≥30 percent in monkeys, rats, mice, and hamsters [because fecal excretion is generally not accounted for, absorption values based on urinary excretion are considered underestimates]), (2) dermal (2 percent for humans; 6 percent for rats), and (3) inhalation routes (98 percent absorption for rats; demonstrated qualitatively in humans). Second, animal studies indicate that for all routes of exposure, DEHP is systemically distributed, including to the testes and fetus; however, distribution has not been reliably evaluated in humans. Third, across species, DEHP is metabolized to MEHP by lipase, for which significant species differences in enzyme activity exist (see additional discussion below). Fourth, DEHP metabolites are excreted primarily in the urine and feces (urinary:biliary excretion ratios vary widely across studies), with blood, serum, or plasma elimination half-lives for MEHP ranging from 2 to 4 hours in humans and marmosets and 1.1 to 9.4 hours in rats. Finally, ATSDR concluded that metabolite excretion profiles observed in humans are similar to those observed in other mammalian species (*i.e.*, monkeys, rats, mice, hamsters, and guinea pigs), although differences in abundance of certain metabolites and glucuronide conjugates have been reported between species.

Several quantitative pharmacokinetic studies have noted species specific differences. For example, Kessler et al. (2004) administered repeated doses 30 or 500 mg/kg/day DEHP to pregnant SD rats and marmosets and reported peak blood concentrations (C_{max}) to be 1.6 to 4.3 times higher in the rat compared to the marmoset, while AUC values were 2.6 to 15.6 times higher in rats compared to marmosets. These results indicate differences in dosimetry that may help explain observed differences in DEHP response between rats and marmosets. Kinetic experiments conducted on human volunteers are available (Kessler et al., 2012; Koch et al., 2012; Koch et al., 2004). Notably, Kessler et al. (2012) found that C_{max} and AUC values for MEHP and DEHP in human serum were much higher than reported for rats and marmosets at similar doses, which led the study authors to conclude that the "MEHP blood burden at a given DEHP dose per kg body weight will be higher in humans than in the animals"; however, this study only included four human volunteers.

Large species differences in tissue lipase activity have been reported. As discussed in Section 3.1.1, a critical first step in phthalate toxicity is the metabolism of the diester parent phthalate to its monoester metabolite by lipases in the intestine or liver. Monoester metabolites are implicated as being the toxic moiety associated with the reproductive toxicity of phthalates. Ito et al. (2005) found lipase activity, measured as the rate of conversion of DEHP to MEHP, to be 2.3 to 29.5 times higher in mice compared to rats in liver and small intestine microsomes and 26.7 to 357 times higher in mice compared to marmosets (Table 3-20). In a follow-up study, Ito et al. (2014) evaluated liver lipase activity in mice and humans, and found mouse lipase activity to be 5.1 times higher than human lipase activity, however, it is worth noting that human lipase activity varied by approximately 10-fold across 28 individuals. Intrinsic clearance (*i.e.*, the ratio of Vmax [maximum velocity]-to-Km [Michaelis constant]) varied significantly across species, indicating species difference in enzyme affinity for DEHP exist. Notably, human liver lipase activity was considerably higher than marmosets (~6.5-fold), and only modestly lower than rats (*i.e.*, less than a factor of 2), indicating liver lipase activity may not vary dramatically between rats and humans. Ito et al. (2014; 2005) also measured the activity of several other enzymes involved in phthalate metabolism (*i.e.*, UDP-glucuronocyltransferase, alcohol dehydrogenase, and aldehyde dehydrogenase); however, the extent of species differences in activity were not as great as for lipase for these enzymes.

Table 3-20. Comparison of Lipase Activity across Species

Species (Strain)	Small Intestine Lipase Activity (pmol/mg)	Liver Lipase Activity (pmol/mg)	Liver *Km* (mmol L^{-1})	Liver *Vmax* (nmol $mg^{-1}min^{-1}$)	Liver *Vmax/Km*
Mouse (CD-1)[a]	11,790	4,964	0.012	3.91	333
Mouse (129/Sv)[b]	–	6,220	0.0076	5.45	714
Rat (SD)[a]	400	2,129	0.006	1.32	227
Marmoset[a]	33	186	1.357	0.49	1.38
Human[b]	–	1,210	0.0144	1.52	106

[a] Source: (Ito et al., 2005)
[b] Source: (Ito et al., 2014)

3.1.6 Data Integration and Weight of Evidence Analysis

Sections 3.1.3.1 to 3.1.3.7 of this document review the available data for the five high-priority and two manufacturer-requested phthalates for several key outcomes associated with phthalate syndrome. As

described in Section 3.1.2, these key outcomes were selected to help inform EPA's development of a cumulative chemical group for CRA based on EPA's current understanding of phthalate syndrome and its underlying MOA.

To support data integration and the weight of evidence analysis, EPA applied modified Bradford Hill criteria, which are typically applied in the context of evaluating the relevance of a non-cancer or a cancer MOA for humans (WHO/IPCS, 2007; U.S. EPA, 2005). Although the purpose of this document is not to establish a MOA for phthalate syndrome, modified Bradford Hill criteria (*i.e.*, temporal and dose-response concordance; strength, consistency and specificity; biological plausibility and coherence) provide a useful structure for discussing the weight of evidence supporting EPA's proposed cumulative chemical group. As discussed in Sections 3.1.3.1 to 3.1.3.7, rat models provide the most available *in vivo* data supporting key outcomes associated with phthalate syndrome, and the discussion of modified Bradford Hill criteria is primarily focused on data from available rat studies. However, inconsistencies, as well as consistencies, observed across species are emphasized throughout the sections that follow.

3.1.6.1 Temporal Concordance

The temporal relationship between phthalate exposure and certain key outcomes associated with phthalate syndrome is generally well recognized. As discussed by NRC (2008) and NASEM (2017), the male programming window in which androgen action drives development of the male reproductive system is from gestation days 15.5 to 18.5 in rats, which corresponds to gestation weeks 8 to 14 in humans (Welsh et al., 2008; Carruthers and Foster, 2005). As discussed in Sections 3.1.3.1 and 3.1.3.2, rat data demonstrate that exposure to DEHP, BBP, DBP, DIBP, DCHP, and DINP (but not DIDP) during the male programming window result in reduced expression of cholesterol transport and steroidogenic genes, as well as reduce fetal testicular testosterone content and/or testosterone production. Time course studies investigating testicular gene expression and testosterone provide somewhat conflicting results regarding temporality. Johnson et al. (2012) gavaged rats with a single dose of 500 mg/kg DBP on GD 19 and observed reductions in gene expression 3 (*Cyp17a1*) to 6 (*Cyp11a1, StAR*) hours post-exposure, while fetal testicular testosterone was not reduced until 18 hours post-exposure, supporting a temporal relationship. In contrast, Thompson et al. (2005) reported a 50 percent reduction in fetal testicular testosterone 1 hour after a single dose of 500 mg/kg DBP on GD 19, while changes in 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. Of note, testosterone levels were reduced by approximately 50 percent until the 6-hour time point and then further declined to approximately 20 percent of control values from the 12-hour time point onwards. This further decline in testosterone correlated with the reduction in mRNA and protein levels of cholesterol transport and steroidogenic genes.

As discussed in Section 3.1.3.3 to 3.1.3.7, rat data indicate that reductions in fetal testicular testosterone during the critical window are associated with development of phthalate syndrome-related effects later in life—including reduced AGD, increased incidence of NR, seminiferous tubule atrophy, hypospadias and other reproductive malformations. In support of these findings, Howdeshell et al. (2015) demonstrate an inverse relationship between reduced fetal testicular testosterone production on gestational day 18 and the frequency and severity of phthalate syndrome-related effects (*i.e.*, decreased AGD, NR, reproductive tract malformations) observed in prepubertal and adult rats well after cessation of exposure. 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 NR, and increase the frequency of reproductive tract malformations and testicular pathology in adult rats. These effects were absent when exposure occurred prior to the male programming window.

Collectively, these studies demonstrate the temporal relationship between gestational exposure during the critical window and the occurrence of adverse effects on the male reproductive system later in life well after cessation of exposure.

3.1.6.2 Dose-Response Concordance

As discussed in Sections 3.1.3.1 to 3.1.3.7, data from rat studies supporting the key outcomes generally exhibit strong dose-response concordance (inconsistencies observed across species discussed in Section 3.1.5). For DEHP, BBP, DBP, DIBP, DCHP and DINP, rat studies consistently demonstrate that gestational exposure during the critical window leads to dose-dependent decreases in fetal testicular mRNA expression of cholesterol transport genes (*i.e., Scarb1, Star*), steroidogenic genes (*i.e., Cyp11a1, Cyp17a1, 3bHSD*), and *Insl3* (Section 3.1.3.1). Additionally, consistent dose-dependent reductions in fetal testicular testosterone were observed for DEHP, BBP, DBP, DIBP, DCHP, and DINP (Section 3.1.3.2).

Consistent with a disruption of steroidogenesis, dose-dependent decreases in male pup AGD (Section 3.1.3.3), increases in nipple/areolae retention in male pups (Section 3.1.3.4) and hypospadias (Section 3.1.3.5) are observed following gestational exposure to DEHP, BBP, DBP, DIBP, and DCHP across available rat studies. Increased incidence of seminiferous tubule atrophy is also observed following gestational and/or perinatal exposure to these phthalates across available rat studies (Section 3.1.3.6). These effects generally occur regardless of rat strain tested; however, NASEM's meta-analysis and BMD analysis of Wistar and SD rat data for DEHP did note several unexplained inconsistencies in strain sensitivity (NASEM, 2017). First, SD rats appeared slightly more sensitive to DEHP than Wistar rats for effects on fetal testicular testosterone (see Table 3-7), while Wistar rats were more sensitive than SD rats for effects on AGD (see Table 3-9). Also, as noted by both EPA and NASEM, dose-dependent increases in hypospadias are observed in SD, but not Wistar, rats administered similar doses of DEHP. In contrast, DBP consistently increased hypospadias in both SD and Wistar rats in a dose dependent manner. Currently, the biological significance of the strain differences in sensitivity to DEHP are unclear.

For DINP, data indicate less consistent dose-related effects on AGD, nipple/areolae retention, and seminiferous tubule atrophy following gestational exposure during the critical window. Two out of six rat studies found that DINP reduced male AGD (Section 3.1.3.3), while two out of three rat studies report a dose-related increase in male nipple/areolae retention (Section 3.1.3.4). For tubular atrophy, one study reported a low incidence of this lesion at 750 mg/kg/day (Gray et al., 2000), while a second study reported that a few rats gestationally exposed to DINP had "areas of tubular degeneration in areas of focal Leydig cell hyperplasia." However, doses at which this effect was observed were not consistently reported (Boberg et al., 2011), and three other studies found no significant incidence of tubule atrophy at similar or higher doses (Section 3.1.3.6). In a study conducted by Clewell et al. (2013b), mild hypospadias were reported in 1 out of 111 control and 2 out of 84 high-dose (*i.e.*, dosed with 720 mg/kg/day DINP) pups; however, the effect was not statistically significant. Hypospadias have not been observed in other studies following gestational exposure to DINP at doses as high as 900 mg/kg/day (Section 3.1.3.5).

Finally, as discussed in Section 3.1.3.7, data are available for DEHP, DBP, DCHP, and DINP that consistently demonstrate dose-response concordance for formation of MNGs. For BBP and DIBP, only a single study evaluating MNGs formation was identified for each phthalate and the available studies only tested a single, relatively high dose (*i.e.*, 600 mg/kg/day DIBP; 750 mg/kg/day BBP) (Spade et al., 2018; Borch et al., 2006a); however, both studies reported effects on MNG formation that were large in magnitude.

To better understand dose-response relationships across phthalates and across evaluated outcomes, EPA conducted preliminary dose-response analyses on gene expression, testosterone, AGD, NR, hypospadias, and seminiferous tubule atrophy data. For this preliminary analysis, ED50 values were calculated (Table 3-21). As can be seen from Table 3-21, 95 percent confidence intervals overlapped across ED50 values for some phthalates and outcomes, which limits this comparative analysis. However, certain trends in potency are apparent. First, for effects on fetal testicular gene expression DEHP, DCHP, and BBP appear to be the most potent, followed by DBP and then DIBP, while DINP is clearly and consistently the least potent. For effects on fetal testicular testosterone production, DCHP, DEHP, and DBP appear to be the most potent, followed by BBP and DIBP, while DINP is the least potent. For effects on male pup nipple/areolae retention, DEHP and DBP appeared to be more potent than DCHP, DIBP and BBP, while for hypospadias, DIBP and DCHP appear to be more potent than DEHP, BBP, and DBP. Finally, for seminiferous tubule atrophy, DCHP and DIBP appear to be more potent than BBP and DEHP, while DBP appears to be the least potent. These preliminary results indicate that although phthalate potency may vary by outcome, further comparative studies using lower effect levels are needed. These results also consistently indicate that DINP is less potent than other phthalates, such as DEHP, which is consistent with how other authoritative agencies have characterized DINP (*i.e.*, as a weak antiandrogen) (EC/HC, 2015b; NICNAS, 2012; U.S. CPSC, 2010f). Finally, it is worth noting that comparative pharmacokinetic studies indicate that differences in potency are not due to differences in dosimetry at the target tissue (*i.e.*, fetal testis) (Clewell et al., 2010).

Collectively, these studies demonstrate dose-response concordance between gestational exposure during the critical window and the occurrence of adverse effects on the male reproductive system.

Table 3-21. Comparison of Rat ED50 Values (mg/kg/day) across Key Outcomes

Key Outcome[a]	DEHP ED50 (95% CI)	DCHP ED50 (95% CI)	DBP ED50 (95% CI)	BBP ED50 (95% CI)	DIBP ED50 (95% CI)	DINP ED50 (95% CI)
Star mRNA	109 (33, 196)	99 (48, 202)	247 (74, 824)	77 (46, 129)	324 (201, 523)	592 (493, 709)
Scarb1 mRNA	120 (62, 178)	62 (40, 96)	295 (111, 779)	50 (20, 121)	287 (159, 519)	594 (440, 802)
Cyp11a1 mRNA	173 (102, 249)	129 (49, 338)	367 (170, 793)	126 (59, 266)	407 (253, 654)	1148 (862, 1,530)
Cyp17a1 mRNA	134 (101, 168)	53 (30, 92)	285 (186, 437)	180 (129, 251)	371 (219, 626)	802 (698, 921)
3bHSD mRNA	242 (80, 503)	95 (37, 244)	530 (288, 974)	164 (72, 372)	595 (325, 1,089)	1016 (750, 1,376)
Insl3 mRNA	158 (104, 215)	162 (97, 270)	237 (149, 376)	167 (65, 434)	414 (261, 656)	1537 (730, 3,236)
Testicular Testosterone	143 (132, 156)	91 (46, 180)	154 (88, 268)	228 (150, 347)	275 (226, 334)	918 (780, 1,081)
↓ Anogenital Distance	1314 (1068, 1846)	1128 (825, 2042)	920 (775, 1149)	813 (685, 1002)	777 (594, 1,177)	–[b]
Nipple/areolae Retention	368 (275, 491)	588 (324, 1067)	331 (240, 463)	749 (551, 2,020)	479 (366, 628)	–[b]
Hypospadias	846 (804, 904)	699 (631, 825)	958 (919, 999)	878 (829, 948)	626 (603, 653)	–[b]

Key Outcome[a]	DEHP ED50 (95% CI)	DCHP ED50 (95% CI)	DBP ED50 (95% CI)	BBP ED50 (95% CI)	DIBP ED50 (95% CI)	DINP ED50 (95% CI)
Seminiferous Tubule Atrophy	472 (438, 508)	380 (350, 412)	628 (576, 683)	417 (392, 444)	344 (313, 377)	–[b]

[a] Rat ED50 values and 95% confidence intervals (95% CI) as reported in Sections 3.1.3.1 to 3.1.3.6.
[b] Rat ED50 values were not estimated for DINP for reduced AGD, NR, hypospadias, or seminiferous tubule atrophy because sufficient dose-response data was not available to support accurate ED50 predictions (see Sections 3.1.3.3 to 3.1.3.6 for more details).

3.1.6.3 Strength, Consistency, and Specificity

As discussed in Sections 3.1.3.1 to 3.1.3.7, rat models provide the most available *in vivo* data supporting key outcomes associated with phthalate syndrome. Available rat studies have been conducted by multiple research groups and are of varying design (*i.e.*, gestational, perinatal, and multigeneration studies). Available rat studies provide remarkably consistent evidence demonstrating that gestational exposure to DEHP, BBP, DBP, DIBP, and DCHP during the critical window of development effects all key outcomes associated with phthalate syndrome (Table 3-22). Although EPA's review focused on studies that evaluated seven key outcomes, EPA extracted data for all phthalate syndrome-related effects reported in each reviewed study (see Appendices B.2 to B.8). As can be seen from Table 3-22, other phthalate syndrome-related effects have been observed following gestational exposure to DEHP, BBP, DBP, DIBP, and DCHP—including decreased absolute reproductive organ and accessory sex gland weight, testicular pathology, epididymal and/or gubernaculum agenesis, undescended testes, sperm effects, and impairment of male fertility and reproductive function. These observations further add to the weight of evidence demonstrating that gestational exposure to DEHP, BBP, DBP, DIBP, and DCHP disrupt development of the male reproductive system in rat models.

For DINP, gestational exposure during the critical window results in consistent reductions in fetal testicular mRNA expression of *Insl3*, cholesterol transport, and steroidogenesis genes (discussed in Section 3.1.3.1). Consistent with a disruption of steroidogenesis at the mRNA level, gestational exposure to DINP also results in consistent reductions in fetal testicular testosterone production (Section 3.1.3.2). However, effects on AGD, NR, and seminiferous tubule atrophy are less consistently observed across available rat studies (Sections 3.1.3.3, 3.1.3.4, 3.1.3.6). In contrast to other high-priority phthalates, gestational exposure to DINP does not appear to cause hypospadias or other severe reproductive tract malformations, such as cryptorchidism (Table 3-22), and did not alter male fertility or reproductive function in available one- and two-generation reproduction studies (Waterman et al., 2000). However, as can be seen from Table 3-22, other effects consistent with phthalate syndrome have been observed following gestational exposure to DINP, including (1) decreased sperm motility (Boberg et al., 2011), (2) epididymal agenesis (Gray et al., 2000), and (3) other testicular pathologies (*e.g.*, Leydig cell aggregation, enlarged diameter seminiferous chords, many gonocytes centrally located in chords) (Li et al., 2015a; Clewell et al., 2013a; Clewell et al., 2013b). These effects provide further evidence that gestational exposure to DINP can have adverse effects on the developing male reproductive system. As was discussed in Section 3.1.6.2, comparative dose-response studies demonstrate that DINP is less potent at disrupting fetal testicular steroidogenesis compared to other high-priority phthalates, and therefore less consistent effects on apical outcomes are not unexpected.

2136 **Table 3-22. Summary of Phthalate Syndrome-Related Effects Observed in Rat Studies[a]**

Phthalate Syndrome-Related Effect	DEHP	BBP	DBP	DIBP	DCHP	DINP	DIDP
↓ Steroidogenic gene and *Insl3* expression	✓	✓	✓	✓	✓	✓	*x*
↓ Fetal testosterone	✓	✓	✓	✓	✓	✓	*x*
↓ Anogenital distance	✓	✓	✓	✓	✓	*i*	*x*
Nipple retention	✓	✓	✓	✓	✓	*i*	*x*
Hypospadias	✓	✓	✓	✓	✓	*x*	*x*
Seminiferous tubule atrophy	✓	✓	✓	✓	✓	*i*	*x*
MNGs	✓	✓	✓	✓	✓	✓	–
↓ Reproductive organ weight[b]	✓	✓	✓	✓	✓	*i*	*x*
Testicular pathology[c]	✓	✓	✓	✓	✓	✓	*x*
Epididymal agenesis	✓	✓	✓	✓	–	✓	*x*
Gubernaculum agenesis	✓	–	✓	–	–	–	*x*
Undescended testes	✓	✓	✓	✓	*x*	*x*	*x*
Sperm effects[d]	✓	✓	✓	–	✓	✓	*x*
↓ Male fertility[e]	✓	✓	✓	–	*x*	*x*	*x*
Appendix	B.2	B.3	B.4	B.5	B.6	B.7	B.8

✓ = Studies available, effects observed.
x = Studies available, no effects observed.
i = Studies available, inconsistent effects observed.
– = No study available.

[a] See reference list (below) for examples of studies demonstrating each observed effect. Rows shaded white indicate key outcomes selected by EPA for in depth review. Rows shaded gray are additional phthalate syndrome-related effects observed during study review of key outcome data. See cited Appendices for study summaries.

[b] May include decreased absolute testis, epididymis, seminal vesicle, and/or prostate weight.

[c] May include, but is not limited to, Leydig cell aggregation, interstitial cell hyperplasia or adenoma, Sertoli cell only tubules, and/or epididymal oligospermia or azoospermia.

[d] May include, but is not limited to, decreased sperm motility and/or concentration.

[e] May include, but is not limited to decreased mating, pregnancy, and/or fertility indices.

References

DEHP: Organ weight (Gray et al., 2009; Lin et al., 2008); testicular pathology (Saillenfait et al., 2009a; Borch et al., 2006b); epididymal & gubernaculum agenesis (Howdeshell et al., 2007; Gray et al., 2000); undescended testes (Saillenfait et al., 2009a; Vo et al., 2009; Culty et al., 2008); sperm & fertility effects (Gray et al., 2009; Vo et al., 2009; TherImmune Research Corporation, 2004)

BBP: Organ weight (Ahmad et al., 2014; Aso et al., 2005; Tyl et al., 2004); testicular pathology (Aso et al., 2005; Tyl et al., 2004); epididymal agenesis (Gray et al., 2000); undescended testes (Tyl et al., 2004; Ema et al., 2003; Gray et al., 2000); sperm and fertility effects (Ahmad et al., 2014; Tyl et al., 2004)

DBP: Organ weight (Clewell et al., 2013b; Mylchreest et al., 2000); testicular pathology (Clewell et al., 2013b; Barlow et al., 2004); epididymal & gubernaculum agenesis (Howdeshell et al., 2007; Mylchreest et al., 1999); undescended testes (Li et al., 2015b; Drake et al., 2009); sperm & fertility effects (Mahood et al., 2007; NTP, 1995)

DIBP: Organ weight (Saillenfait et al., 2008); testicular pathology (Saillenfait et al., 2008; Borch et al., 2006a); epididymal agenesis (Saillenfait et al., 2008); undescended testes (Saillenfait et al., 2008; Saillenfait et al., 2006)

DCHP: Organ weight (Yamasaki et al., 2009; Hoshino et al., 2005); testicular pathology (Li et al., 2016; Ahbab and Barlas, 2015); undescended testes (Saillenfait et al., 2009b); fertility & sperm effects (Hoshino et al., 2005)

DINP: Testicular pathology (Li et al., 2015a; Clewell et al., 2013a; Boberg et al., 2011); epididymal agenesis (Gray et al., 2000); sperm & fertility effects (Boberg et al., 2011; Waterman et al., 2000)

DIDP: See (Hushka et al., 2001)

For DIDP, there is no evidence of effect on the male reproductive system consistent with phthalate syndrome. Three studies have demonstrated no effect on fetal testicular testosterone production and/or steroidogenic gene and *Insl3* mRNA expression in rats gestationally exposed to up to 1,500 mg/kg/day DIDP (Sections 3.1.3.1 to 3.1.3.2). In the available two-generation reproduction studies of DIDP, continuous exposure of up to 400 to 600 mg/kg/day DIDP had no effect on AGD, NR, or hypospadias in male pups of either generation. Additionally, DIDP did not affect any other phthalate syndrome-related outcomes in the available two-generation studies, including reproductive indices (*e.g.*, mating, fertility, gestation and birth index), weight of androgen-sensitive organs (*e.g.*, prostate, testes, epididymis, and SV), sperm parameters (*i.e.*, sperm count, motility, and morphology) or preputial separation (Hushka et al., 2001). Notably, the European Commission (ECJRC, 2003), ECHA (2013), EFSA (2019), Australia NICNAS (2015b), Health Canada (EC/HC, 2015e), and the U.S. CPSC (2010d) have also concluded that DIDP does not induce antiandrogenic effects on the developing male reproductive system.

3.1.6.4 Biological Plausibility and Coherence

As discussed by NRC (2008) and NASEM (2017), androgen action has a conserved role in the development of the male reproductive system across mammalian species, including humans. In rats, exposure to certain phthalates during the critical window can disrupt fetal testicular steroidogenesis leading to reduced testosterone production and a cause spectrum of effects on the developing male reproductive system. In humans, rat phthalate syndrome shows similarities with the hypothesized testicular dysgenesis syndrome, which includes adverse effects such as infertility, decreased sperm count, cryptorchidism, hypospadias, testicular tumors, and reproductive tract malformations (reviewed in (NRC, 2008)). Further, androgen insufficiency is well described in humans. For example, mutations in the gene encoding 5α-reductase can result in male pseudohermaphroditism and delay development of male physical characteristics, resulting in effects ranging from external feminization to male infertility. These effects demonstrate a conserved role for androgen action in humans.

Given the conserved role that androgens play in development of the male reproductive system across mammalian species, it is biologically plausible that *in utero* exposure to phthalates may lead to a disruption of androgen action and cause adverse effects on the developing male reproductive system in humans. Biological plausibility is further strengthened by systematic reviews and meta-analyses of epidemiologic studies conducted by EPA (Radke et al., 2018) and NASEM (2017), both of which found moderate evidence of an association between *in utero* exposure to DEHP and DBP and reduced AGD in male infants (discussed in Section 3.1.4.2). Notably, NRC (2008), NASEM (2017), and other authoritative regulatory agencies have drawn similar conclusions regarding biological plausibility of rat phthalate syndrome in humans and have determined rat models are appropriate for characterizing risk to human health (ECCC/HC, 2020; EFSA, 2019; ECHA, 2017; NICNAS, 2015a; U.S. CPSC, 2014).

3.1.6.5 Uncertainties

Several areas of uncertainty are associated with EPA's current analysis. First, there are differences in species sensitivity to phthalate-induced reproductive toxicity (discussed in Section 3.1.5). Rats and rabbits appear to be sensitive species based on numerous studies in rats and the one gestational exposure study in rabbits, while no effects consistent with phthalate syndrome were observed in one study of marmosets exposed during the critical window. For mice, no effects of fetal testicular steroidogenesis are observed following exposure to DBP or DEHP during the critical window. However, some effects consistent with phthalate syndrome have been observed, albeit inconsistently, including reduced AGD, nipple/areolae retention, decreased testes and accessory sex gland weights, hypospadias, and sperm effects. These effects are presumably occurring in the absence of a disruption of fetal testicular steroidogenesis. Human xenograft and explant studies suggest that the human fetal testis is insensitive to phthalate-induced perturbations of steroidogenesis. As discussed in Section 3.1.4.1, these studies have

limitations and their results must be interpreted with caution. Species differences in metabolism and toxicokinetics have been implicated in playing a role in species differences in sensitivity. For example, monoester metabolites, formed through the enzymatic action of lipase, are thought to be one of the toxic moieties associated with phthalate reproductive toxicity.

As discussed in Section 3.1.5.1, studies have shown that mice and rats have significantly higher lipase activity than marmosets (241- [mice] to 164- [rat] fold higher; Table 3-20). Additionally, comparative pharmacokinetic studies have found peak blood concentrations and AUC values to be 1.6 to 4.3 and 2.6 to 15.6 times higher, respectively, in rats compared to marmosets when administered equivalent doses of DEHP. These difference in metabolism and toxicokinetics may explain observed differences in sensitivity between rats and marmosets. However, lipase activity appears to be significantly higher in humans compared to marmosets, and is within a factor of two of rat lipase activity (Table 3-20). Additionally, kinetic experiments with a small number of human volunteers indicate that MEHP blood levels may be higher in humans compared to rats at comparable doses. These findings raise uncertainty and seem to indicate that observed differences in species sensitivity cannot be fully explained by differences in metabolism and toxicokinetics.

Another source of uncertainty is that the molecular initiating event(s) associated with the phthalate syndrome MOA have not been established (discussed in Section 3.1.1). Establishing the molecular initiating event(s) associated with phthalate syndrome may help to explain the observed differences in species sensitivity.

Another source of uncertainty is lack of inhalation and dermal studies that include an exposure that covers the critical window of development. As discussed in Section 6, EPA is evaluating the oral, dermal, and inhalation exposure routes for the five high-priority and two manufacturer-requested phthalates. Lack of inhalation and dermal studies that include exposure throughout the critical window is a data gap. To address this data gap, EPA may employ route-to-route extrapolation, which can introduce uncertainty into assessments, as it generally does not account for route-specific differences in toxicokinetics (IGHRC, 2006).

3.1.7 Proposed Conclusions on Toxicologic Similarity

The totality of rat data indicates that gestational exposure to DEHP, BBP, DBP, DIBP, and DCHP during the critical window of development leads to a disruption of fetal testicular steroidogenesis, which results in reduced fetal testicular testosterone production, reduced AGD, nipple/areolae retention, and hypospadias. Seminiferous tubule atrophy is also consistently observed following exposure to these five phthalates. Available rat data are remarkably consistent and support temporal and dose-response concordance. For DINP, available rat data also provide consistent evidence that gestational exposure to DINP disrupts steroidogenesis in the fetal testes in a dose-related manner. Comparative dose-response studies indicate that DINP is less potent than other phthalates such as DEHP. Dose-related effects on AGD and NR were also observed for DINP, albeit less consistently than for other phthalates, while severe reproductive tract malformations such as hypospadias have not been reported following gestational exposure. Finally, available data indicate consistent, dose-related increases in incidence of MNGs following gestational exposure to DEHP, BBP, DBP, DIBP, DCHP, and DINP. In contrast, for DIDP, the totality of evidence indicates that gestational exposure to very high doses (*e.g.*, 1,500 mg/kg/day) of DIDP does not disrupt fetal testicular steroidogenesis or cause any other effects consistent with phthalate syndrome in rat models. Based on the totality of data from rat studies, EPA has reached a preliminary conclusion that DEHP, DBP, BBP, DIBP, DCHP, and DINP, but not DIDP, are toxicologically similar.

As discussed above in Section 3.1.6.5, there are several sources of uncertainty that reduce EPA's confidence in this preliminary conclusion, including differences in species sensitivity observed across certain mammalian species that do not appear to be fully explained by differences in toxicokinetics. As discussed in Section 3.1.1, the molecular events associated with the development of phthalate syndrome are unknown. Establishing the molecular events preceding cellular, organ, and organism-level changes may help to further explain species differences in sensitivity. Given the conserved role that androgens play in the development of the male reproductive system across mammalian species, it is biologically plausible that *in utero* exposure to phthalates may adversely affect development of the male reproductive system in humans. Recent systematic reviews and meta-analyses of human epidemiologic data have linked *in utero* exposure to DEHP and DBP to reduced AGD at birth, which further strengthens EPA's conclusion on biological plausibility.

Further, compared to other phthalates such as DEHP, DINP is less potent at disrupting fetal testicular steroidogenesis and subsequent apical outcomes associated with phthalate syndrome are either inconsistently reported (*e.g.*, decreased AGD, NR, seminiferous tubule atrophy) or not reported at all (*e.g.*, hypospadias). These inconsistencies in response are another source of uncertainty and reduce EPA's confidence in the conclusion that DINP is toxicologically similar to DEHP, DBP, DIBP, BBP, and DCHP.

3.2 Evidence of Co-exposure over a Relevant Timeframe

In addition to considerations of toxicological similarity, inclusion and grouping phthalates into a CRA requires consideration of whether co-exposure is occuring over a relevant timeframe for the populations of concern. Relevant timeframe of exposure could mean exposure to multiple chemical in the same timeframe or overlapping of persistent effects from exposure to multiple chemicals. Characterizing co-exposure requires consideration of the source of chemical exposure, populations impacted by exposure, and the possible varying routes and pathways of exposure. Sources of data or information that can help determine whether the general population or subpopulations considered under TSCA are potentially co-exposed to the seven phthalates of interest include

- biomonitoring data showing the presence of multiple phthalates in a human population;
- monitoring data of environmental media including ambient air, drinking water, surface water, and soil showing the co-occurrence of multiple phthalates;
- product formulation information showing multiple phthalates in a single product; or
- workplace monitoring information showing that workers may encounter multiple phthalates in an occupational setting.

The U.S. CPSC and Health Canada applied similar exposure filters for inclusion of individual phthalates into a CRA and concluded that there was evidence of co-exposure to multiple phthalates to the general population, pregnant women, women of reproductive age, and infants, based on biomonitoring and environmental monitoring data (ECCC/HC, 2020; U.S. CPSC, 2014).

Specifically for the U.S. population, U.S. CPSC (2014) utilized U.S. Centers for Disease Control and Prevention (CDC) National Health and Nutrition Evaluation Surveys (NHANES) biomonitoring data from the 2005 to 2006 cycle, which reports urinary concentrations for 15 phthalate metabolites specific to individual phthalate diesters. U.S. CPSC utilized 12 of the reported metabolites to determine exposure of pregnant women in the population to nine phthalate diesters, which included the following toxicologically similar phthalates: BBP, DBP, DEHP, DIBP, and DINP that are being considered for CRA under TSCA. U.S. CPSC also analyzed urinary biomonitoring data collected through the Study for Future Families and found that infants (0 to 37 months of age), as well as their mothers, had measurable levels of BBP, DBP, DEHP, DIBP, and DINP metabolites in their urine (Sathyanarayana et al., 2008b;

Sathyanarayana et al., 2008a). Notably, U.S. CPSC's analysis demonstrated that the general population and relevant subpopulations of concern had similar exposure levels based on measured urinary phthalate metabolites, and metabolites were measured above the analytical limit of detection in close to 100 percent of samples.

Analyses of more recent NHANES urinary biomonitoring data by EPA demonstrate continued co-exposure to the high-priority and manufacturer-requested phthalates. For example, researchers from EPA's Office of Research and Development report that the frequency of detection of most phthalate metabolites associated with exposure to BBP, DBP, DEHP, DIBP, DINP, and DIDP was greater than 97 percent across all NHANES participants from the 2013 to 2014 cycle (Reyes and Price, 2018) (Table 3-23). Similarly, as part of America's Children and Environment program, EPA analyzed a subset of 2015 to 2016 NHANES urinary phthalate metabolite data for BBP and DEHP in women ages 16 to 49 years and children ages 6 to 17 years and demonstrate a greater or equal to 97 percent frequency of detection in these populations for most metabolites. The high frequency of detection of phthalate metabolites in NHANES urinary biomonitoring data provides strong evidence of co-exposure to the high-priority and manufacturer-requested phthalates for the U.S. population. As discussed in Section 3.1.5.1, phthalates have elimination half-lives on the order of several hours and are quickly excreted from the body in urine. Therefore, the presence of phthalate metabolites in NHANES urinary biomonitoring data indicates recent phthalate exposure.

Table 3-23. Summary of Phthalate Metabolite Detection Frequencies in NHANES

Parent Phthalate	Urinary Metabolite	Percentage Below the Limit of Detection		
		2013–2014 NHANES (All Participants; N=2663) [a]	2015–2016 NHANES (Women Aged 16–49; N=585) [b]	2015–2016 NHANES (Children Aged 6–17; N=789) [b]
BBP	Mono-benzyl phthalate (MBzP)	2.4%	3%	2%
DBP	Mono-n-butyl phthalate (MnBP)	1.6%	–	–
DEHP	Mono-2-ethylhexyl phthalate (MEHP)	37.66%	35%	35%
	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	0.3%	0%	1%
	Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)	0.5%	0.4%	1%
	Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)	0.2%	–	–
DIBP	Mono-isobutyl phthalate (MiBP)	2.7%	–	–
DINP	Mono-isononyl phthalate (MiNP)	59.56%	–	–
	Mono-(carboxyoctyl) phthalate (MCOP)	0.1%	–	–
DIDP	Mono(carboxynonyl) phthalate	1.2%	–	–

[a] As reported in Reyes et al. (2018)
[b] As reported in EPA's Detailed Methods for Indicators B9 and B10, prepared is support of America's Children and the Environment program.
– Indicates that the metabolite was not included as part of the analysis.

Of note, although the DCHP metabolite, monocyclohexyl phthalate, was included in NHANES from 1999 to 2010, it has since been excluded from the NHANES survey due to low detection levels and a low frequency of detection in human urine (CDC, 2013a). U.S. CPSC (2014) did not report any exposure to DCHP and stated that current exposure to DCHP individually does not indicate a high level

of concern. Biomonitoring data used by Health Canada (2020) in their cumulative assessment also did not include monitoring for DCHP but based on the in-commerce status and measured presence in dust samples from Canadian homes, DCHP was included in their CRA. Recent human urinary biomonitoring data is not available to support the conclusion that there is co-exposure to DCHP and other high-priority and manufacturer-requested phthalates in the U.S. population. However, there is evidence that exposure to DCHP can occur through various industrial, commercial and consumer uses under TSCA jurisdiction (see COU Table 2-2 in DCHP Final Scope Document (U.S. EPA, 2020e)). Based on exposure to DCHP through the above uses, EPA anticipates there will be co-exposure to DCHP and other phthalates currently undergoing risk evaluation, for certain subpopulations and exposure scenarios. For example, an individual might be exposed to DCHP through an occupational exposure or consumer use, and this exposure may co-occur with other phthalates due to concurrent exposure to DEHP, BBP, DBP, DIBP, and DINP, as demonstrated by NHANES biomonitoring data.

Based on manufacturers reporting to the Chemical Data Reporting (CDR) database, indicating that they produce domestically or import into the U.S. generally above 25,000 lb per site per year, all phthalates currently undergoing risk evaluation under TSCA section 6 are expected to be in commerce. Further details on the sources of phthalate exposure regulated under TSCA is presented in Section 6. Additionally, as described in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c), COUs were identified with expected use by consumer, commercial, and industrial users, further indicating potential for co-exposure.

Based on biomonitoring data and use in commerce, EPA anticipates that there may be co-exposure to BBP, DBP, DCHP, DEHP, DIBP, DINP, and DIDP for certain populations.

3.3 Proposed Cumulative Chemical Group (Step 1 in Conceptual Model [Figure 2-1])

As described in EPA's Draft Proposed Principles of CRA under TSCA, there are two primary considerations for grouping chemicals for inclusion in a CRA, (1) toxicologic similarity, and (2) evidence of co-exposure over a relevant timeframe. The establishment of a cumulative chemical group for purposes of CRA is developed using a weight of evidence narrative that clearly characterizes the strengths and uncertainties of the evidence of toxicological similarity and potential co-exposure for each chemical considered.

As described in Section 3.2, human urinary biomonitoring data indicate that the U.S. population is concurrently exposed to DEHP, DBP, BBP, DIBP, DIDP, and DINP. For DCHP, recent human urinary biomonitoring data are not available; however, DCHP has been detected in house dust samples and has various industrial, commercial and consumer uses that fall under TSCA jurisdiction, which indicates there is potential for humans to be co-exposed to DCHP and the other six phthalates.

As described in Section 3.1.7, the weight of evidence indicates that DEHP, DBP, BBP, DCHP, DIBP, and DINP are toxicologically similar. Data indicate that gestational exposure to these toxicologically similar phthalates during the critical window of development leads to a spectrum of effects on the developing male reproductive system consistent with phthalate syndrome. However, DINP is less potent than other toxicologically similar phthalates. As described in EPA's *Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanisms of Toxicity* (U.S. EPA, 2002), not all chemicals identified as part of common mechanism group need to be carried forward for quantitative CRA. For example, a chemical with low hazard potential may be excluded.

As described in EPA's supplemental mixtures guidance (U.S. EPA, 2000), quantitative CRAs should focus on chemicals and exposure scenarios that are likely to be the largest contributors to risk. Further, uncertainties and biases can be substantial, even for CRAs focusing on a small number of chemicals. EPA considered whether DINP should be excluded from the phthalate cumulative chemical group on the basis of its lower potency. In the phthalate CRA conducted by Health Canada (ECCC/HC, 2020), HQs for DINP were found to be one of the largest contributors to the calculated HI for pregnant women/women of childbearing age and infants due to relatively higher exposure (see Appendix A.2). Thus, although DINP is less potent compared to other high-priority phthalates, it may still significantly contribute to risk for human populations being considered under TSCA due to relatively higher exposure and therefore EPA does not believe available data support the exclusion of DINP from the phthalate cumulative chemical group for CRA.

Based on currently available hazard and exposure data (summarized in Table 3-24), EPA proposes a cumulative chemical group of DEHP, BBP, DBP, DIBP, DCHP, and DINP for human health CRA under TSCA.

Although NHANES urinary biomonitoring data indicates that there is potential for co-exposure to DIDP and other phthalates being evaluated under TSCA (DEHP, BBP, DBP, DIBP, DINP) (Section 3.2), the weight of evidence indicates that DIDP is not toxicologically similar to these phthalates (Section 3.1.7). Available data indicate that DIDP does not cause effects on the developing male reproductive system consistent with phthalate syndrome (Section 3.1.7). As shown in Figure 3-1, chemicals included in a cumulative chemical group should be toxicologically similar and there should be evidence to support co-exposure over a relevant timeframe. Because DIDP does not satisfy both criteria, EPA proposes to exclude DIDP from the phthalate cumulative chemical group.

Table 3-24. Summary of Information Supporting EPA's Proposed Cumulative Chemical Group for CRA under TSCA

Phthalate	High-Priority or Manufacturer-Requested?	Toxicologically Similar?	Evidence of Co-exposure (Biomonitoring)?	Evidence of Exposure through Manufacturing and/or Use (Industrial Commercial, Consumer)?	Include in Cumulative Chemical Group?
DEHP	High-Priority	Yes	Yes	Yes	Yes
BBP	High-Priority	Yes	Yes	Yes	Yes
DBP	High-Priority	Yes	Yes	Yes	Yes
DIBP	High-Priority	Yes	Yes	Yes	Yes
DCHP	High-Priority	Yes	Limited data[a]	Yes	Yes
DINP	Manufacturer-Requested	Yes	Yes	Yes	Yes
DIDP	Manufacturer-Requested	No	Yes	Yes	No

[a] The DCHP metabolite, monocyclohexyl phthalate, was included in NHANES from 1999–2010; however, it has since been excluded from the NHANES survey due to low detection levels and a low frequency of detection in human urine (CDC, 2013a).

4 PROPOSED OPTIONS FOR ADDRESSING PHTHALATE SYNDROME

4.1 Addressing Phthalate Syndrome as a Whole Versus Focusing on the Most Sensitive Effect

NRC laid out two options for addressing phthalate syndrome, including (1) assessing the syndrome as a whole, and (2) focusing on the most sensitive effect associated with the syndrome (NRC, 2008). As discussed further below, EPA considered the applicability of both of these approaches for use in a phthalate CRA under TSCA.

4.1.1 Addressing Phthalate Syndrome as a Whole

As discussed by NRC (2008), when addressing phthalate syndrome as a whole there are two potential approaches that can be used. First, effects associated with phthalate syndrome can be combined by evaluating individual pup level data for the presence or absence of phthalate syndrome-related effects. Individual pups can then be classified as exhibiting phthalate syndrome or not. Under this approach, each dichotomized endpoint is assumed to have an equal level of toxicity, and each pup is simply classified as having the syndrome or not. A second option for addressing phthalate syndrome as a whole is to develop and incorporate a scoring method that adjusts individual pup level data for the severity of each observed effect. For this approach, (1) data for individuals is evaluated, (2) each observed phthalate syndrome-related effect is scored for severity, and (3) a composite toxicity score is developed for each exposed individual.

Recently, researchers in EPA's Office of Research and Development (ORD) developed an ordinal dose-response modeling approach for addressing phthalate syndrome as a whole (Blessinger et al., 2020). Under this approach, data for several phthalate syndrome-related outcomes are evaluated for each individual pup, and individual pups are categorized into ordinal levels based on the expected effect on male fertility. Ordinal levels include, level 0 (no phthalate syndrome-related effects observed), level 1 (≥1 phthalate syndrome-related effect observed with no to moderate impacts on fertility), or level 2 (≥1 phthalate syndrome-related effect observed with severe impacts on fertility). Figure 4-1 shows the phthalate syndrome-related outcomes and associated levels developed by Blessinger et al. Level binning decisions were determined by study authors in partnership with EPA's National Center for Risk Assessment's Reproductive, Developmental, and Neurological Toxicology Workgroup and were consistent with recommendations of toxicologic pathologists (Lanning et al., 2002). Once individual pups are categorized into ordinal levels, benchmark dose (BMD) modeling is conducted to estimate BMD values for ordinal level 1 and 2 data using a benchmark response of 5 percent and 1 percent extra risk, respectively.

Level 1	Level 2
Male developmental reproductive effects	
• Areola/nipple Retention	• Cleft prepuce • Hypospadias (mild, moderate, or severe) • Exposed os penis • Undescended testes (left, right, or both) • Small penis • Vaginal pouch • Prostate absent • Seminal vesicles abnormal
Epididymis histopathology[a]	
• Interstitial mononuclear cells: grade 2–5 • Oligospermia: grade 2–4 • Grade 5 oligospermia, unilateral (i.e., in only one epid), w/o azoospermia in other epididymis; or azoospermia, unilateral, w/o grade 5 oligospermia in other epididymis • Sloughed cells (caput, cauda, or corpus): grade 2–5 • Granulomatous inflammation: grade 2–4[b] • Tubular necrosis: grade 2–5	• Bilateral grade 5 oligospermia or azoospermia
Testis histopathology[a]	
• Interstitial cell hyperplasia: grade 2-4[b] • Tubular necrosis/mineralization: grade 2–5 • Tubular vacuolation/loss of germ cells: grade 2–4[b] • Seminiferous tubule degeneration-atrophy/hypoplasia: grade 2–4 • Seminiferous tubule degeneration-atrophy/hypoplasia: grade 5 in one testis, grade 1–4 or not present in other testis • Loss of seminiferous tubules: grade 2–4[b]	• Seminiferous tubular degeneration-atrophy/hypoplasia: grade 5 in both testes

[a] Unless otherwise indicated, an animal was designated as having the level 1 endpoint if either side (left or right) had the endpoint.
[b] No animals had grade 5 granulomatous inflammation, grade 2–5 interstitial fibrosis, or grade 5 interstitial cell hyperplasia, tubular vacuolation, or loss of seminiferous tubules.

Figure 4-1. Proposed Severity Classifications for Phthalate Syndrome-Related Outcomes (from Blessinger et al. (2020))

Although the ordinal dose-response modeling approach presented by Blessinger et al. provides a relatively straightforward approach for addressing phthalate syndrome as a whole, limitations are apparent. First, the approach requires individual-level pup data that is infrequently available. This would limit the number of studies available to EPA for BMD modeling. Second, the current approach only incorporates a limited number of phthalate syndrome-related endpoints (*e.g.*, a number of malformations, such as testis and epididymal agenesis are not included) and outcomes measured as continuous variables are not included in the scoring system (*e.g.*, decreased AGD, delayed PPS, decreased reproductive and accessory organ weight). In some cases, exclusion of these outcomes may inappropriately lead to a pup being binned into level of 0, when level 1 or 2 is more appropriate. Another consideration is that this approach may not always provide the most sensitive point of departure. For example, to demonstrate the applicability of the approach, Blessinger et al. used pup data from a gestational exposure study in which SD rats were orally dosed with 125 to 625 mg/kg/day DIBP from GD 12 to 21 (Saillenfait et al., 2008). BMDs for phthalate syndrome ordinal levels 1 and 2 were 215 mg/kg (BMDL = 98 mg/kg) and 234 mg/kg (BMDL = 101 mg/kg), respectively, while modeling of azoospermia and sloughed cells gave more conservative BMD_5 values of 117 mg/kg (BMDL = 60 mg/kg) and 112 mg/kg (BMDL = 67 mg/kg).

4.1.2 Focusing on the Most Sensitive Effect

A second option for addressing phthalate syndrome is to focus on the most sensitive effect associated with the syndrome. For this approach, comparative dose-response studies are conducted to identify the most sensitive common phthalate syndrome-related effect across the six toxicologically similar phthalates under consideration. One potential challenge associated with this approach is that no single outcome may be identified as the most sensitive across the six toxicologically similar phthalates. However, failure to identify a single outcome as the most sensitive would likely be more a reflection of the available literature for each phthalate, than biology. For example, across available gestational and perinatal studies there is great deal of variation related to dose selection, exposure timing and duration,

species/strain tested, and measured phthalate syndrome-related outcomes (*e.g.*, fetal testicular testosterone synthesis is sometimes, but not always measured). This variability has generally led to regulatory agencies to select PODs based on different critical effects for use in phthalate CRAs (*e.g.*, see Appendix A). However, previous phthalate CRAs conducted by regulatory agencies have generally utilized the NOAEL/LOAEL approach for determining the critical effect, not more robust dose-response analyses.

4.1.3 EPA's Proposed Approach for Addressing Phthalate Syndrome

As discussed in Section 4.1.1, there are numerous challenges and limitations associated with addressing phthalate syndrome as a whole. Most notably, this approach requires individual pup level data, which is infrequently available and would limit the number of studies available to EPA for BMD modeling. Due to this limitation, EPA is proposing to address phthalate syndrome under TSCA by focusing on the most sensitive effect. As discussed above (Section 4.1.2), one potential challenge with this approach is that no single outcome may be identified as the most sensitive across the six toxicologically similar phthalates. Potential options for addressing this challenge, if encountered, are discussed further in Section 4.4. EPA's proposal to address phthalate syndrome by focusing on the most sensitive effect is consistent with how U.S. CPSC (2014), Health Canada (ECCC/HC, 2020), Australia NICNAS (2015a, 2014a, b, 2013, 2012), Danish EPA (ECHA, 2011), and EFSA (2019) addressed phthalate syndrome (see summary of CRA approaches in Appendices A.1 to A.5).

4.2 Applicability of Dose Addition for Phthalates

As described in EPA's Draft Proposed Principles of CRA under TSCA, several additivity approaches can be used to evaluate multiple chemical substances for cumulative risk to human health, including dose addition, response addition, and integrated addition, as well as approaches that account for toxicologic interactions (U.S. EPA, 2000, 1986). EPA is proposing to rely upon a default assumption of dose addition when conducting CRAs for toxicologically similar chemical substances under TSCA. As described in Section 3.1.7, EPA considers there to be sufficient evidence to conclude that DEHP, BBP, DBP, DIBP, DCHP, and DINP are toxicologically similar and induce effects on the developing male reproductive system consistent with phthalate syndrome. Therefore, EPA is proposing to evaluate DEHP, BBP, DBP, DIBP, DCHP, and DINP for cumulative risk to human health under an assumption of dose addition.

Consistent with EPA's proposal to evaluate phthalates under an assumption of dose addition, other regulatory agencies that have evaluated phthalates for cumulative risk to human health have also done so under an assumption of dose addition (ECCC/HC, 2020; EFSA, 2019; NICNAS, 2015a, 2014a, b; U.S. CPSC, 2014; NICNAS, 2013, 2012; ECHA, 2011). In further support of EPA's proposal to use dose addition, NRC concluded that there is strong evidence to support the use of dose addition for assessing antiandrogenic phthalates, as well as phthalates and other antiandrogens (despite mixed MOAs), for cumulative risk to human health (NRC, 2008). Notably, NRC's conclusion was based upon empirical evidence from multiple *in vivo* phthalate studies (Howdeshell et al., 2008; Howdeshell et al., 2007), *in vivo* studies of antiandrogenic pesticides and pharmaceuticals (Hass et al., 2007; Metzdorff et al., 2007; Birkhøj et al., 2004; Nellemann et al., 2003), and *in vivo* studies of phthalates and antiandrogenic pesticides and pharmaceuticals with mixed MOAs (Rider et al., 2008; Hotchkiss et al., 2004). Although NRC noted that in many cases both dose addition and response addition can accurately predict observed effects, in several cases response addition underestimated the observed effects, while dose addition provided equal or better predictions of observed effects for phthalates, other antiandrogens, and phthalates in combination with other antiandrogens, despite mixed MOAs.

Since NRC published their 2008 report, additional *in vivo* phthalate mixtures studies (Howdeshell et al., 2015; Hannas et al., 2011) and studies of phthalates and other antiandrogens (Conley et al., 2021; Conley et al., 2018; Beverly et al., 2014; Hotchkiss et al., 2010; Rider et al., 2010; Christiansen et al., 2009; Rider et al., 2009) have been published, and results from these studies further support the conclusions of NRC (2008) and EPA's proposal to use dose addition for phthalates. For example, Hannas et al. (2011) report the results of a nine phthalate (*i.e.*, DEHP, DIHP, DIBP, DBP, BBP, DCHP, DPP, di(n)heptyl phthalate, di-n-hexyl phthalate) fixed ratio mixture study. In this study, SD rats were gavaged with dilutions of mixture containing 54 to 650 mg/kg total phthalates on GDs 14 to 18, and then *ex vivo* fetal testicular testosterone production was evaluated. When observed phthalate mixture effects were compared to dose addition and response addition model predictions, the study authors found that dose addition provided the best prediction of the observed mixture effect. In a subsequent fixed ratio mixture study, Howdeshell et al. (2015) gavaged pregnant SD rats with dilutions of a mixture of five phthalates (*i.e.*, BBP, DBP, DEHP, DIBP, DPP) from GD 8 to PND 3. Administered mixture dilutions contained 0, 65, 130, 260, 520 and 780 mg/kg/day total phthalates. Male pups and adult offspring (aged 40 to 46 weeks) were evaluated for 14 phthalate syndrome-related effects, including neonatal mortality, AGD (PND2), nipple retention (PND 13 and adults), hypospadias, epididymal and testicular malformations, SV and ventral prostate agenesis, and absolute testes, epididymal, SV, and ventral prostate weight. Overall, the study authors found that dose addition models accurately predicted 11 out of 14 outcomes and better predicted observed mixture effects compared to response addition models.

Previously, stakeholders have raised concerns over the applicability of dose addition at very low doses (*i.e.*, at doses below the individual chemical LOAELs) (U.S. EPA, 2011). However, two recent publications have addressed this uncertainty (Conley et al., 2021; Conley et al., 2018). Conley et al. (2018) administered an 18 chemical mixture that contained 9 phthalates (DEHP, DPP, DBP, DCHP, BBP, DIBP, diisoheptyl phthalate, dihexyl phthalate, diheptyl phthalate) and 9 antiandrogenic pesticides and pharmaceuticals (p, p'-DDE, linuron, prochloraz, procymidone, pyrifluquinazon, vinclozolin, finasteride, flutamide, simvastatin) with mixed MOAs to rats on GDs 14 to 18. Dosing solutions were prepared as a fixed ratio dilution series based on the LOAEL for antiandrogenic effects for each individual chemical such that the highest dose tested contained each chemical at its LOAEL divided by 5, followed by each chemical at its LOAEL divided by 10, 20, 40, and 80. Antiandrogenic effects (*e.g.*, reduced paired testis, epididymal, LABC weight) were noted at the lowest dose tested (*i.e.*, LOAEL/80). Although, the primary goal of the study was not to evaluate how well dose addition and response addition models predict observed mixture effects, study authors did compare observed mixture effects on AGD in male pups at PND 2 with model predictions by comparing observed and model predicted ED90 and ED60 values. For this outcome, the study authors found that response addition models better predicted the observed mixture ED90, while dose addition models better predicted the observed mixture ED60.

In a subsequent study, Conley et al. (2021) administered a 15 chemical mixture containing 9 phthalates (BBP, DBP, DCHP, DEHP, DIBP, DPP, diheptyl phthalate, dihexyl phthalate, diisoheptyl phthalate) and 6 antiandrogenic pesticides and pharmaceuticals (linuron, p,p'-DDE, prochloraz, procymidone, pyrifluquinazon, vinclozolin) to rats on GDs 14 to 18. Dosing solutions were prepared as a fixed ratio dilution series based on the NOAEL for antiandrogenic effects for each individual chemical such that the highest dose contained each chemical at two-fold its NOAEL, followed by a dilution series of each chemical at its NOAEL and NOAEL divided by 2, 4, 8, 15, 100, and 1,000. Male fetuses (GD 18), pups (PND 2, 9, 13), and adults (PND 120) were then examined for a suite of effects on the male reproductive system associated a disruption of androgen action, including decreased AGD, reduced seminal vesicle weight, and formation of hypospadias. The most sensitive effect was reduced testicular expression of steroidogenic genes at NOAEL/15. For AGD, seminal vesical weight, and hypospadias, ED50 values

were calculated based on the observed mixture effects and predicted using dose addition, response addition, and integration addition models. For all three outcomes, dose addition models provided the most accurate predictions of observed mixture effects.

Mixture studies by Conley et al. demonstrate several key points. First, they provide evidence to support the concept of "something from nothing" since effects were observed at exposure levels below the individual chemical LOAELs (*i.e.,* LOAEL/80 in (Conley et al., 2018)) and NOAELs (*i.e.,* NOAEL/15 in (Conley et al., 2021)). Secondly, these studies provide evidence to support the applicability of dose addition at low doses for mixtures of phthalates and other antiandrogens. Finally, these studies further demonstrate the applicability of dose addition for mixtures of antiandrogens with mixed MOAs. For example, although the tested chemicals disrupt androgen action through multiple molecular initiating events (*e.g.,* finasteride is a 5α-reductase inhibitor, flutamide and vinclozolin are androgen receptor antagonists, linuron inhibits steroidogenic CYPs and is an androgen receptor antagonist, while the molecular initiating event for phthalates is unknown), these chemicals cause common key cellular events and lead to common adverse effects on development of the male reproductive tract in a manner consistent with dose addition.

4.3 Approaches Based on Dose Addition

The final rule for *Procedures for Chemical Risk Evaluation Under the Amended Toxic Substances Control Act* (82 FR 33726, July 20, 2017) provides EPA flexibility to select the most appropriate risk characterization method based on the best available science (TSCA sections 26(h)). As described in EPA's mixture guidances (2000, 1986), several component-based approaches can be used to evaluate two or more chemical substances based on dose additivity. The HI approach and RPFs are two component-based approaches frequently used by EPA. EPA's Office of Land and Emergency Management (OLEM) frequently uses the HI approach for Superfund site risk assessment (U.S. EPA, 1989), while EPA's Office of Pesticide Programs (OPP) often uses the RPF and MOE approaches to evaluate multiple pesticides when implementing the Food Quality Protection Act (U.S. EPA, 2002). The HI and RPF approaches are described briefly below in Sections 4.3.1 and 4.3.2, respectively, and in more detail in EPA's mixture guidances (2000, 1986). EPA is considering the applicability of both the HI and RPF approaches for a phthalates CRA under TSCA.

4.3.1 Hazard Index Approach

The HI approach integrates estimated exposures with toxicity information to characterize the potential for adverse effects. In the HI approach, hazard quotients (HQs) are calculated by dividing an estimate of exposure by a reference value (RfV) for each component chemical in the mixture. These HQs are summed to yield the HI for the mixture (Equation 4-1). For oral and inhalation exposures, EPA's preferred RfVs are the oral reference dose and inhalation reference concentration, respectively, in health risk assessments. Because the HI is dimensionless, exposure estimates and the RfV must have the same units. The HI does not estimate risk, *per se*; it is not expressed as a probability and does not estimate a toxicity measure. Instead, the HI is an indicator of potential hazard. In general, an HI that is greater than or equal to 1 indicates potential concern.

Equation 4-1. Calculating the hazard index

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i}$$

where:

- HI = hazard index (unitless)
- HQ_i = hazard quotient for the i^{th} chemical (unitless)
- E_i = estimated exposure for the i^{th} chemical (mg/kg/day or mg/m^3)

- RfV_i = reference for the i^{th} chemical (*e.g.*, RfD in mg/kg/day or mg/m^3)

4.3.2 Relative Potency Factor Approach

For the RPF approach, chemicals being evaluated require data that support toxicologic similarity (*e.g.*, components of a mixture share a known or suspected common MOA or share a common apical endpoint/effect) and have dose-response data for the effect of concern over similar exposure ranges (U.S. EPA, 2000). RPF values account for potency differences among chemicals in a mixture and scale the dose of one chemical to an equitoxic dose of another chemical (typically called the index chemical [IC]). The chemical selected as the IC is often among best characterized toxicologically and considered to be representative of the type of toxicity elicited by other components of the mixture. Implementing an RPF approach requires a quantitative dose response assessment for the IC and pertinent data that allow the potency of the mixture components to be meaningfully compared to that of the IC. In the RPF approach, RPFs are calculated as the ratio of the potency of the individual component to that of the index chemical using either (1) the response at a fixed dose; or (2) the dose at a fixed response (Equation 4-2).

Equation 4-2. Calculating RPFs

$$RPF_i = \frac{BMD_{R-IC}}{BMD_{R-i}}$$

where:

- BMD = benchmark dose (mg/kg/day or mg/m^3)
- R = magnitude of response (*i.e.*, benchmark response)
- i = i^{th} chemical
- IC = index chemical

After scaling the chemical component doses to the potency of the IC, the scaled doses are summed and expressed as index chemical equivalents for the mixture (Equation 4-3).

Equation 4-3. Calculating index chemical equivalents

$$Index\ Chemical\ Equivalents_{MIX} = \sum_{i=1}^{n} d_i \times RPF_i$$

where:

- Index chemical equivalents = dose of the mixture (mg/kg/day or mg/m^3)
- d_i = dose of the i^{th} chemical in the mixture (mg/kg/day or mg/m^3)
- RPF_i = relative potency factor of the i^{th} chemical in the mixture (unitless)

Noncancer risk associated with exposure to the mixture can then be assessed by calculating an MOE, which in this case is the ratio of the index chemical's non-cancer hazard value (*e.g.*, the BMDL) to an estimate of mixture exposure expressed in terms of index chemical equivalents. The MOE is then compared to the benchmark MOE (*i.e.*, the total uncertainty factor associated with the assessment) to characterize risk. The lower the MOE (margin between the toxicity effect level and the exposure dose), the more likely a chemical is to pose a risk.

4.3.3 Proposed Risk Characterization Approach for Phthalates under TSCA

Both the HI and RPF approaches have been used as part of previous phthalate human health CRAs. For example, Health Canada and Danish EPA employed the HI and risk-characterization-ratio approaches (analogous to HI approach), respectively (ECCC/HC, 2020; ECHA, 2011), while EFSA employed an RPF approach (EFSA, 2019), and U.S. CPSC (2014) employed a hybrid approach that utilized both the HI approach and relative potency assumptions (see Appendices A.1-A.5 for a summary of previous phthalate CRA approaches). However, there are challenges associated with the RPF approach. In 2008, NRC considered the applicability of RPFs for phthalates (NRC, 2008). NRC concluded that RPFs

cannot be recommended for phthalates because phthalates have dose-response curves that have differing slopes and shapes depending on the outcome being evaluated, which would result in differing potency factors depending on the response level at which they are computed.

However, the science has evolved since the NRC made their recommendation against the use of RPFs. RPFs can be applied for chemicals with dissimilar dose-response curves, as the establishment of a known or suspected common MOA shared by members of the class of compounds is considered more fundamental. It is common practice to estimate RPFs closer to the low-dose range of the dose-response function (*i.e.*, at the 5 or 10 percent effect level versus 50 percent) (U.S. EPA, 2016, 2007b, 2000). This practice is intended to reduce possible high-dose influences on estimated RPFs that may arise due to saturation of certain kinetic processes (*e.g.*, receptor binding, metabolic elimination). However, this approach also carries an implicit assumption that dose-response curve shapes will be the same below the selected response level. In this case, special consideration should be given to the choice of IC, as the IC should not have an extreme difference in shape compared to other chemicals under consideration.

As discussed above in Section 3.1.7, available data indicate that DEHP, BBP, DBP, DIBP, DCHP, and DINP are toxicologically similar. Gestational exposure to these phthalates leads to a common syndrome (*i.e.*, phthalate syndrome), and there is evidence that suggests a common MOA (*i.e.*, a disruption of fetal testicular steroidogenesis) for certain, androgen-dependent, aspects of the syndrome. Additionally, robust dose-response data are available across the toxicologically similar phthalates for multiple key outcomes associated with phthalate syndrome. Given the available data, EPA believes there is sufficient information available to support the development of RPFs for phthalates. Therefore, EPA is proposing to use an RPF approach for the phthalate CRA conducted in support of TSCA section 6 risk evaluations.

4.4 Proposed Options for Deriving Relative Potency Factors

As described in OPP's *Guidance on Cumulative Risk Assessment of Pesticide Chemicals that have a Common Mechanism of Toxicity* (U.S. EPA, 2002), RPFs should be developed based on a uniform point of comparison. For chemical substances grouped for CRA, this includes, whenever possible, using the same common effect, same measure of potency, same species/strain and studies that were conducted using relatively comparable methodology. Additionally, consideration should be given to the human relevance of the effect.

To support RPF derivation, EPA considered the strengths and uncertainties associated with the dataset for each evaluated key outcome (Section 4.4.1). Based on this, EPA identified several potential options for deriving RPFs to address phthalate syndrome, which are discussed in Section 4.4.2.

4.4.1 Strengths and Uncertainties of Key Outcomes Datasets for RPF Derivation

4.4.1.1 Decreased Fetal Testicular Testosterone Production

As discussed in Section 3.1.3.2, testosterone is necessary for the proper development of the male reproductive system, and a disruption of testicular testosterone production during the masculinization programming window contributes to the spectrum of effects that make up phthalate syndrome. Further, reduced testosterone production in the fetal testis plays an early role in the phthalate syndrome MOA. Available data clearly and consistently demonstrate that gestational exposure to DEHP, BBP, DBP, DIBP, DCHP, and DINP during the critical window of development leads to a dose-dependent reduction in fetal testicular testosterone production (Table 3-6). Across these six phthalates, there are robust dose-response data available from multiple studies that are similar in design (*i.e.*, utilize the same species/strain of rat, same route/method of exposure, similar exposure durations, similar timing of measure, and similar method of measuring *ex vivo* testosterone production via radioimmunoassay).

Further, several comparative dose-response studies are available that have evaluated fetal testicular testosterone production following exposure to each of the six toxicologically similar phthalates under consideration (Gray et al., 2021; Furr et al., 2014).

There are sufficient dose-response data from multiple studies of similar design to support deriving RPFs for reduced fetal testicular testosterone production. Use of this outcome for deriving RPFs is strengthened by the fact that androgen action has a conserved role in the development of the male reproductive system across mammalian species, including humans. Further, reduced fetal testicular testosterone production has been selected as the critical effect for use in risk characterization in previous phthalate CRAs conducted by several regulatory agencies, including Australia NICNAS, Health Canada, and U.S. CPSC (see Table 3-1 and Appendix A).

4.4.1.2 Decreased Fetal Testicular Expression of Cholesterol Transport and Steroidogenesis Genes

As discussed in Section 3.1.3.1, reduced expression of cholesterol transport and steroidogenesis genes in the fetal testis plays an early role in the phthalate syndrome MOA. It is biologically plausible that reduced steroidogenic gene expression will lead to reduced fetal testicular testosterone production, and some data are available to support the temporal relationship between these outcomes (Section 3.1.6.1, Figure 3-3). Available data provided consistent evidence to support dose-response concordance for DEHP, BBP, DBP, DIBP, DCHP, and DINP for this key outcome (Section 3.1.6.2). For these six phthalates, adequate data are available to support dose-response modeling for changes in expression of *Scarb1*, *StAR*, *Cyp11a1*, *3bHSD*, and *Cyp17a1*. Available gene expression studies have been conducted using similar methodologies (*i.e.*, similar exposure route/method, timing/duration of exposure, timing of outcome assessment) and have most frequently been conducted with SD rats, although some data are available for other strains. Further, several comparative dose-response studies investigating gene expression have been conducted that evaluate all or a subset of the high-priority and manufacturer-requested phthalates (Gray et al., 2021; Hannas et al., 2012; Hannas et al., 2011).

Here, gene expression data are being considered as a measure of the potency of one chemical relative to that of another. One challenge with developing RPFs based on gene expression data is that this type of data is typically not used to derive PODs for use in regulatory risk assessment. Generally, gene expression data are used by EPA as part of the weight of evidence analysis to support the human relevance of an effect or support a hypothesized MOA. However, transcriptomic dose-response modeling approaches that enable transcriptomic PODs to be calculated for use in risk assessment have been proposed, and this is an active area of research at EPA and NTP. For example, NTP has proposed deriving a POD based on transcriptomics dose-response data for "active" gene sets (*i.e.*, at least three genes in the set are altered). The median BMD of affected genes in the active gene set is then derived to get a central-tendency measure of potency (NTP, 2018). Thus, approaches are available that could enable EPA to derive RPFs based on reduced testicular steroidogenic gene expression.

4.4.1.3 Decreased Anogenital Distance

As described in Section 3.1.3.3, decreased male AGD is mechanistically linked to reduced fetal testicular testosterone production and is considered a biomarker of disrupted androgen action. As described in OECD guidance (OECD, 2013), a decrease in male pup AGD that cannot be explained by differences in animal size indicates an adverse effect that is relevant for setting the NOAEL. Consistently, reduced male pup AGD has been selected as the critical (or co-critical) effect for characterizing risk in previous phthalate CRAs (Table 3-1). As can be seen from Table 3-8, there are sufficient data to support dose-response modeling for DEHP, BBP, DBP, DIBP, DCHP, and DINP. Generally, there are available studies of similar design across these six phthalates that would facilitate a

relatively uniform point of comparison (*i.e.*, utilize the same species/strain of rat, same route/method of exposure, similar exposure durations, and similar timing of measure). However, there are several challenges with developing RPFs based on decreased AGD. First, OECD guidance recommends that AGD should be normalized to body weight (preferrable the cubic root of body weight), since animal size can influence AGD. Many of the available studies only report absolute pup AGD. For example, in the case of DIBP only one dose-response study is available, and this study only reports absolute AGD. However, in this case, effects on male pup body weight were only observed at the highest dose tested (625 mg/mg), while effects on AGD were observed starting at lower doses (≥250 mg/kg/day). Thus, absolute AGD may be used for dose-response modeling, but care must be taken to ensure that potentially confounding body weight changes are not occurring at lower doses. Another source of uncertainty stems from the DINP dataset. In contrast to DEHP, BBP, DBP, DCHP, and DIBP where consistent effects on AGD are reported, statistically significant effects on AGD are less consistently reported for DINP across studies that test comparable doses (*i.e.*, DINP reduced AGD in two of six studies). Inconsistency in the DINP dataset reduces EPA's confidence in deriving RPFs based on this outcome.

4.4.1.4 Nipple/Areolae Retention

As discussed in Section 3.1.3.4, male pup nipple/areolae retention is mechanistically linked to a reduction in fetal testicular testosterone during gestation. As described in OECD guidance, nipple/areolae retention is considered a biomarker of a disruption of androgen action and should be considered in setting the NOAEL (OECD, 2013). Consistently, male pup nipple/areolae retention has been selected as the critical (or co-critical) effect for characterizing risk in previous phthalate CRAs (Table 3-1). As can be observed in Table 3-11, there are sufficient data to support dose-response modeling for DEHP, BBP, DBP, DIBP, DCHP, and DINP. Generally, available studies are of similar design (utilize the same species/strain of rat, same route/method of exposure, similar exposure durations, similar timing of measure). However, there are several challenges associated with deriving RPFs for this outcome. First, as discussed in Section 3.1.3.4, there is variability in how publications report nipple/areolae retention (*e.g.*, reported as mean number of nipples/areolas per male, incidence of males with NR, or mean percent of litters with males with NR). Variability in data reporting makes comparisons across studies difficult. However, sufficient studies reported NR as percent of males per litter showing retained nipples/areolas to support EPA's preliminary dose-response modeling. Additionally, although male pup nipple/areolae retention is a biomarker of disrupted androgen action in rodents, it is not directly a human relevant effect. This uncertainty reduces EPA's confidence in deriving RPFs based on nipple/areolae retention in male pups.

4.4.1.5 Seminiferous Tubule Atrophy

Seminiferous tubule atrophy is a pathologic lesion frequently reported in adult animals following gestational and/or perinatal phthalate exposure. As discussed in Section 3.1.3.6, there is some uncertainty underlying the mechanisms associated with phthalate-induced effects on seminiferous tubules; however, available studies consistently demonstrate that exposure to DEHP, BBP, DBP, DIBP, and DCHP lead to a dose-dependent increase in incidence of seminiferous tubule atrophy. Further, this outcome has been selected as the critical (or co-critical) effect for use in risk characterization in previous phthalate CRAs conducted by several regulatory agencies (Table 3-1). There appears to be relatively robust dose-response data available for DEHP, BBP, DBP, DIBP, and DCHP to support dose-response modeling (Table 3-15). However, there are several challenges associated with using this outcome for deriving RPFs. First, available studies have utilized differing exposure durations. For example, the three available studies of BBP and one available study of DCHP are two-generation reproduction studies in which seminiferous tubule atrophy is reported in adult F1 males following continuous exposure to BBP and DCHP throughout gestation, lactation, and the post-weaning period until time of necropsy. In

contrast, the one available study of DIBP reports seminiferous tubule atrophy in adult F1 males after dams were exposed throughout gestation only (*i.e.*, on GDs 12 to 21). For DEHP and DBP, gestational, perinatal, and continuous exposure studies are available. In contrast to DEHP, BBP, DBP, DCHP, and DIBP where seminiferous tubule atrophy is consistently observed, effects on tubular atrophy are inconsistently reported in studies of DINP that test comparable doses. Differences in exposure duration across available studies and inconsistency in the DINP dataset reduces EPA's confidence in deriving RPFs based on this outcome.

4.4.1.6 Hypospadias

Hypospadias are a severe malformation of the reproductive tract in which the urethra does not open on the tip of the penis. As described in Section 3.1.3.5, mechanistic studies provide evidence that link the formation of hypospadias with reduced fetal testosterone production. Data are available to support dose-response modeling for the high-priority phthalates, including DEHP, DBP, BBP, DIBP, and DCHP. However, there are several potential challenges associated with deriving RPFs for this outcome. First, significant increases in incidence of hypospadias have not been observed following gestational exposure to DINP. Additionally, as can be seen from Table 3-13, there are several studies available for DEHP, DBP and BBP that could potentially be used for dose-response modeling; however, data for DIBP and DCHP are limited to a single study for each phthalate. For BBP, the available study is significantly different in design (*i.e.*, a two-generation reproduction study in which hypospadias were reported in the F2 generation) compared to the studies available for other phthalates (*i.e.*, gestational and/or perinatal exposure studies in which hypospadias are observed in adult F1 animals). Limitations in the hypospadias dataset reduce EPA's confidence in deriving RPFs based on this outcome.

4.4.1.7 Incidence of MNGs

As discussed in Section 3.1.3.7, MNG formation may serve as a biomarker of altered Sertoli-germ cell interaction. However, there is uncertainty underlying the MOA associated with MNG formation and the biological significance of MNGs remains unclear. As can be seen from Table 3-17, although increased incidence of MNGs has been observed following gestational exposure to DEHP, BBP, DBP, DIBP, DCHP, and DINP, there is variability in how publications report MNGs. For example, MNGs may be reported as MNGs per testis or seminiferous cross-section, incidence of animals with MNGs in testes, percent seminiferous cords with MNGs, or percentage of germ cells multinucleated. These discrepancies in data reporting make comparisons across studies difficult. Additionally, EPA only identified single studies evaluating MNGs for BBP and DIBP, and these studies both tested a single high-dose of each phthalate, which prohibits further dose-response analysis for BBP and DIBP. Given uncertainties related to biological significance and the MOA underlying MNG formation, as well as data reporting limitations and lack of adequate dose-response data for BBP and DIBP, EPA is not considering MNGs further for RPF derivation.

4.4.2 Proposed Options for Deriving RPFs

EPA is proposing to address phthalate syndrome under TSCA by focusing on the most sensitive effect. One potential challenge with this approach is that no single outcome may be identified as the most sensitive across the six toxicologically similar phthalates. Potential options are under consideration for addressing this challenge, if encountered.

Considering the strengths and uncertainties associated with the datasets for each key outcome, datasets for reduced fetal testicular testosterone production and steroidogenic gene expression appear to have the most robust datasets to support RPF derivation. Confidence in deriving RPFs for these outcomes is further strengthened due to the conserved role that androgen action plays in the development of the male reproductive system across mammalian species, including humans. Furthermore, given what is known

about the phthalate syndrome MOA (discussed in Section 3.1.1), disrupted steroidogenesis during the critical window of development is an upstream effect that appears to be necessary for antiandrogenic effects on the male reproductive system (*i.e.*, phthalate syndrome) to occur. Therefore, reduced fetal testicular testosterone production and steroidogenic gene expression are appropriate measures of toxicological potency because they reflect downstream apical outcomes associated with phthalate syndrome. Datasets for reduced male AGD, NR, seminiferous tubule atrophy, and hypospadias are also relatively robust, however, as discussed above, these datasets have additional uncertainties and challenges that reduce EPA's confidence in using them for RPF derivation. The dataset for MNGs (*i.e.*, no dose-response data for BBP or DIBP) does not appear sufficient to support RPF derivation.

Given the strengths and uncertainties associated with the datasets for each key outcome, EPA is considering several options to derive RPFs based on gestational (*i.e.*, reduced fetal testicular testosterone content and reduced testicular steroidogenic gene expression, Options 1–4) and postnatal effects (*i.e.*, AGD, NR, seminiferous tubule atrophy, hypospadias, Options 5 and 6).

Options under consideration by EPA include the following:

- **Option 1.** For this option, EPA is proposing to derive RPFs based on reduced fetal testicular testosterone production and reduced fetal testicular steroidogenic gene expression. Individual studies of similar design and with sufficient dose-response data would be modelled to estimate BMDs that would be used to derive RPFs. A range of BMRs in the low-end range of the dose response curve would be modeled, including, but not limited to BMRs of 5, 10, 20 percent, as well as the Agency's default of one control standard deviation (U.S. EPA, 2012). Modeling multiple BMRs would allow EPA to consider the consistency of RPFs across a range effect levels.

- **Option 2.** This option is similar to Option 1. RPFs would be derived for testicular testosterone and steroidogenic gene expression and multiple BMRs would be modelled. However, for this option dose-response data from studies of similar design would be combined prior to modeling. One approach to combing data is to conduct a meta-regression, which characterizes dose-response across a group of studies and considers heterogeneity within and across studies through random effects. This approach would help eliminate the estimated random effects of inter- and intra-study variation. The feasibility of this approach for phthalates has been demonstrated by NASEM (2017), who used meta-regression results to estimate BMDs for reduced fetal testicular testosterone production for DEHP, DBP, BBP, DIBP, and DINP.

- **Option 3.** This option is related to Option 1. RPFs would be derived for testicular testosterone and steroidogenic gene expression using data from individual studies and multiple BMRs would be modelled. RPFs derived from gene expression and testosterone data would then be combined to get a composite RPF for each individual phthalate. This option may be preferable over Option 1 if variability in RPF values is observed between the two modeled outcomes.

- **Option 4**. This option is related to Options 2. RPFs would be derived for testicular testosterone and steroidogenic gene expression using combined dose-response data from multiple studies. Multiple BMRs would also be modelled. RPFs derived from gene expression and testosterone data would then be combined to get a composite RPF for each individual phthalate. This option may be preferable over Option 2 if variability in RPF values is observed between the two modeled outcomes.

- **Option 5.** For this option, RPFs would be derived for postnatal effects (*i.e.*, decreased AGD, NR, seminiferous tubule atrophy, and hypospadias) using data from individual studies and multiple BMRs. RPFs would then be combined across postnatal effects to get a composite RPF

for each individual phthalate. For postnatal effects, EPA is proposing to use a composite RPF approach for several reasons. First, as discussed above, there are a number of uncertainties and limitations associated with several of the evaluated postnatal effects and it may not be possible to derive RPFs for the six toxicologically similar phthalates for all four outcomes (*e.g.*, hypospadias are not reported following exposure to DINP). Second, as discussed in Section 3.1.6.2, preliminary dose-response modeling results indicate phthalate potency may vary by outcome. Developing a composite set of RPFs for postnatal effects would help to circumvent these challenges.

- **Option 6.** This option is related to option 5. RPFs would be derived for postnatal effects (*i.e.*, decreased AGD, NR, seminiferous tubule atrophy and hypospadias) using multiple BMRs, however, for this option dose-response data from similarly designed studies would be combined prior to modeling. RPFs would then be combined for each postnatal effect to get a composite RPF for each individual phthalate.

5 PROPOSED POPULATIONS CONSIDERED: STEP 2 IN CONCEPTUAL MODEL (Figure 2-1)

As described in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c), EPA will conduct consumer, occupational, and general population exposure assessments for each individual phthalate. Within these assessments, PESS will be considered, which are "a group of individuals within the general population identified by [EPA] who, due to either greater susceptibility or greater exposure, may be at greater risk than the general population of adverse health effects from exposure to a chemical substance or mixture, such as infants, children, pregnant women, workers, or the elderly" [15 U.S.C. § 2602(12)]. TSCA does not statutorily define what constitutes "greater susceptibility" or "greater exposure," thereby providing EPA with flexibility in how PESS groups are identified.

As discussed throughout Section 3, *in utero* exposure to DEHP, BBP, DBP, DIBP, DCHP and DINP can disrupt testicular steroidogenesis and cause adverse effects on the developing male reproductive system. Postnatal phthalate exposure can also cause male reproductive toxicity; however, the perinatal and peripubertal lifestages are believed to be the most sensitive to phthalate exposure (NRC, 2008). Based on EPA's current understanding of the developmental and reproductive toxicity of phthalates and susceptible populations identified in previous phthalate CRAs (ECCC/HC, 2020; U.S. CPSC, 2014), EPA initially proposes to focus its CRA for phthalates on two groups that may be more susceptible to phthalate syndrome due to lifestages:

- pregnant women/women of reproductive age, and
- male infants, male toddlers, and male children.

It is important to note that although EPA is proposing to focus its CRA efforts on subpopulations susceptible to phthalate syndrome based on lifestages, individual phthalate risk evaluations will consider all relevant lifestages, populations, and PESS.

In addition to potentially being more susceptible to phthalate exposure, these subpopulations and lifestages identified may also have higher exposure to phthalates from factors that may include, but are not limited to, diet, mouthing, and exposure relative to bodyweight. These populations may also be members of the general population living in communities near facilities that emit or release phthalates to water or ambient air resulting in higher phthalate exposure (*i.e.,* fenceline communities). Overburdened communities in which the identified susceptible subpopulations are exposed to higher levels of phthalates will be identified by EPA throughout the risk evaluation process, as appropriate. Additional PESS based on factors that may include but are not limited to race, ethnicity, or socioeconomic status who have higher exposures to phthalates may also be identified throughout the risk evaluation process and incorporated into a CRA as appropriate.

The PESS, initially identified by susceptibility to phthalate syndrome based on lifestage, may be part of the consumers, workers, and general population that are part of the phthalate exposure assessment. EPA's proposed approach focuses on the assessment of cumulative risk to consumers, workers, and general population—specifically fenceline communities that include, but may not be limited to, pregnant women/women of reproductive age, and male infants, toddlers, and children (Figure 5-1).

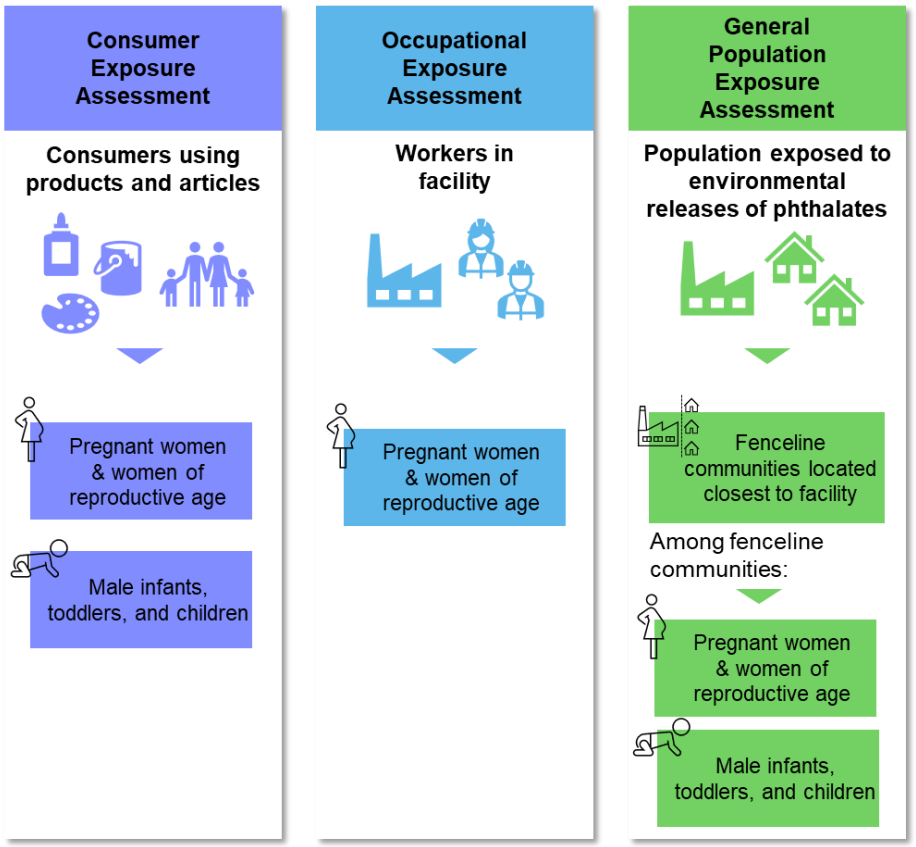

Figure 5-1. Diagram of Initial Proposed Populations Identified Based on Susceptibility to Phthalate Syndrome

6 PROPOSED EXPOSURE AND RISK APPROACH FOR ASSESSING PHTHALATES FOR CUMULATIVE RISK UNDER TSCA: STEPS 3 TO 10 IN CONCEPTUAL MODEL (Figure 2-1)

6.1 Overview

TSCA Section 6(b)(4)(D) requires EPA to identify the hazards, exposures, COUs, and the PESS the Administrator expects to consider in a risk evaluation, which EPA did in the final scope documents for each of the high-priority and manufacturer-requested phthalates. In this section, EPA is providing similar information as it relates to the CRA for phthalates. As discussed in Section 3.3, EPA is proposing to assess DEHP, BBP, DBP, DIBP, DCHP, and DINP (but not DIDP) for cumulative risk to human health under TSCA. This section describes EPA's proposed approach for how exposure from TSCA COUs may be combined with other exposures from other sources (non-attributable and non-TSCA) of phthalate exposure to estimate cumulative exposure needed to determine cumulative risks associated with these phthalates.

This section begins with a summary of information from the final scope documents for each individual phthalate, including COUs (Section 6.2.1) and exposure pathways (Section 6.2.2). Other sources of phthalate exposure are also introduced and discussed in those sections. Section 6.3 describes EPA's proposed scenario-based approach to estimating cumulative phthalate exposure, and a proposed reverse dosimetry approach to support exposure characterization. The proposed scenario-based approach includes estimating TSCA, non-attributable, and non-TSCA exposures for reasonable combinations to determine cumulative risk. A scenario-based method allows for source apportionment of TSCA COU contributions to the total risk. The reverse dosimetry approach considers CDC's NHANES urinary biomonitoring dataset and a single compartment toxicokinetic model to estimate total phthalate exposure. As described in Section 4.3.3, EPA is proposing to use an RPF approach. Therefore, exposure from each individual phthalate will be scaled to the potency of an IC and expressed in terms of IC equivalents. This approach is proposed for consumer (Section 6.4.1), occupational (Section 6.4.2), and general population/fenceline community (Section 6.4.3) exposures. An MOE approach is proposed for use in characterizing cumulative risk.

6.2 Summary of COUs and Pathways for Phthalates from Individual Scope Documents

6.2.1 Conditions of Use Listed in Final Scopes for Individual Phthalate Risk Evaluations (Step 3 in Conceptual Model [Figure 2-1])

As discussed in Section 3.3, EPA's proposed phthalate cumulative chemical group includes BBP, DBP, DCHP, DEHP, DBP, and DINP, but not DIDP. EPA plans to analyze human exposures and releases to the environment resulting from the COUs within the scope of the risk evaluation for each of these phthalates separately, as stated in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c). In each scope document for the individual chemical substance, EPA identified and described the categories and subcategories of COUs which include information related to manufacture, processing, distribution in commerce, use, and disposal that the EPA plans to consider in the risk evaluation. EPA has gathered those COUs and compiled a list across the different phthalates; the COUs associated with industrial, commercial, and consumer uses are summarized in Table 6-1. In addition to these COUs, sites associated with manufacture, processing, distribution, use, and disposal of

each phthalate that emit releases to the surrounding area are considered sources of possible phthalate exposure assessed under TSCA.

Prior to the development of the phthalate CRA, exposure scenarios for TSCA COUs will be completed in individual phthalate risk evaluations.

Table 6-1. Categories of Conditions of Use for High-Priority Phthalates and a Manufacturer-Requested Phthalate

Use	Conditions of Use	DBP	BBP	DEHP	DCHP	DIBP	DINP
Industrial	Adhesive and sealants		X		X	X	X
	Automotive care products		X				X
	Building/construction materials not covered elsewhere		X			X	X
	Castings		X				
	Chemical intermediate		X				
	Fabric, textile, and leather products not covered elsewhere		X			X	
	Finishing agent				X		
	Floor coverings		X			X	
	Fuels and related products					X	
	Hydraulic fluid		X				
	Hydraulic fracturing			X			
	Ink, toner, and colorant products		X		X	X	
	Laboratory chemicals		X	X			
	Paints and coatings		X	X		X	
	Plastic and rubber products not covered elsewhere		X		X	X	
	Plasticizer						X
	Solvent	X					
	Transportation equipment manufacturing			X			
	Adhesives and sealants	X	X	X	X	X	X
Commercial	Air care products					X	X
	Arts, crafts and hobby materials			X			X
	Automotive care products		X	X			X
	Batteries			X			
	Building/construction materials not covered elsewhere		X	X	X		X
	Castings		X				
	Chemical intermediate		X				

Use	Conditions of Use	DBP	BBP	DEHP	DCHP	DIBP	DINP
Commercial	Chemiluminescent light stick	X					
	Cleaning and furnishing care products	X					X
	Dyes and pigments			X			
	Electrical and electronic products			X			X
	Explosive materials	X					
	Fabric, textile, and leather products not covered elsewhere		X	X			X
	Floor coverings	X	X			X	X
	Foam seating and bedding products						X
	Furniture and furnishings not covered elsewhere	X		X			X
	Hydraulic fluid						X
	Ink, toner, and colorant products	X	X		X	X	
	Inspection penetrant kit	X					
	Laboratory chemical	X	X		X	X	X
	Lawn and garden care products			X			
	Lubricants	X					
	Paints and coatings	X	X	X	X	X	X
	Personal care products	X					
	Pigment						X
	Plastic and rubber products						X
	Plastic and rubber products not covered elsewhere	X	X	X	X	X	X
	Solvent						X
	Toys, playground, and sporting equipment			X			X

Use	Conditions of Use	DBP	BBP	DEHP	DCHP	DIBP	DINP
Consumer	Adhesives and sealants	X	X	X	X	X	X
	Air care products					X	X
	Arts, crafts and hobby materials	X	X	X	X		X
	Automotive Care products		X	X			X
	Batteries			X			
	Building/construction materials not covered elsewhere		X	X			X
	Chemiluminescent light stick	X					
	Cleaning and furnishing care products	X	X				X
	Dyes and pigments			X			
	Electrical and electronic products			X			X
	Fabric, textile, and leather products not covered elsewhere	X	X	X		X	X
	Floor coverings	X	X			X	X
	Foam seating and bedding products						X
	Furniture and furnishings not covered elsewhere	X		X			X
	Ink, toner, and colorant products		X		X	X	X
	Lawn and garden care products			X			
	Paints and coatings	X	X	X	X	X	X
	Paper products						X
	Plastic and rubber products						X
	Plastic and rubber products not covered elsewhere	X	X	X	X	X	X
	Reference material and/or laboratory reagent			X			
	Toys, playground, and sporting equipment	X	X	X		X	X

TSCA section 6(b)(4)(D) requires EPA to identify the hazards, exposures, conditions of use, and the PESS the Administrator expects to consider in a risk evaluation. TSCA section 3(2) excludes from the definition of "chemical substance" "any food, food additive, drug, cosmetic, or device (as such terms are defined in section 201 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321]) when manufactured, processed, or distributed in commerce for use as a food, food additive, drug, cosmetic, or device" as well as "any pesticide (as defined in the Federal Insecticide, Fungicide, and Rodenticide Act [7 U.S.C. 136 et seq.]) when manufactured, processed, or distributed in commerce for use as a pesticide." As a result, EPA identified several non-TSCA uses in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c) (*e.g.*, use in food packaging materials, dental sealants and nail polish, fragrances, medical devices, and pharmaceuticals; see Section 2.2.2 of final scope documents for

additional discussion of non-TSCA uses). These non-TSCA uses are excluded from the definition of "chemical substance" in TSCA § 3(2)(B)(vi) and are not included in Table 6-1.

EPA may not in a risk management rule under section 6(a) directly regulate non-TSCA uses; however, incidental effects of 6(a) regulation on non-TSCA uses are not prohibited by TSCA's chemical substance definition. Additionally, as described in EPA's Risk Evaluation Rule (see Procedures for Chemical Risk Evaluation Under the Amended TSCA, 33726 Fed. Reg. 33735 (July 20, 2017), "[t]he potential risks of non-TSCA uses may help inform the Agency's risk determination for the exposures from uses that are covered under TSCA (*e.g.,* as background exposures that would be accounted for, should EPA decide to evaluate aggregate exposures)" (82 FR at 33735). Certain non-TSCA sources may be major pathways of human exposure, and their exclusion from a CRA may lead to an underestimation of risk. For example, previous phthalate CRAs conducted by U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020) found dietary sources to be a major pathway of exposure (see Appendix A.1 to A.2). Therefore, EPA would consider major non-TSCA sources of phthalate exposure as identified during its process as part of a CRA.

6.2.2 Pathways and Routes of Exposure Considered in Risk Evaluation as Stated in Final Phthalate Scopes

As stated in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c), EPA plans to analyze exposure levels for indoor air, ambient air, surface water, groundwater, sediment, human milk, and aquatic biota (*e.g.,* fish) associated with exposure for each of the six phthalates being considered for the CRA. The scope documents for the individual phthalate risk evaluations present an exposure analysis plan based on the exposure from TSCA COUs for the individual risk evaluations. The cumulative assessment, however, will consider exposure from each pathway combined across the phthalates and has unique consideration for building scenarios that are not completed in the individual risk evaluation.

Under TSCA section 6(b)(4)(F), EPA is required to "describe whether aggregate or sentinel exposures to a chemical substance within the conditions of use were considered, and the basis for that determination." In this definition and within the CRA

- aggregate exposure is the combined exposures to an individual from a single chemical substance across multiple routes and across multiple pathways (40 CFR § 702.33), and
- cumulative exposure is the aggregate exposure to multiple agents or stressors (U.S. EPA, 2003).

Because the cumulative exposure assessment will focus on susceptible subpopulations (described in Section 5) the increased exposure and/or susceptibility in these populations may support the need for evaluating aggregate exposures across pathways which can be combined across phthalates to properly characterize cumulative risk. In their aggregate exposure assessments, both U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020) found dietary sources to be major contributors to aggregate exposures in pregnant women and infants (see Appendix A.1 and A.2). Thus, excluding dietary exposure from estimation of aggregate and cumulative exposure may lead to an underestimate of risk. In addition, levels of phthalates are generally detectable in the indoor air, indoor dust, and soil media as demonstrated by (U.S. CPSC, 2014) (see Figure_Apx A-1). Concentration of phthalates in these media may be apportioned to one or more TSCA conditions of use; however, the media concentrations may not be attributable to specific releases. Even where the phthalate exposures may not be attributed to a specific condition of use, the exposures/concentrations found in the media may still pertain to the "chemical substance."

To account for exposures from different sources expected to impact cumulative risk, the CRA may include estimations of the following and appropriate combinations of the following exposures:

- **TSCA COU exposure:** Exposure that can be attributed to a specific TSCA COU (*e.g.*, inhalation exposure during consumer use of an adhesive). Note that exposure scenarios for TSCA COUs will be completed in individual phthalate risk evaluations and evaluated for different populations such as consumers, workers, and general population.
- **Non-attributable exposure:** Exposure from pathways that cannot be attributed to a specific TSCA COU or another specific source. Household dust or human milk are a few examples in which phthalate concentrations measured in those media may result from multiple sources of phthalates that may nor may not be attributed to a TSCA COU or another specific source.
- **Non-TSCA exposure:** Exposure that can be attributed to specific activities that are excluded from the TSCA definition of "chemical substance," under TSCA Section 3(2), such as a pesticide, food, food additive, drug, cosmetic, or medical device.

6.3 Scenario-Building for Pathways of Exposure (Steps 4 and 5 in Conceptual Model)

EPA proposes to combine non-attributable and non-TSCA exposures with exposures from TSCA COUs when appropriate to determine cumulative exposure. The scenario-building needed to estimate the various exposures is described below.

6.3.1 TSCA COUs (Step 4 in Conceptual Model [Figure 2-1])

EPA plans to analyze human exposures and releases to the environment resulting from the COUs stated in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c). COUs for each of these phthalates are shown Table 6-1. Prior to the development of the phthalate CRA, scenario-building and estimations of exposure from TSCA COUs will be completed in individual phthalate risk evaluations.

6.3.2 Estimating Non-attributable and Non-TSCA Exposures (Step 5 in Conceptual Model [Figure 2-1])

EPA outlines the process for estimating the exposure from sources that are not directly attributable to TSCA COUs (non-attributable sources) and attributable to non-TSCA COUs (*i.e.*, excluded from the definition of chemical substance) that will be combined with exposures from TSCA COUs to determine cumulative exposure.

EPA is considering the applicability of two approaches for estimating non-attributable and non-TSCA exposures to DEHP, BBP, DBP, DIBP, DCHP, and DINP—including scenario-based and reverse dosimetry approaches (Figure 6-1).

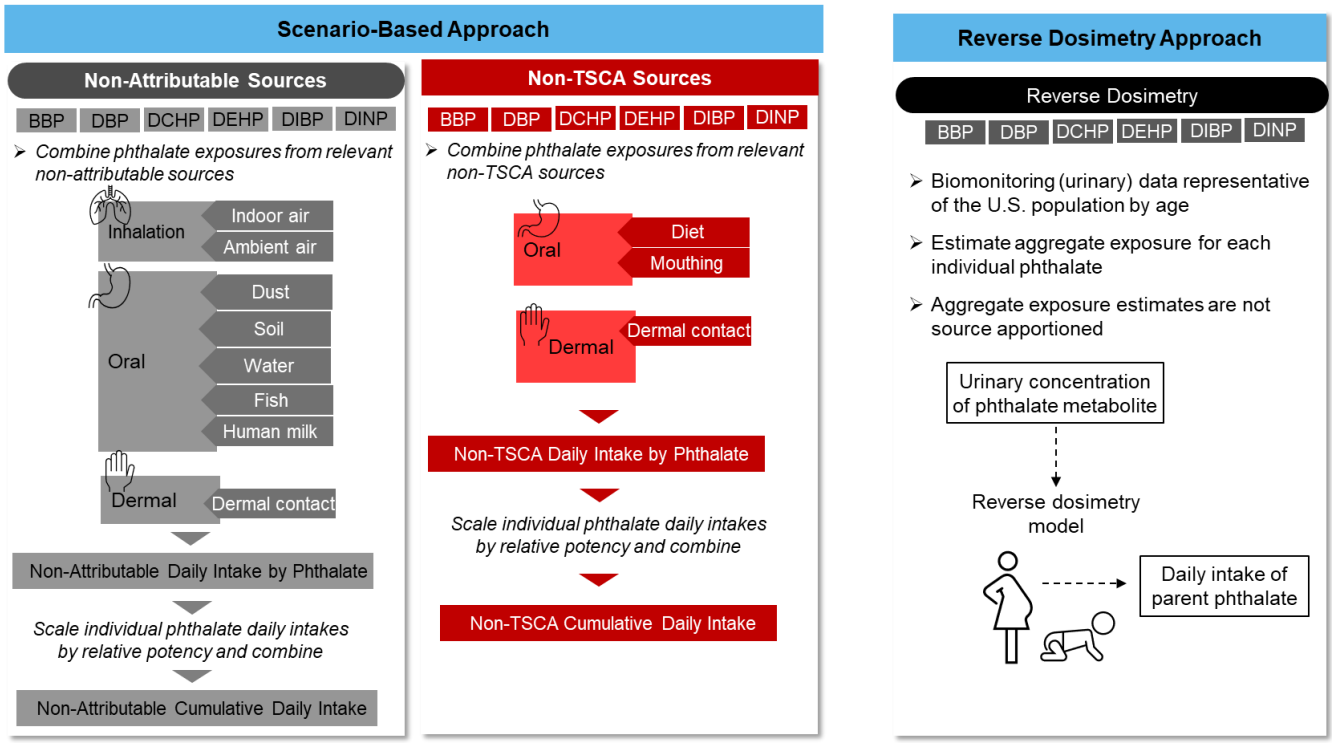

Figure 6-1. Scenario-Based and Reverse Dosimetry Approaches for Estimating Non-attributable and Non-TSCA Exposure

- **Scenario-Based Approach.** This approach involves estimating exposure for specific populations based on distinct behaviors, exposure factors, assumptions, and inferences about how exposure takes place under a specific set of conditions and often relies on monitoring data for determining the concentrations of chemicals in the various exposure media (described further in Section 6.3.2.1). Scenarios can be built for individual pathways of exposure and can be combined to determine cumulative exposure. As shown in Figure 6-1, phthalate exposure estimates (expressed as a daily intake value) from multiple pathways (*e.g.*, ambient air, mouthing, etc.) and exposure routes (*e.g.*, inhalation, oral, dermal) for non-attributable and non-TSCA sources for DEHP, BBP, DBP, DIBP, DCHP, and DINP can be combined with exposure from TSCA COUs to determine cumulative exposure. The availability of current and reliable monitoring data, which the approach relies on, is one limitation of this approach. Further limitations and uncertainties are discussed in Section 6.3.2.4.

- **Reverse Dosimetry Approach.** As shown in Figure 6-1, this approach involves estimating aggregate exposure (expressed as a daily intake value) for each individual phthalate from human urinary biomonitoring data for metabolites unique to each individual parent phthalate to be combined for an estimate of cumulative exposure. As described further in Section 6.3.2.2, reverse dosimetry modeling for phthalates involves use of a single compartment toxicokinetic model and does not distinguish between routes or pathways of exposure, and does not allow for source apportionment (*i.e.*, exposure from TSCA COUs cannot be isolated), which are a limitations of this approach for use under TSCA. Further limitations and uncertainties are discussed in Section 6.3.2.4.

These approaches are based on the needs of the TSCA exposure assessment as well as review of available data and approaches utilized in previous phthalates CRAs (ECCC/HC, 2020; U.S. CPSC, 2014). The scenario-based and exposure reverse dosimetry approaches are described further in Sections 6.3.2.1 and 6.3.2.2, respectively. Based on the limitations and uncertainties associated with each

approach which are described in Section 6.3.2.4. EPA proposes to primarily use a scenario-based approach to estimate non-attributable and non-TSCA exposures that may be combined with exposure from TSCA COUs to determine cumulative risk. EPA proposes to use reverse dosimetry as a comparator for scenario-based exposure estimates as described in Section 6.3.2.5.

6.3.2.1 Scenario-Based Exposure Evaluation for Estimating Non-attributable and Non-TSCA Exposures

The first approach EPA is considering for estimating non-attributable and non-TSCA exposures to phthalates is the scenario-based approach. As shown in EPA's conceptual model for estimating cumulative exposure (Figure 2-1), the approach for determining cumulative exposure would be to combine exposures from TSCA COUs as estimated from scenario-based approaches to exposures from non-attributable and non-TSCA exposures estimated using a scenario-based approach. As described in EPA's *Guidelines for Human Exposure Assessment* (U.S. EPA, 2019a), scenario-based approaches can be used to define exposure for specific populations based on distinct behaviors, exposure factors, assumptions, and inferences about how exposure takes place under a specific set of conditions and often relies on monitoring data for determining the concentrations of chemicals in the various exposure media. Scenario-based assessments estimate exposure based on intensity, duration, and frequency of exposure.

Text Box 6-1. Sources of Exposure Identified in CRAs Conducted by U.S. CPSC and Health Canada

For their phthalate CRAs, both U.S. CPSC and Health Canada used a scenario-based approach employing indirect exposure estimates. U.S. CPSC found the majority of women's exposure to DEHP, DINP, and DIBP was from diet (DCHP was not included in their analysis). Their estimates were in general agreement (within an order of magnitude) with two other studies estimating phthalate exposure using scenario-based exposure assessment methods with differences attributable to differing approaches for dietary exposure estimation (Clark et al., 2011; Wormuth et al., 2006).

Health Canada concluded that the main sources of exposure to the general Canadian population for medium-chain phthalates were food, indoor air, dust, and breast milk (ECCC/HC, 2020).

Both the U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020) phthalate CRAs estimated aggregate exposure for multiple phthalates using a scenario-based approach and calculated a total daily intake value for each individual phthalate (Text Box 6-1). U.S. CPSC (2014) states their scenario-based approach to be a step-by-step approach with four steps including compiling concentrations, compiling human exposure factors, estimating route-specific exposures, and estimating aggregate exposures.

EPA utilizes primarily a scenario-based approach to estimate exposure to TSCA COUs in individual chemical risk evaluations. Major pathways of exposure may vary based on age group; therefore, non-attributable and non-TSCA exposures would be assessed separately for relevant populations. For EPA to utilize a scenario-based exposure assessment to determine non-attributable and non-TSCA exposure levels to all phthalates, EPA could reconstruct an aggregated daily exposure profile for individuals varied by lifestages (women of reproductive age, male infants, toddlers, and children) using similar methods to Health Canada (ECCC/HC, 2020) and U.S. CPSC (2014). In a scenario-based assessment, unique exposure factors including but not limited to ingestion and inhalation rate, body weight, body surface area, and dietary intake differences are applied to determine the non-attributable and non-TSCA exposures to each subpopulation of interest (U.S. EPA, 2021a). For example, given childrens' crawling and hand-to-mouth behaviors, relevant routes of exposure may include oral in addition to inhalation. Because exposures can be estimated for various pathways and populations through unique built scenarios, exposure estimates for non-attributable or non-TSCA pathways can be varied for different populations and combined differently for an aggregated daily exposure profile for specific populations to limit the possibility of "double counting."

Examples of different combinations of exposures from non-attributable and non-TSCA sources are mentioned in Section 6.4.1 for consumers, Section 6.4.2 for occupational, and Section 6.4.3 for a fenceline community.

Concentrations of phthalates measured in various environmental media from relevant and reliable monitoring studies or databases would be considered alongside human exposure factors to determine an estimated aggregate exposure for each phthalate. Exposure via relevant environmental exposure pathways that may not be source-attributable may include, but are not limited to, drinking water, surface water, groundwater, ambient air, indoor air, and soil. Because the scenario-based approach would consider exposures from various phthalates, sources, and pathways separately, EPA could conduct sensitivity analyses to determine relative contributions of the various phthalates and sources of exposure to cumulative risk to inform risk determinations for individual phthalates. Determination of relative contributions to exposure can also help determine the major pathways of exposure for inclusion in the cumulative estimate as shown in Step 6 of the conceptual model (Figure 2-1). Once major pathways of exposure are identified for each individual phthalate, consideration of the magnitude, frequency, and duration of exposure must be considered for a relevant exposure timeframe to determine if co-exposure to multiple phthalates is occuring from the major relevant pathways of exposure.

TSCA section 3(2) excludes from the definition of "chemical substance" "any food, food additive, drug, cosmetic, or device (as such terms are defined in Section 201 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321]) when manufactured, processed, or distributed in commerce for use as a food, food additive, drug, cosmetic, or device." However, as discussed by U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020), dietary intake from food and beverages comprises the majority of daily intake of phthalates and are important sources of exposure to consider in a cumulative assessment. There are several approaches EPA may consider for estimating dietary intake as part of a scenario-based approach for determining non-TSCA exposure.

Text Box 6-2. Approaches Used by U.S. CPSC and Health Canada to Estimate Phthalate Dietary Intake

U.S. CPSC estimated dietary exposure using two datasets of phthalate residues in food items (Bradley et al., 2013; Page and Lacroix, 1995). Additional studies were used for food categorization and consumption estimates, including the U.S. EPA National Center for Environmental Assessment's analysis of food intake and diet composition (Clark et al., 2011; U.S. EPA, 2007a; Wormuth et al., 2006).

Health Canada estimated dietary intake of DIBP, BBP, DBP, and DEHP using the 2013 Canadian Total Diet Study (ECCC/HC, 2020). For other phthalates, the 2013-2014 and 2014-2015 Food Safety Action Plan (Canadian Food Inspection Agency) and/or a dietary exposure study from the United States (Schecter et al., 2013) were used. A United Kingdom total diet study (Bradley et al., 2013) was used to fill in data gaps. The phthalate concentrations were matched to 2004 Canadian Community Health Survey on nutrition (Statistics Canada, 2004) consumption values for each individual food.

First, EPA may identify and evaluate key data sources through a review of the literature for concentrations of phthalates in food products consumed by the U.S. population and food consumption patterns, similar to the approach employed by U.S. CPSC (Text Box 6-2). A second option is to use total diet study data, the method selected by Health Canada (Text Box 6-2). However, currently, there is no national total diet study measuring phthalate residue in U.S. food products. The Food and Drug Administration (FDA) conducts an ongoing Total Diet Study to monitor levels of contaminants in foods eaten by the US population, but phthalates are not measured as part of this study (FDA, 2022). Therefore, EPA may consider if total diet studies of other countries are reflective of the U.S. diet and use that data if appropriate. U.S. food consumption patterns such as the Food Commodity Intake Database and understanding of other nations' phthalate regulations will provide insight into which

nations' total diet studies may be best suited for estimating U.S. intake. EPA's dietary intake assessment may be deterministic or probabilistic based on available data.

EPA's tiered approach to exposure assessment uses a step-by-step, iterative process in which risk assessment advances from relatively simple to increasingly more complex analyses as required by the specific scenario (U.S. EPA, 2019a). Each tier corresponds to increased complexity of exposure, risk, and uncertainty characterization, progressing from screening-level deterministic modeling to advanced deterministic/mechanistic modeling, and ultimately probability modeling (uncertainty and variability assessment) following a similar process found in the WHO/IPCS framework for risk assessment of combined exposure to multiple chemicals (Meek et al., 2011). For example, in the WHO/IPCS framework, the tier of exposure assessment can vary from lower tiers employing simple semi-quantitative estimates of exposure to higher tiers employing probabilistic exposure estimates. After an exposure assessment scenario has been conducted, the results can help inform whether additional refinement of the assessment is needed, either by improving data specificity or by utilizing higher precision analysis techniques.

Data availability will dictate the tier of exposure assessment employed and may vary based on exposure scenario. The limited data available for the U.S. diet, for example, may lead to uncertainties in estimates of total phthalate intake as food and beverages are generally responsible for the majority of total intake in comparison to other sources (Figure_Apx A-1). The recency of food residue data may also introduce uncertainty in exposure estimates that should be reflective of current populations. In general, varying levels of data, both in terms of availability and quality, across phthalates and for the various environmental media concentrations, adds uncertainty to the aggregation of exposure across pathways and across phthalates that may be quantified using differing tiers of assessment.

6.3.2.2 Reverse dosimetry and Biomonitoring Approach for Estimating Non-attributable Exposure

A second approach EPA is considering for estimating non-attributable exposures that may include TSCA and non-TSCA exposure to phthalates is reverse dosimetry. Reverse dosimetry is the process of estimating an external exposure or intake dose to a chemical using biomonitoring data (U.S. EPA, 2019a). Reverse dosimetry modeling does not distinguish between routes or pathways of exposure, instead reverse dosimetry provides an estimate of the total dose (or aggregate exposure) responsible for the measured biomarker.

Urinary biomonitoring data are available to support estimating exposures for most of the high-priority and manufacturer-requested phthalates for various lifestages. CDC's NHANES dataset is a national, statistical representation of the general, non-institutionalized, civilian U.S. population. As can be seen from Table 6-2, monoester metabolites of BBP, DBP, DEHP, DIBP. and DINP in human urine are regularly measured as part of the NHANES biomonitoring program, including during the most recent NHANES survey period for which biomonitoring data is available (*i.e.*, 2017 to 2018). However, DCHP is an exception. The DCHP metabolite, monocyclohexyl phthalate, was included in NHANES from 1999 to 2010; however, it has since been excluded from the NHANES survey due to low detection levels and a low frequency of detection in human urine (CDC, 2013a). NHANES urinary biomonitoring data is also available to support estimating non-attributable exposures for some of the susceptible subpopulations EPA identified in Section 5, including women of reproductive age (all survey years), children aged 6 years or older (all survey years), and children aged 3 to 5 years (only included in two most recent surveys, 2015 to 2016 and 2017 to 2018). However, a limitation of the NHANES dataset is that it does not include biomonitoring data for infants and generally too few pregnant women are sampled to support statistical analysis in survey years after 2005 to 2006 (CDC, 2013b; NCHS, 2012).

Data from other recent studies that include urinary biomonitoring data for DCHP metabolites, infants, and pregnant women may be used to help overcome limitations of NHANES, if identified during systematic review.

Table 6-2. Urinary Phthalate Metabolites Included in NHANES

High-Priority and Manufacturer-Requested Phthalates	NHANES Urinary Metabolite[a]	Associated Parent Compound	NHANES Reporting Years[b]
Butyl benzyl phthalate (BBP)	Mono-benzyl phthalate (MBzP)	BBP	1999–2018
Dibutyl benzyl phthalate (DBP)	Mono-3-hydroxybutyl phthalate (MHBP)	DBP	2013–2018
	Mono-n-butyl phthalate (MnBP)	DBP, BBP	1999–2018
Di-ethylhexyl phthalate (DEHP)	Mono-2-ethylhexyl phthalate (MEHP)	DEHP	1999–2018
	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	DEHP	2001–2018
	Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)	DEHP	2001–2018
	Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)	DEHP	2003–2018
Diisobutyl phthalate (DIBP)	Mono-isobutyl phthalate (MBP)	DIBP	2001–2018
	Mono-2-methyl-2-hydroxypropyl Phthalate (MHiBP)	DIBP	2013–2018
Dicyclohexyl phthalate (DCHP)	Mono-cyclohexyl phthalate (MCHP)	DCHP	1999–2010
Di-isononyl phthalate (DINP)	Mono-isononyl phthalate (MiNP)	DINP	1999–2018
	Mono-oxoisononyl phthalate (MONP)	DINP	2015–2018
	Mono-(carboxyoctyl) phthalate (MCOP)	DINP	2005–2018

[a] NHANES reports uncorrected and creatinine corrected urine concentrations for each metabolite.
[b] 2017–2018 is the most recently available NHANES dataset.

NHANES provides data across the population that can be used to create cumulative distribution functions (percentiles). A major challenge in using the NHANES is selection of the specific metric (*e.g.*, median, arithmetic mean, geometric mean, lower or upper percentiles) that represents the non-attributable exposure. One approach could be to assume that the median exposure represents typical exposure to the U.S. population and may not include those exposed to specific TSCA COUs. Figure 6-2 shows an example cumulative distribution of NHANES where the central tendency might be representative of individual exposures that do not include exposure to TSCA COUs and the upper percentile represents highly exposed individual which may include those exposed to TSCA COUs.

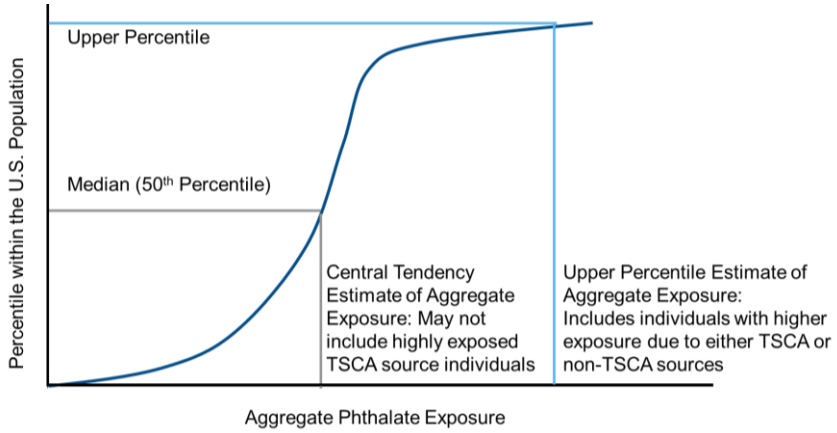

Figure 6-2. Diagram of Hypothetical NHANES Population Distribution of Phthalates and Illustration of Assumptions about Exposure Profiles

Based on the assumption that the median exposure represented by NHANES data may not include individuals exposed to TSCA sources, EPA could combine the median exposures with exposure from TSCA COUs to estimate a cumulative exposure where a portion of the exposure was attributable to TSCA and could be used to inform individual phthalate risk determinations. This approach may be supported by analyses conducted by both U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020) indicating that using reverse dosimetry and scenario-based approaches provide similar (within an order of magnitude) estimates of daily intake for phthalates. Dietary intake, primarily a non-TSCA exposure, comprised the majority of total cumulative daily intake (Figure_Apx A-1), while non-attributable sources such as dust, indoor air, ambient air, and drinking water were smaller contributors to total exposure. This assumption necessarily introduces additional uncertainty in the non-attributable exposure estimates with the potential for "double counting" if estimates from NHANES data already include exposures from TSCA COUs. However, this approach may prevent underestimations of exposures attributable to TSCA COUs that may be unique and not captured in a nationally representative dataset, which has its own limitations discussed below.

A further assumption can then be that the upper percentiles include individuals with TSCA exposures as well as highly exposed individuals and individuals with differences in kinetics that make them more susceptible to phthalate exposure. Using the upper percentile, with that assumption, however, would not allow source apportionment of the TSCA source of exposure to cumulative exposure which is necessary to inform individual phthalate risk determinations under TSCA. Additionally, there may not be data to support NHANES being representative of occupational or fenceline populations.

Reverse dosimetry approaches that incorporate basic pharmacokinetic information are available for phthalates (Koch et al., 2007; Koch et al., 2003; David, 2000) and have been used in previous human health CRAs conducted by U.S. CPSC (2014) and Health Canada (2020). For phthalates, reverse dosimetry can be used to estimate a daily intake (DI) value for a parent phthalate diester based on phthalate monoester metabolites measured in human urine using Equation 6-1 (Koch et al., 2007).

Equation 6-1. Calculating a phthalate daily intake value from urinary biomonitoring data.

$$Phthalate\ DI = \frac{(\mathrm{UE}_{Sum} \times \mathrm{CE})}{\mathrm{Fue}_{sum}} \times MW_{Parent}$$

Where:

- Phthalate DI = The daily intake (μg/kg$_{bw}$/day) value for the parent phthalate diester.

- UE_{sum} = The sum molar concentration of urinary metabolites associated with the parent phthalate diester (in units of µmole per gram creatinine).
- CE = The creatinine excretion rate normalized by body weight (in units of mg creatinine per kg bodyweight per day). CE can be estimated from the urinary creatinine values reported in biomonitoring studies (*i.e.*, NHANES) using the equations of Mage et al. (2008) based on age, gender, height, and race, as was done by Health Canada (ECCC/HC, 2020) and U.S. CPSC (2014).
- Fue_{sum} = The summed molar fraction of metabolites. The molar fraction describes the molar ratio between the amount of metabolite excreted in urine and the amount of parent compound taken up.
- MW_{parent} = The molecular weight of the parent phthalate diester (in units of g/mole).

Using this approach, DI values can be calculated for each of the high-priority and manufacturer-requested phthalates, scaled to the relative potency of an index chemical, and then scaled daily intake values can be summed to yield an estimate of non-attributable exposure expressed as index chemical equivalents.

Controlled human exposure studies have been conducted and provide estimates of the urinary molar excretion factor (*i.e.*, the Fue) to support use of a reverse dosimetry approach (Table 6-3). These studies most frequently involve oral administration of an isotope-labelled (*e.g.*, deuterium or carbon-13) phthalate diester to a healthy human volunteer and then urinary excretion of monoester metabolites is monitored over 24 to 48 hours. Fue values estimated from these studies have been used by both U.S. CPSC (2014) and Health Canada (2020) to estimate phthalate DI values using urinary biomonitoring data. As can be seen from Table 6-3, human Fue values have been estimated for DEHP, BBP, DBP, DIBP and DINP. However, an Fue value is not available for DCHP and the Fue value for DIBP is estimated from a single volunteer (Koch et al., 2012). It may be possible to use analogue data to address these data gaps. For example, U.S. CPSC (2014) used the DBP Fue value to estimate a daily intake value for DIBP using reverse dosimetry. Another uncertainty associated with estimated Fue values is whether or not they are reflective of human variability in phthalate metabolism and excretion. As can be seen from Table 6-3, Fue values were estimated from a relatively small number (N = 1–20) of adult human volunteers, and in some cases the age and gender of volunteers is unknown. It is unclear if these Fue values are reflective of the larger population or susceptible subpopulations based on lifestages identified in Section 5.

Table 6-3. Summary of Studies Providing Estimates of the Urinary Excretion Fractions (F_{ue}) of Phthalate Metabolites

Parent Phthalate	Study Population	Metabolite(s)	Fue[a]	Fue Sum[b]	Reference
DEHP	N = 10 men (20–42 years of age) and 10 women (18–77 years of age)	MEHP	0.062	0.453	(Anderson et al., 2011)
		MEHHP	0.149		
		MEOHP	0.109		
		MECPP	0.132		
	N = 1 man (61 years of age)	MEHP	0.073	0.469	(Koch et al., 2004)
		MEHHP	0.247		
		MEOHP	0.149		
	N = 1 man (61 years of age)	MEHP	0.059	0.627	(Koch et al., 2005)
		MEHHP	0.233		
		MEOHP	0.150		
		MECPP	0.185		
	N = 4 men (28–61 years of age)	MEHP	0.025	0.291[c]	(Kessler et al., 2012)
		MEHHP	0.125		
		MEOHP	0.141		
BBP	N = 14 volunteers (gender and age not provided)	MBP	0.06	0.79	(Anderson et al., 2001)
		MBzP	0.73		
DBP	N = 13 volunteers (gender and age not provided)	MBP	0.69	0.69	(Anderson et al., 2001)
DIBP	N = 1 man (36 years of age)	MiBP	0.703	0.903	(Koch et al., 2012)
		MHiBP	0.1928		
		3OH-MiBP	0.0069		
		MCiPP	Not detected		
DINP	N = 10 men (20–42 years of age) and 10 women (18–77 years of age)	MINP	0.030	0.305	(Anderson et al., 2011)
		MONP	0.063		
		7OH-MMeOP	0.114		
		MCOP	0.099		
	N = 1 man (63 years of age)	MINP	0.0212	0.396	(Koch and Angerer, 2007)
		MONP	0.0997		
		7OH-MMeOP	0.184		
		MCOP	0.0907		

[a] Fue values are presented on a molar basis and were estimated by study authors based on metabolite excretion over a 24-hour period.
[b] Fue sum indicates the sum of Fue values for the measured metabolites.
[c] Fue calculated based on urinary excretion of metabolites over a 22-hour period (Kessler et al., 2012).

Use of reverse dosimetry and urinary biomonitoring data to estimate non-attributable exposures to phthalates is consistent with approaches employed by both U.S. CPSC (2014) and Health Canada (2020). However, there are challenges and sources of uncertainty associated with the use of reverse dosimetry approaches. U.S. CPSC considered several sources of uncertainty associated with use of human urinary biomonitoring data to estimate daily intake values and conducted a semi-quantitative evaluation of uncertainties to determine the overall effect on daily intake estimates (see Section 4.1.3 of (U.S. CPSC, 2014)). Identified sources of uncertainty include: (1) analytical variability in urinary metabolite measurements; (2) human variability in phthalate metabolism and its effect on metabolite conversion factors (*i.e.*, the Fue); (3) temporal variability in urinary phthalate metabolite levels; (4) variability in urinary phthalate metabolite levels due to fasting prior to sample collection; (5) variability due to fast elimination kinetics and spot samples; and (6) creatinine correction models for estimating daily intake values.

In addition to some of the limitations and uncertainties discussed above and outlined by U.S. CPSC, the short half-lives of phthalates can be a challenge when using a reverse dosimetry approach. As discussed in Section 3.1.5.1 and elsewhere (ATSDR, 2022; EC/HC, 2015c), 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. Therefore, spot urine samples, as collected through NHANES and many other biomonitoring studies, are representative of relatively recent exposures. Spot urine samples were used by Health Canada (ECCC/HC, 2020) and U.S. CPSC (2014) to estimate a daily intake values. The short half-lives of phthalates, however, lead to single spot sample that may not be representative of average urinary concentrations that are collected over a longer term or calculated using pooled samples (Shin et al., 2019; Aylward et al., 2016). Multiple spot samples provide a better characterization of exposure, with multiple 24-hour samples potentially leading to better characterization but are less feasible to collect for large studies (Shin et al., 2019). Due to rapid elimination kinetics, U.S. CPSC concluded that spot urine samples collected at a short time (2 to 4 hours) since last exposure may overestimate human exposure, while samples collected at a longer time (>14 hours) since last exposure may underestimate exposure (see Section 4.1.3 of (U.S. CPSC, 2014) for further discussion).

Overall, U.S. CPSC (2014) concluded that factors that might lead to an overestimation of daily intake seem to be well balanced by factors that might lead to an underestimation of daily intake, and therefore reverse dosimetry approaches "provide a reliable and robust measure of estimating the overall phthalate exposure."

6.3.2.3 Comparison of Reverse Dosimetry and Scenario-Based Approaches

As discussed in Sections 6.3.2.1 and 6.3.2.2, Health Canada (ECCC/HC, 2020) and U.S. CPSC (2014) both estimated phthalate daily intake values using reverse dosimetry with human urinary biomonitoring data and scenario-based exposure assessment approaches. Health Canada and U.S. CPSC found that both approaches resulted in daily intake values that were generally similar in magnitude. However, this depended on the recency and quality of data available for use, particularly for data on major exposure pathways like diet.

U.S. CPSC (2014) indicated that a comparison of the intake values estimated from both methods would either reveal the presence of pathways of exposure not captured in their scenario-based approach if estimates from biomonitoring were higher or reveal that worst-case scenarios may not be present in the biomonitoring approach if their estimates from their scenario-based approach was higher. There were no assumptions made that the two methods would yield identical results. U.S. CPSC found their estimates of scenario-based modeled daily intake values to be higher than those estimated using reverse dosimetry and 2005/2006 NHANES biomonitoring data for several phthalates (*i.e.*, BBP and DINP) (Table 6-4),

indicating that their scenario-based assessment included potentially worst-case scenarios. Yet, U.S. CPSC concluded that their results were within an order of magnitude of those from biomonitoring data and were useful in determining contributions of certain products or phthalates within the combined risk. In comparing modeled daily intake values for BBP, DEHP, DINP, DBP, and DIBP estimated using a scenario-based approach to those estimated using NHANES urinary biomonitoring data and reverse dosimetry, U.S. CPSC demonstrated that while results for both approaches were similar in magnitude, intake values estimated from biomonitoring data did vary based on the NHANES cycle used for analysis. This indicates that potential exposure to each of the phthalates may vary over time. For example, trends in decreasing DEHP exposure, and increasing DINP exposure were observed in NHANES data from 2005/2006 to 2012/2013 (Table 6-4).

Table 6-4. U.S. CPSC Estimated Median and 95th Percentile Phthalate Daily Intake Values for Women of Reproductive Age

Scenario	BBP	DEHP	DINP	DBP	DIBP
Median daily intake (µg/kg-day)					
Scenario-based Estimates[a]	1.1	1.6	5.1	0.3	0.1
NHANES 2005/2006[b]	0.26	3.8	1.0	0.69	0.19
NHANES 2007/2008[b]	0.29	4.1	1.5	0.79	0.29
NHANES 2009/2010[b]	0.23	2.0	3.0	0.58	0.32
NHANES 2011/2012[b]	0.19	1.7	5.0	0.33	0.26
NHANES 2012/2013[c]	0.15	1.3	5.0	0.33	0.29
95th Percentile daily intake (µg/kg-day)					
Scenario-based Estimates[a]	2.6	5.6	32.5	5.7	0.5
NHANES 2005/2006[b]	1.1	27.7	10.5	2.6	0.82[d]
NHANES 2007/2008[b]	1.3	31.5	14.6	2.6	1
NHANES 2009/2010[b]	1	10.3[d]	33.7	1.9[d]	0.98
NHANES 2011/2012[b]	0.84	6.4[d]	51.7	1.3	0.94
NHANES 2012/2013[c]	0.97	4.22	53.19	1.14	1.03

[a] Modeled intake estimated from scenario-based assessments. Adapted from Table 2.11 of (U.S. CPSC, 2014).
[b] Intake estimated from NHANES biomonitoring data using reverse dosimetry approach. Adapted from Table 5 of (U.S. CPSC, 2015).
[c] Intake estimated from NHANES biomonitoring data using reverse dosimetry approach. Adapted from Table 2 of (U.S. CPSC, 2017).
[d] Variance estimates can be large at the 95th percentile. Marked estimates are not considered stable. Use caution when drawing conclusions using 95th percentile estimates (U.S. CPSC, 2015).

6.3.2.4 Uncertainties and Limitations of Approaches

Although analyses conducted by both U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020) indicate that the reverse dosimetry and scenario-based approaches provide similar (within an order of magnitude) estimates of daily intake for phthalates, both approaches have strengths, limitations and uncertainties that must be considered to better understand their potential utility for estimating non-attributable and non-TSCA phthalate exposures. Challenges and limitations of both approaches are summarized in Table 6-5 and discussed further below.

Table 6-5. Summary of Uncertainties and Limitations Associated with Use of Scenario-Based and Reverse Dosimetry Approaches

Scenario-Based	Reverse Dosimetry
• Monitoring data sources may not be reflective of current exposure • Lack of data for all phthalates concentrations in all environmental media • Models may utilize conservative assumptions leading to higher exposure estimates • Data availability and quality to determine exposure from different pathways of exposure may vary leading to deterministic estimates for some pathways and probabilistic estimates for other pathways • Uncertainties may compound as individual intake estimates are aggregated across routes and pathways and then combined across phthalates	• Cannot be source apportioned • Relies on use of spot urine samples (may not be representative of average daily exposure due to fast elimination kinetics) • Urinary excretion factors estimated from controlled human exposure studies conducted with a limited number of adult volunteers (may not be reflective of intraspecies variation in toxicokinetics) • No urinary excretion factor is available for DCHP • Lack of current biomonitoring data for DCHP (excluded from NHANES after 2009-10) (systematic review may identify newer data to address this) • Lack of recent infant urinary biomonitoring data (youngest age group in NHANES is children aged 3 to 5 years) (systematic review may identify newer data to address this) • May introduce additional uncertainties when combined with scenario-based exposure estimates for specific TSCA COUs for consumers and workers

As discussed in Section 6.3.2.2, reverse dosimetry is the process of estimating an intake dose for a chemical based on biomonitoring data (U.S. EPA, 2019a). One limitation associated with reverse dosimetry is that this approach cannot be used to distinguish between routes or pathways of exposure and cannot be used to determine source apportionment of TSCA and non-TSCA sources. The inability to source apportion exposure using a biomonitoring approach represents a challenge under TSCA because aggregate exposure estimates may include exposure from non-TSCA and TSCA COUs leading to an overestimate of risk due to "double-counting" if non-attributable and non-TSCA exposure is combined with exposure from another TSCA COU. Additionally, because risk from individual TSCA COUs are estimated using scenario-based approaches in individual chemical risk evaluations, there may be uncertainties introduced when combining those exposure estimates with the aggregate exposure estimated using reverse dosimetry.

Use of a reverse dosimetry approach requires availability of biomonitoring data. In the case of phthalates, CDC's NHANES dataset provides a relatively recent (data available through 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. Further, the NHANES dataset has been used in previous phthalate CRAs conducted by U.S. CPSC (2014) and Health Canada (ECCC/HC, 2020). However, there are several limitations associated with use of the NHANES urinary biomonitoring data. First, NHANES does not include infants, one of the susceptible subpopulations based on lifestages identified by EPA in Section 5 (the youngest age group currently included in NHANES is children aged 3 to 5 years), nor does NHANES currently measure any urinary metabolites for DCHP. The DCHP metabolite, monocyclohexyl phthalate, was included in NHANES from 1999 to 2010; however, it has since been excluded from the NHANES survey due to low detection levels and a low frequency of detection in human urine (CDC, 2013a). These limitations with the NHANES dataset present a challenge for the use of a reverse dosimetry approach for estimating non-attributable phthlate

exposure. However, these limitations may be addressed using biomonitoring data from other recent studies, if identified by EPA during systematic review.

Another source of uncertainty associated with the reverse dosimetry approach is use of spot urine samples, which are collected as part of NHANES and many other human biomonitoring studies. As discussed in Section 3.1.5.1 and elsewhere (ATSDR, 2022; EC/HC, 2015c), 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. Therefore, spot urine samples, as collected through NHANES and many other biomonitoring studies, are representative of relatively recent exposures. The short half-lives of phthalates, however, lead to single spot sample that may not be representative of average urinary concentrations that are collected over a longer term or calculated using pooled samples (Shin et al., 2019; Aylward et al., 2016). Multiple spot samples provide a better characterization of exposure, with multiple 24-hour samples potentially leading to better characterization but are less feasible to collect for large studies (Shin et al., 2019). As discussed by U.S. CPSC, spot urine samples collected at a short time (2 to 4 hours) since last exposure may overestimate human exposure, while samples collected at a longer time (>14 hours) since last exposure may underestimate exposure (see section 4.1.3 of (U.S. CPSC, 2014) for further discussion).

Human variability in phthalate metabolism and excretion is another potential source of uncertainty associated with the reverse dosimetry approach. As discussed in Section 6.3.2.2, reverse dosimetry relies upon molar urinary excretion factors (*i.e.*, the Fue) estimated from controlled human exposure studies to estimate daily intake values from urinary phthalate metabolites. Fue values are available for DEHP, DBP, BBP, DIBP and DINP, however, no Fue value is available for DCHP (Table 6-3). Additionally, the Fue value for DIBP was estimated from a single male volunteer, and may not be reflective of the larger population or certain PESS (*e.g.*, infants, children, women of reproductive age or pregnant women). To overcome this limitation, U.S. CPSC (2014) used the Fue value for DBP to estimate a dietary intake value for DIBP. Finally, as can be seen from Table 6-3, Fue values for DEHP, DBP, BBP and DINP were calculated based on relatively small sample sizes of 10 to 20 adult volunteers and there is some uncertainty related to how reflective these Fue values are of variation in phthalate metabolism and excretion for the broader population, including PESS (*e.g.*, infants, children, women of reproductive age, pregnant women).

As can be seen from U.S. CPSC's analysis of NHANES urinary biomonitoring data in Table 6-4, phthalate daily intake estimates vary by year. The most notable trends appear to be that exposure to DEHP is decreasing, while exposure to DINP is increasing. These trends in exposure are notable, and may lead to differing conclusions regarding risk, depending upon which NHANES survey year is used. Similarly, availability of the most recent data to support scenario-based exposure assessments may have an impact on risk estimates. Dietary intake, for example, comprises a large portion of total estimated intake for all subpopulations and may vary over time; yet, EPA may not have the data to assess the dietary intake reflective of the current U.S. population because of the lack of availability of an ongoing total diet study and may need to rely on older dietary intake data or data from other nations as discussed in Section 6.3.2.1 leading to uncertainties in dietary intake estimates.

Data availability and data quality may affect estimations of exposure for other relevant pathways as well, which is a source of uncertainty for estimating exposure using a scenario-based method. Many of the inputs needed for either deterministic or probabilistic estimates, such as product use, body weight, breathing rate, environmental media concentration, etc. each have associated variabilities and uncertainties. Factors including but not limited to sampling methodology, study age, and location can all

impact uncertainties associated with model inputs. Some of the phthalates may also have more data available than others.

Data availability will dictate whether deterministic or probabilistic methods are appropriate for each pathway. Deterministic models use point estimates as inputs and are most often screening level. Conservative input variables may lead to overestimations of exposure. The type of models used to estimate intake can also vary for different routes or pathways, some of which are better characterized, and each have associated uncertainties.

Combining estimates of daily intake from various routes and pathways and across multiple phthalates also introduces uncertainties. Uncertainties associated with each individual intake estimate may compound when aggregating to estimate a total intake. Furthermore, aggregating exposure estimates quantified using differing tiers of assessment as discussed in Section 6.3.2.1. introduces additional uncertainty. Scenario-based assessments utilize many assumptions of human behavior and can include conservative assumptions to evaluate risk to be protective of populations assessed. As seen in Table 6-4 U.S. CPSC estimated higher intake values using a scenario-based approach for many, although not all, of the phthalates and noted it was potentially due to worst-case assumptions that were carried out for their study (U.S. CPSC, 2014).

6.3.2.5 Proposed Approach for Estimating Exposure from Non-attributable and Non-TSCA Sources

Given the strengths, limitations and uncertainties of scenario-based and reverse dosimetry approaches described in Sections 6.3.2.1-6.3.2.4, EPA believes that the scenario-based approach for estimating non-attributable and non-TSCA phthalate exposure is better suited to support conduct of a phthalate CRA under TSCA. This is in part because the scenario-based approach provides EPA with more flexibility to include and/or exclude major pathways of non-attributable and/or non-TSCA exposure when building cumulative exposure scenarios for consumers (discussed further in Section 6.4.1), workers (Section 6.4.2), and fenceline communities (Section 6.4.3). Furthermore, the scenario-based approach allows for source apportionment of non-attributable and non-TSCA exposures and estimates of exposure from non-attributable or non-TSCA sources can be varied for specific subpopulations and exposure scenarios. In contrast, the reverse dosimetry and biomonitoring approach provides an aggregate exposure estimate for each individual phthalate, which cannot be source apportioned, and may include exposures from TSCA sources, which may lead to double-counting if combined with exposure from specific TSCA COUs.

Therefore, EPA is proposing to use environmental monitoring data and modeling to build scenarios for estimating non-attributable and non-TSCA human exposure to phthalates through relevant pathways of exposure using a scenario-based approach. Under this approach, non-attributable and non-TSCA phthalate exposure will be estimated for the susceptible subpopulations identified in Section 5 by applying exposure factors specific to each lifestage.

Although there are limitations and uncertainties associated with the reverse dosimetry and biomonitoring approach, EPA recognizes the potential utility of this approach to help characterize phthalate exposure, and this information may be utilized by EPA in several ways, including:

- as a comparator for scenario-based daily intake estimates, and
- temporal trends analysis to better understand changes in phthalate exposure over time.

Recent NHANES urinary biomonitoring data is available for most of the high-priority and manufacturer-requested phthalates (with the exception of DCHP). Daily intake values estimated using urinary biomonitoring data and reverse dosimetry can be compared to scenario-based daily intake

estimates to help EPA determine if reasonable scenarios are being considered in their scenario-based assessment. EPA does not anticipate that reverse dosimetry and scenario-based approaches will yield identical results, because both methods have their own sets of uncertainties and limitations (see Section 6.3.2.4); however, as was reported by Health Canada (ECCC/HC, 2020) and U.S. CPSC (2014), both methods are anticipated to provide similar results (U.S. CPSC found that results were within an order of magnitude; see Table 6-4).

Additionally, EPA intends to conduct its own updated analysis of the NHANES dataset starting with the oldest NHANES cycle (*i.e.*, 1999 to 2000) up to the most currently available cycle for each phthalate as statistics and sampling methodology allows. By analyzing each cycle, EPA can examine the temporal trend of phthalate exposure over time in women of reproductive age and other susceptible subpopulations to understand changes in phthalate exposure in the U.S. population. Understanding current phthalate exposure levels in the U.S. population for each phthalate may help inform the Agency's risk determination including identifying which phthalates may be contributing to greater proportions of exposure in women of reproductive age and other susceptible subpopulations over time.

Because NHANES did not include surveillance of children under 6 years of age at the time of their analysis, U.S. CPSC (2014) used data from the Study for Future Families (Sathyanarayana et al., 2008b; Sathyanarayana et al., 2008a) to estimate exposure to children aged 2 to 36 months and to estimate prenatal and postnatal measurements in women (U.S. CPSC, 2014). EPA does not intend to update their analysis to estimate infant daily intake values unless systematic review identifies new or updated sources of biomonitoring data for infants. The lack of recent infant urinary phthalate biomonitoring data is a data gap, which can be overcome by EPA's proposal to primarily rely upon a scenario-based approach to estimate daily intake values for identified susceptible subpopulations based on lifestages.

6.4 Combining Exposure and Estimating Cumulative Risk (Steps 6 to 10 in Conceptual Model [Figure 2-1])

6.4.1 Consumer Exposures and Risk

This section describes EPA's proposed approach for building cumulative exposure scenarios generally for consumers and estimating cumulative risk for consumers. As stated previously in Section 5, EPA proposes to focus its CRA for phthalates on subpopulations that may be more susceptible to phthalate syndrome, which include pregnant women/women of reproductive age, and male infants, male toddlers, and male children who may be impacted by exposure from TSCA consumer COUs (but the proposed approach will be presented as applicable to all consumers). This involves the following steps as outlined in EPA's conceptual model (Figure 2-1):

- **Step 6. Identifying major pathways of exposure.** Determining the major pathways of exposure from TSCA consumer COUs (see purple box in Step 4 of conceptual model in Figure 2-1; completed in individual risk evaluations), non-attributable, and non-TSCA sources. This step would be completed after exposures are estimated for the various pathways of exposure and is dependent on the magnitude of those estimates. Major pathways may vary by relevant population and may also vary by phthalate. Identification of major pathways of exposure to relevant populations may require sensitivity analysis for determining inclusion of a pathway into a cumulative estimate. Description of this process is not detailed in this document as it will be dependent on the identified pathways.
- **Step 7. Determining co-exposure.** Determining likelihood of co-exposure across TSCA consumer COUs, non-attributable sources, and non-TSCA sources (Section 6.4.1.2).

- **Step 8. Convert exposures to index chemical equivalents.** Phthalate exposure from each individual phthalate is scaled to the potency of an index chemical using RPFs and expressed in units of IC equivalents (Section 6.4.1.3).
- **Step 9. Estimating cumulative exposure.** Combining TSCA consumer COU cumulative exposure, the relevant non-attributable cumulative exposure, and the non-TSCA cumulative exposure to estimate cumulative exposure in a reasonable manner (Section 6.4.1.3).
- **Step 10. Estimating cumulative risk.** A cumulative MOE is calculated for comparison to the benchmark MOE (total uncertainty factor associated with the assessment) (Section 6.4.1.4).

As shown in EPA's conceptual model (Figure 2-1), consumers may be exposed to multiple phthalates through use of consumer products associated with TSCA COUs, as well through additional non-attributable and non-TSCA sources (described in Section 6.3.2). Therefore, estimating cumulative risk to consumers will involve combining major sources of phthalate exposure resulting from TSCA consumer COU(s), as well as additional non-attributable and non-TSCA sources that can be reasonably expected to co-occur over a relevant timeframe. Considerations for determining phthalate co-exposure from TSCA consumer COUs are provided in Section 6.4.1.2, and EPA's proposed approach to estimating cumulative risk to consumers is provided in Sections 6.4.1.3 and 6.4.1.4.

6.4.1.1 Data Needs for Consumer Co-exposure Analysis

A consumer exposure assessment in individual phthalate risk assessments will estimate magnitude of exposure to a single phthalate during use of a consumer product, which will depend on the concentration of the phthalate in the product, use patterns (including frequency, duration, amount of product used, room of use) and/or application methods. Common data sources used to complete individual chemical consumer exposure assessments include but are not limited to product formulation data, product use data, EPA's *Exposure Factors Handbook* (U.S. EPA, 2021a), and literature sources reporting on indoor air concentrations or consumer products.

Data sources needed to determine the likelihood of co-exposure to multiple phthalates from a single consumer product or co-exposure to multiple phthalates from the use of multiple products containing a single or multiple phthalates may include, but may not be limited to the following:

- **Product Formulation Data.** Consumers may encounter co-exposure to multiple phthalates through exposures from the presence of multiple phthalates in a single product (*e.g.*, plastic products containing BBP and DBP). The presence of multiple phthalates in a single product may be determined through process information or production formulation data provided by the manufacturer of a product or through publicly available product MSDS (Material Safety Data Sheet) or SDS (Safety Data Sheet) documents.
- **Survey of Consumer Behavior.** Co-exposures to two or more chemical substances from multiple COUs result from what is commonly referred to as the co-occurrence of use (or co-use) and/or co-location of exposure sources. In other words, a determination of co-exposures is dependent on evidence of co-use and/or co-location. In the context of TSCA, co-uses typically refer to scenarios from which an individual (*e.g.*, consumer) may be exposed to two or more COUs such as when a spray and powdered cleaner are used concurrently to clean a bathtub. For consumer co-exposures, which are primarily dependent on co-use data that are rare in the literature, studies which report continuous emissions of chemicals even when products are not in use can be used to determine which products consumers and bystanders may be co-exposed to via specific rooms or space of use and periods of time. Usage surveys may also be used to determine the length of time a product is used to determine if timeframes of exposure to multiple products may overlap.

- **Purchase/Market Data.** If there is limited data on consumer behavior using products concurrently, purchase data may provide insight into whether products are ending up in a single household and potentially leading to co-exposure to multiple phthalates.

6.4.1.2 Co-exposure Resulting from TSCA Consumer COUs (Step 7 in Conceptual Model [Figure 2-1])

Risks from individual phthalates across various exposure routes, presented in the individual risk evaluations, will be combined based on the available evidence of co-exposure to determine a cumulative risk across relevant phthalates. There are several considerations for estimating cumulative risk for consumers, including consumers' use of

- multiple products each containing a different high-priority or manufacturer-requested phthalate;
- multiple products each containing more than one high-priority or manufacturer-requested phthalate; and
- a single product containing multiple high-priority and manufacturer-requested phthalates.

6.4.1.2.1 Survey of Consumer Behavior for Determining Co-exposure

As discussed in EPA's Draft Proposed Principles of CRA under TSCA and stated above in the data needs section (Section 6.4.1.1), in general, there is limited information on the co-use and/or co-location of consumer products to serve as evidence for co-exposure to different chemicals present in multiple consumer products. Some studies have investigated co-use patterns for personal care products, which are regulated by FDA (Safford et al., 2015; Biesterbos et al., 2013). Thus far, only one co-use study by Han et al. has been identified, which considered multiple TSCA-relevant consumer products in its analysis, including laundry detergents, fabric softeners, air fresheners, dishwashing detergents, and all-purpose cleaners. However, the authors found no strong correlation of co-use between any pair of household and personal care products (Han et al., 2020).

6.4.1.2.1 Purchase Data for Determining Co-exposure

Another approach to determine co-use of products has been to use purchase data or presence of certain consumer products in the home to extrapolate combined exposure and risk (Stanfield et al., 2021; Tornero-Velez et al., 2021). Unfortunately, the presence of consumer products in the home is insufficient to paint the realistic picture of daily exposure for consumers. This further emphasizes the importance of co-use data that help to describe consumer use patterns (*e.g.*, which combinations of products are used, how often, how much, etc.) for products currently on the market.
Currently, available co-use studies indicate that there is lack of evidence of co-use specifically for the TSCA COUs shown in Table 6-1. This may in part be because many of the TSCA COUs associated with the phthalates are not necessarily common household products regularly studied for concurrent use.

6.4.1.2.2 Product Formulation Data for Determining Co-exposure

To better understand whether consumers may be exposed to multiple phthalates through the use of a single product containing more than one phthalate, EPA reviewed products associated with TSCA COUs listed in the use report for each phthalate (U.S. EPA, 2021d, 2020f, g, h, i, j). This analysis involved review of product formulation data either from manufacturer websites or Safety Data Sheets (SDS) to determine the presence of a phthalate in a product and the weight or volume percent of that phthalate in the product. The same information was also used to determine if multiple phthalates were listed as being part of a single product. Table 6-6 lists the products and manufacturers identified in each phthalate use report as being associated with a COU that EPA reviewed. This preliminary analysis revealed little evidence to suggest that many consumer products associated with TSCA COUs contain more than one phthalate. Of the products listed in Table 6-6, EPA identified one that contains multiple high-priority and manufacturer-requested phthalates, which is PSI PolyClay Canes and PSI PolyClay Bricks

(containing ≤2.5 percent, but unspecified by weight or volume) each of DEHP, BBP, DBP, and DINP. Although not always interchangeable, many phthalates serve a similar role as a plasticizer in many products (Graham, 1973) and, in the identified products with TSCA uses, the phthalates were often found independently even when identified as being in the same category of products (*e.g.*, paints and coating). One limitation of this preliminary analysis is that it did not include a review of consumer product information for DINP. EPA may update this initial analysis as more up-to-date information for TSCA consumer products is identified.

Table 6-6. Sample of Consumer Products Containing Phthalates

Phthalate	Product [a b c]	Manufacturer [d]
BBP	Sakrete Blacktop Repair Tube	Sakrete of North America
	Concrete Patching Compound	Quikrete Companies
	Mortar Repair Sealant	Quikrete Companies
	DAP Roof & Flashing Sealant, Polyurethane	DAP Products, Inc.
	Pre-Mixed Stucco Patch	Quikrete Companies
	Hercules Plumber's Caulk - White/Linen	HCC Holdings Inc.
	Wilsonart Color Matched Caulk	Wilsonart LLC
	Acrylic Caulk	Momentive Performance Materials - Daytona
	Silicone Fortified Window & Door Sealant	Henry Company
	Air Bloc 33	Henry Company
	PSI PolyClay Canes and PSI PolyClay Bricks [e]	Penn State Industries
	Double Bubble Urethane High Peel Strength D50 Part A (04022)	Royal Adhesives & Sealants
	Dymonic FC Anodized Aluminum	Tremco Canadian Sealants [Canada]
	GE7000	Momentive Performance Materials
	Hydrogel SX	Prime Resins Inc.
	Permatite Acrylic Sealant	Permatite / Division of DSI
	Protecto Sealant 25XL	Protecto Wrap Company
	Spectrem 3 Aluminum Stone - 30 CTG	Tremco Canadian Sealants [Canada]
	Spectrem 4	Tremco U.S Sealants
	STP 17925 Power Steering Fluid & Stop Leak	Armored AutoGroup Inc.
	126VR Disc Brake Quiet 0.25 Fl. Oz Pouch	ITW Permatex
	Steri-Crete SL Component A	Dudick, Inc.
	Stonclad UT Resin Polyol	Stonhard, Division of StonCor Group, Inc.
	ENSURE Sterilization Emulator	SciCan Ltd. [Canada]
	Phthalates in Poly(vinyl chloride)	SPEX CertiPrep, LLC
	Elmer's Model + Hobby Cement	Elmers Products, Inc.
	Accent MBRU 6pk Silver Metallic 2oz	Rust-Oleum Corporation
	Champion Sprayon Acrylic Matte Finish	Chase Products Co.
	6840 Ultra Black	BJB Enterprises, Inc.
	Handstamp - Blue	Identity Group
	Repair and Refinishing Spray	Multi-Tech Products Corp.
	Armacell WB Finish	Mon-Eco Industries, Inc.
	Black Tire Paint Concentrate	Akron Paint and Varnish (dba APV Engineered Coatings)
	IC 1-gl 2pk Gray Shop Coat Primer	Rust-Oleum Corporation

Phthalate	Product [a b c]	Manufacturer [d]
BBP	Klean-Strip Mask & Peel Paint Booth Coating	W. M. Barr
	Lacquer Touch-up Paint - Clear Topcoat	Ford Motor Company
	SK Clear-Seal Satin Sealer 5 Gal	Rust-Oleum Corporation
DBP	3M Bondo Glazing & Spot Putty	3M Company
	SureFlex Multi-Purpose Adhesive, SH-360	Barristo Enterprises, Inc. dba SureHold
	Lanco Seal	Lanco Mfg. Corp.
	PSI PolyClay Canes and PSI PolyClay Bricks [e]	Penn State Industries
	Hydrostop Premiumcoat Finish Coat	GAF
	Hydrostop Premiumcoat Foundation Coat	GAF
	Hydrostop Trafficcoat Deck Coating	GAF
	Pro 1-GL 2PK Flat Aluminum Primer	Rust-Oleum Corporation
	DURALAQ-WB WATERBORNE WHITE ACRYLIC FINISH DULL RUBBED	Benjamin Moore & Co.
	Hydrostop Premiumcoat Foundation Coat Summer	GAF
	Bondo Gray Filler Primer	3M Company
	Pettit XL Vivid 1861 Black	Kop-Coat, Inc. / Pettit Marine Paint
	Accurate Solo 1000, Accurate LT-30, Accurate LT-32, Accurate 2015, Accurate 2495, Accurate 4064, Accurate 4350	Western Powders, Inc.
	Cartridge 9 mm FX Marking, Toxfree primer	General Dynamics - Ordnance and Tactical Systems - Canada Inc. [Canada]
	Rimfire Blank Round - Circuit Breaker	Olin Corporation - Winchester Division, Inc.
	Wizard 31 Epoxy Ball Plug Hardener	Brunswick Bowling Products, LLC
DEHP	765-1553 BALKAMP VINYL REPAIR KIT	Permatex, Inc.
	Chocolate	Wellington Fragrance
	PSI PolyClay Canes and PSI PolyClay Bricks[e]	Penn State Industries
	DUPLI-COLOR BED ARMOR	Dupli-Color Products Company
	DUPLI-COLOR High Performance Textured Metallic Coating Charcoal	Dupli-Color Products Company
	264 BLACK TRUCK BED LNR 6UC	The Valspar Corporation
	RED GLAZING PUTTY 1# TUBE	The Valspar Corporation
	Prime WPC/Prime Essentials/Prime SPC	Carlton Hardwood Flooring
	Lenox MetalMax	Lenox Tools
	6.17 OZ 100040 FH FRESH SCENT PET TW 12PK	Fresh House
	KRYLON Fusion All-In-One Textured Galaxy	Krylon Products Group
	Self-cath pediatric 30 pack	Coloplast Corp.
	3M™ Economy Vinyl Electrical Tape 1400, 1400C	3M
	Pronto Putty	The Valspar Corporation
	Red Glazing Putty 1# Tube	Quest Automotive Products
	BD Loop Goop	Royal Adhesives and Sealants Canada Ltd.
	SCOFIELD® CureSeal 350	Sika Corporation
DCHP	Duco Cement (bottle and tube)1	ITW Consumer - Devcon/Versachem
	Fusor 108B, 109B Metal Bonding ADH PT B	LORD Corporation
DIBP	Blue Label Washable PVA Adhesive	Colorlord Ltd.
	BETAKRIL TEXTURE	Betek Boya ve Kimya Sanayi A.S [Turkey]
	Centerfire Pistol & Revolver and Rifle Cartridges	Companhia Brasileira de Cartuchos (CBC)

Phthalate	Product [a b c]	Manufacturer [d]
DIBP	Art Board	Ningbo Zhonghua Paper Co. Ltd.
	Glitter Boards	DJECO
	Painting - Oh, It's Magic	DJECO

[a] This table includes a sample of products listed in the Use Reports for each DBP, BBP, DIBP, DEHP, DCHP (U.S. EPA, 2021d, 2020f, g, h, i, j).
[b] This table may represent updated information with products listed that are not identified in the published Use Reports.
[c] This is not a comprehensive list of products containing each phthalate nor does the presence of a product on this list indicate its availability in the United States for consumer purchase
[d] Some manufacturers may appear over-represented in this table. This may mean that they are more likely to disclose product ingredients online than other manufacturers, but this does not imply anything about use of the chemical compared to other manufacturers in this sector.
[e] The SDS for PSI PolyClay Canes and PSI PolyClay Bricks, which lists the product as containing multiple phthalates is available here: https://www.pennstateind.com/MSDS/POLYCLAY_MSDS.pdf.

6.4.1.3 Combining Exposure to Consumers to Estimate Cumulative Exposure (Steps 8 and 9 in Conceptual Model [Figure 2-1])

As described in the final scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c), EPA will assess exposures to consumers for each COU outlined in the scope documents. Consumer exposure to phthalate containing products will be assessed for individual COUs and will primarily focus on inhalation and dermal exposures, however, oral exposures may be considered based on the use of the product or the possibility for hand-mouth behavior to occur.

As described above in Section 6.4.1.2, there is currently a lack of evidence that multiple consumer products are used concurrently by consumers. Therefore, EPA is not proposing to combine risk for co-use of multiple consumer products for consumers, unless new information is identified to support doing so. Similarly, due to the initial identification of a single product containing less than or equal to 2.5 percent (unspecified weight or volume) of DEHP, BBP, and DBP, EPA does not anticipate assessing the cumulative exposure to multiple phthalates through the use of a single consumer product, unless new product information is identified to support doing so. EPA believes that assessing risk for individual COUs associated with a single phthalate is adequate because of the numerous product examples containing greater than or equal to 10 percent (weight and volume) of single phthalates, which likely exceeds the cumulative exposure associated with the use of the single identified product. Therefore, EPA is unlikely to combine risk across multiple phthalates for a single consumer COU.

Consumers may have exposures to multiple phthalates through sources other than uses of consumer products. Additional sources of exposure to multiple phthalates are captured in EPA's proposed non-attributable and non-TSCA exposure estimate (Section 6.3.2). Therefore, to estimate cumulative exposure to consumers, EPA proposes to combine non-attributable and non-TSCA exposure for the high-priority and manufacturer-requested phthalates with exposure from use of a single consumer product containing a single phthalate using Equation 6-2. Determining reasonable cumulative exposure scenarios may involve considering the likelihood of co-exposure, the possibility of double counting, and of over- or under-estimating exposures. The estimates for the non-attributable and non-TSCA portion of Equation 6-2 may vary based on the consumer scenario and population being assessed and exposures that comprise each category (*i.e.*, TSCA, non-attributable, non-TSCA) can be adjusted to limit the possibility of "double counting." For example, if the TSCA consumer exposures are known to highly impact indoor air concentrations then the non-attributable indoor air concentrations may need to be adjusted accordingly. Additionally, adults may have different exposure from infants or toddlers based on exposure factors and interaction with different sources of exposure leading to potentially different

estimates in all exposure categories with some phthalates being more or less impactful for different lifestages.

Equation 6-2. Example estimation of cumulative phthalate exposure to consumers[8]

Consumer cumulative exposure (expressed as index chemical equivalents) = non-attributable exposure + non-TSCA exposure + individual consumer COU exposure

Because EPA is proposing to use an RPF approach (Section 4.3.3), phthalate exposure from each individual COU will be scaled to the potency of an IC and expressed as IC equivalents, which will then be summed with non-attributable and non-TSCA exposure (described in Section 6.3.2 and also expressed as index chemical equivalents) to estimate consumer cumulative exposure (expressed as IC chemical equivalents).

6.4.1.4 Estimating Cumulative Risk for Consumers (Steps 10 in Conceptual Model [Figure 2-1])

To estimate cumulative risk for each specific consumer exposure scenario, an MOE (ratio of index chemical POD to consumer cumulative exposure estimate (expressed as index chemical equivalents) calculated using Equation 6-2) would be calculated for comparison to the benchmark MOE (*i.e.*, the total uncertainty factor associated with the assessment) (described in Section 4.3.2). The lower the MOE (margin between the toxicity effect level and the exposure dose), the more likely a chemical is to pose a risk.

6.4.2 Occupational Exposures and Risk

This section describes EPA's proposed approach for building cumulative exposure scenarios for workers and estimating cumulative risk for workers. As stated previously in Section 5, EPA proposes to focus its CRA for phthalates on subpopulations that may be more susceptible to phthalate syndrome which include pregnant women/women of reproductive age who may be impacted by exposure from TSCA occupational COUs, but the proposed approach will be presented as applicable to all workers. This involves the following steps as outlined in EPA's conceptual model (Figure 2-1):

- **Step 6. Identifying major pathways of exposure.** Determining the major pathways of exposure from TSCA occupational COUs (see yellow box in Step 4 of conceptual model (Figure 2-1); completed in individual risk evaluations), non-attributable, and non-TSCA sources. This step would be completed after exposures are estimated for the various pathways of exposure and is dependent on the magnitude of those estimates. Major pathways may vary by relevant populations and may also vary by phthalate. Identification of major pathways of exposure to relevant populations may require sensitivity analysis for determining inclusion of a pathway into a cumulative estimate. Description of this process is not detailed in this document as it will be dependent on the identified pathways.
- **Step 7. Determining co-exposure.** Determining likelihood of co-exposure across TSCA occupational COUs, non-attributable sources, and non-TSCA sources (Section 6.4.2.2).

[8] EPA may consider an alternative to this proposed approach for COU categories such as floor coverings, fabric and textiles, and building materials, which may contribute to concentrations of phthalates in indoor air or dust outside the period of direct product use through ongoing releases. In this case, EPA may consider combining exposure from non-attributable sources, non-TSCA sources, exposure from an individual consumer COU during use and exposure from a consumer COU due to ongoing releases to estimate cumulative exposure. This approach will be considered by EPA as supported by available data.

- **Step 8. Convert exposures to index chemical equivalents.** Phthalate exposure from each individual phthalate is scaled to the potency of an index chemical using RPFs and expressed in units of index chemical equivalents (Section 6.4.2.3).
- **Step 9. Estimating cumulative exposure.** Combining TSCA occupational COU cumulative exposure, the relevant non-attributable cumulative exposure, and the non-TSCA cumulative exposure to estimate cumulative exposure in a reasonable manner (Section 6.4.2.3).
- **Step 10. Estimating cumulative risk.** A cumulative MOE is calculated for comparison to the benchmark MOE (total uncertainty factor associated with the assessment) (Section 6.4.2.4).

As shown in EPA's conceptual model (Figure 2-1), workers may be exposed to multiple phthalates through workplace exposures associated with TSCA COUs, as well through additional non-attributable and non-TSCA sources (described in Section 6.3.2). Therefore, estimating cumulative risk to workers will involve combining major sources of phthalate exposure resulting from TSCA occupational COU(s), as well as additional non-attributable and non-TSCA sources that can be reasonably expected to co-occur over a relevant timeframe.

In order to assess releases and cumulative risks in the workplace, EPA will rely on EPA program data and data discovered through the systematic review process to determine if multiple phthalates are present at occupational sites and/or releasing facilities leading to exposure to workers or fenceline communities (discussed in Section 6.4.3). The following sections will discuss EPA's proposed approach for assessing cumulative risk for workers and cumulative exposure resulting from facility releases. This includes EPA's proposed approach for the following:

- Identifying sites with potential for release and cumulative occupational exposure.
- Quantifying cumulative worker exposure at sites and/or for specific COUs where multiple high-priority and manufacturer-requested phthalates are anticipated to be in use.
- Quantifying cumulative phthalate releases at sites and/or for specific COUs where multiple high-priority and manufacturer-requested phthalates are anticipated to be in use.

6.4.2.1 Data Needs for Releases and Cumulative Occupational Exposure Assessment

An engineering assessment is typically comprised of three primary elements, including: (1) facility estimates, which provide a basis for the scope of release and exposure and estimates; (2) environmental release estimates, which estimate the quantity and release frequency of the chemical into the environment; and (3) occupational exposure estimates, which estimates the chemical exposure to workers and occupational non-users (ONUs) at a facility; and EPA plans to utilize available Agency data and any additional data sources for a cumulative assessment.

Common sources used to complete occupational exposure and environmental release assessments are listed below and are summarized for the high-priority and manufacturer-requested phthalates in Table 6-7:

- Chemical Data Reporting (CDR), to which import and manufacturing sites producing the chemical at or above a specified threshold must report.
- Toxics Release Inventory (TRI), to which facilities handling a chemical covered by the TRI program at or above a specified threshold must report.
- Discharge Monitoring Report (DMR), a periodic report required of National Pollutant Discharge Elimination System (NPDES) permitted facilities discharging to surface waters.
- National Emissions Inventory (NEI), a compilation of air emissions of criteria pollutants, criteria precursors and hazardous air pollutants from point and non-point source air emissions.

- Resource Conservation and Recovery Act (RCRA)Info, to which small and large quantity generators of hazardous waste and treatment, storage and disposal facilities must report.
- National Institute of Occupational Safety and Health: Health Hazard Evaluation (NIOSH HHE), a compilation of voluntary employee, union, or employer requested evaluations of health hazards present at a given workplace.
- Occupational Safety and Health Administration: Chemical Exposure Health Data (OSHA CEHD), a compilation of industrial hygiene samples taken when OSHA monitors worker exposures to chemical hazards.

Table 6-7. Available EPA Program and Common Source Data for Each Phthalate

Chemical	CDR	TRI	DMR	NEI	RCRAInfo	NIOSH HHE	OSHA CEHD
DEHP	✓	✓	✓	✓	✓	✓	✓
DBP	✓	✓	✓	✓	✓	✓	✓
BBP	✓	*x*	✓	*x*	*x*	*x*	*x*
DIBP	✓	*x*	*x*	*x*	*x*	*x*	*x*
DCHP	✓	*x*	*x*	*x*	*x*	✓	*x*
DINP	✓	*x*	*x*	*x*	*x*	✓	*x*

✓ Indicates data available
x Indicates no data available

As can be seen from Table 6-7, the six phthalates selected for release and cumulative occupational exposure assessment represent a challenge due to the limited data available from EPA programs. Only two of the six phthalates are reported to TRI and NEI and only three have recent DMR data. This leaves large data gaps in assessing environmental releases and occupational exposures for certain phthalates and certain COUs that will require alternative methods and data sources to fill.

Additionally, while EPA program data is useful for identifying the presence of multiple phthalates at a single site and quantifying cumulative release and exposure, further data from literature and other sources will be necessary to fully understand how the phthalates are being used at each site. Data needs to complete the cumulative assessment include the following:

- **Chemical reaction pathways and functionality:** Some chemicals can be substituted for others based on cost or availability at processing and use sites. It is anticipated that this may be the case with some of the phthalates. Understanding the chemical reaction pathway of each phthalate within each COU and the functionality it provides the end product will allow for the determination of likelihood that phthalates would be manufactured/processed/used at the same facility; exist in a process at the same time; or be used as occasional replacements for each other.
- **Facility operating schedules:** A site may manufacture, process, or use multiple phthalates over the span of a year, but not at the same time, such as if the site runs separate campaigns using different phthalates for different products. Detailed process information and data on how frequently a worker is exposed to different phthalates (*i.e.*, same day, consecutive days, or more sporadically) can help inform more accurate cumulative exposure profiles and define how cumulative exposures are compared to benchmarks for acute, sub-chronic, or chronic health effects.

- **Facility process descriptions and worker tasks:** At sites where multiple phthalate exposures happen as part of the same operation, it is important to understand whether the phthalates are used simultaneously, consecutively, or separately and whether these tasks are performed by the same worker or workers within the same process units.
- **Market data on phthalate manufacturers and processors:** The EPA programmatic data provides insight into producers and users of selected phthalates at quantities above the reporting thresholds, but market data may enable a more complete understanding of all sites, manufacturers through end-users, working with multiple phthalates. This could include supplier and retailer data, phthalate market use data, and other data that could help generate a list of companies that manufacture or process multiple phthalates.
- **End user product data and compatibility:** An inventory of which phthalates are found in various similar products will be important for understanding the potential for end-user cumulative exposures. An end user, such as for the application of paints, coatings, adhesives, and sealants, may utilize multiple similar products for a given task that could contain different phthalates. Information that can inform such an inventory include SDS, product use data from various sources, or general COU process information.

This represents EPA's initial assessment of data needs and potential data sources. Data needs and source may change during the cumulative assessment of the six phthalates in the phthalate cumulative chemical group.

6.4.2.2 Co-exposure Resulting from TSCA Occupational COUs (Step 7 in Conceptual Model [Figure 2-1])

This section reviews the potential data sources that exist to quantify sites with potential cumulative releases or exposures to the selected phthalates that may lead to cumulative exposures to workers or to fenceline communities (discussed further in Section 6.4.3). Cumulative occupational exposure to the six high-priority and manufacturer-requested phthalates results from multiple phthalates being handled at the same site. There are several considerations for estimating cumulative risk for workers, including considerations of

- **Multiple phthalate direct exposure:** the worker is directly exposed to two or more of the high-priority and manufacturer-requested phthalates in their job, but there are no indirect exposures from additional phthalates.
- **Phthalate direct exposure + indirect workplace exposure:** the worker is directly exposed to one or more of the selected phthalates in their job and indirectly exposed to additional phthalates that are present in the workplace but may not be directly part of their job.

ONU exposures may follow the same mechanisms as described above but are often difficult to quantify due to lack of data. At minimum, a cumulative exposure from all operations contributing to an indirect exposure in a workplace setting can be established to represent a workplace ONU exposure. Depending on the quality of meta data provided, a documented ONU exposure to a given phthalate may be a sufficient estimate of the indirect workplace exposure to that phthalate.

6.4.2.2.1 EPA Program Data for Identifying Sites to Determine Co-exposure

EPA will use its program data as a starting point for identifying sites with cumulative release and exposure potential. EPA program data include CDR, TRI, DMR, NEI, and RCRAInfo listed above and described in further detail in Appendix D.

There may be significant challenges with using EPA programmatic data to identify sites with cumulative release and exposure potential. In CDR, for example, facilities may claim data as confidential business

information or not known or reasonably ascertainable. It should still be possible to identify sites with multiple phthalates, but these claims may make it challenging for EPA to use information claimed CBI, such as number of workers or production volume. Additionally, the CDR, TRI, NEI programs, as well as aspects of the RCRAInfo program, all have reporting thresholds such that sites below this threshold do not report to the program. Furthermore, the TRI, NEI, and RCRAInfo programs only require reporting for specific chemicals and only a limited number of phthalates report to these programs. Facilities not reporting to these programs will need to be identified via other means, such as those described below.

6.4.2.2.2 NIOSH HHE, OSHA CEHD, and Other Literature Sources Data for Identifying Sites to Determine Co-exposure

NIOSH HHEs, OSHA CEHD, and results from systematic review can be used in a similar way to determine the presence of multiple phthalates at a given site. The HHEs and CEHD represent NIOSH and OSHA inspections at specific sites and typically include monitoring data. Systematically reviewed literature may also contain data identifying specific sites or may contain broader data such as process information or product usage data that can inform the potential for unidentified sites to handle multiple phthalates. These systematically reviewed literature sources include EPA generic scenarios (GSs) and emission scenario documents (ESDs), which provide general information for a specific industry or COU.

The site information from these sources will be compared to sites in the other referenced datasets, including CDR, TRI, DMR, NEI, RCRA Info. For example, if a site inspected by NIOSH is reported to have DBP on site in the HHE and that same site reported to TRI for DEHP, it may be assumed that both DBP and DEHP are used at that site. There are several aspects to consider before drawing this conclusion:

- **Temporal:** The two datasets must be reasonably close in time period—a site's operations can evolve over time along with the chemicals used and products manufactured.
- **Non-detects:** Some monitoring regimes test for a spectrum of chemicals regardless of whether they are expected to be present at the site; therefore, the presence of a non-detect in a report does not necessarily mean the presence of that chemical at the site.
- **Biomonitoring data:** Health studies may include urinary biomonitoring data that describes potential exposures to multiple phthalates in the workplace. The study must be clear if the tested metabolites are unique to a given phthalate present in order to be used in determining overlap with other phthalates.

6.4.2.2.3 Product Information Data for Identifying Sites to Determine Co-exposure

A compilation of all phthalate-containing products and the companies producing them may be useful in determining sites that process multiple phthalates. SDSs or ingredient lists for products can inform if the products contain one or more of the high-priority and manufacturer-requested phthalates. The collected product data may indicate if a site produces multiple phthalate-containing products and may potentially inform end-user sites with cumulative exposures to the selected phthalates.

6.4.2.2.4 Identifying Additional Unknown Sites with Release and Exposure Potential to Determine Co-exposure

As discussed previously, there are limitations to the data sources listed above such that they may not be sufficient to fully identify all sites with cumulative release and exposure potential. To account for this uncertainty, EPA plans to evaluate the potential for additional unknown/unidentified sites to have cumulative release and exposure potential. For example, an exposure assessor could expand the analysis of CDR, TRI, NEI, and DMR to include additional phthalates that are not part of the cumulative assessment to gain an understanding of how many phthalates a facility might use in a given COU. These

methods would not identify specific sites with cumulative release and exposure but could be used to identify the potential for additional unknown sites to have multiple phthalates present beyond the ones identified using the above data sources.

Table 6-8 summarizes each unique COU and the applicability of these COUs to the selected phthalates, according to the published scope documents for BBP (U.S. EPA, 2020a), DBP (U.S. EPA, 2020d), DCHP (U.S. EPA, 2020e), DEHP (U.S. EPA, 2020b), DIBP (U.S. EPA, 2020c), and DINP (U.S. EPA, 2021c). COUs will be used for each of the six phthalates to inform the potential for an unknown site within a given COU to handle multiple phthalates. For example, hydraulic fracturing is only applicable to DEHP; therefore, it is unlikely that a hydraulic fracturing site will handle multiple phthalates. However, it is important to note that there may be instances where separate COUs could exist within the same facility. For example, DIBP is the only phthalate that is used in textile finishing, but both DEHP and BBP are used in textile dyeing and DINP and DBP are used in textiles, apparel, and leather manufacturing, all of which could be co-located with the same site. Each COU should be carefully considered when evaluating the potential for a site to have cumulative releases and exposures from multiple COUs and phthalates.

Table 6-8: Conditions of Use for Each High-Priority and Manufacturer-Requested Phthalate

Condition of Use (COU)	DEHP	DINP	DIBP	DBP	BBP	DCHP
Manufacturing	X	X	X	X	X	X
Repackaging	X	X	X	X	X	X
Processing as a reactant	X	X		X	X	X
Incorporation into formulation, mixture, or reaction product	X	X	X			X
Industrial processing (not including formulation)				X		
Plastics compounding	X	X	X	X	X	X
Plastics converting	X	X	X	X	X	X
Use in hydraulic fracturing	X					
Application of finishing agents		X				
Textiles, apparel, and leather manufacturing		X		X		
Application of paints, coatings, adhesives, and sealants	X	X	X	X	X	X
Use of laboratory chemicals	X	X	X	X	X	X
Use of automotive care products	X	X			X	
Use of ink, toner, and colorant products (*e.g.*, printing)		X		X	X	X
Use of cleaning and furnishing care products		X		X	X	
Use of hydraulic fluids		X				
Textile dyeing	X				X	
Textile finishing			X			
Use of air care products		X				
Application of finishing agents in cellulose film production						X
Use of fuels and related products			X			
Manufacturing of plastic foam products		X				
Soldering and welding	X					
Castings					X	
Use of flush fluids		X				
Textiles, apparel and leather manufacturing	X					
Explosives manufacturing				X		
Use of chemiluminescent light sticks				X		
Use of inspection penetrant kits				X		
Use of lubricants				X		

Condition of Use (COU)	DEHP	DINP	DIBP	DBP	BBP	DCHP
N/A – Fabrication or use of final product/articles	X	X	X	X	X	X
Recycling	X	X	X	X	X	X
Waste handling, treatment, and disposal	X	X	X	X	X	X

6.4.2.2.5 Workplace Monitoring Data for Determining Co-Exposure

Inhalation monitoring data that is identified during evaluation of the NIOSH HHEs, OSHA CEHDs, literature, and other sources will be utilized for the cumulative occupational exposure assessment. Data from monitoring activities at sites known or expected to have multiple phthalates are preferred, as these data may represent both direct exposures and indirect workplace exposures. The frequency of this data, however, is anticipated to be low.

Without monitoring data from sites with multiple phthalates, monitoring data for a given phthalate will be used within a given COU to establish direct exposure to that phthalate in that COU. In some instances, there may be no monitoring data, in which case surrogate data from other COUs or from other similar phthalates may be used. Understanding the chemical reaction pathways and use patterns of each phthalate will help to gauge which COUs or phthalate monitoring data is best suited for use as a surrogate. In the absence of suitable surrogate data within the selected phthalates, there may be some literature sources, such as GSs and ESDs, with surrogate data that can be used.

Also, area monitoring data from various workplace locations or ONU monitoring data may be used to establish indirect workplace exposures. Surrogate data as described above may also be used if available.

6.4.2.3 Combining Exposure to Workers to Estimate Cumulative Risk (Steps 8 and 9 in Conceptual Model [Figure 2-1])

With direct and indirect workplace exposures estimated via the methods above, exposure estimates can be combined to estimate cumulative exposures as each occupational scenario requires. This task will rely heavily on establishing worker relationships with the chemicals to which they are exposed and any operational and exposure details provided within the monitoring data assessed in this section. Risks from individual phthalates across various exposure routes, presented in the individual risk evaluations, will be combined based on the available evidence of co-exposure to determine a cumulative risk across relevant phthalates. EPA also plans to develop generic occupational exposure estimates for the unknown sites with cumulative occupational exposure potential identified according to the methodology in Section 6.4.2.2.4.

As previously stated, EPA is defining cumulative occupational exposure as being exposed to more than one high-priority and manufacturer-requested phthalate directly on the job and/or being exposed to a single or multiple phthalates directly on the job in addition to being indirectly exposed to levels of phthalates in the workplace. Cumulative occupational exposure can be used to determine cumulative risk to relevant phthalates occurring in the workplace during the 8-hour workday. Workers may have exposures to multiple phthalates through sources occuring outside the workday. Additional sources of exposure to multiple high-priority and manufacturer-requested phthalates are captured in EPA's proposed non-attributable and non-TSCA exposure estimates (Section 6.3.2). Therefore, to estimate cumulative exposure to workers, EPA is proposing to combine the non-attributable and non-TSCA exposure (as defined in Section 6.3.2) with the cumulative exposure from the workplace to determine a total cumulative exposure for workers using Equation 6-3 and as shown in Figure 2-1.

Equation 6-3. Example estimation of cumulative exposure to occupational subpopulations

Cumulative exposure to occupational subpopulations (expressed as index chemical equivalents) = Non-attributable exposure + Non-TSCA exposure + Cumulative occupational exposure

Determining reasonable cumulative exposure scenarios may involve considering the likelihood of co-exposure, the possibility of double counting, and of over- or under-estimating exposures. The estimates for the non-attributable and non-TSCA portion of Equation 6-2 may differ between a worker and a consumer. Therefore, exposures that comprise each category (*i.e.*, TSCA, non-attributable, non-TSCA) may be adjusted to limit the possibility of "double counting." For example, exposure from a non-attributable source of indoor air, as determined through residential estimates, may need to be adjusted for a worker who spends 8 hours at the workplace and less than 24 hours in the home.

Because EPA is proposing to use an RPF approach (see Section 4.3.3), exposure from each individual phthalate identified as part of the cumulative occupational exposure will be scaled to the potency of an index chemical and expressed as index chemical equivalents, which will then be summed with non-attributable and non-TSCA exposure (described in Section 6.3.2 and also expressed as IC equivalents) to estimate a cumulative exposure for each occupational subpopulation (expressed as index chemical equivalents).

6.4.2.4 Estimating Cumulative Risk for Workers (Step 10 in Conceptual Model [Figure 2-1])

To estimate cumulative risk for each specific occupational exposure scenario, an MOE (ratio of index chemical POD to occupational cumulative exposure estimate (expressed as IC equivalents) calculated using Equation 6-3) would be calculated for comparison to the benchmark MOE (*i.e.*, the total uncertainty factor associated with the assessment) (described in Section 4.3.2). The lower the MOE (margin between the toxicity effect level and the exposure dose), the more likely a chemical is to pose a risk.

6.4.3 General Population (Fenceline Communities) Exposures and Risk

This section describes EPA's proposed approach for building cumulative exposure scenarios for fenceline communities, who are part of the general population. Generally, an assessment of cumulative exposure and risk for a general population may include components of the proposed approach described for consumers, workers, and fenceline communities as they are all part of the general population. Cumulative exposure to a general population may include exposures from multiple phthalates from TSCA, non-attributable, and non-TSCA sources of exposures which are reasonably combined to determine cumulative risk.

The proposed approach focuses on assessing cumulative exposure and risk for the fenceline communities as there are additional consideration for determining cumulative exposure and risk from single or multiple facility releases. Additionally, fenceline communities may have a higher exposure due to proximity to facilities and may be considered a highly exposed subpopulation within the general population assessment conducted in the individual risk evaluations. Risk evaluations for the phthalates will provide exposure estimates for oral, dermal, and inhalation exposures; these exposure estimates are not available at this time and fenceline communities have not yet been identified as having higher exposures.

As stated previously in Section 5, EPA proposes to focus its CRA for phthalates on groups that may be more susceptible to phthalate syndrome which include pregnant women/women of reproductive age, and male infants, male toddlers, and male children who may be impacted by exposure from TSCA releases,

but the proposed approach will be presented as applicable generally to a fenceline community. This involves the following steps for estimating cumulative risk for fenceline communities as outlined in EPA's conceptual model (Figure 2-1):

- **Step 6. Identifying major pathways of exposure:** Determining the major pathways of exposure from TSCA COUs (see green box in Step 4 of conceptual model (Figure 2-1); completed in individual risk evaluations), non-attributable, and non-TSCA sources. This step would be completed after exposures are estimated for the various pathways of exposure and is dependent on the magnitude of those estimates. Major pathways may vary by relevant population and may also vary by phthalate. Identification of major pathways of exposure to relevant populations may require sensitivity analysis for determining inclusion of a pathway into a cumulative estimate. Description of this process is not detailed in this document as it will be dependent on the identified pathways.
- **Step 7. Determining co-exposure:** Determining likelihood of co-exposure across TSCA COUs, non-attributable sources, and non-TSCA sources (Section 6.4.3.2).
- **Step 8. Convert exposures to IC equivalents:** Phthalate exposure from each individual phthalate is scaled to the potency of an index chemical using RPFs and expressed in units of index chemical equivalents (Section 6.4.3.3).
- **Step 9. Estimating cumulative exposure:** Combining cumulative exposure fenceline communities, the relevant non-attributable cumulative exposure, and the non-TSCA cumulative exposure to estimate cumulative exposure in a reasonable manner (Section 6.4.3.3).
- **Step 10. Estimating cumulative risk:** A cumulative MOE is calculated for comparison to the benchmark MOE (total uncertainty factor associated with the assessment).

EPA's *Draft TSCA Screening Level Approach for Assessing Ambient Air and Water Exposures to Fenceline Communities, Version 1.0*, (U.S. EPA, 2022) [hereinafter referred to as EPA's Draft Fenceline Approach], defines fenceline communities as

> Members of the general population that are in proximity to air emitting facilities or a receiving waterbody, and who therefore may be disproportionately exposed to a chemical undergoing risk evaluation under TSCA section (6). For the air pathway, proximity goes out to 10,000 meters from an air emitting source. For the water pathway, proximity does not refer to a specific distance measured from a receiving waterbody, but rather to those members of the general population that may interact with the receiving waterbody and thus may be exposed.

Fenceline communities may have greater exposure to a chemical from being near an emitting source or interacting with a receiving waterbody. EPA's Draft Fenceline Approach focused on single chemical exposures for fenceline communities (U.S. EPA, 2022). There are unique considerations for estimating cumulative risk to fenceline communities, including the estimation of cumulative environmental releases from facilities and combining exposure across facility releases, COUs, and relevant pathways to estimate cumulative risk. These considerations are discussed in the following sections.

6.4.3.1 Data Needs for General Population/Fenceline Community Exposure Assessment

Data needs for conducting environmental release assessments that are utilized for determining the major pathways of exposure and determining the potential for co-exposure to multiple phthalates to fenceline communities were discussed previously in Sections 6.4.2.1 to 6.4.2.2, as well as Appendix D. Briefly, these data sources may include

- EPA programmatic data (i.*e.*, CDR, TRI, DMR, NEI, and RCRAInfo);
- Monitoring data from other agencies such as NIOSH HHEs and OSHA CEHD;
- Systematically reviewed literature that include EPA generic scenarios (GSs) and emission scenario documents (ESD) to provide general information for a specific industry or COU; and
- Surrogate release data from other COUs, other similar phthalates, or other release sites not included in the potential cumulative release.

6.4.3.2 Co-exposure Resulting from TSCA COUs (Step 7 in Conceptual Model [Figure 2-1])

Fenceline communities may be exposed to more than one high-priority and manufacturer-requested phthalate due to TSCA COUs. This may occur when

- a single facility releases more than one phthalate to the ambient air or receiving waterbodies;
- multiple TSCA facilities in close proximity release more than one phthalate to ambient air or receiving waterbodies; and
- a fenceline community is near one or more facilities releasing phthalates but is also being exposed through consumer or occupational COUs.

These fenceline communities near one or more facilities releasing individual phthalates will be identified in individual risk evaluations. Additionally, fenceline communities may be exposed to the high-priority and manufacturer-requested phthalates through non-attributable and non-TSCA sources (described in Section 6.3.2). Because fenceline communities may be exposed to more than one phthalate undergoing TSCA risk evaluation due to facility releases, non-attributable sources, and non-TSCA sources, EPA is proposing to evaluate fenceline communities for cumulative risk (Figure 2-1). Sections 6.4.3.2.1 to 6.4.3.2.2 describe the process for identifying the fenceline communities that may be near a single facility releasing more than one phthalate or near multiple TSCA facilities releasing one or more phthalates.

6.4.3.2.1 Using Reported Release Data to Determine Co-exposure

EPA programmatic data, including TRI, DMR, NEI, and RCRAInfo, will be utilized to quantify environmental releases to the media listed in Table 6-9. Among the four EPA programs in Table 6-9, there is overlap for each release media. However, release estimates for a given site may vary by program. At each site where there is potential for cumulative release of phthalates, release estimates from each programmatic database will be cataloged for each phthalate. In many instances, the program data will not cover all potential releases for a given site due to the limited coverage of the selected phthalates in these programs (Table 6-7). In these instances, relevant release data from literature sources may be utilized. For COUs where no environmental release or estimation data from literature exists, modeling approaches will be considered as described in Section 6.4.3.2.2.

Table 6-9. Media of Release Covered by EPA Programs

Media of Release Covered in Dataset	EPA Program			
	TRI	DMR	NEI	RCRAInfo
Air (fugitive and stack)	✓	*x*	✓	*x*
Surface Water	✓	✓	*x*	*x*
POTW/WWTP	✓	*x*	*x*	✓
Landfill	✓	*x*	*x*	✓
Incineration	✓	*x*	*x*	✓
Energy Recovery	✓	*x*	*x*	✓
Injection	✓	*x*	*x*	✓
Reclamation/Recycling	✓	*x*	*x*	✓
Other	✓	*x*	*x*	✓
✓ Indicates data available *x* Indicates no data available				

Pertinent site location data such as site address and latitude and longitude, as well as wastewater discharge data including receiving water bodies and publicly owned treatment works (POTWs) or wastewater treatment plants (WWTP), allows for analysis of site proximity.

6.4.3.2.2 Surrogate Release Data for Determining Co-exposure

In the absence of suitable release data, EPA will rely on modeling to estimate cumulative environmental releases. Specifically, EPA will first consider surrogate release data from other COUs, other similar phthalates, or other release sites not included in the potential cumulative release or exposure selection specified in Section 6.4.2.2. Understanding the chemical reaction pathways and use patterns of each phthalate will help to gauge which COUs or phthalate release data is best suited for use as a surrogate. In the absence of suitable surrogate data within the selected phthalates, EPA will consider using surrogate data for other similar phthalates or chemicals from literature sources, if available.

In the absence of surrogate data for cumulative environmental releases, mathematical modeling approaches may be utilized. EPA may incorporate the use of Monte Carlo simulation to vary release calculation input parameters to estimate central tendency and high-end releases. Frequently used literature sources will be used to inform the input parameter distributions.

6.4.3.3 Combining Exposure to General Population (Fenceline Communities) to Estimate Cumulative Risk (Steps 8 and 9 in Conceptual Model [Figure 2-1])

There are multiple ways that EPA may consider combining exposures to estimate phthalate cumulative risk to fenceline communities. These possible approaches are described below.

1. Cumulative Exposure to Air Releases

With phthalate environmental releases estimated via the described methods above, release estimates may be combined to estimate cumulative releases from single sites and adjacent sites. EPA proposes to follow tiered methodologies described in EPA's Draft Fenceline Approach (U.S. EPA, 2022) for estimating ambient air and water concentrations of the high-priority and manufacturer-requested phthalates resulting from facility releases in the individual risk evaluations. In considering combining exposures for cumulative risk, as stated in Section 6.4.3.2 cumulative environmental exposure potential of the six high-priority and manufacturer-requested phthalates can result from multiple phthalates being released at the same site or two different sites that handle more than one phthalate being located adjacent

to one another. If cumulative releases are identified from a single site, ambient air concentrations may be aggregated as appropriate across COUs for a single phthalate and the resulting inhalation risk from individual phthalates may be combined to determine cumulative risk from facility air releases using Equation 6-4.

Equation 6-4. Example estimation of cumulative exposure from single facility releases to air

$$Cumulative\ exposure\ (air)\ (expressed\ as\ index\ chemical\ equivalents) = Phthalate\ A\ inhalation\ exposure\ (facility\ \#1) + Phthalate\ B\ inhalation\ exposure\ (facility\ \#1) + \ldots$$

2. Cumulative Exposure to Surface Water

Similarly, as appropriate, surface water concentrations in a receiving water body resulting from a single facility release may be aggregated across COUs for individual phthalates and the resulting dermal and incidental ingestion risk from individual phthalates may be combined to determine cumulative risk from use of the receiving water body using Equation 6-5.

Equation 6-5. Example estimation of phthalate cumulative exposure from single facility releases to surface water

$$Cumulative\ exposure\ (surface\ water)\ (expressed\ as\ index\ chemical\ equivalents) = Phthalate\ A\ incidental\ ingestion\ (facility\ \#1) + Phthalate\ A\ dermal\ exposure\ (facility\ \#1) + Phthalate\ B\ incidental\ ingestion\ (facility\ \#1) + Phthalate\ B\ incidental\ dermal\ exposure\ (facility\ \#1) + \ldots$$

In Equation 6-4 and Equation 6-5, "Phthalate A" and "Phthalate B" could be any of the six toxicologically similar phthalates under consideration. Because EPA is proposing to use an RPF approach (Section 4.3.3), phthalate exposure from each facility release would be scaled to the potency of an index chemical and expressed as index chemical equivalents, and them summed to estimate cumulative exposure.

3. Cumulative Exposure to Air and Water from Multiple Facilities

For scenarios where multiple sites that handle more than one phthalate are located adjacent to one another, cumulative exposure can be calculated similarly as above using Equation 6-6 for releases to air and Equation 6-7 for releases to surface water.

Equation 6-6. Example estimation of cumulative exposure from multiple facility releases to air

$$Cumulative\ exposure\ (air)\ (expressed\ as\ index\ chemical\ equivalents) = Phthalate\ A\ inhalation\ exposure\ (facility\ \#1) + Phthalate\ B\ inhalation\ exposure\ (facility\ \#2) + \ldots$$

Equation 6-7. Example estimation of cumulative exposure from multiple facility releases to surface water

$$Cumulative\ exposure\ (surface\ water)\ (expressed\ as\ index\ chemical\ equivalents) = Phthalate\ A\ incidental\ ingestion\ (facility\ \#1) + Phthalate\ A\ dermal\ exposure\ (facility\ \#1) + Phthalate\ B\ incidental\ ingestion\ (facility\ \#2) + Phthalate\ B\ incidental\ dermal\ exposure\ (facility\ \#2) + \ldots$$

There are unique challenges associated with estimating cumulative risk from exposure to more than one high-priority and manufacturer-requested phthalate for the drinking water pathway. For example, concentrations at the point of actual drinking water intake are difficult to estimate because of the

potential for transport, dilution, and treatment of drinking water from a given distance away from the receiving waterbody. Depending on the available data and methods, cumulative risk from drinking water attributable to TSCA releases may be included as appropriate. Drinking water concentrations may also be included as a non-attributable exposure if it is not able to be attributed to a TSCA COU.

4. Cumulative Exposure to Fenceline Communities Who Are Not Consumers or Workers

Because non-attributable and non-TSCA risk as described in Section 6.3.2, may include exposure pathways such as ambient air, drinking water, and surface water, that may lead to exposures potentially not as high as those for fenceline communities estimated using Equation 6-2, EPA may not utilize the same non-attributable and non-TSCA risk used for consumers or workers. Instead, EPA may consider major exposure pathways to relevant phthalates separately for fenceline communities. Determining reasonable cumulative exposure scenarios may involve considering the likelihood of co-exposure, the possibility of double counting, and of over- or under-estimating exposures. This may mean that the intake estimates for ambient air, drinking water, and surface water which are determined based on facility releases for fenceline communities are combined with risk from other major relevant exposure pathways comprising a unique non-attributable and non-TSCA risk for fenceline communities which does not already include ambient air, drinking water, and surface water, but does include major identified pathways that may include dust (non-attributable) and diet (non-TSCA) to help avoid double counting as shown in Equation 6-8. As stated previously, estimates of cumulative exposure for different lifestages may differ based on exposure factors and interaction with different sources of exposure leading to potentially different estimates in all exposure categories with some phthalates being more or less impactful for different lifestages.

Equation 6-8. Example estimation of cumulative exposure to fenceline populations who are not consumers or workers

Cumulative exposure to fenceline subpopulations (expressed as index chemical equivalents) = Non-attributable exposure (not including ambient air and surface water) + Non-TSCA exposure + Cumulative facility exposure (including ambient air and surface water)

5. Cumulative Exposure to Fenceline Communities Who May Also Be Consumers and Workers

Additionally, individuals who are part of the fenceline communities may be consumers and workers living near the facilities. For these instances, additional combinations of exposure should be considered. For example, cumulative exposure for an individual living near a TSCA facility who is also a consumer of TSCA COUs and works in a facility handling TSCA COUs, would require consideration of exposures from facility releases near the home, workplace exposure over the 8-hour workday, and exposure from the use of consumer products at the home. EPA proposes that for these individuals, cumulative exposure could include the cumulative occupational exposure to TSCA COUs as discussed in Section 6.4.2, exposure from consumer TSCA COUs as discussed in Section 6.4.1, and the estimated cumulative exposure to fenceline populations presented in Equation 6-8 to determine an estimated cumulative exposure to fenceline populations who are also consumers and workers as shown in Equation 6-9.

Equation 6-9. Example estimation of cumulative exposure to fenceline populations who are also consumers and workers

Cumulative exposure to fenceline/consumer/occupational (expressed as index chemical equivalents) = Non-attributable exposure (not including ambient air and surface water) + Non-TSCA exposure + Cumulative facility exposure (including ambient air and surface water)+ Cumulative occupational exposure + Consumer COU exposure

Combining exposures for these populations may require additional data or evidence not already covered in this document to determine the major pathways that contribute to cumulative exposure (Step 6 in conceptual model) to those individuals and support the likelihood of co-exposure to multiple phthalates from the various pathways of exposure (Step 7 in conceptual model). Based on the outlined approaches for the various populations (*i.e.*, consumers [Section 6.4.1], workers [Section 6.4.2], fenceline communities [Section 6.4.3]), reasonable combinations of exposure may be considered, as data allows.

EPA has not identified a proposed methodology, data sources, or lines of evidence to fully develop the cumulative fenceline assessment. In the absence of data or evidence, assumptions may be necessary to determine reasonable combinations of exposure for identified populations, which involve considering the likelihood of co-exposure, the possibility of double counting, and of over- or under-estimating exposures. EPA will be soliciting comments from the SACC and the public on this issue.

7 SUMMARY OF PROPOSED APPROACH AND NEXT STEPS

This document describes EPA's proposed approach for assessing high-priority and manufacturer-requested phthalates for cumulative risk to human health under TSCA. This document was prepared based on the principles of CRA described in EPA's Draft Proposed Principles of CRA Under TSCA. As discussed in Section 2, there are two primary considerations for grouping chemical substances for inclusion in a CRA, including (1) toxicologic similarity and (2) evidence of co-exposure over a relevant timeframe. To determine which high-priority and manufacturer-requested phthalates are toxicologically similar, EPA reviewed data for seven key outcomes associated with phthalate syndrome (*i.e.*, fetal testicular gene expression and testosterone, decreased AGD, NR, hypospadias, seminiferous tubule atrophy, and MNG formation). Based on the weight of evidence, EPA proposes that DEHP, BBP, DBP, DIBP, DCHP, and DINP, but not DIDP, are toxicologically similar and induce effects on the developing male reproductive system consistent with phthalate syndrome (Section 3.1.7). To determine if the U.S. population is co-exposed to multiple phthalates EPA reviewed NHANES urinary biomonitoring data and TSCA industrial, commercial and consumer use data (Section 3.2). Available biomonitoring data demonstrate that the U.S. population is co-exposed to multiple phthalates, including DEHP, BBP, DBP, DIBP, DINP, and DIDP, while co-exposure to DCHP is anticipated to occur through various industrial, commercial and consumer uses under TSCA. These data qualitatively demonstrate that humans are co-exposed to DEHP, BBP, DBP, DIBP, DCHP, DINP, and DIDP. EPA's proposed approach for quantifying phthalate co-exposure is outlined in Section 6. Based on evidence of toxicologic similarity and co-exposure, EPA is proposing to group DEHP, BBP, DBP, DIBP, DCHP and DINP for CRA under TSCA (Step 1 in conceptual model [Figure 2-1]).

As discussed in Section 4.1, NRC presented two options for assessing the risks of phthalate syndrome following exposures to phthalates, including assessing the syndrome as a whole and focusing on the most sensitive effect associated with the syndrome (NRC, 2008). EPA identified a number of challenges associated with addressing phthalate syndrome as a whole and therefore EPA is proposing to address phthalate syndrome by focusing on the most sensitive effect (Section 4.1.3).

As described in EPA's Draft Proposed Principles of CRA under TSCA and in Section 4.2 of this document, several additivity approaches can be used to assess multiple chemical substances for cumulative risk to human health—including dose addition, response addition, and integrated addition as well as approaches that account for toxicologic interactions (U.S. EPA, 2000, 1986). EPA is proposing to rely upon a default assumption of dose addition when conducting CRAs for toxicologically similar chemicals under TSCA. As described in Section 3.1.7, EPA considers there to be sufficient evidence to conclude that DEHP, BBP, DBP, DIBP, DCHP, and DINP are toxicologically similar. Therefore, EPA is proposing to assess these six phthalate for cumulative risk to human health under an assumption of dose addition. In further support of this, EPA identified multiple *in vivo* phthalate mixture studies that provide empirical evidence to support the use of dose additivity models and EPA's proposal to use dose addition is consistent with the recommendations of the NRC (2008).

EPA is considering the applicability of two component-based, dose additive approaches, including the HI and RPF approaches. Based on currently available data, EPA considers there to be sufficient data available to support RPF derivation for the six toxicologically similar TCSA phthalates (Section 4.3.3). To support RPF derivation, EPA considered the strengths and uncertainties associated with the dataset for each of the seven evaluated key outcomes (Section 4.4.1). Given the strengths and uncertainties associated with the datasets for each key outcome, EPA is proposing several options to derive RPFs based on gestational (*i.e.*, reduced fetal testicular testosterone content and reduced testicular steroidogenic gene expression) and postnatal outcomes (*i.e.*, reduced AGD, NR, seminiferous tubule atrophy, and hypospadias) (Section 4.4.2).

EPA will conduct consumer, occupational, and general population exposure assessments for each individual phthalate risk evaluation. The key human populations considered in these exposure assessments include consumers, workers, and the general population, including fenceline communities. Within these populations, there are susceptible subpopulations with greater susceptibility to phthalate syndrome based on lifestages, including pregnant women, women of reproductive age, and male infants, toddlers, and children (Step 2 in conceptual model [Figure 2-1]). These groups are the focus of EPA's CRA (Section 5).

To estimate cumulative exposure for subpopulations with increased susceptibility to phthalate syndrome based on lifestages, EPA is proposing to consider exposures resulting from TSCA COUs (Section 6.2), as well as non-attributable and non-TSCA exposures (Section 6.3.2). Prior to the development of the phthalate CRA, exposure scenarios for TSCA COUs will be completed in individual phthalate risk evaluations (Steps 3 to 4 in conceptual model). EPA is proposing to include non-attributable and non-TSCA exposures as part of the phthalate CRA because certain non-attributable (*e.g.*, dust) and non-TSCA (*e.g.*, dietary) pathways are anticipated to be major contributors to phthalate exposure that contribute to cumulative risk (Section 6.2.2).

EPA is considering two approaches for estimating non-attributable and non-TSCA phthalate exposure, including a scenario-based approach (Section 6.3.2.1) and a reverse dosimetry based approach (Section 6.3.2.2). The scenario-based approach involves estimating non-attributable and non-TSCA exposure to populations of interest based on the concentrations of phthalates in various media, food, and other sources using population specific exposure factors (*e.g.*, inhalation rate, dietary intake, body weight, etc.) (Section 6.3.2.1).The reverse dosimetry approach involves estimating aggregate exposure for each individual phthalate from human urinary biomonitoring data for metabolites unique to each individual parent phthalate as reported in nationally representative datasets, such as NHANES (Section 6.3.2.2). Because the reverse dosimetry approach does not distinguish between routes or pathways of exposure and does not allow for source apportionment, it provides an estimate of total non-attributable phthalate exposure (Section 6.3.2.2). As described in Section 6.3.2.5, EPA is proposing to estimate non-attributable and non-TSCA exposure for DEHP, BBP, DBP, DIBP, DCHP, and DINP from major exposure pathways using a scenario-based approach (Step 5 in conceptual model), while the reverse dosimetry approach, which does not allow for source apportionment, may be used to help characterize phthalate exposure and serve as a comparator for scenario-based intake estimates.

As shown in EPA's draft conceptual model (Figure 2-1), EPA is proposing to assess consumers (Section 6.4.1), workers (Section 6.4.2), and general population/fenceline communities (Section 6.4.3) for cumulative risk from exposure to DEHP, BBP, DBP, DIBP, DCHP, and DINP through TSCA COUs. EPA proposes to identify major pathways of exposure and likelihood of co-exposure to multiple phthalates through various pathways for combining to estimate cumulative exposure to identified susceptible subpopulations based on lifestages (Steps 6 to 7 in conceptual model). To estimate cumulative exposure to consumers (Section 6.4.16.4), EPA proposes to combine the non-attributable and non-TSCA exposure with exposure from individual consumer COUs. To estimate cumulative exposure to workers (Section 6.4.2), EPA proposes to combine the non-attributable and non-TSCA exposure with cumulative occupational exposure from TSCA COUs in a work setting. For cumulative exposure to fenceline communities (Section 6.4.3), EPA proposes estimating cumulative exposures from single or multiple facility releases to ambient air and/or water and combining with non-attributable and non-TSCA exposures. Because EPA is proposing to use an RPF approach (Section 4.3.3), exposure from individual phthalates for each exposure scenario will be scaled to the potency of an index chemical and expressed as index chemical equivalents (Step 8 in conceptual model), which will then be summed to estimate cumulative exposure for each exposure scenario (Step 9 in conceptual model). Cumulative risk

may then be estimated using a margin of exposure (MOE) approach (Section 4.3.3) (Step 10 in conceptual model).

EPA is soliciting comments from the SACC on charge questions and comments from the public for the SACC meeting scheduled on May 8–11, 2023.

REFERENCES

ACC HPP. (2019a). Manufacturer request for risk evaluation Di-isodecyl Phthalate (DIDP). American Chemistry Council High Phthalates Panel.

ACC HPP. (2019b). Manufacturer Request for Risk Evaluation Di-isononyl Phthalate (DINP). (730R19001). American Chemistry Council High Phthalates Panel :: ACC HPP https://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P100YEGF.txt

Adamsson, A; Salonen, V; Paranko, J; Toppari, J. (2009). Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) on steroidogenesis in the fetal rat testis and adrenal gland. Reprod Toxicol 28: 66-74. http://dx.doi.org/10.1016/j.reprotox.2009.03.002

Adham, IM; Emmen, JM; Engel, W. (2000). The role of the testicular factor INSL3 in establishing the gonadal position [Review]. Mol Cell Endocrinol 160: 11-16. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10328887

Ahbab, MA; Barlas, N. (2015). Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalate on fetal testicular development in rats. Toxicol Lett 233: 125-137. http://dx.doi.org/10.1016/j.toxlet.2015.01.015

Ahmad, R; Gautam, AK; Verma, Y; Sedha, S; Kumar, S. (2014). Effects of in utero di-butyl phthalate and butyl benzyl phthalate exposure on offspring development and male reproduction of rat. Environ Sci Pollut Res Int 21: 3156-3165. http://dx.doi.org/10.1007/s11356-013-2281-x

Albert, O; Jégou, B. (2014). A critical assessment of the endocrine susceptibility of the human testis to phthalates from fetal life to adulthood [Review]. Hum Reprod Update 20: 231-249. http://dx.doi.org/10.1093/humupd/dmt050

Anderson, WA; Castle, L; Hird, S; Jeffery, J; Scotter, MJ. (2011). A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. Food Chem Toxicol 49: 2022-2029. http://dx.doi.org/10.1016/j.fct.2011.05.013

Anderson, WAC; Castle, L; Scotter, MJ; Massey, RC; Springall, C. (2001). A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Addit Contam 18: 1068-1074. http://dx.doi.org/10.1080/02652030110050113

Andrade, AJ; Grande, SW; Talsness, CE; Gericke, C; Grote, K; Golombiewski, A; Sterner-Kock, A; Chahoud, I. (2006a). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228: 85-97. http://dx.doi.org/10.1016/j.tox.2006.08.020

Andrade, AJ; Grande, SW; Talsness, CE; Grote, K; Golombiewski, A; Sterner-Kock, A; Chahoud, I. (2006b). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225: 64-74. http://dx.doi.org/10.1016/j.tox.2006.05.007

Arbuckle, TE; Davis, K; Marro, L; Fisher, M; Legrand, M; Leblanc, A; Gaudreau, E; Foster, WG; Choeurng, V; Fraser, WD. (2014). Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environ Int 68: 55-65. http://dx.doi.org/10.1016/j.envint.2014.02.010

Arbuckle, TE; Fisher, M; Macpherson, S; Lang, C; Provencher, G; Leblanc, A; Hauser, R; Feeley, M; Ayotte, P; Neisa, A; Ramsay, T; Tawagi, G. (2016). Maternal and early life exposure to phthalates: The Plastics and Personal-care Products use in Pregnancy (P4) study [Supplemental Data]. Sci Total Environ 551-552: 344-356. http://dx.doi.org/10.1016/j.scitotenv.2016.02.022

Arzuaga, X; Walker, T; Yost, EE; Radke, EG; Hotchkiss, AK. (2020). Use of the Adverse Outcome Pathway (AOP) framework to evaluate species concordance and human relevance of Dibutyl phthalate (DBP)-induced male reproductive toxicity. Reprod Toxicol 96: 445-458.

Ashley-Martin, J; Dodds, L; Arbuckle, TE; Lanphear, B; Muckle, G; Foster, WG; Ayotte, P; Zidek, A; Asztalos, E; Bouchard, MF; Kuhle, S. (2021). Urinary phthalates and body mass index in preschool children: The MIREC Child Development Plus study. Int J Hyg Environ Health 232: 113689. http://dx.doi.org/10.1016/j.ijheh.2021.113689

Aso, S; Ehara, H; Miyata, K; Hosyuyama, S; Shiraishi, K; Umano, T; Minobe, Y. (2005). A two-generation reproductive toxicity study of butyl benzyl phthalate in rats. J Toxicol Sci 30: S39-58. http://dx.doi.org/10.2131/jts.30.S39

ATSDR. (2022). Toxicological profile for di(2-ethylhexyl)phthalate (DEHP) [ATSDR Tox Profile]. (CS274127-A). Atlanta, GA. https://www.atsdr.cdc.gov/ToxProfiles/tp9.pdf

Aylward, LL; Hays, SM; Zidek, A. (2016). Variation in urinary spot sample, 24 h samples, and longer-term average urinary concentrations of short-lived environmental chemicals: implications for exposure assessment and reverse dosimetry. J Expo Sci Environ Epidemiol 27: 582-590. http://dx.doi.org/10.1038/jes.2016.54

Barlow, NJ; McIntyre, BS; Foster, PM. (2004). Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di(n-butyl) phthalate. Toxicol Pathol 32: 79-90. http://dx.doi.org/10.1080/01926230490265894

Beverly, BEJ; Lambright, CS; Furr, JR; Sampson, H; Wilson, VS; McIntyre, BS; Foster, PMD; Travlos, G; Gray, LE, Jr. (2014). Simvastatin and Dipentyl Phthalate Lower Ex Vivo Testicular Testosterone Production and Exhibit Additive Effects on Testicular Testosterone and Gene Expression Via Distinct Mechanistic Pathways in the Fetal Rat. Toxicol Sci 141: 524-537. http://dx.doi.org/10.1093/toxsci/kfu149

Biesterbos, JW; Dudzina, T; Delmaar, CJ; Bakker, MI; Russel, FG; von Goetz, N; Scheepers, PT; Roeleveld, N. (2013). Usage patterns of personal care products: important factors for exposure assessment. Food Chem Toxicol 55: 8-17. http://dx.doi.org/10.1016/j.fct.2012.11.014

Birkhøj, M; Nellemann, C; Jarfelt, K; Jacobsen, H; Andersen, HR; Dalgaard, M; Vinggaard, AM. (2004). The combined antiandrogenic effects of five commonly used pesticides. Toxicol Appl Pharmacol 201: 10-20. http://dx.doi.org/10.1016/j.taap.2004.04.016

Blessinger, TD; Euling, SY; Wang, L; Hogan, KA; Cai, C; Klinefelter, G; Saillenfait, AM. (2020). Ordinal dose-response modeling approach for the phthalate syndrome. Environ Int 134: 105287. http://dx.doi.org/10.1016/j.envint.2019.105287

Blystone, CR; Kissling, GE; Bishop, JB; Chapin, RE; Wolfe, GW; Foster, PM. (2010). Determination of the di-(2-ethylhexyl) phthalate NOAEL for reproductive development in the rat: importance of the retention of extra animals to adulthood. Toxicol Sci 116: 640-646. http://dx.doi.org/10.1093/toxsci/kfq147

Boberg, J; Christiansen, S; Axelstad, M; Kledal, TS; Vinggaard, AM; Dalgaard, M; Nellemann, C; Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol 31: 200-209. http://dx.doi.org/10.1016/j.reprotox.2010.11.001

Boberg, J; Metzdorff, S; Wortziger, R; Axelstad, M; Brokken, L; Vinggaard, AM; Dalgaard, M; Nellemann, C. (2008). Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. Toxicology 250: 75-81. http://dx.doi.org/10.1016/j.tox.2008.05.020

Boekelheide, K; Kleymenova, E; Liu, K; Swanson, C; Gaido, KW. (2009). Dose-dependent effects on cell proliferation, seminiferous tubules, and male germ cells in the fetal rat testis following exposure to di(n-butyl) phthalate. Microsc Res Tech 72: 629-638. http://dx.doi.org/10.1002/jemt.20684

Borch, J; Axelstad, M; Vinggaard, AM; Dalgaard, M. (2006a). Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis. Toxicol Lett 163: 183-190. http://dx.doi.org/10.1016/j.toxlet.2005.10.020

Borch, J; Ladefoged, O; Hass, U; Vinggaard, AM. (2004). Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. Reprod Toxicol 18: 53-61. http://dx.doi.org/10.1016/j.reprotox.2003.10.011

Borch, J; Metzdorff, SB; Vinggaard, AM; Brokken, L; Dalgaard, M. (2006b). Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. Toxicology 223: 144-155. http://dx.doi.org/10.1016/j.tox.2006.03.015

Bradley, EL; Burden, RA; Bentayeb, K; Driffield, M; Harmer, N; Mortimer, DN; Speck, DR; Ticha, J; Castle, L. (2013). Exposure to phthalic acid, phthalate diesters and phthalate monoesters from foodstuffs: UK total diet study results. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 30: 735-742. http://dx.doi.org/10.1080/19440049.2013.781684

Carruthers, CM; Foster, PMD. (2005). Critical window of male reproductive tract development in rats following gestational exposure to di-n-butyl phthalate. Birth Defects Res B Dev Reprod Toxicol 74: 277-285. http://dx.doi.org/10.1002/bdrb.20050

CDC. (2013a). Fourth national report on human exposure to environmental chemicals, updated tables, September 2013. (CS244702-A). Atlanta, GA. http://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Sep2013.pdf

CDC. (2013b). National Health and Nutrition Examination Survey: Sample design, 2007-2010. In Vital and Health Statistics: Series 2, Number 160. Atlanta, GA: U.S. Department of Health and Human Services.

Christiansen, S; Boberg, J; Axelstad, M; Dalgaard, M; Vinggaard, A; Metzdorff, S; Hass, U. (2010). Low-dose perinatal exposure to di(2-ethylhexyl) phthalate induces anti-androgenic effects in male rats. Reprod Toxicol 30: 313-321. http://dx.doi.org/10.1016/j.reprotox.2010.04.005

Christiansen, S; Scholze, M; Dalgaard, M; Vinggaard, AM; Axelstad, M; Kortenkamp, A; Hass, U. (2009). Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ Health Perspect 117: 1839-1846. http://dx.doi.org/10.1289/ehp.0900689

Clark, KE; David, RM; Guinn, R; Kramarz, KW; Lampi, MA; Staples, CA. (2011). Modeling human exposure to phthalate esters: A comparison of indirect and biomonitoring estimation methods. Hum Ecol Risk Assess 17: 923-965. http://dx.doi.org/10.1080/10807039.2011.588157

Clewell, RA; Campbell, JL; Ross, SM; Gaido, KW; Clewell Hj, III; Andersen, ME. (2010). Assessing the relevance of in vitro measures of phthalate inhibition of steroidogenesis for in vivo response. Toxicol In Vitro 24: 327-334. http://dx.doi.org/10.1016/j.tiv.2009.08.003

Clewell, RA; Sochaski, M; Edwards, K; Creasy, DM; Willson, G; Andersen, ME. (2013a). Disposition of diisononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats. Reprod Toxicol 35: 56–69. http://dx.doi.org/10.1016/j.reprotox.2012.07.001

Clewell, RA; Thomas, A; Willson, G; Creasy, DM; Andersen, ME. (2013b). A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. Reprod Toxicol 35: 70-80. http://dx.doi.org/10.1016/j.reprotox.2012.07.008

Conley, JM; Lambright, CS; Evans, N; Cardon, M; Furr, J; Wilson, VS; Gray, LE. (2018). Mixed "Antiandrogenic" Chemicals at Low Individual Doses Produce Reproductive Tract Malformations in the Male Rat. Toxicol Sci 164: 166-178. http://dx.doi.org/10.1093/toxsci/kfy069

Conley, JM; Lambright, CS; Evans, N; Cardon, M; Medlock-Kakaley, E; Wilson, VS; Gray, LE. (2021). A mixture of 15 phthalates and pesticides below individual chemical no observed adverse effect levels (NOAELs) produces reproductive tract malformations in the male rat. Environ Int 156: 106615. http://dx.doi.org/10.1016/j.envint.2021.106615

Corning Hazleton Inc. (1996). Initial submission: Oncogenicity study in rats of di(2-ethylhexyl) phthalate including hepatocellular proliferation and biochemical analysis, w/TSCA cover sheet dated 11/18/96 [TSCA Submission]. (Lab Study ID: CHV 663-134. OTS0558677 88970000055. 8EHQ-1196-13805. TSCATS/444561). Kingsport, TN: Eastman Kodak.

Culty, M; Thuillier, R; Li, W; Wang, Y; Martinez-Arguelles, D; Benjamin, C; Triantafilou, K; Zirkin, B; Papadopoulos, V. (2008). In utero exposure to di-(2-ethylhexyl) phthalate exerts both short-term and long-lasting suppressive effects on testosterone production in the rat. Biol Reprod 78: 1018-1028. http://dx.doi.org/10.1095/biolreprod.107.065649

David, RM. (2000). Exposure to phthalate esters [Letter]. Environ Health Perspect 108: A440. http://dx.doi.org/10.1289/ehp.108-a440a

Do, RP; Stahlhut, RW; Ponzi, D; Vom Saal, FS; Taylor, JA. (2012). Non-monotonic dose effects of in utero exposure to di(2-ethylhexyl) phthalate (DEHP) on testicular and serum testosterone and anogenital distance in male mouse fetuses. Reprod Toxicol 34: 614-621. http://dx.doi.org/10.1016/j.reprotox.2012.09.006

Dostal, LA; Chapin, RE; Stefanski, SA; Harris, MW; Schwetz, BA. (1988). Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl) phthalate and the recovery of fertility as adults. Toxicol Appl Pharmacol 95: 104-121. http://dx.doi.org/10.1016/S0041-008X(88)80012-7

Drake, AJ; van den Driesche, S; Scott, HM; Hutchison, GR; Seckl, JR; Sharpe, RM. (2009). Glucocorticoids amplify dibutyl phthalate-induced disruption of testosterone production and male reproductive development. Endocrinology 150: 5055-5064. http://dx.doi.org/10.1210/en.2009-0700

EC/HC. (2015a). Proposed approach for cumulative risk assessment of certain phthalates under the Chemicals Management Plan. Gatineau, Quebec: Environment Canada, Health Canada.

EC/HC. (2015b). State of the science report: Phthalate substance grouping 1,2-Benzenedicarboxylic acid, diisononyl ester; 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (Diisononyl Phthalate; DINP). Chemical Abstracts Service Registry Numbers: 28553-12-0 and 68515-48-0. Gatineau, Quebec. https://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=47F58AA5-1

EC/HC. (2015c). State of the science report: Phthalate substance grouping: Medium-chain phthalate esters: Chemical Abstracts Service Registry Numbers: 84-61-7; 84-64-0; 84-69-5; 523-31-9; 5334-09-8;16883-83-3; 27215-22-1; 27987-25-3; 68515-40-2; 71888-89-6. Gatineau, Quebec: Environment Canada, Health Canada. https://www.ec.gc.ca/ese-ees/4D845198-761D-428B-A519-75481B25B3E5/SoS_Phthalates%20%28Medium-chain%29_EN.pdf

EC/HC. (2015d). State of the science report: Phthalate substance grouping: Short-chain phthalate esters: 1,2-Benzenedicarboxylic acid, dimethyl ester (DMP). Chemical Abstracts Service Registry Number: 131-11-3. Gatineau, Quebec: Environment Canada, Health Canada. https://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=51624E94-1

EC/HC. (2015e). State of the science report: Phthalates substance grouping: Long-chain phthalate esters. 1,2-Benzenedicarboxylic acid, diisodecyl ester (diisodecyl phthalate; DIDP) and 1,2-Benzenedicarboxylic acid, diundecyl ester (diundecyl phthalate; DUP). Chemical Abstracts Service Registry Numbers: 26761-40-0, 68515-49-1; 3648-20-2. Gatineau, Quebec: Environment Canada, Health Canada. https://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=D3FB0F30-1

ECCC/HC. (2020). Screening assessment - Phthalate substance grouping. (En14-393/2019E-PDF). Environment and Climate Change Canada, Health Canada. https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/screening-assessment-phthalate-substance-grouping.html

ECHA. (2011). 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). Copenhagen, Denmark: Danish Environmental Protection Agency :: Danish EPA. https://echa.europa.eu/documents/10162/c6781e1e-1128-45c2-bf48-8890876fa719

ECHA. (2013). Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006. Helsinki, Finland. http://echa.europa.eu/documents/10162/31b4067e-de40-4044-93e8-9c9ff1960715

ECHA. (2014). Committee for Risk Assessment RAC Opinion proposing harmonised classification and labelling at EU level of Dicyclohexyl phthalate, EC number: 201-545-9, CAS number: 84-61-7. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10328890

ECHA. (2017). Annex to the Background document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP). (ECHA/RAC/RES-O-0000001412-86-140/F; ECHA/SEAC/RES-O-0000001412-86-154/F). https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10328892

ECJRC. (2003). European Union risk assessment report, vol 36: 1,2-Benzenedicarboxylic acid, Di-C9-11-Branched alkyl esters, C10-Rich and Di-"isodecyl"phthalate (DIDP). In 2nd Priority List. (EUR 20785 EN). Luxembourg, Belgium: Office for Official Publications of the European Communities. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC25825/EUR%2020785%20EN.pdf

EFSA. (2019). Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials. EFSA J 17: ee05838. http://dx.doi.org/10.2903/j.efsa.2019.5838

Ema, M; Miyawaki, E; Hirose, A; Kamata, E. (2003). Decreased anogenital distance and increased incidence of undescended testes in fetuses of rats given monobenzyl phthalate, a major metabolite of butyl benzyl phthalate. Reprod Toxicol 17: 407-412. http://dx.doi.org/10.1016/S0890-6238(03)00037-6

Ema, M; Miyawaki, E; Kawashima, K. (1998). Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. Toxicol Lett 98: 87-93. http://dx.doi.org/10.1016/S0378-4274(98)00107-6

FDA. (2022). Total diet study report: Fiscal years 2018-2020 elements data. Washington, DC. https://www.fda.gov/food/fda-total-diet-study-tds/fda-total-diet-study-tds-results

Ferrara, D; Hallmark, N; Scott, H; Brown, R; McKinnell, C; Mahood, IK; Sharpe, RM. (2006). Acute and long-term effects of in utero exposure of rats to di(n-butyl) phthalate on testicular germ cell development and proliferation. Endocrinology 147: 5352-5362. http://dx.doi.org/10.1210/en.2006-0527

Foster, PMD. (2005). Mode of action: Impaired fetal Leydig cell function - Effects on male reproductive development produced by certain phthalate esters [Review]. Crit Rev Toxicol 35: 713-719. http://dx.doi.org/10.1080/10408440591007395

Foster, PMD; Mylchreest, E; Gaido, KW; Sar, M. (2001). Effects of phthalate esters on the developing reproductive tract of male rats [Review]. Hum Reprod Update 7: 231-235. http://dx.doi.org/10.1093/humupd/7.3.231

Furr, JR; Lambright, CS; Wilson, VS; Foster, PM; Gray, LE, Jr. (2014). A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. Toxicol Sci 140: 403-424. http://dx.doi.org/10.1093/toxsci/kfu081

Gaido, KW; Hensley, JB; Liu, D; Wallace, DG; Borghoff, S; Johnson, KJ; Hall, SJ; Boekelheide, K. (2007). Fetal mouse phthalate exposure shows that gonocyte multinucleation is not associated

with decreased testicular testosterone. Toxicol Sci 97: 491-503. http://dx.doi.org/10.1093/toxsci/kfm049

Graham, PR. (1973). Phthalate ester plasticizers-why and how they are used. Environ Health Perspect 3: 3-12. http://dx.doi.org/10.1289/ehp.73033

Grande, SW; Andrade, AJ; Talsness, CE; Grote, K; Chahoud, I. (2006). A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicol Sci 91: 247-254. http://dx.doi.org/10.1093/toxsci/kfj128

Gray, L; Barlow, N; Howdeshell, K; Ostby, J; Furr, J; Gray, C. (2009). Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: added value of assessing multiple offspring per litter. Toxicol Sci 110: 411-425. http://dx.doi.org/10.1093/toxsci/kfp109

Gray, LE, Jr.; Lambright, CS; Conley, JM; Evans, N; Furr, JR; Hannas, BR; Wilson, VS; Sampson, H; Foster, PMD. (2021). Genomic and Hormonal Biomarkers of Phthalate-Induced Male Rat Reproductive Developmental Toxicity Part II: A Targeted RT-qPCR Array Approach That Defines a Unique Adverse Outcome Pathway. Toxicol Sci 182: 195-214. http://dx.doi.org/10.1093/toxsci/kfab053

Gray, LE, Jr.; Ostby, J; Furr, J; Price, M; Veeramachaneni, DNR; Parks, L. (2000). Perinatal exposure to the phthalates DEHP, BBP, and DNIP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58: 350-365. http://dx.doi.org/10.1093/toxsci/58.2.350

Gray, TJB; Rowland, IR; Foster, PMD; Gangolli, SD. (1982). Species differences in the testicular toxicity of phthalate esters. Toxicol Lett 11: 141-147. http://dx.doi.org/10.1016/0378-4274(82)90119-9

Habert, R; Muczynski, V; Grisin, T; Moison, D; Messiaen, S; Frydman, R; Benachi, A; Delbes, G; Lambrot, R; Lehraiki, A; N'Tumba-Byn, T; Guerquin, MJ; Levacher, C; Rouiller-Fabre, V; Livera, G. (2014). Concerns about the widespread use of rodent models for human risk assessments of endocrine disruptors [Review]. Reproduction 147: R119-R129. http://dx.doi.org/10.1530/REP-13-0497

Hallmark, N; Walker, M; McKinnell, C; Mahood, IK; Scott, H; Bayne, R; Coutts, S; Anderson, RA; Greig, I; Morris, K; Sharpe, RM. (2007). Effects of monobutyl and di(n-butyl) phthalate in vitro on steroidogenesis and Leydig cell aggregation in fetal testis explants from the rat: Comparison with effects in vivo in the fetal rat and neonatal marmoset and in vitro in the human. Environ Health Perspect 115: 390-396. http://dx.doi.org/10.1289/ehp.9490

Han, Eu; Choi, K; Sim, S; Choi, J; Uhm, Y; Kim, S; Lim, E; Lee, Y. (2020). Patterns of household and personal care product use by the Korean population: implications for aggregate human exposure and health risk. Environ Sci Eur. http://dx.doi.org/10.1186/s12302-020-00417-3

Hannas, BR; Lambright, CS; Furr, J; Evans, N; Foster, PMD; Gray, EL; Wilson, VS. (2012). Genomic biomarkers of phthalate-induced male reproductive developmental toxicity: A targeted RT-PCR array approach for defining relative potency. Toxicol Sci 125: 544-557. http://dx.doi.org/10.1093/toxsci/kfr315

Hannas, BR; Lambright, CS; Furr, J; Howdeshell, KL; Wilson, VS; Gray, LE. (2011). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicol Sci 123: 206-216. http://dx.doi.org/10.1093/toxsci/kfr146

Hass, U; Scholze, M; Christiansen, S; Dalgaard, M; Vinggaard, AM; Axelstad, M; Metzdorff, SB; Kortenkamp, A. (2007). Combined exposure to anti-androgens exacerbates disruption of sexual differentiation in the rat. Environ Health Perspect 115: 122-128. http://dx.doi.org/10.1289/ehp.9360

Health Canada. (2015). Stakeholder Technical Workshop Document: Approach for using chemical categories and read-across to address data gaps for effects on the developing male reproductive

system: Phthalate Substance Grouping. Ottawa, ON. http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=0FB5F508-1

Heger, NE; Hall, SJ; Sandrof, MA; McDonnell, EV; Hensley, JB; McDowell, EN; Martin, KA; Gaido, KW; Johnson, KJ; Boekelheide, K. (2012). Human fetal testis xenografts are resistant to phthalate-induced endocrine disruption. Environ Health Perspect 120: 1137-1143. http://dx.doi.org/10.1289/ehp.1104711

Hellwig, J; Freudenberger, H; Jäckh, R. (1997). Differential prenatal toxicity of branched phthalate esters in rats. Food Chem Toxicol 35: 501-512. http://dx.doi.org/10.1016/S0278-6915(97)00008-2

Higuchi, TT; Palmer, JS; Gray, LE, Jr.; Veeramachaneni, DN. (2003). Effects of dibutyl phthalate in male rabbits following in utero, adolescent, or postpubertal exposure. Toxicol Sci 72: 301-313. http://dx.doi.org/10.1093/toxsci/kfg036

Hoshino, N; Iwai, M; Okazaki, Y. (2005). A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. J Toxicol Sci 30: 79-96. http://dx.doi.org/10.2131/jts.30.s79

Hotchkiss, AK; Parks-Saldutti, LG; Ostby, JS; Lambright, C; Furr, J; Vandenbergh, JG; Gray, LE, Jr. (2004). A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion. Biol Reprod 71: 1852-1861. http://dx.doi.org/10.1095/biolreprod.104.031674

Hotchkiss, AK; Rider, CV; Furr, J; Howdeshell, KL; Blystone, CR; Wilson, VS; Gray, LE. (2010). In utero exposure to an AR antagonist plus an inhibitor of fetal testosterone synthesis induces cumulative effects on F1 male rats. Reprod Toxicol 30: 261-270. http://dx.doi.org/10.1016/j.reprotox.2010.06.001

Howdeshell, KL; Furr, J; Lambright, CR; Rider, CV; Wilson, VS; Gray, LE, Jr. (2007). Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: Altered fetal steroid hormones and genes. Toxicol Sci 99: 190-202. http://dx.doi.org/10.1093/toxsci/kfm069

Howdeshell, KL; Hotchkiss, AK; Gray, LE. (2017). Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment [Review]. Int J Hyg Environ Health 220: 179-188. http://dx.doi.org/10.1016/j.ijheh.2016.11.007

Howdeshell, KL; Rider, CV; Wilson, VS; Furr, JR; Lambright, CR; Gray, LE. (2015). Dose addition models based on biologically relevant reductions in fetal testosterone accurately predict postnatal reproductive tract alterations by a phthalate mixture in rats. Toxicol Sci 148: 488-502. http://dx.doi.org/10.1093/toxsci/kfv196

Howdeshell, KL; Wilson, VS; Furr, J; Lambright, CR; Rider, CV; Blystone, CR; Hotchkiss, AK; Gray, LE, Jr. (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicol Sci 105: 153-165. http://dx.doi.org/10.1093/toxsci/kfn077

Hushka, LJ; Waterman, SJ; Keller, LH; Trimmer, GW; Freeman, JJ; Ambroso, JL; Nicolich, M; McKee, RH. (2001). Two-generation reproduction studies in rats fed di-isodecyl phthalate. Reprod Toxicol 15: 153-169. http://dx.doi.org/10.1016/S0890-6238(01)00109-5

IGHRC. (2006). Guidelines on route-to-route extrapolation of toxicity data when assessing health risks of chemicals. Bedfordshire, UK: Institute of Environment and Health. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10410579

Ito, Y; Kamijima, M; Hasegawa, C; Tagawa, M; Kawai, T; Miyake, M; Hayashi, Y; Naito, H; Nakajima, T. (2014). Species and inter-individual differences in metabolic capacity of di(2-ethylhexyl)phthalate (DEHP) between human and mouse livers. Environ Health Prev Med 19: 117-125. http://dx.doi.org/10.1007/s12199-013-0362-6

Ito, Y; Yokota, H; Wang, R; Yamanoshita, O; Ichihara, G; Wang, H; Kurata, Y; Takagi, K; Nakajima, T. (2005). Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several

organs of mice, rats, and marmosets. Arch Toxicol 79: 147-154. http://dx.doi.org/10.1007/s00204-004-0615-7

Jarfelt, K; Dalgaard, M; Hass, U; Borch, J; Jacobsen, H; Ladefoged, O. (2005). Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. Reprod Toxicol 19: 505-515. http://dx.doi.org/10.1016/j.reprotox.2004.11.005

Jiang, J; Ma, L; Yuan, L; Wang, X; Zhang, W. (2007). Study on developmental abnormalities in hypospadiac male rats induced by maternal exposure to di-n-butyl phthalate (DBP). Toxicology 232: 286-293. http://dx.doi.org/10.1016/j.tox.2007.01.018

Johnson, KJ; Heger, NE; Boekelheide, K. (2012). Of mice and men (and rats): phthalate-induced fetal testis endocrine disruption is species-dependent [Review]. Toxicol Sci 129: 235-248. http://dx.doi.org/10.1093/toxsci/kfs206

Kessler, W; Numtip, W; Grote, K; Csanády, GA; Chahoud, I; Filser, JG. (2004). Blood burden of di(2-ethylhexyl) phthalate and its primary metabolite mono(2-ethylhexyl) phthalate in pregnant and nonpregnant rats and marmosets. Toxicol Appl Pharmacol 195: 142-153. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/5756441

Kessler, W; Numtip, W; Völkel, W; Seckin, E; Csanády, GA; Pütz, C; Klein, D; Fromme, H; Filser, JG. (2012). Kinetics of di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate in blood and of DEHP metabolites in urine of male volunteers after single ingestion of ring-deuterated DEHP. Toxicol Appl Pharmacol 264: 284-291. [Toxicology and applied pharmacology].

Kim, TS; Jung, KK; Kim, SS; Kang, IH; Baek, JH; Nam, HS; Hong, SK; Lee, BM; Hong, JT; Oh, KW; Kim, HS; Han, SY; Kang, TS. (2010). Effects of in utero exposure to DI(n-Butyl) phthalate on development of male reproductive tracts in Sprague-Dawley rats. J Toxicol Environ Health A 73: 1544-1559. http://dx.doi.org/10.1080/15287394.2010.511579

Koch, HM; Angerer, J. (2007). Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. Int J Hyg Environ Health 210: 9-19. http://dx.doi.org/10.1016/j.ijheh.2006.11.008

Koch, HM; Becker, K; Wittassek, M; Seiwert, M; Angerer, J; Kolossa-Gehring, M. (2007). Di-n-butylphthalate and butylbenzylphthalate - urinary metabolite levels and estimated daily intakes: Pilot study for the German Environmental Survey on children. J Expo Sci Environ Epidemiol 17: 378-387. http://dx.doi.org/10.1038/sj.jes.7500526

Koch, HM; Bolt, HM; Angerer, J. (2004). Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch Toxicol 78: 123-130. http://dx.doi.org/10.1007/s00204-003-0522-3

Koch, HM; Bolt, HM; Preuss, R; Angerer, J. (2005). New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch Toxicol 79: 367-376. http://dx.doi.org/10.1007/s00204-004-0642-4

Koch, HM; Christensen, KLY; Harth, V; Lorber, M; Brüning, T. (2012). Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses. Arch Toxicol 86: 1829-1839. http://dx.doi.org/10.1007/s00204-012-0908-1

Koch, HM; Drexler, H; Angerer, J. (2003). An estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. Int J Hyg Environ Health 206: 77-83. http://dx.doi.org/10.1078/1438-4639-00205

Kortenkamp, A; Faust, M. (2010). Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. Int J Androl 33: 463-474. http://dx.doi.org/10.1111/j.1365-2605.2009.01047.x

Kuhl, AJ; Ross, SM; Gaido, KW. (2007). CCAAT/enhancer binding protein beta, but not steroidogenic factor-1, modulates the phthalate-induced dysregulation of rat fetal testicular steroidogenesis. Endocrinology 148: 5851-5864. http://dx.doi.org/10.1210/en.2007-0930

Kwack, S; Kim, K; Kim, H; Lee, B. (2009). Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment. J Toxicol Environ Health A 72: 1446-1454. http://dx.doi.org/10.1080/15287390903212923

Lambrot, R; Muczynski, V; Lecureuil, C; Angenard, G; Coffigny, H; Pairault, C; Moison, D; Frydman, R; Habert, R; Rouiller-Fabre, V. (2009). Phthalates impair germ cell development in the human fetal testis in vitro without change in testosterone production. Environ Health Perspect 117: 32-37. http://dx.doi.org/10.1289/ehp.11146

Lanning, LL; Creasy, DM; Chapin, RE; Mann, PC; Barlow, NJ; Regan, KS; Goodman, DG. (2002). Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicol Pathol 30: 507-520.

Lee, BM; Koo, HJ. (2007). Hershberger assay for antiandrogenic effects of phthalates. J Toxicol Environ Health A 70: 1365-1370. http://dx.doi.org/10.1080/15287390701432285

Lee, KY; Shibutani, M; Takagi, H; Kato, N; Takigami, S; Uneyama, C; Hirose, M. (2004). Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology 203: 221-238. http://dx.doi.org/10.1016/j.tox.2004.06.013

Lehmann, KP; Phillips, S; Sar, M; Foster, PMD; Gaido, KW. (2004). Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di (n-butyl) phthalate. Toxicol Sci 81: 60-68. http://dx.doi.org/10.1093/toxsci/kfh169

Li, L; Bu, T; Su, H; Chen, Z; Liang, Y; Zhang, G; Zhu, D; Shan, Y; Xu, R; Hu, Y; Li, J; Hu, G; Lian, Q; Ge, RS. (2015a). In utero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis. Toxicol Lett 232: 466-474. http://dx.doi.org/10.1016/j.toxlet.2014.11.024

Li, M; Qiu, L; Zhang, Y; Hua, Y; Tu, S; He, Y; Wen, S; Wang, Q; Wei, G. (2013). Dose-related effect by maternal exposure to di-(2-ethylhexyl) phthalate plasticizer on inducing hypospadiac male rats. Environ Toxicol Pharmacol 35: 55-60. http://dx.doi.org/10.1016/j.etap.2012.10.006

Li, N; Chen, X; Zhou, X; Zhang, W; Yuan, J; Feng, J. (2015b). The mechanism underlying dibutyl phthalate induced shortened anogenital distance and hypospadias in rats. J Pediatr Surg 50: 2078-2083. http://dx.doi.org/10.1016/j.jpedsurg.2015.08.046

Li, X; Chen, X; Hu, G; Li, L; Su, H; Wang, Y; Chen, D; Zhu, Q; Li, C; Li, J; Wang, M; Lian, Q; Ge, R. (2016). Effects of in utero exposure to dicyclohexyl phthalate on rat fetal leydig cells. Int J Environ Res Public Health 13: 1. http://dx.doi.org/10.3390/ijerph13030246

Li, Y; Zhuang, M; Li, T; Shi, N. (2009). Neurobehavioral toxicity study of dibutyl phthalate on rats following in utero and lactational exposure. J Appl Toxicol 29: 603-611. http://dx.doi.org/10.1002/jat.1447

Lin, H; Ge, R; Chen, G; Hu, G; Dong, L; Lian, Q; Hardy, D; Sottas, C; Li, X; Hardy, M. (2008). Involvement of testicular growth factors in fetal Leydig cell aggregation after exposure to phthalate in utero. Proc Natl Acad Sci USA 105: 7218-7222. http://dx.doi.org/10.1073/pnas.0709260105

Lington, AW; Bird, MG; Plutnick, RT; Stubblefield, WA; Scala, RA. (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. Fundam Appl Toxicol 36: 79-89. http://dx.doi.org/10.1093/toxsci/36.1.79

Liu, X; He, D; Zhang, D; Lin, T; Wei, G. (2008). Di(2-ethylhexyl) phthalate (DEHP) increases transforming growth factor-beta1 expression in fetal mouse genital tubercles. J Toxicol Environ Health A 71: 1289-1294. http://dx.doi.org/10.1080/15287390802114915

Lucas-Herald, AK; Mitchell, RT. (2022). Testicular Sertoli cell hormones in differences in sex development [Review]. Front Endocrinol (Lausanne) 13: 919670. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10706127

MacLeod, DJ; Sharpe, RM; Welsh, M; Fisken, M; Scott, HM; Hutchison, GR; Drake, AJ; van Den Driesche, S. (2010). Androgen action in the masculinization programming window and

development of male reproductive organs. Int J Androl 33: 279-287. http://dx.doi.org/10.1111/j.1365-2605.2009.01005.x

Mage, DT; Allen, RH; Kodali, A. (2008). Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. J Expo Sci Environ Epidemiol 18: 360-368. http://dx.doi.org/10.1038/sj.jes.7500614

Mahood, IK; Scott, HM; Brown, R; Hallmark, N; Walker, M; Sharpe, RM. (2007). In utero exposure to di(n-butyl) phthalate and testicular dysgenesis: Comparison of fetal and adult end points and their dose sensitivity. Environ Health Perspect 115: 55-61. http://dx.doi.org/10.1289/ehp.9366

Martino-Andrade, AJ; Morais, RN; Botelho, GG; Muller, G; Grande, SW; Carpentieri, GB; Leao, GM; Dalsenter, PR. (2008). Coadministration of active phthalates results in disruption of foetal testicular function in rats. Int J Androl 32: 704-712. http://dx.doi.org/10.1111/j.1365-2605.2008.00939.x

Masutomi, N; Shibutani, M; Takagi, H; Uneyama, C; Takahashi, N; Hirose, M. (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. Toxicology 192: 149-170. http://dx.doi.org/10.1016/S0300-483X(03)00269-5

McKinnell, C; Mitchell, RT; Walker, M; Morris, K; Kelnar, CJH; Wallace, WH; Sharpe, RM. (2009). Effect of fetal or neonatal exposure to monobutyl phthalate (MBP) on testicular development and function in the marmoset. Hum Reprod 24: 2244-2254. http://dx.doi.org/10.1093/humrep/dep200

Meek, ME; Boobis, AR; Crofton, KM; Heinemeyer, G; Raaij, MV; Vickers, C. (2011). Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regul Toxicol Pharmacol 60. http://dx.doi.org/10.1016/j.yrtph.2011.03.010

Metzdorff, SB; Dalgaard, M; Christiansen, S; Axelstad, M; Hass, U; Kiersgaard, MK; Scholze, M; Kortenkamp, A; Vinggaard, AM. (2007). Dysgenesis and histological changes of genitals and perturbations of gene expression in male rats after in utero exposure to antiandrogen mixtures. Toxicol Sci 98: 87-98. http://dx.doi.org/10.1093/toxsci/kfm079

Mitchell, RT; Childs, AJ; Anderson, RA; van Den Driesche, S; Saunders, PTK; McKinnell, C; Wallace, WHB; Kelnar, CJH; Sharpe, RM. (2012). Do phthalates affect steroidogenesis by the human fetal testis? Exposure of human fetal testis xenografts to di-n-butyl phthalate. J Clin Endocrinol Metab 97: E341-E348. http://dx.doi.org/10.1210/jc.2011-2411

Moody, S; Goh, H; Bielanowicz, A; Rippon, P; Loveland, KL; Itman, C. (2013). Prepubertal mouse testis growth and maturation and androgen production are acutely sensitive to di-n-butyl phthalate. Endocrinology 154: 3460-3475. http://dx.doi.org/10.1210/en.2012-2227

Moore, RW; Rudy, TA; Lin, TM; Ko, K; Peterson, RE. (2001). Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer di(2-ethylhexyl) phthalate. Environ Health Perspect 109: 229-237. http://dx.doi.org/10.2307/3434690

Mylchreest, E; Cattley, RC; Foster, PMD. (1998). Male reproductive tract malformations in rats following gestational and lactational exposure to di(n-butyl) phthalate: An antiandrogenic mechanism? Toxicol Sci 43: 47-60. http://dx.doi.org/10.1006/toxs.1998.2436

Mylchreest, E; Sar, M; Cattley, RC; Foster, PMD. (1999). Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. Toxicol Appl Pharmacol 156: 81-95. http://dx.doi.org/10.1006/taap.1999.8643

Mylchreest, E; Sar, M; Wallace, DG; Foster, PMD. (2002). Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. Reprod Toxicol 16: 19-28.

Mylchreest, E; Wallace, DG; Cattley, RC; Foster, PM. (2000). Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. Toxicol Sci 55: 143-151. http://dx.doi.org/10.1093/toxsci/55.1.143

Nagao, T; Ohta, R; Marumo, H; Shindo, T; Yoshimura, S; Ono, H. (2000). Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: A two-generation reproductive study. Reprod Toxicol 14: 513-532. http://dx.doi.org/10.1016/S0890-6238(00)00105-2

Nardelli, TC; Albert, O; Lalancette, C; Culty, M; Hales, BF; Robaire, B. (2017). In utero and lactational exposure study in rats to identify replacements for di(2-ethylhexyl) phthalate. Sci Rep 7: 3862. http://dx.doi.org/10.1038/s41598-017-03979-0

NASEM. (2017). Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals. In Consensus Study Report. Washington, D.C.: The National Academies Press. http://dx.doi.org/10.17226/24758

NCHS. (2012). The National Health and Nutrition Examination Survey: Sample design, 1999-2006. (Vital and Health Statistics: Series 2, No. 155). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_02/sr02_155.pdf

Nellemann, C; Dalgaard, M; Lam, HR; Vinggaard, AM. (2003). The combined effects of vinclozolin and procymidone do not deviate from expected additivity in vitro and in vivo. Toxicol Sci 71: 251-262. http://dx.doi.org/10.1093/toxsci/71.2.251

NICNAS. (2008a). Existing chemical hazard assessment report: Diethylhexyl phthalate. Sydney, Australia: Australian Department of Health and Ageing. https://www.industrialchemicals.gov.au/sites/default/files/PEC32-Diethylhexyl-phthalate-DEHP.pdf

NICNAS. (2008b). Existing chemical hazard assessment report: Diisobutyl phthalate. Sydney, Australia: National Industrial Chemicals Notification and Assessment Scheme. https://www.nicnas.gov.au/__data/assets/pdf_file/0006/4965/DIBP-hazard-assessment.pdf

NICNAS. (2012). Priority existing chemical assessment report no. 35: Diisononyl phthalate. (PEC35). Sydney, Australia: Australian Government Department of Health and Ageing. https://www.industrialchemicals.gov.au/sites/default/files/PEC35-Diisononyl-phthalate-DINP.pdf

NICNAS. (2013). Priority existing chemical assessment report no. 36: Dibutyl pthalate. (PEC36). Sydney, Australia: Australian Department of Health, National Industrial Chemicals Notification and Assessment Scheme. https://www.industrialchemicals.gov.au/sites/default/files/PEC36-Dibutyl-phthalate-DBP.pdf

NICNAS. (2014a). Di(methoxyethyl) phthalate. Priority existing chemical (PEC) assessment report no. 38. Sydney, Australia: Australian Government Department of Health.

NICNAS. (2014b). Dimethyl phthalate. Priority existing chemical (PEC) assessment report no. 37. Sydney, Australia: Australian Government Department of Health.

NICNAS. (2015a). Priority existing chemical assessment report no. 40: Butyl benzyl phthalate. (PEC40). Sydney, Australia: Australian Government Department of Health and Ageing. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3664467

NICNAS. (2015b). Priority existing chemical draft assessment report: Diisodecyl Phthalate & Di-n-octyl Phthalate. Sydney, Australia: Australian Department of Health and Ageing, National Industrial Chemicals Notification and Assessment Scheme. https://www.industrialchemicals.gov.au/sites/default/files/PEC39-Diisodecyl-phthalate-DIDP-Di-n-octyl-phthalate-DnOP.pdf

NRC. (2008). Phthalates and cumulative risk assessment: The task ahead. Washington, DC: National Academies Press. http://dx.doi.org/10.17226/12528

NTP. (1995). NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice [NTP] (pp. 1-G5). (ISSN 1521-4621 NIH Publication 95-3353). Research Triangle Park, NC. http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox030.pdf

NTP. (2018). National Toxicology Program approach to genomic dose-response modeling. (NTP Research Report No. 5). https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05_508.pdf

OECD. (2013). Guidance document supporting OECD test guideline 443 on the extended one generation reproductive toxicity test. In Series on Testing and Assessment. (No. 151 / ENV/JM/MONO(2013)10). Paris, France: OECD Environment, Health and Safety Publications. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3449546

OECD. (2018). Considerations for assessing the risks of combined exposure to multiple chemicals (No. 296). In Series on Testing and Assessment No 296. Paris, France. http://dx.doi.org/10.1787/ceca15a9-en

Page, BD; Lacroix, GM. (1995). The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985-1989: A survey. Food Addit Contam 12: 129-151. http://dx.doi.org/10.1080/02652039509374287

Parks, LG; Ostby, JS; Lambright, CR; Abbott, BD; Klinefelter, GR; Barlow, NJ; Gray, LE, Jr. (2000). The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 58: 339-349. http://dx.doi.org/10.1093/toxsci/58.2.339

Petrescu, AD; Gallegos, AM; Okamura, Y; Strauss, JF; Schroeder, F. (2001). Steroidogenic acute regulatory protein binds cholesterol and modulates mitochondrial membrane sterol domain dynamics. J Biol Chem 276: 36970-36982. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10492322

Pocar, P; Fiandanese, N; Secchi, C; Berrini, A; Fischer, B; Schmidt, JS; Schaedlich, K; Borromeo, V. (2012). Exposure to di(2-ethyl-hexyl) phthalate (DEHP) in utero and during lactation causes long-term pituitary-gonadal axis disruption in male and female mouse offspring. Endocrinology 153: 937-948. http://dx.doi.org/10.1210/en.2011-1450

Radke, EG; Braun, JM; Meeker, JD; Cooper, GS. (2018). Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence [Review]. Environ Int 121: 764-793. http://dx.doi.org/10.1016/j.envint.2018.07.029

Radke, EG; Braun, JM; Nachman, RM; Cooper, GS. (2020). Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence [Review]. Environ Int 137: 105408. http://dx.doi.org/10.1016/j.envint.2019.105408

Reyes, JM; Price, PS. (2018). An analysis of cumulative risks based on biomonitoring data for six phthalates using the Maximum Cumulative Ratio. Environ Int 112: 77-84. http://dx.doi.org/10.1016/j.envint.2017.12.008

Rider, CV; Furr, J; Wilson, VS; Gray, LE, Jr. (2008). A mixture of seven antiandrogens induces reproductive malformations in rats. Int J Androl 31: 249-262. http://dx.doi.org/10.1111/j.1365-2605.2007.00859.

Rider, CV; Furr, JR; Wilson, VS; Gray, LE, Jr. (2010). Cumulative effects of in utero administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity [Review]. Int J Androl 33: 443-462. http://dx.doi.org/10.1111/j.1365-2605.2009.01049.x

Rider, CV; Wilson, VS; Howdeshell, KL; Hotchkiss, AK; Furr, JR; Lambright, CR; Gray, LE, Jr. (2009). Cumulative effects of in utero administration of mixtures of "antiandrogens" on male rat reproductive development [Review]. Toxicol Pathol 37: 100-113. http://dx.doi.org/10.1177/0192623308329478

Safford, B; Api, AM; Barratt, C; Comiskey, D; Daly, EJ; Ellis, G; McNamara, C; O'Mahony, C; Robison, S; Smith, B; Thomas, R; Tozer, S. (2015). Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul Toxicol Pharmacol 72: 673-682. http://dx.doi.org/10.1016/j.yrtph.2015.05.017

Saillenfait, A; Sabaté, J; Gallissot, F. (2009a). Effects of in utero exposure to di-n-hexyl phthalate on the reproductive development of the male rat. Reprod Toxicol 28: 468-476. http://dx.doi.org/10.1016/j.reprotox.2009.06.013

Saillenfait, AM; Gallissot, F; Sabate, JP. (2009b). Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. J Appl Toxicol 29: 510-521. http://dx.doi.org/10.1002/jat.1436

Saillenfait, AM; Sabaté, JP; Denis, F; Antoine, G; Robert, A; Roudot, AC; Ndiaye, D; Eljarrat, E. (2017). Evaluation of the effects of α-cypermethrin on fetal rat testicular steroidogenesis. Reprod Toxicol 72: 106-114. http://dx.doi.org/10.1016/j.reprotox.2017.06.133

Saillenfait, AM; Sabate, JP; Gallissot, F. (2006). Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats. Toxicol Lett 165: 39-46. http://dx.doi.org/10.1016/j.toxlet.2006.01.013

Saillenfait, AM; Sabaté, JP; Gallissot, F. (2008). Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat. Reprod Toxicol 26: 107-115. http://dx.doi.org/10.1016/j.reprotox.2008.07.006

Saillenfait, AM; Sabaté, JP; Robert, A; Rouiller-Fabre, V; Roudot, AC; Moison, D; Denis, F. (2013). Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. J Appl Toxicol 33: 1027-1035. http://dx.doi.org/10.1002/jat.2896

Sathyanarayana, S; Calafat, AM; Liu, F; Swan, SH. (2008a). Maternal and infant urinary phthalate metabolite concentrations: Are they related? Environ Res 108: 413-418. http://dx.doi.org/10.1016/j.envres.2008.07.002

Sathyanarayana, S; Karr, CJ; Lozano, P; Brown, E; Calafat, AM; Liu, F; Swan, SH. (2008b). Baby care products: possible sources of infant phthalate exposure. Pediatrics 121: e260-268. http://dx.doi.org/10.1542/peds.2006-3766

Schecter, A; Lorber, M; Guo, Y; Wu, Q; Yun, SH; Kannan, K; Hommel, M; Imran, N; Hynan, LS; Cheng, D; Colacino, JA; Birnbaum, LS. (2013). Phthalate concentrations and dietary exposure from food purchased in New York State. Environ Health Perspect 121: 473-494. http://dx.doi.org/10.1289/ehp.1206367

Schwartz, CL; Christiansen, S; Hass, U; Ramhøj, L; Axelstad, M; Löbl, NM; Svingen, T. (2021). On the use and interpretation of areola/nipple retention as a biomarker for anti-androgenic effects in rat toxicity studies [Review]. Front Toxicol 3: 730752. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10492323

Scott, HM; Mason, JI; Sharpe, RM. (2009). Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds [Review]. Endocr Rev 30: 883-925. http://dx.doi.org/10.1210/er.2009-0016

Shin, HM; Bennett, DH; Barkoski, J; Ye, X; Calafat, AM; Tancredi, D; Hertz-Picciotto, I. (2019). Variability of urinary concentrations of phthalate metabolites during pregnancy in first morning voids and pooled samples. Environ Int 122: 222-230. http://dx.doi.org/10.1016/j.envint.2018.11.012

Spade, DJ; Bai, CY; Lambright, C; Conley, JM; Boekelheide, K; Gray, LE. (2018). Validation of an automated counting procedure for phthalate-induced testicular multinucleated germ cells. Toxicol Lett 290: 55-61. http://dx.doi.org/10.1016/j.toxlet.2018.03.018

Spade, DJ; Hall, SJ; Saffarini, C; Huse, SM; McDonnell, EV; Boekelheide, K. (2014). Differential response to abiraterone acetate and di-n-butyl phthalate in an androgen-sensitive human fetal testis xenograft bioassay. Toxicol Sci 138: 148-160. http://dx.doi.org/10.1093/toxsci/kft266

Stanfield, Z; Addington, CK; Dionisio, KL; Lyons, D; Tornero-Velez, R; Phillips, KA; Buckley, TJ; Isaacs, KK. (2021). Mining of Consumer Product Ingredient and Purchasing Data to Identify

Potential Chemical Coexposures. Environ Health Perspect 129: 67006. http://dx.doi.org/10.1289/EHP8610

Statistics Canada. (2004). Canadian Community Health Survey – Nutrition (CCHS). Detailed information for 2004 (cycle 2.2) [Website]. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/7273228

Struve, MF; Gaido, KW; Hensley, JB; Lehmann, KP; Ross, SM; Sochaski, MA; Willson, GA; Dorman, DC. (2009). Reproductive toxicity and pharmacokinetics of di-n-butyl phthalate (DBP) following dietary exposure of pregnant rats. Birth Defects Res B Dev Reprod Toxicol 86: 345-354. http://dx.doi.org/10.1002/bdrb.20199

Thankamony, A; Pasterski, V; Ong, KK; Acerini, CL; Hughes, IA. (2016). Anogenital distance as a marker of androgen exposure in humans. Andrology 0: 1-10. http://dx.doi.org/10.1111/andr.12156

TherImmune Research Corporation. (2004). Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet - Final report. (TRC Study No7244-200; NTP-RACB-98-004). Research Triangle Park, NC: National Toxicology Program, National Institute of Environmental Health Sciences.

Thompson, CJ; Ross, SM; Hensley, J; Liu, K; Heinze, SC; Young, SS; Gaido, KW. (2005). Differential steroidogenic gene expression in the fetal adrenal gland versus the testis and rapid and dynamic response of the fetal testis to di(n-butyl) phthalate. Biol Reprod 73: 908-917. http://dx.doi.org/10.1095/biolreprod.105.042382

Tornero-Velez, R; Isaacs, K; Dionisio, K; Prince, S; Laws, H; Nye, M; Price, PS; Buckley, TJ. (2021). Data Mining Approaches for Assessing Chemical Coexposures Using Consumer Product Purchase Data. Risk Anal 41: 1716-1735. http://dx.doi.org/10.1111/risa.13650

Tyl, RW; Myers, CB; Marr, MC; Fail, PA; Seely, JC; Brine, DR; Barter, RA; Butala, JH. (2004). Reproductive toxicity evaluation of dietary butyl benzyl phthalate (BBP) in rats. Reprod Toxicol 18: 241-264. http://dx.doi.org/10.1016/j.reprotox.2003.10.006

U.S. CPSC. (2010a). Toxicity review of benzyl-n-butyl phthalate. Bethesda, MD: U.S. Consumer Product Safety Commission, Directorate for Hazard Identification and Reduction. https://www.cpsc.gov/s3fs-public/ToxicityReviewOfBBP.pdf

U.S. CPSC. (2010b). Toxicity review of di-n-butyl phthalate. Bethesda, MD: U.S. Consumer Product Safety Commission, Directorate for Hazard Identification and Reduction. https://web.archive.org/web/20190320060443/https://www.cpsc.gov/s3fs-public/ToxicityReviewOfDBP.pdf

U.S. CPSC. (2010c). Toxicity review of Di(2-ethylhexyl) Phthalate (DEHP). Bethesda, MD. http://www.cpsc.gov//PageFiles/126533/toxicityDEHP.pdf

U.S. CPSC. (2010d). Toxicity review of di(isodecyl) phthalate. Washington, DC: Consumer Product Safety Commission (CPSC). http://www.cpsc.gov/PageFiles/126534/toxicityDIDP.pdf

U.S. CPSC. (2010e). Toxicity review of dicyclohexyl phthalate (DCHP). Bethesda, MD: U.S. Consumer Product Safety Commission, Directorate for Hazard Identification and Reduction. https://web.archive.org/web/20190320060432/https://www.cpsc.gov/s3fs-public/ToxicityReviewOfDCHP.pdf

U.S. CPSC. (2010f). Toxicity review of Diisononyl Phthalate (DINP). Bethesda, MD. http://www.cpsc.gov/PageFiles/126539/toxicityDINP.pdf

U.S. CPSC. (2011). Toxicity review of diisobutyl phthalate (DiBP, CASRN 84-69-5). Bethesda, MD: U.S. Consumer Product Safety Commission. https://www.cpsc.gov/s3fs-public/ToxicityReviewOfDiBP.pdf

U.S. CPSC. (2014). Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices). Bethesda, MD: U.S. Consumer Product Safety Commission, Directorate for Health Sciences. https://www.cpsc.gov/s3fs-public/CHAP-REPORT-With-Appendices.pdf

U.S. CPSC. (2015). Estimated phthalate exposure and risk to pregnant women and women of reproductive age as assessed using four NHANES biomonitoring data sets (2005/2006, 2007/2008, 2009/2010, 2011/2012). Rockville, Maryland: U.S. Consumer Product Safety Commission, Directorate for Hazard Identification and Reduction. https://web.archive.org/web/20190321120312/https://www.cpsc.gov/s3fs-public/NHANES-Biomonitoring-analysis-for-Commission.pdf

U.S. CPSC. (2017). Estimated phthalate exposure and risk to women of reproductive age as assessed using 2013/2014 NHANES biomonitoring data. Rockville, Maryland: U.S. Consumer Product Safety Commission, Directorate for Hazard Identification and Reduction. https://web.archive.org/web/20190407045559/https://www.cpsc.gov/s3fs-public/Estimated%20Phthalate%20Exposure%20and%20Risk%20to%20Women%20of%20Reproductive%20Age%20as%20Assessed%20Using%202013%202014%20NHANES%20Biomonitoring%20Data.pdf

U.S. EPA. (1986). Guidelines for the health risk assessment of chemical mixtures. Fed Reg 51: 34014-34025.

U.S. EPA. (1989). Risk Assessment Guidance for Superfund (RAGS): Volume 1: Human health evaluation manual (part A): Interim final [EPA Report]. (EPA/540/1-89/002). Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response. https://www.epa.gov/risk/risk-assessment-guidance-superfund-rags-part

U.S. EPA. (2000). Supplementary guidance for conducting health risk assessment of chemical mixtures (pp. 1-209). (EPA/630/R-00/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533

U.S. EPA. (2001). General principles for performing aggregate exposure and risk assessments [EPA Report]. Washington, DC. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/general-principles-performing-aggregate-exposure

U.S. EPA. (2002). Guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity [EPA Report]. Washington, D.C.

U.S. EPA. (2003). Framework for cumulative risk assessment [EPA Report]. (EPA/630/P-02/001F). Washington, DC. https://www.epa.gov/sites/production/files/2014-11/documents/frmwrk_cum_risk_assmnt.pdf

U.S. EPA. (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA630P03001F). Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf

U.S. EPA. (2007a). Analysis of total food intake and composition of individual's diet based on the USDA's 1994-1996, 1998 continuing survey of food intakes by individuals (CSFII) [EPA Report]. (EPA/600/R-05/062F). Washington, DC. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1065008

U.S. EPA. (2007b). Concepts, methods, and data sources for cumulative health risk assessment of multiple chemicals, exposures, and effects: A resource document [EPA Report]. (EPA/600/R-06/013F). Cincinnati, OH. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=190187

U.S. EPA. (2011). EPA Peer Consultation Workshop on cumulative risk assessment of phthalates: Potential options and methods for evaluating the cumulative hazard associated with six selected phthalates. Arlington, VA. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=214766

U.S. EPA. (2012). Benchmark dose technical guidance [EPA Report]. (EPA100R12001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance

U.S. EPA. (2016). Pesticide cumulative risk assessment: Framework for screening analysis. Washington, DC: Office of Pesticide Programs. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework

U.S. EPA. (2019a). Guidelines for human exposure assessment [EPA Report]. (EPA/100/B-19/001). Washington, DC: Risk Assessment Forum. https://www.epa.gov/sites/production/files/2020-01/documents/guidelines_for_human_exposure_assessment_final2019.pdf

U.S. EPA. (2019b). Proposed designation of butyl benzyl phthalate (CASRN 85-68-7) as a high priority substance for risk evaluation.

U.S. EPA. (2019c). Proposed designation of Di-Ethylhexyl Phthalate (DEHP) (1,2-Benzene-dicarboxylic acid, 1,2-bis (2-ethylhexyl) ester) (CASRN 117-81-7) as a high-priority substance for risk evaluation. Washington, DC: Office of Pollution Prevention and Toxics. https://www.epa.gov/sites/production/files/2019-08/documents/di-ethylhexyl_phthalate_117-81-7_proposeddesignation_082219.pdf

U.S. EPA. (2019d). Proposed Designation of Di-isobutyl Phthalate (DIBP) (CASRN 84-69-5) as High-Priority Substance for Risk Evaluation. https://www.epa.gov/sites/production/files/2019-08/documents/diisobutylphthalate_84-69-5_high-priority_proposeddesignation_082319_0.pdf

U.S. EPA. (2019e). Proposed designation of Dibutyl Phthalate (CASRN 84-74-2) as a high-priority substance for risk evaluation. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/production/files/2019-08/documents/dibutylphthalate_84-74-2_high-priority_proposeddesignation_082319.pdf

U.S. EPA. (2019f). Proposed designation of dicyclohexyl phthalate (CASRN 84-61-7) as a high-priority substance for risk evaluation (pp. 1-21). U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. https://www.regulations.gov/document?D=EPA-HQ-OPPT-2018-0504-0009

U.S. EPA. (2020a). Final scope of the risk evaluation for butyl benzyl phthalate (1,2-benzenedicarboxylic acid, 1-butyl 2-(phenylmethyl) ester); CASRN 85-68-7 [EPA Report]. (EPA-740-R-20-015). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/default/files/2020-09/documents/casrn_85-68-7_butyl_benzyl_phthalate_finalscope.pdf

U.S. EPA. (2020b). Final scope of the risk evaluation for di-ethylhexyl phthalate (1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester); CASRN 117-81-7 [EPA Report]. (EPA-740-R-20-017). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/default/files/2020-09/documents/casrn_117-81-7_di-ethylhexyl_phthalate_final_scope.pdf

U.S. EPA. (2020c). Final scope of the risk evaluation for di-isobutyl phthalate (1,2-benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester); CASRN 84-69-5 [EPA Report]. (EPA-740-R-20-018). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/default/files/2020-09/documents/casrn_84-69-5_di-isobutyl_phthalate_final_scope.pdf

U.S. EPA. (2020d). Final scope of the risk evaluation for dibutyl phthalate (1,2-benzenedicarboxylic acid, 1,2-dibutyl ester); CASRN 84-74-2 [EPA Report]. (EPA-740-R-20-016). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/default/files/2020-09/documents/casrn_84-74-2_dibutyl_phthalate_final_scope_0.pdf

U.S. EPA. (2020e). Final scope of the risk evaluation for dicyclohexyl phthalate (1,2-benzenedicarboxylic acid, 1,2-dicyclohexyl ester); CASRN 84-61-7 [EPA Report]. (EPA-740-R-20-019). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/default/files/2020-09/documents/casrn_84-61-7_dicyclohexyl_phthalate_final_scope.pdf

U.S. EPA. (2020f). Use report for butyl benzyl phthalate (BBP) - 1,2-Benzenedicarboxylic acid, 1- butyl 2(phenylmethyl) ester (CAS RN 85-68-7). (EPA-HQ-OPPT-2018-0501-0035). Washington, DC:

U.S. Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0501-0035

U.S. EPA. (2020g). Use report for di-ethylhexyl phthalate (CAS RN 117-81-7). (EPA-HQ-OPPT-2018-0433-0024). Washington, DC: U.S. Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0433-0024

U.S. EPA. (2020h). Use report for di-isobutyl phthalate (CAS RN 84-69-5). (EPA-HQ-OPPT-2018-0434-0029). Washington, DC: U.S. Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0434-0029

U.S. EPA. (2020i). Use report for dibutyl phthalate (DBP) - (1,2-Benzenedicarboxylic acid, 1,2- dibutyl ester) (CAS RN 84-74-2). (EPA-HQ-OPPT-2018-0503-0023). Washington, DC: U.S. Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0503-0023

U.S. EPA. (2020j). Use report for dicyclohexyl phthalate (CAS RN 84-61-7). (EPA-HQ-OPPT-2018-0504-0030). Washington, DC: U.S. Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0504-0030

U.S. EPA. (2021a). About the Exposure Factors Handbook [Website]. https://www.epa.gov/expobox/about-exposure-factors-handbook

U.S. EPA. (2021b). Final scope of the risk evaluation for di-isodecyl phthalate (DIDP) (1,2-benzenedicarboxylic acid, 1,2-diisodecyl ester and 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich); CASRN 26761-40-0 and 68515-49-1 [EPA Report]. (EPA-740-R-21-001). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/system/files/documents/2021-08/casrn-26761-40-0-di-isodecyl-phthalate-final-scope.pdf

U.S. EPA. (2021c). Final scope of the risk evaluation for di-isononyl phthalate (DINP) (1,2-benzene-dicarboxylic acid, 1,2-diisononyl ester, and 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich); CASRNs 28553-12-0 and 68515-48-0 [EPA Report]. (EPA-740-R-21-002). Washington, DC: Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/system/files/documents/2021-08/casrn-28553-12-0-di-isononyl-phthalate-final-scope.pdf

U.S. EPA. (2021d). Final use report for di-isononyl phthalate (DINP) - (1,2-benzene-dicarboxylic acid, 1,2-diisononyl ester, and 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich) (CASRN 28553-12-0 and 68515-48-0). (EPA-HQ-OPPT-2018-0436-0035). Washington, DC: U.S. Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0436-0035

U.S. EPA. (2022). Draft TSCA screening level approach for assessing ambient air and water exposures to fenceline communities (version 1.0) [EPA Report]. (EPA-744-D-22-001). Washington, DC: Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10555664

van den Driesche, S; Kolovos, P; Platts, S; Drake, AJ; Sharpe, RM. (2012). Inter-relationship between testicular dysgenesis and Leydig cell function in the masculinization programming window in the rat. PLoS ONE 7: e30111. http://dx.doi.org/10.1371/journal.pone.0030111

van Den Driesche, S; McKinnell, C; Calarrão, A; Kennedy, L; Hutchison, GR; Hrabalkova, L; Jobling, MS; Macpherson, S; Anderson, RA; Sharpe, RM; Mitchell, RT. (2015). Comparative effects of di(n-butyl) phthalate exposure on fetal germ cell development in the rat and in human fetal testis xenografts. Environ Health Perspect 123: 223-230. http://dx.doi.org/10.1289/ehp.1408248

Vo, T; Jung, E; Dang, V; Jung, K; Baek, J; Choi, K; Jeung, E. (2009). Differential effects of flutamide and di-(2-ethylhexyl) phthalate on male reproductive organs in a rat model. J Reprod Dev 55: 400-411. http://dx.doi.org/10.1262/jrd.20220

Wang, X; Sheng, N; Cui, R; Zhang, H; Wang, J; Dai, J. (2017). Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring. Chemosphere 172: 260-267. http://dx.doi.org/10.1016/j.chemosphere.2017.01.011

Waterman, SJ; Ambroso, JL; Keller, LH; Trimmer, GW; Nikiforov, AI; Harris, SB. (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. Reprod Toxicol 13: 131-136. http://dx.doi.org/10.1016/S0890-6238(99)00002-7

Waterman, SJ; Keller, LH; Trimmer, GW; Freeman, JJ; Nikiforov, AI; Harris, SB; Nicolich, MJ; McKee, RH. (2000). Two-generation reproduction study in rats given di-isononyl phthalate in the diet. Reprod Toxicol 14: 21-36. http://dx.doi.org/10.1016/S0890-6238(99)00067-2

Welsh, M; Saunders, PTK; Fisken, M; Scott, HM; Hutchison, GR; Smith, LB; Sharpe, RM. (2008). Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest 118: 1479-1490. http://dx.doi.org/10.1172/jci34241

WHO/IPCS. (2007). Harmonization project document no. 4: Part 1: IPCS framework for analysing the relevance of a cancer mode of action for humans and case-studies: Part 2: IPCS framework for analysing the relevance of a non-cancer mode of action for humans. Geneva, Switzerland: World Health Organization. http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1

Wilson, VS; Lambright, C; Furr, J; Ostby, J; Wood, C; Held, G; Gray, LE, Jr. (2004). Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. Toxicol Lett 146: 207-215. http://dx.doi.org/10.1016/j.toxlet.2003.09.012

Wine, RN; Li, LH; Barnes, LH; Gulati, DK; Chapin, RE. (1997). Reproductive toxicity of di-n-butylphthalate in a continuous breeding protocol in Sprague-Dawley rats. Environ Health Perspect 105: 102-107. http://dx.doi.org/10.1289/ehp.97105102

Wohlfahrt-Veje, C; Main, KM; Skakkebaek, NE. (2009). Testicular dysgenesis syndrome: foetal origin of adult reproductive problems [Review]. Clin Endocrinol 71: 459-465. http://dx.doi.org/10.1111/j.1365-2265.2009.03545.x

Wormuth, M; Scheringer, M; Vollenweider, M; Hungerbuhler, K. (2006). What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal 26: 803-824. http://dx.doi.org/10.1111/j.1539-6924.2006.00770.x

Xiao-Feng, Z; Nai-Qiang, Q; Jing, Z; Zi, L; Yang, Z. (2009). Di (n-butyl) phthalate inhibits testosterone synthesis through a glucocorticoid-mediated pathway in rats. Int J Toxicol 28: 448-456. http://dx.doi.org/10.1177/1091581809342596

Yamasaki, K; Okuda, H; Takeuchi, T; Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicol Lett 189: 14-20. http://dx.doi.org/10.1016/j.toxlet.2009.04.023

Yost, EE; Euling, SY; Weaver, JA; Beverly, BEJ; Keshava, N; Mudipalli, A; Arzuaga, X; Blessinger, T; Dishaw, L; Hotchkiss, A; Makris, SL. (2019). Hazards of diisobutyl phthalate (DIBP) exposure: A systematic review of animal toxicology studies [Review]. Environ Int 125: 579-594. http://dx.doi.org/10.1016/j.envint.2018.09.038

Zhang, Y; Jiang, X; Chen, B. (2004). Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to di-n-butyl phthalate in utero and during lactation and determination of its NOAEL. Reprod Toxicol 18: 669-676. http://dx.doi.org/10.1016/j.reprotox.2004.04.009

Zhu, XB; Tay, TW; Andriana, BB; Alam, MS; Choi, EK; Tsunekawa, N; Kanai, Y; Kurohmaru, M. (2010). Effects of di-iso-butyl phthalate on testes of prepubertal rats and mice. Okajimas Folia Anat Jpn 86: 129-136. http://dx.doi.org/10.2535/ofaj.86.129

APPENDICES

Appendix A Phthalate Cumulative Risk Assessment Initiatives

This appendix briefly summarizes the approaches used by U.S. CPSC (Appendix A.1), Health Canada (Appendix A.2), Danish EPA (Appendix A.3), Australia NICNAS (Appendix A.4), and EFSA (Appendix A.5) to assess phthalates for cumulative risk. Table_Apx A-1 provides a summary of high-priority and manufacturer-requested phthalates included in the CRAs conducted by each regulatory body. PODs used in previous phthalate CRAs are summarized in Appendix A.6.

Table_Apx A-1. Summary of Phthalates Included in Previous CRAs

Regulatory Agency	DEHP	DIBP	DBP	BBP	DCHP	DINP	DIDP
U.S. CPSC	✓	✓	✓	✓	–	✓	*x*
Health Canada[a]	✓	✓	✓	✓	✓	✓	*x*
NICNAS[b]	✓	–	✓	✓	–	✓	–
EFSA	✓	–	✓	✓	–	✓	*x*
Danish EPA	✓	✓	✓	✓	–	–	–

[a] Health Canada included 16 phthalates in their CRA, including six high-priority and manufacturer-requested phthalates and 10 phthalates not being considered under TSCA.
[b] Australia NICNAS has conducted five phthalate CRAs, which in addition to the listed phthalates, have also included di(methoxyethyl) (DMEP), dimethyl (DMP), and diethyl (DEP) phthalates.
✓Included in the CRA
x Excluded from the CRA; studies indicate no effects consistent with phthalate syndrome.
– Not considered as part of CRA planning.

A.1 United States Consumer Product Safety Commission

In their report to the U.S. CPSC, the Chronic Hazard Advisory Panel (CHAP) on Phthalates and Phthalate Alternatives assessed five phthalates (*i.e.*, BBP, DBP, DIBP, DEHP, DINP) for cumulative risk (U.S. CPSC, 2014). In considering the best available approach for phthalates, the CHAP concluded that experimental data on combination effects of phthalates from multiple studies provide strong evidence that dose addition produces good approximations of mixtures effects. Male developmental and reproductive effects occurring via an antiandrogenic MOA served as the basis of the CRA. Three sets of antiandrogenic PODs were selected, including (1) the antiandrogenic PODs published by Kortenkamp and Faust (2010); (2) PODs derived based on reduced fetal testosterone data published in Hannas et al. (2011); and (3) PODs identified by the CHAP via a *de novo* literature review. The PODs based on data from Hannas et al. (2011) were derived using relative potency assumptions. DEHP was used as the index chemical. The NOAEL for DEHP-induced testosterone modulated effects was 5 mg/kg/day, and DIBP, DBP and BBP were approximately equipotent (*i.e.*, RPFs for DIBP, DBP, and BBP were all 1), while DINP was 2.3 times less potent than DEHP (DINP NOAEL = 11.5 mg/kg/day DEHP equivalent units). The three sets of PODs used by the CHAP are summarized in Table_Apx A-3.The CHAP considered including DIDP in the CRA but concluded that there is no evidence that DIDP causes phthalate syndrome-related effects in experimental models, so DIDP was excluded from the analysis.

For the exposure assessment, the CHAP assessed cumulative exposure for various groups, including women of reproductive age, pregnant women, and infants (2 to 36 months), using a reverse dosimetry approach with human urinary biomonitoring data. CDC's NHANES urinary biomonitoring data from the 2005 to 2006 cycle was used to estimate cumulative exposure of pregnant women in the general U.S. population. Infants are not included in the NHANES study design, so urinary biomonitoring data from

mother/infant pairs reported in the Study for Future Families (Sathyanarayana et al., 2008b; Sathyanarayana et al., 2008a), which is a multicenter pregnancy cohort study, was used to estimate exposure to infants and provided a second measure of cumulative exposure for pregnant women. In their 2015 report, CHAP revised their original analysis using the 2005/2006 NHANES data based on updates the CDC made to data files, including demographic and phthalates data, available to the public and included newer analysis for 2007/2008, 2009/2010, and 2011/2012 cycles of NHANES data (U.S. CPSC, 2015). In their 2017 report, U.S. CPSC analyzed the 2013/2014 cycle of NHANES data (U.S. CPSC, 2017). In both updated reports, U.S. CPSC stated that analysis for pregnant women were not updated because NHANES datasets following the 2005/2006 cycle did not include an oversampling of pregnant women leading to a small sample size unsuitable for statistical analysis (CDC, 2013b; NCHS, 2012). Reverse dosimetry was used to calculate daily intake values for each parent phthalate using the methodology published by Koch et al. (2007).

CPSC also explored a scenario-based method for determining aggregate exposure from all pathways. To estimate total intake, the CHAP grouped sources and scenarios into the following categories: diet, prescription drugs, toys, child-care articles, personal care products, indoor environment, and outdoor environment. The total exposure from phthalates was assessed for each residue dataset, food categorization scheme, and population (infant, toddler, children, teen, adult) using a deterministic approach, calculating average and 95th percentile total exposure values. Although their scenario-based modeled exposure estimates were not used to estimate cumulative risk, they concluded that their scenario-based modeled estimates were in general agreement with the daily intake values derived from biomonitoring data used for calculating cumulative risk (U.S. CPSC, 2014). Estimated phthalate exposure by individual exposure scenario for women is shown in Figure_Apx A-1.

To characterize risk, the CHAP applied the hazard index (HI) approach. HIs were calculated for each individual based on their own unique phthalate urinary exposure profile, which is in contrast to the standard HI approach in which population-level exposure statistics (*i.e.*, mean, median, 95th percentile) are used. Based on each individual's exposure profile, HIs were calculated using the three different sets of PODs described above and summarized in Table_Apx A-3. Based on 2005 to 2006 NHANES data, approximately 9 to 10 percent of pregnant women had HI values >1.0 indicating risk, while <1 to 4 percent of women and 4 to 5 percent of infants in the SFF dataset had HI values >1.0. In all cases, the HQ for DEHP contributed the most to the calculated HIs, while HQs for DINP, DBP, and BBP were approximately similar, and DIBP consistently had the smallest HQs. CPSC found in their cumulative assessment that the main sources of phthalate exposure for pregnant women/women of reproductive age were food, beverage, and drugs via direct ingestion. Exposure to infants to phthalates was also primarily through diet but exposure to DINP also occurred through mouthing of toys and teethers, and dermal contact with personal care products (U.S. CPSC, 2014).

Source	DEP		DBP		DIBP		BBP		DNOP		DEHP		DINP		DIDP	
	ave.	0.95	ave.	0.95	ave.	0.95	ave.	0.95	ave.	0.95	ave.	0.95	ave.	0.95	ave.	0.95
Total	1.8 E+01	4.0 E+02	2.9 E-01	5.7 E+00	1.5 E-01	5.0 E-01	1.1 E+00	2.6 E+00	1.7 E-01	2.1 E+01	1.6 E+00	5.6 E+00	5.1 E+00	3.3 E+01	3.2 E+00	1.2 E+01
Diet	9.3 E-02	3.6 E-01	7.8 E-02	2.3 E-01	1.3 E-01	4.6 E-01	1.6 E-01	2.5 E-01	1.3 E-01	3.6 E-01	1.4 E+00	4.9 E+00	4.8 E+00	1.5 E+01	3.2 E+00	9.3 E+00
Drugs[a]	1.4 E+01	3.7 E+02														
Personal care, dermal																
Shampoo	1.2 E-02	6.5 E-02														
Soap / body wash	2.3 E-02	4.1 E-02														
Lotion	5.0 E-02	1.8 E-01														
Deodorant	7.4 E-01	1.9 E+01														
Perfume	2.8 E+00	6.2 E+00														
Nail polish	3.4 E-03	1.5 E-02	1.7 E-01	5.4 E+00												
Hair spray	4.7 E-02	1.4 E-01														
Personal care, inhalation[b]																
Deodorant	5.1 E-02	1.3 E+00														
Perfume	2.0 E-01	4.2 E-01														
Hair spray	6.2 E-03	1.8 E-02														
Dermal, PVC[c]																
Toys[d]									8.0 E-03	8.0 E-03	8.0 E-03	8.0 E-03	6.7 E-03	6.7 E-03	1.1 E-03	1.1 E-03
Furniture[e]									0.0 E+00	2.0 E+01			0.0 E+00	1.7 E+01	0.0 E+00	2.9 E+00
Gloves							2.3 E-01	2.3 E-01	3.3 E-02	3.3 E-02	3.3 E-02	3.3 E-02	2.8 E-02	2.8 E-02	4.7 E-03	4.7 E-03
Household-dermal[e]																
Paint/ lacquer							5.4 E-04	1.5 E-03					2.5 E-05	0.0 E+00		
Adhesive							1.0 E-03	3.6 E-03								
Household, inhalation[f]																
Air freshener, spray[b]	1.1 E-01	3.6 E-01	1.6 E-05	2.0 E-05												
Air freshener, liquid	1.5 E-02	4.0 E-02	9.2 E-06	2.4 E-05	6.8 E-06	9.8 E-06										
Paint, spray[b]							6.6 E-01	2.0 E+00					1.5 E-01	3.1 E-01		
Indirect ingestion																
Dust	3.4 E-03	4.3 E-03	1.1 E-02	1.8 E-02	1.2 E-03	2.0 E-03	5.0 E-02	1.1 E-01			2.0 E-01	3.4 E-01	5.2 E-02	4.0 E-01	1.4 E-02	4.4 E-02
Soil			9.3 E-06	4.3 E-05			1.6 E-06	6.9 E-06	3.5 E-06	1.1 E-05	7.2 E-05	3.1 E-04	2.1 E-05	8.1 E-05		
Inhalation, air																
Indoor air	9.5 E-02	2.4 E-01	3.3 E-02	7.4 E-02	1.8 E-02	4.4 E-02	3.8 E-03	8.9 E-03	5.9 E-05	5.9 E-05	1.5 E-02	2.9 E-02				
Outdoor air	1.4 E-03	3.8 E-03	8.4 E-05	3.6 E-04	8.6 E-05	2.6 E-04	7.2 E-05	1.2 E-04	8.4 E-06	8.4 E-06	4.8 E-04	2.9 E-03				
Adult toys[g]									3.8 E-04	8.0 E-02	1.9 E-04	2.6 E-01				

Figure_Apx A-1. Estimated Phthalate Exposure by Individual Exposure Scenario for Women
Adapted from Table E1-S1 in (U.S. CPSC, 2014).

A.2 Health Canada

In June 2020, Health Canada and Environment and Climate Change Canada published their final screening assessment of 28 phthalates (ECCC/HC, 2020). Phthalates were divided into three subgroups (*i.e.*, short-, medium-, and long-chain) based on the length of the carbon backbone in the ester side-group. Based on a structure activity relationship analysis, Health Canada concluded that there was evidence that medium-chain phthalates (but not short- (EC/HC, 2015d) or long-chain (EC/HC, 2015e)) are capable of eliciting effects on the developing male reproductive system through a common MOA (*i.e.*, through a disruption of androgen action) (EC/HC, 2015c; Health Canada, 2015). Based on this finding, Health Canada assessed 16 medium-chain phthalates for cumulative risk to the general Canadian population.[9]

Combined exposure for three sensitive subpopulations (*i.e.*, pregnant women/women of childbearing age, infants, and children) was assessed using two approaches. First, occurrence data for environmental media (*e.g.*, dust, air, drinking water, soil, etc.) and food was used to estimate daily intake values. Second, human urinary biomonitoring data for phthalate metabolites was used to estimate daily intake values for parent phthalates using reverse dosimetry.

Health Canada estimated daily intake for BBP, DBP, DEHP, and DINP through routes of exposure to environmental media and food for the general population, including ambient air, indoor air, drinking water, food and beverages, breast milk, soil, and dust. Exposure scenarios included detailed assumptions regarding daily intake of each phthalate via each route of exposure and separate estimates by varying age groups and categories. Additional pathways were assessed for specific populations and phthalates, including dermal and inhalation (aerosol) exposure to personal care products for adults and infants, and DIBP and DINP in children's toys and articles. However, based on regulatory status of products or determinations of minimal exposures, not all pathways of exposure were included in total intake estimates. This is shown in Appendix D of (ECCC/HC, 2020) (see Tables D-1a, D-2a, D-3a, D6) where central tendency and upper bound estimates of exposure through ambient air, indoor air, drinking water, food and beverages, soil, and dust are combined for each phthalate to estimate aggregate exposure. Health Canada then generated distributions of phthalate exposure using a probabilistic exposure assessment, randomly selecting phthalate concentrations for each food from the matched sources. Exposure estimates from each food were summed for each individual, and a distribution of exposure was generated for all respondents. This process was then iterated 500 times to model the variability of the distribution of exposures.

Health Canada also utilized urinary biomonitoring data for six phthalates (*i.e.*, DEHP, DBP, DINP, DIBP, BBP, DCHP) to estimate daily intake values using reverse dosimetry (ECCC/HC, 2020). To do this, Health Canada utilized urinary biomonitoring data from several sources, including the Canadian Health Measures Survey (Cycle 1 (2007 to 2009) and 2 (2009 to 2011) data); U.S. CDC NHANES (2009 to 2010 survey data); the Maternal Infant Research on Environmental Chemicals study which includes urinary biomonitoring data from 2008 to 2011 for approximately 2,000 Canadian women during their first trimester of pregnancy (Arbuckle et al., 2014); the Maternal Infant Research on Environmental Chemicals – Child Development Plus study, which includes urinary biomonitoring data

[9] Medium-chain phthalates assessed by Health Canada included six high-priority and manufacturer-requested phthalates (DIBP, DCHP, DINP, BBP, DBP, DEHP) and 10 phthalates not undergoing risk evaluation at EPA, including: butyl cyclohexyl phthalate (BCHP, CASRN 84-64-0), dibenzyl phthalate (DBzP, CASRN 523-31-9), cyclohexyl isobutyl phthalate (CHIBP, CASRN 5334-09-8), benzyl 3-isobutyryloxyl-1-isopropyl-2,2-dimethylpropyl phthalate (B84P, CASRN 16883-83-3), benzyl isooctyl phthalate (BIOP, CASRN 27215-22-1), bis(methylcyclohexyl)phthalate (DMCHP, CASRN 27987-25-3), benzyl octyl phthalate (B79P, CASRN 68515-40-2), diisoheptyl phthalate (DIHepP, CASRN 71888-89-6), diisooctyl phthalate (DIOP, CASRN 27554-26-3), and dihexyl ester phthalate (DnHP, CASRN 84-75-3).

from 2013 to 2015 for approximately 200 Canadian children aged 2 to 5 years (Ashley-Martin et al., 2021); and the Plastics and Personal Care Product Use in Pregnancy survey, which includes biomonitoring data from 2009 to 2010 for 80 mother-infant pairs from Ottawa Canada (Arbuckle et al., 2016).

PODs based on antiandrogenic effects on the developing male reproductive system were selected for each phthalate based on both *in utero* exposure and prepubertal/pubertal exposure studies. PODs based on *in utero* exposure were used to characterize risk for pregnant women/women of childbearing age and infants, while PODs based on prepubertal/pubertal exposure were used to characterize risk for children. When phthalate-specific PODs could not be derived, Health Canada used read-across from structurally similar phthalates to fill data gaps. PODs for high-priority and manufacturer-requested phthalates assessed by Health Canada are shown in Table_Apx A-3, while PODs for other phthalates not being assessed under TSCA are summarized in Table F-5 of ECCC/HC (2020).

To characterize cumulative risk, Health Canada used the HI approach. HIs were calculated for pregnant women/women of childbearing age, infants, and children based on 95th percentile daily intake values estimated using human urinary biomonitoring data and occurrence data from environmental media and food. For pregnant women/women of childbearing age HIs were 0.34 (environmental occurrence) and 0.49 (biomonitoring), with HQs for DEHP (36 to 61 percent) and DINP (34–55 percent) being the largest contributors to the HIs. For infants, HIs were 0.83 (environmental occurrence) and 0.37 (biomonitoring), with HQs for DEHP (68 to 69 percent), DINP (14 to 25 percent), and DBP (3.6 to 14 percent) being the largest contributors. Finally, for children, HIs were 0.60 (environmental occurrence) and 0.54 (biomonitoring), with HQs for DEHP (67 to 88 percent), DBP (9.1 to 29 percent), and DINP (1.6 to 2.8 percent) being the largest contributors. Based on these results, Health Canada concluded that phthalates do not currently pose a cumulative risk to the general population in Canada.

A.3 Danish EPA

In 2011, the Danish EPA submitted a proposal for restrictions on four phthalates (*i.e.*, DEHP, BBP, DBP, DIBP) under Annex XV of REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) (ECHA, 2011). At the time of the proposal, all four of the assessed phthalates had already been classified under REACH as Category 1B reproductive toxicants (presumed human reproductive toxicant) with adverse effects on male sexual differentiation during the developmental process (*i.e.*, antiandrogenic effects). To support the proposal for restrictions, combined exposure from DEHP, BBP, DBP, and DIBP from "articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes" were assessed for cumulative risk to human health.

When assessing the four phthalates, Danish EPA relied upon assumptions of dose addition and similar MOA. Selected PODs were based on adverse effects on the developing male reproductive system that were associated with an antiandrogenic MOA. PODs selected for use in the CRA are shown in Table_Apx A-3. For the exposure assessment, combined exposure to DEHP, BBP, DBP and DIBP was estimated for three groups, including 2-year old children, 6- to 7-year old children, and adults. Danish EPA considered a number of exposures routes, including exposure to phthalate containing articles (*e.g.*, erasers, sandals, sex toys), indoor dust, indoor air, and food. Cumulative exposure to phthalates was also assessed using human urinary biomonitoring data of phthalate metabolites, which was converted into a daily intake value for each parent phthalate using reverse dosimetry (Koch et al., 2007). Low median (*i.e.*, the lowest calculated median value), high median (*i.e.*, highest calculated median value), and realistic worst-case scenario (*i.e.*, 95th percentile value) exposure estimates were derived and used to characterize cumulative risk.

To assess risk, the risk characterization ratio (RCR) approach was used. The RCR approach is analogous to the HI approach, in which RCRs are calculated for each chemical in the mixture of interest (*i.e.*, RCR = exposure ÷ derived no effect level (DNEL))[10] and then summed to calculate a cumulative RCR. If the cumulative RCR exceeds 1.0, then risk is considered not to be controlled for the chemicals being assessed. RCR values were generally >1.0 for 2-year old and 6- to 7-year old children in both the high-median and 95th percentile exposures groups based on both biomonitoring data and exposure data for combined articles, food, and indoor dust and air, while adult RCR values exceeded 1.0 only in the 95th percentile exposure groups based on biomonitoring data and combined exposure to articles, food, and the indoor environment. Based on these results, Danish EPA concluded that "for a large part of the population the risk is not sufficiently controlled and the exposure to DEHP, DBP, BBP, and DIBP should be reduced."

A.4 Australia NICNAS

Australia NICNAS has issued Priority Existing Chemical (PEC) Assessment Reports for DINP (NICNAS, 2012), DBP (NICNAS, 2013), di(methoxyethyl) phthalate (DMEP) (NICNAS, 2014a), dimethyl phthalate (DMP) (NICNAS, 2014b), and BBP (NICNAS, 2015a). As part of each PEC assessment NICNAS assessed cumulative risk for a limited number of phthalates, populations, and exposure scenarios. Table_Apx A-2 provides a summary of the phthalates, exposure scenarios, and critical health effects assessed in each PEC report.

CRAs conducted by NICNAS relied upon assumptions of dose addition, no toxicologic interactions, and a similar MOA for each health outcome considered. Systemic effects (*i.e.*, enlarged liver and/or kidney) were assessed as part of the CRA for DINP (NICNAS, 2012), but not other phthalates. Fertility-related effects (*i.e.*, reduced testes weight and/or testosterone) and developmental effects (*i.e.*, reduced pup weight) were assessed as part of the CRAs reported in all five phthalate PEC Assessment Reports. PODs selected by NICNAS are shown in Table_Apx A-3. As can be seen from Table_Apx A-2, a limited number of phthalates were included in each CRA and exposure assessments focused on exposure of a single population group, 6-month old infants, due to use of phthalate containing plasticizers in toys and child-care articles and use of phthalate containing lotions and other cosmetics. To characterize risk from exposure to multiple phthalates, a cumulative margin of exposure approach was used. Cumulative MOEs were compared to a benchmark MOE of 100. Cumulative MOEs for all assessed exposures scenarios were >100, indicating an adequate margin of safety.

[10] DNELs are analogous to oral reference doses or inhalation reference concentrations calculated by the EPA IRIS program, *i.e.*, DNELs and RfDs/RfCs are calculated by dividing a POD by a set of uncertainty factors.

5404 **Table_Apx A-2. Summary of Australia NICNAS Cumulative Phthalate Assessments**

	PEC No. 35: DINP (NICNAS, 2012)	PEC No. 36: DBP (NICNAS, 2013)	PEC No. 37: DMP (NICNAS, 2014b)	PEC No. 38: DMEP (NICNAS, 2014a)	PEC No. 40: BBP (NICNAS, 2015a)
Phthalates Included	- DINP, DEHP, DEP	- DBP, DINP, DEHP, DEP	- DMP, DBP, DINP, DEHP, DEP	- DMEP, DINP, DEHP, DEP	- BBP, DINP, DEP
Critical Health Effect(s)	- Systemic toxicity - Developmental - Fertility-related	- Developmental - Fertility-related	- Developmental - Fertility-related	- Developmental - Fertility-related	- Developmental - Fertility-related
Evaluated Population(s)	- Infants (6-months old)	- Infants (6-months old)	- Infants (6-months old)	- Infants (6-months old)	- Infants (6-months old)
Assessed Exposure Scenarios	- Exposure to DINP in toys and child-care articles + DEP in cosmetics - Exposure to a mixed plasticiser (42% DINP, 1% DEHP) in toys and child-care articles - Exposure to a mixed plasticizer (DINP/DEHP) in toys and child-care articles + DEP in cosmetics	- Exposure to a mixed plasticizer (0.5% DBP, 41.5% DINP, 1% DEHP) in toys and childcare articles - Exposure to a mixed plasticiser (DBP/DINP/DEHP) in toys and childcare articles + DEP in lotions for children	- Exposure to a mixed plasticiser (42.5% DINP, 0.5% DMP) in toys + 0.5% DEP or 0.5% DMP in cosmetics - Exposure to a mixed plasticiser (41.5% DINP, 0.5% DMP, 1% DEHP) in toys + 0.5% DEP or 0.5% DMP in cosmetics - Exposure to a mixed plasticiser (41.5% DINP, 0.5% DBP, 1% DEHP) in toys + 0.5% DMP in cosmetics	- Exposure to a mixed plasticiser (42.5% DINP, 0.5% DMEP) in toys + 0.5% DEP or 0.5% DMP in cosmetics - Exposure to a mixed plasticiser (41.5% DINP, 0.5% DMEP, 1% DEHP) in toys + 0.5% DEP or 0.5% DMP in cosmetics	- Exposure to a mixed plasticizer (42.5% DINP + 0.5% BBP) in toys + 0.5% DEP (or 0.5% DMP) in cosmetics - Exposure to a mixed plasticizer (41.5% DINP + 0.5% BBP + 1% DEHP) in toys + 0.5% DEP (or 0.5% DMP) in cosmetics

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A.5 European Food Safety Authority

In response to a request from the European Commission to update its 2005 risk assessments of DBP, BBP, DEHP, DINP, and DIDP, the EFSA Panel on Food Contact Materials, Enzymes, and Processing Aids (CEP Panel) established a group tolerable daily intake (TDI) value for DBP, BBP, DEHP, and DINP (EFSA, 2019). The group-TDI was derived using an RPF approach in which DEHP served as the index chemical. Using this approach, EFSA relied upon assumptions of dose additivity, no toxicological interactions, and a common MOA (*i.e.*, reduction in fetal testosterone). Effects on the developing male reproductive system were selected as the key health outcome for deriving a group-TDI. RPFs were derived based on PODs summarized in Table_Apx A-3, which are based on a spectrum of effects associated with phthalate syndrome (*i.e.*, the critical effect for BBP was decreased anogenital distance, while the critical effect for DBP was decreased spermatocyte development), instead of a single health effect. Derived RPFs were 1.0 for DEHP (index chemical), 0.1 for BBP, 5.0 for DBP, and 0.3 for DINP, and the group-TDI was 50 µg/kg-d DEHP equivalent units. DIDP was not included in the group-TDI, because EFSA concluded that DIDP does not induce reproductive effects involving a reduction in fetal testosterone.

Dietary exposure to the five phthalates was assessed using data on the levels of occurrence of phthalates in food from the EFSA Chemical Occurrence database and scientific literature. Dietary exposure was estimated for a variety of populations, including infants (<12 months), toddlers (≥12 to <36 months), children (≥3 to <10 years), adolescents (≥10 to <18 years), adults (≥18 to <65 years), elderly (≥65 to <75 years), very elderly (≥75 years), pregnant women, and lactating women. To characterize risk, estimates of combined dietary exposure to DEHP, DBP, BBP and DINP, which ranged from 0.9 to 7.2 and 1.6 to11.7 µg/kg/day for mean and high-end consumers of all age groups, were compared to the group TDI. In the worst-case scenario, dietary exposure contributed up to 23 percent of the group-TDI.

A.6 PODs Used in Previous Phthalate CRAs

Table_Apx A-3. Summary of PODs for High-Priority and Manufacturer-Requested Phthalates Considered in Previous CRAs

Country (Reference)	Hazard Type	Point of Departure Used in Cumulative Risk Assessments[a b]					
		BBP	DBP	DEHP	DIBP	DCHP	DINP
Australia (2015a, 2014a, b, 2013, 2012)	Systemic	–	–	28.9 mg/kg/d (NOAEL, ↑ kidney weight) (Corning Hazleton Inc., 1996)	–	–	88 mg/kg/d (NOAEL, ↑ liver & kidney weight) (Lington et al., 1997)
	Reproductive	10 mg/kg/d[2] (NOAEL, ↓ testosterone in fetal testes) (Lehmann et al., 2004)	10 mg/kg/d (NOAEL, ↓ testosterone in fetal testes) (Lehmann et al., 2004)	4.8 mg/kg/d (NOAEL, ↓ testes weight, seminiferous tubule atrophy in F1& F2) (TherImmune Research Corporation, 2004)	–	–	50 mg/kg/d (NOAEL, ↓ testosterone in fetal testes) (Boberg et al., 2011; Hannas et al., 2011)
	Developmental	50 mg/kg/d (NOAEL, ↓ birth weight in both sexes) (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000)	50 mg/kg/d (NOAEL, ↓ pup weight) (Zhang et al., 2004)	46 mg/kg/d (NOAEL, ↓ pup weight) (TherImmune Research Corporation, 2004)	–	–	50 mg/kg/d (NOAEL, ↓ pup weight) (Waterman et al., 2000)

Country (Reference)	Hazard Type	Point of Departure Used in Cumulative Risk Assessments[a b]					
		BBP	DBP	DEHP	DIBP	DCHP	DINP
Canada (ECCC/HC, 2020)	Antiandrogenic (*in utero* exposure)	50 mg/kg/d (NOAEL, ↓ AGD at birth in F2 males) (Aso et al., 2005; Tyl et al., 2004; Nagao et al., 2000)	10 mg/kg/d (NOAEL, ↓ testicular testosterone, fertility effects, altered spermatocyte development, ↓ tubular & interstitial cell populations, altered seminiferous tubule morphometry) (Ahmad et al., 2014; Boekelheide et al., 2009; Lehmann et al., 2004)	4.8 mg/kg/d (NOAEL, Small and/or aplastic epididymis, TP, other RPS effects in F1 & F2) (TherImmune Research Corporation, 2004)	125 mg/kg/d (NOAEL, ↓ AGD, ↑NR, effects on fertility, ↓ testosterone in fetal testes) (Furr et al., 2014; Saillenfait et al., 2008)	10 mg/kg/d (LOAEL, ↓ AGD, TP, ↑ resorptions) (Li et al., 2016)	10 mg/kg/d (LOEL, MNGs, Leydig cell aggregation) (Li et al., 2015a)
	Antiandrogenic (pre-pubertal exposure)	500 mg/kg/d (LOEL, ↓ sperm count (30%), ↓ sperm motility, ↓BW gain, ↑ relative liver weight) (Kwack et al., 2009)	10–50 mg/kg/d (LOEL, delayed spermatogenesis, ↓ AGD) (Moody et al., 2013; Xiao-Feng et al., 2009)	10 mg/kg/d (NOAEL, ↓ absolute & relative testis weight) (Dostal et al., 1988)	300 mg/kg/d (NOAEL, TP) (Zhu et al., 2010)	18 mg/kg/d (NOAEL, ↓ spermatid head counts, testicular atrophy, ↓ BW gain, ↓ food consumption in F1 males) (Hoshino et al., 2005)	500 mg/kg/d (LOEL, ↓ absolute seminal vesicle and LABC wt) (Lee and Koo, 2007)
Denmark (ECHA, 2011)	Antiandrogenic	50 mg/kg/d (NOAEL, ↓ AGD in F1 & F2 pups) (Tyl et al., 2004)	2 mg/kg/d (LOAEL, ↓ spermatocyte development on PND 21) (Lee et al., 2004)	4.8 mg/kg/d (NOAEL, ↓ testes weight, testicular atrophy in F1 & F2 males) (TherImmune Research Corporation, 2004)	125 mg/kg/d (NOAEL, ↓ AGD, ↑NR) (Saillenfait et al., 2008)	–	–
EFSA (EFSA, 2019)	Antiandrogenic	50 mg/kg/d (NOAEL, ↓ AGD in F1 & F2 pups) (Tyl et al., 2004)	2 mg/kg/d (LOAEL, ↓ spermatocyte development on PND 21) (Lee et al., 2004)	4.8 mg/kg/d (NOAEL, ↓ testes weight, testicular atrophy in F1 & F2 males) (TherImmune Research	–	–	50 mg/kg/d (NOEL, Transient ↓ in fetal testosterone, MNGs) (Clewell et al., 2013a)

Country (Reference)	Hazard Type	Point of Departure Used in Cumulative Risk Assessments[a] [b]					
		BBP	DBP	DEHP	DIBP	DCHP	DINP
				Corporation, 2004)			
United States (U.S. CPSC, 2014)	Case 1 Antiandrogenic PODs from (Kortenkamp and Faust, 2010)	66 mg/kg/d (BMDL, ↓ fetal testosterone synthesis, as reported by (NRC, 2008))	20 mg/kg/d (BMDL, ↓ fetal testosterone synthesis, as reported by (NRC, 2008))	3 mg/kg/d (NOAEL, ↑NR) (Christiansen et al., 2009)	40 mg/kg/d (BMDL, ↓ fetal testosterone synthesis, as reported by (NRC, 2008))	–	750 mg/kg/d (LOAEL, ↓ fetal testosterone synthesis) (Borch et al., 2004; Gray et al., 2000)
	Case 2 Antiandrogenic PODs from (Hannas et al., 2011)	5 mg/kg/d (NOAEL, testosterone modulated effects)	5 mg/kg/d (NOAEL, testosterone modulated effects)	5 mg/kg/d (NOAEL, testosterone modulated effects)	5 mg/kg/d (NOAEL, testosterone modulated effects)	–	11.5 mg/kg/d (NOAEL, testosterone modulated effects)
	Case 3 Antiandrogenic PODs from *de novo* CPSC review	50 mg/kg/d (NOAEL, ↑NR, ↓AGD) (Tyl et al., 2004)	50 mg/kg/d (NOAEL, ↑NR, ↓AGD) (Zhang et al., 2004; Mylchreest et al., 2000)	5 mg/kg/d (NOAEL, ↓spermatocytes & spermatids, reproductive tract malformations, delayed vaginal opening) (Blystone et al., 2010; Andrade et al., 2006a; Grande et al., 2006)	125 mg/kg/d (NOAEL, ↓AGD) (Saillenfait et al., 2008)	–	50 mg/kg/d (NOAEL, ↑NR) (Boberg et al., 2011)

[a] Some CRAs included phthalates not currently undergoing risk evaluation at EPA. PODs for these phthalates are not shown.
[b] NICNAS concluded that fetal testosterone changes are not well characterized for BBP in available studies but considered BBP to be equivalent to DBP in reducing fetal testosterone. Therefore, NICNAS used the DBP NOAEL for reduced fetal testosterone for BBP.
AGD = anogenital distance; BW = body weight; LOAEL = lowest-observed-adverse-effect level; MNG = multinucleated gonocytes; NOAEL = no-observed-adverse-effect level; NR = nipple retention; PND = postnatal day; RPS = rat phthalate syndrome; TP = testicular pathology

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Appendix B Additional Toxicity Information

B.1 Dose-Response Data for Effects on Fetal Testicular Gene Expression and Testosterone Production

Dose Response studies	Positive Phthalates	DOSE	TPROD	Nr0b1	Star	Cyp11a1	Cyp11b2	Hsd3b	Cyp17a1	Lhcgr	Scarb1	Insl3	Dhcr7	Cyp11b1	Rhox10	Wnt7a	Inha
DEHP HARLAN SD	Positive	100	62.3	0.86	0.55	0.68	0.50	0.75	0.59	0.36	0.57	0.65	0.67	0.23	0.80	0.54	1.00
		300	26.5	0.69	0.19	0.29	0.44	0.35	0.28	0.32	0.22	0.28	0.48	0.07	0.88	0.68	0.43
		600	11.1	0.64	0.16	0.19	0.78	0.29	0.07	0.13	0.15	0.17	0.54	0.02	0.88	0.84	0.34
		900	8.9	0.53	0.19	0.19	0.80	0.32	0.07	0.14	0.16	0.17	0.65	0.03	0.66	0.87	0.28
DBP HARLAN SD	Positive	1	129	1.01	1.01	0.87	1.06	1.05	0.81	1.39	1.13	0.82	0.98	0.89	1.01	0.75	0.82
		10	99.1	1.03	0.97	1.03	0.86	1.04	1.05	1.57	1.00	0.97	0.88	0.85	0.93	0.80	1.11
		33	92.2	1.16	1.56	1.38	0.91	1.16	1.06	1.84	1.41	0.86	1.23	1.14	1.08	1.36	1.05
		50	85.5	1.20	1.06	1.24	1.60	0.95	1.00	1.39	1.27	0.83	1.07	1.35	1.33	1.61	0.98
		100	67.6	0.95	0.60	0.89	0.71	0.76	0.79	1.30	0.70	0.77	0.72	0.49	0.80	0.76	0.79
		300	23.2	0.82	0.41	0.58	0.43	0.69	0.51	1.05	0.49	0.40	0.86	0.19	0.91	1.36	0.58
		750	10.7	0.54	0.23	0.21	0.80	0.40	0.13	0.23	0.18	0.24	0.71	0.04	0.59	0.63	0.49
BBP HARLAN SD	Positive	11	108.3	1.10	0.73	0.78	0.55	0.99	1.11	1.19	0.55	1.17	0.59	0.44	1.09	0.81	2.01
		33	89.0	0.73	0.70	0.55	0.32	0.68	0.88	0.68	0.64	0.63	0.58	0.81	0.66	0.44	0.83
		100	73.2	0.81	0.37	0.61	0.65	0.44	0.67	0.55	0.39	0.59	0.57	0.26	0.58	0.67	0.73
		300	33.9	0.91	0.32	0.46	0.41	0.43	0.35	0.57	0.33	0.43	0.61	0.10	0.92	1.01	0.53
		600	24.6	0.64	0.22	0.27	0.42	0.32	0.16	0.20	0.17	0.21	0.53	0.05	0.59	0.89	0.37
		900	15.4	0.55	0.20	0.22	0.15	0.33	0.11	0.19	0.15	0.23	0.72	0.02	0.61	0.72	0.36
DiBP HARLAN SD	Positive	100	86	0.88	0.89	0.99	1.34	0.93	0.99	1.30	0.93	1.03	0.91	1.07	0.90	0.76	0.87
		200	73.9	0.65	0.63	0.71	0.61	0.58	0.61	0.68	0.48	0.62	0.75	0.41	0.93	0.85	0.55
		300	34.2	1.13	0.33	0.42	1.04	0.52	0.39	0.62	0.37	0.47	0.69	0.14	0.79	1.21	0.60
		500	28.7	0.95	0.40	0.47	0.45	0.53	0.48	0.66	0.33	0.45	0.68	0.12	1.12	0.86	0.74
		600	15.8	0.81	0.30	0.28	0.54	0.49	0.31	0.50	0.26	0.31	0.62	0.09	0.83	0.79	0.54
		750	16	0.88	0.45	0.53	0.76	0.58	0.51	0.66	0.48	0.50	0.79	0.41	0.84	1.00	0.69
		900	9.8	0.84	0.26	0.24	1.14	0.40	0.18	0.38	0.24	0.30	0.79	0.08	0.86	0.48	0.59
DINP HARLAN SD	Positive	500	70.5	0.76	0.54	0.71	0.66	0.60	0.64	0.90	0.52	0.73	0.81	0.31	0.94	1.02	0.66
		750	63.1	0.82	0.46	0.71	0.76	0.63	0.56	0.85	0.49	0.79	0.82	0.26	0.89	0.93	0.76
		1000	43.1	0.66	0.33	0.54	0.54	0.46	0.40	0.64	0.34	0.56	0.63	0.15	0.78	0.73	0.63
		1500	31.6	0.65	0.27	0.39	0.58	0.39	0.30	0.62	0.25	0.51	0.64	0.06	0.76	0.89	0.63
DCHP HARLAN SD	Positive	33	74.6	0.86	0.87	1.00	1.38	0.79	0.68	0.20	0.69	0.88	0.91	0.97	1.02	1.14	1.06
		100	38.9	0.61	0.37	0.39	2.08	0.36	0.24	0.16	0.32	0.51	0.85	0.26	1.02	0.84	0.41
		300	26.5	0.63	0.22	0.27	2.57	0.24	0.12	0.09	0.18	0.36	0.64	0.07	0.85	0.97	0.34
		600	20.7	0.60	0.21	0.25	1.40	0.26	0.14	0.16	0.13	0.28	0.64	0.06	1.16	1.14	0.42
		900	24.2	0.48	0.16	0.18	1.06	0.25	0.11	0.13	0.13	0.22	0.53	0.03	0.96	1.09	0.34
DIDP CRSD	Negative	300	120	1.03	1.32	1.24	1.51	1.14	1.26	1.53	1.26	1.22	1.24	1.22	1.08	1.49	1.03
		750	114	1.10	1.11	1.11	0.96	0.97	1.11	1.33	1.02	1.27	1.05	0.84	0.88	1.04	1.25
		1000	105	1.12	1.15	1.17	2.09	1.04	1.18	1.63	1.37	1.00	1.24	1.09	1.31	1.49	1.18
		1500	101	1.08	1.21	1.10	1.38	1.08	1.14	1.26	1.18	1.20	1.16	1.06	1.06	1.42	1.08

Figure_Apx B-1. Dose-Response Data from Gray et al. (2021).
Figure adapted from Gray et al. (2021).
Doses are in units of mg/kg/day. *Ex vivo* fetal testicular testosterone production (TPROD) presented as percent control. mRNA values are presented as fold change versus control. Values highlighted in yellow are statistically significantly different from controls.

B.2 DEHP Study Summaries

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Gray et al., 2021)	Harlan SD rat; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (100) - ↓ fetal testicular expression of *Insl3* (300) & steroidogenic genes (*e.g.*, *StAR* (300), *Cyp11a1* (300), *Cyp11b2* (300), *Cyp17a1* (300), *Dhcr7* (300), *Cyp11b1* (100), *Hsd3b* (300), *Scarb1* (300))
	CRSD rat; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (300) & steroidogenic genes (*e.g.*, *StAR* (300), *Cyp11a1* (600), *Cyp11b2* (600), *Cyp17a1* (600), *Dhcr7* (600), *Cyp11b1* (300), *Hsd3b* (600), *Scarb1* (600))
(Hannas et al., 2011)	SD rats; gavage; GD 14–18; 0, 100, 300, 500, 625, 750, 875 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (625), *StAR* (500), *Cyp11a* (500)
	Wistar rats; gavage; GD 14–18; 0, 100, 300, 500, 625, 750, 875 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (500), *StAR* (500), *Cyp11a* (500)
(Furr et al., 2014)	SD rats; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (100)
(Howdeshell et al., 2008)	SD rats; oral/gavage; GD 8–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300)
(Wilson et al., 2004)	SD rats; oral/gavage; GD 14–18; 0, 750 mg/kg/day; GD 18	- ↓ Testicular *Insl3* mRNA (750/GD 18) - ↓ Testicular testosterone production (750/GD 18)
(Saillenfait et al., 2009a)	SD rats; oral/gavage; GD 12–21, 0, 625 mg/kg/d; PND 70–84	- ↑ Reproductive malformations (small penis, cleft prepuce, hypospadias, cleft phallus with exposed os penis, vaginal pouch, undescended testes) (625/PND 70–84)
	SD rats; oral/gavage; GD 12–21, 0, 500 mg/kg/d; PND 1–120	- ↓ AGD (500/PND 1) - NR (500/PND 12–14, 70–78, 111–120) - Reproductive tract malformations (cleft prepuce, hypospadias, cleft phallus with exposed os penis, undescended testes (unilateral and bilateral), underdeveloped testes, malformed epididymis, absent SV or prostate) (500/PND 70–78, 111–120) Unaffected outcomes - PPS
(Saillenfait et al., 2013)	SD rats; oral/gavage; GD 12–19; 0, 50, 625 mg/kg/d; GD 19	- ↓ fetal testicular testosterone (50) - ↓ fetal testicular expression of steroidogenic genes (*SR-B1* (50), *StAR* (50), *P450scc* (625), *P450c17* (625), *3β-HSD* (625))
(Spade et al., 2018)	SD rats; oral/gavage; GD 17–21; 0, 750 mg/kg/d; GD 21	- ↓ *ex vivo* fetal testicular testosterone production (750) - ↑ Incidence of MNGs (750)
(Borch et al., 2004)	Wistar rats; oral/gavage; GD 7–21; 0, 300, 750 mg/kg/d; GD 21, PNDs 3–190	- ↓ *ex vivo* fetal testes testosterone production and testosterone content (300/GD 21) - ↓ plasma testosterone (750/GD 21) - ↑ luteinizing hormone (750/GD 21) - ↓ AGD (750/PND 3) - NR (750/PND 13)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
		Unaffected outcomes - Serum testosterone (PND 22, 190) or testicular testosterone content (PND 190)
(Parks et al., 2000)	SD rats; oral/gavage; GD 14–PND 2; 0, 750 mg/kg/day; GD 17, 18, 20, PND 2	- ↓ Testicular testosterone production (750/GD 17, 18, 20, PND 2) - ↓ Testicular testosterone (750/GD 17, 18, PND 2) - ↓ absolute testis weight (750/GD 20, PND 2) - ↓ AGD (750/PND 2) - Testicular pathology (Leydig cell hyperplasia, Leydig cell aggregation, ↑ # of gonocytes in seminiferous cord, MNGs) (750/PND 2)
(Borch et al., 2006b)	Wistar rats; oral/gavage; GD 7–21; 0, 10, 30, 100, 300, mg/kg/d; GD 21	- ↓ *ex vivo* fetal testes testosterone production and testosterone content (300/GD 21) - ↓ steroidogenic gene (*SR-B1* (300), *StAR* (100), *P450scc* (300)) and *Insl3* (300) expression - Testicular pathology (↑ gonocytes (100), MNGs (100), vacuolization of Sertoli cells (300), Leydig cell clustering (300) Unaffected outcomes - Plasma testosterone (GD 21)
(Culty et al., 2008)	SD rats; oral/gavage; GD 14–PND 0; 0, 234, 469, 700, 750, 938, 1250 mg/kg/d; PND 21 or 60	- ↓ AGD (1250/PND 60) - Cryptorchidism (938/PND 60) - ↑ Leydig cell volume (234/PND 60) - ↓ serum testosterone (234) Unaffected outcomes - Testis weight (PND 21, 60); germ cell volume (PND 60)
	SD rats; oral/gavage; GD 14–PND 0; 0, 117, 234, 469, 938 mg/kg/d; GD 20 or PND 3	- ↓ *ex vivo* fetal testicular testosterone production (117/GD 20) - ↓ *ex vivo* fetal testicular testosterone production (938/PND 3)
	SD rats; oral/gavage; GD 14–PND 0; 0, 234, 469, 938 mg/kg/d; GD 19, PND 3, 21, 60	- ↓ *Insl3* and steroidogenic (*Cyp11a1*, *Cyp17a1*) gene expression (469 /GD 20) - ↑ mRNA expression of *Cyp11a1*, *Cyp17a1*, *Ins3* reported at PNDs 3, 21, 60 Unaffected outcomes - Star mRNA (GD 19, PNDs 3, 21, 60)
(Vo et al., 2009)	SD rats; oral/gavage; GD 11–21; 0, 10, 100, 500 mg/kg/d; GD 21, PND 1, 63	- ↓ serum testosterone (500/GD 21) - NR (500/PND 13) - Sperm parameters (concentration (500/PND 63), viability (500/PND 63), motility (10/PND 63) - Hypospadias (500/PND 63) - Cryptorchidism (500/PND 63) Unaffected outcomes - Serum testosterone (PND 63); AGD (PND 63); Testis, epididymis, prostate weight (PND 63)
(Lin et al., 2008)	Long–Evans rats; oral/gavage; GDs 2–20; 0, 10, 100, 750; GD 21	- ↓ AGD (750) - ↓ testicular testosterone (750) - ↓ mRNA expression of steroidogenic (*Scarb1* (750), *StAR* (750), *Cyp11a* (100), *Cyp19* (100)) genes and *Insl3* (750) mRNA

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
		- ↓ absolute testes weight (100) - Leydig cell aggregation/Increased # of Leydig cells per cluster (10)
(Martino-Andrade et al., 2008)	Wistar rats; oral/gavage; GDs 13–21; 0, 150 mg/kg/d; GD 21, PND 13, PND 90	- ↓ absolute SV weight (150/PND 90) Unaffected outcomes - Testicular testosterone, seminiferous cord diameter, incidence of MNGs, AGD (GD 21) - NR (PND 13) - PPS - Testis, epididymis, prostate, LABC weight (PND 90) - # spermatids/testis (PND 90)
(Jarfelt et al., 2005)	Wistar rats; oral/gavage; GD 7–PND 17; 0, 300, 750; PND 3, 13, 33, 190	- ↓ AGD (300/PND 3) - NR (300/PND 13) - Sperm parameters (# sperm per cauda epididymis (severely reduced in 3 males at 300 mg/kg); sperm motility (severely reduced in 3 males at 300 and 2 males at 750 mg/kg) (neither effect was statistically significant) - ↓ absolute weight of paired testes (750/PND 22); ventral prostate (300/PND 190); LABC (300/PND 190) - Reproductive malformations (small testis or lack of one testis (300/PND 22, 190), small/malformed epididymis (300/PND 22, 190), malformed SVs (300/PND 22, 190), Cryptorchid testis (750/PND 22, 190), hypospadias (300/PND 22) - Testicular pathology (300/PND 22, 190) Unaffected outcomes - Absolute epididymis (PND 22, 190), prostate (PND 22), SV (PND 22, 190), paired testis (PND 190), LABC (PND 22)
(Gray et al., 2000)	SD rats; oral/gavage; GD 14–PND 3; 0, 750 mg/kg/d; PND 2–mature adults (3–7 months of age)	- ↓ AGD (750/PND 2) - NR (750/PND 13) - Permanent nipples (750/3–7 months) - ↓ absolute testes, LABC, SV, ventral prostate, glans penis, epididymis, cauda epididymis, caput-corpus epididymis weight (750/3–7 months) - Incomplete PPS due to genital malformations (750) - Reproductive tract malformations (cleft phallus, hypospadias, vaginal pouch, SV and epididymal agenesis, fluid filled testis, small testis, testis absent, abnormal gubernaculum) (750/3–7 months) - Undescended testes (750/3–7 months) - Testicular pathology (*e.g.*, MNGs) Unaffected outcomes - Mean age at PPS; serum testosterone (3–7 months)
(Moore et al., 2001)	SD rats; oral/gavage; GD 9–PND 21; 0, 375, 750, 1,500 mg/kg/d; PND 1–112	- ↓ AGD (750/PND 1) - NR (375/PND 14) (% litters with males with NR) - Incomplete PPS (375) - ↑ Litters with undescended testes (750/PND 21) - ↓ absolute testes (750/PND 21, 63), epididymis (750/PND 21, 63. 105), glans penis (750/PND 21, 63, 105) - ↓ epididymal sperm number (750/PND 63)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
		- Anterior prostate agenesis (750) - ↓ masculine sexual behavior (↓ incidence of mounting) (1500/PND 77)
(Howdeshell et al., 2007)	SD rats; oral/gavage; GD 14–18; 0, 500 mg/kg/d; PND 3, PND 14, adult (7–11 months of age)	- ↓ AGD (500/PND 3) - NR (500/PND 14, adult) - ↓ absolute LABC weight (500/adult) - Low incidence of hypospadias, testes and epididymal malformations, SV, vas deferens and gubernacular agenesis reported (500/adult; not statistically significant) Unaffected outcomes - Absolute glans penis, ventral prostate, SV, testes, epididymis weight (500/adult)
(Gray et al., 2009)	SD rats; oral/gavage; GD 8–PND 17; 0, 11, 33, 100, 300 mg/kg/d; PND 2, PND 13, adult (7 months of age)	- ↓ AGD (300/PND 2) - NR (300/PND 13, adult) - ↓ Absolute glans penis, ventral prostate, LABC, Cowper's gland, epididymis weight (300/Adult); SV (100/adult) - ↑ incidence of testicular pathologies and malformations such as hypospadias (11/adult) Unaffected outcomes - Age at PPS; Serum testosterone (adult)
	SD rats; oral/gavage; GD 8–PND 64; 0, 11, 33, 100, 300 mg/kg/d; PND 64	- ↓ Absolute ventral prostate, SV, LABC, Coper's gland, epididymis weight (300/PND 64) - ↓ Epididymal sperm count (300/PND 64) - Delayed PPS (300) - ↑ incidence of testicular pathologies and malformations such as hypospadias (11/PND 64) Unaffected outcomes - Serum testosterone (PND 64)
(Li et al., 2013)	SD rat; oral/gavage; GD 12–19; 0, 500, 750, 1000 mg/kg/d; PND 1, 30, 60	- ↓ AGD (500/PND 1) - ↓ penile length (750/PND 30) - Hypospadias (500/PND 1, 60)
(Christiansen et al., 2010)	Study (S) 1 Wistar rat; oral/gavage; GD 7–PND 16; 0, 10, 30, 100, 300, 600, 900 mg/kg/d; PND 1, 12, 16 Study (S) 2 Wistar rat; oral/gavage; GD 7–PND 16; 0, 3, 10, 30, 100 mg/kg/d; PND 1, 12, 16	- ↓ AGD (10/PND 1; S1) (100/PND 1; S2) (10/PND 1; combined) - NR (10/PND 12; S1) (none/PND 12; S2) (10/PND 12; combined) - Mild external genital dysgenesis (100/PND 16; S1) (3/PND 16; S2) (3/PND 16; combined) - ↓ absolute testis weight (600/PND 16; S1 & combined); ventral prostate (30/PND 16; S1) (10/PND 16; combined); LABC (10/PND 16; S1 & S2 & combined) - ↓ Diameter of seminiferous tubules (300/PND 16; S1 & combined) - Testicular pathology (↓ germ cells, focal Leydig cell hyperplasia) (300, PND 16; S1) Unaffected outcomes - Hypospadias (S1 or S2)
(Andrade et al., 2006b)	Wistar rats; oral/gavage; GD 6–PND 21; 0.015, 0.045, 0.135, 0.405,	- ↑ absolute testis weight (5/PND 22) - ↓ AGD (405/PND 22)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
	1.215, 5, 15, 45, 135, 405 mg/kg/d; PND 1, 13, 22, 33	- NR (405/PND 13) - Delayed PPS (15) - Testicular pathology (*e.g.*, MNGS, acute interstitial hemorrhage/loosening of connective tissue, ↓ germ cell differentiation in seminiferous tubules) (135/PND 1, 22) Unaffected outcomes: - Seminiferous tubule diameter, testes descent, epididymis weight, testicular testosterone (PND 1), malformations of external genitalia (*e.g.*, hypospadias)
(Blystone et al., 2010; TherImmune Research Corporation, 2004)	SD rats; oral/diet; 3-generation (continuous breeding protocol); 1.5 (control), 10, 30, 100, 300, 1000, 7500, 10000 ppm (eq. 0.12, 0.78, 2.4, 7.9, 23, 77, 592, 775 mg/kg/d (F0); 0.09, 0.48, 1.4, 4.9, 14, 48, 391, 543 mg/kg/d (F1); 0.1, 0.47, 1.4, 4.8, 14, 46, 359 mg/kg/d (F2))	- ↓ AGD (7500 ppm/PND 1 (F1, F2, F3)) - NR (7500 ppm/PND 12–13 (F3)) - Delayed PPS (7500 ppm/F1, F3; 10 ppm/F2) - Delayed testes descent (7500 ppm/F1, F3; 30 ppm/F2) - ↓ absolute and/or relative cauda, epididymis, testis weight (7500 ppm/adult (F1, F2, F3)) - Gross necropsy findings (small or aplastic testis, SV, epididymis or cauda) (300 ppm/adult (F1, F2)) - Testicular pathology (seminiferous tubule atrophy, failure of sperm release; sloughed epithelial cells, residual bodies in epididymis) (7500 ppm/adult (F1, F2)) - ↓ sperm/cauda (or mg cauda) & ↓ spermatid/testes (or mg testes) (7500 ppm/adults (F1, F2, F3)) - ↓ Pregnancy index (10,000 ppm/F1 (no F2 litters produced); 7500/F2)
(Pocar et al., 2012)	CD-1 mice; oral/diet; GD 0.5–PND 21; 0, 0.05, 5, 500* mg/kg/d; PND 42 *Only 1 out of 10 high-dose dams produced a litter. This dose group was excluded from most analyses	- ↓ absolute testes weight at 0.05, but not 5 mg/kg/d (PND 42) - ↓ absolute SV weight (0.05/PND 42) - ↓ Sperm count and sperm viability (0.05/PND 42) - ↓ testicular *cyp19a1* mRNA (5/PND 42) Unaffected outcomes - AGD (PND 42); testicular *StAR*, *CYP17a1* mRNA (PND 42)
(Liu et al., 2008)	C57BL/6 mice; oral/gavage; e12–17; 0, 100, 200, 500 mg/kg/d; e19	- ↓ AGD (100) - ↓ urethra length (200) - Hypospadias (100)
(Do et al., 2012)	CD-1 mice; oral/gavage; GD 9–18; 0, 0.0005, 0.001, 0.005, 0.5, 50, 500 mg/kg/d; GD 18	- ↓ absolute testes weight (50) - ↑ serum testosterone (0.0005) Unaffected outcomes - AGD, testicular testosterone
(Gaido et al., 2007)	C57B1/6J; oral/gavage; GD 15–17; 0, 1000 mg/kg/d MEHP; GD 17 (8–hours post dosing)	Unaffected outcomes - Testicular testosterone
	C57B1/6J; oral/gavage; GD 14–16; 0, 500 mg/kg/d MEHP; GD 17 (24–hours post dosing)	Unaffected outcomes - Testicular testosterone

[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation.
AGD = anogenital distance; e = embryonic day; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle

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Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL (mg/kg/day)/timing of evaluation (if different than listed under Study Design))
(Ema et al., 2003)	Wistar rats; oral/gavage; GD 15–17; 0 , 167, 250, 375 mg/kg/d MBP; GD 21	- ↓ AGD (250) - Cryptorchidism (250)
(Gray et al., 2021)	Harlan SD rat; oral/gavage; GD 14–18; 0, 11, 33, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (100) - ↓ fetal testicular expression of *Insl3* (33) and steroidogenic genes (*StAR* (100), *Cyp11a1* (33), *Cyp11b2* (33), *Cyp17a1* (300), *Dhcr7* (11), *Cyp11b1* (11), *Hsd3b* (100), *Scarb1* (33))
	Charles River SD rat; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (600) and steroidogenic genes (*StAR* (600), *Cyp11a1* (600), *Cyp17a1* (600), *Dhcr7* (900), *Cyp11b1* (600), *Hsd3b* (900), *Scarb1* (600))
(Howdeshell et al., 2008)	SD rats; oral/gavage; GD 8–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300)
(Furr et al., 2014)	SD rats; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d (Block 36) or 0, 11, 33, 100 mg/kg/d (Block 37); GD 18	- Block 36: ↓ *ex vivo* fetal testes testosterone production (100) - Block 37: No effect on testosterone
(Gray et al., 2000)	SD rats; oral/gavage; GD 14–PND 3; 0, 750 mg/kg/d; PND 2–mature adults (3–7 months of age)	- ↓ AGD (750/PND 2) - NR (750/PND 13) - Permanent nipples (750/3–7 months) - ↓ absolute testes, LABC, SV, ventral prostate, glans penis, epididymis, cauda epididymis, caput-corpus epididymis weight (750/3–7 months) - Incomplete PPS due to genital malformations (750) - Reproductive tract malformations (cleft phallus, hypospadias, vaginal pouch, SV and epididymal agenesis, fluid filled testis, small testis, testis absent, abnormal gubernaculum) (750/3–7 months) - Undescended testes (750/3–7 months) Unaffected outcomes - Mean age at PPS; serum testosterone (3–7 months)
(Spade et al., 2018)	SD rats; oral/gavage; GD 17–21; 0, 750 mg/kg/d; GD 21	- ↓ *ex vivo* fetal testicular testosterone production (750) - ↑ Incidence of MNGs (750)
(Wilson et al., 2004)	SD rats; oral/gavage; GD 14–18; 0, 750 mg/kg/day; GD 18	- ↓ Testicular *Insl3* mRNA (750/GD 18) - ↓ Testicular testosterone production (750/GD 18)
(Ahmad et al., 2014)	Albino rats; oral/gavage; GD 14–21; 0, 4, 20, 100 mg/kg/d; PND 5, 25, 75	- ↓ 17β-HSD activity (trend/PND 75) - ↓ serum testosterone (100/PND 75) - ↓ absolute epididymis & prostate weight (100/PND 75) - ↓ cauda epididymal sperm count, ↓ sperm motility, ↑ sperm abnormalities (100/PND 75) Unaffected outcomes - AGD (PND 5, 25); testis descent; testis & SV weight (PND 75)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL (mg/kg/day)/timing of evaluation (if different than listed under Study Design))
(Nagao et al., 2000)	SD rats; oral/gavage; 2-generation; 0, 20, 100, 500 mg/kg/day	- ↓ AGD (500/F1 PND 0) - ↓ serum testosterone (500/F0 & F1 adults) - ↓ absolute testes & epididymis weight (500/F1 PND 22) - ↓ absolute testes, epididymis, ventral prostate weight (500/F1 adults) - Testicular pathology (↓ spermatocytes in seminiferous tubules (500/F1 PND 22); atrophy of seminiferous tubules (500/F1 adults); ↓ germ cells in seminiferous tubule (500/F1 adults); edema, interstitium (500/F1 adults); decreased sperm in epididymis, with cell debris (500/F1 adults) - Delayed PPS (500/F1) <u>Unaffected outcomes</u> Mating, fertility, delivery indices (F0, F1); gestation length (F0, F1); absolute reproductive organ weight (testes, epididymides, ventral prostate, SV; F0 adults); absolute SV weight (F1 adults); testicular pathology (F0); sperm motility and concentration (F0, F1 adults); serum testosterone (F1 PND 22); hypospadias (F1), cryptorchidism (F1)
(Aso et al., 2005)	Crj:CD(SD)IGS rats; oral/gavage; 2-generation; 0, 100, 200, 400 mg/kg/day	- Low rate for completed PPS (400/F1) - ↓ absolute epididymis (400/F0 adults; 200/F1 adults) & SV (400/F1 adults) weight - ↑ incidence of small testes (400/F1 adult), softening of testes (100/F1 adult); ↑ incidence of small or hypoplastic epididymides (400/F1 adult) - Testicular pathology (*e.g.*, Leydig cell hyperplasia (400/F0 & 400/F1 adults), diffuse atrophy of testicular seminiferous tubules (400/F1 adults); ↓ spermatozoa in epididymides (400/F0; 100/F1 adults), ↓ germ cells in epididymal lumen (100/F1 adults), bilateral or unilateral partial aplasia or unilateral aplasia of epididymides (400/F1 adults)) - ↓ AGD (100/F2 pups) <u>Unaffected outcomes</u> - Estrous cyclicity, mating index, days required for mating, gestation length, # implantations, fertility index, delivery index, gestation index, # of pups delivered, # of sperm in testis and epididymis, epididymal sperm motility or morphology (F0 and F1 parents); serum hormones (FSH, LH, testosterone, estradiol (F0 and F1 parents); absolute testis and ventral prostate weight (F1 adults); AGD (F1 pups)
(Tyl et al., 2004)	CD rats; oral/diet; 2-generation; 0, 750, 3750, 11,250 ppm (eq. 0, 50, 250, 750 mg/kg/d)	- ↓ Mating and fertility indices (750/F1) - ↓ epididymal sperm concentration & motility (750/F1 adults) - ↓ absolute testes, epididymis, prostate, SV weight (750/F1 adult) - ↓ absolute testes (250/F1 weanlings (PND 21); 750/F2 weanlings (PND 21)) and epididymis weight (750/F1 weanlings (PND 21)) - ↓ AGD (250/F1 and F2 at PND 0) - NR (750/F1 and F2 at PND 11–13) - Delayed PPS (750/F1)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL (mg/kg/day)/timing of evaluation (if different than listed under Study Design))
		- Gross malformations (undescended testes) (750/F1 pups (PND 4)) - Gross malformations (missing epididymis (whole or part), epididymis reduced in size, missing testes, testes reduced in size, and undescended testis(es) (750/F1 weanlings (PND 21)) - Gross malformations (hypospadias, missing reproductive organ or portion(s) of organs and/or abnormal organ size and/or shape) (750/F1 adults) - Gross malformations (missing SVs, missing epididymides) (750/F2 pups (PND 4)) - Testicular pathology (epididymal aspermia, testis dilation, seminiferous tubule degeneration & atrophy) (750/F1 adult) <u>Unaffected outcomes</u> - Mating, fertility, gestation, pregnancy indices (F0); gestational and pregnancy indices (F1); absolute testes, epididymis, prostate, SV weight (F0); epididymal sperm concentration and motility (F0 adults)
[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation. AGD = anogenital distance; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle		

B.4 DBP Study Summaries

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Mylchreest et al., 1998)	SD rats; oral/gavage; GD 3–PND 20; 0, 250, 500, 750 mg/kg/d; PND 1, adults (PND 100)	- ↓ AGD (500/PND 1) - ↓ absolute reproductive organ weight at PND 100 (testis (500), epididymis (750), SVs (500), prostate (750)) - ↑ Reproductive malformations at PND 100 (*e.g.*, hypospadias (250), nonscrotal testes (250), epididymal dysgenesis/agenesis (250), SV agenesis (500)) - Testicular pathology at PND 100 (*e.g.*, degeneration and atrophy of seminiferous tubules (250))
(Mylchreest et al., 1999)	SD rats; oral/gavage; GD 12–21; 0, 100, 250, 500 mg/kg/d; PND 1, 14, adults (3 months of age)	- ↓ AGD (250/PND 1) - NR (250/PND 14) - Delayed PPS (100) - ↓ absolute reproductive organ weight in adults (testis, epididymis, SV (500)) - Reproductive malformations in adults (*e.g.*, hypospadias (500), prostate agenesis (500), epididymal dysgenesis/agenesis (250)) - Cryptorchidism (250/adults) - ↑ Testicular pathology in adults (*e.g.*, degeneration of seminiferous epithelium (250), interstitial cell hyperplasia or adenoma (500))
(Mylchreest et al., 2000)	SD rats; oral/gavage; GDs 12–21; 0, 0.5, 5, 50, 100, 500 mg/kg/d;	- ↓ AGD (500/PND 1) - NR (100/PND 14)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
	PND 1–sexual maturity (PND 100–120)	- ↓ reproductive organ weight (testis, epididymis, prostate, LABC) (500/sexual maturation) - Reproductive tract malformations (absent or malformed epididymis & vas deferens; hypospadias; exposed os penis) (500/sexual maturation) - Testicular pathology (seminiferous tubule degeneration, focal interstitial hyperplasia, adenoma) (500/sexual maturation) Unaffected outcomes - PPS; absolute vas deferens and SV weight
(Mylchreest et al., 2002)	SD rats; oral/gavage; GD 12–21; 0, 500 mg/kg/day; GDs 14, 16, 18, 21	- ↓ fetal testicular testosterone (500/GD 18, 21) - Testicular pathology (Leydig cell hyperplasia (500/GD 16, 18, 21), testis atrophy (500/GD 18, 21), reduced epididymal ducts (500/GD 21), MNGs (500/GD 21))
(Lehmann et al., 2004)	SD rats; oral/gavage; GDs 12–19; 0, 0.1, 1, 10, 50, 100, 500 mg/kg/day; GD 19	- ↓ testicular mRNA & protein expression of Insl3 (500), StAR (50), P450scc (50), CYP17 (500), SR-B1 (50)) - ↓ fetal testicular testosterone (50)
(Wilson et al., 2004)	SD rats; oral/gavage; GD 14–18; 0, 750 mg/kg/day; GD 18	- ↓ Testicular *Insl3* mRNA (750/GD 18) - ↓ Testicular testosterone production (750/GD 18)
(Spade et al., 2018)	SD rats; oral/gavage; GD 17–21; 0, 750 mg/kg/d; GD 21	- ↓ *ex vivo* fetal testicular testosterone production (750) - ↑ Incidence of MNGs (750)
(Howdeshell et al., 2008)	SD rats; oral/gavage; GDs 8–18; 0, 33, 50, 100, 300, 600; GD 18	- ↓ *ex vivo* fetal testicular testosterone production (300)
(Furr et al., 2014)	SD rats; oral/gavage; GDs 14–18; 0, 33, 50, 100, 300; GD 18	- ↓ *ex vivo* fetal testicular testosterone production (100)
(Gray et al., 2021)	Harlan SD rat; oral/gavage; GD 14–18; 0, 1, 10, 33, 50, 100, 300, 750 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (100) - ↓ fetal testicular expression of *Insl3* (100) & steroidogenic genes (*e.g., StAR* (100), *Cyp11a1* (300), *Cyp17a1* (100), *Dhcr7* (750), *Cyp11b1* (100), *Hsd3b* (100), *Scarb1* (100))
(Drake et al., 2009)	Wistar rats; oral/gavage; e13.5–21.5; 0, 100, 500 mg/kg/day; adults (>12 weeks of age)	- ↓ AGD (500/adult) - Cryptorchidism (500/adult) - Hypospadias (500/adult) - ↓ penis length (500/adult) - ↓ absolute testis and ventral prostate weight (500/adult)
	Wistar rats; oral/gavage; e13.5–16.5; 0, 500 mg/kg/day; adults (e17.5)	- ↓ Testicular testosterone (500/e17.5) - ↓ Testicular *Cyp11a1* and *Star* mRNA (500/e17.5)
(Martino-Andrade et al., 2008)	Wistar rats; oral/gavage; GDs 13–21; 0, 100, 500 mg/kg/d; GD 21, PND 13, PND 90	- ↓ testicular testosterone (500/GD 21) - ↑ Seminiferous cord diameter (500/GD 21) - ↑ incidence of MNGs (500/GD 21) - ↓ AGD (500/GD 21) - ↑ NR (500/PND 13) Unaffected outcomes - PPS - Testes, epididymis, prostate, SV, LABC weight (PND 90) - # of spermatids/testis (PND 90)
(Struve et al., 2009)	CD rats; oral/diet; GD 12–19; 0, 112, 582 mg/kg/d (received	- ↓ Testicular testosterone (500/GD 19; 100/GD20)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
	doses); GD 19 (4-h post-dosing) or GD 20 (24-h post-dosing)	- ↓ *Cyp11a1*, *Cyp17a1*, *Scarb1*, *Star* mRNA (100/GD 19); ↓ *Cyp11a1*, *Cyp17a1*, *Scarb1* mRNA (500/GD 20) - ↓ AGD (500/GD 19, 20) - Leydig cell aggregation (100/GD 19, 20), ↑ seminiferous cord diameter (100/GD 19, 20), MNGs (100/GD 19, 20)
(Kuhl et al., 2007)	SD rats; oral/gavage; GD 18; 0, 100, 500 mg/kg/d; GD 19	- ↓ Testicular testosterone (500) - ↓ mRNA expression of *StAR*, *SR-B1*, *Cyp11a1*, *Cyp17* (100)
(Ema et al., 1998)	Wistar rats; oral/diet; GD 11–21; 0, 331, 555, 661 mg/kg/d; GD 21	- ↓ AGD (555) - ↑ incidence of internal malformations, including undescended testes (555)
(Mahood et al., 2007)	Wistar rats; oral/gavage; GD 13.5–20.5; 0, 4, 20, 100, 500 mg/kg/d; GD 21.5	- ↓ Testicular testosterone (100) - ↓ absolute testes weight (500) - ↑ incidence of MNGs (100) - Changes in Leydig cell distribution (*i.e.*, ↓ # of total Leydig cell clusters, ↑ occurrence of medium and large Leydig cell clusters) (100)
	Wistar rats; oral/gavage; GD 13.5–21.5; 0 , 4, 20, 100, 500 mg/kg/d; PND 90	- Increased incidence of infertility (*i.e.*, male produce offspring with untreated females) (500) - ↑ incidence of cryptorchidism (500) - ↑ incidence of Sertoli cell only tubules in cryptorchid testes (100) and increased incidence of Sertoli cell only tubules in scrotal testes (20) - ↓ absolute testes weight (500)
(Barlow et al., 2004)	SD rats; oral/gavage; GD 12–21; 0, 100, 500 mg/kg/d; PND 1, 13, 90, 180, 370 or 540	- ↑ incidence of gross lesions in testes (lesions included atrophy, enlarged, or absent organ), vas deferens (absent organ), SVs (small, malformed or absent lobes), prostate (small or absent), penis (hypospadias of varying severity) (500, PND 180, 370, 540) - Testicular pathology (testicular dysgenesis, germ cell degeneration, rete testes) (500/PND 180, 370, 540) - ↓ AGD (500/PND 1, PND 180) - NR (100/PND 13) (500/PND 180)
(MacLeod et al., 2010)	Wistar rats; oral/gavage; e13.5–15.5; 0, 500; e17.5	- ↓ Testicular testosterone content (31% reduction) (500)
	Wistar rats; oral/gavage; e13.5–20.5; 0, 500; e21.5	- ↓ Testicular testosterone content (60% reduction) (500) - ↓ AGD (500)
	Wistar rats; oral/gavage; e13.5–21.5; 0, 100, 500; PND 25	- ↓ absolute SV (500), ventral prostate (100), and testis (500) weight - ↓ penis length (500) - ↓ AGD (500)
(Li et al., 2009)	Wistar rats; oral/diet; GD 6–PND 28; 0, 0.037, 0.111, 0.333, 1% (eq. to 0, 31, 94, 291, 797 (GD 6–21); 0, 55, 165, 486, 1,484 (PND 0–15); 0, 47, 140, 433, 1,283 (PND 16–28) mg/kg/d); PND 1– 28	- ↓ AGD (291/PND 1) - ↓ relative testes weight (797/PND 28)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Li et al., 2015b)	Wistar rats; oral/gavage; e12.5–20.5; 0, 100, 300, 900; e15.5–PND 63	- ↓ AGD (300/PND 2, 21, 63) - Hypospadias (300/PND 63) - Cryptorchidism (900/PND 63) - ↓ absolute testis weight (300/e17.5) (900/e19.5, 21.5) - ↓ Leydig cell area (300/e15.5, 17.4) (900/e19.5, 21.5) - Leydig cell aggregation (300/e19.5, 21.5) - ↓ Testicular testosterone (300/e17.5) (900/e19.5, 21.5)
(Kim et al., 2010)	SD rats; oral/gavage; GD 10–19; 0, 250, 500, 700 mg/kg/d; PND 11 or 31	- Cryptorchidism (700/PND 11) - Hypospadias (700/PND 11) - NR (500/PND 11) - ↓ AGD (500/PND 11) - ↓ absolute testes, epididymis, ventral prostate, SV, Cowper's gland weight (700/PND 31); ↓ absolute SV weight (500/PND 31) - Degeneration of the seminiferous epithelium (700/PND 31) - ↓ serum DHT and testosterone (700/PND 31) - ↑ expression of estrogen receptor alpha mRNA (500/PND 31); ↓ 5α-reductase mRNA (700/PND 31) - Delayed PPS (250) - ↓ glans penis weight (700/PND 31)
(Ferrara et al., 2006)	Wistar rats; oral/gavage; e13.5–21.5; 0, 500 mg/kg/day; e15.5–21.5, PND 4–90	- MNGs (500/e19.5, 21.5, PND 4) - ↑ incidence of apoptotic gonocytes (500/e15.5, 17.5) - ↓ germ cell # per testis (500/e21.5, PND 4, 8, 15, 25) - ↓ germ cell proliferation index (500, PND 6); ↑ proliferation index (500, PND 25)
(Boekelheide et al., 2009)	SD rats; oral/gavage; GD 12–21; 0 0.1, 1, 10, 30, 50, 100, 500 mg/kg/d; GD 21	- ↓ testis size (qualitative); ↑ Seminiferous tubule size; Leydig cell aggregation (qualitative observations) - ↓ testis volume (50) - ↓ # of cells per testis (30) - ↓ # of seminiferous tubule cross-sections (50) - ↑ MNGs (100)
(van den Driesche et al., 2012)	Wistar rats; oral/gavage; e13.5–20.5; 0, 500, 750; e21.5	- ↑ frequency of large Leydig cell aggregates (500) - ↓ AGD (500) - ↓ Testicular testosterone (500)
	Wistar rats; oral/gavage; e15.5–18.5; 0, 750; e21.5	- ↑ frequency of large Leydig cell aggregates (750) - ↓ AGD (750) - ↓ Testicular testosterone (750)
	Wistar rats; oral/gavage; e19.5–20.5; 0, 500, 750; e21.5	- ↓ Testicular testosterone (500) <u>Unaffected outcomes</u> - Leydig cell aggregation - AGD
(van Den Driesche et al., 2015)	Wistar rats; oral/gavage; e13.5–16.5; 0, 4, 20, 100, 500 mg/kg/d; e17.5	- Impaired Sertoli-germ cell interactions (qualitative imaging) <u>Unaffected outcomes</u> - Germ cell aggregation
	Wistar rats; oral/gavage; e13.5–20.5; 0, 4, 20, 100, 500 mg/kg/d; e21.5	- ↑ Germ cell aggregation (20) - Impaired Sertoli-germ cell interactions (qualitative imaging) - MNGs (500)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
	Wistar rats; oral/gavage; e13.5–21.5; 0, 4, 20, 100, 500 mg/kg/d; PND 4	Unaffected outcomes - Germ cell aggregation - Sertoli-germ cell interactions (qualitative imaging)
(Gaido et al., 2007)	C57Bl/6J mice; oral/gavage; GD 14–16; 0, 1500 mg/kg/d DBP; GD 17 (24-h post final dose)	Unaffected outcomes - Testicular testosterone
	C57Bl/6J mice; oral/gavage; GD 14–16; 0, 1,000 mg/kg/d MBP; GD 17 (24-h post final dose)	Unaffected outcomes - Testicular testosterone
	C3H/HeJ mice; oral/gavage; GD 15–17; 0, 1,000 mg/kg/d MBP; GD 17 (8-h post final dose	Unaffected outcomes - Testicular testosterone
	C57Bl6 mice; oral/gavage; GD 16–18; 0, 250, 500 mg/kg/d DBP; GD 19	- ↑ Seminiferous cord diameter (250/GD 19) - ↑ MNGs & ↑ nuclei/MNG (250/GD 19)
	CD-1 mice; oral/gavage; GD 18; 0, 500 mg/kg DBP; GD 18 (2, 4, and 8 hours post dosing)	Unaffected outcomes - Steroidogenesis related genes (*Scarb1*, *StAR*, *Cyp11a1*, *Cyp17a1*, *Dhcr7)* (microarray experiment)
	CD-1 mice; oral/gavage; GD 14–17; 0, 250 mg/kg/d DBP; GD 17 (2 h post final dose)	Unaffected outcomes - mRNA expression of genes involved in cholesterol and lipid homeostasis and steroidogenesis was not decreased (Microarray experiment).
(Lee et al., 2004)	CD(SD)IGS rats; oral/feed; GD 15–PND 21; 0, 20, 200, 2000, 10000 ppm (eq. to 2–3, 14–29, 148–291, 712–1,372 mg/kg/d); PND 2, 14, 21; PNW 11, 20	- ↓ AGD (712/PND 2) - NR (712/PND 14) - ↓ relative testes weight (712/PND 21) - Testicular pathology (↓ spermatocyte development (2/PND 21), Leydig cell aggregation (148/PND 21), ↓ ductular cross sections of epididymal duct (148/PND 21), ↓ germ cell development (148/PNW 11) Unaffected outcomes - PPS; relative epididymis (PND 21, PNW 11, 20), testes (PNW 11, 20), prostate (PNW 11, 20), SV (PNW 11, 20) weight
(Howdeshell et al., 2007)	SD rats; oral/gavage; GD 14–18; 0, 500 mg/kg/d; PND 3, PND 14, adult (7–11 months of age)	- ↓ AGD (500/PND 3) - ↓ absolute LABC weight (500/adult) - Low incidence of testes and epididymal malformations, vas deferens and gubernacular agenesis (500/adult; not statistically significant) - Testicular degeneration (500/adults) Unaffected outcomes - NR (500/PND 14, adult); absolute testes, glans penis, ventral prostate, SV, epididymis weight (500/adult); hypospadias
(Jiang et al., 2007)	SD rats; oral/gavage; GD 14–18; 0, 250, 500, 750, 1000 mg/kg/d; PND 1, 7, 35, 70	- ↓ serum testosterone (250/PND 70) - ↓ AGD (500/PND 1) - Hypospadias (500) - Cryptorchidism (250) - ↓ relative testis & epididymis weight (500/PND 70)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Clewell et al., 2013b)	SD rats; oral/feed; GD 12–PND 14; 0, 7600 ppm (eq. 642–1,138 mg/kg/d); PND 2, 14, 49–50	- ↓ AGD (642/PND 2, 14) - NR (642/PND 14, 49–50) - Testicular pathology (MNGs, Leydig cell aggregates, ↑ # of gonocytes) (642/PND 2) - ↓ absolute and relative LABC & SV weight (642/PND 49–50) - Reproductive tract malformations (incomplete epididymis, flaccid epididymis, enlarged testis (unilateral); PND 49–50) - Testicular pathology (MNGs, Leydig cell aggregation, ↑ # of gonocytes; PND 2) (tubular/rete dilation, atrophic tubules, MNGs; PND 49–50) Unaffected outcomes - AGD (PND 49–50); testis testosterone (PND 49–50), gubernacular cord length (PND 49); absolute glans penis, cowpers gland weight, testis, epididymis, ventral prostate weight (PND 49–50)
(Zhang et al., 2004)	SD rats; oral/gavage; GD 1–PND 21; 0, 50, 250, 500 mg/kg/day; PND 4, 21, 70	- ↓ AGD (250/PND 4) - Cryptorchidism (500/PND 21) - Testicular pathology (testicular atrophy, epididymal agenesis (250/PND 70) - ↓ absolute epididymis weight (250/PND 70) - Altered sperm parameters at PND 70 (↓ epididymal sperm # (500), ↓ motility (250), ↓ sperm heads per testis (250)
(Wine et al., 1997; NTP, 1995)	CD SD rats; oral/feed; 2–generation (continuous breeding protocol); 0, 0.1, 0.5, 1.0% (eq. 53, 256, 509 mg/kg/d for males and 80, 385, 794 mg/kg/d for females)	- ↓ mating, pregnancy, and fertility indices (509–794/F1) - ↓ absolute testis, relative SV & prostate weight (509/F1) - ↓ epididymal sperm number & total spermatid heads in the testis (509/F1) - Testicular pathology in F1 (degeneration of seminiferous tubules (256), interstitial cell hyperplasia (509), underdeveloped epididymis (509), apparent sperm content reduction (509) Unaffected outcomes - Mating, pregnancy and fertility indices (F0); epididymal sperm motility and percent abnormal (F1)
(Higuchi et al., 2003)	Dutch-Belted rabbits; oral/gavage; GD 15–29; 0, 400 mg/kg/d; PNW 6–25	- Undescended testes in 1/17 pups (400/PNW 12); in same pup malformed prepuce, hypospadias, hypoplastic seminal vesicle and prostate, and agenesis of bulbourethral gland also observed - ↓ paired testes (400/PNW 12) & accessory sex gland weight (400/PNW 12, 25) - Altered sperm parameters (↓ ejaculate volume, ↓ sperm concentration, ↓ total sperm/ejaculate, ↓ morphologically normal sperm, ↑ acrosome-nuclear defects) (400/PNW 22–24) - ↑ incidence of histopathological changes in the seminiferous epithelium (400/PNW 25) - ↓ Serum testosterone (400/PNW 6) Unaffected outcomes - Epididymal weight (PNW 12, 25); agenesis of epididymides; mating ability (PND 22–24); sperm parameters (daily sperm production, caput epididymal sperm reserve, cauda

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
		epididymal sperm reserve) (PNW 22–24); serum testosterone (PNW 12, 25)
(McKinnell et al., 2009)	Marmoset; oral/gavage; GW 7–15; 0, 500 mg/kg/d MBP; PND 1–5 (birth, n=6 offspring) or 18–21 months of age (adult, n = 5)	- ↑ Incidence of clusters of undifferentiated germ cells in 2 out of 6 animals (400/birth) Unaffected outcomes - Reproductive tract malformations (hypospadias, cryptorchidism, small testes/impaired spermatogenesis, focal testicular dysgenesis) - Plasma testosterone (birth) - Absolute testis weight - # of germ cells/testis, germ cell proliferation or differentiation, # Sertoli cells/testis, germ cell:Sertoli cell ratio - MNGs - Germ cell # and proliferation, Sertoli cell #, germ:Sertoli cell ratio
[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation. AGD = anogenital distance; e = embryonic day; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle		

B.5 DIBP Study Summaries

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Howdeshell et al., 2008)	SD rats; oral/gavage; GD 8–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300)
(Hannas et al., 2011)	SD rats; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ expression of steroidogenic genes in fetal testes (*StAR* (300), *Cyp11a* (100))
(Gray et al., 2021)	Harlan SD rats; oral/gavage; GD 14–18; 0, 100, 200, 300, 500, 600, 750, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (300) and steroidogenic genes (*e.g.*, *Star* (200), *Cyp11a1* (300), *Hsd3b* (200), *Scarb1* (200) *Cyp17a1* (200)))
	Charles River SD rats; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (300) - ↓ fetal testicular expression of *Insl3* (300) and steroidogenic genes (*e.g.*, *Star* (600), *Cyp11a1* (600), *Hsd3b* (600), *Scarb1* (600), *Cyp17a1* (600))
(Borch et al., 2006a)	Wistar rats; oral/gavage; GD 7–19; 0, 600 mg/kg/d; GD 19	- ↓ fetal testicular testosterone content & *ex vivo* testicular testosterone production (600, not statistically significant) - ↓ AGD (600) - Testicular pathology (Leydig cell clusters, ↓ staining intensity of StAR in Leydig cells) (600)
	Wistar rats; oral/gavage; GD 7–21; 0, 600 mg/kg/d; GD 20/21	- ↓ fetal testicular testosterone content & *ex vivo* testicular testosterone production (600) - ↓ AGD (600)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
		- Testicular pathology (Leydig cell clusters, Sertoli cell vacuolization, central localization of gonocytes, MNGs, ↓ staining intensity of StAR and P450scc in Leydig cells) (600)
(Boberg et al., 2008)	Wistar rats; oral/gavage; GD 7–19; 0, 600 mg/kg/d; GD 19	- ↓ testicular mRNA expression of *SR-B1, StAR, P450scc, Cyp17, Insl3 (*600)
	Wistar rats; oral/gavage; GD 7–21; 0, 600 mg/kg/d; GD 21	- ↓ testicular mRNA expression of *SR-B1, StAR, P450scc, Cyp17, Insl3 (*600)
(Saillenfait et al., 2006)	SD rats; oral/gavage; GDs 6–20; 0, 250, 500, 750, 1000 mg/kg/d; GD 21	- ↑ incidence ectopic testis (750) - Unilateral or bilateral undescended testes (500) - ↑ incidence of ureter variations (1,000) - ↑ degree of transabdominal testicular migration in relation to the bladder (500)
(Saillenfait et al., 2008)	SD rats; oral/gavage; GDs 12–21; 0, 125, 250, 500, 625 mg/kg/d; PND 1–122	- ↓ AGD (250/PND 1) - NR (250/PND 12–14 & PND 76–122) - Delayed PPS (500) - Reproductive tract malformations ((hypospadias, exposed os penis, nonscrotal testes) (500/PND 76–122) - Underdeveloped or absent testis and/or epididymis (250/PND 76–122); cleft prepuce (625/PND 76–122)) - ↓ absolute weight of testes (625/PND 76–122), epididymis (500/PND 76–122), SV (500/PND 76–122), prostate (250/PND 76–86; 500/PND 111–122) - Testicular pathology (epididymal oligospermia or azoospermia (250/PND 76–86), interstitial cell hyperplasia (500/PND 76–86), tubular necrosis (250/PND 76–86), tubular atrophy/hypoplasia (250/PND 76–86))
(Saillenfait et al., 2017)	SD rats; oral/gavage; GDs 13–19; 0, 250 mg/kg/d; GD 19	- ↓ fetal testes testosterone production (250) - ↓ AGD (250) - ↓ expression of steroidogenic genes (*Hmg-CoAR, Hmg-CoAS, StAR, SR-B1, P450c17*) in fetal testes (250) Unaffected outcomes - mRNA expression of *P450scc, 3B-HSD* in fetal testes; external malformations
(Wang et al., 2017)	ICR mice; dietary; GD 0–21; 0, 450 mg/kg/d; PND 21–80	- ↓ testosterone in serum & testes (450/PND 21) - ↓ absolute testes weight (450/PND 21) - ↓ mRNA and protein levels of steroidogenic genes (450/PND 21 & 80) Unaffected outcomes - AGD (PND 21); absolute epididymal weight (PND 80); testosterone in serum & testes (PND 80); sperm concentration & motility (PND 80)
	ICR mice; dietary; GD 0–PND 21; 0, 450 mg/kg/d; PND 21–80	- ↓ testosterone in serum & testes (450/PND 21 & 80) - ↓ absolute testes weight (450/PND 21) - ↓ mRNA and protein levels of steroidogenic genes (450/PND 21 & 80) - ↓ Sperm concentration & motility (450/PND 80) Unaffected outcomes - AGD (PND 21); absolute epididymal weight (PND 80)

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation. AGD = anogenital distance; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle		

B.6 DCHP Study Summaries

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Hoshino et al., 2005)	SD rats; oral/feed; 2-generation study; 0, 240, 1200, 6000 ppm (equivalent to 16, 80, 402 & 18, 90, 457 mg/kg/d for F0 and F1 males; 21, 104, 511 and 21, 107, 534 mg/kg/d for F0 and F1 females)	- ↓ AGD (511/F1 pups (PND 4)) (107/F2 pups (PND 4)) - NR (511/F1 pups (PND 14)) (107/F2 pups (PND 12)) - Soft small sized testes (457/F1 adults) - ↓ absolute prostate weight (16/F1 adults) - Testicular pathology (seminiferous tubule atrophy (90/F1 adults) - ↓ spermatid head counts in the testes (90/F1 adults) Unaffected outcomes - Mating index, fertility index, gestation length, gestation index, birth index (F0 and F1 mating pairs); pup viability index or physical development (pinna unfolding, incisor eruption, eye opening) (F1, F2 pups); sperm motility (F0, F1 adults), cauda epididymal sperm count (F0, F1 adults), abnormal or tailless sperm (F0, F1 adults), and spermatid head counts in testis (F0 adults); serum hormone (testosterone, FSH, LH) levels (F0, F1 adult males); absolute prostate weight (F0 adults); testicular pathology (F0 adults); PPS (F1)
(Saillenfait et al., 2009b)	SD rats; oral/gavage; GD 6–20; 0, 250, 500, 750 mg/kg/d; GD 21	- ↓ AGD (250) Unaffected outcomes - Testes descent
(Yamasaki et al., 2009)	SD rats; oral/gavage; GD 6-PND 20; 0, 20, 100, 500 mg/kg/d; PND 4–70	- ↓ AGD (500/PND 4) - NR (500/PND 13) - Delayed PPS (500) - ↓ relative prostate & LABC weight (500/PND 70) - ↓ testicular germ cells (500/PND 70) - ↑ incidence of hypospadias (500/PND 49–70) Unaffected outcomes - Relative testis, epididymis, SV weight (PND 70)
(Furr et al., 2014)	SD rats; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d (Block 23) or 0, 33, 100, 300 mg/kg/d (Block 33); GD 18	- Block 23: ↓ *ex vivo* fetal testes testosterone production (100) - Block 33: ↓ *ex vivo* fetal testes testosterone production (100)
(Gray et al., 2021)	Harlan SD rats; oral/gavage; GD 14–18; 0, 33, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (33) - ↓ fetal testicular expression of *Insl3* (100) and steroidogenic genes (*e.g.*, *Star* (100), *Cyp11a1* (100), *Hsd3b* (100), *Scarb1* (100), *Cyp17a1* (100)))

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
	Charles River SD rats; oral/gavage; GD 14–18; 0, 100, 300, 600, 900 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (100) - ↓ fetal testicular expression of *Insl3* (100) and steroidogenic genes (*e.g.*, *Star* (100), *Cyp11a1* (300), *Hsd3b* (100), *Scarb1* (100), *Cyp17a1* (100))
(Ahbab and Barlas, 2015)	Wistar albino rats; oral/gavage; GD 6–19; 0, 20, 100, 500 mg/kg/d; GD 20	- ↓ immunohistochemical staining for 3β-HSD (20) - ↓ serum testosterone (100) - ↓ AGD (20) - Testicular pathology (atrophic seminiferous tubules (20), ↓ germ cells in tubules (20), Sertoli cell only tubules (100), detached cells from tubular wall (20), MNGs (100)) - ↑ number of medium and large Leydig cell clusters (20))
(Li et al., 2016)	SD rats; oral/gavage; GD 12–21; 0, 10, 100, 500 mg/kg/d; GD 21.5	- ↓ testes mRNA for *Star* (10), *Scarb1* (500), *hsd3b1* (10), *hsd17b3* (10), *Insl3* (100), *Lhcgr* (1000) - ↓ testicular testosterone (100) - ↓ AGD (100) - Testicular pathology (focal testis dysgenesis (100), MNGs (100)); ↓ Leydig cell size, cytoplasmic size, nuclear size (10); ↑ # & size of Leydig cell clusters (10))

[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation.
AGD = anogenital distance; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle

B.7 DINP Study Summaries

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Borch et al., 2004)	Wistar rats; oral/gavage; GD 7–21; 0, 750 mg/kg/d; CAS no. 28553-12-0; GD 21	- ↓ Testicular testosterone (750) - ↓ *ex vivo* testicular testosterone production (750) Unaffected outcomes - Plasma testosterone
(Furr et al., 2014)	Harlan SD rats; oral/gavage; GD 14–18; 0, 750 mg/kg/d; CASRN not reported (Block 1 and 5 reported to use DINP from Exxon; Block 7 reported to use DINP from BASF); GD 18 (2 h post final dose)	- ↓ *ex vivo* fetal testes testosterone production (750) (effect reported for Blocks 1, 5, and 7 studies)
(Hannas et al., 2011)	SD rats; oral/gavage; GD 14–18; 0, 500, 750, 1,000, 1,500 mg/kg/d; CASRN 28553-12-0 & 68515-48-0; GD 18	- ↓ *ex vivo* fetal testes testosterone production (500) - ↓ expression of steroidogenesis genes in fetal testes (*i.e.*, *StAR* (1000), *Cyp11a* (1000))
(Gray et al., 2021)	Harlan SD rat; oral/gavage; GD 14–18; 0, 500, 750, 1000, 1500 mg/kg/d; GD 18	- ↓ *ex vivo* fetal testes testosterone production (500) - ↓ fetal testicular expression of *Insl3* (500) and steroidogenic genes (*e.g.*, *Star* (500), *Cyp11a1* (500), *Cyp11b2* (1000), *Hsd3b* (500), *Scarb1* (500))

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Adamsson et al., 2009)	SD rats; oral/gavage; e13.5–17.5; 0, 250, 750 mg/kg/d; CASRN not reported; e19.5	- ↑ Testicular mRNA expression of *GATA-4* (750) and *Insl3* (750) Unaffected outcomes - Testicular testosterone, testicular pathology, testicular protein expression of StAR, P450scc, 3β-HSD, AR; testicular mRNA expression of *Star*, *P450scc, 3β-HSD*, *SF-1*
(Boberg et al., 2011)	Wistar rats; gavage; GD 7–PND 17; 0, 300, 600, 750, 900 mg/kg/d; CASRN 28553-12-0; GD 21, PND 13, PND 90	- ↓ Testicular testosterone (600/GD 21 (no dose-response)) - ↓ AGD (900/PND 13) - NR (750/PND 13) - Testicular pathology (MNGs (600/GD 21); enlarged diameter of seminiferous chords (750/GD 21), gonocytes with central location in chords (750/GD 21)) - ↓ sperm motility (600/PND 90) Unaffected outcomes - Serum testosterone (GD 21), *ex vivo* testicular testosterone production (GD 21); testes testosterone (PND 90); NR (PND 90); AGD (PND 90); reproductive organ weight (PND 90); testicular pathology (PND 90)
(Clewell et al., 2013a)	SD rats; oral/gavage; GD 12–19; 0, 50, 250, 750; CASRN 68515-48-0; GD 19 (2 h post-dosing) or GD 20 (24 h post-dosing)	- ↓ Testicular testosterone (250/GD 19) - Testicular pathology (MNGs (250/GD 20), Leydig cell aggregates (750/GD 20)) Unaffected outcomes - Testicular testosterone (GD 20); AGD (GD 20)
(Clewell et al., 2013b)	SD rats; oral/feed; GD 12–PND 14; 0, 760, 3,800, 11,400 ppm (est. 0, 56, 288, 720 mg/kg/d); CASRN 68515-48-0; PND 2, 14 or 49	- ↓ AGD (720/PND 14) - Testicular pathology (↑ Leydig cell aggregates (720/PND 2), MNGs (288/PND 2)) Unaffected outcomes - Testicular testosterone (PND 2, 49); AGD (PND 2, 49); NR (PND 14, 49); absolute testis and epididymis weight (PND 2, 49); absolute testes, epididymis, SV, ventral prostate, glans penis, LABC, Cowper's Glands weight (PND 49); testicular pathology (PND 49); PPS; reproductive tract malformations (*e.g.*, hypospadias, exposed os penis, undescended testes, epididymal agenesis) (PND 49)
(Masutomi et al., 2003)	SD rats; diet; GD 15–PND 10; 0, 400, 4,000, 20,000 ppm (est. 31–66, 307–657, 1165–2,657 mg/kg/d); CASRN 28553-12-0; PNDs 2, 27, or 77	- ↓ Absolute testes weight (20,000/PND 27) - Testicular pathology (degeneration of Sertoli cells (20,000/PND 77), degeneration of stage XIV meiotic spermatocytes (20,000/PND 77), scattered cell debris in ducts of epididymis (20,000/PND 77))) Unaffected outcomes - AGD (PND 2); PPS; absolute testes weight (PND 77)
(Li et al., 2015a)	SD rats; oral/gavage; GD 12–21; 0, 10, 100, 500, 1,000 mg/kg/d; CASRN not provided; GD 21.5	- ↓ testicular testosterone (1,000) - ↓ testicular gene expression (*Insl3* (10), *Lhcgr* (500), *Star* (500), *Cyp11a1* (100), *Hsd3b1* (100), *Cyp17a1* (100), *Hsd17b3* (1,000)) - Testicular pathology (focal testis dysgenesis (100); MNGs (100); clusters of Leydig cells (10)) Unaffected outcomes

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
		- AGD
(Gray et al., 2000)	SD rats; oral/gavage; GD 14–PND 3; 0, 750 mg/kg/d; CASRN 68515-48-0; PND 2–mature adults (3–7 months of age)	- NR in 2/52 male pups (750/PND 13) - Permanent nipples (750/3–7 months) - Reproductive malformations (small and atrophic testes, fluid filled testes lacking sperm, epididymal agenesis) Unaffected outcomes - Serum testosterone levels, PPS, AGD (PND 2), reproductive malformations (hypospadias, cleft phallus, vaginal pouch, SV agenesis, undescended testes, testis absent, abnormal gubernacular cord (3–7 months)), reproductive organ weight (*i.e.*, testes, LABC, SC, glans penis, ventral prostate, epididymis, cauda epididymis, caput-corpus epididymis)
(Waterman et al., 2000)[2]	SD rats; oral/feed; 1-generation study (10 wks prior to mating–PND 21); 0, 0.5, 1.0, 1.5% (est. 0, 360–923, 734–1731, 1,087–2,246 mg/kg/d); CASRN 68515-48-0	- ↑ absolute testes and epididymis (left only) weight in P0 (1.5%) Unaffected outcomes - Reproductive indices (*e.g.*, mating index, fertility index, gestation index, birth index, sex ratio); absolute epididymis, prostate, SV weight in P0; repro
(Waterman et al., 2000)[3]	SD rats; oral/feed; 2-generation study; 0, 0.2, 0.4, 0.8% (est. 133–153, 271–307, 543–577 mg/kg/d during gestation); CASRN 68515-48-0	Unaffected outcomes (both generations) - Reproductive indices (*e.g.*, mating index, fertility index, gestation index, birth index, sex ratio); absolute testes, epididymis, prostate and SV weight; testicular pathology

[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation.
AGD = anogenital distance; e = embryonic day; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle

B.8 DIDP Study Summaries

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Hellwig et al., 1997)	Wistar rats; oral/gavage; GD 6–15; 0, 40, 200, 1,000 mg/kg/d; GD 21	- No phthalate syndrome-related effects reported (exposure did not cover critical window)
(Waterman et al., 1999)	SD rats; oral/gavage; GD 6–15; 0, 100, 500, 1,000 mg/kg/d; GD 21	- No phthalate syndrome-related effects reported (exposure did not cover critical window)
(Hannas et al., 2012)	SD rats; oral/gavage; GD 14–18; 0, 500, 750, 1,000, 1,500 mg/kg/d; GD 18	Unaffected outcomes - *Ex vivo* testes testosterone production; steroidogenic gene expression
(Furr et al., 2014)	SD rats; oral/gavage; GD 14–18; 0, 500, 750, 1,000, 1,500 mg/kg/d; GD 18	Unaffected outcomes - *Ex vivo* testes testosterone production
(Gray et al., 2021)	Charles River SD rat; oral/gavage; GD 14–18; 0, 300, 750, 1,000, 1,500 mg/kg/d; GD 18	Unaffected outcomes - *Ex vivo* testes testosterone production - Steroidogenic gene expression in the fetal testes

Reference	Study Design [a]	Reported Phthalate Syndrome-Related Effects (LOAEL/LOEL [mg/kg/day]/Timing of Evaluation [if Different than Listed under Study Design])
(Hushka et al., 2001)	SD rats; oral/feed; 2-generation study; 0, 0.2%, 0.4%, 0.8% (Study A)	Unaffected outcomes (both generations) - Reproductive indices (*i.e.*, mating, fertility, gestation and birth index); reproductive organ (*i.e.*, prostate, testes, epididymis, SV) weight; sperm parameters (sperm count, motility, morphology); testicular pathology, gross external abnormalities
(Hushka et al., 2001)	SD rats; oral/feed; 2-generation study; 0, 0.02, 0.06, 0.2%, 0.4% (Study B)	Unaffected outcomes (both generations) - Reproductive indices (*i.e.*, mating, fertility, gestation and birth index); AGD (PND 0); NR (PND 12–13); age at PPS, gross external abnormalities

[a] Study design comprises species/strain, exposure route, exposure duration, doses, and timing of evaluation.
AGD = anogenital distance; GD = gestation day; LABC = levator ani/bulbocavernosus muscle; NR = nipple retention; PPS = preputial separation; PND = postnatal day; PNW = postnatal week; PS = phthalate syndrome; SD = Sprague-Dawley; SV = seminal vesicle

Appendix C Methodology for Preliminary Dose-Response Modeling

C.1 General Approach

- Data from the peer-reviewed literature and cited in summary tables for each key outcome throughout Section 3.1.3 were combined to produce a single dose response curve for each high-priority and manufacturer-requested phthalate for several phthalate syndrome effects (*i.e.*, decreased AGD, nipple/areolae retention, testicular pathology, and hypospadias).
- Studies included in the analysis all exposed the pregnant rat to the phthalate ester during the male programing window (at a minimum). Studies included those that administered the chemical daily from oral gavage on a mg/kg/day basis and those that administered the chemical in the diet on a ppm basis. Studies used Charles River Sprague-Dawley, Wistar and other rat strains. Phthalate ester effects including testicular histology and hypospadias used only data from adult male rat offspring.
- Effects modeled included in male rat offspring included decreased AGD, percent of males per litter with retained nipples, percent with testicular histopathological lesions (*i.e.*, seminiferous tubule atrophy), and percent with hypospadias.
- Data were not modeled for phthalate syndrome effects when no data were available for one of the five high-priority phthalate esters (*i.e.*, DEHP, BBP, DIBP, DBP and DCHP).
- Similar models have been constructed for DINP when effects are equal to or exceed 15 percent of control, but some of the data are not yet peer-reviewed and were not included in the preliminary dose-response analysis.
- Data were fit using GraphPad Prism 8 software to four parameter logistic regression models (4PL). For each effect the top and bottom of the curve was constrained, as appropriate (described in more detail below). Since an RPF approach for CRA is being proposed, the slope was constrained to "shared value for each dataset." This improves the confidence in the ED50 value and 95 percent confidence intervals (95 percent CI) and is a biologically plausible approach because available data indicate that these phthalate esters share a common MOA.

C.2 Anogenital Distance (AGD)

C.2.1 Calculation of Individual Phthalate Ester AGD Dose-Response Models

AGD data from studies published in the literature was entered by study into a GraphPad Prism data file for each individual phthalate ester. All the studies reported AGD for males and some reported the female offspring AGD as well. The age at AGD measurement ranged from late fetal life to 4 days of age, although most studies measured AGD at 1 to 2 days of age. The data for each study was normalized with the control male AGD being 100 percent (the top of the curve) and control female being 0 percent (the bottom of the curve). If the female AGD was not reported then the bottom of the curve was assigned a value of 50 percent of the control male AGD, a value that consistently seen in studies with phthalates and other chemicals. The data from all the studies was combined into a single data set, sorted by dose (in units of mg/kg/day if administered by oral gavage to the dam daily or estimated mg/kg/day if administered in ppm in the diet).

The normalized data for each phthalate ester was entered into a single GraphPad Prism file and 4PL models were run with the bottom constrained to 0 percent, the top constrained to a shared value less than

110 percent, and the slope constrained to a shared value for each phthalate ester, and the ED50 value and 95 percent CI for each PE was estimated.

C.3 Nipple/Areolae Retention in 13 to 14 Day Old Infant Male Rats

C.3.1 Calculation of Individual Phthalate Ester Dose-Response Models

Nipple/areolae data from studies published in the literature was entered by study into a GraphPad Prism data file for each individual phthalate ester. All the studies reported nipple retention for males as percent of males/litter showing any retained areolae/nipples (irrespective if a male had 1 or 12 areolae). Data on the number of nipples (*i.e.,* 1–12) per male were not reported for all five phthalate esters, and this measure of nipple retention was not used for dose-response modeling. The data from all the studies was combined into a single data set, sorted by dose (in units of mg/kg/day if administered by oral gavage to the dam daily or estimated mg/kg/day if administered in ppm in the diet).

The normalized data for each phthalate ester was entered into a single GraphPad Prism file and 4PL models were run with the bottom constrained to a shared bottom greater than 0 percent, the top constrained to 100 percent, and the slope constrained to a shared value for each PE, and the ED50 value and 95 percent CI for each phthalate ester was estimated.

C.4 Testicular Pathology – Seminiferous Tubule Atrophy

C.4.1 Calculation of Individual Phthalate Ester Dose-Response Models

In the phthalate syndrome, histopathology of the testis and epididymis often occur concurrently along with gross malformations of these tissues and these effects are among the more sensitive effects resulting from *in utero* phthalate ester exposure. One of the most frequently reported effects following phthalate ester exposure is seminiferous tubular atrophy/agenesis of the testis in adult male rats (it can be with uni- or bilateral). Histopathology of the epididymis is less frequently reported. Only histopathology scores greater than 1 (minimal effect or single tubule affected) were used in the initial dose-response analysis. Abnormal testis differentiation can result from direct testicular effects of the phthalate ester on the endocrine and paracrine environment disrupting seminiferous tubular development, Leydig cell differentiation and vasculature differentiation of the testis during fetal and neonatal life. Histopathological alterations of the testes also can also result from indirect *in utero* effects post-puberty as a result of excessive fluid back pressure in the testis caused by epididymal abnormalities that prevent fluid and sperm flow from the testis and from testis nondescent associated with gubernacular abnormalities (spermatogenesis does not occur in such testes being temperature sensitive). The data from all the studies was combined into a single data set, sorted by dose (in units of mg/kg/day if administered by oral gavage to the dam daily or estimated mg/kg/day if administered in ppm in the diet).

The normalized data for each phthalate ester was entered into a single GraphPad Prism file and 4PL models were run with the bottom constrained to shared bottom greater than 0 percent, the top constrained to 100 percent, and the slope constrained to a shared value for each PE, and the ED50 value and 95 percent CI for each phthalate ester was estimated.

C.5 Hypospadias

In the phthalate syndrome, malformations of external genitalia are one of the least sensitive effects in some rat strains. All the studies reported incidence of hypospadias for adult F1males following gestational exposure to each of the individual phthalate esters. The data from all the studies was

combined into a single data set, sorted by dose (in units of mg/kg/day if administered by oral gavage to the dam daily or estimated mg/kg/day if administered in ppm in the diet).

The normalized data for each phthalate ester was entered into a single GraphPad Prism file and 4PL models were run with the bottom constrained to 0 percent, the top constrained to 100 percent, and the slope constrained to a shared value for each PE and the ED50 value and 95 percent CI for each phthalate ester was estimated.

Appendix D Occupational Exposure Assessment

EPA Program Data for Identifying Sites

- **CDR:** CDR can be used to identify manufacturing and import sites that handle multiple phthalates. All six phthalates are reported in CDR and represent all import and manufacturing sites producing the chemical at or above a specified threshold. Because CDR reporting is done on a site-by-site basis, any site reporting more than one of the designated phthalates is a site with cumulative exposure and release potential. Both 2016 and 2020 CDR, as well as record relevant data, can be used for each instance of phthalate production including the number of workers, chemical concentration, and volume of chemical.
- **TRI, DMR. and NEI:** These EPA release datasets can be used similarly to the CDR data for determining sites with potential for cumulative exposure and release. Only a portion of the selected phthalates are required to report to some of these programs, however, limiting the dataset utility. Nonetheless, the datasets can be useful for determining cumulative releases between the reporting chemicals. North American Industry Classification System code and other facility reporting parameters will be used to assign COUs to EPA release datasets.
- **RCRAInfo:** This dataset is split into multiple modules, with the two main modules of interest for release assessment being the E-manifest module and the Biennial Report module. All hazardous waste shipments are reported in the E-manifest module and represent movement from the hazardous waste generator to treatment, storage, or disposal facilities. E-manifest reporting does not have a reporting threshold. The Biennial Report is an annual summary of hazardous waste that is generated at a facility, including the quantity and nature of the waste and its disposition (*i.e.*, recycling, treatment, storage, or disposal). Only Large Quantity Generators (LQC) are required to submit a Biennial Report, but LQCs are defined by the overall volume of hazardous waste and is not chemical specific like CDR, TRI, or NEI and therefore the dataset may provide EPA with a better understanding of some sites handling smaller quantities of phthalates. Like many other EPA programs, only a portion of the selected phthalates are required to report, limiting the overall utility of RCRAInfo for release assessments.

Appendix E Glossary of Key Terms

Additivity (U.S. EPA, 2007b, 2000): "when the effect of the combination of chemicals can be estimated directly from the sum of the scaled exposure levels (dose addition) or of the responses (response addition) of the individual components."

Aggregate exposure (40 CFR § 702.33): "means the combined exposures to an individual from a single chemical substance across multiple routes and across multiple pathways."

Best available science (40 CFR § 702.33): "means science that is reliable and unbiased. Use of best available science involves the use of supporting studies conducted in accordance with sound and objective science practices, including, when available, peer reviewed science and supporting studies and data collected by accepted methods or best available methods (if the reliability of the method and the nature of the decision justifies use of the data). Additionally, EPA will consider as applicable:

(1) The extent to which the scientific information, technical procedures, measures, methods, protocols, methodologies, or models employed to generate the information are reasonable for and consistent with the intended use of the information;

(2) The extent to which the information is relevant for the Administrator's use in making a decision about a chemical substance or mixture;

(3) The degree of clarity and completeness with which the data, assumptions, methods, quality assurance, and analyses employed to generate the information are documented;

(4) The extent to which the variability and uncertainty in the information, or in the procedures, measures, methods, protocols, methodologies, or models, are evaluated and characterized; and

(5) The extent of independent verification or peer review of the information or of the procedures, measures, methods, protocols, methodologies or models."

Biomonitoring (U.S. EPA, 2019a): "measures the amount of a stressor in biological matrices."

Category of chemical substances (15 U.S.C. § 2625(c)(2)(A)): "means a group of chemical substances the members of which are similar in molecular structure, in physical, chemical, or biological properties, in use, or in mode of entrance into the human body or into the environment, or the members of which are in some other way suitable for classification as such for purposes of [TSCA], except that such term does not mean a group of chemical substances which are grouped together solely on the basis of their being new chemical substances."

Chemical substance (15 U.S.C. § 2602(2)): "means any organic or inorganic substance of a particular molecular identity, including—(i) any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature, and (ii) any element or uncombined radical. Such term does not include—(i) any mixture, (ii) any pesticide (as defined in the Federal Insecticide, Fungicide, and Rodenticide Act [7 U.S.C. 136 et seq.]) when manufactured, processed, or distributed in commerce for use as a pesticide, (iii) tobacco or any tobacco product, (iv) any source material, special nuclear material, or byproduct material (as such terms are defined in the Atomic Energy Act of 1954 [42 U.S.C. 2011 et seq.] and regulations issued under such Act), (v) any article the sale of which is subject to the tax imposed by section 4181 of the Internal Revenue Code of 1986 [26 U.S.C. 4181] (determined without regard to any exemptions from such tax provided by section 4182 or 4221 or any other provision of such Code) and any component of such an article (limited to shot shells, cartridges, and components of shot shells and cartridges), and (vi) any food, food additive, drug, cosmetic, or device (as

such terms are defined in section 201 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321]) when manufactured, processed, or distributed in commerce for use as a food, food additive, drug, cosmetic, or device."

Condition of use (COU) (15 U.S.C. § 2602(4)): "means the circumstances, as determined by the Administrator, under which a chemical substance is intended, known, or reasonably foreseen to be manufactured, processed, distributed in commerce, used, or disposed of."

Consumer exposure (40 CFR § 711.3): Human exposure resulting from consumer use. This exposure includes passive exposure to consumer bystanders.

Consumer use (40 CFR § 711.3): "means the use of a chemical substance or a mixture containing a chemical substance (including as part of an article) when sold to or made available to consumers for their use."

Cumulative risk (U.S. EPA, 2003): "The combined risks from aggregate exposures to multiple agents or stressors."

Cumulative risk assessment (CRA) (U.S. EPA, 2003): "An analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors."

Dose additivity (U.S. EPA, 2007b, 2003, 2000): "when each chemical behaves as a concentration or dilution of every other chemical. The response of the combination of chemicals is the response expected from the equivalent dose of an index chemical (the chemical selected as a basis for standardization of toxicity of components in a mixture). The equivalent dose is the sum of component doses scaled by their toxic potency relative to the index chemical."

Fenceline exposure: General population exposures occuring in communities near facilities that emit or release chemicals to air, water, or land with which they may contact.

Index chemical (U.S. EPA, 2000): "The chemical selected as the basis for standardization of toxicity of components in a mixture. The index chemical must have a clearly defined dose-response relationship."

Integrated addition: a hybrid additivity approach that incorporates both dose addition and response addition for dichotomous endpoints, thus, producing a mixture estimate that is the probabilistic risk of the adverse endpoint of concern.

Margin of exposure (MOE) (U.S. EPA, 2002): "a numerical value that characterizes the amount of safety to a toxic chemical–a ratio of a toxicological endpoint (usually a NOAEL [no observed adverse effect level]) to exposure. The MOE is a measure of how closely the exposure comes to the NOAEL."

Mixture (15 U.S.C. § 2602(10)): "means any combination of two or more chemical substances if the combination does not occur in nature and is not, in whole or in part, the result of a chemical reaction; except that such term does include any combination which occurs, in whole or in part, as a result of a chemical reaction if none of the chemical substances comprising the combination is a new chemical substance and if the combination could have been manufactured for commercial purposes without a chemical reaction at the time the chemical substances comprising the combination were combined."

Mode of Action (MOA) (U.S. EPA, 2000): "a series of key events and processes starting with interaction of an agent with a cell, and proceeding through operational and anatomical changes causing disease formation."

Non-TSCA exposure: exposure that can be attributed to specific activities that are excluded from the TSCA definition of "chemical substance," under TSCA Section 3(2), such as a pesticide, food, food additive, drug, cosmetic, or medical device.

Occupational exposure: Exposure to a chemical substance by industrial or commercial employees.

Occupational non-users (ONU): Employed persons who do not directly handle the chemical substance but may be indirectly exposed to it as part of their employment due to their proximity to the substance.

Pathways (40 CFR § 702.33): "means the mode through which one is exposed to a chemical substance, including but not limited to: Food, water, soil, and air."

Point of departure (POD) (U.S. EPA, 2002): "dose that can be considered to be in the range of observed responses, without significant extrapolation. A POD can be a data point or an estimated point that is derived from observed dose-response data. A POD is used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures."

Potentially exposed or susceptible subpopulations (PESS) (15 U.S.C. § 2602(12)): "means a group of individuals within the general population identified by the Agency who, due to either greater susceptibility or greater exposure, may be at greater risk than the general population of adverse health effects from exposure to a chemical substance or mixture, such as infants, children, pregnant women, workers, or the elderly."

Reasonably available information (40 CFR § 702.33): "means information that EPA possesses or can reasonably generate, obtain, and synthesize for use in risk evaluations, considering the deadlines specified in TSCA section 6(b)(4)(G) for completing such evaluation. Information that meets the terms of the preceding sentence is reasonably available information whether or not the information is confidential business information, that is protected from public disclosure under TSCA section 14."

Response addition (U.S. EPA, 2007b, 2003, 2000): "When the toxic response (rate, incidence, risk, or probability of effects) from the combination is equal to the conditional sum of component responses as defined by the formula for the sum of independent event probabilities. For two chemical mixtures, the body's response to the first chemical is the same whether or not the second chemical is present."

Routes (40 CFR § 702.33): "means the particular manner by which a chemical substance may contact the body, including absorption via ingestion, inhalation, or dermally (integument)."

Sentinel exposure (40 CFR § 702.33): "means the exposure from a single chemical substance that represents the plausible upper bound of exposure relative to all other exposures within a broad category of similar or related exposures."

Stressor (U.S. EPA, 2019a): "Any chemical, physical or biological entity that induces an adverse response."

Toxicologic interactions (U.S. EPA, 2007b, 2000): "Any toxic responses that are greater than or less than what is observed under an assumption of additivity."

Weight of the scientific evidence (40 CFR § 702.33): "means a systematic review method, applied in a manner suited to the nature of the evidence or decision, that uses a pre-established protocol to comprehensively, objectively, transparently, and consistently, identify and evaluate each stream of evidence, including strengths, limitations, and relevance of each study and to integrate evidence as necessary and appropriate based upon strengths, limitations, and relevance."